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Effect of proline on germination and seedling growth of rice (*Oryza sativa* L.) under salt stress

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Abstract
The present investigation was carried to study the effect of seed priming with 1mM, 5mM and 10mM proline on germination and seedling growth of rice (*Oryza sativa* L.) exposed to five level of salinity *i.e*.0, 50, 100,150 and 200mM NaCl and water. Seeds of ten rice genotypes (IR11, IR12, IR28, USAR2, USAR3, Swarna *sub1*, IR11T172, IR11T220, IRRI147 and IRRI154) were procured from diverse origin and primed with proline and studied under various salinity levels. The experimental design based on completely randomized design with three replicates and performs at the laboratory of crop physiology, NDUA&T, Kumarganj, Faizabad from July 2015 to Dec. 2015. The exposure of rice seeds to increasing concentration of NaCl had drastically reduced germination (%), seed vigour index and α-amylase activity. It is evident from the result that seed treatment with various concentration of proline significantly increased the germination (%), seed vigour index andα-amylase activity of ten rice genotypes under normal as well as salinity condition. Beneficial effect of seed priming with proline on various traits was more pronounced under salinity than normal condition. Further study has demonstrated that rice seeds pre-treated with proline (1mM, 5mM and 10mM) and grown at different NaCl concentrations counteracted the adverse effect of salt. Pre-treatment of proline at a concentration of 1mM was found to be effective and stimulated cellular activities, whereas 10mM proline was found ineffective to improve germination and biochemical activity under high level of salt (150 and 200mM NaCl). Genotypic variability was observed regarding salt tolerance and response of proline as priming agent to improve efficiency to maintained physiological activity occurred during germination under salt stress condition.

Keywords: salinity, rice, proline, germination percent, and α-amylase

Introduction
Rice (*Oryza sativa* L.) With chromosome no. 2n= 24, belongs to the family Graminae. Rice fields covers 11% of Earth's entire arable land. Rice is cultivated worldwide over an area of about 153.51 million hectare with annual production of 650.19 million tonnes. India ranked first in area having 45.2 million hectares and second in production 102 million tonne. Recently India having area 160.2 million hectares and in production 465.4 million tonnes globally. 80% of our country men depend fully or partially on rice as their main cereals food and stable diet. Rice in India was found area 37.48 million hector and production 88 million tonnes during 2014-15.The global rice production for 2014-15 is rise to nearly 475 million tonnes, according to International Grain Council (IGC).The area and production of rice in this state is about 5.94 million hectare and 15.30 million tonnes respectively with an average productivity of 2.57 tonnes per hectare (Anonymous, 2014) [2]. Rice is the second most widely consumed cereal in the world next to wheat. It is the staple food for two thirds of the world's population. Over 2 billion people in Asia alone derive 80% of their energy needs from rice, which contains 80% carbohydrates, 7–8% protein, 3% fat, and 3% fibre. Until recently, rice was considered only a starchy food and a source of carbohydrates and some amount of protein. Rice protein, though small in amount, is of high nutritional value. Seed priming is one of the method which results in modifying the physiological and biochemical nature of seeds so as to get the characters that are favourable for drought tolerance. It is also known as extensive physiological reorganization induced by hydration and dehydration processes. During priming process a number of physico-chemical changes occur, modifying the protoplasmic characters and increasing physiological activity of the embryo and associated structures. Eventually, this results in the absorption of more water due to increase in the elasticity of cell wall and development of a stronger and efficient root system Krishnasamy and Srimathi, (2001) [9]. Soil salinity is serious constraint to increased production, in rice growing countries of the world (Gregorio, 1997) [5]. Soil salinity affects crop growth and yield in a number of ways. In salt affected soils, growth and yield of most crop plants is adversely affected due to high level of soluble salts (causing physiological drought because of decreased osmotic potential of soil solution),

nutritional imbalances and specific ion toxicity predominantly Na^+ and Cl^- or combination of these factors. The growth of plants under saline environment depends on number of morphological, physiological; biochemical and anatomical adaptations, which enable the plant to grow at high salinity. Generally, mechanisms of salt tolerance involve ion exclusion, compartmentation, K^+/Na^+ ratio, Na^+: K^+ selectivity and ion discrimination of leaves.

