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Effect of salinity on biochemical and enzymatic activities of rice (*Oryza sativa* L.)

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Abstract
In the present study deals with the *Oryza sativa* L was selected for the physiological and biochemical studies in laboratory condition for 10 days (2nd, 4th, 6th, 8th and 10th day) that were carried under different salinity levels. Six genotypes of rice crop i.e. tolerant- CSR-36, CSR-43, Usar Dhan-3 and Susceptible- Swarna Sub-1, IR-28, Pusa-44. were used to germinate under varying concentrations of NaCl i.e.0 to 100 mM. The salt stress significantly reduced the germination percentage with increasing salt stress from 0 to 100 mM NaCl. A continuous decrease in carbohydrate content with increase in salt stress was observed in all the varieties. The rate of proline accumulation was observed maximum in Usar dhan-3 at 100 mM NaCl stress. The enzymetic activity of catalase and α-amylase was higher in control than sample induced with salinity stress. This study overall showed better tolerance to salt stress with a lesser extent of antagonistic effect of NaCl on Usar dhan -3 than any other variety.

Keywords: Salt stress, germination, *Oryza sativa*. L, proline

Introduction
Rice (*Oryza sativa* L.) is among the most consumed cereals in the world. Production of rice (*O. sativa* L.) ranks second among food grains, and half of the world's population subsists on rice by receiving the highest (26.2%) calories intake from it (FAO, 2009) [5]. In Asia where it covers half of the arable land used for agriculture in many countries. More than 90% of rice is produced and consumed in Asia and it is the only major cereal crop that is consumed almost exclusively by human. India is the largest rice growing country accounting for about one-third of the world acreage under the crop. About 84% of rice production growth has been attributed to the use of modern technologies. However, salinization of paddy land in the coastal region may develop by advancement of sea or rising of the saline ground water to the upper surface of the soil. This problem is due to high levels of evaporation and salt accumulation in the root zone in arid and semi arid areas (FAO, 1997) [4].

Salinity is one of the major abiotic factors that limits plant growth and productivity in many regions of the world due to increasing use of poor quality of water for irrigation and soil salinization (Munns and Tester 2008, Rozema and Flowers 2008, Kronzucker and Britto 2011, Zhang and Shi 2013) [12, 17, 8, 19]. One of the most detrimental effects of salinity is the accumulation of Na^+ and Cl^- ions in tissues of plants subjected to soils with high concentrations of NaCl (Maathuis *et al*. 2014) [10]. High concentration of Na^+ inhibits uptake of K^+ which is an essential macronutrient for plants growth and development that results in low productivity and may even cause death (Kronzucker *et al*. 2013, Gupta and Huang 2014) [9, 6]. Plants have developed various physiological and biochemical mechanisms to maintain a relatively stable intracellular environment via accumulating various solutes under saline condition (Gupta and Huang 2014, Roy *et al*. 2014) [6, 16]. The osmotic adjustment in plants can maintain the uptake of water and the turgor of cell, allowing regular physiological metabolisms (Radić *et al*. 2013) [14]. Proline, as an important osmoprotectant, contributes to osmotic adjustment, protecting enzymes from oxidative damage under saline condition (Ashraf and Harris 2004, Gupta and Huang 2014) [6]. In addition, under saline condition, the accumulation of other compounds such as soluble sugar, which at higher temperatures are non-toxic to cytoplasmic functions, allowing turgor maintenance and/or protection of macromolecular structure against the destabilizing effects of the decrease in water activity, has also been reported in many plants species (Juan *et al*. 2005) [7].

A major challenge towards world agriculture involves production of 70% more food crop for an additional 2.3 billion people by 2050 worldwide. Salinity is a major stress limiting the increase in the demand for food crops. More than 20% of cultivated land worldwide (~ about 45 hectares) is affected by salt stress and the amount is increasing day by day.

