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Callus induction and plantlets regeneration of two rice (*Oryza sativa* L.) genotypes under saline stress conditions

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ABSTRACT

The present investigation was aimed to obtain salt tolerant genotypes of Inti and Viflor rice cultivars. In this study, callus induction and plant regeneration responsiveness were tested using different concentrations of 0.0-2.0% (w/v) NaCl, for callus induction, and 0.0-1.0% (w/v) NaCl, for plant regeneration, which added in MS based media. Marked variation was observed both in calli proliferation and plant regeneration among the two rice cultivars. Among the cultivars tested, cv. Inti and cv. Viflor produced the highest percentage (91% and 80%, respectively) of callus with size (+) on the MS medium supplemented with 0.75% and 1.0% NaCl, respectively. Plant regeneration of both cv. Inti and cv. Viflor was 62.5% and 75%, respectively, at 0.5% NaCl, but decreased to 12.5% at 1.0% NaCl. The results of the present study showed the decreasing trend in callus induction and plant regeneration with increasing concentrations of NaCl.

Key words: Callus induction, *Oryza sativa*, Plant regeneration, Salt tolerance, Somaclonal variation

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INTRODUCTION

Rice (*Oryza sativa* L.) is the most important food crop in the world and about 70% of the world's poor depend on rice as their major source of food energy[1]. 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, and India is the largest rice growing country accounting for about one-third of the world acreage under the crop[2]. In Latin America and the Caribbean rice is now the most important food grain in most of the tropical areas, where it supplies more calories in people's diets than wheat, maize, cassava, or potatoes, and is also the leading source of protein for the poorest 20% of the population in tropical areas[3]. In this region, total production of paddy rice increased from around 8 million tons in 1961 to more than 28 million tons in 2009, an increase of over 250%; however, there is still a negative balance between production and consumption in the region as a whole[4]. In Peru total production of paddy rice increased from 333 thousand tons in 1961 to around 3 million tons in 2009, an increase of 798%[5].

Despite these encouraging results, it is necessary to increase the levels of production and productivity of rice in Peru. The application of biotechnology in combination with conventional breeding methods may help to increase food production properly, and in that sense, *in vitro* technology, especially plant tissue culture offers many unconventional techniques for crop improvement[6]. In effect, *in vitro* culture of plant cells and tissue has attracted considerable interest over the recent years because it provides the means to study the physiological and genetic processes of plants in addition to offer the potential of assisting in breeding improved cultivars by increasing the genetic variability[7]. The high efficiency of *in vitro* selection system is based on the fact that it is possible to grow millions of cells in flask and achieve a rapid multiplication of cell populations on defined media; however, the efficient plant regeneration through *in vitro* micropropagation, organogenesis and somatic embryogenesis is essential for the successful utilization of biotechnology in rice crop improvement[8].

Various abiotic stresses including high or low temperature, water scarce, high salinity and heavy metals exert drastic antagonistic effects on crop metabolism and thereby plant growth, development and ultimately crop productivity[2]. Amongst these, soil salinity is a major factor limiting the crop production globally, and affecting large areas of the world cultivated land causing significant reductions in crop yield[9]. According to the Food and Agriculture Organization of the United Nations report, 800 million hectares, equivalent to 6.5% of the total land area, are estimated to be affected by high salt concentration [10]. Plants grown under saline conditions for a prolonged period suffer from metabolic dysfunction associated with abnormal ionic exchange, which results in reduced production or even plant mortality[11]. Salinity affects all the major life processes such as growth, photosynthesis, protein synthesis, and lipid metabolism, and in addittion to these interrelated and co-existing impacts, salinity also produces an oxidative stress due to rapid and transient accumulation of reactive oxygen species (ROS), i.e. superoxide and/or hydroxyl radical and singlet oxygen[12, 13]. These ROS cause pigment co-oxidation, lipid peroxidation, membrane destruction, protein denaturation and/or DNA mutation[14].

