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Induction of salt tolerance in tomato (*Lycopersicon esculentum* Mill.) seeds through sand priming

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Abstract

Effect of sand priming on vigour and biochemical changes of two tomato varieties, 205 and 206, at various salinity levels (0, 50, 100 and 150 mM NaCl) were studied. Both varieties differ in their salt tolerance capability and are commonly raised in Zhejiang province. Seeds were mixed with sand particles diameter ranged between 0.5 mm to 2 mm containing 4 % (v/w) water, sealed in plastic box, and then were primed at 25 ºC for 72 h. Final germination percentage (FGP), germination index (GI), vigour index (VI), root length, shoot length, fresh weight and dry weight were studied as tomato seed vigour markers in relation to malondialdehyde (MDA) contents, catalase (CAT), peroxidase (POD), superoxide dismutase (SOD) and ascorbate peroxidase (APX) activities. Sand priming significantly improved FGP, GI, VI and seedling vigour attributes of both tomato varieties under salinity stress. Moreover sand priming treatments significantly enhanced the activities of CAT, POD, SOD and APX whereas reduced the accumulation of MDA contents under salt stress condition. Our results suggested that sand priming can serve as a promising method to enhance tomato seed vigour under salt stress condition probably through augmentation of antioxidant enzymes activities. Generally, var 206 responded better compared with var 205 at higher salinity stress.

Keywords: tomato, antioxidant enzymes, salinity, seed vigour.
Abbreviations: CAT, Catalase. POD, Peroxidase. SOD, Superoxide dismutase, APX, Ascorbate peroxidase. MDA, Malondialdehyde.

Introduction

Tomato is a widely distributed annual vegetable crop which is consumed fresh, cooked or after processing by canning, making into juice, pulp, paste, or as a variety of sauces; being a rich source of phytochemicals such as lycopene, β-carotene, flavonoids, vitamin C and essential nutrients (Beutner et al., 2001). Abiotic stresses are major constraints for global crop production. Among various abiotic stresses, salinity has become a severe threat to ensure food security by affecting about one-third of the irrigated land on earth (Mengel et al., 2001). Salt stress limits plant growth and productivity, mainly by inducing osmotic effects, ion-specific effects and oxidative stress (Okhovatian-Ardakani et al., 2010). Reactive oxygen species (ROS) attack proteins, lipids and nucleic acids, and the degree of damage depends on the balance between formation of ROS and its removal by the antioxidative scavenging systems and it appears to represent an important stress-tolerance trait. Elimination of ROS is mainly achieved by antioxidant compounds such as ascorbic acid, glutathione, thioredoxine and caroteniods, and by ROS scavenging enzymes e.g., superoxide dismutase, glutathione peroxidase and catalase (Noctor and Foyer, 1998). Salinity reduces tomato seed germination and lengthens the time required for germination to such an extent that the establishment of a competitive crop by direct seeding would be difficult in soils where the electrical conductivity of a saturated extract was equal to or above 8 dS m^{-1} (Cuartero and Fernandez-Munoz, 1999). Seed priming (controlled hydration followed by redrying) has been used to reduce germination time, harmonize germination, improve germination rate and improve the crop establishment in many crops under stress conditions. These priming treatments which enhance seed germination include hydropriming (Afzal et al., 2002) osmopriming (Hardegree and Van Vactor, 2000; Rouhi et al., 2011), solid matrix priming (Ghassemi-Golezani et al., 2010) hormonal priming halopriming (Afzal et al., 2009; Nawaz et al., 2011) sand priming (Hu et al, 2006). The beneficial effect of priming has been associated with various biochemical, cellular and molecular events including synthesis of DNA and proteins (Bray et al., 1989). Priming is also thought to increase activity of many enzymes and thus counteracts the effects of seed ageing (Lee and Kim, 2000). Priming treatment significantly enhanced the activities of catalase (CAT), peroxidase (POD), superoxide dismutase (SOD) and soluble sugar content and reduced the malondialdehyde (MDA) accumulation under the salt stress condition in the seedlings (Hu et al., 2006). The faster growth of tomato plants from primed seeds seems to be the result of higher capacity for osmotic adjustment because plants from primed seeds have more Na^+ and Cl^- in roots and more sugars and organic acids in leaves than plants from non-primed seeds (Cayuela et al., 1996). Priming with polyethylene glycol (PEG) has been applied in vegetable seeds at present; however, it is not economical for the poor farmers because of

