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Research Article

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Seed Culture of Rice Cultivars under Salt Stress

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ABSTRACT

Six selected cultivars of rice namely BPT-5204, MTU-7029, Narendra Usar Dhan-3, Rajendra Bhagwati, CSR-30 and Pusa Basmati-1 were screened for salinity tolerance on the basis of in vitro seed germination, callus formation and callus growth on MS basal medium supplemented with 2.0 mg l^{-1} 2,4-D + 1.0 mg l^{-1} KIN under different salt stress (0-2.5%) created by a salt mixture of NaCl, $CaCl_2$, Na_2SO_4 in 7:2:1 ratio. The frequency of seed germination and callus formation gradually decreased with increasing concentrations of salt in the medium. The in vitro callus growth also decreased with increasing concentrations of salt. The salt tolerance index (STI) of the rice cultivars was calculated on the basis of the in vitro observations. Cultivars CSR-30 and Narendra Usar Dhan-3 were found to be salt tolerant, cvs. MTU-7029 and BPT-5204 moderately salt tolerant and cvs. Rajendra Bhawati and Pusa Basmati-1 as salt susceptible respectively.

Keywords: *Rice, Seed culture, Callogenesis, Somaclonal variation, Salinity tolerance.*

INTRODUCTION

Rice is one of the most important food grains with overall production of 718.34 million tonnes from 163.46 million hectares with an average yield of 4395 kg/ha. World-wide India stands 1st in rice area and 2nd in rice-production after China with overall production of 105.24 million tonnes from 42.41 million hectares with the productivity of 2462 kg/ha. Its pericarp and embryo contain 70-80% starch, 7% protein, 1.5% oils. Compared to wheat and maize, it has the highest net protein utilization value. It also produces more calories and carbohydrates per hectare than any other cereal[1].

The productivity of several commercial crops is limited by major abiotic stresses including salinity, drought, water logging and heat. Damages caused by these stresses are responsible for enormous economic loss world-wide. Salinity is a major abiotic stress affecting crops adversely. It is caused by poor water management, high evaporation, heavy irrigation, previous exposure to seawater and regular use of chemical fertilizers.

Salinity is the biggest problem in rice growing areas of many countries[2]. Rice is rated as a salt sensitive crop[3, 4]. Salinity affects all stages of the growth and development of rice plant, but the response of the crop varies with the concentration and duration of exposure to salt and the growth stages. In most of the commonly cultivated rice cultivars, young seedlings are very sensitive to salinity[5, 6]. There are other reports where grain yield is more depressed by salt than the vegetative growth other than that of very young seedlings. Grain yield is a very complex character which comprises of many components and these yield components are related to final grain yield, which are also severely affected by salinity. Panicle length and number of spikelets per panicle are significantly affected by salinity[7-9].

Tissue culture techniques like anther culture, protoplast fusion and culture, leaf culture, root culture, immature embryo culture and mature seed culture are important in rice to create additional variation and to develop novel rice varieties[10, 11].

Somaclones provide a novel and valuable source of genetic variability, which can be exploited for crop improvement particularly, for the development of stress tolerant rice cultivars[12].

In vitro culture of rice seeds under salinity stress and study of seed germination, callus formation and callus growth under salinity stress can indicate the salinity tolerance of the genotype.

MATERIALS AND METHODS

Seeds of six rice cultivars namely BPT-5204, MTU-7029, Narendra Usar Dhan-3, Rajendra Bhagwati, CSR-30 and Pusa Basmati-1were tissue cultured under salt stress. Seeds were soaked in water overnight. Soaked seeds were dehusked and treated with 70% ethyl alcohol for 30 seconds and then washed with distilled water. Thereafter, dehusked seeds were treated with 0.1% mercuric chloride ($HgCl_2$) for 10-15 minutes, rinsed with sterile distilled water thrice and inoculated under laminar air flow.

Growth medium and culture condition

The sterilized dehusked seeds were cultured on MS[13] medium supplemented with 2.0 mg l^{-1} 2,4-D + 1.0 mg l^{-1} KIN under different concentrations of salt mixture (NaCl, $CaCl_2$, Na_2SO_4 in 7:2:1 ratio) ranging from 0-2.5%. The callus formed on medium without salt was also subcultured on the callusing medium with salt stress.

