



Dissimilarity of Somaclonal in surfeit unbiased rice cultivars and inspired assortment appraisal by PCR markers

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Abstract

Submergence tolerance is an important agronomic trait for rice grown in eastern India; where flash flooding occurs frequently and unpredictably during the monsoons. Generation of somaclones for the two submergence tolerant rice cultivar FR13A and FR43B through gamma irradiation and molecular analysis of the somaclones for the variation in the pyruvate decarboxylase (*pdc*) gene was investigated. FR43B showed a relatively higher frequency of callus induction than FR13A. However, the % regeneration of somaclones in both the genotypes gradually decreased with increase in the level of radiation dose. The somaclones of FR43B showed greater tolerance to submergence than FR13A. The doses/concentration of GR 20Kr and GR 25Kr irradiation increased the morphological and yield parameters over those in controls. All the somaclones with the *pdc1* were tolerant to submergence irrespective of the gamma dose there by suggesting that *pdc1* gene is directly linked to submergence tolerance in rice.

Keywords: Pyruvate decarboxylase, gamma irradiation, callus induction frequency, submergence.

INTRODUCTION

Rice is one of most widely cultivated crops in the world and is the staple food of approximately 70% of world's 1.3 billion people (UNCTAD secretariat data base 2009). It provides 21% of global human per capita energy (IRRI, Rice Almanac, 2002). Even with rice providing 35 - 80% of the total calories consumed in Asia and with a reduction in total rice area, the rice production has been keeping pace with the population increase so far. However, in most of the developing countries, increasing population pressure has already strained the food-producing resources (FAO database, 2001). It is estimated that 70% more rice is required from less cropping area, less labor, less water and fewer chemicals by 2025 to make ourselves self sufficient (Khush and Virk, 2000).

Productivity of rice in Eastern India is low because these areas are highly vulnerable to abiotic stresses like flash flood, drought, periodic cycles of submergence; soil problems like soil salinity, alkalinity, low soil fertility and Zn deficiency (Grover et al., 2003). Due to impediments like susceptibility of the cultivars to several biotic and abiotic stresses, frequent changes in cropping intensity,

lack of improved cultural management methods, rice improvement could not make much progress in this region. With shrinking land resources and ever increasing demand of food production, the production of these lands has to be increased substantially to raise the overall rice production in the country.

The Rapid advances in biotechnology have been highly evident in the improvement of crop varieties for the benefit of the world's poorest farmers. Plant tissue culture technology has emerged as an indispensable tool in aid of plant breeding to reach traditional goals and also in the production of industrial products in plants. There is a great potential of cell and tissue culture techniques in plant improvement, provided plants can be readily regenerated in large numbers (Jain et al., 1998). Tissue-culture-induced phenotypic and genotypic variations are collectively termed 'somaclonal variation' (Larkin and Scowcroft 1981). It can result in a range of genetically stable variation, useful in crop improvement (Skirvin et al., 1993; Jain et al., 1998), similar to that induced with chemical and physical mutagens. The occurrence of somaclonal variation is associated with point mutations,

chromosomal rearrangements and recombination, DNA methylation, altered sequence copy number, transposable elements, and seems to be influenced by the genotype, explants type, culture medium, and age of the donor plants (Kumar, 2002; Veilleux and Johnson, 1998; Jain et al., 1998; Jain, 1997). Since somaclonal variation can broaden the genetic variation in crop plants, many plant characters can be altered, including plant height, yield, number of flowers per plant, early flowering, grain quality, resistance to diseases, insect and pests, cold and drought, and salt (Jain et al. 1998). Patnaik et al. (1999) reported a wide variation in quantitative traits such as plant height, plant yield, tiller number, oil content, and qualitative changes in essential oil constituents of plants regenerated from cell suspension cultures of palmarosa grass, *Cymbopogon martinii* (Roxb). Valkonen et al. (1999) obtained nine dwarf di (haploid) slow growing mutants with dark green leaves, short internodes and compact and ball-shaped appearance in tetraploid, long-day adapted potato cultivar 'Pito' (*Solanum tuberosum* subsp. *tuberosum*) through anther culture. In Japan, disease resistant lines of rice, tomato, and tobacco were isolated from somaclones (Nakajima, 1991). Ramos-Leal et al. (1996) obtained sugarcane somaclones resistant to eyespot disease. Several reports have also indicated the value of the selected somaclones in plant breeding such as high yield and shattering resistance in Indian mustard (Katiyar and Chopra, 1995); *Lathyrus sativus* devoid of neurotoxin (Yadav and Mehta, 1995); increased herb and oil yield in *Mentha arvensis*, (Kukreja et al., 1991) and *Cymbopogon martinii* (Patnaik et al., 1999). From the view point of its utilization in crop improvement, perhaps the main attraction of somaclonal variation is the rather frequent occurrence of chromosomal opportunity for introgression of alien gene(s) into the genome of a cultivar across barriers raised by the impossibility of sexual recombination.

