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Seed Priming and Foliar Spray to Mitigate Salt Stress through Regulation of Defence Enzymes in Rice

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ABSTRACT: The investigations were done to find out the suitable seed priming and foliar spray treatments for rice varieties to improve the germination under saline - sodic soil condition. The study revealed that the rice varieties of salt tolerant (TNAU Rice TRY 3), salt sensitive (ADT (R) 49) and moderately salt tolerant (CO 43) varieties given with seed priming and foliar spray treatments with hydrogen peroxide (0.25%), jasmonic acid (75 µM) and sodium nitroprusside (80 µM) enhanced the field emergence, root length, root volume, proline content and defence enzymes *viz.,* peroxidase and catalase activity in all the varieties. Among the treatments, seed priming and foliar spray with SNP @ 80 µM recorded maximum field emergence (94%), root length (9.5 cm), root volume (1.20 cc seedling^{-1}), proline content (449.7 µg g^{-1}) and enzyme activity of catalase (2.83 Min g^{-1}) and peroxidise (3.05 Min g^{-1}) than the other treatments in salt sensitive variety (ADT (R) 49) indicated the suitability of the treatment for enhancing the performance of rice varieties under salt stress condition.

Keywords: Salinity, Seed priming, Salt stress, Sodium nitroprusside, Hydrogen peroxide, Jasmonic acid, Defence enzymes, Rice varieties

The changing climate and soil condition made rice cultivation as a challenging task. Salinity is one of the most brutal environmental factor limiting the productivity of crop plants because most of the crop plants are sensitive to salinity caused by the high concentrations of salt in the soil and the area of land affected by it is increasing day by day. One fifth of irrigated agriculture is negatively affected by high soil salinity [1]. The expected population growth, over 9 billion by 2050, enhances the pressure for agricultural production in marginal saline lands. Generally, saline soil has the electrical conductivity (EC) of 4 dSm^{-1} and exchangeable sodium of 15 per cent. Hence, the yield of the most crop plants gets reduced. It is estimated that 20 per cent of the irrigated land at global level is affected by salinity [2]. About 6.73 million hectares salt affected soils exist in India. Out of which, 2.96 million hectares is saline and remaining 3.77 million hectares area is classified as sodic soils [3].

In saline-sodic soil, the growing plant which suffers mostly due to poor aeration, compact soil structure, low water permeability, imbalanced cations and anions with the toxicity of sodium ions and poor nutrient availability. Salinity also upsets plant hormone level and reduces the utilization of seed reserves [4]. Salinity induced stress inhibited seed germination constraints to achieve uniform seedling stand in rice and ultimately diminishes economic yield and quality of produce [5]. In the context of salinity, it was widely reported that many plant species increased the ability to tolerate salt stress after being exposed to low level of stress for a certain period of time. Rice crop is susceptible to salt stress particularly during the early seedling stage. The quality and yield of rice is greatly affected by environmental stresses such as salinity, drought, heat and cold and this problem can be overcome by either changing the growth environment suitable for the normal growth of the plants or selecting the crop or changing genetic architecture of the plant so that it can be grown in problematic areas. Seed priming is one of the reclamation methods to mitigate the illness of salt stress and also improves the uniformity and rate of emergence of seedlings [6]. Priming treatments are being used to shorten the time between planting and emergence and to protect seeds from biotic and abiotic factors during critical phase of seedling establishment. Such earlier and synchronized emergence often leads to uniform crop stand and improved yield [7]. Preferably, seed priming with inorganic salts as halopriming is effective to improve their germination and crop establishment even under salt stress conditions and several researchers claimed that, halopriming chemicals repairs the plant metabolism and also scavenging the Reactive Oxygen Species (ROS) in the plants enzymatic and non-enzymatic defence systems like peroxidase, catalase and proline *etc*. These defence systems protect the plant cells against the ROS damage [8].

