Emir. J. Food Agric. 2010. 22 (2): 130-139
http://ffa.uaeu.ac.ae/ejfa.shtml

Short Communication

Effect of seed priming on germination and seedling growth of two medicinal plants under salinity

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Abstract: Priming is one of the seed enhancement methods that might be resulted in increasing seed performance (germination and emergence) under stress conditions such as salinity, temperature and drought stress. The objective of this research was to evaluate the effect of different priming types on seed germination of two medicinal plants including pot marigold (*Calendula officinalis*) and sweet fennel (*Foeniculum vulgare*) under salinity stress. Treatments were combinations of 5 levels of salinity stress (0, 2.5, 5, 7.5 and 10 ds m-1) and 5 levels of priming (control, GA_3, Manitol, NaCl and distilled water) with 3 replications. Seeds of pot marigold and sweet fennel were primed for 24 h at 25°C. Results indicated that with increasing salinity, germination traits such as germination percent, rate and plumule length decreased, but seed priming with GA_3 and NaCl showed lower decrease. In all of the salinity levels, primed seeds (except manitol) possessed more germination rate and plumule length than control. The highest radicle fresh and dry weight in pot marigold was seen at 7.5 ds m-1 salinity stress level. It seems that higher germination rate in pot marigold shows higher tolerance to salinity than sweet fennel. Priming with NaCl and GA_3 caused an increase in germination percent of pot marigold and sweet fennel in various range of salinity, but in lower salinity levels percent of germination was higher than upper ones. The result of this experiment is consistent with the hypothesis that under undesirable conditions such as salinity stress, priming with GA_3 and NaCl can prepare a suitable metabolic reaction in seeds and can improve seed germination performance and seedling establishment.

Keywords: germination, pot marigold, priming, salinity stress, sweet fennel.

تأثير عملية التبذير المختبري على الإنبات ونمو نباتين طبيين تحت الملوحة

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ملخص: التبذير ألمختبري يعتبر من أهم الطرق لتعزيز أداء نمو البذور سواء بالنمو أو الإنبات وتحت تأثير ظروف النمو الصعبة كالملوحة ودرجات الحرارة والجفاف . ويهدف البحث إلي تقييم تأثير أنواع مختلفة من التبذير على نمو البذور لنوعين مختلفين من النباتات الطبيبة *Calendula officinalis* و*Foeniculum vulgare* وذلك تحت تأثير الملوحة. المعاملات البحثية مركبة من خمسة مستويات 0.0 و 0.2 و 5.0 و 7.5 و 10.0 dsm-1 وخمس درجات من التبذير ألمختبري وهي معامل القياس وهرمون جبرلين3 وميثانول و Nacl ومياه محلاه وذلك مع موجود 3 مكررات . البذور من النباتيين الطبيين Calendula *officinalis* و *Foeniculum vulgare* قد تم تبذيرهما لمدة 24 ساعة تحت درجة حرارة 25 مئوية، وقد أوضحت النتائج انه مع زيادة تركيز الملوحة تتناقص نسبة الإنبات ويتناقص في استطالة الجزير الرئيسي ولكن مع وجود تركيز جبرلين 3 و Nacl أوضحت أن نسبة التناقص كان اقل. في كل مستويات الملوحة وجد أن البذور المعاملة ماعدا الميثانول بينت نسبة إنبات للبذور واستطالة للجزير اعلي من معامل القياس. وقد كان اكبر وزن جاف واخضر للجذور الرئيسية في النبات Calendula officinalis هي نسبة ملوحة 7.5 dsm-1 وأوضحت التجربة أن نبات *Calendula officinalis* تحمله للملوحة أفضل من نبات *Foeniculum vulgare* . وقد وجد ان التبذير لمستويات الملوحة Nacl و جبرلين 3 أدى إلى زيادة في نسبة الإنبات في النبات *Calendula officinalis* و *Foeniculum vulgare* في مستويات عدة للملوحة ولكن مستويات الملوحة الأقل بينت

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نسبة إنبات اعلي من سواها. وقد اوضحت النتائج النهائية للتجربة أثبات الفرضية انه تحت تأثير الظروف الغير المناسبة مثل الملوحة والتبذير بواسطة الجبرلين 3 و Nacl يمكن أن يؤدى إلى تهيئة الظروف المناسبة للايض النباتي مما يساهم في تحسين ظروف نمو البادرات ونموها في الحقل

Introduction

One of the most important problems facing the farmers in developing countries is the heterogenecity and lack of suitable conditions in soil that causes decreasing in germination percent, heterogeneous emergence, unbalanced seedling growth and competition for environmental resources such as light, nutrients and water. Subsequently, this makes difference in biomass and performance of a species of plants (Roa and Philipse, 1993).

