# Seed pre-sowing treatments and essential trace elements application effects on wheat performance

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Received April 28, 2022; accepted December 17, 2022. Delo je prispelo 28. aprila 2022, sprejeto 17. decembra 2022

### Seed pre-sowing treatments and essential trace elements application effects on wheat performance

Abstract: Current study was conducted to evaluate the effects of different seed priming and foliar spray of micronutrients on bread wheat performance in semi-arid region in Northwest of Iran. Pre-sowing treatments were S1: no pre-sowing treatment (intact seeds), S2: hydro-priming, S3: bio-priming (seed inoculation with plant promoting rhizobacteria consortium: Azotobacter chroococcum + Azospirillum lipoferum), S4: micronutrient seed priming and foliar feeding include, check (0): distilled water spray, Fe: foliar spray of iron, Zn: foliar spray of zinc. All seed priming treatments significantly increased plant height, tiller number, canopy width, total biomass, spike mass, seed number per spike and seed yield compared to intact seeds. A brief comparison of the effect of seed priming and fertilizer treatments showed that the effects of priming treatments on improving growth and seed yield was more obvious than fertilizer treatments. The greatest increase in seed yield and yield components was recorded for plants grown from bio-fortified seeds by essential trace elements. However, comparison of fertilizer treatments showed that growth parameters were significantly affected by Zn application. From the present study, it may be concluded that combined seed priming through pre-sowing hydration, soaking in micronutrients and microbial inoculation is useful to enhance wheat production and agricultural sustainability for smallholder farmers in semi-arid region.

Key words: bio-priming; hydro-priming; nutrient priming; seed yield Učinki obravnavanja semen pred setvijo in dodajanje elementov v sledeh na uspevanje pšenice

Izvleček: Raziskava je bila izvedena za ovrednotenje učinkov predtretiranja semen in foliarnega nanosa mikroelementov na uspevanje krušne pšenice v polsušnih območjih severozahodnega Irana. Predsetvena obravnavanja semena so bila: S1brez obravnavanj (intaktna semena), S2 - predtretiranja z vodo (hydro-priming), S3 - bio-priming (semena inokulirana z mešanico bakterij, ki promovirajo rast rastlin - Azotobacter chroococcum + Azospirillum lipoferum), S4 - semena predtretirana z mikrohranili in kasnejšim foliarnim dodajanjem mikrohranil, kontrola (0): pršenje listov z distilirano vodo, Fe: foliarno pršenje z železom, Zn: foliarno pršenje s cinkom. Vsa predtretiranja so značilno povečala višino rastlin, število stranskih pogankov, širino krošnje (nadzemnega dela rastlin), celokupne biomase, maso klasov, število semen na klas in pridelek semena v primerjavi z intaktnimi semeni. Primerjava učinkov predtretiranja semen in obravnavanj z gnojili je pokazala, da so bili učinki predtretiranj semen na povečanje rasti in pridelka bolj očitni kot učinki gnojenja. Največje povečanje pridelka semen in njegovih komponent je bilo zabeleženo pri rastlinah, ki so zrasle iz semen predtretiranih z mešanico bakterij in esencielnih hranil v sledeh. Primerjava obravnavanj z gnojili je pokazala, da je bilo povečanje rastnih parametrov značilno boljše pri uporabi Zn. Iz te raziskave bi lahko zaključili, da bi bila kombinacija obravnavanja semen s predsetvenim tretiranjem z vodo, namakanjem semen v raztopini mikrohranil in inokulacijo z mikrobi koristna za povečanje pridelave pšenice in trajnosti pridelave pri manjših kmetih v polsušnih območjih.

**Ključne besede:** bio-priming (predtretiranje z mikrobi); vodno predtretiranje; predtretiranje s hranili; pridelek semena

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#### 1 INTRODUCTION

The semi-arid regions of West Asia and North Africa (WANA) are dominated by erratic and unpredictable precipitation, low yield and cereal rain-fed farming system (Rayan, 2011). Poor crop stand establishment is one of the major abiotic restrictions encountered by resource-poor farmers in marginal semi-arid region (Sime & Aune, 2020). The farming system in these regions are characterized by slow seed emergence, low vigor of seedlings, patchy plant stands, and frequent crop failure or yield reduction (Camara et al., 2013). This problem is especially evident in the cereal fields of semi-arid WANA regions, as drought-prone environments, where cereal germination tends to be irregular and can extend over long periods. Late seed germination and low seedling growth rate may result in poor crop stands with high gaps in canopy. This condition is more important especially in semi-arid areas and will lead to loss of moisture through evaporation or an increase in the population of weeds and an increase in the competition for receiving light and nutrients with crop plants (Kaur et al., 2018). Thus accelerating and homogenizing the germination process is a prerequisite for a good crop establishment and helps to increase yield eventually (Samad et al., 2014). However, several physiological approaches and agronomic managements may be employed to increase the crop establishment. Among these approaches, pre-sowing techniques or seed priming are a low cost and safe solution to improve crop stand establishment (Wajid et al., 2018). Between pre-sowing seed treatments priming is a simple method that seeds can adsorb some amount of water in a controlled environment, so that germination processes begin without the appearance of roots. There are various pre-sowing procedures that have been used to improve vegetative growth and seed yield. Depending on the plant species, seed shape and internal components of the seed, various priming treatments can be applied for stimulating the germinative metabolic activities (Paparella et al., 2015). The most commonly used methods are hydro-priming, soaking in a distilled water (Damalas et al., 2019). Hydro-priming is the most cost effective and practical method that needs only water to prime seeds. It easily involves soaking seed in water (usually overnight), surface-drying, and then sowing the same day. Priming promotes germination rate and uniformity due to some kind of metabolic repair of seeds during imbibition, build-up of germination-enhancing metabolites, osmotic modification, and a simple decrease in imbibition lag time (Harris et al., 1999; Ashraf and Foolad, 2005; Roslan et al., 2020). On-farm seed priming is a form of hydropriming, which consists of soaking seeds in water for a number of hours, usually overnight, surface drying them

(to allow limited storage) and sowing soon after. Onfarm seed priming has been reported to improve emergence, crop establishment, and yield besides improving economic benefits in dryland agriculture (Sime & Aune, 2020).

