



## Assessment of Early Drought Tolerance of Algerian Durum Wheat Reveals Superiority of Landraces

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**V**IGOROUS seedling growth is important for good crop establishment, particularly under drought conditions. Our study was set out to identify useful traits and genotypes to enhance early drought tolerance of durum wheat. Two experiments were carried out. In the first one, thirty-five genotypes (Landrace and improved) were tested in a phytotron at germination and early seedling stages, subjected to three osmotic stress levels induced by polyethylene glycol PEG 6000 (0, -3, -6 bar). The second experiment was conducted in the field, with 27 out of the 35 genotypes. Root and shoot traits were measured at seedling stage in both experiments. High PEG 6000 treatment decreased final germination percentage (FGP) by 2.7% and delayed the time to reach 50% germination (t50) by 2.9h. Shoot length was the trait most affected by drought (40% reduction) as compared with other root traits, which even increased under drought, like root to shoot length ratio, root to shoot weight ratio, root dry weight and root number. Coleoptile length (CL) showed a contrasting relationship with other traits, it was negatively correlated in general under no stress, but with positive correlations under stress. Based on drought susceptibility index (DSI), Algerian wheat landraces were the most tolerant compared to modern genotypes. Correlations between traits measured in field and controlled conditions were low. CL could be a potential trait for screening drought tolerant genotypes. Algerian wheat landraces presented a clearly distinct ability for early drought tolerance, and could be a good resource for breeding programs.

**Keywords:** Early growth, Polyethylene glycol, Durum wheat seedlings, Drought susceptibility index.

### Introduction

In Mediterranean-type environments, sowing is typically practiced when soil moisture is ensured by the first rain (Rebetzke et al., 2008). Early growth vigor has been proposed as a trait that could enhance crop water-use efficiency and yield in these environments (López-Castañeda & Richards, 1994; Coleman et al., 2001). One of its possible benefits could occur through increased root growth early in the season (Liao et al., 2004). Early drought restricts germination (Misra et al.,

1990), emergence and early seedling growth (Al-Karaki, 1998), which may lead to crop failure in the West Asia and North Africa (WANA)-region (Abdel-Ghani et al., 2015). In regions characterized by short periods of appropriate soil moisture, seeds with high germination percentage may be advantageous for ensuring a good plant establishment (Brar et al., 1991). Drought stress is a stage specific phenomenon, as it has been described that tolerance at plant establishment phase is poorly correlated with tolerance at other stages (Mano et al, 1996; González et al., 2008;

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Received 25/9/2019; Accepted 28/11/2019

DOI: 10.21608/agro.2019.17341.1182

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Szira et al., 2008). Selection for drought tolerance at early growth stage has been frequently attempted using PEG 6000 to induce water stress, without causing significant physiological damage to crop plants (Carpita et al., 1979; Rauf et al., 2007).

Despite the importance of root system for acquisition of water and nutrients (Blum, 1997; Blum, 2009; Ehdaie et al., 2012), plant breeding focused for a long time almost solely on the above-ground traits, while root traits were relatively neglected because of the practical difficulties of phenotyping at a scale useful to perform selection (Waines & Ehdaie, 2007). In the last decade, more attention has been paid to root phenotyping (Bengough et al., 2004; Nagel et al., 2012; Richard et al., 2015; York et al., 2018), thanks to novel phenotyping methods. Among these, root attributes at seedling stage are important for screening genotypes for early drought tolerance (Chloupek et al., 2010; Sayed, 2011). Some breeders propose to select genotypes with higher root volume combined with maximum length of seminal and adventitious roots (Richards & Passioura, 1981; Grando & Ceccarelli, 1995). Jia et al. (2019) indicated that root system depth and root spread angle are valuable candidate traits for increasing grain yield. Root to shoot ratio and root length at early stages of plant development could also be valuable attributes for improving yield under arid and semi-arid conditions (Dhanda et al., 2004; Shahbazi et al., 2012). Coleoptile length (CL) has also been proposed as an important trait for drought tolerance at plant seedling stage: Long coleoptiles allow deep sowing, which is an adequate practice in water-limited environments in which topsoil dries up fast (Mahdi et al., 1998; Schillinger et al., 1998), enabling growers a longer time window to perform sowing with optimum soil moisture (Gan et al., 1992).

Wheat landraces have been widely replaced by modern varieties (Khlestkina et al., 2004; Reif et al., 2005; Bonnin et al., 2014). Nevertheless, they are still preferred over modern wheats in several parts of the world, mainly for their stable yields in low input conditions, prized end-use qualities, and high straw yield. Farm size, lack of machinery, and lack of fertilizer are also important constraints in growing modern wheats (Karagöz, 2014). Varietal substitution has led to reduction of germination-related traits like shoot, coleoptile and root length and seedling vigor in

Iranian modern varieties, compared to landraces (Ramshini et al., 2016). Bektas et al. (2016) found that shoot biomass, shallow and deep root weight, number of tillers per plant and plant height were significantly greater in landraces than in modern varieties. In several cases, winter cereal landraces have shown better performances than modern varieties, usually under challenging environmental conditions (Yahiaoui et al., 2014; Erice et al., 2019). When root systems were compared, an Algerian wheat landrace (Pelissier) had more root growth than a widely grown modern variety (Ashe et al., 2017).

All these evidences highlight the importance of early drought tolerance, and the potential of landraces to contribute favorable traits in this respect. The aim of our research was to identify traits and genotypes of importance in early stress tolerance, and to explore the potential of Algerian landraces for drought tolerance breeding.

## **Materials and Methods**

### *Plant material*

Thirty-five durum wheat genotypes (landraces and modern cultivars) from different countries (Algeria, France, Italy, Spain, Tunisia), and international breeding programs addressing semi-arid areas, namely the International Maize and Wheat Improvement Center (CYMMIT), the International Center for Agricultural Research in the Dry Area (ICARDA) and the Arab Center for the Studies of Arid zones and Dry lands (ACSAD), were chosen for this study (Table 1). Most of these genotypes have been widely cultivated in Algeria. There are reports of cultivation of the oldest genotype Hedba3 in 1921, whereas the newest ones (Boutaleb and Oued El Berd) were released by the Technical Institute of Field Crops (ITGC, Sétif, Algeria) in 2013, thus this set of genotypes is spanning more than 8 decades (Table 1).

