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Effects of NaCl Stress on Callus Induction and Plant Regeneration from Mature Seeds of Rice (*Oryza sativa* L.)

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ABSTRACT

Two commercial *indica* rice varieties, PAU 201 and PR 116, exhibited appreciable callus induction *in vitro* but the capacity to induce and regenerate callus decreased under salinity stress in both when exposed to sodium chloride (NaCl) at 90 mM. Callus was induced on semi-solid MS medium supplemented with 2.5 mg/l 2,4-dichlorophenoxyacetic acid + 0.5 mg/l kinetin + 560 mg/l proline + 30 g/l sucrose + 8 g/l agar. Callus developed shoots on MS medium supplemented with 2.0 mg/l benzylamino purine + 0.5 mg/l kinetin + 0.5 mg/l α-naphthalene acetic acid + 30 g/l sucrose + 8 g/l agar. The results indicated the need to optimize the protocol for callus induction, maintenance and regeneration before selection process for tolerance to salinity.

Keywords: *Oryza sativa*, *in vitro* screening, callus induction, plant regeneration, salinity

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important staple food crops in the world. More than 90% of rice is cultivated and consumed in Asia. Rice is an important crop, consumed by more than 700 million people as their main food (Bennet 2001). Salinity is considered to be a major environmental factor limiting plant yield (Flowers and Flowers 2005). It is a widespread soil problem in rice-growing countries (Shaheen and Hood-Nowotny 2008). High salt concentrations in the soil of the root zone limit the productivity of nearly 953 mha of productive land in the world (Singh 2009). 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 (Theerakulpisut *et al*. 2005). Moreover, salinity is responsible for degradation of 2 million ha of world agricultural lands every year (Cicek and Cakirlar 2008). Asia has the second largest area under salinity in the world with 6.73 m ha area under Salinity and sodicity in India (Singh 2009). The production and cultivation areas of rice are greatly menaced by soil salinity (Zhang *et al.* 1995). Rice is considered as moderately tolerant to salinity (Shaheen and Hood-Nowotny 2008). Rice growth and development are reduced when exposed to Salinity stress (Cha-um *et al.* 2007; Morsy *et al.* 2007). Rice production in saline soils could be increased considerably if salt-tolerant varieties were to be developed.

Plant tissue culture plays an important role in the production of agricultural and horticultural plants and in the manipulation of plants for improved agronomic performance. *In vitro* culture of plant cells and tissues has attracted considerable interest in recent years because it provides the means to study the physiological and genetic processes of plants in addition to offering the potential to assist in breeding improved cultivars increasing their genetic variability. Regenerated plants are expected to have the same genotype as the donor plant; however, in some cases, somaclonal variants are found among regenerated plants, e.g. in rice (Lutts *et al*. 2001; Araújo and Prabhu 2004; Rasheed *et al*. 2005; Elanchezhian and Mandal 2007). The composition of the medium, mainly the hormonal balance, is another important factor influencing *in vitro* culture initiation and plant regeneration from somatic embryos (Jiang *et al.* 1998). The auxin 2,4-dichlorophenoxyacetic acid (2,4-D), alone or in combination with cytokinins, is widely used to enhance callus induction and maintenance (Castillo *et al.* 1998). Genetic factors are considered to be a major contributor to the *in vitro* response of cultured tissues. Differences in the production of embryogenic calli and regenerated plantlets have been observed, depending on the genotype and explant source (Ganeshan *et al.* 2003). Salinity is the main abiotic stress that can be addressed by *in vitro* selection and the application to other stresses such as heat and drought has also been reported (Lutts *et al.* 1996). These techniques are considered to be an important complement to classical breeding methods (Zalc *et al.* 2004). Many experiments have been conducted for *in vitro* selection of Salinity-tolerant plants, including wheat (Benderradji *et al.* 2007), sugarcane (Mallikarjun *et al*. 2008) and rice (Aditya and Baker 2006; Prajuabmam *et al.* 2009). *In vitro* selection for tolerance to abiotic stress is dependent on the development of efficient and reliable callus induction and plant regeneration systems.

