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# **Effect of different levels of drought stress on the germination and seedling growth parameters of three wheat cultivars seeds**

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## **Abstract**

Seed germination of three varieties of Wheat (*Triticum aestivum* L.) CONDOR, SERI and T.R. evaluated under 5 drought levels (0.0 mPa (control),-0.3 mPa, -0.6 mPa, -0.9 mPa and -1.2 mPa). We used polyethylene glycol (PEG6000) to make drought stress conditions. Germinated seeds were counted daily up to 14 days under laboratory conditions.

Percentage and mean germination time, coefficients of germination, mean germination rate, and uncertainty of germination process as germination parameters. Shoots and roots length; fresh, dry weight of shoots and roots, leaves and roots number, surface area of leaves as seedling growth parameters were studied. The data was analyzed statistically using SPSS software, to identify significant difference among wheat varieties and among treatment.

Comparison between means showed that the highest value for most of germination parameters recorded for SERI variety followed by T.R., and most of seedling growth parameters were recorded for T.R. followed by SERI. The results determined that seeds of all varieties germinate well in the lowest concentration of PEG. Increase the concentration of PEG affect all germination and seedling growth parameters.

**Key words**: drought stress- germination- wheat- PEG 6000- *Triticum aestivum*

### **Introduction**

Wheat (*Triticum aestivum L*.) is a staple food for more than 35% of the world population and it is also the first grain crops in most of developing countries(Metwali, *et al.,* 2011),(Jing and Chang 2003) . Bread wheat is the main food of people in many countries and about 70 % calories and 80 % protein of human is supplied from its consumption(Taregh, *et al.,* 2011). drought stress is one of the major causes of crop loss worldwide, which commonly reduces average yield for many crop plants by more than 50% (Wang, *et al.,* 2003);( Bayoumi, *et al.,* 2008).Depending on which stage of growth a plant experiences drought stress, it reacts quite differently to the stress. Plant affected

by drought at any time, stage such as germination and seedling growth are critical (Pessarakli 1999). With progressive global climatic change and increasing shortage of water resources and worsening eco-environment, wheat production is influenced greatly (Khan and Naqvi 2011).

Drought stress, which is the most serious environmental problem limiting crop production in rain-fed agriculture (Bahieldin *et al.*, 2005), can severely impact plant growth and development, limit plant production and the crop performance(Shao *et al.,* 2009). Drought is a major abiotic factor that limits agricultural crop production. Drought stress affects 40 to 60% of the world's agriculture lands (Shahryari and Mollasadeghi 2011) seriously constraining global crop production(pan *et al.*, 2002). Germination is the first critical phase most affected by drought (Ashraf and Mehmood 1990).

Dhandas et al., indicated that seed vigor index and shoot length are among the most sensitive to drought stress, followed by root length and coleoptiles length(Dhanda, *et al.*, 2004). The rate of seed germination and the final germination percentage as well as the amount of water absorbed by the seeds were considerably lowered with the rise of osmotic stress level (Heikal, *et al.,* 1981). Selection of drought tolerance at early seedling stage is frequently accomplished using simulated drought induced by chemicals like polyethylene glycol (PEG6000) under laboratory conditions.

Previous studies revealed that PEG can be used to modify the osmotic potential of nutrient solution culture and thus induce plant water deficit in a relatively controlled manner (Money, 1989); ( Zhu, 2006). Lu and Neumann; Kulkarni and Deshpande Showed that PEG molecules are inert, no-ionic, virtually impermeable to cell membranes and can induce uniform water stress without causing direct physiological damage. (Lu and Neumann, 1998); (Kulkarni and Deshpande, 2007) PEG as a factor causing drought stress by reducing water potential results in reducing growth in seed germinated and stopping seedling growth so that this effect has been observed more in the shoot than primary roots (Zhu, 2006). Dodd and Donovan also suggested that PEG prevent water absorption by seeds, but penetrable ions by reducing potential inside cell results in water absorption and starting to germinate (Dodd and Donovan, 1999).

Development of stress tolerant varieties is always a main objective of many breeding programs, but success has been limited by adequate screening techniques, and the lack of genotypes that show clear differences in response to various environmental stresses. Therefore, wheat breeders are always looking for means and sources of genetic improvement for grain yield and other agronomic traits. Plant breeders are also adopting new technologies such as molecular markers to increase wheat grain yield under drought stress regions (Khakwani,*et al.,*2011). Agriculture has been affected by environmental stresses such as drought, salinity, extreme temperatures, chemical toxicity and oxidative stress which reduce crop yield fifty percent, approximately and water stress that is caused

by salinity and drought is a prevalent problem in the world. However, plants are affected by drought and salinity similarly (Khayatnezhad, *et al.,* 2010).

