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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 3 (12): 2213-2215, 2000

Effect of Growth Hormones on Callogenesis in Basmati Rice

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Abstract: Tissue culture of Basmati rice was done to study the effects of growth hormones on the formation of callus. The varieties used were "Basmati 370" "Basmati 385" and "Basmati 198". The medium used was Murashige and Skoog (MS-medium). Different plant portions like seeds, roots and shoots were used as explant and were cultured on M5-medium supplemented with different combinations of plant growth hormones. The hormones used were 2,4 Dichiorophexy acetic acid (2,4-D) and Kinetin- Basmati-370 was the best for callus induction. Best callus induction and vigorous growth was observed from seeds cultured on media containing 0.5 mg/l kinetin and 2.0 mg/l 2,4-D.The response of roots and shoot expaints to callogenesis was little as compared to seed explant. The mature seeds of Basmati rice are the best source of callus.

Key words: Callogenesis, kinetin, 2,4,-D, basmati rice, MS-medium, explant

Introduction

Rice is an important food crap of Pakistan which is also exported to earn foreign exchange. Pakistan's annual rice export stands at about 1.15 million tonnes. After Thailand and USA, Pakistan is the biggest rice exporting country in the world. As it is composed of essential food components, it has an important role in our nutrition. Due to its important role played in nutrition, it is now felt that new varieties of rice plants should be evolved.

Somaclonal variations commonly appear after tissue culture involving a callus stage (Larkin and Scowcroft, 1981). Somaclonal variations may be a way of generating useful genetic variations. These variations may produce plants resistant to environmental stresses. Oono (1978) studied some traits of rice somaclones and indicated that there were rather high frequencies of variations. Somaclonal variation describes the phenomenon of enhanced genetic variability and plants regeneration form tissue culture involving callus stage.

When callus is first induced, it consists almost entirely of heterogeneous cells (Fox, 1963). The heterogeneity of cells is greater in early subcultures than in subsequent subcultures (Yamada *et al*., 1971; Watanabe *et al*., 1982).Therefore, selection of cells tolerant of sea water should be made when callus is first induced.

Phenotypic variation occurs in plants regenerated from cultured cells andthus culture of plant tissues is, in itself the simplest form of genetic engineering. The genetic variation exposed constitues the basis of somaclonal variation (Larkin and Scowcroft, 1981) Phenotypically variant plants of rice originating form seed derived callus, including dwarfed and twisted plants were first described. The observation of more commonly occuring variants, Albinos and polyploids derived from anther cultures was described by Oono (1984). Commonly observed variations in tissue-culture-derived plants include the number of tillers per plant, plant height, flag-leaf length, heading date, panicle length, fertility and the number of seeds produced (Oono, 1978; 1981; 1983; Sun, 1980; Sun *et al*., 1981; 1983; Wang *et al*., 1981; Zhao *et al*., 1982; Schaeffer *et al*., 1984; Murai and Kinoshita, 1986). Plant produced through tissue culture involving callus stage often have changes in ploidy or mutations. These may serve as new sources of variation. The mutation rate can be increased by the use of mutagens on cultured cells before regenerating plants (Sung, 1976). By applying tissue culture techniques involving callus stage it is possible to produce improved rice varieties.

Materials and Methods

Rice varieties used were "Basmati-370", Basmati-385" and "Basmati-198". Mature seeds were obtained from Rice Programme, NARC, Islamabad. Sterilant used was Sodium Hypochlorite (Na 0 Cl) Medium used was Murashige and Skoog (1962) medium. Medium was supplemented with 3% (w/v) Sucrose and solidified with 0.8% agar. PH of the medium was adjusted to 5.8.

The medium was poured in test tubes. The test tubes were plugged with cotton plugs. The medium was autoclaved at 15 lbs pressure at 120-125°C for 20 minutes.

The seeds of all rice varieties were de-husked, washed with surf detergent in an autocalved flask in laminar air flow cabinet and then rinsed with sterilised water to remove the detergent. The seeds were surface sterilised in 30% (v/v) Sodium hypo-chlorite for 10 minutes. The seeds were washed at least four times with autoclaved distilled water to remove the sterilant. For callus induction, different plant portions like seeds, roots, were used as explant and cultured on MS medium. MS medium was supplemented with different, combinations of plant growth hormones. For callus induction. in root and shoot explant, the source material (Shoots, roots) was taken from in-vitro seedlings of rice seeds. The explants were inoculated in test tubes under a laminar air flow cabinet. The explants were incubated at 25 ± 2°C with 16 hours photoperiod.

