

Tolerance of Durum Wheat Cultivars to Postanthesis Drought Conditions as Affected by Seed Priming, Bio-Fertilizer and Potassium Application

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ABSTRACT

The present study aimed to investigate the tolerance of different durum wheat (*Triticum turgidum* ssp. *durum* Desf. em. Musn) genotypes to drought stress at heading to maturity stage under the influence of drought alleviation treatments. The field work was carried out at Fuka Research Station, Faculty of Desert and Environmental Agriculture, Alexandria University, Matrouh Branch Egypt, in 2012/2013 and 2013/2014 winter seasons. The experimental design was split-plot, with four replicates where the main plots included six treatments as follows: 1) Spraying with potassium, 2) Priming with calcium chloride, 3) Bio-fertilization with HALEX® 4) Seed hydro-priming, 5) Sowing of dry seed (these five treatments were subjected to drought by holding irrigation at postanthesis) and 6) Control (irrigation all season). The sub-plots included the three durum wheat genotypes. The results revealed insignificant variation for applied treatments on all studied characters, except for 100-grain weight, in the two seasons. Cultivars showed significant variations in LAI (at 80 DAS), dry weight of spikes (at 50 % heading and 30 days later) and number of grains spike⁻¹ in the two seasons. Interaction between drought alleviation treatments and cultivars was insignificant, for all studied characters in the two seasons. Also, wheat plants, irrigated all season, gave the highest 100-grain weight (5.31 and 6.48 g) in the two seasons, respectively. On the other hand, Sohag 3 was superior (0.23 and 0.25) to Marjawi (0.19 and 0.21) in LAI, in the two seasons, respectively. In addition, Beni Suef 5 gave the highest significant dry weight of spikes and number of grains spike⁻¹ in the two seasons. Drought susceptibility index (DIS) values revealed that seed priming with CaCl₂ and water tolerated drought at postanthesis stage and gave comparable grain yield to that of control. Beni Suef 5 and Marjawi showed higher tolerance to drought than Sohag 3.

Key words: Wheat, drought, seed priming, bio-fertilizer, potassium.

INTRODUCTION

Durum wheat (*Triticum turgidum* ssp. *durum* Desf. em. Musn) is one of most grown crops under dry land conditions in the Mediterranean areas and is an important source of human nutrition. The need and importance of durum wheat are increasing due to increase in human population. Durum wheat represents 10% of the wheat grown globally, occupying about eleven million hectares in the Mediterranean areas (Karimizadeh *et al.*, 2013). Drought stress is one of the most important factors, which affect the production of durum wheat in the arid and semi-arid areas because rainfall and temperatures, in such areas, show large and unpredictable fluctuations within and between growing seasons (Mir *et al.*, 2012).

The drought stress can occur at any stage of growth. Most crop cultivars are susceptible to yield losses due to limited water supply during postanthesis period. The ability of a genotype to produce high yield, over a wide range of stress and non-stress environmental conditions, is essential for an ideal cultivar. Although drought may occur at any stage of durum wheat development in Mediterranean regions, climatic frequency studies

have identified one major period when drought is most likely to occur and it is when the durum wheat is in the grain filling phase (Mohammadi *et al.*, 2011).

To reduce the impact of drought on wheat, a variety of materials may be applied before or after sowing. El-Ashry *et al.* (2005) reported that the negative effect of drought on growth of wheat could be decreased by spraying potassium. Similarly, that potassium enhanced drought tolerance in plants by mitigating harmful effects through increasing translocation and by maintaining water balance. Osmotic adjustment has been considered an important phenomenon for mitigating the adverse effects of drought stress in plants (Ashraf, 2010). For that, certain organic (proline, soluble sugars, glycine betaine, proline betaine) and inorganic (potassium ions) compatible solutes are accumulated in the plant cells (Zhang *et al.*, 2009 and Baysal-Furtana *et al.*, 2013). Among these compatible solutes, potassium was very effective in the regulation of plants homeostasis under drought stress. Moreover, potassium uptake and accumulation is increased during drought and plays a major role in stomata opening and closing,

transpiration, photosynthesis, protective protein synthesis, and osmoregulation of plant cells (Raza *et al.*, 2012 and 2013). Potassium also is accumulated in plants to regulate osmotic adjustment under drought or osmotic stress.