The development of crops with elevated levels of salt tolerance is therefore highly desirable[15, 16]; however, improvement in salt tolerance of crop plants remains elusive, due to the fact that salinity affects almost every aspect of the physiology and biochemistry of plants at both whole plant and cellular levels[13].

Numerous studies have been carried out on callus induction and plant regeneration of rice in culture medium supplemented with NaCl. Embryogenic callus of indica rice cv. Basmati 370 induced on MS medium containing 9.05 µM 2,4-D were irradiated at 50 Gy of gamma rays of ^{60}Co for creating genetic variability against salinity, and then irradiated and non-irradiated calli were screened *in vitro* through three consecutive proliferation phases at 4.0, 6.0, 8.0 and 10.0 d/Sm electrical conductivity of NaCl. From gamma ray mutagenized cultures, two putative lines (M_2 generation) with moderate salt tolerance were obtained at seedling stage[17]. *In vitro* experiments on plant regeneration from embryogenic callus cultures were conducted to assess the effect of salt stress with NaCl (0, 25, 50, 75 and 100 mM) on callus induction, survival, fresh weight, regeneration, proline level and total protein content in salt sensitive indica rice cv. IR 64; gradual reduction in regeneration was observed with increasing salt concentrations (25 to 100 mM), and the relative regeneration was maximum at 50 mM [18]. In another study, plant regeneration of rice genotype 'BRRI dhan 38' was 80% at 0 mM NaCl, but decreased to 20% at 100 mM NaCl, and 0% at 150 mM NaCl, and in the rice genotype 'Chini Kanai' plant regeneration on the non-strees medium was 60%, while at 150 mM NaCl it decreased to 20% and there was no regeneration at 200 mM NaCl[19].

In vitro selection of salt tolerant lines and regenerated plants has also been reported in several species. In cotton (*Gossypium hirsutum*), embryogenic callus were subcultured for at least one year and then transferred to modified MS medium supplemented with 5 – 40 g/l NaCl for selecting salt-tolerant, observing that the half-lethal concentration of NaCl was between 5 and 10 g/l, and the lethal concentration of NaCl was 40 g/l[20]. In six cultivated and breeding lines genotypes of tomato (*Lycopersicon esculentum*), hypocotyls segments and true leaves were chosen as explants for callus induction and plant regeneration in MS medium supplemented with different NaCl concentrations (0. 1, 2, 4, 5 or 6 g/l) for eight weeks, showing that the six investigated tomato genotypes differed in their callus growth and regeneration capacities[21]. In *Foeniculum vulgare*, explants of root, hypocotyl and cotyledon of sterilized seedling were transferred to callus and regeneration media with concentrations of 0, 50, 100 and 150 mM of NaCl, and after 4 weeks in the presence of 100 and 150 mM NaCl, the highest frequency of callus induction in hypocotyl and cotyledon explants was recorded on the media supplemented with 1.0 mg/l IAA, 1.0 mg/l 2,4-D and 2.0 mg/l KIN[22]. *In vitro* response of two genotypes of bread wheat (*Triticum aestivum*), Mahon-Demais and Hidhab, to mature embryo culture, callus production, and plant regeneration, was evaluated by exposing them to different concentrations of NaCl (0, 5, 10, and 15 g/l) and under different thermal stress intensities (25, 30, 35, and 40 ^{o}C), being observed significant differences between both genotypes[23]; similarly, in a study evaluating two cultivars of potato (Cardinal and Desiree), *in vitro* direct selection of salt-tolerant callus cultures in MS medium with NaCl (0 to 140 mM) and subsequent plant regeneration, it has been observed more than 50% reduction in relative fresh callus mass in both potato cultivars exposed to 120 mM NaCl, and that the regeneration potential of recurrently-selected callus cultures (100 mM NaCl-treated) on salt-free regeneration medium was not much different as compared to the control (non-selected ones)[13]. These studies suggest that tissue culture is a powerful tool for selection and can be used to improve salt tolerance in plants.

The aim of the present study was to examine the response of two rice genotypes, Inti and Viflor, in callus induction and the response of regenerated plants to NaCl stress using tissue culture technique.