Materials and Methods

The present investigation was carried out in the Department of Crop Physiology, Narendra Deva University of Agriculture & Technology, Kumarganj, Faizabad (U.P.) under laboratory condition during *Kharif* (wet season) of 2015-16. The experiment was conducted in petridish with ten genotypes of rice. The whole experiment was planned under complete randomized design (CRD) with three replications along with twenty treatments. Six hundred Petridish (150×15 mm) were taken and 10 seeds of each entry repeated five times soaked with tap water are used. Petridish kept at room temperature and after germination different parameters were recorded. The observations were recorded on the parameters such as germination percentage, germination rate and α-amylase content in unconditioned and conditioned seeds.

Solutions of desired concentrations of proline were prepared. After that bold and healthy seeds of 10 genotype of rice were primed in the solution of 1, 5 &10 mM proline concentration for 6 to 8 hours. The seeds were dried for six hour in shade after drain out solution. For hydropriming treatment, seeds were soaked in distilled water for 6 to 8 hours. Non primed seeds were taken as untreated control. These seeds were used for recording different observations.

Germination was observed daily according to recommendations by International Seed Testing Association (ISTA, 1993). After final count germination percentage (GP %) was calculated by the following formulae (Raun *et al*., 2002): GP% = Number of total germinated seeds/Total number of seeds tested x 100 Seed vigour index was determined by following Abdul and Anderson, (1970) [1] by multiplying germination of seeds in percentage and total seedling length in mm and expressed in whole number.

Seed vigor index = Σ Plumule length + Radicle length × Germination rate α- amylase activity (enzyme unit g^{-1} fresh weight) was assayed in seed of various days with the help of experimental procedure of Chance and Macehly (1955) [4].

Results and Discussion

Data presented in table 1 showed that normal condition maximum germination was recorded in rice genotypes Usar3 followed by IR11T220, IR11 and Usar2 rest genotypes showed below 80 percent seed germination. These genotypes treated with various concentration of proline, significantly improved seed germination percent of all rice genotypes. Maximum seed germination percent was recorded in 1mM proline concentration. Maximum seed germination percent was recorded in rice genotype IR12 followed by IR11T172, Usar2, IR11, IRRI147 and IR11T220 respectively. Rest genotypes showed below 90 percent seed germination. Germination percent was significantly reduced in all rice genotypes when seeds were exposed to various level of salinity. Seed treatment with 1mM proline significantly toxified the adverse effect of various level of salinity 5mM and 10mM proline did not showed beneficial/significant effect on seed germination of all rice genotype. 1mM proline showed more than 80 percent germination up to 150mM NaCl concentration. In some extent, seed treatment with 5mM proline showed beneficial effect on seeds were exposed to 100mM NaCl concentration.10mM proline did not showed significant effect to ameliorating the adverse effect of salinity induced by salinity. Similar result was found by Deivanai *et al.,* (2011) that pretreatment of proline at a concentration of 1mM was found to be effective, whereas 10mM proline was ineffective in improving plant growth under high level of salt (150 and 200mM NaCl). It has been shown that exogenous application of proline mitigates the adverse effect of salinity by detoxifying the ions and protects the plant cells by maintaining osmotic balance (Ashraf and Foolad, 2007, Hoque *et al*., 2007 and Okuma *et al.,* 2000 and 2004) [3, 6, 11].

The examination of data of seed vigour index of rice genotypes/cultivar treated with different concentration of proline tested in normal condition as well as various levels of salinity have been presented in table 2. Among the rice genotypes, maximum seed vigour was noticed in genotype IR12 followed by Usar3, IR11T172 and IR11. Minimum seed vigour was observed in IR28 followed by IRRI154.Various level of salinity significantly reduced the seed vigour of all rice genotypes. Maximum seed vigour at higher salinity level (200mM NaCl) was recorded in rice genotype IR11T172 followed by Usar3, IR12 and IRRI147. Seed treated with various proline concentration significantly improved the seed vigour of rice genotype grown under various level of salinity. 1mM concentration of proline was found most effective, further higher concentration of proline reduced the seed vigour index. Maximum seed vigour index at 1mM proline was noticed in IRRI147 followed by IR11T220 under 50mM NaCl salinity. Similarly at 200mM NaCl salinity level. Rice genotype Usar 3 showed maximum seed vigour index followed by IR11T172. In the present study the seeds pretreated with proline provide significant evidence for assessing salt tolerance at germination stage. The priming is an effective technique that increases seed vigour and improves germination and seedling growth (Jumsoon *et al*., 1996, Janmohammadi *et al*., 2008 and Prisco and Vieira, 1976) [8, 7, 12].