In sustainable agriculture system, biological fertilizers play an important role in crop production and increasing soil fertility conservation (Sharma *et al.* 2010). Bio-fertilizers are products containing living cells of different types of microorganisms (Chen, 2006) that have an ability to convert nutritionally important elements from unavailable to available form through bio-logical processes and are known to help with expansion of the root system and increase seed germination rate, Behrooz *et al.* (2015) reported that bio-fertilizer had an effective function in improving plant growth characteristics and increasing of products quality of wheat cultivars under dryland conditions.

Seed priming is a pre-sowing strategy for influencing seedling development by modulating pre-germination metabolic activity prior to emergence of the radicle and, generally, enhances germination rate and plant performance (Taylor and Harman, 1990). Priming may increase these factors which can increase crop tolerance to drought and salinity (Wang and Shen, 1991, and Aslam *et al.*, 2013). Calcium plays a vital role in plant response to drought stress. Calcium is of great significance in many plant physiological functioning and responses to abiotic stresses. It acts as a signal transmitter to help in the regulation of plant growth and development under stress. Most importantly, it regulates the opening and closing of stomata and adaptation of plant to drought through ABA homeostasis (Song *et al.*, 2008). In wheat, Ca has been reported to decrease the devastating impact of drought by increasing the concentration of compatible solutes (glycine betaine and proline), which help in the amelioration of stress in the growth of seedlings, improve plant water status and reduce membrane injury (Nayyar, 2003). Various pre-hydration or priming treatments have been employed to increase the speed and synchrony of seed germination. Common priming techniques include osmopriming (soaking seeds in osmotic solutions) such as calcium chloride. Sharma *et al.*, (2010) found that pre-sowing hydration (seed priming) of seed with 3% calcium chloride and 15 ppm gibberellic acid increased emergence count, as compared to the other applied treatments.

The main objective of the present study was to investigate the tolerance of different durum wheat (*Triticum turgidum* ssp *durum* Desf. em. Musn) genotypes to drought stress at heading to maturity stage under the influence of seed priming with water and calcium chloride, application of HALEX[®] bio-fertilizer and spraying with potassium, as methods

for increasing wheat plant tolerance to drought stress.

MATERIALS AND METHODS

Wheat experiments were carried out in the Agricultural Research Station, Alexandria University at Faculty of Desert and Environmental Agriculture (Fuka), Matrouh Branch, located at North West Coast of Egypt (N=31° 04., E=27° 54) during the two winter growing seasons of 2012/2013 and 2013/2014. The main objective of the present study was to investigate the tolerance of different durum (*Triticum turgidum* ssp *durum* Desf. em. Musn) wheat genotypes, one Libyan variety (Marjawi) and two Egyptian cultivars (Beni Suef 5 and Sohag 3), to drought stress at heading to maturity stages (postanthesis) under the influence of seed priming (with distilled water hydropriming) and calcium chloride (osmopriming), HALEX[®] (bio-fertilizer) and spraying with potassium, as methods for increasing wheat plant tolerance to drought stress. The experimental site soil had the following properties: Texture= sandy (calcareous), pH= 8.3, total organic matter= 0.68 %, and Ec= 3.4 dS/m as an average of the two seasons.

The experimental design, used in the two seasons, was split-plot in randomized complete block design (RCBD) with four replications. The main plots included six treatments. i.e 1) Spraying twice with potassium (as liquid, K₂O= 36.5 %) at the rate of 2.38 L/476 L water/ha after thirty days from sowing (DAS) and the second after sixty DAS, 2) Priming with calcium chloride, where grains were soaked for twelve hours, prior to sowing, in a 5:1 solution of 5mg/l of CaCl₂, 3) Bio-fertilization with HALEX[®], (containing a mixture of growth-promoting N-fixing bacteria of Azotobacteriaceae, Azospirillum and Klebsiella) at the rate of 1.20 kg/ha, where wheat grains were mixed well with the biofertilizer directly before sowing, 4) Hydropriming: soaking in distilled water for twelve hours prior to sowing, 5) Sowing of dry seed directly and 6) Control. The first five treatments were subjected to withholding irrigation at early heading, while the control was irrigated throughout the season to fulfill the requirements of durum wheat under the experimental site conditions. While, the sub- plots included the three durum wheat genotypes. Sub-plot size, in both seasons, was 6 m² (3m long * 2m wide) and included ten rows at row spacing of 0.3m. Sowing date was November 27th in the two seasons. Wheat cultivars were sown at the rate of 95.2 Kg/ha. Phosphorus fertilizer, as calcium monophosphate (15.50 % P₂O₅) was added during land preparation at the rate of 37.0 kg P₂O₅/ha. Nitrogen fertilizer was applied at the rate of 142.8 kg N/ha in the form of ammonium nitrate (33.5 % N), in three doses; i.e., 1/5 at sowing, 2/5 at tillering stage and 2/5 at booting stage. Irrigation was applied, using sprinkler irrigation system.

