Pak. J. Bot., 44(2): 671-673, 2012.

OPTIMIZATION OF THE CULTURE CONDITIONS IN THE RICE (*ORYZA SATIVA* CV. BASMATI-370)

ROMESA TAHIR[1], MARIA BATOOL[1], NASIR MAHMOOD MINHAS[1], SHAUKAT ALI[2] SHAHID MASOOD[2] AND SAIF ULLAH AJMAL[1*]

[1]*Department of Plant Breeding and Genetics,PMAS Arid Agriculture University, Rawalpindi, Pakistan.*
[2]*Agricultural Biotechnology Programme, Institute of Agricultural Biotechnology & Genetic Resources, National Agricultural Research Center, Islamabad, Pakistan*
**Corresponding author: gondaldr@yahoo.com*

Abstract

The objective of the present study was to develop an efficient callus induction protocol for a fine rice (*Oryza sativa* L.) variety Basmati-370. Mature grains of this variety were used for callus induction. The Chu's N6 medium was used as basal medium for callus induction and was supplemented with sucrose, Myo-inositol, Casein, agar (4 g L^{-1}, 5 g L^{-1}, 6 g L^{-1}) and growth hormone 2,4-Dichlorophenoxy acetic acid in different concentrations of 2ppm and 2.5ppm. The genotype responded well for all the three concentrations of agar (4 g L^{-1}, 5 g L^{-1}, 6 g L^{-1}) and two concentrations of 2,4-D (2ppm, 2.5ppm), however, the combination of 4 g L^{-1} agar with 2.5ppm 2,4-D gave excellent results with maximum callus induction of 93.42% followed by 4 g L^{-1} agar with 2ppm 2,4-D and 5 g L^{-1} with 2.5ppm 2,4-D which also gave better results with the observed callus induction of 92.75% and 91.11%, respectively.

Introduction

Rice (*Oryza sativa* L.) is an important food crop in the world and feeds over half of the global population (Sasaki, 2005). It is an important source of carbohydrate for human consumption. It provides food and forage and besides this, rice and rice oils are used in cosmetics while starch in textiles. Over 90% of rice is cultivated in Asia (Bano *et al.*, 2005). It covers half of the arable land in many countries in this continent (Cantrell & Hettel, 2004). Rice is the second chief commodity of Pakistan occupying about 11 % of cultivated area in the country. There are two main groups of rice viz. aromatic (Basmati) and coarse (Rashid *et al.*, 2003). Basmati characteristics include aroma, high kernel elongation and fluffiness of the cooked grain. Basmati varieties are highly priced and much in demand for export. The best example of basmati rice is a tall indica variety Basmati-370 which is widely grown (Raina *et al.*, 1987). World population is increasing rapidly and to meet its demands rice production needs to be increased (Tariq *et al.*, 2008; Bashir *et al*., 2010). The conventional methods have considerably increased rice yield but the demand of the day is to utilize all the techniques possible for crop improvement so the use of biotechnology could speed up the process further. Tremendous progress has been made in the area of plant biotechnology during the last decade. One of the important components of biotechnology comprises of the *In vitro* techniques (Tariq *et al.*, 2008). The observation that the plant cells are totipotent was first made by Haberlandt and this observation served as a prerequisite for the beginning of research in the area of tissue culture (Rashid *et al.*, 2003). Tissue culture of dicots is considered simpler as compared to monocots. It is possible to regenerate whole plant from cereal species; such as bread wheat (Redway *et al.*, 1990), maize (Duncan *et al.*, 1985), rice (Yamada *et al.*, 1986) and barley (Luhrs & Lorz, 1987). As the regeneration can be obtained only in limited number of genotypes, the use of tissue culture in rice improvement is limited (Taguchi-Shiobara *et al.*, 1997). The effect of 2,4-D, a growth regulator was studied on callus growth in rice (Gonzalez, 2000). One of the important factors affecting callus growth is the basal medium (Yamada *et al.*, 1967; Al-Khayari *et al.*, 1996). The callus induction frequency of Basmati cultivars was also reported to be genotype specific (Abbasi *et al.*, 2000).

A basic protocol is required for the callus induction and subsequent plant regeneration for the studies related to *In vitro* culture of plants. Present study was conducted to estimate effect of various concentrations of 2, 4-D and agar on callus induction and growth of the rice variety, Basmati-370.

