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SCREENING OF SEEDLINGS OF DURUM WHEAT (*TRITICUM DURUM* DESF.) CULTIVARS FOR TOLERANCE TO PEG-INDUCED DROUGHT STRESS

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Abstract

The effect of drought stress on 11 durum wheat (*Triticum durum* Desf.) cultivars was determined at the germination stage. Cultivars were screened for drought tolerance. Six levels of osmotic stress (0, -0.47, -1.48, -3.02, -5.11 and -7.73 bars) were assessed by applying different concentrations of polyethylene glycol (PEG-8000). There were significant differences between treatments for all seedling characteristics ($p<0.05$, $p<0.001$), except mean daily germination (MDG). All seedling traits also differed significantly ($p<0.001$) among all cultivars. In general, osmotic stress decreased seed germination percentage, germination rate (GR), coleoptile length (CL), shoot length (SL), root length (RL), root/shoot (R/S) length ratio, and root number (RN). Averaged over all osmotic stress levels, Mahmoudi had high MDG (0.55), GR (1.88), CL (4.20 cm), SL (10.45 cm), and RL (9.93 cm), suggesting that this variety was highly tolerant to drought stress. There were high correlation coefficients between different characteristics: SL had a positive and significant ($p<0.01$) correlation with CL (r = 0.83), RL (r = 0.74), and R/S length ratio (r = 0.67). This study showed that, based on morphological traits, preliminary screening at an early stage for drought stress using PEG-8000 may facilitate the choice of an adequate cultivar for growth under water stressed conditions.

Key words: Drought tolerance, Durum wheat, Germination, Osmotic stress.

Abbreviations: DGS, daily germination speed; GP, germination percentage; GR, germination rate; MDG, mean day germination

Introduction

In agricultural production, environmental stresses play a significant limiting role (Ghader, 2014). Abiotic stresses present major challenges in sustaining crop yield (Chen *et al*., 2014). Salinity and drought are two common environmental stresses that affect seed germination and plant growth, especially in arid and semi-arid regions (Mohammadizad *et al*., 2013; Masondo *et al*., 2018). Drought stress decreases crop production while water deficiency reduces plant growth and productivity (Castilhos *et al*., 2014; Gilani *et al.*, 2020). Among different stress factors, drought is ranked first, limiting three quarter, or 454 million ha, of global crop production (Kim *et al*., 2019). In Tunisia, where a gradient in severity and frequency of drought exists from north to south, yield fluctuates considerably and is extremely low in dry years. Yield is also strongly associated with the amount of rainfall (Khakwani *et al.*, 2012; Mansour & Hachicha, 2014). In addition, most cereal cultivation lands are in the northern to north-western areas where the climate varies from semiarid to dry sub-humid (Ferjaoui *et al*., 2014). The national average yield of durum wheat is 14 ± 4 qx/ha while that of bread wheat is 16 ± 5 qx/ha, but yield is higher in the north of Tunisia (18.4 ± 3.8 qx/ha) than at the center and south (11.3 ± 4.7 qx/ha). This variation caused by bioclimatic and farming conditions that are more favorable in the north than in the center and the south of the country (Annabi *et al.*, 2013).

New challenges in modern agriculture include rapid population growth, climate change and the deterioration of arable lands, and enhanced agriculture is vital to face global food demand with stress-tolerant crops present a promising solution (Chen *et al*., 2014; Ulfat *et al.*, 2017). The development and release of new varieties that adapt to water deficit conditions could be a constructive way to surmount unsuitable environmental conditions. A good understanding of factors limiting yield provides an opportunity to identify physiological traits that could increase drought tolerance and yield under rainfed conditions. Drought tolerance can be assessed in crops by using physiological and agro-morphological tests, which serve as indirect selection criteria, thereby accelerating selection methods and hopefully resulting in cultivars with increased yield and productivity under drought-stressed climates (Ahmadizadeh, 2013).

