European Journal of Biotechnology and Bioscience
Online ISSN: 2321-9122, Impact Factor: (RJIF 5.44)
www.biosciencejournals.com
Volume 4; Issue 4; April 2016; Page No. 09-10

Observation of variability pattern in some *in vitro* developed somaclones in rice *landraces* of Gangetic Alluvial Zone

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Abstract
Present investigation aims at isolation of three somaclonal variants after screening two elite cultivars of rice landraces *viz*., Jhingasail and Sarkele aman regenerated from matured seed. The explants were cultured on MS medium supplemented with BAP ($3.5mgl^{-1}$). Both qualitative and quantitative analyses were done on the three somaclonal variants for each cultivars developed through tissue culture method. Remarkable significant variability was observed in these variants over the plants developed through conventional breeding in respect to agro-morphological and biochemical characters. Among three somaclones of Jhingasail, J-3 performed well in respect to maximum traits and SA-3 somaclonal variant of Sarkele amon showed better variability pattern in comparison to other variants. This study would be helpful to the researchers and plant breeder for future crop improvement programme to conserve the landraces and maintain the germplasm through *in vitro* culture method.

Keywords: Rice (O. Sativa L.), somaclonal variation, genetic variability, quantitative traits

1. Introduction
For the geneticist and plant breeder, *in vitro* technology is a potential tool for isolation of somaclonal variation which allow the development and release of new cultivars with genetic variability within a limited time in different economically important plants [1]. Rice (*Oryza sativa* L.) is the staple food crop of more than half of the world's population [2]. To feed the ever growing population, attempts are always being made in all the rice growing stations to increase the yield per hectare but the success of hybridization followed by selection of desirable segregants depends largely on the selection of parents with high genetic variability for various traits. The diversity in crop varieties is considered as a significant parameter for increasing food production, mitigating poverty and promoting economic growth overall contributing to the development of Agriculture. It serves as an insurance against unknown future needs and conditions thereby contributing to the stability of forming systems at local, national and global levels [3]. Also, biofortification of staple food crops for enhanced micronutrient content through genetic manipulation is the best option available to alleviate hidden hunger with little recurring costs [4, 5]. Land races genotypes, wild and weedy relatives are an important source of useful genes [6, 7]. Hence keeping this in view, the present study aims at documentation and reporting of some *in vitro* developed high yielding genetically variable somaclones of two elite rice landraces of west Bengal.

2. Materials and methods
Two cultivars of rice *viz*., Jhingasail and Sarkele aman were collected from Zonal Adaptive Research Station, Krishnagar, Nadia, West Bengal (23˚24´N latitude and 88˚31´E longitude with an altitude of 9.75 meters above mean sea level). The soil reaction gives a slightly acidic pH of 6.0, with low soluble salts (EC of 0.15 dS m-1), medium organic carbon content (0.57%), Total N (0.056%), medium in available P (25.28 kg ha-1) and K (148.77 kg ha-1).
The healthy seeds were taken for the study were surface sterilized using the standard procedure of 0.1% $HgCl_2$ followed by repeated washing with distilled water. The material is then cultured on MS medium supplemented with BAP ($3.5mgL^{-1}$) for generation of shoot. The well-developed regenerated plants with healthy root and shoot were taken out of medium, followed by repeated washing with distilled water for removal of agar and transferred to individual pot having peat:pertite:sand in the ratio of 50:25:25 (v/v). Polythene covered pots were maintained at the temperature of 25-26 ˚C for 21 days for persuing high humid condition. Thirty *in vitro* developed plants were maintained in polybag. After completion of hardening process, the plants were taken into account for recording of data based on various agro-morphological and biochemical traits. The somaclones of Jhingasail were J-1, J-2, and J-3 whereas, that of Sarkele aman were SA-1, SA-2 and SA-3.
Iron Content of the *in vitro* developed somaclones was estimated along with the data recorded from main plant treated as a control. One gram oven dried ground dehusked seed samples were collected in a 150ml conical flask. To this, 25-30ml diacid mixture (HNO_3:$HCLO_4$; 5:4 v/v) was added and kept overnight. Next day, it was digested by heating till clear white precipitates at the bottom. The crystals were dissolved by diluting in doubled distilled water. The contents were filtered through Wattmann no. 42 filter paper. The filtrate was made to 50ml with doubled distilled water. The acid digested samples were used for the determination of Fe contents by Atomic Absorption Spectrophotometer 2380, Perkin Elmer (USA) according to the method of Lindsey and Norwell. Mean values were taken from the measurements of three replicates and standard error of the means was calculated. Difference between means was determined by one way ANOVA.