The aim of this study was to evaluate *in vitro* conditions for callus induction and plant regeneration of two commercial rice varieties PAU 201 and PR 116. Another aim was to screen both rice varieties for their inherent tolerance against salinity.

MATERIALS AND METHODS

The study was conducted in the plant tissue culture laboratory, School of Agricultural Biotechnology, PAU, Ludhiana, India. Seeds of commercial *indica* rice varieties PAU 201 and PR 116 were provided by the rice section of the Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. Manually dehusked seeds were treated with 0.1% bavistin (BASF, India) for 3 h followed by three washes with sterile distilled water (SDW). They were then surface sterilized in 0.1% mercuric chloride (Hi-media, Mumbai, India) for 8-10 min followed by three

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rinses in SDW under a laminar air flow cabinet. Sterilized seeds were cultured in Petri dishes containing semi-solid MS (Murashige and Skoog 1962) medium supplemented with 2.5 mg/l 2,4-D + 0.5 mg/l kinetin (Kin) + 560 mg/l proline + 30 g/l sucrose + 8 g/l agar, all reagents were supplied by Hi-media. The Petri dishes were sealed with parafilm (Hi-media) and placed in a growth chamber in the dark at 25 ± 2°C. After 4 weeks of incubation, the induced callus was subcultured, under the same growth conditions, and onto the same MS medium to which various concentrations (0, 30, 60 90 and 150 mM) of NaCl (Hi-media) were added; the incubation period lasted 2 cycles of 2 weeks each. Resulting callus was excised and transferred into test tubes containing MS medium supplemented with 2.0 mg/l benzylamino purine (BAP) + 0.5 mg/l Kin + 0.5 mg/l α-naphthalene acetic acid (NAA) + 30 g/l sucrose + 8 g/l agar 30 g/l, for shoot initiation, over a 4-week period. Rooting was initiated on half-strength MS medium free of plant growth regulators (PGRs).

The test tubes were placed in a growth chamber under fluorescent light at 5000 Lux and an ambient temperature of 25 ± 2°C. The medium was changed every 15 days and after this period, callus with clearly differentiated shoots was scored as regenerating callus. Each piece of regenerating callus was counted as 1 regardless of the number of shoots. The regenerating calli forming shoots and roots were transferred onto PGR-free basal MS medium and placed in an illuminated chamber to sustain the growth of regenerated plantlets. The pH of all media was adjusted to 5.8 with 0.1 N NaOH (Hi-media) prior to autoclaving. The culture medium was autoclaved at 121°C for 30 min. All reagents used were of analytical grade.

Data was obtained for callus induction efficiency, measured as the number of seeds forming callus/total number of seeds cultured × 100. The difference in callus fresh weight was recorded after NaCl stress (2 subcultures of 2 weeks each i.e., 4 weeks). Similarly, the percentage plant regeneration (number of plantlets/total number of calli) × 100 after NaCl treatment was also recorded (2 subcultures of 4 weeks each i.e., 8 weeks).

For callus induction, 300 seeds were used for one experiment and 10 experiments were conducted. Similarly for shoot regeneration an equal mass of embryogenic calli was placed on regeneration medium. For NaCl treatment the difference in callus fresh weight was repeated 3 times. Statistical analysis was done according to CPCS-1 package representing all data as the mean and standard error.