Statistical data analysis

The experiment was laid out in a factorial CRD design[14] with three replications. The data were subjected to analysis of variance and Duncan's Multiple Range Test (DMRT) for comparing population means. Mean values were compared by two-way ANOVA. The salinity tolerance index, represented as the ratio of the performance of a cultivar at a particular level of salinity to that of control, was calculated on the basis of *in vitro* germination, callus formation and callus growth based on its dry weights, following the method of Reddy and Baidyanath[15].

RESULTS AND DISCUSSION

Plant tissue culture is an important aspect of plant biotechnology and it has led to the development of many varieties of crop plant particularly rice, that are being grown in large areas of the world. One of the important mechanism by which these varieties were developed, is somaclonal variations, which are the variations among regenerated plants in tissue culture[16]. It has provided a novel method for producing variations and selecting the variations at the cell, tissue, organ or the plant level for the desired characters required ultimately for the plant improvement[17]. Somaclonal variations are more when the plants are regenerated through callus phase. Tissue culture technique thus can be used for developing stress tolerance, particularly salt tolerance in crop plants including rice [18-20].

Dehusked seeds of rice are good explants for callus formation and regeneration of plants[21-25]. Dehusked seeds of six selected cultivars of rice namely BPT-5204, MTU-7029, Narendra Usar Dhan-3, Rajendra Bhagwati, CSR-30 and Pusa Basmati-1 were germinated and developed callus on callusing medium MS + 2.0 mg l^{-1} 2,4-D + 1.0 mg l^{-1} KIN with different concentrations of salt mixture (NaCl, $CaCl_2$, Na_2SO_4 in 7:2:1 ratio) ranging from 0-2.5%, after 3-4 days of culture.

Seed germination under salt stress

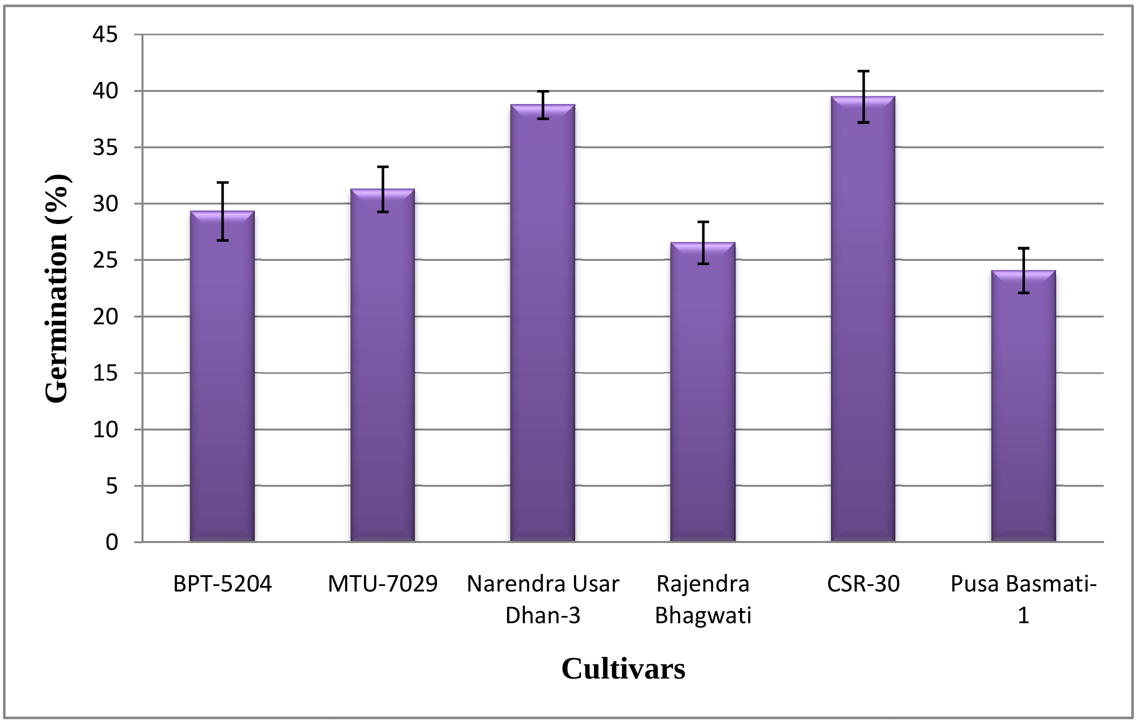
Seed germination was found at par in different cultivars in absence of any salt. Under salt stress, there was a decrease in seed germination with increase in concentration of salt and overall cv. CSR-30 showed the best germination followed by cvs. Narendra Usar Dhan-3, MTU-7029, BPT-5204, Rajendra Bhagwati and Pusa Basmati-1 respectively (Table-1 and Figure-1).