Somaclonal variations can also be characterized by a variety of molecular methods (Henry, 1998). Isozymes were found to be useful markers for somaclonal variation among regenerants from apple rootstocks (Martelli et al., 1993). Xiong et al. (1999) used methylation sensitive amplified polymorphism (MSAP) to detect DNA methylation in rice. This method is sensitive to detect cytosine methylation. However, RFLP and RAPD analysis have been applied widely in the analysis of somaclonal variation (Piccioni et al., 1997; Henry, 1998). Veilleux et al. (1995) used both RAPD and SSR techniques to characterize the genetic composition of anther-derived potato plants. Wolff et al. (1995) used RAPD, SSR, and RFLP markers to evaluate somaclonal variation in vegetatively propagated chrysanthemum cultivars.

Submergence tolerance is an important agronomic trait for rice grown in eastern India, where flash flooding occurs frequently and unpredictably during the monsoons. Flash flooding greatly depresses grain yield in lowland rainfed rice. However, a few tolerant cultivars can withstand complete submergence for 10 to 14 d, such as

FR13A, FR13B, Goda Heenati, Kurkaruppan, BKNFR7 6106-16- 0-1-0 and Thavalu (Mackill et al 1996). FR13A is probably the most frequently used in genetics and physiological studies and as a standard against which other breeding lines are often compared (Mohanty et al 2000). The high tolerance to submergence in FR13A is believed to be due to the activity of pyruvate decarboxylase genes (pdc). pdc is highly important in providing plants with tolerance to anoxic conditions that occur during submergence by flooding (Good and Muench, 1993). During anaerobiosis, the glycolytic intermediate pyruvate is first converted into lactate (Davies et al., 1974). This result in the lowering of the cytoplasmic pH, which in turn activates PDC and inhibits lactate dehydrogenase (Rivoal et al., 1989, Roberts et al., 1984). As a result, pyruvate is non-oxidatively decarboxylated to acet-aldehyde and carbon dioxide by PDC. Reduction of acetaldehyde to ethanol by ADH regenerates NAD⁺, which is then utilized in the glycolytic pathway to maintain carbon flow through this pathway under anaerobic conditions (Good and Muench, 1993). This switching of the energy production pathway from aerobic glycolysis to anaerobic fermentation is one of the major metabolic adaptations that plants undertake when they are submerged or confronted with a lack of oxygen. PDC genes have been isolated from maize (Kelly, 1989; Peschke and Sachs, 1993), yeast (Kellerman et al., 1986), and bacteria (Conway et al., 1987). Recently two pdc cDNAs and two genomic clones have been isolated and characterized in rice (Hossain et al., 1994 a, b, 1996). Similarly, pdc2 the most important PDC gene has been cloned and characterized in rice (Huq et al., 1999).

Keeping in view of the issues mentioned above, the present study is aimed at generation of a large number of somaclones from two cultivars that is, FR 13A and FR 43B that are highly tolerant to submergence followed by molecular analysis of the somaclones for the variation in the pyruvate decarboxylase genes using the sequence specific primers for gene pdc1.

MATERIALS AND METHODS

Plant tissue culture

Seeds from two submergence tolerant rice varieties FR13A and FR13B were used in the present investigation. They were obtained from Central Rice Research Institute, Cuttack, India. 500 seeds from each rice genotype were exposed to 20, 25 and 30 Kilorad (Kr) of gamma-radiation (GR) in a gamma cell (⁶⁰Co source) installed at Indian Agricultural Research Institute (IARI), New Delhi. After irradiation, seeds were dehusked and washed twice with distilled water with a drop of Tween 80 for 5 min and surface sterilized with 0.1% (m/v) aqueous mercuric chloride for 7 - 10 min followed by 5 rinses in sterile distilled water. The seeds were inoculated on semisolid (0.75% agar) MS basal medium (Murashige and Skoog, 1962) containing 2, 4 D 2 mg/l, L-proline 500 mg/l, sucrose 30 gm/l and pH 5.8. The cultures were incubated under dark at 26 ± 2°C for 21 days. After 21 days, callus from individual seeds are cut into bits (3-5mm) and sub cultured on the same medium for another 15 days in dark at 25°C. The matured calli were transferred into 1.1% agar



Figure 1. Rice somatic cell culture. (A) Callus induction from matured seeds. (B) Embryonic callus. (C) Green plant regenerating from embryonic calli. (D) Plant growing in rooting media.

regeneration medium (MS medium supplemented with Kinetin, Napthalene acetic acid and Benzyl Aminopurine in the ratio of 1:1:4; sucrose 30 gm/l; Myoinositol 100mg/l and pH 5.8). It was inoculated for 21 days by providing 16 h light and 8 h dark at 25°C. After 21 days the material was sub cultured on the same medium for another 15 days (Figure 1). The induced shoot material was transferred into 0.7% agar rooting medium (MS medium supplemented with Kinetin and Napthalene acetic acid in the ratio of 1:10; sucrose 30 gm/l; Myoinositol 100mg/l and pH 5.8). After 15 days, the germinated plants were transferred into pots pre-supplemented with farmyard manure for further evaluation.

