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Original Article

EFFECT OF GROWTH REGULATOR CONCENTRATIONS ON CALLUS INDUCTION AND REGENERATION IN JAPONICA RICE VARIETIES THROUGH ANTHER CULTURE

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Abstract: Rice (Oryza sativa L.) is one of the most important food crops of Southeast Asia, which feeds half of the world population. Anther culture is an efficient and convenient technique for rapid production of doubled haploids which are useful in crop breeding programs. The combination of different growth regulators facilitates callus induction on N6 medium and regeneration on MS medium. Highest callus induction frequency was recorded in growth regulator concentrations of 2, 4-D 2.0 mg/l+ Kinetin 1.0 mg/l (34.54%) followed by in growth regulator concentrations 2, 4-D 1.0 mg/l + NAA 2.0 mg/l + Kinetin 0.5 mg/l (33.38%). Highest regeneration frequency was recorded in growth regulator concentrations of Kinetin 0.5 mg/l + BAP 2.0 mg/l + NAA 1.0 mg/l (69.44%) followed by in growth regulator concentrations of Kinetin 2.0 mg/l + BAP 1.0 mg/l + NAA 1.0 mg/l (40.00%). This is clearly indicated that type of growth regulator along with its concentration plays important role in callus induction and regeneration in japonica rice varieties.

Key words: *Oryza sativa* L., Anther culture, Growth regulators

INTRODUCTION

Rice (*Oryza sativa* L., 2n=24) is one of the most important food crops of Asia and it feeds more than 90% of the Asian population. Human population is increasing and cereal crops demand will increase over next epoch [1]. The traditional rice breeding methods are not sufficient to fulfill the demands of growing population. The production of haploids via anther culture represents an alternative biotechnology tool for crop improvement programs. Anther culture is a technique that manipulates microspore cells in immature anthers, to induce haploid callus formation, which are subsequently converted to double haploid embryos. The main advantages of anther culture to shorten the breeding cycle for producing homozygous lines to one generation rather than 8-10 generations [2]. Several critical factors like genotype, growth regulator along with its concentrations, microspore stages, cold pre-treatment and culture medium are influence anthers to promote callus induction and regeneration.

The combination of growth regulator with different concentrations is found diversification in callus induction and regeneration of rice. These growth regulator concentrations affect rice anthers that promote callus induction and regeneration. Inter specific F1 hybrids of indica × japonica produced callus with growth regulator concentrations of 2, 4-D 2.0 mg/l + Kinetin 0.5 mg/l and regeneration in kinetin 0.5 mg/l + BAP 2.5 mg/l + NAA 1.0 mg/l [3]. Multiple portions of spikelets of japonica variety Taipei 309 were used to produce callus with

growth regulator concentrations of 2, 4-D 2.0 mg/l and regeneration in BAP 1.0 mg/l + NAA 0.5 mg/l [4]. Several growth regulator concentrations of 2, 4-D (0.5, 1.0, 2.0, 3.0 mg/l), NAA (0.5, 1.0, 2.0, 3.0 mg/l), IBA (0.5, 1.0, 2.0, 3.0 mg/l) had used in callus induction and Kinetin 1.0 mg/l + BAP 1.0 mg/l + NAA 0.5 mg/l would have used at regeneration in rice cultivars [5]. Author has used different growth regulator concentrations of 2, 4-D (1.5, 2.0, 2.5, 3.0, 3.5 mg/l) and BAP (0, 0.25 mg/l) on callus induction and BAP (1.0, 2.0, 3.0, 4.0 mg/l) and NAA (0, 0.2 mg/l) at regeneration in japonica variety [6]. So, the callus induction and regeneration are observed with different growth regulators concentrations of rice. These achieve the good quality of growth regulator concentrations. With this view, the present studied was carried out in effect of growth regulators concentrations on callus induction and regeneration in japonica rice varieties through anther culture.

MATERIALS AND METHODS

The present experiment was conducted in Plant Tissue Culture Laboratory, Department of Plant Biotechnology, University of Agricultural Sciences, GKVK, and Bangalore, India.

Plant material: Two japonica rice varieties Azucena and Moroberekan were used as the source of explants. These varieties were grown in the field till the time of flowering. Judicious fertilizers and measures of plant protection were adopted to raise healthy plants.

