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Effect of glycine betaine on germination percentage (%) and seed vigour index of rice (*Oryza sativa* L.) under saline condition

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Abstract
The present investigation was conducted during Kharif 2017-18 in laboratory, at experimental study department of Crop Physiology, Acharya Narendra Dev University of Agriculture and technology, Kumarganj, Ayodhya (U.P.). Soil salinity has become a severe threat to ensure food security , salinity-imposed limitations on plant growth are manifested through osmotic and ionic imbalances. At the present, salinity is considered as a serious constraint in increasing the rice production .Seed priming stimulates many of the metabolic processes involved in early phases of germination, resulting in an improved seed performance and provides faster and synchronized germination and more vigorous seedlings. The concentration of Glycine betaine is 50,100 & 150 ppm which influences the germination attributes under salinity. Experiment was laid out in complete randomized design (CRD) with three replications on two genotypes of rice crop *i.e.* Usar dhan-3 and Pusa-44 with different salinity level i.e. (50,100 & 150 mM NaCl) under anaerobic condition. Germination and seed vigour both were delayed and decreased with increasing level of salinity. The adverse effect of salt is more pronounced in susceptible variety (Pusa-44) than the tolerant (Usar dhan-3). It is evident with the result that seed priming with glycine betaine improved germination percentage significantly over control in both saline as well as non-saline conditions.

Keywords: Rice, germination percentage, seed vigour index, salinity, glycine betaine

Introduction
Rice is the most important cereal food crop of India.Rice (*Oryza sativa* L., 2n=24), belongs to the family Poaceae (Graminae). 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. It occupies about 23.3% of gross cropped area of the country and plays vital role in the national food grain supply. Rice is rich in nutrients and contains a number of vitamins and minerals. It is an excellent source of complex carbohydrates the best source of energy. It contains a reasonable amount of protein 6-10%, carbohydrate 70-80%, mineral 1.2-2.0% and vitamins (Riboflavin, Thiamine, Niacin and vitamin E).

Salinity is a common environmental stress seriously affecting crop growth, food production and crop yield in many regions, particularly in arid and semi-arid regions. It is estimated that over 800 million hectares of land in the world are affected by both salinity and sodicity globally (Munns, 2005) [8]. Salt contaminated soils (ECe> 4 dS m^{-1} or 40 mM NaCl or osmotic potential < 0.117 MPa) are defined as saline land, which directly affects plant growth and development in vegetative growth prior to reproductive stage, especially crop species. Some of crop species are susceptible to salt stress (ECe 1.0-1.8 dS m^{-1}), which decline crop growth and productivity about 6-19% *i.e.* rice, corn, bean, eggplant, onion, potato, pepper, sugarcane and cabbage. In general, biochemical, physiological, anatomical and morphological characteristics of plants directly affected by soil salinity. The salt stress affected proline and sugars synthesis an accelerated the rate of biosynthesis an higher concentrations of chlorophyll b than chlorophyll a during vegetative growth is observed in many crop plants. More than 20% of all irrigated land on earth is affected by salinization. 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) [2] and by reducing cellular damage and increasing antioxidant defense systems. Glycine betaine (GB),

a quaternary ammonium compound, is a very effective compatible solute (Rathinasabapathi, 2000; Chen and Murata, 2002) [20, 4] and is found in a wide range of foods (de Zwart *et al*., 2003) [5].

Seed priming has shown beneficial effects on seed germination and seedling development in many plant species. Harris (1996) [6] demonstrated increased germination rate and better establishment in tropical crops such as rice, maize, chickpea and sorghum by water priming. Priming is one of the most important physiological methods which improves the seed performance and provides faster and synchronized germination. The primed seeds give earlier, more uniform and sometime greater germination and seedling establishment and growth (Bradford, 1986) [3].

