Drought stress and osmo-priming effects on physiological and biochemical characteristics of Ocimum basilicum

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Abstract

One of the effects of reducing water content on soil is reduction of growth and development of seedlings and variation of field development. Seed priming technique has been known as a challenge to improve germination and seedling emergence under different environmental stresses. The objectives of this research were to evaluate the effects of osmo-priming on germination characteristics and changes of proline, protein and catalase activity of *Ocimum basilicum* seeds. Results showed that drought stress reduced the germination characteristics and drought stress in -8 bar was the critical stress. Priming treatments were include KNO3, PEG and NaCl by 0, -4 and -8 bar concentrations. The seeds were primed with those materials for 8 and 16 hours. The highest germination characteristics were obtained from nitrate potassium in -8 bar for 16 hours priming. Therefore the best seed treatment under drought stress during germination was obtained from the osmo-primed with -8 bar nitrate potassium for 16 hours. The drought stress increased proline and catalase activity but reduced total protein. Priming treatment increases proline, total protein and catalase activity under drought and control conditions. It is concluded that priming results in improvement in germination components of *Ocimum basilicum* in drought stress conditions and increases the resistance to drought stress with improvement of proline, protein and catalase activity in germination phase.

Keywords: catalase activity; drought stress; *Ocimum basilicum*; priming; proline; protein.

Introduction

Drought and its related stress are among the most important and the most common environmental stresses which have restricted agricultural productions and have reduced the efficiency of semi-arid areas (Kardovani, 1997; Ansari et al., 2012; Ansari et al., 2013). One of the approaches offered to improve irrigation efficiency management is achieving optimal performance with regard to the maximum efficiency of irrigation water utilization (Rahnama, 2003; Ansari and Sharif-Zadeh, 2012). Germination is the first growth stage in plants and is a major and critical stage in the life cycle of plants and a key process in seedling growing (Soltani et al., 2006). Inappropriate environmental conditions for germination and seedling emergence in arid and semi-arid areas are the major causes of poor emergence and weak establishment of seedlings. Environmental stresses such as drought stress will reduce germination characteristics and emergence uniformity. Reduction of germination due to drought stress can be associated with the decrease of water absorption by seeds (Ansari et al., 2012). Different researchers have shown that germination will reduce under the effect of abiotic stresses such as salinity, drought, and cold (Kaya et al., 2008; Patadeh et al., 2011; Ansari et al., 2012).

Seed priming can be taken to counteract the adverse effects of abiotic stress (Ptade et al, 2009. Ashraf and Foolad, 2005). Seed priming techniques have been used to increase germination, improve germination uniformity in more field crops under stressed conditions (Iqbal and Ashraf, 2007. Kaya et al, 2006, Patade et al, 2011. Saglam, et al, 2011). Seed priming increases seed reserve utilization (SRU), seedling dry weight (SLDW) and seed reserve depletion percentage (SRDP) in perisian silk tree. In monocotyledon plants like wheat (Soltani et al, 2006) and mountain ray (Ansari et al., 2012), gibberellic acid (GA) after synthesis in the scutellum migrates in to the aleurone layer.

Osmo priming can contribute to improve seedling emergence in different plant species by increasing the expression of aquaporin's (Gao et al., 1999), improvement of ATPase activity, RNA and acid phosphatase synthesis (Fu et al., 1998), also by improve of amylases, lipases and protease synthesis (Ashraf and Foolad 2005). The priming strategies enhanced activities of free radical scavenging enzymes such as CAT and SOD (Rouhi et al., 2012). The priming strategies enhanced activities of free radical scavenging enzymes such as CAT and APX (Ansari and Sharif-Zadeh, 2012; Rouhi et al., 2012).

The rate of accumulation of active oxygen species during germination is determined through the rate of production and release of them and also the activity of antioxidant system, and the balance between active forms of oxygen and antioxidant system activity determines the extent of the damage (Bailey, 2004). Antioxidant system including antioxidant enzymes and metabolites will cause the elimination of activated oxygen species (Bailey, 2004). Enzymes such as catalase, peroxidase, superoxide dismutase, glutathione reductase, and other enzymes cause the removal and inactivation of different types of reactive oxygen (Bailey, 2004; McDonald, 1999; Kookerja, 2005). Stress increases the activity of antioxidant enzymes (Ansari et al., 2013).