Hence, evaluation of suitability of various chemicals which can be ameliorating salt stress over seed germination, crop establishment and productivity is highly warranted. In the present study, hydrogen peroxide (H_2O_2), jasmonic acid (JA) and Sodium nitroprusside (SNP) were utilised for seed priming and foliar spray treatments to overcome the salt stress condition in rice varieties. Exogenous application of osmolytes, osmoprotectants or plant hormones through foliar or seed is a good option to alleviate the adverse effects of salinity stress on crops [9]. To improve the stress tolerance in plants, controlling the free radicals is the better way [10]. In plants, hydrogen peroxide is one of the major and the most stable ROS and regulates basic processes, such as acclimation, defence and development. H_2O_2 plays dual role in plants that is at low concentration, it act as an acclamatory signal, triggering tolerance to various stresses and at higher concentration it organizes programmed cell death. It also plays an important role in signal transduction for abiotic stress tolerance, although H_2O_2 is toxic at high concentrations [11]. There is an increased production of hydrogen peroxide leads to improve the survival of the crop plants. Sodium nitroprusside is a nitric oxide (NO) donor which is a lipophilic gas and a bioactive molecule that plays an important role in different physiological processes. There is increasing evidence showing that NO acts like a signal molecule in processes such as growth and development, respiratory metabolism, cell death, and ion leakage [12]. On the other side, NO can also mediate plant growth regulators and ROS metabolism and increasingly evident, which is involved in signal transduction and responses to biotic and abiotic stress such as drought, low and high temperatures, UV and ozone exposure, heavy metal, herbicides, cold, and salt stress [13]. Jasmonic acid (JA) are collectively referred to as jasmonates and are important cellular regulators which are involved in diverse developmental processes such as seed germination, root growth, fertility, fruit ripening, and senescence [14]. Jasmonic acid application to the stressed plants reduces the amount of lipid peroxidation and stimulates the synthesis of antioxidant enzymes, enhancing the content and yield [15]. Jasmonic acid spray had pronounced effect on different plants and reduced the harmful effects of salt toxicity and improved plant performance and yield through induction of ROS scavenging enzymes and ion absorption [16]. In view of the above, to mitigate the effect of salt stress by the regulation of defence enzymes the present study was carried out in rice varieties by imposing seed priming and foliar spray treatments with H_2O_2, jasmonic acid and sodium nitroprusside.

MATERIALS AND METHODS

Experiments were carried out in research field of saline-sodic condition at Anbil Dharmalingam Agricultural College and Research Institute, Tamil Nadu Agricultural University, Trichy, Tamil Nadu, India. The seeds of three different rice varieties of salt tolerant (TNAU Rice TRY 3), salt sensitive (ADT (R) 49) and moderately salt tolerant (CO43) varieties were taken for the study and these seeds were given with priming and foliar spray treatments with hydrogen peroxide (0.25%), jasmonic acid (75µM) and Sodium Nitroprusside (SNP) (80µM) along with hydropriming and unprimed seeds as control. The foliar spray treatments with the same chemicals were given along with respective priming treatments on 10^{th} and 20^{th} days after sowing for the young seedlings under nursery condition.

The field emergence were recorded on 25^{th} days after sowing (DAS) and seedling length also recorded by measuring the length of the root and shoot for each treatment on a meter scale. To assess the root volume, the roots of randomly selected ten seedlings from each plot were scooped along with soil and washed in running water carefully. Then the roots were detached from the nodal base of seedlings. The excess moisture on the surface of the roots was removed by wiping with blotter paper. Then, the roots of each seedling were placed in a measuring cylinder containing known volume of water. By measuring the increase in water column, root volume was assessed and the mean expressed in cc seedling^{-1}.

The amino acid, proline content was estimated in fully expanded leaves following the method of Betas [17] and expressed in µg g^{-1} on fresh weight basis. Peroxidase activity was assayed as increase in optical density due to the oxidation of guaiacol to tetraguaiacol [18]. Catalase assay is based on the absorbance of H_2O_2 at 240 nm in UV-range. A decrease in the absorbance is recorded over a time period as described by Aebi [19]. Spectrum analysis was done by UV-Vis spectroscopy to identify the functional group which is formed in the treatments compared with unprimed seeds. The data obtained from the study were analysed statistically by randomized block design [20].

RESULTS AND DISCUSSION

Salinity effects are the results of complex interactions among morphological, physiological, and biochemical processes including seed germination, plant growth, water and nutrient uptake [21]. In the present study, the physiological parameters of field emergence, root length and root volume, biochemical parameters of proline content and enzymes of peroxidase and catalase activity were analysed in the rice nursery after 25 days of stress conditioned field.

