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Hybridization potential *Aegilops* sp. / durum wheat: which interest for the genetic breeding of the drought tolerance?

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ABSTRACT

To study their hybridization potential, two species of the genus *Aegilops* (*Aegilops geniculata* Roth; *Aegilops triuncialis* L.) and two durum wheat (*Triticum durum* Desf.) varieties ('Oued Zenati' and 'Hoggar') were crossed, where *Aegilops* was the female parent. The four cross combinations were tested during five years in order to release the genitors having the most affinity for obtaining interspecific hybrids. The parents were also characterized for their drought stress tolerance during the crossing period. The results confirm the tolerance of *Aegilops* sp. and adaptation of the durum wheat varieties to climatic conditions governing the Algerian cereal zones. 81 hybrids F1 were obtained. Differences in hybridization affinity between the parents were very remarkable. The combination of parents *Aegilops geniculata*/' Oued Zenati' has produced the highest number of hybrids (54 or a rate of 5.23 %), followed by *Aegilops triuncialis*/'Oued Zenati' (18 hybrids or a rate of 3.88 %). The crossing of the two *Aegilops* species with the Hoggar variety produced a small number of hybrids. Among the advantages of this crossing, the obtaining of hybrids in caryopsis without resorting to the embryos rescue. Hybrid seedlings expressed a maternal cytoplasmic heredity. However, no adult plant could have been regenerated.

Key words: *Aegilops*; durum wheat; drought tolerance; interspecific hybridization; genetic breeding

IZVLEČEK

HIBRIDIZACIJSKI POTENCIAL KRIŽANCEV OSTIKE (*Aegilops* sp.) IN TRDE PŠENICE (*Triticum durum* Desf.) PRI VZGOJI KRIŽANCEV ODPORNIH NA SUŠO

Za preučevanje hibridizacijskega potenciala sta bili križani dve vrsti iz rodu ostike (*Aegilops geniculata* Roth.; *Aegilops triuncialis* L.) in dve sorti trde pšenice ('Oued 'Zenati' in 'Hoggar'), pri čemer je bila ostika ženska starševska vrsta. Križanci teh štirih kombinacij so bili preiskušani v obdobju petih let z namenom vzgoje potomcev s čim večjim deležem medvrstnih lastnosti. Starševske vrste so bile v obdobju križanja preučevane glede njihove odpornosti na sušni stres. Rezultati so potrdili odpornost vrst ostike na sušo in prilagoditev sort trde pšenice na podnebne razmere v žitnih območjih Alžirije. Vzgojenih je bilo 81 F1 križancev. Razlike v sposobnosti križanja med starševskimi vrstami so bile opazne. Kombinacija staršev *Aegilops geniculata*/'Oued Zenati'je dala največje število križancev (54 ali 5,23 %), tej je sledila kombinacija *Aegilops triuncialis*/'Oued Zenati' (18 križancev ali 3,88 %). Križanje obeh vrst ostike s sorto Hoggar je dalo le malo križancev. Med prednostimi tega križanja je pridobitev križancev v kariopsi, brez uporabe metode reševanja zarodkov. Hibridne sejanke so pokazale znake maternalnega citoplazmatskega dedovanja. Kljub naporom ni uspelo vzgojiti nobene odrasle hibridne rastline.

Ključne besede: *Aegilops*; trda pšenica; toleranca na sušo; medvrstno križanje; žlahtnenje

