SEED PRIMING APPLICATION EFFECT ON ALLEVIATION OF DROUGHT STRESS IMPACTS DURING GERMINATION IN SUNFLOWER HYBRIDS (*HELIANTHUS ANNUUS* L.)

Saman SHEIDAEI, Hosseın SADEGHI, Bıta OSKOUEI, Mohammad RAHMANI

Seed and Plant Certification and Registration Institute, Iran, Karaj (SPCRI).

ABSTRACT

In order to study the effect of different seed priming techniques on germination and early growth of sunflower under drought stress conditions, a factorial experiment based on completely randomized design with 3 replications was conducted in the laboratory of Seed and Plant Certification and Registration Institute. Seeds of two sunflower cultivars; Azargol and Hysun-36 were pre-treated with 5 treatments including: 2 osmopriming concentrations of Potassium Nitrate (KNO3); 500 and 1000ppm, two hydro-priming with distilled water in two durations of 12 and 18 h and a control treatment without priming. After priming, seeds were placed on different osmotic drought conditions for germination test and early growth evaluation. Osmotic conditions were provided by PEG-6000 in 3 osmotic potential levels; -0.3, -0.6 and -0.9 MPa and one control condition of 0 MPa. Results showed that, The lowest seed germination percentage and early growth occurred at -0.9 MPa for both cultivars and priming with 1000 ppm KNO3 increased seed adaptation to osmotic conditions because the highest germination and growth under osmotic condition observed in this treatment. Hysun-36 showed to be more drought tolerant so that highest germination and growth in osmotic dry condition demonstrated for this cultivar. There were no significant difference in seed germination and early growth performance under osmotic drought between hydro-priming 18h and non-primed control. This results revealed that to gain a better germination and seedling stablishment in dry cultivation, osmo-priming with 1000 ppm KNO3 may be beneficial.

Keywords: sunflower, seed priming, germination percentage, drought stress and seedling vigor index

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the most important oil seed crops in Iran. It is a high yielding oilseed crop, but under scarce conditions, the yield is very lower than its real potential. Among the factors responsible for the low yield, imbalance use of fertilizer, improper plant protection, poor growth and sub optimum plant population are rather important. Suboptimum plant population generally results from poor and erratic germination (Barsa et al., 2003). Recently, salt and drought stress are perhaps the two most important abiotic stresses that limit plant growth and development(Elhafid et al., 1998). A good strategy is the selection of cultivars and species for salinity and drought conditions(Ashraf et al., 1992). But an alternative strategy for the possibilities to overcome salt and drought stresses is by seed treatments with hydro priming or other treatments(Yagmur and Kaydan, 2008). Seed priming is now a widely used commercial process that accelerates the germination rate and improves seedling uniformity in many crops(Halmer, 2003; Taylor and Harman, 1990). Priming allows some of the metabolic processes necessary for germination to occur without germination take place. The beneficial effects of priming have also been demonstrated for many field crops such as wheat, sugar beet, maize, rice, soybean and sunflower (Parera and Cantliffe, 1994; Singh, 1995; Khajeh-Hosseini, 2003; Yari and Sheidaie, 2011; Sadeghian and Yavari, 2004). Seed priming with Potassium Nitrate (KNO_3) had shown good potential to enhance germination, emergence and seedling dry weight of Sunflower(Kaya et al., 2006; Singh and Rao, 1993), corn(Basra et al., 1989) and soybean(Saadateyan et al., 2009). Hydropriming method has also been used successfully in sunflower(Kaya et al., 2006; Saadateyan et al., 2009), wheat(Harris et al., 2001), Rice(Yari and Sheidaie, 2011) and cotton(Casenave and Toselli, 2007). Moreover hydro priming increased germination and seedling growth under salt and drought stresses(Kaya et al., 2006; Saadateyan et al., 2009).

The present study was, therefore, carried out with the objective to evaluate the effects of seed priming treatments under drought stress conditions on germination and the seedling growth of hybrids sunflower.

MATERIALS AND METHODS

The experiment was conducted in the laboratory of the department of seed and plant certification &registration Institute, Iran in 2011. Experimental units were arranged factorials in a completely randomized design (CRD) with three replications.