Data regarding α-amylase activity in seedling of rice genotypes treated with different concentration of proline tested in normal condition as well as various levels of salinity have been presented in table 3. Treatment with different concentration of proline significantly increases theα-amylase activity in all rice genotypes in normal & various level of salinity. In normal condition seed treatment with 1mM conc. of proline was found more effective than higher concentration in all rice varities. Seeds without priming under different salinity level showed significant reduction in α-amylase activity. The maximum α-amylase activity in seeds of rice without proline grown under high salinity level was recorded in IR11T172 followed by IRRI147 and minimum was observed in Swarna*sub1*. Among various proline treatment under normal condition maximum α-amylase activity was found in IR11 followed by Usar3 treated with 1mM concentration of proline and minimum was found in IRRI154. At higher salinity level maximum effect of priming with proline was found in IR11 treated with 1mM concentration of proline followed by IR12 and minimum effect was found in Swarna *sub1* treated with 10mM concentration of proline and grown under 150 mM NaCl. Our findings showed coherence with Roy and Srivastava (1999) [14] reported that amylases are the key enzymes that play crucial role in hydrolyzing the seed's starch reserves thus supplying sugars to the growing embryo.

Table 1: Effect of different concentrations of proline on seed germination of ricegenotype grown under various salinity levels

Varieties\ Treatments	Germination (%)									
	IR11	IR12	IR28	USAR2	USAR3	Swarna*sub 1*	IR11T172	IR11T220	IRRI147	IRRI154
Control	82.00	79.00	70.90	81.17	85.03	72.08	74.78	83.03	74.97	71.00
s1	72.55	64.33	56.15	76.03	68.33	58.10	73.47	72.67	70.37	57.09
s2	60.05	59.33	50.45	71.72	57.04	53.47	61.11	74.77	65.01	48.22
s3	50.85	70.10	43.95	70.33	62.44	44.30	49.55	64.77	59.01	40.33
s4	45.08	59.41	19.00	45.14	64.79	18.09	46.09	65.67	55.77	22.18
p1control	94.00	99.00	76.70	95.43	89.57	76.70	96.77	91.43	92.90	72.30
p1s1	88.00	96.00	72.30	87.77	83.77	72.30	92.10	88.23	87.00	69.00
p1s2	85.00	90.40	65.30	86.57	82.90	65.30	84.43	80.67	85.57	65.00
p1s3	73.00	82.33	53.70	76.67	79.90	53.70	82.10	80.23	79.10	62.00
p1s4	69.00	79.00	37.00	73.33	76.90	33.00	77.33	73.67	78.10	44.00
p2 control	88.70	85.00	73.00	78.77	93.77	71.30	89.67	82.43	85.33	63.00
p2s1	82.30	88.33	74.30	75.43	88.67	71.00	84.77	74.80	80.33	61.00
p2s2	78.00	85.67	62.00	72.67	81.43	60.30	79.90	79.57	77.67	56.70
p2s3	68.30	84.77	52.70	67.10	87.53	52.70	78.57	71.43	73.67	59.70
p2s4	64.00	74.13	24.00	63.23	79.33	24.67	75.90	67.00	70.67	32.00
p3 control	82.30	75.67	71.00	68.90	89.77	67.70	84.00	74.90	72.00	59.30
p3s1	78.70	73.00	62.00	75.43	85.33	60.30	84.43	66.33	68.33	56.00
p3s2	74.00	70.67	56.70	76.57	79.67	56.70	74.00	68.23	66.33	49.00
p3s3	69.00	68.67	44.30	72.10	79.00	44.97	83.77	59.33	68.10	43.30
p3s4	64.00	64.00	33.15	71.20	78.13	35.11	82.43	69.53	67.13	34.14
SEm±	8.66	7.47	8.66	5.11	5.74	7.44	7.43	5.87	4.78	8.08
C.D at 5%	24.84	21.42	24.84	14.67	16.47	21.35	21.31	16.85	13.71	23.19