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1 INTRODUCTION

The cultivation of durum wheat in Algeria represents an economic and social importance (Attab & Brinis, 2012; Chahbar & Belkodja, 2016). The major constraint of this culture in the Mediterranean region is drought; the fluctuations of the rain combined to an intense heat, especially at the end of the cycle, affect sensibly the productivity (Ashraf, 2010; Kosová et al., 2014). The capacity of plants to be acclimatized to the water deficit is associated to their adaptability to the photosynthesis reduction which involves disturbances in multiple biochemical and physiological processes (rate of transpiration, stomatal conductance, effectiveness of water use) and a negative impact on growth (Anjum, 2011; Aissa & Redouane, 2014). The genus *Aegilops*, related to the genus *Triticum*, represents an important source of genes with potential interest for the wheat genetic amelioration (Ashraf, 2010). Indeed, many *Aegilops* species are adapted to various bioclimatic levels, notably arid and semi- arid, and therefore present a tolerance to drought (Molnár et al., 2004; Dulai et al., 2006) and to salinity (Colmer et al., 2006). The interspecific hybrids were significantly exploited in the amelioration of traits presenting simple genetic determinism (Jahier et al., 2006). Addition or substitution lines were developed from interspecific hybrids between wheat and *Aegilops* species (Schneider et al., 2005) allowing the successful introgression of many genes with disease resistance from *Aegilops* species (Schneider et al., 2008; Mujeeb-Kazi et al., 2013). Like the introduction into wheat of an eyespot resistance gene from *Aegilops ventricosa* (Jahier et al., 2006). Recently, similar lines have been created in order to introduce genes that code for: efficient utilization of phosphor by the plant (Wang et al., 2010); high values of zinc and iron of the seeds (Tiwari et al., 2010; Neelam et al., 2011) and amelioration of pastes and breadmaking quality (Wang et al., 2013).Thus, the interspecific hybrids offer remarkable genetic variability for use in wheat genetic breeding programs (Rolland et al., 2014). Many studies report the natural occurrence of interspecific hybrids between wheat and *Aegilops* species which are considered as the female parent (Morrison, et al., 2002; Cifuentes et al., 2006). Nevertheless, these works intended to study the incorporation of transgenes into *Aegilops* species from cultivated wheat varieties. Unfortunately, works dedicated to the introgression of tolerance traits for abiotic stress from *Aegilops* species remain rare (Mujeeb-Kazi et al., 2013). Thus, the objective of this work is the study of the hybridization potential between species of the genus *Aegilops* and durum wheat varieties and the influence of the crossing direction on obtaining interspecific hybrids *Aegilops*/durum wheat. As this study is a part of wheat breeding program to drought tolerance, by wild species as *Aegilops*, the genitors were also characterized for their tolerance to water stress during the crossing period.

2 MATERIALS AND METHODS

The plant material in this study consists of two durum wheat varieties and two tetraploid species of the genus *Aegilops* (Table 1). The seeds were provided by ITGC, El Khroub (Technical Institute of Great Cultivation, Constantine, Algeria), except for *Aegilops geniculata* which is a local natural collection (Constantine).

Table 1: Characteristics of wheat varieties and *Aegilops* species studied (Kellou, 2003 ; Van Slageren, 1994)

Species	Characteristics
Triticum durum Desf.	
Variety Oued Zenati 368 (O.Z)	Selected from the ‘Oued Zenati’ local population, it is a late-variety, adapted to the anterior plains, characterized by a black, long beard spike and high straw.
Variety Hoggar (Hog)	Introduced from Spain, ITGC / Tiaret Selection, 1986. It is adapted to the Highlands and Saharan areas.
Species of the genus *Aegilops* *Aegilops geniculata* Roth*(Ae.gen)* (syn. *Ae.ovata* L.)	Annual species, allo-tetraploid (2n = 4x = 28), its genomic formula is UUMM. It grows in the Mediterranean region, the Middle East and the southern parts of Russia and Ukraine.
Aegilops triuncialis (*Ae.tri*)	Annual species, amphi-tetraploid, (2n = 4x = 28). Its genomic formula is UUCC. It grows in the Mediterranean region.

Experiments on drought tolerance and interspecific hybridization were assured in a greenhouse at the Genetics, Biochemistry and Biotechnology Laboratory of Mentouri Brothers University 1, Constantine, Algeria. For both experiments, the seeds were previously disinfected and pre-germinated in Petri dishes. They were transplanted into pots of 5 kg containing a mixture of ground and sand (2: 1 v / v) at the rate of three seedlings per pot and periodically watered to their field capacity.

2.1 Drought tolerance

At the heading stage, plants were divided in three lots: Control lot (**C**): whose plants were periodically irrigated to saturation; First level stressed lot **(L1)**: abstention of watering for one week (7days); Second level stressed lot **(L2)**: abstention of watering for two weeks (15 days). The treatment of L2 was applied a week before that of the L1 in order to synchronize the samples and the measurements. Three replications per variety and per treatment were applied.

2.1.1 Physiological parameters

The physiological parameters measured are: the relative water content (RWC [%]) calculated from the formula of Clarck & McCaing (1982). Stomatal resistance (SR, [m^2.s mol^{-1}]) is measured using a Porometer (Delta Devices® MK3). The total chlorophyll content (TCC [unit of SPAD “Soil Plant Analysis Development”]) is measured with a chlorophyll SPAD meter (502 of Minolta®).