MATERIALS AND METHODS

Plant Materials

The experiment was conducted in the Tissue Culture and Genetic Resources Laboratory of the Academic Department of Botany, Pedro Ruiz Gallo National University, Lambayeque, Peru. Manually dehusked seeds of rice (*Oryza sativa* L. cv. Inti and cv. Viflor) were surface sterilized by immersion with 70% (v/v) ethanol for 60 sec, and 50% (v/v) comercial bleach containing 5.25% sodium hypochlorite (NaOCl) for 10 min followed by three washings with sterilized distilled water. The floating dehulled seeds were discarded.

Callus induction

After desinfection, the seeds were placed on C1 to C7 medium containing MS[24] salts, vitamins (thiamine.HCl 1.0 mg/l and *myo*-inositol 100 mg/l), 2.0 mg/l 2,4-D, 3.0% sucrose, and NaCl (0.0, 0.25, 0.5, 0.75, 1.0, 1.5 and 2.0%) (Table 1). Observations regarding the morphology of the callus were noted, and non-organogenic and browning calli were discarded.

Plant regeneration

Calli with maximum growth value (++ and +++) were used as source of inocula in the plant regeneration phase. These calli were cultured on R1 to R3 medium containing MS salts, vitamins, 5.0% sucrose, NAA 2.0 mg/l, BAP 1.5 mg/l, 2.0 mg/l KIN and NaCl (0.0, 0.5 and 1.0 mg/l) (Table 1). Emergence of green shoots above 1.0 cm was considered as plant regenerated.

Culture conditions

For callus induction and plant regeneration 25 culture vessels (150x25 mm) were inoculated for each treatment and the experiment was repeated twice. The pH of the medium was adjusted to 5.8±0.1 with 1N NaOH and 1N HCl using electronic pH indicator, and solidified with 0.6% agar before autoclaving at 121 ^{o}C and pressure of 15 lbs psi for 20 min. The cultures were placed in dark at a temperature of 25±2 ^{o}C for callus induction for four weeks, and under both dark (8h) and light (16 h) conditions for plant regeneration for six weeks.

Acclimatization

Well-developed plantlets with sufficient roots systems were successfully transferred to a mixture of soil, sand and organic matter (1:1:1), along with seed derived control plants, and were irrigated with Hoagland solution[25] for 15 days.

Statistical analysis

The experiment was carried out in a completely randomized design with 14 treatment and 25 replications per treatment for callus induction. Statistical significance between mean values was assessed using the analysis of variance (ANOVA) and a conventional Tukey multiple range test at a significance level of =0.05 ($p<0.05$). For plant regeneration the results were expressed in percentage.

RESULTS AND DISCUSSION

For several species, studies have showed that many factors, such as the culture medium composition, genotype, and the type of explant, affect the callus induction and plant regeneration process, therefore, in this study the variation noted in the callus induction capacity and plant regeneration appears to be mainly due to the genotype and the medium composition effect.

Callus induction

Mature dehusked rice seeds of two cultivars, Inti and Viflor, were used for callus initiation. The relative growth of callus was observed at the different treatment levels of 0.0, 0.25, 0.5, 0.75, 1.0, 1.5 and 2.0% NaCl with the supplement of MS medium and 2.0 mg/l 2,4-D. Callus initiation appeared on the surface of the scutella region at the 8th to 10th days after transferring the seeds to culture tubes. The final data on callus induction was recorded after 30 days of inoculation. Results from different concentrations of NaCl showed that the growth rate of calli decreased when concentrations of NaCl increased in the media. It was observed that MS media supplemented with 1.5% NaCl produced the highest percentage of calli, 63 and 70% for Inti and Viflor cultivars, respectively; however, these calli showed poor growth (+, < 5 mm Ø). Calli of moderate (++, 6-10 mm Ø) and good growth (+++, > 10 mm Ø) were observed in the treatments with 0.25 and 0.5% NaCl for the cv. Inti (Fig 1a) and treatments with 0.25 to 1.0% NaCl for cv. Viflor, therefore, calli of cv. Viflor showed greater tolerance to NaCl than calli of cv. Inti. In the control treatment (0.0%) callus induction with moderate to good growth was about 100%, and in both cultivars, Inti and Viflor, the highest rate of phenolized and necrotic callus was observed in treatments supplemented with 1.0 and 1.5% NaCl with 92 and 83%, respectively (Tables 2 and 3). In the other hand, two types of calli were formed:

organogenic calli were soft, white to white-creamy in colour, with knobby appearance, and formed within 3-4 weeks of culture, while non-regenerable calli were friable with brown to black colour. In most of the seeds emerged a small coleoptile and radicle.

Table 1. Treatments tested on callus induction and plant regeneration of two rice cultivars (Inti and Viflor) at different NaCl concentrations

Callus induction (%)		Plant regeneration (%)	
Treatments[a]	**NaCl (%/mM)**	**Treatments[b]**	**NaCl (%/mM)**
C1	0.0/0.0	R1	0.0/0.0
C1	0.0/0.0	R2	0.5/86.2
C1	0.0/0.0	R3	1.0/172.4
C2	0.25/43.1	R1	0.0/0.0
C2	0.25/43.1	R2	0.5/86.2
C2	0.25/43.1	R3	1.0/172.4
C3	0.5/86.2	R1	0.0/0.0
C3	0.5/86.2	R2	0.5/86.2
C3	0.5/86.2	R3	1.0/172.4
C4	0.75/129.3	R1	0.0/0.0
C4	0.75/129.3	R2	0.5/86.2
C4	0.75/129.3	R3	1.0/172.4
C5	1.0/172.4	R1	0.0/0.0
C5	1.0/172.4	R2	0.5/86.2
C5	1.0/172.4	R3	1.0/172.4
C6	1.5/258.3	R1	0.0/0.0
C6	1.5/258.3	R2	0.5/86.2
C6	1.5/258.3	R3	1.0/172.4
C7	2.0/344.8	R1	0.0/0.0
C7	2.0/344.8	R2	0.5/86.2
C7	2.0/344.8	R3	1.0/172.4

[a]MS + vitamins (1.0 mg/l thiamine.HCl and 100 mg/l m-inositol) + 3.0% sucrose + 2.0 mg/l 2,4-D + 0.6% agar.[b]MS + vitamins (1.0 mg/l thiamine.HCl and 100 mg/l m-inositol) + 5.0% sucrose + 2.0 mg/l NAA + 1.5 mg/l BAP + 2.0 mg/l KIN + 0.6% agar.

Table 2. Callus induction of two rice cultivars (Inti and Viflor) on MS medium supplemented with various concentrations of NaCl[1,2]

Treatments NaCl (%)	Cv. Inti						Cv. Viflor					
	Size				Colour		Size				Colour	
	-	+	++	+++	W	B	-	+	++	+++	W	B
C1 0.0	0	4	88	8	100	0	0	8	67	25	100	0
C2 0.25	0	25	75	0	92	8	0	13	71	17	75	25
C3 0.50	0	54	46	0	63	37	13	33	50	4	67	33
C4 0.75	9	91	0	0	50	50	8	58	33	0	58	42
C5 1.0	29	71	0	0	8	92	8	80	13	0	17	83
C6 1.5	37	63	0	0	8	92	29	70	0	0	17	83
C7 2.0	0	0	0	0	-	-	0	0	0	0	-	-

[1]-, without callus formation; +, callus < 5 mm Ø; ++, callus between 6-10 mm Ø; +++, callus> 10 mm Ø.
[2], colour: W, white callus; B, brown callus or phenolized callus