Table- 1: Germination (%) of cultured seeds of rice cultivars on callusing medium with different salt concentrations

Salt (%)	Cultivars									
	BPT-5204	MTU-7029	Narendra Usar Dhan-3	Rajendra Bhagwati	CSR-30	Pusa Basmati-1	Mean	CD	CV	SE(m)
0%	90.73±1.85	94.63±3.04	92.68±1.90	92.97±1.75	92.98±4.64	89.08±3.21	92.17	-	5.49	2.92
0.5%	53.80_b±3.50	55.18_b±2.89	81.44_a±1.91	49.02_b±2.41	81.86_a±1.46	41.12_c±2.24	60.40	7.77	7.15	2.49
1%	24.99_c±4.81	29.96_{bc}±2.53	36.52_{ab}±2.77	14.03_d±1.49	38.07_a±2.06	11.06_d±0.37	25.77	7.76	16.75	2.49
1.5%	6.50_b±0.80	7.97_b±0.21	16.29_a±0.66	3.33_c±0.33	18.51_a±1.85	3.33_c±0.66	9.32	2.87	17.16	0.92
2%	-	-	5.59_a±0.33	-	5.56_a±0.17	-	1.85	0.47	14.28	0.15
2.5%	-	-	-	-	-	-	-	-	-	-
Mean	29.33	31.29	38.75	26.55	39.49	24.09				
CD	7.99	6.24	3.80	5.78	7.12	6.19				
CV	15.15	11.09	5.45	12.09	10.03	13.58				
SE(m)	2.56	2.00	1.22	1.85	2.28	1.98				

Values followed by the same letter in row are not significantly different using Duncan's Multiple Range Test (DMRT) at 5%

Fig.1: Effect of cultivars on germination (%) of cultured seeds on callusing medium with different salt concentrations



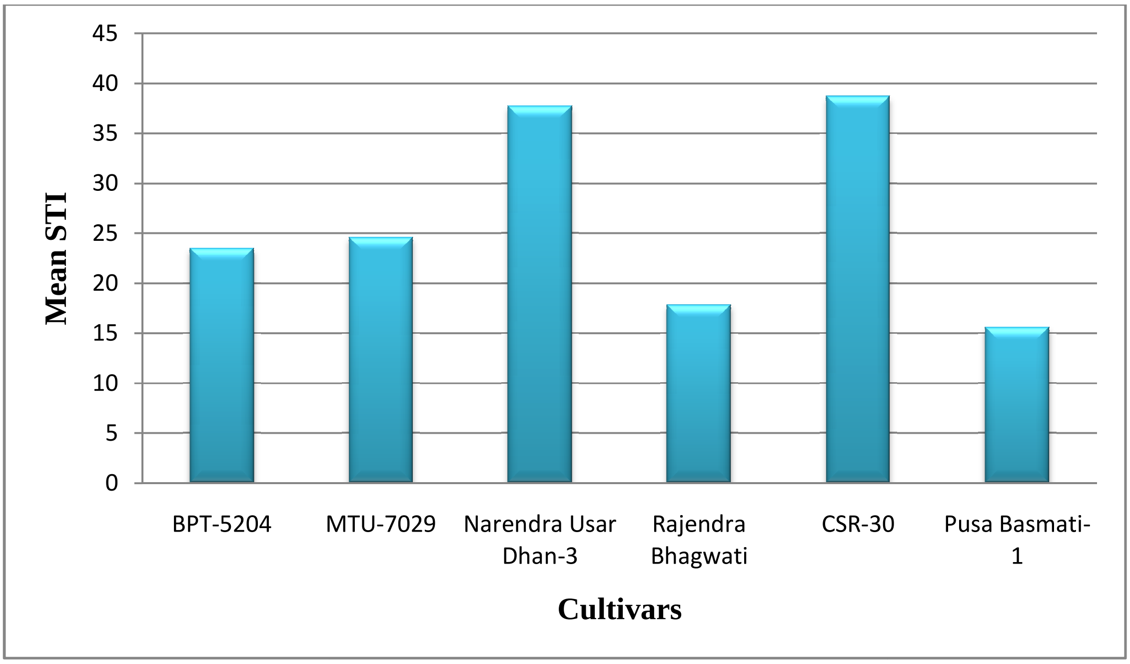
Salinity may affect seed germination in two ways, osmotically, by decreasing the ease with which seeds may take up water; and ionically, by facilitating the uptake of ions in excess amount to be toxic for the embryonic activity [26]. In the presence of high salt concentration in the medium, osmotic potential is negative enough to cause water to diffuse out of tissue. It has been reported that stress environment affects membrane selective efficiency in germinating seed[27], which ultimately results in excess absorption of toxic ion[28]. Bhumbla and Singh[29] have shown the inhibitory effect of salt stress on seed germination. Ali[30] observed in rice varieties of different groups, a concentration dependent decrease in seed germination under sodium chloride stress as found in present investigation. They also observed differences in salinity tolerance among cultivars based on germination frequency and considered it significant because decline in germination also leads to significant reduction in seedling length, early seedling vigour, speed of germination and dry matter production per plant[31]. Kazemi and Eskandari[32] observed delayed and decreased germination of rice seeds under salt stress.

