117 • 1
2021

ACTA AGRICULTURAE SLOVENICA

Acta agriculturae Slovenica • eISSN 1854-1941 • 117 – 1 • Ljubljana, marec 2021

Acta agriculturae Slovenica **eISSN 1854-1941**
Letnik / Volume 117 · Številka / Number 1 · 2021

Acta agriculturae Slovenica izhaja s finančno pomočjo / *is published with the financial support:* Javne agencije za raziskovalno dejavnost Republike Slovenije / *Slovenian Research Agency*.

Acta agriculturae Slovenica je vključena v / *is included into*: Scopus (SJR, SNIP), DOAJ, WOS Zoological Records, CrossRef, CAB Abstracts, FSTA, Google Scholar, dLib, COBISS.

Ovitek: Gomolj krompirja, poškodovan od strun (*Agriotes* spp., Elateridae); (foto: Stanislav Trdan, 1–11)
Cover: Potato tuber damaged by wireworms (Agriotes spp., Elateridae); (photo: Stanislav Trdan, 1–11)

Acta agriculturae Slovenica
Volume / Letnik **117** · Number / Številka **1** · **2021**

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doi:10.14720/aas.2021.117.1.1859 Original research article / izvirni znanstveni članek

Comperative study of aboveground biomass and carbon storage between Tembawang and conventional rubber agroforestry in West Kalimantan Indonesia

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Received September 01, 2020; accepted December 08, 2020.
Delo je prispelo 01. septembra 2020, sprejeto 08. decembra 2020.

Comperative study of aboveground biomass and carbon storage between Tembawang and conventional rubber agroforestry in West Kalimantan Indonesia

Abstract: In the era of intensive oil palm and rubber plantations in Kalimantan, some local communities of Dayak's tribe in West Kalimantan preserved the traditional agroforestry system "Tembawang". In the last two decades, rubber has been planted traditionally by local communities since the expansion of rubber industries. This study aimed to compare tree above ground biomass (AGB) distribution and carbon storage in different DBH (diameter at breast height) classes between Tembawang and conventional rubber plantation in West Kalimantan, Indonesia. Vegetation transect analysis was carried out on two types of traditional agroforestry namely Tembawang and conventional rubber. AGB estimation was based on the existing allometric, carbon storage was estimated from the percentage of biomass. Total AGB of Tembawang was higher than conventional rubber plantation and significantly different ($p < 0.01$). The highest AGB accumulation both Tembawang and conventional rubber was found at above 50 cm diameter class. The aboveground carbon storage from Tembawang and conventional rubber plantation were 90.26 and 42.01 Mg C ha^{-1}, respectively. The highest contribution to carbon storage was found at above 50 cm diameter class, estimated 62.58 % from Tembawang and 49.24 % from conventional rubber. AGB and carbon storage at traditional agroforestry in West Kalimantan were greater than varied different agroforestry system, also the estimated value was closed to tropical secondary forests. Tembawang agroforestry has good potential contribution to carbon storage and conservation of native fruit trees of Kalimantan.

Key words: carbon storage; Dayak's tribe; ethnoecology; plantation; Tembawang

Primerjalna raziskava nadzemne biomase in shranjevanja ogljika med Tembawangom in konvencionalnim pridobivanjem kavčuka v Zahodnem Kalimantanu, Indonezija

Izvleček: V obdobju intenzivnega gojenja oljne palme in plantaž kavčukovca v Kalimantanu so nekatere lokalne skupnosti plemena Dajakov v Zahodnem Kalimantanu ohranile tradicionalni kmetijsko gozdarski sistem (agroforestry), imenovan "Tembawang". Kljub povečevanju industrije kavčuka v zadnjih desetletjih so nekatere lokalne skupnosti ohranile tradicionalno gojenje kavčukovca. V raziskavi sta bili primerjani nadzemna biomasa dreves (AGB) in porazdelitev ogljika v različnih debelinskih razredih dreves (DBH= premer drevesa na prsni višini) med sistemom Tembawang in konvencialno pridelavo kavčuka na plantažah v Zahodnem Kalimantanu, Indonezija. Analiza vegetacije je bila opravljena v dveh transektih tradicionalnega pridobivanja kavčuka imenovenega Tembawang in v konvencionalnih plantažah kavčukovca. Določitev nadzemne biomase je temeljila na alometričnih enačbah, zaloge ogljika so bile ocenjene iz odstotkov biomase. Celokupna nadzemna biomasa je bila v sistemu Tembawang večja kot v konvencionalnih plantažah kavčukovca in značilno različna ($p < 0,01$). Največja akumulacija nadzemne biomase je bila v obeh sistemih, Tembawangu in pri konvencionalni pridelavi kavčuka v debelinskih razredih nad 50 cm. Nadzemna zaloga ogljika je bila v sistemu Tembawang 90,26 Mg C ha^{-1} in 42,01 Mg C ha^{-1}pri konvencionalni pridelavi. Največji delež shranjenega ogljika je bil v debelinskih razredih dreves nad 50 cm, ocenjen na 62,58 % pri Tembawangu in 49,24 % pri konvencionalni pridelavi kavčuka. Nadzemna biomasa in zaloga ogljika sta bili v tradicionalnem kmečkem gozdarstvu Zahodnega Kalimantana večji kot v različnih konvencionalnih sistemih, tudi ocenjene vrednosti so bile blizu tistim v drugotnih tropskih gozdovih. Tembawang sistem kmečkega gozdarstva ima dober potencial za shranjevanje ogljika in ohranjanja samoniklih sadnih dreves v Kalimantanu.

Ključne besede: shranjevanje ogljika; pleme Dayak; etnoekologija; plantaža; Tembawang

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1 INTRODUCTION

Forest ecosystems are an important component of the terrestrial ecosystem. They contain more than half of biodiversity, and highly contribute to stored carbon. Trees are the main component in forest ecosystems containing about 677 Pg or 80 % of the total forest biomass (Kinderman et al., 2008). Forest ecosystems absorb carbon dioxide (CO_2) from the atmosphere through photosynthesis and increase in the average concentration of CO_2 in the atmosphere can be reduced through carbon sequestration (C) (IPCC, 2007).

Carbon sequestration in forest ecosystems has become an important issue both in global climate change, climate discussions and in forest ecosystem studies. Forest conversion into other land-uses such as agriculture, settlement, and also forest degradation can pose a serious threat to sustaining carbon storage. Carbon storage in a forest ecosystem is mainly determined by growth factors, mortality, decomposition, disturbance, forest succession, and climate variations (Gough et al., 2008).

Indonesia has the third-largest area of tropical forest after Brazil and Congo, with an area of 92.133 M ha (FAO, 2020). Furthermore, the potential for carbon stocks in natural forests in Indonesia is large, ranging from 7.5-264.70 t C ha^{-1} (Ministry of Forestry, 2010). In the period of 2000 to 2015, the rate of deforestation of primary forests in Indonesia is also very high (FAO, 2015). Deforestation and forest degradation in Indonesia are generally caused by the settlement expansion driven by population growth, forest logging, and conversion to plantation / agricultural land (Prasetyo et al., 2011).

Kalimantan (Borneo) is the second-largest island in Indonesia, with a vast expanse of natural forests with high diversity and endemicity (Rhee et al., 2004). Among 109 tree families found in Kalimantan, Dipterocarpaceae family was dominated by the forest composition and more than half species in this group were endemic species in Kalimantan (Soepadmo and Wong, 1995). Deforestation in tropical forests in Kalimantan from 2000-2017 reached 6.04 M ha, of which half was caused by land conversion to the plantation industry (Gaveau et al., 2017). According to Carlson et al. (2012), by 2020 the expansion of palm oil industries is projected to contribute about 0.12-0.15 Gt C $year^{-1}$ CO_2 equivalent emissions.

Tembawang represents the term of agroforestry management practices by Dayak's tribe in Kalimantan. This term also represents the other management systems by local communities such as forests, plantations, rice fields, and settlements (Wahyuni, 2002). Tembawang and rubber agroforestry are part of the tropical rainforest in West Kalimantan. This area is a very important natural resource to provide oxygen and preserving carbon. Over the past few decades, the expansion of plantations, agriculture, and settlements in West Kalimantan has been a serious threat to Tembawang and rubber forests. Required anticipation of land-use changes to provide a better climate from assessing population and biomass in Tembawang and traditional rubber agroforestry for enhancing carbon storage. Preliminary research has been conducted in this traditional agroforestry, a total of 43 species and 8 dominant species were found (Rafdinal and Pitopang, 2019). This study aims to compare the standing biomass of Tembawang and conventional rubber agroforestry related to carbon storage and mitigation of climate change.

2 MATERIALS AND METHODS

2.1 DESCRIPTION OF RESEARCH AREA

This study was located at Kayu Lapis Road, Kilometer 21, Nanga Pemubuh Village, Sekadau Hulu District, Sekadau Regency, West Kalimantan Province, Indonesia (Figure 1). Sekadau Hulu is one of 7 districts in Sekadau Regency, has an area of ± 869.7 km^2. Sekadau Hulu District is geographically located in the southern part of Sekadau Regency, consists of 15 villages including Nanga Pemubuh. Nanga Pemubuh village has an area of 89.92 km^2 and is located 55 km from the central district. In general, this region's has relatively high annual rainfall and wet tropical climate. Based on the record of Meteorological, Climatological, and Geophysical Agency of Indonesia (Indonesian Statistics, 2016), the highest rainfall in 2011 occurred in November (826 mm) and the lowest in May (118 mm). The average monthly temperature ranged from 24.8-27.3 °C, and there was no dry season. Soil conditions are generally very heterogeneous, with the pH of soil H_2O in horizon-A ranging from 3.69 to 5.55.

2.2 FOREST INVENTORY

Analysis of vegetation in each sampling site was carried out by a belt transect method. The transects were further divided into 20 m x 20 m plots. In each plot, all individuals with ≥ 10 cm DBH (Diameter at Breast Height) were tagged, measured, and identified. The girth and height of each individual were measured. The plant specimens were identified. Frequency, density, basal area, and Importance Value Index (IVI) were calculated following Misra (1968), Mueller-Dombois and Ellenberg (1974). Trees were grouped into five DBH classes i.e. 10-20 cm, 20-30 cm, 30-40 cm, 40-50 cm, and > 50 cm and the density and Aboveground biomass (AGB) distribution under each DBH class were analyzed.

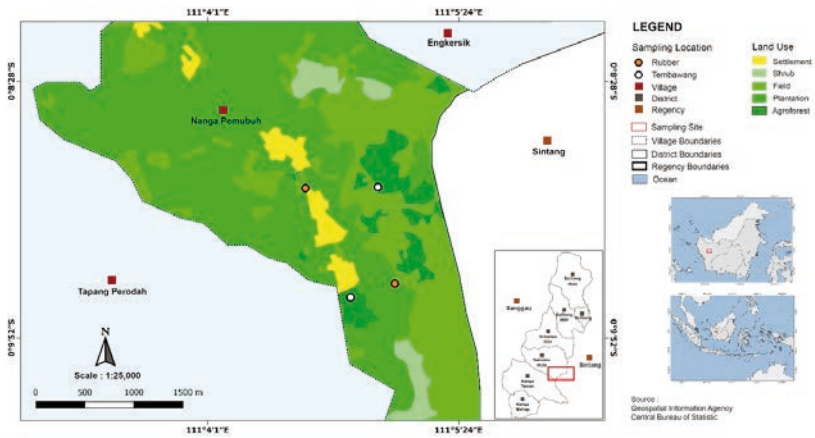

Figure 1: Location and land use of the study site

2.3 ESTIMATION OF ABOVEGROUND BIOMASS

The AGB in different tree diameter classes in each site was estimated using the following mentioned model (Ketterings et al., 2001):

$$AGB = 0.11\,\rho\,D^{2.62}$$

Where:
ρ = wood density
D = diameter at breast height

The aboveground biomass for all diameter classes was summed to calculate the total aboveground biomass in each agroforestry type. The values for the Tembawang and the traditional rubber were compared using t-test analysis.

2.4 ESTIMATION OF CARBON

The aboveground biomass carbon storage was calculated by assuming that the carbon content is 50 % of the total aboveground biomass (Brown, 1997; Cannel and Dewar, 1995; Dixon et al., 1994; Ravindranath et al., 1997; Richter et al., 1995; Schroeder, 1992).

3 RESULTS AND DISCUSSION

3.1 STAND CHARACTERISTICS

Twenty-eight species were recorded from the Tembawang agroforestry, and 31 species were recorded from the conventioal rubber plantation. The density of woody species (≥ 10 cm DBH) was greater in Tembawang (195 trees ha^{-1}) than in traditional rubber agroforestry (190 trees ha^{-1}), but not statistically different ($p > 0.5$). Based on density, *Hevea brasiliensis* Müll. Arg. (57.50 trees ha^{-1}) and *Durio zibethinus* L. (26.25 trees ha^{-1}) were the dominant species in the Tembawang agroforestry and these two species accounted for 31 % of ha^{-1}) and *Durio zibethinus* (18.33 trees ha^{-1}) were the dominant and codominant species, respectively. The basal area was greater in the Tembawang agroforestry (1,560.97 m^2 ha^{-1}) than in the conventional rubber plantation (811.27 m^2 ha^{-1}) (Table 1).

Tembawang and traditional rubber agroforestry in Dayak Tribe were implemented for decades. Both Tembawang and conventionalrubber agroforestry were managed traditionally from generation to generation. The differences in these systems is based on the constituent trees species of each agroforestry type (Rafdinal and Pitopang, 2019). Tembawang is former forest land that is used for planting fruit trees for providing food or traditional ceremonies need by local people, therefore the 3 main constituent species are *Durio zibethinus, Nephelium lappaceum*, and *Artocarpus* sp. However, in the recent decades, Tembawang agroforestry has began to be planted rubber trees by local people, and the latex is sold to industries or companies in the city, therefor now the rubber trees dominated Tembawang agroforestry.

Rubber plantation was generally formed from land clearing by local communities in the decade 2000-2010 in West Kalimantan to support the national rubber industry, rubber latex had high selling value at that time (Indonesia-Investment, 2018). However, some conventional rubber plantations still preserved trees used for daily needs such as wood and fruit trees. Thus, *Durio zibethinus* and *Shorea* species still exist besides the dominant rubber trees.

Table 1: Stand characteristics of Tembawang and conventional rubber agroforestry of West Kalimantan

	Agroforestry	
Variables	Tembawang	Conventional Rubber
Species richness (number of species)	28	31
Stand density (trees ha^{-1})	195	190
Density of dominant tree species:		
Durio zibethinus L.	26.25	18.33
Hevea brasiliensis Müll. Arg.	57.50	38.33
Nephelium lappaceum L.	11.25	10.00
Artocarpus sp.	13.75	10.00
Shorea stenoptera Burck.	12.50	10.00
Artocarpus integer Merr.	10.00	-
Anacardium occidentale L.	-	10.00
Shorea leprosula Miq.	-	13.33
Shorea macrophylla (de Vr.) Ashton	-	10.33
Basal area (m^2 ha^{-1})	1,560.97	811.27

3.2 ABOVEGROUND BIOMASS DISTRIBUTION

Although the young individuals belonging to 10-20 cm DBH class dominated both forests for tree density (Figure 2), the AGB accumulation was greater in the up 50 cm diameter class in Tembawang agroforestry forest and traditional rubber plantation (Figure 3). The contribution of AGB by higher diameter classes generally increases in both agroforestry. The contribution of > 50 cm diameter trees to AGB is greater in Tembawang agroforestry (62.58 %) than in traditional rubber plantations (49.24 %).

Rubber growth is faster than in other trees in these agroforestry systems, latex harvesting of rubber trees was carried out when entering 5-6 years vegetation ages with 45 cm girth or about 15 cm trunk diameter for 25-30 years production period (Damanik et al., 2010). After production age, rubber trees were usually felled and replanted, so that the highest density of rubber agroforestry was dominantly distributed in the lower diameter class, whereas tree density in Tembawang agroforestry was mainly distributed in the higher diameter class, covered by old fruit trees that have been down through generations. Further, the difference in density distribution from diameter class caused the basal area and overall AGB value of Tembawang were greater than rubber plantations.

3.3 TOTAL ABOVEGROUND BIOMASS AND CARBON STORAGE

The total AGB of Tembawang agroforestry (180.51 Mg ha^{-1}) was significantly greater ($p < 0.01$) than traditional rubber plantation (85.01 Mg ha^{-1}). A primary forest can store AGB of up to ± 350 Mg ha^{-1} while a secondary forest varies considerably between 59 - 140 Mg ha^{-1} (Stas, 2014). The value of AGB in a forest ecosystem depends on the composition and structure of the forest. The AGB value of rubber agroforestry was in the range of secondary forest values and was still in the intensive tree crop plantation value range of 60-120 Mg ha^{-1} (Hairiyah et al., 2011). The ABG value of Tembawang agroforestry was higher than the average AGB value of the secondary forest. The composition and structure of Tembawang agroforestry were quite varied and the conditions had been preserved for generations so that it had large AGB potential, but not as large as primary forest.

The aboveground carbon stored by Tembawang and traditional rubber forests was 90.26 and 42.01 Mg C ha^{-1}, respectively and were significantly different ($p < 0.01$). Carbon organic was mostly stored in up 50 cm DBH class in Tembawang (62.58 %) and traditional rubber (49.24 %). The younger (10-20 cm DBH class) trees, which had the highest density in both forests, stored only 3.52 % of total carbon in the Tembawang agroforestry and 5.52 % of traditional rubber (Table 2).

Aboveground carbon storage of Tembawang agroforestry was two times higher than rubber plantations, in terms of carbon storage Tembawang agroforestry was better, but the age of the community and vegetation cycle also to be considered. When compared to other agroforestry systems, the Tembawang agroforestry system stored more carbon. In a comprehensive report

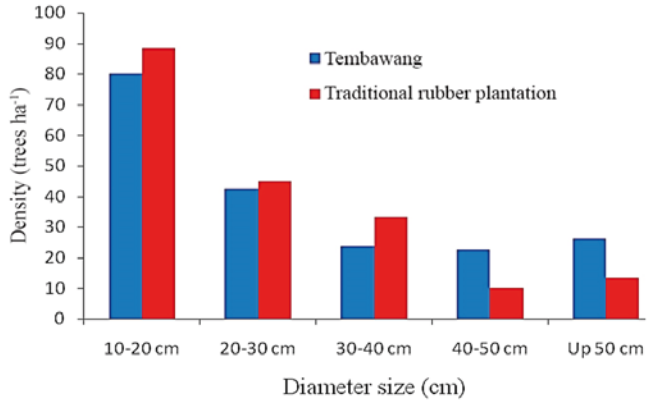

Figure 2: Tree density in different diameter classes in Tembawang forest and traditional rubber of West Kalimantan

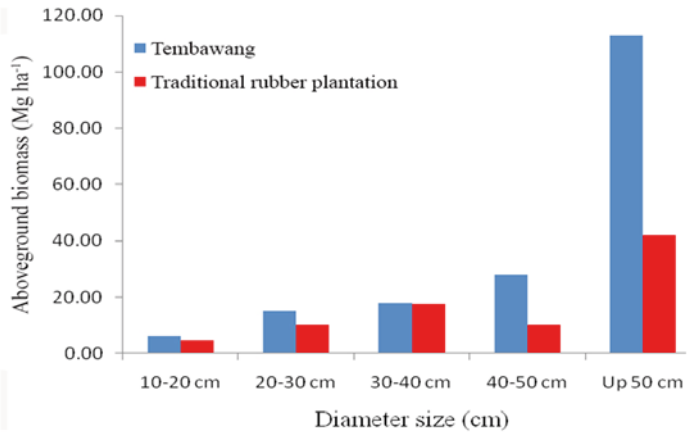

Figure 3: Aboveground biomass at different diameter classes from Tembawang forest and traditional rubber of West Kalimantan

Table 2: Carbon storage in different tree diameter classes in Tembawang forest and conventional rubber of West Kalimantan

	Tembawang Forest		Conventional Rubber Forest	
Diameter Size (cm)	Density (%)	Carbon Storage (%)	Density (%)	Carbon Storage (%)
10-20	41.03	3.52	44.49	5.52
20-30	21.79	8.39	23.68	12.19
30-40	12.18	10.02	17.54	20.94
40-50	11.54	15.49	5.26	12.07
>50	13.46	62.58	7.02	49.24

from Schroeder (1984), agroforestry systems in humid areas had an average value of 50 Mg C ha^{-1}, whereas in another report, Takimoto et al. (2008) reported the value of biomass C storage ranged from 0.7 to 54.0 Mg C ha^{-1} in traditional and improved agroforestry in West Africa. Compared to the dominating palm oil and agarwood industries in Indonesia and Malaysia, with carbon storage values of 14.35 to 37.88 Mg C ha^{-1} (Awang Besar et al., 2020), the carbon storage value of rubber plantation had higher value. In the same DBH range (≥ 10 cm), the carbon storage value in Tembawang agroforestry was in the aboveground carbon storage range of secondary tropical forests in Maluku, Indonesia with an average value of 70.3 Mg C ha^{-1} (Stas, 2014).

4 CONCLUSIONS

The aboveground carbon storage value of the traditional Tembawang and Rubber agroforestry system was estimated at 90.26 and 42.01 Mg C ha^{-1}. The traditional agroforestry system of the Dayak tribe community of Sekadau Regency, West Kalimantan had better impact and potential to preserve carbon than an intensive agroforestry system. The preservation of several species of fruit trees in the agroforestry system was major contribution to carbon storage.

Tembawang agroforestry has high potential for carbon storage compared to rubber plantations and varied different agroforestries, also the value was almost similar to carbon storage potential in secondary tropical forests. Local people's knowledge in traditional agroforestry systems need to be reported. The system could become a model in developing productive agroforestry and minimizing carbon emission.

5 ACKNOWLEDGEMENTS

The research was funded by the Directorate of Research and Community Service Research and Development Directorate-General for Research and Development of the Ministry of Research, Technology, and Higher Education in accordance with the Research Contract No. 107 / SP2H / LT / DRPM / IV / 2017 dated April 3, 2017. The authors wish to thanks to the Directorate of Research and Community Service of the Directorate General of Research and Technology Research and Development of Higher Research, Technology, and Higher Education.

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doi:10.14720/aas.2021.117.1.1845 Original research article / izvirni znanstveni članek

Nutritional indices and biochemical profile of *Helicoverpa armigera* [Hübner (1808)] on different groundnut genotypes

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Received August 20, 2020; accepted December 09, 2020.
Delo je prispelo 20. avgusta 2020, sprejeto 09. decembra 2020.

Nutritional indices and biochemical profile of *Helicoverpa armigera* [Hübner (1808)] on different groundnut genotypes

Abstract: Nutritional indices and biochemical profile of *Helicoverpa armigera* in response to feeding on different groundnut genotypes was studied. The moderately resistant genotypes were ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697. JL 24 was used as the susceptible check. Consumption index (CI), approximate digestibility (AD), efficiency of conversion of ingested food (ECI) and efficiency of conversion of digested food (ECD) were recorded. In addition, the activities of digestive and defensive enzymes of *H. armigera* were studied. *H. armigera* larvae showed significantly lower CI, AD, ECI and EDI when fed on moderately resistant genotypes than the insects fed on JL 24. Serine protease and trypsin activities were low in insects fed on resistant genotypes than the ones fed on JL 24. Further, insects fed on resistant genotypes showed significantly greater glutathione-S-transferase activity than the insects fed on JL 24. A reverse trend was observed for esterase activity. Similar trend was observed for total protein content of the insects. Thus, nutritional quality of host plants affects insect's physiology and could be used as an important indicator of host plant resistance against insect pests and to understand the adaptation of insect pests, if any, to various genotypes/host plants.

Key words: Host plant resistance; nutritional indices; digestive enzymes; groundnut; *Helicoverpa*

Prehranjevalni indeksi in biokemični profil južne plodovrtke, *Helicoverpa armigera* [Hübner (1808)], na različnih genotipih arašidov

Izvleček: V raziskavi so bili preučevani prehranjevalni indeksi in biokemični profil južne plodovrtke (*Helicoverpa armigera*) kot odziv na prehranjevanje na različnih genotipih arašidov. Zmerno odporni genotipi arašidov so bili ICGV 86699, ICGV 86031, ICG 2271 in ICG 1697. Genotip JL 24 je bil uporabljen kot občutljiva kontrola. Določeni so bili prehrambeni indeks (CI), navidezna prebavljivost (AD), učinkovitost pretvorbe pojedene hrane (ECI) in učinkovitost pretvorbe prebavljene hrane (ECD). Dodatno so bile v škodljivcu preučene aktivnosti prebavnih in obrambnih encimov. Gosenice južne plodovrtke so imele značilno manjše vrednosti parametrov kot so CI, AD, ECI in EDI, kadar so se hranile na zmerno odpornih genotipih v primerjavi s tistimi, ki so se hranile na občutljivem genotipu JL 24. Aktivnosti serin proteaze in tripsina so bile manjše pri žuželkah, ki so se hranile na odpornih genotipih v primerjavi s tistimi, ki so se hranile na občutljivem 'JL 24'. Žuželke, ki so se hranile na odpornih genotipih so imele značilno večjo aktivnost glutation-S-transferaze kot žuželke, ki se hranile na 'JL 24'. Nasproten trend je bil opažen v aktivnosti esterase. Podoben trend je bil ugotovljen v vsebnosti celokupnih beljakovin v žuželkah. Hranilna kakovost gostiteljskih rastlin vpliva na fiziologijo škodljivih žuželk in bi jo lahko uporabili kot pomemben kazalnik odpornosti gostiteljskih rastlin proti škodljivim žuželkam. S tem bi razumeli prilagoditve škodljivih žuželk na različne genotipe gostiteljskih rastlin.

Ključne besede: odpornost gostiteljskih rastlin; prehranjevalni indeksi; prebavni encimi; arašidi; *Helicoverpa*

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The study in this manuscript is part of the Ph.D. research work of the Abdul Rasheed WAR (Mentor-Hari C SHARMA)

1 INTRODUCTION

Plants face innumerable challenges from biotic and abiotic stresses, however, biotic stress by insect pests is one of the major stresses the plants face and take a heavy toll on crop yields. Though synthetic insecticides are the main insect pest controlling methods in many crops, they pose a great threat to the non-target organisms. For example, pesticide resistance is developed by insects, pest-resurgence, pesticide residues in food and health hazards in human beings (Isman, 2006; Sharma, 2007). Therefore, there is a need for an alternative environmentally safe crop protection technologies for safe and sustainable crop production. Breeders and entomologists have been developing insect resistant crop cultivars that could withstand insect pressure (Sharma et al., 2003; Smith, 2005; Sharma, 2009; Nair et al., 2020). Groundnut (*Arachis hypogaea* L.) is one of the important oilseed crops across the tropical and subtropical regions. Groundnut crop is affected by a number of biotic and abiotic stresses. Insect pests are the major biotic constraints of groundnut. The economically important insect pests of groundnut include western flower thrips (*Frankliniella occidentalis* Pergande, 1895 and melon thrips (*Thrips palmi* Karny, 1925); leaf miners (*Aproaerema modicella* [Deventer, 1904]); aphids (*Aphis craccivora* Koch, 1854); leafhoppers (*Empoasca dolichi* Paoli, 1930); white grubs (*Holotrichia consanguinea* Blanchard, 1850); pod borer, *Helicoverpa armigera* (Hübner(1808)) and armyworm, *Spodoptera litura* (Fabricius, 1775) (Sharma et al., 2003).

Helicoverpa. armigera (Hübner,[1808]) is a polyphagous lepidopteran pest with wide distribution across Asia, Africa, Australia and southern Europe (Sharma et al., 2003; Sharma, 2005). It causes severe damage to cereals, fruit crops, cash crops, vegetables including groundnut. Host plant resistance plays an important role to ward of insect pests by plants (Howe & Jander, 2008; War et al., 2011). It is a simple, inbuilt and eco-friendly method of managing insect pests (Sharma & Ortiz, 2002; War et al., 2012). Plant defensive traits interfere with host plant selection by the insect pest, deter the insects by producing volatile compounds or by averting oviposition by the insects. Plant defense against insect pests is manifested through morphological (surface wax, lignification, spines, hairs and sclerophylly) and biochemical traits (Dwivedi et al., 1986; Sharma et al., 2009; He et al., 2011; War et al., 2012; Bohinc et al., 2013). Biochemical traits constitute toxic secondary metabolites as a major component of plant defese against insect pests. They are directly toxic to insect pests or recruit the natural enemies of the insect pests (Howe & Jander, 2008; Karban, 2011; War et al., 2011, 2013). In groundnut, toxic secondary metabolites have been reported to hamper growth and development of insect pests (Stevenson et al., 1993; Senguttuvan & Sujatha, 2000; War et al., 2013, 2014).

Several reports have shown the role of plant toxic metabolites affecting insect growth and development. For example, Rao et al. (1998) showed that polyphenols in groundnut plants provide resistance against leaf miner *A. modicella*. Further, plant secondary metabolites such as dihydroxybenzoic acid, vanillic acid, caffeic acid and umbelliferone have been suggested to be involved in resistance against insect pests (War et al., 2016). In groundnut, structural (trichomes) and biochemical traits (phenols, tannins and defensive proteins) are involved in defense against insect pests including *H. armigera* (War et al., 2013, 2016; War & Sharma, 2014). Similarly, Stevenson et al. (1993) reported that in groundnut, plant toxins such as caffeoylquinic acids, quercetin, and diglycosides are the main contributors of insect resistance. They found that chlorogenic acid and rutin are also involved in resistance against *S. litura*. To test the hypothesis that insect resistant plants contain plant defensive traits that affect the insect growth and development by interfering with the nutritional indices of the insects, consumption, digestion and utilization of food and also the biochemical traits of *H. armigera* larvae were studied after feeding on the insect resistant and susceptible groundnut genotypes.

2 MATERIALS AND METHODS

2.1 CHEMICALS

The chemicals used were of analytical grade. Ethylene diamine tetra acetic acid (EDTA), tannic acid, trypsin inhibitor, bovine serum albumin, 1-napthol, glycine, 4-chloronapthol, disodium hydrogen phosphate, sodium dihydrogen phosphate, N-α-Benzoyl-DL-arginine p-nitroanilide (BApNA), glucose, GSH, sodium hydroxide, and sodium carbonate (Na_2CO_3) were procured from Sigma Aldrich (St Louis, Missouri). Acetic acid and 1-chloro-2, 4-dinitrobenzene (CDNB) were obtained from Sisco Research Laboratory (India) and HiMedia Pvt. Ltd (India), respectively.

2.2 *HELICOVERPA ARMIGERA*

Helicoverpa armigera larvae were collected from the field at International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Telangana, India (17° 25'N latitude, 78° 00'E longitude and 545 m.a.s.l.). The field collected insects were reared for one generation on the natural host under laboratory conditions before mixing with the laboratory culture. Under

laboratory conditions, insects were reared on chickpea based artificial diet (Table 1, Armes et al., 1992). The pupae were disinfected in 2 % sodium hypochlorite solution before transferring them to plastic jars containing Vermiculite for adult emergence. Newly emerged adults were immediately transferred to the wooden oviposition cages (30 x 30 x 30 cm) containing 10 % honey or sucrose solution in a cotton swab as food. Eggs were collected on rough surfaces of diaper liners (5 x 15 cm) and thin cotton wool sheets hung inside the cage. The eggs laid on the liners sterilized in 2 % sodium hypochlorite solution. The liners were dried and placed inside the plastic cups. The newly emerged larvae were reared initially in groups of 200 to 250 for five days in 200 ml plastic cups containing 2 to 3 mm layer of artificial diet on the bottom and sides. To avoid cannibalism, the larvae were reared individually in six cells well plates, of which each cell well was 3.5 cm in diameter and 1.5 cm in depth. The cell wells were filled with 7 ml diet for larval development until pupation. After every six months, the laboratory culture was mixed with the field-collected insects to maintain culture heterogeneity. Newly emerged larvae were used for the experiments.

2.3 GROUNDNUT PLANTS

The groundnut genotypes used in this study included four moderate to high levels of insect resistant cultivars (ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697) and a susceptible check (JL 24) (Sharma et al., 2003). The groundnut plants were grown in plastic pots (30 cm diameter and 40 cm deep) containing soil, sand, and farmyard manure (2:1:1 ratio) in a greenhouse at ICRISAT, Patancheru, Telangana, India. The plants were watered as needed and were maintained as per good agricultural practices. Five seeds were sown and later two seedlings were retained in each pot for the experimental purpose at 10 days after seedling emergence. The temperature and relative humidity were maintained at 26 ± 5 °C and 65 + 5 %, respectively, using desert coolers. Leaves (first fully expanded tetrafoliates) from 20-days old plants were used for the experiments.

2.4 CONSUMPTION, DIGESTION AND UTILIZATION OF FOOD BY *HELICOVERPA ARMIGERA*

The detached leaf assay technique described by Sharma et al. (2005) was followed to study consumption, digestion and utilization of food by *H. armigera*. The leaves were brought from glasshouse to lab in ice box. In each 100 ml plastic cup, a single tetrafoliate was embedded in 3 % agar-agar. Third-instar larvae of similar size were starved for 4 h before releasing on the leaves and a single larva was released on one tetrafoliate. After 5 days of feeding, larval mass, leaf damage rating, and dry mass of the residual food was recorded. To calculate the dry mass of the introduced food, fresh mass of the

Table 1: Composition of semi-synthetic diet for *Helicoverpa armigera*

Diet component	Ingredients	Quantity (g) per 1,000 ml diet
Part A	Chickpea flour	300
	Sorbic acid	3.0
	Methyl-p-hydroxybenzoate	5.0
	Ascorbic acid	4.7
	Yeast	48
	Aureomycin powder	11.5
	Cholesterol	1.5
	Formaldehyde (1%)	20 ml
	Multivitamin solution (A,B,D,E,C) drops	10 µl
	Water	450 ml
Part B	Agar-agar	17.3
	Water	800 ml

Diet preparation: The diet was prepared as follows:
1. Measured quantities of part A were mixed.
2. Agar-agar was added to water in a separate container and boiled for 5 min (Part B).
3. Part A and Part B were mixed thoroughly in a blender to get an even consistency.
4. The diet was poured into small plastic cups and allowed to cool under a laminar flow for 1 to 2 h.

food remaining after larval consumption was multiplied by a standard factor determined by maintaining an aliquot of the food under similar conditions in the absence of larvae, weighing it, then drying and reweighing it. The dry and fresh mass of aliquots were used to determine the percentage dry matter. The dry mass was expressed as the percentage dry matter in each genotype. The unconsumed food and frass from each detached leaf assay were removed, weighed and dried at 65 °C for 72 h in a hot-air oven. The dry mass of the food unconsumed by the insects was calculated as the difference between the dry mass of the unconsumed food and the calculated dry mass of the offered food. The difference between the mass of the larvae before and after the feeding period was taken as the larval mass gain.

The nutritional indices such as food consumption, digestion, and efficiency of conversion of the ingested food into body matter were calculated as per Waldbauer (1968) and Sharma & Franzmann (2000).

The consumption index (CI) was calculated as:

$$CI = \frac{Mass\ of\ food\ ingested}{Duration\ of\ feeding\ period\ X\ Mean\ mass\ of\ insect} \times 100$$

Approximate digestibility (AD) of food was calculated as follows:

$$AD = \frac{Mass\ of\ food\ ingested - Mass\ of\ frass}{Mass\ of\ food\ ingested} \times 100$$

Efficiency of conversion of ingested food into body matter (ECI) was calculated as follows:

$$ECI = \frac{Mass\ gained\ by\ the\ larva}{Mass\ of\ food\ ingested} \times 100$$

Efficiency of conversion of digested food (ECD) was calculated as:

$$ECD = \frac{Mass\ gained\ by\ the\ larva}{Mass\ of\ food\ ingested - Mass\ of\ frass} \times 100$$

2.5 BIOCHEMICAL TRAITS OF INSECT PESTS

2.5.1 Total serine protease assay

Insects from the bioassay cups were collected after 5 days of the infestation and dissected. The midguts of the larvae were extracted in in 0.2 M sodium phosphate buffer (pH 7.5) and homogenized in 0.1 M glycine-NaOH buffer (pH 10), containing 1 mM EDTA. The filtrate was passed through three-layered cheese cloth and centrifuged at 10,000 rpm for 20 min at 4 °C. A separate tube was used to collect the supernatant to be used as source for determining the enzyme activity. For the estimation of serine protease activity, azocasein was used as a substrate (Hegedus et al., 2003). To midgut supernatant (0.04 ml), 0.3 ml of 1 % azocasein solution that was prepared in 0.05 M glycine-NaOH buffer (pH 10) was added. The solution was incubated at 28 °C for 15 min. To the reaction mixture, 0.34 ml of 10 % TCA was added and then incubated for 1 h at room temperature. After centrifugation at 12,000 rpm for 10 min, 0.68 ml of 1 M NaOH was added to the supernatant. The absorbance was read at 495 nm and total midgut serine protease activity (SP) was calculated as follows:

$$SP = \frac{Abs(sample) - Abs(blank)}{Incubation\ time\ (min)} \times 1000$$

The total serine protease activity was expressed as tryptic activity (mU) per min of incubation per mg insect protein (mU min^{-1} mg^{-1} protein).

2.5.2 Trypsin assay

Trypsin activity was determined as per Perlmann & Lorand (1970). To the midgut extract of 0.15 ml, 1 ml of 1 mM BApNA (in 0.2 M glycine–NaOH buffer, pH 10), was added. The reaction mixture was incubated at 37 °C for 10 min. The reaction was terminated by adding 0.2 ml of 30 % acetic acid and the absorbance was read at 410 nm. The unit l mol min^{-1} mg^{-1} protein was used to express the trypsin activity.

2.5.3 Esterase (est) assay

For determination of esterase (EST) and glutathione-S-transferase assay (GST) activities, similar procedure was followed for extraction by dissecting the larvae in 0.1 M sodium phosphate buffer (pH 7.5). Homogenization of midguts was carried out in 0.1 M sodium phosphate buffer (pH 7.5) containing 1 mM EDTA. The filtrate was passed through three-layered cheese cloth and centrifuged for 15 min (4 °C) at 12,000 rpm. The supernatant was used for the estimation of EST and GST activities. The EST activity was estimated by adding 0.1 ml enzyme sample diluted 10 times with 0.1 M sodium phosphate buffer to 1.5 mM 1-naphthyl acetate solution. The reaction mixture was incubated at for 30 min at 25 °C and the reaction was stopped by adding Fast Blue B (in 5 % SDS) staining solution. The absorbance was read at 490 nm after 15 min of addition of the stopping solution. The hydrolysed substrate concentration was determined from the standard curve of 1-naphthol. The EST specific activity was expressed as l mol of 1-naphthol formed min^{-1} mg^{-1} protein.

2.5.4 Glutathione-s-transferase (gst) assay

The GST activity was estimated by using 1-chlo-

ro-2,4-dinitrobenzene (CDNB) and reduced GSH as substrates (Habig et al., 1974). To 1 ml of phosphate buffer (pH 7.5), 0.1 ml of CDNB (25 mM) and 1.6 ml of distilled water were added. To this mixture, 0.1 ml of 10 fold enzyme solution diluted with 0.1 M sodium phosphate buffer (pH 7.5) was added. The reaction mixture was incubated for 5 min at 37 °C and 0.1 ml of 20 mM GSH was added. Absorbance was read for 3 min at 30 s interval at 340 nm. The CDNB extinction coefficient of 9.6 mM cm^{-1} was used in calculating the enzyme activity. The specific activity of GST was expressed as nmol of CDNB conjugate formed min^{-1} mg^{-1} protein.

2.5.5 Estimation of protein content

Total protein content of the insects fed on groundnut genotypes was determined by Lowery's method (Lowry et al., 1951). The bovine serum albumin was used as a standard and the protein content was expressed as mg g^{-1} body mass.

2.6 STATISTICAL ANALYSIS

The data was analysed through analysis of variance (ANOVA) using SPSS v15.1 (SPSS, Inc., Chicago, IL, USA). The significant effects of the treatment ($p \leq 0.05$) were separated by the Tukey's test.

3 RESULTS

3.1 FOOD CONSUMPTION AND UTILIZATION BY *Helicoverpa armigera*

The insects fed on genotypes ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697 showed significantly lower CI per unit body mass than the insects on the susceptible check, 'JL 24' (Table 2). Similarly, the insects fed on insect-resistant genotypes, ICGV 86699, ICGV 86031, ICG 1697 and ICG 2271 (36.5 – 45.4%) had lower AD than those fed on 'JL 24' (67.5 %). Further, ECI was significantly lower in larvae fed on genotypes ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697 (21.3 – 28.2 %) than those fed on the susceptible check, 'JL 24' (54.1 %). The ECD in insects fed on the insect-resistant genotypes varied from 23.6 - 30.2 %, while the larvae fed on 'JL 24' showed a high ECD (45.7 %).

3.2 ENZYMES

Table 2: Nutritional indices of *Helicoverpa armigera* larvae fed on groundnut genotypes

	Nutritional indices			
Genotypes	CI (mg/mg/day)	AD (%)	ECI (%)	ECD (%)
ICGV 86699	2.3 ± 0.01^{bc}	36.5 ± 3.8^{c}	21.3 ± 1.5^{b}	27.1 ± 1.3^{bc}
ICGV 86031	2.6 ± 0.03^{bc}	41.2 ± 2.3^{b}	25.5 ± 1.2^{b}	23.6 ± 1.4^{bc}
ICG 2271	3.5 ± 0.02^{b}	44.3 ± 2.9^{b}	28.2 ± 1.8^{b}	30.2 ± 2.5^{b}
ICG 1697	2.9 ± 0.01^{b}	45.4 ± 3.0^{b}	24.7 ± 1.9^{b}	29.3 ± 2.2^{b}
JL 24	4.1 ± 0.04^{a}	67.5 ± 3.7^{a}	54.1 ± 2.3^{a}	45.7 ± 2.7^{a}

Within columns, (means ± SD) followed by same letter(s) do not differ significantly (Tukey's HSD test, $p < 0.05$). CI = Consumption index, AD = Approximate digestibility, ECI = Efficiency of conversion of ingested food and ECD = Efficiency of conversion of digested food.

Table 3: Total serine protease and trypsin activities of *Helicoverpa armigera* larvae fed on resistant and susceptible groundnut genotypes

Genotypes	Serine protease (mU min^{-1} mg^{-1} protein)	Trypsin (µmol min^{-1} mg^{-1} protein)
ICGV 86699	2.32 ± 0.07^{ab}	0.22 ± 0.002^{b}
ICGV 86031	2.11 ± 0.05^{ab}	0.19 ± 0.002^{bc}
ICG 2271	1.78 ± 0.02^{bc}	0.20 ± 0.003^{b}
ICG 1697	1.94 ± 0.04^{bc}	0.18 ± 0.002^{bc}
JL 24	3.26 ± 0.08^{a}	0.41 ± 0.005^{a}

Values (Mean ± SD) with similar letters within a column do not differ significantly at $p \leq 0.05$ (Tukey's HSD test).

3.2.1 Total serine protease and trypsin activity

Lower total serine protease activity was observed in insects fed on genotypes ICGV 86031 ($F_{4,14}$ = 21.7), ICG 2271 ($F_{4,14}$ = 23.1) and ICG 1697 ($F_{4,14}$ = 12.9) than those fed on 'JL 24' ($F_{4,14}$ = 21.3) ($p < 0.05$) (Table 3). Similarly, the trypsin activity was significantly lower in insects fed on insect resistant genotypes ($F_{4,14}$ =10.2, 11.1, 13.8 and 6.2, respectively, for ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697, $p < 0.05$) than in the insects fed on the susceptible check, 'JL 24'.

3.2.2 GST and EST activity

Significantly greater GST activity was observed in insects fed on genotypes ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697 ($F_{4,14}$ = 22.1, 18.2 and 12.8, respectively, $p < 0.05$) than those fed on 'JL 24' (Table 4). The EST activity in insects fed on ICGV 86699, ICGV 86031, ICG 2271 and ICG 1697 was significantly lower ($F_{4,14}$ = 9.5, 9.0 and 11.5, respectively, $p < 0.05$) than the insects fed on the JL 24.

3.2.3 Total protein content

Insect pests exhibited differential protein in insect resistant and susceptible genotypes. The protein content of the insects fed on genotypes ICGV 86699, ICGV 86031, ICG 2271, and ICG 1697 did not differ significantly (7.43, 8.22, 8.70 and 9.01 mg ml^{-1}, respectively), however, it was significantly lower in the insects fed on the susceptible check, 'JL 24' (12.7 mg ml^{-1}) (Table 5).

4 DISCUSSION

The toxic plant secondary metabolites are important weapons employed by plants against insects. The main mode of action of these metabolites is through the antibiosis mechanism, when ingested, these metabolites have detrimental effects on insects and reduce insect growth and development. The low nutritional quality of plant tissues, proteinase inhibitors and other metabolites are some of the antibiosis factors (Bhonwong et al., 2009; Barbehenn et al., 2010; War et al., 2013). These factors affect the insect food intake and its consumption and utilization by insect pests. The antibiosis and antixenosis plant defences against insect pests can be determined by studying the consumption, digestion and utilization of insects fed with specific host plants (Devetak et al., 2013). Imbalance in insect's food constituents will have drastic effects on its growth and development. Nitrogen content of plant tissues is an important limiting factor for growth and development of insect herbivores (Zhong-xian et al., 2007). Since plant tissues are the main source of nutrients for insects, their availability depends on the amount of food ingested and how efficiently it has been converted to body matter.

Table 4: GST and EST activities of *Helicoverpa armigera* larvae fed on resistant and susceptible groundnut genotypes

Genotypes	GST (μmol CDNB min^{-1} mg^{-1} protein)	EST (μmol 1-napthol min^{-1} mg^{-1} protein)
ICGV 86699	1.91 ± 0.01[a]	0.29 ± 0.004[a]
ICGV 86031	1.88 ± 0.03[a]	0.27 ± 0.004[a]
ICG 2271	1.86 ± 0.04[a]	0.31 ± 0.006[ab]
ICG 1697	1.52 ± 0.07[a]	0.30 ± 0.003[a]
JL 24	1.02 ± 0.03[a]	0.39 ± 0.006[a]

Values (Mean ± SD) followed by same letter(s) within a column are not significantly different at $p \leq 0.05$ (Tukey's HSD test).

Table 5: Total protein content (mg ml^{-1}) of *Helicoverpa armigera* larvae fed on fed on resistant and susceptible groundnut genotypes

Genotypes	Protein content (mg ml^{-1})
ICGV 86699	7.43 ± 0.4[a]
ICGV 86031	8.22 ± 0.2[a]
ICG 2271	8.70 ± 0.3[a]
ICG 1697	9.01 ± 0.2[a]
JL 24	12.7 ± 1.3[b]

Values (mean±SD) followed by same followed by letter(s) within a column are not significantly different at $p \leq 0.05$ (Tukey's HSD test).

Our results showed that AD, CI, ECI, and ECD were reduced in *H. armigera* larvae fed on insect-resistant groundnut genotypes and were considerably lower than the insects fed on JL 24. The reduced consumption and utilization of food by *H. armigera* can be attributed to the antibiosis effect of the constitutively produced secondary metabolites including flavonoids, tannins and some defensive proteins (Grayer et al., 1992; Stevenson et al., 1993; Senguttuvan & Sujatha, 2000; Rao, 2003; War et al., 2013). Antibiosis is one of the important modes of host plant resistance against insects that affects the oviposition, survival and growth and development of the target pests (Sharma & Norris, 1991; Sharma et al., 2005; Sujana et al., 2008; Ansari et al., 2011). We observed that even though the consumption index of H. *armigera* was high in some of the insect resistant genotypes such as ICG 1697, ICG 2271 and ICGV 86031, the ECI and ECD were significantly less. This clearly showed that the plant defensive traits pertaining to antibiosis such as toxic secondary metabolites occur in these genotypes. The ingested plant allelochemicals affect the insect's physiological and biochemical traits that in turn impact the post-ingestive nutrient utilization by the pest (Sharma & Norris, 1991; Hasan & Ansari, 2011; Ansari et al., 2011; War et al., 2013). Further, insects excrete some of the plant toxic chemicals along with the faecal matter, which in turn, results in reduced efficiency of food utilization. The results showing the differential responses of insects in ingesting the food material, digestion efficiency and conversion of the ingested food into body matter can be used as important indicators for identifying the host plant resistance against insect pests (Sharma & Norris, 1991; Yazdanfar et al., 2015).

The digestive and the detoxifying enzymes in insect pests showed a differential response on insect resistant and susceptible groundnut genotypes. Serine protease and trypsin activities were reduced in insects fed on the resistant genotypes. This could be attributed to the higher amounts of toxic plant secondary metabolites and/or protease inhibitors in insect resistant genotypes than the susceptible genotype. Reduced serine protease and trypsin activities in insects fed on insect resistant genotypes can be attributed to the antibiosis mechanism of resistance in plants mediated by the toxic secondary metabolites. Further, some plant antioxidative enzymes such as peroxidases are directly toxic to insect pests (Barbehenn et al., 2010). It has also been reported that *H. armigera* larvae when fed on a diet containing plant secondary metabolites show reduced serine protease and trypsin activities (War et al., 2013). Reduced protein digestion, thereby, low levels of amino acids result in reduced growth and developments in insects (Lawrence & Koundal, 2002; Azzouz et al., 2005).

Insect detoxifying enzymes such as GST and EST convert plant allelochemicals into non-toxic or low toxic compounds (Leszczynski & Dixon, 1992; Yang et al., 2005; War et al., 2013). These enzymes are induced in insects in response to plant metabolites. Increased activities of GST were observed in insects fed on resistant genotypes as compared to the ones fed on the susceptible genotype JL 24. The higher toxicity of the compounds is attributed to the higher toxicity of the plant toxic secondary metabolites in the insect resistant genotypes (War et al., 2013). In barley, aphid *Sitobion avenae* (Fabricius, 1775) showed increased levels of GST when fed on plants with greater phenolic content (Leszczynski & Dixon, 1992). The EST activity did not differ significantly in insects fed on the insect resistant genotypes but was significantly higher in the insects fed on the susceptible genotype, JL 24. The higher levels of toxic secondary metabolites in resistant genotypes than the susceptible genotype might have led to the reduced activity of the EST in insects. ESTs are directly involved in the hydroxylation of toxic plant secondary metabolites and insecticides to less toxic compounds (Yang et al., 2005). Positive correlation has been observed in insect midgut serine proteases, trypsin and GST and larval mass and survival (War et al., 2013). Therefore, plant toxic compounds in resistant genotypes that led to the decrease in the levels of these enzymes could be used as important biochemical markers for plant resistance. *Myzus persicae* (Sulzer, 1776) exhibited higher levels of GSTs when fed on brassicaceous host plants containing toxic plant metabolites such as glucosinolates and isothiocyanates (Francis et al., 2005). *H. armigera* fed on artificial diet containing plant toxic metabolites showed higher levels of GST, which has been attributed to the fact that the insect is trying to adapt to the plant toxins (War et al., 2013). They further reported a negative correlation between EST activity and larval growth in *H. armigera*. The total protein content of the larvae fed on insect resistant plants was significantly lower than the larvae fed on the susceptible genotype, JL 24. Reduced protein levels could have resulted from the toxic effects of the plants metabolites on serine proteases, trypsin and other enzymes. Further, plant protease inhibitors and other antinutritional components are some of the important factors that affect protein synthesis in insects (War et al., 2013). Further, essential amino acid reduction drastically affects the insect growth and development (Chen et al., 2005).

It has been reported that *S. litura* larvae fed on banana leaves showed reduced growth and development, digestibility and consumption rate of plant tissues, however, they exhibited a high conversion efficiency of the ingested food with a high rate of conversion of the digested food. This shows that that the insects compensated for the

nutrient intake from the limited plant tissues by more efficiently utilizing the host plant tissues (Zhu et al., 2005). The digestion of the plant tissues by insect pests depends on the activities of various enzymes including serine proteases, trypsin, and others. Several factors affect the AD, CI, ECI, and ECD in insect-plant interaction and it is practically difficult to determine the exact "cause" and "effect" responses of these parameters. The questions that arise here are, is it because of low digestibility that the insects eat low or is it that the digestibility is low as the insect eats more? The efficiency parameters involved in host plant feeding by insect pests are physiologically closely related. Though the factors responsible for the efficient conversion of the digested food are still largely unknown, role of shifts in food selection, insect digestive physiology, body composition and metabolic rates can't be ruled out. It is very important to understanding the basic principles of nutritional ecology to identify the host plant resistance against insect pests.

5 CONCLUSION

This study determined that the AD, CI, ECI, and ECD in *H. armigera* larvae were significantly reduced when fed with the insect-resistant groundnut genotypes. Though high consumption index was observed in some genotypes (ICG 1697, ICG 2271 and ICGV 86031), the lower ECI and ECD, reduced activities of digestive enzymes and increased detoxifying enzyme activities can be attributed to strong antibiosis mechanism of resistance in these genotypes. This study shows that host plant diet directly affects the digestive plasticity, which in turn, influences the development of insect pests including *Helicoverpa armigera*. The information derived from this study would be useful to understand the adaptation of insect pests to various genotypes/host plants and the co-evolution between the insect pests and their host plants.

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doi:10.14720/aas.2021.117.1.1740 Original research article / izvirni znanstveni članek

Assessement of control strategy by spraying low doses of sugars on apple orchard against *Cydia pomonella* (Linnaeus, 1758.)

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Received June 20, 2020; accepted December 10, 2020.
Delo je prispelo 20. junija 2020, sprejeto 10. decembra 2020.

Assessement of control strategy by spraying low doses of sugars on apple orchard against *Cydia pomonella* (Linnaeus, 1758.)

Abstract: Codling moth (*Cydia pomonella* [L.]) is the most serious pest of apple worldwide. Its control still relies largely on insecticide applications. To deal with this situation, it becomes essential to design eco-friendly control systems to minimize chemical treatments. In this context, the effect of spraying of fructose (100 ppm), glucose (100 ppm) and insecticide (Deltamethrin), on the Golden Delicious variety against *C. pomonella* larval damages, was studied in an orchard located in Batna province (Algeria). The results of this study showed that codling moth own four generations in the study area. It is a very important pest with about 59.19 ± 1.15 % of damaged fruits at harvest. The spraying of glucose alone, fructose alone and the chemical insecticide alone causes a significant increase in the percentages of healthy fruit at harvest compared to the untreated control. The use of fructose and glucose has significantly reduced the percentage of damaged fruits at harvest followed by the spraying of the insecticide which produces the lowest percentage. The Abbott's efficacy of glucose treatments was 23.75 ± 2.6 % compared to the insecticide 37.6 ± 2.55 %; and fructose 15.54 ± 3.01 %. The use of sugars is a completely innovative way in the field of plant protection. These first results demonstrate a promising alternative to conventional programs.

Key words: codling moth; glucose; fructose; Deltamethrin; 'Golden Delicious'

Ocenitev nadzorovanja jabolčnega zavijača (*Cydia pomonella* (Linnaeus, 1758)) s škropljenjem z majhnimi odmerki sladkorjev

Izvleček: Jabolčni zavijač (*Cydia pomonella* [L., 1758]) je v svetovnem merilu najpomembnejši škodljivec jablan. Njegovo uravnavanje še vedno v največji meri temelji na uporabi insekticidov. Za odpravo tega je bistveno najti okolju prijazen način uravnavanja, ki bi zmanjšal obravnavanje s kemikalijami. V tem smislu je bil preučevan učinek škropljenja s fruktozo (100 ppm), glukozo (100 ppm) in insekticidom (Deltamethrin) na obseg poškodb zlatega delišesa (Golden Delicious) zaradi ličink jabolčnega zavijača v sadovnjaku, v provinci Batna, Alžirija. Rezultati so pokazali, da ima jabolčni zavijač na tem območju štiri generacije. Je zelo pomemben škodljivec, ki povzroči 59,19 ± 1,15 % poškodovanih plodov ob obiranju. Škropljenje samo z glukozo, fruktozo ali samo s kemijskim incekticidom je značilno povečalo odstotek zdravih plodov v primerjavi s kontrolo brez obravnavanj. Uporabi glukoze in fruktoze sta značilno zmanjšali odstotek poškodovanih plodov ob obiranju, a pri škropljenju z insekticidom je bil njihov odstotek najmanjši. Abbottove učinkovitosti obravnavanj so bile: z glukozo 23,75 ± 2.6 %, z insekticidom 37,6 ± 2.55 %; in s fruktozo 1554 ± 3.01 %. Uporaba sladkorjev je popolnoma nov način zaščite rastlin na prostem. Ti prvi izsledki nakazujejo obetajočo alternativo konvencionalnim programom zatiranja tega škodljivca.

Ključne besede: jabolčni zavijač; glukoza; fruktoza; Deltametrin; 'Golden Delicious'

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1 INTRODUCTION

The economic impact of *C. pomonella* (L., 1758). (Lepidoptera; Tortricidae) on apple trees is continuously increasing. For reasons, the appearance of resistant populations to different insecticides (Charmillot et al., 2007). In addition to its high cost, insecticides present a potential danger to the environment and both the negative side effects on beneficials and occasionally resulting in outbreaks of secondary phytophagous pests. The concept of "Sweet Immunity" postulates that sugar metabolism and signalling influence plant immune networks (Tarkowski et al., 2019). Water-soluble metabolites (sugars and sugar-alcohols) have been already identified from surfaces of apple tree organs that they influence plant site acceptance after alighting and stimulate *C. pomonella* egg-laying (Derridj et al., 1999). Fructose, sorbitol, and myo-inositol are then especially concerned within a blend of six metabolites (Lombarkia & Derridj, 2002).

Soluble carbohydrates and sugar-alcohols on the leaf surface should, however, provide information both about plant physiology and host plant specificity. The concept of exogenous application of sugars every 20 days on apple trees, to modify the egg-laying of *C. pomonella* to reduce the damage it causes, was tested in commercial orchards of several countries (in France, in Italy, in Greece and in Algeria) and over several years it showed an Abbott efficiency of 40 to 59 % (Derridj et al., 2011).

The research hypothesis was that exogenous application of sugars on apple trees would provide satisfactory efficacy and treatments with fructose alone and glucose alone would have less fruit damages than the untreated control.

Codling moth larvae bore deep into the fruit making it unmarketable, it often leads to the fall of the fruit and may go through several generations in a growing season. However, it is often difficult to cope with large yield losses and the problem of reducing codling moth damage is therefore associated with challenges economic benefits for the study area.

Thus, the aim of the present paper is to evaluate the efficacy of sugars in reducing the damages caused by the codling moth.

2 MATERIAL AND METHODS

2.1 STUDY SITE

The experiment was performed in a 'Golden Delicious' apple orchard in the Batna region (eastern Algeria) (35°21′12,4" N, 006°01′ 19,1" E) during 2019 with four different treatments against *C. pomonella*. The treated orchard was managed under common practices of the zone; it had a surface of 0.8 ha with 17 rows (19 trees each). The trees were 9 years old, and the plant spacing is 4 m × 4 m, whereas the height of the trees is approximately 3 m.

2.2 SEXUAL TRAPPING OF ADULTS

Attractive sex pheromone trap (produced and supplied by Russell IPM) was used to monitor the progress of flight (qualitative forecasting), follow the pest dynamics and to estimate the level of pest population of *C. pomonella*. The sexual trap consisted of a plate coated with glue, which was deposited on a capsule containing the specific pheromone ([E, E]-8, 10-dodecadien-1-ol), which attracts the males entering the delta trap and become trapped in the sticky area. The device was attached by a wire to the tree foliage, placed at eye level. The pheromone trap was installed between 17th March and the harvest date (25th September), and it was replaced at 6 weeks intervals. The observations were carried out every 3 days for 6 months (from March until September 2019). For each output, the date of catches and the total number of captured moths were mentioned.

2.3 TREATMENTS

The treatments were adjusted in a randomized Latin square with 4 repetitions. All the modalities (4) are then distributed within each of the four blocks and each block has 3 trees. The efficacy was measured by the extent to which fruit damage attributable to *C. pomonella* caterpillars was reduced by each treatment, compared to the unsprayed control. The applications were performed using electrical pressure sprayer (12 V-12 Ah), capacity of

Table 1: Tested Modalities and their doses

Modalities	Doses	Treatments
1	Control (Untreated)	Control (Untreated)
2	Fructose (Fluka Biochemika)	10 g 100 l^{-1} (100 ppm)
3	Glucose (Fluka Biochemika)	10 g 100 l^{-1} (100 ppm)
4	Decis 25 EC containing 25g l-1 Deltamethrin (Bayer)	(0.5 l) 1000 l^{-1}

16 l. The modalities tested and their doses are reported in Table 1.

The morning treatments (sugars and insecticide) were carried out every 20 days throughout the season from the flowering end until harvest (Derridj et al., 2012).

2.4 DAMAGE ASSESSMENTS

The variable 'percentage of infested and healthy fruits at harvest' is based on the ratio of the total number of infested fruits (damaged) and the total number of healthy fruits per plot. Abbott's formula is very commonly used in field trials. Its efficacy at harvest measures the percentage of *C. pomonella* caterpillars infested fruits versus untreated controls according to the formula T0 – Tt /T0 × 100; where T0 is the percentage of infested fruits in the untreated plots and Tt is the percentage of infested fruits in the treated plots.

2.5 STATISTICAL ANALYSIS

The means between each variable 'percentage of infested fruits, healthy fruits at harvest', and Abbott's efficacy for the four modalities were compared by ANOVA on ranks test, followed by post hoc analysis using Fisher's and Tukey's tests (Table 3). A *p*-value of 0.05 was used to establish significance in all tests. All analyses were performed using SPSS software v. 2016.

3 RESULTS AND DISCUSSION

3.1 SEXUAL TRAPPING OF ADULTS

Considering the entire trapping season, 708 males were captured between March 20 and September 25, the captures per trap per three days varied between 1 and 73, that is on average 25.82 males /trap/week. The pace of the annual flight is shown quad-modal with the first tip of 32 males on April 22, and the second of 73 individuals from July 03, the third of 22 individuals on August 20 and the fourth 18 individuals on September 10 (Figure 1). The gates of the developed generations would then present the following sequences, the first (14.26 % of annual catches) taked place between March 20 (milestone date of the start of the insect's activity) and May first. The second has a significant importance of 56.35 %, from May 4 to July 18. The third (15.96 %) took place between July 21 and August 26. The fourth, scanty (13.41 %) due to diapauses, fully covered the month of September (Table 2).

The determination of generations is based on the following principle: the division a significant and stable increase in catches, followed by a sufficiently long inter-flight (± 30 days) with few catches, indicates a nascent or finishing flight comparable to the start or the end of a generation (Hmimna & El Iraqui, 2015).

3.2 DAMAGE ASSESSMENTS AND TREATMENTS EFFICACY

The spraying of glucose alone, fructose alone and the insecticide induced a significant increase in the percentages of healthy fruit at harvest compared to the untreated control. The analysis of variance (ANOVA) followed by the Tukey test ($p < 0.05$) identified three groups, control (40.80 %, group a), glucose and fructose 55.03 %, 50.29 % respectively (group b) and insecticide 63.19 % (group c) (Table 3).

Similarly, foliar sprays of fructose and glucose made it possible to significantly reduce the percentage of fruit

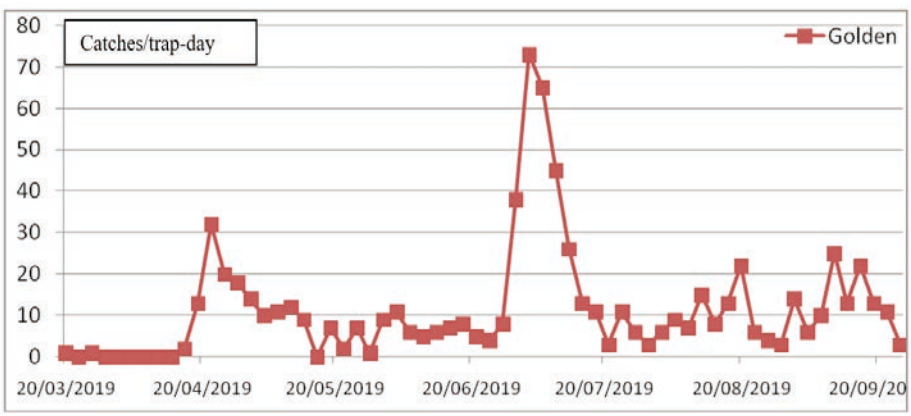

Figure 1: Seasonal flights of adult males of C. pomonella in 'Golden Delicious' orchard

Table 2: The number of catches and their percentage of C. pomonella during the four generations

Generations	G1	G2	G3	G4
Total number of catches	101	399	113	95
% of catches	14.26 %	56.35 %	15.96 %	13.41 %

damaged at harvest compared to the control, the analysis of variance (ANOVA) followed by the Tukey test ($p < 0.05$) revealed three groups, control (59.19 %, group a), glucose and fructose 44.96 % and 49.70 % respectively (group b), followed by the spraying of the insecticide which produces the lowest percentage of 36.80 % (group c), (Table 3).

The Abbott's efficiency of glucose treatments is 23.75 % compared to the insecticide with 37.6 % and fructose generating an average percentage efficiency of 15.54 % (Table 3).

Previous studies in Algeria and in France from 2009 to 2014 summarized by Arnault et al. (2016) have demonstrated the potential of foliar application of sucrose in micro-doses to control *C. pomonella*, and revealed a degree of infested fruits at harvest of the untreated control varied from 12 to 46.8 % in nine experiments were conducted in apple orchards. Compared with this study, the infestation was more important with 59.19 % at harvest which represent a very high pressure of the pest.

The mentioned studies showed that sucrose can induce partial resistance to *C. pomonella* by foliar applications at a very low dose of 0.01 %. The mean Abbott's efficacy obtained for sucrose 100 ppm alone was 41.0 ± 10.0 %, while sucrose in combination with thiacloprid insecticide; enhanced the efficacy of thiacloprid treatment by 18 %. The researchers have concluded that the effect of this combination is not additive but synergistic and rather potentiating.

Furthermore, Arnault et al. (2015) confirmed, in their study conducted in an integrated fruit protection orchard on the Granny Smith variety, the advantage of applying fructose 100 ppm in combination with OrganoPhosphorus OP or Insect Growth Regulator (IGR) insecticides against codling moth. Sprays of fructose at 100 ppm in combination with OP and IGR have significantly reduced the percentage of damaged fruits at harvest compared to the OP and IGR alone (6.5 % and 10 % respectively); efficiency was therefore improved by 35 % when fructose combined with OP and IGR. The same author has specified that in studies leading during 2013 and 2014 in an organic orchards, the foliar applications of fructose at 100 ppm could reduce codling moth damage by 55 % on the Gala variety, knowing that the use of fructose at 100 ppm has significantly reduced the percentage of damaged fruits at harvest compared to the control (3,6 %, 14,9 %) and (2,5 %, 3,9 %) respectively, and the Abbott effectiveness was 76 % (in 2013) and 36 % in 2014). These researches are in good agreement with the results of the present study; this is consistent that the exogenous foliar application of a single sugar can induce plant resistance to the pest. Derridj et al. (2012) argued that foliar application of sucrose alone and/or other compounds from the formulation such as sugars are introduced into the commercial granulovirus products as phago-stimulants for more effective ingestion by larvae can induced modification of the cuticular permeability and, therefore, of the composition of the leaf surface blend and its effects on pest behavior. Burges and Jones (1998) presented the sugars advantages in the formulation of microbial biopesticides; act as phagostimulants, preservatives and dispersants.

It was the main purpose of the paper to draw attention to primary metabolites, in particular, soluble carbohydrates. The sugars applied on the leaves in the morning (best diffusion through the cuticle while the apoplast concentrations are low) are those usually found on apple leaves in the evening. This knowledge of sweet immunity here is even more complex. It seems to be resulted from an induction of partial antixenosis at the *C. pomonella*

Table 3: Percentage of damaged and healthy fruits in apple orchards (n = 12) on different modalities (control, fructose, glucose, insecticides)

Treatments	% damaged fruits (Mean + SEM*)	% healthy fruits (Mean + SEM*)	% Abbott effectiveness (Mean + SEM*)
Control (Untreated)	59,19 ± 1,15 a	40,80 ± 1,15 a	
fructose 100 ppm	49,70 ± 1,20 b	50,29 ± 1,19 b	15,54 ± 3,0 a
glucose 100 ppm	44,96 ± 1,34 b	55,03 ± 1,33 b	23,75 ± 2,6 a
Insecticide (Deltamethrin)	36,80 ± 1,40 c	63,19 ± 1,39 c	37,60 ± 2,55 b

* Values indicated with different letters are significantly different at $p < 0.05$

egg-laying linked to changes in chemical signals of the leaf surface induced by applications of these sugars, confirmed by the findings of Lombarkia (2002) and Lombarkia and Derridj (2008) that have been clearly investigated the important role of primary metabolites and particularly sugars on *C. pomonella*. Egg-laying site preference within the apple tree and its intensity is related to a blend of three soluble carbohydrates (sucrose, D-fructose, and glucose) and three sugar alcohols (sorbitol, quebrachitol, myo-inositol) present at the surface of the apple tree. Quantities and ratios of the six primary metabolites found on the leaf surface may influence host preference of *C. pomonella* as well as their egg-laying behavior, thus they may play a role in the trees' resistance to the codling moth (Lombarkia & Derridj, 2008).

On the other hand, the resistance effect on *C. pomonella* female egg-laying is predominant in the reduction of larval damage, but this does not exclude effects on the neonate larvae before penetrating into the fruit. Derridj et al. (1999) found that soluble carbohydrates and sugar-alcohols present on apple tree surface discriminates apple tree sites such as leaves and leaf side, among the present substances, six were studied in laboratory in their study, fructose, sorbitol and myo-inositol stimulate *C. pomonella* egg-laying and enhance examination of the support by the neonate larvae. Glucose showed more deterrent effect on females and larvae, whereas quebrachitol and myo-inositol arrested neonate larvae at the highest concentrations. In addition, we know that in the formulation of microbial biopesticides, there are simple sugars used for many reasons. It is probable that sugar has physiological effects on the plant. Stacey et al. (1977, 1980), suggested that increases in cotton yield recorded in the presence of sugars may have been due to the physiological effects of the sugar on the plant. Also, sucrose is routinely used with *B. thuringiensis* Berliner 1915 on grape against grape berry moth.

4 CONCLUSIONS

Based on our results, it can be concluded that small molecules such as glucose and fructose can induce partial resistance to *C. pomonella* by foliar applications. This study should stimulate research on this concept for the development of eco-friendly control strategies.

I recommended conducting future researches on the effect of foliar applications of sugars on *C. pomonella* egg-laying and its oviposition site selection.

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doi:10.14720/aas.2021.117.1.1847 Original research article / izvirni znanstveni članek

Exploiting heterosis and combining ability in two-line hybrid rice

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Received August 22, 2020; accepted December 16, 2020.
Delo je prispelo 22. avgusta 2020, sprejeto 16. decembra 2020.

Exploiting heterosis and combining ability in two-line hybrid rice

Abstract: Twenty hybrids were developed from crossing, four environmental genic male sterile (EGMS) lines with five testers in line × tester mating design to magnitude of heterosis over better parent for grain yield and contributing traits in rice (*Oryza sativa* L.). Five hybrids 'WTSC9059' × 'Sakha101', 'WTSC9039' × 'Sakha102', 'WTSC9059' × 'Sakha108', 'WTSC9039' × 'Sakha108' and 'WTSC9039' × 'Sakha101' express superior value for number of panicles, fertility percentage, 100-grain mass, grain yield, apparent heterosis and phenotypic acceptance. The top three heterotic combinations identified for grain yield/ha were 'WTSC9059' × 'Sakha101', 'WTSC9039' × 'Sakha102' and 'Longping' × 'Sakha105' which exhibited 100.00, 71.51 and 66.61 % heterobeltiosis, respectively. The lines 'WTSC9059' and "Longping" and testers 'Sakha101', 'Sakha102' and 'Sakha108' was found to be good general combiner for most of the characteristics and could be extensively used in future hybrid rice breeding program. The grain yield was correlated highly significant and positive with panicle exertion, panicle mass, fertility percentage and appearance of heterosis, otherwise the negative correlation and significant was found with flag leaf area.

Key words: heterosis; combining ability; line × tester; correlation; hybrid rice

Uporaba heteroze in kombinacijske sposobnosti pri dveh linijah hibridnega riža

Izvleček: Dvajset križancev je nastalo iz križanj štirih okoljsko gensko moško sterilnih (EGMS) linij s petimi testerji po principu linija × tester za povečanje heteroze v primerjavi z boljšo starševsko linijo za pridelek zrnja in z njim povezanimi lastnostmi pri rižu (*Oryza sativa* L.). Pet križancev 'WTSC9059' × 'Sakha101', 'WTSC9039' × 'Sakha102', 'WTSC9059' × 'Sakha108', 'WTSC9039' × 'Sakha108' in 'WTSC9039' × 'Sakha101' ima veliko boljše vrednosti za znake kot so število latov, odstotek plodnosti, masa 100 zrn, pridelek zrnja, aparentna heteroza in fenotipska sprejemljivost. Najboljše tri heterotične kombinacije za pridelek zrna na hektar so bile 'WTSC9059' × 'Sakha101', 'WTSC9039' × 'Sakha102' in 'Longping' × 'Sakha105', ki so pokazale 100,00; 71,51 in 66,61 % heterobeltioze. Liniji 'WTSC9059' in 'Longping' in testerji 'Sakha101', 'Sakha102' in 'Sakha108' so bili prepoznani kot dobri splošni kombinatorji za večino lastnosti in bi lahko bili na široko uporabljeni v bodočih križanjih pri žlahtnenju riža. Pridelek zrnja je zelo značilno pozitivno koreliral z močjo lata in njegovo maso, odstotkom fertilnosti in pojavom heteroze, nasprotno je bila negativna korelacija s površino najvišjega, zastavnega lista.

Ključne besede: heteroza; kombinacijska sposobnost; linija × tester; korelacija; hibridni riž

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1 INTRODUCTION

Rice (*Oryza sativa* L.) is one of the world's three major food crops and represents an important component of the world's food supply. Hybrid rice technology has contributed significantly toward food security, environmental protection, and employment opportunities (Gramaje et al., 2020). Generating hybrid rice, which presents a higher grain yield than inbred rice varieties, is one of the most important applications of heterosis in agriculture (Liu et al., 2020). Rice is a self-pollinating crop, and nearly all traditional rice cultivars are inbred lines. Since the 1970s, hybrid seed production has mainly used two-line or three-line hybrid systems (Chang et al., 2016). The three-line system is based on a cytoplasmic male sterile (CMS) line, a restorer line (to produce F_1 hybrid seeds) and a CMS maintainer line (to maintain the CMS line). The two-line hybrid system is based on environmentally sensitive genetic male sterility (Yu et al., 2020)" and it usually uses photoperiod- and thermo-sensitive genic male sterile (PTGMS) lines as maternal parents to produce hybrid seeds. PTGMS lines are sterile under restrictive conditions (high temperatures and long days) but become fertile under permissive conditions such as low temperatures and short days (Lan et al., 2019). Environment-sensitive genic male sterility is controlled by nuclear gene expression, which is influenced by environmental factors such as temperature, day length, or both (Chang et al., 2016). In the two-line system, certain lines, referred to S lines, can be either male sterile (functionally female) or male (produces viable pollen) depending upon temperature and day length. Under temperature / day length combined condition, the S lines are crossed as females to fertile inbred lines to produce hybrid seed, while under separate temperature/day length combination, the same lines are allowed to self-pollinate and produce viable seeds to maintain a source. The success of hybrid rice technology primarily depends on genetic purity, timely availability and the affordability of hybrid seed costs to the farmers (Singh and Ram, 2012).