Salinity and drought stresses are physiologically related, because both induce osmotic stress and most of the metabolic responses of the affected plants are similar to some extent (Kumar, *et al.,* 2011) such as seed soaking in solutions of Polyethylene Glycol (PEG) was expressed as sowing seeds in an osmotic solution that permits seed to absorb water for germination, but inhibits radicle extension via seed coat (Janmohammadi and Sharifzadeh, 2008);(Giri and Schillinger, 2003). The reaction of seed priming is associated with some factors including duration of priming, seed maturity, species and environmental conditions (Armin, *et al.,* 2010).

Since there are differences between species and even different varieties in terms of susceptibility to drought stress, the aim of this study is to investigate the effects of drought stress on germination seedling stage of three wheat genotype. The present study was conduct to evaluate three wheat cultivars for drought resistance at germination and seedling stage. PEG-6000 was used as an osmotic to induce stress conditions.

## **Materials and methods**

In order to study the effects of drought stress, using polyethylene glycol, on germination and seedling growth parameters in wheat, experiment was conducted in Department of Plant Production, Faculty of Agriculture-Soluq Benghazi University, Libya in 2015. The form of experiment was factorial, completely randomized design with five replications. Seeds of three wheat cultivars (Condur, Seri, and T.R.) were used. These cultivars were obtained from al-kufra Agriculture project in Libya, where all obtained from CIMMYT.

Wheat seeds were subjected to five stress level of polyethylene glycol (PEG6000) (ACROS, company) (0.0 mPa (control), -0.3 mPa, -0.6 mPa, -0.9 mPa and -1.2 mPa) prepared according Michel and Kaufmann (Kaufmann and Eckard, 1971);(Michel and Kaufmann, 1973) PEG 6000 was prepared by dissolving the required amount of PEG6000 in distilled water at 25◦C. Wheat grains were disinfected with 5% sodium hypochlorite (NaOCl) solution for 3 minutes. Then seeds were washed three times with distilled water. 20 grains from each cultivars were germinated on two layers of filter paper in plastic Petri dishes filter papers were moistened with respective treatment from PEG6000. Petri dishes were covered and sealed with parafilm to prevent evaporation of moisture (Emmerich and Hardegree, 1991) and incubated under laboratory condition ( $27\pm2$  °C) for 14 days.

279 Seeds were considered germinated when exhibited radicle extension of >2 mm. Every 24 hours germinated seeds were counted daily during the course of the experiment to determine following germination parameters. [*Germination percentage (G %), mean germination time (MGT), Coefficient of variation (CVi), mean germination rate (MGR)*  *and Uncertainty of Germination Process (GU)*]. (ISTA, 1993 & 1999); (Sadeghi *et al.,* 2011); (AOSA, 1983); (Scott, *et al.*, 1984). All of these calculation organized in Excel sheet according to Ranal *et al.* (Ranal and Santana, 2006)& (Ranal *et al.,* 2009).

By harvest seedlings, the experiment was termined 14 days after seeds soaking and traits including [*shoot length, root length, root number, leaf number, leaf surface area, seedling fresh weight, shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight*] were measured , (Almaghrabi, 2012).

## **Experimental design and statistical analysis:**

Effects of two factors were analyzed in these experiments and a completely randomized design with five replications of 20 seeds per replicate. The first factor (Wheat cultivars) had three levels (Condur, Seri and T.R.), the second one (Osmotic potential – Drought levels) had five levels (0.0, -0.3, -0.6, -0.9 and -1.2 *MPa*). The data was analyzed statistically using IBM SPSS statistics software ver. 23, 2015). Analyses of variance (ANOVA) of the obtained data were applied to identify significant difference among



**Figure 1: Effect of Different Concentration of PEG (5 treatments from left to right (0.0, -0.3, -0.6, -0.9 and -1.2** *MPa***)) on Germination of Three Wheat Cultivars (Condur, Seri and T.R.)**

Table (1) show the analysis of variance for the effect of cultivars and drought levels on these parameters of wheat cultivars. From table (1) we can observe no significant differences between mean germination time (MGT) and mean germination rate (MGR). MGT indicate the time for the first germination of the faster seed germinated.