Physiological Parameters

Rice crop is sensitive to salt stress and the sensitivity varies according to growth stage and among the cultivars. A plant growth responds to salinity in two phases: a rapid osmotic phase that inhibits growth of young leaves and a slower, ionic phase that accelerated senescence of mature leaves [22]. Soil salinity may affect the germination by creating osmotic potential which preventing water uptake or may be due to interference of toxic effects of Na^+ and Cl^- ions [23]. In the present investigation, seed priming and foliar spray treatments with hydrogen peroxide, jasmonic acid and sodium nitroprusside were evaluated for improvement in emergence and establishment of rice varieties under saline - sodic soil with an electrical conductivity of 4.13 dSm^{-1} and pH of 8.46 with the exchangeable sodium percentage of 16.93. In general, high amount of pH in the soil leads to accumulation of $CaCO_3$ because of Ca reacts with CO_2 and formed crystalline $CaCO_3$ [24].

In the present study, it was evident that the seed priming and foliar spray with different chemicals of H_2O_2, JA and SNP was found to improve the emergence and establishment of nursery seedlings. The observations made on plant growth parameters revealed that, the seeds primed with priming agents performed better under saline-sodic soil nursery. Regarding varieties, the performance of salt tolerant variety TNAU Rice TRY 3 outperformed over salt sensitive (ADT (R) 49) and moderately salt tolerant variety (CO 43). However, the priming and foliar spray treatments enhanced the performance of salt sensitive variety. Due to seed priming with SNP @ 80 µM the salt tolerant and moderately salt tolerant varieties recorded the maximum field emergence of 97 and 95 per cent and which was 90 per cent for the salt sensitive variety. Seed Priming with SNP alone recorded the maximum field emergence of 92 per cent in salt tolerant variety and which was 86 and 90 percentage for the salt sensitive and moderately salt tolerant variety. The combined effect of seed treatment and foliar spray with H_2O_2 @ 0.25% recorded 84 per cent field emergence and which was 24 and 20 per cent higher than the control and hydropriming in salt sensitive variety (Table 1). Similar trend as that of field emergence

Table 1. Effect of seed priming on field emergence (%) in rice varieties under saline-sodic soil condition

Treatments (T)/ Varieties (V)	TNAU Rice TRY 3	ADT (R) 49	CO 43	Mean
T_1 -Control	73(58.69)*	68(55.55)	71(57.41)	71(57.41)
T_2 –Hydropriming	74(59.34)	70(58.05)	73(58.69)	72(58.05)
T_3 –Seed priming with H_2O_2 (0.25%)	81(64.15)	77(61.34)	79(62.72)	79(62.72)
T_4-Seed priming and foliar spray with H_2O_2 (0.25%)	90(71.56)	84(66.42)	92(73.57)	89(70.63)
T_5 –Seed priming with JA (75 µM)	77(61.34)	72(58.05)	76(60.66)	75(60.00)
T_6 –Seed priming and foliar spray with JA (75 µM)	84(66.42)	80(63.43)	82(64.89)	82(64.89)
T_7 –Seed priming with SNP (80 µM)	92(73.57)	86(68.02)	90(71.56)	89(70.63)
T_8 –Seed priming and foliar spray with SNP (80 µM)	97(80.02)	90(71.56)	95(77.08)	94(75.82)
Mean	84(66.42)	78(61.69)	82(64.89)	81(64.15)
	V	T	V x T	
Sed	0.27	0.44	0.77	
CD (p=0.05)	0.55	0.90	1.55	

*Figures in parenthesis are transformed values was observed for root length. Considering the root volume an important index for measuring the resistant against stress, the seed priming and foliar spray application were found to be effective compared with unprimed control and hydropriming. The improvement was more due to seed priming and foliar spray with SNP on 10th and 20th DAS recorded 19 per cent improvement than the control for the salt sensitive variety and it was 20 per cent for the salt tolerant and moderately salt tolerant varieties (Figure 1).