Materials and Methods

The mature fresh grains of the Basmati-370 were collected from the Plant Genetic Resource Institute, National Agriculture Research Centre, Islamabad. The study was conducted in the same institution in 2008. The grains collected had lower moisture, hence possessed less seed borne contamination. Grains were further stored in glass jars to prevent from insect or moisture damage and any other biotic or abiotic stress. The seeds of Basmati-370 were dehusked by removing the tip from the pointed end and gently pressing from the other end. After dehusking, the grains were surface sterilized by treating first with the 70% (v/v) ethanol solution for 30 seconds-1 minute and were then washed two times for 5 minutes with 50% (v/v) chlorox solution. After that the grains were washed 4-5 times with autoclaved water until the solution became clear in order to remove disinfectants from the grains. The grains were left for drying for 20 minutes. The Chu's N6 medium (Chu *et al.*, 1975) was used as basal medium for callus induction and was supplemented with 3% sucrose (30 g L^{-1}) as a source of carbon, 0.1 g L^{-1} Myo-inositol, 0.4 g L^{-1} Casein, agar (4 g L^{-1}, 5 g L^{-1}, 6 g L^{-1}) and growth hormone 2,4-D (2,4-Dichlorophenoxy acetic acid) in different concentrations (2ppm, 2.5ppm). The pH of the medium was adjusted to 5.75 ± 0.02 with 1N NaOH and 1N HCl using the electronic pH indicator before the addition of agar. After adjusting pH agar was added to the medium and it was placed in oven for boiling. After boiling 8 ml medium per test tube and 20 ml per flask was poured. The medium and other culturing instruments were then autoclaved at temperature of 121°C and pressure of 15 ppsi for 15 minutes. After autoclaving, the medium was allowed to

cool down and solidify at room temperature. Two seeds per test tube were inoculated under aseptic conditions in a laminar flow cabinet. The scutellum side of grains was kept up in such a way that one-third portion of the seed remained above the surface of the medium. The cultures were incubated in dark in an environmentally controlled room, where the temperature was maintained at 25 ± 2°C throughout the growth period. Callus induction frequency was recorded after 2 weeks (15 days) of inoculation. Callus proliferation rate and callus qualities were recorded after 3 weeks (21 days) of inoculation. The frequencies of callus induction (CI) were determined as the percentage of seeds producing calli.

$$CI\ \% = \frac{\text{Number of the seeds producing callus}}{\text{Total number of the seeds}} \text{ X } 100$$

Results

Callus induction is the genotype dependent phenomena. The results for the callus induction in Basmati-370 were quite good. The calli of this variety were large in size. After culturing the swelling of the explants was observed within 2-3 days and initiation of calli was apparent as an off-white coloured tissue on the surface of the embryonic side of the seed within 3-7 days. After 1 week of incubation active cell division was observed. The variety responded well for all the three concentrations of agar (4 g L^{-1}, 5 g L^{-1}, 6 g L^{-1}) and both the concentrations of 2,4-D (2ppm, 2.5ppm), however, as shown in Table 1, the combination of 4 g L^{-1} agar with 2.5ppm 2,4-D gave excellent results with maximum callus induction of 93.42% followed by 4 g L^{-1} agar with 2ppm 2,4-D and 5 g L^{-1} with 2.5ppm 2,4-D which also gave better results with the observed callus induction of 92.75% and 91.11% respectively.

Table 1. Callus induction affected by agar and 2,4-D concentrations.

Agar	2,4-D	Total seeds	Callus induced	CI%
4	2	138	128	92.75
5	2	116	98	84.48
6	2	127	96	75.59
4	2.5	152	142	93.42
5	2.5	135	123	91.11
6	2.5	132	106	80.30