DNA extraction

A modified CTAB protocol (Murray and Thompson, 1980) was used to extract total genomic DNA from expanded young leaves of the regenerated plants in all the treatments and controls. PCR amplification was performed by using SSR markers *pd1* (F- 5'CCC ATC TCA CCA GAC CA3'; R- 5'CTG TTG GCA GCC GAG A3') linked to *pd1* gene (Hossain 1996). The primers were from commercially available primer kit (Sigma Genosys, California, USA). Individual PCR amplifications for each primer were performed using the EP gradient 96 V programmable master cycler (Eppendorf AG, Hamburg, Germany.). The PCR protocol involved a total volume of 10µl reaction mixture containing 35 ng of genomic DNA, 1X PCR buffer (pH 8.3), 200µM dNTP mix, 5 pmol of each of the forward and reverse primers, 2 mM of MgCl₂ and 1 U of *Taq* (*Thermophilus aquaticus*) DNA polymerase (Biotools). The basic PCR program to amplify DNA was as follows: an initial hot start and denaturing step at 94°C for 5 min followed by 35 cycles of a 2 min denaturation at 94°C, a 1 min annealing at 50°C, and a 1 min primer elongation at 72°C. A final extension step at 72°C for 5 min was performed.

Gel electrophoresis

A 10 µl aliquot of the PCR amplified samples was combined with 2

µl of a loading buffer (0.4% Bromo-phenol Blue, 0.4% xylene cyanole and 5 ml of glycerol) and was analyzed directly on 1.5% agarose gels in 0.5X TBE buffer. Electrophoresis was done for about 3 h at 60 volts. The GeneRuler™ 1kb ladder plus (Fermentas Inc, Hanover, MD) was used as a molecular size standard to compare the molecular weights of amplified products. Visualization of the amplified bands was done by staining with ethidium bromide (10 mg/ml) for 20 min and de-staining with double distilled water for 20 min followed by transillumination under short wave UV light (Alpha Innotech).

Submergence screening

Submergence treatment was done in the green house of Central Rice Research Institute following the procedures of Mishra et al., 1996. The plants germinated from the calli were transferred into plastic pots filled with soil with one plant per pot pre-supplemented with farm yard manure. All pots were placed in a metal tray and filled with water. Six days later, the tray containing the pots was placed in a concrete tank inside the glass house and submerged in tap water to a depth of 110 cm. The water was kept slightly overflowing using an outlet at 110 cm level. Beginning from 5 days of submergence, the susceptible cultivar was checked daily. When its leaves were yellowish brown and decaying, water was drained to soil level. Plants were maintained in that condition for seven days for recovery and the number of dead (susceptible) and alive (tolerant) plants were counted. Plants without any green leaves were considered dead and those with at least one newly emerging green leaf were considered alive.

RESULTS

Somatic embryogenesis

The callus generated from the two genotypes that is, FR 13A and FR 43B was studied for its potential of somatic

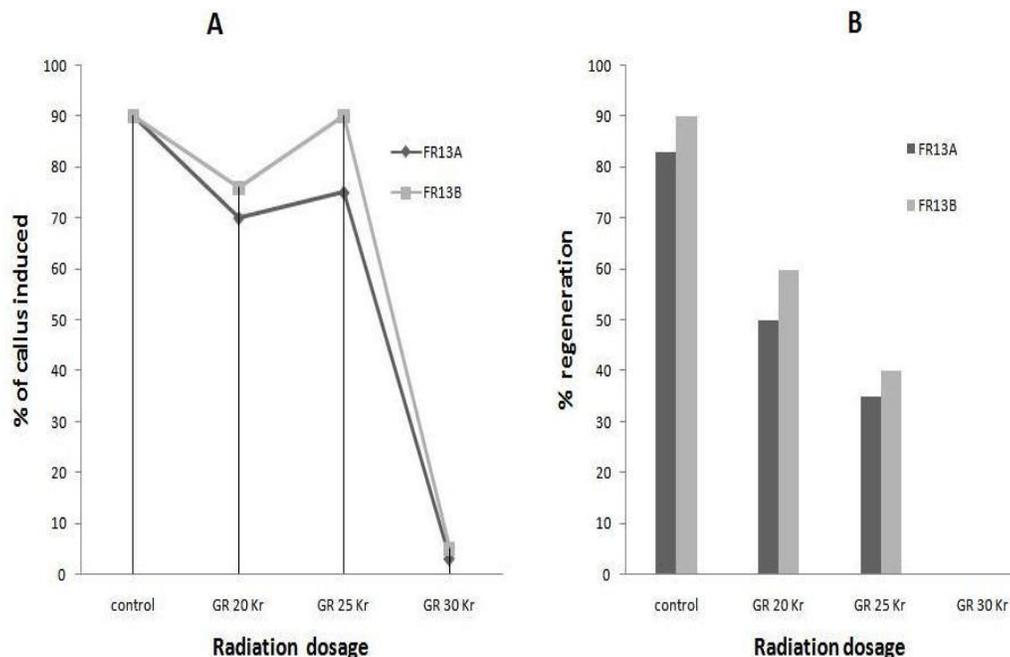


Figure 2. Graph representing the variation in callus induction and plant regeneration due to gamma irradiation of FR13A and FR43B rice genotypes. (A) % of callus induced and (B) % regeneration of plants.

embryogenesis. Two distinct types of callus were observed: i) highly compact, yellowish or creamy type (embryogenic) and ii) loose and white watery type (non-embryogenic). However, the calli induced from medium fortified with 2 mg 2, 4-D (2.0 mg /l⁻¹) and Kn (0.5 mg /l⁻¹) concentration were highly embryogenic compared to the others. In both the genotypes, globular proembryoes were found in the embryogenic portion of primary callus induced after 7 - 10 days on 2, 4-D (2.0 mg /l⁻¹) + Kn (0.5 mg /l⁻¹) medium. These structures have undergone developmental changes into complete globular, heart and torpedo shapes in the subsequent subcultures. The somatic embryoids were observed to be originating from peripheral as well as deep-seated portion of calli, but independent from the surrounding callus tissues. However, when embryogenic calli were subculture on fresh medium with 2, 4-D (2.0 mg /l⁻¹), a major portion of embryoids dissipated to form callus. The embryoids were grown into plants by placing them on media supplemented with cytokinin (Kn 2 mg/l).