Based on *in vitro* seed germination in the presence of salt stress, cultivar CSR-30 showed the highest mean salt tolerance index (STI) of 38.71% followed by cv. Narendra Usar Dhan-3 (37.71%) and can be considered as salt tolerant while Pusa Basmati-1 showed the least mean salt tolerance index (STI) of 15.57% followed by Rajendra Bhagwati (17.84%) and can be considered as salt susceptible. The other cvs. MTU-7029 and BPT-5204 with STI of 24.59% and 23.49% respectively, can be considered as moderately tolerant (Table-2 and Figure-2).

Table-2: Salinity tolerance index (STI) of rice cultivars based on germination frequency on callusing medium with different salt concentrations

Cultivar	Salt concentrations (%)	Germination frequency	STI	Mean STI
BPT-5204	0	90.73	-	23.49
	0.5	53.80	59.29	
	1.0	24.99	27.54	
	1.5	6.50	7.16	
	2.0	-	-	
MTU-7029	0	94.63	-	24.59
	0.5	55.18	58.31	
	1.0	29.96	31.66	
	1.5	7.97	8.42	
	2.0	-	-	
Narendra Usar Dhan-3	0	92.68	-	37.71
	0.5	81.44	87.87	
	1.0	36.52	39.40	
	1.5	16.29	17.57	
	2.0	5.59	6.03	
Rajendra Bhagwati	0	92.97	-	17.84
	0.5	49.02	52.72	
	1.0	14.03	15.09	
	1.5	3.33	3.58	
	2.0	-	-	
CSR-30	0	92.98	-	38.71
	0.5	81.86	88.04	
	1.0	38.07	40.94	
	1.5	18.51	19.90	
	2.0	5.56	5.98	
Pusa Basmati-1	0	89.08	-	15.57
	0.5	41.12	46.16	
	1.0	11.06	12.41	
	1.5	3.33	3.73	
	2.0	-	-	

Fig.2: Salinity tolerance index (STI) of rice cultivars based on germination frequency on callusing medium with different salt concentrations



Callus formation under salt stress

The identification and screening of useful cultivars for embryogenic callus formation and subsequent plant regeneration through *in vitro* system is a vital step in rice genetic improvement programmes including genetic transformation and somaclonal variation[20, 25]. Immature embryos and mature seeds are commonly used for callus induction, because of their availability throughout the year regardless of growing season.