RESULTS AND DISCUSSION

Genotypic callus induction capacity

In order to screen the rice varieties PAU 201 and PR 116 for salinity tolerance *in vitro*, callus induction and regeneration ability of both varieties was investigated. Callus induction from mature seeds was assessed. Further, the response of calli on elevated levels of NaCl was recorded as fresh weight. The response of regenerating callus to NaCl stress was also observed. The percentage callus induction was 44.44 and 53.81%, respectively for PAU 201 and PR 116. PAU 201 appeared to be less responsive to callus induction than PR 116. Embryogenic and non-embryogenic callus formation as well as plant regeneration showed genotypic differences in some cereals, including rice (Khanna and Raina 1998; Hoque and Mansfield 2004; Khalequzzaman *et al.* 2005), wheat (Özgen *et al.* 1998) and barley (Lührs and Lörz 1987). Likewise, in rice, a significant difference in callus induction was found among different genotypes of *indica* rice (Abe and Futsuhara 1986; Peng and Hodges 1989; Seraj *et al.* 1997; Niroula and Bimb 2009). 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 (Hiei *et al.* 1994; Rashid *et al.* 1996; Ikram-ul-Haq *et al*. 2009). In the present study the variation noted in callus induction capacity appears to be mainly due to a genotypic effect, although more cultivars would have to be tested to firmly confirm this hypothesis.

Callus proliferation and plant regeneration response to NaCl stress

The main aim of this experiment was to check the inherent capacity of two rice varieties, PAU 201 and PR 116, to tolerate salinity stress. Both varieties showed a similar response under salt stress conditions. Approximately 100 mg of one-month-old callus was exposed to 5 different concentrations of NaCl. Calli that grew on control medium proliferated normally (i.e. no decrease in callus fresh weight). As the concentration of NaCl in the medium increased, callus fresh weight decreased (**Figs. 1, 3**). In PAU 201 at 90 mM NaCl the fresh weight was 111.23 mg vs. 194.24 mg at 0 mM NaCl and in PR 116 it was 118.26 mg vs. 198.66 mg at 0 mM NaCl. Thus 90 mM resulted in a 42.7 and 40.3% reduction in callus fresh weight of PAU 201 and in PR 116 respectively, compared to the control i.e. non-stressed condition. Furthermore, at higher levels of salt stress, i.e. 150 mM, there was a sharp decrease in fresh weight, reaching 83.68 mg in PAU 201 and 87.82 mg in PR 116. Saline soil contains sufficient salts in the root zone to impair the growth of crop plants. The dominant ion species in salt-affected soils are Na, Ca, Cl, SO_4 and HCO_3. Of these NaCl is the most predominant (Akbar and Ponnamperuma 1982). There is a direct and inseparable relation between the salt and water stress. Since the addition of a salt to water lowers its osmotic potential, the salt stress must expose the plant to a secondary osmotic stress. If a plant or a plant part is transferred from low to high salt medium, it is immediately subjected to osmotic dehydration. Even the leaves of higher plants show a decrease in osmotic (and therefore water) potential although only the roots are in contact with the salt (Kirst 1977). This osmotic dehydration may be the immediate cause of salt injury,

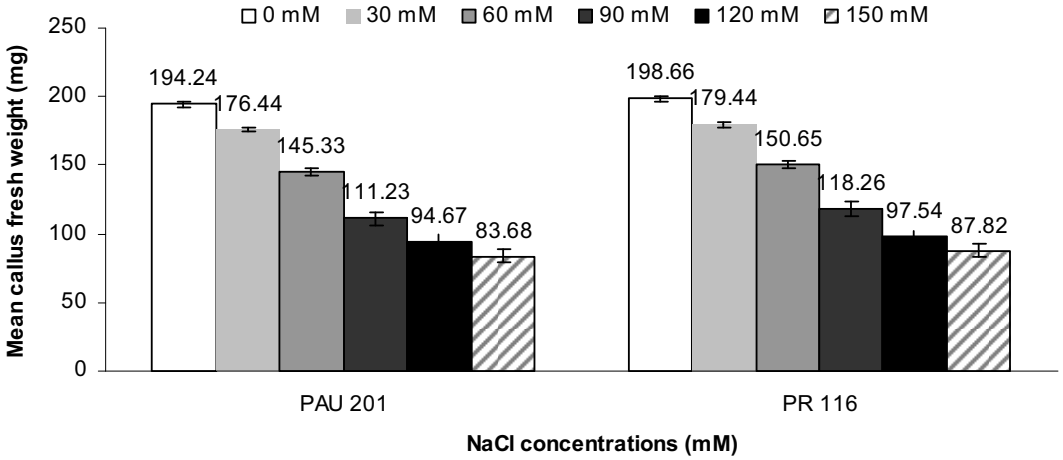

Fig. 1 Decrease in mean callus fresh weight with increase in NaCl concentration.