One of the methods that can overcome to this problem is seed pre-planting treatments called priming that include water absorption at enough level to begin germination events that is accomplished by the subsequent drying. The purpose of priming is increasing germination percent, decreasing mean of germination time and improving growth and vigour of seedling at very wide favor and unfavored environmental conditions. This method is successful in small seed plants and the most medicinal plants that have great economic value with quick and uniform emergence requirement (Ellis and Roberts, 1981).

Seed germination is a major factor limiting the establishment of plants under saline conditions (Ghavami and Ramin, 2007) and is the most critical phase in plant life (El-Keblawy and Al-Rawai, 2005) that greatly influenced by salinity (Misra and Dwivedi, 2004). Salinity is reported to decrease as well as delay in germination of most of the crops. Lower levels of salinity delay germination, whereas higher levels not only reduce the final percentage of germinated seeds (Ghoulam and Fares, 2001), but also can inhibit the seed germination. In addition, salinity imposes other stresses such as ion toxicity on plants, as a result of ion entry in excess of appropriate concentrations and nutrient imbalances, as commonly seen in the displacement of potassium by sodium. In fact, salinity damage is mainly due to altered water relation caused by high salt accumulation in the intercellular spaces (Zhang et al., 2006). Improved seed priming techniques are used to reduce emergence time, accomplish uniform emergence, better allometric (changes in growth of plant parts over time) attributes and requisite stand in many horticultural and field crops (Ashraf and Foolad, 2005; Farooq et al., 2005). These techniques include hydropriming, osmoconditioning, osmohardening, hardening and hormonal priming or soaking prior to sowing (Basra et al., 2005; Ashraf and Foolad, 2005). Effects of priming or pre-treatment of seeds persist under suboptimal field conditions, such as salinity (Wahid et al., 2006; Foti et al., 2008), low or high temperature (Wahid and Shabbir, 2005) and low soil moisture availability (Dul and Tuong, 2002) with different seed priming methods have been completely explained in various plants (Basra et al., 2004; Farooq et al., 2006; Soltani et al., 2001).

So, in order to increase the strength of seeds in salinity stress conditions, examining different methods of priming for accessibility to maximum germination percent and seedling establishment is necessary. The aim of this study was determining the best method of priming under salinity conditions and also its effect on seed germination of two medicinal plants.

Materials and methods

In order to evaluate the effects of seed priming under salinity conditions on germination traits and seedling growth of two medicinal plants, a factorial experiment was conducted at the University of Mohaghegh Ardabili, Ardabil, Iran based on the completely randomized design with three replications. Treatments were seed priming in five levels (control, GA_3, Manitol, NaCl and distilled water) that seeds of pot marigold and sweet fennel were primed in

the solutions for 24 hours. At the first step, 20 seeds were placed in the plastic vessel. Then, priming solutions including 20 mg lit^{-1} gibberellic acid (432k6943, Sigma-Aldrich), 25% Manitol (k91614582, Merck), 3% NaCl (k29042800, Merck) and distilled water were added and after 24 hours the seeds were dried at the laboratory temperature. At the second step 50 seeds were placed in 10 cm Petri dishes and then 5 ml of salt solutions (0, 2.5, 5, 7.5 and 10 ds m^{-1}) were added to every Petri dish. The purpose of using osmotic potential through the second step was to creating artificial salinity stress and evaluation of its effect on seed germination. The regularly counting of germinated seeds was conducted every 24 hours and the appearance of 2 mm or more of radicle was considered as germination. Germination test was ended when the number of germinated seeds was equal in three sequential countings. After the ending of this course the following traits were measured:

1. Germination percent (GP) was evaluated by counting the number of normal seedlings at the end of standard germination test.