The line × tester analysis is a powerful tool to discriminate the good and poor combiners for choosing appropriate parental material in successful hybrid breeding program. It gives reliable information about the nature and degree of gene action and combining ability effects existent in the genetic materials (Akter et al., 2010). Breeding value can be deduced from the general and specific combining abilities for key desirable traits, as well as from the phenotypic and genotypic value of the parental forms for highly inheritable traits. The general combining ability (GCA) of a given parent for a particular trait is defined by the mean value of the trait in the half–sibling progeny of this parent. The specific combining ability (SCA) reflects how a particular pair of parents contributes to the presence of a particular trait level in the hybrid progeny. SCA is defined as interaction between a pair of the parents for a progeny trait. Studies on the genetic basis of heterosis for polygenic traits in various crops have shown that heterosis is the result of partial to complete dominance, overdominance, and epistasis and it may be a combination of all these (Gokulakrishnan and Kumar, 2013). Evidence of real overdominance for quantitative traits is hard to find. However, apparent overdominance caused by nonallelic interaction and linkage disequilibrium is a common contributor to heterosis (Dan et al., 2019 and Hijam et al., 2019). The present study was conducted with objective to determine the heterosis levels in generated hybrids, estimate GCA of the pollinator tester and EGMS lines, determine SCA of the generated hybrid crosses, and estimate the nature and degree of gene actions and heritability.

2 MATERIALS AND METHODS

2.1 GENETIC MATERIALS

A total of 20 testcrosses were developed by applying a line × tester mating design using four EGMS lines (S) and five pollinators tester (P) as described by Kempthorne (1957). The nine parents were cultivated thrice at an interval of 10 days to ensure synchronization in flowering for the purpose of hybridization in Sanya, Hainan Province, China, 2017 rice growing season. The hybrid crosses and their parents were evaluated at the experimental field of the Winall Thriving Seed Co., (WTSC) Farm in 2018 rice growing season in Hefei, Anhui Province, China. The origin, pedigree, salience and feature of nine rice genotypes were illustrated in Table 1. At 2017 rice growing season thirty days after sowing, the seedlings were transplanted by hand into the paddy field with each plot containing 5 rows with 10 plants per row at a space of 20 cm × 20 cm. Twenty-five days after pollination, the F_1 seeds were harvested, dried by air, threshed by hand and stored at room temperature. The F_1 hybrids' seeds and their parents (pollinator lines and EGMS lines) were nursed in the May 1ST 2018. Thirty days later, the seedlings were transplanted into the paddy field with one seedling per hill using a randomized complete block design and three replications for each genotype. Each plot contained 15 rows with 20 plants per row at a space of 20 cm × 15 cm, each test entry consisted 15 rows of 2 m length, the plant area for each genotype was 6 m^2. All practices recommended package for planting, transplanting, N, P, K and Zn fertilizers, water management and plant protection were followed. The soil of the

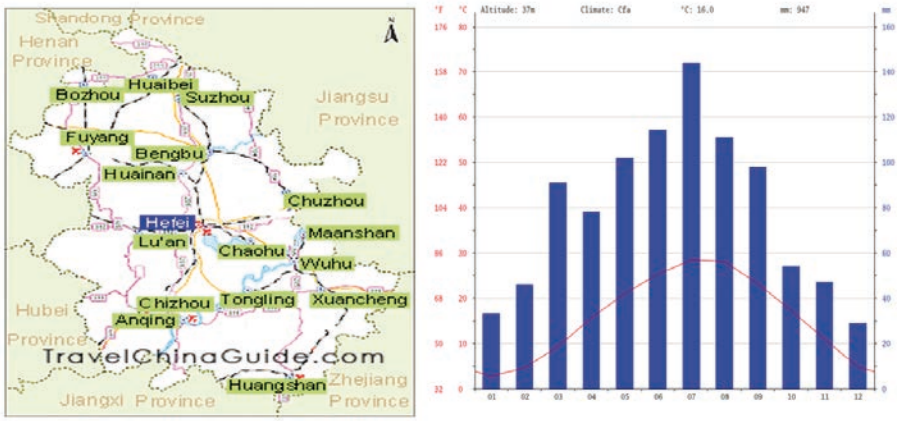

Figure 1: Illustrate of the geographical location and average of temperature and humidity of the experimental site.

experimental site was clay, with organic matter contents of 17.65 %. The geographical location and climate of the experimental site were given in Fig. 1.

2.2 FIELD EVALUATION

After harvest maturity, all the panicles of randomly selected 10 plants were harvested manually, put them into a Nylon mesh bag, sun-dried and threshed. Grain yield (t ha^{-1}, GY) for each plot was recorded from a 33-hill sample, adjusted to a moisture content of 14 % and expressed in kg ha^{-1}. Important agronomic traits including days to 50 % heading (DTH), plant height (cm; PH), SPAD-chlorophyll values (SPAD; CC), flag leaf angle(°; FAG) and area (cm^2; FAR), panicle length (cm; PL), number of panicles per plant (NP), panicle mass (g; PW), fertility percentage (%; FP) and 100-grain mass (g; HGW) according to standard evaluation system (IRRI, 2002). Panicle exertion (PE) refers to the proportion of the panicle that is exerted from the flag leaf sheath to the total panicle length after the full blooming, which is expressed in percentage.

$$PE\ (\%) = (Length\ (cm) of\ exerted\ panicle / Total\ length\ (cm) of\ panicle) \times 100$$

Grain type (GT) as per 1-9 scale, where 1, 3, 5, 9 scale based on length-to-width ratio: slender, > 3.0; medium, 2.1 to 3.0; bold 1.1 to 2.0; and round, < 1.0, respectively. Apparent heterosis (AH) subjective superiority of a hybrid over its parents or a check variety based on visual observation and expressed as vigorous vegetative growth in the field. Phenotypic acceptability (PA), pollen sterility/fertility were monitored and lines were evaluated on a single plant basis for phenotypic acceptability (on a 1–9 scale, where 1 is unacceptable, 3 is poor, 5 is fair, 7 is good and 9 is excellent) according to (Khush et al., 2003).

2.3 STATISTICAL ANALYSIS

2.3.1 Analysis of variance and correlation coefficient of traits

Analysis of variance (ANOVA) was performed using a general linear model

$$Yijk = \mu + Pij + rk + eijk$$

Whereas, Y_{ijk} = the observed value, μ = the population mean, P_{ij} = the mean effect of the *ij*th genotype, R_k = the *k*th replication effect and *eijk* is the experimental error assumed with *ijk*th observation and is assumed to be normally and independently distributed with a mean of zero and variance of σ^2. Mean comparisons among genotypes were evaluated using Tukey's HSD test (p 0.05). Correlation among values of the different traits investigated were performed based on Pearson's product-moment correlation as implemented.

2.3.2 Estimation of heterosis

Heterobeltiosis or better parent heterosis (BPH) was estimated in terms of percent increase or decrease of the F_1 hybrid over its better parent (Fehr, 1987).

$$BPH\ (\%) = [\overline{F1} - \overline{BP}/BP] \times 100$$

Significance of better parent heterosis was determined following the "t" test suggested by (Wynne et al., 1970).

$$BP\ ((t)\ )^{-} = (F1)^{-} - BP/\sqrt{((2/r)EMS\ )}$$

Where, F_1 = Mean of the F_1 hybrid for a trait,
BP = Mean of better parent in the cross, and
EMS = Error mean square.

2.3.3 Combining ability analysis

Estimates of GCA and SCA effects and their variances were computed using line × tester analysis according to (Singh & Chaudhary, 1985). The analysis was done using the Agrobase software statistical package. Additive and dominance genetic variances (σ^2_A and σ^2_D) were calculated by taking inbreeding coefficient (*F*) equal to one; that is, *F* = 1 because both lines and testers were inbred. Significance test for general and specific combining ability effects were performed using *t*-test. The relative mass of additive versus non-additive type of gene actions was calculated as described by (Verma & Srivastra, 2004).

$$(\sigma^{\ 2}_{gca}/\sigma^2_{sca}), and\ (\sigma^2_D\ /\sigma^2_A)^{\ 1/2}$$

2.3.4 Heritability

Broad (H^2) and narrow sense (h^2) heritability for the measured traits were estimated based on Griffiths et al.) (2000).

3 RESULTS

3.1 MEAN PERFORMANCE

Means of the parental genotypes and relative F_1 crosses for the fifteen studied characters showed that the earlier heading was obtained in genotypes 'Longping' × 'Sakha108', 'Sakha107', 'WTSC9059' × 'Sakha105' and 'Longping' × 'Sakha105' while the delayed heading genotypes were 'WTSC9369' × 'Sakha101', 'WTSC9369' × 'Sakha108', 'WTSC9039' × 'Sakha108' and 'WTSC9369' × 'Sakha107'. The tallest plants were observed for 'WTSC9369' × 'Sakha101', 'WTSC9039' × 'Sakha108' and 'WTSC9369' × 'Sakha108' crosses while minimum for 'WTSC9059', 'WTSC9039' and 'Longping' lines. The highest chlorophyll content values were observed for parents 'Sakha105', 'WTSC9039', 'Sakha107' and cross 'WTSC9059' × 'Sakha101', otherwise the lowest values for 'WTSC9369' × 'Sakha107', 'WTSC9039' × 'Sakha107', 'WTSC9369' × 'Sakha105' and 'WTSC9369', respectively. With respect to flag leaf angle (°) the erect flag leaves were found for crosses 'Longping' × 'Sakha108', 'WTSC9369' × 'Sakha108' and 'Longping' × 'Sakha105' while the widest flag leaf angle was found with genotypes 'WTSC9059'

Table 1: Origin, pedigree, salience and feature of nine rice genotypes

No.	Entries	Pedigree	Origin	Salience and feature
1	'WTSC9039'	18925/815 2011SH_7	China	Indica/Japonica type, EGMS line, short grain, plant height 90 cm, grain yield 7-7.20 t ha^{-1}, resistance to blast, sensitive to drought and salinity.
2	'WTSC9059'	Y585/18925 2011SH_8	China	Indica/Japonica type, EGMS line, short grain, plant height 82 cm, grain yield 4-4.50 t/ha, resistance to blast, sensitive to drought and salinity.
3	'WTSC9369'	18925/9451/958/9054	China	Indica/Japonica type, EGMS line, short grain, plant height 88 cm, grain yield 5-5.20 t/ha, resistance to blast, sensitive to drought and salinity.
4	'Longping'	Long ping seed company ltd.	China	Indica/Japonica type, EGMS line, short grain, plant height 80 cm, grain yield 4.30-4.50 t/ha, resistance to blast, sensitive to drought and salinity.
5	'Sakha101'	Giza 176 / Milyang 79	Egypt	Japonica type, pollinator, short grain, plant height 90 cm, high yield 10.5 t/ha, susceptible to blast and sensitive to drought and salinity
6	'Sakha102'	GZ 4096-7-1 / Giza 177	Egypt	Japonica type, pollinator, short grain, plant height 110 cm, high yield 10.0 t/ha, resistance to blast and sensitive to drought and salinity
7	'Sakha105'	GZ 5581-46-3 / GZ 4316-7-1-1	Egypt	Japonica type, pollinator, short grain, plant height 103 cm, high yield 10.0 t/ha, resistance to blast and sensitive to drought and salinity
8	'Sakha107'	Giza 177 / BL1	Egypt	Japonica type, pollinator, short grain, plant height 106 cm, high yield 10.0 t/ha, resistance to blast and tolerance to drought and moderate tolerance to salinity
9	'Sakha108'	'Sakha101'/ HR5824-B-3-2-3 //'Sakha101'	Egypt	Japonica type, pollinator, short grain, plant height 90 cm, high yield 10.5 t/ha, resistance to blast and sensitive to drought and salinity

WTSC , Winall Thriving Seed Company ltd.

Table 2: Mean performance for grain yield and its contributing traits for lines, testers and their crosses

Genotype	Days to heading (day)	Plant height (cm)	Chlorophyll content (SPAD)	Flag leaf angle (°)	Flag leaf area (cm^2)	Panicle exertion (%)	Panicle length (cm)	Number of panicles/ plant	Panicle mass (g)	Fertility Percentage (%)	100-grain mass (g)	Grain yield t ha^{-1}	Grain Type	Apparent heterosis	Phenotypic Acceptability
'WTSC9039'	88.28	69.67	45.00	27.50	37.40	81.01	22.33	8.67	7.67	87.22	3.63	7.02	2.52	4.50	8.00
'WTSC9059'	82.26	64.00	41.00	42.50	42.22	86.08	25.67	11.67	4.00	59.63	2.77	4.51	2.62	6.50	8.25
'WTSC9369'	88.28	78.67	38.00	37.50	30.40	81.01	20.67	11.33	5.33	58.15	3.00	5.02	3.52	5.00	7.90
'Longping'	80.25	75.00	40.00	17.00	23.67	91.14	20.33	8.17	4.47	63.84	2.83	3.51	1.01	5.50	6.25
'Sakha101'	94.29	100.00	40.50	35.50	45.67	100.00	22.00	7.33	2.17	87.79	2.73	7.02	1.21	4.50	8.30
'Sakha102'	79.25	121.00	41.00	35.00	57.53	100.00	22.00	11.33	2.90	86.01	2.97	6.52	1.31	4.50	8.35
'Sakha105'	80.25	107.33	43.50	27.50	45.23	100.00	24.33	11.67	2.77	89.58	3.57	6.02	1.11	5.50	8.25
'Sakha107'	76.24	98.33	45.50	42.50	52.50	100.00	20.33	12.33	2.53	97.00	2.57	7.52	0.91	5.50	8.50
'Sakha108'	90.28	101.00	40.50	35.50	46.50	100.00	23.67	10.67	2.43	94.67	3.30	8.03	1.11	5.50	7.05
'WTSC9039' × 'Sakha101'	88.28	112.67	41.00	20.50	72.37	100.00	25.33	12.00	8.07	94.22	3.70	11.03	1.21	7.50	8.25
'WTSC9039' × 'Sakha102'	80.25	118.67	40.50	22.50	65.63	70.89	29.33	16.67	3.03	51.00	3.07	12.04	1.21	9.00	6.25
'WTSC9039' × 'Sakha105'	81.25	108.67	40.50	25.00	81.20	100.00	27.67	9.33	8.70	93.03	3.70	3.01	0.80	7.50	6.35
'WTSC9039' × 'Sakha107'	82.26	108.00	38.50	35.00	50.93	60.76	25.67	11.67	2.97	20.00	2.70	4.01	0.91	7.50	5.25
'WTSC9039' × 'Sakha108'	114.36	131.00	40.50	37.50	73.33	100.00	27.33	9.00	8.70	93.62	3.97	11.03	1.21	8.00	6.40
'WTSC9059' × 'Sakha101'	100.31	117.00	44.50	34.50	63.82	100.00	25.33	10.67	10.33	95.00	4.13	14.04	1.11	9.00	8.35
'WTSC9059' × 'Sakha102'	78.24	117.67	41.50	40.00	49.77	100.00	25.33	9.67	5.67	95.67	2.86	10.03	1.01	5.50	5.30
'WTSC9059' × 'Sakha105'	76.24	106.67	40.50	40.00	55.83	100.00	26.83	12.67	5.97	82.67	2.72	7.52	1.11	7.50	8.20
'WTSC9059' × 'Sakha107'	107.34	107.33	41.00	26.50	61.28	70.89	23.83	12.33	3.77	50.67	2.65	8.03	1.21	7.50	7.00
'WTSC9059' × 'Sakha108'	100.31	120.67	42.50	24.50	72.00	100.00	27.50	9.00	5.40	95.00	2.48	12.04	1.01	8.50	9.10
'WTSC9369' × 'Sakha101'	110.34	127.67	40.50	25.50	62.67	100.00	24.33	13.33	6.17	92.93	2.76	11.03	1.01	7.50	8.50
'WTSC9369' × 'Sakha102'	86.27	120.00	40.50	30.00	54.13	65.82	24.33	9.67	2.20	41.00	2.00	4.01	2.42	7.25	5.50
'WTSC9369' × 'Sakha105'	85.27	109.67	39.50	30.00	66.40	67.85	29.67	7.00	2.03	45.00	1.73	3.70	1.31	6.00	5.50
'WTSC9369' × 'Sakha107'	114.36	115.33	39.50	26.50	39.62	66.84	25.50	11.00	2.93	35.00	2.65	4.01	2.62	8.25	7.50
'WTSC9369' × 'Sakha108'	111.35	131.33	39.50	15.50	91.00	100.00	27.33	10.67	3.23	71.00	3.07	8.03	0.80	7.30	6.50
'Longping' × 'Sakha101'	101.32	111.67	41.50	27.50	41.67	100.00	22.00	11.00	4.30	91.00	2.20	10.03	0.91	7.15	6.85
'Longping' × 'Sakha102'	79.25	124.00	40.50	40.00	65.65	100.00	24.83	9.67	4.40	97.33	2.55	9.53	1.01	8.25	5.50
'Longping' × 'Sakha105'	76.24	101.67	41.50	17.50	49.27	100.00	23.17	11.00	3.30	94.00	2.45	10.03	1.11	8.20	7.50
'Longping' × 'Sakha107'	107.34	113.33	41.00	20.00	62.68	65.82	24.50	12.67	1.50	28.00	3.00	3.01	1.11	7.30	7.00
'Longping' × 'Sakha108'	75.29	37.33	33.17	9.17	14.93	100.00	8.00	10.67	4.20	80.33	0.70	9.03	1.01	2.40	2.33
LSD 0.05	18.90	19.88	7.00	9.25	15.64	1.95	4.73	3.33	0.93	9.23	0.43	0.08	0.03	1.45	1.33
LSD 0.01	26.72	28.11	9.90	13.08	22.11	2.76	6.69	4.71	1.32	13.05	0.61	0.11	0.04	2.05	1.88

× 'Sakha105' (40 °), 'Longping' × 'Sakha102'(40 °), 'WTSC9059' (42.5 °) and 'Sakha107'(42.5 °). Regardless the small flag leaf area was found for 'Longping' × 'Sakha108', 'Longping', 'WTSC9369' and 'WTSC9039' crosses and the largest area for crosses 'WTSC9039' × 'Sakha101', 'WTSC9039' × 'Sakha108', 'WTSC9039' × 'Sakha105' and 'WTSC9369' x 'Sakha108'. The crosses 'Longping' × 'Sakha101', 'Longping' × 'Sakha102', 'Longping' × 'Sakha105' and 'Longping' × 'Sakha108' had the complete panicle exertion percentage while the partial and lowest values were obtained from hybrid combinations 'WTSC9039' × 'Sakha107', 'WTSC9369' × 'Sakha102', 'Longping' × 'Sakha107' and 'WTSC9369' × 'Sakha107'.

The highest panicle length (cm) was observed with crosses 'WTSC9059' × 'Sakha108', 'WTSC9039' × 'Sakha105', 'WTSC9039' × 'Sakha102' and 'WTSC9369' × 'Sakha105', respectively. However, the lowest panicle length found for genotypes 'Longping' × 'Sakha108', 'Longping', 'Sakha107' and 'WTSC9369'. The maximum number of panicles/ plants were obtained from crosses 'WTSC9059' × 'Sakha105' (12.67), 'Longping' × 'Sakha107' (12.67), 'WTSC9369' × 'Sakha101' (13.33) and 'WTSC9039' × 'Sakha102' (16.67) compared to genotypes 'WTSC9369' × 'Sakha105' (7), 'Sakha101' (7.33), 'Longping' (8.17) and 'WTSC9039' (8.67) with smaller panicles number. The heaviest panicle mass were found for crosses 'WTSC9039' × 'Sakha101', 'WTSC9039' × 'Sakha105', 'WTSC9039' × 'Sakha108' and 'WTSC9059' × 'Sakha101' that ranged from 8.07 g and 10.33 g while the lowest panicle mass was found for genotypes 'Longping' × 'Sakha107', 'WTSC9369' × 'Sakha105', 'Sakha101' and 'WTSC9369' × 'Sakha102' which ranged between 1.5 g to 2.2 g. Maximum fertility percentage was found for genotypes 'WTSC9059' × 'Sakha108' (95 %), 'WTSC9059' × 'Sakha102' (95.67 %), 'Sakha107' (97 %) and 'Longping' × 'Sakha102' (97.33 %) while the minimum fertility was noted with 'WTSC9039' × 'Sakha107' (20 %), 'Longping' × 'Sakha107' (28 %), 'WTSC9369' × 'Sakha107' (35 %) and 'WTSC9369' × 'Sakha102' (41 %). The highest 100-grain mass (g) was found for crosses 'WTSC9039' × 'Sakha101', 'WTSC9039' × 'Sakha105', 'WTSC9039' × 'Sakha108' and 'WTSC9059' × 'Sakha101' which ranged from 3.7 g to 4.13 g. While, lowest 100-grain mass was gained from crosses 'Longping' × 'Sakha108', 'WTSC9369' × 'Sakha105', 'WTSC9369' × 'Sakha102' and 'Longping' × 'Sakha101'. The highest grain yield was found for crosses 'WTSC9369' × 'Sakha101' (11.03 t/ha), 'WTSC9039' × 'Sakha102' (12.04 t/ha), 'WTSC9059' × 'Sakha108' (12.04 t/ha) and 'WTSC9059' × 'Sakha101' (14.04 t/ha), while the lowest grain yield was found with genotypes 'WTSC9039' × 'Sakha105' (3.01 t/ha), 'Longping' × 'Sakha107' (3.01 t/ha), 'Longping' (3.51 t/ha) and 'WTSC9369' × 'Sakha105' (3.7 t ha^{-1}). The short grain type was found with genotype 'WTSC9039' × 'Sakha105', 'WTSC9369' × 'Sakha108', 'Sakha107' and 'WTSC9039' × 'Sakha107', while the longest grain type was found for genotypes 'WTSC9039', 'WTSC9059', 'WTSC9369' × 'Sakha107' and 'WTSC9369'. The highest apparent heterosis was obtained from crosses 'Longping' × 'Sakha102', 'WTSC9059' × 'Sakha108', 'WTSC9039' × 'Sakha102' and 'WTSC9059' × 'Sakha101' which ranged from 8.25 to 9, however the lowest mean values were observed with genotypes 'Longping' × 'Sakha108', 'WTSC9039', 'Sakha101' and 'Sakha102' that ranged between 2.4 to 4.5. Regardless phenotypic acceptability, the desired mean values 8.35, 8.5, 8.5 and 9.1 were found with genotypes 'WTSC9039' × 'Sakha108', 'Sakha105', 'WTSC9059' × 'Sakha108' and 'WTSC9059' × 'Sakha107', respectively, otherwise the lowest mean values were obtained from crosses 'Longping' × 'Sakha108', 'Longping' × 'Sakha107', 'WTSC9039' × 'Sakha105' and 'WTSC9059' × 'Sakha101', respectively.

Pearson's correlation revealed that all yield component traits were positively correlated with GY except for FAR and highly significant for traits PE, PW, FP and AH otherwise the negative correlation and significant was found with AH (Table 3). DTH was correlated significant and positive with FAG and AH. PH correlated significant and positive with CC, FAG, PL, FP, HGMW and AH while negative and significant with GT. CC was correlated significant and positive with FAR, FP, HGWM and PA. The positive and significant correlation was found among FAG and PL, HGW and AH otherwise the correlated negative and significant with GT. PE has positive correlation and significant with PW, FP and GY while negative and significant with GT. The positive correlation and significant was found between PL and HGW, AH and PA. NPP has significant and positive correlation with AH. The significant correlation and positive was found among PW and FP, HGW and GY. FP has positive and negative significant correlation with GY and GT, respectively. The positive and significant correlation was found with HGW, AH and PA.

3.2 HETEROSIS PERCENTAGE

The degree of heterosis showed over better parent (Table 4). Days to heading; heterosis percent over better parent showed highly significant and negative which ranged from -5.00 ('Longping' × 'Sakha105') to 6.18% ('Longping' × 'Sakha108'). Three hybrids out from twenty crosses exhibited negative significant heterosis for days to heading. The plant height, desirable highly significant and negative heterosis over better parent was found for cross 'Longping' × 'Sakha108'. Four hybrids exhibited

Table 3: Correlation between studied agronomic traits

	DTH	PH	CC	FAR	FAG	PE	PL	NPP	PM	FP	HGM	GY	GT	AH
PH	0.44**													
CC	0.05	0.32*												
FAR	-0.16	0.16	0.35*											
FAG	0.35*	0.78**	0.27	0.02										
PE	-0.14	0.03	0.18	0.06	0.09									
PL	0.26	0.71**	0.40*	0.27	0.74**	-0.21								
NPP	-0.01	0.13	0.01	-0.07	0.09	-0.15	0.06							
PW	0.13	0.04	0.21	0.07	0.23	0.40*	0.12	-0.12						
FP	-0.15	0.06	0.36*	0.14	0.12	0.93**	-0.14	-0.18	0.49**					
HGW	0.24	0.36*	0.57**	0.24	0.41*	0.19	0.49**	0.09	0.55**	0.27				
GY	0.16	0.28	0.22	-0.03	0.26	0.53**	0.03	0.24	0.45**	0.61**	0.17			
GT	0.05	-0.35*	-0.06	0.24	-0.40*	-0.44**	-0.06	-0.04	-0.02	-0.36*	0.01	-0.36*		
AH	0.39*	0.68**	0.27	0.01	0.64**	-0.16	0.72**	0.31*	0.24	-0.12	0.33*	0.32*	-0.16	
PA	0.23	0.24	0.68**	0.30	0.20	0.23	0.34*	0.16	0.17	0.29	0.53**	0.15	0.18	0.22

*p = 0.05; **p = 0.01; ***p = 0.001

positively significant heterosis over better parent the degree of heterosis that varied from 1.22 % ('WTSC9059' × 'Sakha102') to 8.54 % ('WTSC9059' × 'Sakha101'). For flag leaf angle, heterosis percent over better parent ranged from -2.82 % ('WTSC9059' × 'Sakha101') to -56.34 % ('WTSC9369' × 'Sakha108'). Eleven hybrids exhibited desirable significant negative heterosis for this trait over better parent. For flag leaf area, fourteen hybrids had desired significant positive heterosis over better parent. 'WTSC9369' × 'Sakha108' (95.70 %) recorded the highest positive heterosis over better parent followed by 'WTSC9039' × 'Sakha105', 'WTSC9039' × 'Sakha101', 'WTSC9039' × 'Sakha108' with 79.53 %, 58.46 % and 57.70 %, respectively. Significant and positive heterosis ranged 8.93 % to 95.70 %. Thirteen crosses out from twenty crosses have complete panicle exertion and the heterosis over better parent equal 0.00 %. For panicle length (cm), fourteen out from twenty crosses showed highly significant and positive heterosis according to over better parent which ranged 4.52 % to 31.35 %. The crosses 'WTSC9039' × 'Sakha101', 'Longping' × 'Sakha101', 'WTSC9369' × 'Sakha101', 'WTSC9059' × 'Sakha105' and 'Longping' × 'Sakha107' gave significant and positive heterosis over better parent that ranged between 2.76 % to 38.41 % for number of panicles/plant. The panicle mass had significant and positive heterosis due to better parent found with eight hybrid crosses 'WTSC9039' × 'Sakha101', 'WTSC9039' × 'Sakha105', 'WTSC9039' × 'Sakha108', 'WTSC9369' × 'Sakha101', 'WTSC9059' × 'Sakha108', 'WTSC9059' × 'Sakha102', 'WTSC9059' × 'Sakha105' and 'WTSC9059' × 'Sakha101' were values 5.22 %, 13.43 %, 13.43 %, 15.76 %, 35.00 %, 41.75 %, 49.25 % and 158.25 %, respectively. Among the 20 hybrids, four hybrids showed significantly positive heterosis for fertility percentage were 'WTSC9039' × 'Sakha101', 'WTSC9059' × 'Sakha101', 'WTSC9059' × 'Sakha102' and 'Longping' × 'Sakha102' which ranged between 7.32 % to 13.16 %. Three hybrids out of twenty crosses showed significant and positive heterosis over better parent including 'Longping' × 'Sakha107' (6.01%), 'WTSC9039' × 'Sakha108'(9.37%) and 'WTSC9059' × 'Sakha101'(49.10%) for 100-grain mass. Eleven out of twenty crosses had significant and positive heterosis over better parent for grain yield with the best hybrids including 'WTSC9059' × 'Sakha101', 'WTSC9369' × 'Sakha101', 'Longping' × 'Sakha105' and 'WTSC9039' × 'Sakha102' with 57.12 %, 66.61 %, 71.51 % and 100.00 %, respectively.

For grain type fifteen from twenty crosses showed significant and negative heterosis over better parent which ranged between -25.57 % to -77.27 %. For apparent heterosis, eighteen crosses recorded desired significant positive heterosis over better parent. Better parent heterosis ranged between 9.09 and 100.00 %. Only one cross 'WTSC9059' × 'Sakha108' had positive and significant heterosis over better parent for phenotypic acceptability.

3.3 COMBINING ABILITY ANALYSIS

Analysis of variance of combining ability revealed

Table 4: Heterosis over better parent for grain yield and associated traits

Crosses	Days to heading	Plant height (cm)	Chlo-rophyll content	Flag leaf angle	Flag leaf area (cm^2)	Panicle exertion	Panicle length (cm)	Number of panicles/ plant	Panicle mass (g)	Fertility Percent-age	100-grain mass (g)	Grain yield t ha^{-1}	Grain Type	Apparent heterosis	Phenotypic acceptability
'WTSC9039' × 'Sakha101'	0.00 ns	61.72**	-8.89**	-25.45**	58.46**	0.00 ns	13.43**	38.41**	5.22**	7.32*	1.93 ns	57.12**	-51.98**	66.67**	-0.60 ns
'WTSC9039' × 'Sakha102'	1.26 ns	70.33**	-10.00**	-18.18**	14.08**	-29.11**	31.35**	47.13**	-60.50**	-41.53**	-15.43**	71.51**	-51.98**	100.00**	-25.15**
'WTSC9039' × 'Sakha105'	1.25 ns	55.98**	-10.00**	-9.09**	79.53**	0.00 ns	13.73**	-20.05**	13.43**	3.85 ns	1.93 ns	-57.12**	-68.25**	36.36**	-23.03**
'WTSC9039' × 'Sakha107'	7.90**	55.02**	-15.38**	27.27**	-2.99*	-39.24**	14.96**	-5.35**	-61.28**	-79.38**	-25.62**	-46.68**	-63.89**	36.36**	-38.24**
'WTSC9039' × 'Sakha108'	29.54**	88.03**	-10.00**	36.36**	57.70**	0.00 ns	15.46**	-15.65**	13.43**	-1.11 ns	9.37**	37.36**	-51.98**	45.45**	-20.00**
'WTSC9059' × 'Sakha101'	21.94**	82.81**	8.54**	-2.82**	39.74**	0.00 ns	-1.32*	-8.57**	158.25**	8.21**	49.10**	100.00**	-57.63**	38.46**	0.60 ns
'WTSC9059' × 'Sakha102'	-1.27 ns	83.86**	1.22*	14.29**	-13.49**	0.00 ns	-1.32*	-17.14**	41.75**	11.23**	-3.70 ns	53.83**	-61.45**	-15.38**	-36.53**
'WTSC9059' × 'Sakha105'	-5.00**	66.67**	-6.90**	45.45**	23.44**	0.00 ns	4.52**	8.57**	49.25**	-7.71*	-23.81**	24.92**	-57.63**	15.38**	-0.61 ns
'WTSC9059' × 'Sakha107'	40.79**	67.70**	-9.89**	-37.65**	16.72**	-29.11**	-7.17**	0.00 ns	-5.75**	-47.76**	-4.33**	6.78 ns	-53.82**	15.38**	-17.65**
'WTSC9059' × 'Sakha108'	21.94**	88.55**	3.66**	-30.99**	54.84**	0.00 ns	7.13**	-22.88**	35.00**	0.35 ns	-24.85**	49.94**	-61.45**	30.77**	10.30**
'WTSC9369' × 'Sakha101'	24.99**	62.29**	0.00 ns	-28.17**	37.22**	0.00 ns	10.59**	17.65**	15.76**	5.85 ns	-8.00**	57.12**	-71.31**	50.00**	2.41 ns
'WTSC9369' × 'Sakha102'	8.86**	52.54**	-1.22*	-14.29**	-5.91**	-34.18**	10.59**	-14.65**	-58.72**	-52.33**	-33.33**	-38.50**	-31.25*	45.00**	-34.13**
'WTSC9369' × 'Sakha105'	6.26**	39.41**	-9.20**	9.09**	46.81**	-32.15**	21.95**	-40.02**	-61.91**	-49.77**	-51.54**	-38.54**	-62.78**	9.09**	-33.33**
'WTSC9369' × 'Sakha107'	50.00**	46.60**	-13.19**	-29.33**	-24.53**	-33.16**	23.37**	-10.79**	-45.03**	-63.92**	-11.67**	-46.68**	-25.57*	50.00**	-11.76**
'WTSC9369' × 'Sakha108'	26.13**	66.94**	-2.47**	-56.34**	95.70**	0.00 ns	15.46**	-5.83**	-39.40**	-25.00**	-6.97**	0.00 ns	-77.27**	32.73**	-17.72**
'Longping' × 'Sakha101'	26.26**	48.89**	2.47**	61.76**	-8.76**	0.00 ns	0.00	34.64**	-3.80**	3.66 ns	-22.26**	42.88**	-24.79 ns	30.00**	-17.47**
'Longping' × 'Sakha102'	0.00 ns	65.33**	-1.22*	135.29**	14.11**	0.00 ns	12.86**	-14.65**	-1.57 ns	13.16**	-14.14**	46.17**	-22.90 ns	50.00**	-34.13**
'Longping' × 'Sakha105'	-5.00**	35.56**	-4.60**	2.94**	8.93**	0.00 ns	-4.77**	-5.74**	-26.17**	4.93 ns	-31.37**	66.61**	0.00 ns	49.09**	-9.09**
'Longping' × 'Sakha107'	40.79**	51.11**	-9.89**	17.65**	19.39**	-34.18**	20.51**	2.76**	-66.44**	-71.13**	6.01**	-59.97**	9.90 ns	32.73**	-17.65**
'Longping' × 'Sakha108'	-6.18**	-50.23**	-18.10**	-46.06**	-67.89**	0.00 ns	-66.20**	0.03 ns	-6.04**	-15.15**	-78.79**	12.45 ns	-9.01 ns	-56.36**	-66.95**

* ns, * and **: non-significant and significant effect at 0.05 and 0.01 probability

significant differences among genotypes, crosses, lines, testers and line × tester interactions. The significant differences among the lines, testers and lines × testers indicated that the genotypes had wide genetic diversity for all traits except days to heading, chlorophyll content, panicle length and apparent heterosis for parents (Table 5). The mean sum of squares for crosses was portioned into lines, testers and line × tester components. In case of lines and testers, significant variances were observed in all traits except number of panicles/ plants. All the line × tester interactions were significant for all traits studied. Variances of SCA were higher than the GCA variances for all traits. This was further supported by low magnitude of σ^2GCA/ σ^2SCA ratios (Table 6). The ratio of GCA and SCA variances were found to be less than unity for all the characters. H^2 ranged from 64.00 % (NPP) to 98.00 % (PE) However, all traits exhibited low h^2, ranging from 1 % with PH, CC and FAN to 14 % with PE and FP.

The relative contribution of lines, testers and interaction of line × tester on expression fifteen traits studied, three traits including panicle length, panicle mass and 100-grain mass showed high contribution of lines ranged from 34.19 to 39.18. Four traits, panicle exertion, panicle mass, fertility percentage and grain yield showed contributed tester ranged from 38.01 to 63.41 % in their hybrids. In other eight traits viz., grain type, phenotypic acceptability, days to heading, flag leaf angle, chlorophyll content, plant height, flag leaf area, number of panicles/ plant, apparent heterosis line × tester interaction contributed the highest depending on the respective cross showing mean percentage of 1.82, 52.72, 53.43, 54.9, 56.55, 60.98, 64.01, 69.28, 70.51, 73.67 and 79.73 % respectively.

3.4 COMBINING ABILITY ESTIMATED

There were significant differences among the genotypes for traits (Table 5), which lead to the combining ability analysis. The increase and decrease in the values of traits desired, positive and negative values of g_i were considered. Plant height, days to heading and flag leaf angle negative GCA and SCA effects were desirable, while in other characters positive GCA and SCA effects were desirable.

3.5 GENERAL COMBINING ABILITY (GCA) EFFECTS

GCA effects of fifteen traits showed that 'WTSC9039' was produced highly significant GCA for

Table 5: Analysis of variance of the line × tester mating design for grain yield and yield components

Source of variance	df	Days to heading	Plant height (cm)	Chloro-phyll content	Flag leaf angle	Flag leaf area (cm^2)	Panicle exertion	Panicle length (cm)	Number of panicles/ plants	Panicle mass (g)	Fertility Percentage	100-grain mass (g)	Grain yield t ha^{-1}	Grain Type	Apparent heterosis	Phenotypic Acceptability
Replications	2	55.24 ns	231.80 ns	19.17 ns	234.93**	138.79 ns	23.74**	10.22 ns	3.882 ns	0.321 ns	25.24 ns	0.03 ns	0.40**	0.05**	0.77 ns	2.08**
Treatments	28	796.34**	1392.19**	89.43**	232.93**	847.79**	617.13**	47.16**	11.51**	16.10**	1717.32**	1.52**	29.3**	1.37**	7.63**	6.35**
Parents	8	110.54 ns	1122.00**	18.37 ns	199.64**	332.39**	218.69**	10.45 ns	9.80*	9.67**	711.20**	0.81**	6.75**	2.64**	1.33 ns	1.65**
Crosses	19	1086.64**	1127.84**	119.05**	223.86**	813.96**	794.74**	58.87**	12.43**	18.60**	2166.88**	1.86**	35.89**	0.66**	6.23**	7.22**
Par. vs. crosses	1	766.97**	8576.30**	94.96**	671.58**	5613.81**	430.08**	118.37**	7.42 ns	19.98**	1224.49**	0.83*	84.42**	4.59**	84.70**	27.51**
Lines	3	1195.71**	1513.61**	113.00**	287.21**	1281.75**	646.08**	132.91**	4.99 ns	40.27**	2022.60**	4.62**	43.59**	1.22**	4.24**	7.85**
Testers	4	1346.00**	510.55**	118.75**	199.47**	178.94	2393.89**	32.52**	11.80*	33.58**	6465.36**	0.79**	93.89**	0.55**	2.81**	9.57**
Lines × testers	12	972.92**	1237.15**	120.67**	216.16**	908.70**	298.85**	49.14**	14.50**	8.19**	770.13**	1.52**	14.63**	0.56**	7.86**	6.27**
Error	56	133.47	147.69	18.31	31.95	91.47	1.42	8.379	4.15	0.32	31.87	0.15	0.002	0.001	0.78	0.65
Total	86															
CV%		13.01	11.54	10.67	19.33	17.61	1.33	12.01	18.88	12.60	7.54	13.96	0.65	10.28	13.22	11.53

* ns, * and **: non-significant and significant effect at 0.05 and 0.01 probability

Table 6: Estimates of genetic variances and heritability for grain yield and yield components

Traits	DTH	PH	CC	FAN	FAR	PE	PL	NPP	PM	FP	HGM	GY	GT	AH	PA
Cov H.S (lines	14.85	18.43	-0.51	4.74	24.87	23.15	5.59	-0.63	2.14	83.50	0.21	1.93	0.04	-0.24	0.11
Cov H.S (tester)	31.09	-60.55	-0.16	-1.39	-60.81	174.59	-1.38	-0.23	2.12	474.60	-0.06	6.61	0.00	-0.42	0.28
Cov F.S	342.70	316.32	33.10	67.22	235.53	356.03	20.77	2.16	8.71	977.28	0.71	16.28	0.26	1.45	2.39
σ^2_{GCA}	3.32	-3.19	-0.05	0.23	-2.77	14.47	0.28	-0.06	0.30	40.77	0.01	0.62	0.00	-0.05	0.03
σ^2_{SCA}	279.82	363.15	34.12	61.40	272.41	99.14	13.59	3.45	2.62	246.09	0.46	4.88	0.19	2.36	1.87
$\sigma^2_{GCA}/\ \sigma^2_{SCA}$	0.01	-0.01	0.00	0.00	-0.01	0.15	0.02	-0.02	0.12	0.17	0.02	0.13	0.02	-0.02	0.01
σ^2_A	6.64	0.001	0.09	0.45	0.01	28.95	0.57	0.002	0.61	81.53	0.02	1.24	0.01	0.001	0.06
σ^2_D	279.82	363.15	34.12	61.40	272.41	99.14	13.59	3.45	2.62	246.09	0.46	4.88	0.19	2.36	1.87
$(\sigma^2_D/\sigma^2_A)^{1/2}$	42.15	-56.91	-362.90	136.40	-49.26	3.43	23.92	-28.54	4.31	3.02	22.90	3.93	31.17	-24.85	34.05
H^2%	83.00	89.00	80.00	86.00	89.00	98.00	82.00	64.00	97.00	93.00	90.00	67.00	75.00	90.00	90.00
h^2%	3.00	1.00	1.00	1.00	1.00	14.00	4.00	1.00	11.00	14.00	4.00	13.00	2.00	1.00	3.00

σ^2_{GCA}, Variance due to general combining ability (GCA); σ^2_{SCA}, variance due to specific combining ability (SCA); $\sigma^2_{GCA}/\ \sigma^2_{SCA}$, GCA variance ratio; σ^2_A, additive genetic variance; σ^2_D, dominance genetic variance; $(\sigma^2_D/\sigma^2_A)^{1/2}$, degree of dominance; H^2, Broad-sense heritability; h^2 narrow-sense heritability.

number of panicles/ plants, days to heading, flag leaf area, panicle length, panicle mass, 100-grain mass and apparent heterosis. Therefore, this line was considered as the best general combiner for the respective traits. Similarly, 'WTSC9059' was identified as good general combiner for chlorophyll content, panicle exertion, panicle mass, fertility percentage, 100-grain mass, grain yield, apparent heterosis and phenotypic acceptability.

Otherwise 'WTSC9369' was good combiner for panicle length, flag leaf angle, flag leaf area and grain type. The line 'Longping' was good combiner for panicle exertion, plant height, flag leaf angle, days to heading, fertility percentage and grain yield. The tester 'Sakha101' identified as good combiner for chlorophyll content, panicle exertion, number of panicles per plant, panicle mass, fertility percentage, 100-grain mass, grain yield, apparent heterosis and phenotypic acceptability. Therefore, this line was considered as the best general combiner for the respective traits**.** The tester 'Sakha102' was good combiner for days to heading, panicle length, number of panicles/ plants, chlorophyll content, grain yield and grain type while"Sakha105" found good combiner for days to heading, plant height, chlorophyll content, flag leaf area, panicle exertion, panicle length and fertility percentage. The tester 'Sakha107' identified as desired combiner for number of panicles/ plants, plant height, grain type and apparent heterosis therefore the tester 'Sakha108' was a good combiner for flag leaf area, panicle exsertion, flag leaf angle, plant height, panicle mass, fertility percentage and grain yield. However, none of parents was observed significant and positive GCA effect for relative water content and number of panicles/plant.

3.6 SPECIFIC COMBINING ABILITY (SCA) EFFECTS

The results of SCA effect of crosses showed that out of twenty hybrid combinations, two of them viz., 'WTSC9039' × 'Sakha107' and 'Longping' × 'Sakha108' produced significant and negative SCA effect for days to heading (Table 8). One and five crosses out from twenty hybrid combination showed significant and negative SCA effects in desirable direction for plant height and flag leaf angle, respectively. For chlorophyll content, five crosses, 'WTSC9039' × 'Sakha108', 'WTSC9059' × 'Sakha108', 'WTSC9369' × 'Sakha108', 'Longping' × 'Sakha105' and 'Longping' × 'Sakha107' showed significant and positive values for SCA effect. Three hybrid combinations 'WTSC9369' × 'Sakha108', 'Longping' × 'Sakha102' and 'Longping' × 'Sakha107' showed significant and positive SCA effect of flag leaf area. Ten out from twenty crosses were significant and positive SCA for panicle exertion. Four out of twenty crosses 'WTSC9059' × 'Sakha108', 'WTSC9369' × 'Sakha108', 'Longping' × 'Sakha102' and 'Longping' × 'Sakha107' showed significant and positive SCA effects for panicle length two hybrid combinations 'WTSC9039' × 'Sakha102' and 'WTSC9059' × 'Sakha105' reported significant and positive SCA effect for number of panicles/plant. For panicle mass, five out from twenty crosses 'WTSC9039' × 'Sakha105', 'WTSC9039' × 'Sakha108', 'WTSC9059' × 'Sakha101', 'WTSC9369' × 'Sakha107' and 'Longping' × 'Sakha102' had significant and positive SCA effect. Eight hybrid crosses showed significant and positive SCA effect for fertility percentage. Six combinations possessed significant and posi-

Table 7: General combining ability estimates of lines and testers for traits studied

	Days to heading	Plant height (cm)	Chlorophyll content	Flag leaf angle	Flag leaf area (cm2)	Panicle exertion	Panicle length (cm)	Number of panicles/ plant
Line								
'WTSC9039'	-1.51 ns	3.78 ns	0.79 ns	0.72 ns	8.98**	-2.11**	2.18**	0.75 ns
'WTSC9059'	1.70 ns	1.85 ns	2.59 ns	5.72**	0.83 ns	5.73**	0.88 ns	-0.12 ns
'WTSC9369'	10.72**	8.78**	0.49 ns	-1.88 ns	3.05 ns	-8.34**	1.34*	-0.65 ns
'Longping'	-10.91**	-14.42**	-3.88**	-4.55**	-12.86**	4.72**	-4.39**	0.02 ns
LSD0.05	4.91	5.16	1.82	2.40	4.06	0.51	1.23	0.87
LSD0.01	6.94	7.30	2.57	3.39	5.74	0.72	1.74	1.22
Tester								
'Sakha101'	9.27**	5.23 ns	2.47*	-0.38 ns	0.42 ns	11.56**	-0.64 ns	0.77 ns
'Sakha102'	-9.79**	8.07*	1.34 ns	5.74**	-0.91 ns	-4.27**	1.07 ns	0.43 ns
'Sakha105'	-11.04**	-5.35 ns	1.09 ns	0.74 ns	3.47 ns	3.52**	1.94**	-0.98*
'Sakha107'	12.03**	-1.02 ns	0.59 ns	-0.38 ns	-6.08**	-22.36**	-0.02 ns	0.93 ns
'Sakha108'	-0.47 ns	-6.93*	-5.49**	-5.72**	3.11	11.56**	-2.35**	-1.15*
LSD0.05	5.49	5.77	2.03	2.68	4.54	0.57	1.37	0.97
LSD0.01	7.76	8.16	2.87	3.80	6.42	0.80	1.94	1.37
Line	Panicle mass (g)	Fertility Percentage	100-grain mass (g)	Grain yield t ha^{-1}	Grain Type	Apparent heterosis	Phenotypic Acceptability	
Line								
'WTSC9039'	1.45**	-1.95 ns	0.67**	-0.03 ns	-0.14**	0.55**	-0.16 ns	
'WTSC9059'	1.38**	11.48**	0.21*	2.07**	-0.12**	0.25 ns	0.93**	
'WTSC9369'	-1.53**	-15.34**	-0.31**	-2.10**	0.43**	-0.10 ns	0.04 ns	
'Longping'	-1.30**	5.81**	-0.57**	0.07*	-0.18**	-0.70**	-0.82**	
LSD0.05	0.24	2.40	0.17	0.06	0.04	0.38	0.34	
LSD0.01	0.34	3.39	0.24	0.08	0.06	0.53	0.49	
Tester								
'Sakha101'	2.37**	20.96**	0.44**	3.28**	-0.15**	0.43*	1.33**	
'Sakha102'	-1.02**	-1.07 ns	-0.14 ns	0.64**	0.21**	0.15 ns	-1.02**	
'Sakha105'	0.16 ns	6.35**	-0.10 ns	-2.19**	-0.12**	-0.06 ns	0.23 ns	
'Sakha107'	-2.05**	-38.91**	0.00 ns	-3.49**	0.26**	0.28 ns	0.03 ns	
'Sakha108'	0.54**	12.67**	-0.20*	1.77**	-0.20**	-0.81**	-0.57**	
LSD0.05	0.27	2.68	0.19	0.07	0.05	0.42	0.39	
LSD0.01	0.38	3.79	0.26	0.09	0.07	0.59	0.54	

ns, and * and **: nonsignificant and significant effect at 0.05 and 0.01 probability.

tive SCA effect for 100-grain mass with combinations of 'WTSC9039' × 'Sakha105', 'WTSC9039' × 'Sakha108', 'WTSC9059' × 'Sakha101', 'WTSC9369' × 'Sakha108', 'Longping' × 'Sakha102' and 'Longping' × 'Sakha107' trait.

Significant and positive SCA effects were observed in nine hybrid combinations for grain yield including 'WTSC9039' × 'Sakha102', 'WTSC9039' × 'Sakha108', 'WTSC9059' × 'Sakha101', 'WTSC9059' × 'Sakha107', 'WTSC9369' × 'Sakha101', 'WTSC9369' × 'Sakha107', 'WTSC9369' × 'Sakha108', 'Longping' × 'Sakha102' and 'Longping' × 'Sakha105'. Among the nine crosses which depicted highly significant positive SCA effects for grain yield showed high heterosis. Ten and seven out from twenty crosses showed significant and positive SCA effect for grain type and apparent heterosis. The crosses

Table 8: Specific combining ability estimates of crosses for traits studied

Crosses	Days to heading	Plant height (cm)	Chloro-phyll content	Flag leaf angle	Flag leaf area (cm^2)	Panicle exertion	Panicle length (cm)	Number of panicles/ plant	Panicle mass (g)	Fertility Percent-age	100-grain mass (g)	Grain yield t ha^{-1}	Grain Type	Apparent heterosis	Phenotypic Acceptability
'WTSC9039' × 'Sakha101'	-10.27 ns	-8.37 ns	-1.67 ns	-7.22*	3.25 ns	2.11**	-1.09 ns	-0.50 ns	-0.60*	2.88 ns	-0.17 ns	-0.47**	0.29**	-0.83 ns	0.42 ns
'WTSC9039' × 'Sakha102'	0.76 ns	-5.20 ns	-1.04 ns	-11.34**	-2.15 ns	-11.18**	1.20 ns	4.50**	-2.24**	-18.30**	-0.22 ns	3.17**	-0.07**	0.96*	0.77*
'WTSC9039' × 'Sakha105'	3.02 ns	-1.78 ns	-0.79 ns	-3.84 ns	9.04 ns	10.15**	-1.34 ns	-1.42 ns	2.25**	16.30**	0.38*	-3.02**	-0.14**	-0.35 ns	-0.38 ns
'WTSC9039' × 'Sakha107'	-19.05**	-6.78 ns	-2.29 ns	7.28*	-11.68*	-3.20**	-1.38 ns	-1.00 ns	-1.28**	-11.47**	-0.72**	-0.72**	-0.42**	-0.68 ns	-1.28**
'WTSC9039' × 'Sakha108'	25.54**	22.13**	5.79**	15.12**	1.53 ns	2.11**	2.62 ns	-1.58 ns	1.87**	10.58**	0.74**	1.04**	0.34**	0.91*	0.47 ns
'WTSC9059' × 'Sakha101'	-1.45 ns	-2.10 ns	0.03 ns	1.78 ns	2.86 ns	-5.73**	0.21 ns	-0.97 ns	1.73**	-9.76**	0.72**	0.44**	0.16**	0.97*	-0.57 ns
'WTSC9059' × 'Sakha102'	-4.46 ns	-4.27 ns	-1.84 ns	1.16 ns	-9.86*	10.09**	-1.50 ns	-1.63 ns	0.46 ns	12.94**	0.03 ns	-0.94**	-0.29**	-2.25**	-1.27**
'WTSC9059' × 'Sakha105'	-5.21 ns	-1.85 ns	-2.59 ns	6.16**	-8.17 ns	2.30**	-0.88 ns	2.78**	-0.42 ns	-7.48*	-0.15 ns	-0.61**	0.14**	-0.05 ns	0.38 ns
'WTSC9059' × 'Sakha107'	2.82 ns	-5.52 ns	-1.59 ns	-6.22**	6.82 ns	-0.92 ns	-1.92 ns	0.53 ns	-0.41 ns	5.77*	-0.31 ns	1.19**	-0.13**	-0.38 ns	-0.62 ns
'WTSC9059' × 'Sakha108'	8.29 ns	13.73*	5.99**	-2.88 ns	8.35 ns	-5.73**	4.08**	-0.72 ns	-1.37**	-1.47 ns	-0.29 ns	-0.07**	0.12**	1.71**	2.08**
'WTSC9369' × 'Sakha101'	-0.44 ns	1.63 ns	-1.87 ns	0.38 ns	-0.52 ns	8.34**	-1.26 ns	2.23*	0.48 ns	14.98**	-0.13 ns	1.60**	-0.48**	-0.19 ns	0.47 ns
'WTSC9369' × 'Sakha102'	-5.46 ns	-8.87 ns	-0.74 ns	-1.24 ns	-7.72 ns	-10.01**	-2.97*	-1.10 ns	-0.10 ns	-14.91**	-0.31 ns	-2.79**	0.58**	-0.16 ns	-0.18 ns
'WTSC9369' × 'Sakha105'	-5.21 ns	-5.78 ns	-1.49 ns	3.76 ns	0.17 ns	-15.77**	1.49 ns	-2.35*	-1.44**	-18.34**	-0.61**	-0.26**	-0.20**	-1.21**	-1.43**
'WTSC9369' × 'Sakha107'	0.81 ns	-4.45 ns	-0.99 ns	1.38 ns	-17.07**	9.10**	-0.72 ns	-0.27 ns	1.67**	16.92**	0.21 ns	1.35**	0.73**	0.71 ns	0.77*
'WTSC9369' × 'Sakha108'	10.30 ns	17.47**	5.09**	-4.28 ns	25.13**	8.34**	3.45*	1.48 ns	-0.62*	1.35 ns	0.83**	0.10**	-0.63**	0.85*	0.37 ns
'Longping' × 'Sakha101'	12.16*	8.83 ns	3.50 ns	5.05 ns	-5.59 ns	-4.72**	2.14 ns	-0.77 ns	-1.61**	-8.10**	-0.42*	-1.57**	0.03*	0.06 ns	-0.32 ns
'Longping' × 'Sakha102'	9.15 ns	18.33**	3.63 ns	11.43**	19.72**	11.10**	3.27*	-1.77 ns	1.88**	20.27**	0.51*	0.56**	-0.23**	1.45**	0.68 ns
'Longping' × 'Sakha105'	7.40 ns	9.42 ns	4.88*	-6.08*	-1.04 ns	3.32**	0.73 ns	0.98 ns	-0.40 ns	9.52**	0.37 ns	3.90**	0.20**	1.60**	1.43**
'Longping' × 'Sakha107'	15.42*	16.75**	4.88*	-2.45 ns	21.92**	-4.97**	4.02**	0.73 ns	0.01 ns	-11.23**	0.82**	-1.82**	-0.18**	0.36 ns	1.13**
'Longping' × 'Sakha108'	-44.13**	-53.33**	-16.88**	-7.95**	-35.01**	-4.72**	-10.15 ns	0.82 ns	0.12 ns	-10.47**	-1.28**	-1.07**	0.18**	-3.46**	-2.93**
LSD 0.05	10.97	11.54	4.06	5.37	9.08	1.13	2.75	1.93	0.54	5.36	0.37	0.04	0.03	0.84	0.77
LSD 0.01	15.51	16.32	5.75	7.59	12.84	1.60	3.89	2.74	0.77	7.58	0.53	0.06	0.04	1.19	1.09

ns, * and **: nonsignificant and significant effect at 0.05 and 0.01 probability.

'WTSC9039' × 'Sakha102', 'WTSC9059' × 'Sakha108', 'WTSC9369' × 'Sakha107', 'Longping' × 'Sakha105' and 'Longping' × 'Sakha107' reported significant and positive SCA effect for phenotypic acceptability.

4 DISCUSSION

Grain yield, being a complex quantitative trait, is controlled and influenced by yield contributing components, such as spikelet number, grain filling and grain mass. The present study the hybrid combinations 'WTSC9369' × 'Sakha101', 'WTSC9039' × 'Sakha102', 'WTSC9059' × 'Sakha108' and 'WTSC9059' × 'Sakha101'had more than 11 t ha^{-1} grain yield/ha^{-1} superior than all parents were used and also the highest in grain yield components, panicle length, fertility percentage, panicle mass and 100-grain mass. These combinations can be exploited as anew hybrids for high yield potentials and good agronomic traits. Overall, hybrids performed better for GY and yield-related traits compared with the parental lines and also showed evident hybrid vigor. The days to heading is important trait that needs to be investigated in every generation and days to maturity and panicle length that needs to be selected in early segregating generation (Ganapati et al., 2020). Jaiswal and Jaiswal 2019 mentioned that the genotype Hubr 16 showed the highest mean value for grain yield and yield contributing traits like panicle length and seeds / panicle followed by Basmati 370. Super hybrid varieties have increased rice yield potential by 12 % compared with ordinary hybrid and inbred varieties. The higher grain yield of super hybrid varieties was attributed to improvement in both source and sink (Zhang et al., 2009). The hybrid varieties produced grain yield of 12 t ha^{-1} in on-farm demonstration fields, 8–15 % higher than the hybrid check varieties (Peng et al., 2008). Agronomic characteristics that are useful for rice breeding, such as large panicles and flowering habit have good panicle uniformity, concentrated flowering periods and good panicle exertion rates, which would be more conducive to hybrid seed production (El-namaky, 2018).

Correlation coefficients are used to measure relationship between two variables as evident from positive correlation of yield components with GY except for FAR and highly significant for PE, PM, FP and AH traits, however, the negative correlation and significant was found with AH (Table 3). (Yu et al., 2020 found the correlation between the introgression rate of the Habataki pedigree and yield related traits, whereas the introgression rate was significantly negatively correlated to 1000-grain mass. Li et al. (2019) found that correlations between agronomic traits and yield depend on the rice variety. The improvement in GY should focus on increasing the FGN, TGW, GP, PH, PL, GPP, SS, decreasing PN and LW. Gramaje et al. (2020) showed that the GCA effects were poorly correlated with specific combining ability (SCA) effects and hybrid performance, while SCA was positively correlated to heterosis estimates for all traits although it was only correlated with Gy for per se performance.

Genetic parameter analysis indicated the presence of significant differences among the lines, testers and lines × testers refer the genotypes had wide genetic diversity among themselves for all traits except days to heading, chlorophyll content, panicle length and apparent heterosis for parents. The significance of variance due to lines and testers confirmed a prevalence of additive variance while significant differences for line × tester among all the characters, indicating the importance of both additive and non-additive types of gene action occur within parents. Additive effects are important for the fixation of the trait and for early selection of the plants. The dominance to additive variance ratio (D/A) could be used for quantitative evaluations at each single QTL through genetic analysis in the heterotic populations (Liu et al., 2020). GCA analysis seeks to facilitate breeding through effective and efficient selection of inbred lines for a cross based on additive and additive × additive gene effects. Moreover, GCA analysis maximize additive gene effect that increases the selection efficiency of breeders in selecting elite inbred parents with better performance (Ullah Zaid et al., 2019)we calculated the GCA effect values of 33 parents of hybrid rice and sequenced them to identify genome-wide single nucleotide polymorphisms (SNPs. The RILs from the cross between the maintainer line Zhenshan97B and the restorer line Minghui63 (both parents of the elite hybrid Shanyou63, the classical hybrid of three-line system) was used to create the subsequent immortalized F_2 population, which enabled the comprehensive evaluations of the genetic contributions of dominance, over-dominance and epistatic effects across the whole rice genome (Liu et al., 2020). Otherwise, dominance effects are not fixable due to segregation and late selection may be fruitful for the selection of genotypes for the next generation of the future breeding studies to develop new rice varieties (Ashfaq et al., 2012). Utharasu & Anandakumar (2013) found that both additive and non- additive gene action were found to control the expression of the traits under study. The magnitude of combining ability revealed non-additive genetic variance was higher than the additive variance for all the studied traits. Gaballah and Abdallah (2015) illustrated non-additive gene effects for grain yield and its components. Hijam et al. (2019) observed importance of both additive and non-additive gene effects for grain yield and yield contributing characters studied.

The variances of SCA were higher than the GCA for all traits (Table 6) and ratio of GCA/ SCA variances were less than unity which reported preponderance of non-additive gene action in the inheritance of respective traits. It interprets greater importance of non-additive gene action in its expression and exploitation for traits through hybrid breeding (Tan et al., 2018). The ratios of GCA/SCA were less than unity in all studied characters except days to maturity and no. of panicles/plant, indicating that dominance gene effects were more important than additive gene effects in the expression of most traits (Zaazaa and Anis, 2014).

Ganapati et al. (2020) reported the plant height, number of filled grain panicle^{-1}, number of grain panicle^{-1} and dominant effects for yield hill^{-1}, yield tiller^{-1} and 1000 grain mass have association to yield, governed by additive gene effect. Bano and Singh (2019) showed that both the additive and non-additive genetic variance exhibited importance for expression of days to 50 % flowering, days to maturity, plant height and effective tillers/ plant.

The relative contribution of lines, testers and their interaction on expression of fifteen traits studied (Fig.3) showed the prevalence of additive gene action in panicle length, panicle mass and 100-grain mass refers to the lines contributed by more positive alleles while the testers contributed by more positive alleles in panicle exertion, panicle mass, fertility percentage and grain yield. Days to heading, flag leaf angle, chlorophyll content, plant height, flag leaf area, number of panicles/ plant, grain type, phenotypic acceptability and apparent heterosis traits contributed by line × tester interaction depending on the respective crosses were subjective to non-additive gene action. Istipliler et al. (2015) revealed the line × tester interaction contributed to combinations variances was found much more than lines and testers, individually.

The relative contribution of line, tester and combinations of line × tester interaction of ten traits were calculated and found that panicle mass contributed the highest (69.53 %) followed by thousand grain mass (63.62 %), yield per plant (54.76 %), panicle per meter square 51.52 % in their hybrids (Akter et al., 2010).

The parents, 'WTSC9059', 'Sakha101', 'Sakha102' and 'Sakha108' were recognized as good general combiners due to their maximum positive GCA effect values for most traits and could be utilized in breeding program to enhance grain yield and its related traits. Eltahawy et al. (2020)but also performance of grain quality traits of F2 bulk population which is the commodity consumed by humans. In order to make GCA improvement for quality traits in parents of hybrid rice by molecular marker assisted selection feasible, genome-wide GCA loci for quality traits in parents were detected through association analysis between the effects of GCA and constructed single nucleotide polymorphism linkage disequilibrium blocks (SNPLDBs reported the general combining ability (GCA) of parents in hybrid rice affects playing vital role in both heterotic level of grain yield and other important agronomic traits. Considering the exhibition of useful GCA effects by the testers were identified as good general combiners for the traits concerned (Hossain et al., 2018).

The crosses 'WTSC9039' × 'Sakha102' and 'WTSC9059' × 'Sakha105' found to be highly significant and positive SCA for number of panicle/plant and could be selected for further improving high heterosis. The hybrid combinations 'WTSC9039' × 'Sakha102', 'WTSC9039' × 'Sakha108', 'WTSC9059' × 'Sakha101', 'WTSC9059' × 'Sakha107', 'WTSC9369' × 'Sakha101', 'WTSC9369' × 'Sakha107', 'WTSC9369' × 'Sakha108', 'Longping' × 'Sakha102' and 'Longping' × 'Sakha105' described highly significant positive SCA effects for grain yield and showed high heterosis. Therefore, could be utilized for future breeding program for development of high yielding genotype. The SCA effects of the crosses for yield and its contributing characters indicated that most of the good specific cross combinations for different characters involved parents with either one or both good GCA effects. Bano and Singh (2019) reported that the three specific combiners for seed yield/plant were 'Kasturi Basmati' × 'Pusa-2517- 2-51-1', 'Pusa Sugandh-5' × 'Type-3' and 'Pusa Sugandh-5' × 'Pusa Sugandh-2'. These crosses were good specific combiners for grain yield / plant. Ganapati et al. (2020) revealed the cross combinations, which expressed high SCA effects for grain yield have invariably exhibited positive SCA effects for one or more yield related traits also.

It appears that heterosis for yield may be through heterosis for individual yield components or alternatively due to multiplication effects of non-additive gene effects of component characters. Generally, high × high, low × high and high × low general combiner parents produced good specific cross combinations. In these crosses additive × additive, dominance × additive and additive × dominance type of gene action was found. In cases, high × high general produced inferior cross combinations indicating epistatic type of gene action for these traits (Anis et al., 2016). The discovery of male sterile lines plays a crucial role in the utilization of rice heterosis and heterosis percent was estimated to know the possible gene action, exploit heterosis for high grain yield and associated traits. The crosses 'WTSC9059' × 'Sakha101', 'WTSC9369' × 'Sakha101', 'Longping' × 'Sakha105' and 'WTSC9039' × 'Sakha102' express superior heterosis values for grain yield. Thus, hybrids could be used for future breeding program for development grain yield and its associated traits.

Gokulakrishnan and Kumar (2013) found the cross combinations 'IR58025A' × 'ASD19', 'IR62829A' × 'ASD16', 'PUSA3A' × 'IR42' promising for seed yield and had high percentage of standard heterosis. (Yu et al., 2020) showed that heterosis in F_1 involved grain number/panicle. The back cross inbreeding lines F_1s showed an increase in grain number/panicle but a decrease in plant height compared with the back cross inbreeding lines. Abdullah et al. (2020) developed a neo-tetraploid rice line Huaduo8 with long panicles and harboring wide compatibility genes for pollen and embryo sac fertility. All the hybrids generated by Huaduo8 produced significant high parent yield heterosis and displayed long panicles.

5 CONCLUSION

The combining ability of five pollinator and four EGMS lines through line × tester analyses for GY and yield related traits. Based on the results could be considering improving the DTH, NOP, Pl, FAR, HGM, FP and GT to increase GY. Specific parents with the potential to produce superior hybrids and improve the existing breeding pool were identified. The variances of SCA were higher than the GCA for all traits and ratio of GCA/ SCA variances were less than unity which reported preponderance of non-additive gene action in the inheritance of respective traits. The prevalence of additive gene action in panicle length, panicle mass and 100-grain mass refer to the lines contributed by more positive alleles while the testers contributed by more positive alleles in panicle exertion, panicle mass, fertility percentage and grain yield. Days to heading, flag leaf angle, chlorophyll content, plant height, flag leaf area, number of panicles/ plants, grain type, phenotypic acceptability and apparent heterosis traits contributed by line × tester interaction depending on the respective crosses were subjective to non-additive gene action. Heterosis was also evident among the crosses although SCA effects did not automatically translate to better hybrid performance.

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doi:10.14720/aas.2021.117.1.1435 Original research article / izvirni znanstveni članek

Bionomics of slender burnished brass (*Thysanoplusia orichalcea* [Fabricius, 1775], Lepidoptera: Noctuidae) on potato (*Solanum tuberosum* L.) in Kashmir

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Received January 11, 2020; accepted December 23, 2020.
Delo je prispelo 11. januarja 2020, sprejeto 23. decembra 2020.

Bionomics of slender burnished brass (*Thysanoplusia orichalcea* [Fabricius, 1775], Lepidoptera: Noctuidae) on potato (*Solanum tuberosum* L.) in Kashmir

Abstract: A study on biology, morphometrics and geometrical progression of *Thysanoplusia orichalcea* was conducted on *Solanum tuberosum*under laboratory conditions. Different stages viz., egg, larva, pupa and adult of *T. orichalcea* were observed for their duration and morphometric measurements. The pest depicted five larval instars and moultedfour times during the entire period. The average pre oviposition and oviposition period was observed to be 3.80 and 2.80 days respectively. Further, fecundity ranges between 381.0-400.0 with an average of 388.0 eggs. The mean incubation period was found to be 4.2 days. The average larval, pre pupal and pupal period was completed in 23.1, 1.4 and 9.5 days respectively. While mean adult longevity on *S. tuberosum* was 7.45 days. The total life cycle was completed in 41.0-51.0 days with an average of 45.65 days. Moreover, mean head capsule width of first, second, third, fourth and fifth larval instar was found to be 0.33, 0.54, 0.88, 1.46 and 2.24 mm respectivelywith Dyar's ratio/ growth ratio of 1.63 mm. The expected head capsule width of first, second, third, fourth and fifth larval instar wasobserved as 0.33, 0.53, 0.86, 1.40 and 2.28 mm respectively.

Key words: biology; Dyar's ratio; fecundity; larval instars; morphometry; *Solanum tuberosum*; *Thysasnoplusia orichalcea*

Bionomija sovke *Thysanoplusia orichalcea* (Fabricius, 1775) (Lepidoptera: Noctuidae) na krompirju (*Solanum tuberosum* L.) v Kašmirju

Izvleček: Raziskava biologije, morfometrije in geometrijske progresije sovke *Thysanoplusia orichalcea* (Fabricius, 1775) je bila izvedena na krompirju (*Solanum tuberosum* L.) v laboratorijskih razmerah. Opazovano je bilo trajanje različnih razvojnih stadijev škodljivca, kot so jajčeca, gosenica, buba in odrasli osebki, na njih so bile opravljene morfometrične meritve. Škodljivec je imel pet razvojnih stopenj gosenic in se je v celotnem obdobju štirikrat levil. Poprečni obdobji pred in med odleganjem jajčec sta trajali 3,8 in 2,8 dni. Samice so v povprečju odložile med 381,0 in 400,0 jajčec, s povprečjem 388,0 jajčec. Poprečno inkubacijsko obdobje je trajalo 4,2 dni. Povprečna obdobja gosenice, obdobja pred za bubljenjem in bube so trajala 23,1, 1,4 in 9,5 dni. Povprečna življenska doba odraslih osebkov na krompirju je bila 7,45 dni. Celoten življenski krog se je zaključil v 41,0-51,0 dneh, v poprečju v 45,65 dneh. Povprečna širina glave je za prvo, drugo, tretjo, četrto in peto stopnjo gosenice znašala 0,33, 0,54, 0,88, 1,46 in 2,24 mm, s količnikom Dyarjevo razmerje/ rastno razmerje 1,63 mm. Pričakovana širina glave je bila za prvo, drugo, tretjo, četrto in peto razvojno stopnjo gosenice 0,33; 0,53; 0,86; 1,40 in 2,28 mm.

Ključne besede: bionomija; Dyarjevo razmerje; plodnost; larvalne stopnje; morfometrija; *Solanum tuberosum*; *Thysasnoplusia orichalcea*

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1 INTRODUCTION

Vegetables are indispensable as they make up a major part of the human diet in many parts of the world. Vegetables play an imperative role in human nourishment since they supply chief phyto nutriceuticals like vitamins, minerals, dietary fiber and phytochemicals (Craig and Beck, 1999; Dias and Ryder, 2011). Amongst various vegetables, Potato (*Solanum tuberosum* L.) lines as the third vital food crop after wheat and rice. In terms of production, it is leading tuber crop all over the globe (Anonymous, 2011a). India generates about 42.34 million tons of potato from 1.86 million hectares of land, thus positions fourth in terms of area and third in terms of potato production in the entire world (Anonymous, 2011a). Around 34.000 t of potatoes are harvested in Kashmir valley from an area of about 1.7 thousand hectares (Anonymous, 2011b). Potato is professed not only as a source of carbohydrates, but also an exceptional basis of essential amino acids.The starch present in the potato augments satiety, enhances glucose tolerance & insulin sensitivity and diminishes plasma cholesterol & triglyceride concentrations (Raben et al., 1994; Cummings, 1996). Despite of the numerous health benefits of potato, its production is still less because of the massive crop damage due to amplified pest menace. Insects also act as vectors for viral diseases, thus further increases the yield losses (Shivalingswami et al., 2002). Due to the lack of resistant characters because of rigorous hybrid cultivation, vegetables are more prone to insect pests and diseases (Dhandapani et al., 2003).

Among various insect pests, *Thysanoplusia orichalcea* (Fab.) commonly called as green semilooper, slender burnished moth and golden wing moth is one of the serious polyphagous pest infesting potato in Kashmir (Bhagat, 2018). The larval stages of *T. orichalcea* are serious defoliators feeding on potato leaves from vegetative to maturity stages. The young larvae puncture holes widely into the lamina of leaves and later instars feed voraciously leaving only the main veins thus plummeting photosynthetic competence. This pest was also found feeding on other crops belonging to familiesCompositae, Cruciferae, Leguminosae, Linaceae, Cucurbitaceae and Chenopodiaceae (Laute et al., 2015). A little has been done on the biology of *T. orichalcea* on potatoin Kashmir though its biology has been worked out on soybean (*Glycine max* (L.) Merr.) and common bean (*Phaseolus vulgaris* L.) in Akola, Maharashtra and Kishtwar, J&K respectively (Laute et al., 2015 and Kotwal & Bhatia, 2016). Therefore, the objective of the current investigation was to study the life cycle of *T. orichalcea* on potatoin the laboratory.

2 MATERIAL AND METHODS

2.1 COLLECTION AND REARING OF *T. orichalcea*

For studying the life cycle and morphometrics of *T. orichalcea* on potato (*S. tuberosum* L.) variety Kufri Jyoti *??*, preliminary culture of different larval instars were gathered from potato fields of Kashmir valley during the year 2018. Each larval instars collected was transferred to separate rearing boxes made of glass at the temperature of 25 °C and 65 % RH in the Entomology Research Laboratory of the Department of Zoology, University of Kashmir. Fresh potato leaves were provided as food for the larval instars on daily basis till the larvae entered into last stage and the excreta was removed regularly.The rearing boxes were monitored regularly for the exuvia. The number and duration of each larval instar was recorded. The last larval instars of *T. orichalcea* were collected from rearing boxes and transferred to pupation chambers having one-third part of it filled with soil, which provides favourable conditions for pupation. The pupae were monitored regularly until adult eclosion. In order to determine the duration of adult moths, freshly eclosed adults were individually placed in rearing boxes lined with filter paper and were provided with 10 % honey solution as food. Ten adults were used for this experiment and the duration of each adult was monitored and documented.. To determine the fecundity, 10 individual pair of adult moths were placed in oviposition chambers immediately after eclosion lined with fresh potato leaves for egg laying and containing cotton swabs soaked in 10 % honey solution as food for adults at the temperature of 25 °C and 65 % RH. Each day the number of eggs produced by these moths was monitored. Further, the data on pre oviposition period, oviposition period and incubation period was also recorded. Moreover, the rearing, pupation and oviposition chambers were covered with muslin cloth fastened with rubber bands.

2.2 MORPHOMETRIC AND GROWTH STUDIES

Ten individuals of each developmental stage viz., eggs, larval instars, pupae and adults were observed under stereozoom binocular microscope and the morphometric measurements were documented with the help of vernier calliper as well as simple scale. For determining the duration of different larval instars, the individual larvae were observed regularly for exuvia and head capsule. The presence of casted head capsule corroborated the moulting. The numbers of larval instars of *T. orichalcea* were determined using Dyar's law (1890). The growth ratio (Dyar's ratio) was obtained by the following formula:

$$Growth\ Ratio = \frac{Head\ capsule\ width\ of\ 2nd\ larvae}{Head\ capsule\ width\ of\ 1st\ larvae}$$

2.3 STATISTICAL ANALYSIS

The data associated with different stages of insect viz., egg, larva, pupa and adult were examined for calculating mean and standard deviation using SPSS 16.0.

3 RESULTS

3.1 FECUNDITY

In the laboratory conditions, average fecundity was observed to be 388.0 (± 8.58) eggs with a range of 381.0 to 400.0 eggs (Table 1). The mean preoviposition period was found to be 3.80 (± 0.83) days with a range of 3.0 to 5.0 days. Moreover, the oviposition period lasted between 2.0 to 4.0 days with an average of 2.8 (± 0.83) days (Table 1).

