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Differential Salinity Tolerance in Calli and Shoots of Four Rice Cultivars

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ABSTRACT

This study was carried out to investigate the effect of NaCl on callus necrosis, the percentage of survival calli and shoot regeneration in rice cultivars, namely Khao Dawk Mali 105, Chai Nat 1, Suphan Buri 1 and Pathum Thani 1, after 60, 90, 120 and 150 days of exposure. After 150 days of exposure, calli showed a decrease in the survival percentage and relative fresh weight against the increasing NaCl concentration. The survival percentages of 55, 52, 44 and 56 were respectively observed in Khao Dawk Mali 105, Chai Nat 1, Suphan Buri 1 and Pathum Thani 1. The relative fresh weights were found to be 0.10, 0.75, 0.23 and 0.01, respectively. Following 120 day exposure, 175 mM NaCl was found to inhibit growth of all rice cultivars. Calli exposed to NaCl exhibited lower percentage of shoot regeneration than control. The percentage of shoot regeneration in Khao Dawk Mali 105, Chai Nat 1, Suphan Buri 1 and Pathum Thani 1 calli receiving 150 mM NaCl were 36, 32, 36 and 28%, respectively. Rooting of selected-shoots was also observed and the findings revealed that, after 30 days of culture, shoots can produce normal roots.

Key words: Khao Dawk Mali 105, Chai Nat 1, Suphan Buri 1, Pathum Thani 1

INTRODUCTION

Over the last 30 years, Thailand has been the largest exporter of rice in the world and rice is one of the most important cereal crops, providing a staple food for Thai population. In the last three years (2006-2008), Thailand's rice exports tend to increase from 7.494-10.216 Mt (Dechachete, 2011). Approximately 9.2 million hectares of rice-growing areas are technically suited for rice production but are left uncultivated or are grown with very low yields because the most problems are affected by pests and diseases, as well as by environmental stress factors, such as drought, chilling temperature, acidified soil and saline soil.

Salinity is one of the most serious factors that adversely affect crop production. Almost three quarters of the surface of the earth is covered by salt water that affects a significant proportion of the world's land surface (Flowers and Flowers, 2005). Salt stress leads to the suppression of plant growth and development, membrane leakage and ion imbalance (Summart *et al.*, 2010). Several physiological pathways such as photosynthesis, respiration, nitrogen fixation and carbohydrate metabolism have been also found affected under high salinity (Amirjani, 2010). Likewise, rice is affected by soil salinity because rice is very sensitive to salt, especially in the seedling stage (Winicov, 1996). The response of rice to salt stress is the exclusion or reduction of Na⁺ uptake and

increased absorption of K^+ to maintain a good balance of Na^+ - K^+ ratio in shoot (Saleem *et al.*, 2005) and accumulation of solutes like proline are important factors that help the plant system to adaptation under salinity stress (Basu *et al.*, 2002).

To date, new techniques such as plant tissue culture and genetic engineering have been utilized worldwide in plant improvement. These techniques help shorten time for creating plants with desirable traits rather than conventional plant breeding (Flowers, 2004). Plant tissue culture techniques have been widely used for micropropagation of clone after breeding, especially in screening for stress tolerance such as salinity tolerance and cold tolerance in plant (Koc *et al.*, 2009). Flowers and Yeo (1995) suggested a possible way for improving salt tolerant ability of crop plants by genetic variation within presented crops by using recurrent selection, mutagenesis or plant tissue culture technique. In the meantime, tissue culture techniques have also been used for developing saline tolerant capability of rice by *in vitro* selection (Koc *et al.*, 2009).

In vitro selection is tissue culture technique that is widely used as a tool for obtaining salt-tolerant plants (Shekhawat and Kumar, 2006) as the fastest and simplest method. The application of *in vitro* culture techniques for selection of salt tolerance in rice has been widely studied (Lutts *et al.*, 1999; Ahmad *et al.*, 2007; Khaleda *et al.*, 2007; Tariq *et al.*, 2008; Rahmzadeh *et al.*, 2008; Evangelista *et al.*, 2009). Likewise, the application of *in vitro* selection for salt-tolerant cell lines and regenerated plants has been reported in several species such as sour orange (Koc *et al.*, 2009), sugarcane (Badawy *et al.*, 2008), bermudagrass (Lu *et al.*, 2007) and wheat (Zair *et al.*, 2003). Lestari (2006) reported that the requirement factor for success in *in vitro* selection technique for salt tolerance are high variation of cells, easy application of *in vitro* selection method and regeneration method of tolerant cells. Apart from that, the response of rice callus culture and shoot regeneration under salt stress is very important factor to improve for salt-tolerance in rice.

This study focuses on the effect of NaCl at the varied concentrations on the morphological changes and necrosis in rice calli, the subsequent percentage of survival calli proliferation and shoot regeneration of four rice cultivars; Khao Dawk Mali 105, Chai Nat 1, Suphan Buri 1 and Pathum Thani 1. This data will be used as criteria for salt tolerance screening for each rice cultivars.