2.1.2 Biochemical parameters

They concerned the determination of soluble sugars content ([SSC µMol 100 mg^{-1} of fresh material] saccharose, glucose, fructose, their methyl derivatives and polysaccharides) by the phenol method of Dubois et al., 1956. The antioxidant activity of peroxidase and catalase is measured on enzymatic extracts, obtained after grinding 0.500 mg of fresh leaves in a phosphate buffer (50 mmol l^{-1} at pH7), centrifugation and filtration of the supernatant. The activity of peroxidase “POX” (EC 1.11.1.7.) is determined at 470 nm using guaiacol as a substrate. The reactional mixture contained 1 ml of hydrogen peroxide H_2O_2 (0.01 N), 1 ml of guaiacol and 1 ml of enzymatic extract. Data was recorded every 20 sec for 2 min. The catalase activity “CAT” (EC 1.11.1.6.) is determined in a reactional mixture containing 1 ml of hydrogen peroxide (0.01N), 1 ml of 50 mmol l^{-1} phosphate buffer and 1 ml of enzymatic extract. The decomposition of H_2O_2 was followed at 240nm (Cakmak & Marschner, 1992), the data were recorded every 15sec for 2min. The enzymatic activity is expressed in µkat mg^{-1} of proteins contained in the plant extract used) (Micro-Katal [µKat]: disappearance of one µmole of substrate per second). The statistical treatment of the results was carried out by the software Minitab version 2017.

2.2 Interspecific hybridization

The interspecific crosses of *Aegilops* sp. / durum wheat varieties were conducted over five years. Three sowing dates were completed for each parent to synchronize their flowering time. Before anthesis, *Aegilops* sp. spikes have been emasculated and wrapped to avoid cross-pollination. They were pollinated with fresh pollen from the durum wheat varieties, without any growth hormones uses after emasculation and pollinisation. The hybrids were harvested in caryopsis.

2.3 *In-vitro* culture

A mature embryo culture of hybrid caryopsis was undertaken to break the dormancy of these seeds. They were disinfected under a laminar flow hood in a 70 % C_2H_5OH solution for 30 sec, rinsed with sterile distilled water and then placed in a dilute 12 % sodium hypochloride solution for 15 min. They were rinsed 5 times with sterile distilled water. The disinfected caryopses were placed in Petri dishes with absorbent paper previously sterilized and they were soaked with sterile distilled water. After 24 h, the mature embryos were removed under binocular and under sterile conditions. They were transferred to MS regeneration medium (Muraschige & Skoog, 1962) supplied with Kinetin (0.25 mg l^{-1}), AIB (Indole butyric acid) (1 mg l^{-1}). The cultures were incubated in a culture chamber in the dark, at a temperature of 25 ± 2 ° C for one week. After the release of coleoptiles, a photoperiod of 16 h day / 8h night was applied. Mature embryos were transplanted to a new medium every four weeks.

3 RESULTS

3.1 Drought tolerance

Tolerance to water stress was evaluated for two *Aegilops* species and two durum wheat varieties.

3.1.1 Physiological parameters

3.1.1.1. Relative water content

The relative water content decreases with the intensity of the stress (Fig. 1a) comparing to the controls (C) whose value varies from 87.90 to 98.08 %, the non-watered plants during one week (L1 of stress) have displayed WRC from 81.81 to 94.11 % and the unsprayed for two weeks (L2 of stress) from 60.90 to 87.15 %. The variance analysis showed very highly significant differences between the genotypes as well as for the treatments at the threshold α = 0.001, the genotype x treatment interaction, proved not significant.

3.1.1.2 Stomatal Resistance

For all the studied genotypes, the SR increases markedly with the severity of the stress (Fig. 1b). The means values range from 1.89 to 27.97 m^2.s mol^{-1} for C plants; from 36.73 to 131.00 m^2.s mol^{-1} for L1; from 81.00 to 175.00 m^2.s mol^{-1} for L2. The *Aegilops* have been remarked by high resistance means and a fast response from L1 (especially for *Aegilops geniculata* "*Ae.gen*") compared to wheat varieties. As a result, the variance analysis of the genotypes as well as the treatments was very highly significant at the threshold α = 0.001, the genotype x treatment interaction is significant at the threshold α = 0.05.