3.2 EGGS

In the laboratory conditions, oviposition occurred during night time at 25 °C and 65 % RH and the eggs were laid on lower surface of the leaves of potato The freshly laid eggs of *T. orichalcea* were green, spherical and shiny. Prior to hatching, egg colour changed to dark brown. The average incubation period of eggs was observed to be 4.2 (± 0.83) days with a range of 3.0-5.0 days (Table 7). The length of the egg varied between 0.67 mm to 0.72 mm with an average of 0.70 (± 0.01) mm and width ranges between 0.59 mm to 0.66 mm with an average of 0.63 (± 0.02) mm (Table 2).

3.3 LARVAE

The current investigation depicted five larval instars in the life cycle of *T. orichalcea*. The larvae shed its exuivae four times during the entire period. The caterpillars flaunted a typical half loop movement and thus attaining a common name as semilooper to its recognition. The neonate larvae emerging from the eggs were creamy white in colour with dark black head, which later on changes to green. The first larval period ranged for about 2.0-3.0 days with a mean duration of 2.4 (± 0.51) days (Table 7). The length and breadth of first larval instar ranged between 2.00-3.00 mm and 0.40-0.50 mm with a mean of 2.50 (± 0.52) and 0.44 (± 0.05) respectively (Table 3). Further, the mean width of head capsule was observed to be 0.33 (± 0.02) with a range of 0.29-0.37 mm before moulting (Table 3).The second larval instars were green in colour and their duration lasted for 3.0-4.0 days with an average of 3.40 (± 0.51) days (Table 7). The length and breadth of 2nd larval instar varied from 8.50-10.50 mm and 1.00-1.40 mm with a mean of 9.50 (± 0.88) and 1.27 (± 0.15) respectively (Table 3). Moreover, the width of head capsule ranged between 0.51-0.59 mm with a mean of 0.54 (± 0.03) (Table 3). The first and second larval instars were found scraping the epidermis of leaf thereby, causing their skeletonization.

The third instar larva displayed two lateral lines running along the plural region. The fourth and fifth larval instars flaunted extra blackish mid dorsal line (Fig 2). From third instar onwards, larvae starts feeding voraciously engulfing the entire leaf including mid rib and vein. The third, fourth and fifth larval period ranges between 4.0-5.0, 5.0-6.0 and 7.0-8.0days with an average of 4.3 (± 0.48), 5.6 (± 0.51) and 7.4 (± 0.51) days respectively (Table 7). The mean length and breadth of third larval instar was 17.35 (± 1.08) and 2.40 (± 0.51) mm with a range of 16.00-18.50 mm and 2.00-3.00 mm respectively (Table 3). In case of fourth larval instar, the average length and

Table 1: Duration of pre oviposition period, oviposition period (days) and fecundity of *T. orichalcea*

S. No.	Stage	Minimum (days)	Maximum (days)	Mean ± S. D.
1.	Pre oviposition period	3.00	5.00	3.80 ± 0.83
2.	Oviposition period	2.00	4.00	2.80 ± 0.83
3.	Fecundity	381.00	400.00	388.0 ± 8.58

Table 2: Measurements of eggs of *T. orichalcea*

S. No.	Variable	N	Minimum (mm)	Maximum (mm)	Mean ± S. D.
1.	Egg length	10	0.67	0.72	0.70 ± 0.01
2.	Egg width	10	0.59	0.66	0.63 ± 0.02

N = Number of observations; SD: Standard deviation

breadth was obtained as 24.75 (± 0.67) mm and 3.95 (± 0.43) mm with a range of 24.0-25.50 mm and 3.50-4.50 mm respectively (Table 3). The length and breadth of fifth larval instar ranged between 32.00-35.00 mm and 4.50-5.50 mm with a mean of 33.30 (± 1.15) and 4.95 (± 0.43) respectively (Table 3). The width of head capsule for third, fourth and fifth larval instar ranged between 0.85-0.90 mm, 1.39-1.50 mm and 2.19-2.28 mm with a mean of 0.88 (± 0.01) mm, 1.46 (± 0.03) mm and 2.24 (± 0.03) mm respectively (Table 3). Further, the total larval period ranged between 21.00 to 26.00 days with an average of 23.10 (± 2.52) days (Table 7).

Growth ratio (Dyar's ratio) for *Thysanoplusia orichalcea* was calculated and the expected widths of head capsule of first, second, third, fourth and fifth larval instar were 0.33, 0.53, 0.86, 1.40 and 2.28 mm respectively (Table 4). The Dyar's ratio was computed as 1.63 mm (Table 4) and authenticated that successive larval instars followed a regular geometrical development (Fig 1).

$$Growth\ Ratio = \frac{Head\ capsule\ width\ of\ 2nd\ larvae}{Head\ capsule\ width\ of\ 1st\ larvae} = \frac{0.54}{0.33} = 1.63\ mm$$

Mean observed head capsule width of first larval instar (N =10) = 0.33 mm

Mean observed head capsule width of second larval instar (N =10) = 0.54 mm

3.4 PRE PUPA

In this stage, the mature 5th larval instar stops feed

Table 3: Morphometric measurements of larval instars of *T. orichalcea*

S. No.	Developmental stage		Minimum (mm)	Maximum (mm)	Mean ± S. D.
1.	First larval instar	Length	2.00	3.00	2.50 ± 0.52
		Breadth	0.40	0.50	0.44 ± 0.05
		Width of head capsule	0.29	0.37	0.33 ±0.02
2.	Second larval instar	Length	8.50	10.50	9.50 ± 0.88
		Breadth	1.00	1.40	1.27 ± 0.15
		Width of head capsule	0.51	0.59	0.54 ± 0.03
3.	Third larval instar	Length	16.00	18.50	17.35 ± 1.08
		Breadth	2.00	3.00	2.40 ± 0.51
		Width of head capsule	0.85	0.90	0.88 ± 0.01
4.	Fourth larval instar	Length	24.00	25.50	24.75 ± 0.67
		Breadth	3.50	4.50	3.95 ± 0.43
		Width of head capsule	1.39	1.50	1.46 ± 0.03
5.	Fifth larval instar	Length	32.00	35.00	33.30 ± 1.15
		Breadth	4.50	5.50	4.95 ± 0.43
		Width of head capsule	2.19	2.28	± 0.03

*Mean of 10 individuals; SD: Standard deviation

Table 4: Comparison of observed mean and expected values of head capsule widths (mm) of the larvae of *T. orichalcea*

		Head capsule width (mm)			Difference (mm)
S. No.	Larval instars	Observed (Mean ± S. D.)	Range	Expected[a]	
1.	First larval instar	0.33 ± 0.02	0.29-0.37	0.33	0.00
2.	Second larval instar	0.54 ± 0.03	0.51-0.59	0.53	0.01
3.	Third larval instar	0.88 ± 0.01	0.85-0.90	0.86	0.02
4.	Fourth larval instar	1.46 ± 0.03	1.39-1.50	1.40	0.06
5.	Fifth larval instar	2.24 ± 0.03	2.19-2.28	2.28	- 0.04

[a] Expected width of head capsule determined by Dyar's ratio. MultiplyingDyar's ratio (1.63) with the observed width of head capsule of first larval instar provides the expected width of head capsule of second instar larva which when multiplied again with Dyar's ratio gives expected width of head capsule of third larval instar and so on.

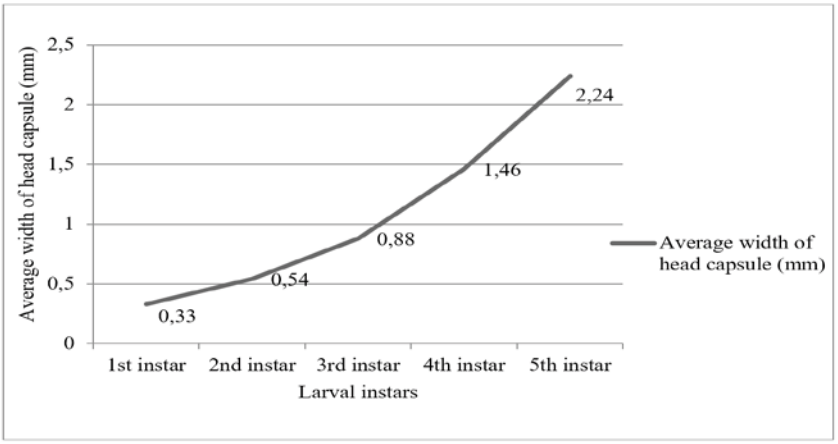

Figure 1: Relation between larval growth and head width of *T. orichalcea*

Table 5: Morphometric measurements of male and female pupae of *T. orichalcea*

S. No.	Variable	N	Minimum (mm)	Maximum (mm)	Mean ± S. D.
1.	Pupal length (Male)	10	17.50	19.00	18.25 ± 0.63
2.	Pupal width (Male)	10	4.50	5.50	5.05 ± 0.43
3.	Pupal length (Female)	10	19.00	24.00	21.70 ± 1.76
4.	Pupal width (Female)	10	5.00	6.00	5.50 ± 0.52

N = Number of observations; SD: Standard deviation

ing and becomes sluggish by contracting its body and appendages (Fig 2). This phase lasted for 1.0-2.0 days with an average of 1.4 (± 0.51) days (Table 7).

3.5 PUPA

The colour of pupa appeared creamish initially which turns into deep brown towards maturity. The pupa is obtect type. Prior to pupation, a mature full grown larva starts to spin white silken cocoon around the body and protects it externally by leaf fold (Fig 2).The male pupa was found slightly smaller than female pupa. The length and breadth of male pupa varies between 17.50-19.00 mm and 4.50-5.50 mm with an average of 18.25 (± 0.63) mm and 5.05 (± 0.43) mm respectively (Table 5). Further, the length and breadth of female pupa varies between 19.00-24.00 mm and 5.00-6.00 mm with a mean of 21.70 (± 1.76) mm and 5.50 (± 0.52) mm respectively (Table 5). The pupal period ranged between 9.0 to 10.0 days with a mean of 9.5 (± 0.52) days at 25 ºC and 65 % RH (Table 7).

3.6 ADULT

The adult moth comes out of puparium by puncturing an escape hole with the aid of some secretions. The forewing of adult moth is olive brown in colour with large conspicuous L shaped metallic golden patch (Fig 2). The male and female moths' possess pectinate and filliform type of antennae respectively. The average male longevity was found to be 8.4 (± 0.51) days with a range of 8.0-9.0 days (Table 7). The female longevity lasted be

Table 6: Morphometric measurements of adult male and female of *T. orichalcea*

S. No.	Variable	N	Minimum (mm)	Maximum (mm)	Mean ± S. D.
1.	Adult length (Male)	10	16.50	19.00	17.65 ± 1.08
2.	Adult width (Male)	10	28.00	31.00	29.70 ± 1.33
3.	Adult length (Female)	10	19.50	22.00	20.60 ± 1.04
4.	Adult width (Female)	10	35.00	40.00	38.00 ± 2.00

Table 7: Developmental period of various life stages of *T. orichalcea*

S. No.	Developmental stage	Minimum* (Days)	Maximum* (Days)	Mean ± S.D.
1.	Incubation period	3.00	5.00	4.20 ± 0.83
2.	First larval instar	2.00	3.00	2.40 ± 0.51
3.	Second larval instar	3.00	4.00	3.40 ± 0.51
4.	Third larval instar	4.00	5.00	4.30 ± 0.48
5.	Fourth larval instar	5.00	6.00	5.60 ± 0.51
6.	Fifth larval instar	7.00	8.00	7.40 ± 0.51
7.	Total larval period	21.00	26.00	23.10 ± 2.52
8.	Pre pupal period	1.00	2.00	1.40 ± 0.51
9.	Pupal period	9.00	10.00	9.50 ± 0.52
10.	Male longevity	8.00	9.00	8.40 ± 0.51
11.	Female longevity	6.00	7.00	6.50 ± 0.52
12.	Average longevity	7.00	8.00	7.45 ± 0.51
13.	Total life cycle	41.00	51.00	45.65 ± 4.88

*Mean of 10 individuals; SD: Standard deviation

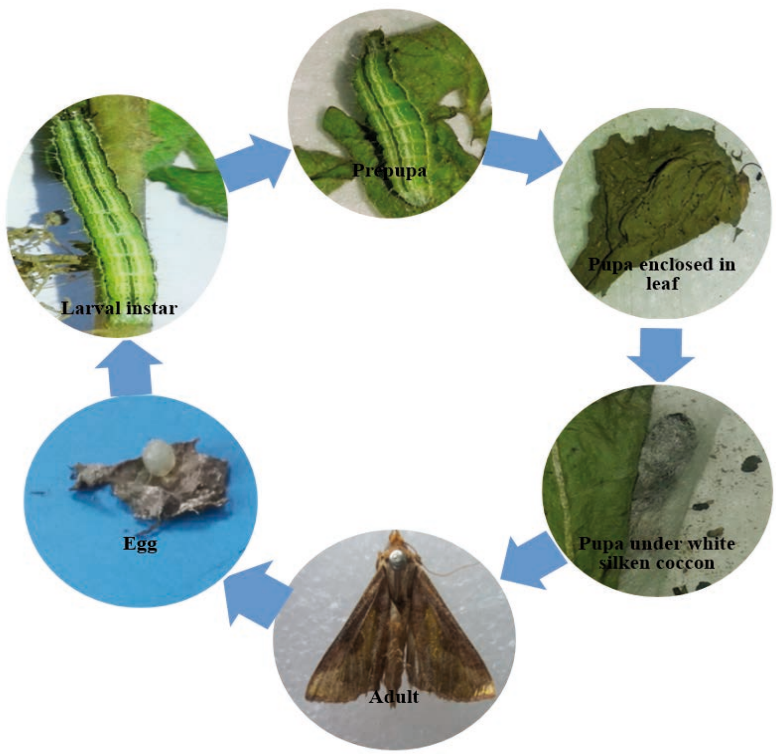

Figure 2: Life Cycle of *Thysanoplusia orichalcea*

tween 6.0-7.0days with a mean of 6.5 (± 0.52) days (Table 7). The average adult (male and female) longevity was found to be 7.45 (± 0.51) days with a range of 7.0-8.0 days (Table 7). Adults are medium in size with male moths slightly smaller than females. The length of adult male moth varies between 16.50-19.00 mm with an average of 17.65 (± 1.08) mm (Table 6). The length of female moth ranges between 19.50-22.0 mm with a mean of 20.60 (± 1.04) mm (Table 6). Further, the width of male and female moth varies between 28.00-31.0 mm and 35.00-40.00 mm with a mean of 29.70 (± 1.33) mm and 38.00 (± 2.00) respectively (Table 6). The average life

cycle ranges between 41.0-51.0 days with an average of 45.6 (± 4.88) days (Table 7).

4 DISCUSSION

Thysanoplusia orichalcea (Lepidoptera: Noctuidae) is one of the serious defoliator feeding on various vegetable crops. In light of the damage caused by *T. orichalcea*, the evaluation of its bionomics could be advantageous for the better management of this obnoxious pest. The present results revealed that the total life cycle lasted from 41.0-51.0 days with an average of 45.6days under temperate conditions of Kashmir valley.On the contrary, the total life cycle of *T. orichalcea* on *Phaseolus vulgaris*was completed in 34.5-43.7 days under semi-temperate conditions of Kishtwar (Kotwal and Bhatia, 2016). Similarly, Laute et al. (2015) found that the total life cycle on soybean was completed in 34.3 days under tropical conditions in Maharashtra. The variation in the total life cycle of *T. orichalcea* may be attributed to different climatic conditions and assessment of life cycle on different host crops. These observations revealed that the longevity in the life cycle of green semilooper declines due to higher temperature at warmer regions compared to temperate climatic conditions of Kashmir.

The current study depicted that the incubation period of *T. orichalcea* on potatolasted for 3.0-5.0 days with an average of 4.2 days. However, Laute et al. (2015) reported the average incubation period of 2.8 days on soybean. The discrepancy observed between the current and previous studies might be due to difference in host plant species and varieties. Since there was no disparity in the number of larval instars and the morphometric measurements between the host plants, this specifies that host plant does not affect these parameters. Laute et al. (2015) and Kotwal & Bhatia (2016) also found the similar results. In the present study, it was found that larval development of *T. orichalcea* on potatowas completed in 21.0-26.0 days with an average of 23.1 days. Conversely, Laute et al. (2015) and Kotwal & Bhatia (2016) revealed total larval period of 16.0-23.0 and 15.0-20.0 days on soybean and common bean respectively. These differences might be due to either the variability or diminution of nutritional quality of the host plant species or ecological distinction with respect to geographical location and time of studies. Perchance of the most realistic implication of the current study is the fact that *T. orichalcea* larval development time differs considerably depending on host plants. Furthermore, the average head capsule width of first, second, third, fourth and fifth larval instar of *T. orichalcea* in the present finding was viewed as 0.33, 0.54, 0.88, 1.46 and 2.24 mm respectively which coincides with the results of Begum, 1999. Thus it was concluded that the average head capsule widths of each larval instar did not intersect and can be employed as precise indication of each larval instar.

Laute et al. (2015) and Kotwal and Bhatia (2016) reported pupal period of 6.0-7.0 and 14.0-15.5 days on soybean and common bean respectively. However, the present investigation showed pre pupal and pupal period ranging between 1.0-2.0 and 9.0-10.0 days with a mean of 1.4 and 9.5 days respectively on potato.The discrepancy monitored between the current and earlier results might be due to the fact that life history parameters are vastly affected by host plant species and test varieties.

Generally, the present study revealed that the longevity of adult male and female moth ranged between 8.0-9.0 and 6.0-7.0 days with an average of 8.4 and 6.5 days respectively on potato.The average longevity of adults (males and females) in the present finding was observed to be 7.45 days with a range of 7.00-8.00 days. Plausibly, the male lived longer than the female. Likewise, other studies also divulged the same results (Goel and Kumar, 1987; Laute et al., 2015 and Kotwal and Bhatia, 2016).

5 CONCLUSIONS

The current investigation on biological description of *Thysanoplusia orichalcea* offers comprehensive information on fecundity, growth and survival, which provides a baseline data for any management practice. Results from these studies can remark the venerable stages of *T. orichalcea* and also may provide information important for predicting the field population phenology. *T. orichalcea* commonly called as semilooper is a polyphagous pest found feeding on many vegetables including potato. The larval instar of this pest represents the main damaging stage. The larva targets the epidermis of leaves thus causing serious defoliation. This influences the growth of plant and in turn affects the production and yield. The annotations on morphometrics and life cycle parameters will turn out to be advantageous in the identification of pest.. By this approach, we can observe different stages of *T. orichalcea* and develop integrated management tactics on potato.

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doi:10.14720/aas.2021.117.1.1964 Original research article / izvirni znanstveni članek

Effects of salt stress on physiological and biochemical responses of three maize genotypes at the early seedling stage

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Received November 12, 2020; accepted December 25, 2020.
Delo je prispelo 12. novembra 2020, sprejeto 25. decebra 2020.

Effects of salt stress on physiological and biochemical responses of three maize genotypes at the early seedling stage

Abstract: Salt stress is one of the major global problems for crop productivity in the arid and semi-arid regions of the world. In this study, variations in some physiological parameters, water relations, and antioxidant systems under salinity (300 mM NaCl) among three maize (*Zea mays* L.) genotypes ('P3167', '32K61', and 'Bora') were investigated. Our result indicated that shoot growth is more sensitive to salinity as compared to root growth. Salt stress led to physiological drought in all maize genotypes as indicated by the significant decrease in relative water content and increase in water deficit index. Salt stress increased SOD activity in all genotypes showing an efficient formation and detoxification of superoxide radical. The constant level of oxidative markers (MDA and H_2O_2) and the increased level of the reduced ascorbate and phenolic may indicate that non-enzymatic antioxidants are responsible for the elimination of oxidative stress. Changes in ascorbate peroxidase and glutathione reductase activities under salinity demonstrated a functional failure in the ascorbate-glutathione cycle, especially in 'P3167' and '32K61'. Based on the presented results we may conclude that the genotype 'Bora' is tolerant to salinity while 'P3167' and '32K61' are sensitive.

Key words: antioxidant system; oxidative stress; phenolics; salinity; soluble sugars

Učinek solnega stresa na fiziološki in biokemični odziv treh genotipov koruze v zgodnji razvojni stopnji semenke

Izvleček: Solni stres je eden največjih globalnih problemov za uspevanje gojenih rastlin v sušnih in polsušnih območjih sveta. V raziskavi so bili preučevani nekateri fiziološki parametri, vodni režim in antioksidacijski system v razmerah slanosti (300 mM NaCl) pri treh genotipih koruze (*Zea mays* L.; 'P3167', '32K61', in 'Bora'). Izsledki so pokazali, da je rast poganjka bolj občutljiva na slanost v primerjavi z rastjo korenin. Solni stres je povzročil fiziološko sušo pri vseh genotipih koruze, ki se je izražala kot značilen upad v relativni vsebnosti vode in povečanju indeksa vodnega deficita. Solni stres je povečal aktivnost SOD pri vseh genotipih, kar kaže na učinkovito razstrupljanje superoksidnega radikala. Stalna raven vsebnosti označevalcev oksidacije (MDA in H_2O_2) in povečana vsebnost reduciranega askorbata ter fenolov lahko nakazujejo, da so neencimski antioksidanti odgovorni za odpravo oksidacijskega stresa. Spremembe v aktivnosti askorbat peroksidaze in glutation reduktaze v razmerah slanosti so pokazale funkcionalni zlom askorbat-glutationskega cikla, še posebej pri 'P3167' in '32K61'. Na osnovi predstavljenih izsledkov lahko zaključimo, da je genotip 'Bora' toleranten na slanost, medtem ko sta 'P3167' in '32K61' občutljiva.

Ključne besede: antioksidacijski sistem; oksidativni stres; fenoli; slanost; topni sladkorji

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1 INTRODUCTION

Salt stress is one of the most prominent agricultural problems for plant productivity in the arid and semi-arid soils in the world. High salinity in soils is responsible for the reduced yield for several crops. Munns and Tester (2008) reported that 45 million hectares of land had been affected by salinity worldwide, and 1.5 million hectares are taken out of cultivation each year due to over-accumulation of salts in the soil. Salinity leads to reductions in several metabolic processes associated with growth, development, and crop productivity (Fayez and Bazaid, 2014). Seed germination, for example, is inhibited by salt stress in several plant species (Ahammed et al., 2018; Gu et al., 2018; Orlovsky et al., 2016; Wilayasinghe et al., 2019; Khayamim et al., 2014). In black gram and mung bean plants, it was observed that salt stress affected water relations as well (Hasan et al., 2019). It was also reported that salt stress reduced root and shoot growth in barley plants depending on genotypes and salt concentration (Doğru and Yılmaz Kaçar, 2019). In plants under optimal growth conditions, the balance between reactive oxygen species (ROS) formation and detoxification is tightly controlled by the antioxidant system (Hameed et al., 2011). However, salt stress may cause the accelerated production of reactive oxygen species (ROS) and oxidative stress in the plant cells as a result of higher leakage of electrons toward O_2 during photosynthetic and respiratory electron transport reactions (Asada, 2006). Mittler (2002) has indicated that much of the damage on plants under salt stress is linked to oxidative stress at the cellular level. Plants that have higher antioxidant enzyme activities were considered salt-tolerant (Gapinska et al., 2008). Higher plants have developed several adaptive mechanisms to cope with oxidative stress under saline conditions such as the increased synthesis of osmoprotectants. Proline and soluble sugars, for example, accumulate in plant tissues and contribute to osmoregulation, structural protection of some biomolecules and membranes, and detoxification of ROS in plants under salt stress (Hare et al., 1998; Ashraf and Foolad, 2007; Abdelkader et al., 2019). Proline may also serve as an organic nitrogen reserve that could be utilized during stress recovery (Sairam and Tyagi, 2004). Among phenolic compounds anthocyanins have been well known to accumulate under salt stress and play an important role in scavenging ROS in plant tissues as well (Petridis et al., 2012; Chunthaburee et al., 2016).

Breeding for salt tolerance in crop plants has usually been limited because of the lack of reliable traits for selection (Yıldırım et al., 2008). Salt tolerance is very complex because multiple genes are involved. In this case, it seems that the most effective way to overcome the salinity problem may be the introduction of salt-tolerant crops. Therefore, this experiment was conducted to determine some physiological and biochemical responses in three maize genotypes grown under salt stress through some growth parameters (root and shoot length), water relations (relative water content end water deficit index), photosynthetic pigment content (chlorophyll a and b), oxidative stress markers (malondialdehyde and hydrogen peroxide content) and some endogenous resistance mechanisms (anthocyanin, free proline, total phenolics, total soluble carbohydrate contents and activities of some antioxidant enzymes).

2 MATERIAL AND METHODS

2.1 PLANT MATERIALS, GROWTH CONDITIONS, AND EXPERIMENTAL DESIGN

Maize (*Zea mays* L.) cultivars ('P3167', '32K61', and 'Bora') were grown in a growth chamber in plastic pots (14 x 14 cm; upper diameter x height) containing Hoagland nutrient solution. The average temperature for day/night was 25/18 °C respectively, relative humidity was 40-50%, the photoperiod for the day/night cycle was 16/8 h respectively, and the maximum photosynthetically active radiation was about 200 µmol m^{-2} s^{-1}. After 20 days of growth, plants were divided into two groups. The first group of plants were control (no salt treatment) and watered with Hoagland nutrient solution until the end of the study. In the second group of plants, salt stress was induced by applying 300 mM NaCl. For every individual genotype, we had 20 pots each of which contains 4 plants per treatment. The seedlings were harvested after 5 days of application and leaves are kept at -80 °C until analysis.

2.2 DETERMINATION OF ROOT AND SHOOT LENGTH

Measurement of root and shoot length were done with a millimetric ruler. The longest root was taken into consideration for measurement. Root and shoot length were expressed as cm $plant^{-1}$.

2.3 DETERMINATION OF LEAF RELATIVE WATER CONTENT (RWC) AND WATER DEFICIT INDEX

Leaf samples were taken and its fresh mass was recorded immediately. The sample was then incubated in deionized water overnight and the turgid mass of the leaf

sample was recorded. The leaf sample was oven-dried at 70 °C for 48 h and the dry mass of the sample was estimated. The relative water content and water deficit index were calculated according to Sairam et al. (2002).

2.4 PHOTOSYNTHETIC PIGMENT AND ANTHOCYANIN ANALYSIS

Photosynthetic pigments were extracted from leaf segments in 3 ml 100 % acetone. The absorbance of the extracts was measured at 644.8 and 661.6 nm using a Shimadzu mini 1240 UV visible spectrophotometer. The concentrations of chlorophyll a and chlorophyll b were calculated according to Lichtenthaler (1987).

Total anthocyanin content was analyzed by the procedure of Mancinelli et al. (1975). Leaf sample (0.1 g) was soaked in 10 ml of a mixture of methanol and 1 N HCL (85/15; v/v) for 72 h at 4 °C. The crude extracts were filtered through a 0.45 µm syringe filter before measurement of total anthocyanin content at 530 and 657 nm. The content was expressed as mg g^{-1} fresh mass.

2.5 MALONDIALDEHYDE (MDA) AND HYDROGEN PEROXIDE (H_2O_2) ANALYSIS

MDA and H_2O_2 content were measured by the method of Heath and Packer (1968) and Ohkawa et al. (1979), respectively. Fresh leaf material (0.1 g) was homogenized in 6 ml of 5 % TCA (4 °C) and centrifuged at 10 000 g for 15 min and the supernatant was used in the subsequent determination. To 0.5 ml of the supernatant were added 0.5 ml of 0.1 M Tris-HCl (pH 7.6) and 1 ml of TCA–TBA reagent. The mixture was warmed at 95 °C for 60 min and then quickly cooled in an ice bath. After centrifugation at 10 000 g for 5 min to remove suspended turbidity, the absorbance of the supernatant at 532 nm was recorded. Non-specific absorbance at 600 nm was measured and subtracted from the absorbance recorded at 532 nm. The concentration of MDA was calculated using its extinction coefficient of 155 mM^{-1} cm^{-1}. For determination of hydrogen peroxide, 0.5 ml of 0.1 M Tris-HCl (pH 7.6) and 1 ml of 1 M KI were added to 0.5 ml of supernatant. After 90 min, the absorbance was measured at 390 nm. A standard curve for hydrogen peroxide was prepared to determine hydrogen peroxide concentration in each sample.

2.6 FREE PROLINE ANALYSIS

Approximately 10 mg powdered dry leaf material was extracted with 4 ml distilled water on a hot plate at 100 °C for 10 min according to Bates et al. (1973). Extracts were filtered and the same procedure was repeated two times. The liquid phase of the homogenate was collected and centrifuged at 3500 rpm for 10 min. Two ml of the supernatant was reacted with 2 ml of acid ninhydrin and 2 ml of glacial acetic acid at 100 °C for 1 h. The reaction mixture was mixed with 4 ml toluene and vortexed for 20 s. The chromophore containing toluene was separated and the absorbance of the pink upper phase was recorded at 520 nm against toluene blank. A standard curve for proline in the range of 0.2-1 µmol ml^{-1} was prepared to determine free proline concentration in each sample.

2.7 TOTAL PHENOLIC COMPOUND ANALYSIS

The total phenolic content of leaves was determined according to Chandler and Dodds (1983). Accordingly, 0.2 g leaf material was powdered in liquid nitrogen and extracted with 80 % methanol. This mixture was placed in a refrigerator at 4 °C for 48 h. homogenates were centrifuged at 4,000 rpm for 10 min. An appropriate amount of supernatant was reacted with 50 % Foline Ciocalteu Reagent (FCR) and 5 % sodium carbonate and kept at room temperature at a dark place for 1 h. The mixture was vortexed and absorbance was read at 725 nm. The total phenolic content of the leaves was calculated by using a standard curve prepared with gallic acid.

2.8 TOTAL SOLUBLE CARBOHYDRATE (TSC) ANALYSIS

TSC content in leaves was measured by the phenol-sulphuric method according to Dubois et al. (1956). For this purpose, leaf material (50 mg) was oven-dried until the constant dry mass was reached. Dried leaf material was powdered in a mortar and pestle and TSS was extracted by 70 % ethanol. After centrifugation of extract at 3,500 rpm for 20 min, a reaction mixture was prepared. This mixture consisted of 1,000 µl supernatant, 300 µl phenol, and 2,000 µl concentrated sulphuric acid. Absorbances of these mixtures were read at 470 nm and the TSC content of the leaves was calculated by a standard curve using sucrose.

2.9 REDUCED ASCORBATE ANALYSIS

The reduced ascorbate content was determined according to Law et al. (1983). For this purpose, leaf material (0.2 g) was extracted by 10 % TCA. After centrifu-

gation of extract at 10,000 rpm for 20 min, a reaction mixture was prepared. This mixture consisted of 400 µl supernatant, 10 % TCA, 5 M NaOH, $NaPO_4$ buffer (150 mM, pH 7.4), 10 mM dithiothreitol, 0.5 % N-ethylmaleimide, 44 % H_3PO_4 and 3 % $FeCl_3$. The mixture was incubated at 37 °C for 60 min and absorbances were read at 525 nm. The reduced ascorbate content was determined by a standard curve.

2.10 ANTIOXIDANT ENZYME ACTIVITIES

For determination of enzyme activities, 0.3 g fresh leaves material from non-acclimated and cold-acclimated leaves were powdered with liquid nitrogen and suspended in a specific buffer with proper pH values for each enzyme. The homogenates were centrifuged at 14,000 rpm for 20 min at 4 °C and resulting supernatants were used for enzyme assay. The protein concentrations of leaf crude extracts were determined according to Bradford (1976), using BSA as a standard.

Activity of superoxide dismutase (SOD; EC 1. 15. 1. 1) was determined by the method of Beyer and Fridovich (1987), based on the photoreduction of NBT (nitro blue tetrazolium). Extraction was in 1.5 ml homogenization buffer containing 10 mM K_2HPO_4 buffer (pH 7.0), 2 % PVP and 1 mM Na_2EDTA. The reaction mixture consisted of 100 mM K_2HPO_4 buffer (pH 7.8), containing 9.9 x 10^{-3} M methionine, 5.7 x 10^{-5} M NBT, 1 % triton X-100, and enzyme extract. The reaction was started by the addition of 0.9 µM riboflavin and the mixture was exposed to light with an intensity of 375 µmol m^{-2} s^{-1}. After 15 min, the reaction was stopped by switching off the light, and absorbance was read at 560 nm. SOD activity was determined by a standard graphic and expressed as unit mg^{-1} protein.

Activita of ascorbate peroxidase (APX; EC 1. 11. 1. 11) was determined according to Wang et al. (1991) by estimating the decreasing rate of ascorbate oxidation at 290 nm. APX extraction was performed in 50 mM Tris–HCl (pH 7.2), 2 % PVP, 1 mM Na_2EDTA, and 2 mM ascorbate. The reaction mixture consisted of 50 mM KH_2PO_4 buffer (pH 6.6), 2.5 mM ascorbate, 10 mM H_2O_2, and enzyme, containing 100 µg proteins in a final volume of 1 ml. The enzyme activity was calculated from the initial rate of the reaction using the extinction coefficient of ascorbate (E = 2.8 mM cm^{-1} at 290 nm).

Activity of glutathione reductase (GR; EC 1. 6. 4. 2) was measured with the method of Sgherri et al. (1994). Extraction was in 1.5 ml of suspension solution, containing 100 mM KH_2PO_4 buffer (pH 7.0), 1 mM Na_2EDTA, and 2 % PVP. The reaction mixture (total volume of 1 ml) contained 100 mM KH_2PO_4 buffer (pH 7.8), 2 mM Na_2EDTA, 0.5 mM oxidised glutathione (GSSG), 0.2 mM NADPH and enzyme extract containing 100 µg protein. The decrease in absorbance at 340 nm was recorded. The correction was made for the non-enzymatic oxidation of NADPH by recording the decrease at 340 nm without adding GSSG to the assay mixture. The enzyme activity was calculated from the initial rate of the reaction after

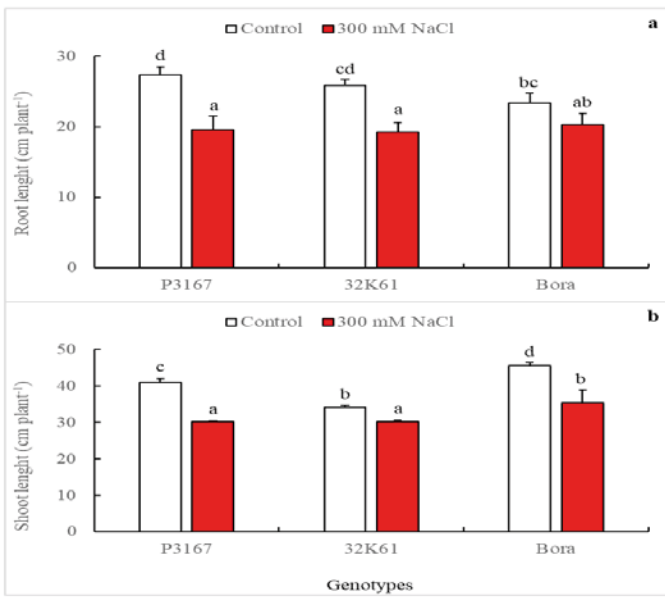

Figure 1: Effect of the salt stress on (a) root length and (b) shoot length of maize plants (Different letters mean significant differences between the treatments according to Duncan's multiple range test ($p < 0.05$))

subtracting the non-enzymatic oxidation using the extinction coefficient of NADPH (E = 6.2 mM cm^{-1} at 340 nm).

2.11 STATISTICAL ANALYSIS

Experiments were a randomized complete block design with three independent replicates. Analysis of variance (ANOVA) was using SPSS 20.0 statistical software for Windows. To separate significant differences between means, the Duncan test was used at $*p = 0.05$.

3 RESULTS

3.1 GROWTH

Root growth was adversely affected by salinity in 'P3167' and '32K61' ($p < 0.05$) while it was not changed by salt stress in Bora ($P > 0.05$) (Fig. 1a). The decrease was around 28 %, 26 %, and 13 % in 'P3167', '32K61', and 'Bora' under 300 mM salinity, respectively. Shoot growth declined by 27 % in 'P3167', 12 % in '32K61', and 22 % in 'Bora', and all these changes were significantly different from respective controls ($p < 0.05$) (Fig. 1b).

3.2 WATER RELATIONS

RWC in leaves significantly decreased in all maize genotypes by salt stress ($p < 0.05$) (Fig. 2a). It was 8 %, 9 %, and 7 % lower than respective controls in 'P3167', '32K61', and 'Bora', respectively. The water deficit index was also adversely affected in all cultivars by salinity. It was found to be 2.75-2.80- and 2.2-fold higher than controls in 'P3167', '32K61', and 'Bora', respectively ($p < 0.05$) (Fig. 2b).

3.3 PHOTOSYNTHETIC PIGMENT AND ANTHOCYANIN

According to the results, there was an inverse relationship between salt stress and photosynthetic pigment content. Chlorophyll a content, for example, significantly decreased by 30 %, 42 %, and 22 % in comparison with controls when salt stress was applied in the growth medium ($p < 0.05$) (Fig. 3a). Similarly, chlorophyll b content in the leaves of genotypes 'P3167' and '32K61' was 40 % and 48 % lower than respective controls and these changes were found to be statistically significant ($p < 0.05$). In the genotype 'Bora', however, chlorophyll b content was decreased by 15 % in comparison with control and this change was not significant ($p > 0.05$) (Fig. 3b).

Salt treatment did not affect total anthocyanin content in the leaves of maize genotypes significantly ($p > 0.05$) (Fig 3c). In the genotypes 'P3167' and '32K61' under salt stress, total anthocyanin content in the leaves were 60 % and 129 % higher than respective controls while salt stress decreased it by 24 % in the leaves of 'Bora'.

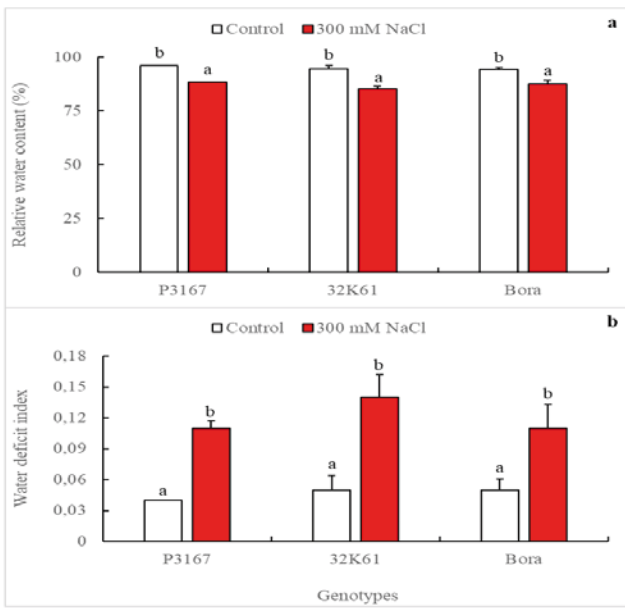

Figure 2: Effect of the salt stress on (a) relative water content and (b) water deficit index of maize plants (Different letters mean significant differences between the treatments according to Duncan's multiple range test ($p < 0.05$))

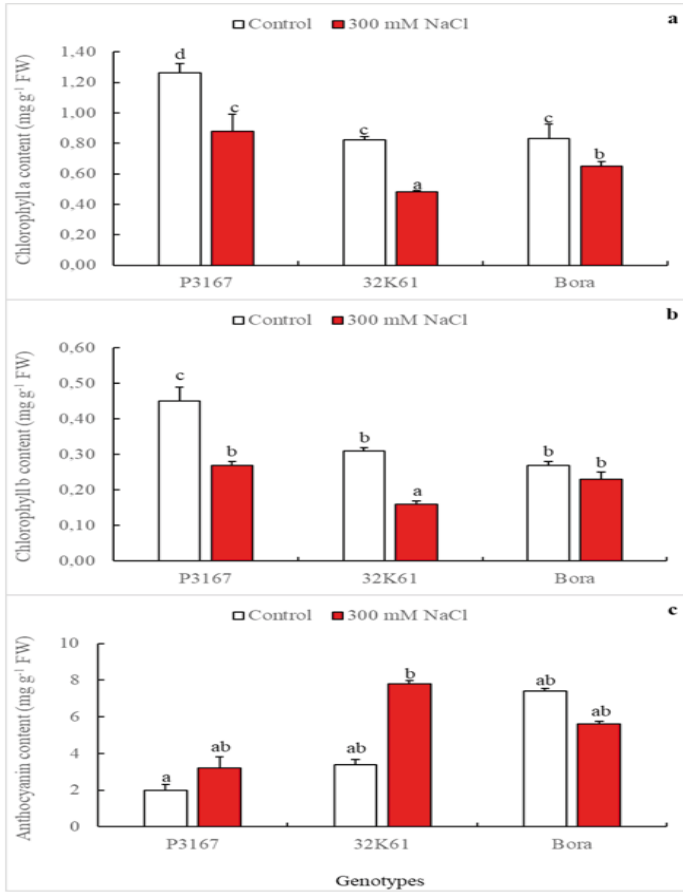

Figure 3: Effect of the salt stress on (a) chlorophyll a, (b) chlorophyll b and (c) anthocyanin content of maize plants (Different letters mean significant differences between the treatments according to Duncan's multiple range test ($p < 0.05$))

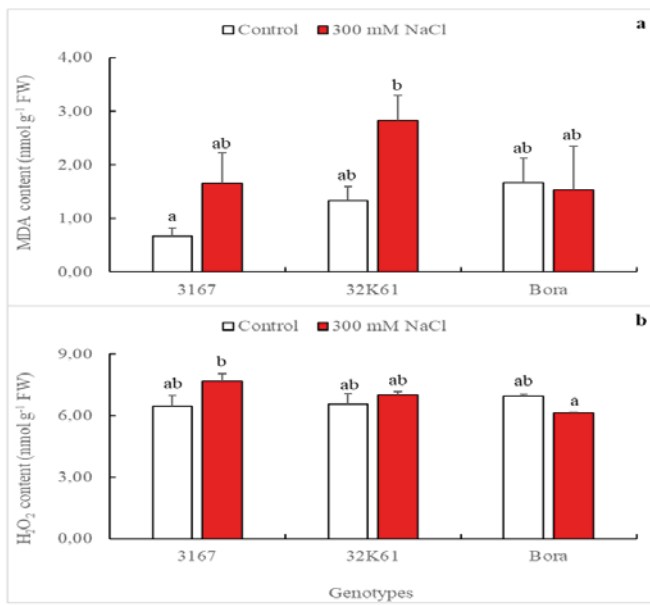

Figure 4: Effect of the salt stress on (a) MDA and (b) H_2O_2 content of maize plants (Different letters mean significant differences between the treatments according to Duncan's multiple range test ($p < 0.05$))

3.4 MALONDIALDEHYDE (MDA) AND HYDROGEN PEROXIDE (H_2O_2)

The rate of MDA accumulation was 146 % and 113 % higher in the salt-stressed 'P3167' and '32K61' leaves while it was found to be 8 % lower in the 'Bora' leaves as compared to control (Fig. 4a). However, these changes were not statistically significant ($p > 0.05$). Similarly, H_2O_2 content in the salt-stressed 'P3167' and '32K61' leaves represented insignificant increases, with 19 % and 7 % in comparison with respective controls, respectively. The H_2O_2 accumulation in the leaves of 'Bora' under salinity was 12 % and insignificantly lower than control ($p > 0.05$).

3.5 FREE PROLINE, TOTAL PHENOLIC, AND TOTAL SOLUBLE CARBOHYDRATE

The rate of free proline accumulation was 13 % and 22 % higher in the salt-stressed 'P3167' and '32K61' leaves while it was found to be 6 % lower in the 'Bora' leaves as compared to control (Fig. 5a). However, these changes were not statistically significant ($P > 0.05$).

Phenolic content in the leaves of 'P3167' and '32K61' was induced by salinity (Fig. 5b) and it was significantly increased by 19 % and 83 %, respectively ($p < 0.05$). In 'Bora', however, change in the phenolic content was not significant and it was increased by 10 % under salt stress ($p > 0.05$).

Higher but insignificant amount of TSC content (54 % higher than control) was measured in the salt-stressed leaves of 'P3167' ($p > 0.05$) (Fig. 5c). In the salt-stressed leaves of '32K61', however, TSC content was not significantly affected but it was 18 % lower than the respective control. Salinity led to the significantly decreased TSC content (75 % of the control) in the leaves of 'Bora' ($p < 0.05$).

3.6 ANTIOXIDANT SYSTEM

Salt stress remarkably increased the reduced ascor-

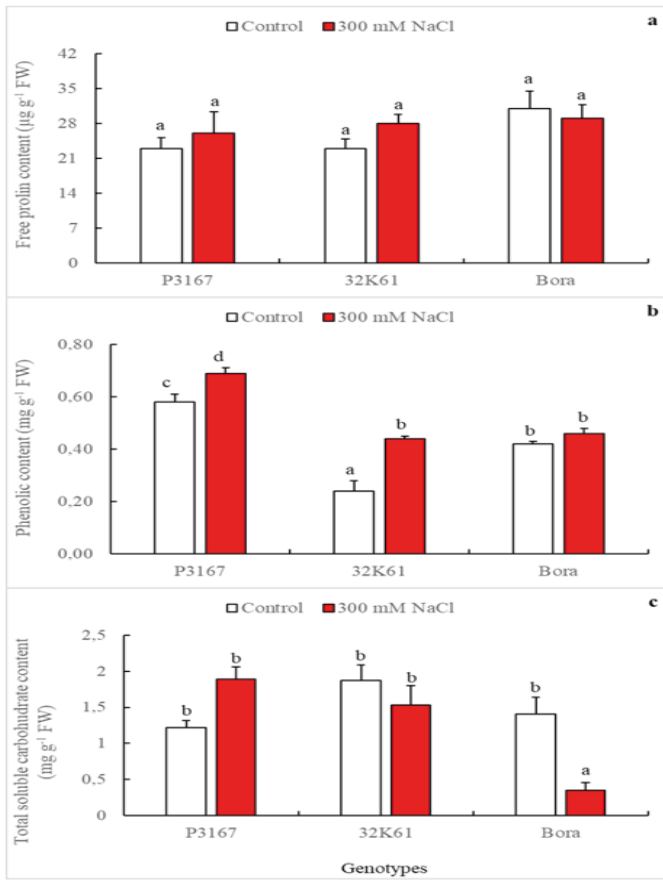

Figure 5: Effect of the salt stress on (a) free proline, (b) phenolic and (c) TSC content of maize plants (Different letters mean significant differences between the treatments according to Duncan's multiple range test ($p < 0.05$))

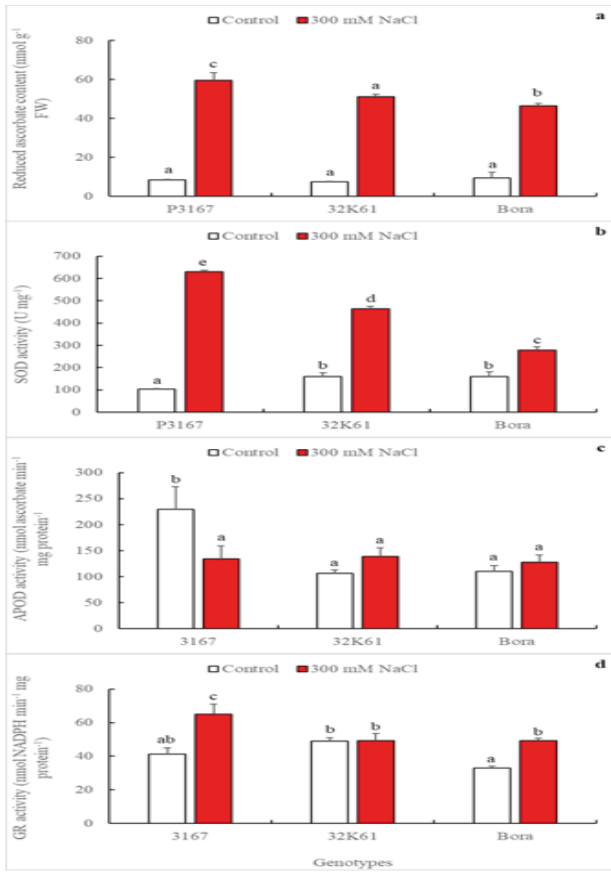

Figure 6: Effect of the salt stress on (a) the reduced ascorbate content, (b) SOD activity, (c) APOD activity and (d) GR activity of maize plants (Different letters mean significant differences between the treatments according to Duncan's multiple range test ($p < 0.05$))

bate content to 7-, 7- and 5-fold in 'P3167', '32K61', and 'Bora', respectively ($p < 0.05$) (Fig. 6a). Similarly, SOD activity was found to be significantly higher than respective controls in all maize genotypes under salt stress ($p < 0.05$) (Fig 6b). SOD activity was 506 %, 191 %, and 75 % higher than controls in 'P3167', '32K61', and 'Bora', respectively. APOD activity was significantly and 42 % lower than the respective control in 'P3167' under salinity ($p < 0.05$) (Fig 6c). In '32K61' and 'Bora', however, insignificant changes were determined in APOD activity as a result of salt stress ($p > 0.05$). It was 31 % and 15 % lower than controls (Fig. 6c). In the case of GR activity, significantly higher values were measured in 'P3167' and 'Bora' (57 % and 50 %, respectively) as compared to control ($p < 0.05$) while it was not affected and was only 1 % higher than control in '32K61' under salinity stress ($p > 0.05$) (Fig 6d).

4 DISCUSSION

Salt stress generally causes reduction in the growth rate in plants as result of the decreased ability of plants to take up water from the soil (Munns, 2002). It was reported that the growth ability of plants under salt stress maight be a reliable criterion to determine the salt tolerance degree of plants (Parida and Das, 2005). In the present study, root and shoot growth of all maize genotypes were negatively affected by salt stress (300 mM NaCl). When genotypes were compared to each other, root growth in the genotypes 'P3167' and '32K61' was found to be more sensitive to salinity while 'Bora' was more tolerant. With regard to organ type, however, shoot growth was more sensitive to salinity in all maize genotypes. These results showed that the roots and shoots of maize genotypes used in this study showed considerable variation with respect to salinity tolerance. These results are also in agreement with the findings of Doğru and Yılmaz Kaçar (2019), who manifested that sensitivity and/or tolerance of different barley genotypes may represent great variation under saline conditions. It was also indicated that salt stress may interfere with mitotic activity in the meristematic cells in plants, depending on the

plant species, genotype, organ type, and exposure time (Munns, 2002). Accordingly, we may conclude that the meristematic cells in the shoot apex are more sensitive to salinity as compared to the root meristems, probably due to more efficient transport of salt ions from roots to shoots, as reported by Zaimoğlu and Doğru (2016). Growth retardation in roots and shoots can be related to the inhibited cell elongation in plants under salt stress (Bendeoğlu et al., 2014). In the present study, it was observed that salt stress decreased RWC and increased water deficit index in the leaves of maize genotypes. Therefore, another possible reason for the reduced growth rate in maize genotypes under salt stress may be associated with the reduced RWC and induced water deficit index in the leaves. In addition, closure of the stomata and further disruption of the transpiration stream as result of water deficit-induced stimulation of abscisic acid synthesis may be indirectly responsible for the reduced growth rate in the salt-stressed maize genotypes used in this study (Polash et al., 2018).

The results of the present study indicated a significant decrease in chlorophyll a content in the salt-stressed maize genotypes which is in agreement with the previous studies of Turan et al. (2007) on *Phaseolus vulgaris* L. and Taffouo et al. (2010) on *Vigna subterranean* L. Chlorophyll b content was found to decrease only in 'P3167' and '32K61'. The decreased chlorophyll level can be attributed to the inhibition of chlorophyll biosynthesis and/or to the degradation by chlorophyllase (Santos, 2004). In addition, the results showed that chlorophyll b molecules were better preserved in the genotype 'Bora' under salt stress. In salt-stressed plants, the reduced chlorophyll content was considered as a typical symptom of oxidative stress (Smirnoff, 1996). On the other hand, the reduced chlorophyll content had been considered as a protective mechanism against oxidative stress as well (Elsheery and Cao, 2008). The results of the present study showed that salt stress did not cause oxidative stress in maize genotypes used in this study, as demonstrated by lower-level MDA and H_2O_2. Accordingly, total anthocyanin content did not represent considerable changes in the salt-stressed maize leaves. Anthocyanins are diverse group of secondary metabolites that could be produced in response to oxidative stress (Chunthaburee et al., 2016). Proline is a water-soluble amino acid and is involved in ROS detoxification (Ashraf and Foolad, 2007). In this study, salt stress did not induce free proline accumulation in the maize cultivars, confirming the hypothesis that maize genotypes are not under oxidative stress as result of salt application. An alternative explanation for this may be that oxidative stress on the maize genotypes under salt stress is eliminated by different defence mechanism as well. A possible defence mechanism that could eliminate oxidative stress on the salt-stressed maize genotypes may be the accumulation of phenolic compounds in leaf tissues. In this study, we observed that salt stress increased the phenolics in the leaves of 'P3167' and '32K61'. This result is in agreement with the previous reports on *Aloe vera* (L.) Burm.f. and radish (Moghbeli et al., 2012; Sakamoto and Suzuki, 2019). Phenolics are believed to prevent the formation of ROS under drought stress (Mayer and Harel, 1991). Parida et al. (2004) have also reported that increases in phenolic content in plant tissues ameliorate the ionic effects of salt stress. Therefore, the enhanced level of phenolic compounds in the leaves of 'P3167' and '32K61' under salt stress may be beneficial to achieve salt tolerance. Phenolic compounds have also been indicated to prevent lipid peroxidation and accumulation of MDA (Potapovich and Kostyuk, 2003), as reported in this study. In the present study, the level of the reduced ascorbate in the leaves of maize genotypes was increased by salinity as reported by several authors previously (Panda and Upadhyay, 2004; Chen et al., 2005). The reduced ascorbate is known to be a detoxifier or neutralizer of superoxide, H_2O_2, and singlet oxygen species. This result may explain the constant level of H_2O_2 content and APOD activity in the leaves of the salt-stressed maize genotypes used in this study. In other words, H_2O_2 accumulation in the salt-stressed leaves of the maize genotypes are prevented by the direct action of the reduced ascorbate instead of APOD activity. The elevated SOD activity in maize genotypes under salt stress may be an indicator of the accelerated rate of superoxide formation and detoxification (Doğru and Çakırlar, 2020a). GR activity increased in the salt-stressed leaves of the 'P3167' and 'Bora' while it did not change in '32K61'. These results could be interpreted as the lower activity of the ascorbate-glutathione cycle because of the absence of harmony between APOD and GR activities (Doğru and Çakırlar, 2020b). TSC content was not affected in the salt-stressed leaves of the 'P3167' and '32K61' while it was decreased in the leaves of 'Bora'. It has been reported that soluble sugars play an important role in osmotic adjustment in plant cells under stressful conditions (Doğru and Ecem Bayram, 2016). According to the results in this study, it could be concluded that TSC is not involved in the osmotic regulation in the salt-stressed 'P3167' and '32K61'. However, TSC may serve as carbon reserves in these genotypes. In the salt-stressed leaves of 'Bora', a lower level of TSC may show that they are used for growth and development.

In conclusion, the present study showed that salt stress reduced the growth rate in the salt-stressed maize genotypes, and shoot growth was more sensitive to salinity in comparison with root growth. In addition, salt stress led to the water deficit (physiological drought) in all genotypes, probably resulting in growth retardation.

In 'P3167' and '32K61', salt stress predominantly and adversely affected chlorophyll a content while 'Bora' retained both chlorophylls a and b. The elevated SOD activity in all maize genotypes under salt stress may indicate an efficient dismutation of superoxide radical. Changes in APOD and GR activities under salinity clearly showed an increased pressure on the ascorbate-glutathione cycle, especially in 'P3167' and '32K61'. The constant level of H_2O_2 and MDA in the salt-stressed leaves of maize genotypes may show that the reduced ascorbate and phenolic compounds may be responsible for avoiding the adverse effects of oxidative stress. Finally, the genotype 'Bora' could be considered as having tolerance to salinity while 'P3167' and '32K61' are sensitive ones.

5 ACKNOWLEDGMENT

This study is supported by Sakarya University Scientific Research Projects Coordination Unit. Project Number: 2010-02-20-009.

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doi:10.14720/aas.2021.117.1.1768 Original research article / izvirni znanstveni članek

Diversity of aphids (Hemiptera: Aphididae) associated with potato crop in Tizi-Ouzou (North of Algeria), with new records

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Received July 08, 2020; accepted December 14, 2020.
Delo je prispelo 08. julija 2020, sprejeto 14. decembra 2020.

Diversity of aphids (Hemiptera: Aphididae) associated with potato crop in Tizi-Ouzou (North of Algeria), with new records

Abstract: Aphids are among the phytophagous pests that cause serious damage to crop plants. In Northern Algeria, we have little information on their diversity. In this context, the study of the diversity of aphids was carried out in three regions of Tizi-Ouzou (North of Algeria) namely Tizi-Ouzou center, Tizi-Rached and Aghribs on the potato crop using yellow traps. The results showed a total richness of 65 aphid species, divided into 36 genera, 9 tribes and 8 sub-families, of which 11 species were identified for the first time in Algeria. These are *Aphis coreopsidis* (Thomas, 1878), *Capitophorus hippophae* (Walker, 1852), *Cavariella theobaldi* (Gillette & Bragg, 1918), *Hyadaphis coriandri* (B. Das, 1918), *Macrosiphoniella linariae* (Koch, 1855), *Monelliopsis pecanis* Bissell, 1983, *Myzus hemerocallis* Takahashi, 1921, *Pseudoregma panicola* (Takahashi, 1921), *Rhopalosiphoninus staphyleae* (Koch, 1854), *Schizaphis eastopi* Van Harten & Ilharco, 1971 and *Ovatus inulae* (Walker, 1849). The field located in the center of Tizi-Ouzou is the richest with 55 species, followed by the field of Tizi-Rached with 30 species, and 24 species have been recorded in Aghribs. During the sampling season, *Hyperomyzus lactucae* (Linnaeus, 1758) and *Brachycaudus helichrysi* (Kaltenbach, 1843) are the most abundant species with 24.44 % and 21.8 % respectively. Three aphid species have been observed on potato leaves, namely *Macrosiphum euphorbiae* (Thomas, 1878), *Aphis gossypii* Glover, 1877 and *Myzus persicae* (Sulzer, 1776). The latter species was observed in all three study regions.

Key words: aphids; diversity; potato; new species

Pestrost listnih uši (Hemiptera: Aphididae) v nasadih krompirja na območju Tizi-Ouzou (Severna Alžirija) z novimi najdbami

Izvleček: Listne uši so med rastlinojedimi škodljivci tisti, ki lahko povzročijo občutne poškodbe na gojenih rastlinah. V severni Alžiriji je o njihovi pestrosti malo znanega. Z ozirom na to je bila izvedena raziskava pestrosti listnih uši na treh območjih Tizi-Ouzou Severne Alžirije in sicer v središču območja Tizi-Ouzou, in na območjih Tizi-Rached in Aghribs v nasadih krompirja z uporabo rumenih pasti. Izsledki so pokazali, da je celokupna pestrost obsegala 65 vrst listnih uši, ki so pripadale 36 rodovom, 9 plemenom in 8 poddružinam, od katerih je bilo 11 vrst novih za Alžirijo. Te vrste so bile *Aphis coreopsidis* (Thomas, 1878), *Capitophorus hippophae* (Walker, 1852), *Cavariella theobaldi* (Gillette & Bragg, 1918), *Hyadaphis coriandri* (B. Das, 1918), *Macrosiphoniella linariae* (Koch, 1855), *Monelliopsis pecanis* Bissell, 1983, *Myzus hemerocallis* Takahashi, 1921, *Pseudoregma panicola* (Takahashi, 1921), *Rhopalosiphoninus staphyleae* (Koch, 1854), *Schizaphis eastopi* Van Harten & Ilharco, 1971 in *Ovatus inulae* (Walker, 1849).Polje v središču območja Tizi-Ouzou je vrstno najbogatejše s 55 vrstami, sledi mu polje na območju Tizi-Rached s 30 vrstami in območje Aghribs s 24 vrstami. Med vzorčenjem sta bili vrsti *Hyperomyzus lactucae* (Linnaeus, 1758) in *Brachycaudus helichrysi* (Kaltenbach, 1843) najpogostejši s 24,44 % in 21,8 % deležem. Na listih krompirja so bile opažene tri vrste listnih uši in sicer *Macrosiphum euphorbiae* (Thomas, 1878), *Aphis gossypii* Glover, 1877 in *Myzus persicae* (Sulzer, 1776), slednja je bila opažena ns vseh treh območjih.

Ključne besede: listne uši; pestrost; krompir; nove vrste

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1 INTRODUCTION

The potato (*Solanum tuberosum* L.), is one of the main food resources in the world. It remains the most consumed vegetable or field crop (Brillaud, 2008; Marchoux, 2008). This crop is subject to many pests, including aphids, they are small sap-sucking insects. The direct damage is caused by these pests to the crop by sap sucking, thus reducing the resources available for growth and development of the plant (Blackman and Eastop, 2000; 2006). However, the most significant damage is caused indirectly by plant pathogenic viruses transmitted by aphids. *Myzus persicae* Sulzer, for example, is known to be a vector of more than 100 plant viruses (Blackman and Eastop, 2000) including Potato Virus Y (PVY), Potato Leafroll Virus (PLRV) or Cucumber Mosaic Virus (CMV) (Lin et al., 2016; Shi et al., 2016).

Aphids are very widespread, more than 4700 species have been recorded in the world (Remaudière and Remaudière, 1997), among them about 450 aphid species have been identified as pests of cultivated plants (Blackman and Eastop, 2000). At least 220 species have been recorded in sub-Saharan Africa (Autrique and Ntahimpera, 1994). One hundred fifty species have been identified in Morocco (Sekkat, 2015). Aphids in Tunisia are represented by one hundred and three species (Bodenheimer and Swirsky, 1957; Blackman and Eastop, 1994; 2000, 2006; Ben Halima-Kamel, 1991, 1995; Ben Halima Kamel and Ben Hamouda, 1993, 1998, 2004, 2005; Boukhris-Bouhachem et al. 1996; Boukhris-Bouhachem et al., 2007). Ninety nine species are recorded from Egypt (Theobald, 1922; Habib and El Kady, 1961; Darwish, 2009), seventy-three species are listed from Libya (Trotter, 1912; 1914; Damiano, 1961; 1962; Blackman and Eastop, 1994; Ahmeid Al-Nagar, 2000; Ahmeid Al-Najar and Nieto Nefrya, 1998).

The Algerian aphid fauna is known partly (Blackman and Eastop, 1994, 2000, 2006; Laamari and Akkal, 2002; Lamaari et al., 2010, 2013; Hidalgo et al., 2012; Laamari, 2016; Laamari et al., 2016; Benoufella-Kitous et al., 2014a, 2014b, 2016, 2019). In Tizi-Ouzou, we have very little data on aphid diversity (Benoufella-Kitous et al., 2014a, 2014b, 2016, 2019). Hence the objective of this study is to evaluate the diversity and abundance of aphids in three potato fields in Tizi-Ouzou region (Northern Algeria).

2 MATERIALS AND METHODS

2.1 THE STUDY REGION

This study was carried out in three localities of Tizi-Ouzou (Fig.1) in open field potato crops: The first, Resita variety, was planted on March 03rd, 2013, over an area of 3000 m^2 is located in Tizi-Ouzou Center (36 ° 43′ 00" N and 4 ° 03′ 00" E) at 200 m altitude. The second, Fabula variety, was planted on December 27th, 2016, on an area of 300 m^2 is located in Tizi Rached (36 ° 40′ N and 4 ° 11′ E) at 412 m altitude and the third, Timate variety, was planted on February 23th, 2017, on an area of 250 m^2 is located in Aghribs (36 ° 48′ 08" N, 4 ° 19′ 22" E) at an altitude of 800 m.

The fields were sampled during the period from April 2nd to June 18th, 2013 for the Resita variety, from February 13th to May 8th, 2017 for the Fabula variety and from April 1st to June 3rd, 2017 for the Timate variety. The experimental protocol followed for the trapping of winged aphids and the visual control of aphids is that

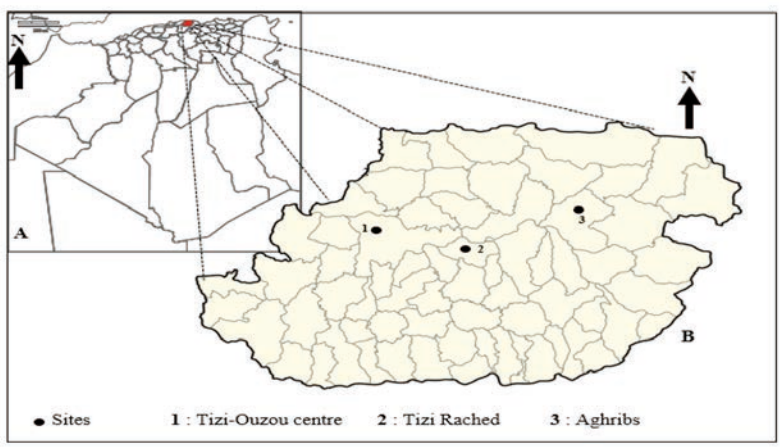

Figure 1: Map of Tizi-Ouzou region and location of study sites, (A) Map of Algeria showing the study region, (B) the study region

described by Atesebeha et al. (2009). Two methods were combined for monitoring aphidofauna.

2.2 TRAPPING OF WINGED APHIDS

To do this, the study field was divided into 9 blocks. In the middle of each block, a yellow Von Moerik trap (Ɵ = 27 cm, h = 10 cm) was placed, filled with water and a few drops of detergent (dishwashing liquid). The attraction of aphids to the yellow color has been known for long time. This color tends to cause these insects to land (Yattara et al., 2013). The contents of the container are necessary for optimum yield (EDES, 2011). Aphids caught in the yellow traps were removed once a week with a brush and stored in test tubes filled with 70 % ethanol.

2.3 VISUAL OBSERVATION OF APHIDS ON POTATO LEAVES

Once a week, in each block of the field, one plant was chosen and the aphids found on the potato leaves were collected in a tube filled with 70 % ethanol, using a brush. Aphids trapped and taken from the plants are sorted and then identified up to the species level. According to Lascaux (2010), the identification of aphids was carried out by observing some morphological characters of the aphid, in particular: the antennae, the frontal tubercles, the tarsi, the cauda, the color and the shape of the sphinculi, the pigmentation of the abdomen and venation of the wings. The samples collected were determined by Dr. Benoufella-Kitous at the Plant Production, Improvement and Protection Laboratory of the Faculty of Biological Sciences and Agronomic Sciences at Mouloud Mammeri University in Tizi-Ouzou. The identification was carried out based on the identification keys of Stroyan (1961), Jacky and Bouchery (1982), Autrique and Ntahimpera (1994), Remaudière et al. (1985) and Leclant (1999) and Blackman and Eastop, (2000; 2006).

3 RESULTS

During the study, 2144 aphids were collected from all three regions (Table 1). More than two thirds of the catches (73.27 %) were made in Tizi-Ouzou. A total of 65 species were caught using yellow traps, divided into 36 genera, 9 tribes and 8 subfamilies: Aphidinae, Anoeciinae, Calaphidinae, Chaitophorinae, Eriosomatinae, Hormaphidinae, Myzocallidinae, and Pterocommatinae, 11 species are new to Algeria.

The Aphidinae subfamily is predominant with 2 tribes, that of Aphidini and that of Macrosiphini. The latter is the richest with 39 species. Tizi-Ouzou centre is the richest in species with 55 species, divided into 32 genera, 7 tribes and 5 subfamilies, namely the Aphidinae, Anoeciinae, Calaphidinae, Eriosomatinae and Myzocallidinae. Followed by Tizi Rached station, with 30 species belonging to three subfamilies, namely Aphidinae, Chaitophorinae and Eriosomatinae. Besides, 24 species were recorded at Aghribs belonging to 4 subfamilies, those of Aphidinae, Eriosomatinae, Hormaphidinae, and Pterocommatinae. Considering all stations, the most abundant species during the sampling season are: *Hyperomyzus lactucae* (24.44 %), *Brachycaudus helichrysi* (21.8 %), *Macrosiphum rosae* (19.93 %) and *Myzus persicae* (13.11 %).

On the other hand, *Macrosiphum euphorbiae*, *Aphis gossypii* and *M. persicae*, were observed on potato leaves. The last two species develop in all potato fields. *M. euphorbiae* was the most observed in Tizi-Ouzou centre. In Aghribs, *M. persicae* was the most abundant, while in Tizi Rached, *A. gossypii* was the most abundant (Table 2).

3.1 DESCRIPTION OF SOME NEW SPECIES

3.1.1 *Aphis coreopsidis*

Apterae are yellow to green with darker legs, antennae and siphunculi. The abdomen has marginal scleritis (Blackman and Eastop, 2020). The siphunculi are black, relatively long and thick the cauda is clear and narrow

3.1.2 *Capitophorus hippophae*

In spring the apterae colonies are pale green, slender. The alatae are greyish-green with a black head and thorax, dark antennae, legs and siphunculi and a a large quadrate dark green patch on the dorsal abdomen (Blackman and Eastop, 2020). Their primary host are species from the genus *Polygonum* , and the secondary host are species from the same genus (Remaudière et al., 1985).

3.1.3 *Hyadaphis coriandri*

The alatea are yellow-green in color, dusted with greyish wax. They have short, dusky, slightly swollen, siphunculi. that are about twice as long as wide (Halbert, 2003). This species colonizes *Hydrocotyle vulgaris* L. (Hydrocotylaceae), *Anethum* sp. *Coriandrum sativum* L., *Daucus* sp. *Foeniculum vulgare* Mill., *Peucedanum* sp.

Table 1: Proportions and relative wealth of aphids trapped in potato fields, the nomenclature of all species is follows that of orders in Fauna Europaea (2020)

Aphid species	Tizi-Ouzou Centre	Aghribs	Tizi Rached
Acyrthosiphon pisum (Harris, 1776)	0.76*	-	-
Anoecia corni (Fabricius, 1775)	0.13	-	-
Aphis coreopsidis (Thomas,1878)[1]	-	-	0.32
Aphis craccivora Koch, 1854	7.51	2.6	4.2
Aphis fabae Scopoli, 1763	9.70	11.8	8.04
Aphis gossypii Glover, 1877	6.87	16.41	6.8
Aphis idaei van der Goot, 1912	3.63	-	1
Aphis nerii Boyer de Fonscolombe, 1841	2.36	0.7	1.61
Aphis pseudocardui Theobald, 1915	-	0.7	-
Aphis spiraecola Patch, 1914	0.95	1.1	1.3
Aphis sp.	0.20	-	0.32
Aulacorthum solani Kaltenbach, 1843	1.78	1.52	2.6
Brachycaudus cardui (Linnaeus, 1758)	0.57	3	1.61
Brachycaudus helichrysi (Kaltenbach, 1843)	1.65	21.8	2.3
B*revicoryne brassicae* (Linnaeus, 1758)	2.23	1.14	0.32
Capitophorus hippophae (Walker, 1852)[1]	0.13	-	-
Cavariella aegopodii (Scopoli, 1763)	0.25	-	0.32
Cavariella pastinacae (Linnaeus, 1758)	0.20	-	-
Cavariella theobaldi (Gillette & Bragg, 1918)[1]	0.13	-	-
Diuraphis noxia (Kurdjumov, 1913)	0.20	-	-
Dysaphis plantaginea (Passerini, 1860)	4.39	1.14	0.64
Dysaphis tulipae (Boyer de Fonscolombe, 1841)	0.20	5.58	1
Dysaphis apiifolia (Theobald, 1923)	0.13	-	-
Dysaphis foeniculus (Theobald, 1923)	-	1.5	-
Eriosoma lanuginosum (Hartig, 1839)	0.13	-	-
Hyadaphis coriandri (B. Das, 1918)[1]	0.95	-	-
Hyadaphis foeniculi (Passerini, 1860)	0.57	-	-
Hyalopterus pruni (Geoffroy, 1762)	0.32	-	-
Hyperomyzus carduellinus (Theobald, 1915)	0.20	-	-
Hyperomyzus lactucae (Linnaeus, 1758)	6.17	6.4	24.44
Hyperomyzus picridis (Börner & Blunck, 1916)	0.13	1.14	-
Lipaphis erysimi (Kaltenbach, 1843)	0.95	0.76	0.64
Macrosiphoniella linariae (Koch, 1855)[1]	2.74	-	-
Macrosiphum euphorbiae (Thomas, 1878)	3.69	1.14	4
Macrosiphum funestum (Macchiati, 1885)	-	-	0.4
Macrosiphum rosae (Linnaeus, 1758)	1.72	8.77	19.93
Megoura viciae Buckton, 1876	1.25	-	-
Metopolophium dirhodum (Walker, 1849)	0.06	-	0 .4
Metopolophium festucae Theobald, 1917	-	3.1	-
Monelliopsis pecanis Bissell, 1983[1]	0.13	-	-
Myzocallis castanicola Baker, 1917	0.20	-	-

Continued			
Myzus ascalonicus Doncaster, 1946	2.04	-	-
Myzus cerasi (Fabricius, 1775)	0.51	-	-
Myzus hemerocallis Takahashi, 1921[1]	0.13	-	-
Myzus persicae Sulzer, 1776	13.11	3.1	9.00
Myzus sp.	-	-	0.32
Nasonovia ribisnigri (Mosley, 1841)	0.45	-	0.32
Ovatus crataegarius (Walker, 1850)	-	-	0.32
Ovatus inulae (Walker, 1849)[1]	0.13	-	-
Pemphigus sp.	1.85	0.3	1.3
Phorodon humuli (Schrank, 1801)	0.45	-	-
Pseudoregma panicola (Takahashi, 1921)[1]	-	0.7	-
Rhopalosiphum insertum (Walker, 1849)	0.13	-	-
Rhopalosiphum maidis (Fitch, 1856)	8.30	-	4.50
Rhopalosiphum padi (Linnaeus, 1758)	7.38	6.10	1.4
Rhopalosiphum rufiabdominale (Sasaki, 1899)	0.64	-	-
Rhopalosiphoninus staphyleae (Koch, 1854)[1]	0.06	-	-
Schizaphis eastopi Van Harten & Ilharco,1971[1]	0.06	-	-
Schizaphis rotundiventris (Signoret, 1860)	0.20	-	-
Sipha maydis Passerini, 1860	-	-	0.4
Sitobion avenae (Fabricius, 1775)	0.38	-	-
Sitobion fragariae (Walker, 1848)	0.70	-	-
Smynthurodes betae Westwood, 1849	0.20	0.7	-
Takecallis taiwana (Takahashi, 1926)	0.10	-	-
Uroleucon sonchi (Linnaeus, 1767)	-	-	0.64
Number of aphid individuals	1571	262	311
Number of species	55	24	30

*The proportions are expressed in percentage.
[1]Species reported for the first time in Algeria.

Table 2: Proportion and relative abundance of species observed in the three potato fields

	Tizi-Ouzou Centre		Aghribs		Tizi Rached	
Species	Aphids[a]	Frequency	Aphids[a]	Frequency	Aphids[a]	Frequency
A. gossypii	492	16 %	64	25.4 %	403	67.3 %
M. euphorbiae	2438	78 %	-	-	133	22.2 %
M. persicae	196	6 %	188	74.60 %	63	10.5 %
Total	3126	100 %	252	100 %	599	100 %

a: number of aphids.