### *Phytotron experiment*

The experiment was conducted at the Biotechnology Research Center (CRBt), Constantine, Algeria. Fifteen healthy seeds of the same size of each genotype, were weighted, surface sterilized with 0.5 % of sodium hypochlorite (NaClO) and rinsed for six times with distilled water. They were then germinated on Whatman (type1) filter paper soaked with 10 ml of PEG 6000 solutions in Petri dishes. PEG 6000 was

used to induce osmotic stress at two levels, -3 and -6 bar, following the method suggested by Michel & Kaufmann (1973), while distilled water without PEG 6000 was used as control treatment. Petri dishes were transferred to a phytotron for 8 days, in darkness, at constant 25°C and 70% relative

humidity. The experimental design was a split plot design with two replications (each consisting of 15 seeds of a genotype in a Petri dish), where the whole plot was PEG treatment and the subplot was the genotype.

**TABLE 1. Type, origin and date of release information's of the 35 genotypes of durum wheat used in this study.**

Genotype	Abv	Type	Origin	Year of release	Phytotron	Field
Beliouni	Bel	Landrace	Algeria	1958	×	
Bidi 17	Bid	Landrace	Algeria	1930	×	×
Djenah Khotifa	Dje	Landrace	North Africa	1955	×	
Gloire de Mongolfier	Glo	Landrace	Algeria	1960	×	×
Guemgoum R'khem	Gue	Landrace	Algeria	1960	×	×
Hedba 3	Hed	Landrace	Algeria	1921	×	×
Mohammed Ben Bachir	MBB	Landrace	Algeria	1930	×	×
Oued Zenati 368	OZ	Landrace	Algeria	1936	×	×
Langloise	Lan	Landrace	Algeria	1930	×	×
Sbaa Aldjia	Sba	Landrace	Tunisia	-	×	
Acsad 65	Acs	Improved	ACSAD	1984	×	×
Altar	Alt	Improved	CYMMIT	1984	×	×
Aures	Aur	Improved	Algeria	2013	×	
Boutaleb	Bot	Improved	Algeria	2013	×	
Capeiti	Cap	Improved	Italy	1940	×	×
Cirta	Cir	Improved	Algeria	2000	×	×
El Maather	ELM	Improved	Algeria	-	×	
GTA Dur	GTA	Improved	CIMMYT	1972	×	×
INRAT 69	INR	Improved	Tunisia	1969	×	×
Korifla	Kor	Improved	ICARDA	1987	×	×
Mansourah	Man	Improved	Algeria	2012	×	×
Massinissa	Mas	Improved	Algeria	2012	×	×
Megress	Mgs	Improved	Algeria	2007	×	×
Mexicali 75	Mex	Improved	CIMMYT	1975	×	×
Miki-2	Mik	Improved	ICARDA	2008	×	
Montpellier	Mon	Improved	France	1965	×	×
Ofanto	Ofa	Improved	Italy	1990	×	×
Oued El Berd	OEB	Improved	Algeria	2013	×	×
Polonicum	Pol	Improved	France	1973	×	×
Simeto	Sim	Improved	Italy	1988	×	×
Sitifis	Sit	Improved	Algeria	2011	×	×
Tejdid	Tej	Improved	Algeria	-	×	
Vitron	Vit	Improved	Spain	1987	×	×
Waha	Wah	Improved	ICARDA	1986	×	×
ZB × Fg	ZBF	Improved	Algeria	1983	×	×

×: Indicate the presence of the corresponding genotype in the experiment.

Germination date was recorded when the radicle reached at least 2mm in length. Germinated seeds were counted every 24h for 8 days. Final germination percentage (FGP) and the time needed to reach 50 % germinated seeds (t50) were recorded. Time to reach 50% germination was calculated based on the following formula proposed by Coolbear et al. (1984) and modified by Farooq et al. (2005):

$$t_{50} = t_i + [(N/2 - n_i)(t_j - t_i)] / (n_j - n_i)$$

where N is the final number of seeds that germinated and  $n_i$  and  $n_j$  were the cumulative number of seeds germinated by adjacent counts at times  $t_i$  and  $t_j$  when  $n_i < N/2 < n_j$

At the end of the experiment, seedlings were preserved in a 30% ethanol solution until the rest of the traits were recorded in five representative seedlings chosen from each Petri dish: mean value of shoot length (SL), coleoptile length (CL), root number (RN), total root length (TRL), maximum root length (MRL), root dry weight and shoot dry weight (RDW and SDW, respectively), and total plant biomass (TPB). Additionally, several indices were calculated: root to shoot ratio for weight and length (RSW and RSL respectively), seedling vigor index (SVI) and drought susceptibility index (DSI). The drought susceptibility index (DSI) for TPB was calculated according to Fischer & Maurer (1978) using the following formula, originally developed for yield:

$$DSI = (1 - Y_D/Y_P) / (1 - X_D/X_P)$$

where,  $Y_D$  corresponds to the mean genotypic TPB under stress,  $Y_P$  corresponds to the mean control TPB for each genotype,  $X_D$  is the TPB mean of all genotypes under stress, and  $X_P$  is the mean TPB of all genotypes under control conditions.

The SVI based on seedling weight (hereafter,  $SVI_w$ ) was obtained using the following formula:

$$SVI_w = (RDW + SDW) \times FGP$$

The SVI based on seedling length (hereafter,  $SVI_L$ ) was calculated using the following formula (Abdul-Baki & Anderson, 1973):

$$SVI_L = (MRL + SL) \times FGP.$$

where, MRL: Maximum root length, SL: Shoot length, FGP: Final germination percentage

#### Field experiment

Twenty-seven out of the 35 wheat genotypes were sown on 28 Nov 2016 in a randomized complete block design with two replications under rainfed conditions. The rainfall throughout Nov was 29.7 l/m<sup>2</sup> for 7 days, so soil humidity was appropriate for seed germination. Sowing density was 300 seeds/m<sup>2</sup> in six row plots of 1.2m width and 2.5m long (3m<sup>2</sup>), at the Technical Institute of Field Crops (ITGC), Sétif, Algeria. Five seedlings per replicate were carefully harvested 10 days after emergence; roots were gently cleaned from soil by washing with tap water. The same traits measured in phytotron experiment were recorded in the field, except t50, SVI and DSI.

#### Data analyses

The analyses of variance were carried out by REML (Restricted Maximum Likelihood) procedure of Genstat 18 (Payne et al., 2009), taking replications as random factor, and genotype, treatment, genotype by treatment and the comparison of landraces vs. improved varieties (named 'type' effect), as fixed factors. Multiple means comparison was carried out using an LSD at 0.05 level of significance.

Broad-sense heritability ( $h^2$ ) was calculated on entry mean basis using the REML procedure in Genstat 18, as follows:

$$h^2 = \sigma^2_g / (\sigma^2_g + (\sigma^2_e/r))$$

where  $\sigma^2_g$  is the genotypic variance,  $\sigma^2_e$  is the error variance and  $r$  is the number of replications.

