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Research Article

EFFECT OF SEED PRIMING ON RESERVE MOBILIZATION, WATER UPTAKE AND ANTIOXIDATIVE ENZYME ACTIVITIES IN GERMINATING SEEDS OF GROUNDNUT UNDER SALINITY STRESS

PAL A.[1]*, YADAW S.K.[2], PAL A.K.[3] AND GUNRI S.[4]

[1,2,3]Department of Plant Physiology, Faculty of Agriculture, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, 741252, West Bengal
[4]Department of Agronomy, Faculty of Agriculture, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, 741252, West Bengal
*Corresponding Author: Email-apapurbapal@gmail.com

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Abstract- An experiment was carried out to study the effect of seed priming on germination behavior, reserve mobilization, solute accumulation and antioxidative enzyme activities in germinating seeds of groundnut under salinity stress. Seeds of groundnut cv.TG-51 were treated with various priming agents *viz.,* gibberellic acid 50 ppm, hydrogen peroxide 60 mM, ascorbic acid 100 ppm, salycilic acid 25 ppm, mannitol 2.5% and sodium chloride 50 mM for 14 hours and were subjected to salinity stress (200mM NaCl). Results indicated that the primed seeds showed significant improvement in germination speed and growth of embryonic axis over the unprimed ones under salinity treatment. This might be attributed to higher water uptake ability and enhanced rate of reserve mobilization because of seed priming before germination. The priming treatments also showed enhanced accumulation of proline along with higher activities of antioxidant enzymes GPOX and CAT and alleviated levels of lipid peroxidation in the embryonic axis which might contribute to osmotic regulation and mitigation of oxidative stress under salinity stress during seed germination. Among all the priming agents, GA_3 50 ppm, mannitol 2.5% and NaCl 50mM especially produced encouraging results.

Keywords- Antioxidative enzyme, Groundnut, Reserve mobilization, Salinity stress, Seed priming.

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Introduction

Groundnut (*Arachis hypogaea* L.) is the fourth most important source of edible oil and the third most important source of protein in the world. In India, it accounts for approximately 50% of oilseed production [1]. Still the productivity falls below the level of demand. Different growth stages of this crop are often subjected to various types of abiotic stress like drought, salinity, high temperature etc. which may cause yield loss. 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 [2,3]. Seed germination is the most critical stage in crop growth cycle and it is very much sensitive to salinity stress. Poor germination in saline soils leads to poor crop stand and productivity. Pre-sowing priming of seeds have 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) [4]. Many 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 ROS over unprimed seeds under the salinity stress [5-9]. With this background, the present research work was envisaged to observe the effect of seed priming on embryonic growth, reserve mobilization, water uptake and antioxidative enzyme activities in germinating seeds of groundnut under salinity stress.

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 were soaked for 14 hours in three different concentrations each of six priming agents *viz.,* gibberellic acid (GA_3), hydrogen peroxide (H_2O_2), Ascorbic Acid (AA), salycilic acid (SA), mannitol and sodium chloride (NaCl). After that the primed seeds were washed with distilled water thoroughly followed by air drying. Finally, they could germinate in presence of 200 mM NaCl at a temperature of 28±1°C and relative humidity around 80%. Unprimed (2 hour's water soaked) seeds were set similarly for comparison. From the data on growth of embryonic axis recorded at different periods of germination (data not shown), concentrations of each of the six priming agents were finally selected for further physiological studies. Speed of germination of the primed and unprimed seeds under salinity along with unstressed control seeds was calculated from daily count of germinated seeds upto 5th day as per the method of Maguire [10].

The water uptake of the seeds and the reserve mobilization of the cotyledons were determined at an interval of 12 hours upto 72 hours of germination following the method of Harb, 2013 [11]. To determine the extent of membrane damage under salinity stress in both the primed and unprimed seeds, lipid peroxidation was measured in terms of thiobarbituric acid reactive substances (TBARS) content following the method of Heath and Packer (1968) [12], The proline content along with the activities of two important antioxidant enzymes like catalase (CAT) and guaiacol peroxidase (GPOX) in the embryonic axis at different periods of germination were determined as per Mohanty and Sridhar (1982) [13], Goth (1991) [14] and Siegel and Galston (1967) [15]., respectively. The mean data in all the cases were subjected to statistical analysis following completely randomized design (CRD).

