http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 7 (5): 861-864, 2004
ISSN 1028-8880

Response to High Frequency Callus Induction Ability from Root Regions of Germinated Embryo in Indica Rice

[1]T.L. Aditya, [2]M.E. Hoque and [3]M. Khalequzzaman
[1]Plant Breeding Division, Bangladesh Rice Research Institute, Gazipur-1701, Bangladesh
[2]Biotechnology Division, Bangladesh Rice Research Institute, Gazipur-1701, Bangladesh
[3]Genetic Resources and Seed Division, Bangladesh Rice Research Institute, Gazipur-1701, Bangladesh

Abstract: High frequency callus induction ability was achieved from well-germinated embryos (on hormone free MS media) of eight indica rice genotypes by culturing on MS medium supplemented with 2 mg l^{-1} 2,4-D and solidified with 0.3% phytagel. When mature seeds were directly cultured, the highest frequencies of callus induction were observed for two genotypes (IR51491-AC5-4 and BR24) out of eight genotypes tested. However, embryogenic callus induction ability of different genotypes significantly differed. Calli from scutellar tissue formed yellow, compact, friable and globular type embryogenic calli designated as embryogenic (E) type I. In contrast when callus produced from root regions of germinated embryo all genotypes showed high callus induction ability and formed soft-friable, pale yellow, shiny and nodular calli designated as embryogenic type II. In both case crystalline, hard, white with suppressed root primordia were formed which were designated as non-embryogenic (NE) calli. The simple procedures described in this paper provide an efficient protocol for reproducible callus induction method of Bangladeshi rice varieties/line, which is pre-requisite for a good regeneration system and a source of fine embryogenic cell suspension cultures for protoplast isolation and genetic transformation method.

Key words: Induction frequency, mature embryo, root, indica rice

INTRODUCTION

A callus is a largely unorganized, continued proliferation of undifferentiated parenchyma cells from parent tissue on clearly defined semi-solid media. From a functional point of view the most important characteristics of callus are that this abnormal growth has the potentiality to develop normal roots, shoots and embryoids, which form plantlets. Faruhashi and Yatazama[1] first observed that exogenous supply of 2,4-D stimulated/triggered the production of callus from rice stem and roots of rice seedlings[2]. Callus has been initiated from various explants of rice including immature embryo[3,4], mature embryo[5,6], roots[7], anthers[8] mature endosperm[9], stem base[10] and young coleoptiles[11]. High frequency callus production was achieved when mature seeds of indica rice was cultured on MS based media supplemented with strong auxin, such as, 2,4-D[5,12,13].

The genotype of the donor plant is the most important factor in callus induction in rice. Several workers observed variations in culture responses among indica rice genotypes[5,11]. Therefore, the choice of genotype for *in vitro* studies has led a dramatic influence on the probability of successful culture initiation and plant regeneration.

Vasil and Vasil[14] defined two types of callus as embryogenic (type I and type II) and nonembryogenic callus. Finch *et al.*[15] recognised embryogenic callus by its compact and nodular or loosely globular, dry appearance and a yellow/white colouration whereas non-embryogenic callus was observed often mucilaginous and made up of suppressed root primordia. Callus cultures on periodical subcultures result in somaclonal variation and genetic instability, causing polyploidy, aneuploidy, mutations, chromosome translocation, gene amplification, etc.[16].

The objectives of the study were to establish reproducible callus induction ability derived from root regions of well-germinated mature embryos of eight Bangladeshi indica rice compared with calli derived from scutellar tissue and select the best genotypes for further tissue culture procedures such as regeneration ability, protoplast culture and genetic transformation.

MATERIALS AND METHODS

Cultivars and explant preparation: Mature dehulled rice seeds (*Oryza satriva* L.) of eight indica cultivars were used in this study. Out of eight genotypes four namely BR24, BR26, BRRI dhan29 and BRRI dhan40 were

Corresponding Author: Dr. Tamal Lata Aditya, Senior Scientific Officer, Plant Breeding Division, Bangladesh Rice Research Institute, Gazipur-1701, Bangladesh E-mail: brrihq@bdonline.com

developed by Bangladesh Rice Research Institute (BRRI). One homozygous breeding line IR51491-AC5-4 was originated from International Rice Research Institute currently testing in BRRI for salt tolerance. Patnai, Pokkali, Binnatoa were moderately salt tolerant land races cultivated in coastal areas of Bangladesh. Dehulled seeds were immersed in distilled water with one drop of Tween twenty to clean the materials and washed with sterile distilled water. After that seeds were surface sterilized by dipping in 70% (v/v) ethanol for 3 min followed by 25 min in a sodium hypochlorite solution (commercial bleach) by rigorous shaking. Seeds were rinsed for three times by sterile distilled water after each sterilization procedure.

