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Enhancing seedling growth and enzyme activity by priming rice seed's with proline under saline condition

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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 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 radicle length and Plumule length and increase proline activity. It is evident from the result that seed treatment with various concentration of proline significantly increased the radicle and plumule length and proline 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).

Keywords: Salinity, rice, proline, radicle length, and plumule length.

Introduction
Rice is the most important food crop of the developing world and is the staple food of more than half of the world's population. It is especially important crop of Asia, where more than 90% of world rice is grown and consumed and where more than half of world's people live. Rice farming is about 10,000 year old and largest single use of land for producing food. Rice fields covers 11% of Earth's entire aerable land. Rice (*Oryza sativa* L.) is the principal source of food for more than a third of the world's population and is one of the most widely grown crops in coastal areas inundated with sea water during high tidal period, although it is usually considered moderately susceptible to salinity.

Osmoconditioning (Seed priming) is a technique that has proved effective for improving germination, seedling emergence and yield of many early planted, small seeded vegetables and flower crops. Priming or control hydration refers to conditioning of seeds in aerated solution with high solute content, which keeps the seed in a partially hydrated state (Chong *et al.* 2002) [5]. Seed are important and costly input in farming, rapid and uniform field emergence is two essential pre-requisites to increase yield, quality and ultimately profit of annual crops. Rate and uniformity of seedling emergence are key component of seed yield quality in both irrigated and rainfed environments. Seed priming is a specific seed treatment given to seed before sowing especially in adverse soil moisture conditions and highly recommended and successful in rainfed agriculture. Priming of seed is physiological pre-conditioning which is widely used in various crops which include subjecting of seeds to cycles of wetting, drying and incubation at low temperature. Seed priming is normally practiced with water and can be improved further by selection of inorganic chemicals and botanicals.

Soil salinity is serious constraint to increased production, in rice growing countries of the world (Gregorio, 1997) [8]. 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.

Salt stress induces the accumulation of reactive oxygen species (ROS) in plant cells. The excess production of ROS is toxic to plants and causes oxidative damage to cellular constituents, leading to cell death. Inorganic nutrients such as N, P and K play essential roles in plant metabolism. In addition to its role as an osmoprotectant, proline counteracts the adverse effects of various stresses on plants by affecting the uptake and accumulation of inorganic nutrients (Ali *et al*. 2008) [3] and by reducing cellular damage and increasing antioxidant defense systems.

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 radical length, plumule length and proline content in unconditioned and conditioned seeds. Three normal seedlings from all the replication of each treatment were taken randomly and the mean length of plumule and radicle length (mm) from the base to the tip was recorded at two days intervals. Free proline content in leaves was estimated spectrophotometrically according to the methods of Bates *et al.* (1973) [4].

Results and Discussion

Data for the progressive change in radical length 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 1. Treatment with different concentration of proline significantly increases the radical length in all rice genotypes in normal & various level of salinity. Seeds without priming under different salinity level cause reduction in radicle length. The maximum radical length in seeds of rice without proline grown under high salinity level was recorded in IR11T220 (1.70) followed by IRRI-147 (1.63) and minimum was noted in IR-28 (0.91). Among various proline treatment under normal condition maximum radical length was observed in IRRI-147(10.13) treated with 1mM proline concentration followed by IR11T220 (9.50) treated with 1mM concentration of proline and minimum was found in IRRI-154(0.66) treated with 1mM concentration of proline. At higher salinity level maximum effect of priming with proline was found In IRRI-147 (10.13) treated with 1mM concentration of proline followed by IR11T220 (9.50) and minimum effect was found in IR-28(0.40) treated with 1mM concentration of proline and grown under 150 mM NaCl. Seed treatment with high concentration of proline (5&10mM) did not showed significant effect on radicle of rice genotypes grown under normal conditions well in various salinity condition. The radicle length of seedlings grown in salt solutions also showed decreasing trend indicating that the salt stress not only affect germination but also the growth of seedlings which indicates that synthetic ability of seed was affected. This is in conformity with the findings of Djanaguiraman *et al*., (2003) [7] and Hakim *et al*., (2010) [9] they observed that radicle length was conspicuously affected by salt. Similar finding reported found by Deivanai *et al.,* (2011) [6] that exogenous proline application had significant impact on radicle elongation in genotypes of rice.