Microorganisms are the crucial contributors in numerous ecological processes, viz., nutrient cycling, improving the supply of essential elements, plant growth and development (Sarkar et al., 2021). Seed bio-priming is a pre-sowing routine where seeds are treated with some beneficial microbial cells (Singh et al., 2020). Bio-priming with plant promoting rhizobacteria (PGPR) can increase plant growth compared to intact plants by improving the nutrients availability, improving plant resistance and tolerance to disease and some abiotic stresses (Roslan et al., 2020). Managing agrochemicals for crop production always remains a classic challenge for us to maintain the doctrine of sustainability and it seems that bio-priming may provide a maintainable and reasonable solution. It has been reported that seed inoculation with beneficial microorganism can increase accumulation of some certain organic molecules (sugar, proline, polyamines) and secondary metabolites (polyphenols, flavonoids) as a defense mechanism under biotic or abiotic stress (Sarkar & Rakshit, 2020). Seed inoculation with beneficial microbial cells is a practical skill that can make integrated nutrient management plans more efficient (Devika et al., 2021). Further, it is also suggested that seed priming in nutritional solution improves plant growth and the nutrient status (micro and macronutrients) under unfavorable conditions, which are crucial for optimizing yield and quality status of grains (Singhal et al., 2021).

On the other hand, semi-arid fields of WANA are subjected to severe risk of desertification due to poor and shallow soil and low organic matter content, high pH, low water holding capacity and plant nutrients. Unavailability of crop nutrients in appropriate amount and form to crops is one of the major crop productivity constraints in the developing countries (Lal, 2013). Soil organic matter and nutrients have been severely depleted owing to continuous cultivation and non-application of sufficient and proper fertilizers, ignoring of fallowing in crop rotations and prevalence of severe erosion and the productivity of these soils has consequently declined. Zinc and iron deficiencies are common throughout the developed and developing world and lack of these essential trace elements can limit the growth and productivity of a wide range of crops, including wheat. It seems that in order to ensure food security and increase crop yield, in addition to pre-sowing seed treatments, some fertilizer managements should be considered in these areas. We hypothesized that on-farm seed priming (soaking seeds in water for a predetermined duration before sowing), seed inoculation with PGPR (*Azotobacter and Azospirillum*), foliar spray of micro-nutrient fertilizers or their combination increases the agro-economic benefits in wheat production. Each of the technologies can separately increase wheat productivity. However, their combination can further improve economic yields of crop plants and financial returns, thus it can be considered as a potentially viable option for resource-poor farmers. The objective of this study was, therefore, to estimate the individual and combined effects of on-farm priming, essential trace elements on wheat agronomic performances, in the semiarid northwest of Iran.

#### 2 MATERIALS AND METHODS

#### 2.1 SITE DESCRIPTION

Experiment was implemented at the Research Farm of University of Maragheh, Northwest Iran (latitude 37°23' N, longitude 46°16' E and altitude 1485 m. The site has a semi-arid, moderate cool, Mediterranean climate with mean annual rainfall around 380 mm/year. Summer (May to September) has a mean maximum temperature of 31 °C and mean minimum temperature of 18 °C. Winter (December to the end of March) has mean minimum temperature of -2 °C and a maximum of 11 °C. Between the driest and the most humid months, the difference in precipitation is close to 64 mm. The variation in mean annual temperature is around 25.2 °C. Rainfall from June to October is relatively light, and the highest rate of evapotranspiration then occurs. Application of supplemental irrigation is necessary during the dry spell. Generally, wheat-barley-fallow cropping system is adapted by the majority of the farmers in studied areas. Soil sample taken from a maximum depth of 30 cm (in composite) was analyzed for its physio-chemical characteristics. The soil of the field was a clay loam containing 0.43 % of organic matter (OM) with pH 7.65 and electrical conductivity (EC) of 0.84 ds·m<sup>-1</sup> (Table 1). The soil of experimental sit had low content of Zn (0.73 ppm) and Fe (1.62 ppm).

#### 2.2 SEED PRIMING AND ESSENTIAL TRACE ELE-MENTS APPLICATION

The experiments were arranged as split-plot  $(4 \times 3)$ , based on the randomized complete block design (RCBD) with three replications. Four seed priming treatments as main plots and three micronutrients foliar spray assigned to sub-plots. Seeds of wheat genotype Sardari were used in this experiments which were collected from Dryland Agricultural Research Institute (DARI), Maragheh, Iran. The best soaking duration and optimal concentration of essential trace elements for priming wheat seed were determined in a series of laboratory experiments at Plant Production Department, University of Mragheh. Seeds and priming solutions ratio was kept as mass/volume 1:5 (w/v). Seed priming technique was practiced by soaking wheat seeds in respective solutions of distilled water as hydro-priming (8 h), micronutrient as nutrientpriming (16 h,  $\Psi$ s = -0.80 MPa), bio-priming with plant promoting rhizobacteria consortium (107 CFU ml-1: Azotobacter chroococcum Beijerinck 1901 + Azospirillum lipoferum Reinhold (Beijerinck 1925) Tarrand et al. 1979 (Approved Lists 1980) for 8 h). Untreated wheat seeds were considered as non-primed control. For nutrient seed priming Iron, Zn and Mn were. Iron was used as Fe-EDTA (8.5 mM Fe; Fermolife, Iran), Zn and Mn used as 4 mM Zn (ZnSO4·H2O; Henan Lihao Chem Plant, China) + 2.5 mM Mn (MnSO<sub>4</sub>·7 H<sub>2</sub>O; Henan Lihao Chem Plant, China) solution. Then seeds were rinsed with plenty of distilled water and were left to air-dry until their mass reach about the initial ones. Also micronutrient fertilizers (iron nano chelate, zinc nano chelate and distilled water as control) applied three times during initiation of tillering stage, booting and milky stage as foliar spray with a concentration of 1000 ppm.