The response of wheat cultivars to drought stress has been examined extensively because soil drought represents the main constraint for successful crop production. Plants can readily modify their metabolic and physiological processes, as well as the morphology of the above-ground parts and the root system in response to water deficit (Gallé *et al*., 2013; Castilhos *et al*., 2014). Seed germination is the most sensitive stage to abiotic stress (Jian *et al*., 2016) and involves multiple morphological and physiological alterations take place during germination. In unfavorable conditions, seeds may enter secondary dormancy to sustain their ability to germinate, and when conditions are favorable, such seeds are able to germinate. Under semi-arid conditions, low humidity is a limiting factor during germination (Ahmadizadeh, 2013; Hafeez *et al*., 2017; Li *et al*., 2018). Thus, there is a need to screen drought-tolerant genotypes in these areas (Mickky & Aldesuquy, 2017). Coleoptile length is an important factor controlling the emergence of seedlings from deep sowing depths (Prabhakar *et al*., 2013; Lee *et al*., 2017). Seedling emergence is highly sensitive to water deficit and early drought hinders successful crop establishment by negatively affecting seedling emergence (Shahbazi *et al*., 2012; Hellal *et al*., 2018). Toklu *et al*., (2015) noted that seedling growth, coleoptile length and seed germination are fundamentals for successful stand establishment of yield plants. Studying the effects of water stress on the growth and development of roots and shoots also has merit as water stress is the most important abiotic constraint to increase grain yield in rainfed wheat-growing areas. Geneticists and breeders also breed plants with root traits that improve productivity under drought (Comas *et al*., 2013; Shan *et al*., 2015). Wheat plants experience drought stress when the water supply to roots becomes too little to support growth or when transpiration becomes very high due to wind and temperature (Bin Abdul Hamid, 2012). Germination and seedling characteristics such as germination percentage, germination rate, and seedling growth are the most viable criteria used for selecting drought tolerance in crops at the seedling stage (Jabbari *et al*., 2013; Basha *et al*., 2015). The genotypic ability for a root/shoot length ratio contributes to drought tolerance, which is genetically determined (Khan *et al*., 2020). The efficiency of soil water uptake by the root system is thus a key factor in determining the rate of transpiration and drought tolerance (Ahmadizadeh, 2013; Tardieu *et al*., 2017). Roots capture water and nutrients, besides anchoring the plant in the ground, and is logically seen as the most important organ to improve crop adaptation to water deficit (Vadez, 2014).

The germination test in a high osmotic potential solution is one of the most important laboratory methods to screen the drought tolerance of a crop plant (Ahmadizadeh, 2013). At an early seedling stage, polyethylene glycol (PEG) is used to induce plant water deficit (Muscolo *et al*., 2014). PEG is a non-toxic and non-penetrable osmotic agent that lowers water potential in mediumthat is employed to imitate drought stress in plants (Toosi *et al*., 2014). PEG-6000 has often been used to induce water deficit and preserve uniform water potential through an experimental period (El Siddig *et al*., 2013). Seed germination as a function of water potential is often tested with soil adjusted to desired water potentials or by using PEG solutions (Prabhakar *et al*., 2013).

In view of the importance of the germination phase and early plant formation under drought conditions, the present study was carried out to screen Tunisian durum wheat cultivars for drought tolerance by inducing water stress by applying PEG at the germination and seedling growth stages, and to quantify the association between drought-associated seedling traits.

Materials and Methods

Plant materials: Grains of 11 durum wheat (*Triticum durum* Desf.) cultivars (Maâli, Mahmoudi, Om rabiaa, Karim, Nasr, Salim, Maghrbi, Ben bechir, Souri, Agiliglabre, and Jnehkottifa) were obtained from the Genetic Laboratory of Cereal Crops, Agronomic Institute of Tunisia. Kernels were initially surface sterilized with 12% sodium hypochlorite for 5 min and then rinsed twice with distilled water for 5 min each rinse. Ten grains of each cultivar were placed in 90-mm diameter plastic Petri dishes (Amazon, Orléans, France) on a single sheet of filter paper (90-mm diameter; Fioroni, Paris, France). Petri dishes (Sterilin Ltd., Cambridge, UK) were covered with lids to prevent the loss of moisture by evaporation.

Grains were germinated under controlled conditions in a dark growth room for 10 days, at 50% relative humidity and at an average day/night temperature of 22 ± 2°C (El Siddig *et al*., 2013). Seeds were considered to be germinated when the emerging radicle had protruded 5 mm, at which point germinated seeds were removed (Prabhakar *et al*., 2013).

Polyethylene glycol solutions: PEG (PEG-8000, molecular weight 8000 g mol^{-1}, > 99.0% purity; Sigma-Aldrich, St. Louis, USA) aqueous solutions with different water potentials (-0.47, -1.48, -3.02, -5.11 and -7.73 bars) were established by dissolving 50, 100, 150, 200 and 250 g of PEG in 1000 ml of distilled water. The control was distilled water (0 bars). Water potential (Ψ) was calculated by an equation that relates PEG concentration to water potential (Michel, 1983):

$$\Psi = 1.29\,[PEG]\,[PEG]^2T - 140\,[PEG]^2 - 4\,[PEG]$$

where Ψ is the water potential of each treatment (bars), [PEG] is the concentration of PEG solution [g PEG (g H_2O) $^{-1}$] and T is temperature (°C).

Five ml of each concentration of PEG solution, or distilled water for the control, was added after every 2 days to a separate Petri dish to maintain the required water potentials of each treatment constant.

Measurements: After 10 days of treatment, coleoptile length (CL) was assessed by measuring the distance from the grain to the tip of the coleoptile in each replicate for all 10 seedlings (Prabhakar *et al.*, 2013). Shoot length (SL), root length (RL), ratio of root/shoot (R/S) length, and root number (RN) were determined from five samples in each of the six humidity levels (five water potentials plus one control) from each replication, based on established germination protocols (Moayedi *et al.*, 2009; Mohammadizad *et al.*, 2013). In addition, the following parameters were assessed daily: number of germinated seeds, germination percentage (GP), mean daily germination (MDG; i.e., the mean number of seeds that germinated each day), daily germination speed (DGS), and germination rate (GR). These parameters were calculated as follows:

GP = (total number of germinated grains / total number of observed grains) × 100;
MDG = final emergence/10 (Jajarmi, 2008);
DGS = 1/MDG (Maguire, 1962);
GR = (n1× t1) + (n2 × t2) + (n3 × t3) + ... (ni × ti)/T (Olmez *et al.*, 2006):

where n = number of days in which grains germinated; t = number of germinated grains in each counting day; T = total number of germinated grains.