its high cost. Sand as a priming matrix can be used as an alternative which is simple, highly effective and affordable for resource poor farmers an ideal priming medium needs to attain some characteristics i.e inert media, easy separation from seeds, no damage to seeds and high ability of holding water. Sand as a priming medium satisfies above mentioned conditions. Very little works on sand priming in field crops has been reported yet (Hu et al., 2002; Hu et al., 2006). However, scanting information is available for usage of sand priming to improve tomato seed germination under salt stress conditions in relation to antioxidant defence mechanism. Therefore, the objective of present investigation was to explore benefits (if any) of sand priming to enhance tomato seed germination through modulation in antioxidant enzyme activities under salt stress.

Results

Germination and seedling vigour evaluation

Salinity stress significantly reduced final germination; however, sand priming improved final germination of both tomato varieties. Final germination of both varieties was significantly increased when treated with sand priming at various levels of salinity stress (Table 1). Sand priming improved germination and vigour index in both varieties at all salinity levels. While root (2.53 cm) and shoot lengths (1.26 cm) of var 205 were significantly improved after sand priming in combination with high salt stress (150 mM) as compares to high stress salt (150 mM) treatments alone. Whereas seeds primed with sand exhibited more shoot fresh weight (12.83 mg and 26.33 mg) and dry weight (2.50 mg and 5.03 mg) in 205 and var 206 respectively under high salt stress (150 mM) (Table 1).

Antioxidant enzyme activities and malondialdehyde contents

The results shoed that the MDA content in shoots of both tomato varieties was significantly reduced after sand priming in comparison to high-salt stress of (150 mM). The activity of antioxidant enzymes was significantly changed after exposure to high salt stress, CAT, POD, SOD and APX activities increased in sand primed seeds of both tomato varieties (Fig 1). At the same time, the differences between control and sand primed seedlings were considerable. The activities of CAT increased (4.5 %) POD (15.3 %), SOD (6.6 %) and APX (4.1 %) after sand priming in combination with high salt stress in var 206 as compared with var 205 (Fig 1). Maximum CAT activity was recorded in sand priming in combination with 50 mM NaCl followed by sand priming in combination with 100 mM NaCl, whereas an increased activity of POD was recorded in sand priming at 50 mM NaCl followed by 100 mM NaCl in var 206. Meanwhile the highest SOD and APX activity was recorded in seedlings raised from sand priming at 50 mM NaCl and 100 mM NaCl in var 206 compared with var 205.