Data regarding plumule length 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 2. Treatment with different concentration of proline significantly increased the plumule length 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 cause reduction in plumule length. The maximum plumule length in seeds of rice without proline grown under high salinity level was found in Usar-3 (0.80) followed by IRRI-147 (0.60) and minimum was noticed in SwarnaSub-1 (0.20). Among various proline treatment under normal condition maximum plumule length was obtained in Usar-3(3.57) treated with 1mM proline concentration followed by IR-12 (3.47) treated with 1mM concentration of proline and minimum was found in IR-28 (1.40) treated with 1mM concentration of proline. At higher salinity level maximum effect of priming with proline was found in Usar-3 (1.93) treated with 1mM concentration of proline followed by IR-12 (1.67) and minimum effect was found in IR-28(0.30) treated with 10mM concentration of proline and grown under 200 mM NaCl. Similar result was found by Deivanai *et al.,* (2011) [6]. The observed increase in plumule length could be attributed to positive effect of exogenous proline application which stimulated cell elongation and division. The physiological effect of this amino acid on cell elongation was supported by Ozdemir *et al.,* (2004) [10] under salt stress in rice.

Data recorded on proline content in seed 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 3. Treatment with different concentration of proline significantly increases the proline content in all rice genotypes in normal & various level of salinity. In normal condition seed treatment with 5mM conc. of proline was found more effective than other concentration of proline. Seeds without priming under different salinity level cause reduction in proline. The maximum proline content in seeds of rice without proline treatment grown under high salinity level was recorded in IR-12 (312.30) followed by IR11T172 (309.70) and minimum was found in IR-28 (191.30). Among various proline treatment under normal condition maximum proline was found in Usar-3(355.90) followed by IR-12(338.40) treated with 5mM concentration of proline and minimum was found in IRRI-154 (101.90) treated with 10mM proline concentration. At higher salinity level maximum effect of priming on proline was found in IR-11 (618.00) followed by IR-12 (574.80) & minimum effect was found in SwarnaSub-1(128.60) treated with 1mM concentration of proline and

grown under 50 mM NaCl. Proline has been assigned the role of cyst salute, a storage compounds or a protective agent for cytoplasm enzymes and cellular structure (Pandey and Ganapathy, 1985) [11]. Proline content significantly increased in rice seedlings exposed to increasing salt stress. Proline content significantly increased in rice seedlings when exposed to various concentration of NaCl salinity. It is well documented that proline accumulates in larger amount than any other amino acids under water deficit and regulates osmotic potential of the cell (Ali *et al*., 1999 and Abraham *et al*., 2003) [2, 1].

Table 1: Effect of different concentration of proline on radical length of rice genotype under various salinity levels