The procedure for callus induction from the mature rice seeds has already been reported[26]. When incubated on the high 2,4-D medium, the growth of plumule and radicle was suppressed, although the scutellar epithelium and mesocotyl tissues proliferated[27, 28, 29]. Moreover, the literature reports several studies on induction of embryogenic and organogenic calli in rice seeds, supplemented or not with NaCl. In cv. IR 64 *indica* rice embryogenic callus induction was observed in MS medium supplemented with 13.5 µM 2,4-D, 1.3 µM KIN, and four different concentrations of NaCl (25, 50, 75 and 100 mM), and callus induction frequency as well as fresh weight decreased significantly with increasing NaCl concentrations in the medium; there was no significant reduction in callus survival rate as evidenced by gradual reduction in the percentage of survival at 100 mM when compared to callus induction at the same concentration[18]. In our study, the highest concentrations of NaCl tested (0.75, 1.0, 1.5 and 2.0%) were much higher than 100 mM NaCl. In another study, callus induction in six rice cultivars were tested using different concentrations of NaCl, from 0.1 to 0.3% (w/v); the highest number of callus induction (8 to 39%) in all genotypes on the media supplemented with 2.0 mg/l 2,4-D was recorded in the presence of 0.1% NaCl, while the highest dose of NaCl salt (0.3%) inhibited callus induction when compared to 0.1 and 0.2% NaCl media combinations[30]. Similarly to the study of Priya *et al*. [18], in this work the NaCl concentrations used

were quite low (0.1 to 0.3%) and the percentage of callus induction were also low, showing that these genotypes were highly susceptible to salinity. In addition, in two rice genotypes, 'BRRI Dhan 38' and 'Chini Kanai', the maximum percentage (75%) of callus induction was observed in MS medium supplemented with 5.0 mg/l 2,4-D for 'BRRI Dhan 38' and 3.0 mg/l 2,4-D for 'Chini Kanai', and approximately 100.0 mg of one month-old embriogenic callus was exposed to each (0.0, 50, 100.0, 150.0 and 200 mM) NaCl concentration; with the increment in NaCl concentration there was a gradual decrease in callus fresh weight[19]. Likewise, in three rice genotypes collected from the coastal area of Sandwip, Bangladesh, callus induction was observed on MS medium supplemented with 2.5 mg/l 2,4-D and different concentrations of NaCl from 0.2 to 1.5%, and was formed only in the medium containing 0.2, 0.5 and 1.0% NaCl with 76.4, 66.6 and 31.8%, respectively[31]. These results were similars to those reported in our study.

Table 3. Analysis of variance and means comparison of callus induction of two rice cultivars (Inti and Viflor) on MS medium supplemented with various concentrations of NaCl

Treatments	NaCl (%)	Callus growth (mm)
VFC1	0.0	3.16 a
INC1	0.0	3.04 a
VFC2	0.25	3.04 a
INC2	0.25	2.70 ab
INC3	0.5	2.45 bc
VFC3	0.5	2.42 bcd
VFC4	0.75	2.25 bcde
VFC5	1.0	2.04 cdef
INC4	0.75	1.97 cdfg
INC5	1.0	1.87 defg
VFC6	1.5	1.70 efg
INC6	1.5	1.62 fg
INC7	2.0	1.0 h
VFC7	2.0	1.0 h
C.V. = 22.41%; ALS = 0.5515		

Different letters indicate significant difference between means at P<0.05.

Table 4. Plantlet regenerationof two rice cultivars (Inti and Viflor) on MS medium supplemented with various concentrations of NaCl[1]