Discussion

Successful callus induction is a prerequisite for the application of transformation techniques in crop plants. Callus induction potential is affected by the concentration of plant growth regulator and basal salts of the culture medium. In this study, Basmati-370 was evaluated for its response to tissue culture. Mature seeds of the variety were used for the callus induction. The genotype responded well for callus induction on N6 medium supplemented with different concentrations of 2,4-D. Three different levels of agar were used in combination with two different levels of 2,4-D. The variety Basmati-370 responded well for all the three concentrations of agar (4 g L^{-1}, 5 g L^{-1}, 6 g L^{-1}) and both the concentrations of 2,4-D (2ppm, 2.5ppm), however, as shown in Table 1 the combination of 4 g L^{-1} agar with 2.5ppm 2,4-D gave excellent results with maximum callus induction of 93.42% (Fig.1A) followed by 4 g L^{-1} agar with 2ppm 2,4-D (Fig. 2A) and 5 g L^{-1} agar with 2.5ppm 2,4-D (Fig. 2B). The results showed that the best concentration of agar on which the maximum callus induction was observed was 4 g L^{-1}. When the concentration of 2,4-D was kept constant alongwith other medium contents the callus induction was maximum at 4 g L^{-1} agar and decreased with increase in the concentration of agar as shown in Table 1. The best concentration of 2,4-D was found to be 2.5ppm as it performed better with different concentrations of agar than 2ppm 2,4-D as evident from the Table 1. Regarding the combinations of agar with 2,4-D, the 4 g L^{-1} agar performed well with both the concentrations of 2,4-D (Fig. 1A and Fig. 2A) while the 5 g L^{-1} agar performed better with 2.5ppm (Fig. 2B) as compared to 2ppm 2,4-D (Fig. 1B) as shown by the callus induction frequency. The concentration of agar 6 g L^{-1} also had good callus induction frequency with both the concentrations of 2,4-D (Fig. 1C and Fig. 2C) but there was no distinction made to be notified. The appearance of dense tissue was an indication of cell division resulting in tissue clusters within 2 weeks of incubation. A distinct feature of callus cell culture in cereals is the colour and surface morphology. The appearance of the compact, nodulated callus that is milky white to yellow in colour is the first visible indication of embryogenic callus development in most of the cereals (Fig. 1 and Fig. 2). It was observed that the embryogenic callus production was effected by 2,4-D concentration. In plant tissue culture a desirable genotype is expected to possess high callus induction frequency and Basmati-370 was a desirable genotype in this regard.

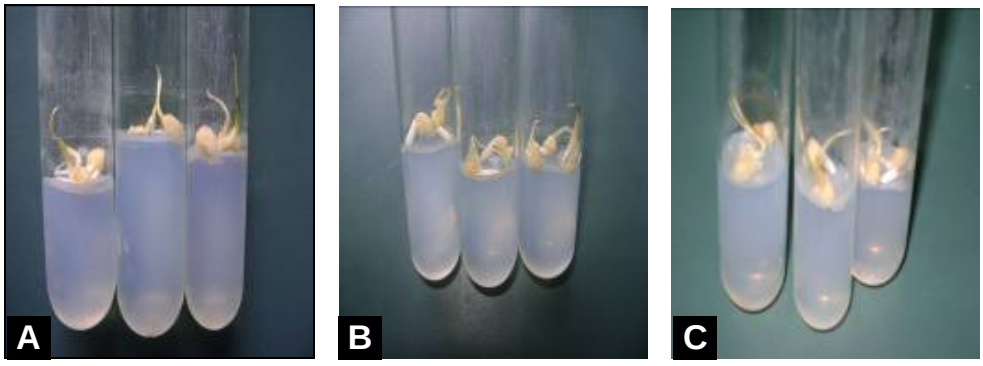

Fig. 1. Callus induction affected by agar and 2,4-D concentrations (A) 4 g L^{-1} agar with 2ppm 2,4-D, (B) 5 g L^{-1} agar with 2ppm 2,4-D, (C) 6 g L^{-1} agar with 2ppm 2,4-D.

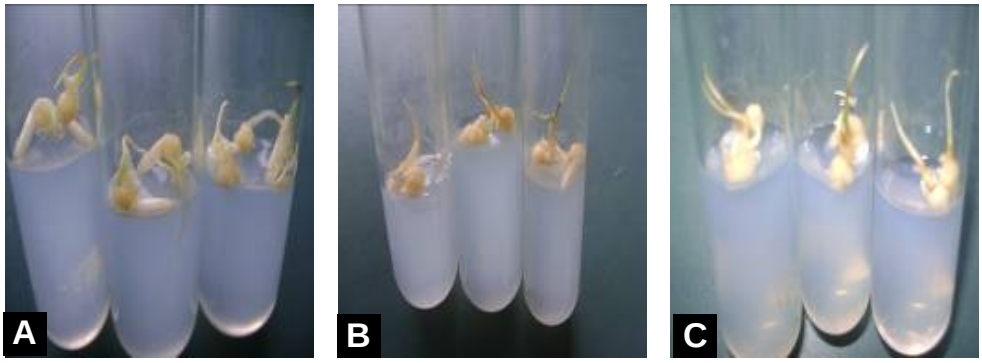

Fig. 2. Callus induction affected by agar and 2,4-D concentrations (A) 4 g L^{-1} agar with 2.5ppm 2,4, (B) 5 g L^{-1} agar with 2.5ppm 2,4-D, (C) 6 g L^{-1} agar with 2.5ppm 2,4-D.