Results

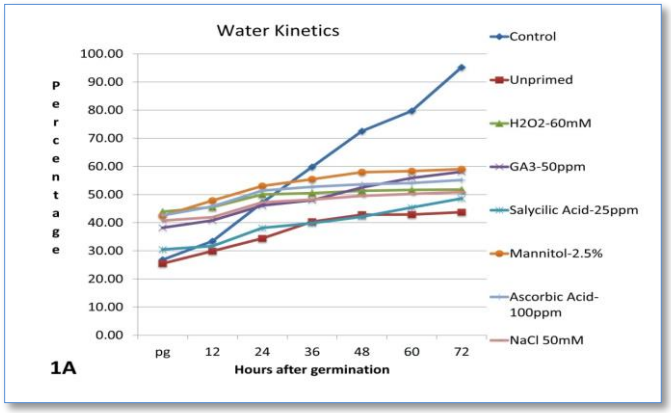
The perusal of data indicated that the unprimed seeds registered substantially slower speed of germination as well as reduced growth of embryonic axis under salinity stress as compared to the unstressed control seeds [Table-1]. Seeds primed with 2.5% mannitol, 25 ppm salycilic acid and 60mM H_2O_2 showed comparatively high speed of germination than 100 ppm ascorbic acid, 50 ppm GA_3 and 50mM NaCl treated seeds under saline condition and there was on an average 1.40-1.50 times improvement over the un-primed seeds. This was closely followed by priming with antioxidant ascorbic acid.

Table-1 *Effect of seed priming on speed of germination and growth of embryonic axis in germinating seeds of groundnut cultivar TG-51 under salinity stress*

Treatments	Speed of germination	Embryonic axis Fresh weight (mg)	Embryonic axis Dry weight (mg)	Embryonic axis Length (mm)
Control (unstressed)	6.73 a	166.040 a	35.260 a	49.250 a
Unprimed	4.49e	94.300g	22.700e	19.330e
H_2O_2 (60mM)	5.83c	110.967 e	27.067c	35.330b
Salycilic acid (25ppm)	6.39 b	142.033 bc	31.700 b	26.330 d
Ascorbic acid (100ppm)	5.74 d	147.967 b	34.067 a	32.670 c
GA_3 (50ppm)	5.97c	140.567c	25.567d	34.000bc
Mannitol (2.5%)	6.39 b	117.667 d	26.133 cd	26.000 d
NaCl (50mM)	4.58e	103.733f	25.900cd	26.330d
C.D. 5%	0.297	6.279	1.419	1.493

Means with the same letter within a column are not significantly different at the P≤0.05 level (LSD)

Data on water uptake percentage of whole seeds at different hours of germination indicated that salinity stress adversely affected the water uptake with unprimed seeds registering the most severe inhibition [Fig-1A]. Out of all the priming treatments, soaking of seeds by mannitol 2.5% solution registered the highest water uptake (59.02%) at 72 hours of germination closely followed by GA_3 50ppm (58.09%) and ascorbic acid 100 ppm (55.16%) treatments. The enhanced rate of water uptake by whole seeds under mannitol and GA_3-induced priming also resulted in increased mobilization of reserves from cotyledon during critical stages of germination [Fig-1B]. The reserve mobilization in unprimed cotyledons progressed at a very diminishing rate throughout the germination periods and there was around 60% decrease in mobilization at 72 hours of germination under salinity stress as compared to the unstressed control. In the present experiment, the unprimed seeds also exhibited much lower accumulation of proline in the embryonic axis at all the stages of germination under salinity stress and finally registered around 62.57% less content at 72 hours of germination in comparison with the unstressed control seeds [Fig-2A]. On the contrary, all the priming treatments enhanced accumulation of osmolyte, with 50mM NaCl, 60 mM H_2O_2 and 100 ppm ascorbic acid showing the highest positive effects. The cell membrane permeability of the growing embryo was measured in terms of lipid peroxidation that involved the determination of the content of thiobarbituric acid reactive substances (TBARS).