Treatments NaCl (%)		Cv. Inti			Cv. Viflor		
Callus induction	Plant regeneration	Shoot Ind. (%)	Roots Format. (++/+++) (%)	Browning (%)	Shoot Ind. (%)	Roots Format. (++/+++)	Browning (%)
0.0	0.0	62.5	100.0	38.0	75.0	100.0	37.5
0.0	0.5	62.5	100.0	62.0	75.0	100.0	62.5
0.0	1.0	50.0	100.0	75.0	62.5	100.0	75.0
0.25	0.0	50.0	100.0	87.0	50.0	100.0	75.0
0.25	0.5	37.5	100.0	87.5	50.0	90.0	75.0
0.25	1.0	37.5	100.0	87.5	37.5	70.0	75.0
0.5	0.0	37.5	100.0	87.5	37.5	80.0	75.0
0.5	0.5	25.0	60.0	100.0	37.5	80.0	75.0
0.5	1.0	12.5	50.0	100.0	25.0	90.0	100.0
0.75	0.0	25.0	40.0	100.0	25.0	70.0	100.0
0.75	0.5	12.5	30.0	100.0	25.0	70.0	100.0
0.75	1.0	12.5	20.0	100.0	12.5	40.0	100.0
1.0	0.0	12.5	0.0	100.0	12.5	0.0	100.0
1.0	0.5	0.0	0.0	100.0	12.5	0.0	100.0
1.0	1.0	0.0	0.0	100.0	0.0	0.0	100.0
1.5	0.0	0.0	0.0	100.0	0.0	0.0	100.0
1.5	0.5	0.0	0.0	100.0	0.0	0.0	100.0
1.5	1.0	0.0	0.0	100.0	0.0	0.0	100.0
2.0	0.0	0.0	0.0	100.0	0.0	0.0	100.0
2.0	0.5	0.0	0.0	100.0	0.0	0.0	100.0
2.0	1.0	0.0	0.0	100.0	0.0	0.0	100.0

[1]*Shoot. ind., shoot induction; Roots Format., roots formation*

Plantlet regeneration

In this experiment calli derived from the different concentrations of NaCl (0.0, 0.25, 0.5, 0.75, 1.0, 1.5 and 2.0%) were cultured on MS medium supplemented with 5.0% sucrose, 2.0 mg/l NAA, 1.5 mg/l BAP, 2.0 mg/l KIN and NaCl (0.0, 0.5 and 1.0 %) for plantlet regeneration. The mean percentage of shoots produced per regenerating calli was gradually decreased in both cultivars when the concentration of NaCl increased in the medium. Plant regeneration of cv. Inti was 62.5% at 0.0/0.0% (callus induction/plant regeneration) and 0.0/0.5% of NaCl, but

decreased to 12.5% at 0.75/0.5%, 0.75/1.0% and 1.0/0.0% of NaCl, and 0.0% from 1.0/0.5% of NaCl. In cv. Viflor, plant regeneration on the non-stress medium was 75% at 0.0/0.0% and 0.0/0.5% of NaCl (Fig 1b), but decreased to 12.5% at 0.75/1.0%, 1.0/0.0 and 1.0/0.0% of NaCl, and 0.0% from 1.0/1.0% of NaCl (Table 4). In our study cv. Viflor was slightly more NaCl tolerant than cv. Inti.

The plant regeneration frequencies of explants and genotypes are influenced by varios factors such as the culture methods, the media and the culture conditions [32, 33], and in the rice regeneration efficient protocols should be specifically developed for particular explants and varieties. In general, the findings of regeneration decrements obtained in the present work were in agreement with many researches which reported negative response of plant regeneration to NaCl [34]. In two rice genotypes, 'BRRI Dhan 38' and 'Chini Kanai', MS media supplemented with 2.0 mg/l KIN, 1.0 mg/l NAA and 2.0 mg/l BA produced a higher percentage of plant regeneration (80 and 60%, respectively, at 0.0 mM NaCl), but decreased to 20% at 100 mM NaCl in both genotypes; at 150 mM NaCl plant regeneration was 20% for 'Chini Kanai' and 0% for 'BRRI Dhan 38', therefore, 'Chini Kanai' was more salt tolerant than 'BRRI Dhan 38'[19]. In another study on *in vitro* plant regeneration from embryogenic callus cultures of cv 'IR 64' indica rice, a gradual reduction in regeneration was observed with increasing salt concentrations (25 to 100 mM) with maximum at 50 mM, and significant increase in proline content (9.2 fold in the leaves) after 30 days[18]. In Basmati rice (cv. Basmati 370), NaCl adapted irradiated embryogenic callus at 50 Gy of gamma rays of $_{60}$Co for creating genetic variability against salinity (at 4.0 and 6.0 d/Sm electrical conductivity of NaCl), showing 2.0 – 4.75% regeneration frequency on MS regeneration medium containing 5.37 µM NAA and 9.29 µM KIN[35]. In another similar study, the mean number of shoots produced per regenerating embryogenic calli was gradually decreased in all the deepwater rice cultivars when the concentration of NaCl (0.1 – 0.3%) increased in the medium [30]. All of these results were also similars to those reported in our study.

