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Effect of chloride and sulfate salinity on *in vitro* regeneration of rice

Md. Riyadh Arefin*[1], Mohammad Mehfuz Hasan Saikat[2], Mr. Umakanta Sarker[2], M. Abdul Karim[3], Md. Sadiqur Rahman[4]

[1]*Botany Division, Bangladesh Tea Research Institute, Srimangal, Moulvibazar, Bangladesh*

[2]*Department of Genetics and Plant Breeding, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur, Bangladesh*

[3]*Department of Agronomy, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur, Bangladesh*

[4]*Seed Technology Discipline, Bangladesh Agricultural Research Institute (BARI), Akbarpur, Moulvibazar, Bangladesh*

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Abstract

Rice is the seed of the monocot plants *Oryza sativa* (Asian rice) or *Oryza glaberrima* (African rice). As a cereal grain, a great source of protein; it is the most widely consumed staple food for a large part of the world's human population, especially in Asia. Among the various factors limiting rice yield, salinity is one of the oldest and most serious environmental problems in the world. Rice yield can be reduced by up to 50% when grown under moderate (6dS/m) salinity level. Salinity in aquatic ecosystem is determined by the total amount of dissolved salts present in it. Four types of cations prevail in saline water such as Sodium (Na^+), Potassium (K^+), Calcium (Ca^{++}) and Magnesium (Mg^{++}) and 3 anions such as Chloride (Cl^-), Carbonate (CO_3^-) or Bi-Carbonate (HCO_3^-) and Sulphate (SO_4^{2-}). Even though Cl is an essential element for plant growth, but higher concentration may restrain plant growth or cause toxicity to some plants. Higher concentration of Chloride ions also causes leaf tip burn, disruption of membrane function, obstruction in internal solute balance that hampers nutrient uptake. On the other hand, Sulphur is an important factor in plant feeding but high sulphate concentrations may affect plant development and crop yield. In NaCl and Na_2SO_4 salts, Sodium (Na^+) is common but differs only by two anions: Chloride (Cl^-) and Sulphate (SO_4^{2-}). The present investigation was conducted through *in vitro* regeneration protocol of ten rice genotypes to observe the effect of these two salts and to identify which salt causes more inhibitor.

* **Corresponding Author:** Md. Riyadh Arefin ✉ riyadh.btri@gmail.com

Introduction

Rice is the seed of the monocot plants *Oryza sativa* (Asian rice) or *Oryza glaberrima* (African rice). As a cereal grain, a great source of protein; it is the most widely consumed staple food for a large part of the world's human population, especially in Asia (Datta *et al.,* 2003).

Among the various factors limiting rice yield, salinity is one of the oldest and most serious environmental problems in the world (Islam *et al*., 2007). Rice yield can be reduced by upto 50% when grown under moderate (6dS/m) salinity level (Zhang *et al.,* 2002). Salinity in aquatic ecosystem is determined by the total amount of dissolved salts present in it. Four types of cations prevail in saline water such as Sodium (Na^+), Potassium (K^+), Calcium (Ca^{++}) and Magnesium (Mg^{++}) and 3 anions such as Chloride (Cl^-), Carbonate (CO_3^-) or Bi-Carbonate (HCO_3^-) and Sulphate (SO_4^{2-}) (Baten *et al.,* 2015). Even though Cl is an essential element for plant growth, but higher concentration may restrain plant growth or cause toxicity to some plants. Higher concentration of Chloride ions also causes leaf tip burn, disruption of membrane function, obstruction in internal solute balance that hampers nutrient uptake (Lantzke, 2004). On the other hand, Sulphur is an important factor in plant feeding but high sulphate concentrations may affect plant development and crop yield (Kowalska, 2005).

In NaCl and Na_2SO_4 salts, Sodium (Na^+) is common but differs only by two anions: Chloride (Cl^-) and Sulphate (SO_4^{2-}). The present investigation was conducted through *in vitro* regeneration protocol of ten rice genotypes to observe the effect of these two salts and to identify which salt causes more inhibitor.

Materials and methods

Experimental location and materials

The experiment was conducted in the Advanced Plant Breeding Laboratory, Department of Genetics and Plant Breeding, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Salna, Gazipur-1706, Bangladesh.

The following rice genotypes including a salt tolerant variety (BINA 8) were used as experimental materials in the present investigation.

Salt concentrations and media preparation

The healthy, disease free and dehusked seeds were used as explants for the study. MS (Murashige and Skoog, 1962) medium supplemented with two salts (NaCl and Na_2SO_4) was used for callus induction and the callus pieces at appropriate size were transferred to MS regeneration medium.

For callus induction, the MS (Murashige and Skoog, 1962) medium supplemented with 2, 4-D (2mg/l), sucrose (30g/l), agar (10g/l) and six levels of salt concentrations was taken for each salt. For plant regeneration the MS (Murashige and Skoog, 1962) medium supplemented with sucrose (30g/l), agar (l0g/1), NAA (l mg/L), BAP (1 mg/l) and six levels of salt concentration was used for each salt. Six levels of NaCl and Na_2So_4 salt solution (0, 0.1, 0.2, 0.4, 0.6 and 0.8%) were used in this experiment to observe the effect of salts.