Statistical analysis of data: A completely randomized design was performed in a factorial experiment with three repetitions. The factors were durum wheat cultivars and water potential. Analysis of variance (ANOVA) was conducted for seed germination and growth measurements' traits. The treatment means were compared by Duncan’s multiple range test (p<0.05, p<0.01 and p<0.001) using SPSS software (version 16.0).

Results and Discussion

Emergence and related traits seed germination: The germination percentage of 11 durum wheat cultivars as a function of time under six water potentials is shown in Table 1 (on days 7, 8, 9 and 10, columns are vacant as no seeds germinated further on those days). Water potential and cultivar had a significant effect on seed germination. Abido & Zsombik (2018) also noted that water potential affected the GP of seven Hungarian wheat varieties. Shereen *et al*., (2019) found that GP was significantly reduced in eight rice genotypes as water stress levels increased. Relative to the control, germination decreased as water potential with the increase of water. Also, in this study, water stress delayed and prevented seed germination. Water stress at the germination stage could result in the delay, decrease or even complete prevention of germination (Moral *et al.*, 2015).

Differences in seed germination among cultivars were observed. This genetic variation could be used to screen new wheat varieties adapted to unfavorable conditions in arid and semi-arid regions (Chachar *et al.*, 2014). Om rabiaa showed significantly higher seed germination (96.11%) than other cultivars under most water potentials. Jnehkottifa had the lowest seed germination (42.78%). Except for Maâli, Souri, Agiliglabre and Jnehkottifa, all other cultivars showed more than 80% seed germination at -5.11 bars. However, at -7.73 bars, only Om rabiaa and Nasr reached this range, indicating that the critical water potential for these cultivars lies between -5.11 and -7.73 bars. Nevertheless, some cultivars did not show any decrease in seed germination over all osmotic stress levels (Table 1). These results corroborate those of Baloch *et al*., (2012), in which more than half of 16 spring wheat cultivars grown under osmotic stress did not show any reduction in seed germination even though GP alone was not able to assess osmotic stress tolerance since it could not clearly discriminate the 16 cultivars.

Mean daily germination, daily germination speed and germination rate: There was a significant effect of water potential on MDG and GR (Table 2). Prabhakar *et al*., (2013) also observed that water potential influenced GR. Based on mean values, maximum GR and DGS were observed in the control (0 bars). GR is significantly reduced when osmotic stress increases (Ezzat-Ahmadi *et al.*, 2014). In contrast, osmotic stress caused an increase in MDG. Abdoli & Saeidi (2012) also found that stress caused by water deficiency augmented MDG of nine wheat cultivars from 1.91 to 2.05 days. In 96 wheat genotypes, Dodig *et al*., (2014) showed that MDG increased after exposure to PEG-induced osmotic stress, delaying MDG by 14 days when osmotic potential was -0.4 MPa compared to the control treatment (distilled water). Ahmad *et al*. (2017) claimed that such a response is due to more time being required to germinate under drought stress. There was also considerable variation in MDG, DGS and GR between cultivars (Table 2). Mujtaba *et al*., (2016) found highly significant differences among six wheat genotypes for the same traits. Om rabiaa, which is considered to be tolerant to drought (Brini *et al*., 2007), had the highest MDG and GR but a low DGS. Comparing the two new cultivars (Mâali and Salim), Salim had significantly higher MDG and GR than Mâali (Table 3).

Seedling growth

Coleoptile length: The differences among water potential treatments and among cultivars for CL were highly significant, and there was a highly significant interaction between water potential and cultivars (Table 4). Prabhakar *et al*., (2013) reported that CL could affect seed emergence since it was linked to emergence capacity from deep sowing depths, accounting for 60% of the differences between varieties in their study. High genetic variation was observed in CL. Numerous studies have reported an association between long coleoptiles and increased seedling emergence, weed restraint and improved grain yield (Farhad *et al*., 2014). The shortest CL was observed at the highest water potential (-7.73 bars). Mahmoudi had significantly longest coleoptiles among all cultivars (Table 5). The tested cultivars responded differently to osmotic stress (Table 5). Compared to normal conditions, CL was increased in Maâli, Mahmoudi and Maghrbi at -0.47, -1.48 and -3.02 bars, in Karim, Salim, Agiliglabre and Ben Bechir at -0.47 bars, in Nasr and Souri at -0.47 and -1.48 bars, and in Jnehkottifa under high stress levels (-3.02 and -5.11 bars). Farhad *et al*., (2014) reported that water deficit could increase CL.

