Research Journal of Agriculture and Biological Sciences, 5(4): 423-427, 2009

In Vitro Investigation on Salt Tolerant Characteristics of Rice Seedlings (*Oryza sativa* L.)

[1]Anotai Prajuabmon, [1]Piyada Theerakulpisut, [2]Boonyuen Kijwijan and [1]Nooduan Muangsan

[1]Genomics and Proteomics Research Group for Improvement of Salt-tolerant Rice,
[2]Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand

Abstract: Effects of salt stress on some physiological and biochemical characteristics were investigated in three rice cultivars differing in salt-tolerance ability (*Oryza sativa* L. cvs. Pokkali, Leuang Anan and KDML105). Seven-day-old rice seedlings germinated on MS medium were subjected to NaCl at concentrations of 0, 50, 100, 150 and 200 mM for 15 days. The results showed that all three cultivars of rice seedlings grown under high salinity had shoot and root length, fresh and dry weight of shoot, and relative growth rate of shoot decreased, whereas the Na^+/K^+ ratio and proline content of leaf were increased. Pokkali accumulated the lowest amount of proline whereas KDML 105 was the highest. In addition, Pokkali showed the lowest Na^+ / K^+ ratio whereas Leuang Anan was the greatest.

Key words: Salinity, Salt Tolerance, *In Vitro* Culture, Rice, Seedlings

INTRODUCTION

Salinity is considered to be the major environmental factor limiting plant yield, especially in arid and semi-arid region [19]. This leads to huge losses in term of arable land and productivity as most of the economically important crop species are very sensitive to soil salinity [13]. For adaptation to saline environment, plants have evolved internal systems to grow and develop. Reduction in growth under saline conditions is a consequence of several physiological responses, including modification of ion balance, water status, mineral nutrition, stomatal behavior, photosynthetic efficiency, and carbon allocation and utilization. Salinity also can cause progressive loss of chlorophyll content, leading to a corresponding reduction of light absorption by leaves [19]. In addition, a large number of plants accumulate proline in response to osmotic stress caused by salinity [22].

Rice (*Oryza sativa* L.) is an important crop, which more than 700 million people consume as their main food [23]. This crop, as other important crops evolving in glycophytic habitat, is moderately sensitive to salt in the field. The yield potential of many rice cultivars is largely limited due to the excess of salt in the soil, especially in the South and South-East Asia [4].In Northeastern of Thailand, 35% of land area faces varying degrees of salinity problems from the accumulation of NaCl generated by the underground salt dome resulting in low crop productivity[21]. This will require acceleration in rice production. Solving this problem will entail development of rice varieties, which have higher yields, excellent grain quality, and resistance to biotic and abiotic stresses [17].

This work focused on the effects of NaCl on seedlings of three rice cultivars grown *in vitro* culture. The tolerant, moderately tolerant, and sensitive to salinity cultivars, Pokkali, Leuang Anan, and KDML105, respectively, were selected to study physiological and biochemical characteristic changes. The data from this study will be useful for screening to select salt tolerant cultivars by using *in vitro* culture technique.

MATERIALS AND METHODS

Plant Materials and Stress Conditions: Three cultivars of rice (*O. sativa* L.), namely Pokkali (a traditional salt tolerant cultivar from India), Leuang Anan (moderately salt tolerant) and Khao Dawk Mali 105 (KDML 105; salt sensitive) were used in this study. Manually dehusked seeds of rice were surface sterilized with 70% ethanol for 3 minutes and followed with 15% Clorox for 20 minutes, then rinsed three times with sterile distilled water. Sterilized seeds were germinated on 6% agar solidified MS media [15] for 7 days, and then the nearly-size seedlings were selected and transferred to culture on MS media added NaCl at concentrations of 0 (control), 50, 100, 150 and 200 mM for 15 days. Seedlings were cultured *in vitro* under conditions 12 hours photoperiod and at 25 ± 2 °C room temperature.