Material Methods

The present investigation was conducted in laboratory, at experimental study 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). Experiment consists of three NaCl concentrations (0, 50, 100 mM) and three glycine betaine concentration i.e. 50.100 and 150 ppm. Two varieties; one tolerant (Usar Dhan 3) and one susceptible (Pusa 44) used in experiment and sixteen treatments *viz*; Control (0 mM NaCl + 0 ppm GB(distilled water), GB1 (50ppm), GB2 (100ppm), GB3 (150ppm), S1 (50mM NaCl), S2 (100mMNaCl), S3 (150mMNaCl), Gb1+S1 (50ppm+50mMNaCl), Gb1+S2 (50ppm+100mM NaCl), Gb1+S3 (50ppm+150mM NaCl), Gb2+S1 (10ppm +50mM NaCl), Gb2+S2 (100ppm+100mM NaCl), Gb2+S3 (100ppm+150mM NaCl), Gb3+S1 (150ppm+50mM NaCl), Gb3+S2 (150ppm+100mM NaCl), GB3+S3 (150ppm + 150mM NaCl).Seed germination was recorded as rate as well as percentage during successive period of germination. It was recorded alternately up to 10 days. The germination percentage was calculated using the following formula:

Germination percent =Number of seed germinated/ Total number of seeds×100

The vigour index of seedlings was calculated by adopting the method suggested by Abdul-Baki and Anderson (1973) [1] and expressed in whole number.

Seed Vigour index = Germination (%) × [Shoot length + Root length]

Result and Discussion

Germination is an important stage of seedling establishment and therefore it plays a key role in crop production. It is a complex phenomenon involving many physiological and biochemical changes that lead to the activation of embryo. Salinity induces numerous disorders in seeds during germination. Firstly, it reduces the imbibitions of water because of lower osmotic potential of the medium (Munns & Tester, 2008) [7] and secondly it causes mineral imbalances and toxicity (Rajendran *et al*., 2009) [9]. Salinity delayed as well as reduced seed germination in both the genotypes. The effect of salinity was more pronounced in susceptible variety (Pusa-44) than the tolerant (Usar Dhan-3). Priming with glycine betaine improved germination percent significantly over control. The increased germination percentage was observed at day 2 upto 150 ppm concentration of GB (glycine betaine). The interaction of GB to salinity and genotypes was better in Pusa-44 than in Usar Dhan-3. However, at day 6 Usar dhan-3 attain 100% germination in control whereas at day 10 both the varieties attain 100% germination. Maximum germination percent was noticed in Usar dhan-3 i.e.100% at 150 ppm concentration of GB at day 6 followed by Pusa-44 87.76 % at 150 ppm concentration. Minimum effect of GB on germination per cent on both the varieties was observed at 50 ppm concentration i.e. 32.75%, 42.58%, 63.86%, 87.52% and 93.13% at 2^{nd},4^{th},6^{th},8^{th} and 10^{th} day, respectively. It indicates that hormone concentration possibly became super-optimal at this point.

Various level of salinity significantly reduced the seed vigour of all rice genotypes. Seed treatments with various level of glycine betaine had significant beneficial effect on seedling vigour of both rice genotypes. Maximum increase in seedling vigour was obtained at 150 ppm GB concentration in non saline condition, further seed vigour was decrease with respective increasing concentration of salt. Maximum seed vigour index was found in 150 ppm concentration in Usar dhan-3 i.e.318.51 followed by Pusa-44 i.e. 201.17.Minimum value of seed vigour index was noticed at the lowest concentration on GB i.e. 50 ppm which is shown by mean value of presented in table 2 at day 2,4,6,8 and 10 the was 55.31, 119.80, 401.50, 712.67 and 910.78, respectively. Moreover all concentration of GB significantly improved seedling vigour in comparison to non treated seed.

