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Effect of seed priming on germination behavior, oxidative stress and antioxidant enzyme activities in groundnut (*Arachis hypogaea L*) under salinity stress

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ABSTRACT
Salinity is an important abiotic stress which significantly affects seedling, vegetative and reproductive growth and yield of groundnut. An experiment was carried out to study the effect of seed priming on germination behavior, solute accumulation and antioxidative enzyme activities in germinating seeds of groundnut under salinity stress. Seeds of groundnut cv.TG-51 were treated with three different concentrations each of various priming agents for 14 hours and were subjected to 200mM NaCl salinity stress. Results indicated that the priming with gibberellic acid 50 ppm, hydrogen peroxide 60 mM, ascorbic acid 100 ppm, salycilic acid 25 ppm, mannitol 2.5% and sodium chloride 50 mM showed significant improvement in different germination parameters studied along with length of embryonic axis at 72 hours of germination over the water-soaked unprimed ones under salinity treatment. Further physiological studies with these selected concentrations of priming agents revealed that in general, the cotyledon showed much higher extent of membrane damage under salinity than embryonic axis with water-soaked unprimed seeds registering the maximum damage. Seed treatment with hydrogen peroxide 60 mM caused minimum lipid peroxidation of the membrane in both embryonic axis and cotyledon at different hours of germination and this might be attributed to much higher guaiacol peroxidase (GPOX) activity induced by this priming treatment. Seed priming with salicylic acid 25 ppm recorded the highest mean activity of catalase (CAT) enzyme, total phenol content in embryonic axis and cotyledon. However, NaCl 50 mM and mannitol 2.5% induced much higher accumulation of proline in the cotyledon, especially, at 48 and 72 hours of germination under salinity stress. In embryonic axis, the effect of hydrogen peroxide 60 mM was much higher in inducing proline accumulation than any other priming agents. Overall mean comparison indicated higher activities of GPOX and CAT enzymes along with higher content of proline and phenol in the cotyledon than embryonic axis in germinating seeds of groundnut. The priming treatments showed enhanced accumulation of proline along with higher activities of antioxidant enzymes GPOX and CAT and phenol content which might regulate osmotic adjustment and mitigate oxidative stress under salinity stress during seed germination.
***Keywords**: Antioxidative enzyme, Groundnut, Salinity stress, Seed priming*

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INTRODUCTION

Different growth stages of plant are often subjected to various types of abiotic stress like drought, salinity, high temperature etc. which may cause yield loss. Soil salinization is a fast growing problem of agriculture with about 23% of the world's cultivated land being saline(Wang *et al.,* 2012).Salinity stress has been widely reported to negatively affect germination, growth and yield of many crop plants.Salt stress induces ionic stress and osmotic stress in plant cells. Germination and early seedling growth stage are the most critical stage of plant development and salinity stress at these stages may result in serious damage in seedling establishment. Different approaches like conventional breeding and selection, transgenics production (Zhao *et al.,* 2006), exogenous application of osmolytes, osmoprotectants or plant hormones (Ashraf *et al.,* 2008) and pre-sowing seed treatments with priming agents (Afzal *et al.,* 2006) have been employed for alleviation of salinity stress in crops.

In India, groundnut is an important oilseed, food and feed crop grown in an area of 6.45 million ha with atotal production of 6.57 million tons (FAO, 2007). Groundnut occupies nearly 28.3% of the cultivated area and contributes 31.7% of the production of the total oilseeds in the country. Among many reasons ascribed for the lower productivity of groundnut, salinity is an important abiotic stress which significantly affects seedling, vegetative and reproductive growth, seed quality and yield (Girdhar *et al.*, 2005 and Nithila *et al*., 2013). Root zone salinity increases as a result of continuous use of saline water for irrigation because of limited or non-availability of good quality water in majority of groundnut growing areas.

Seed priming as a pre-sowing technique can improveradicle emergence, germination rate, germination vigor,seedling establishment and yield by making changes inmetabolic activities in the seeds of many crops (Bodsworthand Bewley, 1981; Dell Aquila and Tritto, 1991 and Taylor andHarman, 1990).It has been found to be an easy, low cost, low risk and effective approach to enhance plant tolerance to the stressful environments (Ashraf and Foolad, 2005). A number of priming strategies include seed treatments with osmotica, inorganic salts or hormones. These seed pre-treatments are reported to induce pre-germination changes, which usually have beneficial effects on seed germination percentage, germination speed, reserve mobilization and uniformity of seedling growth and development and enhanced activities of antioxidative enzymes for scavenging Reactive Oxygen Species (ROS) over unprimed seeds under the salinity stress (Kaur *et al.,* 2002; Kaya *et al.,* 2006; Ahmed and Farag, 2011; De Souza *et al*., 2014 and Younesi and Moradi, 2014).With this background, the present research work was envisaged to observe the effect of seed priming on germination behavior, oxidative stress, solute accumulation and antioxidant enzyme activities in groundnut.