Discussion

Priming hastens and synchronizes seedling emergence and is capable of enhancing seedling tolerance to biotic and abiotic stresses including salinity stress during the critical phase of seedling establishment and consequently can ensure uniform crop stand and yield improvement (Afzal et al., 2009). Sand priming significantly improved germination potential and stand establishment of both tomato varieties as indicated by higher final germination, germination index, vigour index and increased biomass of primed seeds as compared to unprimed seeds of both tomato varieties under varying salinity environments (Table 1). This earlier synchronized and faster germination can be attributed to the enhanced synthesis of DNA, RNA and proteins during priming operations (Bray et al., 1989). Significant enhancement in final germination after sand priming could possibly be the aftermath of an array of physiological processes e.g. reserves food material breakdown, increased cell division and expansion of embryonic axis etc. Moreover this earlier and synchronized germination of sand primed seeds could be ascribed to increased metabolic activities in sand primed seeds as compared to unprimed seeds. Salinity stress inhibits overall plant growth. However, normally root length is more influenced than shoot length (Jamil et al., 2006). Shoot and root lengths increased with the application of different sand priming treatments under salinity stress (table 1). This increased shoot and root lengths as compared to high salt stress may be due to enhanced cell wall extensibility of the primed seeds. Higher fresh and dry weights are reported to correlate with the earlier start of germination. Resultant increased fresh and dry weights in sand primed seeds are in conformity with the findings of earlier researchers (Chookhampaeng et al., 2008). The improvement in seedling fresh and dry weights might possibly be the out come of increased cell division within the apical meristem induced by sand priming operations.Salinity can trigger oxidative stress in plant tissues through increased production of reactive oxygen species, which can induce membrane lipid peroxidation. So, accumulation of malondialdehyde can serve as an important oxidative stress indicator. MDA contents are reported to increase with the increase in salt stress in various horticultural crops (Khan and Panda, 2008). The reduction of MDA accumulation in the seedlings of sand primed seeds might be associated with better membrane repair during sand priming process and inductive responses of antioxidant enzymes which can provide protection against oxidative damage (Fig. 1e). In order to combat reactive oxygen species which augment salinity induced oxidative stress plants have developed some particular mechanisms such as induction of antioxidant enzymes (SOD, CAT, POD, APX etc) and non-enzymatic antioxidants (ascorbic acid and reduced glutathione etc). Similarly in our study significant increases in activities of some antioxidant enzymes such as SOD, CAT, POD, APX in sand primed seeds in combination with salt stress as compared to only salt stressed seeds in both tomato varieties was noticed. Such induction of antioxidant enzymes might have triggered inherent antioxidant defense mechanism in the sand primed seeds which might be responsible for rejuvenation of vigour in tomato (Fig. 1). Such enhancement of seed germination through priming has also been associated with stimulation of antioxidant activities in various crops by previous researchers (Bailly et al., 1997; Chiu and Sung, 2002).

Materials and methods

Sand priming

Seeds of two tomato varieties ‘205’ and ‘206’ were obtained from Zhejiang Academy of Agricultural Sciences.198, Shiqiao Rd, Hangzhou, Zhejiang, P, R, China. The reason for choosing these two tomato varieties is that they vary in salt tolerance capability and are under common cultivation in the Zhejiang province with initial moisture contents of 8.23 % and 8.13 %, respectively. Sand particle diameter ranged

Table 1. Effect of sand priming on germination and seedling vigour of two tomato varieties 205 and 206 in response to different concentrations of NaCl.

Variety	Priming treatment	FGP (%)	GI	VI	Root length (cm)	Shoot length (cm)	Fresh wt (mg/plant)	Dry wt (mg/plant)
205	Control	84.66 ab*	52.28 bc	174.12 bc	4.93 c	3.32 ab	39.13 b	10.53 b
	Sand priming	94.33 a	93.67 a	324.68 a	5.60 a	3.62 a	41.53 a	11.96 a
	50 mM NaCl	76.66 b	46.83 c	160.57 c	5.43 ab	3.39 ab	32.30 c	6.80 d
	100 mM NaCl	15.10 d	4.86 e	15.59 e	4.96 c	3.26 ab	28.56 d	6.70 d
	150 mM NaCl	2.20 d	0.22 e	0.04 e	0.10 e	0.06 d	4.5 f	1.20 f
	SP+ 50 mM NaCl	85.00 ab	57.25 b	195.76 b	5.33 ab	3.41 ab	38.96 b	9.30 c
	SP+ 100 mM NaCl	50.20 c	21.52 d	67.57 d	5.10 bc	3.13 b	31.00 c	6.83 d
	SP+ 150 mM NaCl	9.33 d	0.78 e	0.97 e	2.53 d	1.26 c	12.83 e	2.50 e
	LSD at 0.05	6.583	3.251	16.145	0.159	0.207	0.842	0.556
206	Control	87.10 a	58.49 c	174.23 b	5.16 a	2.98 b	44.67 a	15.06 a
	Sand priming	94.66 a	102.11 a	312.32 a	5.36 a	3.06 ab	41.63 b	11.90 b
	50 mM NaCl	68.46 b	36.36 d	102.19 c	5.03 ab	3.04 ab	35.40 c	8.16 d
	100 mM NaCl	5.33 de	1.086 e	3.4033 d	4.63 b	3.13 ab	31.63 d	7.23 e
	150 mM NaCl	0.00 e	0.00 e	0.00 d	0.00 d	0.00 d	0.00 f	0.00 g
	SP+ 50 mM NaCl	87.11 a	81.20 b	270.24 a	5.20 a	3.36 a	42.36 b	10.20 c
	SP+ 100 mM NaCl	40.46 c	38.14 d	120.26 c	5.10 a	3.16 ab	33.43 d	6.80 e
	SP+ 150 mM NaCl	17.76 d	13.15 e	21.68 d	3.06 c	1.60 c	26.33 e	5.03 f
	LSD at 0.05	7.062	7.182	19.88	0.207	0.173	0.859	0.366