Seed material: Seed materials of Sunflower hybrids(Hysun-36 and Azargol), which selected on the basis of their area that planted in Iran.

Priming techniques: Priming media were used such as distilled water, 1000 ppm Potassium Nitrate (KNO3); 500ppm Potassium Nitrate (KNO_3); and control. All priming media were prepared in distilled water. Seed was fully immersed in KNO_3 priming media at a temperature of 25°C for durations of 2 hours and immersed in distilled water of 25°C for durations of 12 and 24 hours under dark conditions. Seed were removed from priming media at the same time, then rinsed thoroughly with distilled water and lightly dried using blotting paper and then allowed to dry on paper towels at room temperature. Control treatment consists of untreated seed.

Osmotic stress of PEG_{6000}: Three drought stresses with different osmotic potentials of -0.9,-0.6 and -0.3MPa were arranged as described by Michel and Kaufmann(1973). The osmotic potential of control solution was 0 MPa. The osmotic potentials of PEG_{6000} were read with a Wescor Vapour Pressure Osmometer-5520.

Seed germination test and seedling growth: The treated and untreated seeds were then transferred to Petri dishes (50 seeds per Petri dish with three replications) containing two (whatman No.2) filter paper moistened with 10ml of control solution or the same solution added with PEG6000. Petri dishes were placed in germinator at 25±1°C under dark conditions. The Petri dishes were controlled in one day intervals for solutions content. Germination was recorded daily up to day 7 after the start of the experiment. A Seed was considered germinated when radical emerged by about 2mm in length (ISTA, 2003).

Mean germination time, germination percentage, germination rate and vigor seedling index were calculated as described in Ellis and Roberts(1981).

MGT (day) $=\sum NiDi/N$ (1)

Where *Di* is the number of days after sowing, *Ni* is the number of seeds germination on i^{th} day, N is the total number of germinated seeds.

Germination Percentage (GP) was calculated as $GP = \frac{\text{Total seeds germination}}{\text{Total number of seeds}} \times 100$ (2)

Germination Rate (GR) $=\sum Ni/\sum TiNi$ (3)

Where Ni is the number of newly germinated seeds at time Ti.

The Seedling Vigor Index(SVI) was calculated as the product of seedling dry weight by germination percentage.

Seedling Vigor index(VI)=SDW×GP (4) Where SDW is seedling dry weight at the end of test and GP% is the final germination percentage. Final germination percentage, seedling length, radical length, stems length, seedling dry weights were recorded 7 days after cessation of the experiment. MSTATC computer software was used to carry out statistical analysis (Russel and Eisensmith, 1983**)**. The significance between the means were compared using Least Significant Difference(LSD) values($P<0.05$).

RESULTS

Germination: Germination percentage was influenced by seed priming treatments and drought osmotic stress conditions. The results indicated that Hysun-36 hybrid showed a significantly less decline in germination percentage under drought stress conditions in comparison to Azargol. Germination percentage under drought stress (-0.3, -0.6 and -0.9MPa of PEG) was increased by Seed primed with KNO_3 and water(12h) compared to untreated seeds(Table1). However, hydro priming seeds for 18h indicated no significant differences with untreated ones in percent germination(Table1).

Mean Germination Time: A significant three-way Interaction (hybrid, seed treatment and stress) was found for MGT ($P<0.01$)(Table2). There was no significant difference in MGT among primed seeds under control drought stress condition (0 MPa PEG) for both Azargol and Hysun-36hybrids. Seed primed with 1000ppm KNO_3 reduced the time to start germination and MGT under -0.6 and -0.9 MPa osmotic potential of PEG compared with other treatments in Azargol hybrid. Also seed treated with 500 ppm KNO_3 and 1000ppm KNO_3 shortened the time to seed germination compared with other treatments in Hysun-36 hybrid. Whereas, hydro primed seeds for 18h had negative significant effect on MGT at (-0.3, -0.6 and -0.9 MPa) and -0.9 MPa of PEG in Azargol and Hysun-36hybrids, respectively (Table2).