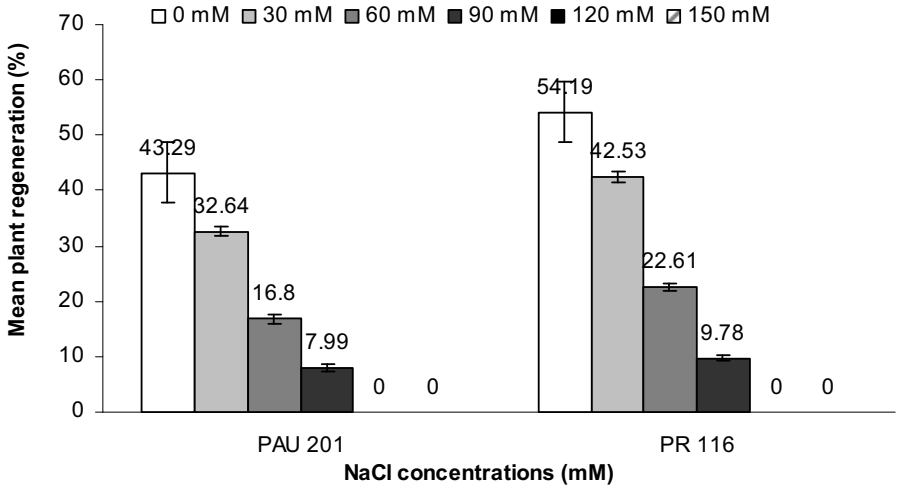

Fig. 2 Decrease in mean plant regeneration percent with increase in NaCl concentration.

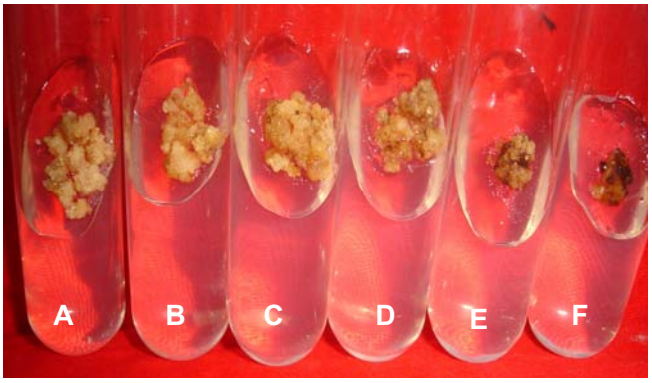

Fig. 3 *In vitro* screening of rice callus for salt stress using different concentrations of NaCl. (**A**) Control, (**B**) 30 mM NaCl, (**C**) 60 mM NaCl, (**D**) 90 mM NaCl, (**E**) 120 mM NaCl, (**F**) 150 mM NaCl.