Table 2: Effect of different concentration of proline on seed vigour index of rice genotypes grown under various salinity levels

Varieties\Treatments	Seed vigour index									
	IR11	IR12	IR28	USAR2	USAR3	Swarna*sub1*	IR11T172	IR11T220	IRRI147	IRRI154
Control	450.00	476.69	354.85	435.00	467.00	365.15	453.14	447.00	442.00	361.01
s1	301.00	329.17	187.69	337.00	337.00	217.90	341.05	309.00	313.00	198.00
s2	213.15	236.76	115.90	209.00	241.00	132.36	238.00	217.05	225.00	122.15
s3	155.00	171.92	75.05	146.00	163.00	82.16	166.00	161.00	165.11	79.37
s4	98.90	119.47	37.98	91.00	121.00	53.11	127.00	103.00	112.00	42.15
p1control	1171.90	1212.80	370.60	1154.20	1188.60	385.90	1172.90	1140.50	1231.40	400.20
p1s1	701.10	727.50	301.40	679.20	740.70	315.90	727.60	750.70	921.10	310.50
p1s2	626.20	657.60	209.10	623.70	661.60	222.10	645.70	621.30	731.00	234.00
p1s3	423.40	448.50	148.30	429.20	503.60	157.40	510.50	499.40	595.30	194.30
p1s4	446.20	473.70	62.00	454.80	554.60	75.60	500.00	327.50	417.60	88.00
p2 control	945.80	945.40	267.70	893.20	1027.40	275.80	990.50	900.10	1032.40	256.20
p2s1	562.60	586.10	200.70	586.10	661.20	203.50	617.20	736.20	784.00	187.10
p2s2	421.20	442.40	152.90	434.50	496.10	160.90	465.50	529.10	585.00	162.40
p2s3	323.40	337.10	126.40	330.30	391.10	136.90	388.10	426.60	463.60	167.10
p2s4	249.60	265.10	51.00	258.70	329.00	59.00	313.60	324.00	357.70	68.00
p3 control	623.00	642.00	163.30	626.10	666.70	169.20	624.90	642.00	746.20	160.20
p3s1	448.40	473.20	117.80	448.50	490.20	144.80	491.70	435.20	610.70	123.20
p3s2	291.10	318.30	115.20	308.60	326.40	122.80	318.30	356.10	410.40	116.00
p3s3	259.90	281.10	31.00	268.10	296.70	39.90	302.80	301.30	399.50	49.00
p3s4	87.50	82.80	40.60	154.20	188.60	45.90	172.90	140.50	231.40	49.00
SEm±	25.98	31.75	13.28	19.05	22.52	16.74	21.36	15.01	17.90	15.01
C.D at 5%	74.53	91.10	38.09	54.66	64.60	48.03	61.28	43.06	51.34	43.06

Table 3: Effect of different concentration of proline on α-amylase activity in seed of rice genotype grown under various salinity levels.

Varieties\ Treatments	α-amylase(enzyme unit g^{-1} fresh weight)									
	IR11	IR12	IR28	USAR2	USAR3	Swarna*sub1*	IR11T172	IR11T220	IRRI147	IRRI154
Control	14.30	12.43	7.08	11.22	12.03	7.11	11.77	12.21	11.92	7.45
s1	12.12	11.21	6.32	10.98	11.77	6.55	11.09	11.23	11.33	6.45
s2	10.98	9.90	5.50	9.90	10.77	6.21	10.66	10.98	10.34	6.12
s3	9.65	8.08	5.05	8.88	9.09	5.98	9.98	9.88	9.90	5.98
s4	8.09	7.66	4.98	7.98	7.90	4.88	8.88	8.32	8.44	5.56
p1control	18.50	17.44	7.88	17.59	17.71	7.40	17.59	17.37	17.37	7.44
p1s1	17.37	17.08	7.43	17.54	16.85	7.12	17.14	16.61	16.89	6.98
p1s2	17.23	16.49	7.24	16.24	16.82	6.66	16.20	16.43	16.42	6.75
p1s3	16.67	16.04	6.37	15.37	16.66	6.30	15.34	15.44	15.99	6.55
p1s4	16.40	16.23	6.55	14.43	14.41	6.26	14.30	14.42	14.51	6.21
p2 control	18.37	17.11	7.85	16.22	17.31	7.30	16.19	17.18	17.21	7.11
p2s1	17.47	16.94	7.80	15.44	16.58	6.60	15.51	16.47	16.72	6.88