Table 1. Total seed germination (%) of 11 Tunisian durum wheat cultivars at different water potentials.

Water potential (bars)	Cultivar	Days								
		2	3	4	5	6	7	8	9	10
0	Mâali	53	67	73	80	83	nf	nf	nf	nf
	Mahmoudi	67	83	93	97	97	nf	nf	nf	nf
	Om rabiaa	73	100	100	100	100	nf	nf	nf	nf
	Karim	67	87	90	90	90	nf	nf	nf	nf
	Nasr	40	90	97	97	97	nf	nf	nf	nf
	Salim	33	97	97	97	97	nf	nf	nf	nf
	Maghrbi	63	87	90	93	93	nf	nf	nf	nf
	Ben Bechir	73	80	83	87	87	nf	nf	nf	nf
	Souri	47	67	70	73	73	nf	nf	nf	nf
	Agiliglabre	3	30	40	40	47	nf	nf	nf	nf
	JnehKottifa	0	37	43	43	53	nf	nf	nf	nf
-0.47	Mâali	73	73	77	80	80	nf	nf	nf	nf
	Mahmoudi	90	90	93	97	97	nf	nf	nf	nf
	Om rabiaa	93	97	100	100	100	nf	nf	nf	nf
	Karim	73	90	90	90	90	nf	nf	nf	nf
	Nasr	97	97	97	97	97	nf	nf	nf	nf
	Salim	100	100	100	100	100	nf	nf	nf	nf
	Maghrbi	87	90	90	90	90	nf	nf	nf	nf
	Ben Bechir	47	63	63	63	63	nf	nf	nf	nf
	Souri	47	67	67	67	67	nf	nf	nf	nf
	Agiliglabre	7	57	60	63	63	nf	nf	nf	nf
	JnehKottifa	13	40	43	43	43	nf	nf	nf	nf
-1.48	Mâali	73	77	77	77	77	nf	nf	nf	nf
	Mahmoudi	83	83	87	93	93	nf	nf	nf	nf
	Om rabiaa	83	93	93	93	93	nf	nf	nf	nf
	Karim	90	100	100	100	100	nf	nf	nf	nf
	Nasr	67	97	97	97	97	nf	nf	nf	nf
	Salim	67	97	100	100	100	nf	nf	nf	nf
	Maghrbi	73	77	83	83	83	nf	nf	nf	nf
	Ben Bechir	77	77	77	77	77	nf	nf	nf	nf
	Souri	33	47	50	50	50	nf	nf	nf	nf
	Agiliglabre	7	23	30	30	30	nf	nf	nf	nf
	JnehKottifa	7	30	40	40	40	nf	nf	nf	nf

Table 1. (Cont'd.).

Water potential (bars)	Cultivar	Days								
		2	3	4	5	6	7	8	9	10
-3.02	Mâali	57	67	73	73	73	nf	nf	nf	nf
	Mahmoudi	63	73	83	90	93	nf	nf	nf	nf
	Om rabiaa	97	100	100	100	100	nf	nf	nf	nf
	Karim	83	87	87	90	90	nf	nf	nf	nf
	Nasr	97	97	97	97	97	nf	nf	nf	nf
	Salim	83	93	93	93	93	nf	nf	nf	nf
	Maghrbi	83	90	90	90	90	nf	nf	nf	nf
	Ben Bechir	67	70	70	70	70	nf	nf	nf	nf
	Souri	33	40	40	40	40	nf	nf	nf	nf
	Agiliglabre	13	30	37	43	43	nf	nf	nf	nf
	JnehKottifa	17	33	33	33	33	nf	nf	nf	nf
-5.11	Mâali	43	63	67	70	73	73	73	nf	nf
	Mahmoudi	73	87	97	100	100	100	100	nf	nf
	Om rabiaa	20	63	77	80	90	90	90	nf	nf
	Karim	40	67	87	87	93	93	97	nf	nf
	Nasr	40	67	87	87	93	93	97	nf	nf
	Salim	10	67	80	90	90	93	93	nf	nf
	Maghrbi	0	47	60	80	90	93	93	nf	nf
	Ben Bechir	50	83	90	90	93	93	93	nf	nf
	Souri	47	63	63	63	63	63	63	nf	nf
	Agiliglabre	0	17	33	33	43	47	47	nf	nf
	JnehKottifa	0	27	30	40	40	43	43	nf	nf
-7.73	Mâali	10	27	37	40	50	53	53	nf	nf
	Mahmoudi	27	53	57	60	63	70	77	nf	nf
	Om rabiaa	0	33	67	70	90	90	93	nf	nf
	Karim	3	33	50	67	70	70	70	nf	nf
	Nasr	7	47	60	67	80	83	83	nf	nf
	Salim	0	17	23	37	43	50	50	nf	nf
	Maghrbi	0	27	30	40	53	60	60	nf	nf
	Ben Bechir	13	37	40	43	50	53	53	nf	nf
	Souri	0	30	33	43	43	43	43	nf	nf
	Agiliglabre	0	13	33	37	43	47	47	nf	nf
	JnehKottifa	0	13	30	37	40	43	43	nf	nf
[1] DMRT	Ψ	136.82^{***}	74.51^{***}	43.30^{***}	28.44^{***}	17.15^{***}	492.95^{***}	497.35^{***}		
	Variety	60.30^{***}	47.69^{***}	41.98^{***}	39.95^{***}	39.58^{***}	6.01^{***}	6.54^{***}		

[1] Duncan's multiple range test (DMRT) was conducted for each day among water potentials (Ψ) and varieties
[2] nf: no further germination
[3] Level of significance: * p<0.05; *** p<0.001

Table 2. Analysis of variance (F value) of mean day germination (MDG), daily germination speed (DGS) and germination rate (GR) of 11 durum wheat cultivars for six water potentials.