## Results

### Effect of PEG-induced drought stress on the assessed traits

The differences between treatments were significant for t50 (Time to reach 50% germination), due to the slower germination at -6 bar, but not for FGP. Genotypes were significantly different for both t50 and FGP. However, interactions between genotypes and treatments were found only for t50 (Table 2).

Both drought treatments increased t50 (Table 2), but only significantly at the high drought stress level (2.9h, 7.4% at -6 bar). As the high

treatment (-6 bar) effect was more pronounced on germination traits, from here on we will only report its results, referred to as the 'drought stress treatment', unless stated otherwise.

Under drought conditions, 24 genotypes showed a decrease in FGP while 11 genotypes showed no change or even increased their FGP (Supplementary Table 1). The opposite occurred for t50, 24 genotypes increased the time to 50% germination, and 11 showed accelerated germination under drought, or no change (Suppl. Table 1).

Drought had a significant effect on all of seedling traits, except for CL, RDW and TPB. Genotypes were significantly different for CL, MRL, RSL, RSW,  $SVI_w$  and  $SVI_L$ . It is remarkable that there was no significant interaction between genotypes and treatment (Table 3).

Phenotypic mean values of seedling traits were higher under control than under stress conditions (-6 bar) except for RN, RDW, RSL and RSW. In general, the ranges of values were wider under control conditions, except for CL, RN, RSL and RSW (Table 3). The highest reduction due to PEG stress was observed for the mean value of SL (40.26%) followed by SDW (19.26%), TRL (13.19%) and MRL (12.62%), whereas mean of TBP (8.25%) and CL (2.26%) were reduced the least. In contrast, RSL, RSW, RDW and RN means were increased under PEG treatment by 47.53, 38.66, 8.62 and 7.46%, respectively. For root to shoot length ratio (RSL) and root to shoot weight ratio (RSW), the mean values were

greatly increased under PEG treatment, which was a consequence of the great reduction of SL and SDW respectively. In general, the coefficient of variation values (CV) were similar between traits under both conditions except for RN which was the smallest one (10.99 and 10.66 for non-stress and stress conditions, respectively). CV values were greater under control than under stress conditions; only SL and CL had slightly higher CV values under stress conditions (Table 3). The DSI based on TPB showed negative and positive values. Genotypes with negative values were considered drought tolerant, and genotypes having positive values were considered as drought susceptible. Wheat genotypes presenting the lowest negative DSI values were almost all landraces, whereas modern ones presented positive DSI values (Table 4).

#### *Effect of field compared to phytotron conditions*

Under field conditions, ANOVA analyses showed a significant difference (0.05) for CL, highly significant difference (0.001) for RSW and very highly significant difference (<0.001) for RN and RDW (Table 5).

The comparison between the mean values for seedling traits recorded in the field and under phytotron non-stress and stress conditions, showed lower mean values in the field for all measured traits, except for SDW which was superior under field compared to both controlled conditions (stress and non-stress) and also for SL and TPB where the phenotypic mean values in the field were superior but only to those of stress (Table 5).

**TABLE 2. Summary statistics and means comparison for the 35 wheat genotypes under PEG treatments (0, -3 and -6 bar) for final germination percentage (FGP) and time to reach 50% germination (t50).**

	Min	Max	Mean (SE)	CV%	Reduction %
FGP					
Control, 0 bar	53.3	100.0	89.1a (7.09)	14.2	
PEG -3 bar	33.3	100.0	90.1a (9.03)	14.6	-1.1
PEG -6 bar	20.0	100.0	86.7a (11.68)	16.8	2.7
t50					
Control 0 bar	0.7	3.5	1.6b (0.32)	27.5	
PEG -3 bar	0.8	2.5	1.6b (0.24)	17.7	-1.2
PEG -6 bar	1.4	3.5	1.7a (0.18)	18.7	-7.4

**TABLE 3. Ranges, means, standard error (SE), coefficient of variation (CV) and significance of the analysis of variance for 35 wheat genotypes evaluated under optimum (non-stress) and drought stress conditions (-6 bar), for seedling traits.**

	Control					Drought stress (-6 bars)					Combined ANOVA (control and stress)			
	Min	Max	Mean	CV	SE	Min	Max	Mean	CV	SE	Reduction%	Genotype (Geno)	Treatment (Treat)	Geno×Treat
CL (cm)	1.84	5.42	3.92	18.23	0.66	1.45	5.85	3.83	22.01	0.66	2.26	***	ns	ns
SL (cm)	3.78	17.36	11.97	23.45	2.51	1.14	10.86	7.15	24.27	2.51	40.26	ns	***	ns
MRL (cm)	2.90	20.48	12.50	35.06	3.15	3.90	16.46	10.93	21.69	3.15	12.62	**	**	ns
TRL (cm)	7.02	71.26	39.88	40.74	26.9	9.06	57.06	34.62	25.61	26.9	13.19	ns	*	ns
RN	3.40	5.80	4.71	10.99	0.51	2.80	6.00	5.06	10.66	0.51	-7.46	ns	***	ns
SDW (mg)	0.60	15.10	8.00	36.50	0.0022	1.40	9.90	6.50	24.92	0.0022	19.26	ns	***	ns
RDW (mg)	1.60	10.90	5.80	40.47	0.0017	2.10	9.70	6.30	20.73	0.0017	-8.62	ns	ns	ns
TPB (mg)	3.90	21.70	13.90	34.74	0.0037	3.50	19.30	12.80	21.01	0.0037	8.25	ns	ns	ns
RSL	0.50	1.84	1.07	30.80	0.32	0.71	3.42	1.58	23.00	0.32	-47.53	**	***	ns
RSW	0.27	1.08	0.73	26.34	0.18	0.49	1.55	1.00	19.53	0.18	-38.66	*	***	ns
SVI <sub>w</sub>	0.29	2.16	1.26	39.56	0.35	0.22	1.56	1.11	26.70	0.35	11.64	**	*	ns
SVI <sub>L</sub>	251	3188	2193	33	502.99	307	2312	1586	28	502.99	27.00	***	***	ns

- CL: Coleoptile length, SL: Shoot length, MRL: Maximum root length, TRL: Total root length, RN: Root number, SDW: Shoot dry weight, RDW: Root dry weight, TPB: Total plant biomass, RSL: Root to shoot length, RSW: Root to shoot weight, SVI<sub>w</sub>: Seedling vigor index based on seedling weight, SVI<sub>L</sub>: Seedling vigor index based on seedling length.