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Table 1: Effect of different concentration of glycine betaine on germination percentage in seeds of rice genotypes grown under different NaCl level at 2^{nd} day

	GB0	GB0		GB1	GB1		GB2	GB2		GB3	GB3		
	V1	**V2**	**Av**	**V1**	**V2**	**Av**	**V1**	**V2**	**Av**	**V1**	**V2**	**Av**	**Overall Mean**
S0	40.00	30.00	35.00	45.00	35.00	40.00	50.00	40.00	45.00	55.00	45.00	50.00	42.50
S1	0.00	0.00	0.00	40.00	30.00	35.00	44.00	35.00	39.50	48.00	40.00	44.00	29.63
S2	0.00	0.00	0.00	35.00	25.00	30.00	38.00	30.00	34.00	41.00	35.00	38.00	25.50
S3	0.00	0.00	0.00	32.00	20.00	26.00	35.00	26.00	30.50	38.00	28.00	33.00	22.38
	10.00	7.50	8.75	38.00	27.50	32.75	41.75	32.75	37.25	45.50	37.00	41.25	30.00
			Variety	S	GB	VxS	VxGB	SxGB	VxSxGB				
		SEM	0.247	0.349	0.349	0.494	0.494	0.698	0.988				
		CD	0.698	0.987	0.987	1.395	1.395	1.973	2.790				

4th day

	GB0	GB0		GB1	GB1		GB2	GB2		GB3	GB3		
	V1	V2	Av	V1	V2	Av	V1	V2	Av	V1	V2	Av	Overall mean
S0	52.00	39.00	45.50	58.50	45.50	52.00	65.00	52.00	58.50	71.50	58.50	65.00	55.25
S1	30.00	25.00	27.50	52.00	39.00	45.50	57.20	45.50	51.35	62.40	52.00	57.20	45.39
S2	25.00	20.00	22.50	45.50	32.50	39.00	49.40	39.00	44.20	53.30	45.50	49.40	38.78
S3	20.00	15.00	17.50	41.60	26.00	33.80	45.50	33.80	39.65	49.40	36.40	42.90	33.46
	31.75	24.75	28.25	49.40	35.75	42.58	54.28	42.58	48.43	59.15	48.10	53.63	43.22
		Variety	S	GB	VxS	VxGB	SxGB	VxSxGB					
	SEM	0.325	0.460	0.460	0.650	0.650	0.919	1.300					
	CD	0.918	1.299	1.299	1.836	1.836	2.597	3.673					

6th day

	GB0	GB0		GB1	GB1		GB2	GB2		GB3	GB3		
	V1	V2	Av	V1	V2	Av	V1	V2	Av	V1	V2	Av	Overall mean
S0	78.00	58.50	68.25	87.75	68.25	78.00	97.50	78.00	87.75	100.0	87.75	93.88	81.97
S1	45.00	37.50	41.25	78.00	58.50	68.25	85.80	68.25	77.03	93.60	78.00	85.80	68.08
S2	37.50	30.00	33.75	68.25	48.75	58.50	74.10	58.50	66.30	79.95	68.25	74.10	58.16
S3	30.00	22.50	26.25	62.40	39.00	50.70	68.25	50.70	59.48	74.10	54.60	64.35	50.19
	47.63	37.13	42.38	74.10	53.63	63.86	81.41	63.86	72.64	86.91	72.15	79.53	64.60
		Variety	S	GB	VxS	VxGB	SxGB	VxSxGB					
	SEM	0.333	0.472	0.472	0.667	0.667	0.943	1.334					
	CD	0.942	1.332	1.332	1.884	1.884	2.664	3.768					

8th day

	GB0	GB0		GB1	GB1		GB2	GB2		GB3	GB3		
	V1	V2	Av	V1	V2	Av	V1	V2	Av	V1	V2	Av	Overall mean
S0	88.00	95.00	91.50	95.00	95.00	95.00	100.0	95.00	97.50	100.0	95.00	97.50	95.38
S1	90.25	85.50	87.88	95.00	90.25	92.63	95.00	90.25	92.63	95.00	90.25	92.63	91.44
S2	85.50	80.75	83.13	85.50	80.75	83.13	85.50	80.75	83.13	87.40	82.65	85.03	83.60
S3	77.90	73.15	75.53	81.70	76.95	79.33	80.75	76.00	78.38	83.60	78.85	81.23	78.61
	85.41	83.60	84.51	89.30	85.74	87.52	90.31	85.50	87.91	91.50	86.69	89.09	87.26
		Variety	S	GB	VxS	VxGB	SxGB	VxSxGB					
	SEM	0.267	0.377	0.377	0.534	0.534	0.755	1.067					
	CD	0.754	1.066	1.066	1.508	1.508	2.132	3.016					