Results and Discussion

All the three varieties were good for callus induction. Celli from seed tissue culture were obtained which were light green in colour. Most of the calli were soft and granular (Table 1-3) Compact and loose, calli were also obtained.Basmati-370 was the best among all the three varieties and calli were 6btained which were light green, creamy and granular, Best callus induction and vigorous growth was noted on media containing 0.5 mg/l kinetic and 2.0 mg/l 2,4-D (Table 1-3). This result is in accordance with tissue culture done by M. Mahaswaran who did rice seed tissue culture of IR-50 on MS media. Chen and Lin (1976) reported that the callus induction frequency depends upon the medium as well as the variety used, Calk with shoots and roots were also obtained. Celli developed from seeds of all the 3 varieties were sub-cultured on the same medium supplemented with same hormones. Brown calli also developed by sub-culturing. The %age of light green calli was still higher as compared to that of brown calli.

Table 1: Callogenesis in mature seed explant of Bas-370 using Ms-mdeium

Hormones (mg/l)		Callus		
2,4 D	Kinetin	Texture	Growth	Induction frequency %
1.0	0.25	Granular	+ +	79.1
1.0	0.50	Granular	+ +	75.0
1.0	0.00	Granular	+ +	62.5
2.0	0.25	Granular	+ +	75.0
2.0	0.50	Granular	+ +	83.3
2.0	0.00	Granular	+ +	75.0
4.0	0.25	Granular	+ +	75.0
4.0	0.50	Granular	+ +	62.5
4.0	0.00	Granular	+ +	75.0

*Growth: + Weak = + + Normal = + + + Vigorous

Table 2: Callogenesis in mature seed explant of Bas-385 using MS-mdeium

Hormones (mg/l)		Callus		
2,4 D	Kinetin	Texture	Growth	Induction frequency %
1.0	0.25	Granular	+ +	75.0
1.0	0.50	Granular	+ +	70.8
1.0	0.00	Granular	+ +	78.8
2,0	0.25	Granular	+ +	75.0
2.0	0.50	Granular	+ +	79.1
2.0	0.00	Granular	+ +	70.8
4.0	0.25	Granular	+ +	70.8
4.0	0.50	Granular	+ +	66.6
4.0	0.00	Granular	+ +	66.6

*Growth: + Weak = + + = Normal = + + + Vigorous

Table 3: Callogenesis in mature seed explant of Bas-198 using MS-mdeium

Hormones (mg/l)		Callus		
2,4 D	Kinetin	Texture	Growth	Induction frequency %
1.0	0.25	Granular	+ +	66.6
1.0	0.50	Granular	+ +	66.6
1.0	0.00	Granular	+ +	66.6
2.0	0.25	Granular	+ +	70.8
2.0	0.50	Granular	+ +	75.0
2.0	0.00	Granular	+ +	66.6
4.0	0.25	Granular	+ +	70.8
4.0	0.50	Granular	+ +	62.5
4.0	0.00	Granular	+ +	41.6

*Growth: + Weak = + + Normal = + + + Vigorous

Table 4: Callogenesis in shoot and root explants of Basmati rice varieties

Variety	Explant	Age of explant (days)	Peak callus forming period (days)	Callus induction frequency %
Basmati 370	Shoot	10	14	25.1
		20	15	16.7
	Root	10	16	16.7
		20	17	12.5
Basmati 385	Shoot	10	15	8.3
		20	15	8.3
	Root	10	18	8.3
		20	-	-
Basmati 198	Shoot	10	15	8.3
		20	-	0.0
	Root	10	18	4.2
		20	-	0.0

A little contamination appe-ared by subsequent sub-culturing. Root and shoot explants respond very little when induced for callogenesis (Table 4). Medium was optimised for maximum callus induction for root and shoot explants. The optimal requirement of hormones for maximum callogenesis for all the three varieties was 2 mg/l 2-4, D and 0.5 mg/l Kinetin. Auxin are essential as the inducing, compounds for, DNA replication (Yasuda and Yamada, 1970, Yamada *et al*., 1971, Yasuda *et al*., 1974). The most effective among the common auxins used for callus induction in rice is 2, 4-D (Sekiya *et al*., 1977). Usually we transfer cereal callus to a medium containing no auxins when we want to regenerate plant lets from callus. Kinetin is used in callogenesis as it has a role to accelerate organogenesis when callus is transferred to the regenerating medium. Age of root and shoot explant plays an important role in callogenesis. 10 days old young shoots and roots respond more as compared to the older one (20 days old) (Table 4). As the response of roots and shoot explants to callogenesis was little as compared with seed explants, it is concluded that mature seeds are better source of callogenesis in *Oryza sativa* since there was a good percentage of callus induction in seed explant when cultured on MS medium supplemented with appropriate concentration of growth hormones.

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