Culture of seed on callus induction media

Culture of seed by one-step procedure: In one-step method eight sterilized seeds were aseptically germinated in Petridishes (9 cm dia, sterilin UK) containing 25 ml MS medium[17] supplemented with 3% (w/v) sucrose, 2 mg l^{-1} 2,4-D at pH 5.8 and semisolidified with 0.3% phytagel designated as MS2 callus induction media.

Culture of seed by two-step procedure: In two-step procedure eight sterilized seeds were germinated in same type of Petridishes on hormone-free MS medium with 3% sucrose and 0.8% agar at 25±1°C in the dark. After one week well-germinated seeds were transferred into same MS2 media as used in one-step procedure. Eight Petridishes were represented as eight replications for each genotype. Cultures were incubated at 25±1°C in the dark.

Assessment of callus growth: Assessment of callus growth was measured on fresh weight (fr.wt.) basis, expressed as a percent weight gain after a certain period of time by using 5-week-old calli. Firstly, the known weight of callus tissue was aseptically transferred onto 10 cm glass tube containing 10 ml of MS1 (MS medium+1 mg l^{-1} 2,4-D) callus induction media. After six weeks, the callus pieces from glass tubes were removed with a sterile spatula and re-weighed under same sterile conditions.

Determination of different parameters

Callus initiation (%): With regard to one-step procedure of callus initiation, about 2-3 weeks later, the number of seeds forming callus in each treatment (genotype represented as treatment) was counted and converted to percentage. On the other hand for two-step procedure, after 4-5 weeks when primary and some others secondary roots were between 2-5 cm long, callus induction frequency was recorded by counting seeds forming calli in each individual treatment. The percentage of callus initiation was recorded by using the following formula:

$$\text{Callus initiation (\%)} = \frac{\text{Number of callus producing seed}}{\text{Total number of seed plated}} \times 100$$

Weight gain (%): Weight gain was calculated from initial and final fresh weight by using the following formula:

$$\text{Weight gain (\%)} = \frac{\text{Final fresh weight} - \text{Initial fresh weight}}{\text{Final fresh weight}} \times 100$$

Experimental design and statistical analysis: Completely Randomized Design (CRD) was used for conducting experiment and data were analyzed for one way ANOVA by using SAS package as developed by Computer software programme. LSD test was applied for the evaluation of statistical significance of differences. Qualitative parameters e.g. texture and nature of callus were assessed by visual observation by a scoring system on a scale of 1 to 5; e.g. 1-pale yellow to yellow, friable, shiny and nodular; 2-yellow, soft- friable with nodular surface; 3-yellow, compact, dry with nodular surface; 4-bright yellow, dry and crystalline; 5-bright yellow, hard, 'rooty' and mucilaginous. Browning and necrosis of calli were assessed by also a scoring system on a same scale as above e.g. 1-yellow to pale yellow; 2-light brown; 3-brown; 4-deep brown; 5-necrosis or completely black.

RESULTS AND DISCUSSION

Assessment of callus induction frequency: Standardization of callusing performance as callus induction frequency was achieved by seed germination and subsequent callus initiation. Embryo germination was recorded to be high as 90% for all the genotypes tested. Statistical analysis showed highly significance variations ($P<0.01$) among the genotypes for callus induction frequency. Callus induction frequency was in the range of 36.25-76.88% for one- step procedure and 53.13-84.38% for two-step procedure (Table 1). BRRI dhan40 and BRRI dhan29 showed low callus induction frequency by one-step procedure but they improved their induction frequency by two-step procedure (Table 1). All the genotypes performed better in two-step procedure except Patnai and BR23. Differences in callus induction frequency among different cultivars were noticed in different studies in rice tissue culture[5,11]. Differences in callus induction frequency ($P<0.01$) among different genotypes were observed in the current study and supported previous studies. This is fact that the callusing

Table 1: Callusing performance of eight indica rice genotypes on MS basal callus induction media

	Callus initiation (%)		Weight gain in callus(%)	
Genotype	One-step	Two-step	One-step	Two-step
IR 5149-AC5-4	69.00ab	78.13ab	77.00c	87.00ab
BRRI dhan40	36.25d	53.13d	80.63bc	90.00a
Patnai	67.38ab	59.38c	73.50c	87.50ab
BR24	76.88a	84.38a	85.63ab	86.50b
BR26	70.50ab	59.38c	90.50a	95.50a
Pokkali	62.75abc	68.75bc	87.38ab	97.00a
Binnatoa	58.00bc	76.56ab	91.25a	98.30a
BRRI dhan.29	50.25dc	78.12ab	74.13c	82.36c
P value	<0.01	<0.01	< 0.01	0.05
LSD at 5%	14.51	14.18	8.59	4.42

Means are average of 8 replications. Means followed by same letter(s) are not significantly different from each other.