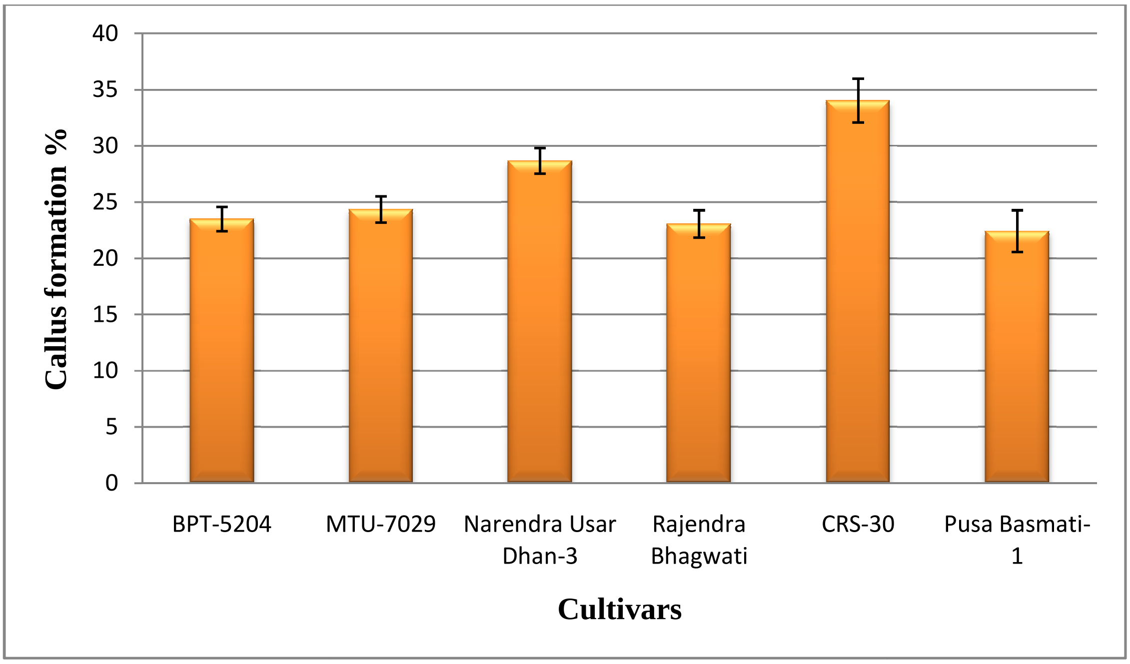
Dehusked cultured seeds developed callus after 4-5 days of culture following germination. There was gradual decrease in callusing frequency of cultured seeds with increasing salt concentrations in the medium. Only seeds of cvs. CSR-30 and Narendra Usar Dhan-3 formed small callus in low frequency in presence of 2% salt. There was no callus formation from cultured seeds on medium with 2.5% salt. Seeds of cultivars that were found to be salt tolerant on the basis of *in vitro* germination produced more callus compared to those that were found to be susceptible. The best frequency of callus formation in presence of salt stress was found in the cultured seeds of cv. CSR-30 followed by cvs. Narendra Usar Dhan-3, MTU-7029, BPT-5204, Rajendra Bhagwati and Pusa Basmati-1 respectively. (Table-3 and Figure-3).

Table-3: Callus formation (%) of cultured seeds of rice cultivars on callusing medium with different salt concentrations

Salt mixtures	Cultivars									
	BPT-5204	MTU-7029	Narendra Usar Dhan-3	Rajendra Bhagwati	CSR-30	Pusa Basmati-1	Mean	CD	CV	SE(m)
0%	68.51_c±1.85	70.85_c±2.13	85.37_{ab}±2.04	80.69_b±1.75	96.48±1.75	89.07_a±0.19	81.82	5.45	3.70	1.75
0.5%	49.78_a±1.51	47.03_{ab}±1.61	49.89_a±1.61	41.80_b±1.61	63.64±1.62	33.81_c±3.02	47.65	5.94	6.93	1.90
1%	16.99_{bc}±1.08	22.42_a±1.03	21.87_{ab}±0.82	12.47_{cd}±1.66	25.17_a±4.06	9.13_d±0.86	18.00	6.09	18.80	1.95
1.5%	5.68_b±0.29	5.78_b±0.23	9.25_a±0.45	3.36_c±0.74	13.29±0.60	2.50_c±0.38	6.64	1.51	12.64	0.48
2%	-	-	5.59_a±0.33	-	5.56_a	-	1.85	0.47	14.28	0.15
2.5%	-	-	-	-	-	-				
Mean	23.49	24.34	28.66	23.05	34.02	22.41				
CD	3.36	3.66	3.54	3.81	6.04	5.81				
CV	7.96	8.36	6.88	9.20	9.88	14.45				
SE(m)	1.08	1.17	1.13	1.22	1.94	1.86				

Values followed by the same letter in row are not significantly different using Duncan's Multiple Range Test (DMRT) at 5%

Fig. 3: Effect of cultivars on callus formation (%) of cultured seeds on callusing medium with different salt concentrations



The difference in callusing frequency of cultured seeds on medium with salt concentrations can be an indicator for *in vitro* screening of cultivars for salt tolerance[33, 24]. The ability of cultured seeds of some cultivars to produce callus in higher frequencies under salt stress is mainly dependent on their genotype[34]. Callus is comprised mainly of masses of undifferentiated cells and is good starting material for *in vitro* manipulation. Moreover, calli induced from scutellar tissue of mature seeds are an excellent source of cells for *in vitro* regeneration [35-37]. A significant difference in callusing frequency of cultured seeds was found among genotypes of rice[38-41].