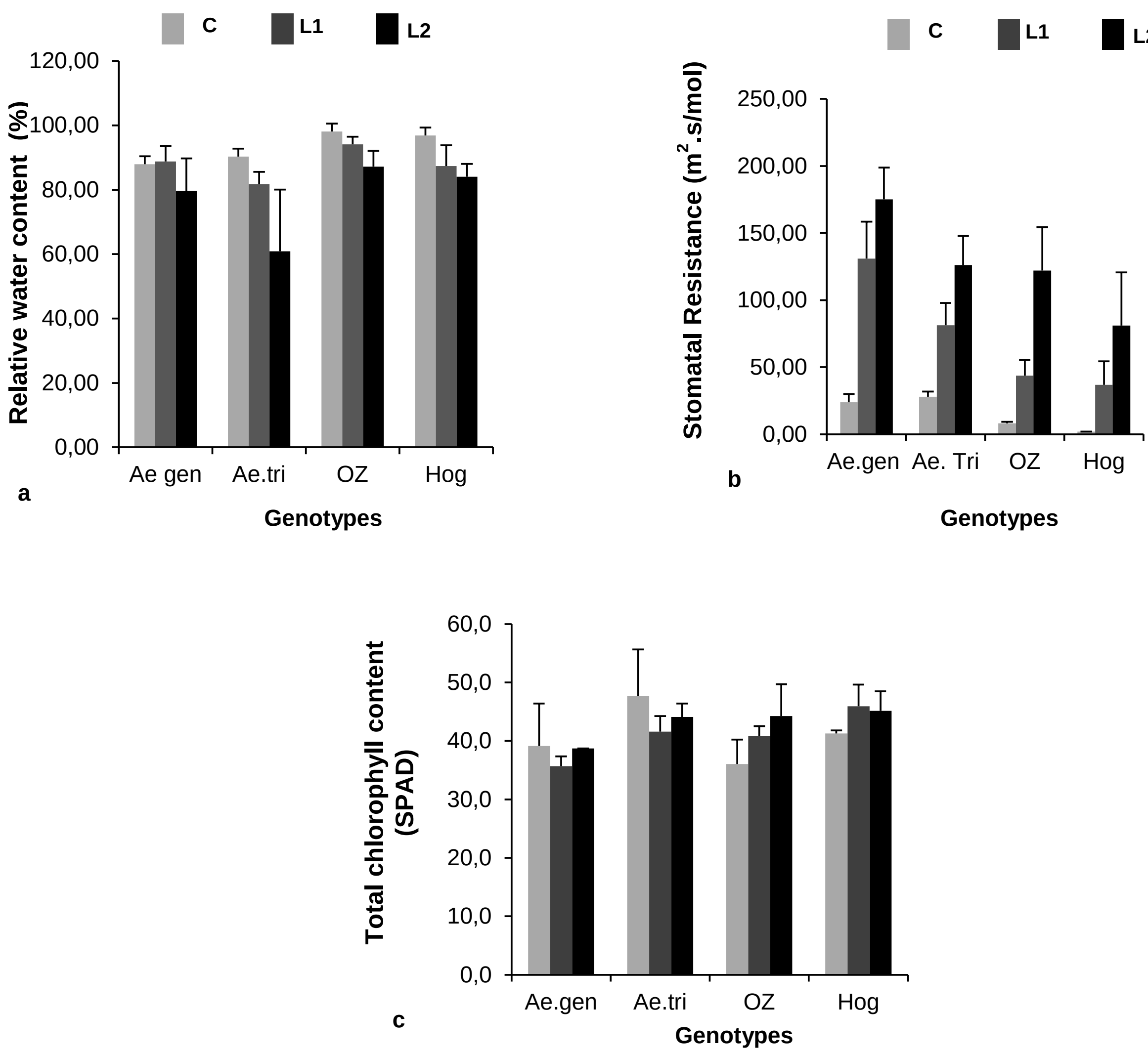


Figure 1: Water stress effect on physiological parameters: a. Relative water content; b. Stomatal resistance; c. Total chlorophyll content.

3.1.1.3 Total chlorophyll content

Ae.gen and *Ae.Tri*, present values of 39.1 and 47.7 SPAD for C that decrease at L1 to 35.7 and 41.6 SPAD but increase at L2 to 38.7 and 44.1 SPAD, respectively (Fig. 1c). O.Z has an increase in TCC with stress levels, compared to C whose value is 36.1 SPAD, TCC reaches 40.9 SPAD at L1, then 44.3 at L2. For Hog, the value of C is 41.3SPAD, the TCC increases to 45.9 SPAD at L1 and then decreases slightly at 45.1 SPAD at L2. As a result, the analysis of variance revealed a significant difference between the genotypes studied at $\alpha = 0.05$ threshold, whereas the treatments as well as the genotype x treatment interaction proved to be insignificant.

3.1.2 Biochemical parameters

3.1.2.1 Soluble sugar content

The recorded SSC revealed different stress behaviours (Fig. 2a). For *Ae.gen* and *Ae.tri*, respectively, the SSC is 0.765 and 0.813 µmol for C, it reaches 0.793 and 0.864 µmol at L1, but decreases at 0.790 and 0.756 µmol at L2. For O.Z, a decrease in SSC is observed at L1 (0.839 µmol) compared with C (0.863 µmol), then an increase at L2 (0.883 µmol). Hog, has a SSC of 0.980 µmol for C which decreases for L1 to 0,799 µmol and remains constant at this value at L2. The variance analysis revealed highly significant differences between the genotypes as well as the genotype x treatment interaction at $\alpha = 0.01$ threshold, whereas the treatments proved to be insignificant.