Fig. 1a-b: Callusing performance of indica rice; a) callus derived from scutellum tissue; b) callus derived from primary and secondary root regions

responses of explants depend on the choice of genotype (Table 1). This might be due to the differences in physiological status of original mature seeds.

Assessment of callus growth on fresh weight basis: Variations in the response for callus growth on fresh weight basis were clearly observed when averages of wait gain as percentages were compared (Table 1). In the current study, percent weight gain was higher for all genotypes in the case of two-step procedure than one-step procedure. In both case significant differences ($P<0.01$ or $P=0.05$) were observed among the genotypes for weight gain. The reason for increased weight gain in two-step procedure than one-step procedure was mainly due to more water content and phenotype (sticky and soft-friable) of root calli than calli derived from scutellar axis. Similar types of calli was found by Mukhopadhyay *et al.*[7]. Assessment of callus growth and productivity provides useful data on the basis of which a decision can be made to retain a cell line with a high growth rate, a high productivity, or comparison between two parameters.

Response to callus initiation and characterization of texture and nature of callus: In case of one-step procedure the initiation of callus started 14 days after inoculation on callus induction medium for IR51491-AC5-4, BR24, BR26 and Binnatoa whereas in two-step procedure all genotypes responded to callus initiation by 21-24 days except BRRI dhan29 (Table 2). BRRI dhan29 responded to initiation by 24 days in one-step procedure and by 28 days in two-step-procedure. The response to initiation of callus was faster in embryonic or scutellar axis than that of root regions since in the one-step procedure mature seeds were directly cultured on callus induction medium.

In one-step procedure, 14 days after culture the cells of scutellar tissue produced a continuous meristematic zone behaved as a proliferating unit, forming typical nodular calli designated as embryogenic (E) type I (Fig. 1a). Genotypes IR51491-AC5-4, Pokkali and Binnatoa formed yellow, compact and sometimes crystalline in texture by one step-culture (Table 2). On the other hand genotypes BR24 and BR26 produced soft, friable and globular type calli which were designated as embryogenic (E) type II. In case of one-step procedure approximately 50% of the calli were embryogenic, friable (E type I) and contained distinct, white, globular somatic embryos on its nodular surface while 20% of calli (E type II) were soft-friable and loosely globular and suitable for fine cell suspension. Same types and nature of calli were described by Vasil and Vasil[14].

More than 70% of embryogenic calli (type II) were produced from root regions of one individual seed by two-step procedure. More or less all calli were designated as embryogenic type II described with friable, soft friable, shiny, loosely globular and yellow to pale yellow in colouration (Fig. 1b). Non-embryogenic calli (NE) were also formed together with embryogenic calli with respect of genotypes IR51491-AC5-4, BRRI dhan40 and Patnai. In the present study callus induction frequency was satisfactory on MS based media in the presence of auxin (2,4-D at 1 or 2 mg l^{-1}) without any cytokinin or other additives such as BAP, kinetin, ABA, tryptophan, casein hydrolysate, which agreed with previous studies[18,19]. In contrast several researchers used MS based induction media supplemented with any kind of above additives[12,20]. Results from their study showed that no increase in callus ability than the current study. The current study indicated to use MS basal media as standard and unique culture media for callus induction ability.

In conclusion, root regions of germinated embryos was found to be better for high frequency callus induction while seed scutellum axis performed better for embryogenic callus formation. Identifying genotypes with high callus ability and phenotype of calli were the most

Table 2: Responsiveness to callus initiation, texture, nature of callus with their fate after 8-10 weeks of culture derived from mature seed scutellum and root regions of well-germinated seeds

	Days response to callus initiation		Texture and nature of callus		Fate of callus (8 – 10 week old)	
Genotype	One-step	Two-step	One-step	Two-step	One-step	Two-step
IR 51491-AC5-4	14	21	3	2	2	1
BRRI dhan40	21	24	4	2	4	2
Patnai	17	21	3	3	4	2
BR24	14	21	2	1	5	3
BR26	14	23	2	2	5	3
Pokkali	16	21	2	2	4	2
Binnatoa	14	24	1	1	2	1
BRRI dhan29	24	28	2	2	2	2

One-step and two-step : Callus induction methods Scoring was recorded for overall visual observation of each genotype

important factors to provide an efficient regeneration system and a source of good embryogenic cell suspension cultures for somatic hybridization and genetic transformation methods.