Sources \ Variance	Df	MDG	DGS	GR
Water potential	5	3.06^{*}	1.93	14.86^{***}
Cultivar	10	10.93^{***}	3.91^{***}	12.03^{***}
Water potential× cultivar	50	1.13	0.71	0.96

Level of significance: * p<0.05; ***p<0.001

Table 3. Mean day germination (MDG), daily germination speed (DGS) and germination rate (GR) of 11 durum wheat cultivars for six water potentials.

		Source of variance		
		MDG	DGS	GR
Water potential (bars)	0	0.09 b	1.81 a	2.06 a
	-0.47	0.36 a	1.21 ab	1.39 bc
	-1.48	0.30 a	1.75 ab	1.30 c
	-3.02	0.36 a	1.12 b	1.66 b
	-5.11	0.39 a	1.21 ab	2.15 a
	-7.73	0.28 a	1.37 ab	1.21 c
Varieties	Mâali	0.00 d	1.16 c	1.50 bc
	Mahmoudi	0.55 a	1.00 c	1.88 ab
	Om rabiaa	0.66 a	1.00 c	2.22 a
	Karim	0.44 ab	1.00 c	1.88 ab
	Nasr	0.61 a	1.00 c	2.16 a
	Salim	0.61 a	1.50 bc	2.00 a
	Maghrbi	0.27 bc	1.11 c	1.94 a
	Ben bechir	0.11 cd	1.16 c	1.27 cd
	Souri	0.00 d	1.72 bc	0.83 e
	Agiliglabre	0.00 d	2.27 ab	1.27 cd
	Jnehkottifa	0.00 d	2.70 a	0.94 de

Means with similar letter(s) in each trait are not significantly different at p<0.05 (Duncan's multiple range test)

Table 4. Analysis of variance (F value) of shoot and root-related characters of 11 durum wheat cultivars for six water potentials.

Sources \ Variance	df	CL (cm)	SL (cm)	RL (cm)	R/S length ratio	RN
Water potential	5	374.74^{***}	420.17^{***}	165.54^{***}	84.27^{***}	134.94^{***}
Cultivar	10	49.76^{***}	18.68^{***}	9.98^{***}	5.25^{***}	3.39^{***}
Water potential× cultivar	50	8.37^{***}	3.85^{***}	2.77^{***}	1.32	4.05^{***}

CL, coleoptile length; SL, shoot length; RL, root length; R/S, root/shoot length ratio; RN, root number

Table 5. The response of five growth parameters of 11 durum wheat cultivars to six water potentials.

Variance sources		CL (cm)	SL (cm)	RL (cm)	R/S length ratio	RN
Water potential (bars)	0	3.38 a	10.44b	11.06 a	1.30 a	5.59 a
	-0.47	3.49 a	11.17 a	10.31 a	0.93b	5.47 ab
	-1.48	3.42 a	10.59 ab	10.61 a	1.01b	5.72 a
	-3.02	3.31 a	8.66c	6.68c	0.64c	5.41b
	-5.11	2.49b	4.78d	7.46b	1.22 a	5.01c
	-7.73	0.54c	0.00e	2.45d	0.00d	2.78d
Cultivars	Mâali	2.45de	6.86def	7.90c	0.96b	5.21 abc
	Mahmoudi	4.20 a	10.45 a	9.93 a	0.77bcd	4.76de
	Om rabiaa	2.72c	7.16cde	8.23c	0.93b	5.01bcde
	Karim	2.59cd	7.33cde	7.98c	0.89bc	4.94bcde
	Nasr	2.10f	6.12f	7.98c	0.93b	5.27 ab
	Salim	2.43de	6.47ef	7.59c	0.77bcd	4.62e
	Maghrbi	2.28ef	6.92def	9.38 ab	1.19 a	4.85cde
	Ben bechir	2.60cd	7.42cd	8.58bc	0.93b	5.08 abcd
	Souri	2.95b	9.24b	8.30c	0.70cd	5.43 a
	Agiliglabre	2.99b	7.94c	7.56c	0.70cd	4.85cde
	Jnehkottifa	3.14b	7.76cd	5.63d	0.61d	4.89bcde

Means with similar letter(s) in each trait are not significantly different at p<0.05 (Duncan's multiple range test). CL, coleoptile length; SL, shoot length; RL, root length; R/S, root/shoot length ratio; RN, root number

Table 6. Interaction effect of osmotic stress and cultivars on seedling traits in 11 durum wheat cultivars.