Somaclonal variations

The experiment was conducted combining both mutation and somatic cell culture techniques for enhancing the range and magnitude of somaclonal variation which can be used for selecting desirable genotypes having good

agronomic characters including yield and high levels of tolerance to submergence. The various parameters that were analyzed in this regards are as follows:

Callus induction frequency: The primary callus was initiated from the scutellar region of the explants after 5 - 7 days of inoculation from both the genotypes in all the formulations tested. The two genotypes showed similar callus induction potential under normal condition. However, the two genotypes showed a varied callus induction frequency when irradiated. Of the two genotypes tested, FR43B showed a relatively higher frequency of callus induction than FR13A. The % of callus induced in FR43B was found similar (that is, 90%) under control and treated (25 Kr) conditions. Similarly, in FR13A, the callus induction frequency was higher when untreated as compared to irradiated conditions. The callus induction potential in both the genotypes increased with increase in the level of irradiation from 20 - 25 Kr but it decreased drastically at 30 Kr (Figure 2).

The % regeneration of plants in both the genotypes gradually decreased with increase in the level of gamma radiations. A total of 290 FR13A plants were obtained which includes 220 untreated plants, 45 plants of GR 20 Kr and 25 plants of GR 25 Kr. Similarly, 416 FR43B plants were obtained that includes 236 untreated plants, 130 plants with GR 20Kr and 48 plants of GR 25 Kr (Table 1). When radiation treatment was increased up to

Table 1. Callus induction frequency (%) of the submergence tolerant rice varieties FR13A and FR13B.

	No. of seed germinated	% of callus induced	% of regeneration	No. of plant obtained
FR13A				
Control	256	90	83	220
20Kr	200	70	50	45
25Kr	120	75	35	25
30Kr	80	3	0	0
FR13B				
Control	260	90	90	236
20Kr	200	76	60	130
25Kr	150	90	40	48
30Kr	80	5	0	0