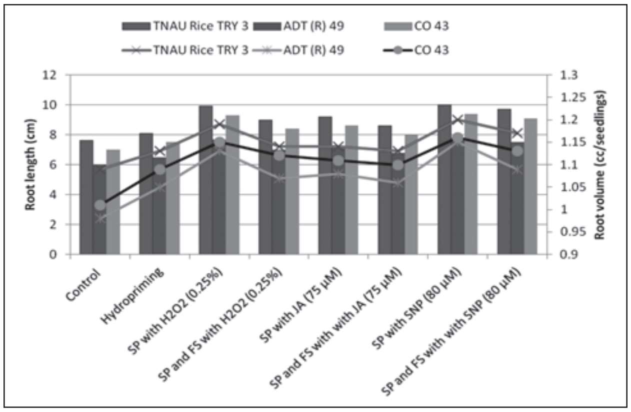


Figure 1. Effect of seed priming and foliar spray on root length (cm) and root volume (cc/seedling)

The use of salts in priming solution or addition of signalling molecule like H_2O_2 can be used as adaptation strategy to improve salt tolerance in crops and in wheat, seed priming improved salt tolerance at initial germination stage was due to reduced oxidative damage, expression of stress proteins and further activation of metabolic repair [7]. Improved salt tolerance from priming is the result of higher capacity for osmotic adjustment since plants from primed seeds accumulate Na^+ and Cl^- in roots and more sugars and organic acids in leaves [25]. During stress conditions, seed priming improves the germination and seedling growth in sunflower [26] and soybean [27].

Proline Content (µg g^{-1})

An increase in the proline content seems to be related to salt tolerance. Proline contribution to the osmotic potential, however, was high throughout salt tolerance in salt tolerance genotypes. In the present study, higher proline accumulation was noticed in the salt tolerant variety (461.4 µg g^{-1}) than salt sensitive (365.8 µg g^{-1}) and (428.1.0 µg g^{-1}) moderately salt tolerant variety (Table 2). Among the treatments, application of SNP @ 80 µM through seed priming and foliar spray recoded the maximum value (449.7 µg g^{-1}) followed by seed priming with SNP @ 80 µM (442.4 µg g^{-1}) where as unprimed control recorded the least value of 372.1 µg g^{-1}. The higher accumulation of proline showed that there is correlation between proline accumulation and osmotic potential. Proline can protect the plants from stress through different mechanisms. [28]. The exogenous application of H_2O_2 confers tolerance to salt stress in rice by enhancing proline accumulation [29].

Antioxidant Enzymes

Peroxidase activity (Min g^{-1})

In the present study, the seed priming and foliar application with chemicals and bioactive molecules regulates the antioxidant enzymes *viz*., catalase and peroxidise. Among the varieties salt tolerant recorded the highest peroxidase activity of 3.85 (Min g^{-1}) over salt sensitive (2.25 Min g^{-1}) and moderately salt tolerant variety (3.05 Min g^{-1}) when observed after giving foliar

Table 2. Effect of seed priming and foliar spray on proline content (µg g^{-1}) in rice varieties under saline-sodic soil condition

Treatments (T)	TNAU Rice TRY 3	ADT (R) 49	CO 43	Mean
T_1 -Control	410.6	305.8	400.0	372.1
T_2 -Hydropriming	429.4	320.1	418.6	389.4
T_3 -Seed priming with H_2O_2 (0.25%)	460.9	370.2	429.7	420.3
T_4-Seed priming and foliar spray with H_2O_2 (0.25%)	481.6	390.1	438.4	436.7
T_5 -Seed priming with JA (75 µM)	452.7	361.0	424.0	412.6
T_6 -Seed priming and foliar spray with JA (75 µM)	465.2	377.1	430.2	424.2
T_7 -Seed priming with SNP (80 µM)	490.3	396.3	440.7	442.4
T_8 -Seed priming and foliar spray with SNP (80 µM)	500.3	405.7	443.2	449.7
Mean	461.4	365.8	428.1	418.4
	V	T	VxT	
SEd	3.36	5.49	9.50	
CD (p=0.05)	6.76	11.04	19.12	