Measured characters:

To monitor the effect of drought on wheat growth, and the effect of applied treatments to overcome the damaging effects of drought on plant growth, the following characters were measured from random samples taken at designated periods for each character:

1. Plant dry weight (g), at 30 and 60 days after sowing.
2. Leaf area index (LAI) = (LA2 + LA1) / 2* (1/ground area), at 40 and 80 days after sowing (DAS), according to Gardner *et al.* (1985)
3. Dry weight of spikes (g), at 50 % heading and 30 days later.

At harvest, the followings traits were measured:

4. Spike length (cm): as an average of five random spikes/sub-plot.
5. Number of grains/spike: as an average of five random spikes/sub-plot.
6. 100-grain weight (g): as an average of three 100-grain samples/sub-plot.
7. Grain yield (t/ha): calculated by converting the grain yield obtained from the inner six rows of each sub-plot to t/ha.
8. Harvest index (H.I %): was computed, using the following equation:
H.I % = Grain yield / Biological yield * 100
9. Grain protein content (%): The protein content of the whole grains was determined, according to the Kjeldahl method (A.O.A.C, 1980) applying a factor of N × 5.7.
10. Drought susceptibility index (DSI), according to Fischer and Maurer (1978), as follows:

$$DSI = 1 - \frac{Y_s}{Y_w} / 1 - \frac{\bar{Y}_s}{\bar{Y}_w}$$

Where,

Y_s: Yield under stress condition.

Y_w: Yield under all season irrigation.

\bar{Y}_s : Mean of genotypes or treatment under stress condition.

\bar{Y}_w : Mean of genotypes or treatment under all season irrigation.

$$\left(1 - \frac{\bar{Y}_s}{\bar{Y}_w}\right) = \text{Environmental stress intensity.}$$

Data were subjected to the proper analysis according to Gomez and Gomez (1984) using SAS (Statistical Analyses Systems) ver. 9.1 (2000). Means were compared, using the least significant difference (LSD) value at 5% level of probability.

RESULTS AND DISCUSSION

Analysis of variance (Table 1) indicates that applied treatments, to overcome the damaging effect of drought on plant growth, were insignificantly different, in the two sampling dates and the two seasons for plant dry weight, LAI and dry weight of spikes. Cultivars showed significant variation in LAI at 60 DAS in the two seasons and dry weight of

spikes at 50% heading and thirty days later in the two seasons. Interaction was insignificant between drought alleviation treatments and cultivars, for all studied characters in the two seasons. That implies a similar behavior for cultivars, as influenced by the applied treatments. Means, presented in Table (2), showed that, though insignificant, in early stages of growth, hydropriming showed higher plant dry weight and LAI, compared to the faster germination and emergence of seedlings, due to imbibing of water and activation of pre-germination processes. Taylor and Harmen (1990) reported that seed priming modulated pre-germination activity prior to emergence of radical and enhanced germination rate and plant performance. Similarly, Kamyar and Hamdollah (2012) concluded that hydropriming improved seed germination under drought salt conditions. Concerning dry weight of spikes, drought alleviation treatments, especially seed priming with water or CaCl₂, and application of bio-fertilizer, gave comparable values to all season irrigation (control) in the two sampling dates and the two seasons. That implies a beneficial effect for those treatments in overcoming the damaging effect of drought on spike growth and grain development, especially at the later stage (second sample taken after thirty days from 50 % heading). Similar findings were reported by Attia *et al.* (2005) for bio-fertilizer effects and Siddiqui *et al.* (2011) for osmopriming with CaCl₂, concluding that both biological fertilizers and calcium played an important role in meliorating the adverse effects of drought on wheat plants.

