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In vitro Evaluation and Regeneration of Three Local Boro Rice (*Oryza sativa* L.) Cultivars under NaCl Stress

JAYANTA ROY[1]
MD. MONIRUL ISLAM
S.M. ABDULLAH AL MAMUN
Agrotechnology Discipline
Khulna University, Bangladesh

Abstract:

Dehusked seeds of three local boro rice cultivars viz. Koijor, Nayanmony and Bapui native to Khulna District of Bangladesh were cultured on MS medium supplemented with different levels and combinations of 2, 4- D and NAA for callus induction. Rice cultivar Koijor and the medium fortified with 2 mgL^{-1} 2, 4-D + 0.5 mgL^{-1} NAA responded best to callusing. Six weeks old calli were transferred onto regeneration medium containing three levels of NaCl salt viz. 5, 10 and 15 gL^{-1} respectively with a view to isolate salt tolerant rice somaclones. Plant regeneration of plated calli varied with salt concentrations and with an increase in the salt concentration plant regenerated frequency decreased. Maximum plant regeneration was noticed on the medium supplemented with 0 gL^{-1} NaCl salt and lowest on the medium containing 15 gL^{-1} NaCl salt. But the rice cultivars Koijor and Nayanmony produced regenerated plants in all salt media with variable degree of frequency. Calli of rice cultivar, Bapui did not respond to regeneration at all.

Key words: Rice, Callus, 2,4- D, Plant regeneration, NaCl salt.

Introduction

Boro rice (*Oryza sativa* L.) is the most important crop in South Asia. Due to higher yield, farmers prefer to cultivate this crop.

In Bangladesh boro rice occupied nearly 41.72% of the 4.81 million ha of rice harvested area and contributed 55.35% of the 18.75 million tons of rice produced in 2011-2012. The country average yield in 2011-2012 was 3.9 tha^{-1} [1].

Most of the lands remain fallow in the dry season (January–May) because of soil salinity and the lack of suitable irrigation water [2, 3]. About 1.0 million ha of arable lands are affected by varying degrees of salinity [4]. Soil and water salinity limits boro rice cultivation in coastal regions of Bangladesh. So development of saline tolerant rice cultivars is a crying need for salt affected areas as because rice is the main cereal in Bangladesh. To meet this challenge rice scientists have been working extensively to develop and introduce new and modern salt tolerant rice varieties for increasing yield to ensure food security of ever growing population of Bangladesh. Various methods such as hybridization, genetic engineering, mutation breeding, tissue culture etc. are the useful tools to develop salt tolerant varieties for specific purposes. Tissue culture techniques have been widely used in plant breeding, especially in selection of stress tolerant crop varieties. Tissue culture is a useful tool that gives rise genetic variability through genetic modifications during the process of *in vitro* culture, that leads to produce somaclonal variation [5, 6, 7]. Production of callus formation and its subsequent regeneration under salt stress may facilitate selection of salt tolerant rice genotypes [8]. Once greater Khulna District of Bangladesh was a reservoir of rice land races with valuable genetic resources. Rapid urbanization and replacement of land races with modern high yielding rice cultivars most of the local races were extincted. They are in the verge of extinction. The rest need spatial care for not only to protect from genetic erosion but also explore and exploit their genetic potential for combating salinity, drought, submergence or any other adverse environmental hazards. Under the above circumstances the

present research work was taken to study the following objectives

- ✓ Optimization of auxin(s) concentration for maximum callus induction in dehusked rice seed culture
- ✓ Isolation of rice somaclones from plated calli under different levels of NaCl salt

Materials and Methods

Manually dehusked seeds of three local boro rice cultivars, native to Khulna district *viz.* Koijor, Nayanmony and Bapui were aseptically inoculated on callus inducing MS (Murashige & Skoog, 1962) medium containing following combination and concentration of auxins: 1.0 mgL^{-1} 2, 4-D + 0.0 mgL^{-1} NAA; 1.5 mgL^{-1} 2, 4-D + 0.0 mgL^{-1} NAA; 2.0 mgL^{-1} 2, 4-D + 0.0 mgL^{-1} NAA; 1.0 mgL^{-1} 2, 4-D + 0.5 mgL^{-1} NAA; 1.5 mgL^{-1} 2, 4-D + 0.5 mgL^{-1} NAA; 2.0 mgL^{-1} 2, 4-D + 0.5 mgL^{-1} NAA; 1.0 mgL^{-1} 2, 4-D + 1.0 mgL^{-1} NAA; 1.5 mgL^{-1} 2, 4-D + 1.0 mgL^{-1} NAA and 2.0 mgL^{-1} 2, 4-D + 1.0 mgL^{-1} NAA. The cultures were kept in the dark condition for four week in the dark followed by 2 weeks under sixteen hours photoperiod at 25±1°C.