#### 2.3 SOWING, IRRIGATION, WEED CONTROL AND HARVESTING

The experimental field was ploughed once in early autumn and harrowed twice to bring the soil to fine tilth one week before planting in the third decade of February. The recommended dose of fertilizer (150 kg N and 80 kg  $P_2O_5$  ha<sup>-1</sup>) was applied in the form of urea and triple superphosphate at the time of seed bed preparation. Sowing was done on March 8, 2020. Each experimental plot was 8 m<sup>2</sup> consisting of twelve rows, 4 m long and 15 cm apart. Seeds were sown 2 cm apart at 5 cm depth. Following seed sowing, light irrigation was done. Weeds were controlled by systemic selective chlorophenoxy herbicides including 2, 4-D and MCPA (U 46 Combi Fluid-Nufarm, Portugal). Plants were grown under irrigated condition that received both natural rainfall and four time irrigation irrigations (surface or flood irrigation) were applied during planting, jointing, milking stage and grain filling stage. The amount of irrigation water was calculated to restore water content in the root zone to field capacity. Depth of net irrigation water fraction was ~124 mm. Rest of the agronomic operations like soil preparation and weed management etc. were kept alike for all experimental units.

						Organic		CEC				
Soil	Sand	Silt	Clay	Zn	Fe	matter	EC		(Cmolc	Κ	Р	Ν
texture	(%)	(%)	(%)	(ppm)	(ppm)	(%)	(ds.m <sup>-1</sup> )	pН	kg-1)	(mg kg <sup>-1</sup> )	(mg kg <sup>-1</sup> )	(%)
Clay loam	25	31	44	0.73	1.62	0.43	0.84	7.65	18.2	287.3	14.8	0.17

Table1: Physico-chemical properties of field soil (depth of 0-30 cm), Maragheh, in Northwest of Iran

#### 2.4 DATA COLLECTION AND ANALYSIS

Plant height, canopy width, number of tillers per plant, spike length, 1000- grain mass and mass, number of grains per plant, straw yield, total biomass, yield per plant, and biological yield were taken at maturity stage. Chlorophyll content (SPAD values) of flag leaves were recorded using a SPAD-502 meter (Konica-Minolta, Japan) at heading stage. Twelve independent SPAD measurements were made per treatment, using several different plants (Ling et al., 2011). Harvest index (HI) was calculated according to the following formula: Harvest index (%) = Grain yield / Biological yield × 100. All data were subjected to variance analysis (ANOVA) for each character to determine crop parameter response to seed priming and foliar application of essential trace elements. Statistical analysis of the data was performed using GLM procedure of SAS 9.1 version software package (SAS Institute Inc., Cary, NC, USA). The least significant difference (LSD) at 5 % was used to compare between the means. Pair-wise Pearson's correlation coefficient was calculated among twenty agronomic traits. Cluster analysis was performed for the traits and treatment combinations. The principal components analysis (PCA) based on Everitt & Dunn (1992) was used.

#### 3 RESULTS

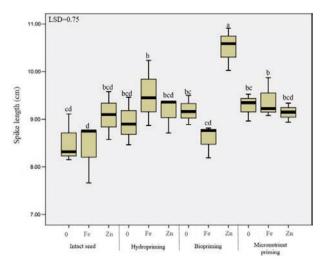
Analysis of variance (ANOVA) showed that plant height was strongly affected by seed priming treatments (p < 0.01) and micronutrient fertilizers also affected this trait (p < 0.05). Mean comparison of plant height sowed that the highest plant height was recorded in plants grown from b*i*o-primed seed (controlled seed hydration and inoculation with PGPR consortium containing *Azotobacter* and *Azospirillum*) and hydro-primed seed. The mentioned pre-sowing treatments increased the plant

Treatments		PH	TL	SL	CW	BT	CHF			
Seed priming										
	$S_1$	68.55°	2.93 <sup>b</sup>	9.42ª	$17.08^{b}$	7.12 <sup>c</sup>	34.72 <sup>c</sup>			
	$S_2$	$74.98^{ab}$	3.84 <sup>ab</sup>	9.27 <sup>a</sup>	17.80ª	9.92 <sup>b</sup>	39.86 <sup>ab</sup>			
	S <sub>3</sub>	$78.07^{a}$	4.75 <sup>a</sup>	9.20ª	18.02ª	11.28ª	41.90 <sup>a</sup>			
	$S_4$	72.97 <sup>b</sup>	4.70 <sup>a</sup>	8.66 <sup>b</sup>	18.11ª	11.41ª	37.30 <sup>b</sup>			
Fertilizer	0	71.18 <sup>b</sup>	3.66 <sup>b</sup>	9.47ª	17.54 <sup>b</sup>	9.76 <sup>b</sup>	32.18 <sup>b</sup>			
	Fe	73.45 <sup>ab</sup>	3.91 <sup>ab</sup>	8.97 <sup>b</sup>	17.57 <sup>b</sup>	11.51ª	38.26 <sup>a</sup>			
	Zn	76.30 <sup>a</sup>	4.59ª	9.43 <sup>b</sup>	18.15ª	11.10ª	41.40 <sup>a</sup>			
			Statistical significance							
S		**	**	**	*	**	**			
F		*	ns	*	*	*	**			
S*F		ns	ns	**	ns	ns	*			
CV		5.32	13.85	4.90	3.77	10.29	6.21			