References

Abbasi, F.M., H. Rashid and A. Quraishi. 2000. Regeneration efficiency and embryogenic callus production of three cultivars of rice. *Pak. J. Agric. Res*., 16: 97-99.

Al-Khayri, J.M., C.E. Shambin, R.W. McNew and E.J. Anderson. 1996. Callus induction and plant regeneration of U.S. rice genotypes as affected by medium content. *Theor. Appl. Genet*., 58: 87-90.

Bano, S., M. Jabeen, F. Rahim and I. Ilahi. 2005. Callus induction and regeneration in seed explants of rice (*Oryza sativa* cv. Swat-II). *Pak. J. Bot*., 37(3): 829-836.

Bashir, M.U., N. Akbar, A. Iqbal and H. Zaman. 2010. Effect of different sowing dates on yield and yield components of direct seeded coarse rice (*Oryza sativa* L). *Pak. J. Agri. Sci*., 47: 361-365.

Cantrell, R.P. and G.P. Hettle. 2004. New challenges and technological opportunities for rice-based production systems for food security and poverty alleviation in Asia and the Pacific. *FAO Rice Conference, FAO, Rome, Italy*.

Chu, C.C., C.C. Wang, C.S. Sun, K.C. Hsu, K.C. Yen, C.Y. Chu and F.Y. Bi. 1975. Establishment of an efficient medium for anther culture of rice through comparative experimentation on the nitrogen source. *Scientia. Sinica*., 13: 659-668.

Duncan, D.R., M.E. Williams, B.E. Zehr and J.M. Widholm. 1985. The production of callus capable of plant regeneration from immature embryos of numerous *Zea mays* genotypes. *J. Planta*, 165: 322-332.

Gonzalez, M.C. 2000. Effect of different growth regulators on *In vitro* culture of rice. *Cultivors-Tropicales*, 21: 27-28.

Luhrs, R. and H. Lorz. 1987. Initiation of morphogenic cell suspension and protoplast cultures of barley. *J. Planta*, 175: 71-81.

Raina, S.K., P. Sathish and K.S. Sarma. 1987. Plant regeneration from *In vitro* cultures of anthers and mature seeds of rice (*Oryza sativa* L.) cv. Basmati-370. *J. Plant Cell Rep*., 6: 43-45.

Rashid, H., S.N.R. Bokhari, Z. Chaudhry and S.M.S. Naqvi. 2003. Studies on genotype response to callus induction from three Basmati cultivars of rice (*Oryza sativa* L.). *Pak. J. Bio. Sci*., 6(5): 445-447.

Redway, F.A., V. Vasil, D. Lu and I.K. Vasil. 1990. Identification of callus types for long-term maintenance and regeneration from commercial cultivars of wheat (*Triticum aestivum* L.). *Theor. Appl. Genet*., 79: 609-617.

Sasaki, T. 2005. The map-based sequence of the rice genome. *Nature*, 436: 793-800.

Taguchi-Shiobara, F., S.Y. Lin, K. Tanno, T. Komatsuda, M. Yano, T. Sasaki and S. Oka. 1997. Mapping quantitative trait loci associated with regeneration ability of seed callus in rice, *Oryza sativa* L. *Theor. Appl. Genet*., 95: 828-833.

Tariq, M., G. Ali, F. Hadi, S. Ahmad, N. Ali and A.A. Shah. 2008. Callus induction and *In vitro* plant regeneration of rice (*Oryza sativa* L.) under various conditions. *Pak. J. Biol. Sci*., 11(2): 255-259.

Yamada, Y., T. Nishi, T. Yasuda and E. Takahasi. 1967. The sterile culture of rice callus, *Oryza sativa* L. and its application. In: *Advances in germfree Research and Gnotobiology*. (Eds.): M. Miyakawa and T. D. Luckey. CRC Press, Cleveland, pp. 377-386.

Yamada, Y., Z.Q. Y and D.T. Tang. 1986. Plant regeneration from protoplast derived callus of rice (*Oryza sativa* L.). *J. Plant Cell Rep*., 5: 85-88.

(Received for publication 2 January 2010)