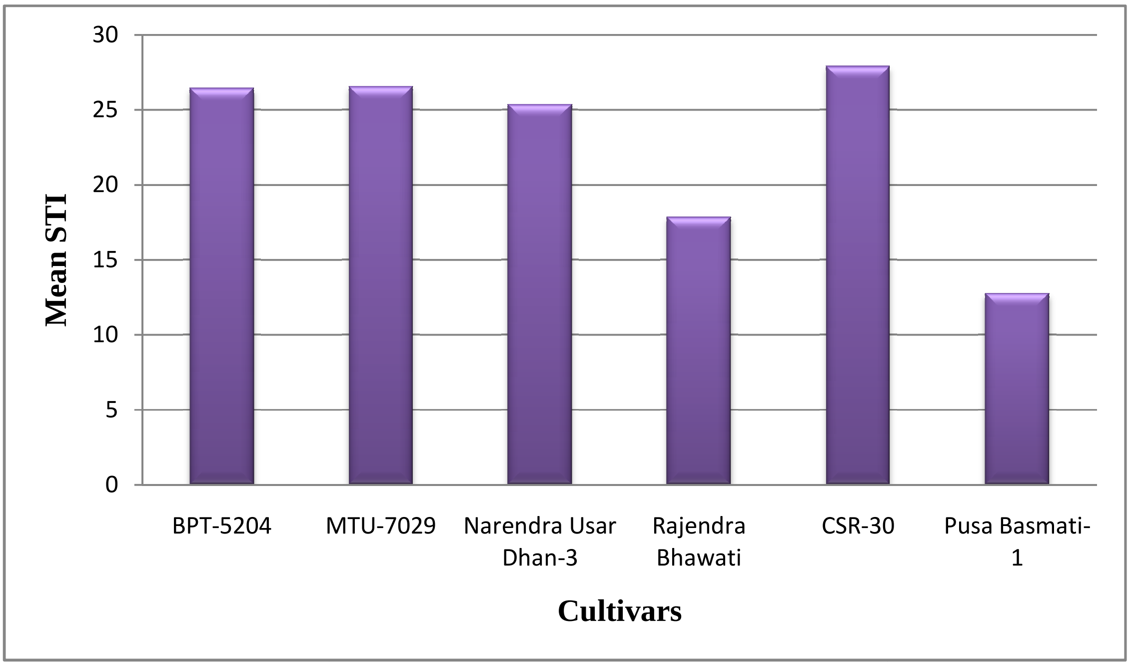
Considering the frequency of *in vitro* callus formation from the cultured seeds under salt stress, cultivar CSR-30 showed the highest mean salt tolerance index (STI) of 27.89% and can be considered as salt tolerant while Pusa Basmati-1 showed the least mean salt tolerance index (STI) of 12.75% followed by Rajendra Bhagwati (17.85%) and can be considered as salt susceptible. The other cvs. MTU-7029, BPT-5204 and Narendra Usar Dhan-3 with STI of 26.54%, 26.43% and 25.35% respectively, can be considered as moderately tolerant (Table-4 and Figure-4).

Table-4: Salinity tolerance index (STI) rice cultivars based on callus formation frequency on callusing medium with different salt concentrations

Cultivar	Salt concentrations (%)	Callus formation frequency	STI	Mean STI
BPT-5204	0	68.51	-	26.43
	0.5	49.78	72.66	
	1.0	16.99	24.79	
	1.5	5.68	8.29	
	2.0	-	-	
MTU-7029	0	70.85	-	26.54
	0.5	47.03	66.38	
	1.0	22.42	31.64	
	1.5	5.78	8.15	
	2.0	-	-	
Narendra Usar Dhan-3	0	85.37	-	25.35
	0.5	49.89	58.44	
	1.0	21.87	25.61	
	1.5	9.25	10.83	
	2.0	5.59	6.54	

Rajendra Bhagwati	0	80.69	-	17.85
	0.5	41.80	51.80	
	1.0	12.47	15.45	
	1.5	3.36	4.16	
	2.0	-	-	
CSR-30	0	96.48	65.96	27.89
	0.5	63.64	26.08	
	1.0	25.17	13.77	
	1.5	13.29	5.76	
	2.0	5.56	-	
Pusa Basmati-1	0	89.07	-	12.75
	0.5	33.81	37.95	
	1.0	9.13	10.25	
	1.5	2.50	2.80	
	2.0	-	-	

Fig. 4: Salinity tolerance index (STI) of rice cultivars based on callus formation frequency on callusing medium with different salt concentrations