REFERENCES

1. Furuhashi, K. and M. Yatazama, 1964. Indefinite culture of rice stem node callus. Kagaka, 34: 623.
2. Yatazawa, M., K. Furuhashi and T. Suzuki, 1967. Growth of callus tissue from rice roots *in vitro*. Plant Physiol., 18: 363-373.
3. Zhang, S., 1995. Efficient plant regeneration from indica (group 1) rice protoplasts of one advanced breeding line and three varieties. Plant Cell Report, 15: 68-71.
4. Chand, S. and A.K. Sahrawat, 2001. Stimulatory effect of partial desiccation on plant regeneration in indica rice (*Oryza sativa* L.). J. Plant Biochem. Biotechnol., 10: 43-47.
5. Khana, H.K. and S.K. Raina, 1998. Genotype×culture media interaction effects on regeneration response of three indica cultivars. Plant Cell Tissue and Organ Culture, 52: 145-152.
6. Yang, Y.S., Y.D. Zheng and Y.L. Chen, 1999. Improvement of plant regeneration from long-term cultured calluses of Taipei 309, a model rice variety in *in vitro* studies. Plant Cell Tissue and Organ Culture, 57: 199-206.
7. Mukhopadhyay, A., D. Minhas and A. Grover, 1997. Callusing from rice root explants: Adventitious root formation precedes callus initiation response. Curr. Sci., India, 73: 465-469.
8. Sugimoto, K., H. Miyake and Y. Takeoka, 1999. Somaclonal variation in regenerants derived from anther culture of rice (*Oryza sativa* L.). Plant Prod. Sci., 2: 71-76.
9. Bajaj, Y.P.S., 1991. Biotechnology in rice (*Oryza sativa* L) improvement. In: Bajaj, Y.P.S. (Ed.). Biotechnology in Agriculture and Forestry. Rice. Springer-Verlag Berlin Heidelberg, 14: 1-18.
10. Finch, R.P., A. Baset and I.H. Slamet, 1992. *In vitro* shoot culture of wild oryzae and other grass species. Plant Cell Tissue and Organ Culture, 30: 31-39.
11. Oinam, G.S. and S.L. Kothari, 1993. Genotypic differences in embryogenic callus formation and plant regeneration in indica rice. IRRN 18: 3.
12. Kunanuvatchaidach, R., I.D. Godwin and S.W. Adkins, 1995. High efficiency plant regeneration from callus induced on mature indica rice caryopses. Australian J. Bot., 43: 337-348.
13. Seraj, Z.I., Z. Islam, M.O. Faroque, T. Devi and S. Ahmed, 1997. Identification of the regeneration potential of embryo derived calluses from various indica rice varieties. Plant Cell Tissue and Organ Culture, 48: 9-13.
14. Vasil, I.K. and V. Vasil, 1992. Advances in cereal protoplast research. Physiol. Plantarum, 85: 403-410.
15. Finch, R.P., P.T. Lynch, J.P Jotham and E.C. Cocking, 1991. Isolation, Culture and Fusion of Rice protoplasts. In: Bajaj, Y.P.S. (Ed.). Biotechnology in Agriculture and Forestry. Rice. Springer-Verlag Berlin Heidelberg, 14: 253-268.
16. Larkin, P.R. and W.R. Scowcroft, 1981. Somaclonal variation- a novel source of variability from cell culture for plant improvement. Theor. Appl. Gen., 60: 197-214.
17. Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plantarum, 15:473-497.
18. Oinam, G.S. and S.L Kothari, 1995. Totipotency of coleoptile tissue in indica rice. Plant Cell Report, 14: 245-248.
19. GonŸalez, M.C., 2000. Effects of different growth regulators on *in vitro* culture of rice cv. Amistad-82. Cultivos Tropicales, 21: 27-28.
20. Chatterjee, B. and P.D. Gupta, 1998. Induction of somaclonal variation by tissue culture and cytogenetic analysis in *Oryza sativa* L. Biol. Plantarum, 40: 25-32.