Germination Rate (GR): A significant three-way Interaction(hybrid, seed treatment and stress) was found for GR ($P<0.05$). Seeds primed with 1000ppm KNO_3 and hydro priming enhanced rate of germination under control stress condition (0 MPa of PEG) in Azargol hybrid. Also seed treated with KNO_3(especially 1000ppm KNO_3) improved rate of germination at higher concentration of PEG for both Azargol and Hysun-36hybrids.

Root length: Results of comparison means of hybrid and osmotic stresses showed that Azargol hybrid had longer root length than Hysun-36 hybrid under control drought stress condition(0 MPa of PEG). In contrast; increasing concentration of PEG improved length of root in Hysun-36 hybrid. The result of this experiment showed that priming with 1000ppm KNO_3 and hydro priming(12h) increased root length under drought osmotic stress conditions when concentration of PEG increased.

Shoot length: Shoot length was significantly influenced by hybrid and osmotic stresses ($P<0.01$). Results of comparison means of hybrid and drought osmotic stress conditions showed that the highest shoot length was attained from Azargol hybrid under control drought stress condition(0MPa of PEG), but Hysun-36hybrid had longer Shoot length than Azargol hybrid under drought osmotic stress at -0.3 MPa of PEG. The results of comparison means of seed priming treatments and osmotic stresses indicated that in the conditions of 500 ppm KNO_3 and 1000ppm KNO_3 treatments under control drought stress condition(0 MPa of

PEG) the longest status was shown, but by increasing concentration of PEG, there were no significant differences in Shoot length among seed priming treatments(Data not shown).

Seedling dry weight: Results of comparison means of hybrid and drought osmotic stress conditions showed that Hysun-36hybrid had more Seedling dry weight in comparison to Azargol hybrid under drought osmotic stress conditions ($P<0.01$). Seedling dry weight was significantly affected by seed priming treatments. Seed subjected with 500ppm KNO_3, 1000ppm KNO_3 and hydropriming(12h) increased seedling dry weight compared with untreated seeds while hydro priming for 18h had negative effect on Seedling dry weight compared to untreated seeds(Table3).

Seedling Vigor Index: The results of comparison means of hybrid and osmotic stresses indicated that seedling vigor index of Hysun-36hybrid at -0.3 and -0.6MPa concentration of PEG was higher compared to Azargol hybrid. The results showed that by increasing drought osmotic stress, Hysun-36 hybrid have more potential resistance in germination stage compared with Azargol hybrid, under drought osmotic stress conditions. Results of priming media comparison means showed that higher seedling vigor index was recorded from applying 1000ppm KNO_3 priming treatment. The lowest Seedling Vigor Index was observed in untreated seeds. These findings indicated that primed sunflower seeds with KNO_3 could positively affect on seedling Vigor Index(Table 3).

DISCUSSION

In many crops pre-soaking or priming causes improvement in germination and seedling establishment (Harris et al. 2001). Increases in the seedling correlated with higher water uptake by primed seed resulted in higher seedling growth. The beneficial effects of KNO_3 on germination were found in this study .Final germination was higher from1000ppm KNO_3, Suggesting no toxicity of KNO_3 due to ion accumulation in the embryo, which is in support with the earlier findings (Demir and Venter, 1999; Kaya et al., 2006; Singh and Rao, 1993). Also seeds primed with 1000ppm KNO_3 and hydropriming(12h) improved rate of germination under drought osmotic stress conditions. these finding are line with Mvale et al(2003) reported that osmopriming seed improved germination rate in sunflower seeds. Also osmopriming has been shown to activate processes related to germination, through affecting the oxidative metabolism such as increasing superoxide dismutase(SOD) and peroxidase (POD)(Jie, 2002). Moreover, the present study revealed that seed treated with 500ppm KNO_3 and 1000ppm KNO_3 shortened the time to seed germination compared with other treatments. These finding are in line with Demir and avaenter(1999) who states that seed primed with KNO_3 reduced MGT and had positive effect on germination percentage in sunflower seeds. In contrast, hydro priming seeds for 18 hour had negative effect on MGT under -0.9 MPa osmotic potential of PEG. This could be explained by more rapid water uptake than the amount of water for germination in these hybrids. Also the results showed that by increasing drought osmotic stress, Hysun-36hybrid have more potential resistance in germination stage compared with Azargol hybrid. Increasing concentration of PEG improved length of root in Hysun-36hybrid.These findings support the earlier work of Beckman et al.(1993), who reported that increasing in length of root in switch grass by seed priming treatments.