- \*, \*\* and \*\*\*: Significant difference at 0.5, 0.01 and 0.001 level, respectively.



**TABLE 4. Thirty five wheat genotypes ranked on drought susceptibility index (DSI), calculated from total plant dry biomass (TPB, mg per seedling).**

Genotype	Type	TBP/control	TBP/stress	DSI
Langloise	Landrace	8.70	16.00	-10.17
Djenah Khoteifa	Landrace	7.72	13.14	-8.51
Sbaa Aldjia	Landrace	6.75	11.32	-8.20
Gloire de Mongolfier	Landrace	11.68	17.52	-6.06
Guemgoum	Landrace	11.46	16.25	-5.06
MBB	Landrace	10.95	14.31	-3.72
Oued Znatie	Landrace	11.09	13.57	-2.71
Polonicum	Improved	10.56	12.62	-2.36
Hedba 03	Landrace	10.26	11.97	-2.02
INRAT 69	Improved	14.09	16.19	-1.81
Aures	Improved	13.55	14.75	-1.07
Mexicalli 75	Improved	10.41	11.19	-0.91
Megress	Improved	11.94	12.60	-0.67
Waha	Improved	12.65	13.23	-0.56
Beliouni	Landrace	10.18	10.29	-0.13
Vitron	Improved	13.73	12.69	0.92
Altar 14	Improved	11.73	10.73	1.03
Bidi 17	Landrace	14.62	13.15	1.22
Acsad 65	Improved	15.28	13.72	1.24
Miki-2	Improved	13.51	12.03	1.33
Tejdid	Improved	15.21	12.73	1.98
ZB/Fg	Improved	13.78	11.36	2.13
Gta Dur	Improved	14.32	11.41	2.46
Oued El Berd	Improved	16.19	12.70	2.61
Wahbi	Improved	19.81	14.60	3.19
Stitfis	Improved	17.42	12.61	3.35
Montpellier	Improved	17.68	12.56	3.51
Cirta	Improved	17.24	12.09	3.62
Ofanto	Improved	18.07	12.64	3.64
Mansourah	Improved	15.38	10.49	3.85
Korifla	Improved	20.30	13.62	3.99
El Maather	Improved	19.96	12.31	4.64
Massinissa	Improved	20.19	11.93	4.96
Capeiti	Improved	13.46	7.79	5.10
Semito	Improved	19.21	11.09	5.12

**TABLE 5. Ranges, means, coefficient of variation (CV) and analysis of variance for 27 wheat genotypes under control, stress and field conditions, with ANOVA analysis of field data for seedling traits.**

Trait	Non-stress					Stress					Field					ANOVA
	Mean	Min	Max	CV	Mean	Min	Max	CV	Mean	Min	Max	SE	CV	Redu. % C	Redu. % S	
CL	3.92	1.84	5.42	19.59	3.87	2.26	5.85	22.01	3.19	1.56	4.70	0.55	19.82	18.64	17.39	*
SL	12.03	4.04	17.36	21.77	7.13	1.14	10.36	24.03	8.23	5.80	11.38	1.18	15.57	31.56	-9.20	ns
MRL	12.86	2.90	20.48	33.21	10.80	3.90	15.16	20.34	5.27	3.40	8.04	0.97	18.11	59.00	43.00	ns
TRL	40.64	7.02	71.26	39.47	34.46	9.06	57.06	25.54	16.29	8.14	26.90	3.88	25.08	59.91	44.69	ns
RN	4.67	3.40	5.80	11.08	5.05	2.80	6.00	10.18	4.33	3.20	5.40	0.38	11.84	7.22	15.42	***
SDW	8.00	0.60	15.10	35.74	6.50	1.40	9.90	25.18	9.30	6.40	11.70	0.0012	13.37	-16.08	-34.84	ns
RDW	6.00	1.60	10.90	37.62	6.30	2.10	9.70	20.67	4.60	2.50	7.90	0.0008	23.34	22.54	28.44	***
TPB	14.1	4.80	21.70	32.78	12.8	3.50	19.30	20.95	14.00	10.90	17.60	0.0016	11.52	0.95	-8.18	ns
RSL	1.10	0.53	1.83	30.21	1.57	0.71	3.42	24.22	0.64	0.45	0.88	0.12	18.53	41.51	83.78	ns
RSW	0.74	0.27	1.07	24.98	1.00	0.49	1.55	20.42	0.50	0.28	0.76	0.09	20.57	31.68	67.02	**
SVI <sub>w</sub>	1.28	0.29	2.16	35.80	1.12	0.22	1.56	26.25	0.88	0.51	1.43	0.20	22.34	31.08	18.92	ns
SVI <sub>L</sub>	2247.13	251.33	3188.00	30.47	1593.45	307.00	2312.00	27.20	868.81	523.71	1547.36	225.58	24.69	61.34	32.25	ns

- CL: Coleoptile length, SL: Shoot length, MRL: Maximum root length, TRL: Total root length, RN: Root number, SDW: Shoot dry weight, RDW: Root dry weight, TPB: Total plant biomass, RSL: Root to shoot length, RSW: Root to shoot weight, SVI<sub>w</sub>: Seedling vigor index based on seedling weight, SVI<sub>L</sub>: Seedling vigor index based on seedling length, Redu. % C: Mean value reduction compared to control, Redu. % S: Mean value reduction compared to stress.

- \*, \*\*, \*\*\*: Significant difference at 0.5, 0.01 and 0.001 level, respectively.



The ranges of variation for seedling traits observed in the field were smaller than those found under stress and non-stress conditions for all traits, for example TRL (cm): field= (8.14–26.90), control= (7.02– 71.26), stress= (9.06–57.06), MRL (cm): field= (3.4– 8.04), control= (2.90– 20.48), stress= (3.90– 15.16), CL (cm): field= (1.56– 4.70), control= (1.84– 5.42), stress= (2.26– 5.85) and for RN: field= (3.2–5.4), control= (3.4– 5.8), stress= (2.8– 6) (Table 5).