such as the depression of growth and yield in rice by high salt concentrations regardless of the source, i.e. whether it was NaCl, $CaCl_2$, or Na_2SO_4 (Ehrler 1960). NaCl is an ionic and penetrating stress agent and produces specific ionic toxicities in plant culture studies (Murillo-Amador *et al.* 2006; Aqeel Ahmad *et al.* 2007; Mokhberdoran *et al.* 2009; Daneshmand *et al.* 2010). In addition, it can be easily taken up by the cultured cells and can cause ionic as well as osmotic effects (Turner and Jones 1980; Neumann 1997). *In vitro* studies on salt tolerance were also carried out with callus and seedlings of two *indica* rice varieties, CSR27 (salt-tolerant) and HBC19 (salt-sensitive) (Thach and Pant 1999). Calli of both varieties were transferred to salinized MS medium containing 0 (control), 0.5, 1.0, 1.5 and 2.0% NaCl (w/v) for 4 weeks. 14-day old seedlings were also exposed to salt treatment in half-strength MS salts solution with 0 (control), 0.25, 0.50, 0.75, 1.0 and 1.50% NaCl (w/v) for 1 week (Thach and Pant 1999). Further, Aditya and Baker (2006) studied the selection of salt-tolerant somaclones from four Bangladeshi *indica* rice genotypes through *in vitro* and *ex vitro* NaCl stress applied step-wise and non-step-wise. Caulogenesis was initiated under 4 different levels of non-step-wise NaCl stress (50, 100, 150 and 200 mM) and subsequent plant regeneration was observed under the same levels of NaCl stress. Rus *et al.* (2000) reported a decrease in the relative growth rate and water content of calli proliferated in saline medium compared to calli incubated in salt-free medium. Abebe *et al.* (2003) observed a 37% reduction in the growth of calli in the presence of 100 mM NaCl stress.

In order to check the efficiency of callus regeneration in the presence of salt stress, calli were exposed to elevated levels of NaCl by placing salt directly in the regeneration medium. This experiment was done to check the inherent capacity of control calli to regenerate on medium containing salt. One-month-old calli were grown on plant regeneration medium supplemented with 5 concentrations of NaCl for two cycles of 2 weeks each. Plants regenerated normally in non-stressed medium but as the concentration of NaCl in the medium increased there was a concomitant decrease in the percentage plant regeneration for both PAU 201 and PR 116 (**Fig. 2**). The percentage plant regeneration was 43.29% at 0 mM NaCl decreasing to 7.99% at 90 mM NaCl and reaching 0% at 120 mM for PAU 201. In PR 116, the percentage plant regeneration on non-stressed medium was 54.19% decreasing to 9.78% at 90 mM NaCl; there was no regeneration at 120 mM NaCl. Therefore 90 mM NaCl stress was taken to be as the new standard for screening transgenic calli for plant regeneration. In an *in vitro* experiment with bread wheat (*Triticum aestivum* L.) cv. 'Mahon Demias' and 'Hidhab' there was appreciable callus induction but significantly differences in the capacity of both to proliferate callus and regenerate under salinity stress, even though 'Mahon Demias' appeared to be more tolerant than 'Hidhab' (Benderradji *et al.* 2007). The efficiency of callus proliferation differed significantly between these two bread wheat genotypes at each of the tested salt stress levels. 'Mahon Demias' was not affected by 5 g/l NaCl-salt while 'Hidhab' was. At higher salt stress levels 'Mahon Demias' reacted moderately, while 'Hidhab' showed a sharp decrease in the callus proliferation capacity, reaching 25.0% at 15 g/l NaCl. 'Mahon Demias' showed a curvilinear response to the level of salt stress, while the response of 'Hidhab' was linear. González *et al.* (2001) mentioned that NaCl inhibited *Triticum* regeneration. In tomato, a positive correlation between the salt response at the cellular and whole plant levels was found when calli were used (Rus *et al.* 1999). In a recent study by Prajuabmom *et al.* (2009), 7-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 decreased shoot and root length, fresh and dry weight of shoots, and a relatively lower shoot growth rate.

Salinity is considered as a major factor limiting plant development and crop productivity and salinization continues to increase particularly in arid and semi-arid regions. Salinity tolerance is a polygenic trait difficult to select in classical breeding procedures under field conditions (Richards 1996). Alternative strategies include the regeneration of plants with improved salt tolerance after *in vitro* selection of salt-tolerant cells and/or construction of transgenic plants over-expressing genes expected to increase salt tolerance (Bohnert and Jensen 1996; Wincov 1996; Abebe *et al.* 2003). *In vitro* tissue culture is an important means to improve crop tolerance and yield through genetic transfor-

mation as well as induction of somaclonal variation. So it is important to devise an efficient protocol of callus proliferation in order to start *in vitro* selection for salt and drought stress tolerance and to broaden the opportunities for genetic manipulation of rice through tissue culture, including trying various explants and media.