**Table 2**: Effect of seed priming and foliar spray of micronutrients on morphological and growth characteristics of Common wheat

 (*Triticum aestivum* L.) in Northwest of Iran

S: seed priming treatment, S1: no pre-spwing treatment (intact seeds), S2: hyropriming, S3: Biopriming (hydropriming + seed inoculation with biofertilizer containing *Azotobacter* and *Azospirillum*), S4: micronutrient seed priming, F: fertilizer treatment, Check (0): without fertilizer application, Fe: foliar spray of iron, Zn: foliar spray of zinc. CV: coefficient of variation (%), PH: plant height, TL: tiller number, SL: spike length (cm), CW: canopy width (cm), BT: biomass (g), CHF: flag leaf chlorophyll content (SPAD unit). Values in a column with the same letter (s) or without any letter (s) do not differ significantly, whereas values with dissimilar letters are statistically different. Ns = not significant, \* = significant at 5 % level of probability



**Figure 1**: The effect of different seed priming treatments and foliar application micronutrients on spike length of spring wheat. Vertical bars in each column are standard error. Between the columns with different names there are statistically significant differences

height by 13 % and 6 %, respectively, compared to the control. Among fertilizer treatments, the highest plant height was observed under zinc foliar application condition, which was about 7 % higher than the control. Evaluation of tiller number per plants showed that biopriming and micronutrients priming relatively increased the number of tillers per plant by up to 60 % compared to the plants grown from intact seeds (control). However, hydro-priming treatment could also have a significant effect on this trait and increase the number of tillers by 31 % compared to the control. Assessment of exogenous application of micronutrient fertilizers indicated that the greatest effect on tiller number was related to zinc application (Table 2).

A similar trend also was recorded for response of canopy width to priming treatments and foliar application of essential trace elements. So that the plants grown from primed seeds had an average of about 6 % larger canopy width compared to the control. Alternatively, the utilization of zinc significantly increased the canopy width compared to the control (4 %). However, the effect of iron foliar application on canopy width was not significant and there was no significant difference between iron application and control (Table 2).

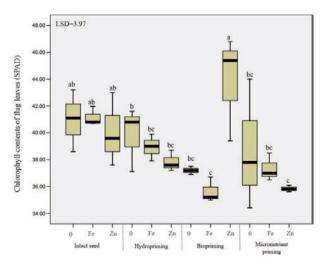
Evaluation of spike length showed that although seed treatments caused a significant increase in length of this organ, this increase was more noticeable in bioprimed seed and under foliar application of zinc fertilizer (37 %) when compared with control conditions (Figure 1). Zinc foliar application in non-priming conditions, iron foliar application in hydro-priming and nutrient priming conditions were more effective than other foliar spraying treatments.

Assessment of plant biological mass showed that seed priming significantly affected this parameter, so that plants grown from primed seed had 50 % more biological mass than plants grown from intact seeds. Likewise, foliar application of Fe and Zn increased the biological mass by 18 % and 13 % (Table 2). Evaluation of chlorophyll content showed that seed priming increased the chlorophyll content of flag leaf. However, the highest chlorophyll content was recorded in plants grown from bio-primed seeds and sprayed with zinc, followed by plants grown from hydro-primed seeds and sprayed with iron (Figure 2).

Evaluation the effects of seed priming and micronutrient fertilizers on seed yield components are presented in Table 3. The effect of priming treatments on yield components was significant, however, the effect of foliar application of micronutrient was not very significant. Plants grown from primed seeds (hydro-priming, biopriming and nutrient priming) showed the higher spike mass, seed mass per spike, fertile spikelet number and spike number in unit area when compared with control.

Assessment of 1000-seed mass showed that although all seed priming treatments increased the 1000seed mass, the highest 1000-seed mass were recorded in plants grown from bio-primed and hydro-primed seeds under zinc and iron foliar application conditions. It is noteworthy that the application of zinc under bio and hydro-priming conditions had the greatest effect on grain mass and significantly increased this seed yield component (Figure 3).

Examination of the number of seeds per spike



**Figure 2**: Influence of pre-sowing seed treatment and micronutrient fertilizer on chlorophyll content of flag leaf in spring wheat in semi-arid region of Iran