spray with SNP @ 80 µM on 10th and 20th days after sowing. With respect to treatments, combination of seed treatment and foliar spray with SNP @ 80 µM recorded higher peroxidase activity of 3.05 Min g^{-1} followed by seed priming with SNP @ 80 µM (3.0 Min g^{-1}) and whereas the unprimed control recorded least content of 2.35 Min g^{-1}. Similar results were found in the cucumber seeds, which is highly sensitive to salinity that can be alleviated through NO by the improvement of antioxidant enzymes (Table 3). It is well documented that abiotic stresses exert at least in part of their effects by causing oxidative damage. Production of ROS is increased under saline conditions and ROS mediated membrane damage has been demonstrated to be a major cause of the cellular toxicity by salinity in rice. Excessive production of ROS is toxic to the plants and causes oxidative damage to the cellular constituents, leading to cell death. Plants possess enzymatic and non-enzymatic antioxidant defense systems to protect their cells against the damaging effects of ROS. Antioxidant molecules having its capability to alleviate the cellular damage caused by ROS. Antioxidant enzymes are related to the resistance to various abiotic stresses including salinity. [30].

Catalase activity (Min g^{-1})

Under saline-sodic condition among the varieties, salt tolerant variety recorded highest catalase activity (Table 4) of 2.98 Min g^{-1} over salt sensitive (2.15 Min g^{-1}) and moderately salt tolerant variety (2.45 Min g^{-1}). With respect to treatments, seed priming and foliar spray with SNP @ 80 µM recorded higher catalase activity of 2.83 Min g^{-1} followed by seed priming with SNP @80 µM (2.79 Min g^{-1}). The unprimed seed recorded the lowest value (2.05 Min g^{-1}). The exogenous application of SNP markedly increased the activity of peroxidase, catalase and super oxide dismutase activities in wheat plants.

Table3. Effect of seed priming and foliar spray on peroxidase activity (Min g^{-1}) in rice varieties under saline-sodic soil condition

Treatments (T)	TNAU Rice TRY 3	ADT (R) 49	CO 43	Mean
T_1 -Control	3.26	1.43	2.35	2.35
T_2 -Hydropriming	3.36	1.95	2.65	2.65
T_3 -Seed priming with H_2O_2 (0.25%)	3.70	2.13	2.88	2.90
T_4-Seed priming and foliar spray with H_2O_2 (0.25%)	3.79	2.20	2.95	2.98
T_5 -Seed priming with JA (75 µM)	3.60	2.07	2.80	2.82
T_6 -Seed priming and foliar spray with JA (75 µM)	3.73	2.16	2.90	2.93
T_7 -Seed priming with SNP (80 µM)	3.82	2.22	2.97	3.00
T_8 -Seed priming and foliar spray with SNP (80 µM)	3.85	2.25	3.05	3.05
Mean	3.64	2.05	2.82	2.84
	V	T	VxT	
SEd	0.20	0.03	0.06	
CD (p=0.05)	0.39	0.06	0.11	

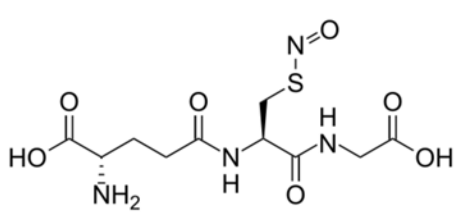
Table 4. Effect of seed priming and foliar spray on catalase activity (Min g^{-1}) in rice varieties under saline-sodic soil condition

Treatments (T)	TNAU Rice TRY 3	ADT (R) 49	CO 43	Mean
T_1 -Control	2.50	1.65	2.01	2.05
T_2 -Hydropriming	2.67	1.83	2.18	2.23
T_3 -Seed priming with H_2O_2 (0.25%)	2.89	2.21	2.50	2.53
T_4-Seed priming and foliar spray with H_2O_2 (0.25%)	3.30	2.34	2.61	2.75
T_5 -Seed priming with JA (75 µM)	2.80	2.10	2.40	2.43
T_6 -Seed priming and foliar spray with JA (75 µM)	2.94	2.26	2.53	2.58
T_7 -Seed priming with SNP (80 µM)	3.33	2.37	2.66	2.79
T_8 -Seed priming and foliar spray with SNP (80 µM)	3.39	2.41	2.70	2.83
Mean	2.98	2.15	2.45	2.52
	V	T	VxT	
SEd	0.02	0.03	0.06	
CD (p=0.05)	0.04	0.07	0.11	