2. Germination rate (GR) was calculated by following formula:

$$(\Sigma \frac{ni}{di})$$

where ni is the number of germinated seeds in every counting and di is the day of counting (Ellis and Roberts, 1981).

3. Mean of germination time (MGT) that is the reverse of germination rate ($\Sigma \frac{di}{ni}$).

4. Seedling dry and fresh weight (plumule and radicle).

5. Plumule and radicle length.

The normality test and analysis of variance over data conducted by the MSTATC and SPSS software and comparison of means by Duncan's multiple range test.

Results

Analysis of variance results showed that the effect of salinity was significant on the whole studied traits except of the dry and fresh weights of plumule to radicle ratio. The effect of priming on sweet fennel just was significant on GP, GR and plumule and radical length, but about pot marigold in addition to these traits, MGT and dry and fresh weight of plumule were significant, too. Also, interaction between salinity and priming on GR and GP in sweet fennel and on GP, MGT and plumule length in pot marigold was significant (Table 1). In both species MGT increased by raising salinity levels, such that the lowest MGT was at control level and the highest was at 10 ds m^{-1} salinity level (Table 2).

Also, in pot marigold the highest MGT achieved at 10 ds^{-1} salinity level and priming with manitol, but the lowest MGT was obtained at control salinity level and priming with GA_3 (Table 3). Probably, this is the result of the effect of gibberellic acid under salinity conditions that can decrease the negative effects of sodium ions through germination.

GP decreased by increasing salinity, but by comparing sweet fennel and pot marigold, it is clear that in pot marigold, GP of seeds had not significant difference with control up to 5 ds m^{-1}. This shows the tolerance of pot marigold at the primary stages of germination to salinity stress. The highest GP in pot marigold and sweet fennel obtained under control salinity level and priming with GA_3 and the lowest amount at 10 ds m^{-1} salinity level and priming with manitol. In sweet fennel priming with manitol and GA_3 had no significant difference (Table 3).

In both species the longest plumule observed at control salinity. Salinity stress probably decreases cell division and its expansion in aerial parts of plant. Also, the longest plumule in pot marigold obtained in the interaction of control salinity and priming with GA_3. The shortest plumule obtained at 10 ds m^{-1} salinity level and priming with manitol (Table 3). By increasing salinity to 10 ds m^{-1}, fresh and dry weight of plumule decreased.

Table 1. Analysis of variance for germination traits and other seed traits in pot marigold and sweet fennel under salinity and priming treatments.

MS											
Treatments	**Df**	**GR**	**MGT**	**GP**	**PL**	**RL**	**PFW**	**RFW**	**PDW**	**RDW**	**PDW/RDW**	**PFW/RFW**
Pot marigold												
Salinity	4	68.97	0.256**	486.22**	13.02**	3.14*	0.0007*	0.00048*	0.00038*	0.00023*	30.95	28.05
Priming	4	13.33**	0.098**	340.02**	3.14**	3.04**	0.0014**	0.00017	0.00075**	0.00008	18.93	17.16
Salinity× priming	16	3.06	0.033**	89.90**	0.58*	0.32	0.0003	0.00003	0.00018	0.00001	6.88	6.23
Error	50	2.22	0.008	29.65	0.29	0.73	0.0002	0.00014	0.00011	0.00006	21.65	19.63
CV (%)		15.97	19.90	8.95	21.96	15.77	12.60	13.16	12.61	13.64	17.71	17.71
Sweet fennel												
Salinity	4	25.78**	0.22**	26.9296**	61.34**	32.02**	0.0145**	0.0042**	0.00819**	0.00233**	6.504	6.280
Priming	4	0.89*	0.01	164.31**	3.17**	3.09**	0.0003	0.00005	0.00017	0.00003	0.573	0.553
Salinity× priming	16	1.01**	0.01	110.26**	0.43	0.47	0.0001	0.00002	0.00005	0.00001	0.745	0.720
Error	50	0.27	0.007	16.41	0.42	0.27	0.001	0.00030	0.00102	0.00016	3.74	3.616
CV (%)		12.88	11.23	5.63	20.59	18.42	12.30	19.6	12.19	19.08	9.23	9.23

*and ** indicate significant difference at 5% and 1% probability level, respectively. GR- Germination rate, MGT- Mean of Germination time , GP- Germination percent, PL-Plumule length, RL-Radicle length, PFW-Plumule fresh weight , RFW-Radicle fresh weight, PDW-Plumule dry weight, RDW-Radicle dry weight.