MATERIALS AND METHODS

The experiment was carried out in the laboratory of Department of Plant Physiology, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia. Seeds of groundnut cv. TG-51 weresurface sterilized by dipping in 0.1% mercuric chloride solution for three minutes and washed thoroughly with distilled water followed by proper drying. These surfaces sterilized seeds were primed with three different concentrations each of various priming agents viz, gibberellic acid (GA_3),hydrogen peroxide (H_2O_2), Ascorbic Acid (AA), Salycilic Acid (SA), Mannitol and Sodium Chloride (NaCl)for 14 hours. After that the primed seeds were washed with distilled water thoroughly and air dried. Finally they were allowed to germinate in presence of 200 mM NaCl at a temperature of 28±1°C and relative humidity around 80%. A water-soaked NaCl-stressed set along with an unstressed control was similarly set for germination for comparison of data. The germination count was done at an interval of 12 hours upto 60 hours. From the data, speed of germination, mean daily germination (MDG), peak value (PV) and germination value (GV) were calculated following the formula of Czabator (1962). Mean germination time (MGT) wascalculated as per Ellis and Roberts (1981). The length of the protruding embryonic axis was measured after dissecting the seeds at different intervals. On the basis of germination behavior, a particular concentration producing the best resultswas selected in case of each of the priming agents for further physiological studies. To determine the extent of membrane damage in both cotyledon and embryonic axis of primed seeds and water soaked control seeds under salinity stress, the lipid peroxidation was measured in terms of thiobarbituric acid reactive substances (TBARS) content following the method of Heath and Packer (1968), while the activities of two important antioxidant enzymes,*viz*, Catalase (CAT) and Guaiacol Peroxidase (GPOX), were determined as per Goth (1991) and Siegel and Galston (1967), respectively. The contents of proline and phenol in the cotyledon and embryonic axis of germinating seeds were determined as per Mohanty and Sridhar (1982) and McDonald *et al*. (2001), respectively.The experiment was set up following completely randomized design (CRD) with three replications.The mean data were analyzed using INDOSTAT version 7.1 software.

RESULTS AND DISCUSSIONS

Analysis of variance indicated that all the treatments showed highly significant variations among them for all the parameters related to germination behavior of seeds of groundnut cv. TG 51 along with the length of embryonic axis recorded at 72 hours of germination. Perusal of the data exhibited that priming of seeds with different solutions resulted in significant improvement in germination parameters under salinity stress in most of the cases over that of water-soaked stressed seeds (Table 1). Almost all the priming treatments also registered significant increase in length of embryonic axis measured at 72 hours of germination over that of water-soaked NaCl-treated seeds. The results corroborated well the early findings ofHeydecker (1974) and Harris *et al*. (1999) who emphasized the importance of seed priming to help in rapid and uniform germination and emergence of seeds even under adverse environmental conditions.Out of three different concentrations used for each of the priming agent in the present

experiment, 60 mM H_2O_2, 50 ppm GA_3, 25 ppm salicylic acid (SA), 2.5% mannitol and 50 mM NaCl were found to have the best results. These concentrations were finally selected for conducting further studies on the biochemical changes occurring in the cotyledon and embryonic axis of germinating seeds due to priming treatments at different periods of germination.

All the treatments recorded highly significant differences among them for the biochemical parameters under study at all stages of germination in both cotyledon and embryonic axis. Comparison of mean values indicated that the cotyledon hadhigher content of proline than the embryonic axis at 48 hours and 72 hours of germination but not at 24 hours (Table 2). The content of proline increased linearly along with the progress of germination in all the cases except for embryonic axis of water-soaked stressed seeds where it declined sharply. Out of all the priming agents, H_2O_2 60 mM registered much higher accumulation of proline in the embryonic axis, while NaCl 50 mM and mannitol 2.5% had much content of this osmolyte in the cotyledon especially, at 48 and 72 hours of germination. Accumulation of proline as a compatible solute for osmotic adjustment during salinity stress was reported earlier by Saha *et al.*(2010) and Kahrizi *et al.*(2012). The promotive effects of seed priming in osmotic adjustment were also reported earlier by Chen and Arora (2011) and Zhang *et al*. (2015). Unlike proline the pattern of changes in phenol content in cotyledon and the embryonic axis followed a sigmoidal curve in case of all the priming treatments with 48 hours of germination recording the highest content (Table 2). However, the content of phenol in case of water soaked saline-treated-seeds followed a different trend with the content being decreased linearly along with the progress in germination. In general, the cotyledon recorded higher content of phenol than the embryo at all intervals of study. Out of all the treatments,salicylic acid 25 ppm recorded the highest mean in both the parts of the germinating seeds and it was followed by H_2O_2 60 mM in embryonic axis and NaCl 50 mM in cotyledon. The increased accumulation of phenolic compounds under abiotic stress might be attributed to their ROS scavenging potential as well to their ability to inhibit lipid peroxidation by trapping the lipid alkoxyl radical (Millic *et al.,*1998 and Sakihama *et al.,*2000).