Plants on the basis of adaptive evolution can be classified roughly into two major types: the halophytes (that can withstand salinity) and the glycophytes (that cannot withstand salinity and eventually die). Majority of major crop species belong to this second category. Thus salinity is one of the most brutal environmental stresses that hamper crop productivity worldwide. Salinity stress involves changes in various physiological and metabolic processes, depending on severity and duration of the stress, and ultimately inhibits crop production. Initially soil salinity is known to represses plant growth in the form of osmotic stress which is then followed by ion toxicity. During the initial phases of salinity stress, water absorption capacity of root systems decreases and water loss from leaves is accelerated due to osmotic stress of high salt accumulation in soil and plants, and therefore salinity stress is also considered as hyperosmotic stress (Munns, 2005) [11]. Osmotic stress in the initial stage of salinity stress causes various physiological changes, such as interruption of membranes, nutrient imbalance, impairs the ability to detoxify reactive oxygen species (ROS), differences in the antioxidant enzymes and decreased photosynthetic activity, and decrease in stomatal aperture. Salinity stress is also considered as a hyperionic stress. One of the most detrimental effects of salinity stress is the accumulation of Na+ and Cl− ions in tissues of plants exposed to soils with high NaCl concentrations. Entry of both Na+ and Cl− into the cells causes severe ion imbalance and excess uptake might cause significant physiological disorder(s). High Na+ concentration inhibits uptake of K+ ions which is an essential element for growth and development that results into lower productivity and may even lead to death. In response to salinity stress, the production of ROS, such as singlet oxygen, superoxide, hydroxyl radical, and hydrogen peroxide, is enhanced. Salinity-induced ROS formation can lead to oxidative damages in various cellular components such as proteins, lipids, and DNA, interrupting vital cellular functions of plants.

Material Methods

The present investigation was conducted at Department of Crop Physiology, A.N.D. University of Agriculture & Technology, Kumarganj, Ayodhya (U.P.) during *Kharif* season of 2017-18. The experiment was conducted in laboratory condition (Petridish). Three tolerant varieties (CSR 36, CSR 43, Usar Dhan 3 and three susceptible varieties (Swarna Sub 1, IR 28, Pusa 44) used in experiment. Experiment consists of three NaCl concentrations (0, 50, 100 mM) and eighteen treatments *viz*; CSR 36+control (Distilled water), CSR 36+S1 (50 mM NaCl), CSR 36+S2(100 mM NaCl), CSR 43+Control (Distilled water), CSR 43+S1 (50 mM NaCl), CSR 43+S2 (100 mM NaCl), Usar Dhan 3+control(Distilled water),Usar Dhan 3+S1 (50 mM NaCl), Usar Dhan 3+S2: 50 ppm +100 mM NaCl, Swarna Sub 1+control(Distilled water), Swarna Sub 1+S1 (50 mM NaCl), Swarna Sub 1+S2 (100 mM NaCl), IR 28+Control (Distilled water), IR 28+S1 (50 mM NaCl), IR 28+S2 (100 mM NaCl), Pusa 44+control(Distilled water), Pusa 44+S1 (50 mM NaCl),Pusa 44+S2: (100 mM NaCl). Change in carbohydrate content, proline accumulation, catalase and α-amylase activity were recorded as rate as well as percentage during successive period of germination. It was recorded alternately up to 10 days.

Determination of carbohydrate content in rice seeds (mg g^{-1} dry wt.)

Carbohydrate content of seeds at day 2,4,6,8 and 10 was determined according to the method described by Yemm and Willis (1954) [18].

Estimation of Proline content (μg g^{-1} fresh wt.)

Proline was estimated spectrophotometically according to the method of Bates *et al.*, (1973) [2]. Proline content in leaf tissue was calculated using the formula:

$$\text{Proline content}\left(\mu\frac{g}{g}\text{fr. wt.}\right)=\frac{36.23\ \times \text{Od } 520 \times \text{Volume of the aliquot made (ml)}}{\text{Volume of the aliquot (ml)} \times \text{Weight of the sample (ml)}}$$

Catalase activity in rice seeds (enzyme unit g^{-1} fresh wt min^{-1})

Catalase activity can be assayed colorimetrically according to method given in analytical biochemistry. Catalase facilitates the dismutation of H_2O_2 to water and O_2 according to the reaction.

$$H_2O_2 \xrightarrow{\text{Catalase}} H_2O + ½\, O_2$$

The enzyme plays an important role in association with SOD as well in photorespiration and glycolate pathway.