Corresponding Author: Nooduan Muangsan, Assistant Professor at Department of Biology, Faculty of Science, Khon Kaen University, Thailand
E-mail: nooduan@kku.ac.th

Measurement of Length and Weight of Shoots and Roots: Fifteen days after salinization, plants were randomly sampled for morphological characteristic observation and separated into shoots and roots which were measured of length by a ruler. The fresh weight of each sample was taken, and then the samples were oven-dried at 60 °C for 72 hours for the determination of dry weight.

Measurement of Relative Growth Rate (RGR): RGR was determined by the methods outlined by Dionisio-Sese and Tobita [6]. Plants were randomly selected and separated into shoots and roots to estimate growth by dry weight measurements with the samples oven-dried at 60 °C for 72 hours. RGR was calculated from the increase in dry weight of plant at the beginning and at the end of the salt treatment, using the equation RGR = $(In\ DW_f - In\ DW_i) / (t_f - t_i)$ where DW is the sample dry weight, t is the time and subscripts denote initial and final sampling, that is, 0 and 15 days after salinity treatment.

Chlorophyll Extraction and Measurement: To determine total chlorophyll content, leaves of each plant were weighed and frozen at –80 °C. Extraction buffer, N, N'-Dimethylformamide (DMF), was added to frozen tissues in a microcentrifuge tube and placed at 4 °C in darkness for at least 48 hours. The absorption spectrum at 647 and 664.5 nm of the extracted liquid (1 ml) in a quartz cuvette using DMF as a blank was measured with a Milton Roy Spectronic 1001 Plus, UV-visible recording spectrophotometer. Chlorophyll content was calculated as follows with using extinction coefficients reported by Inskeep and Bloom [8].

Chlorophyll a (ug/g FW) = [12.70(A664.5) – 2.79(A647)] x V / W

Chlorophyll b (ug/g FW) = [20.70(A647) – 4.62(A664.5)] x V / W

Total chlorophyll (ug/g FW) = [17.90(A647) + 8.08(A664.5)] x V/W

V = leaf extract volume (ml)

W = leaf fresh weight (g)

Measurement of Proline Content: Proline content was analyzed according to the procedure of Bates et al. [1]. Approximately 0.1 g of fresh weight of shoots was homogenized in 5 ml of 3% aqueous sulfosalicylic acid and the homogenate was filtered through Whatman No. 1 filter paper. Two ml of the filtrate were reacted with 2 ml of acid ninhydrin and 2 ml of glacial acetic acid in a test tube 1 hour at 100 °C and the reaction was terminated in an ice bath about 30 minutes. The reaction mixture was extracted with 4 ml toluene, mixed vigorously with test tube stirrer for 15 second. The toluene phase containing the chromophores was aspirated and warmed to room temperature about 10 minutes. The absorbance was measured at 520 nm with a spectrophotometer (Model 340 Sequoia-Turnerl) using toluene for a blank. Purified proline was employed to standardize the procedure for quantifying sample values. The proline content was determined as standard curve and the concentration was expressed as µg/g FW.

Determination of Na^+ and K^+ concentrations: For measurement of Na^+ and K^+ concentrations, plants were washed with distilled water and oven-died for 72 hours at 60 °C. Each dried samples were weighted to obtain 0.1 g and then dried samples were ground in a mortar. The samples were sent to analyze Na^+ and K^+ concentrations at Department of Land Resources and Environment, Faculty of Agriculture, Khon Kaen University. Briefly, the samples were digested in nitric acid (HNO_3), sulfuric acid (H_2SO_4) and perchloric acid ($HClO_4$) using Flame photometry method.

Statistical Analysis: Analysis of variance (ANOVA) was performed on all measurements. Significant differences between means were determined using the Duncan's multiple range test (DMRT) at the $P \leq 0.05$ (n=5) level.