Table1. Effect of seed priming treatments on germination percentage of hybrids sunflower under drought stress conditions

Seed priming treatments	Osmotic potentials(MPa) 0	-0.3	-0.6	- 0.9
Control	96.17 a	72.00 d	41.67 g	18.67 j
500ppm (KNO_3)	92.33 ab	73.67 cd	54.33 e	34.33 h
1000ppm (KNO_3)	96.17 a	78.83 c	54.33 e	46.17 fg
distilled water(12h)	94.67 a	78.50 c	49.67 ef	27.67 i
distilled water(18h)	91.83 ab	73.33 cd	45.5 fg	19.50 j

*Means with same letter are not significantly different at *LSD* ($P<0.05$)

Table2. Effect of seed priming treatments on Mean Germination Time of hybrids sunflower under drought stress conditions

		Osmotic potentials(MPa)			
Hybrid	treatments	0	-0.3	-0.6	-0.9
Azargol	Control	1.69 P	2.25 l	3.24 h	4.63 d
Azargol	500ppm (KNO_3)	1.64 P	2.19 lmn	3.22 h	4.70 cd
Azargol	1000ppm (KNO_3)	1.67 P	2.13 lmno	2.95 i	3.93 ef
Azargol	distilled water(12h)	1.63 P	2.21 lm	3.21 h	4.68 cd
Azargo	distilled water(18h)	1.68 P	2.61jk	3.75 fg	5.11 b
Hysun-36	Control	1.98 o	2.75 ij	4.0 1e	5.31 b
Hysun-36	500ppm (KNO_3)	1.98 o	2.53 k	3.76 fg	4.84 c
Hysun-36	1000ppm (KNO_3)	1.99 mno	2.53 k	3.64 g	4.71 cd
Hysun-36	distilled water(12h)	1.98 no	2.92 i	4.04 e	5.3 b
Hysun-36	distilled water(18h)	1.01mno	2.95 i	4.01 e	5.65 a

*Means with same letter are not significantly different at *LSD* ($P<0.05$)

Table3. Effect of seed priming treatments on seedling dry Weight (g) and Vigor index of sunflower

Seed priming treatments	Seedling dry Weight(g)	Vigor index
Control	25.79 b	19.45 c
500ppm (KNO_3)	28.15 a	22.5 ab
1000ppm (KNO_3)	28.44 a	23.84 a
distilled water(12h)	27.64 a	22.38 ab
distilled water(18h)	26.93 ab	21.22 b

* Means with same letter are not significantly different at LSD ($P<0.05$)

CONCLUSIONS

Overall it could be concluded that suitable priming the sunflower seeds was 1000ppm KNO_3 resulted in higher germination percentage and seed vigor under drought osmotic stress conditions. Therefore, priming with KNO_3 may be an efficient method to overcome seed germination problems and to improve seedling growth in field, especially under drought conditions. Hydro priming for 18 h had negative effect on Seedling dry weight compared to untreated seeds. It was concluded that increasing of hydro priming time may have negative impact on germination and seedling growth in hybrids of sunflower used in this experiment. Also Hysun-36hybrid have more potential resistance under drought stress conditions in germination stage compared with Azargol hybrid and it could be suitable for planting at this conditions.