Stage of panicle harvest: Panicles were selected at the pre-flowering stage, when young panicles were still enclosed within the leaf sheath. Panicles were collected between 6.00 and 9.00 am and washed with water and sprayed with 70% (v/v) ethanol. Panicles with a distance of 12-13 cm between flag leaf and subtending leaf for Azucena (Fig. 1) and 14-15 cm for Moroberekan were selected (Fig. 2). These panicles were kept in refrigerator for cold pretreatment at 5°C for 8 days. In order to identify the stage of pollen development, anthers of rice was stained with 2% (v/v) acetocarmine and observed under light microscope. The panicles with mid uni-nucleate pollen were used for anther culture (Fig. 3).

Sterilization, preparation of explants for callus induction: The panicles were surface sterilized by immersion in 70% (v/v) ethanol for 20 seconds followed by 0.2% $HgCl_2$ for 10 minutes. The treated panicles were washed 3-4 times with sterile distilled water. Later the anthers were isolated from spikelet avoiding any mechanical damage, followed by inoculation into the bottle (Fig. 4), each containing 10 ml of solidified induction solid N6 medium containing 5% maltose and 0.8% agar callus induction. The medium was supplemented with different concentrations of phytohormones (2, 4-D at 0, 1.0, 2.0 mg/l), (NAA at 0, 1.0, 2.0 mg/l) and (kinetin 0, 0.5, 1.0 mg/l). The cultures were sealed with parafilm and kept in dark at 23±2°C. The bottles were examined periodically at weekly interval for 10-20 weeks, to observe the progress in respect of callus formation.

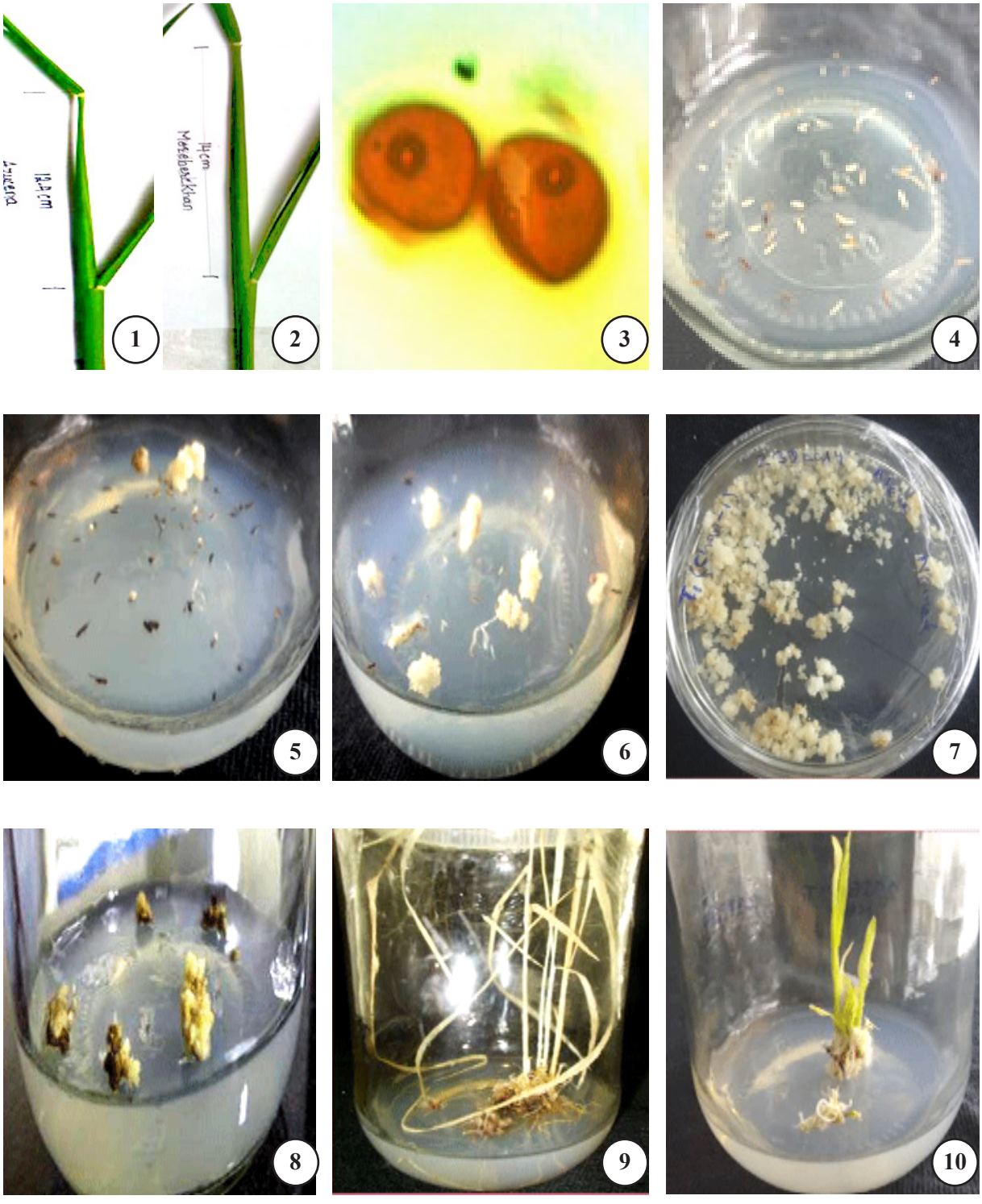
Regeneration: Embryogenic calli were transferred into the MS basal medium supplemented with different concentrations of growth regulators (kinetin at 0, 0.5, 1.0, 2.0 mg/l), (BAP at 0, 1.0, 2.0 mg/l), (NAA at 0, 0.5, 1.0 mg/l) and sucrose 3% (w/v). The cultures media were incubated in a culture room maintained at 23 ± 2°C, with a relative humidity of 50-60% and 16 hour photoperiod at a photon flux density of 3000 lux from white cool fluorescent tubes. Observation of regeneration was taken after 21-77 days of incubation.