Table 5. Plantlets of two rice cultivars (Inti and Viflor), on MS medium supplemented with various concentrations of NaCl, established in the field

Treatments NaCl (%)	Cv. Inti		Cv. Viflor	
	Nº	(%)	Nº	(%)
C1R1	6	9.52	17	9.23
C1R2	2	3.17	1	0.54
C1R3	0	0.0	7	3.80
C2R1	9	14.28	24	13.04
C2R2	12	19.04	53	28.80
C2R3	17	26.98	11	5.97
C3R1	1	1.57	7	3.80
C3R2	5	7.93	42	22.82
C3R3	0	0.0	0	0.0
C4R1	7	11.11	9	4.89
C4R2	0	0.0	10	5.43
C4R3	2	3.17	2	1.08
C5R1	0	0.0	0	0.0
C5R2	1	1.58	1	0.54
C5R3	1	1.58	0	0.0
C6R1	0	0.0	0	0.0
C6R2	0	0.0	0	0.0
C6R3	0	0.0	0	0.0
C7R1	0	0.0	0	0.0
C7R2	0	0.0	0	0.0
C7R3	0	0.0	0	0.0
Total	**63**	**100.0**	**184**	**100.0**

Salt tolerance and somaclonal variation

Marked variation was observed both in *in vitro* plant regeneration and established plants in field among the cultivars. From a total of 247 regenerated and established in field plants, 184 (74.49%) corresponded to cv. Viflor and 63 (25.51%) to cv. Inti with a ratio of 3:1 (Table 5). In the cv. Inti the highest percentage of regenerated and established in field plants were observed in the C2R3, C2R2 and C2R1 treatments with 26.98, 19.04 and 14.28%, respectively, while in the cv. Viflor it was observed in the C2R2 and C3R2 treatments with 28.80 and 22.82%, respectively. In addition, two plantlets of cv. Inti and one plantlet of cv. Viflor were regenerated with the extreme treatments C5R2 (1.0/0.5% NaCl) and C5R3 (1.0/1.0% NaCl) and successfully established in field. The probable reason of this variation could be due to the genetic characters influenced by heredity[30].

Our results were in agreement with many researchers who reported a negative response of plant regeneration to NaCl [2, 35, 36]. The major inhibitory and deleterious effects of salinity on plant growth and yield have been attributed to osmotic effect, ion toxicity and nutritional imbalance leading to reduction in photosynthetic efficiency and other physiological disorders[37, 38]. The drastic increase in the concentration of Na^+ and Cl^- in tissues,

following plant exposure to salinity, led to toxicity as it was evidenced by reduced plant growth[22, 39]. Ionic toxicity occurs because of the high concentrations of Na^+ and Cl^- in the cells citoplasm, which disturb several biochemical and physiological processes, and therefore osmotic stress is induced by the lowering of the water potential causing turgor reduction and celular water loss[40].