Callus growth under salt stress

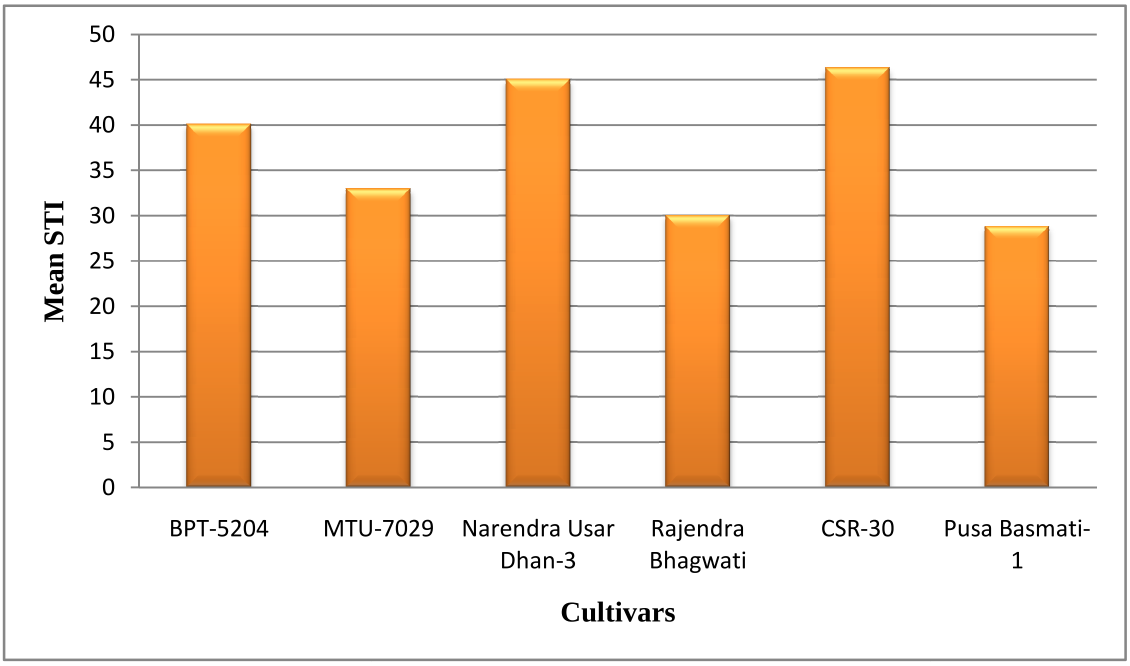
The developed callus of all the six cultivars of rice on callusing medium without salt stress, were subcultured on the same callusing medium with different salt concentrations to increase the frequency of induction of salt tolerance. The continued growth of callus even in the presence of high concentrations of salt (upto1.5-2%), indicated the induction of salt tolerance. The callus growth decreased with increased salt stress. This showed that the salt had an inhibitory effect on the growth of callus. The increasing salt concentrations created an osmotic or ionic shock resulting in adverse effect on growth of plant cells and tissues. This was also supported by[42, 43]. Shankhdhar [44] also reported the dramatic decrease in the fresh weight of the callus with increasing salt concentrations. The reduction might be as a result of reduced water availability in the culture medium due to increased salt concentration.

Based on callus growth in the presence of salt stress as indicated by their dry weight, the salt tolerance index (STI) of the six selected rice cultivars was estimated. Cultivar CSR-30 showed the highest mean salt tolerance index (STI) of 46.33% followed by cv. Narendra Usar Dhan-3 (45.06%) and can be considered as salt tolerant while Pusa Basmati-1 showed the least mean salt tolerance index (STI) of 28.80% followed by Rajendra Bhagwati (30.03%) and can be considered as salt susceptible. The other cvs. BPT-5204, MTU-7029 with STI of 40.10%, 32.97% respectively, can be considered as moderately tolerant (Table-5 and Figure- 5).