The seeds showed higher content of thiobarbituric acid reactive substances (TBARS) in embryonic axis and cotyledon in water soaked seeds under salinity stress than unstressed control indicating oxidative stress-induced membrane damage (Table 3). Several reports have described involvement of ROS together with their signalling roles during seed germination (Kumar *et al*., 2015). It has been suggested that after imbibition, the resurgence of mitochondrial respiration in the seed might result in electron donation to oxygen as an electron acceptor, leading to ROS production (El-Maarouf-Bouteau and Bailly, 2008). As a consequence of ROS generation, the lipid peroxidation in germinating seeds might show temporary increase and this might be further enhanced under abiotic stress resulting in oxidative stress. The comparative analysis of mean values in cotyledon and embryonic axis indicated that the greater membrane damage under salinity could be mitigated by different priming agents. The finding was well consistent with Younesi and Moradi (2014) and Zhang *et al.* (2015). Among the six priming treatments, H_2O_2 60mM was found to be very effective in such mitigation and it reduced the overall TBARS content by 62.84% and 61.94% in embryonic axis and cotyledon, respectively, over that of water soaked stressed seeds. This might indicate the involvement of ROS as second messenger in initiation of signaling pathway for counter-balancing the excess generation of ROS during salinity stress.

Accumulation of ROS takes place as a result of various environmental stresses and to protect against these toxic oxygen intermediates, plant cells contain both enzymatic and non-enzymatic scavenging components. Among the enzymatic antioxidants, catalase (CAT)and peroxidase have been found to be very important in scavenging the ROS. CAT is suggested to be involved in mass scavenging of H_2O_2 unlike peroxidase which is suggested to be involved in fine regulation of H_2O_2 (Asada, 1992 and Mittler, 2002). Results showed that the activities of both these enzymes in embryonic axis as well as in cotyledon were enhanced in the primed seeds under salinity stress during all the stages of germination, while the water soaked NaCl-stressed seeds showed much reduced activities of these two important enzymes (Table 4). The findings were corroborative of the early reports of Farhoudiand Lee (2012) and Younesi and Moradi (2014). In the present experiment, the activity of guaiacol peroxidase (GPOX) in both the cotyledon and embryonic axis registered a consistent pattern of change in all the cases with the activity being increased gradually as the germination processed progressed (Table 4).But no such consistent pattern of change was revealed by the activity of CAT in either of the seed parts. In general, cotyledon recorded much higher activities of both these scavenging enzymes than the embryonic axis. Out of all the priming treatments, salicylic acid 25 ppm was most effective in inducing the higher activity of CAT while H_2O_2 60 mM was most effective in triggering GPOX activity in both cotyledon and embryonic axis.

CONCLUSION

Distinguishing the cellular metabolisms at germination level appears to play an important role in the acclimation of crops to salt stress. Our findings show significant differences between the water-soaked primed and stressed seed under salinity stress in terms of germination behavior and biochemical parameters.Summarizing the data it might be concluded that soaking of groundnut seeds with selective concentrations of different priming agents like osmoticum NaCl and mannitol, growth regulators gibberellic acid and salicylic acid, antioxidants ascorbic acid and reactive oxygen species H_2O_2significantly improved the germination speed and embryonic growth of groundnut seeds under salinity stress. The pre-sowing seed treatment with these agents induced higher accumulation of proline and phenol as well as higher activity of antioxidative enzymes under salinity stress and this might attribute for mitigation of salt stress during germination of groundnut seeds.