The results in Table (3) showed that Sohag 3 was superior to Marjawi in LAI, in the second sample, in the two seasons, but was similar to Beni Suef 5. That may be attributed to the genetic constitution differences between cultivars. On the other hand, Beni Suef 5 was significantly superior to Marjawi and Sohag 3 in dry weight of spikes in the two sampling dates in the two seasons. That may indicate the higher tolerance, to imposed drought, of Beni suef 5, compared to the two other genotypes at postanthesis stage, resulting in higher accumulation of dry matter in the spike and higher dry weight of spikes. Similar findings were reported by Keyvan (2010) and Khayatnezhad *et al.* (2011) who found variation between and within groups of bread and durum wheat genotypes for drought tolerance, depending on the studied characters. Similarly, Bowne *et al.* (2012) reported differences between three wheat cultivars in their tolerance to drought at terminal stages of growth.

Concerning grain yield components; i.e., number of spikes m⁻², number of grains spike⁻¹ and 100-grain weight, analysis of variance in (Table 4) revealed that the applied treatments had a significant effect on 100-grain weight in the two seasons.

Table 5: Means of different treatments for no. spikes m⁻², no. grains spike⁻¹ and 100-grain weight in 2012/13 and 2013/14 seasons.

Treatments	No. spikes m ⁻²		No. grains spike ⁻¹		100-grain weight (g)	
	2012/13 Season	2013/14 Season	2012/13 Season	2013/14 Season	2012/13 Season	2013/14 Season
Spraying K	169.74 a ⁽¹⁾	207.00 a	35.87 a	43.75 a	3.88 cd	4.74 c
Priming with CaCl ₂	156.01 a	190.25 a	37.72 a	46.00 a	4.79 abc	5.85 ab
Bio-fertilizer	161.40 a	196.83 a	39.97 a	48.75 a	4.70 abc	5.74 ab
Seed hydropriming	161.06 a	196.42 a	40.51 a	49.41 a	5.20 ab	6.35 ab
Dry seed	134.68 a	164.25 a	34.50 a	42.08 a	3.68 bd	4.37 c
Control	159.55 a	194.58 a	42.84 a	52.25 a	5.31 a	6.48

(1) Means followed by the same letter (s) are not significantly different at 0.05 level.

Table 6: Means of cultivars for no. spikes m⁻², no. grains spike⁻¹ and 100-grain weight in 2012/13 and 2013/14 seasons.

Cultivars	No. spikes. m ⁻²		No. grains. spike ⁻¹		100-grain weight (g)	
	2012/13 Season	2013/14 Season	2012/13 Season	2013/14 Season	2012/13 Season	2013/14 Season
Marjawi	147.43 a ⁽¹⁾	179.8 a	37.20 b	45.37 b	4.93 a	6.02 a
Sohag 3	160.17 a	195.33 a	33.10 c	40.37 c	4.60 a	5.62 a
Beni Suef 5	163.59 a	199.50 a	45.40 a	55.37 a	4.60 a	5.62 a

(1) Means followed by the same letter (s) are not significantly different at 0.05 level.

Table 7: Mean squares for analysis of variance for grain yield, harvest index and protein percentage in 2012/ 13 and 2013/14 seasons.

S.O.V.	d.f	Grain yield (ton ha ⁻¹)		Harvest index		Protein (%)	
		2012/13 Season	2013/14 Season	2012/13 Season	2013/14 Season	2012/13 Season	2013/14 Season
Treatments (T)	5	0.281 N.S	0.348 N.S	12.36 N.S	15.27 N.S	0.569 N.S	2.695 N.S
Error (a)	15	0.102	0.127	9.08	11.21	2.159	3.519
Cultivars (C)	2	0.028 N.S	0.035 N.S	3.87 N.S	4.788 N.S	2.177 N.S	4.281 N.S
T *C	10	0.036 N.S	0.045 N.S	10.54 N.S	13.01 N.S	1.751 N.S	3.656 N.S
Error (b)	36	0.025	0.031	7.38	9.12	2.023	2.771

N.S: Not significant at 0.05 level of probability.