Treatments		SPM	SNP	SMS	FSN	SNM	TSM	SY	HI	
Seed priming	5									
	S <sub>1</sub>	1.60 <sup>b</sup>	21.80 <sup>d</sup>	1.22 <sup>b</sup>	38.65 <sup>b</sup>	368.51 <sup>b</sup>	53.44 <sup>bc</sup>	2536.4°	49.76 <sup>ab</sup>	
	S <sub>2</sub>	1.82ª	24.02 <sup>b</sup>	1.40 <sup>a</sup>	44.80 <sup>a</sup>	506.32ª	54.59 <sup>ab</sup>	2665.0 <sup>bc</sup>	49.19 <sup>ab</sup>	
	S <sub>3</sub>	1.82ª	26.41ª	1.41ª	45.04ª	526.23ª	55.64ª	2906.3 <sup>ab</sup>	50.23a	
	$S_4$	1.81ª	23.73 <sup>bc</sup>	1.40 <sup>a</sup>	46.12 <sup>a</sup>	568.83ª	52.53°	3073.2ª	48.26 <sup>b</sup>	
Fertilizer	0	1.72 <sup>b</sup>	22.26 <sup>c</sup>	1.32 <sup>b</sup>	43.05 <sup>ab</sup>	455.56ª	53.08 <sup>b</sup>	2611.4ª	49.84 <sup>a</sup>	
	Fe	1.73 <sup>b</sup>	23.95 <sup>b</sup>	1.39 <sup>ab</sup>	42.63 <sup>ab</sup>	483.34ª	54.60ª	2874.2ª	48.36 <sup>a</sup>	
	Zn	1.85ª	25.03 <sup>a</sup>	1.43 <sup>a</sup>	45.28ª	538.57ª	54.48ª	2900.1ª	49.74ª	
		Statistical significance								
S		*	*	*	**	*	**	*	*	
F		ns	*	ns	ns	ns	*	ns	ns	
S*F		*	*	**	ns	ns	**	*	ns	
CV		10.29	9.37	9.12	8.35	9.26	5.43	18.66	5.24	

 Table 3: Impact of seed pre-sowing treatment and micronutrients on seed and yield component of common wheat in semi-arid

 region of Maragheh

S: Seed priming treatment, S1: no pre-sowing treatment (intact seeds), S2: hyropriming, S3: hydropriming + seed inoculation with biofertilizer (*Azotobacter* and *Azospirillum*), S4: micronutrient seed priming, F: fertilizer treatment, Check (0): without fertilizer application, Fe: foliar spray of iron, Zn: foliar spray of zinc. CV: coefficient of variation (%), SPM; spike mass, SNP: seed number per spike, SMS: seed mass per spike, FSN: fertile spikelet number, SNM: spike number in unit area, TSM: thousand seed mass (g), SY: seed yield (kg ha<sup>-1</sup>), HI: harvest index (%). Values in a column with the same letter (s) or without any letter (s) do not differ significantly, whereas values with dissimilar letters are statistically different. Ns = not significant, \* = significant at 5 % level of probability, \*\* = significant at 1 % level of probability

showed that seed bio-priming and application of zinc produced the highest amount of this trait (Figure 4). Bio-priming, hydro-priming and nutrient priming had the greatest effect on the number of seed per spike, respectively. However, the efficiency of foliar spraying treatments at different levels of priming was different and the highest foliar application efficiency was observed in

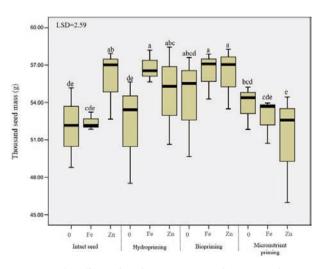
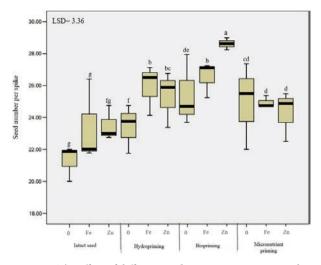


Figure 3: The effects of seed invigoration techniques and iron and zinc fertilizers on seed mass of spring wheat

hydro-priming and bio-priming conditions. Seed yield evaluation showed that the highest amount was recorded in bio-primed plants by zinc foliar application. Although all priming treatments increased the seed yield, the greatest effect was related to bio-priming and hydro-priming treatments. Foliar spraying had the greatest effect on bioprimed plants (Figure 4).

Although the pre-sowing seed treatments significantly improved seed yield when compared with control (intact seed), the best performance was related to plants grown by trace fertilizers. Evaluation of seed yield showed that the highest amount was recorded under biopriming condition along with application of essential trace elements (Zn and Fe) and followed by Zn received plant under hydro-primed condition (Figure 5).

Principal component analysis (PCA) was employed to provide an overview of the capacity to distinguish combined treatments. First principal component clearly separated the non-primed seeds from other priming techniques (Figure 6). PCA could separate the foliar treatments by second component. PCA evidently separated the micronutrients application from control. Also second principal component segregated the Zn application along with bio-priming was very close to seed yield component. Also PCA provided the correlation coefficient between any two traits by the cosine of the angle



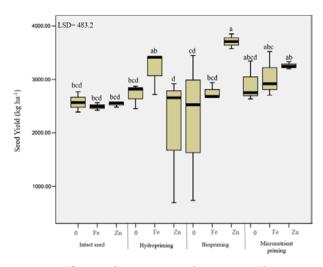
**Figure 4**: The effect of different seed priming treatments and foliar application micronutrients seed number in spike of spring wheat in semi-arid region in northwest Iran

between their vectors of related traits. A strong positive association between seed yield, chlorophyll content of flag leaf, seed mass, ground cover, fertile spikelet number and seed mass indicated by the small obtuse angles between their vectors ( $r = \cos 0 = +1$ ).