The plants in environmental stress like drought, salinity, heat, etc. with stocking the osmotic adjusting material will defeat the stress (Ashraf et al, 2003). Proline is a soluble acid amine in water that will be increased in the environmental stresses and the increase in its amount is the indicator of plant resistance in opposes to stress condition. Proline will protect the protein and cell membrane from intense density damage of Ione (Rajender 1987). Safarnezhad et al. (1996) by examining the effect of osmotic stress on alpha alpha genotype showed that the resistible genotype indicated more and quicker reaction in terms of proline concentration to sensitive species. Proline concentration in the time of drought stress in other plants also reported (Kidonbi 1990; Seraj, 2002; Smith 2002).

Ocimum is a genus of aromatic annual and perennial herbs and shrubs in the family Lamiaceae, native to the tropical and warm temperate regions of all 6 inhabited continents, with the greatest number of species in Africa. Most culinary and ornamental basils are cultivars of *Ocimum basilicum* and there are many hybrids between species. Thai basil (*O. basilicum var. thyrsiflora*) is a common ingredient in Thai cuisine, with a strong flavour similar to aniseed, used to flavour Thai curries and stir-fries. Therefore, the study aimed was to determine the effect of drought stress on germination characteristics and biochemical changes of *O. basilicum*.

Material and methods

The study was conducted in the laboratory of, Azad University of Shahrekord brench, Iran.

Determine the critical drought potential: Drought tolerance of O. basilicum seeds was tested with using solutions of 0, -0.2, -0.4, -0.6 and -0.8 Mpa Polyethylene glycol (PEG-6000) concentrations. 3 replicates of 50 seeds for each treatment were used. For drought stress 5 ml polyethylene glycol 6000 (Michelle Kaufmann) during the experiment period was added to Petri dishes with different levels at temperature of 25 ±1°C until the germination didn't occur anymore in dark conditions.

After test time expiration, for determination the critical drought potential, indexes such as: germination percentage, normal seedling percentage, germination rate, seedling length, mean time to germination, seedling dry weight and seed vigor were calculated. According to these results critical potential, was -0.8 Mpa.

For determined the best priming treatment, seeds of O. basilicum were pretreated with PEG, KNO3 and NaCl at concentrations of 0, -0.4 and -0.8 Mpa for 8 and 16 hours at 15±1°C. After priming, samples of seeds were removed and rinsed 2 times in distilled water and then dried at 25±1°C to the original moisture level.

Seeds of primed and unprimed (control) were placed at critical potential at germinator, in dark condition. Germination was considered to have occurred when the radicles were 2 mm long. Three replicates of 50 seeds for each treatment were used. Germinated seeds were recorded every 24 h. After test time expiration, some germination characteristics correlating such as: germination percentage, normal seedling percentage, germination rate, seedling length, mean time to germination, seedling dry weight and seed vigor were evaluated.

After the completion of germination period plant leaves were harvested and proline content in plant tissues was measured using the approach of Bates et al. (1973). Proline concentration in milligram per gram (mg/g) of fresh leaf tissue was determined by using a standard curve.

Catalase activity was measured at 25°C via spectrophotometric measurement and by Del Rio et al. (1991) method and with the wavelengths of 240 nm.

The data were analyzed by MSTAT-C software and the means were compared using the minimum significant difference (Duncan) test. Diagrams were drawn by Excel software.

Results and Discussion

The effect of drought stress on germination characteristics

Results of variance analysis of the effect of different levels of drought stress on all traits of O. basilicum were significant in a probable 1% level (table 1). This matter shows the difference between different levels of drought stress in different germination indexes such as germination percentage, germination rate, mean time to germination, normal seedling percentage, seedling length, seed vigor and seedling dry weight. In different plants also it is reported that drought stress has a significant effect on germination indexes (Ansari and et al, 2012, 2013; soltani and et al, 2006; Gamze et al, 2005).

Table1- Variance analysis of the effect of different levels of drought stress on seed germination indexes of *O. basilicum*. Gp: germination percentage. GR: germination rate. MTG: mean time of germination. NSP: normal seedling percentage. Sl: seedling length. SVI: seed vigor. SDW: seedling dry weight.

