



E-ISSN: 2278-4136

P-ISSN: 2349-8234

www.phytojournal.com

JPP 2024; 13(4): 423-427

Received: 15-07-2024

Accepted: 19-08-2024

Dr. Muneshwar Prasad Mandal

Asstt. Prof. -cum- Jr. Scientist
Department of Plant Physiology
and Biochemistry, Bhola Paswan
Shastri Agricultural College,
Purnea, Bihar, India

Dr. Mukul Kumar

Asstt. Prof. -cum- Jr. Scientist
Department of Plant Physiology
and Biochemistry, Mandan
Bharti Agriculture College,
Agwanpur, Saharsa, Bihar, India

Dr. Kiran

Asstt. Prof. -cum- Jr. Scientist
FBS&H, Dr. Rajendra Prasad
Central Agricultural University,
Pusa, Samastipur, Bihar, India

Dr. Ashish Ranjan

Jr. Scientist -cum- Asstt. Prof.
Department of Horticulture,
Bhola Paswan Shastri
Agricultural College,
Purnea, Bihar, India

Dr. DK Mahto

University Prof. -cum- Chief
Scientist, Department of
Agronomy, Bhola Paswan
Shastri Agricultural College,
Purnea, Bihar, India

Corresponding Author:**Dr. Mukul Kumar**

Asstt. Prof. -cum- Jr. Scientist
Department of Plant Physiology
and Biochemistry, Mandan
Bharti Agriculture College,
Agwanpur, Saharsa, Bihar, India

Impact of salt stress on biochemical processes in rice seedlings

Dr. Muneshwar Prasad Mandal, Dr. Mukul Kumar, Dr. Kiran, Dr. Ashish Ranjan and Dr. DK Mahto

DOI: <https://doi.org/10.22271/phyto.2024.v13.i4e.15035>

Abstract

Rice is generally characterized as a salt-sensitive crop (Flowers and Yeo, 1981) [11]. But there are large number of varieties that show variations in salt tolerance (Datta, 1972; Yeo and Flowers, 1982) [6, 44]. The response of four rice genotypes against 0, 8, and 12 dSm⁻¹ salinity levels were studied at the early seedling growth stage. Data were analyzed statistically for Protein metabolism and Enzyme activities. Salinity decreased the protein content, peroxidase, and catalase activity but increased protease activity, proline content, and amino acids in all genotypes and the magnitude of reduction increased with increasing salinity stress. Rice genotypes Kishori, Rajshree, Marcha and Jeeravati showed greater salt tolerance and susceptibility during germination. Kishori and Rajshree are tolerant and Marcha & Jeeravati are susceptible rice genotypes against salt stress. The result suggested that Kishori, Rajshree, Marcha, and Jeeravati might be used for further study of the salinity effect on growth processes and its physiological consequences at an advanced stage of growth.

Keywords: Rice, salt stress, protein metabolism, proline, enzyme activity

Introduction

Rice (*Oryza sativa* L.) is the world's most important crop and more than half of the world's population depends on it for food (calories and protein especially in developing countries) grain. Asia accounts for almost 90 per cent of area and production of the world's rice and total area being 168 million ha and production being about 722 million ton with a productivity of 4.29 ton/ hectare (FAO-2012) [12]. Worldwide total area under salinity is 953 m. ha which covers 8 per cent of the land surface (Amirjani *et al.* 2010) [1]. The coastal areas are particularly vulnerable to salinity due to saline water intrusion and rise in the sea level as a consequence of global warming. Soil salinity is a major constraint, limiting agricultural productivity in nearly 20 per cent of cultivated area and half of the irrigated area worldwide (Zhu, 2001) [45].

Salt stress is one of the most significant abiotic stresses and affects every aspect of plant physiology and metabolism. During salt stress, Na⁺ enters the cells and accumulates to a concentration that induces ionic and osmotic stress in plants. Plant cells respond and adapt to these adverse conditions through signalling networks (Lee *et al.* 2004) [20]. Soil salinity is a major factor limiting the crop production globally (Kumar *et al.* 2010) [19]. Soil salinity affects large areas of the world's cultivated land causing significant reductions in crop yield (Tavakkoli *et al.* 2011) [40].

Rice is sensitive to salinity at the seedling stage and becomes tolerant at the vegetative phase and very susceptible at the reproductive phase in terms of grain yield. It is necessary to identify the sensitivity and tolerance level of varieties at early seedling stages for successful crop production in a saline environment. Therefore the objective of this study was to find out the salt tolerance of rice genotypes.