Pituranthos sp. *Steganotaenia* sp. (Umbelliferae) (Remaudière et al., 1985).

3.1.4 *Myzus hemerocallis*

Apterae are pale yellowish green or greenish, they measure between 1.6 and 2.4 mm (Blackman and Eastop, 2020). They attack basal parts of *Hemerocallis* spp. and *Agapanthus umbellatus*. L'Herit.

3.1.5 *Pseudoregma panicola*

Apterae are brownish black or dark brownish red-violet, secreting columns of dense white ; the adults measure between 1,1 and 1,9 mm (Blackman and Eastop, 2020). This species colonize underside of bambou leaves *Arundinaria alpina* K. Schum. (Remaudière et al., 1985).

3.1.6 Rhopalosiphoninus staphyleae

In spring, the Apterae on *Staphylea* are yellowish-white or pale yellow with a translucent whitish spot on anterior part of dorsal (Blackman and Eastop, 2020). This species lives on *Viola tricolor* L. (Violaceae).

3.1.7 *Schizaphis eastopi*

The abdomen is devoid of pigmentation, siphunculi are black, cylindrical with apical constriction. The cauda is clear, small than the siphunculi. This species lives on *Typha domingensis* Pers., *Typha angustifolia* L. and *Typha capensis* (Rohrb.) N.E. Br. (Typhaceae) (Remaudière et al., 1985).

4 DISCUSSIONS

Based on the trapping results of winged aphids caught using yellow traps installed in three potato fields in the Tizi-Ouzou region, 65 species were identified, after comparison of our results and those of Laamari et al. (2010; 2013), 11 species are new for Algeria, 27 species were collected in Tizi-Ouzou in Draâ Ben Khedda on the bean (Benoufella-Kitous et al., 2014a). Benoufella-Kitous et al. (2014b) affirmed the presence of 28 species on citrus fruits in Oued-Aissi (Tizi-Ouzou, Algeria). On the food legumes, 55 species have been recorded in Tala Amara (Tizi-Ouzou) (Benoufella-Kitous et al., 2016) and 43 species in Tizi Rached (Benoufella-Kitous et al., 2019).

Most of these aphid species are pests of cultivated plants, including *M. persicae*, an effective vector, especially of potato viruses. The difference in the specific richness of one region to another can be explained by the difference in the floristic composition which has a direct influence on the aphid richness. Among the plant species recorded in large numbers in the study stations: *Picris echioides* L., *Sonchus oleraceus* L., *Solanum nigrum* L., *Malva sylvestris* L. and *Aven asterilis* L. which can host up to 20 aphidian species (Laamari et al., 2010; Moussadegh et al., 2016).

B. helichrysi is the most abundant species in Aghribs with 21.8 %. This abundance can be explained by the presence of its primary host plant: thevine crop. The secondary host of this species is represented by the Asteraceae (sow-thistle, lettuce), where it is found in colonies of several individuals living inside of the leaves, sometimes in mixed colonies with *Nasonovia ribisnigri* (Hullé et al., 1999). In Tizi-Ouzou centre, *M. persicae* is the most dominant with 206 individuals with a frequency of 13.11 %. Similar results have been noted in China by Lopes et al. (2012). These authors highlighted the predominance of this aphid over other captured species. Also in Mali, Yattara et al. (2013), report the presence of this species with 21.2 % on potato. This dominance is explained by the presence of its primary host: the peach tree *Prunus persica* (L.) Batsch, and also its secondary host which is the potato.

The species *A. coreopsidis* was reported for the first time in Saudi Arabia by Hussain et al. (2015), in spring this species has a several secondary hosts such as Compositae, Asteraceae, Malvaceae and Lamiaceae family (Blackman and Eastop, 2020), and several species of these families are found around the field of Tizi Rached. *C. hippophae* has as secondary host Polygonaceae such as *Polygonum persicaria* L. (Forbes and Chan, 1985). The presence of *R. staphyleae* in the Tizi-Ouzou centre is probably due to the presence of flowering plants and also weeds belonging to the Chenopodiaceae family which are secondary hosts of this aphid.

Three species of aphids are found on potato leaves, namely *M. euphorbiae*, *A. gossypii* and *M. persicae*. According to Hullé et al. (1999), the latter three species are characteristic of this crop. *M. persicae* is reported by Laamari and Akkal (2002) in Setif (Algeria). Laamari (2004), in the Guellal region of Biskra (Algeria), notes the presence of *M. euphorbiae* and *M. persicae* on a potato crop. In Kati and Sikasso (Mali), Yattara et al. (2013) highlights the presence of *A. gossypii* and *M. persicae* on this crop for three consecutive years. Lopes et al. (2012) report the presence of the latter two species on potato in China. The difference between the abundance of the three species observed on the leaves of potato crops in all fields, could

be due to the arrival order of the winged adults, and could be to the competition phenomenon, the aphids can respond differently when they are feeding on the same plant. This dietary variation is related to the capacity of aphid species to encourage the plant to produce a richer diet, especially on amino acid (Telang et al., 1999).

5 CONCLUSION

This preliminary study, which focuses on the diversity of aphids associated with potato crop in three localities of the Tizi-Ouzou region (North of Algeria), has shown the existence of 65 species, which 11 species are reported for the first time in Algeria. The presence of aphids and their diversity is favored by the presence of their host plant. In the three study fields several weeds and fruit trees are present, this would explain the abundance of these pests within these fields. Therefore, it would be interesting to continue several studies in different regions, in order to identify the host plants of each aphid species. The identification of their host constitutes an important scientific database in order to contribute to the establishment of an aphid list with trophic relationships (aphid-host plant).

6 ACKNOWLEDGEMENT

We would like to thank the three anonymous reviewers for their helpful suggestions and their insightful and constructive critiques.

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doi:10.14720/aas.2021.117.1.1523 Original research article / izvirni znanstveni članek

Effect of selected Rwandan wheat varieties on the physicochemical characteristics of whole wheat flour

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Received February 18, 2020; accepted January 13, 2021.
Delo je prispelo 18. februarja 2020, sprejeto 13. januarja 2021.

Effect of selected Rwandan wheat varieties on the physicochemical characteristics of whole wheat flour

Abstract: The present study aimed to evaluate the effect of the wheat varieties newly introduced in Rwanda on the physicochemical characteristics of their whole wheat grains in order to know their potentials for processing. Gihundo wheat grain variety had the highest values for extraction yield (99.20 %), contents of ash (1.47 %) and total dietary fiber (15.97 %), water absorption capacity (89.00 %), dough development time (7.62 min) and brightness (84.67 %). For the same physicochemical characteristics, whole flour from Nyaruka wheat variety showed the lowest values for extraction yield (96.20%), water absorption capacity (80.00 %), dough development time (6.33 min) and brightness (80.33), while whole flour from Reberaho wheat variety exhibited the lowest values for the contents of ash (0.98 %) and total dietary fiber (12.44 %). The protein content ranged between 10.00 % and 10.85 % for whole flours from all wheat varieties. The results showed that whole flour from Gihundo wheat grain variety exhibited high values for most of the physicochemical characteristics determined in comparison to the other three varieties. It is important to select grains or flour from these wheat varieties newly introduced in Rwanda based on the individual cultivar because their derivative products could have a more desired quality.

Key words: physicochemical characteristics; whole wheat flour; wheat varieties

Vpliv izbranih ruandskih sort pšenice na fizikalno-kemijske lastnosti polnozrnate pšenične moke

Izvleček: Cilj raziskave je bil proučiti vpliv v Ruandi na novo vpeljanih sort pšenice na fizikalno-kemijske lastnosti pripadajočih celih zrn z namenom prepoznave njihovega potenciala za nadaljnjo predelavo. Zrna iz pšenice sorte Gihundo so imela največji izplen moke pri mletju (99,20 %), polnozrnata moka je vsebovala največ pepela (1,47 %), največ skupne prehranske vlaknine (15,97 %), imela je najboljšo sposobnost vezave vode (89,00 %), najdaljši razvoj testa (7,62 min) in bila je najbolj svetla (84,67). Za iste fizikalno-kemijske lastnosti je polnozrnata moka iz sorte Nyaruka dosegla najmanjši izplen meljave (96,20 %), najslabšo sposobnost vezave vode (80,00 %), najkrajši razvoj testa (6,33 min) in bila je najbolj temna (80,33), medtem, ko je polnozrnata moka iz sorte Reberaho vsebovala najmanj pepela (0,98 %) in skupne prehranske vlaknine (12,44 %). Vse štiri pšenične polnozrnate moke so vsebovale med 10,00 % in 10,85 % beljakovin. Rezultati so pokazali, da je imela polnozrnata moka iz pšenice sorte Gihundo v primerjavi z mokami iz zrn ostalih treh sort boljše vrednosti za večino fizikalno-kemijskih parametrov. Pomembno je izbrati pšenična zrna ali moko iz tistih zrn na novo vpeljanih sort v Ruandi, ki imajo najboljše lastnosti, saj lahko to vodi k večji kakovosti izdelkov.

Ključne besede: fizikalno-kemijske lastnosti; polnozrnata pšenična moka; sorte pšenice

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1 INTRODUCTION

Whole wheat flours have been gaining an increase in demand and utilization for their nutritive value and health benefits in different food products for human consumption worldwide. AACC (2000) defines whole grain flour as flour which consists of the intact, ground, cracked or flaked caryopsis, whose principal anatomical components (the starchy endosperm, germ, and bran) are present in the same relative proportions as they exist in the intact caryopsis. Whole grain flour contains more vitamins, minerals, antioxidants, bioactive phytochemicals and dietary fiber than refined flour (Chung et al., 2009; Adom and Sorrells, 2005; Slavin, 2004). Due to health promoting effects of phytochemicals and dietary fiber, an increased consumption of whole grains is recommended (Seal, 2015).

While the extraction yield affects the (dough) farinograph test properties of whole wheat flours, these phytochemical compounds can also affect the quality of the end-use products such as low loaf volume and dense crumb structure, grainy, nutty and bitter flavors (Heinio, 2009; Chang and Chambers, 1992), and darker crumb and crust color (Lebesi and Tzia, 2011). Among the phytochemicals, carotenoids and phenolic compounds such as anthocyanins can act as antioxidants and impact the color and flavor of the food product (Rao and Rao, 2007). On average, the total carotenoid content was 2.57 mg kg^{-1} for the whole wheat grain, 1.92 mg kg^{-1} for the endosperm, 9.11mg kg^{-1} for the germ and 0.74 mg kg^{-1} for the bran. Those values for the endosperm, the germ and the bran were calculated based on seed fraction mass proportions to whole grain (Ndolo and Beta, 2013). Therefore, depending on the varieties, the physicochemical characteristics of whole wheat grains would significantly differ and influence the processing and quality of end-products. Some physicochemical attributes of the grains and flours of these new wheat varieties released in Rwanda in 2017 have not yet been determined so far in order to provide industries and consumers wheat with preferred specific quality traits and functionality (MINAGRI, 2017; Newtimes, 2017; RAB, 2017). This may often result in promising wheat cultivars that can be rejected by a farmer, seller, processor or consumer (Battenfield et al., 2016). With regard to this, the present study aimed to determine the physicochemical characteristics of whole wheat grains which can influence the quality of their based products.

2 MATERIALS AND METHODS

2.1 COLLECTION OF THE SAMPLES

Four (4) dry wheat grain varieties namely TAI, EN161, Eagle10 and Korongo with their local names Gihundo, Kibatsi, Nyaruka, and Reberaho, respectively, were collected from the stores of Rwanda Agriculture and Animal Resources Development Board (RAB) located at Kinigi, Musanze district, Rwanda. These wheat varieties were grown in RAB farms at the same location and under the same agro ecological conditions in the crop year 2019.The wheat grains were sampled in the same year, packed in high density polyethylene bags and stored at room temperature prior to milling.

2.2 PREPARATION OF THE RAW SAMPLES

2.2.1 Wheat grains milling

Before milling, the wheat grains were conditioned to 15.5 % moisture content by the addition of distilled water and were left for at least 24 h at ambient conditions in a closed plastic container for the absorption of the moisture (Mishra, 2016) in order to get a fine particle size whole wheat flour. AACC (2003) method was used to calculate the amount of water to be added for wheat grains tempering:

$$ml = \left(\left(\frac{100 - \%\, moisture}{100 - 15.5\,\%}\right) - 1\right) x\ grams\ of\ wheat\ grains$$

The conditioned wheat grains of each variety were wholly milled for 15 min by using a laboratory hammer mill (CM 1090 Cemotec, 2009, China). All bran and germ were mixed with the flour. The flour was packed in high density polyethylene envelop and stored at -20 °C until the day of analysis.

2.3 DETERMINATION OF PHYSICOCHEMICAL CHARACTERISTICS

2.3.1 Moisture content of whole wheat flour

Moisture content of the whole wheat flour was determined using moisture analyser (Model: HE 53/01, Mettler Toledo, China).

2.3.2 Ash content of whole wheat flour

Ash content of the whole wheat flour was determined by AOAC (2005) method using a muffle furnace (Lindberg/Blue 1100°C Box furnace BF 51800 series Ashville, NC).

2.3.3 Total fat content of whole wheat flour

Total fat content was determined by AOAC (2005) method using Soxhlet extraction procedure.

2.3.4 Total protein content of whole wheat flour

Total protein content was determined by AOAC (2005) method using the Kjeldahl procedure.

2.3.5 Total dietary fiber content of whole wheat flour

Total dietary fiber content was determined by AOAC (2005) method where the extraction was done using petroleum ether.

2.3.6 Total carbohydrate content of whole wheat flour

Total carbohydrate was determined by AOAC (1990) method.

$$Total\ carbohydrate = 100 - (Protein\ \% + Fat\ \% + Ash\ \% + Moisture\ \%).$$

2.3.7 Bulk density of whole wheat flour

The bulk density was determined as described by Khalid et al. (2017). The flour samples were gently filled into 10 ml graduated plastic cylinders. The bottom of the cylinder was gently tapped on a laboratory bench covered with foam several times until there was no further diminution of the sample level. The mass of the sample was calculated and the bulk density was calculated as mass of sample per unit volume of sample (g ml^{-1}).

2.3.8 Oil absorption capacity of whole wheat flour

The oil absorption capacity was determined as described by Khalid et al. (2017). Each sample of 0.5 g was mixed with 6 ml of mustard oil in pre-weighed centrifuge tubes. The contents were vortexed for 1 min to disperse the sample in the oil. The samples were then kept for 30 min in vertical positions and then subjected to centrifugation of 3000 rpm for 15 min (3-18KS, Sigma Laborzentrifugen GmbH, Germany). The layer of the oil was removed by pipette and the tubes were kept in inverted position for 10 min to drain the oil before reweighing. The gain in mass was expressed as grams of oil absorbed per gram of flour.

2.3.9 Wheat grain hardness

The wheat hardness was determined as described by Manley (1995).The ground grain samples were obtained by passing the whole wheat grain (15.5 % moisture content) through a Model 3100 hammer mill (Falling Number AB, Huddinge, Sweden) equipped with 1mm screen. The wheat hardness was calculated as the % of ground wheat grain (10 g) forcing through 75 micrometer air jet sieve in 90 s. The mass less than 4.0 g indicated a hard wheat, while 4.0 g or more indicated a soft wheat.

2.3.10 Extraction yield of whole wheat flour

The flour extraction yield was determined as a percentage of all bran and germ mixed with the flour from the mass of grains milled (AACC, 2000):

$$\%\ flour\ yield = \frac{Mass\ of\ whole\ wheat\ flour\ collected}{Mass\ of\ wheat\ milled}\ X\ 100$$

2.3.11 Farinograph test of whole wheat flour

The farinograph parameters were determined using CW Brabender Instruments, inc., Germany following the AACC method (AACC, 2000). The flour sample was placed into a mixing bowl and distilled water was added up to the optimum dough consistency (500 BU). The water absorption, dough development time, dough stability time and mixing tolerance index during mixing were evaluated from the farinograph.

2.3.12 Color of whole wheat flour

The color was determined by a color reader (CR-10, Konica Minolta,inc. Japan) on the basis of L*, a* and b* values.

2.4 STATISTICAL ANALYSIS

Data in triplicate were subjected to one-way analysis of variance (ANOVA) using SAS System for Windows (version 9.3, SAS Institute, Cary, NC). Treatment means were separated using the Tukey post hoc test and least significant difference was accepted at $p \leq 0.05$.

3 RESULTS AND DISCUSSION

3.1 EFFECT OF WHEAT VARIETY ON GRAIN HARDNESS, EXTRACTION YIELD AND ON THE PROXIMATE COMPOSITION OF WHOLE WHEAT FLOURS

The extraction yield was between 96.20 % and

Table 1: Effect of wheat variety on grain hardness, extraction yield and on the proximate composition of whole wheat flours

Wheat variety grains	Grain hardness (g)	Extraction yield (%)*	Moisture content (%)	Total protein content (% dmb)	Ash content (% dmb)	Total fat content (% dmb)	Total carbohydrate content (% dmb)	Total dietary fiber content (% dmb)
Gihundo	$3.03^{a} \pm 0.10$	$99.20^{c} \pm 0.12$	$11.73^{b} \pm 0.25$	$10.85^{a} \pm 0.15$	$1.47^{c} \pm 0.31$	$1.61^{c} \pm 1.11$	$74.34^{a} \pm 1.19$	$15.97^{c} \pm 0.99$
Kibatsi	$3.36^{b} \pm 0.01$	$97.70^{b\pm} 0.04$	$11.56^{a} \pm 0.02$	$10.45^{a} \pm 1.03$	$1.31^{b} \pm 0.98$	$1.76^{d} \pm 0.23$	$74.92^{b} \pm 1.56$	$14.97^{b} \pm 0.22$
Nyaruka	$3.86^{c} \pm 0.02$	$96.20^{a} \pm 0.10$	$12.07^{c} \pm 0.10$	$10.00^{a} \pm 0.61$	$1.38^{b} \pm 0.93$	$1.46^{b} \pm 0.01$	$75.09^{c} \pm 0.99$	$15.01^{bc} \pm 0.11$
Reberaho	$3.82^{c} \pm 0.21$	$96.50^{a} \pm 0.13$	$12.09^{c} \pm 0.01$	$10.30^{a} \pm 0.95$	$0.98^{a} \pm 0.22$	$1.38^{a} \pm 2.09$	$75.28^{d} \pm 0.19$	$12.44^{a} \pm 0.04$

Values are means ±SD of 3 replications. Treatment means followed by different letters in the same column are significantly different at $p \leq 0.05$. dmb: Dry mass basis. *Percentage of all bran and germ mixed with the flour from the mass of grains milled.

99.20 % and grain hardness was from 3.03 g to 3.86 g. The results for extraction yield were in the range with those reported by Cauvain (2015). As the product was the whole wheat flour, the wheat milling extraction was supposed to be 100 %. However, the flour yield was not 100 % due to that some quantities of the flour were held, disappeared in parts of the mill and were not collected into the vessel. During brushing the flour off the parts of the hammer mill, some few quantities of it were also transported/conveyed by air. The highest flour yield of Gihundo variety may be due to the low grain hardness (Table 1), producing a lower quantity of damaged starch and tailings during the milling process. The extraction yields of Nyaruka and Reberaho wheat varieties were not significantly different ($p > 0.05$) and were the lowest. The level of extraction yield indicated the particle size of the wheat flour. This means that when the yield was high, the particle size of the flour was small. The results showed that all wheat grains were possibly hard as their results were below 4 g or 40 % (Manley ,1995).

The results for total protein content were between 10.00 % and 10.85 %. There was no significant difference in protein content ($p > 0.05$) among whole wheat flours from the four wheat varieties, may be because they were grown at the same location and under the same agro ecological conditions. Wheat flours from all varieties could possibly fall under all purpose flour category as their protein contents were not below 9 % or above 12 % (Chu, 2004). Moisture content ranged from 11.56 % to 12.09 %. The moisture contents of the wheat flours from Nyaruka and Reberaho varieties were not significantly different ($p > 0.05$) and were higher than those from the other two wheat varieties. Increase in moisture content could have been associated with increase in fibre (Maneju and Udobi, 2011). Excess moisture content above 12 % may be detrimental as it can lead to mould growth, toxin formation, insect infestation, sprouting in storage (Ezeama , 2007) and has the effect of reducing the protein content as a percentage of total mass. On the other hand, grain with excessively low moisture would result in a hard grain with low flour yield (Mishra, 2016).

The content for ash was between 0.98 % and 1.47 % and the content for total dietary fiber was from 12.44 % to 15.97 %. The ash content results were in line with 0.42 % found by Khalid (2017) and from 1.46 to 1.56 % reported by Mishra (2016). Khalid et al. (2017) worked on the proximate composition of the native and irradiated whole wheat flour from wheat grains var. WH-1021, while Mishra (2016) compared the proximate composition of whole wheat flours and wheat flours made from five wheat cultivars (Advance, Prevail, Select, Brick and Forefront). The results for dietary fiber levels were similar to those found by Bressiani et al. (2016), where they were between 12.45 % and 15.95 % in the study done on the effect of particle size on the damaged starch content, proximate composition, gluten content, phenolic content and free sulfhydryl groups of fine whole wheat flour, medium whole wheat flour and coarse whole wheat flour from BRS Guabiju, wheat variety. There was a significant difference ($p \leq 0.05$) of the ash and dietary fiber contents among whole wheat flours from all wheat varieties. The ash content may have differed due to mineral content which varied according to wheat cultivar (Bressiani et al.,2016) and the amount of minerals in flour which increases with extraction rate (Scade, 1975). The extraction yield may have influenced the levels of ash and dietary fiber of whole wheat flour because generally when extraction yield is high, the ash and dietary fiber contents are high as well. It is explained by the fact that the bran portion which is the main source of ash and dietary fiber could be highly concentrated in flour when there was a high extraction yield. Therefore, it could be the reason why the whole wheat flour from Gihundo variety had the highest ash and dietary fiber contents, while the whole wheat flour from Reberaho variety had the lowest ash and dietary fiber contents. The high ash content of the flour indicates that whole grain flour could be an important source of minerals. Increased dietary fibre content of the flour has several health benefits; it aids in the diges-

tion of the bread in the colon and reduces constipation often associated with bread produced from white wheat flour (Jideani, 2009). Dietary fibre plays a significant role in the prevention of several diseases such as cardiovascular diseases, diverticulosis, constipation, irritable colon, cancer and diabetes (Slavin, 2005).

The minimum and maximum total fat contents were 1.38 % and 1.76 %, respectively. Fat content differed significantly ($p \leq 0.05$) from one type of whole wheat flour to another one. Flour from Kibatsi had the highest fat content, while flour from Reberaho wheat variety had the lowest fat content. Wheat grains with a high amount of germ could have given whole wheat flours with a high amount of fat (Mishra, 2016). Fat plays a significant role in the shelf life of food products and as such relatively high fat content could be undesirable in food products. This is because fat can promote rancidity in foods, leading to development of unpleasant and odorous compounds. Bread loaf volume can increase with free polar fat contents that are naturally present in a particular cultivar, mostly when utilized samples are from pure wheat breeding lines (Chung et al.,1982). In the study of investigating the relationships between textural qualities of noodles and flour lipids, Qiyu and Siyuan (2009) found that as the free lipid content of the flour was increased, hardness of noodles linearly increased, reaching a maximum at a level of 1.84 g 100 g^{-1} flour, thereafter falling to a low value. The same author reported that cohesiveness of noodles was significantly ($p \leq 0.05$) decreased due to removal of free lipid while the highest cohesiveness value was obtained at a free lipid content of 1.24 g 100 g^{-1} flour. A higher free lipid content in flour would reduce cohesiveness of noodles. According to these findings, whole wheat breads made from the analysed wheat varieties would be harder and less cohesive.

Total carbohydrate content ranged between 74.34 % and 75.28 %. There was a significant difference ($p \leq 0.05$) in carbohydrate contents among whole wheat flours from all wheat varieties, where whole wheat flours from Reberaho and Gihundo varieties had the highest and the lowest carbohydrate contents, respectively. B-vitamins and minerals from the wheat bran and germ of the whole wheat breads avail carbohydrate for proper assimilation in humans (Connection, 2017). Whole wheat flour from Reberaho wheat variety could be a good source of metabolisable energy and could assist also in fat metabolism because of its high carbohydrate content (Ifie, 2011).

3.2 EFFECT OF WHEAT VARIETY ON THE PHYSICAL PROPERTIES OF WHOLE WHEAT FLOURS

The range for oil absorption capacity was from 1.24 g g^{-1} to 1.45 g g^{-1}. These results were in line with the ones obtained by Khalid et al. (2017). Oil absorption capacity changed with the types of whole wheat flour, where Gihundo and Nyaruka varieties gave whole wheat flours with the highest and the lowest oil absorption capacity, respectively. The high amount of dietary fiber in Gihundo wheat flour (Table 1) might have been responsible for the high oil absorption capacity of the flour (Chou and Huang, 2003). The high oil absorption capacity makes the flour suitable in facilitating enhancement in flavor and mouthfeel when used in food preparations (Kaushal and and Kumar, 2012).

The results for bulk density of the whole wheat flours were between 0.32 g ml^{-1} and 0.56 g ml^{-1}. These results were near to those found by Offia et al. (2015). The bulk density of whole wheat flours from Kibatsi, Nyaruka and Reberaho did not differ significantly ($p > 0.05$). Flour from Gihundo wheat variety could be dense in comparison to flours from other wheat varieties, and it meant that Gihundo grain endosperm could be filled out better than the endosperm from the other variety grains. The high bulk density of flours suggests their suitability such as thickener in food products and for use in food preparations since it helps to reduce paste thickness which is an important factor in convalescent and child feeding (Kaushal and Kumar, 2012). In contrast, low bulk density would be an advantage in the formulation of complementary foods (Akpata and Akubor, 1999).

The results for least gelation concentration were in the range of 40.36 % and 70.23 %. Whole wheat flours

Table 2: Effect of wheat variety on the physical properties of whole wheat flours

Wheat variety grains	Oil absorption capacity (g g^{-1})	Bulk density (g ml^{-1})	Least gelation concentration (%)
Gihundo	1.45^{d}± 0.01	0.56^{b}± 0.13	70.23^{d}± 0.03
Kibatsi	1.31^{c}± 0.10	0.35^{a}± 0.09	56.23^{c}± 0.02
Nyaruka	1.24^{a}± 0.12	0.34^{a}± 0.01	40.36^{a}± 0.15
Reberaho	1.28^{b}± 0.01	0.32^{a}± 0.02	47.42^{b}± 0.21

Values are means ± SD of 3 replications. Treatment means followed by different letters in the same column are significantly different at $p \leq 0.05$.

from Gihundo and Nyaruka varieties were the highest and the lowest in least gelation concentration, respectively. The lower the least gelation concentration, the better is the gelating ability of the protein ingredient and the swelling ability of the flour is enhanced (Kaushal and Kumar, 2012). The low least gelation concentration of wheat flour may be an asset as an additive to other gel forming materials in food products.

3.3 EFFECT OF WHEAT VARIETY ON THE FARINOGRAPH TEST PROPERTIES OF WHOLE WHEAT FLOURS

The range for water absorption capacity was between 80 % and 89 %. The range for those results included 0.85 g g^{-1} (85 %) found by Khalid et al. (2017) for wheat grains var. WH-1021 in the study on physico-chemical properties of native and irradiated whole wheat flour. Gihundo variety followed by Kibatsi variety gave whole wheat flours with high values of water absorption capacity in comparison to Nyaruka and Reberaho varieties. The last two were not significantly different ($p > 0.05$) in water absorption capacity. Maghirang et al. (2006) reported that an increase or decrease in water absorption capacity may be explained by differences in flour extraction yield and presumably higher starch damage content as well, after finding that some wheat varieties with differences in grain protein content had similar water absorption requirement. Similarly, to this report, the total protein contents of whole wheat flours from all wheat varieties (Table 1) were not significantly different ($p \leq 0.05$), but the flours with high extraction yield (Table 1) showed high water absorption capacity. Sluimer (2005) also showed that water absorption increased with increasing extraction rate. It could be the reason why the high extraction yield (Table 1) impacted the whole wheat flours from Gihundo and Kibatsi varieties to require a high amount of water to center the farinograph curve on the 500-Brabender Unit (BU) line compared to flours from other remaining wheat varieties. The small particle size of bran and high amount of fiber in the flour might have been responsible for high water absorption capacity as reported by Chou and Huang (2003). Water absorption is a key parameter in the purchase of flour for breadmaking (Webb and Owens, 2003).The high water absorption capacity of the flours may assure product cohesiveness (Houson and Ayenor, 2002).

The time for dough development was between 6.33 min and 7.62 min and for stability, it was between 10.72 min and 11.65 min. Gihundo variety followed by Kibatsi variety produced whole wheat flours with long development time in comparison to Nyaruka and Reberaho varieties. The last two were not significantly different ($p > 0.05$) in development time. As the dough development time is the time taken from when water is added up to when the dough reaches maximum consistency, the results indicated that whole wheat flours from Nyaruka and Reberaho varieties took short time for optimum mixing compared to flours from Gihundo and Kibatsi varieties. The stability time shows the maximum consistency for a dough and is a good indication of its strength. Flours from Nyaruka and Gihundo varieties took the longest and the shortest time, respectively, for dough stability. Thus, the dough obtained from Nyaruka wheat variety lasted long with high consistency, while similar consistency for the dough from Gihundo wheat variety lasted a short time. The results for dough development time (DDT) and dough stability time ranged in the results found by Bressiani et al. (2016). In the time evaluation of the effect of particle size on mixture properties, extensional properties and paste properties of refined flour and whole grain wheat flour from BRS Guabiju, wheat variety, those authors reported between 7.60 min and 12.93 min for DDT, between 11.6 min and 12.8 min for dough stability for fine whole grain wheat flour, medium whole grain wheat flour and coarse whole grain wheat flour. For the flours which showed low dough development and stability time, they could have been affected by weakened gluten network with fiber-rich bran particles (Bae et al., 2014).

Table 3: Effect of wheat variety on the farinograph test properties of whole wheat flours

Wheat variety	Water absorption capacity (%)	Dough development time (min)	Dough stability time (min)	Mixing tolerance index (BU)
Gihundo	89.00^{c}± 0.09	7.62^{c}± 0.07	10.72^{a}± 0.03	22.88^{d}± 0.11
Kibatsi	85.00^{b}± 0.03	6.73^{b}± 0.12	12.09^{c}± 0.02	24.73^{c}± 0.01
Nyaruka	80.00^{a}± 0.10	6.33^{a}± 0.01	13.12^{d}± 0.10	20.17^{a}± 0.25
Reberaho	81.00^{a}± 0.15	6.79^{a}± 0.04	11.65^{b}± 0.12	21.02^{b}± 0.02

Values are means ± SD of 3 replications. Treatment means followed by different letters in the same column are significantly different at $p \leq 0.05$.

Mixing tolerance index (MTI) was from 20.17 BU to 22.88 BU. Mixing tolerance index highly differed ($p \leq 0.05$) in whole wheat flours obtained from all wheat varieties. Whole wheat flour from Gihundo and Kibatsi varieties showed the highest and the lowest values of mixing tolerance index, respectively. This indicated that dough from Gihundo variety whole wheat flour was hard and dough from Kibatsi variety whole wheat was soft during mixing. The hardness of dough mixing could contribute to firm product texture. Mishra (2016) who compared the farinograph parameters among ground whole wheat flours and flour incorporated with treated bran, found the results in same range, where MTI was between 18.13 BU for ground whole wheat flours from five wheat cultivars (Advance, Prevail, Select, Brick and Forefront).

3.4 EFFECT OF WHEAT VARIETY ON THE COLOR OF WHOLE WHEAT FLOURS

The color ranged between 80.33 and 84.67, 1.4 and 2.30 and 10.14 and 10.67 for L*, a* and b* values, respectively. Gihundo variety followed by Kibatsi variety produced whole wheat flours with high L*values in comparison to Nyaruka and Reberaho varieties. The last two were not significantly different ($p > 0.05$) in L* values. The results indicated that whole wheat flour from Gihundo variety was brighter than the remaining flours. The whole wheat grain with high flour extraction yield produces a fine size flour containing high amounts of bran and germ, which later can make the flour to be less bright (Maghirang et al., 2006). This assumption was not the case for whole wheat flours from Gihundo and Kibatsi varieties which demonstrated the highest yield extraction (Table 1). Thereby, the flour brightness was considered to mainly be caused by the wheat cultivar (Maghirang et al., 2006). Redness (a*) and yellowness (b*) values were not significantly different in whole wheat flours from all wheat varieties ($p > 0.05$). Anthocyanins and carotenoids, located mainly in the germ and bran, may have contributed to the colors of the whole wheat flour (Schwinn, 2004; Rao and Rao, 2007). Anthocyanins are phenolic compounds (flavonoids), responsible for most blue to blue-black, and red to purple colors of diverse plant organs (Schwinn, 2004). Meanwhile, carotenoids are responsible for the yellow, orange and red colors in various plants (Rao and Rao, 2007). The beneficial effect of wheat bran against colon cancer is attributed to the presence of a high concentration of polyphenolic compounds, which are likely released into the colon as a result of bacterial fermentation (Monica and Whole Grain Connection, 2017). The obtained L*, a* and b*values were very close to those found by Mishra (2016). The author obtained 82.08, 1.71 and 10.64 for L*, a* and b* average values, respectively, for whole grain flour from five hard red spring wheat cultivars.

4 CONCLUSION

The results showed that the wheat varieties affected the extraction yield and other qualities of their whole wheat grain flours. The proximate composition was significantly different among whole wheat flours from the wheat grain varieties, except protein content. Gihundo and Nyaruka wheat grain varieties have the highest and the lowest extraction yields, respectively. The farinograph test parameters of the doughs were impacted by wheat varieties. The whole wheat flour brightness was considered to mainly be caused by the wheat cultivar. Beyond the quantity and economic reasons that most of bakeries qualify wheat grains by their extraction yields, it is also important to consider other qualities such farinograph (dough) test characteristics, color and proximate composition to select grains or flour from these wheat varieties newly introduced in Rwanda based on the individual cultivar because their derivative products could have a more desired quality for competitive markets. For their nutritive value and health benefits, whole wheat flours have been gaining an increase in demand and utilization in different food products for human consumption worldwide.

Table 4: Effect of wheat variety on the color of whole wheat flours

Wheat variety grains	Extraction yield (%)	L*	a*	b*
Gihundo	99.20^{c}±1.40	84.67^{c}±1.04	1.50^{a}±3.01	10.67^{a}± 3.07
Kibatsi	97.70^{b}±0.22	82.67^{b}± 2.08	1.90^{ab}±0.27	10.23^{a}± 0.54
Nyaruka	96.20^{a}±1.01	80.33^{a}±1.04	2.10^{b}± 1.04	10.47^{a}±1.50
Reberaho	96.50^{a}±1.82	80.52^{a}± 3.03	2.30^{b}±0.58	10.14^{a}±1.04

Values are means ± SD of 3 replications. Treatment means followed by different letters in the same column are significantly different at $p \leq 0.05$. % Percentage of all bran and germ mixed with the flour from the weight of grains milled.

5 ACKNOWLEDGEMENT

Authors sincerely thank Borlaug Higher Education for Agricultural Research and Development (BHEARD) program for its financial support to the present research work.

Authors also thank Rwanda Agriculture and Animal Resources Development Board (RAB), Musanze station, Musanze district, Rwanda to provide the wheat grain samples.

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doi:10.14720/aas.2021.117.1.1297 Original research article / izvirni znanstveni članek

Planting date effects on maize (*Zea mays* L.) growth and development in the rainforest of southwestern Nigeria

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Received October 05, 2019; accepted January 18, 2021.
Delo je prispelo 05. oktobra 2019, sprejeto 18. januarja 2021.

Planting date effects on maize (*Zea mays* L.) growth and development in the rainforest of southwestern Nigeria

Abstract: This study was conducted to determine the optimum planting date for maize (*Zea mays* L.) to cope with the negative impacts of climate change in the marginal rainforest agro-ecology as typified at Ile-Ife, SW Nigeria. Five maize varieties were planted weekly, in 3-replicate randomized complete block design experiments at the Obafemi Awolowo University Teaching and Research Farm, throughout the 2016 and 2017 cropping seasons. The varieties were monitored for seedling and adult plant traits including grain yield with its components. Statistical analysis showed significant effect of planting dates (DOP) on all traits. The first few DOPs in March and April had the highest grain yield which reduced with delayed planting till June and increased again mid July/August before finally dropping off thereafter. The higher yield in the earlier dates each year, was due to early flowering and taller plants with higher ear placement. Planting after the first few rains in March/April was the optimum for the first cropping season, and late July to mid-August was best for the second cropping season in this agroclimatic zone. Planting beyond these periods results in poor grain yield and pre-disposes the crop to terminal drought, which could result in complete crop failure.

Key words: agronomy; climate change impact and adaptation; drought; *Zea mays* L.

Vplivi datuma setve na rast in razvoj koruze (*Zea mays* L.) v območjih deževnega gozda jugozahodne Nigerije

Izvleček: Raziskava je je bila izvedena za določitev optimalnih datumov setve koruze (*Zea mays* L.) za spoprijemanje z negativnimi posledicami podnebnih sprememb na mejnih območjih deževnega gozda v Ile-Ife, jugozahodna Nigerija. Pet sort koruze je bilo posejanih tedensko v popolnem naključnem bločnem poskusu s tremi ponovitvami na raziskovalnem posetvu Obafemi Awolowo University Teaching, v rastnih sezonah 2016 in 2017. Pri vseh sortah so bile spremljane lastnosti rastlin od sejank do odraslih rastlin, vključno s pridelkom in njegovimi komponentami. Statistična analiza je pokazala značilen vpliv datuma setve (DOP) pri vseh lastnostih. Prvi datumi setve v marcu in aprilu so imeli največji pridelek zrnja, ki se je nato zmanjševal do setve v juniju in spet povečeval do setve v sredini julija in avgusta, nakar je končno upadel. Večji pridelek pri zgodnejših datumih setve je bil vsako leto povezan z zgodnejšim cvetenjem, višjimi rastlinami in z višjim položajem storžev. Setev po prvih deževjih v marcu in aprilu je bila optimalna za prvi del rastne sezone, setev koncem julija do sredine avgusta pa je bila najboljša za drugi del rastne sezone v teh agroekoloških razmerah. Setve izven teh obdobij do dale majhen pridelek zrnja zaradi izpostavljenosti suši, kar bi lahko vodilo k popolni izgubi letine.

Ključne besede: agronomija; vpliv podnebnih sprememb in prilagoditve; suša; *Zea mays* L.

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1 INTRODUCTION

Maize (*Zea mays* L.) is a popular crop in sub-Saharan Africa (SSA) and its popularity in the region has continued to grow because it provides a cheap source of calories for the relatively poor population, who process it to different food forms in addition to consuming it boiled or roasted. A good number of industries, including the livestock industries also depend on maize for the production of feed and other products as a result of which the demand for the grains remains high (Talabi et al., 2017). Unfortunately, in Nigeria today, there is a considerable reduction in the area of land available for agricultural production due to increased urbanization. There is also continous decline in soil fertility, resulting from persistent cultivation of a piece of land over several or many years. Furthermore, there is the impact of climate change which shows as poor condtions of climate, including drought, and a reduction in the effective growing season due to delayed onset of rainfall followed by early rainfall recession (Fakorede and Akinyemiju, 2003). These are key factors limiting maize production from keeping up with the high demands for its grains.

In Nigeria as in many other countries of the world, maize has received relatively large research attention from agronomists, plant breeders and other crop scientists over the years. Genetic improvement has been made on many important aspects of the crop, including the development of high yielding, disease resistant, drought tolerant and nutritionally fortified varieties (Badu-Apraku and Fakorede, 2017). However, yield could be further enhanced with improved cultural practices, including optimum planting dates. Studies have shown that planting maize outside its optimum dates of planting results in reduced yield (Fakorede, 1985). Investigations had started since the late 1970s at the Obafemi Awolowo University (formerly University of Ife), Ile-Ife to determine the optimum planting dates for maize in the marginal rainforest agro-ecology of Ile-Ife in Southwestern Nigeria. Fakorede (1985) found at Ile-Ife that yield decreased by 30, 38 and 34 kg ha^{-1} for each day by which sowing was delayed after the first planting in March of 1978, 1980 and 1981 respectively. He suggested early planting of maize with the first few rains in the year to ensure optimum yield. Oluwaranti et al. (2008) evaluated the yield performance of different maturity groups of maize varieties at different planting dates (DOP) at the same location and found that grain yield and yield components decreased with delayed planting in the late season. Planting date studies in the savanna regions of Nigeria have shown similar results. In the Sudan savanna zone, for example, Jibrin et al. (2012) found late June to early July as the optimum planting window for maize; grain yield decreases drastically with delayed planting beyond this period.

Rainfall is the primary climatic factor that determines the timing of most agronomic practices, especially planting operations in Nigeria. False start of rainfall and unpredictable frequent occurrence of short duration drought during the growing season, both of which have become more severe over time, are now features of the rainforest agro-climatic zone of SW Nigeria typified by Ile-Ife, a location at 7° 28' N, 4° 33' E and 244 m asl in the zone. Such weather anomalies have been attributed to climate change (Fakorede and Akinyemiju, 2003). Reduced seedling emergence and low vigor, culminating in missing stands, are usually associated with the weather anomalies that characterize early planted maize in the zone. Despite these negative impacts, early planted maize under research as well as farmer practices produce higher yields than plantings delayed till when the environments are seemingly more favorable. Based on this observation, Fakorede (1985) hypothesized that cultivars for early planting would be more productive if they emerged rapidly, with high seedling and vegetative vigor under relatively hot, dry conditions. Furthermore, most of the DOP studies thus far in Nigeria involved open-pollinated varieties (OPVs), although Fakorede's (1985) study included some varietal hybrids, a type of hybrid that has never been commercialized in Nigeria. The varieties were also not screened for drought and or heat tolerance. However, drought and or heat tolerant OPVs belonging to different maturity groups (extra early, early, intermediate, late), as well as conventional types of hybrid (single-cross, double-cross, 3-way cross hybrids) also of different maturity groups, are now available and are cultivated by farmers in Nigeria. In addition, most DOP studies conducted so far in the rainforest agroclimatic zone of Nigeria involved relatively few dates within each planting season. The study by Oluwaranti et al. (2008) which was conducted during a 4-year period, showed contrasting trends in the early season, a clear indication of the need for more investigations. Studies conducted in advanced countries, such as that by Jong et al. (1982) inter alia suggested that the larger the sampling size of the environment, the more reliable the results obtained for purposes of prediction and recommendation of agronomic practices.

The primary objective of the present study, therefore, was to further investigate the trends in growth, grain yield and yield components of maize planted at weekly interval, starting with the first few rains in March till the recession of rainfall in November for a 2-year period. A secondary objective was to investigate the presence of genotype x DOP interaction with the goal of recommending varietal types adapted to specific planting periods in the agroclimatic zone.

2 MATERIALS AND METHODS

The study was carried out at the Teaching and Research Farm of Obafemi Awolowo University, Ile-Ife (OAU T&R Farm) in years 2016 and 2017. In each experiment, five maize varieties (four OPVs and one single-cross hybrid), well adapted to the tropical rainforest environments, were planted in 3-replicate randomized complete block design. The OPVs included 'White DT STR SYN1 – TZL Comp. 1–W', 'TZL Comp. 4 DT F_2,' 'TZL Comp. 1 C6/DT – SYN – 1 – W' all of which were drought tolerant (DT) and of intermediate/late maturity, 'ACR 94 TZE Comp 5 C_3' (early maturing), and 'Oba Super 1', an intermediate-late single-cross hybrid obtained from Premier Seeds, Zaria. The four OPVs were obtained from the IITA Maize Improvement Program, and all five varieties are white-grained, high yielding and have been released for commercial production in Nigeria and several other West and Central African (WCA) countries.

The experiments were planted weekly from March to November each year. Each planting date (DOP) represented the individual environment. However, there were some weeks when planting could not be done due to some logistic problems, but 56 plantings (28 each year) were effected, out of which 54 (data unavailable to calculate emergence parameters for the first DOP in March each year due to rainfall anomalies) were analysed for seedling emergence and vigour. Only 42 DOPs (20 in 2016 and 22 in 2017) attained maturity and were analyzed for flowering, plant and ear heights, and grain yield. Each plot contained six or four rows which were 5 m long and 0.75 m apart; within row spacing was 0.5 m resulting in plot size of about 15 m^2 and 22.5 m^2 for the four and six-row plots. Prior to planting, the experimental land was ploughed and harrowed and seeds were treated with Apron* which contains thiamethoxam, mefenoxam (metalaxyl-M) and difenoconazole, to control damage by soil-borne diseases and insect pests. Three seeds were planted per hill and thinning was done at 9 days after planting (DAP) to two plants per stand giving an estimated plant population density of 53, 333 plants ha^{-1}. Fertilizer was applied immediately after thinning at the rate of 60 kg ha^{-1} each for N, P_2O_5 and K_2O. Primextra, which contains atrazine (2-chloro-4- (ethyl amino)-6-isopropylamino-s-triazine) and alachlor (N-(methyl-2-methoxy-ethyl)-2-ethyl-8-methyl-chloroacetanilide) as active ingredients was applied as post-planting, maize pre-emergence herbicide at the rate of 5 l ha^{-1}. Further weed control was done using paraquat (N,N′-dimethyl-4,4′-bipyridinium dichloride), carefully applied as a post-emergence, non-selective and contact herbicide at the rate of 3.0 l ha^{-1}.

Emergence counts were made daily from five to nine days after planting (DAP); from which emergence percentage (*E %*) and emergence index (*EI*) were computed as follows (Fakorede and Agbana (1983):

$$E\,\% = \frac{Seedlings\ emerged\ in\ X\ DAP}{Total\ no.\ of\ seeds\ sown} * 100$$

$$EI = \frac{\Sigma(Plants\ emerged\ in\ a\ day)*(DAP)}{Plants\ emerged\ 9\ DAP}$$

The dates of the appearance of the first flower (tassel) and number of days to 50 % tasselling, anthesis (pollen shed) and incipient silk extrusion were recorded for each plot in both years. Plant and ear heights (PHT and EHT) were obtained by measuring the distance from the soil surface to the first branch at the base of the tassel, and the node bearing the top ear for 10 random plants in each plot and their means taken as PHT and EHT per plot, respectively. Data were collected on grain yield and yield components (ear number and mass, ear length and kernel moisture content). The grain yield data were adjusted to 15 % moisture content. Variance analysis was done on all data using PROC GLM of Statistical Analysis System (SAS, 2000). The linear additive model for the ANOVA was: $Y_{ijk} = \mu + \alpha_i + \beta_{j(i)} + \Sigma_k + \alpha\Sigma_{(ik)} + \Sigma_{ijk}$, in which Y_{ijk} is the observed measurement of the k^{th} genotype grown in the j^{th} rep under the i^{th} environment; μ is the grand mean; α_i is the main effect of the i^{th} environment, i = 1,2,….., 42 or 54; $\beta_{j(i)}$ is the effect of the j^{th} replication nested within the i^{th} environment, j = 1,2,3; Σ_k is the effect of the k^{th} genotype, k = 1,2,….5; α $\Sigma_{(ik)}$ is the first order interaction of the i^{th} environment with the k^{th} genotype, and Σ_{ijk} is the random error (residual) term. Furthermore, correlation and regression analyses were done for all data to determine the trends in response of maize to planting dates. The measured traits (emergence, flowering, PHT, EHT and yield) were regressed on DOP in linear model: $Y = a + bX$ and polynomial model: $Y = a + b1X^1 + ...bnX^n$, *Y* and X are the measured traits and DOP, respectively; a and b are the intercept and slope/coefficient of the regression, respectively; n is the order of the polynomial (quadratic, cubic, quartic). Pearson correlation analysis was done where the coefficient.

$$\mathbf{r} = \frac{\Sigma\,(\boldsymbol{X} - \bar{\mathbf{x}})\,(\mathbf{Y} - \bar{\mathbf{Y}})}{\sqrt{[\Sigma(\boldsymbol{X} - \bar{\mathbf{x}})\mathbf{2} * \Sigma(\boldsymbol{Y} - \bar{\mathbf{Y}})\mathbf{2}]}}.$$

3 RESULTS

Results from the variance analysis revealed highly significant DOP effect for all traits (Table 1). Emergence, adult plant traits and grain yield along with its components were highly significant ($p \leq 0.01$) for DOP and other sources of variation.Fairly high emergence for the

Table 1: Mean squares from the ANOVA of seedling vigour traits, yield and other adult plant traits of five maize varieties evaluated in 54 or 42 DOP at the Obafemi Awolowo University Teaching and Research Farm, Ile-Ife in 2016 and 2017 cropping seasons.

				Adult plant traits								Other yield components			
Source	DF	E% @ 9 DAP	EI (days)	DF	Silking	ASI	PHT	EHT	Yield (t/ha)	DF	EN	DF	EL	ED	KRN
DOP† (D)	53	3256.58**	3.55**	41	234.38**	47.18**	5105.87**	2083.048**	7.61**	39	411.35**	40	23.08**	50.79**	3.26**
Rep within D	108	268.92**	0.57**	84	15.64**	5.70**	379.56**	171.189**	0.49**	80	32.52**	82	1.98	3.65	0.74
Variety (V)	4	14332.64**	7.33**	4	878.69**	83.09**	6672.62**	1853.702**	4.76**	4	572.83**	4	23.42**	4.31	10.04**
D x V	212	607.57**	0.37**	164	6.67*	3.36**	227.88	142.98**	0.45**	156	65.61**	160	2.65**	2.39	0.77
Error	433	69.59	0.21	336	5.01	2.22	187.99	91.20	0.22	320	21.47	328	1.53	2.82	0.64
Total	809	516.49	0.56	629	27.37	6.43	585.77	256.423	0.83	599	63.51	614	3.43	5.96	0.92
CV,%		11.97	6.90		3.42	32.45	9.3	16.11	30.67		23.06		10.08	36.43	6.01

*,** F - statistically significant at 5 % and 1 % level of probability, respectively.
† - DOP = date of planting, E% @ 9 DAP = emergence percent at 9 DAP, EI = emergence index in days, silking is in days, ASI = anthesis-silking interval in days, PHT and EHT are plant and ear heights in cm, EN = number of ears per plot, EL = ear length (cm),
ED = ear diameter (cm), KRN = number of kernel rows per ear.

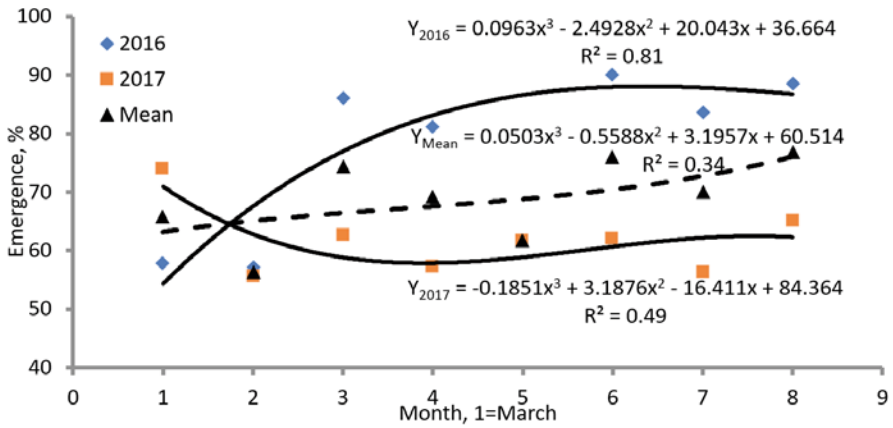

Figure 1: Mean values by months for emergence percentage (%) at 9 DAP of five maize varieties evaluated in 54 environments at the OAU T&R Farm in 2016 and 2017 seasons.

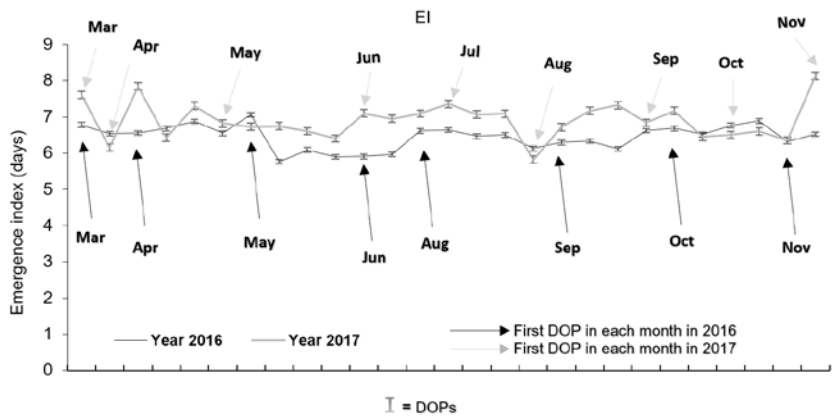

Figure 2: Mean values for the emergence index (in days) of five maize varieties evaluated in 54 environments at the OAU T&R Farm in 2016 and 2017 seasons.

first DOPs in March each year was followed by a period of low emergence in late March/April (Figure 1). Thereafter emergence increased steadily on the average from May and was fairly uniform mid-season when rainfall is expected to be high, until around October when it decreased steadily, even though the R^2 value (34 %) was quite low despite the high R^2 value (81 %) observed in 2016. Emergence was generally higher and faster in 2016 than 2017 with an opposite trend between the years for EI (Figures 1 and 2).

There was really no definite pattern for silking with respect to DOP over the two years, except for the earliness in silking observed in the first couple of environments in 2016. Similar trends occurred for tasseling and anthesis. The interval between anthesis and silking, ASI, showed a similar trend to the other flowering traits, but for a wider margin where differences occurred between the two years (Figure 3).

Plant and ear heights generally showed a trend where they appeared to be fairly uniform mid-seasons, although planting very early or at the latter part of the season produced contrasting results. However, plants were taller and ears placed higher in most dates in 2016 (Figure 4).

Grain yield was highest for the first few DOPs in March and April (Figures 5 and 6) even though other environments planted later in March and April each year had some of the poorest yield performances compared to the other months – which, in turn, showed no definite trends with DOPs in 2016 scoring higher yield on the average (1.65 ± 0.78 t ha^{-1}) than DOPs in 2017 (1.39 ± 0.68 t ha^{-1}). Combined analysis of both years confirmed higher average yield in March and a steady decline in

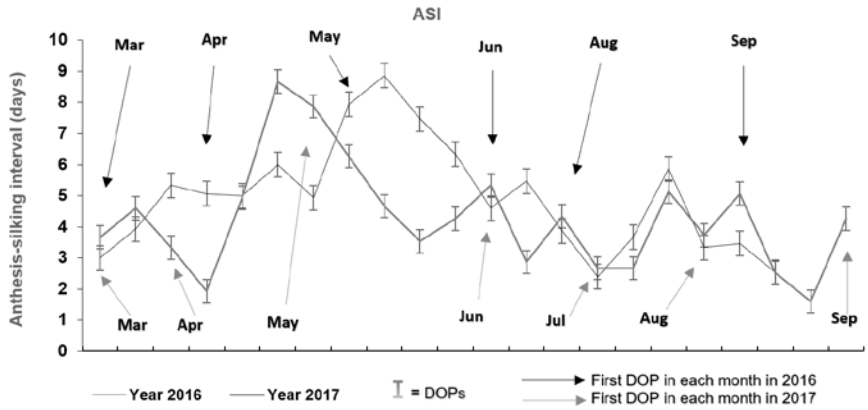

Figure 3: Mean values for the days from anthesis to silking of five maize varieties evaluated in 42 environments at the OAU T&R Farm in 2016 and 2017 seasons.

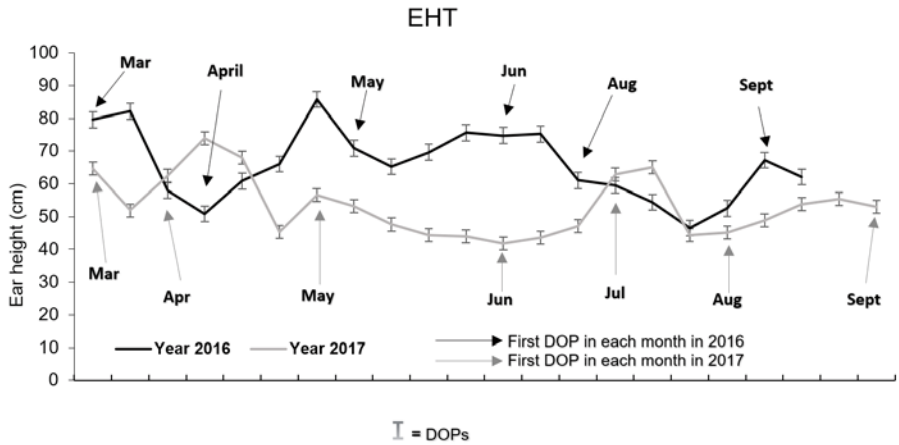

Figure 4: Mean values for ear height (cm) of five maize varieties evaluated in 42 environments at the OAU T & R Farm in 2016 and 2017 seasons.

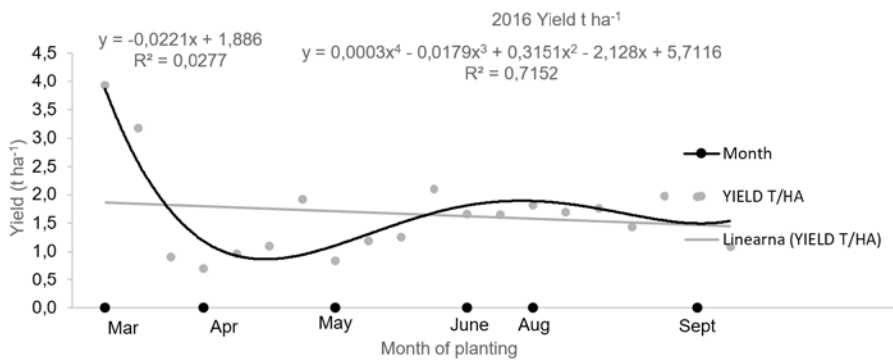

Figure 5: Mean grain yield (t ha^{-1}) of five maize varieties evaluated over 20 DOPs at the OAU T&R Farm in 2016 cropping season.

subsequent months up until July when yield picked up declining again in September plantings (Figure 7).

Figures 5 and 6 indicate a somewhat linear decrease in yield with delays in planting, although the extremely low R^2 values (2016 = 2 %, 2017 = 17 %) especially for 2016 indicate that the linear yield response to DOP is almost certainly a decoy. The R^2 values increased significantly (2016 = 72 %, 2017 = 62 %) when cubic and especially quartic polynomials were plotted for the yield response to DOP with the high grain yield of the early DOPs in March of 2016 decreasing to the lowest level in April/May, and later increasing very slightly mid-season, and was somewhat uniform thereafter. In 2017, yield was highest in March/April, lowest mid-season, increased slighlty in late July/August before hitting another low in September. The R^2 from the regression analysis of both years combined was at 45 % when a cubic polynomial was plotted for yield in response to DOPs but the linear response of yield to DOPs was very low at R^2 = 5 %.

There were no correlations of seedling vigour traits with flowering and other adult plant traits including grain yield despite the fact that E %, PHT and EHT were all higher in 2016 than 2017 (Table 2). However, earlier flowering and shorter ASI as well as taller plants and higher ear placement increased yield significantly.

4 DISCUSSION

Results of this study decidedly reinforces the position that maize growth and productivity change in response to variation in planting date, as indicated by results of earlier studies (Fakorede, 1985; Oluwaranti et al., 2008) as well as common observations in farmers' fields. The present study, therefore, provides an additional sci-

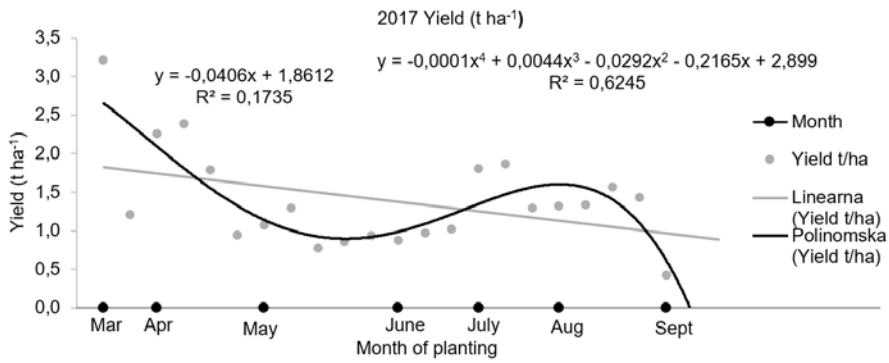

Figure 6: Mean grain yield (t ha^{-1}) of five maize varieties evaluated over 22 DOPs at the OAU T&R Farm in 2017 cropping seasons.

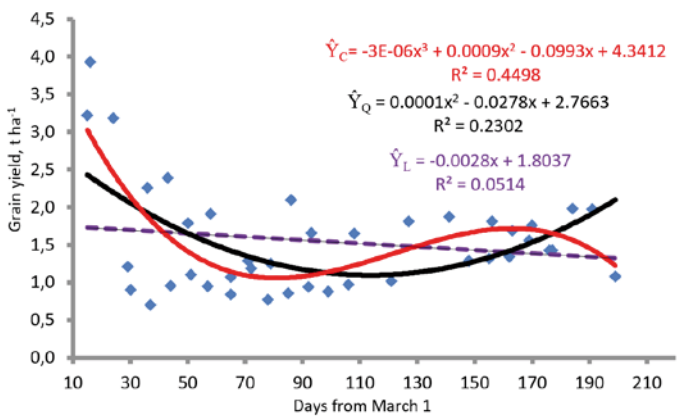

Figure 7: Mean grain yield (t ha^{-1}) by DOPs of five maize varieties evaluated over 42 different planting dates at the OAU T&R Farm in 2016 and 2017.

Table 2: Correlation coefficients among agronomic traits and grain yield of five maize varieties planted over several environments at OAU T&R Farm, Ile-Ife in 2016 and 2017 cropping seasons.

	EI	Days to tassel	Days to anthesis	Days to silk	ASI, days	PHT, cm	EHT, cm	YIELD, t/ha
E%_9	-0.43**	-0.15	-0.22	-0.12	0.12	0.14	0.22	0.26
EI		0.18	0.28	0.11	-0.23	-0.10	-0.23	-0.10
Days to tassel			0.98**	0.90**	0.18	-0.50**	-0.58**	-0.40**
Days to anthesis				0.90**	0.12	-0.58**	-0.67**	-0.45**
Days to silk					0.55**	-0.51**	-0.56**	-0.54**
ASI, days						-0.06	0.01	-0.37*
PHT, cm							0.92**	0.63**
EHT, cm								0.62**

*, ** Significantly different from zero at 0.05 and 0.01 level of probability, respectively.

entific proof the optimum DOP to maximize maize production in the rainforest agro-climatic zone of SW Nigeria is very early in the growing season. Highly significant mean squares were associated with the DOP source of variation for all traits at different growth stages, with the DOP sum of squares accounting for the largest proportion of the total sum of squares in each case, ranging from about 41 % for E % and EI to 59 % for grain yield. Environmental conditions vary widely from day to day and from one environment to the other due to temporal and spatial variations in climatic factors such as temperature, precipitation, solar radiation, evapotranspiration, relative humidity, and edaphic factors relating to soil conditions. In this study, climatic factors varied widely among the environments (Fayose and Fakorede, 2021), probably aggravated by the impact of climate change which had been reported as affecting maize growth and production at the location (Fakorede and Akinyemiju, 2003). For instance, the "August break" normally distinct between early and late seasons at Ile-Ife, was not experienced in 2016 and there was a delay in the onset of rainfall in 2017 beyond what had previously been experienced at the location. These probably resulted in the large differences observed among the DOPs for most traits assayed in this study. For that reason, analysis of the data for the individual years separately was justified (Fig. 1).

Emergence is expected to be lower early in the season (because of low soil moisture) and later increase to an optimum mid-season before declining. This trend was more noticeable in 2016 as emergence in 2017 did not follow a discernible pattern. Also, flowering, PHT and EHT showed no particular trend from one DOP to another. Grain yield on the other hand, was significantly higher with the first few DOPs each year which might seem to support the results of the study by Fakorede (1985) who found that early planting ensured optimum maize grain yield and steady decrease in yield occurred with delays in planting. This makes sense because there is usually low cloud cover during the early DOPs thereby allowing optimum solar radiation, a necessary condition for maize optimum yield, to reach the surface. However, there were dates in March and April that performed very poorly in comparison to later DOPs which might seem to be contradictory to the findings of the aforementioned study. This, could be attributable to the relatively poor edaphic conditions probably resulting in inadvertently inadequate agronomic practices and the attack by army worm, *Spodoptera frugiperda* (Smith, 1797), which was a problem at a point during the study. Oluwaranti et al. (2008) observed similar results to those of Fakorede (1985) in the late season at the same location.

Analysis of the yield in the present study may have indicated no discernible trend in yield performance for both years under consideration. Beyond the early DOPs, however, a polynomial response (cubic) of yield to DOP was observed in which yield was highest very early in the season, dropping to its lowest value around mid-season before rising ever so slightly later and flattening out or somewhat decreasing afterwards. Further analysis of DOPs combined for both years showed a clear trend where average yield decreased steadily in each successive month till June and picked up in July/August before finally dropping off in September in what is clearly a cubic response of yield to DOPs. Year 2016 had higher emergence, earlier flowering, higher ear placement, and taller plants than 2017. This may be due to the higher amount of rainfall received during the growing season at the OAU T&R Farm in 2016 (> 1000 mm) compared to 2017 (675 mm). According to Fakorede and Agbana (1983) and Fayose and Fakorede (2014), varieties with high E % and low values for EI and ERI tend to accumulate dry matter at a higher rate than slowly emerging

varieties resulting in vigorous plants, thereby increasing yield directly or indirectly. This perhaps, is one of the reasons why yield was also higher in 2016 than 2017. Early flowering, short ASI, and taller plants were indeed associated with increased grain yield in this study even though seedling vigour had no significant relationship with yield. Seedling vigour could therefore be assumed to have influenced yield indirectly.

There was also significant DOP x Variety interaction for yield and other traits monitored in this study except PHT, indicating that the different maize varieties responded differently to the different climatic conditions. This suggests the tendency of certain varieties to perform better when planted in specific DOPs. Such varieties when planted in other DOPs outside their favoured DOP would struggle to reproduce the good performance. Each of the varieties indeed performed differently in one environment relative to others as indicated by the ANOVA. The hybrid, 'Oba Super 1' had the poorest average performance across DOPs on the one hand, whearas 'TZL Comp. 4 DT F_2' and 'TZL Comp. 1 C6/DT – SYN – 1 – W' had the best average performances on the other hand. It would seem therefore that drought tolerant late maturing varieties would have better adaptation to this agro-ecology especially in the early season, which provides a window long enough for the partitioning of enough dry matter for the grain filling stage which, in turn, is extended to ensure higher grain return. However, the perfornance of 'White DT STR SYN1 – TZL Comp. 1 – W' was unlike the two varieties despite sharing similar characteristics, which casts a cloud on the earlier hypothesis. Nevertheless, 'TZL Comp. 1 C6/DT – SYN – 1 – W' for its consistency across DOPs would seem the best of the varieties evaluated in this study for planting in the marginal rainforest agro-ecology of Ile-Ife in SW Nigeria. The gxe effect could be deeply investigated, however, with a larger sample size and that would give a larger degrees of freedom, allowing more room for comparison, with the goal of recommending the varieties that are best adapted to the rainforest agro-ecologies.

5 CONCLUSIONS

In conclusion, despite the prevailing climate change scenarios which has no doubt impacted agricultural activities especially crop production in the past few decades, this study established that planting maize early in the season ensures optimum grain yield. Yield decreased steadily as long as planting is delayed up till June. For optimum yield, planting should be done with the first few rains in March as long as soil moisture could be maintained either with supplementary irriagation or better soil management and other agronomic practices in case of the occurrence of drought of any duration, which is very probable with the present reality of climate change. Planting should not be delayed beyond early to mid April in the early season. Planting in the late season should be done around late July to mid-August. Planting beyond this period reduces yield and predisposes the plants to terminal drought.

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doi:10.14720/aas.2021.117.1.1829 Original research article / izvirni znanstveni članek

Salt and drought stress exhibits oxidative stress and modulated protein patterns in roots and leaves of date palm (*Phoenix dactylifera* L.)

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Received August 13, 2020; accepted January 20, 2021.
Delo je prispelo 13. avgusta 2020, sprejeto 20. januarja 2021.

Salt and drought stress exhibits oxidative stress and modulated protein patterns in roots and leaves of date palm (*Phoenix dactylifera* L.)

Abstract: The formation of new proteins under the influence of harsh environmental conditions is a plant adaptation reaction. Two-year-old date palm tissue culture-derived plants from 'Barhee' grown in the field were subjected to salt stress (70 g l^{-1} NaCl) and dehydration-induced by applying 70 g l^{-1} polyethylene glycol or without irrigation and withholding irrigation (0 g l^{-1}) for one month. The soluble carbohydrate content increased in response to salinity and polyethylene glycol treatment in leaves compared to the control and drought treatment without irrigation. Proline increased in all treatments. Malondialdehyde and hydrogen peroxide increased under salinity. Salinity treatment increased the activity of ascorbate peroxidase and catalase enzyme. Salinity and polyethylene glycol treatments increased abscisic acid, whereas the indoleacetic acid level decreased. The protein pattern of roots and leaves in one-dimensional polyacrylamide gel electrophoresis showed that the stress conditions led to new protein bands' appearance and other proteins' disappearance. A comparison of protein patterns between the control and stress treatments revealed that the relative intensity of proteins in roots and leaves were more associated with salinity treatment than the drought. The results may be clearing important the molecular mechanism of tolerance under the influence of extreme environmental stress.

Key words: abscisic acid; ascorbate peroxidase; lipid peroxidation; malondialdehyde; polypeptide

Solni in sušni stres se izražata kot oksidacijski stres in vplivata na vzorce beljakovin v koreninah in listih dateljeve palme (*Phoenix dactylifera* L.)

Izvleček: Tvorba novih beljakovin je pod vplivom neugodnih okoljskih razmer prilagoditveni odziv rastlin. Dvoletne dateljeve palme, vzgojene v tkivnih kulturah iz sorte 'Barhee', posajene na prostem, so bile izpostavljene solnemu stresu (70 g l^{-1} NaCl) in dehidraciji z uporabo 70 g l^{-1} polietilen glikola ali brez zalivanja za en mesec (0 g l^{-1}). Vsebnost topnih ogljikovih hidratov se je v listih povečala kot odziv na slanost in obravnavanje s polietilen glikolom v primerjavi s kontrolo in obravnavanjem s sušo brez zalivanja. Vsebnost prolina se je povečala v vseh obravnavanjih. Vsebnosti malondialdehida in vodikovega peroksida sta se povečali v razmerah slanosti. Obravnavanje s slanostjo je povečalo aktivnosti encimov askorbat peroksidaze in katalaze. Obravnavanji s slanostjo in polietilen glikolom sta povečali vsebnost abscizinske kisline, a zmanšali vsebnost indolocetne kisline. Vorec beljakovin v koreninah in listih določen z enodimenzionalno poliakrilamidno gelsko elektroforezo je pokazal, da so stresne razmere vodile k novemu vzorcu beljakovin v stresnih razmerah in izginjanju drugih. Primerjava vzorcev beljakovin med kontrolo in stresnimi obravnavanji je pokazala, da je relativna jakost pojavljanja beljakovin v koreninah in listih bolj povezana z obravnavanjem s slanostjo kot s sušo. Rezultati bi lahko bili pomembni pri pojasnitvi molekularnega mehanizma tolerance pod vplivom ekstremnega okoljskega stresa.

Ključne besede: abscizinska kislina; askorbata peroksidaza; peroksidacija lipidov; malondialdehid; polipeptid

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1 INTRODUCTION

Protein pattern analysis is a helpful instrument for testing plants' reactions to abiotic stress (Piasecka et al., 2019). Changes in natural conditions influence the quality and amount of plant proteins. Protein groups react significantly to ecological stress, for example, drought (Hellal et al., 2018)a laboratory experiment was conducted in a factorial randomized complete design with four replications. The controlled experiment included ten of Egyptian barley cultivars namely; (Giza 123, 124, 125, 126, 127, 129, 130, 134, 135 and 2000 and salt stress (Patankar et al., 2018)the molecular basis of this tolerance is complex and poorly understood. Therefore, this study aimed to identify the genes involved in salinity tolerance using a basic yeast functional bioassay. To achieve this, a date palm cDNA library was overexpressed in Saccharomyces cerevisiae cells. The expression levels of selected genes that make yeast cells tolerant to salt were subsequently validated in the leaf and root tissues of date palm seedlings using a quantitative PCR method. About 6000 yeast transformant cells were replica printed and screened on a synthetic minimal medium containing 1.0 M of NaCl. The screening results showed the presence of 62 salt-tolerant transformant colonies. Sequence analysis of the recombinant yeast plasmids revealed the presence of a group of genes with potential salt-tolerance functions, such as aquaporins (PIP. Salt stress is a major limiting factor in plant development and can lead to water stress (Elsheery et al., 2020; Shareef et al., 2020). Salinity reduces the plant's ability to absorb water (Naeem et al., 2013). Acosta-Motos et al. (2017) explained the mechanism of a direct effect of salinity on plants by triggering specific morphological, physiological, and biochemical changes. Determination of stress resistance involves useful molecules that induce adaptation reactions of the developing plant under stress conditions, such as specific proteins (Ghatak et al., 2017; Mohamed et al., 2018).

The root is the vital organ that first experiences salt stress in the plant (Komatsu & Hossain, 2013). Some proteins respond to salt stress induced in the roots more than other plant parts. The main proteins produced by salinity or drought stress are linked to carbohydrates and energy metabolism (Xu et al., 2015). Paul et al. (2015) stated that the main class of specific proteins resulting from stress ultimately leads to oxidative stress. These defense proteins accumulate in plant roots and play an initial role in plant endurance to drought (Omar et al., 2018).

A plant's tolerance to water stress or its avoidance depends on multiple mechanisms activated by different molecular signals in the roots, stimulated by abscisic acid such as SnRK2-interacting calcium sensor and AREB/ABFs-ABRE-binding protein/ABRE-binding factor to send the message to the leaf to start the endurance mechanisms (Chae et al., 2007)we have isolated a dehydration-inducible gene (designated OSRK1. Abscisic acid represents the long-distance signal that moves from the roots to the leaves, increasing its concentration in the leaves due to expressing genes responsible for ABA biosynthesis, which leads to stomatal closure (Lokhande & Suprasanna, 2012; De Smet & Zhang, 2013).

Drought and salinity are essential environmental issues prevailing in date palm (*Phoenix dactylifera* L.) cultivation regions where they can negatively impact growth and productivity (Al-Khayri & Al-Bahrany, 2004; Al-Bahrany & Al-Khayri, 2012; Shareef, 2019). Several types of genetic markers have been used to characterize date palm varieties under different stress conditions based on phenotypic (Elsafy et al., 2015), biochemical (Yaish et al., 2015)little is known about the underlying molecular mechanisms that contribute to its salt tolerance. Only recently, investigators have uncovered microRNA-mediated post-transcriptional gene regulation, which is critical for typical plant development and adaptation to stress conditions such as salinity. To identify conserved and novel miRNAs in date palm and to characterize miRNAs that could play a role in salt tolerance, we have generated sRNA libraries from the leaves and roots of NaCl-treated and untreated seedlings of date palm. Deep sequencing of these four sRNA libraries yielded approximately 251 million reads. The bioinformatics analysis has identified 153 homologs of conserved miRNAs, 89 miRNA variants, and 180 putative novel miRNAs in date palm. Expression profiles under salinity revealed differential regulation of some miRNAs in date palm. In leaves, 54 of the identified miRNAs were significantly affected and the majority (70%, and molecular markers (Al Kharusi et al., 2017). Ponnaiah et al. (2019)crude protein (CP has shown that changes in the levels of proteins of pearl millet under salinity and drought conditions have helped to identify the genes involved in controlling tolerance to these stress conditions. In a previous experiment, El Rabey et al. (2015) used date palm seedlings to analyze proteomics for salinity and sensitive protein associated with water stress resulting from gene expression of salinity and dehydration in leaves. The current experiment aimed to analyze the protein pattern change and oxidative stress response in offshoots from tissue culture (true to type) date palms' leaves and roots exposed to drought and salinity.