MATERIALS AND METHODS

Plant materials and seed preparation and disinfection: Four rice cultivars, namely Khao Dawk Mali 105 (KDML105), Chai Nat 1, Suphan Buri 1 and Pathum Thani 1, were used in the study. KDML105 was obtained from Khon Kaen Rice Seed Center, Khonkaen province while Suphan Buri 1, Chai Nat 1 and Pathum Thani 1 were obtained from Lop Buri Rice Seed Center, Lop Buri province. Seeds of four rice cultivars were dishusked and surface-sterilized for 5 min in 70% (v/v) ethyl alcohol, followed by 30% (v/v) Clorox® with 2-3 drops of Tween-20 as a wetting agent for 30 min. Seeds were then rinsed three times with sterile distilled water for 5 min. Sterile seeds of four rice cultivars were cultured for 30 days on NN medium added with suitable concentration of 2,4-D and organic compound for callus induction as follow:

- KDML105: NN+2 mg L⁻¹ 2,4-D+300 mg L⁻¹ casein hydrolysate
- Chai Nat 1: NN + 2.5 mg L⁻¹ 2,4-D+500 mg L⁻¹ L-proline
- Suphan Buri 1: NN+1.5 mg L⁻¹ 2,4-D+300 mg L⁻¹ casein hydrolysate+1,000 mg L⁻¹ L-proline
- Pathum Thani 1: NN+2.5 mg L⁻¹ 2,4-D+500 mg L⁻¹ L-proline

The pH of medium was adjusted to 5.7 before adding 4 g L⁻¹ (w/v) gelrite (Phytigel; Sigma) and autoclaved at 15 pound pressure, 121°C for 15 min. The cultures were incubated at 25±2°C under 16 h photoperiod.

Effect of NaCl on callus cultures: Thirty-day-old calli were cultured on suitable callus induction medium supplemented with varied concentrations of NaCl (0, 25, 50, 75, 100, 125, 150, 175, 200, 225 and 250 mM NaCl) to observe the effect of salt stress on rice callus cultures. After 60, 90, 120 and 150 days of culture, the percentage of survival calli, browning and necrosis were recorded. The percentage of survival calli (%) was recorded as number of proliferated calli/number of calli plated×100. Calli were weighed before transferring to medium supplemented with NaCl (W0) and weighed again at the end of selection (W1). Relative Fresh Weight (RFW) of callus was calculated as:

$$\text{RFW} = \frac{\text{W1}-\text{W0}}{\text{W0}}$$

The characteristics and morphology of calli were scored and recorded. For scoring, a rating scale ranging from 1 to 9 was applied according to Babu *et al.* (2007) as shown in Table 1.

Effect of NaCl on plant regeneration in rice: All adapted calli were transferred to MS medium added with 30 g L⁻¹ (w/v) sucrose, 4 g L⁻¹ (w/v) gelrite (Phytigel; Sigma), suitable concentrations of plant growth regulators and organic compounds for enhancing shoot regeneration in each cultivar as follows:

- KDML105: MS+3 mg L⁻¹ BA+300 mg L⁻¹ casein hydrolysate
- Chai Nat 1: MS+5 mg L⁻¹ BA+0.5 mg L⁻¹ NAA+300 mg L⁻¹ casein hydrolysate
- Suphan Buri 1: MS+3 mg L⁻¹ BA+0.5 mg L⁻¹ NAA+500 mg L⁻¹ L-proline
- Pathum Thani 1: MS+5 mg L⁻¹ BA+300 mg L⁻¹ casein hydrolysate

NaCl at the same concentrations (0-250 mM) was added into the regeneration medium. Callus cultures were maintained for three months in culture room under 16/8 h light/dark period at 25±2°C. The regenerated plants were transferred to hormone-free MS medium for root formation. Regeneration Frequency (RF) was measured to calculate as:

$$\text{RF} (\%) = \frac{\text{No. of embryo-derived calli showing green shoots}}{\text{Total number of calli transferred}} \times 100$$

Table 1: Characteristics scoring of injury symptoms of calli under salt stress

Rating scale	Description for scoring the symptoms of injured calli
1	Completely turned black or dark brown, dead
3	Yellow to brown in color, more than 75% of calli turned brown
5	Pale yellow to white in color but appeared in some proliferated calli
7	Yellow to pale yellow, water soaked areas interspersed with pale yellow friable calli
9	Greenish-yellow to yellow in color, healthy, nodular and friable calli

Statistical analysis: The statistical significance of the differences among treatments was calculated using Duncan's multiple range tests.

