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Durum wheat response (*Triticum durum* Desf.) to drought stress under laboratory conditions

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Abstract*: Drought stress is the main problem limited durum wheat production in Tunisia. In this order, laboratory experiments of 11 durum wheat varieties were carried to i) evaluate their response at seedling level to water stress induced by different concentrations of polyethylene glycol (PEG-6000) (0, 100, 150, 200 and 400 g/l) and to ii) screen drought tolerant varieties. Wheat varieties were raised in hydroponic and nourished with Hoagland solution. Results showed significant differences between treatments for all seedling characteristics in hydroponic culture. Except for relative water content, seedling traits differed significantly among varieties ($p<0.05$, $p<0.01$ and $p<0.001$). PEG treatment significantly decrease seedling shoot length, seedling root length, root to shoot ratio, relative water content, chlorophyll (a), chlorophyll (b) and total chlorophyll, but it increase proline content. For PEG concentrations over than 200 g/l, all studied varieties were not able to withstand and continue their development. Under imposed drought stress condition, Mahmoudi variety had great performances for most traits (seedling shoot length, seedling root length, root to shoot ratio, chlorophyll (a) and proline content). It could be believed as a drought tolerant variety*.

Keywords: *physiological traits, durum wheat, water stress.*

Abbreviation: *chlorophyll (a) (Chl (a)), chlorophyll (b) (Chl (b)), (t)), proline content (Pro), root to shoot ratio (R/S), seedling root length (SRL), seedling shoot length (SSL) and total chlorophyll (t) (Chl (t))*

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I. Introduction

Face to the augmented demand for food supply (70 % by 2050) and the climate change, there is a need to increase cereal production (Semenov et al., 2014). In the world, especially in Africa, climate change has significantly caused drought stress (Gupta et al., 2017; Kang et al., 2009). It is among the most important factor that limits global wheat productivity (Mwadzingeni et al., 2016). Drought losses in plant yields are higher than those from other abiotic stress (Alqudah et al. 2008). It affects different plant morphological and physiological functions (biomass, shoot and root growth…) (Al-Maskri et al., 2016; Widuri et al., 2018). Drought stress has also a detrimental effect on relative water content, gas exchange and chlorophyll content, proline concentration root and shoots traits (Al-Shaheen and Soh, 2016; Jackson et al., 1996). Under water stress condition, proline accumulation in plants is related to water stress tolerance (Sirusmehr and Vazirimehr 2016), it is the most biochemical traits of drought tolerance (Singh et al., 2014). It contributes to maintain plant production (Vendruscolo et al., 2007). In fact, these Biochemical analysis such as glycine betaine and proline Were used as complementary tool to select drought tolerant genotypes (Mwadzingeni et al., 2016). Therefore, the employ of morphological and/or physiological traits to screen and develop cultivars for water stress areas could be involved as selection criteria through traditional plant breeding programmes (Jatoi et al., 2011). Thus, there was a serious requirement to integrate different knowledge and methodologies in breeding programs for drought tolerance (Mwadzingeni et al., 2016).

The hydroponics culture screening technique could be a simple, fast and efficient method leads to screen hundred of seedlings (Soni et al., 2014). Application of osmotic compounds such as polyethylene glycol (PEG) in a hydroponic solution imposes osmotic stress, which changes plant water status (Grzesiak et al. 2003 and Burnett 2005). In fact, Mohammadkhani and Heidari (2008) reported that PEG-6000 has long been utilized under laboratory conditions, which reflect stress imposed by the soil, for screening drought tolerant genotypes. Diverse seedling traits (shoot length, root to shoot ratio, relative water content, chlorophyll and proline content, etc) were used to screen drought tolerant genotype.

In this regard, the present work aims to i) measure the effect of water stress on durum wheat seedling growth and physiological traits ii) to screen drought tolerant durum wheat varieties by growing plants in hydroponic medium containing different polyethylene glycol (PEG-6000) concentrations.

II. Material And Methods

Plant material

Eleven durum wheat (*Triticum durum* Desf.) varieties: Maâli, Mahmoudi, Om Rabiaa, Karim, Nasr, Salim, Maghrbi, Ben Bechir, Souri, Agili glabre and Aziz were used.

Laboratory experiment and growing conditions

Laboratory experiment was conducted in the Genetic and Plant Breeding Laboratory, in the National Agronomic Institute of Tunisia. Homogenous seeds of each variety were surface sterilized with 12 % sodium hypochlorite solution, washed twice with distilled water and then soaked overnight at room temperature to germinate during 7 days in plastic tray containing two sheets of Whatman no. 1 filter paper moistened with water. At 2 leaf stage, durum wheat seedling were transferred and fixed with cotton to plastic boxes containing Hoagland nutrient solution, containing necessary nutrients for plant growth (Hoagland and Arnon, 1950), continuously aerated using an air compressor. Seedlings were grown in greenhouse at 25 °C in a 16-h light/8-h dark photoperiod regime and 65-75 % relative humidity. Each genotype was randomly in each column in each treatment.