10th day

	GB0	GB0		GB1	GB1		GB2	GB2		GB3	GB3		
	V1	V2	Av	V1	V2	Av	V1	V2	Av	V1	V2	Av	Overall mean
S0	6.69	5.51	6.10	7.63	6.30	6.97	8.51	6.74	7.63	10.12	7.79	8.96	7.41
S1	6.01	4.81	5.41	6.65	4.54	5.60	6.94	5.72	6.33	8.21	6.81	7.51	6.21
S2	5.14	4.42	4.78	5.69	4.35	5.02	9.29	4.82	7.06	7.21	6.36	6.79	5.91
S3	4.67	3.39	4.03	5.29	3.83	4.56	5.74	3.49	4.62	6.19	5.39	5.79	4.75
	5.63	4.53	5.08	6.32	4.76	5.54	7.62	5.19	6.41	7.93	6.59	7.26	6.07
		Variety	S	GB	VxS	VxGB	SxGB	VxSxGB					
	SEM	0.059	0.084	0.084	0.119	0.119	0.168	0.237					
	CD	0.168	0.237	0.237	0.335	0.335	0.474	0.671					

Table 2: Effect of different concentration of glycine betaine on seed vigour index in seeds of rice genotypes grown under different NaCl level at 2nd day

	GB0	GB0		GB1	GB1		GB2	GB2		GB3	GB3		
	V1	V2	Av	V1	V2	Av	V1	V2	Av	V1	V2	Av	Overall mean
S0	85.20	45.60	65.40	76.95	44.45	60.70	96.50	52.40	74.45	110.00	60.75	85.38	71.48
S1	0.00	0.00	0.00	61.60	36.00	48.80	95.92	56.00	75.96	132.96	77.60	105.28	57.51
S2	0.00	0.00	0.00	84.00	49.00	66.50	72.20	42.90	57.55	103.73	61.60	82.67	51.68
S3	0.00	0.00	0.00	62.08	28.40	45.24	89.60	52.26	70.93	76.38	43.12	59.75	43.98
	21.30	11.40	16.35	71.16	39.46	55.31	88.56	50.89	69.72	105.77	60.77	83.27	56.16
		Variety	S	GB	VxS	VxGB	SxGB	VxSxGB					
	SEM	0.609	0.861	0.861	1.218	1.218	1.723	2.436					
	CD	1.721	2.433	2.433	3.441	3.441	4.867	6.883					

4th day

	GB0	GB0		GB1	GB1		GB2	GB2		GB3	GB3		
	V1	V2	Av	V1	V2	Av	V1	V2	Av	V1	V2	Av	Overall mean
S0	145.60	100.62	123.11	226.40	166.99	196.69	336.70	230.36	283.53	476.19	290.75	383.47	246.70
S1	53.70	36.50	45.10	171.60	101.40	136.50	243.67	169.72	206.69	335.09	226.20	280.64	167.23
S2	32.50	15.20	23.85	112.39	65.65	89.02	188.71	114.66	151.68	261.70	173.81	217.76	120.58
S3	18.60	3.90	11.25	74.46	39.52	56.99	140.60	81.46	111.03	201.06	113.93	157.50	84.19
	62.60	39.06	50.83	146.21	93.39	119.80	227.42	149.05	188.23	318.51	201.17	259.84	154.68
		Variety	S	GB	VxS	VxGB	SxGB	VxSxGB					
	SEM	1.906	2.695	2.695	3.812	3.812	5.391	7.624					
	CD	5.385	7.615	7.615	10.769	10.769	15.230	21.538					