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Table 1. Effect of seed priming on germination behavior and growth of embryonic axis under salinity stress in groundnut cv TG 51

Treatment	Speed of Germination	Mean Germination Time	Peak Value	Mean Daily Germination	Germination value	embryonic axis length (mm) at 72 hours
Control (without salinity)	6.94	26.67	5.56	4.17	23.15	52.33
Control (with salinity)	4.49	45.00	2.08	1.67	3.47	19.33
H_2O_2 20mM	4.75	46.15	2.56	2.08	5.34	28.67
H_2O_2 40mM	5.07	43.08	3.21	2.78	8.90	28.33
H_2O_2 60mM	6.52	30.77	5.13	2.78	14.25	35.33
GA_3 25ppm	5.24	40.00	3.21	2.78	8.90	25.67
GA_3 50ppm	7.20	25.45	6.06	4.17	25.25	34.00
GA_3 100ppm	4.06	47.69	1.50	2.78	4.16	17.33
SA 25ppm	6.38	29.40	4.42	4.17	18.40	26.33
SA 50ppm	5.30	38.18	1.52	2.78	4.21	22.67
SA 100ppm	5.14	44.00	3.33	2.08	6.94	17.00
Mannitol 0.5%	6.31	30.91	4.55	2.78	12.63	19.33
Mannitol 1%	5.13	38.46	1.92	2.78	5.34	14.33
Mannitol 2.5%	6.67	28.00	5.00	4.17	20.83	26.00
AA 25ppm	4.31	48.00	1.67	2.08	3.47	28.33
AA 50ppm	4.72	40.00	2.50	2.78	6.94	22.00
AA 100ppm	5.74	33.40	2.21	2.78	6.13	32.67
NaCl 25mM	6.06	30.91	2.27	4.17	9.47	20.67
NaCl 50mM	7.20	25.45	6.06	4.17	25.25	26.33
NaCl 100mM	4.23	47.27	1.52	2.08	3.16	15.00
SEm(±)	**0.11**	**0.75**	**0.06**	**0.07**	**0.24**	**0.59**
CD(P= 5%)	**0.22**	**1.52**	**0.13**	**0.14**	**0.48**	**1.19**

Table 2. Effect of seed priming on proline and phenol content in embryonic axis and cotyledon of groundnut cv TG 51under salinity stress

Treatment	Proline(μm/g fresh weight)								Phenol(mM galic acid/gm fresh weight)							
	Embryonic axis				Cotyledon				Embryonic axis				Cotyledon			
	Hours of germination				Hours of germination				Hours of germination				Hours of germination			
	24 hours	**48 hours**	**72 hours**	**Mean**	**24 hours**	**48 hours**	**72 hours**	**Mean**	**24 hours**	**48 hours**	**72 hours**	**Mean**	**24 hours**	**48 hours**	**72 hours**	**Mean**
Control (without salinity)	2.36	2.88	3.69	**2.98**	1.86	3.33	4.01	**3.07**	6.21	3.83	3.78	**4.61**	3.06	7.49	7.50	**6.01**
Control (with salinity)	2.10	1.78	1.38	**1.75**	0.77	0.83	1.17	**0.92**	5.09	2.23	2.25	**3.19**	9.70	7.30	5.75	**7.58**
H_2O_2 60mM	1.37	1.88	3.31	**2.19**	0.66	0.96	3.17	**1.60**	4.73	6.19	3.29	**4.74**	3.63	11.32	4.25	**6.40**
GA_3 50ppm	0.45	1.08	2.40	**1.31**	1.23	2.25	2.61	**2.03**	4.13	4.20	3.30	**3.88**	4.24	8.17	4.53	**5.65**
SA 25ppm	1.02	1.15	1.36	**1.18**	0.94	1.95	2.85	**1.91**	4.36	6.82	5.10	**5.43**	9.51	12.89	7.23	**9.88**
Mannitol 2.5%	0.86	1.25	2.64	**1.58**	0.45	2.41	3.84	**2.23**	4.11	5.29	3.50	**4.30**	3.86	11.26	5.10	**6.74**
AA 100ppm	0.68	1.22	2.92	**1.61**	0.54	1.19	2.67	**1.47**	3.80	5.00	4.36	**4.39**	4.65	10.03	5.15	**6.61**
NaCl 50mM	0.84	0.87	3.33	**1.68**	1.28	3.56	4.17	**3.00**	3.64	4.60	2.58	**3.61**	6.16	10.62	6.57	**7.78**
Mean	**1.21**	**1.51**	**2.63**		**0.97**	**2.06**	**3.06**		**4.51**	**4.77**	**3.52**		**5.60**	**9.89**	**5.76**	
	SEm (±)	CD(P=5%)			SEm (±)	CD(P=5%)				SEm (±)	CD(P=5%)			SEm (±)	CD(P=5%)	
Treatment	0.02	0.04			0.03	0.06				0.05	0.10			0.08	0.17	
Stage	0.01	0.03			0.02	0.03				0.03	0.06			0.05	0.10	
Treatment ×Stage	0.04	0.08			0.05	0.10				0.09	0.18			0.14	0.29	