Materials and Methods

Seeds of different rice genotypes used in the study were collected from Department of Plant Breeding and Genetics, RAU, Pusa, Samastipur. Healthy uniform seeds of all varieties were surfaced sterilized with 0.1% mercuric chloride solution for four minutes and thoroughly washed with distilled water and surface dried using tissue paper. Twenty-five seeds for each genotype in each treatment were allowed to germinate on a filter paper in 9 cm diameter petridishes. Each filter paper was moistened with salt solutions (NaCl: CaCl₂: Na₂SO₄ in the ratio of 7:2:1) of 0 dSm⁻¹ salt concentrations according to the treatments. 8 ml appropriate solution was applied to each Petri dish. The Petri plates were arranged in completely randomized design (CRD) with three replicates for each treatment.

The number of seeds sprouted and germinated was counted daily upto 10 days. The proteins were extracted from the plant material and their estimation was carried out after Lowry *et al.* (1951) [22]. Total free amino acids were estimated using the methods prescribed by Lee and Takahasi (1966) [21]. The method developed by Bates *et al.* (1973) [4] for three quantification of proline was used. Determination of enzyme activities: Enzymes like amylase, peroxidase, catalase and protease activity were assayed in fresh samples of 10 day-old seedling. Amylase activity was determined by the method of Mc Cready *et al.* (1962) [26]. The activity of peroxidase was determined by the method of Palmiano and Juliano (1973) [30]. The activity of catalase was determined by the method of Kar and Mishra (1976) [16]. The protease activity was determined as described by Dubey and Rani (1990) [10].

Results and Discussion

Table 1 indicated that with increasing salt stress, soluble protein content in fresh 10-day-old seedling continuously decreased. The data of soluble protein content showed that in all four genotypes, protein content decreased and the decrease was maximum in Jeeravati (21.24 mg) and was minimum (34.98 mg) in Kishori. The mean values of the soluble protein content of tolerant genotypes were found higher in comparison to that of susceptible group of genotypes and in tolerant group of the mean value of 40.13 mg, whereas in the susceptible group, it was only 33.24 mg. Effect of stress was seen significant, as the mean value was 44.80 mg in 0.0 dSm⁻¹, its value reduced to 28.12 mg at 12.0 dSm⁻¹ salt stress. Interaction of stress and genotypes was found highly significant at 5 per cent level.

As regards to soluble total proteins of 10-day-old seedling, tolerant and susceptible genotypes showed a decreasing trend with increasing stress levels. However, protein value was higher in tolerant genotypes as compared to susceptible genotypes. It was high in control treated plants and significantly diminished in plants grown under salt stress. At (150 mM) salt stress, protein content was reduced in all varieties. It showed that rice plants were severely affected at high salt concentration. Susceptible genotypes registered comparatively higher values for the soluble protein implying their salt tolerance behaviour. Jha and Singh (1997) [15] reported higher protein content of stressed seedling of tolerant rice cultivars as compared to susceptible cultivars. The proteins that accumulate under salt stress conditions may provide a storage form of nitrogen that is re-utilized in post-stress recovery (Singh *et al.*, 1974) [37] and also play a role in osmotic adjustments. The total soluble protein content determines the dry matter accumulation of crops. Since it represents the efficiency of the RuBPase, the carboxylating enzyme in C₃ plants (Plaut, 1974) [32]. Vijayaraghavan (1994) [43] had reported a reduction in soluble protein content under salt stress situation. Soluble protein decreased in susceptible cultivars in comparison to tolerant cultivars under salinity stress (Mandal and Kumari 2013) [25].

Observations pertaining to table 1 suggest that in 10-day-old seedling of different genotypes of rice, the total free amino acids value was found least (32.81mg) in Rajshree. In this table, susceptible group of genotypes dominated over the tolerant group in respect of amino acids content. The mean value of total free amino acids in tolerant group was 32.65 mg whereas in susceptible group, it was 38.36 mg. Salt stress affected significantly the total free amino acids content. The value of 0.0 dSm⁻¹ stress (25.66 mg) increased to 36.21 mg at 8.0 dSm⁻¹ and reached to 44.65 mg at 12.0 dSm⁻¹. The data

were highly significant for stress, genotypes and interactions at 5 per cent level.

