The Iraqi Journal of Agricultural Sciences 43 (2) : 100-107, (2012) Hamza

SEED PRIMING OF BREAD WHEAT TO IMPROVE GERMINATION UNDER DROUGHT STRESS.

Jalal H. Hamza

Department of Field Crop Sciences / College of Agriculture / University of Baghdad

ABSTRACT

Experiments were conducted during 2011 in the laboratories of the Davy's building, School of Biomedical and Biological Sciences, University of Plymouth, UK. The experiment aimed to improve the germination of wheat bread seed (*Triticum aestivum* L. cv. Caxton) under drought stress via priming, and to determine the levels of drought stress that make seed priming not recommended. Primed and non primed seeds (control) and several levels of negative potentials (0, -0.5, -1, -1.5 and -2 MPa) as drought stress were tested. A completely randomized design with four replicates was used. The results showed that primed seed gave faster ending of germination (LDG), little difference in germination speed between the fast and slow germination members of a seed lot (TSG), faster population of seeds had germinate (MGT), highest first count (FG), highest final count (FIG), highest rapidity of germination which increases when the number of germinated seeds increases and the time required for germination decreases (CVG), highest and faster percentage of germination on each day of the germination period (GRI) compared with control treatment (non primed). Increasing of negative potentials up to -2 MPa led to an increase in LDG, TSG, and MGT, and a decrease in FG, FIG, CVG, and GRI compared with zero potential. Primed seeds had positive effect on (LDG), (TSG), (MGT), (FG), (CVG) and (GRI) compared with non primed seed under the same negative potentials. Whereas improved the (FIG) up to -1 MPa. We conclude, that technique of seeds priming can be use to improve the viability and vigour of seeds under drought stress ≤ -1 MPa through the escaping mechanism. On other hand, it's not recommend if the drought stress > -1 MPa.

مجلة العلوم الزراعية العراقية – 43(3): 100-107، (2012) حمزة

تنشيط بذور حنطة الخبز لتحسين انباتها تحت اجهاد الجفاف.

جلال حميد حمزة

قسم علوم المحاصيل الحقلية / كلية الزراعة / جامعة بغداد

المستخلص

نفذت تجربة مختبرية خلال العام 2011 في مختبرات كلية العلوم في جامعة بليموث في بريطانيا. هدفت الدراسة الى تنشيط بذور حنطة الخبز (*Triticum aestivum* L. cv. Caxton) لتحسين انباتها تحت اجهاد الجفاف ، وكذلك لتحديد مستوى اجهاد الجفاف الذي يكون عنده اثر تنشيط البذور سلبياً. نفذت تجربة عاملية وفق التصميم العشوائي الكامل باربع مكررات. العامل الاول عبارة عن بذور منشطة وغير منشطة (معاملة المقارنة) ، والعامل الثاني عبارة عن عدة مستويات من الجهود الازموزية السالبة (0 و −0.5 و −1 و −1.5 و −2 ميكاباسكال) والتي تمثل اجهاد الجفاف. اظهرت النتائج ان تنشيط البذور اعطى اسرع نهاية للانبات (LDG) واقل فرق في سرعة الانبات بين الانبات السريع والبطيء لكمية البذور (TSG) واقل متوسط لزمن الانبات (MGT) واعلى نسبة انبات في العد الاول (FG) واعلى نسبة انبات في العد النهائي (FIG) واعلى سرعة انبات والتي تزداد عند زيادة نسبة الانبات ونقصان الوقت اللازم للانبات (CVG) واعلى واسرع انبات في كل يوم من مدة الانبات (GRI) مقارنة بمعاملة المقارنة (البذور غير المنشطة). ان زيادة الجهود السالبة لغاية −2 ميكاباسكال ادى الى زيادة (LDG) و (TSG) و (MGT) وانخفاض (FG) و (FIG) و (CVG) و (GRI) مقارنة مع الجهد صفر. تنشيط البذور كان له تأثيراً ايجابياً لتحسين كل من (LDG) و (TSG) و (MGT) و (FG) و (CVG) و (GRI) مقارنة مع البذور غير المنشطة تحت الجهود الازموزية السالبة نفسها ، في حين ان هذا التأثير الايجابي لتنشيط البذور كان لغاية الجهد الازموزي السالب −1 ميكاباسكال في نسبة الانبات في العد النهائي (FIG). نستنتج ان استخدام تقنية تنشيط البذور يمكن ان يحسن حيوية وقوة البذور تحت ظروف اجهاد الجفاف ≤ −1 ميكاباسكال من خلال الهروب منه ، ومن ناحية اخرى لانوصي بتنشيط البذور اذا كان اجهاد الجفاف > −1 ميكاباسكال.