LITERATURE

Ashraf M, Bokhariand H, Cristiti SN.1992. Variation in osmotic adjustment of lenti(Lens culimaris Medic.)in response to drought.Acta Bot Neerlandica 41:51-62

Basra SMA, Mehmood MN, Afzal I, Basra AS, Dhillon R, Malik CP. 1989. Influence of seed pretreatment with plant growth regulators on metabolic alterations of germinating maize embryos under stressing temperature regimes. Ann Bot (London) 64:37-41

Basra SMA, Zia MN, Mehmood T, Afzal I, Khaliq A. 2003. Comparision of different invigoration techniques in wheat *Triticum aestivum*L. Seeds.Pak J Arid Agr 5:11-17

Beckman JJ, Moser LE, Kubikand K, Waller S. 1993. Big Bluestem and Swithgrass Estblishment as Infuenced by Seed Priming.University of Nebraska-Lincoln Agronomy and Horticulture Department

Casenave EC, Toselli ME. 2007. Hydropriming as a pre-treatment for cotton germination under thermal and water stress conditions. Seed Sci Technol 35:88-98

Demir I, Venter HA. 1999. The effect of priming treatments on the performance of watermelon (Citrillus lanatus (Thunb.) Matsum. &Nakai) seeds under temperature and osmotic stress. Seed Sci Tech 27: 871-875

Elhafid R, Smith H, Karrou M, Samir K.1998. Physiological responses of spring durum wheat cultivars to early-season drought in a Mediterranean Environment. Ann Bot 81:363-370

Elis RH, Roberts EH. 1981.The quantification of ageing and survival in orthodox seeds. Seed Science and Technology 9:377-409

Halmer P.2003. Methods to improve seed performance. In:Benech-Arnold RL, Sanchez RA Seed Physiology, Application to Agriculture. Food Product Press New York

Harris D, Raghuwanshi BS, Gangwar JS, Singh SC, Joshi KD, Rashidand A, Hollington P.A. 2001. Participatory evaluation by farmers of on farm seed priming in wheat in India,Nepal and Pakistan. Experimental Agriculture 37:403-415

ISTA. 2003. International Testing Association ISTA. Handbook on Seedling Evaluation. 3rd ed.

Jie L, Ong She L, Dong Mei O, Fang L, Hua En W. 2002. Effect of PEG on germination and active oxygen metabolism in wild rye(Leymus chinesis) seed. Acta prataculture Sinica 11:59-64

Kaya MD, Okcu G, Atak M, Cikili Y, Kolsarici O. 2006. Seed treatments to overcome salt and drought stress during germination in sunflower(*Helianthus annuus* L.). European Journal of Agronomy 24:291-295

Khajeh-Hosseini MA, Powell A, Bingham IJ. 2003. The interaction between salinity stress and seed vigour during germination of soybean seeds. Seed Sci Technol 31:715-25

Michel BE, Kaufmann M.R. 1973. The osmotic potential of polyethylene glycol 6000. Plant Physiol 51: 914-916

Mwale SS, Hamusimbi C, Mwansa K. 2003. Germination, emergence and growth of sunflower (*Helianthus annuus* L.) in response to osmotic seed priming. Seed Sci Technol 31:199-206

Parera CA, Cantliffe DJ. 1994. Pre-sowing seed priming. Hort Rev 16:109-41

Russel DF, Eisensmith SP. 1983. MSTATC. Crop soil science department. Michigan state

Saadateyan B, Solamani F, Ahmadvand G, Vojdane S. 2009. Efeect of hydro priming on germination index in sunflower under salt and drought stress conditions. National Conference on Oil Seed Crops in Iran 23-24 September 622-624

Sadeghian SY, Yavari N. 2004. Effect of water-deficit stress on germination and early seedling growth in sugar beet. J Agron CropSci 190:138-44

Singh BG, Rao G.1993. Effect of chemical soaking of sunflower(*Helianthus annuus* L.) seed on vigour index. Indian J Agric Sci 63:232-3

Singh BG. 1995. Effect of hydration-dehydration seed treatments on vigour and yield of sunflower. Indian J Pl Physiol 38:66-8

Taylor AG, Harman GE.1990. Concepts and technologies of selected seed treatments. Annu Rev phytopathol 28:321-329

Yagmur, M, Kaydan D.2008. Alleviation of osmotic stress of water and salt in germination and seedling growth of triticale with seed priming treatments. African Journal of Biotechnology 7:2156-2162

Yari L, Sheidaie S. 2011. Effect of seed priming on seed germination's behavior of rice (*Oryza sativa* L.). International Journal of AgriScience 1(1): 54-45