Table 1 showed increasing trend of amino acids accumulation with salt stress. It may be due to proteolytic activity in rice seedling by exposure to salt stress. The increase of proteolytic activity and decrease of protein synthesis which directly causes accumulation of free amino acids, might lower the need of ammonium incorporation to form amino acids and indirectly cause the excessive accumulation of ammonium (Nguyen, 2005) [29]. Protein plays a role in acclimation to anaerobic conditions created by salt stress. Acclimated seedlings maintain a higher energy status during anoxia, and this is associated with a greater ability to synthesize ATP through glycolysis and ethanolic fermentation. Down regulation of photosynthetic-related proteins and those related to protein biosynthesis in potatoes and soybeans confirms that these proteins are impacted by salt stress. In contrast, upregulation of osmotin like proteins, heat shock protein, calreticulin and some new proteins in potato and upregulation of late embryogenesis abundant proteins, alcohol dehydrogenase, and annexin in soybean suggest that these proteins might be involved in salt tolerance in these two important crops. Babu *et al.*, 2006 [3] told that the genotypic difference in soluble protein content could be related to grain yield. Maximum reduction in protein content was recorded in rice seedlings with 12dSm⁻¹. Protein serves as respiratory substrate when the carbohydrates and other compounds are inadequate. The depletion of biochemical constituents such as protein contents under the influence of higher concentration of NaCl might cause inhibition in germination and growth characteristics of rice. A marked reduction in the protein content of the rice plants under stress was observed with increasing salt concentrations (Muhammad, 2012) [27].

The value of proline as shown in Table 1 reflected that with increasing salt stress, the proline content of 10-day-old seedling increased. Among all the four genotypes of rice, proline content was maximum in tolerant group of genotypes and its mean value 26.27 mg whereas with an average value (20.84 mg) in susceptible group of genotypes. The proline content was maximum 26.44 mg in Kishori (T. group) and minimum 20.66 mg in Jeeravati (S. group). Again, in two tolerant genotypes, the proline content of Kishori was relatively higher than Rajshree and proline content of susceptible genotypes Marcha had lower values as compared to Jeeravati. The effect of salt stress was also found significant which caused increase in proline content and the mean value was found 17.15 mg at 0.0 dSm⁻¹, 22.78 mg at 8.0 dSm⁻¹ and 30.72 mg dSm⁻¹ at 12.0 dSm⁻¹. The interaction effect of stress and genotypes was found highly significant.

Observation pertaining to proline content showed that salt stress promoted the accumulation of proline in both type of genotypes, tolerant and susceptible in 10-day-old seedlings. Tolerant genotypes maintained high accumulation of proline as compared to susceptible genotypes. Proline can serve as a protector of enzymes denaturation, a reservoir of nitrogen and carbon or as a stabilizer of the machinery for protein synthesis (Hamada & Khulaed, 1995) [13]. Hence, proline accumulation in plant can be taken as an index to identify the tolerant genotypes. Pessarakkali (1999) [31] reported that accumulation of proline depends on the extent of stress. Proline was considered as salt tolerant mechanism and serve in osmotic regulation (Aslam *et al.*, 1989) [2]. The proline accumulation may be due to either non- incorporation of free amino acid proline into protein synthesis due to salt stress or the breakdown of the existing protein molecules into various

constituent amino acids with proline being predominant (Somani, 1991 and Mukherjee, 1974) [38, 28].

In higher plants proline is biosynthesized by either the glutamate or the ornithine pathway. The glutamate pathway is considered the major route especially in response to osmotic stress (Kishor *et al.*, 2005). Generally, proline protects plants from stress through different process including by the adjustment of cellular water detoxification of ROS, protection of membrane integrity and stabilization of enzymes and proteins, thus it can be beneficial to plants in adopting to stress (Maggio *et al.*, 2002. Trovato *et al.*, 2008) [23, 42]. Proline has been reported to detoxify free radical activity by scavenging two ROS (OH^\cdot and O_2^\cdot) in certain *in vitro*, detection systems (Blokhina *et al.*, 2003) [5] from these results, the endogenous pool of proline might help to explain the relatively high overall osmotic and antioxidant tolerance of rice seedling. Proline accumulation in salt-stressed seedlings increased to a significantly higher degree than in seedlings without salt stress, especially in salt-tolerant genotypes. Proline & anthocyanins substances relative important in plant defense mechanisms against salt stress. A salinity stress of 12 dSm^{-1} resulted into higher proline content than the control plants of tolerant genotypes against in susceptible genotypes at the same stress level (Sunita *et al.*, 2011) [39].