Introduction

Priming can contribute to improve germination rate and seedling emergence in different plant species (2, 6, 7). Salehzade et al. (19) primed wheat seeds with polyethylene Glycol (PEG), KNO3 solutions (-0.3, -0.6 and -0.9 MPa) for 12 h, and reported that priming treatments gave minimum time to 50% germination, and an increase in seedling dry weight with PEG at -0.6 MPa.

Drought is much more extensive loss of water, which can potentially lead to gross disruption of metabolism and cell structure, inhibits cell enlargement more than cell division, and leads to stomatal closure and limitation of gas exchange (5, 12, 21). There was decrease in FIG and seedling growth with increase in osmotic potentials (0, 15, 20 and 25% PEG), the maximum value for FIG, GRI, shoot length, root length, coleoptile length, fresh shoot weight, dry shoot weight, fresh root weight and dry root weight was observed in control (distilled water) and minimum value was observed in 25% PEG (18). A significant decrease was observed with increase in negative potentials (0, -0.3, -0.6, -0.9, -1.2, -1.5 MPa) in all germination indices in wheat, the length of stem among the other traits had more sensitivity to drought stress, negative potentials reduces the radical length at more than -0.3 MPa, FIG and CVG lessened when negative potentials exceeded more than -0.9 MPa (11). Abd El-Moneim et al. (1) reported that seed germination decreased as osmotic potential (0, -0.5, -1, -1.5 MPa) became more negative, inhibition of seed germination was greatest under the highest negative potential (-1.5 MPa), and cumulative germination after ten days ranged from 52.6 to 97.9% for the control compared to 27.4 to 69.8% at -1.5 MPa indicating more pronounced differences among genotypes at the highest negative potentials. Osmotic stress 10 and 20% PEG reduced FIG, growth rate, vigor index, dry weight and length of seedling and increased leaf cells electrolyte leakage, the effect of 20% PEG was more severe (15). The objectives of this study were improve germination of bread wheat seed (*Triticum aestivum* L. cv. Caxton) under drought stress via priming, and to determine the levels of drought stress that make seed priming not recommended.

Materials and methods

A Lab. experiment was conducted at the laboratory of the Davy building, School of Biomedical and Biological Sciences, University of Plymouth, UK in 2011. The design of this was completely randomized design with four replicates. Primed and non primed seeds (control) and five levels of negative potentials as drought stress were tested by germination indices in wheat.

Solutions of Poly Ethylene Glycol (PEG) of molecular weight 6000 or above does not enter the apoplast or plant cells, water is withdrawn from the cell and the cell wall without damaging cell content (17, 22). Hence, PEG-6000 or above can mimic dry soil conditions more closely than solutions using low molecular weight osmotica which can be taken up by plant cells and can be toxic for plant growth. Therefore we used PEG for two purposes. First, it controlled water movement to the seed at priming stage. Second, it helped us getting negative potentials during seed germination and seedling growth.

Primed seeds were prepared by immersed (250 g) of seeds in 300 ml of liquid having negative potential (-1 MPa) for 6 hr at 20 ºC in plastic containers covered with caps to prevent evaporation loss in dark incubator. After priming, samples of seeds were removed and rinsed many times in tap water and then dried to the original moisture (circa 12%) by subject it to 20 ±1 ºC and 50% RH for 24 hr. Digital Promimeter was used for measuring and monitoring the moisture content during the drying period. This treatment of negative potential (-1 MPa) was prepared by dissolving calculated amounts of PEG 6000 in distilled water (21.9 g PEG 6000.100^{-1} ml) (as priming treatment). Negative potential of solutions were measured by OSMOMET advice.