The results of the present study indicated that two rice varieties PAU 201 and PR 116 have good callus induction ability. This experiment gave us an idea about the inherent tolerance of rice genotypes for salt tolerance. We have transferred the *Osgly II* gene which encodes for the glyoxalase II enzyme, into commercial rice variety PAU 201 (unpublished data) and next we will check the transgenic calli and plantlets for tolerance to salt stress. The results of this study will serve as a base for future screening experiments of transgenic plant material.

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REFERENCES

Abe T, Futsuhara Y (1986) Genotypic variability for callus formation and plant regeneration in rice. *Theoretical and Applied Genetics* **72**, 3-10

Abebe T, Guenzi AC, Martin B Cushman JC (2003) Tolerance of mannitol-accumulating transgenic wheat to water stress and salinity. *Plant Physiology* **131**, 1748-1755

Aditya TL, Baker DA (2006) Selection of salt tolerant somaclones from *indica* rice through continuous *in vitro* and *ex vitro* sodium chloride stress. *Indian Journal of Plant Physiology* **11**, 349-357

Akbar M, Ponnamperuma FN (1982) Saline soil of south and southeast Asia – Potential rice cultivars. In: *Proceedings of the 3rd International Symposium on Genetic Aspects of Plant Mineral Nutrition*, Braunschweig, Germany, 37 pp

Aqeel Ahmad MS, Javed F, Ashraf M (2007) Iso-osmotic effect of NaCl and PEG on growth, cations and free proline accumulation in callus tissue of two *indica* rice (*Oryza sativa* L.) genotypes. *Plant Growth Regulation* **53**, 53-63

Araújo LG, Prabhu AS (2004) Partial resistance to blast in somaclones of rice cultivar CICA-8. *Fitopatologia Brasileira* **29**, 394-398

Benderradji L, Bouzerzou H, Djekoun A, Yekhlef N, Benmahammed A (2007) Effects of NaCl stress on callus proliferation and plant regeneration from mature embryos of bread wheat (*Triticum aestivum* L.) cultivars Mahon Demias and Hidhab. *Plant Tissue Culture and Biotechnology* **17**, 19-27

Bennet J (2001) Significance of abiotic stress in agriculture. In: *JIRCAS Workshop on Genetic Engineering of Crops Plants for Abiotic Stress*, 5-7 September 2001, Bangkok, Thailand

Bohnert HJ, Jensen RG (1996) Metabolic engineering for increased salt tolerance – The next step. *Australian Journal of Plant Physiology* **23**, 61-666

Castillo AM, Egan B, Sanz JM, Cistue L (1998) Somatic embryogenesis and plant regeneration from barley cultivars grown in Spain. *Plant Cell Reports* **17**, 902-906

Cha-um S, Supaibulwatana K, Kirdmanee C (2007) Glycinebetaine accumulation, physiological characterizations and growth efficiency in salt-tolerant and salt-sensitive lines of *indica* rice (*Oryza sativa* L. ssp. *indica*) in response to salt stress. *Journal of Agronomy and Crop Science* **193**, 157-166

Cicek N, Cakirlar H (2008) Effects of salt stress on some physiological and photosynthetic parameters at three different temperatures in six soybean (*Glycine max* L. Merr.) cultivars. *Journal of Agronomy and Crop Science* **194**, 34-46

Daneshmand M, Arvin MJ, Kalantari KM (2010) Physiological responses to NaCl stress in three wild species of potato *in vitro*. *Acta Physiologiae Plantarum* **32**, 91-101

Ehrler W (1960) Some effects of salinity on rice. *Botanical Gazette* **122**, 102-104

Elanchezhian R, Mandal AB (2007) Growth analysis of somaclones generated from a salt-tolerant traditional 'Pokkali' rice (*Oryza sativa*). *Indian Journal of Agricultural Sciences* **77**, 184-187