α-amylase activity in rice seeds (enzyme unit-mg g^{-1} fresh wt.) :

α-amylase activity in leaf of test species recorded with the help of experimental procedure of Chance and Macehly (1955) [3].

Result and Discussion

Effect of NaCl on Carbohydrate content

Carbohydrate content in the embryo axis decreased significantly with increase in NaCl level.Carbohydrate content was more in Usar dhan-3 (52.32) than in CSR-36 (45.57) with 0 NaCl concentration. irrespective of salinity levels. The data pertaining to carbohydrate content presented in (Table-1) indicate that carbohydrate declined with the increase in salt concentration. Maximum soluble carbohydrate at 100mM NaCl concentration was observed in Usar Dhan-3 followed by CSR-43 and CSR-36 and lower concentration was noticed in IR-28 at par with IR-36 and Swarn Sub-1 respectively at 10th day of germination. Similar trends were also observed at 2nd, 4th, 6th, and 8th days of germination.

Table 1: Effect of salinity on carbohydrate (mg g^{-1} dry wt.)

2nd Day

NaCl level	Varieties						Average
	V1	V2	V3	V4	V5	V6	
0	43.56	43.64	44.84	42.70	42.85	42.90	43.42
50	41.82	41.89	43.05	40.99	41.14	41.18	41.68
100	39.73	39.80	40.89	38.94	39.08	39.12	39.59
Average	41.70	41.78	42.93	40.88	41.02	41.07	41.56
		Varieties (V)		NaCl levels (N)		VxN	
SEm±		1.118		0.791		1.937	
CD at 5%		3.207		2.268		5.555	

4th Day

NaCl level	Varieties						Average
	V1	V2	V3	V4	V5	V6	
0	44.60	44.78	45.31	43.10	43.20	43.40	44.07
50	42.82	42.99	43.50	41.38	41.47	41.66	42.30
100	40.68	40.84	41.32	39.31	39.40	39.58	40.19
Average	42.70	42.87	43.38	41.26	41.36	41.55	42.18
		Varieties (V)		NaCl levels (N)		VxN	
SEm±		0.476		0.337		0.825	
CD at 5%		1.365		0.965		2.365	

6th Day

NaCl level	Varieties						Average
	V1	V2	V3	V4	V5	V6	
0	45.30	46.78	51.03	43.89	44.36	44.78	46.02
50	43.49	44.91	48.99	42.13	42.59	42.99	44.18
100	41.31	42.66	46.54	40.03	40.46	40.84	41.97
Average	43.37	44.78	48.85	42.02	42.47	42.87	44.06
		Varieties (V)		NaCl levels (N)		VxN	
SEm±		0.325		0.23		0.563	
CD at 5%		0.932		0.659		1.615	

8th Day

NaCl level	Varieties						Average
	V1	V2	V3	V4	V5	V6	
0	45.48	46.96	51.92	44.00	44.52	44.85	46.29
50	43.66	45.08	49.84	42.24	42.74	43.06	44.44
100	41.48	42.83	47.35	40.13	40.60	40.90	42.21
Average	43.54	44.96	49.70	42.12	42.62	42.94	44.31
		Varieties (V)		NaCl levels (N)		VxN	
SEm±		0.313		0.221		0.542	
CD at 5%		0.898		0.635		1.555	

10th Day

NaCl level	Varieties						Average
	V1	V2	V3	V4	V5	V6	
0	45.57	47.02	52.32	44.15	44.67	44.98	46.45
50	43.75	45.14	50.23	42.38	42.88	43.18	44.59
100	41.56	42.88	47.72	40.26	40.74	41.02	42.36
Average	43.63	45.01	50.09	42.27	42.76	43.06	44.47
		Varieties (V)		NaCl levels (N)		VxN	
SEm±		0.294		0.208		0.509	
CD at 5%		0.843		0.596		1.46	

Effect of NaCl on Proline Accumulation:

Accumulation of proline under saline condition in rice genotypes probably impart certain degree of tolerance against adverse effect of salinity. In the present study, the seedling of rice accumulated more proline content with increase in salinity level as compare to control. 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) [13]. 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.