* Small letters behind data mean differences between treatments of the same variety in the same column (α=0.05, LSD). FGP = Final germination percentage, GI = Germination index, VI = Vigour index. SP = Sand priming.

between 0.5 mm to 2 mm with coherence nil to very slight, cannot be moulded, single grains adhere to fingers, nil to slight turbidity when puddled. The sand was washed with water and then dried at 130 °C for 4 h. Each gram of tomato seed was mixed with 40 g of sand containing 4 % (v/w) water and sealed in plastic box. Tomato seeds were primed at 25 °C for 72 h and after priming were separated from sand and dried at room temperature for 24 h. In this experiment, the seeds which were used as control (CK) were not sand primed. They were used as such without any priming treatment.

Germination and seedling vigour evaluation

Saline solutions of 0, 50, 100 and 150 mM NaCl concentrations were prepared. The primed and unprimed seeds (CK) were placed in 9 cm diameter petri dishes containing two layers of moistened blotters with 4 ml of respective saline solutions. After that, seeds were kept in a germination chamber at 25 °C under alternating cycle of 12 h of light and 12 h darkness for 10 days. In the first 4 days, 1 ml 0.8 % NaCl solution was supplied in the petri dishes. Three replicates of 75 seeds each for each treatment were used. Seeds were considered germinated when a 2 mm long radicle protruded through the seed coat. Final germination percentage was calculated on the tenth day after sowing.

Germination index (GI= $\sum \frac{Gt}{Tt}$,

where Gt is number of germinated seeds in the time t day, Tt is time corresponding to Gt in days) was calculated as described by (Hu et al., 2006).

Tomato seedlings were harvested after two weeks and washed with deionized water. These seedlings were initially used to measure root and shoot lengths and later on used for fresh and dry weight measurement. Dry weight was determined after oven drying the samples at 80 °C for 24 h.

Measurement of antioxidant enzyme activities and malondialdehyde contents

Whole seedlings with fourteen-day-old of tomato were used according to the method of guaiacol to measure the antioxidant enzyme activities and contents of malondialdehyde (Zhu and Zhong, 1990). About 0.3 g sample was homogenized in 4 ml extraction buffer consisting of 50 mM phosphate (pH 7.8). This homogenate was centrifuged at 4 °C for 20 min at 12,000 rpm and the resulting supernatant was used for determination of various enzymes activity and MDA contents by using a spectrophotometer (UV-2450, SHIMADZU, Japan).

CAT activity was measured by reduction in absorbance at 240 nm due to the decline of extinction H_2O_2. The 3 ml reaction mixture containing 2.8 ml phosphate buffer (25 mM, pH 7.0), 0.1 ml H_2O_2 (0.4 %) and 0.1 ml enzyme extract was used. The reaction started with the addition of H_2O_2 (Cakmak and Marschner, 1992). The enzyme activity was calculated in terms of l mol of H_2O_2 g^{-1} FW min^{-1} at 25 ± 2 °C.