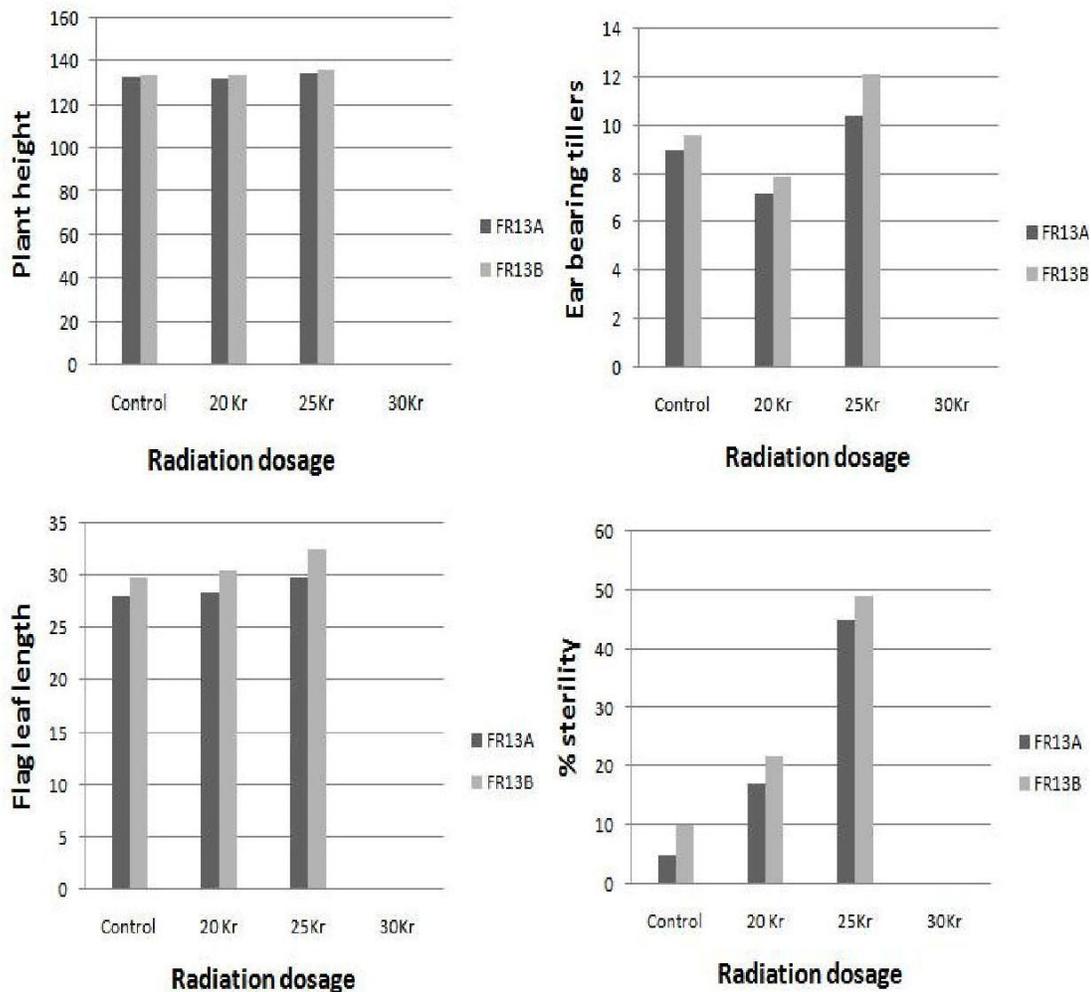


Figure 3. Graph representing the variation in morphological characters due to gamma irradiation of FR13A and FR43B rice genotypes.

30 kr, all the calli derived from 30 kr treated grain showed no regeneration at all in both the genotypes (Figure 3).

When the shoots were 1 cm in height, plantlets were transferred into the rooting medium and the newly gene-

Table 2. Variation in morphological characters of FR13A and FR13B for five traits: plant height (PH), tillers per plants (TP), panicle length (PL), flag leaf length (FLL) and percentage sterility. These results are for all the two range of gamma radiations.

	PH (cm)	EBT (nos)	PL (cm)	FLL (cm)	GL (cm)	GB (cm)	% Sterility
FR13A							
Control	132.73 (3.58)	9 (0.33)	24.26 (0.77)	28.02 (1.13)	9.34	2.72	5
20 Kr	132.12 (1.36)	7.2 (0.26)	22.32 (0.33)	28.31(0.59)	9.12	2.52	17
25 Kr	134.26 (1.39)	10.4 (0.34)	26.56 (0.40)	29.93 (0.58)	9.39	2.82	45
FR13B							
Control	133.6 (3.22)	9.6 (0.35)	26.93 (0.56)	29.92 (0.55)	9.62	2.83	10
20 Kr	133.96 (1.25)	7.9 (0.22)	27.32 (0.21)	30.56 (0.89)	9.03	2.41	22
25 Kr	136.21 (1.32)	12.1 (0.30)	28.12 (0.35)	32.43 (0.58)	9.61	2.89	49

rated plants were transferred into the pots for further evaluation.

Morphological variations: A range of morphological variation was observed in the newly generated plants of both the genotypes with different radiation dosage. Seven important characteristics (plant height (PH), ear bearing tillers (EBT), maximum panicle length (MPL), grain length (GL), grain breadth (GB), flag leaf length (FLL) and % sterility) were selected to analyze the influence of gamma irradiation treatments on performance of plants. The doses/concentration of GR 20 and GR25 Kr irradiation increased the morphological and yield parameters over those in controls. The highest panicle length was shown by FR13A at GR 25 Kr which is slightly more than the untreated FR13A. Similarly, the highest ear bearing tillers was shown by FR43B at GR 25 Kr (11.2). The panicle length of FR43B at GR 25 Kr was slightly higher than the control. No remarkable difference was noticeable in the grain length and grain breadth of both the genotypes. At 31.63cm, FR43B at GR25 Kr has the longest flag leaf. Similarly, the flag leaf length of FR13A 25 Kr was more than control. However, a visible change in the % sterility was noted. The % sterility of both the genotypes was 5-10% when untreated but it gradually increased with the increase in the radiation dosage. The highest %sterility was shown by FR13A with GR 30Kr (that is, 65%) (Table 2).

Submergence tolerance: The somaclones generated from the seeds irradiated with different dose of gamma radiation from both FR13A and FR43B were tall. In the present study, the difference between tolerant and susceptible was very distinct after 14 days of submergence. All the untreated controlled somaclones of FR13A and 201 out of 236 somaclones from FR43B were completely tolerant to submergence. In FR13A, 33 clones out of 45 at GR 20 Kr and 12 out of 25 clones at GR 25 Kr were tolerant to submergence. Similarly, in FR43B, 23 out of 130 at GR 20 Kr and 9 somaclones out of 48 at GR 25 Kr were tolerant. Rests of the somaclones were completely susceptible.

After submergence, elongation of all plants was observed in both the genotypes. The control plants of both FR13A and FR43B elongated as much as 30 cm during submergence. The depth of water, however, was sufficient to keep all plants completely submerged. However, a minor difference in the rate of elongation was observed with increase in radiation dosage. The average elongation of FR13A with GR 20 Kr and 25 Kr was 31.2 cm and 35 cm respectively (Table 3) . Similarly, the elongation of FR43B with 20 Kr and 25 Kr was 30.2 and 33.5cm respectively. The submergence tolerance testing at 30 Kr dosages could not be performed because there was no germination of calli at 30 Kr dosages in both the genotypes.