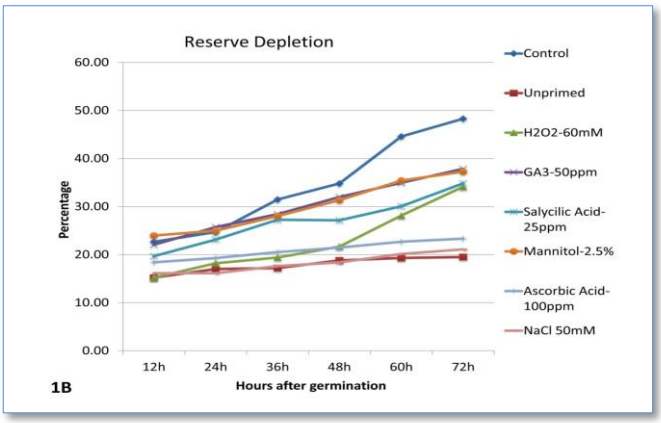
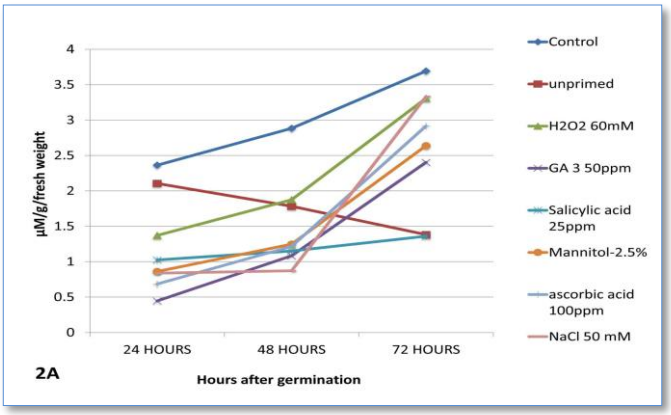
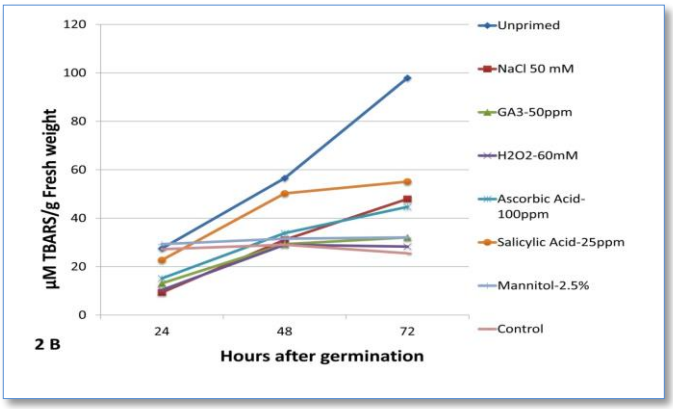
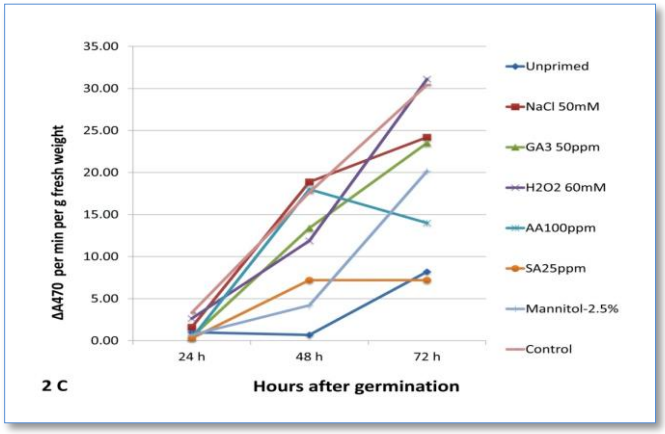


Fig-1 Effect of seed priming on water uptake (A) and reserve mobilization (B) in germinating seeds of groundnut cultivar TG 51 under salinity stress.

Because of ROS generation, the lipid peroxidation in germinating seeds may show temporary increase and this is further enhanced under abiotic stress resulting in oxidative stress. In the present experiment, the content of TBARS in embryonic axis was much higher in the unprimed seeds under salinity stress than unstressed control indicating oxidative stress-induced membrane damage [Fig-2B] Among the six priming treatments, hydrogen peroxide 60mM, GA_3 50ppm and mannitol 2.5% registered the minimum TBARS content at 72 hours of germination in the embryonic axis indicating least damage under oxidative stress. On the contrary, salycilic acid (25ppm), ascorbic acid (100ppm) and NaCl (50mM) showed moderate effect. When plants are subjected to saline stress, activities of many antioxidant enzymes are enhanced to eliminate ROS (Ruiz-Lozano 2003). Guaiacol peroxidase (GPOX) and catalase (CAT) are two important antioxidant enzymes that convert H_2O_2 to water. Among the priming agents, H_2O_2 60mM registered the highest activity of the GPOX enzyme under salinity stress at 72 hours of germination followed by NaCl 50mM and GA_3 50 ppm, while GA_3 50 ppm along with salicylic acid 25 ppm and NaCl 50mM exhibited the highest activity of CAT [Fig-2C and 2D].