POD activity was measured with guaiacol as the substrate in a total volume of 3 ml (Zhang, 1992). The 3 ml reaction mixture consisted of 2.7 ml phosphate buffer (25 mM, pH 7.0), 0.1 ml guaiacol (1.5 %), 0.1 ml H_2O_2 (0.4 %) and 0.1 ml of enzyme extract. Increase in the absorbance due to oxidation of guaiacol (E = 25.5 mM^{-1} cm^{-1}) was measured at 470 nm. The enzyme activity was calculated in terms of l mol of guaiacol oxidized g^{-1} FW min^{-1} at 25 ± 2 °C.

SOD activity was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazo-lium (NBT) (Rao and Sresty, 2000). NBT reaction solution contained 50 mmol L^{-1} phosphate buffer (pH 7.8), 13 mmol L^{-1} methionine, 75 µmol L^{-1} NBT, 2 µmol L^{-1} riboflavin, 0.1 mmol L^{-1} EDTA. The reaction mixture was 3.1 ml, which contained 3 ml NBT reaction solution and 0.1 ml of enzyme extract. Reaction was started by adding 2 µmol L^{-1} riboflavin

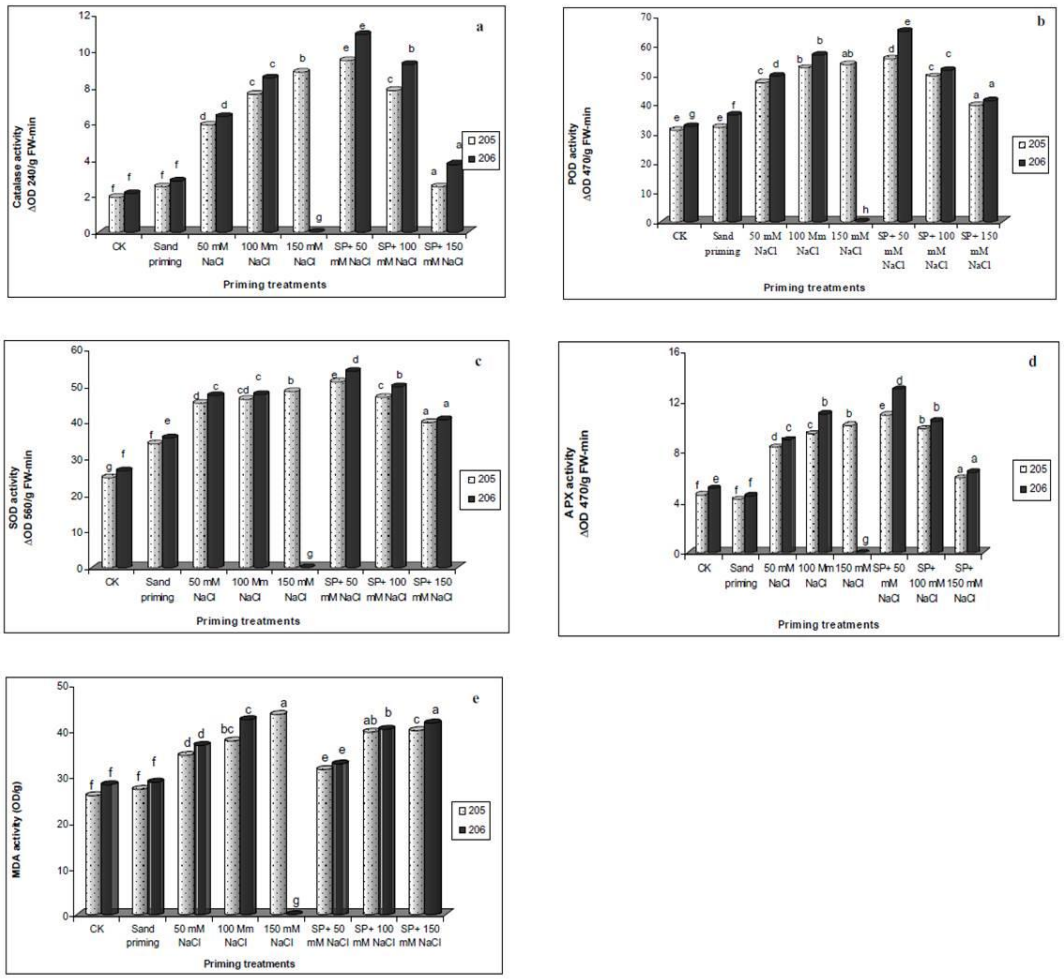

Fig 1. Effect of sand priming on (a) Catalase (CAT) (b) Peroxidase (POD) (c) Superoxide dismutase (SOD) (d) Ascorbate peroxidase (APX) activities and (e) Malondialdehyde (MDA) contents in leaves of two tomato varieties 205 and 206. Data represents the average of three replicates. SP means sand priming and CK means control.