Varieties / Treatments	Radicle length (mm)									
	IR-11	**IR-12**	**IR-28**	**USAR-2**	**USAR-3**	**S SUB1**	**IR11T172**	**IR11T220**	**IRRI147**	**IRRI154**
Control	3.60	3.81	3.00	3.69	3.49	3.11	3.23	3.89	3.97	2.99
s1	2.69	2.83	2.11	2.80	2.57	2.23	2.41	2.98	2.70	2.07
s2	2.19	2.34	1.51	2.23	2.11	1.59	2.03	2.17	241	1.47
s3	1.67	1.80	1.27	1.71	1.70	1.40	1.77	1.91	1.75	1.11
s4	1.42	1.51	0.91	1.60	1.57	0.97	1.56	1.70	1.63	0.92
p1control	8.03	9.3	2.8	9.1	9.4	2.9	9.3	9.5	10.13	0.66
p1s1	4.5	5	2.6	4.8	5	2.7	4.9	5.4	7.4	3.3
p1s2	4.2	4.9	2.2	4.7	5	2.3	4.9	5.1	6.1	2.8
p1s3	3.5	3.6	2.03	3.4	3.9	2.1	3.8	4.2	5.2	2.4
p1s4	4.7	5.1	1.67	5	5.3	1.62	5.2	2.9	3.9	1.78
p2 control	7.2	7.9	1.9	7.7	8.06	2	8	8.3	9	2.2
p2s1	3.8	4.3	1.7	4.5	4.8	1.8	4.7	6.06	6.7	2.1
p2s2	2.9	3.3	1.5	3.4	3.7	1.6	3.6	4.3	5.3	1.9
p2s3	2.6	3.1	1.7	3.2	3.4	1.8	3.3	4.03	4.3	1.7
p2s4	2.5	3.1	1.12	3.2	3.5	1.21	3.4	3.7	3.9	1.37
p3 control	5.1	5.7	1.4	5.8	5.9	1.5	5.4	6.4	7.1	1.9
p3s1	3.6	4.4	1.2	4.5	4.8	1.6	4.6	5.1	6.1	1.6
p3s2	2.7	3.1	1.7	3.3	3.4	1.8	3.3	4	4.33	1.4
p3s3	2.5	3.03	0.4	3.1	3.3	0.56	3.2	3.6	4.3	1.9
p3s4	0.8	0.6	0.80	9.1	9.4	0.90	9.3	9.5	10.13	0.66
SEm±	**0.173**	**0.289**	**0.225**	**0.156**	**0.237**	**0.127**	**0.289**	**0.087**	**0.115**	**0.144**
C.D at 5%	**0.497**	**0.828**	**0.646**	**0.447**	**0.679**	**0.364**	**0.828**	**0.248**	**0.331**	**0.414**

Table 2: Effect of different concentration of proline on plumule length of rice genotype grown under various salinity levels

Varieties / Treatments	Plumule length(mm)									
	IR-11	**IR-12**	**IR-28**	**USAR-2**	**USAR-3**	**S SUB1**	**IR11T172**	**IR11T220**	**IRRI147**	**IRRI154**
Control	1.40	2.07	0.90	1.87	2.10	1.00	1.87	1.87	2.00	1.10
s1	0.93	1.47	0.70	1.30	1.57	0.80	1.30	1.30	1.53	0.80
s2	0.87	1.03	0.33	0.83	1.13	0.37	0.83	0.83	1.00	0.47
s3	0.47	0.97	0.30	0.77	1.00	0.40	0.77	0.77	0.93	0.47
s4	3.37	0.60	0.22	0.40	0.80	0.20	0.40	0.40	0.60	0.25
p1control	3.37	3.47	2.03	3.27	3.57	2.13	3.27	3.27	3.33	2.23
p1s1	3.17	3.27	1.57	3.07	3.20	1.67	3.07	3.07	3.07	1.70
p1s2	2.77	2.87	1.00	2.67	2.63	1.10	2.67	2.67	2.53	1.20
p1s3	2.50	2.60	0.73	2.40	2.27	0.83	2.40	2.40	2.40	0.93
p1s4	1.57	1.67	0.37	1.47	1.93	0.47	1.47	1.47	1.50	0.57
p2 control	2.63	2.97	1.77	2.77	3.07	1.87	2.77	2.77	2.87	1.97
p2s1	2.20	2.73	1.00	2.53	2.80	1.07	2.53	2.53	2.63	1.17
p2s2	1.73	2.30	0.97	2.10	2.40	1.07	2.10	2.10	2.20	1.17
p2s3	0.90	1.83	0.70	1.63	1.93	0.80	1.63	1.63	1.80	0.90
p2s4	1.97	1.00	0.34	0.80	1.13	0.36	0.80	0.80	1.00	0.44
p3 control	1.72	1.80	1.40	1.81	1.82	1.51	1.77	1.75	1.63	1.49
p3s1	1.53	1.63	0.93	1.65	1.64	0.99	1.63	1.61	1.51	0.89
p3s2	1.21	1.31	0.61	1.34	1.32	0.69	1.40	1.33	1.11	0.66
p3s3	1.00	1.10	0.49	1.12	1.09	01.52	1.19	1.07	0.96	0.49
p3s4	0.80	0.95	0.30	0.96	0.90	0.33	0.98	0.90	0.86	0.36
SEm±	**0.517**	**0.453**	**0.321**	**0.173**	**0.231**	**0.058**	**0.115**	**0.133**	**0.064**	**0.142**
C.D at 5%	**1.483**	**1.300**	**0.920**	**0.497**	**0.663**	**0.166**	**0.331**	**0.381**	**0.182**	**0.406**