Flowers TJ, Flowers SA (2005) Why does salinity pose such a difficult problem for plant breeders? *Agricultural Water Management* **78**, 15-24

Ganeshan S, Baga M, Harwey BL, Rossnagel BG, Scoles GJ, Chibbar RN (2003) Production of multiple shoots from thiadiazuron-treated mature embryos and leaf-base/apical meristems of barley (*Hordeum vulgare* L.). *Pant Cell, Tissue and Organ Culture* **73**, 57-64

González JM, Friero E, Jouve N (2001) Influence of genotype and culture medium on callus formation and plant regeneration from immature embryos of *Triticum turgidum* Desf. cultivars. *Plant Breeding* **120**, 513-517

Hiei Y, Ohta S, Komari T, Kumashiro T (1994) Efficient transformation of rice (*Oryza sativa* L.) mediated by *Agrobacterium* and sequence analysis of the boundaries of the T-DNA. *Plant Journal* **6**, 271-282

Hoque EH, Mansfield JW (2004) Effect of genotype and explant age on callus induction and subsequent plant regeneration from root-derived callus of *indica* rice genotypes. *Plant Cell, Tissue and Organ Culture* **78**, 217-223

Ikram-ul-Haq, Zhao CX, Zahid M, Cheruth AJ, Mohamed MA (2009) Effect of physical desiccation on plant regeneration efficiency in rice (*Oryza sativa* L.) variety super basmati. *Journal of Plant Physiology* **166**, 1568-1575

Jiang W, Cho MJ, Lemaux PG (1998) Improved callus quality and prolonged regenerability in model and recalcitrant barley (*Hordeum vulgare* L.) cultivars. *Plant Biotechnology* **15**, 63-69

Khalequzzaman M, Haq N, Hoque ME, Aditya TL (2005) Regeneration efficiency and genotypic effect of 15 *indica* type Bangladeshi rice (*Oryza sativa* L.) landraces. *Plant Tissue Culture* **15**, 33-42

Khanna HK, Raina SK (1998) Genotype × culture media interaction effects on regeneration response of three *Indica* rice cultivars. *Plant Cell, Tissue and Organ Culture* **52**, 145-153

Kirst GO (1977) Coordination of ionic relations and mannitol concentrations in the euryhaline alga *Platymonas subcordiformis* after osmotic shocks. *Planta* **135**, 69-75

Lührs R, Lörz H (1987) Plant regeneration *in vitro* from embryogenic cultures of spring- and winter-type barley (*Hordeum vulgare* L.) varieties. *Theoretical and Applied Genetics* **75**, 16-25

Lutts S, Kinet JM, Bouharmont J (1996) Effects of salt stress on growth, mineral nutrition and proline accumulation in relation to osmotic adjustment in rice (*Oryza sativa* L.) cultivars differing in salinity resistance. *Plant Growth Regulation* **19**, 207-218

Lutts S, Kinet JM, Bouharmont J (2001) Somaclonal variation in rice after two successive cycles of mature embryo derived callus culture in the presence of NaCl. *Biologia Plantarum* **44**, 489-495

Mallikarjun K, Hanchinal RR, Nadaf HL (2008) Ethyl Methane Sulphonate (EMS) induced mutation and selection for salt tolerance in sugarcane *in vitro*. *Indian Journal of Plant Physiology* **13**, 44-48

Mokhberdoran F, Nabavi Kalat SM, Sadrabadi Haghighi R (2009) Effect of temperature, iso-osmotic concentrations of NaCl and PEG agents on germination and some seedling growth yield components in rice (*Oryza sativa* L.). *Asian Journal of Plant Sciences* **8**, 409-416

Morsy MR, Jouve L, Hausman JF, Hoffmann L, Stewart JM (2007) Alteration of oxidative and carbohydrate metabolism under abiotic stress in two rice (*Oryza sativa* L.) genotypes contrasting in chilling tolerance. *Journal of Plant Physiology* **164**, 157-167