After six weeks of inoculation of seeds calli were plated on freshly prepared MS medium supplemented with 0.1 mgL^{-1} IBA, 0.5 mgL^{-1} Bavistin, 3 mgL^{-1} BAP and different levels of NaCl salt (0, 5.0, 10.0 and 15.0 gL^{-1}) to allow plant regeneration. The cultures were kept under fluorescent light in a growth chamber at 25 ± 1 ^{0}C temperature and 16 hours photoperiod with 2000-3000 lux light intensity. After 4 weeks the plated calli with meristemoid spots were sub cultured on the same regeneration medium.

The regenerated plants with small amount of roots or without roots were transferred in liquid MS medium containing IBA 1.0 mgL^{-1} for allowing massive rooting. Well rooted plants were then transferred to a small pot containing sterilized sand supplemented with liquid MS media after removal of agar

attached with roots and treated the plants with an antifungal solution Bavistin @ 0.1%. The plants were then watered with distilled water. Each pot was covered with polythene after spraying water inside it and closed to check evapotranspiration. The pots were kept in green house for 10 days under continuous care.

Established plants from small pots were transferred to large pots containing puddle field soil and were kept in net house. They were frequently watered and kept under observation for three weeks. In the present experiment, data on the following parameters were collected for evaluation:

I. Callus induction frequency (%) *in vitro*
II. Regeneration frequency (%) *in vitro*
III. Survivability (%) of regenerated plants *ex vitro*

Results and Discussion

Surface sterilized dehusked seeds were inoculated on MS medium containing different concentrations of 2, 4-D and NAA. Mean frequency of callus formation varied significantly with different varieties (Table 1). Maximum frequency of callus induction (80.89%) was obtained for the cultivar Koijor which was statistically similar with that of Nayanmony but was significantly higher than Bapui (74.44%), whereas callus induction in Nayanmony and Bapui was indifferent. Genotypic dependent callus induction were also reported [9, 10].

Table 1: Effect of cultivars on callus induction frequency in rice seed culture

Cultivar	No. of seeds inoculated	No. of seeds producing callus	Callus induction frequency (%)
Bapui	450	335	**74.44**
Nayanmony	450	340	75.56
Koijor	450	364	**80.89**
$LSD_{0.01}$			5.38
CV (%)			8.29
Level of Significance			0.01

Significant effect of different growth regulators on callus induction noticed (Table 2). The frequency of callus in different growth regulators varied from 88% to 67.33%. Among the different concentrations of auxins, MS medium supplemented with 2.0 mgL^{-1} 2, 4-D in combination with 0.5 mgL^{-1} NAA was found the most effective for callus induction (88%). The lowest frequency was recorded for the treatment combination of 1.0 mgL^{-1} 2, 4 –D + 0.0 mgL^{-1} NAA (67.33%) and 1.0 mgL^{-1} 2, 4-D + 0.5 mgL^{-1} NAA (68.67%) followed by 1.5 mgL^{-1} 2, 4- D + 1.0 mgL^{-1} NAA (71.33%). It was reported that the highest callus induction percentages in rice seed culture were observed in the medium containing 2.0 mgL^{-1} 2, 4-D and these results are in agreement with the present findings [11].

Table 2: Effect of growth regulators on callus induction frequency in rice seed culture

Auxin combinations (mgL^{-1})		No. of inoculated seeds	No. of seeds responded to callus	Callus induction frequency (%)
2, 4- D	NAA			
1.0	0.0	150	101	**67.33 e**
1.5	0.0	150	120	80.00 bc
2.0	0.0	150	123	82.00 b
1.0	0.5	150	103	68.67 e
1.5	0.5	150	121	80.67 bc
2.0	0.5	150	132	**88.00 a**
1.0	1.0	150	113	75.33 cd
1.5	1.0	150	107	71.33 de
2.0	1.0	150	119	79.33 bc
CV (%)				8.29
Level of Significance				0.01

*Frequency of callus accompanied by the same letter (s) did not differ significantly at 1% level as per DMRT

The interaction effect of cultivars and growth regulators differed significantly for callus induction frequency (Table 3).