Callus induction and regeneration

The dehusked seeds were sterilized and the seeds were air dried in the Laminar Air Flow cabinet for ten minutes and were transferred on callus induction media. This process was done for each genotype. The culture was inoculated with the treated seeds in the dark at 25 ± 2°C for callus induction. After 3-6 weeks of inoculation, seeds of the responsive varieties started to produce callus. Callus induction frequency was calculated on the basis of the number of seeds producing callus. Some seeds produced more than one callus, but for this calculation, all calli originating from one were considered as one. Calli with a size of at least 2 mm were transferred to regeneration medium (MS basal semisolid medium), and were incubated in a temperature controlled growth room at 25 ± 2°C under 16-hour light photoperiod with a light intensity of about 2000-3000 lux for plant regeneration. Day to day observations was carried out to note the response. Regenerated plants were counted on the basis of the number of callus producing plantlets.

Statistical analysis of data

The data for the characters under study were statistically analyzed wherever applicable. The experiment was conducted in growth room and arranged in Factorial Completely Randomized Design (CRD) with three replications. The Analyses of Variance for different characters were preformatted and means were compared by the Duncan's Multiple Range Test (DMRT).

To observe the effect of different treatments of the experiment, data were collected on the following parameters:

$$\text{Percent callus induction} = \frac{\text{Number of seeds induced calli}}{\text{Number of seeds incubated}} \text{X } 100$$

$$\text{Percent plant regeneration} = \frac{\text{Number of calli with plantlets}}{\text{Number of incubated calli}} \text{X } 100$$

Results

Effect of genotype and salt

Different rice cultivars responded differently to various levels of NaCl and Na_2SO_4 salt. For both NaCl and Na_2SO_4 salts, BINA 8 gave the highest response (Table 2).

The lowest callus induction response was observed in Binaphul (54.67%) with NaCl and BR11 (57.29) with Na_2SO_4. Plant regeneration were also effected by different rice genotypes and salts (Table 3).

In case of NaCl, the highest response was given by BINA 8 (38.27) while others gave statistically similar results. Again, BINA 8 (38.92) responded very well in Na_2SO_4 salt, while BRRI dhan51 (26.51) and Gopalbhog (25.74) showed poorest response which were statistically similar.

Table 1. Rice genotypes used in this experiment.

Sl. No.	Rice genotypes	Sl. No.	Rice genotypes
1.	BR10	6.	BINA 8
2.	BR11	7.	Uknimadhu
3.	BRRI dhan49	8.	Binaphul
4.	BRRI dhan51	9.	Gopalbhog
5.	BRRI dhan52	10.	Straw

Effect of salt and salt concentrations

Responses related to salt x salt concentration interaction are presented in Table 4. Percentage of callus induction was the highest (97.93%) at no salt concentration media. The lowest callus induction (1% & 2.7%) was found at 0.80% with both NaCl and Na_2SO_4.

Table 2. Effect of different rice genotypes and salts on callus induction (%).

Variety/genotype	NaCl	Na_2SO_4
BR10	59.75 ab	62.25 ab
BR11	54.79 b	57.29 c
BRRI dhan49	57 b	60.33 bc
BRRI dhan51	55.83 b	59.17 bc
BRRI dhan52	57 b	62 abc
BINA 8	65 a	66.17 a
Uknimadhu	58 b	62.17 ab
Gopalbhog	57.17 b	60.5 bc
Binaphul	54.67 b	62.17 ab
Straw	59.83 ab	59 bc
LSD at 5% level of significance	5.0987*	4.7918*

*Significant at 5% level of significance

Table followed by the same letter in a column do not differ significantly by DMRT.

Again, highest plant regeneration percentage (77.05%) was recorded at no salt concentration in both NaCl and Na_2SO_4 while the lowest was recorded (0.15%) at 0.80 % salt concentration in both NaCl and Na_2SO_4 supplemented MS media.

Effect of salt

The highest callus induction (61.10%) and plant regeneration (30.21%) were observed in the medium containing Na_2So_4 while the lowest callus induction (57.90%) and plant regeneration (29.17%) were observed in the NaCl containing media (Table 5).

Discussion

Effect of genotype and salt

The best performer variety in terms of plant regeneration and callus induction was found on Na_2So_4 supplemented media. Ping *et al.* (2006) examined NaCl tolerance by observing callus growth state, germination rate and seedling height under solid medium in 13 genotypes of rice.

Table 3. Effect of different rice genotypes and salts on plant regeneration (%).