RESULTS AND DISCUSSIONS

To analyze the physiological and biochemical changes of rice to salt stress, we used an *in vitro* culture system to grow rice seedlings under various concentrations of NaCl, because this has provided the best control system. Under these levels of salt stress, 22-day-old rice seedlings had suffered morphological damage. However, they exhibited 100% survival when cultured under tissue culture system in the absence of salt stress (0 mM NaCl) and in the presence of salt stress (50, 100, 150, 200 mM NaCl), indicating that seedlings cultured under the tissue culture system responded less sensitively to salt-stress.

As shown in Table 1, the shoot and root length of in vitro seedlings of all three rice cultivars decreased with increasing of NaCl concentrations from 0 to 150 mM. At 200 mM NaCl the shoot length of both Luang Anan and KDML105 were adversely effected and only half length of that in the 0 mM NaCl control group, whereas the shoot length of Pokkali had slightly decreased. Similarly, the shoot fresh weight and dry weight of three rice cultivars showed the decrease at higher salinity levels. Slightly induction was obtained in root fresh weight and root dry weight of seedlings exposed to NaCl stress from 0 to 150 mM. At 200 mM NaCl, these parameters were reduced.

Table 1: Effects of NaCl on growth of three rice cultivars treated with NaCl at 0, 50, 100, 150 and 200 mM for 15 days.