#### *Landraces vs. improved genotypes*

ANOVA analyses revealed a significant effect of type (landrace vs. improved) and type by treatment interaction on most traits measured except SL, RN, SDW for type effect (Table 6). Landraces showed higher coleoptile length than improved genotypes under control and stress conditions. For all other traits improved genotypes were superior or equal to landraces under control but the opposite was observed under stress (Table 6). Across treatment, landraces tended to increase all traits under stress except SL and CL, which were reduced by 40.44 and 8.85%, respectively. RDW of landraces was the most increased trait (traits per se) under stress (69.26%) (Table 6, Fig. 1). On the other hand, improved genotypes showed the largest decreases for most traits under stress. SL and SDW were the most affected by stress (reduced by 38.99 and 28.38%, respectively), but a slight increase was observed for CL (0.96%). Root number was increased for both improved genotypes and landraces under stress by 6.66 and 9.57%, respectively (Table 6). Under stress, landraces and improved cultivars increased their root length and root biomass compared to shoot part (increase in RSL and RSW) (Fig. 1). Seedling vigor index, based on seedling length ( $SVI_L$ ) or on seedling weight ( $SVI_W$ ), were significantly higher for improved cultivars under control conditions but not under stress conditions. Landraces tended to have a higher  $SVI_W$  under stress, compared to improved genotypes (Table 6).

The comparison between landraces and improved genotypes in field revealed significant differences only for SDW and RSW. Landraces presented higher SDW values and improved genotypes had a better RSW ratio (Suppl. Table 2).

#### *Broad heritability in the field compared to*

#### *controlled conditions*

Overall, heritability calculated from field data was inferior than that obtained under control conditions and was higher than under stress. Under control conditions, broad heritability was higher than under drought stress for most traits (Suppl. Table 3). MRL presented appreciable heritability under stress (0.98) and field conditions (0.99). CL was more heritable (0.65) under control than other conditions (0.25). RDW had higher heritability values under all conditions than SDW. RSW displayed very high heritability value under control (0.98) followed by field (0.50) and stress (0.17) (Suppl. Table 3).

#### *Traits relationship*

Pearson correlation coefficients between seedling traits measured in both control and drought stress conditions ranged from very weak correlation (0.07) for TRL and  $SVI_W$  to highly significant ones (0.30–0.50) for FGP, t50, CL, RSL, and  $SVI_L$  (values in the diagonal, Table 7).

In the control treatment, many significant correlations were found, TPB was positively correlated with all traits, except RSW and t50, and was greatly influenced by SDW and RDW. A high correlation was also found between TRL and MRL. Negative correlations were observed for t50 and CL with all other traits, indicating that genotypes with earlier germination and/or shorter coleoptile tended to have higher seedling traits values. RDW had a positive correlation with SDW (0.76).

Under stress, a high correlation was found between TPB and SL (0.75). Correlation between TPB with TRL and MRL (0.74 and 0.63 respectively) was less pronounced under stress conditions than under non-stress. RN, SDW and RDW had a similar correlation with TPB as found in non-stress. Remarkably, CL had a positive correlation with all seedling traits except RSW and RSL, whereas these correlations were negative at the control conditions, which means that seedlings having a longer coleoptile tended to be more tolerant (vigorous) under stress by producing more TPB. In addition, TPB under stress was negatively correlated with RSL (-0.44) and RSW (-0.43), whereas these correlations were positive under control conditions, indicating that, under no stress, seedlings invested more in root growth, and under stress they invested more in shoot growth (Table 7).

**TABLE 6. Ranges, means, percentage of reduction (Redu. %) and analysis of variance for landraces and improved genotypes under control and stress (PEG, -6) conditions.**

Trait	Type	Control			Stress			Redu. %	ANOVA		
		Min	Max	Mean	Min	Max	Mean		T	Trt	T × Trt
t50 (day)	Improved	0.70	2.38	1.49	1.46	2.10	1.66	-11.28	***	*	ns
	Landrace	1.00	3.50	1.90	1.44	3.50	1.90	-0.39			
FGP %	Improved	60.00	100.00	92.32	20.00	100.00	88.11	4.56	**	ns	ns
	Landrace	53.33	100.00	81.11	46.67	100.00	83.21	-2.60			
CL (cm)	Improved	1.84	5.20	3.68	2.26	5.85	3.72	-0.96	***	ns	ns
	Landrace	3.28	5.42	4.50	1.45	5.42	4.11	8.85			
SL (cm)	Improved	3.78	17.36	11.81	1.14	10.86	7.20	38.99	ns	***	ns
	Landrace	4.94	15.44	12.23	2.83	12.08	7.28	40.44			
MRL (cm)	Improved	2.90	20.48	13.57	3.90	16.46	10.83	20.25	**	**	**
	Landrace	3.90	16.76	9.83	6.20	13.76	11.18	-13.73			
TRL (cm)	Improved	7.02	71.26	44.08	9.06	57.06	33.84	23.22	*	*	***
	Landrace	13.20	56.72	29.38	15.20	48.04	36.56	-24.43			
RN	Improved	3.40	5.80	4.78	2.80	6.00	5.10	-6.66	ns	***	ns
	Landrace	3.40	5.60	4.55	3.30	5.80	4.98	-9.57			
SDW (mg)	Improved	0.58	15.05	8.62	1.38	9.48	6.17	28.38	ns	***	***
	Landrace	2.34	11.82	6.46	2.27	9.94	7.18	-11.18			
RDW (mg)	Improved	2.36	10.90	6.59	2.14	9.72	6.20	5.91	***	ns	***
	Landrace	1.60	6.84	3.88	2.55	9.40	6.56	-69.29			
TPB (mg)	Improved	4.78	21.67	15.38	3.52	17.94	12.38	19.51	**	ns	***
	Landrace	3.94	17.84	10.34	4.82	19.34	13.75	-32.98			
RSL	Improved	0.54	1.84	1.15	0.72	3.42	1.60	-39.78	*	***	ns
	Landrace	0.50	1.57	0.90	0.96	2.19	1.54	-71.68			
RSW	Improved	0.32	1.08	0.76	0.49	1.55	1.03	-35.48	**	***	ns
	Landrace	0.27	1.04	0.64	0.72	1.45	0.94	-47.76			
SVI <sub>w</sub>	Improved	0.29	2.16	1.43	0.22	1.47	1.09	24.05	***	*	***
	Landrace	0.32	1.44	0.83	0.23	1.56	1.17	-41.02			
SVI <sub>L</sub>	Improved	251.33	3188.00	2380.32	307.00	2312.00	1583.95	33.46	**	***	**
	Landrace	614.40	2850.00	1726.13	421.17	2290.00	1591.56	7.80			

- T50: Time to reach 50% germination, FGP: Final germination percentage, CL: Coleoptile length, SL: Shoot length, MRL: Maximum root length, TRL: Total root length, RN: Root number, SDW: Shoot dry weight, RDW: Root dry weight, TPB: Total plant biomass, RSL: Root to shoot length, RSW: Root to shoot weight, SVI<sub>w</sub>: Seedling vigor index based on seedling weight, SVI<sub>L</sub>: Seedling vigor index based on seedling length.

- \*, \*\* and \*\*\*: Significant difference at 0.5, 0.01 and 0.001 level respectively.