Data analysis: The callus induction frequency and plant regeneration frequency was analyzed by factorial completely randomized design (FCRD) of square root transformation with correction factor 0.5 %. The presented figures of the table were derived from mean of the treatment

RESULTS AND DISCUSSION

Growth regulator concentration produces different type of callus, colour of callus, callus growth that helps in recognizing good growth regulator. It improves a callus induction frequency and regeneration frequency that helps in development of novel rice variety in short time period.

Effect of growth regulator concentrations on callus induction in japonica rice varieties: Significant difference was observed to growth regulator concentrations on callus induction in japonica rice varieties (Table 1). The cultured anthers started turning brown after 3-4 weeks of culturing and



Explanation of Figures:

Figures 1 to 10 are anther culture in japonica rice variety. **Fig. 1:** Panicle harvest stage of Azucena, **Fig. 2:** Panicle harvest stage Moroberekan. **Fig. 3:** Pollen development stage observed under light microscope at 40x (mid uninucleate). **Fig. 4:** Anthers inoculated on N6 medium. **Fig. 5:** Callus appeared from cut end of swelled anther wall (8 weeks after culture). **Fig. 6:** Callus obtained from anthers of Azucena after 8 weeks of culture **Fig. 7:** Callus obtained from anthers of Moroberekan after 8 weeks of culture. **Fig. 8:** Callus turned in green colour after 15 days of culture. **Fig. 9:** Regeneration from androgenic callus of Azucena. **Fig. 10:** Regeneration from androgenic callus of Moroberekan.

microcalli appeared on the surface of anthers after 5-8 weeks of inoculation (Fig. 5). Mean of auxin concentrations ranged 5.75% to 19.94% with control observed on callus induction. Among auxin concentrations, higher callus induction was recorded in 2, 4-D (19.94%) followed by 2, 4-D + NAA (19.28%). This may be due to degeneration of binucleate pollen and promotion of rapid cell proliferation and formation of non-embryogenic callus by 2, 4-D [7]. 2, 4-D and NAA indicates that the two synthetic auxins are about equally effective in inducing callus formation and in supporting callus growth, but callus formed in the presence of 2, 4-D is less capable of plant regeneration than that formed on medium with NAA. The inhibitory effect is probably due to preculture of the callus on the induction medium [8].Thus, 2, 4-D + NAA are preferred from the point of callus induction. So, these findings reported at cross rice cultivar of Safri-17 x PB 3 & Safri 17 × RYT 3275 in 2, 4-D [9] and anthers of japonica rice cultivar Xiushui 11 in 2, 4-D [11] and callus induction in japonica genotype (IR 77734-93-2-3-2, IR 78554-145-1-3-2) containing 2, 4-D + NAA [12].