The treatments which used to get negative potentials during seed germination and seedling growth were prepared by dissolving separately calculated amounts of PEG 6000 in distilled water (0, 17.0, 21.9, 25.3, 28.2 g PEG 6000.100^{-1} ml) to get (0, -0.5, -1, -1.5, -2 MPa), respectively.

Fifty seeds from each treatment with four replicates were placed in 140 mm diameter Petri dishes on two layers of Whatman No.1 filter paper moistened with 40 ml of each negative potential. The Petri dishes were placed in dark incubator at 20 ºC. Seeds were considered germinated when they exhibited radicle extension > 2 mm. The germination percent was daily recorded up to 8 days.

Several attributes were studied such as, FDG (d) which means the day on which the first germination event occurred, lower FDG values indicate a faster initiation of germination (13). LDG (d) which means the day on which the last germination event occurred, lower LDG values indicate a faster ending of germination (13). TSG (d) which means the time in days between the first and last germination event occurring in a seed lot, the higher TSG value, the greater difference in germination speed between the fast and slow germinating members of a seed lot (13). Germination percentage (%) as first count (FG) (after 4 days) and germination percentage (%) as final count (FIG) (after 8 days) (3, 10). Germination (%) = 100 * (n/N). Where n is the number of germinated seed and N is the number of sowed seeds. Coefficient of velocity of germination (CVG) gives an indication of the rapidity of germination. It increases when the number of germinated seeds increases and the time required for germination decreases. Theoretically, the highest possible CVG is 100. This would occur if all seeds germinated on the first day. It can be calculated by equation 1 (14). Germination rate index (GRI) reflects the percentage of germination on each day of the germination period. Higher GRI values indicate higher and faster germination. It can be calculated by equation 2 (13). Mean germination time (MGT) which means the lower MGT is the faster a population of seeds has germinated. It is calculated by equation 3 (13). These indicators were calculated to investigate the effects of priming on the germination of seed under drought stress.

Equation 1: $CVG\ (\%.d^{-1}) = 100 * \Sigma Ni / \Sigma (NiTi))$

Equation 2: $GRI\ (\%.d^{-1}) = \Sigma\ (Ni / i)$

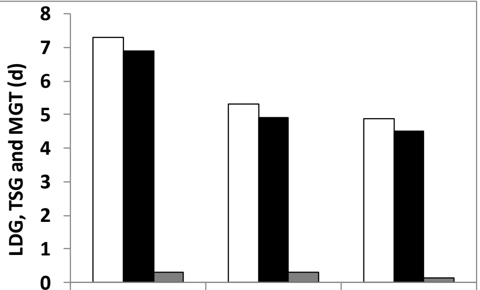
Equation 3: $MGT\ (d) = \Sigma\ (NiTi) / (\Sigma Ni)$

Where N is the percentage of germinated seed in day i, and Ti is the sequence of day from sowing seed. All data were subjected to analysis of variance (ANOVA) using SPSS software (version 17) and comparisons of means were made using the least significant difference test (LSD) at 5 % level of probability (16).

Results and Discussion

The effect of priming on attributes studied

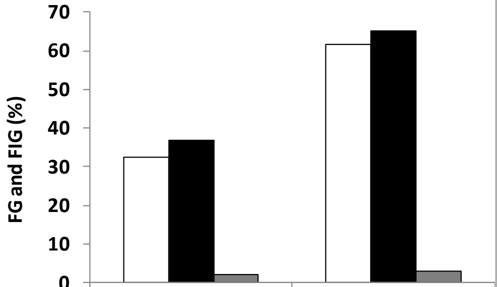
The statistical analysis showed that all attributes studied had significant positive response to the effect of priming compared with the non primed seeds, except the first day germination which was after 3 days for all treatments, and that means the initiation of germination was the same. Primed seed gave faster ending of germination (LDG) (6.90 d), little difference in germination speed between the fast and slow germinating members of a seed lot (TSG) (4.90 d), faster population of seeds had germinate (MGT) (4.50 d) (figures 1), highest FG (36.8 %), highest FIG (65.1 %) (figures 2), highest rapidity of germination which increases when the number of germinated seeds increases and the time required for germination decreases (CVG) (22.45 % d^{-1}), highest and faster percentage of germination on each day of the germination period (GRI) (16.19 % d^{-1}) (figures 3) compared with control treatment (Non primed) which gave (7.30 d, 5.30 d, 32.40 %, 61.60 %, 21.01 % d^{-1}, 14.38 % d^{-1}, and 4.87 d, respectively). The improvement in attributes studied in primed seeds (figures 1, 2, 3), agree with other results which showed that seed priming can lead to fast, uniform of germination, seedling growth, establishment and higher yield in many crops such as maize, wheat and rice (4, 8, 9, 20).