Osmotic stress levels (0, -0.47, -1.48, -3.02 and -5.11 bars) were imposed by five various PEG-6000 concentrations (0, 100, 150, 200 and 400 g/l). The solution was renewed every 48 h. After 20 days, plants were retained.

Data

Data was recorded on seedling shoot length, seedling root length, root to shoot length ratio, relative water content (RWC), chlorophyll a, b and total and free proline content.

Relative water content was estimated according to Turner (1986) and evaluated from the equation bellow:

$$RWC = ((FW - DW)/(TW - DW)) \times 100$$

Where fresh weight leaves (FW) were taken and weighed immediately from each treatment and genotype. Then, leaves were floating in distilled water for 4 h in the light at room temperature and weighed to record leaves turgid weight (TW). Dry weight (DW) was determined after drying leaves at 70°C for 24 h.

Chlorophylls a and b were determined by the method described by Horii et al. (2007) where 3 ml of 99.5% methanol was added to 50 mg of fresh leaf tissue and incubated for 2 h in dark. Samples were homogenized and centrifuged at 10000 rpm for 10 min.

Absorbance of the supernatant was read at 650 nm and 665 nm by the UV spectrophotometer (Genesys10 SUV-Vis spectrophotometer). Methanol (99.5%) was used as a blank. Chl a, Chl b and Chl T contents were calculated using the following equations:

$$\text{Chlorophyll a } (\mu g/mL) = 16.5 \times A665 - 8.3 \times A650$$

$$\text{Chlorophyll b } (\mu g/mL) = 33.8 \times A650 - 12.5 \times A665$$

$$\text{Total chlorophyll } (\mu g/mL) = 25.8 \times A650 + 4.0 \times A665$$

Proline content

Proline accumulation was assayed by the method of Troll and Lindsley, (1955), modified by Monneveux and Nemmar (1986). After cutting into small pieces, 100 mg of fresh leaves were introduced into a test tube, in which 2 ml of 40% methanol were added, the whole was heated in a water bath at 85 ° C for 60 minutes. The tubes are closed to avoid the volatilization of the alcohol. After cooling, 1 ml of the solution is removed and placed in test tubes to which 1 ml of acetic acid (CH_3COOH), 1 ml of a mixture containing (12 ml of distilled water, 30 ml of acetic acid, 80 ml of ortho-phosphoric acid (H_3PO_4 density 1.7) and 25 mg of ninhydrin were added. Then, tubes were placed in water bath for 30 minutes at 100°C. Solution turns gradually to red, after cooling, 5 ml of toluene are added to the mixture, following a great agitation for 15 second, two layers are observed: the superior which contains the proline is recovered and dehydrated by the addition of anhydrous Na_2SO_4. Finally, the optical density was determined by a spectrophotometer (Genesys10 SUV-Vis spectrophotometer) at 528 nm. Proline concentration was calculated on fresh weight using L-proline standard curve.

The proline concentration was determined from a standard curve and calculated on a fresh weight basis using the following formula (Bates et al., 1973):

$$\text{Proline (mg g-1 FW)} = [(\mu g \text{ proline/ml x ml toluene}) / (\text{g sample}/5)] / 1000$$

Statistical analysis

Variance analysis were carried out using SPSS software ver. 16.0 and means comparison were determined by Duncan's multiple range test ($p<0.05$, $p<0.01$ and $p<0.001$).

III. Results And Discussion

Effect of water stress on different seedling traits

The results of variance analysis for studied traits under various PEG-6000 concentrations indicated a highly significant ($p<0.001$) effect of drought stress and PEG treatments × varieties interaction and the presence of a considerable genotypic variation ($p<0.05$, $p<0.01$ and $p< 0.001$) except for RWC (table 1).

Table 1: Variance analysis of seedling shoot length (SSL), seedling root length (SRL), root to shoot ratio (R/S), relative water content (RWC), chlorophyll (a) (Chl (a)), chlorophyll (b) (Chl (b)), total chlorophyll (t) (Chl (t)) and proline content (Pro) in eleven durum wheat varieties under five different drought stress levels

Variance Sources	Df	SSL (cm)	SRL (cm)	R/S	RWC (%)	Chl (a) (ug/ml)	Chl (b) (ug/ml)	Chl (t) (ug/ml)	Pro (mg/g FW)
PEG treatments	4	2360.98***	1055.15***	716.12***	669.00***	355.62***	125.18***	287.75***	1433.86***
Varieties	10	20.67***	41.63***	24.41***	1.64 ns	3.00**	2.31*	2.62**	89.23***
PEG treatments × varieties	40	7.30***	10.19***	8.09***	3.72***	4.06***	2.90***	3.68***	59.16***

Level of significance: ns = no significant; $p<0.05$=*; $p<0.01$=**; $p<0.001$=***.