The proline content was significantly increased in all rice genotypes sown various NaCl Concentrations. Genotypic variability was also observed among rice genotypes. Maximum proline accumulation was noticed in Usar Dhan-3 (235.55) followed by CSR-43 (213.55), CSR-36 (176.52), Pusa-44 (170.77), IR-28(163.61) and Swarn Sub-1 (156.53). Similar trend was obtained at 2nd, 4th, 6th and 8th (Table-2).

Table 2: Effect of NaCl on Proline accumulation (μg g^{-1} fresh wt.)

2th Day

NaCl level	Varieties						Average
	V1	V2	V3	V4	V5	V6	
0	160.80	198.41	214.82	139.71	143.68	158.64	169.34
50	168.96	210.58	223.63	145.39	151.63	162.68	177.14
100	173.54	204.36	234.36	149.96	157.38	169.85	181.57
Average	167.76	204.45	224.27	145.02	150.89	162.92	176.02
		Varieties (V)		NaCl levels (N)		VxN	
SEm±		0.103		0.073		0.178	
CD at 5%		0.295		0.208		0.51	

4th day

NaCl level	Varieties						Average
	V1	V2	V3	V4	V5	V6	
0	162.98	201.36	219.72	142.98	148.47	159.76	
50	170.97	206.59	228.97	148.14	156.66	165.96	
100	175.14	208.78	238.18	151.27	160.81	168.12	183.71
Average	169.69	205.57	228.95	147.46	155.31	164.61	178.60
		Varieties (V)		NaCl levels (N)		VxN	
SEm±		0.084		0.059		0.145	
CD at 5%		0.24		0.17		0.416	

6th Day

NaCl level	Varieties						Average
	V1	V2	V3	V4	V5	V6	
0	165.81	204.41	223.78	146.05	151.57	160.79	175.40
50	172.01	209.65	231.03	150.21	158.25	166.99	181.36
100	176.18	211.84	243.02	154.20	163.74	172.16	186.86
Average	171.33	208.63	232.61	150.15	157.85	166.65	181.21
		Varieties (V)		NaCl levels (N)		VxN	
SEm±		0.100		0.071		0.173	
CD at 5%		0.286		0.202		0.496	

8th Day

NaCl level	Varieties						Average
	V1	V2	V3	V4	V5	V6	
0	168.97	208.55	229.24	150.38	153.64	163.94	179.12
50	175.18	211.79	235.52	154.56	160.83	169.94	184.64
100	179.35	218.99	248.75	158.71	167.99	174.32	189.52
Average	174.50	213.11	234.17	154.55	160.82	169.40	184.43
		Varieties (V)		NaCl levels (N)		VxN	
SEm±		0.118		0.084		0.205	
CD at 5%		0.339		0.24		0.588	

10th Day

NaCl level	Varieties						Average
	V1	V2	V3	V4	V5	V6	
0	171.99	210.65	230.29	153.41	158.76	165.97	181.85
50	176.20	213.10	236.80	156.59	162.96	171.18	186.14
100	181.37	216.90	237.57	159.58	169.12	175.16	190.28
Average	176.52	213.55	235.55	156.53	163.61	170.77	186.09
		Varieties (V)		NaCl levels (N)		VxN	
SEm±		0.084		0.059		0.145	
CD at 5%		0.241		0.17		0.417	

Effect of salinity on Catalase activity

Catalase significantly increased in rice seedlings exposed to increasing salt stress. Under normal condition maximum catalase activity was found in table 3 Usar dhan-3 (38.44). At 0 (control) mM NaCl concentration catalase activity range was extend under normal condition i.e. 35.50-36.62 unit g^{-1} fresh wt min^{-1} whereas at higher salinity level (100 mM) it was ranged 38.92-40.15 unit g^{-1} fresh wt min^{-1}. The catalase activity was minimal in control and significantly increased in rice seedlings when exposed to various concentration of salinity.