2 MATERIALS AND METHODS

2.1 PLANT MATERIAL AND STRESS CONDITIONS

Two-year-old date palm plants 'Barhee' regenerated

from tissue culture were used in this experiment, which was conducted at Alharthah, Basrah, Iraq (30°38'47.1"N 47°45'08.2"E) during 2019. The plants were grown in silty clay loam soil with an organic fertilizer in September and expanded the offshoot cultivation area. On 1 October, the treatments were conducted. The minimum temperature was 18 °C, the maximum 32 °C, the relative humidity was 30 %, and the light intensity 1350 μmol m^{-2} s^{-1}. Five replicates of each treatment were investigated. The treatments consisted of regular irrigation, (control) withholding irrigation for one month, induced drought stress with field capacity was 12.5 % (20 ml depth of water), induced drought stress using 70 g l^{-1} polyethylene glycol (PEG), and salinity stress with 70 g l^{-1} sodium chloride. Field capacity was 75 % (120 ml depth of water) for PEG and salt treatment. After 30 days, samples were washed with distilled water, dry matter taken, whereas fresh samples frozen in liquid nitrogen.

2.2 ESTIMATION OF TOTAL CARBOHYDRATE CONTENT

The amount of total soluble sugar was estimated spectrophotometrically at 490 nm following the phenol sulfuric acid reagent method of Dubois et al. (1951). The leaves and roots (100 mg) segments were homogenized in 80 % ethanol and centrifuged at 2000 x g for 25 min. The supernatant (1 ml) was mixed with 0.05 % phenol (2 ml) (pH 6) and 98 % sulfuric acid (2 ml). The blends were hatched at 80 °C for 20 min in a water bath. Total soluble carbohydrate content was calculated using a standard curve of glucose and expressed as mg g^{-1} F.M.

2.3 DETERMINATION OF PROLINE

Proline was extracted and estimated, according to Bates et al. (1973). Root and leaf (0.5 g dry matter) samples were homogenized separately in cold aqueous sulfosalicylic acid 3 % (10 ml). The homogenate was sieved through Whatman No. 2 paper. In a test tube, 2 ml of the filtrate was blended with acidic ninhydrin (2 ml) and glacial acetic acid (2 ml) and incubated in a 100 °C water bath for 1 hr. After quick cooling in an ice bath, toluene (4 ml) was included, and the mixture was shaken by hand. The toluene phase, containing the chromophore, was aspirated, and the absorbance of this phase, using samples of 1 ml, was determined at 520 nm.

2.4 MEASUREMENT OF LIPID PEROXIDATION

Lipid peroxidation was determined by evaluating malondialdehyde (MDA). MDA was estimated dependent on the strategy of Esterbauer & Cheeseman (1990). A blend of 0.6 g fresh plant tissue and TCA 5 % (5 ml) was centrifuged at 12000 x g for 25 min. The supernatant was blended in thiobarbituric acid 0.67 % (2 ml) and heated for 30 min at 100 °C in a water bath. Sample absorbance was evaluated at 450, 532, and 600 nm using a blank containing all reagents. MDA content of the sample was calculated using the formula:

$$C\ (\mu mol\ g^{-1}) = 6.45\ (A532 - A600) - 0.56\ A450.$$

2.5 ESTIMATION OF HYDROGEN PEROXIDE

Leaves and roots (500 mg) samples were crushed in trichloroacetic acid (TCA) 5 %, and the mixture was utilized for the assurance of H_2O_2 by the technique of Sagisaka (1976). The response blend with 50 % of TCA, 10 mmol ammonium sulfate ferrous, 2.6 mol potassium thiocyanide, and plant separate, the 480 nm used to read absorbance.

2.6 EXTRACTION OF ANTIOXIDANT ENZYMES

Leaves and roots (200 mg) samples were absorbed in fluid nitrogen and crushed in an extraction buffer (2.0 ml): 100 mmol potassium phosphate (pH 7.8), 0.1 mmol ethylene diamine tetraacetic acid (EDTA), and 10 mmol ascorbic acid. 13000 xg for 15 min at 4 °C used to centrifuged the mixture. The catalase activity (CAT) and ascorbate peroxidase (APX) was measured by collecting the supernatant. All proteins were resolved with a similar support arrangement. Also, the evaluation was made utilizing the Bradford (1976) strategy. CAT activity determined by the technique depicted by Azevedo (1998) was utilized with specific alterations. Its operation was checked by a spectrophotometer assessing the H_2O_2 at 240 nm for 2 min in a response medium containing 100 mmol potassium phosphate buffer (pH 7.0), 12.5 mmol H_2O_2, and 50 μl of plant separate incubated at 28 °C. As a new solution, a similar response medium free of separate was utilized. The activity of APX was resolved following Nakano & Asada (1981) by observing the ascorbate's oxidation pace at 290 nm. The response medium was incubated at 28 °C. It was comprised of 100 mmol potassium phosphate buffer (pH 7.0), 0.5 mmol ascorbic acid, and 0.1 mmol H_2O_2. The reduction in absorbance was observed for 2 min from the beginning of the response.

2.7 HORMONES ANALYSIS

Five grams of a fresh tissue sample, which was homog-

enized in 70 % methanol, was stirred overnight at 4 °C. The extract was filtered through Whatman filter paper (No.1) and evaporated under a vacuum. The pH of the aqueous phase was adjusted to 8.5, using 0.1 mol phosphate buffer. Later the aqueous phase was partitioned twice using methanol. A rotary evaporator removed the methanol phase. The aqueous phase pH was adjusted to 2.5, using 1 N hydrochloric acid (HCl). The injection of the concentrate determined phytohormones into a reversed-phase HPLC, C18 column in an isocratic elution mode utilizing a portable stage comprising of acetone: water (26:74) with 30 mmol phosphoric acid as per Tang et al. (2011). The pH was kept up at 4, utilizing 1 N sodium hydroxide. The temperature was kept at 25 °C. The flux rate was 0.8 ml min^{-1}, and the elution of the phytohormones was observed at 208 and 265 nm for indoleacetic acid and abscisic acid, respectively.

2.8 EXTRACTION OF PROTEINS AND GEL ELECTROPHORESIS

Proteins were extracted by homogenizing the 300 mg of solidified dried leaf in 1 ml of extraction buffer [0.2 mol, tris-hydroxymethyl aminomethane (Tris) + 0.001 mol ethylenediamine tetra acetic acid + (Na_2 + EDTA) + 12 % glycerol + 0.01 M dithiothreitol (DTT) + 0.05 mmol phenylmethylsulfonyl fluoride (PMSF)] and homogenized by a mortar and pestle. At that point, the samples were centrifuged at 15,000 ×g for 15 min; the buffer consisted of 0.125 M Tris HCl (pH 6.8) + 4 % SDS + 20 %, glycerol + 10 % b-mercaptoethanol + 0.01 % bromophenol blue. Protein samples were denaturized by heat in the water bath at 90 °C for 3 min. Protein electrophoresis was performed in SDS polyacrylamide gel following procedures described by Laemmli (1970). The protein bands demonstrated clear changes, which were investigated by ImageJ programming.

2.9 STATISTICAL DESIGN AND ANALYSIS

The experiment was conducted according to the completely randomized blocks design for four treatments: control, drought, polyethylene glycol, salinity, with five replications per treatment. The mean values ± the standard error were displayed. The data were subjected to analysis of variance (ANOVA) utilizing IBM (SPSS Statistics V23.0), and Duncan's multiple range test at $p < 0.05$ were separated by the means.

3 RESULTS

3.1 ANTIOXIDATIVE AND OXIDATIVE RESPONSE TO DROUGHT AND SALINITY STRESS

Drought and salinity treatments altered soluble carbohydrate and proline in roots and leaves (Fig. 1a,b). Af-

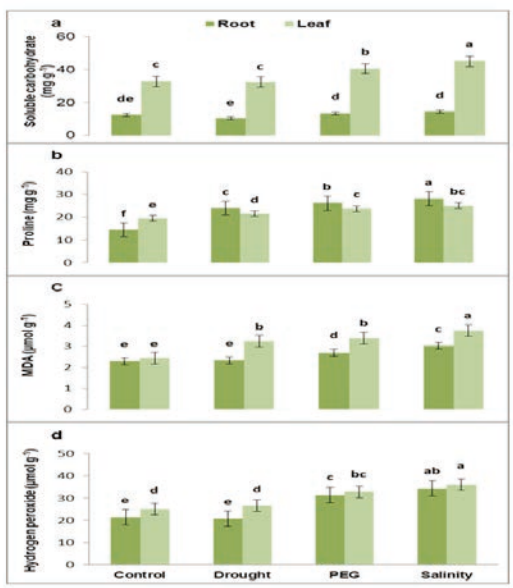

Figure 1: Oxidation change in response to drought, PEG and salinity stress, (a) Soluble carbohydrate, (b) Proline, (c) Malondialdehyde (MDA), (d) Hydrogen peroxide. Data are shown as means (of five replicates) ±S.E. Different letters indicate significant differences between treatments of leaves and roots ($p < 0.05$)

ter one month of treatment, the soluble carbohydrates' content increased significantly in response to salinity and PEG treatments in leaves related to the control and drought treatment. In contrast, without irrigation, the drought treatment decreased carbohydrates' content considerably in the roots (Fig. 1a).

Proline in leaves and roots increased significantly in all treatments related to the control (Fig. 1b). Salinity treatment increased MDA in leaves and roots related to the control (Fig. 1c). In contrast, drought treatment showed no significant difference in MDA compared to the control in roots. Salinity treatment increases the hydrogen peroxide in roots and leaves related to other treatments. In contrast, drought treatment had no significant difference in H_2O_2 related to the control in both roots and leaves (Fig. 1d).

3.2 ENZYMES ACTIVITY AND HORMONAL LEVELS IN RESPONSE TO DROUGHT AND SALINITY STRESS

Salinity treatment increased APX enzyme activity by 16.46 and 49.19 % higher than the control in roots and leaves, respectively. At the same time, the drought treatment reduced APXenzyme activity by 20.81 and 15.65 % compared to the control in roots and leaves, respectively (Fig. 2a). The CAT enzyme activity decreased significantly in response to drought in the roots and leaves related to the control. In contrast, the salinity treatment significantly increased CAT activity in leaves related to the control treatment (Fig. 2b).

Salinity and PEG treatments increased ABA's level in the leaves significantly by 47.86 and 216.02 % compared to the control, respectively. Also, both the salinity and PEG treatments increased ABA's level significantly in the roots by 38.85 and 33.63 % related to the control, respectively (Fig. 2 c). In the roots, the IAA level decreased significantly in response to salinity, 32.85 % compared to the control. Whereas, in leaves, the IAA level decreased significantly in response to drought treatment, where 46.60 % reduction was observed compared to the control (Fig. 2d).

3.3 PROTEIN PATTERN CHANGES IN RESPONSE TO DROUGHT AND SALINITY STRESS

The protein patterns of roots and leaves in one-dimensional polyacrylamide gel electrophoresis (SDS-PAGE) and subsequent examination by the ImageJ program demonstrated some striking contrasts (Fig. 3). The root protein patterns and relative intensity of protein expression showed that the appearance of protein bands 7 and 8 (molecular mass 59.492 and 37.410 kD, respectively) was up-managed by drought, while the expression of protein bands 4 and 5 (molecular mass 97.400 and 97.391 kD, respectively) was down-controlled under drought. The relative intensity of protein expression in protein bands 4, 5, and 7 was up-managed, while protein

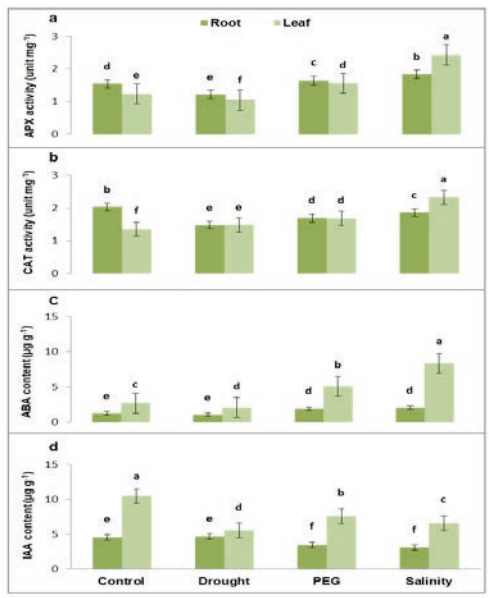

Figure 2: Enzymes activity and hormonal levels in response to drought, PEG and salinity stress, (a) Ascorbate peroxidase activity (APX), (b) Catalase activity (CAT), (c) Abscisic acid (ABA), (d) Indoleacetic acid (IAA). Data are shown as means (of five replicates) ± S.E. Different letters indicate significant differences between treatments of leaves and roots ($p < 0.05$)

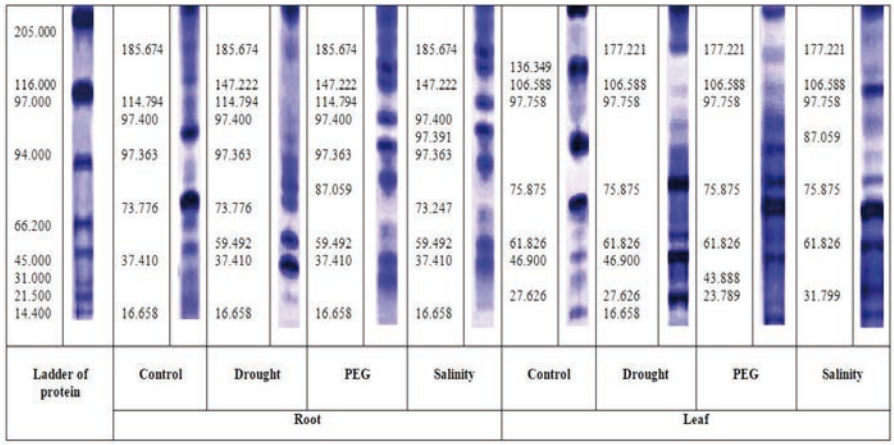

Figure 3: Analysis of protein patterns by one-D SDS-PAGE extracted from root and leaf, showing protein pattern changes in response to drought, PEG, and salinity stress

bands 1, 3, 8, and 9 (molecular mass 185.674, 114.794, 37.410, and 16.658 kD, respectively) were down-controlled by PEG. The relative intensity of protein expression in protein bands 4, 5, and 7 was up-managed, while protein bands 1, 3, 8, and 9 (molecular mass 185.674, 114.794, 37.410, and 16.658 kD, respectively) were down-controlled by PEG and salt stress except for protein band 3 which was not present in the salt stress treatment. The appearance of novel protein bands (including band 1 and 7 with molecular mass 147.222 and 59.492 kD, respectively) by drought, EPG, and salt stress related to the control, whereas band 6 (molecular mass 87.059 kD) was detected at PEG treatment and band 4 (molecular mass 97.391 kD) was found in the presence of salt stress treatment.

The leaf protein patterns and relative intensity of protein expression showed that the appearance of protein band 2 (molecular mass of 106.588 kD) was up-managed by drought. In contrast, the expression of protein bands 3, 4, 5, 6, and 7 (molecular mass 97.758, 75.875, 61.825, 46.900, and 27.626 kD, respectively) was down-controlled under drought. The relative intensity of protein expression in protein band 2 was up-managed, while protein bands 3, 4, and 5 were down-controlled by PEG. The relative intensity of protein expression in protein band 3 was up-managed, while protein bands 2, 5, and 6 were down-controlled by salt stress. The appearance of new protein bands, including band 1 with a molecular mass of 177.221 kD by drought, EPG, and salt stress compared to the control.Whereas band 1 (molecular mass 136.349 kD) was not present in all treatments. The appearance of novel protein bands, including band 8 with a molecular mass of 16.658 kD, responded to drought. The protein bands 6 and 7 with a molecular mass of 43.888

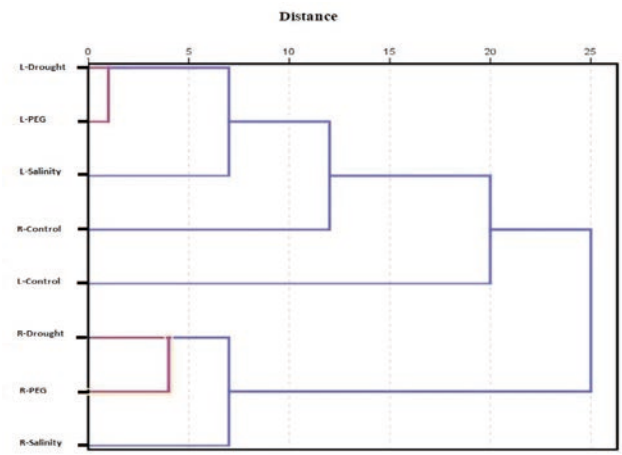

Figure 4: Dendrogram of hierarchical clustering to root (R) and the leaf (L) of date palm offshoot under drought, polyethylene glycol (PEG), and salinity stress by using protein patterns

and 23.789 kD responded to EPG. Also, bands 4 and 7 (with molecular mass 87.059 and 31.799 kD, respectively) appearance by salt stress (Fig. 3). Comparing the protein pattern in the control and all stress treatments revealed that the relative intensity in roots and leaves was associated more with salinity treatment than other tested factors.

3.5 CLUSTER ANALYSIS OF PROTEIN PATTERN TO ROOT AND LEAF IN RESPONSE TO DROUGHT AND SALINITY STRESS

Hierarchical cluster analysis of the protein pattern to root and leaf in response to drought and salinity stress (Fig. 4) showed two distinct clusters. The first group included drought, PEG, salinity, and control treatments to leaf (L-drought, L-PEG, L-Salinity, L-Control) and root control (R-Control). A significant similarity was observed between the drought treatments (L-drought) and PEG (L-PEG) in the leaf's protein pattern. At the same time, the second group included drought, PEG, and salinity treatments (R-Drought, R-PEG, R-Salinity), which showed the similarity between the treatments drought and PEG in the protein pattern of the root.

4 DISCUSSION

Salt and water stress are among the most severe environmental stress factors determining plant growth and development (Elsheery & Cao, 2008). The relationship between new proteins and tolerance to extreme environmental conditions is essential for environmental stress research. Drought and salinity treatments alter soluble carbohydrates' and proline concentration in roots and leaves (Fig. 1). Soluble carbohydrates contribute to osmotic adjustment during stress and protect giant molecules and membranes' structure during extreme environmental conditions (De Lacerda et al., 2005; Helaly et al., 2018). Carbohydrates play an essential role as an antioxidant resulting from plant tissue damage (Al Hassan et al., 2015). Soluble carbohydrates contribute to IAA building, especially glucose, which increases the IAA primers, such as (ANT and TRP) (Sairanen et al., 2013). The root is known to be a sink organ of carbohydrates. The carbohydrates are reduced under drought treatment (Fig. 1a). Decreased polysaccharides' content in the root under the influence of dehydration results from sugars' consumption in the process of respiration (Jaleel et al. 2009).

Proline concentration in leaves and roots increased significantly in all treatments than the control (Fig. 1b). Proline increases in plants that are more tolerant to harsh environmental conditions. One of the most important adaptations to the surrounding conditions is the accumulation of proline under the influence of drought or salt stress (Amini & Ehsanpour, 2005). Proline has a vital role in protecting the plant, promoting growth, and effective antioxidants in water stress conditions on the date palm (Dhawi & Al-Khayri 2008; Butt et al. 2020). Also, proline helps protein inversion and a regular signal that activates multiple planting adaptation responses to extreme environmental conditions (Khedr et al., 2003). Increasing the proline concentration provides for protein building because it is an amino acid and improves endurance by maintaining cell water potential, osmotic balance, and membrane stability by preventing leaching.

Salinity treatment resulted in a significant increase in MDA and H2O2 in leaves and roots (Fig. 1 c,d). The high MDA and H2O2 content shows increased oxidative stress and reflect tissue damage due to environmental stress (Ben Abdallah et al., 2017). The accumulation of sodium and chloride causes plant tissue damage, thus activating oxidative stress (Baghalian et al., 2008). MDA modifies many proteins into the photosynthetic center II (PSII) to stimulate the plant to adapt to environmental stress (Chen et al., 2018). Proteins are significant focuses for radicals and two-electron oxidants in biological systems because of their plenitude and high rate constants for the response (Davies, 2016). Hydrogen peroxide's most essential property is the capacity to cross cell membranes freely, which superoxide generally cannot do (Kurutas, 2016). High-level H2O2 can stimulate new enzymatic proteins such as APX and CAT enzymes that scavenge free radicals (Sofo et al., 2015).

Salinity treatment increased APX and CAT activity in leaves and roots (Fig. 2 a,b). APX activity increased under saline stress conditions due to gene expression that contributes to the maintenance of enzyme activity and physiological processes (Omar et al., 2012; Khan et al., 2020b). Gene expression of the enzyme is regulated during plant development responds to environmental stress (Huseynova et al., 2013). The effectiveness of APX directly contributes to the protection of plants against extreme environmental conditions (Khan et al., 2020a). Also, the APX enzyme's high activity can reduce the damage of reactive oxygen species (ROS) in the roots and thus increase the conversion of ROS to H2O2 and the use of the enzyme to maintain root cells (Huang et al., 2019).

Dehydration leads to reduced growth in general and thus affects gene expression resulting in reduced synthesis of enzymes. Different enzyme activity under various stress factors confirms the validity of the antioxidant system (Khan et al., 2019). The high activity of the CAT enzyme in the leaves is attributed to the stability of the tolerance of environmental conditions in plants (Kawa-

mura & Muraoka, 2018). Reduced enzyme activity under drought stress compared to the standard conditions showed the role of H2O2 as a signal transmitted from the root to the leaf and thus reduced the concentration of H2O2 in the roots (Fig. 1d).

Salinity and PEG treatments increased ABA levels in the leaves and roots (Fig. 2c). The ABA signaling pathway receives and transmits hormonal stimulation to activate several events towards the plant's ability to adapt to environmental conditions (Vishwakarma et al., 2017). ABA signaling pathway affects building many proteins that lead to plant adaptation to extreme environmental conditions (Mittler & Blumwald, 2015). The accumulation of ROS contributes to collecting the ABA required to activate the gene expression process (Vishwakarma et al., 2017).

Salinity and drought treatments decreased the level of IAA in the leaves and roots (Fig. 2d). IAA is involved in stimulating the construction of proteins such as H+-ATP (Salehin et al., 2019). Environmental stress modifies IAA transmission and its placement in the roots, so the auxin concentrations in the roots decrease when the plant is exposed to stress (Tanimoto, 2005). The reduction of IAA in the roots and leaves due to the imbalance in the growth regulators and the enzymes' inhibition is responsible for building auxin.

Comparing protein patterns in the control and stress treatments revealed that the relative intensity in roots and leaves was more significantly associated with salinity treatment than other factors (Figs. 3, 4). Cluster analysis showed the convergence of the effect of PEG and drought stress on protein patterns, whether in leaf or root, while distinguishing the impact on drought and salinity. Plant growth and adaptation to environmental conditions are strongly influenced by protein metabolism (Ghatak et al., 2017). Various investigations have indicated that the construction of new proteins is associated with changes in plant environmental conditions such as salinity (Yaish et al., 2015) drought (El Rabey et al., 2015), which causes an increase or decrease in polypeptides. The results indicate that the environmental conditions of stress led to the emergence of new proteins and other proteins' disappearance, accompanied by unmatched stress proteins' appearance. The formation of new proteins under dehydration's influence refers to creating these proteins earlier on cell death programming as we observed shrinkage and stiffness in plants prone to dehydration.

5 CONCLUSION

One of the most challenging difficulties in recent literature related to plant endurance is to explain the molecular basis of adaptation to environmental stress factors. A change in gene expression by drought stress leads to a cascade of changes, including the protein patterns. Exposure of date palm offshoots to dehydration creates specialized proteins whose primary function is to close the conveying channels in roots, close stomata, shrink cells, reduce vital processes, and maintain water in the tissues critical to maintaining plant survival. Exposure the plant to salinity builds multiple specialized proteins that improve the tolerance mechanism by modifying the roots' selective channels, closing the stomata, and activating the defense mechanism against oxidative stress.

6 ACKNOWLEDGMENT

The authors are grateful to Prof. Dennis V. Johnson of Cincinnati, Ohio, USA, for this manuscript's critical review and editorial enhancement.

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doi:10.14720/aas.2021.117.1.1935 Original research article / izvirni znanstveni članek

Analysis of gliadin patterns and diversity in *Triticum polonicum* L. accessions from Ethiopia

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Received October 19, 2020; accepted January 24, 2021.
Delo je prispelo 19. oktobra 2020, sprejeto 24. januarja 2021.

Analysis of gliadin patterns and diversity in *Triticum polonicum* L. accessions from Ethiopia

Abstract: Gliadins from 25 accessions represented by 350 individual seed samples were analysed by acid-polyacrylamide gel electrophoresis (A-PAGE) with the objective of identifying gliadin band patterns and examine the extent of diversity in *Triticum polonicum* L. collections from Ethiopia. Seventy polymorphic bands and 68 different patterns were identified. Eighteen different mobility bands and 16 patterns were identified in *ω*-gliadin region, 22 bands and 20 patterns in *γ*-gliadin region, 12 bands and 22 patterns in *β*-gliadin region and 18 bands and 10 patterns in *α*-gliadin region. The average genetic diversity calculated from the data of the four gliadin zones of the analysed samples was 0.15. The γ region have the highest diversity (H = 0.193), followed by ω regions (H = 0.177) and β region (H = 0.168) and the lowest diversity was observed in α region (H = 0.127). Cluster analysis based on genetic distances resulted in grouping of the analysed accessions in to seven main groups. Though the level of diversity was relatively lower than other tetraploid wheat species from Ethiopia, the findings are indicative of the existence of variation in the collections which can be exploited for wheat improvement.

Key words: A-PAGE; genetic diversity; gliadin; *Triticum polonicum*

Analiza vzorcev gliadinov in raznolikosti akcesij poljske pšenice (*Triticum polonicum* L.) v Etiopiji

Izvleček: V raziskavi so bili s kislo poliakrilamidno gelsko elektroforezo (A-PAGE) analizirani gliadini 25 akcesij poljske pšenice v 350 vzorcih semen z namenom določiti vzorce gliadinskih prog in ugotoviti obseg raznolikosti te pšenice v Etiopiji. Določeno je bilo 70 polimorfnih prog in 68 različnih vzorcev. Osemnajst različnih mobilnih prog in 16 vzorcev je bilo določenih v območju *ω*-gliadinov, 22 prog in 20 vzorcev v območju *γ*-gliadinov, 12 prog in 22 vzorcev v območju *β*-gliadinov in 18 prog in 10 vzorcev v območju *α*-gliadinov. Poprečna genetska raznolikost, izračunana na osnovi podatkov analiziranih vzorcev za ta štiri območja gliadinov je bila 0,15. Območje γ je imelo največjo raznolikost (H = 0,193), sledili sta mu območji ω (H = 0,177) in β (H = 0,168), najmanjša raznolikost je bila ugotovljena v območju α (H = 0,127). Analiza grozdov na osnovi genetske oddaljenosti je združila analizirane akcesije v sedem glavnih skupin. Čeprav je raven raznolikosti nekoliko manjša kot pri drugih tetraploidnih vrstah pšenice v Etiopiji, so odkritja pokazala obstoj spremenljivosti v zbirkah, ki bi jih lahko uporabili pri izboljšanju pšenice.

Ključne besede: A-PAGE; genetska raznolikost; gliadin; *Triticum polonicum*

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1 INTRODUCTION

Focus given mainly to increasing grain yield and crop productivity has resulted in an increase in micronutrient deficiency in food grains (Garg et al., 2018). Agronomic and genetic biofortification are being used as a means to improve the deficiency of micronutrients in modern varieties. Exploitation of underutilized species is one option to meet the demand for enhanced nutrition requirements. Evaluation of minor wheat species may provide breeders with important sources of genes suitable for biotic and abiotic stress tolerance and nutrition improvement. Among the many underutilized crops *Triticum polonicum* L., commonly known as Polish wheat, offers valuable agronomical and nutritional benefits that can be incorporated in to wheat varieties currently in use. *Triticum polonicum* L., 2n = 28, AABB), is a species which is grown sporadically in Africa (Ethiopia and Algeria), Europe (Spain, Italy, Ukraine and Russia) and some countries in the Asia temperate (USDA, 2020). Lately, *T. polonicum* is gaining attention due to its desirable traits. In comparison with durum and bread wheat, it has abundant protein and ash content and low fat content (Bieńkowska et al., 2019a) and it has good content of iron phosphorus, zinc, sulphur, magnesium, and boron, in addition to a lower content of aluminum (Bieńkowska et al., 2019b). Starch with up to 43.2 % amylase content was also reported in the grain of *T. polonicum* (Rodrıguez-Quijano et al., 2003). Foods with high amylase content take longer to digest hence to improve diet, cereals with high amylase content are needed (Vaziri et al., 2014). It is also shown to have potential and useful attributes in durum wheat breeding for drought tolerance (Hakimi et al., 1998).

Since most of the new varieties of wheat are notably poorer in mineral content breeders have specified *T. polonicum* as a genetic resource with good potential to enhance wheat's nutrition, specifically for genetic biofortification of durum and bread wheat (Bieńkowska et al., 2019b). The species is also reported to show satisfactory resistance to *Fusarium* head blight (Wiwart, 2013), and could be a potential source of semi-dwarfing genes for the development of durum wheat cultivars (Watanabe, 2004). *T. polonicum* can be used in wheat breeding programs since it can be crossed and produce fertile and genetically stable hybrids with *T. aestivum* L. and *T. durum* Desf. (Akond et al., 2006; Hakimi et al., 1997).

Gliadins are mainly monomeric proteins that are not affected by environment (Lookhart & Finney, 1984) and coast effective hence they make good tool for identifying genetic differences and determine quality (Bushuk & Zillman, 1978; Payne, 1987). In prior studies high and low molecular weight glutenin subunits composition of Ethiopian tetraploid wheat (Hailu et al., 2006) and allelic variation of gliadin coding loci of improved durum wheat varieties from Ethiopia (Hailegiorgis et al., 2017) have been reported. *T. polonicum* is one of the minor tetraploid wheat species cultivated in Ethiopia with limited importance, usually grown mixed with *T. durum* and *T. aestivum* (Demissie & Habtemariam, 1991) with earlier reports of cultivation in pure stands as well (Engels & Hawkes, 1991). It is a neglected crop in terms of conservation, research and utilization. The diversity of gliadin patterns in Ethiopian tetraploid wheat has not been reported so far. Genetic diversity study in conserved genetic resources of the species is important to better utilize and manage the resource. This study examined gliadin pattern and diversity of some *T. polonicum* collections from Ethiopia.

2 MATERIALS AND METHODS

2.1 PLANT MATERIAL AND GLIADIN EXTRACTION

Twenty five *T. polonicum* accessions from Ethiopia obtained from the Ethiopian Biodiversity Institute were used for this study. Fourteen seeds from each accession (a total of 350 individual seed samples) were used for gliadin profiling and diversity assessment. Each wheat grain was crushed using mortar and pestle and gliadin was extracted with 70 % (v/v) ethanol allowing the mixture to stand at 40 °C for 45 minutes and at +4 °C until electrophoresis. Aliquot of the supernatant was mixed with sample buffer (18 % sucrose containing methyl green tracking dye) before loading 25 µl of the extract on the gel.

2.2 A-PAGE

Glidins were fractionated using acid -polyacrylamide gel electrophoresis (A-PAGE), containing acrylamide (7.5 %) and bis-acrylamide (0.375 %) with ferrous sulfate (0.009 %) and ascorbic acid (0.00018 %) polymerized by the addition of hydrogen peroxide (15 %). Electrophoresis was performed at 50 V, 150 V and 250 V for 10 min each, at 350 V for 60 min and at 550 V for 80 minutes in 0.05 M aluminum lactate (pH 3.1) buffer. The cultivar *Icaro* was used as a standard in each gel run to compare electrophoretic bands and patterns. At the end of the run, gels were fixed using 12 % trichloroacetic acid for 15 minutes and stained overnight in staining solution containing 0. 04 % (w/v) Coomassie brilliant blue R-250 in 100 ml ethanol and 80 ml 12 % trichloroacetic acid.

After destaining using tap water, gels were photographed on a white illumination source for documentation. Each sample was run twice to confirm the gliadin profile.

2.3 DATA ANALYSIS

Banding pattern of each individual seed was compared within and among accessions and the standard cultivar to distinguish the different gliadin bands and patterns. Each different band of individual seeds displayed by gliadin loci was scored for its presence (1) or absence (0). The data obtained were entered as a binary data matrix and used to perform genetic diversity measures using GenAlEx 6.5 (Peakall & Smouse, 2012). Gene diversity was computed as the expected heterozygosity (H) based on band frequencies of gliadins. Genetic distance matrix was used to perform cluster analysis using the unweighted pair-group method for the arithmetic average (UPGMA) (Sneath & Soakal, 1973) clustering method by TFPGA version 1.3 (Miller, 1997).

3 RESULTS AND DISCUSSION

3.1 GLIADIN ELECTROPHORETIC PATTERNS

Twenty five different accessions of *T. polonicum* represented by 350 samples were analyzed by A-PAGE. Electrophoretic profile for representative accessions is shown in Fig.1. Assuming that the bands with the same relative mobility represent the same subunit, a total of 70 different bands and 68 gliadin patterns were detected. The average number of gliadin bands per accession was 24 and it ranged from 14 to 37. These bands were grouped into patterns at each of the four gliadin zones (α, β, γ and ω gliadins). The patterns within each gliadin group of α, β, γ and ω were identified by comparing banding patterns of each sample with each other and the standard cultivar.

Gliadin patterns in the four gliadin regions observed in the analysed samples is depicted in Fig. 2. Based on the observed gliadin profiles, 10 different gliadin patterns and 18 different mobility bands were identified in the α-gliadin region which showed limited variation compared to the other zones. Each α-gliadin pattern showed two to five bands. The most frequent pattern was pattern 1, observed in 84 % of the analysed accessions followed by pattern 2, 3 and 5 each observed in 16 % of the accessions. Twenty two different gliadin patterns were identified in the β-gliadin zone each pattern showing three to six bands. The most frequent pattern was pattern 1 detected in 30 % of the accessions followed by pattern 20 observed in 20 % of the accessions. In γ-gliadin zone, 20 different patterns having one to five bands in each pattern were detected. Pattern 1 was the most frequent one appearing in 68 % of the accessions. In ω-gliadin zone, 16 different patterns having three to seven bands were observed. Pattern 1 was the most frequent pattern encountered in 44 % of the accessions followed by pattern 11 appearing in 20 % of the accessions. The number of gliadin bands and patterns, 70 and 68 respectively, detected in the present study is higher than that of Pan et al. (2007) who reported 48 gliadin bands and 65 patterns in *T. polonicum* accessions from different countries. In other studies, Zaefizadeh et al. (2010) reported 66 polymorphic bands and 81 patterns in durum wheat landraces and Ojaghi & Akhundova, (2010) reported 48 bands and 47 patterns in double haploid *T. aestivum*. When comparing the different gliadin regions, α-gliadin region showed the lowest number of band patterns which is similar to find-

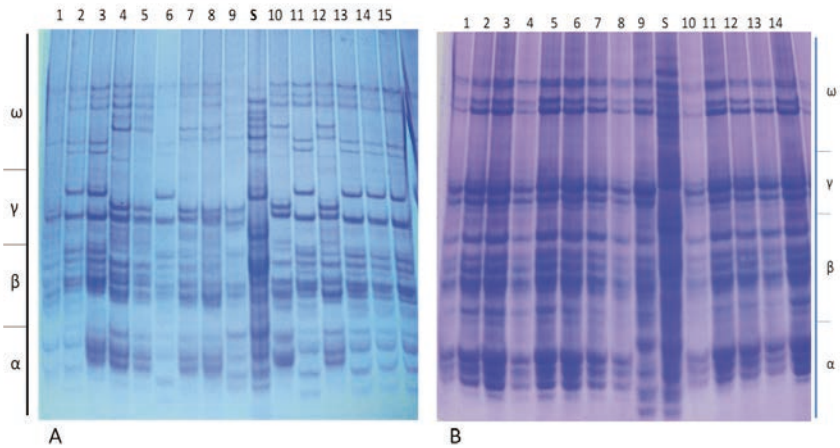

Figure 1: Gliadin electrophoretic patterns on A-PAGE for representative *T. polonicum* accessions showing within accession variation (A) and no variation within accession (B).

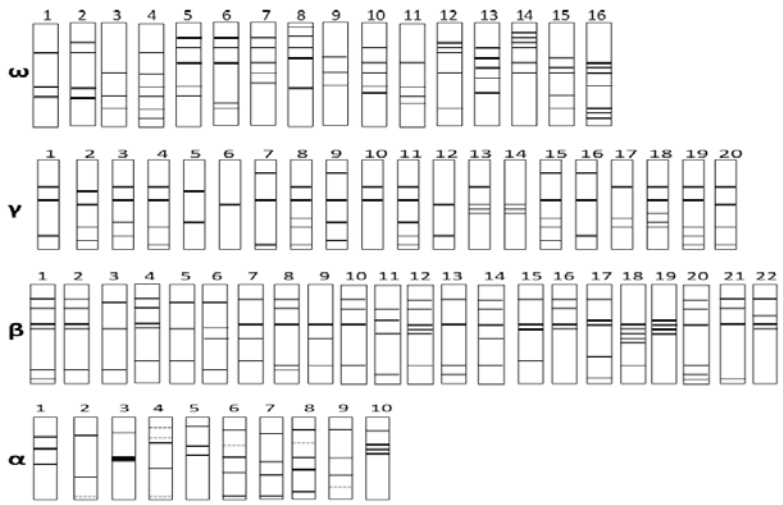

Figure 2: Electrophoregram of different gliadin patterns in α, β, γ, and ω gliadin regions observed in the analysed samples.

ings in studies on other wheat species (Ram et al., 2005; Aliyeva et al., 2012).

3.2 GENETIC DIVERSITY

A total of 70 bands were scored among the studied samples. The overall frequencies of these bands ranged from 0.007 to 0.871 among all the populations. The band with the highest frequency was detected in γ region while bands with the lowest frequency were detected in γ and α gliadin regions. The majority of the bands (74.28 %) showed frequency of < 25 %. The number of bands within each accession ranged from 14 to 37. Within accessions, the percentage of polymorphic bands ranged from 0.00 to 47.14 and the gene diversity (H) ranged from 0.00 to 0.153. The highest value was recorded in accession 23 (accession no. 226725) while five accessions (209774, 222726, 222632, 222242 and 226441) showed no within accession variation.

The overall gene diversity in the samples based on the patterns observed for each of the four gliadin regions showed that the γ region has the highest diversity (H = 0.193), followed by ω regions (H = 0.177) and β region (H = 0.168) and the lowest diversity was observed in α region (H = 0.14). In all the *T. polonicum* samples (350 entries) all bands were polymorphic and the mean gene diversity estimate was 0.167. The genetic diversity is relatively lower than other tetraploid wheat from Ethiopia examined using seed storage protein (Hailu et al., 2006) and microsatellite markers (Teklu et al., 2006; Yifru et al., 2006). Assessment of agromorphological characteristics of *T. polonicum* from Ethiopia had also indicated that phenotypic variation is relatively small except for the apical tooth and awn length (Demissie & Habtemariam, 1991). Limited cultivation of the species might have caused genetic erosion resulting in low genetic variation. Hailu et al (2006) reported 100 % genetic erosion of *T. polonicum* in Tigray and Gojam regions of Ethiopia and 84.4 % genetic erosion throughout the country. Yifru et al. (2006) also reported a much localized use of *T. polonicum* which might result in its loss. Tetraploid wheat used to occupy 60 % of the total wheat area (Tessema and Belay 1991). However, because of broader adaptability and yield advantage, bread wheat has been expanding in area of production substituting traditional durum and emmer wheat (Tsegaye & Berg, 2007). Recent reports estimate that tetraploid wheat accounts for around 40 % of the total wheat production (Brasesco et al., 2019), however, detail data on production of the different tetraploid wheat species is lacking.

3.3 ANALYSIS OF RELATIONSHIP

Nei's genetic distance (1972) among the accessions ranged from 0.00 to 0.538. The lowest genetic distance was observed among accessions 18, 19 and 21 (0.00) followed by accession 2 and 3 (0.0002). The highest distance was observed between accessions 22 and 24 (0.538) and accession 22 and 15 (0.529). The UPGMA clustering, based on Nei's genetic distance, for the 25 accessions revealed seven major groups (Fig. 3) containing one to 10 accessions per group. Group A comprises of accessions that showed no within accession diversity while group C contains accessions with the relatively high values of gene diversity, percentage of polymorphic bands and number of bands per accession.

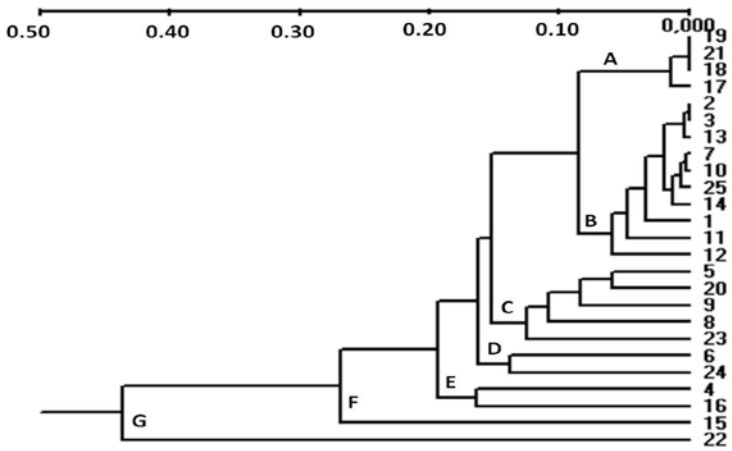

Figure 3: Dendrogram showing relationship among 25 *T. polonicum* accessions based on Nei's genetic distance.

4 CONCLUSION

Despite lower diversity revealed in the analysed accessions, the findings reported here have important implications for *T. polonicum* conservation and utilization in the country. In the Ethiopian genebank there are very few *T. polonicum* accessions. However farmers cultivate *T. polonicum* mixed with other wheat species hence wheat accessions conserved as Triticum spp also include *T. polonicum* as shown by activities that splits these mixed accessions in to their respective species. Splitting mixed species and additional collection of the species from potential growing areas need to be conducted so that the species can be used in improving wheat varieties in nutrient composition and disease resistance. Analysis of nutrient content and diversity analysis using molecular markers need also be carried out in order to reveal further the potential value of the species.

5 ACKNOWLEDGEMENT

This research was financially supported by the Ethiopian Biodiversity Institute. The author acknowledges the help of Ms Meaza Alemseged in sample preparation.

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doi:10.14720/aas.2021.117.1.1252 Original research article / izvirni znanstveni članek

The effects of zinc biofortification of seeds and NPK fertilizer application on the growth and yield of sesame (*Sesamum indicum* L.)

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Received August 28, 2019; accepted Janjuary 28, 2021.
Delo je prispelo 28. avgusta 2019, sprejeto 28. januarja 2021.

The effects of zinc biofortification of seeds and NPK fertilizer application on the growth and yield of sesame (*Sesamum indicum* L.)

Abstract: Soils of the southern Guinea savanna zone of Nigeria are inherently infertile due to shortened fallow period and the continuous use of inorganic fertilizer which depletes the soil of micronutrients such as zinc over time. A field trial was carried out at the Teaching and Research Farm of the University of Ilorin, Nigeria during the 2016 and 2017 cropping seasons to evaluate the effect of zinc and NPK fertilizer on the growth, yield and zinc concentration of seeds of sesame. The experiment was laid out as a factorial fitted into a randomized complete block design (RCBD), replicated thrice. The treatment consisted of four levels of $ZnSO_4$ (0, 5, 10 and 15 kg ha^{-1}) and four levels of NPK 15:15:15 (0, 100, 200 and 300 kg ha^{-1}). Data collected were subjected to analysis of variance (ANOVA) and means were separated using new Duncan multiple range test at 5 % level of probability. Results obtained showed significant effects of Zn and NPK rates on plant height, number of leaves, yield per plot and yield per hectare. The application of 15 kg ha^{-1} Zn and 300 kg ha^{-1} (15:15:15) NPK resulted in high yield and high zinc content of seeds.

Key words: bio-fortification; sesame; growth; yield

Učinki biofortifikacije semen s cinkom in uporaba NPK gnojil na rast in pridelek sezama (*Sesamum indicum* L.)

Izvleček: Tla v južno gvinejski savanski coni Nigerije so nerodovitna zaradi prekratkih obdobij prahe in stalne uporabe mineralnih gnojil, kar s časom povzroča obubožanje tal na mikrohranilih kot je zink. Poljski poskus je bil izveden na učnem in raziskovalnem posestvu Univerze v Ilorinu, Nigerija, v rastnih sezonah 2016 in 2017 za ovrednotenje učinka cinka in NPK gnojil na rast in pridelek ter vsebnost cinka v semenih sezama. Poskus je bil faktorski, popolni bločni poskus (RCBD) s tremi ponovitvami. Obravnavanja so obsegala štiri ravni cinka kot $ZnSO_4$ (0, 5, 10 in 15 kg ha^{-1}) in štiri ravni NPK 15:15:15 (0, 100, 200 in 300 kg ha^{-1}). Variabilnost zbranih podatkov je bila analizirana z ANOVA, poprečja so bila ločena z multiplim Duncanovim testom rangov pri 5 % verjetnosti. Izsledki so pokazali značilne učinke Zn in NPK na višino rastlin, število listov, pridelek na ploskev in pridelek na hektar. Uporaba 15 kg ha^{-1} Zn in 300 kg ha^{-1} (15:15:15) NPK je dala največji pridelek in največjo vsebnost cinka v semenih.

Ključne biofortifikacija; sezam; rast; pridelek

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1 INTRODUCTION

Low input agriculture is no longer sustainable due to increasing population, thus the use of inorganic fertilizer for the improvement of growth and yield to prevent hunger and starvation has become imperative (Graham et al., 2001), hence, farmers in the Guinea savanna zone of Nigeria cultivate their crops intensively relying on nitrogenous and phosphate fertilizers to increase crop growth and yield. However, the continuous use of inorganic fertilizer can deplete the soil of valuable micronutrients and sometimes can cause zinc antagonism (Haas et al., 2016) and these valuable micro nutrients are vital for crop growth and human health. Subsequently, soils low in mineral composition produce seeds and vegetative parts with even lower mineral contents. Roughly one third of the world's population suffers from hidden hunger which results from deficiencies of vitamins (particularly A and C) and minerals (such as zinc, iodine and iron), which leads to illness that is more severe in children than adults (GNR, 2003; Kennedy et al., 2004). Zinc is involved in many enzymatic reactions in crops such as carbohydrate metabolism and protein synthesis and its shortfall results in discoloration of leaves, impaired growth and delayed sexual development in human (Haas et al., 2016; Vootman & Bindraban, 2015; Haas et al., 2012). Cakmak (2008) reported that over thirty percent of the diet of the world`s population is deficient in zinc. This is attributed to crop production in areas with low soil mineral availability and consumption of crops with inherently low mineral content, low fish intake and other animal products in their diet due to poverty (Graham et al., 2001). Majority of rural dwellers in the southern Guinea savannah zone of Nigeria depends on cereals as staple food, have no access to fruits, vegetables and cannot afford fish and animal products because of poverty. Black et al. (2013) had reported severe zinc deficiency in children under five years old in some parts of Africa including Nigeria due to low zinc in their soil. In addition, this low zinc concentration in soil solution is due to its limited mobility in the soil (Cakmak, 2014); hence difficulty in plant roots to absorb it for its growth, development and its availability in plant tissues. One of the ways of overcoming this pitfall is through biofortification using zinc.

Biofortification is a process of making essential nutrients bio-available to crops during crop growth using agronomic and genetic methods (Bouis et al., 2011). Several methods have been used for the biofortification of crops, namely; breeding and agronomic approach using inorganic fertilizer (Cakmak, 2008). Rice has been biofortified with zinc in Bangladesh using breeding method (Chowdhury, 2014) whereas Zou et al. (2012) reported an increase in the concentration of zinc by up to 20 parts per million in wheat seeds in India and Pakistan by foliar spray, but in maize and rice, foliar spray of zinc resulted in only a small effect (Phattarakul et al., 2012). Suresh et al. (2013) reported a significant improvement in growth and yield of sesame using 25 kg ha^{-1} $ZnSO_4$ but no report of its concentration on the seeds. However, biofortified staple foods cannot provide high level of minerals and vitamin supplements compared to industrially fortified foods, but they can help by increasing the daily adequacy of micronutrient intake in humans (Bouis et al., 2011). The biofortification of local staples will help to bridge this micronutrients and vitamins gaps (Hotz & McClafferty, 2007) thereby reducing disease prevalence among the rural populace. The soils of the Guinea savannah zone of Nigeria are deficient in zinc (Aduloju, 2000) and farmers hardly apply zinc fertilizer. Developing biofortified crops can improve the efficiency of growth in these soils with depleted or very low mineral composition (Borg et al., 2009). Cakmak (2008) reported the daily requirement of zinc for different age groups (10 mg for children, 12 mg for women and 15 mg for men) whereas Pavlotska (2007) noted that nursing mothers require 25 mg per day of zinc. The objective of this study was to determine the effect of zinc and NPK fertilizer on the growth and yield of sesame.

2 MATERIALS AND METHODS

2. 1 THE EXPERIMENTAL SITE

Field experiment was conducted at the Teaching and Research Farm of the University of Ilorin, Ilorin, Nigeria during the 2016 and 2017 cropping seasons. The study area is situated in the southern Guinea savannah on (Latitude 04°35^{1}N and Longitude 08°29^{1}E, 307 m above sea level). The average rainfall of the area is between 1000–1540 mm which is bimodal in occurrence.

2. 2 EXPERIMENTAL LAYOUT AND SOWING OF THE SEEDS

The land was ploughed and harrowed before marking out into plots. The size of each plot was 3 m × 3 m with a 0.50 m avenue between the plots. The seeds of sesame (Ex-Sudan cultivar) which were obtained from the National Cereal Research Institute of Nigeria, Badeggi, Niger State, Nigeria were sown on the 15th of July, 2016 and repeated on July 15; 2017 using the drilling method at a depth of 2.5 cm and the seeds were covered lightly with soil to prevent desiccation by sunlight. The seed-

lings were later thinned three weeks after sowing to two plants per stand at a spacing of 30 × 30 cm between and within rows.

2. 3 EXPERIMENTAL DESIGN

The experiment was laid out in a factorial arrangement fitted into a randomized complete block design (RCBD) replicated thrice. The factors were NPK (15:15:15) fertilizer rates (0, 100, 200, 300 kg ha^{-1}) and $ZnSO_4$ levels (0, 5, 10, 15 kg ha^{-1}). The NPK fertilizer was applied 10 cm away from the plant using the side band placement method while the zinc sulphate was applied mid-morning by foliar method using a knap sack sprayer at three weeks after sowing. Zinc is commonly applied to crops as $ZnSO_4$ (Cakmak, 2008), as this will help to increase the micronutrient content in edible parts (Prasad et al., 2015).

2. 4 SOIL AND SEED ANALYSES

Soil samples from the experimental plot were collected from the top soil at a depth of 0–30 cm from a 2.5 x 2.5 m grid, bulked; then a composite was taken for physical and chemical analyses before and at the end of the cropping seasons. The soil samples collected were air dried ground and passed through a 2 mm sieve. The sieved soil samples were taken to the laboratory for chemical analysis, as described by Carter & Gregorich (2007). Soil organic carbon was determined by the procedure of Walkley & Black using the dichromate wet oxidation method (Nelson & Sommers, 1996). Organic matter was estimated by multiplying carbon (C) by 1.724. Total nitrogen was determined by Micro-Kjeldahl digestion and distillation techniques (Bremner, 1996), and available phosphorus was determined following Bray No 1 (1N NH_4F + 0.5N) extractant by vanado-molybdo-phosphoric acid method (Kuo, 1996), Soil pH was measured (soil: water ratio, 1:2) using a glass electrode; particle-size analysis was done using the hydrometer method (Gee & Or, 2002). Textural class was determined using a textural triangle (Brady & Weil, 1999; Hunt & Gilkes, 1992) and extraction of exchangeable bases was done by using IN ammonium acetate, exchangeable K and Na were determined by using flame photometry while calcium and magnesium were analyzed by atomic absorption spectrophotometry.

The total zinc concentrations in the seed samples were determined by wet digestion method as described by Minaleshewa et al. (2010) and concentrations in the extracts were determined by flame atomic absorption Spectrometry while its concentration in the soil was determined by the method of (Maclean & Langille, 1976).

2. 5 DATA COLLECTION AND ANALYSIS

Data were collected on growth parameters (plant height, number of leaves, and leaf area index), dry matter accumulation, yield parameters (number of capsules per plant, length of capsule, yield per net plot and yield per hectare). The plant height was assessed by measuring the plant from the ground level to the terminal point using a measuring tape, while the number of leaves was assessed visually by counting the green leaves of the five tagged plants in the net plot (60 plants) whereas the leaf area was calculated based on the work of Silva et al. (2002) using:

$$S = 0.3552 \times C^2$$

where S = leaf area in cm^2 and C = leaf longitudinal length x breadth while the leaf area index was estimated as;

$$\frac{leaf\ area}{ground\ cover}$$

The data on yield components were collected on number of capsules per plant, length of capsule, seed mass per net plot and seed mass per hectare. Data on number of capsules per plant was estimated by counting, the length of capsule was calculated by the use of digital Vernier caliper, while the yield per net plot (60 plants) was carried out by weighing the seeds by using a sensitive balance whereas the yield per hectare was extrapolated from the yield per net plot and the data collected were subjected to general analysis of variance (ANOVA) using Genstat statistical software (17th edition). Treatment differences were compared using the new Duncan Multiple range test at 5 % level of probability.

3 RESULTS AND DISCUSSION

3.1 PHYSICAL AND CHEMICAL PROPERTIES OF SOIL

The data on physical and chemical properties of soil of the experimental site is presented in Table 1. The result showed that the soil pH was slightly acidic to moderately acidic before and after the experiment. The organic matter content was low throughout the experimental period. The chemical soil properties such as total nitrogen content, available phosphorus and exchangeable cations such as potassium, calcium and magnesium were very

Table 1: Physical and chemical properties of soil of the experimental site before and after cropping in 2016 and 2017

Year	Soil pH	Org C (%)	Org. Matter (%)	Total N mg g^{-1}	P mg g^{-1}	K cmol kg^{-1}	Ca cmol kg^{-1}	Mg cmol kg^{-1}	Zn mg kg^{-1}
2016a	6.1	0.82	1.41	0.07	6.67	0.31	5.05	0.28	0.56
2016b	5.9	0.54	0.93	0.04	5.42	0.32	4.39	0.21	0.54
2017	5.8	0.50	0.86	0.03	3.28	0.30	4.23	0.19	0.53

a Before initial cropping, b. End of first year cropping.

low; and available micronutrients such as zinc was very low before and at the end of the cropping seasons. These low soil nutrients of the study site are below the critical levels for optimum crop growth and yield using soil data rating as suggested by Linsday & Norvell (1978); FDALR (1990) which is as follows; organic carbon, 1–1.4 %, total nitrogen, 0.151-0.200 %; available phosphorus, 7.0–20 mg kg^{-1}; exchangeable potassium, 0.3–0.6 cmol kg^{-1}; exchangeable calcium, 5-10 cmol kg^{-1}; exchangeable magnesium 1-3 cmol kg^{-1} and available zinc 0.6 –1.0 cmol kg^{-1}. The low nutrient status of the experimental site, which is typical of Alfisols could be attributed to burning of crop residues, short fallow period and seasonal bush fire which deprives the soil of valuable organic matter. This is the reason why the cultivation of crops is done solely with the application of external inputs such as nitrogenous fertilizer which has led to the depletion of valuable micronutrients such as zinc over time. Eifediyi et al. *(*2016) had reported that soils of the study area are deficient in macro and micro nutrients which are essential for crop growth and development.

3.2 METEOROLOGICAL DATA

The rainfall data of the study site for 2016 and 2017 is presented in Figure 1. The data on rainfall indicated that no precipitation was experienced in the month of February during the two cropping seasons. But in 2016, September recorded the highest rainfall of 332 mm while the highest rainfall in 2017 was recorded in the month of August (302.7 mm). The rainfall in the study area has changed drastically in the past few decades and thus in sharp contrast to the rainfall pattern in the past which is bimodal in nature which starts in late march with the first peak in July, an August break and the second peak in September. This variation in rainfall could be attributed to the effect of climate change (Odjugo, 2011). The data on temperature is shown in Figure 2. The highest temperature in 2016 was experienced in the month of February while in 2017; the highest temperature was recorded in the month of March. The temperature of the study area has been on the increase in the past few years which can also be attributed to the effect of climate change (Ajibade, 2002).

The relative humidity is shown in Figure 3. The highest relative humidity was recorded in the month of September in 2016 while in 2017, it was in August.

3.3 GROWTH PARAMETERS

The effect of Zn and NPK fertilizer application rate

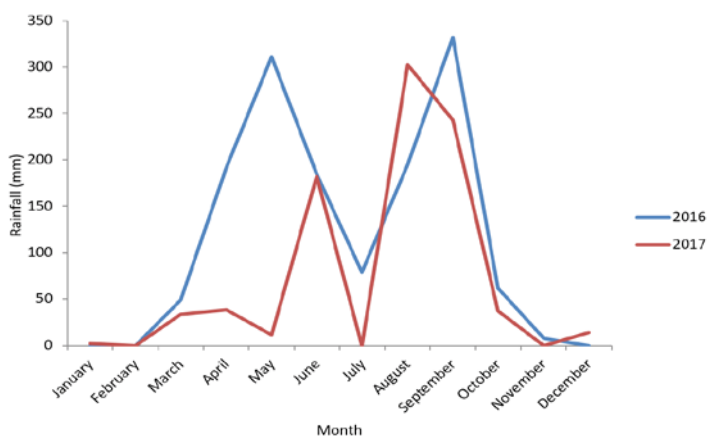

Figure 1: Rainfall pattern of the study area in 2016 and 2017.

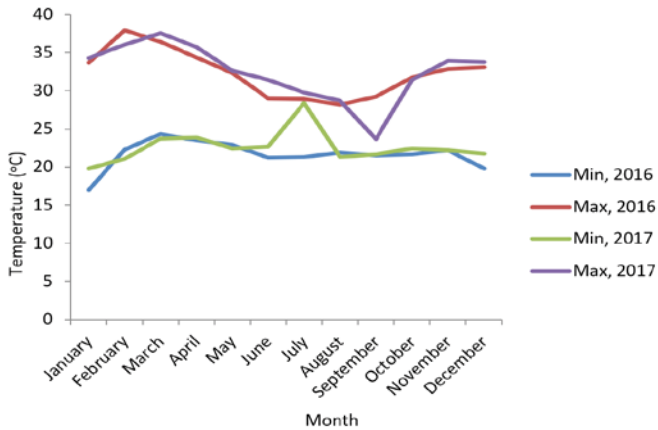

Figure 2: Temperature pattern of the study site in 2016 and 2017.

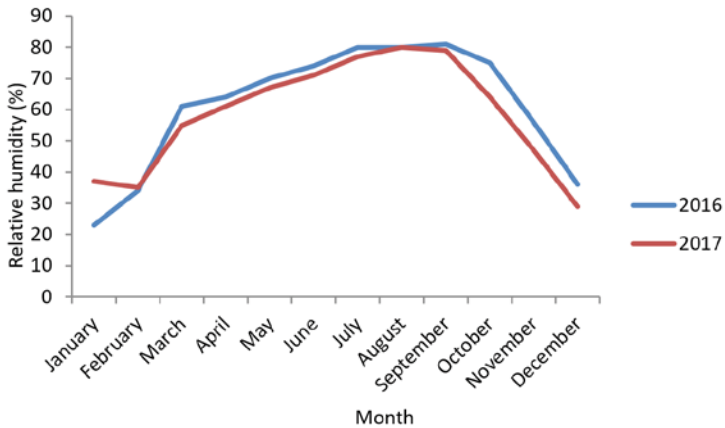

Figure 3: Relative humidity pattern of the study site in 2016 and 2017.

on the plant height and number of leaves of sesame at 4, 6, 8 and 10 weeks after sowing (WAS) are presented in Tables 2 and 3. The data revealed that the zinc applied at the rate of 15 kg ha^{-1} produced the highest plant height and number of leaves at the four sampling periods. There was no significant difference observed in the first season (2016) but in the second season (2017), significant differences ($p < 0.05$) were observed between the different rates. A trend was observed when the different rates of application of NPK 15:15:15 fertilizer was applied at the different sampling dates of 4, 6, 8 and 10 WAS with the 300 kg ha^{-1} producing the tallest plants and the highest number of leaves. No significant difference was observed in the first year (2016), but in the second season (2017), significant differences ($p < 0.05$) were observed at the four sampling periods. The positive response of crop to the application of fertilizer most especially zinc in the increase in growth attributes could be attributed to the important role it plays in enzymatic reactions, regulation of auxin synthesis (Marschner,1995) metabolic processes, and oxidation-reduction reactions and for many enzymes which are needed for nitrogen metabolism (Hafeez et al., 2012; Omidian et al., 2012).

The effect of Zn and NPK fertilizer application rate on the leaf area index of sesame at 4, 6,8 and 10 weeks after sowing (WAS) is presented in Table 4. The data revealed that the zinc applied at the rate of 15 kg ha^{-1} produced the highest leaf area index at the four sampling periods, significant differences ($p < 0.05$) were observed between the different rates in the two seasons at 4 WAS. There was no significant difference observed in the first and second seasons at the 6, 8 and 10 WAS. A similar trend was observed when NPK fertilizer was applied, with the 300 kg ha^{-1} producing the highest leaf area index throughout the period of assessment in both seasons. Significant differences ($p < 0.05$) were observed between the different rates in the two seasons at 4 WAS. Increase in leaf area index with increase in rate of zinc fertilizer

Table 2: Effects of Zn and NPK fertilizer on the plant height of sesame at 4, 6, 8 and 10 weeks after sowing

	4 WAS		6 WAS		8 WAS		10WAS	
	Season							
Treatments	1	2	1	2	1	2	1	2
Zn (kg ha^{-1})								
0	34.0	28.17	76.2	73.1	98.2	90.6	107.1	100.0
5	39.4	30.75	81.4	75.2	102.0	93.3	110.9	102.8
10	40.9	32.08	81.6	85.5	103.4	110.8	114.9	120.8
15	46.8	34.67	87.4	80.2	108.3	102.3	117.6	114.4
Sed ($p < 0.05$)	ns	0.740	Ns	5.21	ns	6.27	ns	5.19
NPK (kg ha^{-1})								
0	29.9	18.25	69.3	50.8	86.3	69.3	96.7	80.8
100	40.0	25.00	79.4	79.8	101.1	104.3	111.9	112.7
200	40.4	36.50	84.2	87.4	107.9	109.6	116.7	117.5
300	50.8	45.92	93.8	95.8	116.7	113.7	125.2	124.0
Sed ($p < 0.05$)	6.48	0.938	8.56	6.28	7.68	6.40	7.01	6.15
Interaction	ns	1.785	Ns	12.06	ns	12.73	ns	11.85

Season 1 = data on 2016 cropping season; season 2 = data on 2017 cropping season

Table 3: Effects of Zn and NPK fertilizer on the number of leaves of sesame at 4, 6, 8 and 10 weeks after sowing

	4 WAS		6 WAS		8 WAS		10WAS	
	Season							
Treatments	1	2	1	2	1	2	1	2
Zn (kg ha^{-1})								
0	17.58	24.0	41.3	41.3	55.0	53.3	62.7	60.8
5	18.92	24.0	41.4	41.4	57.7	58.1	66.7	64.5
10	21.92	28.42	52.1	52.1	66.2	65.8	76.3	74.2
15	22.83	30.42	57.1	57.1	68.8	78.1	77.6	75.6
Sed ($p < 0.05$)	ns	1.28	3.63	3.63	3.77	3.13	3.89	3.89
NPK (kg ha^{-1})								
0	12.42	13.00	28.1	28.1	45.1	49.3	54.9	53.1
100	20.00	22.33	53.2	53.2	66.2	65.2	72.3	70.3
200	20.25	30.33	54.1	54.1	64.0	68.2	74.6	72.2
300	28.58	41.17	56.4	56.5	71.6	72.5	81.4	79.4
Sed ($p < 0.05$)	2.11	1.28	3.63	3.63	3.77	3.13	3.89	3.89
Interaction	ns	2.57	Ns	7.26	7.78	6.27	ns	ns

Season 1 = data on 2016 cropping season; season 2 = data on 2017 cropping season

application may be due to the fact that zinc exerts a great influence in basic plant life processes, such as nitrogen metabolism, uptake of nitrogen and protein quality, photo synthase-chlorophyll synthesis, carbon anhydrase activity, resistance to abiotic and biotic stresses, protection against oxidative damage (Alloway, 2004; Potarzycki & Grzebkisz., 2009;Tekale et al., 2009). The increase in the leaf area index as a result of zinc application is in agreement with the report of Badshah et al. (2013) who reported significant increase in leaf area with foliar application of zinc.

The effect of Zn and NPK fertilizer application

Table 4: Effects of Zn and NPK fertilizer on the leaf area index of sesame at 4, 6, 8 and 10 weeks after sowing

	4 WAS		6 WAS		8 WAS		10WAS	
	Season							
Treatments	1	2	1	2	1	2	1	2
Zn (kg ha^{-1})								
0	2.75	2.64	3.75	3.64	4.47	4.58	4.62	4.73
5	2.76	2.65	3.90	3.75	4.50	4.61	4.66	4.77
10	2.94	2.83	3.98	3.86	4.52	4.63	4.70	4.81
15	3.32	3.22	4.49	4.38	6.01	6.12	5.10	5.21
Sed ($p < 0.05$)	0.386	0.386	ns	ns	ns	ns	ns	ns
NPK (kg ha^{-1})								
0	2.17	2.06	3.59	3.86	4.71	4.82	4.51	4.62
100	2.70	2.59	3.63	3.52	4.79	4.90	4.56	4..67
200	2.82	2.72	4.01	3.90	4.96	5.07	4.82	4.93
300	4.08	3.97	4.88	4.77	5.05	5.16	5.19	5.30
Sed ($p < 0.05$)	0.386	0.386	ns	ns	ns	ns	ns	ns
Interaction	0.771	0.771	ns	ns	ns	ns	ns	ns

Season 1 = data on 2016 cropping season; season 2 = data on 2017 cropping season

Table 5: Effects of Zn and NPK fertilizer on the dry matter accumulation (g) of sesame at 4, 6, 8 and 10 weeks after sowing in the 2016 and 2017 cropping seasons

	4 WAS		6 WAS		8 WAS		10 WAS	
	Season							
Treatments	1	2	1	2	1	2	1	2
Zn (kg ha-^{1})								
0	0.56	0.58	1.61	1.60	2.04	1.79	2.62	2.50
5	0.58	0.63	1.68	1.68	2.87	2.36	3.84	3.72
10	0.71	0.68	2.04	1.89	2.61	2.62	3.91	3.79
15	0.71	0.73	1.90	2.03	2.89	2.64	3.97	3.85
Sed ($p < 0.05$)	0.060	0.014	0.187	0.187	ns	ns	0.355	0.355
NPK (kg ha-^{1})								
0	0.45	0.41	1.63	1.63	2.29	2.04	3.08	2.96
100	0.58	0.56	1.69	1.69	2.65	2.40	3.42	3.30
200	0.61	0.71	1.84	1.84	2.73	2.48	3.59	3.47
300	0.91	0.95	2.05	2.05	2.75	2.50	4.25	4.13
Sed ($p < 0.05$)	0.060	0.014	0.187	0.187	0.320	0.320	0.355	0.355
Interaction	0.119	0.029	0.374	0.374	ns	ns	0.710	0.710

Season 1 = data on 2016 cropping season; season 2 = data on 2017 cropping season

rate on the dry matter accumulation of sesame at 4, 6, 8 and 10 weeks after sowing (WAS) is presented in Table 5. The data revealed that the zinc applied at the rate of 15 kg ha^{-1} accumulated the highest mass at the four sampling periods. Significant differences ($p < 0.05$) were observed between the different rates at 4, 6 and 10 WAS, but there was no significant difference observed in the first and second seasons at the 8 WAS. A trend was observed when the different rates of application of NPK 15:15:15 fertilizer was applied at the different sampling dates of 4,

6, 8 and 10 WAS with the 300 kg ha^{-1} producing the highest dry matter mass. However, significant differences (p < 0.05) were observed at the four sampling periods. There was also an increase in the dry matter accumulation and dry matter is an important parameter in the study of crop canopies. Increase in dry matter as the rate of application of Zn and NPK fertilizer increased could be attributed to the role performed by nitrogen and potassium in improving plant metabolism, enhancing plant meristematic activity and increasing the rate of photosynthesis (Alloway, 2004).

3.4 YIELD AND YIELD COMPONENTS OF SESAME

The effects of zinc and NPK fertilizer on the yield components of sesame is presented in Table 6. The data showed that the zinc applied at the rate of 15 kg ha^{-1} produced the highest number of capsules per plant and length of capsules which were significantly (p < 0.05) different from the other rates in the two years of study (2016 and 2017). The application of 300 kg ha^{-1} also produced the highest number of capsules and length of capsules in the two years of study which were significantly different (p < 0.05) from the other rates. There was also a significant zinc and NPK fertilizer interaction.

The data on the yield of net plot and yield per hectare showed a similar trend as what was observed in the number of capsules per plant. The highest net yield of (452 g and 454 g) and yield per hectare (502.2 kg and 505.1 kg) were produced by applying zinc at the rate of 15 kg ha^{-1} which were significantly different from the other rates in 2016 and 2017 cropping seasons respectively. Similarly, the application of NPK fertilizer at 300 kg ha^{-1} gave the highest yield (net plot, 555.5 g and 554.3 g in 2016 and 2017 respectively) while the yield per hectare (617.1 kg and 615.8 kg in 2016 and 2017 cropping seasons respectively) which were significantly different from the other fertilizer rates. There was also a significant interaction between the zinc and NPK fertilizer application.

Increasing the rate of zinc from 5 to 10 and 15 kg ha^{-1}, significantly increased yield and yield components. This is in agreement with Cakmak, (2000) and Omidian et al. (2012) who reported that zinc application increased the yield components of sesame as a result of the role it plays in cell division, cell enlargement and synthesis of protein. Zinc also regulates the membrane function and provides resistance to environmental stress in crop plant. The foliar spray of Zn increased the number of capsules and number of seeds per capsule (Bakry et al., 2012). This may be due to the involvement of zinc in photosynthesis, chlorophyll production, pollen function and fertilization (Pandey et al., 2006). Saedi (2002) stated that the foliar application of zinc promoted the vegetative and flowering stages of crops due to increase in the plant metabolism and photosynthesis. This increase in sesame yield may also be due to the abundant efficacy of en-

Table 6: Effects of Zn and NPK fertilizer on the yield components of sesame in the 2016 and 2017 cropping seasons

	Number of capsules		Length of capsules (cm)		Yield of net plot (g)		Yield (kg ha^{-1})		Zinc concentration (mg kg^{-1})	
	Season									
Treatments	1	2	1	2	1	2	1	2	1	2
Zn (kg ha^{-1})										
0	61	62	3.23	3.27	353.6	354.1	392.9	393.4	9.48	8.93
5	62	64	3.44	3.33	378.2	380.4	420.2	422.6	14.20	16.53
10	75	76	3.33	3.44	411.4	412.2	457.1	458.0	16.73	20.45
15	100	101	3.41	3.52	452.0	454.6	502.2	505.1	20.55	21.95
Sed (p < 0.05)	8.55	8.63	0.904	0.094	10.16	11.20	11.29	12.45	0.979	0.637
NPK ((kg ha^{-1})										
0	54	56	3.13	3.22	238.7	242.6	265.2	269.6	11.30	9.45
100	74	74	3.35	3.38	368.2	370.0	409.1	411.1	16.33	17.59
200	75	76	3.42	3.45	432.9	434.4	481.0	482.7	16.60	20.08
300	95	96	3.50	3.50	555.4	554.3	617.1	615.8	17.75	20.78
s.e.d (p < 0.05)	8.55	8.63	0.904	0.094	10.16	11.20	11.29	12.45	0.979	0.638
Interaction	17.09	17.27	0.181	0.189	20.32	22.40	22.57	24.89	1.957	1.275

Season 1 = data on 2016 cropping season; season 2 = data on 2017 cropping season

zyme activities which influence plant pigments because zinc is an important component of all classes of enzymes (CIMMYT, 2006; Malakouti, 2007). Mohsen et al. (2009) also suggested that foliar spray of zinc increased the seed yield of safflower (*Carthamus tinctorius* L.). Similarly, foliar application of zinc sulfate significantly improved the seed yield of canola (*Brassica napus* L.) (Omidbeigi, 2005), safflower (*Carthamus tinctorius* L.) (Movahedy et al., 2009), lentil (*Lens culinaris* Medik.) (Nakhzari et al., 2011) and soybean (*Glycine max* (L.) Merr.) (Jamson et al., 2009).

Zinc concentration in sesame seed also increased as the rate of application of Zn and NPK fertilizer increased. This increase in the zinc concentration may be attributed to the fact that zinc has favorable effects on the metabolism of plant which might be responsible for greater metabolite accumulation in the reproductive organs (Babaeian et al.2011).

4. CONCLUSION

The study revealed that the application of zinc at 15 kg ha^{-1} and NPK 15:15:15 at the rate of 300 kg ha^{-1} improved the growth, yield attributes and zinc concentration of sesame seeds. The increase in the zinc content of sesame seeds will therefore help to alleviate zinc deficiency in human diet. Farmers should therefore be enlightened on the need for appropriate combination of zinc and NPK fertilizers combination for optimum zinc absorption in seeds in order to minimize deficiency in human diet.

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doi:10.14720/aas.2021.117.1.1622 Original research article / izvirni znanstveni članek

Induction of defence related enzymes and biocontrol efficacy of *Trichoderma harzianum* in tomato plants infected with *Fusarium oxysporum* and *Fusarium solani*

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Received April 18, 2020; accepted January 27, 2021.
Delo je prispelo 18. aprila 2020, sprejeto 27. januarja 2021.

Induction of defence related enzymes and biocontrol efficacy of *Trichoderma harzianum* in tomato plants infected with *Fusarium oxysporum* and *Fusarium solani*

Abstract: Fusarium wilt of tomato plants caused by *Fusarium oxysporum* Schlecht. emend. Snyder & Hansen and *Fusarium solani* (Mart.) Sacc. are serious problem limiting tomato production worldwide. Biological control has emerged as one of the most promising alternatives to chemical fungicides. The biological control capability of a *T. harzianum* isolate against *F. solani* and *F. oxysporum* has been investigated. It inhibited colony growth of two *Fusarium* species by more than 80 % in dual culture tests. Results of greenhouse experiments revealed that disease severity in the tomato plants co-inoculated with *T. harzianum* was significantly lower than plants only infected with the *Fusarium* pathogens. Tomato plants inoculated with the antagonistic *T. harzianum* isolate, showed enhanced peroxidase and polyphenol oxidase activities in greenhouse experiments and increased resistance to *F. solani* and *F. oxysporum*. The *T. harzianum* isolate indirectly affected the *Fusarium* pathogens by enhancing plant defence.