Cultivars	Length (cm)		Fresh weight (mg)		Dry weight (mg)		Relative growth rate (RGR)	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Pokkali								
0 mM	23.56 ± 0.92 ^{ab}	4.90 ± 0.59 ^{a}	178.1 ± 13.97 ^{a}	17.1 ± 1.38 ^{a}	24.3 ± 2.08 ^{a}	4.2 ± 0.57 ^{a}	0.114 ± 0.010 ^{a}	0.052 ± 0.010 ^{b}
50 mM	22.90 ± 0.55 ^{ab}	2.98 ± 0.96 ^{a}	156.6 ± 20.03 ^{a}	13.2 ± 2.35 ^{a}	22.5 ± 2.40 ^{a}	3.9 ± 0.28 ^{a}	0.115 ± 0.005 ^{a}	0.046 ± 0.007 ^{b}
100 mM	25.04 ± 0.97 ^{a}	3.46 ± 0.86 ^{a}	153.6 ± 11.84 ^{a}	11.9 ± 1.37 ^{a}	22.4 ± 1.82 ^{a}	3.7 ± 0.30 ^{a}	0.111 ± 0.009 ^{a}	0.050 ± 0.006 ^{b}
150 mM	21.62 ± 0.53 ^{b}	0.74 ± 0.17 ^{b}	146.7 ± 5.43 ^{a}	14.9 ± 0.51 ^{a}	25.7 ± 1.04 ^{a}	4.6 ± 0.26 ^{a}	0.128 ± 0.009 ^{a}	0.078 ± 0.005 ^{a}
200 mM	18.44 ± 0.93 ^{c}	0.36 ± 0.04 ^{b}	117.4 ± 8.80 ^{a}	13.5 ± 0.88 ^{a}	20.9 ± 1.46 ^{a}	4.7 ± 0.64 ^{a}	0.110 ± 0.007 ^{a}	0.068 ± 0.006 ^{b}
Luang Anan								
0 mM	16.88 ± 0.71 ^{b}	4.26 ± 1.20 ^{a}	107.9 ± 12.00 ^{b}	20.1 ± 1.80 ^{a}	13.9 ± 1.80 ^{b}	3.2 ± 0.39 ^{b}	0.103 ± 0.016 ^{a}	0.076 ± 0.027 ^{a}
50 mM	19.56 ± 0.29 ^{a}	5.90 ± 1.19 ^{ab}	125.9 ± 5.18 ^{ab}	21.4 ± 0.90 ^{a}	16.1 ± 0.84 ^{ab}	3.5 ± 0.41 ^{b}	0.112 ± 0.004 ^{a}	0.056 ± 0.011 ^{a}
100 mM	18.64 ± 0.49 ^{a}	2.92 ± 1.21 ^{bc}	137.4 ± 8.46 ^{a}	26.3 ± 2.28 ^{a}	18.9 ± 1.11 ^{a}	4.8 ± 0.22 ^{ab}	0.117 ± 0.006 ^{a}	0.105 ± 0.011 ^{a}
150 mM	15.38 ± 0.49 ^{b}	1.02 ± 0.33 ^{c}	105.2 ± 8.57 ^{b}	30.1 ± 5.71 ^{a}	18.7 ± 1.60 ^{a}	6.7 ± 1.04 ^{a}	0.120 ± 0.009 ^{a}	0.080 ± 0.011 ^{a}
200 mM	9.04 ± 0.83 ^{c}	0.46 ± 0.12 ^{c}	73.8 ± 4.20 ^{c}	20.3 ± 3.02 ^{a}	13.4 ± 0.59 ^{b}	5.0 ± 0.68 ^{ab}	0.097 ± 0.004 ^{a}	0.076 ± 0.013 ^{a}
KDML 105								
0 mM	19.00 ± 0.76 ^{a}	0.76 ± 0.41 ^{a}	130.8 ± 9.34 ^{a}	27.7 ± 1.78 ^{b}	20.1 ± 1.41 ^{a}	5.3 ± 0.33 ^{b}	0.107 ± 0.005 ^{b}	0.081 ± 0.002 ^{ab}
50 mM	18.74 ± 0.73 ^{a}	1.38 ± 0.37 ^{a}	141.2 ± 11.07 ^{a}	23.4 ± 1.55 ^{b}	21.0 ± 1.75 ^{a}	4.8 ± 0.27 ^{b}	0.119 ± 0.004 ^{ab}	0.066 ± 0.006 ^{b}
100 mM	18.62 ± 0.40 ^{a}	0.92 ± 0.54 ^{a}	136.7 ± 10.11 ^{a}	25.8 ± 2.72 ^{b}	22.6 ± 2.16 ^{a}	5.6 ± 0.50 ^{b}	0.125 ± 0.005 ^{a}	0.075 ± 0.008 ^{b}
150 mM	14.62 ± 0.86 ^{b}	0.98 ± 0.35 ^{a}	116.8 ± 9.77 ^{a}	34.0 ± 2.00 ^{a}	20.0 ± 1.39 ^{a}	6.9 ± 0.32 ^{a}	0.111 ± 0.003 ^{b}	0.095 ± 0.003 ^{a}
200 mM	7.94 ± 0.71 ^{c}	0.20 ± 0.05 ^{a}	67.5 ± 8.24 ^{b}	22.4 ± 2.00 ^{b}	14.1 ± 1.30 ^{b}	5.8 ± 0.42 ^{b}	0.085 ± 0.002 ^{c}	0.098 ± 0.007 ^{a}

* Values are expressed as the means ± S.E. (n = 5). Different letters for each concentration indicate significant differences at 0.05 probability level as determined by Duncan's multiple range tests.

Table 2: Chlorophyll A, chlorophyll B, total chlorophyll, proline content and Na+/K+ ratio of seedlings of three rice cultivars treated with NaCl at 0, 50, 100, 150 and 200 mM for 15 days.