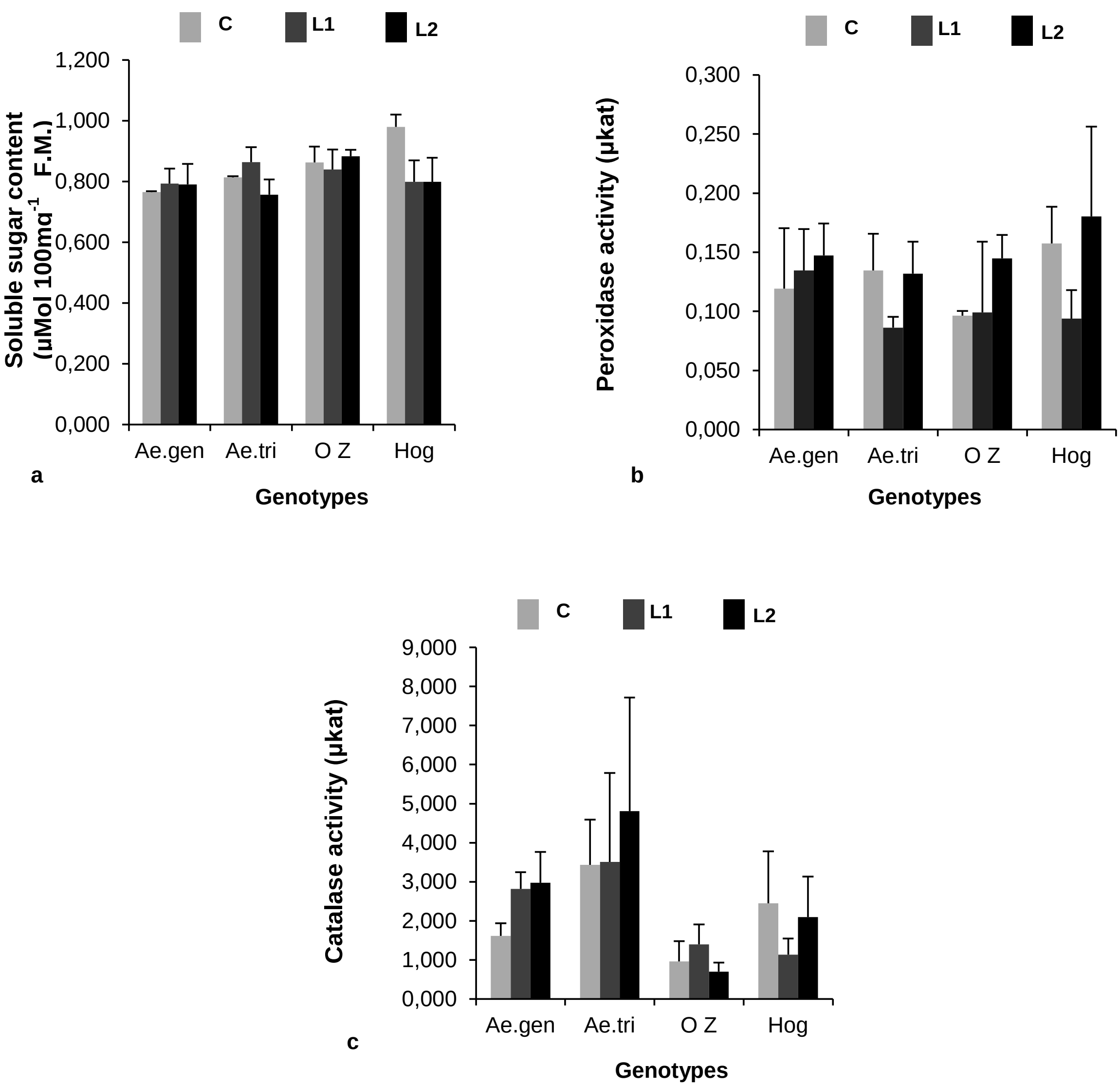


Figure 2: Water stress effect on biochemical parameters: a. Soluble sugar content; b. Peroxidase activity; c. Catalase activity

3.1.2.2 Peroxidase activity

Aegilops sp. show an increase in this activity in stressed plants compared to control plants which have a value of 1.617 and 3.436 µKat mg^{-1}, at L1 the value reaches 2.820 and 3.511 µKat mg^{-1}, at L2 it is 2.977 and 4.808 µKat mg^{-1} respectively, for *Ae.gen* and *Ae.tri*, this latter presents the most important values of POX activity (Fig. 2b). The O.Z control present a POX activity of 0.959 μKat mg^{-1}, which increases for L1 to 1.398 μKat mg^{-1} but decreases at 0.699 μKat mg^{-1} for L2. Whereas in the Hog variety, the POX activity decreases for L1 at 1.132 μKat mg^{-1} compared to C whose activity is 2.447 μKat mg^{-1} and then increases at L2 to 2.098 μKat mg^{-1}. The analysis of variance was very highly significant between the different genotypes (α = 0.001), whereas the treatments as well as the genotype x treatment interaction proved to be insignificant.