Molecular analysis

Leaves from all the somaclones FR13A and FR13B were collected and DNA isolated for molecular analysis prior to screening for submergence tolerance. The DNA from each genotype was PCR amplified and checked for the presence of Pdc1 gene. It was seen that in 33 out of 45 clones, the primer amplified the pdc1 sequence from FR13A-20 kr derived plants and 12 samples out of 25 were amplified from FR13A derived from 25 kr treatment (Figure 4). When the amplification was done with FR43B somaclones, only 23 out of 130 samples from FR43B- 20 kr treatment and only 9 out of 48 samples derived from FR43B-25 kr treatments were amplified (Figure 5). All the somaclones with the pdc1 were tolerant to submergence irrespective of the radiation dose. This may be clear to suggest that pdc1 gene is directly linked to submergence in rice.

DISCUSSION

Genetic variation is an essential component of any conventional crop breeding programme. The adoption of new technologies such as plant tissue culture and recombinant DNA may help in achieving some of the goals to increase food production. There is a great potential of cell

Table 3. Reaction of FR13A and FR43B rice genotypes to submergence.

Genotypes	somaclones screened	Tolerant	Susceptible
FR13A (control)	220	220	0
FR13A-20Kr	45	33	12
FR13A-25Kr	25	12	13
FR43B (Control)	236	201	35
FR43B-20Kr	130	23	107
FR43B-25Kr	48	9	39

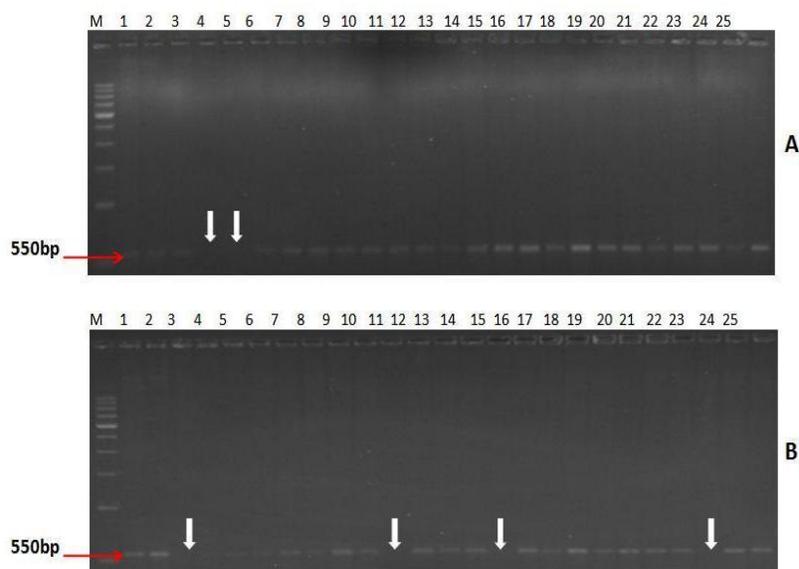


Figure 4. PCR amplification of *pdc1* sequence in the somaclones of FR13A. A. Amplification of FR13A-20Kr; B. Amplification of FR13A-25Kr. The arrow marks represent the genotypes without *pdc1* gene.

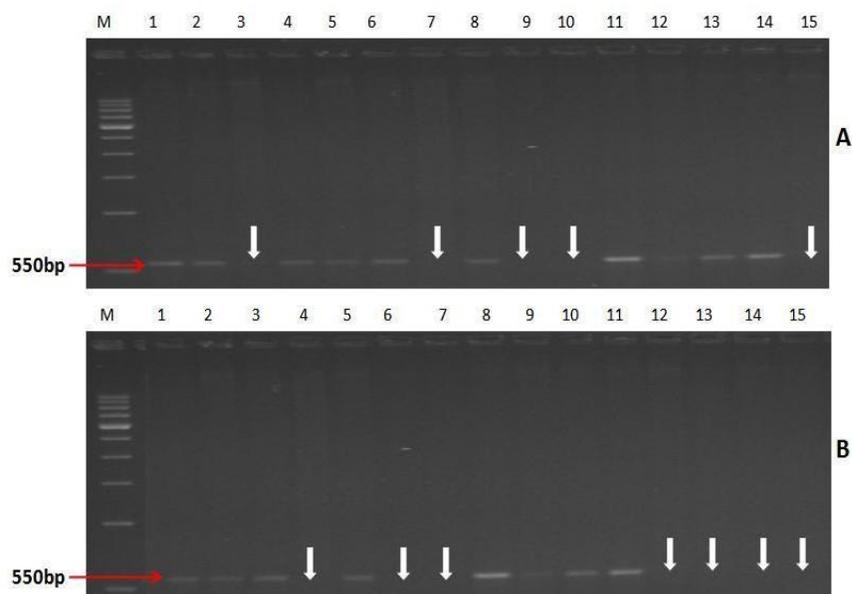


Figure 5. PCR amplification of *pdc1* sequence in the somaclones of FR43B. A. Amplification of FR43B- 20Kr; B. Amplification of FR43B-25Kr. The arrow marks represent the genotypes without *pdc1* gene.

and tissue culture techniques in plant improvement, provided plants can be readily regenerated in large numbers (Jain et al., 1998). Induction of somatic embryogenesis is an attractive approach in plant tissue culture, as the somatic embryos being single cell origin, are more suitable for genetic, breeding and mutation research.