Table 3: Effect of cultivars × growth regulators interaction on callus induction in rice seed culture

Cultivars	Auxin combinations (mgL^{-1})		No. of seeds inoculated	No. of seeds responded to callus induction	Callus induction frequency (%)
	2, 4 –D	NAA			
	1.0	0.0	50	30	60 gh
	1.5	0.0	50	41	82 bcd
	2.0	0.0	50	41	82 bcd
	1.0	0.5	50	36	72 ef
Bapui	1.5	0.5	50	38	76 cdef
	2.0	0.5	50	40	80 cde
	1.0	1.0	50	36	72 ef
	1.5	1.0	50	34	68 fg
	2.0	1.0	50	39	78 cde
	1.0	0.0	50	34	68 fg
	1.5	0.0	50	38	76 cdef
	2.0	0.0	50	42	84 bc
	1.0	0.5	50	29	**58 h**
Nayanmony	1.5	0.5	50	41	82 bcd
	2.0	0.5	50	45	90 ab
	1.0	1.0	50	37	74 def
	1.5	1.0	50	36	72 ef
	2.0	1.0	50	38	76 cdef
	1.0	0.0	50	37	74 def
	1.5	0.0	50	41	82 bcd
	2.0	0.0	50	40	80 cde
	1.0	0.5	50	38	76 cdef
	1.5	0.5	50	42	84 bc
Koijor	2.0	0.5	50	47	**94 a**
	1.0	1.0	50	40	80 cde
	1.5	1.0	50	37	74 def
	2.0	1.0	50	42	84 bc
CV (%)					8.29
Level of Significance					0.05

* Frequency of callus accompanied by the same letter (s) did not differ significantly at 5% level as per DMRT

Callus induction frequencies varied from 58% to 94%. Maximum callus formation (94.00%) was recorded for Koijor followed by Nayanmony (90.00%) when seeds were cultured on MS medium supplemented with 2, 4-D 2.0 mgL^{-1} + 0.5 mgL^{-1} NAA and lowest (58 %) for 2, 4-D 1.0 mgL^{-1}+ 0.5 mgL^{-1} NAA followed Bapui aus treated with 2, 4- D 1.0 mgL^{-1} + NAA 0.0 mgL^{-1}. It was reported that, MS + 2, 4-D (2 mgL^{-1}) was most suitable medium for target cultivars to callus formation [12]. It was also confirmed by many researchers and they postulated

that rice genotype, media formulation, concentration of plant growth regulators (PGR) and genotype × PGR interaction would play important role in callus induction from inoculated explants *in vitro* [13, 14, 15, 16, 17].

After four weeks of inoculation, calli were subcultured on MS medium containing 3 mgL^{-1} BAP, 1.0 mgL^{-1} IBA and 0.5 mgL^{-1} Bavistin supplemented with various level of NaCl salt *viz.* 0 gL^{-1},5 gL^{-1}, 10 gL^{-1} and 15 gL^{-1}, respectively.

The effect of salt concentrations on the plant regeneration was highly significant. It was noticed that calli of the cultivar Bapui did not respond to regeneration at all. Other two cultivars regenerated variably with the level salt concentrations. Both the cultivars showed gradual decline in regeneration potentiality with increasing salt concentrations (Table 4). Maximum regeneration frequency was recorded for Nayanmony (46.67%) and Koijor (48.33%) in the medium supplemented with 5 gL^{-1} NaCl salt. It was also reported that 55% plated calli of indica rice cultivar showed regeneration and with an increased in salt concentration regeneration potential decreased.

Table 4: Plantlet regeneration from plated calli under various levels of NaCl salt in two rice cultivars

Cultivars	NaCl (gL^{-1})	No. of calli plated onto regeneration media	No. of calli responded to plant regeneration	Regeneration frequency (%)
Nayanmony	5	60	28	**46.67a**
	10	60	17	28.33bc
	15	60	9	**15.00c**
Koijor	5	60	29	**48.33a**
	10	60	19	31.17b
	15	60	11	**18.33bc**
Level of Significance				0.01

***** Frequency of regeneration accompanied by the same letter (s) did not differ significantly at 1% level as per DMRT

Well rooted plantlets were transplanted in plastic pot (10cm × 7cm) containing coco dust mixed with vermicompost (3:1), and were kept in hardening room maintaining humidity >90% and temperature 30 °C; light intensity for 1st week 3000 lux and gradually increased up to 4000 lux in 6th week. Data were recorded for survivability of the *ex vitro* transferred plantlets (Table 5). About 50% of the plantlets were survived from both the cultivars.