RESULTS

Effect of NaCl on callus cultures: Thirty-day-old calli of the rice cultivar; KDML105, Suphan Buri 1, Chai Nat 1 and Pathum Thani 1 were cultured on suitable callus induction medium supplement with various concentration of NaCl and the morphology of Thirty-day-old calli in four rice cultivar were compact and greenish-yellow as shown in Fig. 1. Therefore, after cultured on medium added with NaCl, it was found that rice calli of all cultivars treated with 25-150 mM NaCl showed different responses. The viability of calli was observed only in treatment with 0-150 mM NaCl. Under these conditions, calli on the lower part of clumps attaching medium turned brown while the upper part remained the same. It was evident that surviving calli fully developed and formed whole clumps (Fig. 2). Calli of all rice cultivars treated with 175-250 mM NaCl were pale yellow in color and some developed necrosis and finally died (Fig. 3).

The results showed a decrease in the viability of calli when NaCl concentrations were elevated (Fig. 4). It was clear that the percentage of surviving calli of KDML105, Suphan Buri 1 and Pathum Thani 1 were 4, 8 and 4, respectively while the callus growth of Chai Nat 1 treated with 175 mM NaCl was inhibited. The growth of rice calli of all cultivars was inhibited at 200 mM NaCl. The percentages of survival calli of KDML105, Chai Nat 1, Suphan Buri 1 and Pathum Thani 1 treated with 150 mM NaCl were 52, 52, 44 and 56, respectively. Apart from that, the percentage of callus viability was also affected by times of sub-culture. The interaction between period of culture and varied concentrations of NaCl was significantly different ($p < 0.05$) as shown in Table 2.



Fig. 1(a-d): Callus morphology of thirty-day-old calli of (a) KDML 105, (b) Chai Nat 1, (c) Suphan Buri 1 and (d) Pathum Thani 1 cultured on medium for 90 days. Bar = 5 mm

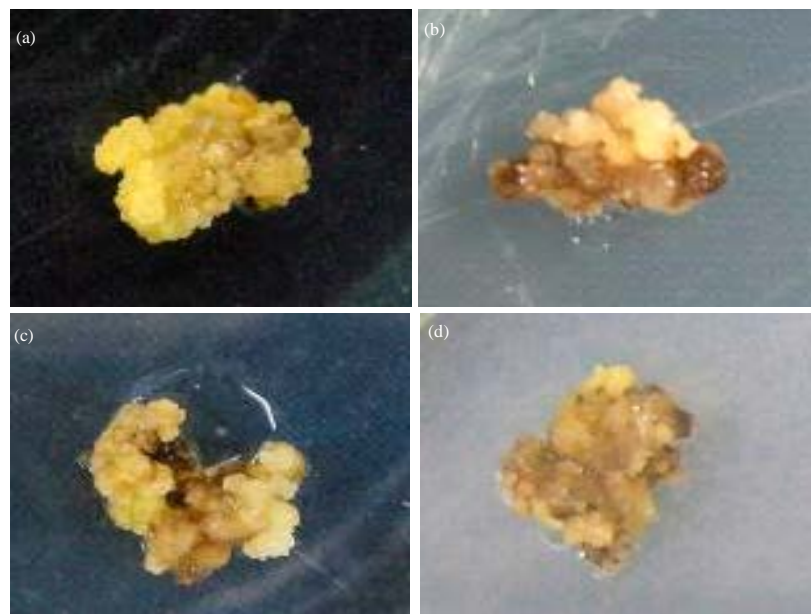


Fig. 2(a-d): Callus morphology with signs of necrosis in rice (a) KDML 105, (b) Chai Nat 1, (c) Suphan Buri 1 and (d) Pathum Thani 1 after cultured on medium supplemented with 175 mM NaCl; Bar = 5 mm

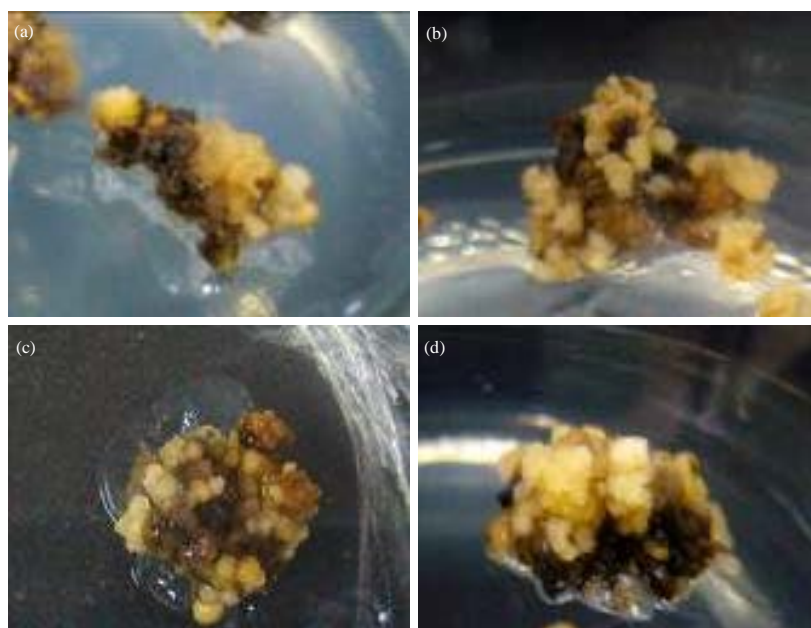


Fig. 3(a-d): Calli of the rice cultivars (a) KDML 105, (b) Chai Nat 1, (c) Suphan Buri 1 and (d) Pathum Thani 1 turned dark brown and eventually died after culture on medium supplemented with 175 mM NaCl for 150 days. Bar = 5 mm