In the present study, an attempt was made to generate plants using somatic cell culture approach from two genotypes that is, FR13A and FR43B. The experiment was successful as plantlets could be generated from the callus developed from the mature seeds. Experiment was also undertaken by exposing the somaclones to different level of gamma radiation. The callus induction frequency was optimum and the production of plantlets from the mutagen treated grains was successful at both 20K and 25 Kr radiation dose. The callus induction frequency was relatively higher in FR43B than in FR13A. There was a steady increase in the callus induction potential with increase in radiation dose from 20 - 25 Kr. However, there was no plant germination at 30 Kr in both the genotypes. This may be due to the development of deleterious mutations at high dose of gamma radiation (Cheema and Atta, 2003).

A qualitative variation in the morphological traits was also visualized with different level of gamma radiation treatments. The plant height increased between 2 - 3 cm in the irradiated somaclones as compared to the untreated plants. This finding is highly important in the sense that both the genotypes FR13A and FR43B are submergence tolerant and increase in the plant height will always be considered a beneficial mutation. Moreover, this also resulted in increased panicle length and flag leaf length a feature often seen in water stress conditions. As usual, the % sterility increased with increase in radiation dosage. This is in accordance with earlier data.

The submergence testing of both genotypes produced predictable results. All the untreated somaclones of FR13A and FR43B survived complete submergence. There was a decrease trend in the survivability of the somaclones with increase in gamma radiation dose. This is because high radiation may be mutating the DNA sequence responsible for expression of submergence tolerance. When the somaclones were analyzed for the presence of a specific sequence *pd1* for submergence tolerance, only a proportion of the somaclones amplified in both the genotypes. The plant without *pd1* gene amplification must have a distortion of the DNA in the said sequence due to gamma radiation. This can be proved by performing RTPCR expression analysis or western blotting technique. Further studies on these somaclones both at the sequence level (by sequencing the gene portions involved) and also at the plant level is required to see that whether these plants still retain the submergence tolerance of the parental genotype or not. The extension of these studies will greatly help in understanding the sequence information as well as its relationship in

the expression of the genes concerned with submergence tolerance.

REFERENCES

- Bouman BAM, Hengsdijk H, Hardy B, Bindraban PS, Tuong TP, Ladha JK (2002). International Workshop on Water-wise Rice Production, International Rice Research Institute, (Los Baños, Philippines).
- Cheema AA, Atta BM (2003). Radiosensitivity studies in Basmati rice. *Pak J. Bot.* 35(2):197-207.
- Conway T, Osman YA, Konnan JI, Hofhan EM, Ingram LO (1987). Promoter and nucleotide sequences of the *Zymomonas mobilis* pyruvate decarboxylase. *J. Bacteriol.* 69:949-954.
- Davies DD, Grego S, Kenworth P (1974). The control of the production of lactate and ethanol in higher plants. *Planta* 118:297-310.
- Food insecurity in the world (2001). FAO database 2001. Website: <http://www.fao.org/DOCREP/003/Y1500E/Y1500E00.HTM>.
- Good AG, Muench DG (1993). Long-term anaerobic metabolism in root tissue. Metabolic products of pyruvate metabolism. *Plant Physiol.* 1163-1168.
- Grover A, Agrawal PK, Kapoor A, Katiyar-Agrawal S, Agrawal M, Chandramouli A (2003). Addressing abiotic stresses in agriculture through transgenic technology. *Curr. Sci.* 84(3): 355-367.
- Henry RJ (1998). Molecular and biochemical characterization of somaclonal variation, in SM Jain, DS Brar and BS Ahloowalia (eds), Somaclonal variation and induced mutations in crop improvement, Kluwer Academic Publishers, Dordrecht, Germany pp. 485-499.
- Hossain MA, Huq E, Grover A, Dennis ES, Peacock WJ, Hodges TK (1996). Characterization of pyruvate decarboxylase genes from rice. *Plant Mol. Biol.* 31:761-770.
- Hossain MA, Huq E, Hodges TK (1994a). Sequence of a cDNA from *Oryza sativa* (L.) encoding the pyruvate decarboxylase 1 gene. *Plant Physiol.* 106:799 - 800.
- Hossain MA, McGee JD, Grover A, Dennis E, Peacock WJ, Hodges TK (1994b). Nucleotide sequence of a rice genomic pyruvate decarboxylase gene that lacks introns: a pseudo-gene? *Plant Physiol.* 106: 1697-1698.
- Huq E, Harrington S, Hossain MA, Wen F, McCouch SR, Hodges TK (1999). Molecular characterization of *pd2* and mapping of three *pdc* genes from rice. *Theor. Appl. Genet.* 98: 815-824.
- Jain SM, Brar DS, Ahloowalia BS (1998). Somaclonal Variations and Induced Mutations in Crop Improvement. Kluwer Academic Publishers, Dordrecht.
- Jain M (1997). Micropropagation of selected somaclones of *Begonia* and *saintpaulia*. *J. Biosci.* 22(5): 585-592.
- Katiyar R K, Chopra VL (1995). A somaclone of *Brassica juncea* is processed into a variety and is released for commercial cultivation in India. *Cruciferae Newslett.* 17: 86-87.
- Kellerman E, Seeboth PG, Hollenberg CP (1986). Analysis of the primary structure and promoter function of a pyruvate decarboxylase gene (*PDC1*) from *Saccharomyces cerevisiae*. *Nucleic Acids Res.* 14: 8963-8977
- Kelly PM (1989). Maize pyruvate decarboxylase mRNA is induced anaerobically. *Plant Mol. Biol.* 13: 213-222.
- Khush GS, Virk PS (2000). Rice Breeding: Achievement and future strategies. *Crop Improv.* 27:115-144.
- Kukreja AK, Dhawan OP, Mathur AK, Ahuja PS, Mandal S (1991). Screening and evaluation of agronomically useful somaclonal variations in Japanese mint (*Mentha arvensis* L.). *Euphytica* 53: 183-191.
- Kumar U (2002). Methods in plant tissue culture.
- Larkin P, Scowcroft N (1981). Somaclonal variation: a novel source of variability from cell for plant improvement. *Theor Appl Genet.* 60: 197-214.
- Mackill DJ, Coffman WR, Garrity DP (1996). Rainfed lowland rice improvement. IRRI, Los Banos.
- Martelli GI, Greco B, Mezzetti P, Rosatti (1993). Isozymic analysis of somaclonal variation among regenerants from apple root stock leaf tissue. *Acta Hort.* 336: 381-387.