	Chlorophyll A (µg/g FW)	Chlorophyll B (µg/g FW)	Total Chlorophyll (µg/g FW)	Proline (µg/g FW)	Na^+/K^+ ratio
Pokkali					
0 mM	266.7 ± 93.16 ^{a}	301.3 ± 29.24 ^{a}	567.9 ± 115.77 ^{a}	45.17 ± 3.19 ^{c}	0.284
50 mM	182.0 ± 18.36 ^{a}	291.9 ± 27.73 ^{a}	473.7 ± 46.05 ^{a}	73.13 ± 11.46 ^{c}	0.526
100 mM	202.3 ± 49.68 ^{a}	288.4 ± 41.99 ^{a}	490.5 ± 91.54 ^{a}	91.51 ± 7.16 ^{c}	0.757
150 mM	173.0 ± 5.54 ^{a}	274.7 ± 6.28 ^{a}	447.5 ± 11.75 ^{a}	196.28 ± 22.69 ^{b}	0.750
200 mM	175.4 ± 13.85 ^{a}	256.0 ± 9.39 ^{a}	431.2 ± 22.97 ^{a}	381.82 ± 73.96 ^{a}	1.705
Luang Anan					
0 mM	321.8 ± 61.54 ^{a}	381.2 ± 36.67 ^{a}	702.8 ± 83.29 ^{a}	70.39 ± 13.80 ^{c}	0.321
50 mM	132.1 ± 11.30 ^{c}	210.9 ± 16.75 ^{b}	342.9 ± 28.05 ^{b}	69.45 ± 10.69 ^{c}	0.808
100 mM	174.4 ± 21.43 ^{bc}	234.0 ± 8.86 ^{b}	408.3 ± 28.84 ^{b}	92.50 ± 10.99 ^{bc}	1.272
150 mM	270.7 ± 40.10 ^{ab}	346.5 ± 58.06 ^{a}	617.0 ± 94.95 ^{a}	212.14 ± 7.92 ^{b}	1.907
200 mM	323.6 ± 60.61 ^{a}	298.4 ± 22.74 ^{ab}	621.8 ± 70.08 ^{a}	525.86 ± 92.60 ^{a}	2.659
KDML 105					
0 mM	246.1 ± 16.55 ^{a}	384.5 ± 25.56 ^{a}	630.4 ± 41.94 ^{a}	55.78 ± 2.40 ^{b}	0.312
50 mM	285.4 ± 36.59 ^{a}	389.7 ± 31.09 ^{a}	674.8 ± 67.63 ^{a}	89.04 ± 38.90 ^{b}	0.594
100 mM	256.0 ± 28.29 ^{a}	365.7 ± 26.89 ^{a}	621.6 ± 54.17 ^{a}	95.03 ± 20.93 ^{b}	0.993
150 mM	315.1 ± 18.92 ^{a}	402.1 ± 10.80 ^{a}	717.0 ± 22.17 ^{a}	190.56 ± 26.21 ^{b}	1.546
200 mM	361.7 ± 36.03 ^{a}	359.7 ± 37.87 ^{a}	721.2 ± 63.14 ^{a}	684.17±130.17 ^{a}	1.965

***** Values are expressed as the means ± S.E. (n = 5). Different letters for each concentration indicate significant differences at 0.05 probability level as determined by Duncan's multiple range tests

The relative growth rate of shoot based on dry weight of three rice cultivars decreased with increasing salt concentration in the medium. The relative growth rate of salt tolerant cultivar Pokkali was the least effected with 200 mM, while KDML105 was the most effected, suggesting that RGR of shoot correlated positively with salt tolerance ability. In the roots, RGR was quite different from that of the shoots. KDML105 showed the highest RGR of root (Table 1).

When cultured with salt stress, in vitro rice seedlings of KDML105 and Luang Anan showed slightly increase in concentrations of the pigments, chlorophyll a, chlorophyll b and total chlorophyll, whereas those were reduced in Pokkali in the presence of salt stress (Table 2).

In response to NaCl treatment, rice leaves accumulated higher amount of proline in higher salt concentrations. The leaf proline contents in Pokkali, Leuang Anan and KDML 105 were 45.17, 70.39 and 55.78 µg/g (FW), respectively, for control plants and increased to 381.82, 525.86 and 684.17 µg/g (FW), respectively, for plants treated with NaCl at 200 mM. Under high salinity treatment, KDML 105 accumulated the greatest amount of proline and Pokkali was the lowest (Table 2). Likewise, the concentration of Na^+ in all plant cultivars was dramatically increased, whereas that of K^+ decreased (data not show). Leuang Anan showed an increased accumulation with the highest values Na+/K+ ratio, whereas the most tolerant Pokkali had the lowest Na^+ /K^+ ratio (Table 2). It was clearly that NaCl caused high Na+ accumulation in the leaves of rice upon salt treatment, but the tolerant Pokkali accumulated at the lower level. This might due to its mechanism to blocking influx of Na+ or eliminate extra Na+ [2, 24].