The observations pertaining to amylase activity as presented in Table 2 in 10-day-old seedling of rice genotypes indicated that amylase activity significantly reduced under increased salt stress conditions. The mean value of tolerant group of genotypes was higher (117.24 units/mg protein) when compared with the susceptible group of genotypes where it was (99.70 units/mg protein). In the seedlings, the maximum activity was recorded at 0.0 dSm^{-1} (141.70 units/mg protein) which declined subsequently at 8.0 dSm^{-1} (103.82 units/mg protein) and ultimately to 79.88 units/mg protein at 12.0 dSm^{-1} . The interaction of stress and genotypes was also found highly significant.

On the other hand, the amylase specific activity showed decreasing trend with rise in salinity stress. Though, the values of amylase activity were higher in tolerant genotypes as compared to susceptible genotypes the amylase specific activity was lower at higher salt stress (Krishnamurthy *et al.*, 1987) [18] and it was higher in tolerant genotypes as compared to susceptible genotypes (Dubey, 1983) [9]. Salinity caused decrease in water uptake followed by inhibition in the activity of hydrolyzing enzyme α -amylase. The cultivars differing in salt tolerance have shown to differential behaviour of amylolytic enzymes during germination of seeds (Dubey, 1983) [9]. In the present study the decreased sugar levels might be due to its less mobilization from endosperm to growing organs such as shoots and roots to maintain osmotic balance in stressed plants. Since major osmotically active substances of glycophytes are potassium salts, salts of organic acids and sugars (Hellebust, 1976; Shereen *et al.*, 2011) [14, 36].

The observation as mentioned in Table 2 indicated that peroxidase activity of 10-day-old seedling decrease with increase in salt stress. Here, tolerant group of genotypes maintained higher activity as its mean value was 155.41 units/mg protein as compared to susceptible one (137.24 units/mg protein). Of four genotypes taken together, Kishori maintained highest value 155.82 units/mg protein. However, the lowest was recorded in Jeeravati, where its value was 136.65 units/mg protein. Salt stress significantly decreased the activity of peroxidase, it went from 179.57 units/mg protein under 0.0 dSm^{-1} stress to 115.78 units/mg protein at

12.0 dSm^{-1} . In the table, interaction of stress and genotypes was found highly significant.

The peroxidase specific activity of 10-days-old seedling plant showed decreasing trend in tolerant and susceptible genotypes. Higher peroxidase activity was observed in tolerant genotypes as compared to susceptible genotypes. The findings are in consonance with reports of Malik and Shaikat, 1986 [4]. Peroxidase are wide spread in plant tissues are often particularly evident in cell walls. Its synthesis and recreation are modulated by numerous environmental factors such as salt stress, drought, temperature, light and plant regulator. Peroxidase plays a significant role in process of plant growth and development and possess poly functionality (Titov, 1975; Dhanyalakshmi, 2013) [41, 7].

The observation pertaining to catalase activity as compared in Table 2 in 10-day-old seedling of rice genotypes that catalase activity significantly reduced under increased salt stress condition. The mean value of tolerant group of genotypes was higher (81.18 units/mg protein) when compared with the susceptible group of genotypes where it was (69.06 units/mg protein). In the seedlings, the maximum activity was recorded at 0.0 dSm^{-1} (92.12 units/mg protein) which declined subsequent at 8.0 dSm^{-1} (75.37 units/mg protein) and ultimately to 57.88 units/mg protein at 12.0 dSm^{-1} . The interaction of stress and genotypes was also found highly significant.

The observation pertaining to catalase activity as compared to 10-day-old seedling of rice genotypes where it significantly reduced under increased salt stress condition. In the seedlings, the maximum activity was recorded at 0.0 dSm^{-1} (92.12 units/mg protein) which declined subsequent at 8.0 dSm^{-1} (75.37 units/mg protein) and ultimately to 57.88 units/mg protein at 12.0 dSm^{-1} . The interaction of stress and genotypes was also found highly significant.