and placing the reaction tubes under 15 W fluorescent lamps for 15 min. A complete reaction mixture without enzyme extract served as a control. The photoreduction of NBT was measured at 560 nm and one unit of SOD was defined as being present in the volume of extract that caused inhibition of the photo- reduction of NBT by 50 %.

APX activity was measured according to Nakano and Asada (1981). The assay depended on the decrease in absorbance at 290 nm as ascorbate was oxidized. The 3 ml reaction mixture consisted of 2.7 ml phosphate buffer (25 mM, pH 7.0), 0.1 ml ascorbate (7.5 mM), 0.1 ml H_2O_2 (0.4 %) and 0.1 ml of enzyme extract. The reaction started by addition of H_2O_2. The enzyme activity was calculated in terms of l mol of AsA g^{-1} FW min^{-1} at 25 ± 2 °C.

MDA concentration was determined as 2-thiobarbituric acid (TBA) reactive metabolites (Zhang et al., 2007). About 1.5 ml extract was homogenized in 2.5 ml of 5 % TBA made in 5 % trichloroacetic acid (TCA). The mixture was heated at 95 °C for 15 min, and then quickly cooled on ice. After centrifugation at 5,000 *g* for 10 min, the absorbance of the supernatant was measured at 532 nm. Correction of non-specific turbidity was made by subtracting the absorbance value measured at 600 nm. The concentration of MDA was calculated in terms of n mol of g^{-1} FW.

Data analysis

The main effect of salt treatment (from both NP- and Sand primed-seeds) was tested by one-way analysis of variance (ANOVA). Percentage data were arcsin-transformed before analysis according to $\hat{y}$ = arcsin [sqr (x/100)]. Means were compared between treatments by LSD (least significant difference) at the 0.05 confidence level using Statistix 8.1 software (Copy right 2005, Analytical Software, USA).

Conclusions

Our data suggests that sand priming can serve as a promising tool for alleviation of salt stress in tomato through enhancing seedling vigour and triggering of antioxidant enzymes defense response.

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References

Afzal I, Ashraf S, Qasim M, Basra SMA, Shahid M (2009) Does halopriming improve germination and seedling vigour in marigold (*Tagetes spp*.). Seed Sci Technol 37: 436-445

Afzal I, Basra SMA, Ahmad N, Cheema MA, Warraich EA, Khaliq A (2002) Effect of priming and growth regulator treatment on emergence and seedling growth of hybrid maize (*Zea mays*). Int J Agric Biol 4: 303-306

Bailly C, Benamar A, Corbineau F, Come D (1997) Changes in superoxidedismutase, catalase and glutathione reductase activities in sunflower seeds during accelerated ageing and subsequent priming. pp. 665-672. In: Ellis RH, Black M, Murdoch AJ, Hong TD (ed) Basic and applied aspects of seed biology. Dordrecht: Kluwer Academic Publishers

Beutner S, Bloedorn B, Frixel S, Blanco IH, Hoffman T, Martin H (2001) Quantitative assessment of antioxidant properties of natural colorants and phytochemicals: carotenoids, flavonoids, phenols and indigoids. The role of β-carotene in antioxidant functions. J Sci Food Agric 81: 559-568

Bray CM, Davison PA, Ashraf M, Taylor, RM (1989) Biochemical changes during priming of leek seeds. Ann Bot 63: 185-193

Cakmak I, Marschner H (1992) Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascorbate peroxidase, and glutathione reductase in bean leaves. Plant Physiol 98: 1222-1227