p2s2	17.33	16.83	7.66	14.41	16.62	6.44	14.41	16.14	16.42	6.46
p2s3	16.20	15.55	6.67	14.28	16.45	6.15	14.31	15.40	16.01	6.12
p2s4	15.32	14.40	6.33	13.59	14.31	5.39	13.55	15.12	14.15	5.88
p3 control	15.69	15.53	7.97	15.36	17.20	7.21	15.49	13.21	16.77	7.02
p3s1	15.35	14.57	7.67	14.65	16.60	6.58	14.68	13.36	16.26	6.71
p3s2	14.46	14.30	7.52	14.60	16.26	6.53	14.67	13.82	15.93	6.40
p3s3	14.23	13.57	6.83	13.61	15.66	6.52	13.67	14.45	15.00	6.00
p3s4	14.15	13.32	6.08	13.34	13.49	5.31	13.44	14.72	13.49	5.45
SEm±	0.58	1.73	0.50	1.16	1.44	0.45	1.59	1.36	1.66	0.35
C.D at 5%	1.66	4.97	1.44	3.31	4.14	1.28	4.56	3.89	4.77	1.01

References

1. Abdul BAA, Anderson JD. Viability and leaching of sugars from germinating barley. Crop Sci, 1970; 10:31-34.
2. Anonymous. Food and agriculture organization of the united nations, 2014; xvii:3.
3. Ashraf M, Foolad MR. Roles of glycinebetaine and proline in improving plant abiotic stress tolerance. Environ. Expt. Bot, 2007; 59:206-216.
4. Chance B, Maehly AC. Assay of Catalase and Peroxidases. Methods in Enzymology, 1955; 2:764-775.
5. Gregorio GB. Tagging salinity tolerance genes in rice using amplified fragment length polymorphism (AFLP). Ph.D. Dissertation. Laguna, Philippines, 1997, 118.
6. Hoque MA, Okuma E, Banau MNA, Nakamura Y, Shimoishi Y, Murata N. Exogenous proline mitigates the detrimental effects of salt stress more than exogenous betain by increasing antioxidants enzyme activity. J Plant Physiol, 2007; 64:553-561.
7. Janmohammadi M, Dezfuli PM, Sharifzadeh F. Seed invigoration techniques to improve germination and early growth of inbred line of maize under salinity and drought stress. Gen. Appl. Plant Physiol, 2008; 34:215-226.
8. Jumsoon K, Jeuonlai C, Ywonok J. Effect of seed priming on the germinability of tomato (*Lycopercicon esculentum* Mill.) seeds under water and saline stress. Journal of Korean Society of Horticultural Sciences. 1996; 37:516-521.
9. Krishnasamy V, Srimathi P. Seed management for rainfed agriculture-land use planning and watershed management, 2001, 140-144.
10. Okuma E, Murakami Y, Shimoishi Y, Tada M, Murata Y. Effects of exogenous application of proline and betaine on the growth of tobacco cultured cells under saline conditions. Soil and Plant Nutrition, 2004; 50:1301-1305.
11. Okuma E, Soeda K, Tada M, Murata Y. Exogenous proline mitigates the inhibition of growth of *Nicotiana tabacum* cultured cell under saline conditions. Soil Sci. and Plant Nutri, 2000; 46:257-263.
12. Prisco JT, Vieira GHF. Effects of NaCl salinity on nitrogenous compounds and proteases during germination of *ligrra sinensis* seeds. Physiol. Plant, 1976; 36:317-320.
13. Raun S, Xue Q, Thlkowska K. Effect of seed priming on germination and health of rice (O*ryza sativa* L) seeds. Seed Sci. Technol. 2002; 30:451-458.
14. Roy NK, Srivastava AK. Effect of presoaking seed treatment on germination and amylase activity of wheat (*Triticum aestivum* L.) under salt stress conditions. Rachis, 1999; 18:46-51.