S.O.V	df	GP	GR	MTG	NSP	SL	SVI	SDW
Drought stress	4	2270.4**	173.76**	7.5**	3634.27**	54.61**	910556.95**	0.02**
Error	10	6.4	0.29	0.23	6.67	0.17	374.88	0.00005
C.V%	-	3.4	4.03	7.91	4.31	4.16	2.7	5.77

**significant at 1% probability level

Our results showed that with the increase in different levels of drought stress, germination characteristics such as germination percentage, germination rate, normal seedling percentage, seedling length, seed vigor index and seedling dry weight were significantly reduced but mean time to germination was significantly increased (table 2).

The highest germination percentage with the means of 93.33 %, germination rate with the means of 21.89 seed per day, normal seedling percentage with the means of 94.67 % related to control condition but the highest seedling length with the means of 13.93 cm, seed vigor with the means of 1254 and seedling dry weight with the means of 0.2 gr were related to -

0.2 Mpa condition (Table 2), the following of these results the lowest mean time to germination were observed in control conditions (table 2).

Drought stress affect the germination and germination rate of seeds and decreased germination characteristic. Maybe the reason of the reduction in the germination rate in the levels above the drought stress is the reduction in water potential and consequently reduction in the rate of sucking water. So that the seeds will suck water with a lower rate and the metabolic activities of seed will become slower and consequently cell development and root release will become slower and germination rate will be reduced. The reduction in normal seedling percentage with the increase of drought stress levels can be because of the reduced normal seedling growth and in this way results in the reduction in the normal seedling percentage. Hiaso and Acevedo (1974) reported that, since the increase in stress level, the metabolic activities for increasing the division activity and cell development will be reduced, it is possible that the reason for the reduction in the rootled and shoot growth is the reduction in cell division in stress condition. Seed vigor is an index of germination that is the product normal seedling percentage and seedling length or seedling dry weight. In this experiment the seed vigor index was produced from normal seedling percentage in seed vigor (Ansari and et al, 2013). Hence, the seed vigor with the reduction of normal seedling percentage and seedling length with the increase in drought stress will be reduced. The reason for the reduction of seedling dry weight with the increase in salinity stress is the reduction in seedling growth. Generally with the increase in drought stress the power for sucking water with seeds will be reduced and the needed time for sucking water will be increased and consequently the start of germination processes will be reduced and also there will a disorder it and the time to the start of germination will be increased there for reduced germination rate (Gill et al., 2002). Other reports also indicated that drought stress will cause a reduction in germination characteristics in many of the plants (Kaya et al., 2006; Ansari et al., 2012; Soltani et al., 2006).

Table 2. Mean comparison of the effect of different levels of drought stress on seed germination indexes of *O. basilicum*. Gp: germination percentage. GR: germination rate. MTG: mean time of germination. NSP: normal seedling percentage. Sl: seedling length. SVI: seed vigor. SDW: seedling dry weight.

Drought stress (Mpa)	GP	GR	MTG	NSP	SL	SVI	SDW
0	93.33[a]	21.89[a]	3.95[d]	94.67[a]	13.23[a]	1243.87[a]	0.19[a]
-0.2	92[a]	19.2[b]	5.03[c]	90[a]	13.93[a]	1254[a]	0.2[a]
-0.4	77.33[b]	14.4[c]	6.49[b]	66[b]	11.57[b]	762.27[b]	0.12[b]
-0.6	47.33[c]	7.375[d]	7.28[a]	36.67[c]	7.47[c]	267.67[c]	0.08[c]
-0.8	32[d]	3.99[e]	7.74[a]	13.33[d]	3.83[d]	50.53[d]	0.014[d]

The determination of the best treatment priming

Our results for the effect of priming on germination characteristics showed that the effect of priming, time of priming, priming level, priming and priming level interaction effects and time of priming and priming level interaction levels on all traits of O. basilicum were significant (table 3). The effect of priming and time of priming interaction effects and he effect of priming, time of priming and priming level interaction effects on germination percentage, normal seedling percentage and seed vigor index were significant, also effect of priming and time of priming interaction effects on seedling dry weight was significant (Table 3). Ansari et al. (2012, 2013) reported that priming increased germination characteristics.