- T: Type, Trt: Treatment.

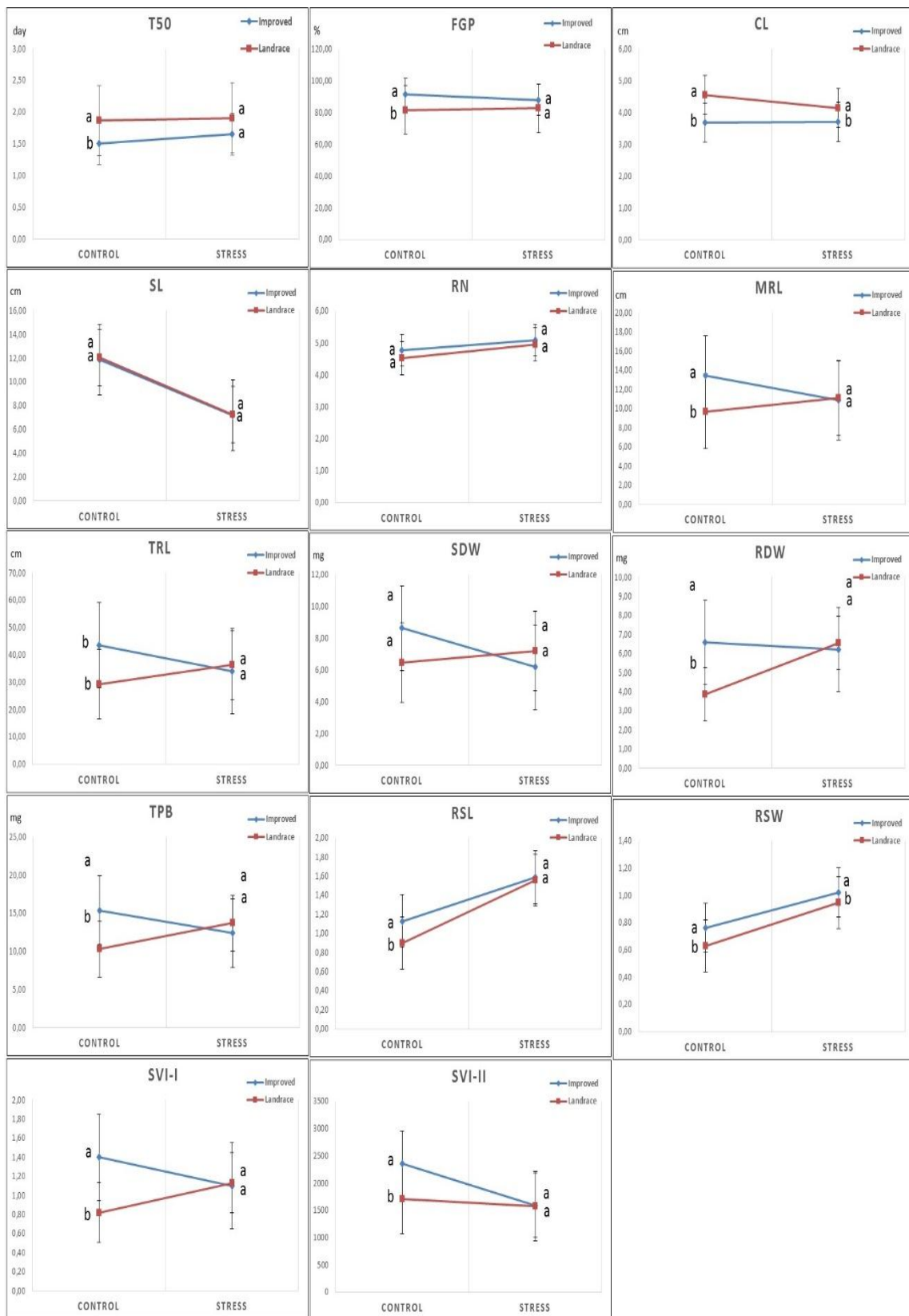


Fig. 1. Seedling traits variation across stress and control conditions for landraces (red line) and improved genotypes (blue line) (Bars represent  $\pm$  standard error).

TABLE 7. Pearson correlation coefficients of the assessed seedling traits under non-stress (below diagonal) and stress conditions (-6 bars) (above diagonal) and between the two conditions (cells with gray color).

	Stress													
	t50	FGP	SL	CL	RN	TRL	MRL	SDW	RDW	RSW	RSL	TPB	SVI <sub>w</sub>	SVI <sub>L</sub>
t50	0.45***	-0.51	-0.24	-0.27	-0.02	-0.31	-0.36	-0.16	-0.22	-0.03	-0.03	-0.20	-0.39	-0.46
FGP	-0.53	0.50***	0.21	0.23	0.12	0.27	0.43	0.11	0.22	0.07	0.09	0.17	0.62	0.73
SL	-0.06	0.06	-0.02	0.37	0.35	0.69	0.70	0.79	0.58	-0.57	-0.65	0.75	0.67	0.74
CL	0.08	0.05	-0.06	0.42***	0.32	0.40	0.34	0.40	0.19	-0.42	-0.24	0.34	0.39	0.37
RN	-0.10	0.02	0.40	-0.34	-0.13	0.40	0.25	0.40	0.52	-0.16	-0.43	0.49	0.40	0.25
TRL	-0.36	0.45	0.47	-0.35	0.53	0.07	0.86	0.64	0.76	-0.16	-0.14	0.74	0.71	0.73
MRL	-0.36	0.47	0.47	-0.28	0.43	0.96	0.15	0.56	0.62	-0.20	-0.04	0.63	0.71	0.89
SDW	-0.05	0.11	0.65	-0.41	0.53	0.70	0.68	-0.14	0.73	-0.68	-0.53	0.95	0.79	0.54
RDW	-0.34	0.35	0.39	-0.45	0.54	0.88	0.87	0.76	0.10	-0.04	-0.26	0.91	0.81	0.53
RSW	-0.39	0.31	-0.34	-0.16	0.13	0.34	0.34	-0.17	0.47	0.20	0.63	-0.43	-0.30	-0.25
RSL	-0.34	0.47	-0.24	-0.25	0.13	0.67	0.73	0.25	0.64	0.64	0.32**	-0.44	-0.27	-0.18
TPB	-0.20	0.24	0.57	-0.45	0.57	0.83	0.81	0.95	0.93	0.13	0.46	-0.20	0.85	0.57
SVI <sub>w</sub>	-0.35	0.52	0.48	-0.39	0.51	0.88	0.87	0.85	0.93	0.24	0.58	0.95	-0.07	0.81
SVI <sub>L</sub>	-0.45	0.68	0.61	-0.16	0.39	0.88	0.91	0.64	0.76	0.22	0.52	0.74	0.86	0.30**

- T50: Time to reach 50% germination, FGP: Final germination percentage, CL: Coleoptile length, SL: Shoot length, MRL: Maximum root length, TRL: Total root length, RN: Root number, SDW: Shoot dry weight, RDW: Root dry weight, TPB: Total plant biomass, RSL: Root to shoot length, RSW: Root to shoot weight, SVI<sub>w</sub>: Seedling vigor index based on seedling weight, SVI<sub>L</sub>: Seedling vigor index based on seedling length.