Cayuela E, Perez-Alfocea F, Caro M, Bolarin MC (1996) Priming of seeds with NaCl induces physiological changes in tomato. Plant Physiol 96: 231-236

Chiu KY, Sung JM (2002) Effect of priming temperature on storability of primed sh-2 sweet corn seed. Crop Sci 42: 1996-2003

Chookhampaeng S, Pattanagul W, Theerakulpisut P (2008) Effects of salinity on growth, activity of antioxidant enzymes and sucrose content in tomato (*Lycopersicon esculentum* Mill.) at the reproductive stage. ScienceAsia 34: 69-75

Cuartero J, Fernandez-Munoz R (1999) Tomato and salinity. Sci Hort 78: 83-125

Ghassemi-Golezani K, Afsaneh C, Safar N, Mohammad M (2010) Influence of hydro-priming duration on field performance of pinto bean (*Phaseolus vulgaris* L.) cultivars. Afri J Agri Res 5 (9): 893-897

Hu J, Xie, XJ, Wang ZF, Song WJ (2006) Sand priming improves alfalfa germination under high salt concentration stress. Seed Sci Technol 34: 199-204

Hu, J, Zhu ZY, Song WJ, Wang JC (2002) Effect of sand priming on the germination and the physiological changes in direct-sowing rice (*Oryza sativa* L.). In: Proceedings of the international forum on seed science & technology and seed industry development, pp.185-191, China Agricultural Science and Technology Press, Beijing.

Jamil M, Lee DB, Jung KY, Ashraf M, Lee SHC, Rha ESH (2006) Effect of salt (NaCl) stress on germination and early seedling growth of four vegetables species. Central Europian Agricalture 7(2): 273-282

Khan MH, Panda SK (2008) Alterations in root lipid peroxidation and antioxidative responses in two rice cultivars under NaCl-salinity stress. Acta Physiol Plant 30: 81–89

Lee SS, Kim JH, (2000) Total sugars, α-amylase activity and germination after priming of normal and aged rice seeds. Korean J Crop Sci 45: 108-111

Mengel K, Kirkby EA, Kosegarten H, Appel T (2001) Principles of plant nutrition. Kluwer Academic Publishers, Dor- drecht, The Netherlands

Nakano Y, Asada K (1981) Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplast. Plant Cell Physiol 22: 867–880

Nawaz A, Muhammad A, Muhammad AP, Afzal I (2011) Effect of halopriming on germination and seedling vigour of tomato. Afri J of Agri Res 6(15): 3551-3559.

Noctor G, and C. Foyer C (1998) Ascorbate and Glutathione: Keeping active oxygen under control. Annu Rev. Plant Physiol. Plant Mol. Biol., 49: 249-279.

Okhovatian-Ardakani AR, Mehrabanian M, Dehghani F, Akbarzadeh A (2010) Salt tolerance evaluation and relative comparison in cuttings of different pomegranate cultivars. Plant, Soil Environ 56: 176–185

Rao KVM, Sresty TVS (2000) Antioxidant parameters in the seedlings of pigeon pea (*Cajanus cajan* (L.) Millspaugh) in response to Zn and Ni stresses. Plant Sci 157: 113–128

Rouhi HR, Ali AS, Farzad SZ, Reza TA, Mohammad AA, Goudarz A (2011) Study of diferent priming treatments on germination traits of Soybean seed lots. Not Sci Biol 3(1): 101-108

Zhang S, Hu J, Zhang Y, Xie XJ, Knapp A (2007) Seed priming with brassinolide improves lucerne (*Medicago sativa* L.) seed germination and seedling growth in relation to physiological changes under salinity stress. Aust J Agri Res 58: 811–815

Zhang XZ (1992) The measurement and mechanism of lipid peroxidation and SOD, POD and CAT activities in biological system. In: Zhang XZ (ed) Research methodology of crop physiology. Agriculture Press, Beijing, pp 208–211

Zhu GL, Zhong WH (1990) Experimental handbook of plant physiology. Peking University Press, Peking, China