Table 2: Analysis of variances for the percentage of survival calli (%) on medium supplemented with varied concentrations of NaCl for 60, 90, 120 and 150 days

Source of variation	Df	Mean square	F-value
Days	3	111295.15	300.55*
NaCl	10	40440.45	1084.10*
Cultivars	3	1183.63	3.48**
Days×NaCl	30	1772.81	26.25*
Days×Cultivars	9	370.30	5.48*
NaCl×Cultivars	30	37.30	0.55**
Days×NaCl×Cultivars	90	67.52	0.41**
Error	704	162.95	

*Significant at p<0.05; **Non significant at p<0.05

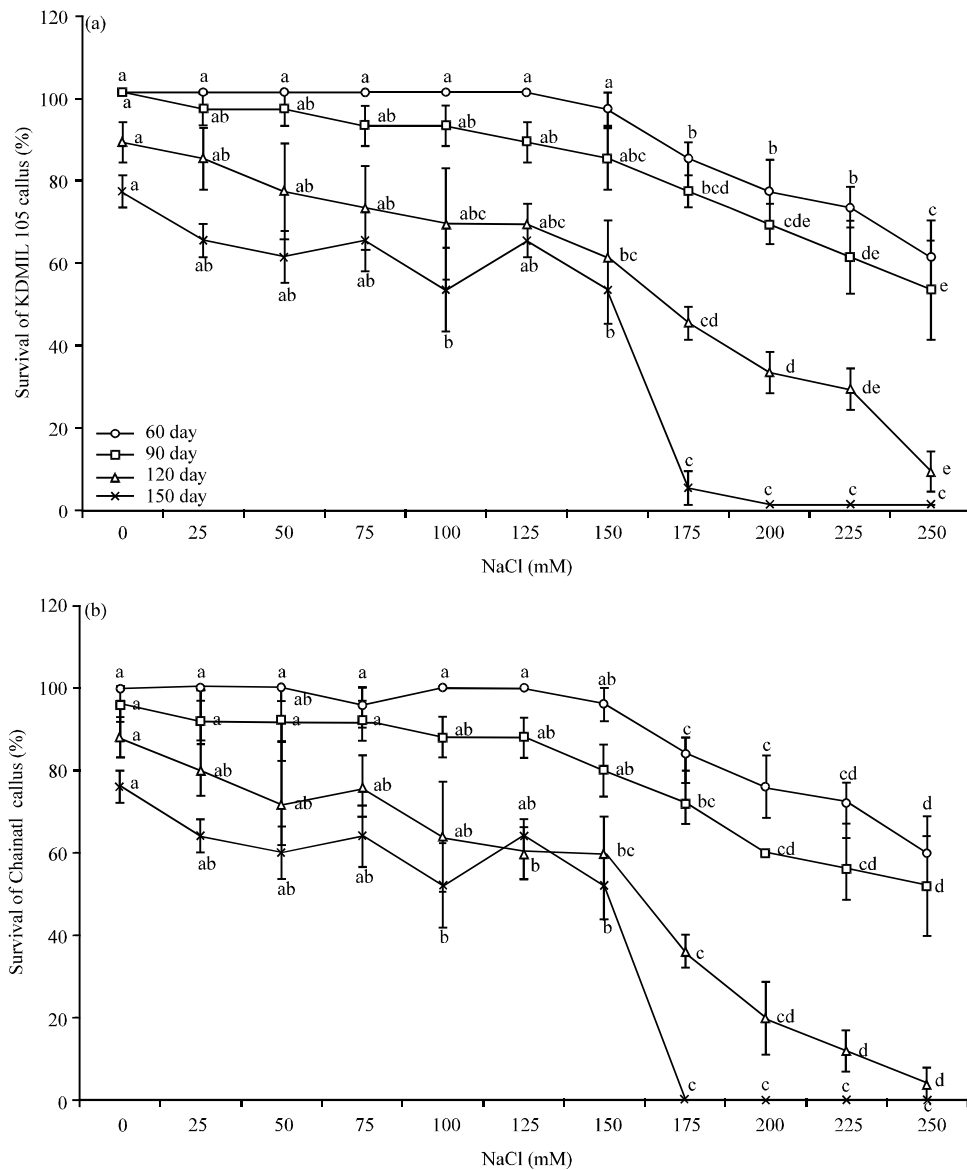


Fig. 4(a-d): Continue

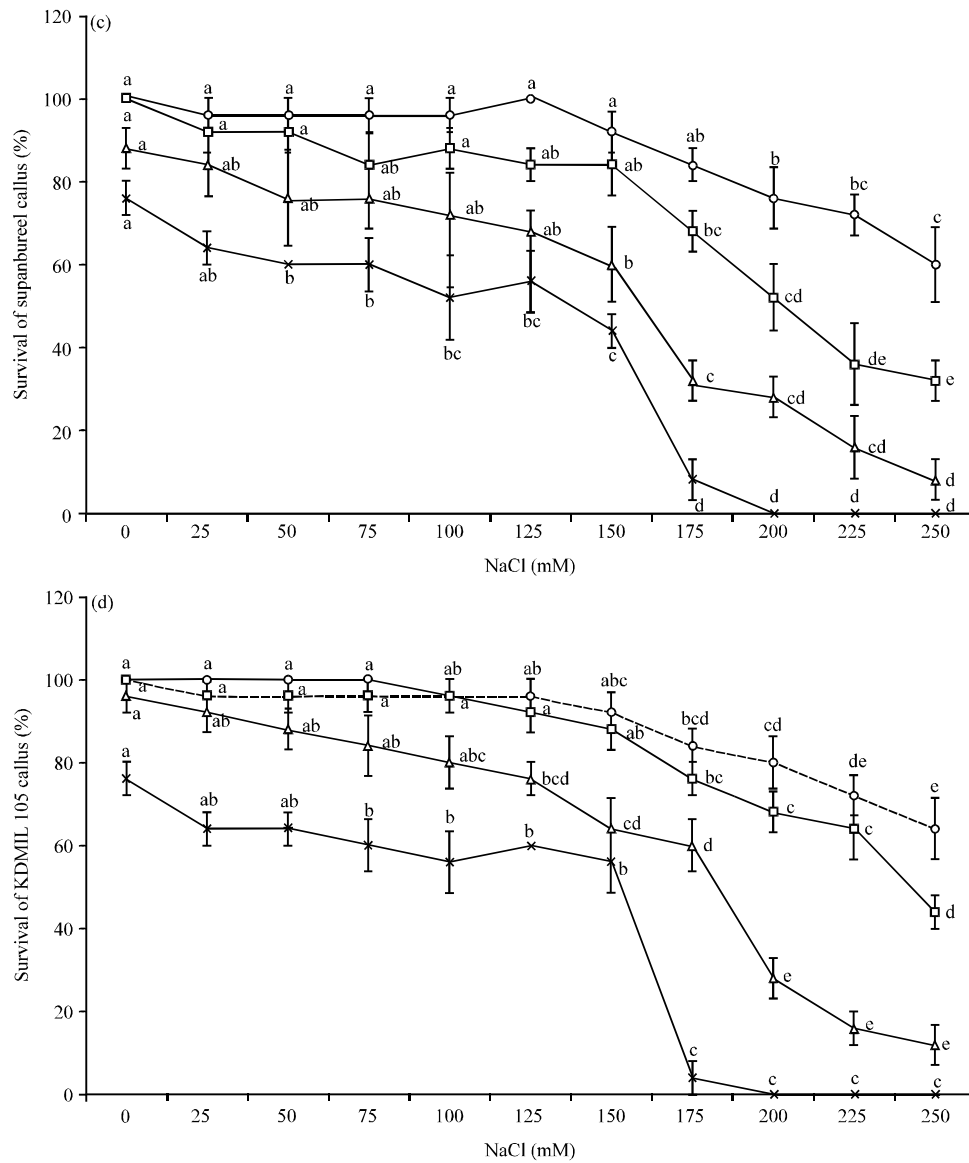


Fig. 4(a-d): Percentage of survival calli (%) in four rice cultivars, (a) KDMIL 105, (b) Chai Nat 1, (c) Suphan Buri 1 and (d) Pathum Thani 1 cultured in medium supplemented with varied concentrations of NaCl for 60, 90, 120 and 150 days. Different letters indicate the significant difference

The results showed a decrease in relative fresh weight (RFW) of calli in all rice cultivars as salt concentrations were elevated. RFWG of the rice cultivars Khao Dawk Mali 105, Chai Nat 1, Suphan Buri 1 and Pathum Thani 1 were 0.10, 0.75, 0.23 and 0.01, respectively. At the end of culture, the results showed that calli can not proliferate or survive after cultured on medium amended with 175-250 mM NaCl. Analysis of variance for relative fresh weight, as shown in Table 3, showed significant differences ($p < 0.05$) among various concentrations of NaCl.