Variety/ genotype	NaCl	Na_2SO_4
BR10	30.28 b	31.68 abc
BR11	30.10 b	31.37 abc
BRRI dhan49	28.92 b	28.80 bc
BRRI dhan51	26.52 b	26.51 c
BRRI dhan52	30.85 b	34.13 ab
BINA 8	38.27 a	38.92 a
Uknimadhu	27.06 b	29.61 bc
Gopalbhog	25.78 b	25.74 c
Binaphul	27.31 b	27.87 bc
Straw	26.57 b	27.47 bc
LSD at 5% level of significance	6.8324*	7.4905*

*Significant at 5% level of significance

Table followed by the same letter in a column do not differ significantly by DMRT.

The results showed that NaCl tolerance was different among different genotypes of rice. Muhammad *et al.* (2013) selected salt-tolerant soma clone from 3 local rice varieties (Rajashail, Katicota, and BRRI-22). The best responded regarding the callus induction frequency, the regeneration frequency and the number of shoot per callus were obtained from Rajashail and Katicota among three varieties. Qamaruz *et al.* (2011) found two genotypes MR219 and MR219 consistently performed the best in both callus culture (93.51 and 92.22%) and plant regeneration capacity (27.03 and 26.34%), respectively.

Table 4. Effect of different salts at various levels of concentration on callus induction (%) and plant regeneration (%).

Treatment		Callus induction (%)	Plant regeneration (%)
Salt	Salt concentrations (%)		
NaCl	0	97.93 a	77.05 a
	0.10	89.00 b	42.18 b
	0.20	73.00 c	36.76 c
	0.40	51.50 d	17.91 d
	0.60	35.00 e	1.00 e
	0.80	1.00 f	0.15 e
	LSD at 5% level of significance	3.9494*	5.2924*
Na_2SO_4	0	97.93 a	77.05 a
	0.10	92.50 b	45.70 b
	0.20	78.00 c	36.76 c
	0.40	55.00 d	19.35 d
	0.60	40.50 e	2.25 e
	0.80	2.70 f	0.15 f
	LSD at 5% level of significance	3.7117*	5.8026*

*Significant at 5% level of significance

Table followed by the same letter in a column do not differ significantly by DMRT

Similarly, in this experiment, BINA 8 performed very well in both NaCl and Na_2SO_4 containing media because it is a salt tolerant variety.

Effect of salt and salt concentrations

Adil *et al.* (2009) found a reverse relationship between salt and salt concentration. Nahar *et al.* (2013) studied to evaluate the effect of salt stress on two rice genotypes (BRRI dhan38 and Chini Kanai). Plant regeneration of BRRI Dhan38 was 80% at 0 mM NaCl, but decreased to 20% at 100 mM NaCl. There was no plant regeneration at 150 mM NaCl for BRRI dhan38 and Chini Kanai respectively. Puspasree (2013) reported that calluses were subjected to NaCl stress at 5 levels (0.2, 0.4, 0.6, 0.8 and 1.0%) for 3 successive passages. Among the cultivars, IR 50 showed the maximum percentage of callus induction (92.0) and Dasal showed the minimum (78.5).

Table 5. Mean effect of different salts on callus induction (%) and plant regeneration (%) in rice genotypes.

Treatment		Callus induction (%)	Plant regeneration (%)
Salt	NaCl	57.9 b	29.17 b
	Na_2SO_4	61.1 a	30.21 a
	LSD at 5% level of significance	3.0368*	1.0268*

*Significant at 5% level of significance

Table followed by the same letter in a column do not differ significantly by DMRT.

There was a decrease in callus score with increase in salt concentration and number of passages. Forkan *et al.* (2013) reported that callus was grown on agar solidified media containing 0.2, 0.4, 0.6, 0.8 and 1.0% Na_2SO_4 salt. The callus growth decreased with increasing Na_2SO_4 concentration in the medium. Generally, saline condition decreases the growth rate of rice plant. Again, amongst different doses of salt concentration, the highest dose most adversely affects the growth. It was clearly observed from the Table 4 that, increasing level of salt concentration decrease the response of callus induction and plant regeneration both.

Effect of salt

Na_2So_4 gave better response for *in vitro* plant regeneration of rice than NaCl salinity. Two salts (NaCl & Na_2So_4) were used to see their inhibitory differences in this experiment. Both callus induction and plant regeneration were affected by NaCl and Na_2So_4. Among the two salts, Na^+ is common in NaCl and Na_2So_4 but differ only by two anions: Chloride (Cl^-) and Sulphate (SO_4^{2-}). So, Cl^- containing salts are more inhibitory in *in vitro* response of rice while better response can be expected from Na_2So_4 salt.

Conclusion

The response of callus induction and plant regeneration were decreased with the increasing level of salt concentration. Being a salt tolerant variety, BINA 8 gave best response in both NaCl and Na_2SO_4. But individual effect of NaCl and Na_2So_4 on callus induction and plant regeneration was different. The responses were least affected in the medium containing Na_2So_4. It thus appears that Cl^- containing salts are more inhibitory in *in vitro* response of rice.

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