Salinity slowed down growth of seedlings of three rice varieties. The reduction of growth under salt stress conditions has also been reported in callus [3, 16, 2] as well as in seedlings [25, 10, 18, 5]. The reduction of the growth could be due to osmotic stress as a well as salt injury [20, 14]. The accumulation of proline in plants under salt stress has been reported in many plant species [16, 12, 7, 11].

In conclusion, with increasing NaCl levels, salinity caused marked decreases in growth (shoot and root length, fresh and dry weight of shoot, and RGR of shoot), but not root dry weight and root RGR of all 3 cultivars. Pokkali was least effected than Leuang Anan and KDML105 cultivars in all NaCl concentrations when compared with the control groups. Rice leaves accumulated higher amount of proline in higher salt concentrations, Pokkali accumulated the lowest amount of proline and KDML 105 was the greatest. The Na^+ /K+ ratio in plants of all cultivars was dramatically increased. The most tolerant Pokkali showed the lowest Na+/K+ ratio similar to previous reports [21, 9], whereas Leuang Anan had the greatest Na^+ / K^+ ratio. All of these results suggest that the tissue culture system at 200 mM NaCl can be used as a system for screening salt tolerance in rice.

ACKNOWLEDGEMENTS

This work was supported by Geonomics and Proteomics Research Group for Improvement of salt-tolerant Rice, Faculty of Science, Khon Kaen University. Thanks are also for Khon Kaen Rice Research Center and Biotechnology Research and Development Office Department of Agriculture for the generous supply of rice seeds.

REFERENCES

1. Bates, L.S., R.P. Waldren and I. D. Teare, 1973. Rapid determination of free proline for water stress studies. Plant and Soil, 39: 205-207.
2. Babu, S., A. Sheeba, P. Yogameenakshi, J. Anbumalarmath and P. Rangasamy, 2007. Effects of salt stress in the selection of salt tolerant hybrids in rice (*Oryza sativa* L.) under in vitro and in vivo condition. Asian Journal of Plant Science, 6(1): 137-142.
3. Binh, D.Q., F. Fábián and L.E. Heszky, 1993. Responses to continuous and discontinuous NaCl stress of long-term cultured rice (Oryza sativa L.) cells. Acta Bio Hung, 44(2-3): 197-210.
4. Bihn, D.Q., L.E. Hesky and I. Simon-Kiss, 1994. *In vitro* studied on salt tolerance in rice (*Oryza sativa* L.). Cashier Option Méditerranéenes, 8: 53-54.
5. Cha-um, S., K. Supaibulwatana and C. Kirdmanee, 2005. Phenotypic responses of Thai jasmine rice to salt-stress under environmental control of in-vitro photoautotrophic system. Asian Journal of Plant Science, 4: 85-89.
6. Dionisio-Sese, M.L. and S. Tobita, 1998. Antioxidant responses of rice seedlings to salinity stress. Plant Science, 135: 1-9.
7. Igarashi, Y., Y. Yoshika, Y. Sanada, K. Yamaguchi-Shinozaki, K. Wada and K. Shinozaki, 1997. Characterization of the gene for delta1-pyrroline-5-carboxylate synthetase and correlation between the expression of the gene and salt tolerance in Oryza sativa L. Plant Mol Biol., 33: 857–865.
8. Inskeep, W.P. and P.R. Bloom, 1985. Extinction coefficients of chlorophyll a and b in N,N-dimethylformamide and 80% acetone. Plant Physiology, 77: 483-485.