Key words: fungal pathogens; antagonistic fungus; peroxidase; polyphenol oxidase; plant growth and biological control

Vzpodbuditev aktivnosti encimov povezanih z obrambo in učinkovitost biokontrole z glivo *Trichoderma harzianum* Rifai v paradižniku okuženem z glivama *Fusarium oxysporum* Schlecht. emend. Snyder & Hansen in *Fusarium solani* (Mart.) Sacc.

Izvleček: Fuzarijska venenja paradižnika, ki jih povzročata glivi *Fusarium oxysporum* Schlecht. emend. Snyder & Hansen in *Fusarium solani* (Mart.) Sacc. so resen problem, ki omejuje svetovno pridelavo paradižnika. Biološka kontrola se je pokazala kot najbolj obetajoča alternativa kemičnim fungicidom. V raziskavi je bila preučevana sposobnost biološkega uravnavanja gliv *F. solani* in *F. oxysporum* z izolati glive *Trichoderma harzianum*. Izolati so zavrli rast kolonij obeh vrst iz rodu *Fusarium* za več kot 80 % v preiskusih dvojnih kultur. Izsledki iz poskusov v rastlinjaku so pokazali, da je bila obolelost pradižnika značilno manjša, če je bil ta inokuliran hkrati z obema patogenima glivama iz rodu *Fusarium* in z glivo *T. harzianum*. Ratline paradižnika, ki so bile inokulirane z izolati antagonistične glive *T. harzianum* so imele v poskusih v rastlinjaku povečani aktivnosti peroksidaze in polifenol oksidaze ter povečano odpornost proti patogenima glivama *F. solani* in *F. oxysporum*. Izolati iz glive *T. harzianum* so neposredno vplivali na patogena iz rodu *Fusarium* s povečanjem obrambe rastlin.

Ključne besede: paradižnik; patogene glive; antagonistične glive; rast in biološka kontrola; peroksidaza; polifenol oksidaza

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1 INTRODUCTION

Tomato plants can be attacked by different soil-borne fungi, which cause severe diseases such as wilt and root rot (Morsy et al., 2009). *Fusarium* species are the most common pathogens, which can live in organic materials and nearly all soil types (Boyaci et al., 2010). They can attack the vascular system in infected plants, block water transport through xylem by inducing vessel plugging, leading to foliage wilt and finally plant death (Portal et al., 2018; Malandrakis et al., 2018).

The attempts to minimise the use of fungicides make it necessary to develop economical and effective methods to obtain biopesticides that are based on endogenous microorganisms (de los Santos-Villalobos et al., 2012). *Trichoderma* (Hypocreales) accommodates various soil-borne and pathogen-antagonistic species (Verma et al., 2007). *Trichoderma harzianum* is well known for its antagonistic effect on pathogenic fungi. It is the most potent biocontrol agent that inhibits the growth of *Fusarium* species. Mechanisms of biocontrol agent are based on antifungal metabolites, mycoparasitism, competition for nutrients and induced resistance (Perello et al., 2003; Silva et al., 2019).

Moreover, an increased activity of polyphenol oxidase and peroxidase in response to the infection by the pathogen is considered to play an active role in contributing to disease resistance in certain plant hosts (Vidhyasekaran, 2004). Pradeep and Jambhale (2002) and Vidhyasekaran (2004) observed that plants show increased activities of polyphenol oxidases and peroxidases in response to pathogen infections and the ability of a plant to resist disease. The present study investigated the effectiveness of *T. harzianum* to induce systemic resistance against *Fusarium solani* and *Fusarium oxysporum* when infecting tomato plants.

2 MATERIALS AND METHODS

2.1 ANTAGONISTIC ACTIVITY

Trichoderma harzianum isolate UPM40 was obtained from the plant protection department, faculty of agriculture, University Putra Malaysia and was evaluated in vitro for its antagonistic activity against two *Fusarium* isolates in dual culture method (Rahman et al., 2009). DNA barcoding strategies based on ITS sequences were used to identify one of the *Fusarium* as a member of the *F. solani* (KM039055) and the other as a member of the *F. oxysporum* species complex (KM039054) (from previous study Rashid et al., 2016). Isolate UPM40 was identified as a member of *Trichoderma harzianum* species complex on the basis of morphological characters. Discs of 6 mm diameter of the antagonist and pathogens were cut from the edge of actively growing colonies on potato dextrose agar (PDA Difco™) and placed at opposite sides (4.5 cm from each other) on PDA plates. Each antagonist/pathogen combination was set up in triplicates. The plates were incubated at 24 ± 2 °C. The antagonistic effect of the test fungus was estimated by measuring their radial growth in comparison to control plates using the following formula: I = [(C-T) / C] X 100 where: I = % of inhibition in the mycelia growth, C = the growth of pathogens in control plates and T = the growth of pathogens in the dual culture plates.

2.2 SOIL PREPARATION AND EXPERIMENTAL DESIGN

Soil comprising clay, sand and organic matter (1:1:1 v:v) from Erbil Governorate (pH 5.5-6) was air dried and sieved (5 mm), sterilised twice in an autoclave at 121 °C for one hour and allowed to cool for 24 hours before use.

The tomato ('Baccarat 322') seeds were surface disinfected by soaking them in 95 % ethanol for 10 seconds, followed by 3 % sodium hypochlorite for 1 minute and then washed 6 times with sterile distilled water. Two tomato seeds were planted per plastic cell in seedling growing trays. The seedlings were trimmed down to one seedling per cell after two weeks. The one-month-old tomato plants were then moved to clean pots (two plants/pot). On a daily basis, these plants were watered with tap water. The independently repeated experiment was conducted in a completely randomised design.

2.3 IN VIVO TEST

A Neubauer haemocytometer was used to adjust the *T. harzianum* spore concentration to 2×10^5 spore/ml (working solution). The soil with one-month-old tomato plants was drenched with 100 ml of working solution (Mahato et al., 2018). After 24 hours, the same amount of 2×10^5 spore/ml pathogen suspension was added to the soil per pot.

2.3.1 Chlorophyll content measurement

The chlorophyll content was measured in ten repetitions per leaf (5 plants per treatment) with a CL-01 Chlorophyll Content Metre after 30 days of inoculation. This device measured the relative chlorophyll content via dual wavelength optical observance (620 and 940 nm).

2.3.2 Sample collection for the biochemical analysis

The roots of the treated and untreated tomato plants were collected 10, 20 and 30 days after the treatment application; they were washed in running tap water and stored in a deep freezer (-80 °C) until they were used for the biochemical analysis.

2.3.3 Total protein content measurement

Protein extracts were generated from roots and protein concentrations were determined using the method described by Arora and Wisniewski (1994), of which the latter was based on Bradford assays.

2.3.4 Quantification of polyphenol oxidase (PPO) activity

The reaction mixture consisted of 0.5 ml of the enzyme extract and 2.3 ml of 0.1M phosphate buffer (pH = 6.1). Both were mixed in a cuvette and adjusted to the zero absorbance of a spectrophotometer (Mahadevan and Sridhar, 1982). Catechol solution (0.1 M, 0.2 ml) was added and the reactants were quickly mixed. The enzyme activity, measured as the change in absorbance per minute (ÄA/min) at 400 çm, was measured immediately after the addition of the catechol solution.

2.3.5 Quantification of peroxidase (PO) activity

Peroxidase activity was determined according to Sreedevi et al. (2011). The peroxidase enzyme activity was determined from the roots of the un-inoculated and inoculated tomato plants. About 0.5 g of freshly harvested material was ground in a pre-chilled mortar with 20 ml of 0.1 M cold ice phosphate buffer (pH 7.1) and centrifuged at 2000 rpm for 10 minutes. The supernatant was made up to 25 ml and used for the assay. Freshly prepared pyrogallol (0.2 M) reagent (0.1 ml), 1.0 ml of the enzyme extract, and 1.4 ml of the 0.1M phosphate buffer (pH, 7.1) were mixed in a cuvette, and the mixture was immediately adjusted to the zero absorbance of a spectrophotometer. The H_2O_2 solution (0.5 ml of 0.01M) was added to it, and the content was mixed by inverting the cuvette. The enzyme activity was recorded according to the changes in absorbance per minute (ÄA /min/ä) at 430 cm.

3 RESULTS AND DISCUSSION

3.1 ANTAGONISTIC ACTIVITY

The results indicated that *Trichoderma harzianum* (T. h) reduced the radial growth of *F. solani* (F. s) colony by 82.31 % and *F. oxysporum* (F. o) by 80.25 %.

3.2 IN VIVO EXPERIMENT

Trichoderma treated plants showed increased plant height, fresh shoot mass and root dry mass when co-inoculated with F. o (20.6 cm, 0.79 g and 0.15 g) or F. s (27 cm, 0.66 g and 0.17 g). Plants treated only with F. o had 10.17 cm and 14.00 cm height (0.31 g) shoot mass and (0.06 g) root mass. In addition, plants inoculated only with T. h showed the highest mean values for plant height, shoot dry mass and root dry mass (32.33 cm, 1.02 g and 0.18 g) compared to other treatments and control plants (Table 1). The increased growth response caused by *Trichoderma* isolate may be due to the modification of the rooting system (Chao et al., 1986). The results further indicate that *T. harzianum* had a significant role in reducing disease incidence. Therefore, the results of the study are similar to the findings of Abd- El-Khair et al. (2011), Otadoh et al. (2011) and Alwathnani et al. (2012).

Table 1: Effect of *T. harzianum, F. oxysporum* and *F. solani* on shoot height (cm), shoot and root dry mass (g) on tomato plants

Treatments	Shoot height (cm)	Shoot dry mass (g)	Root dry mass (g)
Control	22.67a	0.76a	0.14a
T. h	32.33b	1.02c	0.18b
F. o	10.17c	0.17d	0.06c
F. s	14.00d	0.31e	0.06c
T. h + F. o	20.67e	0.79a	0.15d
T. h + F. s	27.00f	0.66b	0.17be

C: control; F. o: F. oxysporum; F. s: F. solani and T. h: T. harzianum.

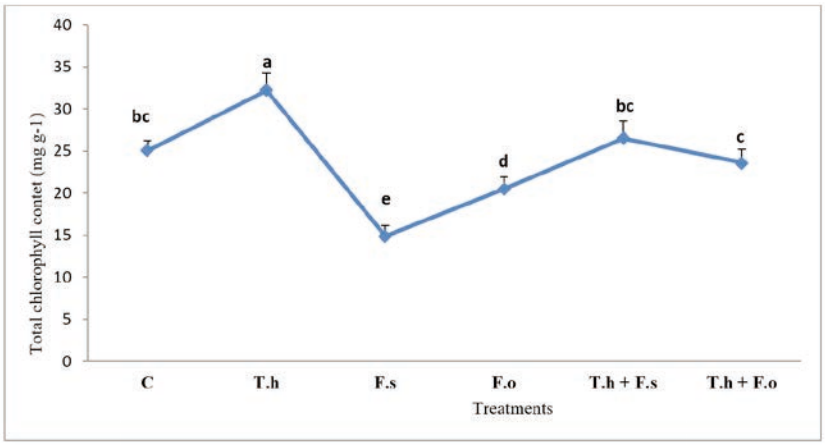

Figure 1: Effect of *T. harzianum* on chlorophyll content of the tomato plants inoculated with *F. oxysporum* and *F. solani*.

One of the important physiological indicators is the photosynthesis rate, which is related to the chlorophyll content in plant leaves in normal conditions. In the present study, the chlorophyll content was found to increase significantly in all plants treated with T. h compared to those inoculated with F. s or F. o. The chlorophyll content also significantly went up in plants treated with T. h + F.s (26.5) or T. h + F. o (23.5) in comparison to the F. o and F. s only (14.86 and 20.5) inoculated plants (Figure 1). Previous studies have claimed that applying biocontrol agents to infected plants increases mineral levels [(nitrogen (N), phosphorous (P), potassium (K) and magnesium (Mg)], chlorophyll biosynthesis and photosynthetic activity (Henry et al., 2009; Morsy et al., 2009; Alwathnani et al., 2012).

The total soluble protein content significantly decreased in tomato plants: F. s (0.082, 0.076 and 0.075) and F. o (0.084, 0.074 and 0.072) after 10, 20 and 30 days, respectively (Figure 2). The significant decrease in the protein content in the tomato tissues as a result of the pathogen infection may be due to certain activities related to a hypersensitive response (Chandra and Bhatt, 1998). *Trichoderma harzianum* also increased the total protein content in the infected tomato plants (T. h + F. s and T. h + F. o). The defence reaction occurs due to an accumulation of pathogenesis-related (PR) proteins such as chitinase, phenylalanine ammonia lyase and peroxidase (Kloepper et al., 1992). This has also been previously reported by Houssien et al. (2010).

Tables 2 and 3, show that the activity of peroxidase and polyphenol oxidase, increased significantly compared to the controls. The activity increased during the treatment period from day 10 to day 30. Also *Fusarium* species are able to produce metabolites, which play a vital role in tissue browning due to their ability to oxidise phenols to quinones (Ramadoss, 1991). Except the tomato plant inoculated only with the antagonistic *T. harzianum*, there was an increase in the activity of the polyphenol ox-

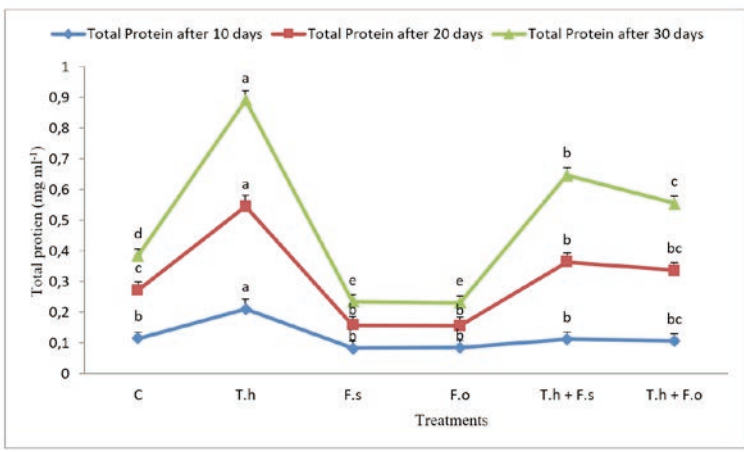

Figure 2: Effect of *T. harzianum* on the total protein content (mg g^{-1}) of the tomato plants inoculated with *F. oxysporum* and *F. solani*..

Table 2: Effect of *Trichoderma harzianum* on the peroxidase activity of the tomato plants inoculated with *F. oxysporum* and *F. solani*

	Peroxidase activity min g^{-1} fresh mass of tomato leaves		
Treatments	After 10 days	After 20 days	After 30 days
Control	0.18 a	0.17 a	0.15 a
T.h	0.16 a	0.43 b	0.36 b
F.o	0.25 b	0.34 c	0.59 c
F.s	0.27 bc	0.47 cd	0.62 cd
T.h+ F.o	0.27 bc	1.20 f	1.87 f
T.h+ F.s	0.29 cd	1.29 fg	1.95 f

Table 3: Effect of *T. harzianum* on the polyphenol oxidase activity of the tomato plants inoculated with *F. oxysporum* and *F. solani*

	Polyphenol oxidase activity min g^{-1} fresh mass of tomato leaves		
Treatments	After 10 days	After 20 days	After 30 days
Control	0.16 a	0.18 a	0.13 a
T.h	0.17 a	0.31 b	0.17 a
F.o	0.22 b	0.35 ab	0.69 b
F.s	0.21 b	0.39 bc	0.75 bc
T.h+ F.o	0.29 c	1.28 d	2.71 d
T.h+ F.s	0.27 c	1.29 d	2.55 de

idase and peroxidase up to the 20th day. After this time, the activity decreased or remained stable. At the initial stage of infection, there is probability of the host plant to secrete more defence enzymes (Ojha et al., 2012). The activity increased gradually from the 20th day up to the 30th day in the plants infected with T. h. + F. o. and T. h. + F. s.. The highest activities for both treatments 1.87 and 1.95 min g^{-1} for fresh mass of peroxidase and 2.71 and 2.55 min g^{-1} for fresh mass of polyphenol oxidase were recorded after the 30th day of inoculation. This is the most likely related to the fact that in tomato plants, when inoculated with a pathogen and an antagonist, the host plant secretes more of the phenol oxidase enzyme for defence, but at the later stages of infection, the antagonist blocks the activity of the pathogen. Various antioxidant enzymes, such as peroxidases and polyphenol oxidases can participate in the reactive oxygen metabolism of the species during infection (Morkunas and Gmerek, 2007). Other researchers have observed increased activity in these enzymes in the host tissues in response to pathogenic infections (Abo-Elyousr et al., 2008, Christopher et al., 2010; Ojha et al., 2012).

4 CONCLUSION

The experiments demonstrated that the *T. harzianum* is effective and can induce systemic and localized resistance against *Fusarium* infection in terms of changes of the plants' polyphenol oxidase activity, peroxidase activity and the total phenolic content. These mechanisms help to develop resistance in tomato plants. The current study suggests using *T. harzianum* strain (UPM40) to manage *Fusarium* diseases in tomato plants.

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doi:10.14720/aas.2021.117.1.1378 Original research article / izvirni znanstveni članek

The role of exogenous silicon to mitigate Al_2O_3 nanoparticle-induced toxicity in barley (*Hordeum vulgare* L.)

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Received October 12, 2019; accepted January 28, 2021.
Delo je prispelo 12. oktobra 2021, sprejeto 28. januarja 2021.

The role of exogenous silicon to mitigate Al_2O_3 nanoparticle-induced toxicity in barley (*Hordeum vulgare* L.)

Abstract: In this study, we used silicon (Si, in the form of K_2SiO_3, 2 mM) to alleviate the toxicity of aluminum oxide (Al_2O_3) nanoparticles (NPs) in barley (*Hordeum vulgare* L.). Using Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS) analyses, we showed that the Al_2O_3 NPs were taken up by barley plants. Barley growth was negatively affected by the addition of 3 g l^{-1} nano-Al_2O_3, whereas the diminishing effect of NPs on barley growth was not obvious when 1 g l^{-1} nano-Al_2O_3 was applied, indicating that the nano-Al_2O_3 action is dependent on nano-Al_2O_3 dose. Si pretreatment ameliorated toxic effects of high nano-Al_2O_3 on root growth. Si pretreatment did not decrease nano-Al_2O_3 entry into roots but reduced nano-Al_2O_3 accumulation in the shoot. The restriction of the root-to-shoot translocation of nano-Al_2O_3 was one of the important mechanisms for Si to mitigate high nano-Al_2O_3 toxicity. The occurrence of oxidative stress was found under 3 g l^1 nano-Al_2O_3 treatment, as evaluated by the accumulation of malondialdehyde (MDA). Exogenous addition of Si could alleviate toxicity symptoms induced by Al_2O_3 nanoparticles by reducing lipid peroxidation via enhancing antioxidant activity of catalase as well as by limiting the root-to-shoot translocation of nano-Al_2O_3. These data provide the first direct evidence that the Si pretreatment ameliorates nano Al_2O_3 phytotoxicity in plants.

Key words: *Hordeum vulgare* L.; malondialdehyde; nano-Al_2O_3; nanotoxicity; silicon

Vloga dodajanja silicija za preprečevanje strupenosti nano delcev Al_2O_3 pri ječmenu (*Hordeum vulgare* L.)

Izvleček: V raziskavi je bil uporabljen silicij (Si), v obliki 2 mM K_2SiO_3, za preprečevanje strupenosti nano delcev aluminijeva oksida (Al_2O_3;NPs) pri ječmeni (*Hordeum vulgare* L.). Analiza z ICP-MS je pokazala, da so bili nano delci Al_2O_3 privzeti v rastline. Na rast ječmena je negativno vplival dodatek 3 g l^{-1} nano delcev Al_2O_3, medtem, ko rast ječmena ni bila občutno zmanjšana pri dodatku 1 g l^{-1} nanodelcev Al_2O_3, kar kaže, da je učinek nano delcev Al_2O_3 odvisen od doze. Predhodno obravnavanje rastlin s silicijem je oblažilo toksičen učinek velikih koncentracij nano delcev Al_2O_3 na rast korenin. Predhodno obravnavanje s Si ni zmanjšalo privzema nano delcev Al_2O_3 v korenine ampak zmanjšalo njihovo kopičenje v poganjkih. Omejitev translokacije nano delcev Al_2O_3 iz korenin v poganjke se je izkazala kot pomemben mehanizem preprečevanja njihove toksičnosti s silicijem. Pojav oksidacijskega stresa pri obravnavanju z 3 g l^{-1} nano delci Al_2O_3 je bil ovrednoten s kopičenjem malondialdehida (MDA). Dodajanje silicija lahko prepreči nastanek toksičnih znakov, ki jih povzročajo nano delci Al_2O_3 preko zmanjšanja peroksidacije lipidov s povečevanjem aktivnost katalaze kot tudi z omejevanjem njihove translokacije iz korenin v poganjke. Ti izsledki so prvi neposreden dokaz, da predobravnavanje s silicijem zmanjšuje strupenost nano delcev Al_2O_3 pri rastlinah.

Ključne besede: *Hordeum vulgare* L.; malondialdehid; nano-Al_2O_3; nanotoksičnost; silicij

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1 INTRODUCTION

Aluminum (Al) toxicity is one of the main stress factors limiting plant growth and crop yields in acid soils (Wang et al., 2004), which now account for ~40 % of the earth's arable land (Ma et al., 2001; Rahman et al., 2018). To alleviate damaging effect of Al toxicity as well as to prevent root growth inhibition, some plant species used diverse mechanisms such as releasing organic acids that chelate Al, transporting of organic acid anions out of the root cells and forming complexes with organic acids in their leaves, that enable them to grow on acid soils (Ma et al., 2001). Barley is considered to be most sensitive to Al toxicity among cereal species (Wang et al., 2006). Because of the fact that the yield of barley was reduced in acid soils (Fujii et al., 2012), the understanding of the physiological and biochemical mechanisms improving Al tolerance of this species is very important.

Nowadays aluminum oxide (Al_2O_3) nanoparticles (NPs) are one of the most used NPs and developed for applications in cosmetic fillers producing, materials packaging, tools cutting, glass and metal production, etc (Hanemann and Szabó, 2010). Thus these NPs can enter to the waste water streams and may predominantly be applied to agricultural fields (Colvin 2003; Navarro et al. 2008). Regarding to nanotoxicology, many studies have been published concerning the different cytotoxic effects of such nanoparticles on mammalian, animals and bacteria (Wiesner et al., 2006; Lin and Xing, 2007), and only a few studies have focused on the toxicity of NPs to plants (Lee et al. 2008, 2010). Recently, root growth inhibition by 2 g l^{-1} nano-Al_2O_3 was reported for soybean, cabbage, and carrot (Yang and Watts, 2005), tobacco (Burklew et al., 2012) and wheat (Yanik and Vardar, 2015). Since quantitative methods for determining nanoparticles in plant tissues have not been considered, in this study, uptake and accumulation of nano-Al_2O_3 nanoparticles were quantified by Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS) analyses. In this study, we used exogenous Silicon (Si) to mitigate toxicity symptoms induced by Al_2O_3 NPs in barley plants.

Silicon is a beneficial mineral element for plants. A number of studies have been demonstrated that Si is beneficial for the growth of plants, especially those belonging to the family Poaceae (Broadley et al., 2012). Si can mitigate the effects of various environmental stresses such as salinity, drought, chilling, UV radiation (Collin et al., 2014; Zhu and Gong, 2014; Habibi, 2016) and Al and Mn toxicity (Zargar et al., 2019). Exogenous application of Si exhibits the capacity to enhance the plant growth and yield as well as stress tolerance under metal toxicity by reducing the metal uptake and transport in plants (Adrees et al., 2015), formation of silicon bodies in the cell wall (Prabagar et al., 2011) and enhancing the activities of antioxidant enzymes (Habibi, 2014; Shen et al., 2014; Dorneles et al., 2019).

This research was conducted to study the effects of Si application on the amelioration of nano-Al_2O_3 toxicity. According to the best of our knowledge, there is no information in literature regarding the ameliorating effect of Si on nano-Al_2O_3 toxicity in plants. To address this issue, we examined in some detail the biochemical mechanisms by which nano-Al_2O_3 influences the growth, photosynthetic pigments and antioxidant capacity in barley plants. Since the Si alleviates elemental aluminum-induced damages resulting in better plant growth under aluminum toxicity, we hypothesize that Si can also mitigate nano-Al_2O_3 toxicity damages.

2 MATERIALS AND METHODS

2.1 CHARACTERIZATION AND PREPARATION OF NANOPARTICLE SUSPENSION FOR TREATMENT

Aluminum oxide nanoparticles (Al_2O_3 Nanopowder, alpha, 99 %, 80 nm, Hydrophilic) were obtained from US Research Nanomaterials, Inc. The morphology and diameter of Al_2O_3 NPs were also evidenced by scanning electron microscope (SEM, Seron Technology, AIS2100 model) as shown in the Figure 1. After dispersing NPs in distilled water, the solution was sonicated through ultrasonication (230 V/50–60 Hz) for 30 min in order to obtain a homogeneous mixture.

2.2 GROWTH CONDITIONS AND EXPOSURE OF NANOPARTICLES TO PLANT

Seeds of barley (*Hordeum vulgare* 'Bahman') were germinated on filter paper moistened with distilled water. Ten-day-old seedlings were transported to modified Hoagland nutrient solution (Johnson et al., 1957) containing 6 mM KNO_3, 4 mM $Ca(NO_3)_2$, 2 mM $NH_4H_2PO_4$, 1 mM $MgSO_4$, 50 µM H_3BO_3, 2 µM $MnSO_4$, 2 µM $ZnSO_4$, 0.5 µM $CuSO_4$, 0.5 µM H_2MoO_4 and 0.02 mM $FeSO_4$-EDTA for 25 days prior to the treatment procedure. At 25 days after germination, Al_2O_3 nanoparticles (0, 1 and 3 g l^{-1}) and Si (K_2SiO_3, 2 mM) were applied together with the nutrient solution described above. Plants were grown under a temperature regime of 22-25/17-19 °C, relative humidity of 60-65 % and daily pho-

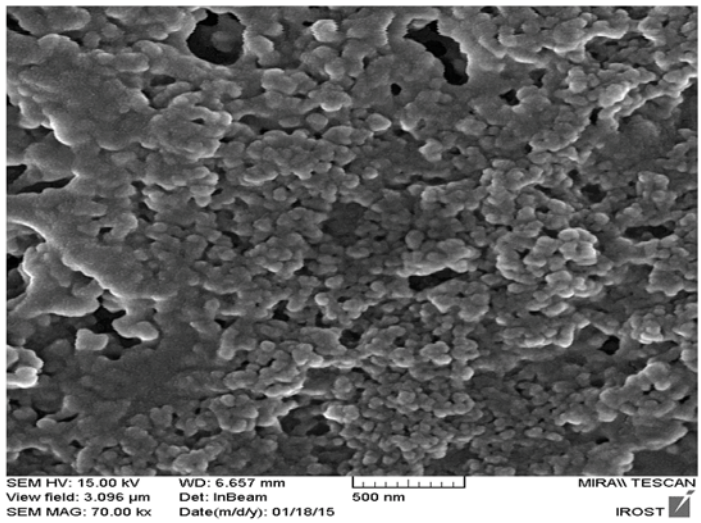

Figure 1: Scanning electron microscopy image of Al_2O_3 nanoparticles when nano-Al_2O_3 was mixed with hydroponic solutions.

ton flux density of about 300-350 µmol m^{-2} s^{-1} throughout the experimental period.

2.3 HARVEST PLANTS

Plants were harvested 14 days after applying the nanoparticles. Fully expanded and mature leaves were utilized for measurement of enzymatic analysis. Shoots and roots were washed with distilled water, blotted dry on filter paper and after determination of fresh mass (FM) they were dried for 48 h at 70 °C for determination of dry mass (DM). Plants height and tap root length were measured using a ruler.

2.4 DETERMINATION OF ALUMINUM

According to Yanik and Vardar (2015), shoot and root samples were oven-dried at 80 °C for 24 h, and mixed with 8 ml 65 % HNO_3 at 175 °C. To quantify Al_2O_3 NPs concentration in shoot and roots, we used Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS) analyses; and the measured concentration of elemental Se in shoot and root samples was normalized by the dried mass of the shoot and root.

2.5 DETERMINATION OF TOTAL CAROTENOIDS AND CHLOROPHYLLS *a* AND *b*

Leaf concentration of chlorophyll and carotenoids was measured according to Lichtenthaler and Wellburn (1985). After extraction of fresh pigments in the cold acetone, the samples stand for 24 h in the dark at 4 °C.

2.6 ASSAY OF ANTIOXIDATIVE ENZYMES AND MALONDIALDEHYDE (MDA) CONTENT

The activities of superoxide dismutase (SOD, EC 1.15.1.1) and catalase (CAT, EC 1.11.1.6) were determined according to methods described elsewhere (Habibi and Hajiboland, 2012). Lipid peroxidation was estimated from the amount of malondialdehyde (MDA) formed in a reaction mixture containing thiobarbituric acid according to methods described elsewhere (Habibi and Hajiboland, 2012).

2.7 STATISTICAL ANALYSES

Experiment was done in complete randomized block design with 4 independent replications. Statistical analysis was carried out using Sigma Stat (3.5) with Tukey test. Results were given as mean ± standard deviation (SD). Differences between treatments were considered to be significant, when a p value was less than 0.05 ($p < 0.05$).

3 RESULTS AND DISCUSSION

3.1 EFFECT OF DIFFERENT NANO-AL_2O_3 CONCENTRATIONS ON ITS UPTAKE AND ACCUMULATION USING ICP-MS

Exogenous Al_2O_3 NPs application increased en-

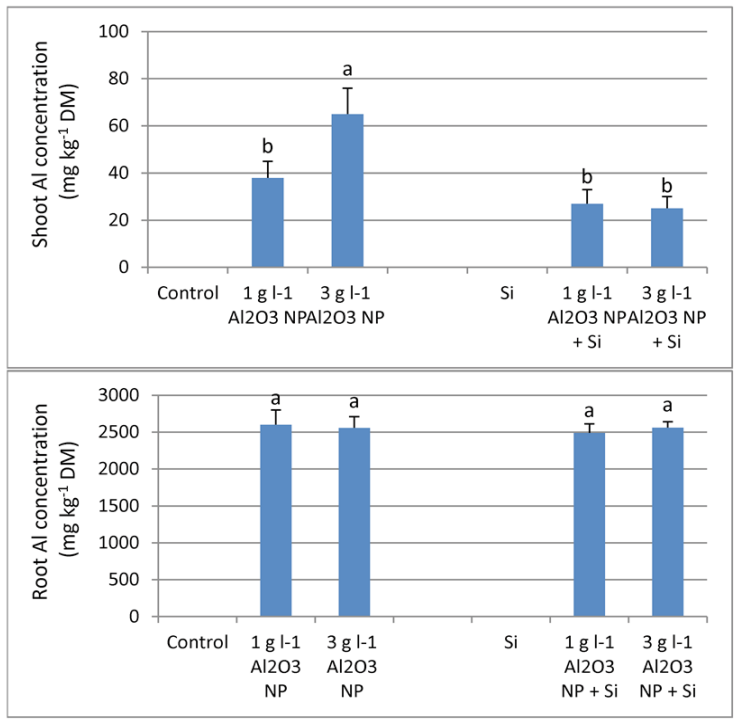

Figure 2: Al_2O_3 nanoparticles accumulation in barley that was recovered by ICP-MS in shoot and root sections of treated plants. Error bars indicate the standard deviation. Bars indicated with the same letter are not significantly different ($p < 0.05$).

dogenous Al_2O_3 NPs contents in shoot and roots of barley plants (Figure 2). Al_2O_3 NPs concentration in plants grown without NP addition was under the analyzing limits. The highest content of Al was found in roots of plants. The most effective uptake and transport of Al was observed for 3 g l^{-1} Al_2O_3 NPs. These results agreed with Asztemborska et al. (2015) who reported that Al_2O_3 NPs was taken up by *Zea mays* L. plants. Indeed, most research have mainly focused on assessing the nature of the safety and toxicity of these nanoparticles (Yanik and Vardar, 2015), but the uptake and entry of nanoparticles into plant systems is still poorly comprehended (Li et al., 2015). In this study, the relatively high concentrations of Al found in shoots grown in the presence of Al_2O_3 NPs strongly indicated the transport of intact particles of Al_2O_3 from the root to the shoot in barley plants.

3.2 Si PRETREATMENT REDUCED NANO-AL_2O_3 ACCUMULATION IN THE SHOOT

Si pretreatment did not reduce nano-Al_2O_3 entry into roots but decreased nano-Al_2O_3 accumulation in the shoot (Figure 3). Recently, authors proposed the formation of aluminosilicate complexes in the cell wall (Prabagar et al., 2011; Adrees et al., 2015). Similarly, possible retention of aluminum in the cell wall has been studied in relation to ameliorating effect of Si on aluminum toxicity in maize (Wang et al., 2004). These authors reported that Si causes higher aluminum tolerance in plants via binding of aluminum to the cell wall. Our results indicated significant effect of supplemental Si on nano-Al_2O_3 concentration in shoots. They are in agreement with the findings of Dorneles et al. (2016) who reported a decrease in aluminum concentration by Si application in the shoots of potato plants. However, there is no information in literature regarding the ameliorating effect of Si on nano-Al_2O_3 toxicity in plants.

3.3 NANO-AL_2O_3 ACTION IS DEPENDENT ON NANO-AL_2O_3 DOSE

Barley growth was negatively affected by nano-Al_2O_3 levels up to 1 g l^{-1}. Although no change was observed in 1 g l^{-1}, NP treatment at 3 g l^{-1} decreased the shoot and root fresh mass, and root elongation with regard to controls (Figure 3). Moreover, shoot and root dry mass was affected negatively by high concentration of nano-Al_2O_3 during the experiment (Figure 4). In this study, the highest applied concentration of Al_2O_3 was about 1.5 times higher than that reported to be toxic (2 g l^{-1}) for

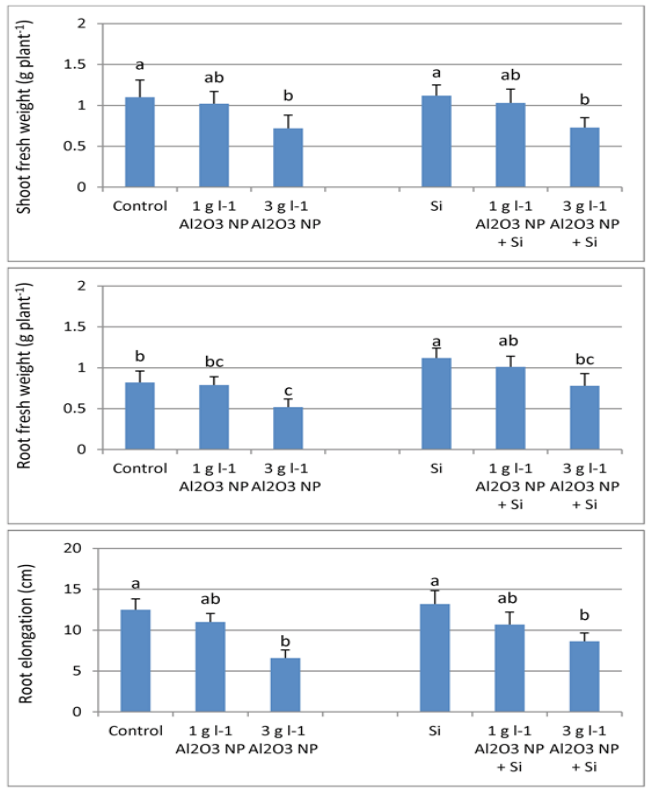

Figure 3: Effects of different concentration of Al_2O_3 NPs on the shoot and root fresh mass, and root elongation of barley seedlings exposed to 2 mM Si for 14 days. Error bars indicate the standard deviation. Bars indicated with the same letter are not significantly different ($p < 0.05$).

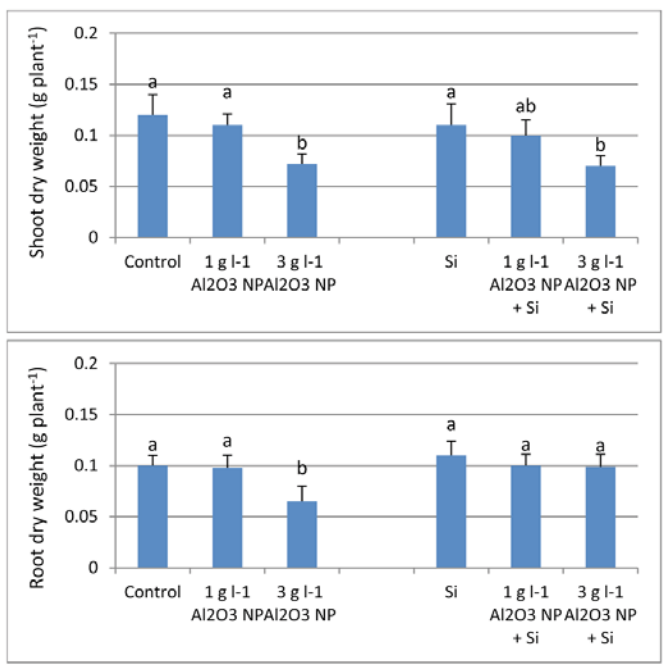

Figure 4: Effects of different concentration of Al_2O_3 NPs on the shoot and root dry mass of barley seedlings exposed to 2 mM Si for 14 days. Error bars indicate the standard deviation. Bars indicated with the same letter are not significantly different ($p < 0.05$).

corn, cucumber, soybean, cabbage, and carrot (Lee et al., 2010). Additionally, our results are in agreement with the findings of Burklew et al. (2012) who reported that root growth and development decreased as the concentration of aluminum oxide nanoparticles increased. Probably, higher concentrations of nano-Al_2O_3 adsorb on the root surface, and interrupt the root functions (Asztemborska et al., 2015). Our results clearly indicated that the diminishing effect of exogenously applied nano-Al_2O_3 on growth parameters of barley plants was dependent on doses of nano-Al_2O_3 used.

3.4 Si PRETREATMENT AMELIORATED TOXIC EFFECTS OF NANO-AL_2O_3 ON ROOT GROWTH

In current study, root growth was reduced by 3 g l^{-1} nano-Al_2O_3, whereas this reduction was alleviated by application of exogenous Si (Figure 4). Exogenous addition of Si can mitigate toxicity symptoms induced by aluminum stress in many plant species (Hammond et al., 1995; Singh et al., 2011; Shen et al., 2014) by enhancing antioxidant protection via modifying the activities of antioxidant enzymes (Shen et al., 2014; Dorneles et al., 2019), and by apoplastic binding of aluminum via formation of aluminosilicate complexes in the cell wall (Wang et al., 2004; Adrees et al., 2015). However, the mechanisms of Si-mediated alleviation of nano-Al_2O_3 stress are still unknown.

3.5 Si PRETREATMENT DID NOT CHANGE THE CONCENTRATION OF PHOTOSYNTHETIC PIGMENTS

Leaf photosynthetic parameters including chlorophyll *b* and carotenoid contents were not significantly influenced under nano-Al_2O_3 stress with or without Si treatment (Figure 5). However, a consistent tendency of chlorophyll *a* to decrease in response to high levels of nano-Al_2O_3 was observed. It has been reported that Si markedly mitigates Al-induced reduction in photosynthetic parameters in peanut plants (Shen et al., 2014). In contrary, our results indicated that the photosynthetic

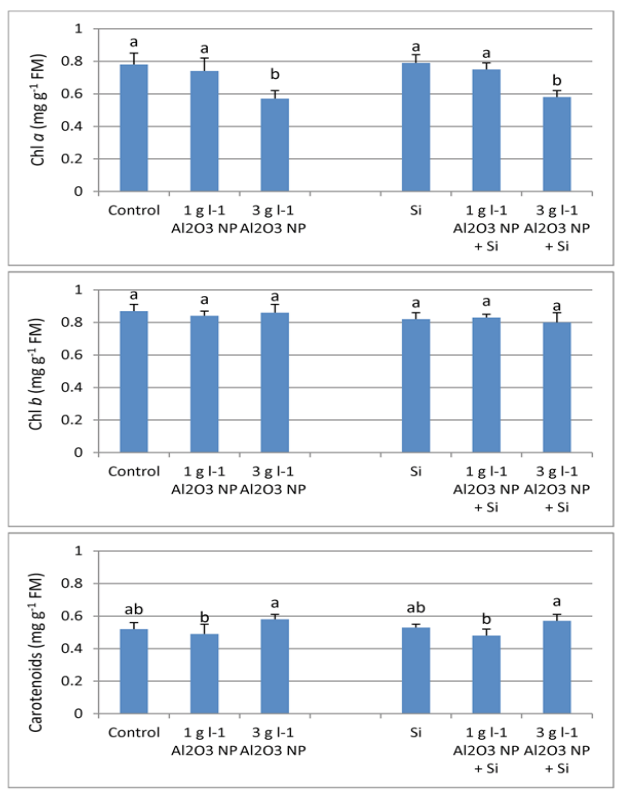

Figure 5: Effects of different concentration of Al_2O_3 NPs on the chlorophyll (Chl) a, b and carotenoid contents in leaves of barley seedlings exposed to 2 mM Si for 14 days. Error bars indicate the standard deviation. Bars indicated with the same letter are not significantly different ($p < 0.05$).

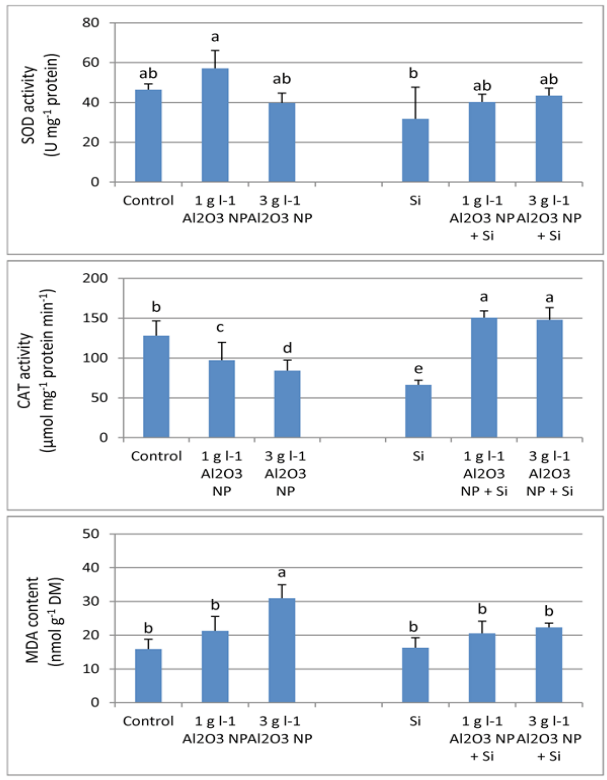

Figure 6: Effects of different concentration of Al_2O_3 NPs on the activity of superoxide dismutase (SOD) and catalase (CAT), and malondialdehyde (MDA) content in leaves of barley seedlings exposed to 2 mM Si for 14 days. Error bars indicate the standard deviation. Bars indicated with the same letter are not significantly different ($p < 0.05$).

pigment concentration was not influenced by Si under nano-Al_2O_3 stress.

3.6 Si PRETREATMENT MITIGATED AL_2O_3 NANOPARTICLE-INDUCED OXIDATIVE STRESS IN BARLEY PLANTS

The activity of SOD was not influenced even under the highest nano-Al_2O_3 levels applied (Figure 6). However, a consistent tendency of CAT activity to decrease in response to nano-Al_2O_3 was observed. We observed that CAT activity was enhanced by Si application in the nano-Al_2O_3-stressed seedlings, which was consistent with the ability of Si to decrease the content of MDA in this plant. We found that the application of 3 g l^{-1} nano-Al_2O_3 was toxic, because it caused the accumulation of MDA, a marker for the ROS-mediated cell membrane damage. However, the MDA content was reduced with Si under nano-Al_2O_3 stress. Similarly, increasing the activity of antioxidant enzymes and mitigating the Al-induced damage to membrane lipids was reported in potato genotypes grown with silicon (Dorneles et al., 2019). Indeed, exogenous addition of Si can mitigate toxicity symptoms induced by aluminum stress via modifying the activities of antioxidant enzymes (Shen et al., 2014; Dorneles et al., 2019; Zargar et al., 2019); however, the mechanisms of Si-mediated inhibition of membrane lipids peroxidation and enhancing the activities of antioxidant enzymes under nano-Al_2O_3 toxicity are still unknown and must be further explored. Furthermore, we showed that Si ameliorated the negative effect of high nano-Al_2O_3 on productivity in barley plants by reducing lipid peroxidation and enhancing antioxidant activity of CAT (Figure 6).

4 CONCLUSION

In summary, we showed that the toxic effect of nano-Al_2O_3 was in a dose-dependent manner. Nano-Al_2O_3 treatment at 3 g l^{-1} decreased the shoot and root mass, and root elongation with regard to controls; however, no changes were observed in 1 g l^{-1}. Si pretreatment ameliorated toxic effects of 3 g l^{-1} nano-Al_2O_3 on root growth. This Si-mediated alleviation of nano-Al_2O_3 toxicity was in parallel with the enhanced antioxidant protection via modifying the activities of antioxidant enzymes and the

restriction of the root-to-shoot translocation of nano-Al_2O_3, as well as lower MDA production.

5 ACKNOWLEDGEMENT

We thank Ali Maleki, University of Kharazmi for providing Scanning Electron Microscopy image of Al_2O_3 nanoparticles.

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doi:10.14720/aas.2021.117.1.1844 Original research article / izvirni znanstveni članek

Variability of some isolates of *Prunus necrotic ringspot virus* and *Prune dwarf virus* infecting sour and sweet cherry in Ukraine

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Received August 20, 2020; accepted February 10, 2021.
Delo je prispelo 20. avgusta 2020, sprejeto 10. februarja 2021.

Variability of some isolates of *Prunus necrotic ringspot virus* and *Prune dwarf virus* infecting sour and sweet cherry in Ukraine

Abstract: *Prunus necrotic ringspot virus* (PNRSV) and *Prune dwarf virus* (PDV) are the most common pathogens in stone crop orchards. These diseases are easily transmitted with pollen and hence rapidly spread in orchards leading to stunting of trees, their increased susceptibility to abiotic stress factors and, eventually, to significant yield losses. In Ukraine, only monitoring studies on the spread of these viruses were conducted until now. However, phylogenetic comparison of Ukrainian isolates was lacking. In this work, total RNA was isolated from plant samples tested positive for PNRSV and PDV by ELISA. The part of viral CP gene sequences were amplified and sequenced with their subsequent phylogenetic analysis. It was determined that PNRSV isolates from Ukraine analyzed in this study belong to different groups – PV-96 (MT828889) and PV-32 (MT892676) with a maximum identity level of 100 % with known isolates from NCBI GenBank. PDV isolates (MT828888 and MT828887) showed high identity with each other (99.6 %), and Slovakian isolate from sweet cherry was shown as the most related to them with identity of 95.3 %.

Key words: PNRV; PDV; ELISA; RT-PCR; sweet cherry virus; phylogenetic analysis

Variabilnost izolatov virusa nekrotične obročkaste pegavosti breskve (PNRSV) in virusa pritlikavosti slive (PDW) na višnji in češnji v Ukrajini

Izvleček: Virus nekrotične obročkaste pegavosti breskve (PNRSV) in virus pritlikavosti slive (PDW) sta najbolj pogosta patogena v sadovnjakih koščičastega sadja. Bolezni se z lahkoto prenašata s cvetnim prahom in se tako hitro širita v sadovnjakih, kar vodi k slabitvi dreves, povečuje njihovo občitljivost na abiotske stresorje in včasih k znatni izgubi pridelka. V Ukrajini so bile do sedaj izvedene le raziskave o razširjenosti teh virusov, ni pa bilo njihovih filogentskih raziskav.V tej raziskavi so bili z ELISA testom analizirani izolati iz vzorčenih rastlin, pozitivnih na PNRSV in PDV. Del zaporedij virusovega CP gena je bilo namnoženih in sekvenciranih za njihovo kasnejšo filogenetsko analizo. Ugotovljeno je bilo, da izolati PNRSV virusov iz Ukrajine, analizirani v tej raziskavi, pripadajo različnim skupinam in sicer skupinama PV-96 (MT828889) in PV-32 (MT892676) z največjo stopnjo istovetnosti 100 % z znanimi izolati iz NCBI GenBank. PDV izolata (MT828888 in MT828887) sta pokazala veliko medsebojno istovetnost (99.6 %), in s slovaškim izolatom iz češnje, ki se je izkazal najbolj soreden z njima z istovetnostjo 95.3 %.

Ključne besede: PNRV; PDV; ELISA; RT-PCR; češnjev virus; filogenetska analiza

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1 INTRODUCTION

Sour cherry (*Prunus cerasus* L.) and sweet cherry (*Prunus avium* L.) are traditional fruit crops in Ukraine. They have an important role in the structure of fruit and berry crop production. According to the State Statistics Service of Ukraine (http://www.ukrstat.gov.ua/), the volume of sour and sweet cherry production in Ukraine in 2019 amounted to 236.13 thousand tons. However, virus diseases are one of the limiting factors for increasing yields.

The most common sour and sweet cherry viruses in the world are *Prunus necrotic ringspot virus* (PNRSV) and *Prune dwarf virus* (PDV) (Caglayan et al., 2011). According to the preliminary data, prevalence of these viruses in mother plant orchard of cherries in Ukraine is 11.8 % for PNRSV and 8.7 % for PDV (Pavliuk et al., 2018). Both viruses belong to *Ilarvirus* genus, Bromoviridae family (Pallas et al., 2012). In fruit trees, infection by these viruses leads to a variety of symptoms, the manifestation of which depends on climatic conditions and the type of host plant (Kamenova et al., 2019).

PDV causes chlorotic rings and spotting, deformation (thinning and curling) of leaf blades, and shortening of internodes (Kamenova et al., 2019). PNRV induce similar symptoms to PDV, and is also manifested by necrotic and chlorotic spots, mosaic and deformation of leaves, flowers and fruits, but often remains latent (Sokhandan-Bashir et al., 2017). PNRSV symptoms may vary depending on the time passed since the initial infection. Immediately after the virus enters the plant ("shock phase"), the most acute necrotic symptoms are typically observed. In the following chronic course of the disease, the symptoms may be partially masked or slightly altered. Also, in the initial infection, the virus delays the flowering on individual branches or of the whole tree, and young leaves are often smaller than those of healthy plants. The spotting pattern may vary depending on the cultivar (Verderevskaya & Marinesku, 1985; Nyland et al., 1976; Wells & Kirkpatrick, 1986).

Mixed infection of plants with PDV and PNRSV leads to the development of more severe symptoms (Caglayan et al., 2011). These two viruses are often found in combination as both are readily transmitted by pollen.

These pathogens are particularly dangerous for sour and sweet cherry, peach, and almond (Umer et al., 2019). Crop yield is reduced due to the decrease in the number of fruit buds caused by these two viruses (Kamenova et al., 2019). Under the influence of these pathogens, peach yield can be reduced by 60 % (Umer et al., 2019). Infection of *Prunus* spp. plants by PNRSV reduces tree growth by 30 %, and reduces yield by 20-56 %, also making the trees very susceptible to low temperatures (Amari et al., 2007).

In susceptible crops, PDV monoinfection can cause yield losses of up to 99 %, also significantly reducing fruit quality (Çağlayan et al., 2011).

In addition to transmission by grafting and other agronomic measures, spreading by pollen is a typical way of transmission (Çağlayan et al., 2011; Pallas et al., 2012). Due to their ability to transmit by pollen, PDV and PNRSV can spread rapidly in stone crop orchards and cause significant economic damage (Amari et al., 2007; Çağlayan et al., 2011; Kamenova et al., 2019; Nemeth, 1986; Umer et al., 2019).

As other ilarviruses, these pathogens have segmented genome consisting of three fragments of single-stranded positive sense RNA (Pallas et al., 2012; Roosinck et al., 2005). PNRSV has isometric virions (Jakab-Ilyefalvi et al., 2011) while PDV can form two types simultaneously – icosahedral and bacilli forms (Kozieł et al., 2017).

RNA1 and RNA2 encode replicase subunits, while RNA3 encodes coat protein (CP) and movement protein (MP). The CP is synthesized from subgenomic RNA (RNA4) derived from RNA3 (Pallas et al., 2012). CP of ilarviruses forms a shell for packaging of genome segments and participates in its activation (Pallas et al., 2013). RNA3 length is 1683 nt for PDV and 1943 or 1951 nt for PNRSV. Ilarvirus MP is required for virus cell-to-cell trafficking (Pallas et al., 2012). For phylogenetic analysis of ilarviruses, CP or MP gene sequences are typically used (Codoñer & Elena, 2006), or partial sequences of *RdRp* (Maliogka et al., 2007).

Viral genome variability contributes to the emergence of new highly virulent isolates, so there is a need for phylogenetic analysis, which allows to determine the variability of the genome and to establish its genetic identity with already known isolates. The aim of the research was to analyse part of CP gene of Ukrainian PNRSV and PDV isolates sampled from sour and sweet cherry trees, and to establish their phylogenetic relationships with known isolates by comparing the nucleotide sequences.

2 MATERIALS AND METHODS

In order to identify the symptoms of viral infection, a visual examination of the mother plantations was performed during the period of the most pronounced manifestation of symptoms – May-June of 2019 year. In order to confirm the presence of viral infection, leaf samples were taken from four spots in tree canopy.

Samples were taken (Table 1) from plants with symptoms typical for to PDV and PNRSV infections, as well as from asymptomatic plants. The latter were select-

ed from trees near to those having the most expressed symptoms. Positive samples previously tested by ELISA were confirmed by RT-PCR. One sample from each infected cultivar was taken for sequencing. These samples included those from sour cherry cultivars Kseniia (PNRSV), Boguslavka (PDV), and a sample of sweet cherry Nizhnist cultivar (PDV and PNRSV).

Extraction of total RNA was performed using RNeasy Plant Mini kit (Qiagen, UK) according to the manufacturer's recommendations. 75 mg of fresh tissue was used for extraction. Extracted RNA was stored at -20 °C. Extraction quality was checked by DeNovix DS-11 spectrophotometer (DeNovix, USA), at wavelength of 260, 260/230, 260/280 nm.

PNRSV and PDV identification was carried out by RT-PCR using primer pairs amplifying a part of CP gene: *PNRSV–10F (*5'-TTC TTG AAG GAC CAA CCG AG AGG-3'*)/PNRSV–10R (*5'-GCT AAC GCA GGT AAG ATT TCC AAG C-3'*)* with expected fragment size of 348 bp, and *PDV–17F (*5'-CGA AGT CTA TTT CCG AGT GGA TGC-3'*)/PDV–12R (*5'-CAC TGG CTT GTT TCG CTG TGA AC-3'*)* with the expected fragment size of 303 bp (Massart et al., 2008.) In order to control RNA amplification, internal control with *nad5-f/nad5-r* primers with expected fragment size of 181 bp was used (Menzel et al., 2002).

In order to conduct RT-PCR, commercial kit Verso 1-Step RT-PCR Kit ReddyMix (Thermo Scientific, USA) was used according to the manufacturer's recommendations. The following components were taken per reaction mixture with a volume of 20 µl: 2X 1 Step PCR ReddyMix – 10 µl, Verso Enzyme Mix – 0,4 µl, RT Enhancer – 1 µl, Primer forward – 0,4 µl (10 mmol), Primer reverse – 0,4 µl (10 mmol), H_2O – 6,6 µl, RNA – 50 ng for one reaction. The same reaction was performed to check RNA quality with *nad5-f/nad5-r* primers. Amplification was performed in «Eppendorf Mastercycler **P**ersonal» programming thermostat (Eppendorf AG, Germany) with the following parameters: 50 °C – 15 minutes, 95 °C – 2 minutes, 40 amplification cycles (95 °C – 20 s, 55 °C – 30 s, 72 °C – 15 s), and 72 °C – 5 minutes.

The presence of amplification fragments was checked by separating the PCR products in 2 % agarose gel, with TBE buffer with the addition of ethidium bromide 0.5 μl^{-1}.

Obtained amplicons of domestic isolates of the *Ilarvirus* genus were sequenced by Sanger sequencing method. The sequences of PNRSV and PDV capsid protein gene were compared with the known sequences in GenBank (www.ncbi.nlm.nih.gov) using function BLAST 2.10.0 software. For phylogenetic analysis, 25 PNRSV and 30 PDV isolates were selected from the GenBank (www.ncbi.nlm.nih.gov), that were extracted from different crops and geographic origin. Multiple sequence alignments were performed by Clustal W algorithm of MEGA 10 (Kumar et al., 2018). Sequences were cut corresponding to the length of our fragment. Construction of the phylogenetic tree was carried out using MEGA 10 software by the methods Neighbour-Joining (NJ) (Saitou & Nei, 1987) using Kimura two parameter. The statistical significance was calculated using 500 bootstrap replicates (Felsenstein, 1985). Standard error of the pairwise identities of groups was calculated using STATISTICA software.

3 RESULTS AND DISCUSSION

Careful examination of the trees revealed leaf de-

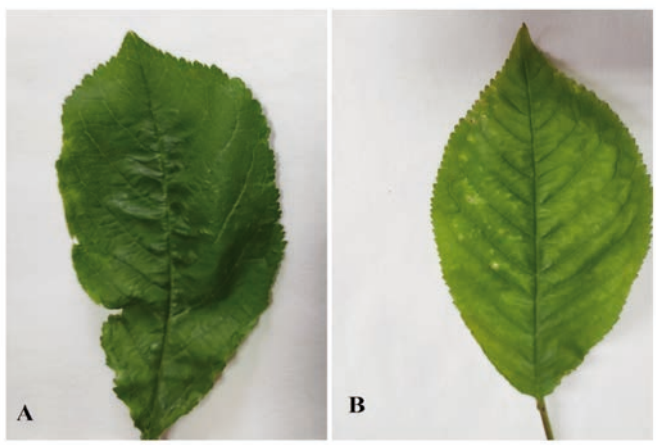

Figure 1: Chlorosis and deformation of the leaf. A – 'Boguslavka' (PDV infected), B – 'Nizhnist' (PDV and PNRSV infected) (virus isolates recovered from these samples were used for phylogenetic analysis)

formations along the veins (Fig. 1A), dark brown spots with small holes and chlorotic areas. It should be noted that chlorosis was the most common symptom among all surveyed trees. These symptoms are characteristic of PDV infection and have been repeatedly described by a number of authors (Massart et al., 2008; Smith et al., 1988; Sanchez et al., 2015; Kamenova et al., 2019).

Chlorotic spots and small necrotic holes were detected on sweet cherry of 'Nizhnist' cultivar (Fig. 1B). The presence of both viruses in the plant was confirmed by ELISA and RT-PCR.

The course of viral diseases is often latent and displays no symptoms of infection. This is especially true when the disease becomes chronic. Therefore, the examination of 'Kseniia' sour cherry trees did not reveal any symptoms of virus infection, although ELISA and RT-PCR diagnostics confirmed virus presence.

Despite many trees showing symptoms similar to virus infection, the presence of pathogens was not confirmed in all samples. This is because the symptoms of virus infection can be very similar to the effects of abiotic factors, lack of nutrients, insect damage etc.

After preliminary ELISA testing, in order to study the molecular characteristics samples were selected: 'Kseniia' infected with PNRSV, 'Bohuslavka' – with PDV and 'Nizhnist' with mixed infection of these viruses (Table 1).

Isolates were named based on the position of infected trees in the orchard. RT-PCR method confirmed the presence of viruses in the tested samples (Fig. 2). As a result, the sequences of isolates PNRSV 4-1 (828889), PNRSV 16-15 (MT892676), PDV 1-67 (MT828888), PDV 16-15 (MT828887) were obtained.

Sequenced fragments of the PNRSV coat protein gene after its alignment had 295 nucleotides corresponding to nts 1601-1891 in RNA3. This part contains a 177 nt part of the coding region (nts 1601- 1777) and a part of UTR. The sequenced fragment of the genome of the Ukrainian isolates PNRSV 4-1 and PNRSV 16-15, which is the C-terminal end of the CP gene, is extremely conserved as it includes the dimerization region at position of 1694-1744 nt (198-215 amino acids) (Aparicio et al., 2006). It should be noted that the presence of a non-coding sequence does not affect the phylogenetic grouping of isolates.

Table 1: Sour and sweet cherry samples tested for PDV and PNRSV by ELISA method

Cultivar	Number of tested samples (n)	ELISA test			Location
		PDV	PNRV	Mixed infection	
Nizhnist (sweet cherry)	6	-	-	1	Zaporizhzhya
Vidrodzhennia (sour cherry)	7	-	-	-	Zaporizhzhya
Boguslavka (sour cherry)	11	4	-	-	Kyiv
Kseniia (sour cherry)	7	-	3	-	Kyiv
Malva (sour cherry)	4	-	-	-	Kyiv
Total	35	4	3	1	-

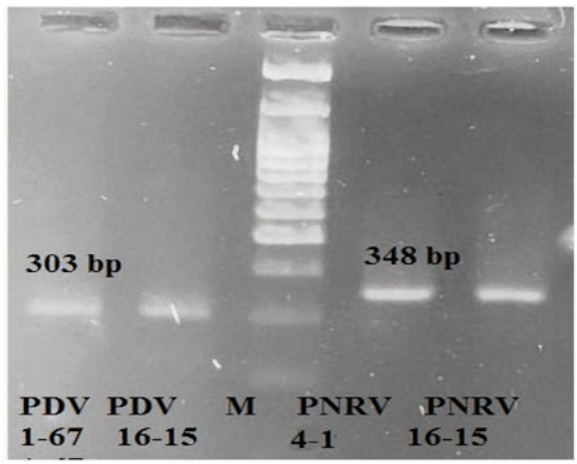

Figure 2: Fragments amplified from extract of infected sour and sweet cherry samples, M – SibEnzyme 100bp molecular weight marker

Based on the molecular characteristics of the nucleotide sequences of the CP and MP genes, PNRSV isolates are divided into three groups: PV32, PE5 and PV96 (Pallas et al., 2012). According to the results of the analysis, Ukrainian isolates extracted from cherry cultivars were attributed to two different groups. PNRSV 4-1 isolate fell into PV96 group while PNRSV 16-15 – into PV32 group. Isolates belonging to the PV96 group are characterized by asymptomatic course of infection, while PV32 can cause severe disease symptoms, and PE-5 can cause both mild and severe symptoms. However, information on pathogenicity of isolates is limited, so virus group affiliation and respective symptoms do not necessarily correlate (Hammond, 2003). In our case, trees of 'Kseniia' infected by the isolate of PV96 group did not show infection symptoms, while the plant of 'Nizhnist' the symptoms were apparent. In the latter case, we cannot say for sure that such manifestation of symptoms depends on the aggressiveness of the isolate, as this sample was also infected with PDV.

The identity between the nucleotide sequences of the whole investigated fragment of PNRSV 4-1 and PNRV 16-15 isolates was 97.9 %, but was 100 % at the amino acid level. The non-coding section turned out to be the most variable 95.5 % identity between the isolates. The identity between the coding fragments was 98.7 %. The difference of 1.3 % between domestic isolates is caused by two nucleotide T/C substitutions that occurred at positions 1642 and 1714 nt.

During the construction of the phylodendrogram, three main clusters were formed. The first cluster (Fig. 3) consisted of 12 isolates representing the PV-96 group, including PNRSV 4-1 isolate. The identity of this isolate with other from the same group ranged from 100 to 99.3 % at the nucleotide level. The most similar isolates were sAJ133205.1 (peach, Italy), AJ133208.1 (nectarine, Spain), JQ005044.1 and JQ005057.1 (sweet cherry and peach, Canada), MH730938.1 (peach, China). The range of pairwise identities within this group was 99.7-99.3 %. This indicates nucleotide substitutions that occur in the middle of the group.

PNRSV 16-15 isolate from Ukraine belongs to PV-32 group in its molecular characteristics. In addition to the Ukrainian isolate, the group includes 10 isolates from the NCBI GenBank. Of this group, the most similar isolates were AJ133213.1 (plum, Italy), MF069040.1 (sweet cherry, Czech Republic), AY948440.1 (rose, In-

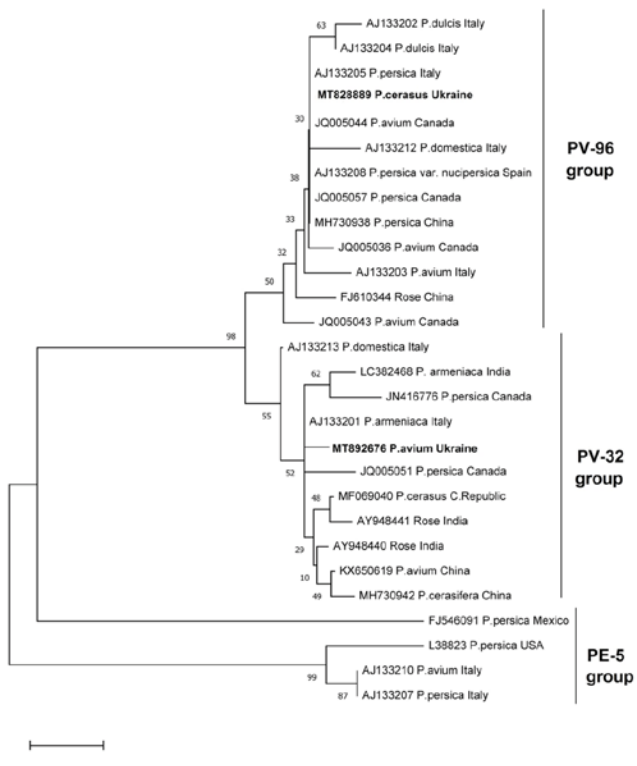

Figure 3: Phylogenetic tree of PNRV isolates, constructed on the basis of sequences of fragments of the CP gene and the UTR using the Neighbour-Joining algorithm option of MEGA 10. Bootstrap analysis of 500 replicates was performed. Sequences obtained in our study are highlighted in bold. The scale bar represents the number of substitutions per nucleotide.

dia), KX650619.1 (sweet cherry, China). Other members of this group were 99.6-98.6% identical to PNRSV 16-15.

Comparison of the nucleotide sequences of the isolates detected nucleotide substitutions, both within the group and between groups. These substitutions were synonymous and did not affect the amino acid sequence. All isolates included in PV 96 and PV32 groups were 100 % identical at the amino acid level.

When comparing this section of the representatives of the two groups, a total of 13 nucleotide substitutions were found which did not affect the amino acid sequence.

The PE-5 group stood out separately. Isolates of this group were characterized by low identity with PV groups. The identity of Ukrainian isolates with representatives of PE-5 was 90.3-91.5 % with PNRSV 4-1 and 89.8-90.7 % with PNRSV 16-15

The analysis showed that the grouping of isolates does not depend on the geographical origin and the host plant. The same conclusions were made in previous studies (Aparicio et al., 1999; Aparicio & Pallás, 2002; Scott et al., 1998), as CP is highly conservative and does not depend on the host plant or geographical area (Sala-Rejczak & Paduch-Cichal, 2013).

Sequenced fragment of the coat protein of PDV with a size of 262 nt, which corresponds to the position of 1340-1601 nt in RNA3. This section encodes 87 amino acids at the position 43-129. The studied fragment is part of ORF3b (Kozieł et al., 2017; Bahman et al., 1994).

When comparing the fragment of the nucleotide sequence of PDV 1-67 and PDV 16-15 isolates, the identity was at the level of 99.6 %, while for the amino acids – 98.8 %. A single nucleotide substitution in PDV 1-67 isolate – A/T at position 1455 resulted in the replacement of the amino acid F (phenylalanine) / Y (tyrosine) at position 81. It should be noted that these substitutions were absent in all other isolates. Also, Ukrainian isolates were characterized by the presence of C nucleotide at position 1519, while all other isolates had T in this position. However, this replacement was synonymous and did not change the protein composition. The range of pairwise identities of PDV 1-67 and PDV 16-15 to other isolates was quite wide and ranged from 95.3 to 85.9 % at the nucleotide level, which indicates a fairly high variability of the genome of isolates. Amino acid sequence identity was slightly higher at 97.7-86.5 %, indicating that some amino acid substitutions were still synonymous. In total, in the analysis of all studied isolates, 24 positions in which amino acid substitutions took place were calculated.

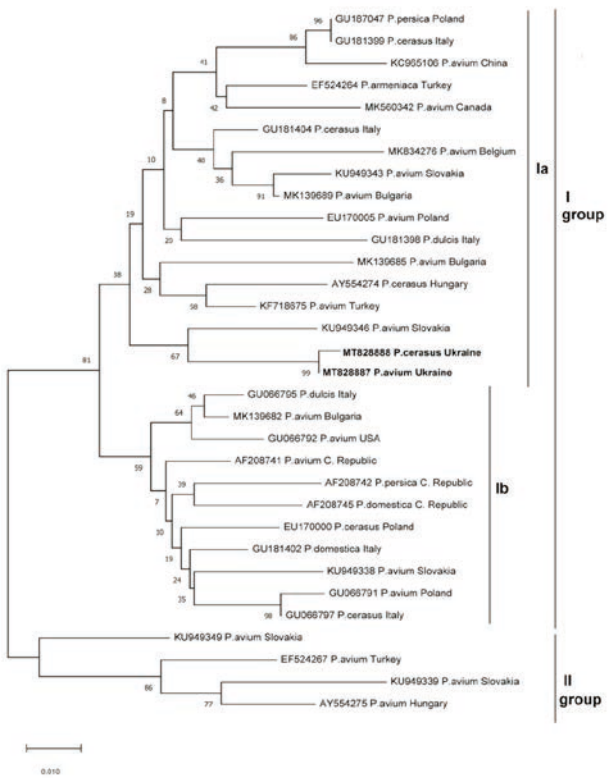

Figure 4: Phylogenetic tree of PDV isolates, constructed on the basis of sequences of fragments of the CP gene using the Neighbour-Joining algorithm option of MEGA 10. Bootstrap analysis of 500 replicates was performed. Sequences obtained in our study are highlighted in bold. The scale bar represents the number of substitutions per nucleotide.

When constructing a phylogenetic tree, all isolates were divided into two main groups (Fig. 4). The first group included two subgroups. Subgroup Ia consisted of 17 isolates, which differed in geographical origin and host plant. The Ia subgroup also included Ukrainian isolates. KU949346 isolate (sweet cherry, Slovakia) was the closest in amino acid and nucleotide sequences. The level of identity by nucleotide sequences was 95.3 % with PDV 16-15 and 94.9 % with PDV 1-67 and 98.8 and 97.7 % by amino acid sequences, respectively. Identity of this subgroup with other isolates varied between 94.4-88.3 %, while the mean pairwise identity within this subgroup was 93.81 %. Subgroup Ib included 11 isolates. The range of pairwise identities with PDV 1-67 and PDV 16-15 was 93.6-91.4 % by nucleotide sequences. Isolates of this group were more similar to each other – 95.97 %. The overall mean identity of all isolates of the first group was 93.41 % (s_e = 0.3).

The second group was formed only by isolates of sweet cherry as the host plant while the geographical distribution of these isolates was different. In most previous studies, no grouping based on the host plant or geographical origin was observed (Vaskova et al., 2000; Pallas et al., 2012, Öztürk & Çevik, 2015, Predajňa et al., 2017). While study of PDV isolates from Turkey revealed four groups. The first and second groups contained only isolates of cherry and almond, respectively, while the third and fourth contained isolates extracted from different host plants (Ulubaş Serçe et al., 2009). Identity of this group with PDV 1-67 and PDV 16-15 isolates ranged from 90.9 to 85.9 % by nucleotide sequences. In general, this group was the most distant in nucleotide sequences with the mean identity of the isolates 93.67 % (s_e = 0.6).

Construction of the phylodendrogram based on amino acid sequences showed similar results of isolate grouping into two groups. The same results were obtained in a previous study, which analyzed the amino acid sequences of the CP gene of isolates contained in the GenBank (Pallas et al., 2012).

Thus, PDV isolates that differ in molecular characteristics may circulate on the territory of Ukraine, although they are extracted from host plants of the same species and have a common geographical origin.

4 CONCLUSIONS

Based on of nucleotide sequence analysis of PNRSV, it was determined that isolates belonging to PV-96 and PV-32 groups circulate in Zaporizhzhya and Kyiv regions, respectively.

The manifestation of symptoms may differ based on the group explaining the fact that some infected PNRSV trees show no signs of viral infection.

Clustering of PNRSV isolates confirms the presence of three groups that do not relate to the host plant and geographical distribution. Nucleotide substitutions affecting clustering of isolates have been established.

Phylogenetic analysis revealed that PDV isolates formed two groups with two subgroups, and in our case the second group included isolates only from sweet cherry from different countries.

High similarity of Ukrainian PDV isolates with each other and the distance with known isolates may indicate the existence of a separate geographical origin based group.

This research should be continued with the involvement of a wider range of isolates from other regions of Ukraine as well as other crops.

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doi:10.14720/aas.2021.117.1.1656 Original research article / izvirni znanstveni članek

Discrimination of drought tolerance in a worldwide collection of safflower (*Carthamus tinctorius* L.) genotypes based on selection indices

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Received May 11, 2020; accepted February 28, 2021.
Delo je prispelo 11. maja 2020, sprejeto 28. februarja 2021.

Discrimination of drought tolerance in a worldwide collection of safflower (*Carthamus tinctorius* L.) genotypes based on selection indices

Abstract: Improvement of elite safflower genotypes for drought-tolerance is hampered by a deficiency of effective selection criteria. The present study evaluated 100 genotypes of safflower in terms of their drought tolerance over a period of three years (2016–2018) under both non-stress and drought-stress conditions. The eight drought-tolerance indices of tolerance index (TOL), mean productivity (MP), geometric mean productivity (GMP), stress susceptibility index (SSI), stress tolerance index (STI), yield stability index (YSI), drought resistance index (DI), and harmonic mean (HARM) were calculated based on seed yield under drought (Y_s) and non-drought (Y_p) conditions. A high genetic variation was found in drought tolerance among the genotypes studied. The MP, GMP, and STI indices were able to discriminate between tolerant and drought-sensitive genotypes. Plots of the first and second principal components identified drought-tolerant genotypes averaged over the three study years. Cluster analysis divided the genotypes into three distinct groups using the drought tolerance indices. Ultimately, eight genotypes (namely, G_3, G_{11}, G_{13}, G_{24}, G_{33}, G_{47}, G_{58}, and G_{61}) from different origins were detected as more tolerant to drought stress suitable for use in safflower breeding programs in drought-affected areas. The most tolerant and susceptible genotypes could be exploited to produce mapping populations for drought tolerance breeding programs in safflower.

Key words: cluster analysis; drought stress; principal component analysis; selection index; yield; safflower

Abbreviations: TOL: Tolerance; MP: mean productivity; SSI: drought susceptibility index GMP: geometric mean productivity; YSI: yield stability index; DI: drought resistance index.