Rice genotype were tested under laboratory condition to assess the effect of NaCl on catalase enzyme activity during seed germination. Catalase activity was increased with the increment of NaCl concentration in growing medium. At 10th day, maximum catalase activity (38.72 g^{-1}) was recorded in (100mM, NaCl) followed by 37.17 $g^{-1}$and 35.32 g^{-1} control (0 mM, NaCl) and 50 mM NaCl concentration respectively in all genotypes. Genotypic variability was also noticed among genotypes. Maximum average mean catalase activity was obtained in Usar Dhan-3 (38.44) followed by CSR-43 (37.89) and CSR-36 (37.26) and minimum catalase activity was noticed in Swarna Sub-1 (35.49 g^{-1}) followed by IR-28(36.02 g^{-1}) and Pusa-44 (37.33 mg g^{-1}).

Table 3: Effect of NaCl on catalase activity (g^{-1} fresh wt min^{-1})

2nd Day

NaCl level	Varieties						Average
	V1	V2	V3	V4	V5	V6	
0	33.04	33.40	33.69	30.83	31.20	31.52	32.28
50	34.78	35.16	35.46	32.46	32.84	33.18	33.98
100	36.23	36.62	36.94	33.81	34.21	34.56	35.40
Average	34.68	35.06	35.36	32.37	32.75	33.09	33.88
		Varieties (V)		NaCl levels (N)		VxN	
SEm±		0.259		0.183		0.449	
CD at 5%		0.743		0.525		1.287	

4th Day

NaCl level	Varieties						Average
	V1	V2	V3	V4	V5	V6	
0	34.15	34.77	35.21	32.30	32.80	33.05	33.71
50	35.95	36.60	37.07	34.00	34.52	34.79	35.49
100	37.45	38.12	38.61	35.42	35.96	36.24	36.97
Average	35.85	36.49	36.96	33.91	34.43	34.69	35.39
		Varieties (V)		NaCl levels (N)		VxN	
SEm±		0.591		0.418		1.023	
CD at 5%		1.694		1.198		2.934	

6th Day

NaCl level	Varieties						Average
	V1	V2	V3	V4	V5	V6	
0	34.99	35.94	36.48	33.87	34.62	34.85	35.13
50	36.84	37.83	38.40	35.65	36.44	36.68	36.97
100	38.37	39.41	40.00	37.14	37.96	38.21	38.52
Average	36.73	37.73	38.29	35.56	36.34	36.58	36.87
		Varieties (V)		NaCl levels (N)		VxN	
SEm±		0.861		0.609		1.491	
CD at 5%		2.469		1.746		4.276	

8th Day

NaCl level	Varieties						Average
	V1	V2	V3	V4	V5	V6	
0	35.49	36.05	36.57	33.53	33.91	35.35	35.15
50	37.35	37.95	38.50	35.29	35.69	37.21	37.00
100	38.91	39.53	40.10	36.76	37.18	38.76	38.54
Average	37.25	37.84	38.39	35.19	35.59	37.11	36.90
		Varieties (V)		NaCl levels (N)		VxN	
SEm±		0.934		0.661		1.618	
CD at 5%		2.679		1.895		4.641	

10th Day

NaCl level	Varieties						Average
	V1	V2	V3	V4	V5	V6	
0	35.50	36.10	36.62	33.81	34.32	35.56	35.32
50	37.36	38.00	38.54	35.59	36.12	37.43	37.17
100	38.92	39.58	40.15	37.07	37.63	38.99	38.72
Average	37.26	37.89	38.44	35.49	36.02	37.33	37.07
		Varieties (V)		NaCl levels (N)		VxN	
SEm±		0.839		0.593		1.453	
CD at 5%		2.406		1.701		4.167	

Effect of salinity on α-amylase activity

α-amylase activity in embryo decreased progressively with increasing level of salinity in all the genotypes (Table 4). Salinity induced greater inhibition of α-amylase in Usar Dhan-3 than in CSR-36.Whereas, under salinity condition it was significantly increase at increase in NaCl levels. The enzyme activity was average ranged from 7.03-8.38 under different salinity level. Our findings showed coherence with Roy and Srivastava, (1999) [15] 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.