Table 3: Effect of different concentration of proline on proline content in seed of rice genotype under various salinity levels

Varieties / Treatments	Proline (µmol g/FW)									
	IR-11	**IR-12**	**IR-28**	**USAR-2**	**USAR-3**	**S SUB 1**	**IR11T172**	**IR11T220**	**IRRI147**	**IRRI154**
Control	213	225.9	106.2	232.2	228.0	102.0	214.0	241.2	209.2	102.2
s1	221	241.7	126.0	249.6	241.4	119.3	237.2	265.1	232.5	129.0
s2	232	276.8	137.5	273.2	269.3	141.3	265.8	279.3	261.8	137.2
s3	298	305.2	169.0	291.4	281.1	164.0	279.6	301.0	299.1	187.5
s4	307	312.3	191.3	307.1	305.3	193.7	309.7	312.4	307.7	200.0
p1control	299	305.4	197.3	300.8	322.4	116.5	262.1	326.6	266.8	113.0
p1s1	391	359.8	263.7	333.6	334.9	128.6	327.2	377.4	315.5	135.1
p1s2	483	400.6	281.4	378.5	393.1	141.4	385.8	399.7	350.3	178.8
p1s3	532	466.7	342.5	475.1	463.4	178.2	438.3	449.7	438.3	278.8
p1s4	618	574.8	353.3	548.7	544.4	291.6	529.9	539.6	484.3	295.6
p2 control	323	338.4	206.5	330.1	355.9	140.8	266.5	282.9	283.8	104.5
p2s1	395	391.5	229.1	387.0	409.9	249.5	312.9	358.7	329.6	146.8
p2s2	488	485.2	285.8	417.7	465.7	272.8	399.9	413.5	379.5	186.8
p2s3	590	577.3	350.3	515.0	469.0	309.6	470.1	428.8	443.3	283.6
p2s4	618	603.3	365.8	566.5	534.8	311.0	541.5	523.8	520.9	302.2
p3 control	283	273.4	142.5	250.2	310.5	106.0	243.6	290.7	219.6	101.9
p3s1	319	300.5	155.9	363.6	327.7	132.9	297.9	321.6	281.7	119.5
p3s2	412	380.3	249.4	373.3	419.6	180.2	313.2	370.8	326.7	142.8
p3s3	442	490.5	335.1	455.5	452.0	278.3	446.7	414.7	432.1	248.5
p3s4	601	526.5	340.4	562.2	516.1	265.6	543.0	480.5	554.4	288.2
SEm±	**18.475**	**19.053**	**31.754**	**38.682**	**45.033**	**50.807**	**32.332**	**43.879**	**56.580**	**12.702**
C.D at 5%	**53.001**	**54.657**	**91.096**	**110.971**	**129.190**	**145.753**	**92.752**	**125.877**	**162.316**	**36.438**

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