Table 8: Means of different treatments for grain yield, harvest index, drought susceptibility index and protein percentage in 2012/13 and 2013/14 seasons.

Treatments	Grain yield (ton ha ⁻¹)		Harvest index (HI)		Drought susceptibility index		Protein (%)	
	2012/13 Season	2013/14 Season	2012/13 Season	2013/14 Season	2012/13 Season	2013/14 Season	2012/13 Season	2013/14 Season
Spraying K	0.41 a ⁽¹⁾	0.54 a	11.14 a	14.67 a	1.576	1.625	12.00 a	12.51 a
Priming with CaCl ₂	0.59 a	0.78 a	12.11 a	15.94 a	0.591	0.598	11.96 a	12.77 a
Bio-fertilizer	0.53 a	0.71 a	12.27 a	16.15 a	0.923	0.897	12.37 a	13.38 a
Seed hydropriming	0.66 a	0.88 a	12.44 a	16.37 a	0.214	0.171	12.42 a	13.45 a
Dry seed	0.39 a	0.52 a	12.57 a	16.55 a	1.687	1.711	12.44 a	13.54 a
Control	0.69 a	0.92 a	13.80 a	18.17 a	-	-	12.13 a	13.11 a

(1) Means followed by the same letter are not significantly different at 0.05 level.

Table 9: Means of cultivars for grain yield, harvest index, drought susceptibility index and protein percentage in 2012/13 and 2013/14 seasons.

Cultivars	Grain yield (ton ha ⁻¹)		Harvest index		Drought susceptibility index		Protein (%)	
	2012/13 Season	2013/14 Season	2012/13 Season	2013/14 Season	2012/13 Season	2013/14 Season	2012/13 Season	2013/14 Season
Marjawi	0.52 a ⁽¹⁾	0.69 a	12.01 a	15.81 a	0.902	0.89	12.73 a	13.44 a
Sohag 3	0.55 a	0.73a	12.68 a	16.69 a	1.272	1.32	11.70 a	12.83 a
Beni Suef 5	0.58 a	0.76 a	12.47 a	16.41 a	0.734	0.75	12.23 a	13.11 a

(1) Means followed by the same letter are not significantly different at 0.05 level.

Cultivars had significant effect on number of grains spike⁻¹ in the two seasons. The interaction was insignificant between treatments and cultivars for all studied characters in both seasons. Means of the number of spikes m⁻² and number of grains spike⁻¹ (Table 5) showed that such traits were insignificantly different in the two seasons. This may be due to the complete (full) establishment before the impact of the drought, which was imposed at 50% heading. However, it was noted that spraying with potassium, at vegetative stage, increased number of spikes m⁻², compared to the other treatments and control in the two seasons. That may be attributed to the role of potassium in overcoming drought effects in all critical stages of growth through osmoregulation and improving plant growth (Aowne *et al.*, 2012). On the other hand, seed hydropriming showed a relatively higher number of grains spike⁻¹, compared to the other treatments and control (Table 5). That may be due to the early establishment of hydroprimed seedlings, leading to early flowering and, thus, escaping the damaging effects of drought on grain formation and development. Similar findings were reported by Garg (2010). Regarding 100-grain weight, wheat plants, irrigated all season, gave the highest values for that character. However, seed priming with calcium chloride, water and bio-fertilization with HALEX[®] gave statistically similar values to control in the two seasons. That may be attributed to the effect of those treatments in overcoming the drought stress at grain filling stage. On the other hand, spraying potassium gave significantly lower 100-grain weight and was comparable to dry seed treatment. That may be attributed to the potassium role in stomatal closure under drought stress conditions, leading to lower photosynthesis activity and translocation of photosynthates to individual grains, compared to the other treatments. These findings were in accordance with those reported by Nayyar (2003) and Siddiqui *et al.* (2011) for priming with CaCl₂, Garg (2010) for hydropriming and Bassel *et al.* (2001) for bio-fertilization.