	LDG	TSG	MGT
□ Non primed seed	7.30	5.30	4.87
■ Primed seed	6.90	4.90	4.50
▪ LSD 0.05	0.29	0.29	0.15

Studied attributes

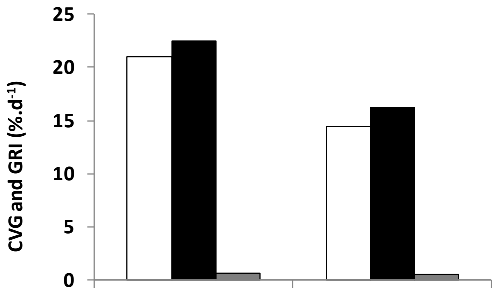
Figure 1. Effects of seed priming on LDG, TSG and MGT (d).



	FG	FIG
□ Non primed seed	32.40	61.60
■ Primed seed	36.80	65.10
▪ LSD 0.05	1.92	2.79

Studied attributes

Figure 2. Effects of seed priming on FG and FIG (%).



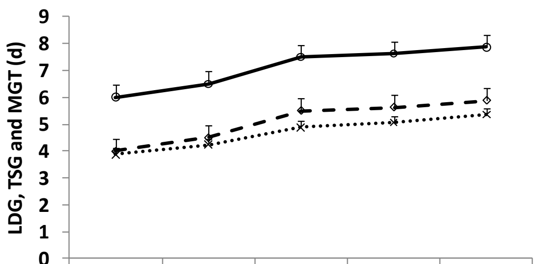
	CVG	GRI
□ Non primed seed	21.01	14.38
■ Primed seed	22.45	16.19
▪ LSD 0.05	0.61	0.51

Studied attributes

Figure 3. Effects of seed priming on CVG and GRI (%.d^{-1}).

The effect of drought stress on attributes studied

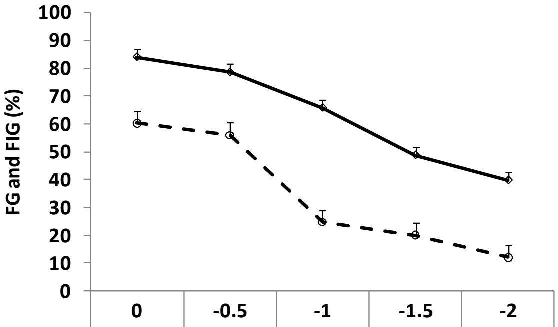
The statistical analysis showed that all attributes studied had significant negative response to the effect of drought stress (negative potentials) compared with zero potential, except the first day germination had no response. Increasing of negative potentials up to -2 MPa led to an increase in LDG, TSG, and MGT (7.88, 5.88, 5.73 d respectively) compared with zero potential (6.00, 4.00, 3.88 d, respectively) (figures 4). Also increasing negative potentials led to a decrease in the values of FG, FIG, CVG, and GRI (12.00, 39.75 %, 18.82 and 8.17 % d^{-1}, respectively) compared with zero potential (60.25, 84.00 %, 25.80 and 22.88 % d^{-1}, respectively) (figures 5, 6). The deterioration in attributes studied was coincided with the increasing in negative potentials (figures 4, 5, 6). Our finding agree with Abd El-Moneim et al. (1) who reported that seed germination decreased as osmotic potential (0, -0.5, -1, -1.5 MPa) became more negative and inhibition of seed germination was greatest under the lowest osmotic potential, -1.5 MPa.