Table 2. Comparison of means for salinity and priming effects on pot marigold and sweet fennel germination traits.

Salinity (ds m-1)	GR	MGT	GP	PL	RL	PFW	RFW	PDW	RDW
Pot marigold									
Control	7.85 a	0.138 c	64.46 a	3.47 a	2.77 a	0.050 a	0.006 b	0.036 a	0.004 b
2.5	7.68 a	0.138 c	66.06 a	3.19 a	2.29 a	0.043 a	0.008 b	0.031 a	0.006 b
5	6.00 b	0.185 c	62.40 ab	2.65 b	2.74 a	0.047 a	0.005 b	0.034 a	0.003 b
7.5	4.20 c	0.225 b	59.66 b	1.68 c	2.48 a	0.048 a	0.018 a	0.035 a	0.013 a
10	2.96 d	0.451a	51.60 c	1.34 c	1.65 b	0.032 b	0.004 b	0.023 b	0.003 b
Priming									
Control	5.40 b	0.234 b	57.80 dc	2.14 dc	1.89 b	0.038 b	-	0.028 b	-
GA_3	6.38 ab	0.196 b	66.66 a	2.88 ab	2.30 b	0.052 a	-	0.038 a	-
Manitol	4.29 c	0.371 a	54.86 d	1.88 d	2.25 b	0.036 b	-	0.026 b	-
NaCl	6.67 a	0.162 b	64.26 ab	2.93 a	3.12 a	0.057 a	-	0.041 a	-
distilled water	5.96 ab	0.204 b	60.60 bc	2.49 bc	2.35 b	0.037 b	-	0.027 b	-
Sweet fennel									
Control	5.44 a	0.191 c	87.58 a	5.46 a	4.72 a	0.013 b	0.050 a	0.018 b	0.067 a
2.5	5.22 a	0.193 c	79.75 b	5.09 a	3.49 b	0.031 a	0.049 a	0.042 a	0.066 a
5	4.10 b	0.247 bc	74.16 c	2.64 b	3.19 b	0.005 bc	0.014 b	0.007 bc	0.019 b
7.5	3.49 c	0.293 b	64.93 d	1.55 c	1.63 c	0.002 c	0.006 b	0.003 c	0.008 b
10	2.24 d	0.489 a	53.01 e	1.04 d	1.10 d	0.0009 c	0.003 b	0.001 c	0.004 b
Priming									
Control	4.01 ab	-	71.78 b	2.62 d	2.19 c	-	-	-	-
GA_3	4.23 a	-	70.66 b	3.49 ab	3.14 a	-	-	-	-
Manitol	3.72 b	-	67.35 c	2.81 cd	2.56 bc	-	-	-	-
NaCl	4.36 a	-	76.32 a	3.73 a	3.32 a	-	-	-	-
distilled water	4.17 a	-	73.32 b	3.12 bc	2.93 ab	-	-	-	-

Different letters at each column indicate significant differences at 5% probability level.
GR-Germination rate (seed per day), MGT-Mean of germination time (day) for single seed, GP-Germination percent (%), PL-Plumule length (cm), RL-Radicle length (cm), PFW-Plumule fresh weight(gr), RFW-Radicle fresh weight (gr), PDW-Plumule dry weight (gr), RDW-Radicle dry weigth (gr)

Table 3. mean comparison for salinity and priming interaction on germination traits and plumule length in pot marigold and sweet fennel.