Table 5: Frequency of plant survivability of the regenerated plants *ex vitro*

Cultivars	Salt concentration (g L^{-1})	Frequency of the survivability (%)
Nayanmony	5	**55.55**
	10	54.84
	15	**41.67**
Koijor	5	**53.19**
	10	48.28
	15	**40.00**
Level of significance		NS

NS= Not significant

Conclusions

Dehusked seeds of three local boro rice cultivars native to south Khulna region named Bapui, Nayanmony and Koijor were cultured on MS medium supplemented with different concentrations and combinations of 2, 4- D and NAA for callus induction with a view to select and optimize suitable combination and concentration of these PGR for maximum callusing in different rice cultivars. Six weeks old calli were transferred onto regeneration media containing three levels of NaCl salt *viz.* 5, 10 and 15 gL^{-1}, respectively to isolate salt tolerant somaclones. The well rooted plantlets were gradually acclimatized with natural environment.

Results on callus induction showed that callus induction ability of the cultivar Koijor (80.89%) was significantly higher than other two cultivars, Bapui (74.44%) and Nayanmony

(75.56%). In case of growth hormone, 2, 4-D 2.0 mgL^{-1} with NAA 0.5 mgL^{-1} produced the best results, 88% of the cultured seeds responded to callusing. Genotype × PGR interaction indicated that for all the three cultivars most suitable combination of PGR was 2, 4-D 2.0 mgL^{-1} + NAA 0.5 mgL^{-1}, although effect of 2, 4-D @ 2.0 mgL^{-1} was also found to be closer to previous combination. In this experiment only two types of auxin were used. It is suggested to study with other sources of auxin to maximize callusing for the rice cultivars studied.

The screening of calli for plant regeneration under NaCl stress consisted of two passages each with 30 days. After second passage, it was found that calli of the Bapui cultivar did not respond at all to regeneration. The other two cultivars produced regenerated plant with various levels of NaCl salts. Moreover maximum regenerated plants were noticed with 5 gL^{-1} NaCl salt and with increasing salt concentration regeneration rate decreased.

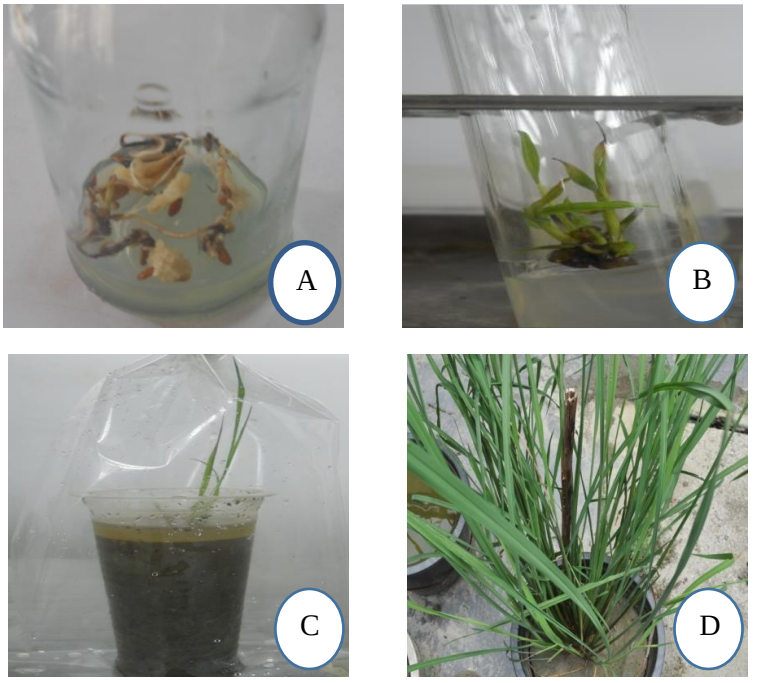

Fig. 1. Dehusked seeds of rice were cultured on MS supplemented with different concentrations and combinations of 2, 4-D and NAA for callus induction (A). The calli were cultured for shoot induction on regeneration media supplemented with different concentrations of NaCl salt (B). The regenerated plantlets were transferred to polythene covered small pots containing mixture of sand and vermicompost (C). Well acclimatized regenerated plants after transferring in large pots in field (D).