3.1.2.3 Catalase activity

The CAT activity (Fig. 2c) shows, for *Ae.tri* and Hog variety, respectively, a decrease at L1 (0.086 and 0.094 μKat mg^{-1}) in comparison with the C plants (0.135 and 0.157 μKat mg^{-1}), then an increase at L2 (0.132 and 0.180 μKat mg^{-1}). For O.Z, the activity increases slightly at L1 (0.099 μKat mg^{-1}) compared to C (0.096 μKat mg^{-1}), it increases considerably at L2 (0,145 μKat mg^{-1}). For *Ae.gen*, the increase in enzymatic activity with stress levels is more remarkable than in O.Z, the CAT is 0.119 μKat mg^{-1} for C and it reaches 0.135 μKat mg^{-1} at L1 then 0.147 μKat mg^{-1} at L2. For this parameter, the treatments appeared significant at α = 0.05 threshold, while the genotypes as well as the genotype x treatment interaction proved to be insignificant. The comparison between the two enzymes activities reports higher values of POX than those of CAT (Fig. 2b and c). The correlations between physiological and biochemical parameters are shown in Table 2, where we recorded two significant positive correlations (α = 0.05) between SSC and SR, and between WRC and SSC. A highly significant negative correlation (α = 0.01) between WRC and SR is also observed.

Table 2: Linear correlations matrix of physiological and biochemical parameters

	TCC	SSC	CAT	POX	SR
SSC	0.027				
CAT	0.242	0.092			
POX	0.159	-0.082	0.158		
SR	-0.070	-0.352*	0.166	0.317	
RWC	-0.169	0.350*	-0.145	-0.280	-0.481**

$p \le \alpha = 0.05$: (*)significant differences. $p \le \alpha = 0.01$: (**)highly significant differences

The grouping of the four genotypes, with a dendrogram using the single linkage and the Squared Pearson distance and for a minimum similarity level of 50 % (Fig. 3), enabled to distinguish four homogeneous groups: the first is represented by *Ae.gen*, the second by *Ae.tri*, the third consists of O.Z and the fourth group of Hog.

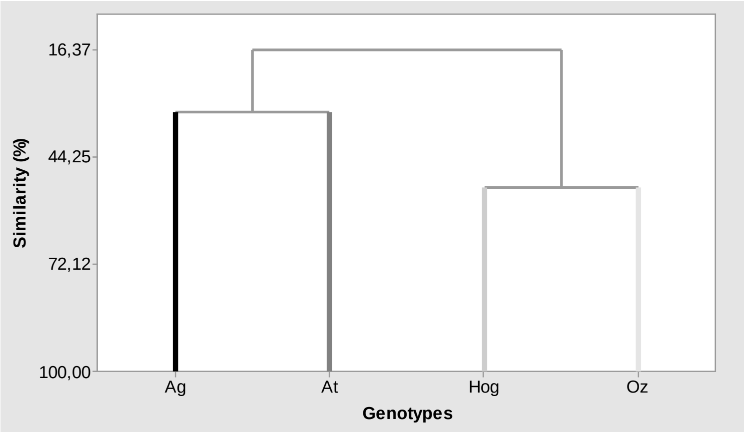


Figure 3: Grouping dendrogram of studied genotypes. **Ag:** *Ae. geniculata;* **At:** *Ae. triuncialis*; **Hog:** Hoggar; OZ: Oued Zenati

3.2 Interspecific hybrids obtaining

The interspecific hybridization between two species of the genus *Aegilops* as the female parent, with the two durum wheat varieties, allowed us to obtain 81 hybrids. Table 3, summarizing the five-year results for the four possible combinations of crossing, represents the cross-ability between the genitors, expressed as a percentage of the number of hybrids obtained reported to the number of pollinated flowers.

Table 3: Five-year hybridizations results according to genitors combinations

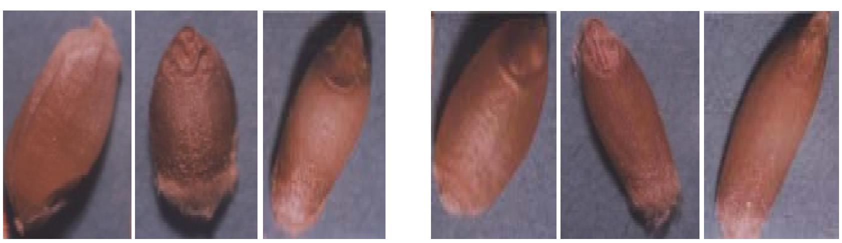
Crosses	NS	NSK	NPF	NFS	NHS	Cross-ability%
Ae.gen /OZ	192	524	1032	714	54	**5. 23**
Ae.gen/ H	38	97	194	157	4	**2. 06**
Ae.tri /OZ	66	237	464	361	18	**3.88**
Ae.tri/H	47	171	336	269	5	**1.49**

NS: Number of pollinated spikes. NSK: Number of pollinated spikelets. NFP: Number of pollinated flowers. NFS: Number of fruit set. NHS: Number of hybrids seeds.