		Pot marigold			Sweet fennel	
Salinity (ds m-1)	**Priming**	**GR**	**GP**	**PL**	**GR**	**GP**
	Control	0.12 bc	65.33 abcd	2.5 bcdefghi	5.5 ab	88.66 abcd
	GA3	0.10 c	78 a	4.4 a	6.2 a	94.06 a
	Manitol	0.15 bc	63.66 abcde	3 abcdef	4.7 abcdef	89.93 abc
Control	NaCl	0.19 bc	53.33 bcde	3.1 abcde	4.2 bcdefg	74.66 defgh
	distilled water	0.11 bc	70 ab	4.1 ab	5.5 ab	90.60 ab
	Control	0.14 bc	62 abcde	3.2 abcde	3.1 fghi	80.26 abcdefg
	GA3	0.12 bc	71.33 ab	3.7 abc	3.9 bcdefgh	77.86 bcdefgh
	Manitol	0.17 bc	58.33 abcde	2.6 bcdefghi	3.4 defghi	75.30 cdefgh
	NaCl	0.11 bc	67.66 abc	3.3 abcde	5.5 ab	84.76 abcde
2.5	distilled water	0.13 bc	63 abcde	2.9 abcdefg	3 fghi	80.56 abcdefg
	Control	0.18 bc	59.66 abcde	2.1 cdefghij	5 abcde	74.50 defgh
	GA3	0.15 bc	66.33 abc	2.9 abcdefgh	4.5 abcdefg	71.33 efghi
	Manitol	0.28 bc	54 bcde	1.9 defghij	5.4 abc	67 ghij
	NaCl	0.12 bc	70 ab	3.4 abcd	3.2 efghi	82 abcdef
5	distilled water	0.16 bc	62 abcde	2.8 abcdefgh	4.7 abcdefg	76 bcdefgh
	Control	0.27 bc	56.33 bcde	1.5 efghij	2.1 hi	64.63 hijk
	GA3	0.23 bc	63.66 abcde	1.8 defghij	5.6 ab	63.06 hijk
	Manitol	0.35 bc	53.33 bcde	1 ij	2.2 hi	59.23 ijkl
	NaCl	0.17 bc	66.66 abc	2.6 bcdefghi	5.2 abcd	72.66 efghi
7.5	distilled water	0.24 bc	58.33 abcde	1.2 hij	1.6 i	65.06 hijk
	Control	0.43 b	45.66 de	1.2 hij	4 bcdefgh	50.86 kl
	GA3	0.36 bc	54 bcde	1.3 fghij	2.8 ghi	47 l
	Manitol	0.89 a	45 e	0.7 j	3.8 bcdefgh	45.30 l
10	NaCl	0.20 bc	63.66 abcde	2.1 cdefghij	2.3 hi	67.53 fghij
	distilled water	0.36 bc	49.66 cde	1.3 ghij	3.5 cdefgh	54.36 jkl

Different letters at each column indicate significant differences at 5% probability level.
GR-Germination rate (seed per day), GP-Germination percent (%), PL-Plumule length (cm)

The effect of salinity on radicle length in pot marigold at control and 2.5, 5, 7.5 ds m^{-1} had no significant difference (Table 2). This shows that salinity at 7.5 ds m^{-1} does not change radicle length, fresh and dry weight of plumule and radicle, while it decreases the plumule length. The maximum fresh and dry weight of radicle in pot marigold was seen at 7.5 ds m^{-1}. In both species of studied medicinal plants the maximum seed GR obtained at priming with NaCl that had no significant difference with GA_3 and distilled water and in both species the minimum seed GR obtained with manitol priming (Table 2). The maximum MGT obtained in priming with manitol (Table 2). By definition, MGT is associated to the time length (day) that radicle exits. However, small numerical value, shows the more GR. The maximum GP in pot marigold obtained in priming with GA_3 that had no significant difference by priming with NaCl (Table 2). In sweet fennel, the maximum GP obtained at priming with NaCl (Table 2). The effect of priming on radicle and plumule length was significant in both species. The maximum radicle and plumule length in both studied species was seen in priming with NaCl that had no significant difference with GA_3 priming (Table 2). In pot marigold, the maximum dry and fresh weight of plumule obtained at priming with NaCl that had no significant difference with GA_3 priming.