Odkrivanje tolerance na sušo v mednarodni zbirki genotipov žafranike (*Carthamus tinctorius* L.) na osnovi izbranih indeksov

Izvleček: Izboljšanje elitnih genotipov žafranike na prenašanje suše ovira pomanjkanje učinkovitih selekcijskih kriterijev. V raziskavi je bilo ovrednoteno 100 genotipov žafranike glede na njihovo prenašanje suše v obdobju treh let (2016–2018) v razmerah brez stresa in razmerah sušnega stresa. Izračunanih je bilo osem indeksov tolerance na sušni stres kot so tolernca na stres (TOL), poprečna produktivnost (MP), geometrijska poprečna produktivnost (GMP), indeks stresne občutljivosti (SSI), indeks stresne tolerance (STI), indeks stabilnosti pridelka (YSI), indeks odpornosti na sušo (DI), in harmonično poprečje na osnovi pridelka semena v sušnih (Y_s) in nesušnih (Y_p) razmerah. Med preučevanimi genotipi je bila ugotovljena velika genetska variabilnost v toleranci na sušo. Z indeksi MP, GMP, in STI je bilo mogoče razlikovati na sušo tolerantne in občutljive genotipe. Polja prve in druge glavne komponente so določila na sušo tolerantne genotipe v vseh treh letih raziskave. Klasterska analiza je z uporabo indeksov tolerance na sušo razdelila genotipe v tri jasno ločene skupine. Na koncu je bilo ugotovljenih osem genotipov (G_3, G_{11}, G_{13}, G_{24}, G_{33}, G_{47}, G_{58}, in G_{61}) različnega izvora, ki so bili bolj tolerantni na sušo in so primerni za uporabo v žlahtniteljskih programih žafranike na od suše ogroženih območjih. Na sušo najbolj prilagojene genotipe žafranike bi lahko uporabili za odkrivanje populacij, ki bi bile primerne pri žlatnjenju žafranike na sušo.

Ključne besede: klasterska analiza; sušni stres; analiza glavnih komponent; selekcijski indeks; pridelek; žafranika

Okrajšave: TOL: toleranca; MP: poprečna produktivnost; SSI: indeks občutljivosti na sušo; GMP: geometrijska poprečna produktivnost; YSI: indeks stabilnosti pridelka; DI: indeks odpornosti na sušo.

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1 INTRODUCTION

Droughts due to alterations in rainfall patterns and climate change form a most devastating factor in food production on a global scale (Blum, 2018; Anjum et al., 2017). This warrants a Blue Revolution in agriculture concentrated on increasing productivity per unit of water to produce more crops per drop of water. Recently, an important target in crop breeding programs is the development of drought-tolerant genotypes that possess a high capability for adaption to arid and semi- arid climates (Kirigwi et al., 2004; Basu et al., 2016).

The challenges in understanding the mechanisms involved in plant behavior under water scarcity include: 1) mutagenic control of drought tolerance, 2) genetic variability and differences among species in responding to changes in water availability, and 3) interactions with other factors such as drought stress duration and intensity (Varshney et al., 2018). Moreover, breeding programs are adversely affected by the high interaction of genotype × environment, low heritability of drought tolerance traits, lack of efficient selection particularly under field conditions, and the difficulties associated with simultaneous selection, sharp climate changes, and unpredictable rainfall in different regions (Ashraf, 2010; Rauf et al., 2016; Blum, 2018). Since the genotypes with a high yield under optimum conditions may not be drought tolerant (Blum, 2018), many studies preferred selection under both stress and non-stress conditions (Fernandez, 1992).

To have a high and durable yield in a drought-prone environment drought-tolerant genotypes are needed (Abdolshahi et al., 2012). The capacity of genotypes to perform reasonably well in drought-stressed environments is the paramount reason for their stable production (Raman et al., 2012). To decrease the impacts of abiotic stress without any substantial yield loss, researchers tend to develop drought-tolerant genotypes based on prior evaluation and identification of drought-tolerant germplasm. The high cost of drought soil amelioration has encouraged breeders to use selection indices as an economic and efficient method for resolving the problems associated with drought stress breeding (Vieira et al., 2016).

In this regard, a variety of selection indices to identify stress-tolerant cultivars have been proposed that some of the important and most applicable of them include: Tolerance (TOL) (Rosielle $ Hamblin, 1981) (Table 1) , mean productivity (MP) (Rosielle & Hamblin, 1981) (See Table 1), stress susceptibility index (SSI) (Fischer and Maurer, 1978) (See Table 1), geometric mean productivity (GMP) (Kristin et al., 1997) (See Table 1), stress tolerance index (STI) (Fernandez, 1992) (See Table 1), yield stability index (YSI) (Gavuzzi et al., 1997) (See Table 1), and drought resistance index (DI) (Lan, 1998) (See Table 1). Our literature review have reported on the efficiency of different selection indices for selecting drought-tolerant genotypes in different crops as like as rice (Raman et al., 2012); canola (Khalili et al., 2012);sunflower (Gholinezhad et al., 2014); maize (Hao et al., 2011) and bread wheat (Abdolshahi et al., 2012).

Safflower (*Carthamus tinctorius* L.) is an annual oil

Table 1: Different drought tolerance indices used for screening safflower genotypes

Index name	Equation	Reference
Mean productivity	$MP = \frac{Y_s + Y_p}{2}$	Rosielle and Hamblin, 1981
Tolerance index (TOL)	$TOL = Yp - Ys$	Ficsher and Maurer, 1978
Geometric Mean Productivity (GMP)	$GMP = \sqrt{(Yp)(Ys)}$	Kristin et al., 1997
Stress Susceptibility Index (SSI)	$SSI = \frac{1 - \left(\frac{Ys}{Yp}\right)}{1 - \left(\frac{\overline{Ys}}{\overline{Yp}}\right)}$	Rosielle and Hamblin, 1981
Stress Tolerance Index (STI)	$STI = \frac{Ys \times Yp}{\overline{Yp}^2}$	Fernandez, 1992
Yield Stability Index (YSI)	$YSI = \frac{Ys}{\overline{Y_p}}$	Gavuzzi et al., 1997
Drought Resistance Index (DI)	$DI = \frac{Ys \times \left(\frac{Ys}{Yp}\right)}{\overline{Ys}}$	Lan, 1998
Harmonic Mean	$HARM = 2(Y_p \times Y_s)/(Y_p + Y_s)$	Kristin et al., 1997

seed crop with diverse industrial and pharmaceutical application that is grown commercially in Iran (Golkar & Karimi, 2019). The deep roots of safflower make it a drought-tolerant plant viable under the drought stress conditions in arid climates (Mirzahashemi et al., 2014; Hussain et al., 2016).

Drought stress is one of the most devastating abiotic stresses that poses a serious threat to worldwide safflower production (Hussain et al., 2016). Given the declining water resources in the arid and semi- arid regions of the world due to consecutive droughts, increased safflower cultivation can be an economic and valuable alternative to other drought-tolerant genotypes. In this regards, some studies is known about drought tolerance of local Iranian cultivars (Omidi et al., 2012; Bahrami et al., 2014; Mirzahashemi et al., 2014). Despite of current efforts intended for assessing tolerance criteria based on tolerance indices in safflower, little has been reported at maturity (Bahrami et al., 2014). Furthermore, this tolerance undoubtedly appears to be stage-dependent and must be evaluated at the yielding phase.

Variations in drought patterns such as differences in location, year, and drought intensity as well as genotypic differences call for safflower genotypes with different levels of drought tolerance to be cultivated in different areas. However, the differences in the genotypes recommended might have stemmed from the variability in the drought tolerance potential of safflower genotypes. Moreover, climate changes increase drought frequency in some regions but drought is a global issue. Given the broad distribution of safflower around the world, it is the objective of the present study to identify drought-tolerant genotypes from a new worldwide collection based on drought selection indices. The new identified genotypes could be used for cultivation in arid regions of the world.

2 MATERIALS AND METHODS

2.1 PLANT MATERIAL

One hundred safflower genotypes originating from different geographical regions of the world were selected for screening drought tolerance (Table S1). The exotic genotypes were obtained from Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Germany. Iranian genotypes were taken from the genotype inventory at the Agricultural Research Center, Isfahan, Iran.

2.2 FIELD EXPERIMENT AND IRRIGATION REGIMES

An experiment was carried out in three consecutive years from early March 2016 to the end of 2018 at Lavark Research Farm, affiliated to Isfahan University of Technology, 40 Km southwest of Isfahan (32° 32′N, 51° 23′ E, 1630 m above sea level), Iran. Mean annual precipitation and temperature at this site are 149 mm and 15.4°C, respectively. The soil was silty clay loam with a bulk density of 1.3 g cm^{-3} in the top 50 cm and a pH level of 7.4-7.9. The field experimental design was a square lattice design (10 by 10) with two replications for each different irrigation (drought stress and non-drought) regimes in each year. The seeds were planted in rows of 3 m long and spaced 25 cm from each other to yield a plant density of 40 plants m^{-2} in the plots. All the plants received the first irrigation before planting. After this period, irrigation was applied every week until the budding stage. From budding stage to full maturity stage, the non-stress treatment involved irrigation when 50 % of the total available water was depleted from the root zone, but in the drought stress conditions, irrigation was applied when 85 % of the total available water was depleted from the root zone.

The irrigation interval (number of days between two irrigations) during the growing season (budding to full maturity) was variable because of the variation in evapotranspiration (ET). Soil samples were taken from a depth of 0 to 60 cm of the soil from both drought and non- drought plots to determine the soil water content and calculate the irrigation water content on the basis of a 60 cm rooting depth. Soil samples were taken before each irrigation when evaporation from a Class A pan indicated 70 and 140 mm of evaporation under normal and drought-stress conditions, respectively.

Then, irrigation depth was determined using the formulae: $I = [(\theta_{FC} - \theta_i)/100]\ D \times B)$; where, I represents irrigation depth (cm), θ_{FC} (–0.03 MPa) is soil gravimetric moisture percentage at field capacity (22 %), θi (–1.5 MPa) is soil gravimetric moisture percentage at irrigation time (10 %), D is root-zone depth (50 cm), and B is soil bulk density at the root zone (1.3 g cm^{-3}) (Clarke et al., 2008). The volume of irrigation water applied was monitored at each irrigation by calculating the depth of water over a Parshall flume which was calculated as: $Id = I \times p$, where p is the fraction of I that can be depleted from the root zone. Then $I_g = (I_d/Ea) \times 100$, which E_a is irrigation efficiency (%), assumed to be 65 % on the average. The differences in available water related to different mean for temperature in growing seasons across three years of study. No growth regulators or fungicides were applied. Surface application of 130 (kg ha^{-1}) N and 25 (kg ha^{-1}) P was carried out in both treatments with an additional 55 kg ha^{-1} of N during the rosette stage. Plants were harvested in the middle row at maturity and seed yield was recorded in each plot. Ten different selection indices were calculated using the equations reported in Table 1. In these equations, Y_S represents the

yield of genotypes under stress; Y_p, the yield of genotypes under normal conditions (kg ha^{-1}); and denote mean yields of all the genotypes under stress and non-stress conditions, respectively.

2.3 STATISTICAL ANALYSIS

A combined analysis of variance (ANOVA) was performed using SAS software (SAS. Ver. 9.3.1), for seed yield and selection indices using GLM procedure. Principal Component Analysis (PCA) and 3D biplot diagrams were exploited to identify tolerant and susceptible genotypes using R software (Ver. 3.6.1). Correlations between seed yield in the non-stress and drought-stress treatment as well as the relevant drought tolerance indices were determined using SAS PROC CORR and Heat Map Graph (R software ver 3.6.1). The safflower genotypes were classified using the seed yields obtained from each of the water treatments and drought tolerance indices data using the Ward algorithm based on the squared Euclidean distances in the R Software (Ver. 3.6.1).

3 RESULTS

Analysis of variance indicated the non-significant effect of year on all the studied traits (Table 2). A highly significant ($p < 0.01$) variation in seed yield was observed among the studied genotypes under both (stress and non-stress) conditions and for all the tolerance indices examined (Table 2). The genotype × year interaction effect was not significant for any of the indices, except for DI and HARM (Table 2). The significant genotype × environment interaction for both DI and HARM, indicating considerable variability among the genotypes across different years and different irrigation treatments for these selection indices.

Table 3 reports the ten highest and the lowest seed yields, Y_p and Y_s, for the studied genotypes. Clearly, the highest Y_p values were obtained for G_{13} (5680 kg ha^{-1}) (from Iran) and G_{61} (5310 kg ha^{-1}) (from Morocco), but the least Y_p value was obtained for G_{79} (900 kg ha^{-1}). Under stress conditions, the highest seed yield (Y_s) was obtained in genotypes 47 (3038 kg ha^{-1}) and 24 (2670 kg ha^{-1}), but the lowest (590 kg ha^{-1}) was observed in G_{79} (Table 3). G_{13} recorded the highest values of TOL (4130), SSI (1.61), and HARM (1.14) indices, whereas G_{86} (from Tajikistan) recorded the least values for TOL (260), SSI (0.24), and HARM (0.13). The highest (0.87) and the lowest (0.27) values of YSI were obtained for G_{86} and G_{13}, respectively. Finally, the genotypes 47 and 25 had the highest (1.55) and lowest (0.20), respectively, mean values of the DI index (Table 3).

The correlation coefficients among Y_p, Y_s, and other drought tolerance selection indices were calculated to determine the most desirable drought tolerance criteria (Table 4). It was found that seed yield and YSI exhibited negative (- 0.5**) and positive (0.34**) correlations under the non-stress and stress conditions, respectively. Seed yield under the non-stress treatment showed positive and significant correlations with all the selection indices, except for YSI (-0.50**) and DI (Table 4). Seed yield under the stress treatment showed positive and significant correlations with MP, GMP, STI, and DI but negative and significant ones with SSI and HARM (Table 4).

Principal Component Analysis (PCA) as a representative for distinguish the relationships among the indices revealed that the first component (PC_1) explained 54 % of the total seed yield variation and exhibited positive correlations with Yp, MP, GMP, and STI (Figure 1). PC_2 explained 44 % of the total yield variation and had higher positive correlations with DI, YSI, and Ys but higher negative correlations with SSI and HARM (Figure 1).

Table 2: Combined analysis of variance for seed yield under non-stress (Y_p) and stress (Y_s) conditions and different susceptibility indices in safflower genotypes growing under drought stress and normal conditions evaluated in 2016 and 2018

S.O.V	DF	Y_p	Ys	SSI	YSI	TOL	MP	GMP	STI	DI	HARM
Year (Y)	2	3947374.2	77841	0.00019	0.05	133849.6	29145.2	303800.5	0.047	0.34	0.2
Block/ Year	3	282227.2	117545.25	0.36	0.06	696640.8	25726.0	14486.5	0.003	0.13	0.18
Genotype (G)	99	3305183.4**	844416.53**	0.40**	0.08**	2177268.3**	1530482.9**	1260048.7**	0.53**	0.34	0.23**
G × Y	198	89064.9	80535.49	0.071	0.01	143568.0	48908.2	55476.1	0.028	0.05**	0.54**
Residual	297	89064.9	154372.9	0.12	0.025	350774.0	122390.6	125950.5	0.06	0.09	0.07

* and **, significant at $p < 0.05$ and $p < 0.01$, respectively. Abbreviations: DF: degree of freedom; Y_p: seed yield under non-stress; Y_s: seed yield under stress; SSI: stress susceptibility index, YSI: yield stability index; TOL: stress tolerance; MP: mean productivity; GMP: geometric mean productivity; STI: stress tolerance index; DI: Drought Resistance Index; and HARM: Harmonic mean

Table 3: Ten highest and lowest values for seed yield under non-stress conditions (Y_p), Seed yield under stress conditions (Y_s), and different selection indices among the 100 different safflower genotypes investigated

Ten highest indices	YP¥ (kg ha^{-1})	YS (kg ha^{-1})	SSI	YSI	TOL	MP	GMP	STI	DI	HARM
	5680(G13)	3080(G47)	1.613(G13)	0.8724(G86)	4130(G13)	3985(G47)	3820.2(G47)	2.2172(G47)	1.5516(G47)	1.1488(G13)
	5310(G61)	2670(G24)	1.58(G61)	0.8552(G50)	3770(G61)	3615(G13)	3233.2(G24)	1.6356(G11)	1.3097(G24)	1.1104(G61)
	4910(G58)	2390(G11)	1.5697(G25)	0.84(G99)	3140(G58)	3425(G61)	3159.8(G11)	1.5709(G24)	1.1934(G94)	1.1069(G76)
	4890(G47)	2385(G3)	1.5629(G76)	0.8148(G38)	2800(G76)	3380(G11)	3046.4(G3)	1.4443(G3)	1.1672(G97)	1.1029(G25)
	4520(G33)	2350(G97)	1.5285(G18)	0.8042(G81)	2760(G33)	3340(G58)	2957.2(G13)	1.33(G13)	1.15(G69)	1.0677(G18)
	4370(G11)	2167.5(G48)	1.5057(G59)	0.7948(G94)	2750(G18)	3305(G24)	2931(G58)	1.2916(G58)	1.1144(G48)	1.03(G59)
	3980(G27)	2160(G2)	1.4701(G27)	0.7841(G41)	2690(G27)	3142.5(G3)	2846.3(G61)	1.2356(G61)	1.1077(G99)	1.0095(G27)
	3980(G18)	2130(G63)	1.4483(G66)	0.7749(G12)	2450(G59)	3140(G33)	2824.3(G97)	1.215(G97)	1.0974(G63)	0.9724(G66)
	3940(G24)	2118.3(G94)	1.4284(G58)	0.7713(G69)	2440(G25)	2880(G97)	2817.8(G33)	1.2063(G33)	1.0941(G86)	0.97(G21)
	3900(G3)	2100(G69)	1.4258(G21)	0.7668(G37)	2300(G66)	2845(G2)	2759(G2)	1.1386(G2)	1.0793(G11)	0.9508(G58)
Ten lowest indices	Y_P (kg ha^{-1})	Y_S (kg ha^{-1})	SSI	YSI	TOL	MP	GMP	STI	DI	HARM
1	2660(G8)	930(G78)	1.3358(G95)	0.7411(G10)	1915(G39)	2660(G57)	2568.3(G48)	1.0026(G48)	1.0463(G96)	0.8926(G95)
2	1510(G81)	920(G95)	0.5084(G69)	0.3546(G21)	410(G71)	1210(G67)	1167.2(G64)	0.209(G31)	0.3026(G79)	0.2604(G69)
3	1460(G64)	920(G87)	0.4939(G12)	0.3461(G66)	380(G99)	1200(G64)	1161.3(G31)	0.2038(G64)	0.3007(G95)	0.2553(G12)
4	1450(G67)	910(G37)	0.4824(G41)	0.3334(G27)	340(G38)	1175(G71)	1155.7(G71)	0.2032(G71)	0.3003(G66)	0.2536(G41)
5	1380(G89)	890(G21)	0.4545(G94)	0.3204(G59)	339(G5)	1160(G89)	1138.2(G89)	0.1942(G89)	0.2884(G18)	0.2367(G94)
6	1380(G71)	860(G31)	0.4392(G81)	0.3093(G18)	320(G81)	1150(G9)	1071.8(G9)	0.173(G9)	0.2834(G87)	0.2299(G81)
7	1350(G82)	801(G5)	0.4147(G38)	0.2939(G76)	310(G79)	1070(G82)	1046.6(G37)	0.1647(G37)	0.2647(G59)	0.208(G38)
8	1210(G37)	790(G82)	0.3429(G99)	0.2904(G25)	300(G50)	1060(G37)	1026(G82)	0.1582(G82)	0.2497(G21)	0.1803(G99)
9	1140(G5)	780(G9)	0.3284(G50)	0.2884(G61)	300(G37)	970.5(G5)	952.4(G5)	0.1363(G5)	0.2346(G76)	0.1587(G50)
10	900(G79)	590(G79)	0.2846(G86)	0.2719(G13)	260(G86)	745(G79)	718.9(G79)	0.0788(G79)	0.2024(G25)	0.1392(G86)

Table 4: Correlation coefficients between seed yield (kg ha^{-1}) of safflower genotypes under non-stress (Yp) and stress (Ys) conditions and each of the stress susceptibility indices averaged over three years

	Y_p	Y_s	SSI	YSI	TOL	MP	GMP	STI	HARM	DI
Y_p¥	1									
Y_s	0.59**	1								
SSI	0.50**	-0.34**	1							
YSI	-0.50**	0.34**	-0.99**	1						
TOL	0.86**	0.10	0.83**	-0.83**	1					
MP	0.95**	0.80**	0.24*	-0.24*	0.67**	1				
GMP	0.88**	0.89**	0.08	-0.08	0.52**	0.98**	1			
STI	0.86**	0.88**	0.06	-0.06	0.50**	0.96**	0.98**	1		
HARM	0.53**	-0.32**	0.99**	-0.99**	0.86**	0.27**	0.10	0.08	1	
DI	0.17	0.89**	-0.70**	0.70**	-0.31**	0.47**	0.61**	0.61**	-0.67**	1

* and ** Significant at $p < 0.05$ and $p < 0.01$; respectively; ns, not significant.
Abbreviations ¥: Y_p: Seed yield under non- stress condition; Y_s: Seed yield under stress condition; SSI: stress susceptibility index, YSI: yield stability index, TOL: stress tolerance, MP: mean productivity, GMP: geometric mean productivity, STI: stress tolerance index, HARM: harmonic mean, DI: drought resistance index.

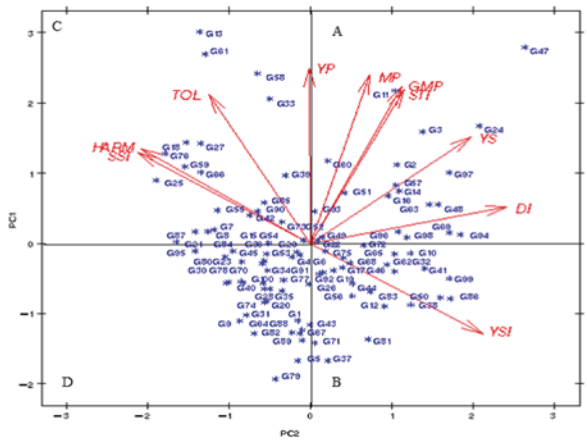

Figure 1: Biplot drawn based on the first and second components obtained from principal component analysis using seed yield of safflower genotypes under non stress (Y_p) and stress (Y_s) conditions. Abbreviations: stress susceptibility index (SSI), yield stability index (YSI), stress tolerance (TOL), mean productivity (MP), geometric mean productivity (GMP), stress tolerance index (STI), drought resistance index (DI); harmonic mean (HARM), and conditions in 100 safflower genotypes.

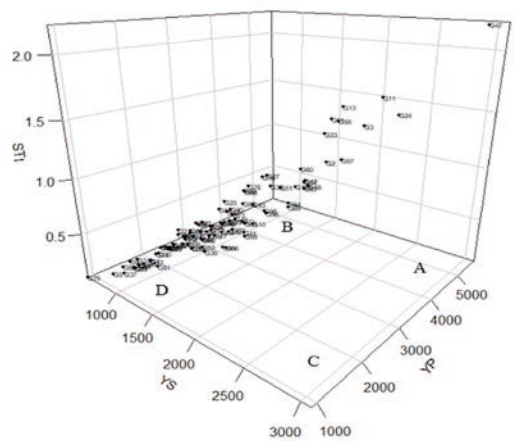

Figure 2: Three-dimensional diagram for identifying drought-tolerant genotypes based on seed yield under non-stress (Y_p) and stress (Y_s) conditions as well as the stress tolerance index (STI).

Because of the positive and significant correlation of STI with seed yield under both conditions, a three-dimensional graphs based on the STI index were drawn (Figure 2). These graphs split the genotypes into four groups, each of which represents one combination of the genotypes. The genotypes 47, 24, 97, 3, and 11 (Group A) are those with high yields under drought and non-stress environments. Those in Group B (e.g., G_{13}, G_{59}, and G_{61}) consisted of genotypes with high yields in a normal environment but low seed yields under drought conditions. No genotype was, however, detected as one with a high yield in a stressful environment (Group C). The genotypes with low yields under both environmental conditions were assigned to Group D (e.g., G_3, G_{11}, G_{79}, and G_5).

3.1 CLUSTER ANALYSIS

A dendrogram was drawn based on the cluster analysis using seed yield under drought and non-drought conditions along with the selection indices TOL, MP, GMP, STI, SSI, YSI, DI, and HARM (Figure 3). The cluster analysis performed classified the 100 genotypes of safflower investigated into three distinct groups consisting of 7, 44, and 49 genotypes. The genotypes in the smallest group (1) including G_{13}, G_{61}, G_{33}, G_{58}, G_{47}, G_{11}, G_{24}, and G_3 showed the highest seed yield under both non-stress and drought stress conditions (Figure 3). The genotypes clustered in Group 2 (i.e., G_{51}, G_{93}, G_{39}, G_{60}, G_2, G_{57}, G_{14}, G_{16}, G_{97}, G_{48}, G_{63}, G_{99}, G_{50}, G_{86}, G_{10}, G_{65}, and G_{81}) recorded medium levels of seed yield under drought stress. The

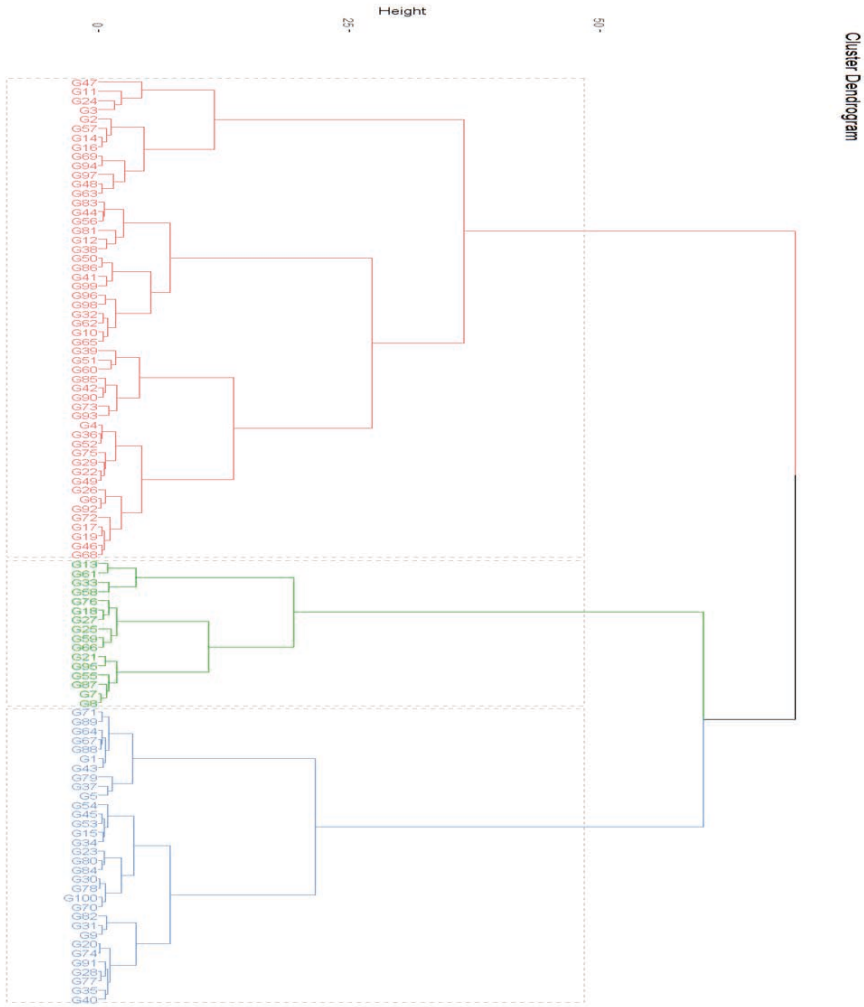

Figure 3: Discrimination of drought tolerance in a worldwide collection of safflower (*Carthamus tinctorius* L.) genotypes based on selection indices.

third group consisted of genotypes with a low productivity under either environmental conditions (i.e., G_{79}, G_{37}, G_{89}, G_{55}, G_{21}, and G_{25}).

4 DISCUSSION

This study evaluated drought tolerance in a world collection of safflower accessions under the effects of year and genotype. The analysis of variance showed a large genetic variation in drought tolerance among the accessions studied as an unpredictable factor affecting seed yield in the genotypes from different geographical regions. Year factor was not found to have any significant effect on seed yield or selection indices; hence, the indices selected for this germplasm can be effectively used if seed yield is adequately heritable. Considering the fact that traits involved in drought tolerance mechanisms are polygenic ones, the requirement to screen tolerant genotypes has encouraged plant breeders to seek a reliable index. In response to this need, the present study evaluated eight different selection indices (i.e., MP, GMP, TOL, SSI, STI, YSI, DI, and HARM) for use in the estimation of seed yield under drought stress. Based on the correlation analysis performed, the positive and significant correlation between TOL and Y_p (0.86^{**}) implies that the genotypes superior in terms of seed yield (such as G_{79} and G_9) showed greater reductions in seed yield under drought conditions. Also, the non-significant correlation between TOL and Y_s (0.10) revealed the failure of the TOL index

to identify the most tolerant genotypes, confirming the results reported by Rizza et al. (2004). The greater TOL values indicated the higher sensitivity of the genotypes investigated to drought stress; thus, smaller values of this index is favored. The positive and significant correlation between Y_p and SSI (0.50**) and that between Y_p and HARM (0.53**) demonstrated that the genotypes with higher values for Y_p or the SSI index exhibited a higher sensitivity to drought stress (Table 2). On the other hand, the negative and significant correlation between SSI and Ys (-0.34**) or that between HARM and Y_s (-0.32**) implied that the superior genotypes under drought stress recorded lower values for SSI and HARM. Hence, the SSI and HARM indices are able to discriminate superior safflower genotypes (the ones with lower values of SSI or HARM indices) in drought prone areas. Studying spring wheat, Guttieri et al. (2001) maintained that SSI values >1 and <1 might indicate above-average and below-average susceptibility to drought stress, respectively. The most suitable index for selecting stress-tolerant genotypes is an index that establishes a positive and strong correlation with seed yield under both stress and non-stress conditions (Fernandez, 1992). To select drought-tolerant genotypes, based on the most desirable indices, use is made of the correlation coefficient of each index with Y_p and Y_s (Golabadi et al., 2006; Ebrahymian et al., 2012; Abdolshahi et al., 2012; Naghavi et al., 2013). Seed yield was found to have a highly significant positive correlation with GMP, MP, STI, and HARM indices under both the environmental (drought and non- drought conditions) conditions examined (Table 2). Based on the correlation analysis conducted in this study, GMP, STI, and MP were found to favor genotypes with a high-yield potential under stress conditions (Table 2), which agrees with the findings reported Sio-Se Mardeh et al. (2006), Hao et al. (2011), and Ebrahimiyan et al. (2012). Given the fact that G_{47} recorded the highest values for MP and STI, this genotype was identified as the most productive and stable safflower ones from among the ones investigated under both stress and non-stress conditions. The results of the present study indicating the capability of the selection indices GMP, MP, and STI to identify genotypes satisfactorily under both conditions are consistent with those reported for GMP and MP in mungbean (Fernandez, 1992); STI and GMP in rice (Raman et al., 2012); safflower (Bahrami et al., 2014) *Brassica napus* L. (Khalili et al., 2012) and durum wheat (Ilker et al., 2011); as well as GMP, STI, and MP in tall fescue (Ebrahymian et al., 2012) and maize (Hao et al., 2011). GMP is often used by plant breeders interested in calculating relative performance since drought stress might vary in severity both under field conditions and over different years (Fernandez, 1992). In the present study, GMP established significant and positive correlations with TOL, Y_p, and Y_s (Table 3). DI, which is commonly accepted as an index to identify genotypes with high yields under both stress and non-stress conditions (Lan, 1998), showed only a highly significant and positive correlation with Y_s (0.89**) (Table 4), demonstrating that selection of safflower genotypes with high DI values might be useful for severe drought-stricken regions but that the genotypes selected based on this index do not have very high yields or yields equivalent to those of genotypes currently cultivated under normal irrigation. Seed yield under non-drought conditions (Yp) was positively correlated with Y_S, confirming previous reports on safflower (Bahrami et al., 2014) other crop species such as bread wheat (El-Rawy and Hassan, 2014), corn (Naghavi et al., 2013) and bread wheat (Abdolshahi et al., 2012). It may also be noted that the satisfactory responses shown by some genotypes under stress conditions could be ascribed to the good adaptation mechanisms in these genotypes (Naghavi et al., 2013). The impacts of the different indices in each PC indicate that PC_1 and PC_2 could be identified as yield potential and stress susceptibility groups, respectively. The genotypes (such as G_{47}, G_{11}, G_3, and G_{24}) recording high values for both PC_1 and PC_2 may be considered as superior ones for seed yield under both experimental conditions; hence, they are designated as stable genotypes (Figure 1A). The genotypes recording low PC_1 but high PC_2 values included those also with high values of DI, YSI, and seed yield under drought stress, but low values of SSI and HARM values (Figure 1B). The genotypes (such as G_{13} and G_{61}) recording high PC_1 but low PC_2 values included genotypes with high values for GMP, STI, MP, and seed yields under non-stress conditions (Figure 1C). On the other hand, the majority of the genotypes with low PC_1 and PC_2 values were identified as susceptible genotypes; these included G_{79}, as the most tolerant one, and the genotypes G_9, G_{82}, and G_{89} (Figure 1D), that were recognized as unstable genotypes. The majority of the genotypes investigated (more than 60 %) were classified in Groups B and D (Figure 1). This biplot may also be used for identifying contrasting genotypes (genotypes in group A *versus* D) for planning fine mapping populations for safflower genome studies of drought tolerance. Based on our cluster analysis, the genotypes assigned to Group 3 were recognized as the most tolerant ones to be used as parents for improving drought tolerance in safflower breeding programs. Thus, the genotypes in Group 1 and Group 3 were identified as drought tolerant and drought susceptible, respectively. Cluster analysis has been widely used not only to discriminate high distance genotypes but also to determine genetic diversity based on similar traits under drought stress conditions (Golabadi et al., 2006; Mohammadi et al., 2011; Naghavi et al., 2013).The results of the

present cluster analysis of the genotypes investigated were consistent with the PCA results obtained. Thus, drought-tolerant genotypes recording high PC_1 and PC_2 values as well as those assigned to Groups of 1 and 3 in the cluster analysis can be used as extreme parental genotypes with the highest genetic distance to develop new hybrid varieties in safflower aimed at production of drought-tolerant cultivars. However, further evaluation of genotypes using drought tolerance indices across multiple locations is required to confirm their stability for developing improved safflower genotypes.

5 CONCLUSION

From the results obtained, it may be concluded that it is preferable to use simultaneously different drought tolerance indices for screening drought-tolerant safflower genotypes. The results of different multivariate analyses revealed that STI, MP, and GMP, in this descending order, were not only capable of efficient selection of high seed-yield genotypes under both the environmental conditions examined but also of discrete identification of drought-tolerant from drought-sensitive safflower genotypes. The G_{47} genotype (Spanish origin) was identified as the most drought-tolerant one with the highest seed yield under both drought and non-stress conditions. Based on the results obtained in this study, the elite genotypes (i.e., G_{24}, G_{13}, G_3, G_{11}, G_{33}, G_{58}, and G_{61}) may be recommended as promising cultivars for cultivation in drought-affected areas or as appropriate donor parents in safflower hybridization programs. These genotypes may also be exploited for improving seed yield and stability in safflower for cultivation in drought prone regions through appropriate selection methods.

6 ACKNOWLEDGMENT

The authors would like to thank Research Institute for Biotechnology and Bioengineering, Isfahan University of Technology, Isfahan, Iran. Research Center, Isfahan, Iran, is also acknowledged for their financial support as a research project number # 97/ 70649.

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Table supplementary 1: Characteristics of the 100 different genotypes of safflower used in this study

Genotype code	Genotype name	Geographical origin	Genotype code	Genotype name	Geographical origin	Genotype code	Genotype name	Geographical origin
G1	A2	Iran (Azerbayejan)	G34	Car159	Germany	G67	Car64	Slovakia
G2	Ac- Stirling	Canada	G35	Car160	Russia	G68	Car67	Germany
G3	AC-sunset	Canada	G36	Car161	Russia	G69	Car68	Germany
G4	Arak 2811	Iran (Markazi)	G37	Car169	Hungary	G70	Car70	Lybyan
G5	C111	Iran(Isfahan)	G38	Car175	India (Kusum)	G71	Car72	North Korea
G6	Car118	India	G39	Car181	India	G72	Car74	North Korea
G7	Car 116	India	G40	Car188	Poland	G73	Car75	North Korea
G8	Car 9	Slovaki	G41	Car19	Poland	G74	Car76	North Korea
G9	Car100	Italy	G42	Car190	Iran (Isfahan)	G75	Car77	North Korea
G10	Car106	Spain	G43	Car198	Azerbaijan	G76	Car78	Hungary
G11	Car114	India	G44	Car199	Korean republic	G77	Car79	Japan
G12	Car117	Sudan (tozi)	G45	Car200	unknown	G78	Car80	North Korea
G13	K21	Iran (Kordestan)	G46	Car201	Sudan	G79	Car83	Tajikistan
G14	Car124	Pakistan	G47	Car210	Spain	G80	Car86	Tunisia
G15	Car125	Russia	G48	Car211	Germany	G81	Car87	Romania
G16	Car126	Belgium	G49	Car214	Poland	G82	Car89	Tunisia
G17	Car127	Germany	G50	Car215	Germany	G83	Car94	Spain
G18	Car129	Germany	G51	Car216	Germany	G84	GE62918	Germany
G19	Car130	Morocco	G52	Car217	Germany	G85	Gila	USA
G20	Car131	Paraguay	G53	Car218	Germany	G86	Hartman	USA
G21	Car132	Germany	G54	Car219	Germany	G87	IL111	Iran (Aur-oumieh)
G22	Car135	Portugal	G55	Car221	Germany	G88	Isf-14	Iran (Isfahan)
G23	Car137	Pakistan	G56	Car224	Germany	G89	Isf28	Iran(Isfahan)
G24	Car138	Poland	G57	Car226	Germany	G90	K21	Iran (Kord-estan)
G25	Car146	Egypt	G58	Car227	Germany	G91	KMS 36	Iran (karaj)
G26	Car147	Pakistan	G59	Car228	Germany	G92	Mex.17-45	Mexico
G27	Car148	Pakistan	G60	Car230	Germany	G93	Mex.7-147	Mexico
G28	Car151	India	G61	Car24	Morocco	G94	Mex.7-38	Mexico
G29	Car152	Iraq	G62	Car37	Sudan	G95	Mex-13-216	Mexico
G30	Car155	Russia	G63	Car42	Sudan	G96	Mex2-138	Mexico
G31	Car156	Pakistan	G64	Car49	Spain	G97	Mex22-191	Mexico
G32	Car157	Morocco	G65	Car55	Poland	G98	Mex6-97	Mexico
G33	Car158	Paraguay	G66	Car56	Nebraska 8 (USA)	G99	PI 301055	Turkey
						G100	Saffire	Canada

doi:10.14720/aas.2021.117.1.1716 Original research article / izvirni znanstveni članek

Does women's intra-household bargaining power have effect on child welfare? Evidence from farm households in Ogun state, Nigeria

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Received June 06, 2020; accepted March 03, 2021.
Delo je prispelo 06. junija 2020, sprejeto 03. marca 2021.

Does women's intra-household bargaining power have effect on child welfare? Evidence from farm households in Ogun state, Nigeria

Abstract: This study examines whether greater women's household bargaining power is associated with the improvement in children's welfare in Ogun State, Nigeria. Using data from 320 farm households with a Logit regression model, the study revealed that 31.86 % of children under-five years of age were stunted, 32 % were underweight and 16.2 % were wasted. Children growing up healthy were 62 %, implying that one – third of under-five children in the study area still experience nutrition deficiency. About 3.33 % and 1.05 % children simultaneously experienced stunting and wasting together, which perhaps suggests a harsh deprivation environment. In addition, 63.33 % of women in the study area had low bargaining power implying that they lack control over important decisions in their households. Women who enjoy decision-making power in their households, particularly with large purchasing power, are associated with having children with better height-for-age, mass-for-age, and mass-for-height ratios. Women's inequality as relates to intra-household bargaining power negatively affects children's welfare and leads to chronic malnutrition. As a policy recommendation, it is therefore, important to enhance women's status, which, with time will lead to more investment in their children's education, health, and overall welfare.

Key words: bargaining power; child welfare; mass index; Ogun state

Ali ima enakopravnost žensk v gospodinstvih vpliv na dobrobit otrok? Primeri iz kmečkih gospodinjstev iz države Ogun, Nigerija

Izvleček: V raziskavi je bilo preučevano, kako je enakopravnost žensk v gospodinjstvih povezana z izbolšanjem dobrobiti otrok, v državi Ogun, Nigerija. Z regresijskim logit modelom so bili obdelani podatki iz 320 kmečkih gospodinjstev. Raziskava je odkrila, da je bilo 31,86 % otrok , v starosti pod pet let, zaostalih v razvoju, 32 % jih je imelo premajhno maso in 16,2 % jih je bilo podhranjenih in bolnih. Otrok, ki so doraščali zdravo je bilo 62 %, kar kaže na to, da so trije od petih otrok še vedno podhranjeni. Okrog 3,33 % in 1,05 % otrok se hkrati sooča z zaostankom v razvoju in podhranjenostjo, kar kaže na zelo nevzpodbudno okolje za razvoj otrok. Dodatno ima na preučevanem območju 63,33 % žena majhno enakopravnost, kar kaže na pomankljivost njihovega odločanja o pomembnih zadevah v gospodinjstvih. Žene, ki imajo večjo moč odločanja v gospodinjstvih, še posebej tiste z večjo kupno močjo, imajo otroke, ki so višji glede na njihovo starost, močnejši in z večjim razmerjem med maso in višino. Ženska neenakopravnost je povezana z njihovo neenakopravnostjo v gospodinjstvu, kar vpliva negativno na dobrobit otrok in vodi h kronični podhranjenosti. Priporočilo bi torej bilo, da je pomembno izboljšati položaj žena, kar bi s časom vodilo v več vlaganja v izobraževanje njihovih otrok, njihovega zdravja in v splošno izboljšanje stanja.

Ključne besede: enakopravnost; dobrobit otrok; masni indeks; država Ogun

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1. INTRODUCTION

Globally, the significance of women's empowerment in intra-household decision-making on household welfare has been a topical issue and debate among scholars. The debate centres on the need to enhance women's bargaining power and increase their participation in intra-household decision-making (Grown et al., 2005; Malhotra and Schuler, 2005). This subject is also captured in the Millennium Development Goals (MDGs) and the Sustainable Development Goals (SDGs) in order to achieve international development. The need to enhance women's empowerment and achieve economic growth also led to the formation of the World Bank's Gender Action Plan in 2006 (Zuckerman, 2007). Policymakers and development practitioners are of the opinion that women's empowerment is a necessity for societal development (Pratley, P. (2016). Based on this, an empirical research study in this nature is essential in order to be able to suggest to policymakers an effective policy to empower women both in the rural and urban areas for the purpose of achieving desired outcome of empowering women and promote their bargaining power in all economic fronts. It is important to note that lack of women's empowerment manifests in various ways, including in the labour market (increase unemployment), increase poverty and limited economic opportunities (Guvuriro and Booysen, 2019). In line with the need for women's empowerment, Yusof and Duasa (2010) and Doepke and Tertt (2014) revealed that women's empowerment can influence the allocation of resources including those that concern children and the one that relates to national development. This study finds that women remain economically disadvantaged in Nigeria when compared to their men counterparts. They are also disadvantaged in the intra-household decision-making power and are sometimes prevented from actively participate in cash-based work or labour market. This development accounts for the significance and motivation of this study, especially on the need to examine whether greater women's household bargaining power or empowerment will lead to the improvement in children's welfare in Nigeria.

According to Duflo (2000), there is a correlation between women's greater involvement in household decision-making and the allocation of spending in the family and among farm households, which benefit children. Duflo revealed that an increase in the women's decision-making power in the family would positively affect the expenditure allocation and overall children's wellbeing. This means that as the share of household income controlled by women increases, the welfare of the children is enhanced (World Bank, 2011). This is because an improvement in women's intra-household bargaining power and resources allocation lead to improved welfare outcomes of children (Wang, 2014).

According to the data released by UNICEF/WHO/World Bank Group (2017), about 151 million of children under-five years suffer from malnutrition and 67 million were wasted globally in 2017 due to lack of women's empowerment. Studies have revealed that among the public health problems that resulted from lack of women's empowerment, malnutrition remains the highest among children in developing countries including Nigeria. Malnutrition affects the mental reasoning and physical development of children, increases the danger of infections and contributes to the child's illness and death (Pelletier and Frongillo, 2003). According to De Onis and Blössner (2003), stunting, wasting and underweight are the three major symptoms of a child's malnutrition. Stunting and wasting are an indication of chronic and acute malnutrition while underweight is a combination of both acute and chronic malnutrition (De Onis and Blössner 2003). All these problems and symptoms are because of lack of women's empowerment.

Various studies have shown that malnutrition contributes to the global burden of diseases. According to Liu et al. (2015), malnutrition accounts for 50 % of all the global death rate among children under the age of five. The WHO (2016) also revealed that about 99 million of under-five year's children were either stunted, wasted or underweight globally while approximately 6 million children were stunting and wasting at the same time (Khara et al., 2018). Based on these figures, 55 % of all stunted children were from Asia and 39 % from Africa, while 69 % of the stunting and wasting children were from Asia and 27 % from Africa (UNICEF/WHO/World Bank Group, 2017) since women are lagging behind in terms of empowerment and involvement in households' decision-making.

In Nigeria, and especially among farm households, malnutrition is one of the most important factors that contribute to child mortality among under-five year's children. This occurs most among the farm households. According to UNICEF/WHO/World Bank (2017), mortality rates of about 128 deaths per 1000 births were recorded in 2017 while 37 % of under-five children are stunted. Because of deficiency in women's empowerment and poor intra-households decision-making power, there is also the problem of maternal nutrition in which 11 % and 25 % prevalence of undernutrition and overweight/obesity were recorded among women (National Population Commission and ICF International, 2014). This problem is more prevalent among farm households and as a result, children in rural areas are more likely to be stunted than those in urban areas (Herrador et al., 2014). It is against this view and background that this study

seeks to examine whether greater women's household bargaining power is associated with the improvement in children's welfare using farm households in Ogun State as a case study. This study has meaningful implications for policy and contributes to the growing literature, which finds evidence supporting increased female bargaining power as a method to promote equality and improve child health outcomes. The rest of the article is as follows. The next section presents the empirical review of the literature. Section 3 presents the methodology employed in analyzing the research data. Section 4 contains the women's bargaining power and the questions that were contained in the questionnaire. Section 5 presents the results and discussion and section 6 concludes the study.

Many studies have established strong evidence of women's empowerment and higher intra-households decision-making power to translate into improved child wellbeing and societal development. For example, Gokhale et al. (2004) and Chen and Li (2009) show a strong link between women's education, decision-making and child survival or better living condition. Angel-Urdinola and Wodon (2010) also revealed that women's earning and income generation contribute to women's empowerment and intra-households' decision-making. According to Urdinola and Wodon, wage gap is a sign of discrimination against women and this can hinder children's access to education, healthcare and other social-economic wellbeing.

Richards et al. (2013) carried out a study on gendered intra-household bargaining as a social determinant of child health and nutrition in the low and middle-income countries. They revealed that "intra-household bargaining power operates through inter-linked mechanisms that shape how resources are channelled to children in terms of nutrition and health inputs which lead to better child health and nutrition outcomes". Tolhurst et al. (2008) advocated a gender transformatory approach, which aims to promote women's empowerment and intra-household decision-making power needed in order to achieve gender relationships and quality wellbeing for the children.

Behrman and Skoufias (2006) highlighted the significance of income in achieving women's empowerment and greater intra-households decision-making. Based on their view, income in the hands of women will lead to a complete income pooling in the households and heighten the development of the entire family. Gummerson and Schneider (2013) supported this view and revealed that women are the more responsible managers of financial resources in the family. This is also in line with Behrman and Skoufias (2006) who stated that "policies targeted towards women can generate immediate consequences by either improving a women's voice in the household and/or contributing to an improvement in human capital investments in children".

Expanding on the link between improved women's bargaining power and resource allocation, Doss (2013) revealed that there is a sufficient evidence to support the view that women's bargaining power does affect resource allocation and developmental outcomes. This means that there is a link between women's empowerment or bargaining power and the development of the society. Invariably, there is a link between the relative decision-making power at the household level and greater expansion of the development of the society. Martínez (2013) shared similar view and indicated that increased women's bargaining power would promote equality and improve child welfare. While carrying out a study of Women's intra-household bargaining power and child welfare outcomes, Saaka (2018) also revealed that women's empowerment is associated with benefits for women and their children.

However, despite the numerous studies on the women's intra-household bargaining power and children's welfare outcomes around the world, there is scanty research on the subject in Nigeria. Realizing this empirical gap in the literature, this study is undertaken to contribute to the debate on whether greater women's household bargaining power is associated with the improvement in children's welfare in Nigeria. It is expected that the findings of the study will help enlighten policymakers on the wisdom of formulating policies that enhance women's empowerment and increase their bargaining power within the household as well as increase spending on long-term durable goods (education and nutrition of children).

2. METHODOLOGY

2.1 STUDY AREA

The study area is Ogun State in South West Nigeria. The state was created in February, 1976 with Abeokuta as the capital. It has a land of approximately 1.7 million hectares and occupies about 1.9 % of Nigeria's total land and about 2.5 % of the country's population. It has 20 local government areas spread across the four main agricultural zones of the state- Egba, Ijebu, Remo, and Yewa/Awori. The state shares an international boundary with Benin Republic to the west and Oyo state to the north, Lagos state to the south, and Ondo state to the east. In addition, the state has a population of 3.7 million based on 2006 national population census. There are two distinct seasons in the state namely, the rainy season and the

dry season and in terms of farming activities, the state is well known for livestock and cocoa production.

Fasina et al. (2020) revealed that the death of newborn babies resulting from malnutrition and neonatal mortality is very high in the state. There is poor access to quality education and health care facilities especially among the rural farm households. Poor care-seeking behaviour of families due to unemployment and poor salaries are high in the area. Ogunlesi and Ogunlesi (2012) also revealed that low health care education and the use of home remedies are the common living status of farm households in the study area.

2.2 STUDY DATA AND METHODS OF DATA COLLECTION

The data for the study were collected through a well-structured questionnaire and interview from the rural farmers. Data were collected on the socio-economic characteristics such as age, age at marriage, number of years in marriage, gender, marital status, educational status, and other variables of mother and child (such as employment status, monthly income, land ownership, livestock ownership and access to credit). The mass and height of children were collected through the anthropometric measurement procedures and counter-checked using their vaccination cards.

2.3 SAMPLE AND SAMPLING TECHNIQUE

This study employed the multistage sampling technique; at stage one, two agricultural zones of Ijebu and Ilaro were selected out of the four zones in the state. At stage two, 50 % of the blocks were selected from each zone. This gives three blocks for Ijebu and two blocks for Ilaro and translates to five blocks. At stage three, four cells each per block were randomly selected to give twenty cells. At stage four, sixteen (16) farm households were selected from each cell, which gave three hundred and twenty (320) households. Women in the productive age (15-49 years) and under-five children were targeted by the study. In total, 141 boys and 281 girls, aged 0-59 months from 320 households were considered for the study.

2.4 ANALYTICAL TECHNIQUES AND RESEARCH DESIGN

Both descriptive and quantitative techniques were used to analyse the data. The choice of both approaches was to ensure that the results are valid and reliable. It is also based on Vetter (2017) that a descriptive research design can answer the questions of who, what, where, when and how. These were the concepts used in the questionnaire in framing the research questions of this study. The descriptive analysis using percentages and frequency analysis were used to describe the sociodemographic characteristics of the variables employed while logit regression model was used to analyze the data and determine the factors related to child malnutrition.

2.4.1 Welfare Estimates of Children under the Age of Five: Analysis of Anthropometric Measurements

The welfare status of children under the age of five is usually measured using three indices: mass-for-height (wasting) which reflects acute growth disturbances, height-for-age (stunting) which reflects long-term growth faltering and mass-for-age (underweight) which is a combination of both long and short-term effects. Anthropometric data were analyzed to generate welfare indicators of children under the age of five. The study adopted the method of Abera et al. (2017) to convert mass, height, and age of children into height-for-age (HAZ), mass-for-age (MAZ), and mass-for-height (MHZ) in order to assess malnutrition. At the end, the variables for stunting, underweight, and wasting were classified as 1 for stunted and 0 for not stunted, 1 for underweight and 0 for not underweight, and 1 for wasted and 0 for not wasted. On the other hand, the WHO (2006) child-growth standard was employed for the anthropometric measurements of children.

In line with WHO (2008) and Yalew et al. (2014), the minimum dietary diversity and breastfeeding were used in the study. Seven food groups containing grains, roots and tubers, legumes and nuts, dairy products (milk, yogurt, and cheese), flesh foods (meat, fish, poultry, and liver/organ meats), eggs (vitamin-A rich fruits and vegetables) and other fruits and vegetables were used as good meal frequency.

2.4.2 Women's bargaining power

This study made use of the women's decision-making processes within the household as proxy for women's bargaining power. Their ability to make decision about food preparation and consumption (Patel et al., 2007), decision about family asset or investment (Reggio, 2011), decision about gender roles, children education, number of children and health care decision (Allendorf, 2007). This study assumed that women who participate in the

intra-households decision-making have relative bargaining power. This means that women who partake in family decision-making can be said to have greater bargaining strength than those ones that are excluded.

This study was based on farm households and specifically on married female households. In line with Anderson et al. (2017), this study restricts sample to female's spouses who are living together as husband and wife. The reason for this selection was that when there is no spouse, only the one parent makes decisions. Therefore, female spouses were interviewed taking into consideration that the wife's present husband is the father of the children. This helps to ascertain how they reach important socioeconomic decisions in the family. In order to assess the allocation of household decision-making power, twenty-two (22) questions about household and farm activities were contained in the questionnaire. Using a likert scale ranging from 1 to 10, respondents were asked to choose the best option that align with their views over a given decision.

Questions included in the questionnaire include the following:

This study adopts questions from Doss (2013), Anderson et al. (2017) and Mengesha and Merkeb (2020). In a scale of 1 to 10, how is decision-making in the family shared between yourself and your spouse? The decision-making power is in the following regards:

(a) Asset ownership
- Land ownership, by plot or parcel, at the individual level?
- Rights associated with the land, by plot/parcel and individual owner?
- Documentation of land ownership, including names on documents?
- Ownership of dwelling?
- Ownership of livestock?

(b) Crop and livestock
- What types of crop to be cultivated on the farm?
- Where to sell crops?
- What types of livestock to be raised on the farm?
- When to sell off the livestock?
- How to spend money raised from the sale of crops?
- How to spend money raised from the sale of livestock?

(c) Decisions about children
- What number of children to have?
- What foods to feed the children?
- Whether to send children to school?
- Type of health practices?

(d) Advance decisions
- Whether to buy a new high-yield seed or use the ordinary seeds?
- Whether to buy new farm equipment or use the old tools?
- What types of information or training the household needs?
- Who to attend farm training?

(e) Broad decision-making authority: livelihood versus overall
- Overall decision-making for the household?
- Can you travel to visit your family and friends?
- Can you go to the market alone?
- Can you go to a health clinic for your own health needs?
- Can you take your children to a health clinic alone?

The report of decision-making authority is measured on a scale (0–10) that is assigned to the choice that each spouse choose for themselves and their spouse. The statistics for each of the variables used are summarized in Table 1.

Decision-Making Index (DMI) was used to measure women bargaining power. The whole responses for each respondent (husband and wife) were calculated. However, this study focuses on farm households women bargaining power. The result gotten is the respondent's score on the women decision-making index. The closer the value of the index is to 1, the higher the women bargaining power. This is as stated below:

$$Women\ Decision\ Making\ Index = {}^{Number\ of\ Cards\ Allocated\ to\ Women_i}/_{Total\ Number\ of\ Cards} \quad (1)$$

2.4.1 Estimation model

In this study, the welfare outcome of children under the age of five is determined by the mother's bargaining power in the family, the child's and women's socioeconomic characteristics, and other household factors. This study focuses on three main welfare outcomes: underweight, stunting and wasting of children under the age of five. The corresponding econometric model is specified as follows:

$$Y_i = \beta_0 + \beta_1 P_i + \beta_2 X_i + \varepsilon_i \quad (2)$$

Where Yi is a measure of the children welfare outcomes, Pi is the women's bargaining power, Xi is the variables of children under the age of five and women's characteristics which are variables that contribute to improvements in child welfare outcomes.

3. RESULTS AND DISCUSSION

3.1 SOCIOECONOMIC CHARACTERISTICS OF WOMEN

The results of the socio-demographic characteristics

Table 1: Women's socioeconomic characteristics

Characteristics	Frequency	Percent	Mean
Age			
≤ 30	176	55.00	
31-40	104	32.50	
41 - 49	40	12.50	
Mean age			29
Age at marriage			
≤ 20	201	62.81	
21-25	86	26.88	
26-30	18	5.63	
> 30	15	4.68	
Mean			8
Number of marriages			
Once	277	86.56	
More than once	43	13.44	
Number of years in marriage			
1-5	67	20.94	
6-10	188	58.75	
11 and above	65	20.31	9
Education			
No formal education	171	53.44	
Primary	94	29.38	
Secondary	44	11.75	
Tertiary	11	3.44	
Body Mass Index (kg m^{-2})			
Underweight	73	22.81	
Healthy weight	148	46.25	
Overweight/grade I obesity	64	20.00	
Obese/grade II obesity	35	10.94	
Land ownership	38	11.88	
Livestock ownership	102	31.88	
Access to credit	77	24.06	
Employment status			
Working	288	90.00	
Full housewife	32	10.00	
Monthly income			
Less than ₦50,000	231	72.19	
₦51,000-₦100,000	76	23.75	
₦101,000 and above	13	4.06	
Mean			₦41,540

Source: Author's field survey, 2019

of the respondents, as presented in Table 1, show that the dominant age group for women in the study area was less than 30 years, which comprises 55 % of women respondents with a mean age of 29 years. Of 320 respondents, 62.81 % were women under 20 years of age when married and the mean age at marriage was 18 years. This clearly shows that the study concentrated on women in the reproductive age group (15-49 years) and evidence of early marriage is common in rural farm households in Nigeria. About 43 women (13 %) have married more than once and 67 (21 %) women have been married for less than 5 years. Besides, 53.44 % have no formal education. It shows a lower literacy rate among rural women farm households. Overall, nutritional status of 46.25 % of the mothers is normal, that is, they have normal Body Mass Index (BMI) while 28.1 % mothers suffer from lack of energy or thinness and 20 % of women are overweight. In addition, 12 % of the female respondents own land while 32 % own livestock. However, 24 % have access to credit.

3.2 INFANT AND YOUNG CHILD FEEDING PRACTICES AMONG FARM HOUSEHOLD'S CHILDREN UNDER FIVE YEARS OF AGE

Table 2 gives the results of the feeding practices among the farm households. While breastfeeding was popular among farm households children (0-23 months), young children's feeding like the minimum dietary diversity was still inadequate and lacking. For example, about 87 % of the children (6-24 months) were inadequately fed with the minimum recommended meal frequency and 77.08 % lack the minimum dietary diversity. 86.12 % of newly born babies do not timely received milk and colostrum. The findings also show that exclusive breastfeeding is done for 0-5 months (33.535) which is less than the six months recommended by WHO/UNICEF. Children were given other complementary foods, which exposed them to unhealthy feeding and sicknesses.

3.3 ANTHROPOMETRY RESULTS OF THE WELFARE OF FARM HOUSEHOLD'S CHILDREN UNDER FIVE YEARS OF AGE

The WHO (2006) growth standard was employed for anthropometric measurements. As stated under section 3.4.1, the variables for stunting, underweight, and wasting were classified and calculated for farm household's children of 0 to 59 month. As shown in Table 3, the incidence of underweight among children was 32 %, and boys (38.15 %) are more likely to be underweight than girls (26.43 %). On the other hand, the incidence of stunting was recorded in which about 32 % of the children have stunted growth. The result shows that stunting rises with age, climaxing at slightly above 35 % among children in their second and third year of life. Similar results were found in the previous studies of Yimer (2000); Asfaw and Giotom (2000) in Ethiopia. Severe stunting among farm households with children of age 12-23 months (38.71 %) was also recorded with boys (38.48 %) more likely to be stunted than girls (30.12 %). This means that male children are more likely to be stunted and underweight than their female counterparts. This finding is consistent with a meta-analysis in sub-Saharan Africa (Wamani et al., 2007), a study in the Northern Ethiopia (Alemayehu et al., 2015), and research in Myanmar (Mya et al., 2019). With underweight and stunting as a chronic malnutrition problem, Omilola (2010) revealed that "stunted children may never regain height lost due to stunting, and most children will never gain the corresponding body mass".

Finally, wasting was found to be the least prevalent malnutrition among farm households' children in the study. It was half (16.2 %) the rate of prevalence in underweight and stunting. However, this value is higher than the prevalence rate of 11 % recorded in Nigeria in 2003 but lower than the figure of 18 % recorded in 2013 (National Population Commission, 2014).

In line with Dabale and Sharma (2014), wasting was higher among boys (16.5 %) than in girls (15.2 %). The highest incidence of wasting was seen among children aged 0–23 months as compared to lowest figure seen among children aged 36–47 months. This is in line with

Table 2: Feeding practices of farm household's under-five year's old children

Feeding practices across categories	Frequency	Percent
Ever breastfed (0-23 months)	414	98.10
Breastfed exclusively (0-5 months)	141	33.53
Meal frequency (6-24 months)	369	86.61
Not timely receive milk and colostrum within one hour of birth	363	86.12
Not meet the minimum dietary diversity	326	77.08

Source: Author's field survey, 2019

Table 3: Prevalence of underweight, stunting and wasting in children 0 to 59 months of age

Prevalence of overall, moderate and severe underweight status (Mass-for-Age Z-score) in children 0 to 59 months of age			
Characteristics	% Prevalence of underweight	% Prevalence of moderate underweight	% Prevalence of severe underweight
Overall	32.00	27.65	4.35
Sex			
Boy	38.15	31.74	6.41
Girl	26.43	21.01	5.42
Age			
0-5 Months	28.17	18.62	9.55
6-11 Months	39.26	32.96	6.30
12-23 Months	36.83	29.27	7.56
24-35 Months	30.11	24.50	5.61
36-47 Months	32.54	26.81	5.73
48-59 Months	29.62	25.18	4.44
Prevalence of overall, moderate and severe stunting status (Height-for-Age) in children 0 to 59 months of age			
Characteristics	% Prevalence of stunting	% Prevalence of moderate stunting	% Prevalence of severe stunting
Overall	31.82	23.76	8.06
Sex			
Boy	38.48	29.78	8.70
Girl	30.12	25.88	4.24
Age			
0-5 Months	30.11	24.87	5.24
6-11 Months	32.65	28.82	3.83
12-23 Months	38.71	29.85	8.86
24-35 Months	35.27	30.82	4.45
36-47 Months	33.54	29.09	4.54
48-59 Months	32.03	24.09	7.94
Prevalence of wasting (Mass-for –Height) in children 0 to 59 months of age			
Characteristics	% Prevalence of wasting	% Prevalence of moderate wasting	% Prevalence of severe wasting
Overall	16.20	10.30	5.72
Sex			
Boy	16.50	10.30	5.72
Girl	15.20	10.00	5.20
Age			
0-5 Months	16.60	11.40	5.20
6-11 Months	16.70	11.60	5.10
12-23 Months	16.40	10.10	6.30
24-35 Months	15.80	11.20	4.60
36-47 Months	15.10	10.90	4.20
48-59 Months	15.30	10.25	5.05

Source: Author's field survey, 2019.

Akombi et al. (2017), which posited that the incidence of wasting and severe wasting was higher in the age group of 0-5 months and 6-23 months.

Table 4: Extent of wasting and stunting in under five year old children

	No stunting	Moderate stunting	Severe stunting	Total
No wasting	62.44	17.38	4.16	83.98
Moderate wasting	4.12	3.33	2.85	10.30
Severe wasting	1.62	3.05	1.05	5.72
Total	68.18	23.76	8.06	100.00

Source: Author's field survey, 2019

3.4 EXTENT OF (DUAL MALNUTRITION DEFICITS) WASTING AND STUNTING IN CHILDREN UNDER FIVE YEARS OF AGE

Table 4 shows the combined burden of stunting and wasting of children less than five year of age. Children growing up healthy are 62.44 %, implying that one – third of farm household's children less than five years of age in the study area still experiences nutrition deficiency. Children suffering from the simultaneous occurrence of stunting and wasting are 3.33 % while 1.05 % are undergoing severe stunting and wasting together. This might not be unconnected with the environmental condition and harsh deprivation of the farm household's children, especially in the rural area. As a policy recommendation, it is therefore, advisable that good nutrition be introduced to the children and treatment for malnutrition illnesses be carried out among the farm household's children in the study area. This recommendation is in line with Babatunde et al. (2011) that suggested that government should enact food policy that will increase daily per capita household calorie supply especially in the rural area.

3.5. THE PATTERN OF WOMEN BARGAINING POWER

Table 5 shows that 63.33 % of women in the study area have low bargaining power, while 26.25 % have moderate bargaining power and 10.42 % have high bargaining power. This implies that the men were adequate in more indicators than the women; the women were adequate in more indicators than the man in 10 % of households; and the man and the women are equally adequate in 26 % of households. On average, the male respondents are adequate in 63 % more indicators (approximately two indicators) than the female respondent in the same household. Therefore, women lack control over important decisions in their households.

3.5.1 Effects of women's bargaining power on farm household's welfare of the children less than five years of age

Table 6 presents the results of the logit regression model. Women farm households' involvement in productive activities have a positive effect on their child's welfare outcomes. When they are employed or involved in other income generating activities, there is a lower likelihood of their being underweight, stunted and wasted. Belch and Willis (2002) revealed that women (farm households) with more financial resources wield more power in household decision-making and have stronger bargaining power in the family. In addition, Getahun and Villanger (2017) also revealed that wives that are employed have high bargaining power, as they are able to contribute to household expenditures and this improves their participation in the intra-household decision-making. Women's incomes provide them with the bargaining power and control over major decision like schooling and consumption expenditures (Doss, 2013). It explains how they can live on during family challenges or marriage break-up, and reflects how the children can be well taken care of without the man. This means that higher

Table 5: Pattern of women bargaining power

Level of bargaining power	Frequency	Percentage
Low bargaining power (the decision is taken mainly by men) (DM1 =0.33)	203	63.33
Moderate bargaining power (the decision is shared) (DMI = 0.34 - 0.66)	84	26.25
High bargaining power (the decision is taken mainly by women) (DMI0.67)	33	10.42
Total	320	100

Source: Field survey, 2019.

Table 6: Logit regression results of the effects of farm households women's bargaining power on welfare of under-five years old children

	Underweight		Stunting		Wasting	
Variables	Coefficient	t-value	Coefficient	t-value	Coefficient	t-value
Children's characteristics						
Sex (male dummy)	1.09*	1.95	0.73**	2.28	0.73	1.28
Age (years)	2.52**	2.04	1.98**	2.33	-1.98**	-2.33
Birth order	11.01***	3.43	5.82**	2.19	5.82**	2.19
Birth mass (kg)	-0.33***	-3.82	-0.33***	-4.45	-0.12***	-5.12
Multiple birth	1.21*	1.89	-0.42	-1.48	-0.42	-1.42
Mother's characteristics						
Mother's BMI (overweight dummy)	-0.22***	-2.62	-0.06**	-2.13	-0.06**	-2.13
Maternal stature (short dummy)	2.35**	2.28	1.13***	2.94	-1.13	-1.02
Age at first marriage	-3.50***	6.12	-2.14**	2.31	1.11	0.98
Educational attainment (number of years of schooling)	-0.18***	-5.11	-0.23**	-2.02	-0.34***	4.19
Employment status (working dummy)	-0.72*	-1.91	-0.35**	-2.31	-0.34***	-3.32
mother's first child (male dummy)	0.09	0.83	0.09	0.92	0.09	0.92
Income	0.16	1.38	0.13	1.44	0.13	1.46
Bargaining power	-7.68***	-3.77	-5.23***	-3.25	-5.21***	-3.25
Frequency of antenatal visit	0.02	0.14	-0.17**	-2.14	-0.17	-1.14
Constant	0.04**	2.02	0.03***	5.12	0.01*	1.99
LR Chi^2 = 412.04						
Prob > Chi^2 = 0.0000						
Pseudo R^2 = 0.2514						
Log likelihood = -1664.24						

Note: ***, **, & * implies significant at $p < 0.001$, $p < 0.05$, & $p < 0.01$ respectively.

incomes or earnings increase women's bargaining power and ability to participate in intra-households decision-making especially among the rural farmers.

Age also plays a significant role in women's empowerment. McElroy (1990) contends that "spousal age measures how well each family member can do in the marriage or remarriage market". Therefore, newer wives may have more bargaining power during negotiations. Equally, older wives may have greater bargaining power and influence on decision-making that positively contribute to the welfare of their children (Chari et al., 2017) and increase the nutritional status of their children. This is because older wives would have more influence on their children's welfare, leading to a higher bargaining power.

The coefficient on the variable for bargaining power that captures mother participation in household's decision-making was significant at the 1 % and positively related to child's health outcomes. This means that the higher the women's bargaining power, the lower the likelihood of the under-five children in the farm household to be underweight, stunted and wasted. Therefore, women with greater decision-making power in the household, especially concerning large purchases, are more empowered and associated with having children with better nutrition, which will translate to better height-for-age, mass-for-age and mass-for-height ratios.

4. CONCLUSION

This paper examines whether greater women's household bargaining power is associated with the improvement in children's welfare in Ogun State, Nigeria. It examines the relationship between farm households women's intra-households decision-making power and child's welfare outcomes. Using cross-sectional farm household data from Abeokuta-Ogun State, the findings revealed that an increase in mother's bargaining power can benefit the child and increase his or her welfare out-

comes. The study shows that women with greater intra-household decision-making power are associated with having children with better height-for-age, weight-for-age, and weight-for-height ratios.

Women farm households' involvement in productive activities have a positive effect on their child's welfare outcomes. The broader social economic implication is that when women are employed or involved in other income generating activities, there is a lower likelihood of their children being underweight, stunted and wasted. This means that wives that are employed have high bargaining power, as they are able to contribute to household expenditures and this improves their participation in the intra-household decision-making (Getahun and Villanger, 2017). Women's incomes provide them with the bargaining power and control over major decision like schooling and consumption expenditures.

This study is however, limited in scope as it was carried out in Southwest part of the country. The researchers believe that further studies (future researchers) could scale-up the scope and focus on other part of the country particularly those that fall outside of the Southwest region, as the finding is very relevant and germane for developing countries and particularly in Nigeria where gender inequality persists when it comes to intra-household decision-making in the family. The paper deals with relevant social issue and contributes to existing literature on women's intra-households bargaining power using the farm households as a case study. There was an established relationship between women's empowerment and child welfare. Overall, it can be concluded that women's empowerment and their enhancement in intra-households decision-making are crucial elements to achieving better children welfare and sustainable development. Therefore, policies and programmes should be focused on increasing women's farm households' status and empowerment, as this will lead to more investment in their children's education, health, and overall welfare.

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Primerjava tradicionalnih in sodobnih metod za določanje gospodarsko pomembnih vrst strun (Coleoptera: Elateridae)

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Received October 05, 2020; accepted December 26, 2020.
Delo je prispelo 05. oktobra 2020, sprejeto 26. decembra 2020.

Primerjava tradicionalnih in sodobnih metod za določanje gospodarsko pomembnih vrst strun (Coleoptera: Elateridae)

Izvleček: Strune so ličinke hroščev pokalic (Coleoptera: Elateridae) in so znane kot gospodarsko pomembni škodljivci. Poznavanje vrstne diverzitete strun na določenem območju je pomembno, saj se ekološke zahteve posameznih vrst razlikujejo, to pa vpliva na izbor ustreznih načinov zatiranja. Morfološko določanje strun je zahtevno in pogosto je razlikovanje med posameznimi vrstami skoraj nemogoče. Molekularne metode zato ustrezno dopolnjujejo morfološke metode, in sicer se za molekularno določanje strun najpogosteje uporablja mitohondrijski gen za citokrom oksidazo I. V članku navajamo molekularne, morfološke in vedenjske metode za določanje strun, prav tako izpostavljamo tudi prednosti in slabosti naštetih metod. Na koncu članka je priložen poenostavljen morfološki določevalni ključ za določevanje gospodarsko pomembnejših vrst strun rodu *Agriotes* v slovenskem jeziku.

Ključne besede: strune; morfološko določanje; molekularno določanje; vedenjski vzorci

Comparison of traditional and modern methods for identification of economically important wireworm species (Coleoptera: Elateridae)

Abstract: Wireworms are larvae of click beetles (Coleoptera: Elateridae) and are well known pests of economic importance. Knowing the species diversity in a particular area is important, as the ecological requirements and consequent management strategies of individual species vary. Morphological identification of wireworms is challenging; separating between individual species is often almost impossible. Molecular methods therefore complement morphological methods. Mitochondrial gene for cytochrome oxidase I is most commonly used for molecular identification of wireworms. In this study we list molecular, morphological and behavioural methods for wireworm identification and also highlight the advantages and disadvantages of these methods. At the end of the article, a simplified morphological identification key for determining economically important wireworm species of the genus *Agriotes* in Slovenian language is attached.

Key words:wireworms; morphological identification; molecular identification; behavioural patterns

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1 UVOD

Strune so polifagne ličinke hroščev pokalic (Coleoptera: Elateridae) in so na svetovni ravni znane kot eni najpomembnejših talnih herbivorov na kmetijskih zemljiščih. Družina Elateridae je ena izmed vrstno najštevilčnejših družin hroščev, vendar njeni filogenetski odnosi še vedno niso dorečeni (Gur'yeva, 1974; Kundrata in Bocak, 2011; Lawrence in Newton, 1995). Večina najpomembnejših kmetijskih škodljivcev v Evropi pripada rodu *Agriotes* (Furlan, 2014; Tóth in sod., 2003), v Severni Ameriki rodovom *Hypnodius, Agriotes, Limonius, Ctenicera, Aeolus* in *Melanotus* (Morales-Rodriguez in sod., 2014; Saguez in sod., 2017), v Aziji rodovoma *Agriotes* in *Melanotus* (Oba in sod., 2015), v Avstraliji pa rodovom *Conoderus, Agrypnus, Heteroderes, Arachnodima* in *Hapatesus* (Calder, 1996).

Strune lahko uvrstimo v kriptične vrste, saj je predstavnike določenih vrst med seboj težko oziroma praktično nemogoče ločiti na podlagi morfoloških znakov. Zaradi zapletene sistematike in življenjskega kroga v tleh so bile strune do nedavnega obravnavane kot en sam škodljivec. Temu primerni so bili tudi varstveni ukrepi, kjer so smernice narekovale enake načine zatiranja, ne glede na vrstno sestavo strun na določeni lokaciji. Vendar je potrebno upoštevati, da so strune talne žuželke z raznolikimi prehranjevalnimi navadami in njihova vloga v ekosistemu ni vedno jasna. Na primer vrsta *Glyphonyx bimarginatus* Schaeffer, 1916 je ena izmed pomembnejših škodljivcev sladkornega trsa na Floridi (Cherry, 2007), vendar ne velja za pomembnega škodljivca krompirja (Langdon in Abney, 2017). Vrsti *Conoderus exsul* (Sharp, 1877) in *Agrypnus variabilis* (Candeze, 1857) sta na Havajih in v Avstraliji pomembna škodljivca sladkornega trsa, na Novi Zelandiji pa sta vrsti pomembna plenilca ličink bojevniške muhe *Inopus rubriceps* (Macquart, 1847), ki je škodljivec trav na pašnikih (Robertson in Pottinger, 1979; Williams in Galbreath, 1987). Danes, ko družba zahteva okolju prijaznejše načine zatiranja škodljivcev in je uporaba biotičnih agensov v porastu (Ansari in sod., 2009; Kabaluk in sod., 2007; Kleespies in sod., 2013), v ospredje prihaja potreba po znanju o posebnih ekoloških zahtevah posameznih vrst (Hermann in sod., 2013) in njim ustreznih metodah za učinkovito integrirano varstvo pridelkov.