Table 3- Variance analysis of the effect of priming on seed germination indexes of *O. basilicum* under -0.8 Mpa conditions. Gp: germination percentage. GR: germination rate. MTG: mean time of germination. NSP: normal seedling percentage. Sl: seedling length. SVI: seed vigor. SDW: seedling dry weight.

S.O.V	df	GP	GR	MTG	NSP	SL	SVI	SDW
Priming (A)	2	144.52**	19.43**	0.99**	129.19**	0.43**	5569.43**	0.0001**
Time of priming (B)	1	864**	45.23**	3.52**	785.85**	0.88**	19937.29**	0.0006**
Priming level (C.)	2	716.07**	25.51**	1.5**	766.52**	1.78**	21781.09**	0.0004**
A×B	2	12.67**	0.45ns	0.44ns	22.52**	0.07ns	1456.26**	0.00001*
A×C	4	61.3**	5.88**	0.54**	49.19**	0.11*	1963.34**	0.00003**
B×C	2	34.89**	5.76**	0.83**	33.19**	0.17*	1283.46**	0.00008**
A×B×C	4	12.56*	0.2ns	0.29ns	11.85**	0.02ns	617.18**	0.000003ns
Error	36	4.3	0.3	0.14	1.26	0.04	61.57	0.000003
C.V%	-	4.39	6.8	5.82	4.64	4.53	7.41	7.24

*, ** and ns shows significant at 5, 1% probability level and non-significantly.

Results related to priming, time of priming and priming level showed that priming increased germination percentage, normal seedling percentage and seed vigor index as compared to the control, and the highest germination percentage (66%), normal seedling percentage (44.67%) and seed vigor index were related to priming by nitrate potassium -0.8 Mpa for 18 h (Fig 1, 2 and 3). Results showed that longer of time and concentrations for treatments priming cause increase germination than lowest time and concentration.

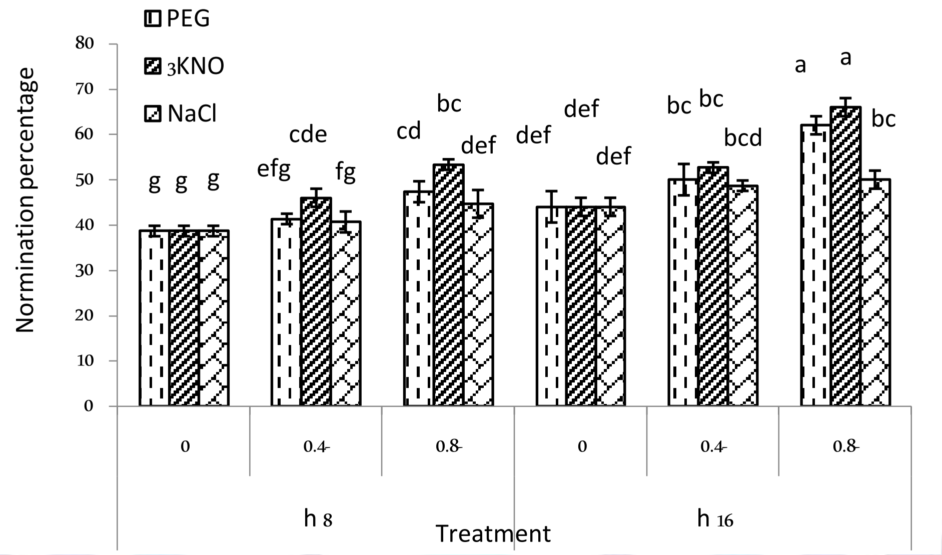

Figure 1- the effect of different treatments priming on germination percentage of *O. basilicum* under -0.8 Mpa of drought stress.