# **Table 1: Analysis of variance for effect of cultivars and drought levels on germination parameters of three wheat cultivars.**

*Df: degree of freedom and \*\*: significant at 5 %. ns: nonsignificant*

Table (2) showed that the faster time for emerging of seed was 3.76 , 4.12 and 4.51 day for Condur variety followed by Seri and T.R. the treatments do not showed any difference effect on mean germination time (no significant difference between treatment). In all cultivars the final germination percentage was decrease with drought stress increase, the highest germination at control and started to decrease after that. Condur cultivar had higher final germination percentage regarding of drought stress. Drought stress at germination stage can results in delayed and reduced germination.

No significant difference between varieties and treatments on mean germination time (MGT), on the base of results the effect of cultivars and treatments on the Coefficient of Variation CVi (coefficient germination time) was significant and SERI gave higher coefficient of variation followed by T.R.; high germination time was observed at - 0.3 *mPa* with 4.47 days. All germination parameters indicate that stress factor like PEG negatively affect plant growth.

According to ANOVA (table 3), PEG levels (drought levels) were significant for all the traits except for root number. The difference between the cultivars was statistically significant for all the traits except for leaf number. There was significance in the interaction between cultivars and drought levels for all parameters except root number, root fresh weight and leaf surface area.



### **Table 2: Effect of different drought levels on germination parameters**

	$-0.9$ $mPa$	0.2668	0.2572	0.2092	0.244a	
	$-1.2$ mPa	0.2400	0.2852	0.2242	0.250 a	
	Mean	0.2711A	0.2515AB	0.2260 B		
	<b>LSD</b>					
	$0.0$ mPa	1.3182	2.0540	2.3354	1.90a	
<b>Uncertainty of</b> <b>Germination</b>	$-0.3$ mPa	1.3400	2.0944	2.0976	1.84a	
<b>Process</b>	$-0.6$ mPa	1.8514	1.9808	1.7684	1.87a	0.347
bit(GU)	$-0.9$ mPa	1.5100	2.1124	1.8430	1.82a	
	$-1.2$ mPa	0.3170	1.8320	1.5850	1.24b	
	Mean	1.2673 B	2.0147A	1.9258A		
	<b>LSD</b>					

*Values in mean column (between treatments) & rows (between cultivars) sharing same letter are statistically no-significant at 5%.*

The presence of increased concentration of PEG during seedling growth inhibits the traits of this stage, maximum shoot length was recorded in T.R. (16.168 cm.) followed by Seri and Condur (15.454 and 15.166 cm.) at level of - 0.3 *mPa*. Whereas the maximum root length recorded in T.R. (19.309 cm.) followed by Condur 14.506 cm. results showed that the highest root length was at level -0.9 *mPa.* Shoot length increase slightly at level of - 0.3 *mPa* in all cultivars and then reduced with the increase in PEG concentration. Results showed increase in root length as an adaptation with water deficient cause of PEG presence. Reduction in shoot length under drought condition result of an inhibition of cell division and elongation (Fraser, *et al.*, 1990); (Kamran *et al.,* 2009); (Chachar *et al.*, 2014a);( Chachar *et al.*, 2014b) and (Chachar *et al.,* 2016).

For seedling and shoot fresh weight (g/shoot), the shoot fresh weight values were decreased with increasing water stress in all examined cultivars, greatest shoot fresh weight recorded in T.R. cultivar 0.522 g/shoot as average at control level, followed by Condur. The reduction in shoot fresh weight was attributed to lower number and development of smaller leaves with increased PEG concentration. (Chachar *et al.*, 2014a);(Khakwani, *et al.,* 2011).

Also the greatest root fresh weight recorded in T.R. cultivar followed by Seri 0.1068 and 0.0868 g/root respectively. Mean values for shoot and root dry weight in different PEG concentration were recorded for shoot 0.098, 0.062, 0.033, 0.034 and 0.026 and for root 0.056, 0.026, 0.022, 0.015 and 0.011 in control, -0.3, -0.6, -0.9 and -1.2 *MPa* respectively, with no significant difference between drought levels up to -0.3 *MPa*.

Maximum shoot dry weight of T.R. cultivar under control condition 0.224g/shoot, minimum shoot dry weight was observed with Condur cultivar. Maximum root dry weight of T.R. cultivar under control condition 0.1360g/root, minimum root dry weight was observed with Condur cultivar. The decrease of shoot and root dry weight was reported by *(Kamran et al.,* 2009); (Ahmad *et al.,* 2013); (Chachar *et al.*, 2014a); ( Chachar *et al.*, 2014b) and (Chachar *et al.,* 2016) Who found that water stress had a significant effect on shoot and root dry weight. Root biomass is very important traits while selecting drought tolerant genotypes (Steven *et al.,* 2016)

Same observation noted for leaf surface area increase of drought level reduce leaf surface area of all wheat cultivar, the maximum leaf surface area recorded with T.R. variety with 4.44 cm<sup>2</sup> followed by Condur and Seri with 3.1734 and 3.1332 cm<sup>2</sup> respectively at control condition. The values decrease to 50% approximately with PEG concentration increase.