REFERENCES

[1]. BBS, Bangladesh Bureau of Statistics, Statistical Year Book of Bangladesh, Bangladesh Bureau of Statistics, Ministry of Planning, Govt. of The People's Republic of Bangladesh; Dhaka, Bangladesh.pp.23-34, 2012.

[2]. Karim, Z., S. G. Hussain and V. Ahmed. 1990. Salinity problems and crop intensification in the coastal regions of Bangladesh. Soils publication No. 33, BARC, p.17.

[3]. Mondal, M. K. 1997. Management of soil and water resources for higher productivity of the coastal saline rice lands of Bangladesh. PhD thesis, University of the Philippines, Los Banos, Philippines.

[4]. Islam, M. R. 2004. (Ed.) Where Land Meets the Sea: A Profile of the Coastal Zone of Bangladesh, The University Press Limited, Dhaka.

[5]. Larkin, P. J. and W.R. Scowcroft. 1981. Somaclonal variation: a novel source of variability from cell cultures for plant improvement. Theoretical and Applied Genetics, 60: 197–214.

[6]. Sun, Z. X. and K. L. Zheng. 1990. Somaclonal variation in rice, in Y.P.S. Bajaj (Ed), Biotechnology in agriculture and forestry, SpringIer-Verlag, Berlin). 11: 288-325.

[7]. Kaeppler, S. M., R. L. Phillips and P. Olhoft. 1997. Molecular basis of heritable tissue culture-induced variation in plants, in: Jain et al. (Ed.), Somaclonal variation and induced mutations in crop improvement. Current Plant Science and

Biotechnology in Agriculture, (Kluwer Academic Publishers, Dordrecht, Netherlands).32: 465–484.
[8]. Tam, D. M. and N.T. Lang. 2003. In vitro selection for salt tolerance in rice, Omonrice. 11: 68-73.
[9]. Diah, R and H. Anzai. 2006. Studies on callus induction, plant regeneration and transformation of javanica rice cultivars, Plant Biotechnology. 23: 521-524.
[10]. Fredeslinda, C. E., R. A. Rhodora and B.U. Lilian. 2009. Callusing and regeneration potential of rice (Oryza sativa L.) genotypes towards the development for salt tolerance. Philippine Journal of Science. 138 (2):169-176.
[11]. Libin, A., P. J. King., K. H. Ong., J. K. Chubo and P. Sipen. 2003. Callus induction and plant regeneration of Sarawak rice (Oryza sativa L.) variety Biris. African Journal of Agricultural Research. 7(30): 4260-4265.
[12]. [8] Tam, D. M. and N.T. Lang. 2003. In vitro selection for salt tolerance in rice, Omonrice. 11: 68-73.
[13]. Pandey, S. K., B. Ramesh and P.K. Gupta. 1994. Study on effect of genotypes and culture medium on callus formation and plant regeneration in rice (Oryza sativa L.), Indian Journal of Genetics and Plant Breeding.54 (3): 293-299.
[14]. Shankhdhar, D., S. C. Shankhdhar and R. C. Pant. 2002. Development of somatic embryos in rice. Indian J. Plant Physiol., 7(3): 211-214.
[15]. Naqvi, S. M., S. Razia and H. Rashid. 2005. Tissue culture studies in Oryza sativa L. cvs. Basmati 385 and Super Basmati. Pakistan journal of Botany, 37(4): 823-828.
[16]. Kabir, A. H., I. Mahfuz, M. A. Razvy, M. B. Ahmed and M.F. Alam. 2008. Indirect organogenesis and somaclonal variation in four rice cultivars of Bangladesh. Journal of Applied Sciences Research, 4(4): 451-458.
[17].Jaseela, F., V. R. Sumitha and G.M. Nair. 2009. Somatic embryogenesis and plantlet regeneration in an agronomically important wild rice species Oryza nivara. Asian Journal of Biotechnology, 1(2): 74-78.

[18]. Sangita, B., G. Gangopadhyay and B. B. S. Mukherjee. 1981. Salt tolerance in rice in vitro: Implication of accumulation of Na^+, K^+ and proline, Plant cell, Tissue and Organ culture, 69(1): 55-64.