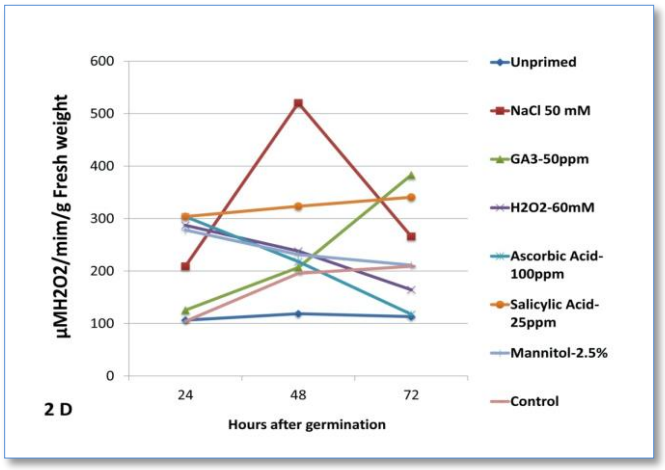


Fig. 2 Effect of seed priming on proline content (A), lipid peroxidation (B) and activities of GPOX (C) and CAT (D) enzymes in growing embryonic axis of groundnut cultivar TG 51 under salinity stress

Discussion

Rapid germination and successful emergence of seedling is important determinant of assessing seedling vigor. It is reported that seed priming is one of the most important technique to help rapid and uniform germination and emergence of seeds and to increase seed tolerance to adverse environmental conditions [16]. In this experiment result of speed of germination in groundnut for the priming was consistent with some early findings in different crops [5-8, 17]. The accelerated germination and embryonic growth of primed seeds might be attributed to higher water-uptake ability than unprimed ones [18] as well as due to enhanced metabolic energy and increased rate of cell division during early phases of seed germination [19]. The synchronization and promotion of germination with seed priming may take place for several reasons, but changes in metabolite levels are important events during seed priming. Present experiment showed that the primed seeds except those treated with salicylic acid, showed high percentage of water uptake upto 24 hours of germination and after that a steady increase upto 48 hours followed by plateau under most of the treatments under saline condition. The changes in percentage of reserve depletion in the cotyledon along with growth of embryonic axis were monitored at 12 hours interval till 72 hours of germination. The data indicated substantial differences among the treatments for both the characters. It is suggested that the controlled hydration procedure followed by re-drying at the time of seed priming period help the seeds to complete many of the physiological and anatomical changes that characterize phase II of typical water uptake events [7]. This causes seeds to germinate faster upon rehydration even under salinity stress. This reduction in mobilization of reserve from cotyledon was found to be detrimental to the biochemical and molecular changes needed for normal growth of embryonic axis during seed germination. In agreement, studies on wheat showed highly significant decrease in reserve mobilization in response to abiotic stress [11, 20]. The promotive effects of seed priming in osmotic adjustment were also reported earlier [21-22]. Both salt and drought stresses could induce oxidative stress, as indicated by the increase level of lipid peroxidation. Several reports have described involvement of reactive oxygen species (ROS) in the early imbibition period, together with their signalling roles during seed germination [23]. It is 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 [24]. The graph showing comparative analysis which indicated the greater membrane damage under salinity can be mitigated by different priming agents mainly by hydrogen peroxide (60mM), GA3 (50ppm) and mannitol (2.5%) This iincreased lipid peroxidation is often noted as a symptom of stress related injury, caused by the accumulation of ROS. However, comparative analysis indicated alleviation of lipid peroxidation under salinity stress by seed priming. The finding was well consistent with Younesi and Moradi (2014) and Zhang *et al.* (2015) [9, 22]. Results of present experiment showed that the activities of both Guaiacol peroxidase (GPOX) and catalase (CAT) enzymes in embryonic axis were enhanced in the primed seeds under salinity stress during all the stages of germination under study, while the unprimed ones showed much reduced activities of these two important enzymes. The findings were corroborative of the early reports of Farhoudi (2012) and Younesi and Moradi (2014) [25,9].

Conclusion

It might be concluded that better osmorgulation by organic solutes like proline, enhanced activities of antioxidant enzymes like GPOX and CAT and lesser extent of lipid peroxidation because of seed priming improved the potential of groundnut seeds for salt tolerance during seed germination and initial seedling establishment stage. Seed priming with GA_3 50 ppm, mannitol 2.5% and NaCl 50mM especially produced significant results.

Abbreviations

c.v-cultivar, mM-milimolar, ppm-parts per million, %-percentage, ºC-degree centigrade, mg-miligram, mm-milimeter, LSD-Latin Square Design, ROS-Reactive Oxygen Species, CAT-Catalase, GPOX-Guaicol peroxidase. C.D.-Critical Difference

Author Contributions: All author equally contributed

Conflict of Interest: None

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