	0	-0.5	-1	-1.5	-2
LDG	6.00	6.50	7.50	7.63	7.88
TSG	4.00	4.50	5.50	5.63	5.88
MGT	3.88	4.23	4.89	5.06	5.37

Drought stress (negative potentials)

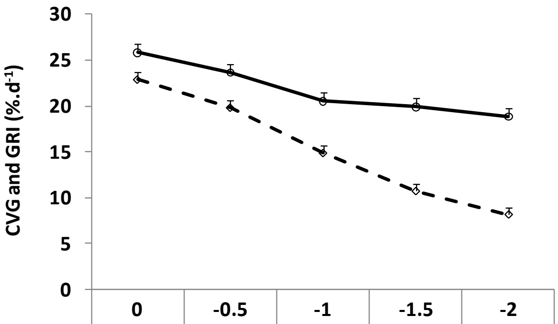
Figure 4. Effects of drought stress on LDG, TSG and MGT (d). Bars are value of LSD at 0.05



	0	-0.5	-1	-1.5	-2
FG	60.25	56.00	24.75	20.00	12.00
FIG	84.00	78.50	65.75	48.75	39.75

Drought stress (negative potentials)

Figure 5. Effects of drought stress on FG and FIG (%). Bars are value of LSD at 0.05



	0	-0.5	-1	-1.5	-2
CVG	25.80	23.64	20.50	19.89	18.82
GRI	22.88	19.81	14.87	10.72	8.17

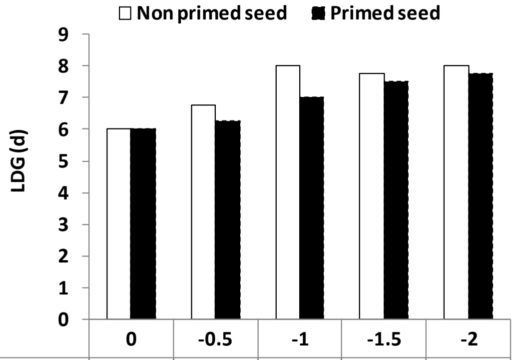
Drought stress (negative potentials)

Figure 6. Effects of drought stress on CVG and GRI (%.d^{-1}). Bars are value of LSD at 0.05

The effect of interaction between seed priming and drought stress on attributes studied

The result showed that both factors affected all attributes studied, except the FDG. It was the same (3rd d) under the effect of seed priming, drought stress (negative potentials) and their combinations. Despite an apparent superiority of primed seed compared with non primed seed under the same negative potentials, the statistical analysis showed no significant effect for the interaction between primed seed and drought stress on LDG, TSG and FG (figures 7, 8, 9).

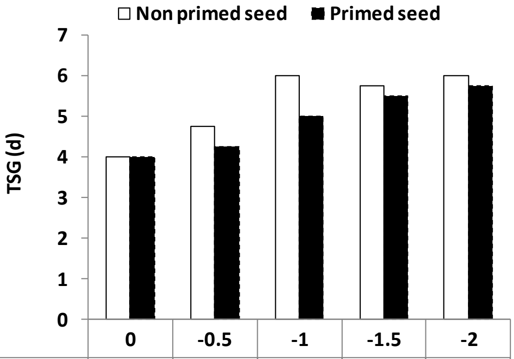
A significant effect for the interaction between primed seed and drought stress was found on MGT, FIG CVG and GRI (figures 10, 11, 12, 13).



	0	-0.5	-1	-1.5	-2
Non primed seed	6.00	6.75	8.00	7.75	8.00
Primed seed	6.00	6.25	7.00	7.50	7.75

Drought stress (negative potentials)

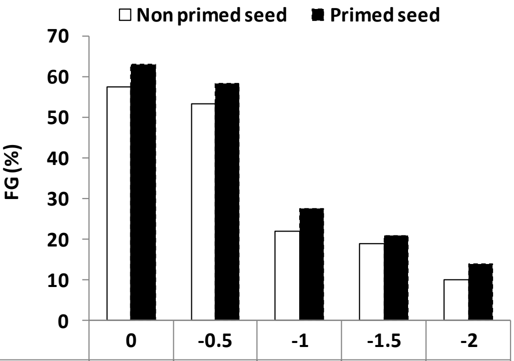
Figure 7. Effects of interaction between seed priming and drought stress on LDG (d). LSD at 0.05 is N.S