#### 4 DISCUSSION

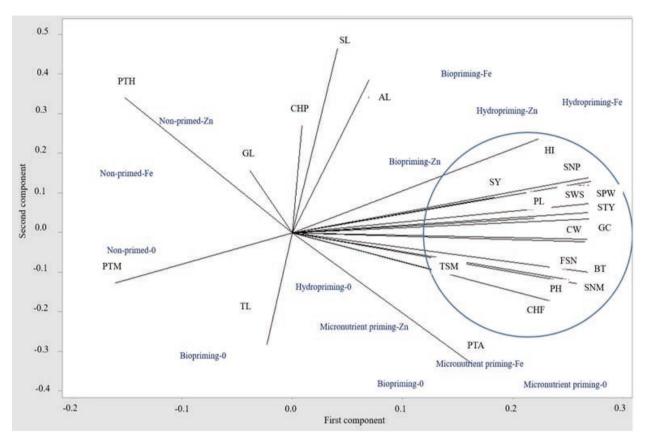
Seed priming is used to achieve rapid and uniform seed germination and seedling emergence to enhanced crop production performance. In the present study, we found that seed bio-priming and hydro-priming increase height and tiller number as compared to control treatment. The results were similar to those of earlier studies, which reported that seed priming enhanced seedlings fresh mass, hypocotyl, when compared with unprimed seedlings (Sarkar and Rakshit, 2020; Anwar et al., 2020). These findings are suggested that seed priming incredibly enhanced seedlings growth. Considering the key role of plant hormones auxin and gibberellin in regulating plant height and tiller production, it seems that these treatments have increased height by accelerating the plant initial growth as well as changes in phyto-hormone levels as well as hormone ratios (Zhuang et al., 2019).

Results revealed that canopy width improved by both priming treatments and Zn foliar application. Canopy width as one of the most important vegetative traits can affect yield. Increasing crop canopy structure can improve canopy photosynthetic productivity and thereby crop yield potential (Feng et al., 2016). The findings of the current study are consistent with those of Wajid et al. (2018) who showed that different seed priming approach



**Figure 5**: Influence of pre-sowing seed treatment and micronutrient fertilizer on seed yield of spring wheat in semi-arid region of Iran

increased the leaf area in wheat. The increase in canopy width is probably due to the acceleration of germination and rapid establishment of the seedling and the optimal use of ecological conditions. This can be due to a wide range of factors such as hormones and faster exploitation of growth factors such as water, nutrients and light. Increasing the canopy width can affect the source-sink relationship. Traits such as plant height, canopy width, number of tillers, chlorophyll content can directly play a role in increasing the rate of photosynthesis and also improve the ability of the source to supply photo-assimilates for growing sinks. Besides, traits such as spike length, number of florets per spikelet and number of seeds, as well as grain size or mass are traits that are directly related to the size of the sink and indirectly reflect the activity of the sink. It seems that seed priming affected both sink and source activity and size in current study. Sink- source relations can regulate biomass production and assimilate allocation in plants (Burnett, 2019). In plants grown from non-primed seed the reduction in the photosynthetic area resulted in reducing the assimilation formation and translocation towards the sink, which led to reduce the seed yield and related attributes. Furthermore, the balance between carbon- and nitrogen-containing metabolites is an important indicator of source-sink status. The rapid expansion of root systems in primed seeds allows the plant to absorb nitrogen, which can lead to more photosynthesis and more activity of the source as well as the sink. Further absorption of nitrogen can cause further supply of free amino acid to sink. The ratio of free amino acids to sucrose expresses the relative availability of nitrogen and carbon, with a high ratio indicating an excess of available nitrogen and a low ratio indicating



**Figure 6**: Plot of the first two PCAs showing relation among various agronomical traits of wheat. PL: peduncle length, SL: spike length, AL: length of the awn, SPM: Spike mass, BT: biomass, FSN: fertile spikelet number, SNP: seed number per spike, SES: seed mass per spike, STY: straw yield, GL: grain length, TL: tiller number, CW: canopy width, SY: seed yield, GC: ground cover, CHF: chlorophyll content of flag leaf, CHP: chlorophyll content of penultimate leaf, TSM: thousand seed mass, PTH: day from planting to heading, PTA: day from planting to anthesis, PTM: day from planting to maturity

an excess of available carbon. This balance is attuned to enable plants to improve their growth and development. Also there was found Zn application affected both source and sink size. This finding supports previous research into this brain area which links Zn and Sink- source relations (Wang et al., 2021).

The results of this study showed a significant superiority of bio-priming in improving the evaluated traits. Bio-priming is an important method to reinforce the mechanisms of seed-microbe-soil plant interactions. However, many mechanisms behind these associations and mode of action are still not well understood and need further investigation. This superiority is supported by Mirshekari et al. (2012) who writes that seed bio-priming with PGPR consortium (*Azotobacter* + *Azospirillum*) significantly increased yield component of barley including dry matter accumulation, seed mass, harvest index, biological yield, and seed yield. It seems that PGPR capable of colonizing the rhizosphere or plant roots facilitate nutrient availability for plant uptake (Jacoby et al., 2017). Seed bio priming

8 | Acta agriculturae Slovenica, 119/1 – 2023

can improve plant performance through modification of soil enzymes such as cellulose, protease, catalase, dehydrogenase, acid phosphatase, alkaline phosphatase, phytase and amylase enzymes (Mengual et al., 2014). Seed bio-priming also can induce plant growth by alternation of the phytohormone contents (indole-3-acetic acid, gibberellic acid, and salicylic acid) or decreasing the content of abscisic acid (Sarkar et al., 2021). Furthermore, seed bio-priming through inoculation with beneficial root-associated bacteria increases speed and uniformity of germination and modified the seedling nutritional condition by increasing ascorbic acid, protein, flavonoid, and total phenolic contents; and also via reorganization of defense process such as improving antioxidant potential by increasing hydroxyl radical scavenging activity, free radical scavenging activity, redox capacity, and iron chelating capacity affected seed yield (Jain et al., 2014). The latter case is of great importance in semi-arid regions, so that the wheat plant in these regions at the end of its development period are faced with drought and heat stress.