- Mishra SB, Senadhira D, Manigbas NL (1996). Genetics of submergence tolerance in rice (*Oryza sativa* L.). *Field Crops Res.* 46(1-3):177-181.
- Mohanty HK, Mallik S, Grover A (2000). Prospects of improving flooding tolerance in lowland rice varieties by conventional breeding and genetic engineering. *Curr. Sci.* 78(2): 132-140.
- Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol Plant* 15:473-497.
- Murray M, Thompson WF (1980). Rapid isolation of molecular weight plant DNA. *Nucleic Acid Res.* 8: 4321-5
- Nakajima K (1991). Biotechnology for crop improvement and production in Japan. Paper presented at the Regional Expert Consultation on the Role of Biotechnology in Crop Production, FAO Regional Office for Asia and the Pacific, Bangkok 21: 18-21.
- Patnaik J, Sahoo S, Debata BK (1999). Somaclonal variation in cell suspension culture-derived regenerants of *Cymbopogon martinii* (Roxb.) Wats var. motia. *Plant Breed.* 118: 351-354.
- Peschke VM, Sachs MM (1993). Multiple pyruvate decarboxylase genes in maize are induced by hypoxia. *Mol. Gen. Genet.* 240: 206-212.
- Piccioni E, Barcaccia G, Falcinelli M, Standardi A (1997). Estimating alfalfa somaclonal variation in axillary branching propagation and indirect somatic embryogenesis by RAPD fingerprinting. *Int. J. Plant Sci.* 158: 556-562.
- Ramos Leal R, Maribona RH, Ruiz A, Konerva S (1996). Somaclonal variation as a source of resistance to eyespot disease of sugarcane. *Plant Breed* 115: 37-42.
- Rivoal J, Ricard B, Pradet A (1989). Glycolytic and fermentative enzyme induction during anaerobiosis in rice seedlings. *Plant Physiol. Biochem.* 27: 43-52.
- Roberts JKM, Callis J, Wemmer P, Walbot V, Jardetzky O (1984). Mechanism of cytoplasmic pH regulation in hypoxic maize root tips and its role in survival under hypoxia. *Proc. Natl. Acad. Sci. USA* 91: 3379-3383.
- Skirvin RM, Norton M, McPheeters KD (1993) Somaclonal variation: has it proved useful for plant improvement? *Acta Horti.* 336:333-340.
- Toenniessen GH (1996). Progress in rice biotechnology. In: Proceedings of the fifth annual meeting national rice biotechnology network. IARI, New Delhi pp 1-11
- UNCTAD secretariat database (2009). <http://www.unctad.org/infocomm/anglais/rice/market.htm>.
- Valkonen JPT, Moritz T, Watanabe KN, Rokkm V-M (1999). Dwarf (di)haploid potato mutants obtained from tetraploid potato cultivar (*Solanum tuberosum* ssp. *tuberosum*) via anther culture are defective in gibberellin biosynthesis. *Plant Sci.* 149:51-57.
- Veilleux RE, Johnson AAT (1998). Somaclonal variation: molecular analysis, transformation interaction, and utilization. *Plant Breed. Rev.* 16: 229-268.
- Veilleux RE, Shen LY, Paz MM (1995) Analysis of the genetic composition of anther-derived potato by randomly amplified polymorphic DNA and simple sequence repeats. *Genome* 38: 1153-1162.
- Wolff K, Zietkiewicz E, Hofstra H (1995) Identification of *Chrysanthemum cultvars* and stability of DNA fingerprint patterns. *Theor. Appl. Genet.* 91: 439-447.
- Xiong LZ, Xu CG, Maroff MAS, Zhang QF (1999). Patterns of cytosine methylation in an elite rice hybrid and its parental lines, detected by a methylation-sensitive amplification polymorphism technique. *Mol. Gen. Genet.* 261: 439-446.
- Yadav VK, Mehta SL (1995). *Lathyrus sativus*: A future pulse crop free of neurotoxin. *Curr. Sci.* 68: 288-292.