	0	-0.5	-1	-1.5	-2
Non primed seed	4.00	4.75	6.00	5.75	6.00
Primed seed	4.00	4.25	5.00	5.50	5.75

Drought stress (negative potentials)

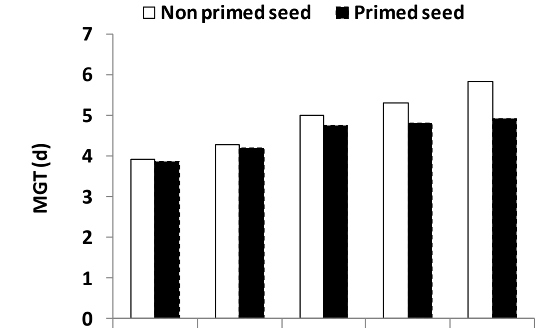
Figure 8. Effects of interaction between seed priming and drought stress on TSG (d). LSD at 0.05 is N.S



	0	-0.5	-1	-1.5	-2
Non primed seed	57.50	53.50	22.00	19.00	10.00
Primed seed	63.00	58.50	27.50	21.00	14.00

Drought stress (negative potentials)

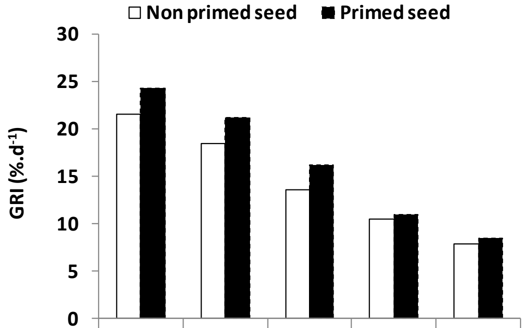
Figure 9. Effects of interaction between seed priming and drought stress on FG (%). LSD at 0.05 is N.S



	0	-0.5	-1	-1.5	-2
Non primed seed	3.92	4.28	5.01	5.31	5.83
Primed seed	3.85	4.18	4.76	4.80	4.91

Drought stress (negative potentials)

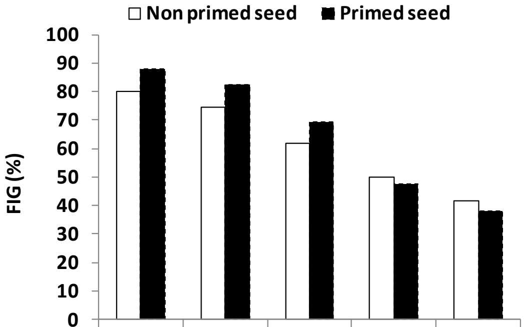
Figure 10. Effects of interaction between seed priming and drought stress on MGT (d). LSD at 0.05 is 0.33



	0	-0.5	-1	-1.5	-2
Non primed seed	21.52	18.48	13.58	10.45	7.89
Primed seed	24.23	21.15	16.15	10.99	8.45

Drought stress (negative potentials)

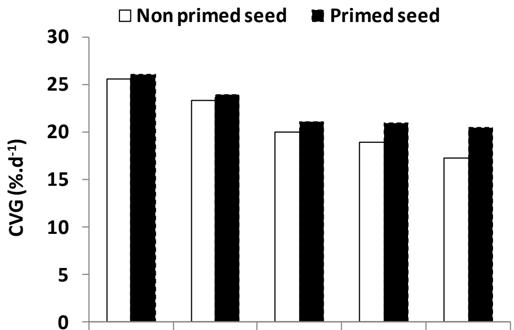
Figure 13. Effects of interaction between seed priming and drought stress on GRI (%.d^{-1}). LSD at 0.05 is 1.15



	0	-0.5	-1	-1.5	-2
Non primed seed	80.00	74.50	62.00	50.00	41.50
Primed seed	88.00	82.50	69.50	47.50	38.00