Obstoječi morfološki določevalni ključi ter molekularne analize so pogosto omejeni na posamezno geografsko območje oziroma se osredotočajo le na izbrane taksonomske skupine. Vendar je potrebno upoštevati, da se zaradi spremenjenih kmetijskih praks in vnosa tujerodnih vrst spreminja sestava (kompleks) vrst na posameznih območjih. Primer je vnos evropskih vrst *Agriotes obscurus* (Linnaeus, 1758) in *Agriotes lineatus* (Linnaeus, 1767) v Britansko Kolumbijo v začetku 20. stoletja (King, 1950). Vrsti sta tako do leta 2000 že veljali za pomembna škodljivca vrtnin ter okrasnih in krmnih rastlin v Severni Ameriki (Vernon in sod., 2001).

Ena od metod za določanje strun je njihovo gojenje do stadija imaga, saj je morfološko določanje odraslih osebkov enostavnejše in zanesljivejše (Leseigneur, 1972; Lohse, 1979; Jiang, 1999; Laibner, 2000; Cate, 2007; Prosvirov, 2013). Ta metoda pri strunah ni najbolj praktična, saj le-te potrebujejo več let, da zaključijo svoj življenjski krog v tleh, hkrati pa se dolžina larvalnega stadija med vrstami močno razlikuje, na primer pri vrstah *Agriotes* spp. štiri do sedem let (Furlan, 1998; Miles, 1942), pri vrsti *Limonius californicus* (Mannerheim) štiri do enajst let (EPPO, 2005) ter eno leto pri vrsti *Aelous mellilus* (Say, 1836) (Jewett, 1940). Prav tako identifikacija le odraslih predstavnikov ne odslikava realne vrstne sestave strun v specifičnem času in prostoru (Benefer in sod., 2012).

Vrste iz rodu *Agriotes* spadajo med najpomembnejše škodljivce v Srednji Evropi, kjer je bilo do sedaj zabeleženo dvajset vrst iz omenjenega rodu (Cate, 2007; Furlan in Tóth, 2007), kar devet od teh pa predstavlja pomembne talne škodljivce na kmetijskih zemljiščih: *A. lineatus, Agriotes brevis* Candeze, 1863, *Agriotes ustulatus* (Schaller, 1783), *Agriotes sputator* (Linnaeus, 1758), in *A. obscurus*, ki so hkrati tudi najpogostejše vrste v Sloveniji (Milevoj in sod., 2005), ter *Agriotes litigiosus* (Rossi, 1792), *Agriotes proximus* Schwarz, 1891, *Agriotes rufipalpis* Brullé, 1832 in *Agriotes sordidus* (Illiger, 1807). Morfološki ključi (Schaerffenberg, 1940; Cocquempot in sod., 1999; Klausnitzer, 2013; Heimbach in sod., 2020) omogočajo identifikacijo le devet od vsega dvajsetih vrst iz rodu *Agriotes*.

2 MORFOLOŠKA IDENTIFIKACIJA

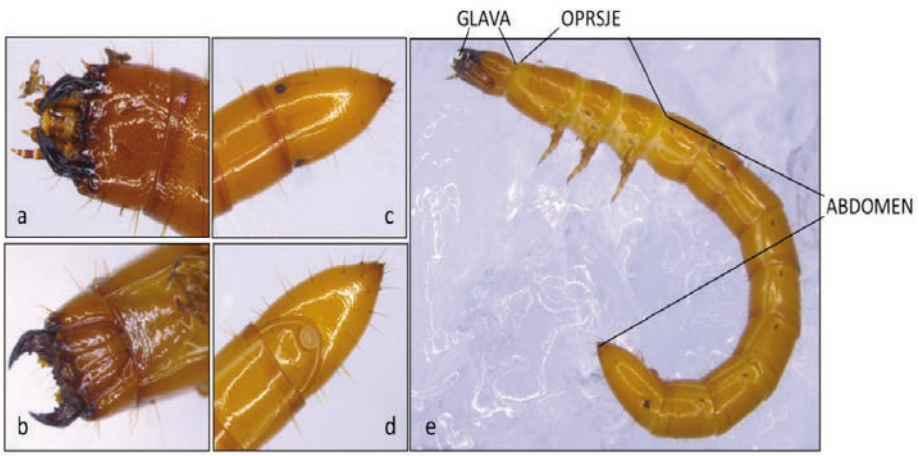
Čeprav so ličinke znotraj družine Elateridae precej raznolike, jih od ličink drugih družin razlikujemo po naslednjih znakih: (1) trije pari pravih nog na oprsju, sestavljeni iz 5 členov (peti segment predstavlja terminalni krempeljc); (2) hitinizirano telo; (3) zgornja ustna (ang. labrum) odsotna, oziroma zlita z oglavnim ščitom (ang. clypeus) in glavno kapsulo; (4) maksila (ang. maxilla) in spodnja ustna (ang. labium) podaljšani in zliti v samostojno enoto; (5) frontalni šiv v obliki lire; (6) tipalke iz treh členov; (7) dihalnice biforalne oblike (tj. z dvema vhodoma); (8) parni izrastki na konici abdomna – analne vilice (ang. urogomphi); (9) telo ravno, z devetimi abdominalnimi segmenti; (10) deseti abdominalni segment slabše viden, saj leži ventralno glede na deveti segment. Na njem je lociran anus (Hyslop, 1917; Glen in sod., 1943; Klausnitzer, 2013). Vizualno so strunam podobne

ličinke hroščev iz družine črnivcev (Tenebrionidae), še posebno iz rodu *Eleodes*. Družini zanesljivo med seboj ločimo po tem, da imajo ličinke črnivcev frontalni šiv v obliki črke Y, imajo dobro razvito zgornjo ustno, bolj izrazite tipalke, prvi par nog na oprsju je pogosto daljši od drugih parov ter na devetem abdominalnem segmentu so pogoste ščetine in manjši trni (Rogers in sod., 1978; Glen in sod., 1943).

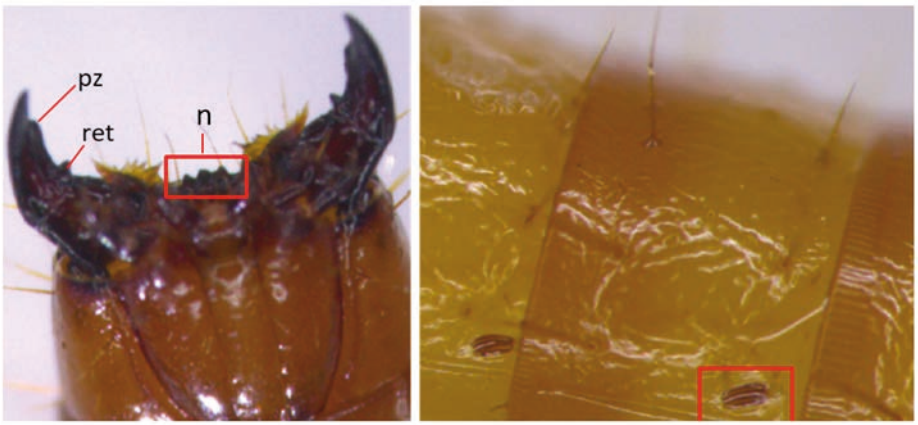
Določanje strun je težavno zaradi velike morfološke podobnosti med vrstami, hkrati pa grejo ličinke med razvojem skozi večje število larvalnih stopenj (8-16), med katerimi prihaja do morfoloških sprememb znotraj iste vrste (Pic in sod., 2008; Klausnitzer, 2013). Te spremembe so rezultat prehranjevanja in premikanja v tleh ter posledičnih poškodb zunanjega skeleta oz. hitinjače. Strukture, ki so podvržene takšnim spremembam je najbolje opazovati takoj po levitvi, saj se njihova izrazitost proti koncu larvalnega stadija zmanjšuje (Glen in sod., 1943). Glen in sod. (1943) za najpomembnejše strukture za zanesljivo identifikacijo rodov in vrst strun navajajo (Slika 1, Slika 2): (1) deveti abdominalni segment, in sicer prisotnost/odsotnost kavdalne zareze, prisotnost/odsotnost »očesc« (ang. muscular impressions, eye-spots), oblika analnih vilic, prisotnost/odsotnost ščetin, oblika stranskih robov; (2) struktura glavne kapsule, nosnica (ang. nasale) je vidna pod mikroskopom, pomembna pa je prisotnost/odsotnost strukture ter oblika zobcev. Nosnica je podvržena fizičnim poškodbam in je zato pogosto obrabljena; (3) mandibule so pomemben morfološki znak, in sicer prisotnost/odsotnost retinakuluma in pomožnega zobca na notranji strani mandibule ter kót, ki ga pomožni zobec tvori z osjo mandibule. Tudi mandibule so podvržene fizičnim poškodbam, hkrati pa lahko pri merjenju kotov tako majhnih objektov pride do napak; (4) prisotnost/odsotnost izrazitih telesnih zarez in vdolbin. Te strukture so manj izrazite na mladih ličinkah in ličinkah, ki so se nedavno levile; (5) oblika ventralnega dela predprsja (ang. presternum), in sicer je lahko sestavljen iz več delov oziroma zlit v en sklerit; (6) prisotnost/odsotnost ščetin; (7) prisotnost/odsotnost oči.

Za zanesljivo določitev je potrebno opazovati dobro razvite ličinke, saj se pri mladih ličinkah obarvanost, število ščetin, značilnosti nosnice, kavdalne zareze in analnih vilic lahko močno razlikujejo; pri vrstah *A. sordidus, A. sputator, A. lineatus* in *A. obscurus* morajo ličinke za določitev doseči dolžino vsaj 1 cm (Pic in sod., 2008).

Mnogi morfološki določevalni ključi (Tabela 1) datirajo v prvo polovico 20. stoletja in so zaradi tega taksonomsko zastareli. Pogosto so napisani v različnih jezikih, na primer v nemščini (Beling, 1883, 1884; Schaerffenberg, 1940; Korschefsky, 1941; Klausnitzer, 2013), danščini (Schiødte, 1870), francoščini (Pic in sod., 2008), angleščini (Glen in sod., 1943; Becker, 1956; Eidt, 1954; Etzler, 2013; Glen, 1950; Lanchester, 1946; Riley in Keaster, 1979; Wilkinson, 1963; Heimbach in sod., 2020). Prav tako so klasični dihotomni ključi nemalokrat zahtevni za uporabo za nestrokovnjake, posebno če ključi temeljijo na besednem opisovanju lastnosti in morfoloških struktur. Uporabnikom prijaznejši so slikovni določevalni ključi, ki prikažejo sliko za vsak iskan morfološki znak. Takšni ključi za določevanje strun so redki; Etzler (2013) je ustvaril kombinacijo slikovnega in dihotomnega dolo-



Slika 1: Struna rodu *Agriotes* sp. a: glava, dorzalno. b: glava, ventralno. Dobro vidne mandibule in nosnica. c: deveti abdominalni segment, dorzalno. Dobro vidna »očesca«. d: deveti abdominalni segment, ventralno. Dobro viden anus. e: struna, lateralno. Slike: Eva Praprotnik.
Figure 1: Wireworm of the genus *Agriotes* sp. a: head, dorsal. b: head, ventral. Mandibles and nasale clearly distinguishable. c: ninth abdominal segment, dorsal. Muscular impressions ("eye-spots") clearly distinguishable. d: ninth abdominal segment, ventral. Anus clearly distinguishable. e: wireworm, lateral. Photo: Eva Praprotnik.



Slika 2: Levo: glava, ventralno. pz – pomožni zobec, ret – retinakulum, n – nosnica (nasale). Desno: dihalnica biforalne oblike. Slike: Eva Praprotnik.
Figure 2: Left: head, ventral. pz – preapical tooth , ret – retinaculum , n – nasale. Right: biforous spiracle. Photo: Eva Praprotnik.

čevalnega ključa za gospodarsko pomembne vrste strun, ki se pojavljajo v ameriški zvezni državi Montana in na pacifiškem severozahodu.

Strokovnjaki v Evropi se z izzivom določevanja strun in iskanjem zanesljivih morfoloških znakov ukvarjajo že dolgo časa. Beling (1883, 1884) in Schiødte (1870) sta opisala ličinke družine Elateridae, dihotomni določevalni ključ Schaerffenberga (1940) je namenjen določanju 15 najpomembnejših vrst strun v Nemčiji, delo Korschefskyja (1941) povzema biologijo družine Elateridae in sistematiko njihovih ličink, določevalni ključi Cocquempota in sod. (1999), Heimbacha in sod. (2020) in Pica in sod. (2008) se osredotočajo na rod *Agriotes,* najbolj celovit določevalni ključ pa je Klausnitzerjev (2013), saj v njem dobimo opise in skice za celotno družino Elateridae Evrope. Literatura o strunah Severne Amerike je prav tako obširna, saj nam Glen in sod. (1943) podajo dihotomni določevalni ključ za več kot 30 vrst strun Kanade, 7 let pozneje pa detajlni določevalni ključ plemena Lepturoidini Severne Amerike (Glen, 1950). Wilkinson (1963) nadgradi in spiše določevalni ključ za 25 vrst strun, ki se pojavljajo na kmetijskih zemljiščih Britanske Kolumbije. Določevalna ključa Eidta (1954) in Beckerja (1956) se osredotočata na rod *Agriotes,* Lanchester (1946) se osredotoča na rod *Limonius*, Riley in Keaster (1979) pa na rod *Melanotus*. Stibick je napisal pregled skupine Hypnoidinae, ki jo filogenetsko uvršča v samostojno poddružino in je eden redkih, ki je naredil pregled na svetovni ravni, in sicer Severne in Južne Amerike (Stibick, 1976; Stibick, 1978), Evrazije (Stibick, 1979), Indije (Stibick, 1980b) in Nove Zelandije (Stibick, 1980a).

Morfološko določanje strun je pogosto nezanesljiva metoda in zahteva veliko znanja in izkušenj, pa vendar so prve filogenetske študije družine Elateridae v veliki meri temeljile prav na morfoloških značilnostih ličink ter fosilnih ostankih (Becker, 1956). Takšno filogenetsko drevo temelji na izvornih (pleziomorfnih) in izpeljanih (apomorfnih) znakih, ki so se pri vrstah, ki jih med seboj primerjamo, pojavili v različnih kombinacijah v različnem časovnem obdobju (Rieppel, 2020). Hyslop (1917) je na podlagi morfoloških lastnosti odraslih hroščev in ličink izrisal filogenetsko shemo v geološki časovni lestvici, ki je do razvoja molekularnih metod veljala za najpopolnejši prikaz evolucijske zgodovine družine Elateridae. Evolucija družine je v biološkem smislu šla v smeri krajšanja življenjske dobe odraslih hroščev in posledično daljšanja obdobja, ki ga preživijo v stadiju ličink. Larvalni stadiji so tako tekom filogeneze doživeli znatnejše morfološke spremembe kot pa stadiji imaga (Dolin, 1978).

3 MOLEKULARNO DOLOČANJE

V zadnjem desetletju se za določanje morfološko težko ločljivih vrst uveljavlja vse več molekularnih metod (Tabela 1), ki v veliki meri temeljijo na izolaciji DNK in pomnoževanju specifičnih odsekov z uporabo metode verižne reakcije s polimerazo (ang. polymerase chain reaction - PCR). Veliko le-teh temelji na identifikaciji odraslih hroščev (Han in sod., 2016; Kundrata in sod., 2016; Oba in sod., 2015), čeprav so ličinke tiste, ki povzročajo znatno škodo v kmetijskih in gozdnih ekosistemih.

DNK črtno kodiranje je metoda, pri kateri namnožimo DNK fragmente, ki so specifični za določen takson. Pri živalih se za določanje najpogosteje uporablja zaporedje v genu za mitohondrijsko citokrom c oksidazo (COI) (Hebert in sod., 2003). DNK črtno kodiranje je pomembno orodje za določanje kriptičnih vrst, torej

Tabela 1: Metode za določanje strun, vključno z njihovimi prednostmi in slabostmi
Table 1: Methods of wireworm identification, including their advantages and disadvantages

Metode	Preučevani organizem	Prednosti	Slabosti	Referenca
Morfološko določanje	15 najškodljivejših vrst strun v Nemčiji	prisotne skice in fotografije morfoloških struktur	ključ v nemškem jeziku, uporaba zahtevna za nestrokovnjake, poudarek le na ekonomsko pomembnih vrstah	Schaerffenberg, 1940
	več kot 30 vrst strun Kanade	ključ v angleškem jeziku, natančne skice morfoloških struktur	uporaba zahtevna za nestrokovnjake, poudarek le na ekonomsko pomembnih vrstah	Glen, in sod. 1943
	vrste plemena Lepturoidini Severne Amerike	ključ v angleškem jeziku, natančne skice morfoloških struktur	uporaba zahtevna za nestrokovnjake	Glen, 1950
	45 vrst, ki se pojavljajo v ameriški zvezni državi Montana in pacifiškem severozahodu	podroben slikovni ključ v kombinaciji z dihotomnim ključem	poudarek le na ekonomsko pomembnih vrstah	Etzler, 2013
	pregled skupine Hypnoidinae	pregled poddružine na svetovni ravni	zahteven dihotomni ključ	Stibick, 1976, 1978, 1979, 1980a, 1980b
	25 vrst strun, ki se pojavljajo na kmetijskih površinah Britanske Kolumbije	ključ v angleškem jeziku, prisotne skice morfoloških struktur	uporaba zahtevna za nestrokovnjake, poudarek le na ekonomsko pomembnih vrstah	Wilkinson, 1963
	6 ekonomsko pomembnih vrst strun rodu *Limonius*	ključ v angleškem jeziku, prisotne skice morfoloških struktur, podroben opis morfoloških struktur	uporaba zahtevna za nestrokovnjake, poudarek le na ekonomsko pomembnih vrstah	Lanchester, 1946
	nearktične vrste rodu *Agriotes*	ključ v angleškem jeziku, obravnava ličinke in odrasle primerke, prikaz razširjenosti vrst na zemljevidu	uporaba zahtevna za nestrokovnjake	Becker, 1956
	družina Elateridae v Evropi	celovit ključ za identifikacijo celotne družine, podrobne skice morfoloških struktur	ključ v nemškem jeziku, uporaba zahtevna za nestrokovnjake	Klausnitzer, 2013
	10 vrst rodu *Melanotus* Severne Amerike	ključ v angleškem jeziku, prisotne skice morfoloških struktur	uporaba zahtevna za nestrokovnjake	Riley in Keaster, 1979; Riley, 1983
	4 vrste rodu *Agriotes* iz Nove Škotske	ključ v angleškem jeziku, podrobne skice in opisi morfoloških struktur	uporaba zahtevna za nestrokovnjake, poudarek le na ekonomsko pomembnih vrstah	Eidt, 1954
	4 vrste rodu *Agriotes*	poenostavljen in kratek določevalni ključ	ključ v francoskem jeziku, poudarek le na ekonomsko pomembnih vrstah	Pic, in sod. 2008
	več kot 15 rodov družine Elateridae	podrobna sistematika družine Elateridae, prisotne skice morfoloških struktur	ključ v nemškem jeziku, uporaba zahtevna za nestrokovnjake	Korschefsky, 1941
	4 vrste rodu *Agriotes* v Franciji	prisotne skice morfoloških struktur, prikaz razširjenosti vrst na zemljevidu	ključ v francoskem jeziku, poudarek le na ekonomsko pomembnih vrstah	Cocquempot, in sod. 1999
	ključ za identifikacijo 11 rodov strun, s poudarkom na vrstah iz rodu *Agriotes*	ključ v angleškem jeziku, prisotne fotografije morfoloških struktur	uporaba zahtevna za nestrokovnjake	Heimbach, in sod. 2020

nadaljevanje tabele 1

Molekularno določanje analiza mitohondrijskega gena *COI* v kombinaciji z multipleks PCR	17 vrst rodu *Agriotes* iz Srednje Evrope	poceni in zanesljiva metoda, pri kateri je verjetnost lažno pozitivnih rezultatov majhna	omejeno število začetnih oligonukleotidov znotraj ene PCR reakcije	Staudacher, in sod. 2011
analiza mitohondrijskega gena *COI* in jedrnega gena EF1-α	rod *Melanotus* iz bambusovih gozdov Južne Kitajske	analiza večjega števila lokusov poda zanesljivejše rezultate	analiza le mitohondrijskih genov ne poda vedno zanesljivih filogenetskih rezultatov	Zhang, in sod. 2019
analiza mitohondrijskega gena *COI*	30 ekonomsko pomembnih vrst v Montani, ZDA	poceni in zanesljiva metoda, številni optimizirani protokoli	analiza le mitohondrijskih genov ne poda vedno zanesljivih filogenetskih rezultatov	Etzler, in sod. 2014
analiza mitohondrijskega gena *COI*	10 vrst rodu *Melanotus*, 1 vrsta rodu *Conoderus*	poceni in zanesljiva metoda, številni optimizirani protokoli	analiza le mitohondrijskih genov ne poda vedno zanesljivih filogenetskih rezultatov	Lindroth in Clark 2009
analiza mitohondrijskega gena *COI*	7 vrst rodu *Agriotes* iz Francije	poceni in zanesljiva metoda, številni optimizirani protokoli	analiza le mitohondrijskih genov ne poda vedno zanesljivih filogenetskih rezultatov	Pic, in sod. 2008
analiza mitohondrijskega gena *COI*	3 vrste rodu *Agriotes*	poceni in zanesljiva metoda, številni optimizirani protokoli	analiza le mitohondrijskih genov ne poda vedno zanesljivih filogenetskih rezultatov	Lehmus in Niepold, 2015
T-RFLP	3 vrste rodu *Agriotes*	poceni, enostavna in zanesljiva metoda	metoda je občutljiva na variabilnost izolirane DNK med posameznimi vzorci. Uporaba različnih barvil vpliva na mobilnost DNK fragmentov v kapilarni elektroforezi	Ellis, in sod. 2009
analiza *16S* rRNK	15 ekonomsko pomembnih vrst v Kanadi	poceni in hitra metoda, ustrezna metoda za filogenetske študije	metoda se v največji meri uporablja za študije prokariontskih organizmov	Benefer, in sod. 2013
Vedenjske lastnosti intenziteta tonične negibnosti	*A. lineatus* in *A. obscurus*	v kombinaciji z morfološko identifikacijo lahko zmanjšamo možnost napake pri identifikaciji	temperatura ima velik vpliv na vedenje strun, za identifikacijo potrebujemo žive primerke	Ritter, in sod. 2016

dveh ali več vrst, ki so med seboj težko ločljive le na podlagi morfoloških lastnosti. Hkrati nam metoda omogoči prepoznavo novih povezav med odraslimi hrošči in prej vrstno nedoločljivimi ličinkami (Etzler in sod., 2014).

Pic in sod. (2008) so prvi uporabili molekularno metodo za določanje strun. Pomnoževali so COI regijo mitohondrijske DNK za določitev 7 vrst rodu *Agriotes*, ki velja za najpogostejši rod pokalic v Franciji. Metoda kombinacije molekularnega in morfološkega določanja se je izkazala za uspešno pri štirih najškodljivejših vrstah *A. sordidus, A. sputator, A. lineatus* in *A. obscurus*, vendar z eno izjemo. Tri ličinke, ki so jih raziskovalci morfološko opredelili kot *A. sputator,* so se na kladogramu razvrstile ob zaporedju odraslega predstavnika vrste *Agriotes gallicus* Lacordaire, 1835. Ker je larvalni stadij *A. gallicus* skorajda neznan, predstavlja molekularno določanje edini način za razlikovanje ličink *A. gallicus* in *A. sputator*.

Lindroth in Clark (2009) sta prav tako pomnoževala COI regijo mitohondrijske DNK za prepoznavo in filogenetski pregled gospodarsko pomembnih vrst strun srednjega zahoda ZDA iz rodu *Melanotus* in *Conoderus*. Dobljeni filogenetski odnosi se v veliki meri skladajo z morfološkimi podatki določevalnega ključa po Riley in Keaster (1981). Podan je zgled vrste *Melanotus depressus* (Melsheimer, 1844), ki se od ostalih vrst omenjenega rodu znatno razlikuje, saj je edina brez parnih prečnih prog na posteriornih segmentih. S tem se skladajo tudi rezultati filogenetske analize, saj vrsta zavzema izhodiščni položaj za vse ostale preučevane vrste znotraj rodu *Melanotus*.

Zhang in sod. (2019) so za molekularno določanje treh vrst strun iz rodu *Melanotus* iz Južne Kitajske, ki se prehranjujejo s poganjki bambusa, uporabili podatke mitohondrijskega gena *COI* in jedrnega gena *EF1-α*. Namreč analiza filogenetskih odnosov in določitev vrst, ki temelji le na mitohondrijskem genu *COI* včasih ne zadostuje in poda zavajajoče rezultate zlasti pri ozko sorodnih vrstah (Rubinoff in Holland, 2005; Shaw, 2002).

Tudi Etzler in sod. (2014) so za določanje gospodarsko najpomembnejših vrst strun na območju ameriške zvezne države Montana uporabili analizo *COI* gena. Filogenetska analiza vrste *Hypnoidus bicolor* (Eschscholtz, 1829) je pokazala veliko raznolikost med posameznimi kladi (več kot 3%), kar lahko nakazuje na prisotnost dveh ali celo treh kriptičnih vrst, ki jih na podlagi morfoloških znakov uvrščamo v eno samo vrsto. Benefer in sod. (2013) so z analizo *16S rRNK* gena prav tako pokazali veliko genetsko raznolikost omenjene vrste, v korelaciji z geografsko razdaljo, in sicer do 4,6 %. Za primerjavo, med vrstami *A. lineatus*, *A. sputator* in *A. obscurus* genetska raznolikost znaša 3,7 % (Benefer in sod., 2012), oziroma 4,9 % (Pic in sod., 2008). Status *H. bicolor* je pomemben zaradi vse večjega negativnega vpliva vrste v agroekosistemih, saj se njena številčnost po prepovedi nekaterih kemičnih insekticidov povečuje (Parker in Howard, 2001). Staudacher in sod. (2011) so razvili preprost protokol za določanje 17 vrst iz rodu *Agriotes* z metodo DNK črtnega kodiranja, v kombinaciji s PCR multipleksom pa so določili 9 najpomembnejših in hkrati najpogostejših vrst. Princip delovanja PCR multipleksa je enak klasični PCR reakciji, s tem da lahko naenkrat pomnožujemo več različnih DNK segmentov v vzorcu, in sicer z uporabo večjega števila začetnih oligonukleotidov v reakcijski mešanici. Zaradi prihranjenega časa in denarja uporaba metode PCR multipleksa narašča, še posebej pri raziskavah z velikim številom vzorcev (Gauthier, 2010; Lagisz in sod., 2010). Potrebno pa je upoštevati, da je število začetnih oligonukleotidov, ki jih lahko uporabimo znotraj ene mulitipleks PCR reakcije omejeno in morajo biti optimizirani na delovanje pri enaki temperaturi podaljševanja novo sintetiziranih nukleotidnih zaporedij, saj lahko v nasprotnem primeru pride do neenakovrednega pomnoževanja posameznih fragmentov (Sint in sod., 2012). Filogenetska analiza celotnega *COI* gena je pokazala veliko podobnost med vrstama *A. lineatus* in *A. proximus*. Ti dve vrsti prav tako privablja enak feromon (Subchev in sod., 2005), morfološke razlike odraslih osebkov so minimalne, ličinke vrste *A. proximus* pa tudi še niso morfološko opisane. Ta dejstva sprožijo vprašanje ali je smiselno obravnavati *A. lineatus* in *A. proximus* kot dve ločeni vrsti (Staudacher in sod. 2011).

Lehmhus in Niepold (2015) sta pomnoževala mitohondrijski gen *COI* za identifikacijo vrst *A. lineatus, A. sputator* in *A. obscurus,* Ellis in sod. (2009) pa so za identifikacijo teh treh vrst pomnoževali *16S* regijo mitohondrijske DNK in uporabili tehniko polimorfizma dolžine končnih restrikcijskih fragmentov (ang. Terminal restriction fragment length polymorphism - T-RFLP). Osnova metode je pomnoževanje specifičnega odseka DNK, pri čemer sta oba, lahko tudi le en začetni oligonukleotid, označena s fluorescentnim barvilom (Walker in sod., 2017). Pomnoženim odsekom dodamo encim restrikcijska endonukleaza, ki razreže odseke na specifičnih mestih, sama dolžina končnih odsekov pa je vrstno specifična. Metoda je poceni, ponovljiva in zanesljiva za določitev nekaterih najpomembnejših vrst strun, vendar je hkrati občutljiva na variabilnost med vzorci (količina izolirane DNK), uporaba različnih barvil pa vpliva na mobilnost DNK fragmentov v kapilarni elektroforezi (Prakash in sod., 2014).

4 DOLOČANJE NA PODLAGI VEDENJSKIH LASTNOSTI

Za določanje žuželk lahko v kombinaciji z morfo-

loškimi značilnostmi uporabimo tudi vedenjske lastnosti (Beaudoin-Ollivier in sod., 2000). Ritter in sod. (2016)so spremljali protipredatorsko vedenje pri različnih vrstah strun iz rodu *Agriotes*. Strune se v primeru odvzema iz substrata lahko odzovejo s tremi vedenjskimi oblikami, in sicer: 1) tonična negibnost (hlinjenje smrti); 2) faza orientacije (gibanje na površju); 3) faza kopanja v substrat. Ugotovili so, da je pri 20 °C tonična negibnost statistično značilno različna med vrstama *A. lineatus* in *A. obscurus*, in sicer ličinke, ki ostanejo negibne več kot 4,35 s pripadajo vrsti *A. lineatus*, tiste, ki so negibne manj kot 4,35 s pa vrsti *A. obscurus*. Pri morfološki določitvi, ki temelji na številu ščetin nad dihalnico (*A. lineatus* 1 ščetina in *A. obscurus* 2 ščetini (Lehmhus in Niepold, 2015)), je možnost napačne določitve 19,1 %. Če hkrati spremljamo še čas tonične negibnosti, se ta napaka zmanjša na 2,2 %. Slabost izbrane metode je, da za določanje potrebujemo žive primerke. Hkrati je prisoten velik vpliv temperaturnih sprememb, saj te različno vplivajo na protipredatorsko vedenje posameznih vrst strun (Staudacher in sod., 2013), zato so za razrešitev teh vprašanj pomembne dodatne etološke študije.

5 ZAKLJUČEK

Prepoznavanje raznolikosti kriptičnih vrst na določenem območju je pomembno za uporabo ustreznih smernic za varstvo kmetijskih pridelkov. Nezmožnost rutinskega in zanesljivega razlikovanja med posameznimi vrstami strun predstavlja veliko omejitev pri raziskovanju in upravljanju kmetijskih ekosistemov. Pri določanju strun je pomembna kombinacija različnih metod, še posebej pri vrstah, ki jih na podlagi morfoloških lastnosti težko ločimo med seboj. Dihotomni ključi so za nestrokovnjake lahko zahtevni za uporabo, zato lahko s kombinacijo morfoloških in molekularnih metod izboljšamo in razširimo obstoječe identifikacijske ključe. Analiza mitohondrijskega gena *COI* je dovolj zanesljiva za vrstno določitev strun, za boljše razumevanje med- in znotrajvrstnih odnosov pa so potrebne dodatne filogenetske študije, tudi na globalni ravni. Hkrati metoda ni vedno vsem dostopna in ni uporabna za rutinske preiskave kmetijskih svetovalcev.

Za lažje razumevanje in določanje smo pripravili morfološki določevalni ključ za ekonomsko pomembnejše vrste strun. Pri uporabi morfoloških določevalnih ključev se je potrebno zavedati, da obstaja velika verjetnost napake pri določitvah, zlasti pri opazovanju živih primerkov.

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Priloga 1: Morfološki identifikacijski ključ za najpogostejše vrste iz rodu *Agriotes* v Sloveniji (Povzeto po: Schaerffenberg, 1940; Klausnitzer, 2013, Heimbach, in sod. 2020)
Appendix 1: Morphological identification key for the most common species of the genus *Agriotes* in Slovenia (See: Schaerffenberg, 1940; Klausnitzer, 2013, Heimbach, et al. 2020)

Za ličinke iz rodu *Agriotes* je značilno cilindrično, močno hitinizirano telo, deveti abdominalni segment je nedeljen, stožčasto zašiljen, na bazi tega segmenta pa so prisotna »očesca«.
1 Tergiti so vzdolžno valovito nagubani. Deveti abdominalni segment se konča s topo, bolj ali manj izrazito bradavico. Med vrhom mandibule in retinakulumom ni dodatnih zobcev. Srednji zobec pri nosnici je daljši od zunanjih dveh. Ličinka vitka in intenzivno rumene barve, maksimalna dolžina 9 mm, širina 0,6 mm…….. *Agriotes pallidulus*
1* Tergiti nagubani, gladki ali z vdolbinicami. Konica devetega analnega segmenta koničasta in hitinizirana. Mandibule pred vrhom razširjene ali s prisotnim pomožnim zobcem med vrhom mandibule in retinakulumom. Zobci nosnice enako dolgi (Slika 2)..…... .....……...……..…..…2
2 Prisotne granulacije na začetku segmentov in med kolčkom (ang. coxa). Pomožni zobec z osjo mandibule tvori pravi kot 90 °. Od 1.-8. segmenta: nad dihalnico je ob večji ščetini prisotna še manjša ščetina. Maksimalna dolžina 18 mm, širina 1,4 mm …… ……..... ……. *Agriotes sputator*
2* Odsotne granulacije na začetku segmentov in med kolčkom……...……...……...3
3 Mandibule pred vrhom razširjene, vendar brez pomožnega zobca. Pore, iz katerih izraščajo ščetine 9. abdominalnega segmenta, so rahlo izbočene. Maksimalna dolžina 25 mm, širina 1,9 mm…… *Agriotes ustulatus*
3* Med vrhom mandibule in retinakulumom prisoten pomožni zobec……...……...…….4
4 Od 1.-8. segmenta: nad dihalnico je manjša ščetina odsotna. Pomožni zobec z osjo mandibule tvori kot 60 °. Maksimalna dolžina 27 mm, širina 2 mm……...……...……...… *Agriotes lineatus*
4* Od 1.-8. segmenta: nad dihalnico je ob večji ščetini prisotna še manjša ščetina. Pomožni zobec z osjo mandibule tvori kot 120 °. Maksimalna dolžina 28 mm, širina 2 mm……...… *Agriotes obscurus*

doi:10.14720/aas.2021.117.1.2106 Review article / pregledni znanstveni članek

Načini zatiranja marmorirane smrdljivke (*Halyomorpha halys* [Stål, 1855], Hemiptera, Pentatomidae)

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Received Februay 27, 2021; accepted March 03, 2021.
Delo je prispelo 27. februarja 2021, sprejeto 03.03. 2021.

Načini zatiranja marmorirane smrdljivke (*Halyomorpha halys* [Stål, 1855], Hemiptera, Pentatomidae)

Izvleček: Marmorirana smrdljivka (*Halyomorpha halys* [Stål, 1855]; [Hemiptera, Pentatomidae]) je predstavnik družine ščitastih stenic. Gre za invazivno, tujerodno in polifagno vrsto, ki izvira iz Vzhodne Azije. Sredi 90-ih je bila vnesena v ZDA ter leta 2004 v Evropo. Danes se pojavlja v večini evropskih držav. V novih okoljih se hitro prilagaja in uspešno razmnožuje, pri čemer razvije najmanj en popoln rod letno. Z naraščanjem populacij postaja moteč dejavnik v urbanem okolju ter v zadnjem času eden od najpomembnejših škodljivcev v kmetijski pridelavi. V Sloveniji smo stenico prvič našli leta 2017 v Šempetru pri Gorici. V slabih dveh letih se je razširila po celotni Sloveniji ter začela povzročati škodo v kmetijski pridelavi. V preglednem članku smo zbrali raziskave tujih in domačih raziskovalcev, vezanih na preučevanje različnih načinov zatiranja marmorirane smrdljivke. V članku so predstavljeni tako kemični načini zatiranja, uporaba insekticidnih mrež, atraktantov in repelentov, privabilnih posevkov kot tudi načini biotičnega varstva z uporabo plenilcev in parazitoidov.

Ključne besede: *Halyomorpha halys;* kemično zatiranje; atraktanti; repelenti; privabilni posevki; insekticidne mreže; biotično varstvo rastlin; Slovenija

Management methods for marmorated stink bug (*Halyomorpha halys* [Stål, 1855], Hemiptera, Pentatomidae)

Abstract: The brown marmorated stink bug (*Halyomorpha halys* (Stål, 1855); [Hemiptera, Pentatomidae]) is an invasive, alien and polyphagous insect species native to East Asia. It was introduced to the United States in the mid-1990s and to Europe in 2004. Today it is present in most European countries. In new environments, it adapts quickly and reproduces successfully, developing at least one complete generation per year. With the growth of populations, it is becoming a disturbing factor in the urban areas and recently one of the most dangerous pests in agricultural production. It was first discovered in Slovenia in 2017 in Šempeter near Gorica. In less than two years, it spread to the entire territory od Slovenia and began to cause damage to agricultural production. In a review paper, we have collected research by foreign and domestic researchers related to the study of different ways of controlling the brown marmorated stink bug. The article presents chemical methods of control, the use of insecticide nets, the use of attractants and repellents, trap crops, as well as methods of biotic protection using predators and parasitoids.

Key words: *Halyomorpha halys;* chemical control; attractants; repellents; trap crops; insecticidal nets; biological control; Slovenia

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1 UVOD

Na področju omejevanja številčnosti marmorirane smrdljivke (*Halyomorpha halys*) in preprečevanje škode v kmetijski pridelavi v tem trenutku nimamo na razpolago prav veliko ukrepov, kot tudi ne učinkovitih rešitev. Opravka imamo z izjemno »robustnim« škodljivcem, ki se ga ne da enostavno obvladovati z insekticidi. Marmorirana smrdljivka je predstavnik družine Pentatomidae. Gre za invazivno, tujerodno in polifagno vrsto, ki izvira iz Vzhodne Azije (Panizzi in sod., 2000; Lee in sod., 2013a). Vrsta je bila sredi 90-ih vnesena v ZDA (Hoebeke in Carter, 2003; Morrison in sod., 2017) ter leta 2004 v Evropo (Leskey in sod., 2012a; Rice in sod., 2014). Danes se pojavlja v večini evropskih držav. V novih okoljih se hitro prilagaja in uspešno razmnožuje, pri čemer razvije najmanj en popoln rod letno (Leskey in sod., 2012a). Z naraščanjem populacij postaja moteč dejavnik v urbanem okolju ter v zadnjem času eden najbolj nevarnih škodljivcev v kmetijski pridelavi (Inkley, 2012). V Sloveniji smo jo prvič odkrili leta 2017, in sicer v Šempetru pri Gorici. V slabih dveh letih se je razširila na območju celotne Slovenije ter začela povzročati škodo v kmetijski pridelavi (Rot in sod., 2018).

Vrsta je polifag. Prehranjuje se s preko 150 različnimi rastlinskimi vrstami iz številnih družin, med katerimi prevladujejo metuljnice in rožnice (Morrison in sod., 2017). Med sadnimi vrstami so njeni najpomembnejši gostitelji hruška, jablana, breskev, leska, kaki in aktinidija (Leskey in sod., 2012a). Škodo povzroča tudi na vinski trti, na plodovkah (paradižnik, paprika, jajčevec, kumare) in stročnicah (fižol). V pridelavi poljščin so najbolj ogroženi posevki soje ter koruze. Prehranjuje se tudi na številnih okrasnih rastlinah in grmovnicah ter prosto rastočih drevesnih vrstah, kot so: veliki jesen (*Fraxinus excelsior L.*), visoki pajesen (*Ailanthus altissima [Mill.] Swingle*), pavlovnija (*Paulownia tomentosa* [Thunb.] Steud.), jerebika (*Sorbus aucuparia L.*), lovorikovec (*Prunus laurocerasus L.*), oslez (*Hibiscus spp.*), vrtnica (*Rosa sp.*), navadna bodika *(Ilex aquifolium L.)* in številnih druge (Nielsen in Hamilton, 2009; Leskey in sod., 2012a; Morrison in sod., 2017).

V ZDA, kjer je marmorirana smrdljivka zastopana najdlje, je škoda, ki jo je povzročila v kmetijski pridelavi ocenjena na nekaj milijard dolarjev. V letu 2010 je pridelovalcem jabolk na območju Srednjega Atlantika povzročila škodo v višini 34 mio. $, sočasno so pridelovalci breskev beležili 50 % izgube pridelka (Leskey in sod., 2012a; Morrison in sod., 2017). V Evropi je do sedaj povzročila največ škode v Italiji, v pridelavi hrušk in jabolk (Cesari in sod., 2015). V deželi Emiglia Romagna, v provinci Modena je bilo v letu 2015 zaradi močnega napada marmorirane smrdljivke poškodovanih od 30 do 50 % hrušk (Cesari in sod., 2015). Z Madžarske poročajo o veliki škodi v pridelavi fižola in paprike, v Gruziji in Abhaziji je zaradi marmorirane smrdljivke ogrožena pridelava lešnikov (Morrison in sod., 2017). Poleg škode v kmetijski pridelavi je po vsej Evropi postala moteč dejavnik v urbanem okolju. Zaradi specifičnega načina prezimovanja, v jesenskem času stenice množično priletajo v bližino človeških bivališč. V iskanju skupnega zimskega zavetja izločajo agregacijske feromone ter se množično zbirajo na fasadah hiš, kar povzroča prave invazije žuželk (Inkley, 2012).

V nasadih se škodljivec pojavlja od cvetenja do zorenja plodov gostiteljskih rastlin. Obdobje, v katerem povzroča škodo, je izjemno dolgo. Kemično zatiranje marmorirane smrdljivke, vezano na pragove škodljivosti, zahteva veliko število škropljenj, kar negativno vpliva na agroekosistem, obremenjuje okolje in je v popolnem nasprotju z vzpostavljenim sistemom integriranega varstva (Morrison in sod., 2017). Dolgoročno gre pričakovati, da se bodo domorodni koristi organizmi sčasoma prilagodili na novega tujerodnega škodljivca ter ga začeli omejevati na dopustno raven. Izkušnje iz tujine kažejo, da je obvladovanje marmorirane smrdljivke izjemno zahteven proces, ki mora združevati številne ukrepe varstva rastlin (Morrison in sod., 2017). Temeljiti morajo na zanesljivih metodah spremljanja populacije škodljivih organizmov, predvidevanju nastanka škode ter pravočasni napovedi in izvedbi ukrepov.

2 SPREMLJANJE POPULACIJSKE DINAMIKE MARMORIRANE SMRDLJIVKE

Za spremljanje populacijske dinamike različnih vrst domorodnih stenic se v praksi navadno uporabljajo različne detekcijske metode, kot so uporaba metuljnice, otresanje rastlin, feromonske vabe in UV oz. črne svetilke (svetilke, ki oddajajo vijolično in modro kratkovalovno sevanje) (Krupke in sod., 2001; Leskey in Hogmire, 2005; Kamminga in sod., 2009; Borges in sod., 2011). Za spremljanje populacijske dinamike marmorirane smrdljivke so se v praksi sprva uporabljale piramidalne pasti z uporabo agregacijskega feromona (metil [2E, 4E, 6Z]-dekatrienoat) stenice *Plautia stali* Scott (Nielsen in sod., 2011; Laskey in sod., 2012b; Joseph in sod., 2013). Raziskovalci so ugotovili, da omenjeni feromon deluje na marmorirano smrdljivko kot kairomon (Aldrich in sod., 2007; Khrimian in sod., 2008). Nadaljnje raziskave so pokazale, da feromon (metil [2E, 4E, 6Z]-dekatrienoat) ne vpliva na gibanje marmorirane smrdljivke spomladi in zgodaj poleti in je zato uporaben le pri pozno poletnem in jesenskem lo-

vljenju stenic (Leskey in sod., 2012b). Khrimian in sod. (2014) so ugotovili, da dvokomponentno privabilo, ki ga sestavljata agregacijski feromon (murgantiol) ter sinergistično sredstvo metil-dekatrienoat (MDT) vplivata tako na ličinke kot tudi na odrasle osebke marmorirane smrdljivke skozi celotno rastno dobo. Njihovo raziskavo so potrdili tudi rezultati nekaterih sorodnih raziskav (Weber in sod., 2014; Leskey in sod., 2015; Morrison in sod., 2017; Rice in sod., 2018), v katerih so se predvsem ukvarjali z ugotavljanjem razmerij različnih vrst agregacijskih feromonov in njihovim učinkom na lovljenje populacij marmorirane smrdljivke. Poznavanje agregacijskih feromonov marmorirane smrdljivke predstavlja podlago za strategijo »privabi in ubij (angl. "attract and kill"), kjer s feromoni privabimo na izbrano rastlinsko vrsto škodljivca in ga nato z uporabo drugih tehnik (kemično, biotično varstvo, mehansko z uporabo insekticidnih sesalcev) zatremo (Morrison in sod, 2016). Iz ZDA poročajo tudi o spremljanju populacijske dinamike z uporabo svetlobnih vab (bela, črna in modra dolgovalovna svetloba), vendar so potrdili uspešnost uporabe zgolj pri lovljenju odraslih osebkov (Nielsen in sod., 2013).

3 UPORABA ETERIČNIH OLJ

Rastlinska eterična olja predstavljajo alternativni način zatiranja škodljivih žuželk v kmetijstvu (Isman, 2006). Eterična olja so vir aktivnih, močnih presnovkov, ki lahko vplivajo na bionomijo, vedenje in fiziologijo žuželk. Poleg tega imajo kratko obstojnost v okolju in majhno toksičnost za sesalce in so zaradi široke uporabe kot dišave in arome navadno na voljo v velikih količinah po razumnih cenah (Isman, 2006). Dosedanje raziskave so pokazale, da eterična olja navadno delujejo kot repelenti za različne skupine žuželk (Moore in sod., 2007; Zhang in sod., 2013).

Zhang in sod. (2014) so preučevali vpliv različnih eteričnih olj (nageljnove žbice, limonina trava, poprova meta) in ugotovili, da prav vsa vplivajo odvračalno na ličinke kot tudi odrasle osebke marmorirane smrdljivke. V nadaljevanju so kitajski raziskovalci naredili kemično analizo eteričnih olj in določili več kot 20 aktivnih snovi. Nekatere med njimi (evgenol, karvon, menton, pulegon, metil salicilat, trans/cis-citral, metil benzoat in β-kariofilen) so v primerjavi z atraktanti (MDT) zmanjšali ulov marmorirane smrdljivke v lovilnih posodah za 72 do 99 %. Raziskovalci zaključujejo, da lahko kombinacija repelentov in atraktantov, razporejenih na kmetijskih in urbanih zemljiščih, predstavlja t.i. »odvrni in privabi (angl. "push and pull") strategijo zatiranja marmorirane smrdljivke.

4 KEMIČNO ZATIRANJE MARMORIRANE SMRDLJIVKE

Marmorirana smrdljivka postaja vedno večji problem v kmetijstvu, zato raziskovalci širom sveta iščejo učinkovite ukrepe z željo po omejitvi njene številčnosti in posledično zmanjšanju gospodarske škode na gojenih rastlinah. Leskey in sod. (2012c) poročajo o neučinkovitosti insekticidov, ki se navadno uporabljajo za zatiranje nekaterih drugih vrst stenic v ZDA. Lee in sod. (2013a) so pripravili pregled literature, vezane na učinkovitost različnih vrst insekticidov, ki jih v Aziji uporabljajo za zatiranje marmorirane smrdljivke. Ugotovljeno je bilo, da so nekateri insekticidi iz skupine kloriranih ogljikovodikov, organskih fosforjevih estrov, karbamatov, piretroidov in neonikotinoidov pokazali zelo veliko učinkovitost pri kontaktnem zatiranju marmorirane smrdljivke (Bae in sod., 2008). Žal večina teh pripravkov ni registriranih v ZDA in Evropi za zatiranje škodljivca oz. so bile nekatere aktivne snovi celo zakonsko umaknjene iz trgovskih polic. Zato raziskovalci iščejo ustrezne novejše rešitve na področju kemičnega zatiranja marmorirane smrdljivke.

V raziskavah, ki so jih opravili v ZDA, so tako v obdobju med leti 2008 in 2017 različne skupine raziskovalcev opravile serije laboratorijskih preizkušanj učinkovitosti različnih vrst insekticidov pri zatiranju marmorirane smrdljivke (Nielsen in sod., 2008; Kuhar in sod., 2012; Leskey in sod., 2012c; Lee in sod., 2013b; Kuhar in Kamminga, 2017). Nielsen in sod. (2008) so potrdili veliko toksičnost piretroidov (bifentrin, beta-ciflutrin, lambda cihalotrin, ciflutrin in fenpropatrin) pri zatiranju marmorirane smrdljivke. Zadovoljivo učinkovitost so pokazali tudi nekateri neonikotinoidi (dinetofuran in tiametoksam). Nielsen in sod. (2008) so tudi potrdili, da je zatiranje ličink marmorirane smrdljivke bolj učinkovito kot zatiranje odraslih osebkov. V sorodni raziskavi so Leskey in sod. (2012c) potrdili učinkovitost piretroidov (bifentrin, fenpropatrin, permetrin), organskih fosforjevih estrov (dimetoat, malation, metidation, klorpirifos, acefat), karbamatov (metomil) in kloriranih ogljikovodikov (endosulfan). Krawczyk in sod. (2011) so potrdili veliko učinkovitost aktivnih snovi bifentrin, metomil, endosulfan in večine neonikotinoidov (razen a.s. tiakloprid) pri zatiranju vseh razvojnih stadijev marmorirane smrdljivke. Kuhar in sod. (2012) so potrdili učinkovitost acefata, oksamila, zeta-cipermetrina, fenpropatrina, lambda-cihalotrina in acetamiprida pri zatiranju ličink marmorirane smrdljivke. Njihovi rezultati so potrdili tezo Nielsen in sod. (2008), da so ličinke bolj občutljive na delovanje insekticidov kot odrasli osebki marmorirane smrdljivke. Lee in sod. (2013b) poročajo o 100 % učinkovitosti

Preglednica 1: Učinkovitost insketicidov pri zatiranju marmorirane smrdljivke (*Halyomorpha halys*) v laboratorijskih razmerah.
Table 1: Performance of insecticides against the brown marmorated stink bug (*Halyomorpha halys*) in laboratory bioassays.

Insekticid	Učinkovitost	Insekticid	Učinkovitost
Organski fosforjevi estri		Neonikotinoidi	
Dimetoat	4	Dinotefuran	3,3
Malation	4	Klotianidin	2,8
Metidation	4	Tiametoksam	2,8
Klorpirifos	3	Acetamiprid	2,3
Metal paration	3	Imidakloprid	1,5
Acefat	2,5	Tiakloprid	1,5
Formetanat	2		
Azinfos-metil	1	Klorirani ogljikovodiki	
Diazinon	1	Endosulfan	4
Fosmet	1		
		Druge skupine insekticidov	
Karbamati		Spinetoram	1
Metomil	3,3	Abamektin	1
Oksamil	2	Piriproksifen	1
Karbaril	1,5	Priflukuinazon	1
		Novaluron	1
Piretroidi		Tlfenpirad	1
Etofenproks	4	Indoksakarb	1
Bifentrin	3,8	Spirotetramat	1
Permetrin	3	Klorantranilprol	1
Fenpropatrin	2,8	Ciantranilprol	1
Lambda-cihalotrin (LC)	2,8	Flonikamid	1
Ciflutrin	2,7		
Beta-ciflutrin (BC)	2,5	Kombinirana uporaba	
Cipermetrin	2,5	Tiametoksam + klorantranilprol	4
Gama-cihalotrin	2	Beta ciflutrin + imidakloprid	3,7
Zeta-cipermetrin (ZC)	2	LC + tiametoksam	3,7
Esfenvalerat	1	Bifentrin + imidacloprid	3
		ZC + bifentrin	3
		LC + klorantranilprol	1

Legenda: učinkovitost pripravka 1 (< 50%), 2 (50-69 %), 3 (70-89 %), 4 (90-100 %) (Nielsen in sod., 2008; Krawczyk in sod., 2011; Leskey in sod., 2012c; Kuhar in sod., 2012; Lee in sod., 2013b; Kuhar in Kamminga, 2017).

aktivnih snovi metomil, acefat, klorpirifos, dimetoat, malation, metidation, bifentrin, fenpropatrin, permetrin, dinotefuran in endosulfan pri zatiranju odraslih osebkov marmorirane smrdljivke. Pregled učinkovitosti posameznih aktivnih snovi pri zatiranju marmorirane smrdljivke v laboratorijskih razmerah je predstavljen v preglednici 1.

Pridobljeni laboratorijski rezultati so skupine raziskovalcev vodili tudi na preizkušanje učinkovitosti insekticidov pri zatiranju marmorirane smrdljivke na prostem (Bergmann in Raupp, 2014; Leskey in sod., 2014; Monneyham in sod., 2016; Kuhar in Kamminga, 2017). Učinkovitost insekticidov v laboratorijskih preizkusih je dostikrat večja od tiste, ki jo dosežemo v poskusih na prostem zaradi številnih omejujočih dejavnikov, ki jih v laboratoriju lahko reguliramo. Ker se marmorirana smrdljivka lahko premika na daljše razdalje (Lee in Leskey, 2015) kot tudi med različnimi gostiteljskimi rastlinami

Preglednica 2: Insekticidi, ki so pokazali visoko učinkovitost pri zatiranju marmorirane smrdljivke (*Halyomorpha halys*) na prostem.
Table 2: Insecticides that provided a significant reduction of the brown marmorated stink bug (*Halyomorpha halys*) in field efficacy experiments.

Skupina insekticidov		
Organofosfati	Neonikotinoidi	Kombinirana uporaba
Acefat	Dinotefuran	Tiametoksam + klorantranilprol
Fosmet	Klotianidin	BC + imidakloprid
	Tiametoksam	LC + tiametoksam
Karbamati		Bifentrin + imidacloprid
Metomil	Klorirani ogljikovodiki	ZC + bifentrin
Oksamil	Endosulfan	LC + klorantranilprol
		BC + spinetoram
Piretroidi	Druge skupine insekticidov	Bifentrin + abamectin
Etofenproks	Flupiridafuron	Binfetrin + klorantranilprol
Bifentrin	Diflubenzuron	Metomil + fosmet
Permetrin	Indoksakarb	Metomil + ZC
Fenpropatrin	Klorantranilprol	
Lambda-cihalotrin (LC)	Ciklaniprol	
Beta-ciflutrin (BC)	Flonikamid	
Gama-cihalotrin		
Zeta-cipermetrin (ZC)		
Esfenvalerat		

(Krawczyk in sod., 2011; Kuhar in sod., 2012; Herbert in sod., 2013; Nielsen in Rucker, 2013; Frank, 2014; Walgenbach in Schoof, 2015; Morrison in sod., 2016; Kuhar in Kamminga, 2017).

(Zobel in sod., 2016), je ključnega pomena, da pride v stik z letalno dozo aktivne snovi na tretirani rastlini (Morrison in sod., 2016). Le tako je možno doseči zadovoljivo raven učinkovitosti izbranih insekticidov pri zatiranju marmorirane smrdljivke. Leskey in sod. (2014) so ugotovili, da je sveži nanos insekticida na rastlino znatno bolj letalen za marmorirano smrdljivko kot nekaj dni star insekticidni ostanek na rastlini. Omenjeni raziskovalci so to ugotovitev potrdili na primeru aktivnih snovi fenpropatrin ter dinotefuran. Obe aktivni snovi sta se izkazali kot zelo učinkoviti, če je marmorirana smrdljivka prišla v stik z aktivno snovjo takoj, ko je bila le-ta nanesena. Ob poznejšem stiku (po 24h) z aktivno snovjo je bil učinek le-te zanemarljiv. Leskey in sod. (2014) so ugotovili, da insekticidni ostanki na rastlini delujejo na marmorirano smrdljivko odvračalno (vplivajo na zmanjšano hranjenje), ne povzročijo pa njene smrtnosti. Bargmann in Raupp (2014) sta v sorodni raziskavi potrdila pomen takojšnjega stika marmorirane smrdljivke z insekticidom na primeru aktivnih snovi karbaril, acetamiprid in permetrin. Ostanki aktivnih snovi permetrin in karbaril so v njuni raziskavi vplivali na več kot 80 % smrtnost ličink marmorirane smrdljivke 48 h po nanosu, medtem ko aktivna snov acetamiprid ni imela učinka na smrtnost le-teh. Mooneyham in sod. (2016) so preučevali učinek devetih registriranih insekticidov, ki se uporabljajo za zatiranje škodljivcev na stavbah. Ugotovili so, da aktivne snovi lambda-cihalotrin (LC), LC + tiametoksam, beta-ciflutrin (BC), BC + imidacloprid učinkujejo (več kot 80 % smrtnost odraslih osebkov marmorirane smrdljivke) tudi po desetih dneh po nanosu na okenske okvirje. Skupine različnih raziskovalcev so v obdobju med leti 2011 do danes izvedle številne raziskave vezane na preizkušanje učinkovitosti insekticidov pri zatiranju marmorirane smrdljivke na prostem (Krawczyk in sod., 2011; Kuhar in sod., 2012; Herbert in sod., 2013; Nielsen in Rucker, 2013; Frank, 2014; Walgenbach in Schoof, 2015; Morrison in sod., 2016; Kuhar in Kamminga, 2017). Na splošno so se številni insekticidi, ki so bili učinkoviti v laboratorijskih preizkusih, dobro izkazali tudi na prostem. Aktivne snovi, ki so bile najučinkovitejše, so bile iz skupine piretroidov (beta-ciflutrin, bifentrin, permetrin, fenpropatrin, lambda-cihalotrin, zeta-cipermetrin), neonikotinoidov (dinotefuran, klotianidin in tiametoksam), karbamatov (metomil) in kloriranih ogljikovodikov (endosulfan) (Preglednica 2).

5 UPORABA INSEKTICIDNIH MREŽ

Uporaba mrež za zaščito ljudi pred škodljivimi žuželkami je dobro znana in razširjena praksa, katerih uporaba sega v konec 17. stoletja (Da San Gallo, 1679). V kmetijstvu se zaščitne mreže v zadnjem času uporabljajo tako v rastlinjakih kot tudi na prostem z namenom, da zaščitimo rastline pred škodljivimi organizmi (Sauphanor in sod., 2012). Novost v pristopu so t.i. insekticidne mreže, kjer je na mrežo nanesen izbrani insekticid, ki poveča učinkovitost zatiranja škodljivca oz. omejevanja poškodb in posledične škode na gojenih rastlinah (Hill in sod., 2007).

Zaenkrat je najbolj učinkovit način preprečevanja škode, ki jo povzroča marmorirana smrdljivka, ravno uporaba insekticidnih mrež (Kuhar in sod., 2017; Sabbatini Peverieri in sod., 2018). V pridelavi sadja se za ta namen uporabljajo protitočne mreže, nadgrajene s stranskimi mrežami, ki popolnoma preprečijo dostop stenicam do rastlin. V določenih primerih se na mreže lahko nanese tudi insekticid (Kuhar in sod., 2017). Popolno zamreženje nasadov je potrebno izvesti takoj po cvetenju sadnega drevja (Leskey in Nielsen, 2018). V raziskavi, ki so jo opravili v Italiji (Sabbatini Peverieri in sod., 2018), raziskovalci poročajo o veliki učinkovitosti insekticidne mreže, ki je bila tretirana z a.s. alfa-cipermetrinom, pri zatiranju oz. omejevanju škode, ki jo povzroči marmorirana smrdljivka. Potrdili so več kot 70 % smrtnost odraslih osebkov marmorirane smrdljivke, ki so prišli v stik z insekticidno mrežo. Poskus je bil sicer opravljen v laboratorijskih razmerah. Kuhar in sod. (2017) poročajo o 80 % smrtnosti odraslih osebkov marmorirane smrdljivke, ki so prišli v stik z insekticidno mrežo, ki je bila tretirana z a.s. deltametrin. V poskusu na prostem, so Sabbatini Peverieri in sod. (2018) v nasadu breskev preučevali insekticidno mrežo prepojeno z alfa-cipermetrinom in deltametrinom. Mrežo so dodatno prepojili tudi z atraktantom (murgantiol + MDT). Raziskava je pokazala veliko umrljivost vseh razvojnih stadijev marmorirane smrdljivke in posledično občutno zmanjšanje škode na rastlinah. Gre za zgled uporabe strategije »privabi in ubij«, ko z atraktantom privabimo škodljivca, ki nato pride v stik z mrežo, ki je prepojena z insekticidom. Prav tako so italijanski raziskovalci preučevali tudi vpliv zaščitnih mrež brez dodatka insekticida na pojav škode, ki jo naredi marmorirana smrdljivka (Candian in sod., 2018). Rezultati raziskave so pokazali 45 % zmanjšanje škode v breskovih nasadih v primerjavi z netretiranimi nasadi, ter za 20 % zmanjšanje škode v primerjavi z nasadi, ki so bili tretirani z insekticidom deltametrin (Candian in sod., 2018). V nobeni od omenjenih raziskav raziskovalci niso izpostavili vpliva insekticidnih mrež na neciljne organizme kot tudi ne na koristne vrste žuželk.

6 METODA PRIVABILNIH POSEVKOV

Metoda privabilnih posevkov je pogosto uporabljena v primerih, ko za zatiranje škodljivega organizma ni registriranega fitofarmacevtskega sredstva, ko je pripravek predrag oziroma tedaj, ko glavni posevek ni odporen na napad ali okužbo škodljivih organizmov. Pri metodi privabilnih posevkov izkoriščamo lastnosti za škodljivce dovzetnih rastlin (Trdan in sod., 2005; Cook in sod., 2006). Privabilne posevke posadimo oziroma posejemo med rastline glavnega posevka ali v njegovo bližino, z namenom, da bi na dovzetne rastline privabili škodljivce in obenem zmanjšali njihovo številčnost na glavnem posevku (Cook in sod., 2006).

Marmorirana smrdljivka je polifag, saj se hrani z različnimi rastlinskimi vrstami. Soergel in sod. (2015) so preučevali vpliv privabilnih posevkov pri pridelavi paprike. Ugotovili so, da je bil ulov marmorirane smrdljivke na sončnicah (*Helianthus annuus* L.) v primerjavi s papriko občutno večji. V sklopu raziskave sicer niso ugotovili zmanjšanja poškodb na papriki, kar avtorji pripisujejo predvsem dejstvu, da marmorirane smrdljivke niso zatirali na privabilnih posevkih. Znano je, da je marmorirana smrdljivka zelo mobilna vrsta (Lee in Leskey, 2015), zato predvidevajo, da so se osebki, ki so se v večjem številu pojavili na sončnicah pozneje vrnili na papriko in na plodovih povzročili gospodarsko škodo. Do podobnih rezultatov so prišli tudi Mathews in sod. (2017), ki so preučevali vpliv mešanih privabilnih posevkov (sončnica in navadni sirek) na zmanjšanje škode, ki jo povzroči marmorirana smrdljivka na papriki. Ulov marmorirane smrdljivke je bil na privabilnih posevkih za 4 x večji kot na glavni rastlini, vendar do zmanjšanja poškodb na plodovih ni prišlo. V sorodni raziskavi so Nielsen in sod. (2016) preučevali različne rastlinske vrste, kot potencialne privabilne posevke. Poleg sončnice so v raziskavo vključili tudi navadni sirek (*Sorghum bicolor* L.), biserno proso (*Pennisetum glaucum* [L.]), jedilni oslez (*Abelmoschus esculentus* Moench) in njivski grah (*Pisum sativum* var. *arvense* [L.]). Ulov marmorirane smrdljivke je bil na navadnem sirku za več kot 4 x večji v primerjavi z ostalimi preučevanimi rastlinami. V nadaljevanju so preizkusili različne metode zatiranja škodljivca na privabilnih posevkih – kemično (azadirahtin), uporaba sesalcev za mehansko odstranjevanje žuželk z rastlin in ožiganje rastlin. Ožiganje rastlin se je izkazalo kot najbolj učinkovito (Nielsen in sod., 2016). Iz dosedanjih rezultatov raziskav je razvidno, da samostojna uporaba metode privabilnih posevkov ni zadostno učinkovit ukrep pri zmanjšanju škode, ki jo povzroči marmorirana smrdljivka na glavni rastlini, temveč je potrebno metodo kombinirati z uporabo drugih varstvenih ukrepov.

7 BIOTIČNO VARSTVO

Biotično zatiranje marmorirane smrdljivke trenutno temelji predvsem na ugotavljanju ustreznih agensov, ki bi se v prihodnje lahko uporabljali v programih biotičnega varstva rastlin. Parazitske osice iz reda kožekrilcev (Hymenoptera), so znane kot najbolj učinkovit naravni sovražnik marmorirane smrdljivke. Svoja jajčeca odla-

Preglednica 3: Naravni sovražniki marmorirane smrdljivke (*Halyomorpha halys*).
Table 3: Natural enemies of the brown marmorated stink bug (*Halyomorpha halys*).

Vrsta naravnega sovražnika	Tip	Razvojni stadij marmorirane smrdljivke
Anastatus	parazitoid	jajčeca
Anastatus bifastiatus	parazitoid	jajčeca
Anastatus mirabilis	parazitoid	jajčeca
Anastatus pearsalli	parazitoid	jajčeca
Anastatus reduvii	parazitoid	jajčeca
Arilus cristatus	plenilec	ličinke, imago
Arma chinensis	plenilec	
Astata bicolor	plenilec	ličinke
Astata unicolor	plenilec	ličinke, imago
Astochia virgatipes	plenilec	
Bicyrtes quadrifasciatus	plenilec	ličinke
Bogosia	parazitoid	imago
Geocoris	plenilec	jajčeca, ličinke
Gryon japonicum	parazitoid	jajčeca
Gryon obesum	parazitoid	jajčeca
Harmonia axyridis	plenilec	jajčeca
Isyndus obscurus	plenilec	
Misumenops tricuspidatus	plenilec	
Ooencytrus	parazitoid	jajčeca
Ophiocordyceps nutans	patogen	
Orius	plenilec	jajčeca
Telenomus chlorupus	parazitoid	jajčeca
Telenomus podi	parazitoid	jajčeca
Trichopoda pennipes	parazitoid	ličinke, imago
Trissolcus brochymenae	parazitoid	jajčeca
Trissolcus cultratus	parazitoid	jajčeca
Trissolcus edessae	parazitoid	jajčeca
Trissolcus euschisti	parazitoid	jajčeca
Trissolcus flavipes	parazitoid	jajčeca
Trissolcus itoi	parazitoid	jajčeca
Trissolcus japonicus	parazitoid	jajčeca
Trissolcus mitsukurii	parazitoid	jajčeca
Trissolcus scutellaris	parazitoid	jajčeca
Trissolcus semistriatus	parazitoid	jajčeca
Trissolcus thyantae	parazitoid	jajčeca
Trissolcus utahensis	parazitoid	jajčeca

(Arakawa in Nakamura, 2003; Li in Liu, 2004; Yang, 2009; Haye in sod., 2015; Abram in sod., 2017, Dieckhoff in sod., 2017; Costi in sod., 2019).

gajo v jajčeca gostitelja in povzročajo njihov propad. V Aziji, v izvornem okolju marmorirane smrdljivke, je razširjena vrsta *Trissolcus japonicus* (Ashmead, 1904), ki je znana kot njen najbolj učinkovit parazitoid (Yang, 2009). V Severni Ameriki in Evropi je bilo ugotovljenih več različnih rodov jajčnih parazitoidov, potencialnih naravnih sovražnikov *H. halys*, ki pripadajo trem družinam: Scelionidae (*Telenomus*, *Trissolcus*, *Gryon* spp.), Eupelmidae (*Anastatus* spp.) in Encyrtidae (*Ooencyrtus* spp.). Osice iz rodov *Telenomus* in *Trissolcus* (Scelionidae) so znane kot specializirani jajčni parazitoidi ščitastih stenic (Hemiptera: Pentatomidae), medtem ko so parazitoidi iz rodov *Anastatus* in *Ooencyrtus* bolj generalisti in napadajo žuželke iz različnih družin (Abram in sod., 2017, Dieckhoff in sod., 2017). Raziskave, opravljene v Švici, so potrdile zastopanost vrst *Anastatus bifasciatus* (Geoffroy), *Trissolcus cultratus* (Mayr), *Trissolcus semistriatus* (Nees), *Trissolcus scutellaris* (Thomson), *Trissolcus cultratus* Mayr in *Telenomus chloropus* (Thomson) (Haye in sod., 2015). Rezultati triletnega spremljanja zastopanosti domorodnih jajčnih parazitoidov v Emigli Romagni v Italiji, so potrdili zastopanost vrste *A. bifasciatus*. Stopnja parazitizma je znašala od 1 do 3 % (Costi in sod., 2019). V preglednici 3 navajamo naravne sovražnike marmorirane smrdljivke. V Evropi je v teku več raziskav z namenom preizkušanja učinkovitosti domorodnih naravnih sovražnikov marmorirane smrdljivke. Kot obetavna sta se pokazala jajčna parazitoida *Ooencyrtus telenomicida (Vassiliev 1904)* [Hymenoptera] in *Anastatus bifasciatus* [Hymenoptera] (Costi in sod., 2019). Prvi je učinkovitost izkazal zlasti v laboratorijskih poskusih, medtem ko je v poljskih poskusih pokazal omejeno delovanje. Nadaljujejo se tudi raziskave vrste *A. bifasciatus*, ki je v laboratorijskih poskusih pokazala manjšo učinkovitost pri parazitiranju *H. halys* v primerjavi z vrsto *O. telenomicida*, vendar pa je zaradi bioloških značilnosti najresnejši kandidat za klasično biotično varstvo marmorirane smrdljivke (Dieckhoff in sod., 2017). *A. bifasciatus* se razvija in razmnožuje v temperaturnem območju 15-32 °C, letno razvije do 3 rodove v obdobju od junija do oktobra, kar se ujema s periodo ovipozicije pri marmorirani smrdljivki. Samice se hranijo na jajčecih gostitelja, hkrati vanje odlagajo lastna jajčeca, kar povzroči smrt gostitelja. Na podlagi obetavnih rezultatov začetnih raziskav, nameravajo raziskovalci Univerze iz Modene in Mednarodnega centra za kmetijstvo in bioznanosti (CABI), ki deluje v Švici, razviti protokole za masovno gojenje parazitoida *A. bifasciatus*, ki bodo podlaga za morebitno komercialno rabo (Costi in sod., 2019).

Eno od možnih alternativ predstavlja tudi uporaba entomopatogenih gliv in entomopatogenih ogorčic pri zatiranju marmorirane smrdljivke. Gouli in sod. (2012) so v laboratorijskih razmerah preučevali učinkovitost različnih sevov entomopatogene glive *Beauveria bassiana* (Bals.-Criv.) Vuill. ter *Metarhizium anisopliae* (Metchnikoff) Sorokin pri zatiranju odraslih osebkov marmorirane smrdljivke. Vsi sevi *B. bassiana* so se v poskusu izkazali za bolj učinkovite od glive *M. anisopliae*. S sevom *B. basssiana* GHA (Botanigard®, Mycotech Europe Limited; Metrob d.o.o.) so dosegli med 85 in 100 % smrtnost odraslih osebkov marmorirane smrdljivke. Burjanadze in sod. (2020) so v laboratorijskih razmerah preučevali učinkovitost različnih vrst entomopatogenih ogorčic (*Heterorhabditis bacteriophora* Poinar, *Steinernema borjomiense* n.sp. in *Steinernema apuliae* sp.n.). Z ogorčico *H. bacteriophora* so dosegli okoli 80 % smrtnost odraslih osebkov marmorirane smrdljivke.

8 ZAKLJUČEK

Na področju omejevanja številčnosti marmorirane smrdljivke in preprečevanje škode v kmetijski pridelavi v tem trenutku nimamo na razpolago prav veliko ukrepov, kot tudi ne učinkovitih rešitev. Kemično obvladovanje marmorirane smrdljivke vezano na pragove škodljivosti, zahteva veliko število škropljenj, kar negativno vpliva na agroekosistem, obremenjuje okolje in je v popolnem nasprotju z vzpostavljenim sistemom integriranega varstva. Proti škodljivcu imamo trenutno v Sloveniji registrirana zgolj dva kemična pripravka; Karate Zeon 5 CS in Mospilan 20 SG, ki imata kot aktivno snov lambda-cihalotrin in acetamiprid, kar ju uvršča pod širokospektralne insekticide. Uporaba le teh je samo kratkoročna rešitev, saj lahko stenica s časom razvije odpornost. Ne-ciljno delovanje teh sredstev prizadene tudi koristne organizme, kot so naravni sovražniki. Slednjim pripisujemo velik pomen pri obvladovanju marmorirane smrdljivke v Sloveniji. Dolgoročno gre pričakovati, da se bodo domorodni koristi organizmi sčasoma prilagodili na novega tujerodnega škodljivca ter ga začeli omejevati na dopustno raven. Trenutno potekajo raziskave, kjer raziskovalci iz KGZS – KGZ Nova Gorica preučujejo zastopanost jajčnih parazitoidov marmorirane smrdljivke v Sloveniji. Potrdili so zastopanost treh vrst; *Anastatus bifasciatus*, *Trissolcus mitsukurii* ter *Ooencyrtus telenomicida* (Rot in sod., neobjavljeno). Raziskovalna skupina sočasno preučuje tudi učinkovitost parazitiranja omenjenih vrst. Rezultati raziskav domorodnih jajčnih parazitoidov stenic bodo prispevali k boljšemu poznavanju domorodne koristne favne ter njenega odziva ob vnosu in naselitvi nove tujerodne vrste (marmorirane smrdljivke). Eno izmed alternativnih rešitev bi lahko predstavljalo tudi sajenje privabilnih rastlin, s katerimi odvrnemo pojav škodljivca na glavni rastlini in njegovo zatiranje na privabilni rastlini z uporabo biotičnih agensov (entomopatogene glive, ento-

mopatogene ogorčice), sesalcev za mehansko odstranjevanje žuželk z rastlin in ožiganja rastlin.

Izkušnje iz tujine kažejo, da je obvladovanje marmorirane smrdljivke izjemno zahteven proces, ki mora združevati številne ukrepe varstva rastlin. Temeljiti morajo na zanesljivih metodah spremljanja populacije škodljivca, predvidevanju nastanka škode ter pravočasni napovedi in izvedbi ukrepov. Poznavanje bionomije marmorirane smrdljivke v lokalnih razmerah, je predpogoj za načrtovanje ukrepov za prebivalce v naseljih ter preprečevanje škode v kmetijski pridelavi.

9 ZAHVALA

Prispevek je nastal v sklopu projekta CRP V4-2002, ki je financiran s strani Javne agencije za raziskovalno dejavnost Republike Slovenije (ARRS) in Ministrstva za kmetijstvo, gozdarstvo in prehrano Republike Slovenije (MKGP). V projektu poleg vodilnega partnerja (UL – Biotehniška fakulteta) sodelujejo tudi KGZS – KGZ Nova Gorica in Maribor, UM – Fakulteta za kmetijstvo in biosistemske vede, Inštitut za hmeljarstvo in pivovarstvo Slovenije, Kmetijski inštitut Slovenije in UL – Fakulteta za strojništvo.

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