Cultivars	Water potential (bars)	CL	SL	RL	RN
Mâali	0	2.54	9.04	11.41	6.07
	-0.47	3.11	10.08	8.99	5.60
	-1.48	3.25	8.80	10.67	5.80
	-3.02	3.06	8.04	6.54	5.60
	-5.11	2.40	5.22	7.52	5.13
	-7.73	0.39	0.00	2.28	3.07
Mahmoudi	0	5.21	15.49	13.99	5.27
	-0.47	5.34	14.66	11.69	5.00
	-1.48	5.51	13.20	14.64	5.87
	-3.02	5.65	11.92	8.91	5.27
	-5.11	2.59	7.48	7.08	5.00
	-7.73	0.95	0.00	3.30	2.20
Om rabiaa	0	3.78	10.63	11.13	5.67
	-0.47	3.61	11.21	11.05	5.47
	-1.48	3.57	9.67	10.73	5.47
	-3.02	3.31	8.47	7.89	5.47
	-5.11	1.73	2.99	6.87	5.40
	-7.73	0.34	0.00	1.75	2.60
Karim	0	3.48	10.19	11.20	6.00
	-0.47	3.47	11.24	10.17	5.00
	-1.48	3.45	9.73	9.34	5.87
	-3.02	3.32	9.39	7.98	5.53
	-5.11	1.65	3.45	7.09	5.20
	-7.73	0.18	0.00	2.16	2.07
Nasr	0	2.89	9.55	10.29	6.93
	-0.47	2.92	10.38	11.74	6.47
	-1.48	2.93	9.33	9.35	7.07
	-3.02	2.41	6.45	7.21	5.80
	-5.11	1.23	1.03	7.35	3.80
	-7.73	0.22	0.00	1.97	1.60
Salim	0	3.25	10.97	8.15	5.13
	-0.47	3.28	10.93	11.56	5.13
	-1.48	3.33	9.47	11.02	5.73
	-3.02	2.79	6.36	8.21	5.73
	-5.11	1.77	1.10	5.49	4.53
	-7.73	0.18	0.00	1.14	1.47
Maghrbi	0	2.72	9.27	12.36	5.27
	-0.47	3.06	9.30	11.74	5.60
	-1.48	3.06	10.84	12.55	5.40
	-3.02	2.73	7.82	8.93	4.80
	-5.11	2.04	4.35	9.17	4.73
	-7.73	0.11	0.00	1.54	3.36
Ben bechir	0	2.87	9.41	12.35	5.20
	-0.47	3.04	9.76	11.50	5.13
	-1.48	2.77	10.71	12.25	5.53
	-3.02	2.66	9.33	6.14	5.33
	-5.11	3.79	5.37	7.03	5.33
	-7.73	0.48	0.00	2.24	3.95
Souri	0	3.59	9.95	10.67	6.20
	-0.47	3.64	11.89	8.93	6.20
	-1.48	3.69	12.85	10.77	6.23
	-3.02	3.19	8.73	4.65	5.32
	-5.11	2.46	6.07	4.34	5.16
	-7.73	1.18	0.00	3.79	3.53
Agiliglabre	0	3.46	10.91	11.07	5.49
	-0.47	3.77	12.29	9.80	5.93
	-1.48	3.08	10.49	8.78	5.36
	-3.02	3.86	9.52	4.31	4.87
	-5.11	2.85	4.43	7.13	4.76
	-7.73	0.97	0.00	4.32	2.72
Jnehkottifa	0	3.41	9.52	9.08	4.28
	-0.47	3.16	11.20	6.32	4.67
	-1.48	3.01	11.45	6.64	4.60
	-3.02	3.43	9.30	2.79	5.78
	-5.11	4.91	5.11	6.41	6.07
	-7.73	0.94	0.00	2.57	4.00

CL, coleoptile length; SL, shoot length; RL, root length; RN, root number

Shoot length, root length, root/shoot length ratio and root number: The results of ANOVA for SL, RL, R/S length ratio and RN are described in Table 4. For all these traits there were highly significant differences among water potentials and among cultivars. Except for R/S length ratio, there was a significant interaction between osmotic stress levels and cultivars (Table 5). Water deficit significantly affected root-related traits. Adda *et al*., (2005) and Faisal *et al*., (2017) also showed a significant effect of water stress on durum and bread wheat root characters.

Early and rapid elongation of roots is an important indicator of drought resistance (El Siddig *et al*., 2013). RL, SL and RN decreased as water potential increased (Table 4). Rana *et al*., (2017) reported a decrease in RL and SL, which was an obstacle to cell division in shoot and root elongation, and to seed reserve utilization. RL increased in Nasr at -0.47 bars, in Maâli, Mahmoudi, Souri and Maghrbi at -1.48 bars, and in Salim under both osmotic stress levels, -0.47 and -1.48 bars. Baloch *et al*. (2012) found elongated roots in wheat cultivars under drought stress. Except for Om rabiaa, low and moderate osmotic stress (> -7.73 bars) enhanced RN in different cultivars. Moreover, most cultivars displayed an increase in SL when PEG was applied at 50 and 100 g/L, which might be due to an increase in RL. Compared to shoots, the physiological activities of roots are less sensitive to water deficit (Ahmad *et al*., 2017).