9. Kong-ngnen, K., P. Theerakulpisut, S. Bunnag and M. Kosittrakun, 2002. Salt tolerance in rice: Glasshouse screening, field experiment and salt-induced polypeptides. KKU Research Journal (Graduate Student Issue), 1(2): 26-32.
10. Lee, K.S., W.Y. Choi, J.C. Ko, T.S. Kim and G.B. Gregorio, 2003. Salinity tolerance of japonica and indica rice (*Oryza sativa* L.) at the seedling stage. Planta, 216(6): 1043-6.
11. Lutts, S., V. Majerus and J.M. Kinet, 1999. NaCl effects on praline metabolism in rice (*Oryza sativa*) seedlings. Physiologia Plantarum, 205: 450-458.
12. Madan, S., H.S. Nainawatee, R.K. Jain and J.B. Chodhury, 1995. Proline and praline metabolizing enzymes in vitro selected NaCl-tolerant Brassica juncea L. under salt stress. Annals of Botany, 76: 51-57.
13. Mahajan, S. and N. Tuteja, 2005. Cold, salinity and drought stress: An overview. Archives of Biochemistry and Biophysics, 444: 139-158.
14. Munns, R. and M. Tester, 2008. Mechanisms of salinity tolerance. Annu. Rev. Plant Biol., 59: 651-81.
15. Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum, 15(3): 473–497.
16. Sabu, A., T.E. Sheeja and P. Nambisan, 1995. Comparison of praline accumulation in callus and seedlings of two *Oryza sativa* L. differing in salt tolerance. Indian Journal of Experimental Biology, 33: 139-141.
17. Saharan, V., R.C. Yadav, N.R. Yadav and K. Ram, 2004. Studies on improved Agrobacterium-mediated transformation in two *indica* rice (*Oryza sativa* L.). African Journal of Biotechnology, 3(11): 572-575.
18. Shereen, A., S. Mumtaz, S. Raza, M.A. Khan and S. Solargi, 2005. Salinity effects on seedlings growth and yield components of different inbred rice lines. Pakistan Journal of Botany, 37(1): 131-139.
19. Šiler, B., D. Mišić, B. Filipović, Z. Porović, T. Cvetić and A. Mijović, 2007. Effect of salinity on in vitro growth and Photosynthesis of common cemtuaty (*Centaurium erythraea* Rafn.). Archives Biological Science, 59(2): 129-134.
20. Tavakkoli, E., P. Rengasamy and G. McDonald, 2008. A critical analysis of osmotic and ionic effects of salinity in two barley cultivars. In the Proceedings of the 14th Australian Agronomy Conference, Adelaide South Australia.
21. Theerakulpisut, P., S. Bunnag and K. Kong-ngern, 2005. Genetic diversity, salinity tolerance and physiological response to NaCl of six rice (*Oryza sativa* L.) cultivars. Asian Journal of Plant Science, 4(6): 562-573.
22. Tsai, Y.C., C.Y. Hong, L.F. Liu and C.H. Kao, 2004. Relative importance of Na^+ and Cl^- in $NaCl^-$ induced antioxidant system in roots of rice seedling. Physiologia Plantarum, 122: 86-94.
23. Wanichananan, P., C. Kirdmanee and C. Vutiyano, 2003. Effect of salinity on biochemical and physiological characteristics in correlation to selection of salt tolerance in Aromatic rice (*Oryza sativa* L.). ScienceAsia, 29: 333-33.
24. Yokoi, S., F.J. Quintero, B. Cubero, M.T. Ruiz, R.A. Bressan, P.M. Hasegawa and J.M. Pardo, 2002. Differential expression and function of Arabidopsis thaliana NHX Na+/H+ antiporters in the salt stress response. Plant Journal, 30: 529–539.
25. Zeng, L. and M. Shanon, 2000. Effects on seedling growth and yield components of Rice. Crop Science, 40: 996-1003.