Most morphological and physiological durum wheat indicators decease with the increase of drought stress levels. Highest value of SSL (37.89 cm), SRL (20.08 cm), R/S (0.53), RWC (75.67 %), Chl (a) (21.87 ug/ml), Chl (b) (7.00 ug/ml) and Chl (t) (29.13 ug/ml) were observed under control treatment (without PEG) (table 2). However, a highly significant increase was observed in Pro content while increasing PEG concentration, highest value (3.36 mg/g FW) was obtained under a severe stress (150 g/l PEG). In fact, water stress enhances proline levels. These results corroborate those obtained by Khalilzadeh et al. (2016) and Sultan et al. (2012) in wheat genotypes and by Moharramnejad et al. (2015) in maize. After 20 days and under highest PEG concentrations (200 and 400 g/l), the total of 11 varieties is not able to withstand and continue their development. Gallé et al. (2013) found that, in two wheat cultivars, water stress decrease relative growth rate of leaves and roots. Also, similar are the results of Soni et al. (2014) where drought stress decreased most growth parameters (shoot length, root length, shoot fresh weight, root fresh weight). Leaf RWC decrease significantly when the water stress was prolonged in wheat cultivars (Sultan et al., 2012 and Liu et al., 2013) and in triticale (Kaydan and Mehmet, 2008). In bread wheat, Guo et al. (2013) found that Chl (a) and Chl (b) content decreased with increasing PEG concentration, each parameter was less than in the control treatment. In the present experiment, symptoms of drought stress were observed under PEG treatment in durum wheat leaves. In fact, chlorophyll content determination is frequently used to establish environmental stresses impact, as its pigment content is usually associated to visual symptoms and plant photosynthesis productivity (Jain et al., 2013).

Table 2: Means comparison of eleven durum wheat varieties under five different drought stress levels.

		Variance sources							
		SSL (cm)	SRL (cm)	R/S	RWC (%)	Chl (a) (ug/ml)	Chl (b) (ug/ml)	Chl (t) (ug/ml)	Pro (mg/g FW)
PEG treatments (g/l)	0	37.89 a	20.08 a	0.53 a	75.67 a	21.87 a	7.00 a	29.13 a	0.52 c
	100	29.00 b	10.02 b	0.50 b	60.96 b	17.96 b	6.87 a	25.06 b	0.67 b
	150	18.32 c	9.32 b	0.34 c	37.58 c	97.09 c	2.82 b	12.64 c	3.36 a
	200	0.00 d	0.00 d	0.00 d	0.00 d	0.00 d	0.00 c	0.00 d	0.00 d
	400	0.00 d	0.00 d	0.00 d	0.00 d	0.00 d	0.00 c	0.00 d	0.00 d
Varieties	Maâli	14.25 e	7.08 d	0.30 b	36.27 ab	10.96 ab	5.04 a	16.16 a	1.35 d
	Mahmoudi	21.20 a	13.13 a	0.39 a	36.21 ab	12.28 a	3.68 b	16.10 a	3.02 a
	Om rabiaa	17.23 b	11.32 b	0.38 a	31.42 b	10.53 ab	3.85 ab	14.52 ab	1.01 f
	Karim	15.31 cd	5.80 ef	0.21 cd	35.42 ab	8.98 bc	2.62 b	11.72 b	0.88 fg
	Nasr	16.88 bc	8.49 c	0.31 b	33.72 b	9.92 abc	3.08 b	13.13 ab	2.55 b
	Salim	16.73 bc	9.10 c	0.33 b	35.91 ab	10.62 ab	3.57 b	14.33 ab	2.31 c
	Maghrbi	14.25 de	6.10 def	0.23 c	40.33 a	10.93 ab	3.38 b	14.44 ab	1.49 d
	Ben bechir	16.86 bc	6.35 de	0.22 cd	35.04 ab	7.99 c	2.61 b	10.70 b	1.04 ef
	Souri	20.47 a	7.24 d	0.21 cd	33.75 b	9.82 c	2.92 b	12.85 ab	1.28 de
	Agili	18.30 b	7.06 d	0.24 c	31.06 b	9.28 bc	3.04 b	12.43 ab	0.70 g
	glabre Azizi	16.99 b	5.07 f	0.18 d	34.10 b	7.63 c	2.88 b	10.61 b	1.01 f

Means with similar letter(s) in each trait are not significantly different

Screening of drought tolerant varieties

Results shows that, among different studied varieties, Mahmoudi had highest SSL (21.20 cm), SRL (13.13 cm), R/S (0.39), Chl (a) (12.28 ug/ml) and Pro (3.02 mg/g FW). A high proline accumulation, under water stress condition is associated with drought stress tolerance genotypes (Bilal et al., 2015). Consequently, Mahmoudi variety seems to be a drought tolerant variety.

IV. Conclusion

In the present study, water stress induced by PEG decreased seedling growth. Durum wheat varieties differ notably in their response to drought stress. Mahmoudi showed most great performances, it could the most drought tolerant varieties. Thus, hydroponic test could be useful tool to a preliminary screening of durum wheat response water to stress but it is necessary to confirm results with a field experiment.

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