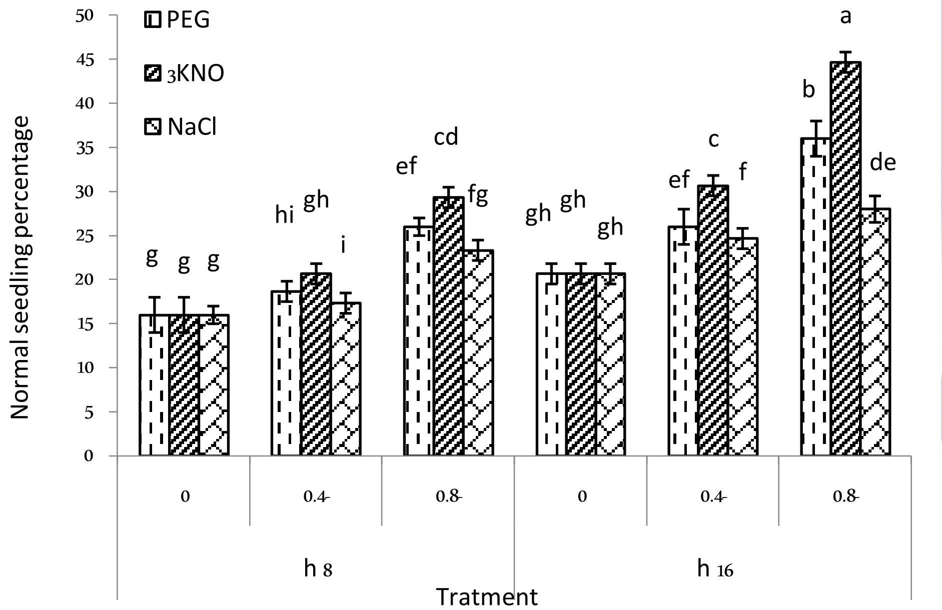

Figure 2- the effect of different treatments priming on normal seedling percentage of *O. basilicum* under -0.8 Mpa of drought stress.

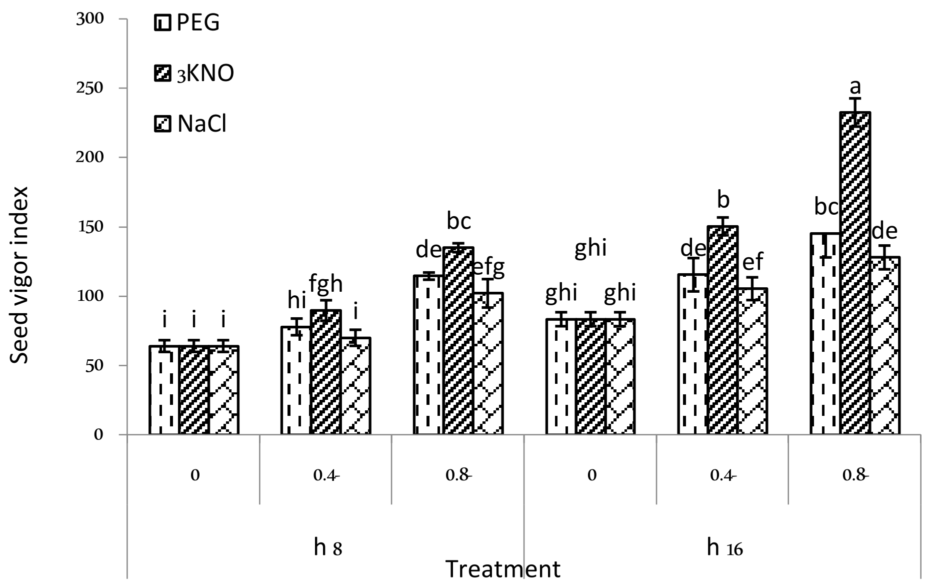

Figure 3- the effect of different treatments priming on seed vigor of *O. basilicum* under -0.8 Mpa of drought stress.

Mean comparison of the main factor of priming on germination characteristics showed that the highest germination rate (9.23 seed per day), seedling length (4.51 cm) and seedling dry weight (0.026 gr) were attained from priming with nitrate potassium, the highest mean time germination (5.95) was related to priming with PEG (Table 4). The effect of main factor of time of priming and priming level on germination rate, seedling length and seedling dry weight showed that the highest germination rate, seedling length and seedling dry weight for time of priming were related to 16 h and for priming level were related to -0.8 Mpa (Table 4). The highest mean time to germination for time of priming was related to 8 h and for priming level was related to 0 Mpa (Table 4).