The scavenging enzyme SOD, Catalase and Peroxidase decline in proportion with the magnitude of stress, it may be due to sparing of free radicals which seems to be accumulated causing greater damage of membranes leading to poor seedling growth. The data showed, an increase in protease activity with the increasing salt stress. It was found 19.03 units/mg protein at 0.0 dSm^{-1} however, at 12.0 dSm^{-1} it was 38.75 units/mg protein. In the tolerant group of genotypes, the mean value of protease activity was 25.94 units/mg protein whereas in susceptible group of genotypes, it was 31.37 units/mg protein. The mean value of Kishori was recorded least (25.69 units/mg protein) among the tolerant group of genotypes, however, it was highest in the case of Marcha (31.22 units/mg protein) among the susceptible group of genotypes. Among the two susceptible genotypes, the protease activity of Jeeravati was higher than Marcha. The interaction of stress and genotypes was found highly significant.

High values of protease activity of susceptible cultivars compared to tolerant ones and their activation under salinization appears to be characteristic feature of these genotypes (Table 2). These findings are closely related to our observations on protease specific activity in the axis showing higher activity with stress and supported by the findings of Reddy and Vora (1985) [34] in bajra, Sheoran and Garg (1978) [35] in mung seedlings. It has been suggested that salinity reduces the synthesis of macromolecules such as RNA, DNA and proteins (Prisco and O' Leary 1972) [33] and increase their degradation by affecting the hydrolytic enzymes, especially nucleases and proteases (Dubey *et al.*, 1987; Krishnamurthy *et al.*, 1987) [8].

Table 1: Effect of salt stress on protein metabolism in 10-day-old rice seedlings

Salt stress (Conc.)	Genotypes	Soluble protein (mg/g dry wt.)	Free amino acid (mg/g dry wt.)	Proline content (mg/g dry wt.)
0.0dSm ⁻¹	Kishori	45.48	26.12	17.44
	Rajshree	45.00	25.98	17.35
	Marcha	44.59	25.59	16.98
	Jeeravati	44.15	24.96	16.84
	Mean	44.80	25.66	17.15
8.0 dSm ⁻¹	Kishori	41.05	32.22	25.39
	Rajshree	40.11	33.22	24.98
	Marcha	34.00	39.15	20.62
	Jeeravati	33.38	40.08	20.14
	Mean	37.13	36.21	22.78
12.0 dSm ⁻¹	Kishori	34.98	38.95	36.48
	Rajshree	34.19	39.23	35.96
	Marcha	22.06	49.24	25.46
	Jeeravati	21.24	51.17	24.99
	Mean	28.12	44.65	30.72
CD at 0.05	Stress (S)	0.29	0.42	0.49
	Genotypes (G)	0.34	0.48	0.56
	S x G	0.59	0.84	0.97

Table 2: Effect of salt stress on Enzyme activities in 10-day-old rice seedlings

Salt stress (Conc.)	Genotypes	Amylase activity (U/mg protein)	Peroxidase activity (U/mg protein)	Catalase activity (U/mg protein)	Protease activity (U/mg protein)
0.0dSm ⁻¹	Kishori	142.05	180.00	92.74	19.33
	Rajshree	141.96	179.95	92.44	19.05
	Marcha	141.45	179.33	91.94	18.98
	Jeeravati	141.34	179.00	91.35	18.77
	Mean	141.70	179.57	92.12	19.03
8.0 dSm ⁻¹	Kishori	114.15	151.24	80.76	25.34
	Rajshree	113.25	150.08	70.86	26.06
	Marcha	94.05	137.20	70.86	30.22
	Jeeravati	93.84	136.00	69.99	31.10
	Mean	103.82	143.63	75.37	28.18
12.0 dSm ⁻¹	Kishori	96.44	136.22	71.13	32.39
	Rajshree	95.58	134.98	70.14	33.45
	Marcha	64.52	96.95	45.29	44.45
	Jeeravati	62.99	94.96	44.96	44.72
	Mean	79.88	115.78	57.88	38.75
CD at 0.05	Stress (S)	0.77	0.82	0.57	0.40
	Genotypes (G)	0.89	0.95	0.66	0.47
	S x G	1.54	1.64	1.14	0.81

Conclusion

The study demonstrates that soil salinity significantly impacts rice seedling physiology, with varying effects across different genotypes. Increased salt stress led to a notable decline in soluble protein content and enzyme activities, particularly in susceptible rice varieties. Conversely, salt-tolerant genotypes exhibited higher levels of soluble proteins, free amino acids, and proline, which are crucial for stress adaptation and osmotic regulation. Proline accumulation, in particular, was significantly higher in tolerant genotypes, highlighting its role as a protective mechanism against salt stress. The results emphasize the importance of identifying and developing salt-tolerant rice varieties to mitigate the adverse effects of salinity and enhance crop productivity in affected regions.

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