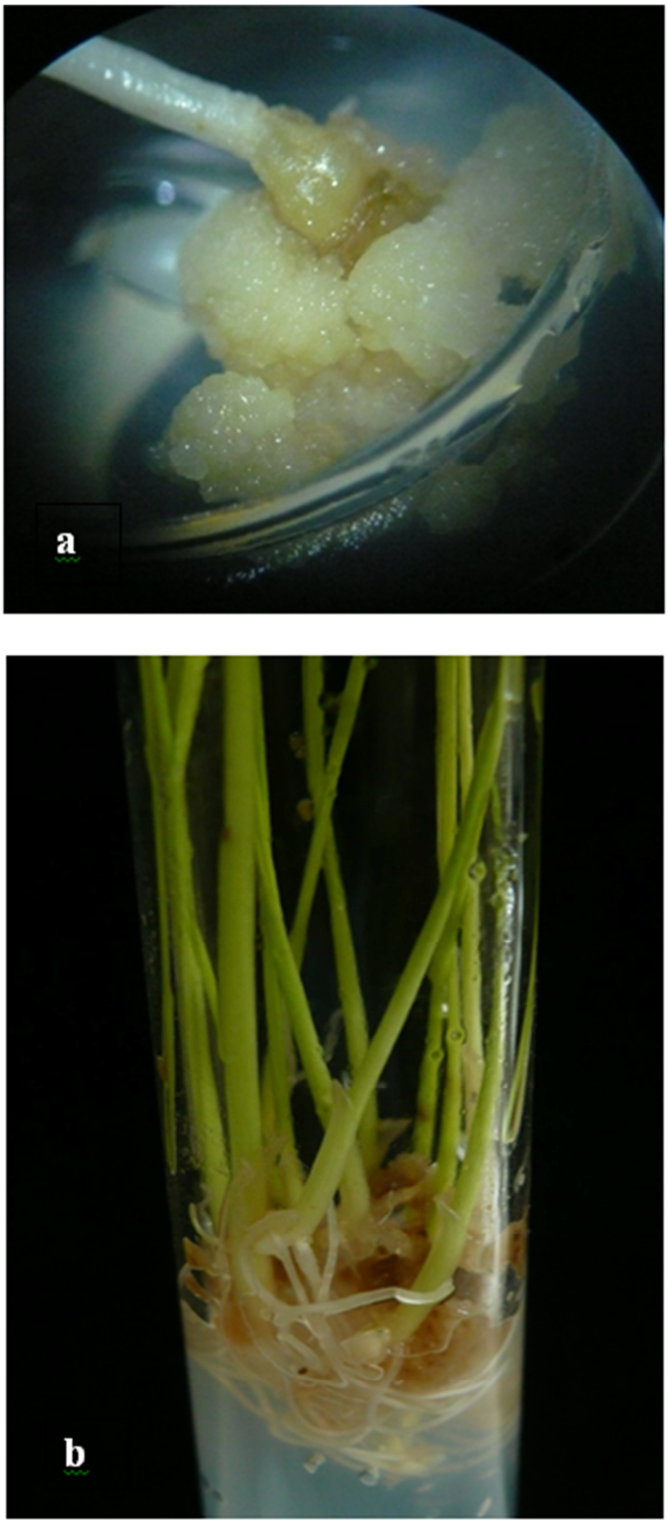

Figure 1. Effect of NaCl on callus induction and plant regeneration of two rice cultivars. a. Callus induction of cv. Inti (MS + 2.0 mg/l 2,4-D + 0.25% NaCl) and b. Plant regeneration of cv. Viflor (MS + 2.0 mg/l NAA, 1.5 mg/l BAP, 2.0 mg/l KIN + 0.5% NaCl)

In the other hand, the concept of *in vitro* selection is to exploit the genetic variation, known to occur in plants by screening cell cultures for resistance to diseases and pests or tolerance to herbicides or any abiotic stress as drought

and salinity. This process typically involves subjecting cells in cultures to a suitable selection pressure and recovering any variant cell lines that are resistant or tolerant to that particular stress [13]. This source of genetic variability is a phenomenon called somaclonal variation[41], manifested as cytological abnormalities, frequent qualitative and quantitative phenotypic mutation, sequence change, and gene activation and silencing[42].

CONCLUSION

On the basis of these experiments, it can be concluded that salinity toxicity is not exclusively a whole-plant phenomenon, but rather appears to be a cellular phenomenon which can be studied in cultured cells, suggesting that in both cases, salinity markedly suppresses the growth of rice. Likewise, from these findings it is concluded that the frequency of callus formation and plant regeneration, from scuttelar tissue of seeds, is influenced by the composition of culture media in the presence of several NaCl concentrations and also by the regenerating capacity of organogenic callus influenced by the genotype.

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