In addition, the characteristics scoring of symptoms of injured calli of all rice cultivars cultured in medium supplemented with various concentrations of NaCl were scored and recorded. At the end

Table 3: Mean of relative fresh weight (RFW) of callus and the characteristics scoring of symptoms of injured calli in four rice cultivars under NaCl stress cultured for 150 days

NaCl (mM)	KDML105		Chai Nat 1		Suphan Buri 1		Pathum Thani 1	
	RFW	Score	RFW	Score	RFW	Score	RFW	Score
0	5.76 ^a	7.4 ^a	4.82 ^a	7.0 ^a	5.14 ^a	6.6 ^a	4.21 ^a	7.8 ^a
25	3.87 ^b	7.0 ^{ab}	3.22 ^b	6.2 ^{ab}	4.93 ^a	5.8 ^{ab}	3.29 ^{ab}	6.6 ^{ab}
50	1.78 ^c	6.6 ^{abc}	2.90 ^b	6.2 ^{ab}	3.66 ^{ab}	5.4 ^{ab}	2.33 ^{bc}	6.6 ^{ab}
75	1.60 ^c	5.8 ^{bcd}	1.76 ^c	6.2 ^{ab}	2.09 ^{bc}	5.4 ^{ab}	1.32 ^d	5.8 ^{bc}
100	1.31 ^c	5.4 ^{cd}	1.10 ^c	5.0 ^{bc}	1.03 ^c	4.2 ^{bc}	0.43 ^{de}	5.4 ^{bc}
125	0.68 ^c	4.6 ^d	0.89 ^c	4.6 ^c	0.83 ^c	4.6 ^{bc}	0.14 ^{de}	5.0 ^{bc}
150	0.10 ^f	4.6 ^d	0.75 ^c	3.8 ^c	0.23 ^c	3.4 ^d	0.01 ^e	4.6 ^c
175	ND	1.4 ^e	ND	1 ^d	ND	1.8 ^{de}	ND	2.6 ^d
200	ND	1 ^e	ND	1 ^d	ND	1 ^e	ND	1 ^d
225	ND	1 ^e	ND	1 ^d	ND	1 ^e	ND	1 ^d
250	ND	1 ^e	ND	1 ^d	ND	1 ^e	ND	1 ^d
Significance	*	*	*	*	*	*	*	*

Means followed by the same letter in each column are not significantly different using Duncan's multiple range test at 95%, Level of significance is represented by (*) at $p < 0.05$, ND: A particular value could not be determined due to callus necrosis

of selection, the finding showed that mean of scoring in the rice cultivars KDML 105, Chai Nat 1, Suphan Buri 1 and Pathum Thani 1 was 4.6, 3.8, 3.4 and 4.6, respectively. It was also found that callus necrosis and fresh weights were also affected during sub-cultured. Calli became necrosis especially in 175 mM NaCl or higher concentrations.

Effect of NaCl on plant regeneration in rice: In all cultivars, selected calli after the fourth sub-culture were transferred to MS regeneration medium supplemented with varied concentrations of NaCl (0-150 mM). The percentage of shoot regeneration decreased when NaCl concentrations increased (Fig. 5). Calli cultured on regeneration medium supplemented with 25-75 mM NaCl appeared to develop normal shoots. Selected calli cultured in the presence of 100 and 125 mM NaCl showed a slight decrease in the percentage of shoot regeneration.

Calli cultured on medium added with 150 mM NaCl showed shoot regeneration but the percentage of shoot regeneration is low. The percentages of shoot regeneration of KDML105, Chai Nat 1, Suphan Buri 1 and Pathum Thani 1 cultured on medium supplemented with 150 mM NaCl were 36, 32, 36 and 28, respectively. The average number of shoots per callus was not significantly different ($p > 0.05$) in the cultivars KDML105 and Chai Nat 1 among different concentrations of NaCl as shown in Fig. 4b. However, in the rice cultivars Suphan Buri 1 and Pathum Thani 1, it was found that the average number of shoots per callus was significantly different ($p < 0.05$). It was observed that mean of number of shoots per callus slightly decreased when NaCl concentrations were elevated. The average numbers of shoots per callus cultured on 150 mM NaCl were 4, 4, 3.4 and 3.6 in the rice cultivars KDML105, Chai Nat 1, Suphan Buri 1 and Pathum Thani 1, respectively.

It was found that calli derived from medium supplemented with 175 mM NaCl appeared green in color at the first month but failed to develop shoots. It was noted that a significant reduction of shoot regeneration was observed in calli treated with high levels of NaCl. Rooting of regenerated plants from selected calli was also observed. After 30 days of culture, survival shoots in all rice cultivars were transferred to MS medium without NaCl for root induction. It was found that shoots can produce normal roots as shown in Table 4.