The positive response of many traits to Zn application under non-priming condition without may to some extent indicate a severe deficiency of this element in the soil of the site study. Due to the central role of applied rhizobacteria for bio-priming (Azotobacter and Azospirillum) in nitrogen assimilation, it seems that Zn can interact with mentioned PGPR. Zn is an essential micronutrient in plant growth and development. The major role of Zn in plants is to act as the cofactor for enzymes involved in N metabolism, such as alcohol dehydrogenase. This enzyme play a critical role in plant growth, development, adaptation with anaerobic soil conditions. Therefore, Zn deficiency reduces anaerobic root metabolism and seedlings' capacity under restriction of oxygen in the soil (Tuiwong et al., 2022). However, our finding revealed that utilization of Zn fertilizers alone is not be sufficient for increasing wheat performance in semi-arid region, so that the application of suitable seed priming and micronutrient nano-fertilizer application had the best effect on both vegetative growth and seed yield. This may be the result of synergistic relationships between the fertilizer management and priming treatments. Overall, seed priming improves wheat performance under semi-arid conditions through improved germination metabolism and accelerating crop stand establishment, resulting in accelerated growth and development even under unfavorable condition of semi-arid regions.

#### 5 CONCLUSIONS

Our results showed that seed priming significantly improved wheat plants growth characteristics and yield component. On-farm pre-sowing seed treatments, whereby seeds were soaked in water for a predetermined duration followed by surface-drying (to facilitate handling) and inoculated with bio-fertilizers (PGPR) before sowing, resulted in the highest seed yield and best performance. On the other hand micronutrient deficiencies especially Zn and Fe are common in studied site as a representative of semi-arid Mediterranean region. The present study found that foliar application zinc significantly improved vegetative growth and yield components when compared to control. However, seed treatment in solutions containing essential trace elements can be successfully used to improve wheat quality attributes like tiller number, spike number and seed yield. Keeping in view all results, we concluded that among the seed priming treatments bio-priming (inoculation with PGPR consortium containing Azotobacter and Azospirillum) and hydro-priming along with foliar application of zinc was the most effective and recommendable for wheat field in studied region. Despite the efforts so far reported to further improve seed priming, novel ideas and cutting-edge investigations need to be brought into this technological sector of agri-seed industry. Further research work is needed to optimize the effectiveness of pre-sowing seed inoculation with different bio-fertilizer and with using of more varieties of wheat in combination of other agents/ factors.

#### 6 ACKNOWLEDGEMENTS

This research was supported by a grant-inaid for scientific research from the University of Maragheh and funded by the Ministry of Science, Research and Technology, Iran.

#### 7 REFERENCES

- Anwar, A., Xianchang, Y. U., & Yansu, L. I. (2020). Seed priming as a promising technique to improve growth, chlorophyll, photosynthesis and nutrient contents in cucumber seedlings. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 48(1), 116-127. https://doi.org/10.15835/nbha48111806
- Ashraf, M., & Foolad, M.R. (2005) Pre-sowing seed treatment—a shotgun approach to improve germination growth and crop yield under saline and non-saline conditions. *Advanced Agronomy*, 88, 223-271. https://doi.org/10.1016/ S0065-2113(05)88006-X
- Burnett, A. C. (2019). Source-sink relationships. In: eLS. John Wiley & Sons, Ltd: Chichester. https://doi.org/10.1002/9780470015902.a0001304.pub2
- Camara, B. S., Camara, F., Berthe, A., & Oswald, A. (2013). Microdosing of fertilizer – a technology for farmers' needs and resources. *International Journal of AgriScience*, *3*, 387–399.
- Damalas, C. A., Koutroubas, S. D., & Fotiadis, S. (2019). Hydropriming effects on seed germination and field performance of faba bean in spring sowing. *Agriculture*, 9(9), 201. https://doi.org/10.3390/agriculture9090201
- Devika, O.S., Singh, S., Sarkar, D., Barnwal, P., Suman, J., & Rakshit, A. (2021). Seed priming: a potential supplement in integrated resource management under fragile intensive ecosystems. *Frontiers in Sustainable Food Systems*, 5. https://doi.org/10.3389/fsufs.2021.654001
- Everitt, B.S., & Dunn, G. (1992). Applied multivariate data analysis. New York: Oxford University Press.
- Feng, G., Luo, H., Zhang, Y., Gou, L., Yao, Y., Lin, Y., & Zhang, W. (2016). Relationship between plant canopy characteristics and photosynthetic productivity in diverse cultivars of cotton (*Gossypium hirsutum* L.). *The Crop Journal*, 4(6), 499-508. https://doi.org/10.1016/j.cj.2016.05.012
- Harris, D., Joshi, A., Khan, P.A., Gothkar, P., & Sodhi, P.S. (1999). On-farm seed priming in semi-arid agriculture: Development and evaluation in maize, rice and chickpea in India using participatory methods. *Experimental Agriculture*, 35, 15–29. https://doi.org/10.1017/S0014479799001027
- Jacoby, R., Peukert, M., Succurro, A., Koprivova, A., &

Kopriva, S. (2017). The role of soil microorganisms in plant mineral nutrition—current knowledge and future directions. *Frontiers in Plant Science*. *8*, 1617. https://doi. org/10.3389/fpls.2017.01617