Drought stress (negative potentials)

Figure 11. Effects of interaction between seed priming and drought stress on FIG (%). LSD at 0.05 is 6.23



	0	-0.5	-1	-1.5	-2
Non primed seed	25.58	23.35	19.96	18.91	17.23
Primed seed	26.02	23.93	21.04	20.87	20.40

Drought stress (negative potentials)

Figure 12. Effects of interaction between seed priming and drought stress on CVG (%.d^{-1}). LSD at 0.05 is 1.37

Results showed that the decrease in LDG, TSG, MGT, and the increase in FG, CVG and GRI had associated with primed seed compared with non primed seed under the same negative potentials.

Results showed that how priming made seeds tolerant to the drought stress through the improvement of seed's behavior and decrease the time of LDG, TSG and MGT compared with non primed seed under the same negative potentials (figures 7, 8, 10).

Results of FG, CVG and GRI showed that priming had improved the performance of seeds through enhancement the speed of germination compared with non primed seed when both exposed to the same negative potentials (figures 9, 12, 13). Priming of seeds before planting may hastened the stages of metabolism, development and division of embryo cells to germinate in shorter time with higher rate compared with non primed seeds.

Speed of germination alone is insufficient unless accompanied with the highest final percentage of germination. Our results showed that speed of germination accompanied with the highest final percentage of germination was only possible when the osmotic potentials were 0 or -0.5 or -1 MPa, but not under negative potentials -1.5 or -2 MPa. By comparing results in figure 11 vs figures 9, 12 and 13 we noticed that the activation process requires the availability of others factors of growth like moisture. This means that there is a need of providing adequate moisture in time to complete the process of germination till the final count, not only to get high speed of germination.

Results in figure (11) showed that primed seed had adequate available moisture in time up to -1 MPa to imbibed seeds and activates the metabolism to give the highest percentage of germination at final count compared with non primed seed under the same negative potentials. Then the final percentage of germination began to decline, because the effect of priming under the negative potentials (-1.5 or -2 MPa) had reversed impact compared with non primed seeds under the same negative potentials. Therefore, non primed seeds gave final count higher than primed seed under the same levels (-1.5, -2 MPa). This might be due to the low rate of metabolism in non primed seeds compared with primed seed during germination. This coincides with a slow seeds' imbibing under high osmotic potential of water which gave the seed the advantage to use the available water in time in spite of being under high negative potentials.

We conclude, that technique of seeds priming can be used to improve the viability and vigour of seeds under drought stress through the escaping mechanism, which happens by reduce some of the need of seeds from moisture to complete the process of germination, as seeds completed a part of metabolic processes during the pre-activation that preceded seeds planting. Also, the technique of seeds priming is ineffectiveness under negative potentials higher than -1 MPa, because it gave negative impact by reducing the final percentage of germination compared with non primed seed. Therefore, seed priming is not recommend if the drought stress (negative potential) more than -1 MPa.

References

1.Abd El-Moneim, D.A., I.N. Mohamed, A.H. Belal and M.E. Atta. 2010. Screening bread wheat genotypes for drought tolerance: Germination, radical growth and mean performance of yield and its components.Options Méditerranéennes, 95: 301-305.

2.Ashraf, M. and M.R. Foolad. 2005. Pre-sowing seed treatment-a shotgun approach to improve germination growth and crop yield under saline and none-saline conditions. Advan. Agron., 88: 223-271.

3.Association of Official Seed Analysts. 1988. Rules for Testing Seeds. J. of Seed Technol., 12(3): 14-20.

4.Basra, S.M.A., M. Farooq, R. Tabassam and N. Ahmad. 2005. Physiological and biochemical aspects of pre-sowing seed treatments in fine rice (Oryza sativa L.). Seed Sci. Technol., 33: 623-628.

5.Farooq, M., S.M.A. Basra, A. Wahid, Z.A. Cheema, M.A. Cheema and A. Khaliq. 2008. Physiological role of exogenously applied glycinebetaine in improving drought tolerance of fine grain aromatic rice (Oryza sativa L.). J. Agron. Crop Sci., 194: 325–333.