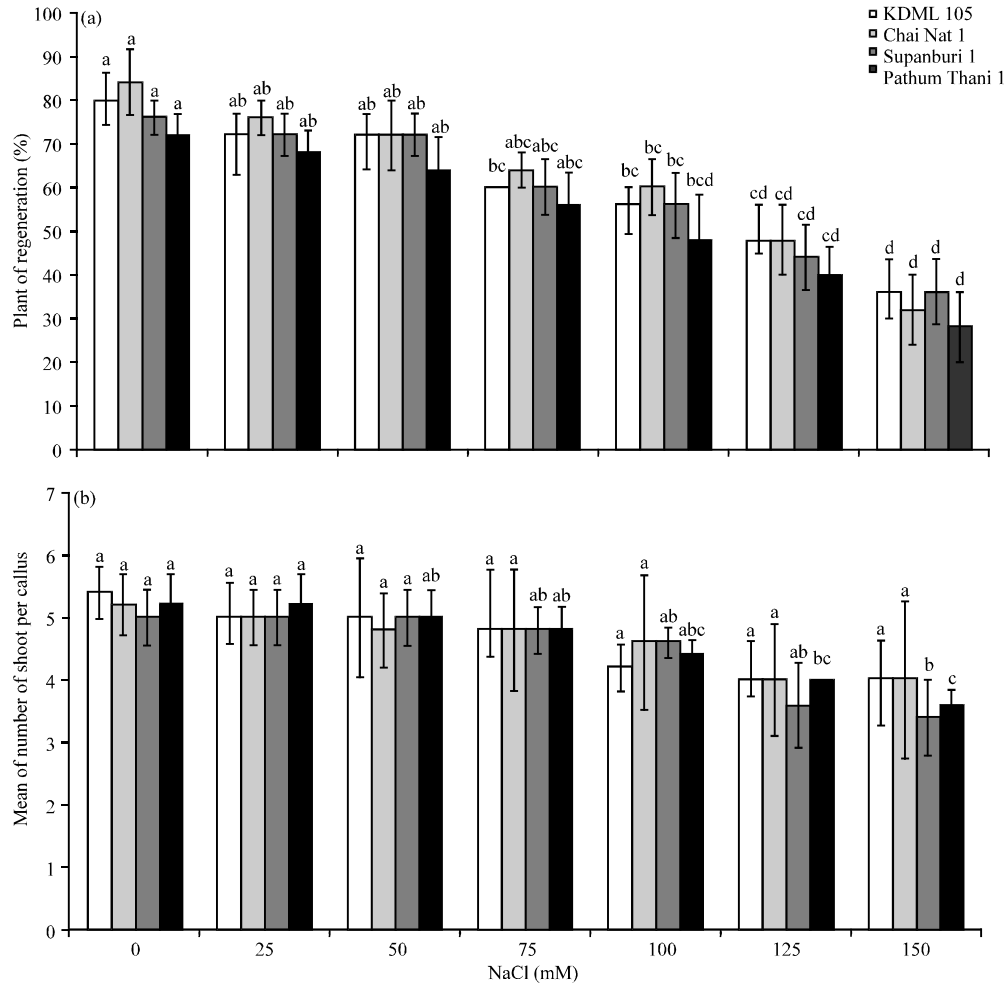


Fig. 5(a-b): Percentage of shoot regeneration of calli (a) the average number of shoots per callus and (b) in four rice cultivars cultured on MS medium supplemented with 0-150 mM NaCl. Different l

Table 4: Percentage of root formation and mean of number of roots/clump of shoots after cultured on MS medium for 30 days

Cultivars	Root formation (%)	Mean of number of roots/clump
KDML105	98 ^a	9.9 ^a
Chai Nat 1	97 ^a	9.2 ^a
Suphan Buri 1	96 ^a	8.7 ^a
Pathum Thani 1	98 ^a	9.5 ^a
Significance ^s	NS	NS

Means followed by the same letter in each column are not significantly different using Duncan's multiple range test at 95%, ^sLevel of significance is represented by (*) at p<0.05, NS: Non-significant

DISCUSSION

Responses of callus growth and shoot regeneration in rice cultivars, namely Khao Dawk Mali 105, Chai Nat 1, Suphan Buri 1 and Pathum Thani 1, were observed in the present study. The results showed that Thirty-day-old calli cultured on suitable medium were greenish-yellow and friable callus. After calli were transferred to medium supplemented with 0-250 mM NaCl, there

was a change in the characteristics and morphology of calli and the effect of NaCl stress were recorded at 60, 90, 120 and 150 days after sub-cultured. In the present study, it was found that callus browning and necrosis increased when NaCl concentrations were elevated.

The findings evidently showed that calli of all rice cultivars grew well under varied concentrations of NaCl, up to 150 mM. However, at the concentration of 175 mM, callus growth was completely inhibited. Calli grown on callus proliferation medium supplemented with NaCl were greenish-yellow but turning brown as NaCl concentrations increased. These outcomes were well-supported by Khorami and Safarnejad (2011) reporting that an increase in NaCl resulted in elevated concentration of Na⁺ and Cl⁻ in the cell cytoplasm, leading to toxicity to disturb plant growth. Badawy *et al.* (2008) found that NaCl resulted in callus necrosis and growth reduction of sugarcane. Htwe *et al.* (2011) also reported that addition of NaCl into culture medium caused an increase in callus necrosis in five rice genotypes. Wu *et al.* (2005) reported that callus browning can be an indicator of necrosis or tissue damage, or the production of stress response compounds such as phenolic compound. Likewise, Laine and David (1994) reported that browning of callus culture occurs due to oxidation of phenolic compounds by polyphenoxidase peroxidase or exposure to air. Koc *et al.* (2009) explained that many abiotic and biotic factors induce the synthesis and accumulations of phenolic compound in plant. Naz *et al.* (2008) revealed that an increase in phenolic content is normally associated with an increase in enzymes that regulate the synthesis of phenolic compound while the intensity of browning is also related with the hyperactivity of oxidative enzyme. In addition, there are also other factors that may affect the incidence of brown callus. Time and repeated sub-culture also have an effect on callus browning and necrosis. In the present study, it was found that, at the third time of sub-culture, calli cultured on 175 mM NaCl or higher concentrations turned brown and eventually died. This finding was in agreement with Koc *et al.* (2009).