Table 3. Effect of seed priming on lipid peroxidationin embryonic axis and cotyledon of groundnut cv TG 51under salinity stress

Treatment	Lipid peroxidation(μM of TBRAS content /gm fresh weight)							
	Embryonic axis				**cotyledon**			
	Hours of germination				**Hours of germination**			
	24 hours	**48 hours**	**72 hours**	**Mean**	**24 hours**	**48 hours**	**72 hours**	**Mean**
Control(without salinity)	27.19	29.02	25.49	**27.23**	19.94	58.04	89.59	**55.85**
Control(withsalinity)	27.51	56.53	97.91	**60.65**	43.15	123.90	140.06	**102.37**
H_2O_2 60mM	10.34	29.02	28.27	**22.54**	27.26	40.12	49.97	**39.12**
GA_3 50ppm	13.12	29.27	32.05	**24.81**	31.29	95.89	128.95	**85.38**
SA 25ppm	22.71	50.22	55.11	**42.68**	33.82	97.60	119.74	**83.72**
Mannitol 2.5%	29.27	31.54	32.05	**30.95**	35.65	74.69	102.71	**71.02**
AA 100ppm	15.14	33.82	44.67	**31.21**	24.48	44.67	58.55	**42.56**
NaCl 50mM	9.34	31.04	47.95	**29.44**	50.47	68.89	98.67	**72.68**
Mean	**19.33**	**36.31**	**45.44**		**33.26**	**75.48**	**98.53**	
		SEm (±)	CD(P=5%)			SEm (±)	CD(P=5%)	
Treatment		0.45	0.90			1.00	2.01	
Stage		0.27	0.55			0.61	1.23	
Treatment ×Stage		0.77	1.55			1.73	3.49	

Table 4.Effect of seed priming on catalase and guiacol peroxidase activity in embryonic axis and cotyledon under salinity stress in groundnut cvTG 51

Treatment	Catalase (µmof residual H_2O_2 /g fresh weight)								GPOX(change in A470 per min per g fw)							
	Embryonic axis				cotyledon				Embryonic axis				cotyledon			
	Hours of germination				Hours of germination				Hours of germination				Hours of germination			
	24 hours	48 hours	72 hours	Mean	24 hours	48 hours	72 hours	Mean	24 hours	48 hours	72 hours	Mean	24 hours	48 hours	72 hours	Mean
Control (without salinity)	104.10	195.59	208.98	169.56	371.05	404.23	417.65	397.64	3.32	17.64	30.44	17.13	4.98	14.62	14.30	11.30
Control (with salinity)	106.33	118.61	113.03	112.66	207.47	242.45	371.47	273.80	1.00	0.68	8.20	3.29	1.44	6.56	6.88	4.96
H_2O_2 60mM	287.09	237.99	164.35	229.81	384.30	517.65	471.55	457.83	2.64	11.88	31.12	15.21	7.28	13.95	26.85	16.03
GA_3 50ppm	125.30	206.75	383.04	238.36	249.85	473.42	570.49	431.25	0.44	13.40	23.52	12.45	1.60	12.38	19.58	11.19
SA 25ppm	303.82	323.49	340.64	322.65	349.85	728.93	771.47	616.75	0.28	7.20	7.20	4.89	5.60	10.50	12.54	9.55
Mannitol 2.5%	278.16	231.30	211.22	240.22	349.55	442.55	271.46	354.52	0.68	4.20	20.16	8.35	2.24	14.37	26.62	14.41
AA 100ppm	303.82	217.91	117.49	213.07	349.67	542.55	372.25	421.49	0.28	18.00	14.00	10.76	3.96	5.73	10.21	6.63
NaCl 50mM	208.98	520.28	265.88	331.71	416.23	586.95	290.99	431.39	1.60	18.88	24.20	14.89	6.40	17.92	18.37	14.23
Mean	214.70	256.49	225.58		334.75	492.34	442.17		1.28	11.49	19.85		4.19	12.00	16.92	
	SEm (±)	CD(P =5%)			SEm (±)	CD(P =5%)				SEm (±)	CD(P =5%)			SEm (±)	CD(P =5%)	
Treatment	2.77	5.56			5.74	11.54				0.15	0.30			0.14	0.28	
Stage	1.69	3.41			3.51	7.07				0.09	0.18			0.08	0.17	
Treatment ×Stage	4.79	9.64			9.94	19.99				0.26	0.51			0.24	0.48	

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