Interactive effects of priming and time of priming on seedling dry weight showed that the highest seedling dry weight (0.03 gr) was related to priming by nitrate potassium and 16 h (table 5). Results of interactive effects of time of priming and priming level on germination rate, mean time to germination, seedling length and seedling dry weight showed that the highest germination rate (10.48 seed per day), seedling length (4.83 cm) and seedling dry weight (0.34 gr) were related to 16 h priming and -0.8 Mpa (Table 5). The highest mean time to germination (6.26) was related to 8 h priming and 0 Mpa (Table 5).

Seed priming is one of the methods that can be taken to counteract the adverse effects of abiotic stress (patade et al., 2009. Ashraf and foolad., 2005). The purpose of seed priming is to a partially hydrated with water, or various chemical solutions like polyethylene glycol (Osmo priming) or salts like CaCl2, CaSo4 and NaCl (Halopriming) the seeds to a point where germination processes are begun but not completed (Asfraf and Foolad., 2005), followed by drying of seeds to the original moisture level (McDonald, 2000). Seed priming techniques have been used to increase germination, improve germination uniformity, improve seedling estabilishment and stimulate vegetative growth in more field crops such as: wheat (Iqbal and Ashraf, 2007), sunflower seeds (Kaya et al, 2006), Chickpea (Kaur et al, 2002), Capsicum (Patade et al, 2011), lenti (Saglam et al, 2011) and soybean (Sadeghi et al, 2011), under stressed conditions. Also similarity indicated, in maize (Foti et al, 2008), cucumber (Ghasemi-Golezani and Esmaeilpour, 2008). Priming has also been shown to induce nuclear DNA synthesis in the radicle tip cells in several plant species including: tomato (Liu et al. 1997), pepper (Capsicum annuum) (Lanteri et al., 1993), maize (Zea mays) (Garcia et al. 1995), and leek (Ashraf and Bray, 1993; Clark and James, 1991). Osmopriming can cantibute to improve seedling emergence in different plant species by increasing the expression of aquaporins, improvement of ATPase activity, RNA and acid posphathase synthesis (Fu et al., 1998), also bye improve of amylases, lipases and protease synthesis (Ashraf and Foolad 2005). Prime treatments combined had effect on germination and growth seedling than the Treated separately in some treatments. The effect of treatments priming on germination and seedling growth were related to concentrate, time of priming, and temperature for priming and palant species (Ansari et al., 2012; Ansari et al., 2013; Ansari and Sharif Zadeh, 2012).

Table 4. Mean comparison of the effect of priming, time of priming and priming level on germination characteristics of *O. basilicum* under -0.8 Mpa drought stress. GR: germination rate. MTG: mean time of germination. Sl: seedling length. SDW: seedling dry weight.

Traetment		GR	MTG	SL	SDW
Priming	PEG	7.99b	5.95a	4.29bc	0.023b
	KNO3	9.23a	5.78c	4.51a	0.026a
	NaCl	7.32c	5.89b	4.21c	0.021c
Time of priming	8 h	7.29b	6.03a	4.21b	0.02b
	16 h	9.06a	5.71b	4.47a	0.027a
Priming level	0	6.74c	6.2a	4.02c	0.019c
	-0.4	8.29b	5.85b	4.36b	0.023b
	-0.8	9.5a	5.57c	4.64a	0.029a

Table 5. Mean comparison of the effect of priming, time of priming and priming level on germination characteristics of *O. basilicum* under -0.8 Mpa drought stress. GR: germination rate. MTG: mean time of germination. Sl: seedling length. SDW: seedling dry weight.

Treatment		GR	MTG	SL	SDW
PEG	8 h	-	-	-	0.02de
	16 h	-	-	-	0.026ab
KNO3	8 h	-	-	-	0.022cde
	16 h	-	-	-	0.03a
NaCl	8 h	-	-	-	0.019e
	16 h	-	-	-	0.024bc
PEG	0 Mpa	6.73g	6.24a	4.02f	0.019g
	-0.4 Mpa	7.72ef	6.1b	4.3e	0.022ef
	-0.8 Mpa	9.52c	5.51e	4.57c	0.028bc
KNO3	0 Mpa	6.73g	6.24a	4.02	0.019g
	-0.4 Mpa	9.72bc	5.66d	4.62bc	0.027cd
	-0.8 Mpa	11.24a	5.43f	4.9a	0.033a
NaCl	0 Mpa	6.73g	6.24a	4.02f	0.019g
	-0.4 Mpa	7.44f	5.78c	4.15g	0.02fg
	-0.8 Mpa	7.75def	5.77c	4.47d	0.025d
8 h	0 Mpa	6.45f	6.26a	4f	0.018f
	-0.4 Mpa	6.9e	6.11c	4.18d	0.02e
	-0.8 Mpa	8.52c	5.72d	4.46c	0.023c
16 h	0 Mpa	7.03d	6.13bc	4.03ef	0.021de
	-0.4 Mpa	9.68b	5.58e	4.53b	0.026b
	-0.8 Mpa	10.48a	5.42f	4.83a	0.034a