	$\boldsymbol{D}$ $\mathcal{f}$	Mean Square									
Source of variance		Shoot length $\left( cm\right)$	Root length $\left( cm\right)$	Root Number	Leaf Number	Leaf Surface $Area(Cm^2)$	seedling fresh $weight$	Shoot Fresh Weight	Shoot Dry Weight	Root Fresh Weight	Root Dry Weight
Cultivars	$\overline{2}$	44.739*	$89.468^\ast$	$1.764*$	$0.240^{ns}$	$13.253*$	$0.007^\ast$	$0.694*$	$0.061^{\ast}$	$0.023*$	$0.011^\ast$
Drought Levels	$\overline{4}$	128.591 $\ast$	108.744 $\ast$	$0.316^{ns}$	$0.400*$	$10.417*$	$0.014*$	$0.066^\ast$	$0.013*$	$0.006*$	$0.005^\ast$
Cultivar X S Drought Levels	8	23.522*	27.544*	$0.520^{ns}$	$0.240*$	$0.714^{ns}$	$0.002*$	$0.013*$	$0.012^\ast$	$0.002^{ns}$	$0.004*$
Error	6 $\theta$	2.402	5.524	0.584	0.094	1.061	0.001	0.003	0.001	0.001	0.001
Total	7 $\overline{4}$										

**Table 3: Analysis of variance for effect of cultivars and drought levels on growth parameters of three wheat cultivars.**

*Df: degree of freedom and \*: significant at 5 %. ns: nonsignificant*

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## **Conclusion**:

Water deficit, induced by polyethylene glycol (PEG) affected germination and seedling development. Wheat germination was affected at level at -0.3 *mPa*. The drought stress significantly reduced the seed germination shoot/root fresh and dry weight. Among cultivars tested T.R. variety is the tolerant one which have the potential to perform well under drought conditions followed by Seri and Condur which exerts sensitivity to water stress conditions at seedling stage parameters. We recommend T.R. variety in any breeding programed to obtains drought tolerant cultivars.



### **Table 4: Effect of different drought levels on growth parameters of three wheat cultivars**

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*Values in mean column sharing same letter are statistically no-significant at 5%.*

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**أتثري مستوايت خمتلفة من اجلفاف على معايري النمو واإلنبات لثالثة أصناف من القمح**

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## **امللخص**

مت تقييم إنبات ثالثة أصناف من القمح (.L aestivum Triticum (وهي CONDOR و SERI و. $T.R$  تحت خمسة مستويات من الجفاف ( 0.0 (الشاهد)، – 0.3 ، –0.6 ، –0.9 و  $-1.2$  ميجابيكسل)، وذلك ابستخدام البويل اثيلني جليكول 6000(6000PEG (glycol polyethylene ، وذلك لتوفري ظروف اجلفاف، مت عد البذور النامية يوميا إىل اليوم 14 حتت ظروف اإلنبات املعملية. تم أخذ القياسات لعملية الإنبات بقياس النسبة المئوية للإنبات ومتوسط زمن الإنبات ومعامل الإنبات ومتوسط سرعة اإلنبات، كما مت قياس الطول والوزن الرطب واجلاف لكل من اجملموع اخلضري واجملموع اجلذري وللنبتة كاملة، إضافة إىل عدد األوراق وعدد اجلذور ومساحة سطح الورقة كمقاييس للنمو، ومت حتليل هذه النتائج إحصائيا ابستخدام برانمج SPSS لتحديد الفروق املعنوية بني األصناف املدروسة وكذلك املعاجلات املستخدمة. مقارنة املتوسطات أظهرت أن فرقا معنواي عاليا قد سجل يف معظم مقاييس اإلنبات املدروسة كانت مع الصنف SERI متبوعا ابلصنف .R.T، أما معايري النمو سجل الفرق املعنوي فيها لصنف .R.T متبوعا ابلصنف SERI. وقد أثبتت النتائج أن بذور األصناف الثالثة أنبتت بشكل جيد عند املستوايت املنخفضة من البويل اثيلني جليكول 6000 والذي أثر على كل مقاييس اإلنبات والنمو.