In the interaction between 2, 4-D and Kinetin concentrations on callus induction, highest callus induction frequency was recorded in growth regulator concentrations of 2, 4-D 2.0 mg/l + kinetin 1.0 mg/l (34.54%) (Fig. 6). Combination of 2, 4-D with kinetin was produced multiple calli from number of anthers was observed along with synchronous growth of developing calli [13]. Combination of 2, 4-D with kinetin was more effective in producing embryogenic and organogenic calli [12]. 2, 4-D was essential for callus induction and kinetin was useful to promote callus differentiation or callus growth. This callus induction also found in rice cultivars [14].

In the interaction between 2, 4-D + NAA and Kinetin concentrations on callus induction, highest callus induction frequency was recorded in growth regulator concentrations of 2, 4-D 1.0 mg/l+ NAA 2.0 mg/l + Kinetin 0.5 mg/l (33.38%) (Fig. 7). Application of 2, 4-D and NAA in combination with kinetin could lead to an increase of callus induction and plant regeneration [15]. This callus induction reported in japonica variety Azucena [16].

Effect of growth regulators concentrations on regeneration in japonica rice varieties: Many factors such as growth regulator along with its concentrations, culture medium, culture environment, explant and genotype of donor are responsible for plant regeneration from androgenic calli of japonica rice. In the present investigation, there was a significant difference observed to growth regulator concentrations on regeneration in japonica rice varieties (Table 2). After 15 days of culture, calli started differentiating into nodular structure and turned into green colour (Fig. 8). Mean of cytokinin concentrations ranged 19.65% to 34.72% with control observed on regeneration. Among cytokinin concentration, Kinetin + BAP treatments showed highest regeneration (34.72%). The combination of Kinetin + BAP were increased the length of shoots and the number of shoots [17]. Kinetin was found more effective for shoot proliferation and regeneration in rice compared with BAP [18]. Kinetin was enhanced the callus proliferation and

Table 1: Effect of growth regulator concentrations on callus induction in *japonica* rice varieties

Auxin concentration (mg/l)		Kinetin (mg/l)		Mean for auxin concentrations
		0.5	1	
2,4-D	(Control) 0	0.00	0.00	0.00
	1	2.94	8.55	5.75
	2	5.33	34.54	19.94
NAA	1	8.32	11.52	9.92
	2	3.94	10.95	7.45
2,4-D + NAA	1 + 1	8.23	2.36	5.30
	1 + 2	33.38	5.18	19.28
	2 + 1	21.19	14.08	17.64
	2 + 2	13.35	17.71	15.53

Table 2: Effect of growth regulator concentrations on regeneration in *japonica* rice varieties

Cytokinin concentration (mg/l)		NAA (mg/l)		Mean for cytokinin concentrations
		0.5	1	
Kinetin	(Control) 0	0.00	0.00	0.00
	0.5	0.00	0.00	0.00
	1	25.00	14.29	19.65
	2	16.67	25.00	20.84
Kinetin + BAP	0.5 + 1	0.00	0.00	0.00
	0.5 + 2	0.00	69.44	34.72
	1 + 1	0.00	0.00	0.00
	1 + 2	0.00	0.00	0.00
	2 + 1	0.00	40.00	20.00
	2 + 2	0.00	0.00	0.00

regeneration by influencing mitosis, cytokinesis, total protein synthesis, lipid biosynthesis, vascular differentiation and differentiation of mature chloroplasts from protoplastids [19]. BAP was more effective in inducing and sprouting of large number of shoots [20]. This regeneration reported in F2 hybrids of Oryza sativa L. subsp. japonica [21].

In the interaction between Kinetin + BAP and NAA concentrations on regeneration, highest regeneration frequency was recorded in growth regulator concentrations of Kinetin 0.5 mg/l+ BAP 2.0 mg/l + NAA 1.0 mg/l (69.44%) (Fig. 9) followed by in growth regulator concentrations of Kinetin 2.0 mg/l + BAP 1.0 mg/l+ NAA 1.0 mg/l (40.00%) (Fig. 10). Combination of auxin and cytokinin is known to affect the regeneration of rice plants from embryogenic calli [22].