Similar results were found in the sesame seeds, the seeds were exposed to the salinity stress then the increase of antioxidant enzymes *viz.*, peroxidase, catalase and superoxide dismutase were observed [31]. Recent studies trying to understand antioxidative system function in germination showed a substantial increase in CAT and SOD activities in maize seeds, as a result of the ROS induced antioxidant gene expression [32]. Franklin [33] reported that seed priming with H_2O_2 improved the antioxidant activity may explain the increase tolerance to salt stress of plants. The beneficial effect of H_2O_2 is it reserves the harmful effects of salinity on growth by alleviating salinity induced membrane damage which was associated to the ability of H_2O_2 to induce antioxidant enzymatic defences, especially catalase activity. Foliar spray with phytohormones such as jasmonic acid influence a wide variety of physiological and developmental responses and antagonistically regulate the expression of salt stress inducible proteins associated with salt stress in rice. Thus, they act as a modulator by suppressing or enhancing the stress response of plants [34].

In the present study the effectiveness of SNP may be due to increased activity of POX, CAT and proline. The SNP at higher concentration act as stress inducer and at lower concentration stimulate the activity of defence enzymes and thereby improving the emergence and establishment of seedlings under salt stress condition [35]. SNP having its capability of producing Nitrous Oxide (NO) seems to depend on its interaction with sulfhydryl-containing molecules. Cysteine and glutathione are the sulfhydryl-containing amino acid which is already present in the rice seeds [36]. Actually, the reaction between SNP and thiols was described several decades ago and is used as a test for their identification, but still, SNP is the most widely studied iron nitrosyl compounds. The effect has been justified through UV-Vis spectroscopy.

SNP reacts with the cysteine amino acid which is already present in the rice seeds. Finally, formed the temporary nitric oxide storage compound like s-nitrosocysteine



S-nitrosocysteine

Temporary nitric oxide storage compound

which slowly release NO during germination. Periodical release of NO at lower rate in-turn trigger the defence related enzymes within the seed under salt stress condition.

The present study to alleviate effect of salt stress through seed priming and foliar spray indicated that the seeds haloprimed with SNP @ 80 µM recorded increased ranges of all the physiological parameters when compared to hydroprimed and unprimed seeds. In this regard, seed priming and foliar spray with SNP @ 80 µM recorded increased field emergence, root length and root volume than the other priming treatments irrespective of varieties. Compared with hydropriming and unprimed seeds, the primed seeds performed better. Seed priming with H_2O_2 played an important role in signal transduction for abiotic stress tolerance, although H_2O_2 is toxic at high concentrations. Meanwhile Seed priming with H_2O_2 having the capacity to enhance the multi-resistance to heat, drought, chilling and salt stress. Seeds primed with Jasmonic acid have proved effective in improving plant stress tolerance but it depends on the type of species and concentration.

The present study revealed that the salt tolerant variety, TNAU Rice TRY 3 maintained better seedling growth under salinity conditions. This might be attributed to the inherent mechanism of tolerant variety in the regulation of entire germination process. The performance of moderately salt tolerant (CO 43) and salt sensitive variety ADT(R) 49 were improved due to the seed priming and foliar spray with SNP followed by H_2O_2 and Jasmonic acid on 10th and 20th DAS. Seed priming and foliar spray with these chemicals influences not only physiological processes and also influences on plant defence enzymes like peroxidase (POX), catalase (CAT) and superoxide dismutase (SOD), because salinity also influences on enzymes already present in the plant ecosystem. In addition to seed treatments, foliar application of nutrients and bioactive molecules protected the young seedlings from various stress conditions. Foliar application of H_2O_2 and JA modulates several physiological responses, leading to improved resistance against abiotic stresses. Foliar application of SNP enhanced the plant growth parameters, antioxidant enzymes, high accumulation of proline and also increased the crop performance under salinity condition. The out performance of SNP was due to the formation of S-nitrosocysteine in rice seed which trigger the defence related enzymes within the seed under salt stress condition which was confirmed through UV-Vis Spectroscopy. Hence, seed priming and foliar spray with SNP @ 80 µM could be recommended to alleviate the effect of salt stress in rice even under high salt concentration.

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