The effect of different treatments on biochemical changes

Results of variance analysis of the effect of different treatment on prolin, catalase and protein of O. basilicum were significant in a probable 1% level (table 6).

Table 6. Variance analysis of the effect of different treatment on prolin, catalase and protein of *O. basilicum*.

S.O.V	df	Prolin	Protein	Catalase
Treatment	3	119.92**	1368.7**	47.39**
Error	8	5.2	86.51	0.47
C.V%	-	16.86	13.87	7.01

**significant at 1% probability level

Drought stress increased prolin and catalase but reduced protein (Table 7). The used of priming increased protein and prolin under control and stress conditions (Table 7). The highest value of prolin and catalase activity were attained from nitrat potassium under drought stress, priming under control and drought stress conditions increased protein and the highest value of protein was attained from nitrate potassium (Table 7).

Generally in stress condition the plant will prepare its resistance to oppose this condition by increasing proline amount. The plants in different environmental conditions will synthesis solutes with low molecules weight that is generally called compatible solutes. These compatible solutes consist of acid amine (proline, glycine), sugar, sugar alcohol, ions, organic acids, amides, amines, betaine groups that are synthesis in reaction to stress and do not interfere with normal biochemical reactions of cell. Khakeshvar Moghadam et al (2011) reported that drought stress will has a significant effect on the amount of proline of shoot and the root of dill. Slama et al (2005) mentioned that the amount of the proline of grass was increased three times than the control one under the effect of drought stress. It is suggested that the reason for this matter is the provocation of biosynthesis activity of proline and the control of catabolic enzyme. Dehkordi and Balouchi (2012) reported that stress conditions reduced protein and priming increased protein under drought and control conditions. Safarnejad et al. (1996) investigated the effect of osmotic stress on alfalfa genotypes and reported that resistant genotypes show more and faster reactions than susceptible species in terms of proline accumulation, i.e. resistant species have more proline accumulation at high levels of stress (Jiroosi et al., 1996). Proline accumulation during drought stress has been reported in other plants, as well (Seraj, 2002; Safarnejad et al., 2004). In many other researches it has also has been reported that drought stress increases the catalase activity (Ansari et al., 2013; Jiang and Zhang, 2001). Among different ecotypes of alfalfa it has been found that the ecotype which has more antioxidant enzyme activity under stress conditions can tolerate the drought stress conditions better than the others (Sadat Asilan et al., 2010). Stress is one of the most important factors which lead to the production of free radicals of oxygen and hydrogen peroxide (Higdos et al., 2001). Catalse converts this molecule to water and oxygen and during the reaction, ascorbate acts as the donor of hydrogen (Noctor and Foyer, 19998).

Table 7. The effect of different treatments on prolin, catalase and protein of *O. basilicum*.

Treatment	Prolin	Protein	Catalase
Control	0.07c	0.69b	4.91d
KNO3	0.08b	0.97a	9.1c
Stress	0.09ab	0.49d	11.1b
KNO3+Stress	0.11a	0.53c	14.42a

Conclusion

The results of the experiment showed that drought stress led to the decrease of germination characteristics. The highest germination characteristics were obtained from nitrate potassium in -8 bar for 16 hours priming. Therefore the best seed treatment under drought stress during germination was obtained from the osmo-primed with -8 bar nitrate potassium for 16 hours. The drought stress increased proline and catalase activity but reduced total protein. Priming treatment increases proline, total protein and catalase activity under drought and control conditions. It is concluded that priming results in improvement in germination components of Ocimum basilicum in drought stress conditions and increases the resistance to drought stress with improvement of proline, protein and catalase activity in germination phase.

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