CONCLUSION

The present study revealed that growth regulator concentration promotes huge variation in callus induction and regeneration of japonica rice. This will help in choosing growth regulator concentration that helps in reducing breeding cycle. These findings will be of immense value in the application of in vitro androgenesis for rice crop improvement.

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REFERENCES

[1] Kathuria, H. Giri, J. Tyagi, H. and Tyagi, A.: Crit. Rev. Plant Sci., 26: 65-103 (2007).
[2] Senadheera, D.Z., Zapatha-Aris, F.J., Gregorio, G.B., Alejar, M.S., Cruz, H.C.,Padolina, T.F. and Galvez, A.M.: Field Crops Res.,76: 103-110 (2001).
[3] Balachandran, S.M., Sarma, N.P. and Siddiq, E.A.: Curr. Sci., 77(7): 962-967 (1999).
[4] Afza, R., Shen, M.E.I., Zapata, F.J., Xie, J., Fundih, K., Lee, K., Mucino, E.B. and Kodym, A.: Plant Sci., 153: 155-159 (2000).
[5] Bidhan, R. and Asit, B.M.: Afri. J. Biotechnol., 4(3): 235-240 (2005).
[6] Saroj, S.K. and Ajinder, K.: Res. J. Biotechno., 8(12): 96-101 (2013).
[7] Trejo-Tapia, G., Amaya, U.M., Morales, G.S., Sanchez, A.D.J. and Bonfil, B.M.: Plant Cell Tiss. Org. Cult., 71: 41-46 (2002).
[8] Huang, C.R. Wu, Y.H. and Chen, C.C.: Effects of plant growth substances on callus formation and plant regeneration in anther culture of rice. In: Proceedings of the International Rice Genetics Symposium, Rice Genetics, IRRI, Manila, Philippines, pp 763-761(1985).
[9] Minj, A.K. Sahu, M. Chopkar, R. Jha, Z. and Verulkar, S.B.: J. Cell Tissue. Res., 16(3): 5899-5904 (2016).
[10] Ali, S., Qing-Zhong, X. and Xian-Yin, Z.: Rice Sci., 11(5-6): 345-349 (2004).
[11] Faruq, G., Shamsuddin, F., Jenifer, A., Jusoh, S.H. and Khalid, N.N.: Ind. J. Sci. Tech., 7(11): 1861-1870 (2014).
[12] Wang, M., Bergen, S.V. and Duijin, B.V.: Plant Physiol., 124: 523-530 (2000).
[13] Lenka, N. and Reddy, G.M.: Proc. Ind. Nat. Sci. Acad., 860(1): 87-92 (1994).
[14] Shukla, R., Dube, A. and Koshy, E.P.: The Bioscan, 9(3): 1077-1080 (2014).
[15] Islam, M., Adhikary, S.K., Gain, P., Rahman, M. and Siddique, N.E.A.: Pak. J. Biol. Sci., 7(3): 331-334 (2004).
[16] Dalpat, L., Shashidhar, H.E., Gowda, P.H.R. and Ashok, T.H.: Int. J. Agrl. Environ. Biotechnol., 7(2): 213-218 (2014).
[17] Lee, K.S., Jeon, H.S. and Kim, M.Y.: Plant Cell Tissue Org. Cult., 71: 9-13 (2002).
[18] Ritu, M. and Shilpi, S.: Int. J. Biotech Trds. Tech., 11(1): 16-24 (2015).
[19] Verma, D. Joshi, R., Shukla, A. and Kumar, P.: Ind. J. Exp. Bot., 49: 958-963 (2011).
[20] Pawar, B.D., Bahurupe, J.V., Kale, P.B. and Markad, N.R.: Int. Quatr. J. Environ. Sci., 9(1-2): 231-233 (2015).
[21] Vladislavovna, I.M.: Science Time, 10(10): 160-167 (2014).
[22] Rueb, S. Leneman, M., Schilperoort, R.A. and Hensgens, L.A.M.: Plant Cell Tissue Org. Cult., 36: 259-264 (1994).