6th day

	GB0	GB0		GB1	GB1		GB2	GB2		GB3	GB3		
	V1	V2	Av	V1	V2	Av	V1	V2	Av	V1	V2	Av	Overall mean
S0	535.08	322.34	428.71	766.94	429.98	598.46	923.33	553.80	738.56	1142.00	669.53	905.77	667.87
S1	237.15	137.25	187.20	602.94	297.77	450.35	713.00	451.13	582.07	991.22	565.50	778.36	499.49
S2	168.75	74.10	121.43	449.09	193.05	321.07	557.97	329.94	443.96	734.74	452.50	593.62	370.02
S3	90.00	42.53	66.26	351.31	120.90	236.11	439.53	241.33	340.43	610.58	319.41	465.00	276.95
	257.75	144.05	200.90	542.57	260.42	401.50	658.46	394.05	526.25	869.64	501.74	685.69	453.58
		Variety	S	GB	VxS	VxGB	SxGB	VxSxGB					
	SEM	5.718	8.087	8.087	11.437	11.437	16.174	22.874					
	CD	16.156	22.848	22.848	32.312	32.312	45.696	64.623					

8th day

	GB0	GB0		GB1	GB1		GB2	GB2		GB3	GB3		
	V1	V2	Av	V1	V2	Av	V1	V2	Av	V1	V2	Av	Overall mean
S0	937.20	862.60	899.90	1200.8	977.55	1089.1	1036.0	778.05	907.03	1126.0	841.70	983.85	969.99
S1	601.97	416.39	509.18	758.10	451.25	604.68	829.35	610.99	720.17	1000.35	784.27	892.31	681.58
S2	648.95	438.47	543.71	764.37	519.22	641.80	870.39	669.42	769.90	745.52	433.09	589.30	636.18
S3	514.92	305.04	409.98	616.84	413.22	515.03	693.64	550.24	621.94	708.93	334.32	521.63	517.14
	675.76	505.62	590.69	835.03	590.31	712.67	857.35	652.18	754.76	895.20	598.35	746.77	701.22
		Variety	S	GB	VxS	VxGB	SxGB	VxSxGB					
	SEM	6.457	9.131	9.131	12.913	12.913	18.262	25.827					
	CD	18.241	25.797	25.797	36.483	36.483	51.595	72.966					

10th day

	GB0	GB0		GB1	GB1		GB2	GB2		GB3	GB3		
	V1	V2	Av	V1	V2	Av	V1	V2	Av	V1	V2	Av	Overall mean
S0	1181.00	949.00	1065.00	1376.00	1074.00	1225.00	1452.00	1233.00	1342.50	1827.00	1398.00	1612.50	1311.25
S1	1036.45	729.00	882.73	1194.00	734.35	964.18	1292.00	952.85	1122.43	1519.00	1181.80	1350.40	1079.93
S2	831.60	641.75	736.68	952.20	624.75	788.48	1295.10	725.90	1010.50	1221.76	988.32	1105.04	910.17
S3	676.50	426.58	551.54	809.26	521.64	665.45	895.05	534.40	714.73	993.52	787.67	890.60	705.58
	931.39	686.58	808.99	1082.87	738.69	910.78	1233.54	861.54	1047.54	1390.32	1088.95	1239.63	1001.73
		Variety	S	GB	VxS	VxGB	SxGB	VxSxGB					
	SEM	11.225	15.874	15.874	22.449	22.449	31.748	44.898					
	CD	31.712	44.847	44.847	63.424	63.424	89.695	126.848					

Conclusion

The decrease in seed germination percentage and seed vigour of rice genotypes under higher salinity was due to reduced rate of water imbibitions and disturbed metabolic status of the germinating seeds as evident by proline accumulation, decreased enzyme activity and carbohydrate content and enhanced salt accumulation. Seed priming effectively modulated the adverse effect of salinity showing better growth. economical For rapid screening of salt tolerant rice genotypes seed germination percentage (%) and seed vigour index could be picked up as the probable indices under existing conditions. Although 150 ppm concentration of glycine betaine showed the best result but 100 ppm concentration was also at par and is more economical.

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