- \* , \*\* and \*\*\*: Significant difference at 0.5, 0.01 and 0.001 level, respectively.

- Values in bold and with asterisks are different from 0 with a significance level alpha=0.05. Cells with gray color are correlations between stress and non-stress for the same trait.

Correlation coefficients calculated between traits measured in field showed a high correlation of TPB with SDW (0.75), a positive moderate correlation between TPB and RDW, TRL and MRL (0.70, 0.52 and 0.50, respectively), and a weak correlation with RN (0.30). RDW was highly correlated with TRL (0.57), moderately correlated with RN, MRL (0.57, 0.53, respectively) and weakly correlated with SL (0.29), RSW (-29) and RSL (-35). CL presented strong correlation with SL (0.72) and a weak correlation with RN and TRL (0.42 and 0.34, respectively) (Suppl. Table 4).

The correlation between traits measured in the field and under controlled conditions showed low and non-significant correlations among traits, except a weak significant correlation was observed between field and stress for SDW (0.33) (Suppl. Table 5).

### **Discussion**

Drought stress at an early growth stage is a major limiting factor of wheat production in many parts of the world (Dhanda et al., 2004). Rebetzke et al. (2007) demonstrated that a good seedling emergence is important for achieving high wheat yields. Final germination percentage and time to reach 50% of germination are two important traits for plant establishment, especially under early drought conditions.

In this study, wheat genotypes behaved similarly under control and stress conditions for FGP but not for t50. The significant effect of treatment by genotype interaction in this last variable indicated that the genotypes responded differently across treatments, suggesting that the selection for this trait should be performed under target conditions (either under control or PEG stress), same as concluded by Abdel-Ghani et al. (2015).

Genotypes presenting a better FGP under stress were not necessarily the same genotypes having better t50 and vice versa. Only 4 (Bellouini, Capeiti, Gloire de Mongolfier and Miki-2) of the 9 most tolerant genotypes were considered tolerant for both FGP and t50, and these could be the best candidates to become drought-tolerant parents in a breeding program. Despite their results for other traits showed large variation, they could still have good breeding potential due to their ability to perform better under stress than under the control treatment. Gloire de Mongolfier could

be singled out as the most promising genotype when taking into account all its rankings. It was particularly good under PEG stress regarding biomass related traits like SDW, RDW, TPB and  $SVI_w$  (Suppl. Table 6), and was the fourth most tolerant genotype regarding DSI (Table 4). Many genotypes decreased their FGP and delayed their t50 under drought stress, as expected for PEG-induced drought, which is reported to affect seed germination by reducing water availability (Al-Karaki, 1998; Kaya et al., 2006). Conversely, some genotypes improved their FGP and t50 under drought stress, which could be explained by an already described osmo-priming effect of PEG (Al-Karaki, 1998; Kaya et al., 2006). Some varieties widely grown under Algerian conditions, like Waha and Vitron, were among the most susceptible cultivars based on FGP and t50, indicating room for improvement for these two traits.

No interactive effect was found for all seedling growth traits, wheat genotypes ranked similarly under control and stress conditions for all seedling traits. SL was the most sensitive to drought stress (reduction 40.26%) while CL was the least affected trait. Our results differ from those of Zarei et al. (2007), who found that root length was the most sensitive trait to drought stress induced by PEG in wheat.

In our experiment, genotypes tended to invest more resources in growing roots than shoots under stress conditions, compared to the control. Dhanda et al. (2004), in a similar study, found that root to shoot length ratio increased by 40% under stress conditions. In some cases, the absolute root biomass of plants in drying soil may increase relative to well-watered conditions (Sharp & Davies, 1985). The possible causes of increased root to shoot length ratio under water stress may be the limited supply of water and nutrients to the shoot, and changes in resource allocation due to changes in hormone messages induced in roots when they encounter drought stress (Davies & Zhang, 1991).

Heritability, trait range and coefficient of variation, all decreased under stress conditions for most traits, as also found by Dhanda et al. (2004), indicating a reduction of expression or variation under stress conditions. More gain from selection might be expected for FGP, t50, CL and RSL (under control conditions), for FGP and t50 (under stress conditions) and for RDW, RSW and CL (in the field).



Seedling vigor index based on either length or weight of seedling are useful traits as they are correlated with other seedling traits. Time to reach 50% of germination ( $t_{50}$ ) correlated negatively with other traits, reflecting the importance of faster germination rate, indicating that faster germinating genotypes will be more vigorous. Remarkably, under non-stress, CL displayed negative correlations with all traits whereas they were positive under stress conditions, except for RSL and RSW ratio. This finding indicates that plants with longer coleoptile tended to be more tolerant by promoting more biomass under stress, contrarily to plants with shorter coleoptiles, which were yielding more biomass under optimal conditions. In our study, most landraces were ranked ahead of modern ones for CL (Suppl. Table 6), also manifested as the significant higher CL mean observed in landraces (as a group). Furthermore, CL expresses consistently across treatments, suggesting that this trait could be a potential target for indirect selection under either condition. An advantage for its use in breeding is its high narrow-sense heritability, as found by Shahbazi et al. (2012). Genotypes with longer coleoptile are appropriate for deep sowing to reach soil moisture in semi-arid regions, something which was often avoided by growers of dwarfing gene cultivars (Rebetzke et al., 2007). Currently, alternative dwarfing genes (e.g. Rht8), which reduce plant height without affecting coleoptile length, are available for use in wheat breeding (Rebetzke et al., 2007).

A positive correlation was found between root length (total and maximum) and shoot length under both conditions, indicating that increase in root length will increase shoot length, and *vice versa*, confirming results reported by Kan et al. (2002) and Baalbaki et al. (1999). Based on the drought susceptibility index (DSI), genotypes could be clearly separated into landraces and modern cultivars, with landraces showing increased drought tolerance. Six widely grown Algerian landraces were listed among the most tolerant genotypes (Beliouni, Djenah Khoteifa, MBB, Bidi 17, Oued Znatie, and Guemgoum R'khem), which suggest their potential as donors of early drought tolerance. The importance of this difference, according to breeding history of the accessions, led us to focus on the comparison between landraces and improved cultivars, which is discussed next.