Results presented in Table (6) indicated that the three studied cultivars had similar values for number of spikes m⁻² and 100-grain weight. However, Beni Suef 5 had a significantly higher number of grains spike⁻¹, followed by Marjawi, whereas Sohag 3 gave the significantly lowest number of grains spike⁻¹ in the two seasons. These differences among cultivars may be related to their tolerance to drought conditions, which were controlled by the genetic constitution of the genotype. Smith and Griffiths (1993) reported natural genetic variation in traits related to drought tolerance in durum wheat. In addition, Abdullah *et al.* (2011) reported variation in drought tolerance of Syrian durum wheat genotypes and concluded that tolerant genotypes suffered less

reduction in grain yield components compared to sensitive genotypes.

Analysis of variance in (Table 7) indicated insignificant variations in grain yield ha⁻¹, harvest index and protein content, as influenced by the applied treatments, cultivars, and their interaction. However, in both seasons, data in (Table 8) revealed that priming with CaCl₂ and hydropriming gave a comparable grain yield to the control. Drought susceptibility index (DSI) values revealed that these two treatments suffered less damage from imposed drought at pre-anthesis growth stage, compared to the other applied treatments. Untreated dry seed and spraying with potassium gave high values for DSI, indicating the low tolerance of wheat plants to drought stress. These findings may elucidate the role of seed priming, either osmopriming with CaCl₂ or hydropriming, in overcoming the damaging effects of drought through regulation of several biological processes in wheat plants, such as osmoregulation, photosynthesis, stomatal closure, etc. in accordance with the findings reported by Nayyar (2003), Sharma *et al.* (2010) and Aslam *et al.* (2013).

The findings, reported in the present investigation, revealed the possibility of alleviating the damaging effects of drought, occurring at grains formation and development stage in durum wheat through hydropriming, which enhances plants establishment early in the season, thus, they may escape, or tolerate, terminal drought conditions. However, bio-fertilization and osmopriming, with calcium chloride enhance vegetative growth in early stages, combined with regulation of biological processes, such as photosynthesis and transpiration, which increases the wheat plant tolerance, to drought conditions. The results, also, indicated the importance of growing the suitable cultivar which has a genetic ability to tolerate drought.

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الملخص العربي

تأثير نقع البذور والتسميد الحيوي والرش بالبوتاسيوم على تحمل أصناف القمح الصلب للجفاف في مرحلة طرد السنابل

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أجريت الدراسة لمعرفة تأثير عدة معاملات على تحمل ثلاثة أصناف من القمح الصلب للجفاف بعد مرحلة طرد السنابل عن طريق عدة معاملات. تم تنفيذ التجربة بكلية الزراعة الصحراوية والبيئية - فوكة - جامعة الإسكندرية - فرع مطروح خلال الموسمين الشتويين ٢٠١٢/٢٠١٣ و ٢٠١٣/٢٠١٤. أستخدم تصميم القطع المنشقة لمرة واحدة في أربع تكرارات حيث وضعت معاملات تحمل الجفاف في القطع الرئيسية وهي: ١- الرش بالبوتاسيوم ٢- النقع في كلوريد الكالسيوم ٣- التسميد الحيوي (HALEX®) ٤ - نقع البذور في الماء ٥- زراعة البذور جافة ٦- الري الى نهاية الموسم (تم تعريض النبات في المعاملات الخمس الى الأجهاد بوقف الري بعد مرحلة طرد السنابل). أظهرت نتائج الدراسة الى وجود أختلافات بسيطة في جميع الصفات المدروسة باستثناء وزن المائة حبة في كلا الموسمين (٥,٣١ و ٦,٤٨ جم) على التوالي. ومن ناحية أخرى كان الصنف "سوهاج ٣" (٠,٢٣ و ٠,٢٥) والصنف "مرجوى" (٠,١٩ و ٠,٢١) متفوقا في صفة دليل مساحة الأوراق في كل من الموسمين على التوالي، بالإضافة الى ذلك سجل الصنف بنى سوف ٥ أعلى عدد للحبوب بالسنبلة في كلا الموسمين. كما أظهرت الدراسة أيضاً ان مؤشر تحمل الجفاف (DSI) قد سجل أعلى قيمة مع معاملة نقع الحبوب في الماء وكلوريد الكالسيوم وأعطت أعلى محصول للحبوب لتلك المعاملات مقارنة بمعاملة الكنترول، كما أظهر الصنفان "بنى سوف ٥" و"المرجوى" أعلى تحمل للجفاف مقارنة بالصنف "سوهاج".^٣