Analyses were done using statistical package for social sciences (SPSS) for window, version 13.0.

3. Results & Discussion

On the basis of biochemical and different agro-morphological traits, (Table 1) three somaclonal variants, like, J-1, J-2 and J-3 of Jhingasail and three somaclonal varients such as, SA-1, SA-2 and SA-3 of Sarkele aman were isolated after screening thirty plants developed *in vitro* from seed. Out of nine agro-morphological parameters like, culm number/plant, leaf length, leaf breadth, plant height, panicle length, grain length, grain breadth, grain length/breadth ratio, yield /plant and one biochemical traits like iron content taken for the study, somaclones of SA-3 performed well in almost all the traits except leaf breadth. In respect to plant height and culm number /plant, J-1 and J-2 showed remarkable increase in mean value than J-3 which showed significant improvement in respect to other important traits.

Research findings suggested that somaclonal variability among *in vitro* regenerated plants may be due to the fact of epigenetic factor. In this present study, the shoot or plants regenerated through *in vitro* culture performed most significantly than 'the plants developed through seed sowing at field directly [8]. Thus, these improved somaclones of rice landraces may be used as a potential donor for the future rice breeding to the farmers to save the nation from increasing risk of hunger. Findings from the performance of somaclonal variants in comparison to control plant led to the conclusion that for each case, somaclones showed better performance than control plant of Jhingasail and Sarkele aman respectively. Regarding grain length and yield per plant, J-3 and SA-3 performed very well than others. Iron deficiency is the most prevalent micronutrient deficiency worldwide effecting physical growth, mental development and learning ability during child adolescence. Considering this, Iron content was estimated from seed sample. Remarkable variability was found in this biochemical trait. It was found that J-3 and SA-3 showed better iron content of 79.1 and 81.1µg/g respectively than other plants (Table 1).

3.1 Tables and Figures

Table 1: Field data of three somaclones of two rice landraces for various agro-morphological and biochemical trait

Name of the Landrace & Somaclones	Plant height (cm)	Culm No./plant	Leaf Length (cm)	Leaf breadth (cm)	Panicle Length (cm)	Grain Length (mm)	Grain Breadth (mm)	Grain L/B ratio (mm)	Yield/plant (t ha^{-1})	Iron Content (µg/g)
Jhingasail	34	10.5	61.0	1.9	24.5	8.39	3.3	2.53	3.2	74.0
J-1	35.5	11.0	61.8	1.95	25.0	8.62	3.2	2.69	3.3	75.1
J-2	35.9	12.2	62.0	2.0	24.9	9.0	3.3	2.72	3.5	74.9
J-3	34.8	11.9	63.1	2.1	25.1	8.89	3.1	2.86	3.8	79.1
Sarkele aman	43.0	15.0	49.0	1.6	30.5	7.3	4.2	1.73	3.0	78.5
SA-1	44.0	15.9	50.0	1.9	31.0	7.9	4.0	1.9	3.3	80.1
SA-2	44.5	16.4	49.8	2.0	31.0	7.99	3.9	2.04	3.2	79.8
SA-3	45.1	16.8	50.2	1.8	32.2	8.0	3.8	2.10	3.7	81.1

4. Conclusions

The somaclonal variability observed in this above investigation proved that the *in vitro* developed seedlings do have more potentiality and these can be used as an elite planting material to the plant breeder for selection of desirable characters in crossing with suitable parent. From this study it may be concluded that landraces are the treasure trove of different beneficial genes and these genes should be conserved properly so that these can be used as a potential donor for future plant breeding.

5. Acknowledgments

The author is thankful to Dr. S. Ahmed, Principal, Sripat Singh College, Jiaganj, Murshidabad for providing necessary laboratory facilities, Prof. P.D. Ghosh (K.U.), Dr. A. Ghosh (Z.A.R.S., Krishnagar, Nadia; IAS, C.U.) and Prof. P.K. Sahu (BCKV) for necessary help and assistance.

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