The varying response of genotypes to PEG treatment, due to differential genetic sensitivity to water deficit, is very important for plant breeders as drought-tolerant genotypes can be screened and tagged at the seedling stage without extensive and expensive field trials (Meher *et al.*, 2017; Rana *et al.*, 2017). Durum wheat genotypes forming longer roots under water limitation exhibit an adaptive response by increasing water uptake capacity by seeds. There is little evidence to support that drought-tolerant cultivars uniformly display advantageous traits such denser shoot and root dry matter, and longer shoot and root lengths under water stress (Bin Abdul Hamid, 2012).

Many plants respond to drought by increasing the proportion of assimilate diverted to growth and thus, increase the S/R ratio and the volume of soil water available to the plant (Riaz *et al*., 2013; Ahmad *et al*., 2017). The simple supposition is that deeper and more abundant root systems can tap extra water from the soil profile, thus moderatingthe effects of drought (Vadez, 2014). Based on means values of all cultivars (Table 5), the R/S length ratio was high in the control (0 bars) with no significant differences in length under stress (-5.11 bars) and there was no interaction between water potential and cultivars (Table 6). The higher R/S length ratio under water stress may be attributed to longer roots under stress, probably due to the induction of root-to-shoot hormonal signaling while the root system is subjected to drought stress (Bin Abdul Hamid, 2012). It could also be associated with higher dry matter and soluble sugar content in roots, due to an increase in enzyme activity (Xu *et al.*, 2015).

Mahmoudiformed the longest shoots and roots but had a low R/S length ratio and RN (Table 5). Root traits associated with sustaining plant productivity under drought include small roots with fine diameters, long roots, and dense roots (Comas *et al.*, 2013).

Table 7. Correlation coefficients among germination and seedling characters of 11 wheat cultivars under water stress.

	CL (cm)	SL (cm)	RL (cm)	R/S	NR	MDG	DGS	GR
LC (cm)	1	0.83**	0.67**	0.44**	0.67**	0.16*	-0.12	-0.29**
SL (cm)		1	0.74**	0.46**	0.67**	0.14*	-0.07	-0.40**
RL (cm)			1	0.73**	0.64**	0.31**	-0.25**	-0.25**
R/S				1	0.58**	0.31**	-0.23**	-0.09
NR					1	0.24**	-0.22**	-0.27**
MDI						1	-0.77**	0.65**
DGS							1	-0.57**
GR								1

**correlation is significant at p<0.001; * correlation is significant at p<0.05. CL, coleoptile length; SL, shoot length; RL, root length; R/S, root/shoot length ratio; RN, root number; MDG, mean day germination; DGS, daily germination speed; GR, germination rate