- Jain, A., Singh, S., Sarma, B.K., & Singh, H.B. (2012). Microbial consortium-mediated reprogramming of defence network in pea to enhance tolerance against *Sclerotinia sclerotiorum*. *Journal of Applied Microbiology*. 112 (3), 537–550. https:// doi.org/10.1111/j.1365-2672.2011.05220.x
- Kaur, S., Kaur, R., & Chauhan, B. S. (2018). Understanding crop-weed-fertilizer-water interactions and their implications for weed management in agricultural systems. *Crop Protection*, 103, 65-72. https://doi.org/10.1016/j.cropro.2017.09.011
- Lal, R. (2013). Climate change and soil quality in the WANA region. In *Climate Change and Food Security in West Asia* and North Africa (pp. 55-74). Springer, Dordrecht. https:// doi.org/10.1007/978-94-007-6751-5\_3
- Ling, Q., Huang, W., & Jarvis, P. (2011). Use of a SPAD-502 meter to measure leaf chlorophyll concentration in *Arabidopsis thaliana. Photosynthesis Research*, 107(2), 209-214. https://doi.org/10.1007/s11120-010-9606-0
- Mengual, C., Schoebitz, M., Azcon, R., & Roldan, A. (2014). Microbial inoculants and organic amendment improves plant establishment and soil rehabilitation under semiarid conditions. *Journal of Environmental Management*, 134, 1–7. https://doi.org/10.1016/j.jenvman.2014.01.008
- Mirshekari, B., Hokmalipour, S., Sharifi, R.S., Farahvash, F., & Ebadi-Khazine-Gadim, A. (2012). Effect of seed biopriming with plant growth promoting rhizobacteria (PGPR) on yield and dry matter accumulation of spring barley (*Hordeum vulgare* L.) at various levels of nitrogen and phosphorus fertilizers. *Journal of Food, Agriculture and Environment*, 10(3/4), 314–320.
- Paparella, S., Araújo, S.S., Rossi, G., Wijayasinghe, M., Carbonera, D., & Balestrazzi, A. (2015). Seed priming: State of the art and new perspectives. *Plant Cell Reports*, 34, 1281–1293. https://doi.org/10.1007/s00299-015-1784-y
- Ryan, J. (2011). Rainfed farming systems in the West Asia– North Africa (WANA) Region. In *Rainfed Farming Systems* (pp. 365-393). Springer, Dordrecht. https://doi. org/10.1007/978-1-4020-9132-2\_15
- Roslan, M.A.M., Zulkifli, N.N., Sobri, Z.M., Zuan, A.T.K., Cheak, S.C., & Abdul Rahman, N. A. (2020). Seed biopriming with P-and K-solubilizing *Enterobacter hormaechei* sp. improves the early vegetative growth and the P and K uptake of okra (*Abelmoschus esculentus*) seedling. *PloS ONE*, 15(7), e0232860. https://doi. org/10.1371/journal.pone.0232860
- Samad, A., Khan, M. J., Shah, Z., & Tariq Jan, M. (2014). Determination of optimal duration and concentration of zinc

and phosphorus for priming wheat seed. Sarhad Journal of Agriculture, 30(1), 27-34.

- Sarkar, D., Rakshit, A. (2020). Safeguarding the fragile ricewheat ecosystem of the IndoGangetic Plains through biopriming and bioaugmentation interventions. *FEMS Microbiology Ecology*, 96(12), fiaa221. https://doi.org/10.1093/ femsec/fiaa221
- Sarkar, D., Singh, S., Parihar, M., Rakshit, A. (2021). Seed biopriming with microbial inoculants: A tailored approach towards improved crop performance, nutritional security, and agricultural sustainability for smallholder farmers. *Current Research in Environmental Sustainability*, 3, 100093. https:// doi.org/10.1016/j.crsust.2021.100093
- Sime, G., & Aune, J. B. (2020). On-farm seed priming and fertilizer micro-dosing: Agronomic and economic responses of maize in semi-arid Ethiopia. *Food and Energy Security*, 9(1), e190. https://doi.org/10.1002/fes3.190
- Singh, P., Singh, J., Ray, S., Rajput, R.S., Vaish-A., Singh, R.K., & nav, Singh, H.B. (2020).Seed biopriming with antagonistic microbes and ascorbic acid induce resistance in tomato against Fusarium wilt. Microbiological Research, 237, 126482. https://doi.org/10.1016/j.micres.2020.126482
- Singhal, R. K., Pandey, S., & Bose, B. (2021). Seed priming with Mg  $(NO_3)_2$  and  $ZnSO_4$  salts triggers physio-biochemical and antioxidant defense to induce water stress adaptation in wheat (*Triticum aestivum L.*). *Plant Stress*, 2, 100037. https://doi.org/10.1016/j.stress.2021.100037
- Tuiwong, P., Lordkaew, S., Veeradittakit, J., Jamjod, S., & Promu-thai, C. (2022). Seed priming and foliar application with nitrogen and zinc improve seedling growth, yield, and zinc accumulation in rice. *Agriculture*, 12(2), 144. https://doi. org/10.3390/agriculture12020144
- Wajid, M., Khan, M. A., Shirazi, M. U., Summiya, F., & Saba, M. (2018). Seed priming induced high temperature tolerance in wheat by regulating germination metabolism and physio-biochemical properties. *International Journal of Agriculture and Biology*, 20(9), 2140-2148. Doi: 10.17957/ IJAB/15.0747
- Wang, L., Xia, H., Li, X., Qiao, Y., Xue, Y., Jiang, X., Yan, W., Liu, Y., Xue, Y. and Kong, L., (2021). Source–sink manipulation affects accumulation of zinc and other nutrient elements in wheat grains. *Plants*, 10(5), 1032. https://doi.org/10.3390/ plants10051032
- Zhuang, L., Ge, Y., Wang, J., Yu, J., Yang, Z., & Huang, B. (2019). Gibberellic acid inhibition of tillering in tall fescue involving crosstalks with cytokinins and transcriptional regulation of genes controlling axillary bud outgrowth. *Plant Science*, 287, 110168. https://doi.org/10.1016/j.plantsci.2019.110168