For selection of salt-tolerant rice cultivars, callus appearance was scored and recorded. It was found that more than 75% of calli turned brown but some can be proliferated for salt tolerance. These results were in agreement with Babu *et al.* (2007) reporting that callus growth and score of callus growth decreased while salt concentrations were elevated. Urechean (2003) reported that 40 mM NaCl represents a shock dose for most of the callus initiated from immature embryos, resulting in browning or necrosis in maize calli. Apart from that, percentage of survival and relative fresh weight of calli in all rice cultivars also declined as NaCl concentrations trembled. Summart *et al.* (2010) reported that fresh and dry weight slightly decreased after calli were cultured under NaCl stress for six days. Ahmad *et al.* (2007) reported that relative growth rate of the calli of indica rice was significantly reduced due to an increase in NaCl concentrations. Shankhdhar *et al.* (2000) reported that the fresh weight of six cultivars of rice calli decreased with an increase of salt concentrations. Badawy *et al.* (2008) reported that relative fresh weight callus of sugarcane decreased with an increase of NaCl concentrations in culture medium. Zair *et al.* (2003) reported that growth of wheat calli also decreased with an increase of NaCl at the concentration above 2.5 g L⁻¹. In addition, Htwe *et al.* (2011) reported that addition of 150, 200 and 250 mM NaCl in culture medium inhibited the growth of calli. However, after 150 days of culture, rice calli were completely inhibited at concentrations between 175 and 250 mM.

In addition, the frequency of shoot regeneration in four rice cultivars in the present study showed that NaCl at high level reduced regenerating capacities of calli. A sudden reduction in regeneration frequency at 175 mM or over in all rice cultivars probably indicated severely damaged occurrence on cell proliferation and growth. However, Lutts *et al.* (1999) reported that the highest

dose of salt at 100 mM completely inhibited plant regeneration in rice and Htwe *et al.* (2011) reported that the concentration of NaCl at 150 mM started to significantly inhibit the growth of regeneration of rice. Kim *et al.* (1988) explained that loss of regenerative potential of rice calli is resulted from long-term culture and period of time for sub-culture. There are several reports indicating that NaCl induced a decrease in shoot regeneration in rice (Kim *et al.*, 1988; Lutts *et al.*, 1999; Htwe *et al.*, 2011). Khaleda *et al.* (2007) also reported that a decrease in shoot regeneration of deep water rice depends on concentrations of NaCl. Khorami and Safarnejad (2011) explained that NaCl has an effect on plant growth and regeneration by lowering water potential of the medium, so cultured explants are unable to take up water and nutrients from the medium. Saffan (2008) reported that the osmotic effect resulting from salinity stress may cause trouble in the water balance of the plant, including a reduction of turgor and an inhibition of growth in plants. Huang and Liu (2002) suggested that an increase in glucose content may potentially alter regeneration-related factors in rice. There are several studies regarding addition of organic compounds to increase regeneration frequency of plants under salt stress. Lutts *et al.* (1999) reported that addition of tryptophan in regeneration medium stimulated shoot regeneration and increased continuous in survival rates of plant regeneration in all rice cultivars at all NaCl concentrations (0, 50 and 100 mM).

Several factors are associated with increased survival growth of plants under *in vitro* salt stress. Malash and Khatab (2008) suggested that drought pre-treatment at seedling stage could enhance salt tolerance of adult tomatoes. Munir and Aftab (2009) reported that less necrosis of sugarcane callus was observed in PEG-pretreated calli rather than in non-pretreated calli subjected to the same salt concentrations. Barakat and Abdel-Latif (1996) reported that the stepwise method of an increase of NaCl in the medium was more effective for plant regeneration in wheat. Miki *et al.* (2001) reported that rice shoot bud clumps can grow strongly better when subjected to step up salt treatment with 0.5, 1.0, 0.15 and 2.0% NaCl at 3 weeks intervals than when cultured in a single step treatment with 1.5 or 2.0% of NaCl. Likewise, Ndayiragije and Lutts (2006) reported that application of exogenous 1 mM Putrescine can stimulate rice callus growth under salt stress in relation to a decrease in both Na⁺ and Cl⁻ accumulation.

CONCLUSION

In short, the results obtained in the present study confirm that NaCl has a profound effect on callus cultures and shoot regeneration. The percentage of survival calli, relative fresh weight, callus necrosis and the percentage of shoot regeneration decreased with increasing degrees of NaCl. The findings also suggest that somaclonal variation for salt tolerance can be applied to rice improvement in the future.

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