Table-5: Callus growth of rice cultivars on callusing medium with different salt concentrations and their salinity tolerance index (STI) based on dry weight of callus

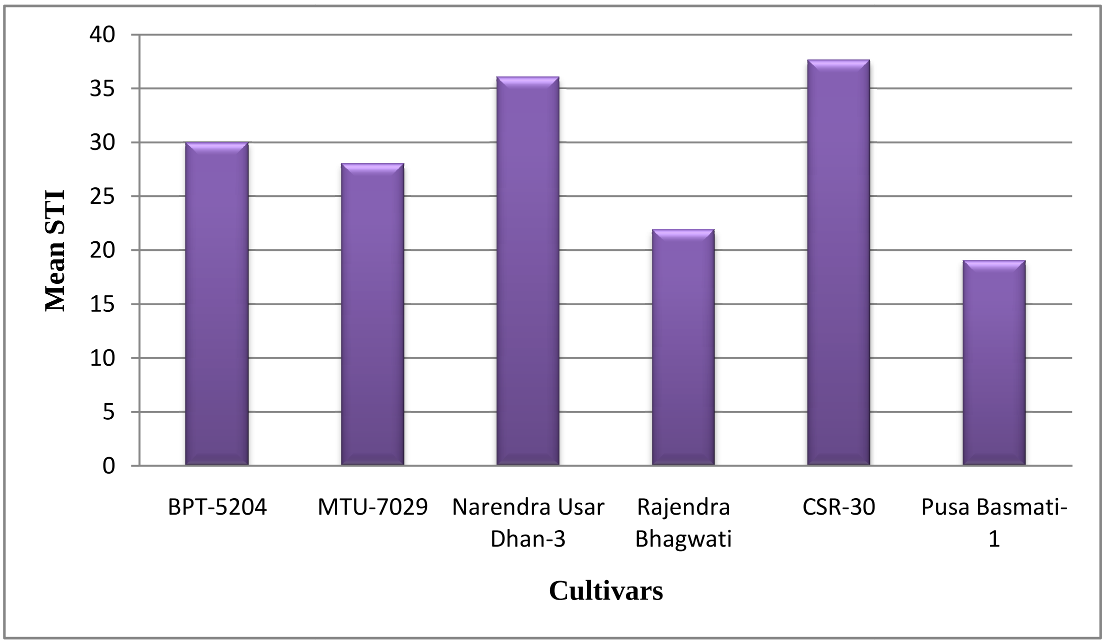
Cultivar	Salt concentrations (%)	Callus growth			
		Final callus weight (mg)		Salinity tolerance index (STI)	
		Fresh wt	Dry wt	STI	**Mean STI**
BPT-5204	0	1296	129		40.10
	0.5	1012	101	78.29	
	1.0	576	60	46.48	
	1.5	356	46	35.65	
	2.0	-	-	-	
MTU-7029	0	1388	166		32.97
	0.5	1086	143	86.14	
	1.0	484	56	33.73	
	1.5	189	20	12.04	
	2.0	-	-		
Narendra Usar Dhan-3	0	1610	162		45.06
	0.5	1308	140	86.42	
	1.0	808	99	61.11	
	1.5	305	30	22.84	
	2.0	165	16	9.87	
Rajendra Bhagwati	0	1363	149		30.03
	0.5	1027	118	79.19	
	1.0	421	42	28.18	
	1.5	182	19	12.75	
	2.0	-	-	-	
CSR-30	0	1731	225		46.33
	0.5	1513	181	80.44	
	1.0	1077	140	62.66	
	1.5	663	72	32.00	
	2.0	201	24	11.42	
Pusa Basmati-1	0	1187	118		28.80
	0.5	795	83	70.33	
	1.0	351	36	30.50	
	1.5	157	17	14.40	
	2.0	-	-	-	

Fig. 5: Salinity tolerance index (STI) of rice cultivars based on callus growth on callusing medium with different salt concentrations



Finally the six selected rice cultivars were ranked for their salt tolerance level on their mean salinity tolerance index (STI) based on *in vitro* seed germination, callus formation and callus growth (dry weight) under salt stress. Cultivars CSR-30 (37.64%) and Narendra Usar Dhan-3 (36.04%) were found to be the most salt tolerant, cvs. BPT-5204 (30.00%) and MTU-7029 (28.03%) to be moderately tolerant and cvs. Rajendra Bhagwati (21.90%) and Pusa Basmati-1 (19.04%) to be salt sensitive respectively (Figure-6). The formation of callus and their continued growth at higher levels of salt stress indicated the induction and formation of salt tolerant cells and calluses. Cultivars CSR-30 and Narendra Usar Dhan-3 are known to show high to moderate tolerance to salt stress[45-47]. Cv. BPT-5204 is moderately salt tolerant[24] while Pusa Basmati-1 is salt sensitive cultivar[47]. The status of selected rice cultivars with respect to salt tolerance was substantiated by the tissue culture screening through seed culture under salt stress.

Fig. 6: Mean salinity tolerance index (STI) of rice cultivars based on *in vitro* seed germination, callus formation and callus growth (dry weight) under salt stress



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