Murashige T, Skoog F (1962) A revised method for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* **15**, 473-479

Murillo-Amador B, Troyo-Diéguez E, García-Hernández JL, López-Aguilar R, Ávila-Serrano NY, Zamora-Salgado S, Rueda-Puente EO, Kaya C (2006) Effect of NaCl salinity in the genotypic variation of cowpea (*Vigna unguiculata*) during early vegetative growth. *Scientia Horticulturae* **108**, 423-431

Neumann P (1997) Salinity resistance and plant growth revisited. *Plant Cell and Environment* **20**, 1193-1198

Niroula RK, Bimb HP (2009) Effect of genotype and callus induction medium on green plant regeneration from anther of Nepalese rice cultivars. *Asian Journal of Plant Sciences* **8**, 368-374

Özgen M, Türet M, Altinok S, Sancak C (1998) Efficient callus induction and plant regeneration from mature embryo culture of winter wheat (*Triticum aestivum* L.) genotypes. *Plant Cell Reports* **18**, 331-335

Peng J, Hodges TK (1989) Genetic analysis of plant regeneration in rice (*Oryza sativa* L.). *In Vitro Cellular and Developmental Biology – Plant* **25**, 91-94

Prajuabmon A, Theerakulpisut P, Kijwijan B, Muangsan N (2009) *In vitro* investigation on salt tolerant characteristics of rice seedlings (*Oryza sativa* L.). *Research Journal of Agriculture and Biological Sciences* **5**, 423-427

Rasheed S, Fatima T, Bashir TH, Riazuddin S (2005) Rapd characterization of somaclonal variation in *indica* basmati rice. *Pakistan Journal of Botany* **37**, 249-262

Rashid H, Yokoi S, Toriyama K, Hinata K (1996) Transgenic plant production mediated by *Agrobacterium* in *indica* rice. *Plant Cell Reports* **15**, 727-730

Richards RA (1996) Defining selection criteria to improve yield under drought. *Plant Growth Regulation* **20**, 57-166

Rus AM, Panoff M, Perez-Alfocea F, Bolarin MC (1999) NaCl responses in tomato calli and whole plants. *Journal of Plant Physiology* **155**, 727-733

Rus AM, Rios S, Olmos E, Santa Cruz A, Bolarin MC (2000) Long-term culture modifies the salt responses of callus lines of salt-tolerant and salt-sensitive tomato species. *Journal of Plant Physiology* **157**, 413-420

Seraj ZI, Islam Z, Faruque MO, Devi T, Ahmed S (1997) Identification of the regeneration potential of embryo derived calli from various *indica* rice varieties. *Plant Cell, Tissue and Organ Culture* **48**, 9-13

Shaheen R, Hood-Nowotny RC (2008) Carbon isotope discrimination: Potential for screening salinity tolerance in rice at the seedling stage using hydroponics. *Plant Breeding* **124**, 220-224

Singh G (2009) Salinity-related desertification and management strategies: Indian experience. *Land Degradation and Development* **20**, 367-385

Thach TN, Pant RC (1999) *In vitro* study on salt tolerance in rice. *Omonrice* **7**,

80-88

Turner NC, Jones MM (1980) Turgor maintenance by osmotic adjustment: A review and evaluation. In: Turner N-C, Kramer B-J (Eds) *Adaptation of Plants to Water and High Temperature Stress*, Willey Interscience, New York, pp 89-103

Wincov I (1996) Characterization of rice (*Oryza sativa* L.) plants regenerated from salt-tolerant cell lines. *Plant Sci*ence **113**, 105-111

Zalc JM, Wicr HB, Kidwell KK, Steber CM (2004) Callus induction and plant regeneration from mature embryos of a diverse set of wheat genotypes. *Plant Cell, Tissue and Organ Culture* **76**, 277-281

Zhang GY, Guo Y, Chen SL, Chen SY (1995) RFLP tagging of a salt tolerance gene in rice. *Plant Science* **110**, 227-234