Disscussion

By increasing salinity levels, GP decreased in both medicinal plants (Table 2). Decrease in GP of seeds due to the effect of increasing salt concentration maybe resulted from decreasing osmosis potential of solution, increasing toxic ions and changing in the remobilization balance of seed reservoirs. Decreasing germination percent by the effect of increasing salt concentration is in consistence with the results of Xiao-Fang et al. (2000). In pot marigold 2.5 ds m^{-1} salinity level showed the highest mean of GP (66.06) that was higher than control (64.46), but had no significant difference. In some plants such as halophytes or salt resistant plants, the existence of sodium ions even at low amounts could have positive effect on seed germination, and even could increase GP than control (Sabahat and Ajmal Khan, 2004). Distilled water has zero osmosis potential and some seeds show lower GP at this potential. The most researchers believe that the decrease of GR is the result of decreasing water potential and seed accessibility to water (Rdhan and Yanaht, 1982).

GR in pot marigold without salinity stress was 44% more than sweet fennel. In sweet fennel the highest GR obtained at control salinity level and priming with GA_3 (Table 3). It is considered that this maybe the result of difference between two studied seed lot vigour. Although, comparing two different plant species by the view of studied traits is not correct, but this work show that the seeds of different species have different responses in germination at similar conditions.

By increasing salinity to 10 ds m^{-1}, fresh and dry weight of plumule decreased. It is probably due to decreasing in remobilization of reservoirs from cotyledons to embryo axis. The factors that affect the growth rate of embryo axis, also are affecting the mobility of reservoirs and its remobilization from cotyledons to embryo axis (Akita and Cabuslay, 1990). Mer et al. (2000), observed that by increasing salinity, plumule length in wheat, barley, pea and cabbage seeds decreased. They pointed out that decreasing the growth of young seedlings by increasing salinity, was because of the most decreasing of water absorption by radicle, and subsequently by accumulation of soluble salts in cells, water potential of root cells decreases and biological processes occur in roots even in low water potentials.

The maximum fresh and dry weight of radicle in pot marigold was seen at 7.5 ds m^{-1}. It looks that pot marigold by attention to its higher GR, is more tolerant at germination stage in comparison with sweet fennel. Halophyte plants, because of their special root anatomical structure is less affected under salinity stress (Gulzar and Ajmalkhan, 2001).

In both species the minimum seed GR obtained with manitol priming. It looks that manitol may increase osmosis potential and force seeds under the moisture deficit that, in these conditions the activity of enzymes will decrease and the rest of metabolic activities will confront with problems. In other words, at higher osmosis potential, the moisture will be accessible for seed and its germination will decrease. It is reported that in sunflower, watermelon and melon seeds, priming with salt (NaCl) causes an increase in GR and rapid establishment of seedlings from treated seeds (Demir Kaya et al., 2006; Foti et al., 2008). Ruan and Tylkowska (2002), reported that osmo-priming of rice seeds by salt solution in compare with polyethilenglicole, increases the germination index and decreases germination time significantly. Priming with GA_3 will accelerate metabolic reactions before germination process and make possible seed germination under salinity stress conditions with low moisture (Varma et al., 1984).

GA_3 increases the synthesis of hydrolytic enzymes at aleuron layer and by the activity of these enzymes, storage compounds convert to transferable ones (sucrose and glucose) and transfer to embryo (Sedghi et al., 2008). The main factor in transferring of reservoirs is their solubility in water that with decreasing moisture because of salinity stress, their remobilization to embryo will not possible (Varier and Yaduraju, 1996).

Pre-treatment with NaCl and GA_3 accelerates some metabolic processes even in low potential. This causes improvement at metabolic activities in germination especially under salinity stress conditions and subsequently the weight of radicle and plumule increases in less time.

Conclusion

The results of experiment showed that priming with NaCl and GA_3 improves germination indices and seedling growth at studied species. Priming with NaCl and GA_3 increased GP in sweet fennel and pot marigold seeds under salinity that this increase was more at low levels of salinity. It looks that priming with NaCl will increase the activity of super oxide dismutase (SOD) and peroxidase and by increasing respiration rate, will improve GP and GR (Jie et al., 2002). The germination of priming seeds was begun earlier than control seeds, and subsequently these seeds will establish more quick under salinity stress, and will exit earlier. Thus, they will be less time under the attack of pest and soil pathogens. Whilst, the primed seeds with NaCl and GA_3 had more GR than control, at certain time they produce more dry matter under salinity stress. Since priming with NaCl, specially is simple and cheap, we can propose this method to farmers, so they can increase percent and homogeneity of emergence of medicinal plants under environmental stresses.

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