6.Fu, J.R., X.H. Lu, R.Z. Chen, B.Z. Zhang, Z.S. Liu, Z.S. Li and D.Y. Cai. 1988. Osmoconditioning of peanut Arachis hypogaea (L.) seeds with PEG to improve vigour and some biochemical activities. Seed Sci. Technol., 16: 197-212.

7.Gao, Y.P., L. Young, P. Bonham-smith and L.V. Gusta. 1999. Characterization and expression of plasma and tonoplast membrane aquaporins in primed seed of Brassica napus during germination under stress conditions. Plant Mol. Biol., 40: 635-444.

8.Ghiyasi, M., A.A. Seyahjani, T. Mehdi, A. Reza and S. Hojat. 2008b. Effect of osmopriming with polyethylene glycol (8000) on germination and seedling growth of wheat (Triticum aestivum L.) seeds under salt stress. Res. J. Biol. Sci., 3: 1249-1251.

9.Ghiyasi, M., M.P. Miyandoab, M. Tajbakhsh, H. Salehzade and M.V. Meshkat. 2008a. Influence of different osmopriming treatments on emergency and yield of maize (Zea mays L.). Res. J. Biol. Sci., 3: 1452-1455.

10.International Seed Testing Association. 1993. International Rules for Seed Testing. Seed Sci. Technol., 21: 142-168.

11.Jajarmi, V. 2009. Effect of water stress on germination indices in seven wheat cultivar. World Acad. Sci. Eng. Techno., 49: 105-106.

12.Jaleel, C.A., P. Manivannan, A. Wahid, M. Farooq, H.J. Al-Juburi, R. Somasundaram and R. Panneerselvam. 2009. Drought stress in plants: a review on morphological characteristics and pigments composition. Int. J. Agric. Biol., 11(1): 100–105.

13.Kader, M.A. 2005. A comparison of seed germination calculation formulae and the associated interpretation of resulting data. J. and Proceeding of the Royal Society of New South Wales, 138: 65-75.

14.Kader, M.A. and S.C. Jutzi. 2004. Effects of thermal and salt treatments during imbibition on germination and seedling growth of sorghum at 42/19°C. J. Agron. Crop Sci., 190: 35-38.

15.Maghsoudi, K. and M.J. Arvin. 2010. Salicylic acid and osmotic stress effects on seed germination and seedling growth of wheat (Triticum aestivum L.) cultivars. Plant Ecophysiology, 2: 7-11.

16.Quinn, G.P. and M.G. Keough. 2005. Experimental Design and Data Analysis for Biologists. 4th edn, Chapter 8, pp. 173-207. Cambridge University press, The Edinburgh Building, Cambridge CB2 2RU, UK.

17.Ranjbarfordoei, A., R. Samson, P. Van Damme and R. Lemeur. 2000. Effects of drought stress induced by polyethylene glycol on pigment content and photosynthetic gas exchange of Pisttacia khinjuk and P. mutica. Photosynthetica, 38: 443-447.

18.Rauf, M., M. Munir, M. Ul Hassan, M. Ahmad and M. Afzal. 2007. Performance of wheat genotypes under osmotic stress at germination and early seedling growth stage. African J. of Biotech. 6(8): 971-975.

19.Salehzade, H., M.I. Shishvan, M. Ghiyasi, F. Forouzin and A.A. Siyahjani. 2009. Effect of seed priming on germination and seedling growth of wheat (Triticum aestivum L.). Res. J. Biol. Sci., 4(5): 629-631.

20.Sharafzadeh, F., H.H. Zolleh, H. Mohamadi and M. Janmohamadi. 2006. Study of osmotic priming effects on wheat (Triticum aestivum L.) Germination in different temperatures and local seed masses. J. Agron., 5: 647-650.

21.Smirnoff, N. 1993. The role of active oxygen in the response of plants to water deficit and desiccation. New Phytol., 125: 27–58.

22.Van den Berg, L. and Y.J. Zeng 2006. Response of South African indigenous grass species to drought stress induced by ployethylen glycol (PEG) 6000. S. Afr. J. Bot., 72: 284-286.