Maximum means value of α-amylase activity was obtained in 0 NaCl (7.94) followed by 50 (7.62) and 100 (7.18) mM NaCl concentration respectively at 10th day of germination. Maximum mean value of α-amylase activity was obtained in Usar Dhan-3 (8.38 g^{-1}) followed by CSR-43 (7.93 g^{-1}) and CSR-36 (7.86 g^{-1}) in control (0, NaCl) and lower concentration α-amylase activity was noticed in Swarna Sub-1 (7.03 g^{-1}) followed by IR-28(7.12 g^{-1}) and Pusa-44 (7.27 g^{-1}).

Table 4: Effect of NaCl on α-amylase activity (enzyme unit g^{-1} fresh wt.)

2nd Day

NaCl level	Varieties						Average
	V1	V2	V3	V4	V5	V6	
0	8.12	8.18	8.64	7.24	7.31	7.42	7.82
50	7.80	7.85	8.29	6.95	7.02	7.12	7.51
100	7.41	7.46	7.88	6.60	6.67	6.77	7.13
Average	7.77	7.83	8.27	6.93	7.00	7.10	7.48
		Varieties (V)		NaCl levels (N)		VxN	
SEm±		0.178		0.126		0.308	
CD at 5%		0.51		0.361		0.884	

4th Day

NaCl level	Varieties						Average
	V1	V2	V3	V4	V5	V6	
0	8.14	8.21	8.69	7.28	7.36	7.49	7.86
50	7.81	7.88	8.34	6.99	7.07	7.19	7.55
100	7.42	7.49	7.93	6.64	6.71	6.83	7.17
Average	7.79	7.86	8.32	6.97	7.05	7.17	7.53
		Varieties (V)		NaCl levels (N)		VxN	
SEm±		0.196		0.138		0.339	
CD at 5%		0.561		0.397		0.972	

6th Day

NaCl level	Varieties						Average
	V1	V2	V3	V4	V5	V6	
0	8.15	8.22	8.70	7.29	7.38	7.52	7.88
50	7.82	7.89	8.35	7.00	7.08	7.22	7.56
100	7.43	7.50	7.93	6.65	6.73	6.86	7.18
Average	7.80	7.87	8.33	6.98	7.07	7.20	7.54
		Varieties (V)		NaCl levels (N)		VxN	
SEm±		0.16		0.113		0.278	
CD at 5%		0.46		0.325		0.797	

8th Day

NaCl level	Varieties						Average
	V1	V2	V3	V4	V5	V6	
0	8.17	8.25	8.73	7.32	7.41	7.55	7.91
50	7.84	7.92	8.38	7.03	7.11	7.25	7.59
100	7.45	7.52	7.96	6.68	6.76	6.89	7.21
Average	7.82	7.90	8.36	7.01	7.09	7.23	7.57
		Varieties (V)		NaCl levels (N)		VxN	
SEm±		0.189		0.134		0.328	
CD at 5%		0.543		0.384		0.941	

10th Day

NaCl level	Varieties						Average
	V1	V2	V3	V4	V5	V6	
0	8.21	8.28	8.75	7.34	7.44	7.59	7.94
50	7.88	7.95	8.40	7.05	7.14	7.29	7.62
100	7.49	7.55	7.98	6.69	6.79	6.92	7.24
Average	7.86	7.93	8.38	7.03	7.12	7.27	7.60
		Varieties (V)		NaCl levels (N)		VxN	
SEm±		0.167		0.118		0.29	
CD at 5%		0.48		0.339		0.831	

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Conclusion
Total carbohydrate content in embryo decreased progressively with increase in salinity level as compared to control or 0 mM NaCl. In general, minimum carbohydrate content in all the rice varieties in comparison to control. Tolerant genotype Usar dhan-3 possesed higher sugar content than the susceptible genotype Pusa-36.

Salinity resulted in the accumulation of proline content which improves progressively with increase in salinity level from 0 mM to 100 mM. Although genotype Usar dhan-3 possesed higher proline content under all the salinity level. Stress developed by NaCl showed more harmful effect on α-amylase activity. The activity of α-amylase in the embryo of both the genotypes decreased significantly with increasing level of salinity. Whereas the activity of catalase enzyme increased with increase in salinity level in all the rice varieties either tolerant or susceptible one.

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