The results show differences in hybridization affinity between parents. The combination of *Ae.gen* and O.Z produced the largest hybrids number (54 a rate of 5.23 %). The crossing between *Ae.tri* and O.Z, comes second in hybrids production (18 a rate of 3.88 %). Combinations of *Ae.gen* and Hog as well as *Ae.tri* and Hog gave a small number of hybrids.

3.3 Mature embryo culture and plantlet regeneration

All the hybrids were collected in caryopsis resembling the female parent *Aegilops* sp. (Fig. 4), of different sizes (very noteworthy for hybrids whose parent is *Ae.tri* characterised by long caryopsis), mostly with a normal endosperm, only a few were scalded.



a **b** **c** **d** **e** **f**

Figure 4: Harvested hybrid caryopsis photographs in comparison with those of respective parents. a-Hog, b- Hybrid Ae.gen/Hog, c-Ae.gen, d- O.Z, e- Hybrid Ae.tri/OZ, f- Ae.tri.

Several cold stratifications as well as the scarification of the seeds did not allow the break dormancy of hybrids, observed under natural conditions. Only mature embryos culture allowed germination and regeneration of hybrid seedlings (Table 4). The embryos collected were of different sizes, some very small not exceeding one millimeter in diameter.

Table 4: Germination rate and number of hybrid seedlings regenerated by mature embryo culture

Hybrids	Cultured embryos	Germination %	Regerenated seedlings	Adult plants
Ae gen/.O.Z	7	100%	2	0
Ae gen/Hog	5	100%	2	0
Ae tri/Hog	2	100%	1	0
Ae tri/O.Z	1	100%	1	0
Total	15	100%	6	0

From fifteen embryos, six seedlings were regenerated in a relatively average rate of regeneration of 40 %, however, no adult plant is obtained. After successful germination (100 %) of all hybrids, those with normal growth (Fig. 5a) regenerated the seedlings. While for others, anomalies have been detected leading to precocious death causes by lack of root system edification (Fig. 5b); lack of the coleoptile development (Fig. 5c); and albino coleoptiles regeneration (Fig. 5d).

Figure 5: Hybrid mature embryo photographs of MS medium germination. **a-***Ae.tri*/OZ, two weeks of culture. **b-** *Ae.gen*/OZ, two weeks of culture. **c-***Ae.tri*/Hog, one week of culture. **d-** Another hybrid *Ae.gen/*OZ, four weeks of culture

The death of seedlings, at the acclimation stage, occurring at different stages of growth (the most advanced is that of tillering for the hybrid *Ae.gen* / Hog) is due to the weak growth of the seedlings (stunted plantlets, leaves with very small surface, weak root system) (Fig. 6). However, hybrid seedlings also exhibited morphological features of female parents *Ae.gen* and *Ae.tri* in the early stages of development, similar to twisted pre-foliation and leaf color.

a **b** **c** **d**

Figure 6: Regenerated hybrid plantlets. **a:** *Ae.gen*/O.Z. **b**: *Ae.tri*/Hog. **c:** *Ae.gen*/Hog (six weeks of culture). **d-** *Ae.gen*/Hog (twelve weeks of culture).

4 DISCUSSION

Obtaining interspecific hybrids offers significant variability. It is the crucial step in any program of genes introgression from wild species. However, its success depends not only on the choice of genitors that suits the objectives of the program, but also on crossing affinity. Thus, the study of drought tolerance confirms the high potential of *Aegilops* species to tolerate water stress, particularly that of *Ae.geniculata*, which corroborates with the works of (Rekika et al., 1998; Zaharieva et al., 2001; Baalbaki et al., 2006) and the adaptation of the OZ variety to Mediterranean stress type (Ali Dib et al., 1992; Meziani et al., 1993). The RWC and the SR had a significant impact on the other physiological and biochemical parameters of this study, SR - RWC (r = - 0.481 **); SSC - RWC (r = 0.350 *); SR and SSC (r = - 352 *) (Table 2). In fact, the ability to maintain elevated

RWCs in a situation of water stress is related to the osmotic adjustment capacity or to the high elasticity of the plant tissues (Bousbaa et al., 2013). The opening or closing of stomata, sensitive to the concentrations of abscisic acid produced by the roots, is the most element affected by the water stress of all those in relation to the water of the plant (Anjum et al., 2011; Shang et al., 2016). By closing its stomata, the plant saves the available water and preserves cell integrity, thus constituting one of the best strategies for water stress tolerance (Djekoun & Ykhlef, 1996; Ykhlef et al., 2000; Bousbaa et al., 2013; Shang et al., 2016). Consequently, the closure of the stomata leads to a decrease of the photosynthesis (Maurino & Peterhansel, 2010; Gallais, 2015) and an increase of the reactive oxygen species where peroxidase plays an important role in their elimination, especially in conditions of water stress (Anjum et al., 2011).