#### *Type effect*

One of the most interesting findings of this

study was the clear differences between landraces and improved genotypes for several traits (Fig. 1). Landraces had longer coleoptiles than improved cultivars, which is an advantageous trait for deep sowing practice. Ramshini et al. (2016) found that coleoptile length was significantly decreased in improved cultivars compared to old ones. They also found a significant difference between these two groups, with higher means observed in old cultivars for SL, RSL, SDW, TPB and  $SVI_1$ , where as shoot length was significantly higher in modern cultivars. This effect could be influenced by the use of semi-dwarf alleles in modern cultivars, which has been shown to reduce early growth root length (Wojciechowski et al., 2009). Other studies found an overall reduction of root size in modern cultivars, compared to landraces (Waines & Ehdaie, 2007). Some reports hypothesized that lower root to shoot ratio of improved cultivars early in the growing season may explain their increased harvest index, due to the reduced investment in root growth (Siddique et al., 1990). However, the optimum root size for grain yield has not been thoroughly investigated in wheat or most crop plants (Waines & Ehdaie, 2007).

For most other traits, improved cultivars showed higher values than landraces only under control conditions. Landraces seemed to be more tolerant than improved cultivars since they increased trait performances under stress, as confirmed by the DSI result (Table 4, Fig. 1). Several researches have already noted an outstanding performance of landraces. For instance, Ash et al. (2017) found that durum wheat variety Strong field produced only about half of the root biomass of the wheat landrace Pelissier, at maturity in greenhouse trials under well-watered conditions. Bektas et al. (2016) found that wheat landraces were superior for root biomass, shallow root weight, deep root weight, number of tillers and plant height compared to improved cultivars. Some Spanish barley landraces also outperformed modern cultivars under low site productions (Yahiaoui et al., 2014).

#### *Field conditions effect*

Closing the gap between field and controlled experiment conditions is a current trend which aims at extrapolating results obtained under artificial conditions to real (field) conditions. In this study, the ranges of variation and mean values of seedling traits in the field were less than what those observed under controlled conditions, except for SDW and TPB. This could be partly explained by



the effect of soil impedance, which hampers root growth, and the effect of temperature and humidity of the soil as well. This suggestion is supported by that the SDW values obtained in field were superior to under controlled conditions which may be explained by more space dedicated in field than in Petri dishes. Correlations established between traits in field and controlled experiment showed no interesting results and the two conditions of experiment were too different for all traits. The only weak correlation was found for SDW (Suppl. Table 5).

### **Conclusion and Perspective**

A good range of variation was observed for most seedling traits under controlled conditions, which could be useful in wheat breeding programs. Longer coleoptile length could be a potential trait for selection of drought tolerant genotypes especially at early growth stage in semi-arid environments, although pleiotropic effects on final shoot and root development and grain yield should be studied in parallel.

After these results, Algerian wheat landraces, which have been cultivated for a long time in the region, could be introduced in durum wheat breeding programs to breed for drought tolerance at the early growth stage. Some widely cultivated modern varieties were listed among the most susceptible genotypes like Waha, Vitron and Wahbi. These varieties, which already have good agronomic performance overall, could be further improved by enhancing their FGP and/or t50.

Further work is required to correlate root traits at seedling stage and root/agronomic traits at adult stage, to find proxy traits, which allow performing selection at early plant stage. Crosses between tolerant genotypes and susceptible genotypes identified in this study can generate populations appropriate for QTL mapping to identify genomic regions related to interesting seedling traits, and with good breeding potential.

*Acknowledgment:* We would like to thank Ali Debbi, researcher at Research Center for Biotechnology (CRBT), for providing equipments for the realization of the experiment. Funding from the project AGL2016- 80967-R of the Spanish Ministry of Science and Innovation for paper publication.

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### تقييم التحمل المبكر للجفاف لسلاسلات من قمح الديورم الجزائرية

قوة نمو البادرات مهمه للحصول على محصول جيد، خاصة تحت ظروف الجفاف. لذلك تم إجراء هذه الدراسة لتحديد صفات وتراكيب وراثية مفيدة في تعزيز التحمل المبكر للجفاف في قمح المكرونة (القمح الصلب). تم إجراء تجربتين، التجربة الأولى تمت في الصوبة لإختبار 35 تركيب وراثي (أصناف قديمه وحديثه) في مرحلة الإنبات والمراحل المبكرة للبادره، حيث تم تعريض التركيب الوراثية لثلاثة مستويات من الإجهاد الأسموزي استحدثت باستخدام مادة البولي إيثيلين جليكول (PEG 6000) بتركيزات؛ صفر، -3 و -6 بار. أما التجربة الثانية تم إجراءها في الحقل باستخدام 27 تركيب وراثي من الخمسة و الثلاثين. في كلتا التجربتين تم تقدير صفات الجذر والسويقة في عمر البادره.

أظهرت النتائج أن المعاملة العالية من البولي إيثيلين جليكول أدت إلى إنخفاض نسبة الإنبات النهائي بنسبة 2.7% وكذلك تأخر وقت الوصول إلى 50% من الإنبات 2.9 ساعة. وكانت صفة طول السويقة (Shoot length) الأكثر تأثراً بالجفاف (إنخفضت بنسبة 40%) على عكس بعض صفات الجذر التي زادت حتى تحت الجفاف، مثل نسبة طول الجذر إلى طول السويقة، نسبة وزن الجذر إلى وزن السويقة، الوزن الجاف للجذر، وعدد الجذور. وأظهرت صفة طول غمد الريشة (Coleoptile length) علاقة متغيرة مع الصفات الأخرى، حيث كان مرتبطاً سلبياً بشكل عام تحت عدم الإجهاد، ولكن كان مرتبطاً إيجابياً تحت الإجهاد. بناءً على دليل الحساسية للجفاف، كانت أصناف القمح الجزائرية القديمة أكثر تحملاً للجفاف مقارنة بالأصناف الحديثة. الارتباط بين الصفات التي تم تقديرها تحت ظروف الحقل والصوبة كان منخفض. صفة طول غمد الريشة (Coleoptile length) يمكن أن تكون صفة مهمه لتحديد التركيب الوراثية المحتملة للجفاف. أصناف القمح الجزائرية القديمة أظهرت قدرة مميزة وبشكل واضح على تحمل الجفاف المبكر، لذلك يمكن أن تكون مصدر جيداً لبرامج التربية.