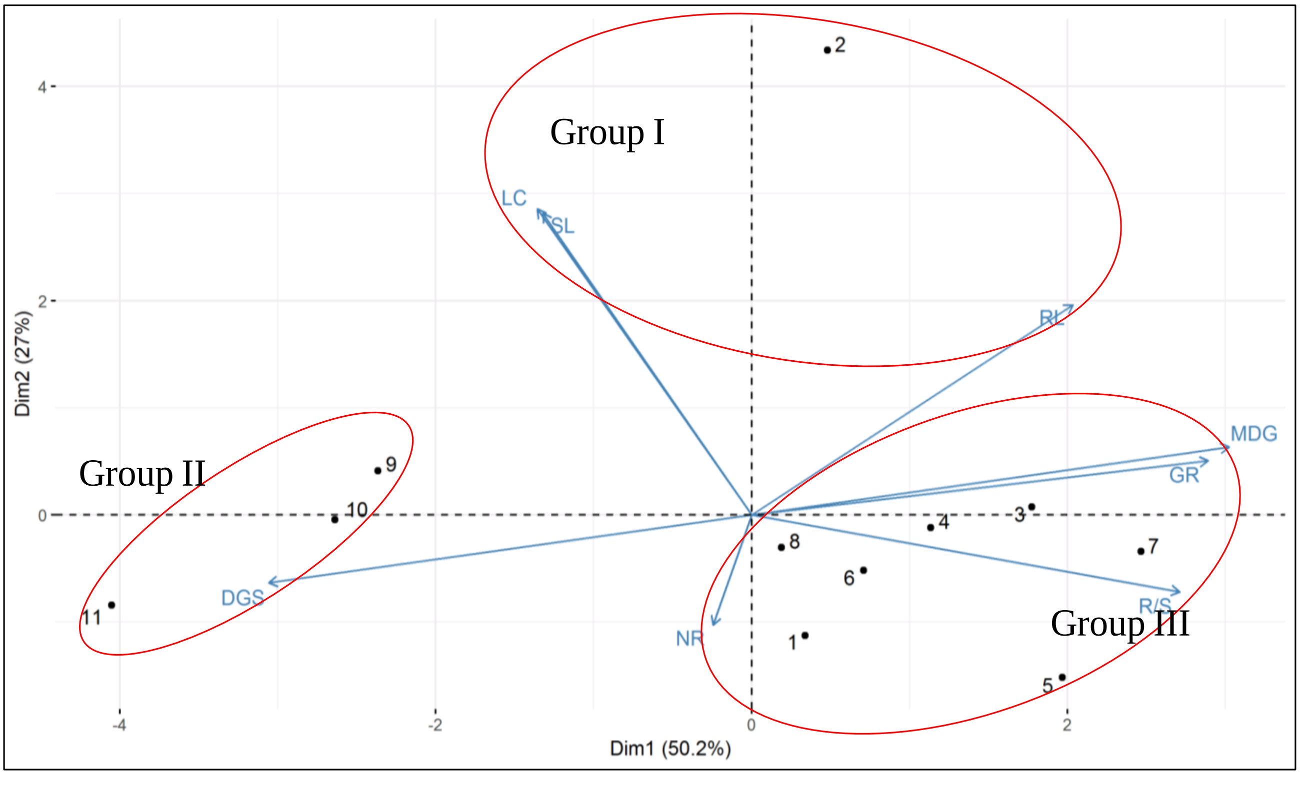

Fig. 1. Biplot of principal component analysis of 11 durum wheat cultivars and studied traits. 1: Maâli, 2: Mahmoudi, 3: Om rabiaa, 4: Karim, 5: Nasr, 6: Salim, 7: Maghrbi, 8: Ben bechir, 9: Souri, 10: Agiliglabre, 11: Jnehkottifa. CL, coleoptile length; SL, shoot length; RL, root length; R/S, root/shoot length ratio; RN, root number; MDG, mean day germination; DGS, daily germination speed; GR, germination rate.

Relationship between germination and seedling growth characters

Correlation analysis: There was a positive and significant correlation between MDG and CL, SL, RL and R/S length ratio (Table 7). However, a negative and highly significant correlation was observed between DGS and RL, R/S length ratio and RN. GR showed a negative and highly significant correlation with CL, SL, RL, RN and DGS, and a positive correlation with MDG. The correlation between GR and R/S length ratio was not significant. Rauf *et al*., (2007) found a significant and positive correlation between GR, CL, SL and RL but a non-significant and negative correlation between GR and R/S length ratio in 16 wheat cultivars. In fact, PEG-induced coleoptile growth under osmotic stress was correlated with agro-morphological traits such as plant height and 1000-kernel weight of 114 durum wheat cultivars derived from a field-grown trial (Nagel *et al*., 2014). SL showed a positive and highly significant correlation with CL (r = 0.83), RL (r = 0.74), R/S length ratio (r = 0.46) and RN (r = 0.67). In addition, RL showed a positive correlation with CL (r = 0.67). Khan *et al.* (2013) also noted that RL was significantly correlated with CL (r = 0.82) in wheat. The highest correlations between SL and CL (r = 0.83) and SL and RL (r = 0.74) may suggest that selection for these characters can be useful in breeding programs. Similar results were obtained by Hellal *et al*., (2018) in barley cultivars, for three germination periods (3, 5 and 7 days), in which SL and RL were highly correlated with germination period (r = 0.820, r = 0.829, r = 0.886, and r = 0.871, r = 0.919, r = 0.968).

Principal component analysis: The relationships between different studied traits and cultivars are graphically presented as a PCA analysis (Fig. 1), as it is the most suitable multivariate method (Beheshtizadeh *et al*., 2013). The first two components PCA1 (strongly associated to DGS) and PCA 2 (linked to CL and SL) accounted for 77.2% of total variation. Mahmoudi (group I) was distinguished from other cultivars, showing greatest performance for SL, CL and RL. The latter (RL) was highly correlated to MDS and GR, as assessed by the acute angle of their vectors. Mahmoudi is thus the most drought-tolerant cultivar for germination and seedling traits. Souri, Agiliglabre and Jnehkottifa, which formed group II, had highest DGS, implying greater germination ability. Group III includes Maâli, Mahmoudi, Om rabiaa, Karim, Nasr, Salim, Maghrbi and Ben bechir, which showed a variable response to drought stress.

Conclusion

Germination and seedling growth are the first and most important stages of a plant life cycle and are most susceptible to drought stress. In this study, high osmotic water potentials had negative effects on several traits in 11 durum wheat cultivars, but germination percentage was the least affected. Correlation and PCA analysis revealed that coleoptile root and shoot length were the most correlated traits. The latter could be a useful indicator for preliminary screening of potentially drought-tolerant cultivars. The results of this study revealed that the 11 wheat cultivars responded differently to water stress levels in terms of germination and seedling growth-related characters. When considering performance of these indices under drought stress conditions, at early stages of growth, var. Mahmoudi proved to be the most suitable cultivar for culture in semi-arid regions.

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