The few works dedicated to interspecific crosses where species of the genus *Aegilops* are taken as female parent, report the weakness of obtaining such hybrids, which seems, more favourable in field conditions (Guadagnuolo et al., 2001). Many criteria influence the acquisition of fertile hybrids and backcross progenitor for introgression between two genera, including genetic relationships, ploidy level, and hybridization direction (Waines & Hegde, 2003). The success of obtaining hybrids depends largely on the parental genotypes involved in the crossing. The differences in hybridization affinity of *Aegilops* species and wheat varieties are highly observed (Guadagnuolo et al., 2001; Waines & Hegde, 2003; Stone & Peeper, 2004; Hadzhiivanova et al., 2012; Ykhlef et al., 2007). In many studies, the common sharing of the D genome between the bread wheat and the wild parent allowed the pairing of homeologous chromosomes and obtaining fertile hybrids (Snyder et al., 2000; Schoenenberger et al., 2005; Martins et al., 2015). The effect of the Ph1 locus is only suppressed in some diploid *Aegilops* species (Al-Kaff et *al*, 2007), so allowing the pairing of homeologous chromosomes in the hybrid (Waines & Hegde, 2003). The lethality of hybrids, manifested by meristem tissues anomalies from germination to a weak growth at advanced stages of development, are often reported in interspecific hybridization, resulting from incompatibilities between the nuclear and cytoplasmic genomes, due to complementarities or epistasis interactions between genes (Tikhenko et al., 2008; Matsuoka et al., 2007; Mizuno et al., 2010). In the case of *Poaceae*, a paternal heredity of chloroplast DNA has never been observed (Guadagnuolo et al., 2001), so in our study, the maternal cytoplasmic heredity explains the morphological characters of resemblance between hybrids and the female *Aegilops* parent. Differences in establishment of pre-or post-zygotic hybridization barriers between parents manifest according to the direction of hybridization, which make easier the obtaining of hybrids in one of the directions (Riesberg & Carney, 1998). Following our results, interspecific hybridization where *Aegilops* is the female parent have the advantage of obtaining caryopsis hybrids (Guadagnuolo et al., 2001; Cifuentes et al., 2006) compared to the reciprocal hybridization durum wheat /*Aegilops* where interspecific hybrids were obtained only by embryos rescue (Hadzhiivanova et al., 2012; Ykhlef et al., 2007). Our study is a contribution to the identification of genitors and mechanisms that facilitate interspecific hybrids obtaining. We have focused on the cross-ability of the O.Z variety, widely used and adapted to the Mediterranean stress type, with the *Ae.gen* and *Ae.tri* species, and the quality of hybrids obtained in the *Aegilops* /durum wheat direction. Thus, research within these two species of accessions, that are more favourable for obtaining hybrids, is promising success and less expensive hybridization.

5 CONCLUSION

We have undertaken in this study, the hybridization of two species of the genus *Aegilops* and two durum wheat varieties. The characterization of the genitors for their drought tolerance during the hybridization period, confirms our choice of the genitors, where we noticed the superiority of the *Aegilops* for water stress tolerance comparing to the wheat and the adaptation of durum wheat varieties to the climate of Algerian cereal zones. The duration of the stress applied seems average. Therefore, in a short term the plants reacted by a fast closing of the stomata which remedied the loss of water by transpiration and consequently maintained a high RWC favourable to the good cellular functioning. Obtaining hybrids, even with a low rate of 3.9 %, indicates the possibility of interspecific hybridization between *Aegilops* species and durum wheat, taking *Aegilops* as the female parent. Their success is affected by several parameters where the genotype of the involved parents and the degree of relationship are important criteria, because of the existence of genes that inhibit homeologous pairings between parental genomes as well as the establishment of genes that cause lethality and the sterility of hybrids in some species during speciation. The obtaining of caryopsis in very good condition and without recourse to the embryos rescue, confirms that genetic mechanisms of post zygotic isolation have been expressed in the hybridization direction where *Aegilops* is considered as the female parent. The study and understanding of these mechanisms and the identification of their responsible genes will overcome these barriers and facilitate the acquisition of hybrids in order to succeed the introgression programs of interesting genes from wild species. We lead cytogenetic and molecular studies to characterize hybrids and to elucidate potential problems that led to the loss of hybrid seedlings during the acclimation phase.

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