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Callus Induction And Plant Regeneration Of Selected Genotypes In Indica Rice Through Anther Culture

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Abstract

Anther culture is considered as an important biotechnological technique in plant breeding as it offers great potential of obtaining genetically diverse and homozygous diploid plants within a short time period compared to conventional breeding. The objective of this study was to find out highly responsive rice varieties for anther culture. Genotypes of Bg 358, Bg 379-2, Bw 361, Bw 363, Bw 364, Bw 267-3, Bw 272- 6b and At 306 were selected for this study based on their yield and other desirable characters such as grain quality, tolerance to iron toxicity, resistance to pest and disease etc. Two different culture media which have been prepared by modifying N6 medium with two concentrations of NAA (2.5 mg/l and 2mg/l) with 2mg/l of 2, 4 – D and 0.5 mg/l of Kinetin were used. Calli were formed in Bg 379/2, Bw 361, Bw 363, Bw 267-3, Bw 272-6b and At 306 varieties after 6 – 8 weeks of culturing in both NAA concentrations. Among them highly responsive varieties were Bg 379/2 and Bw 272-6b, which showed 2.667 and 7.0 mean callus formation frequencies respectively. The variety Bg 358 and Bw 364 did not form any reliable callus even after 9 weeks of culturing. All responsive varieties had higher callus initiation ability in medium with 2.5mg/l of NAA. Though concentrations of NAA were significantly affected for the callus initiation, there was no significant (P = 0.2855) interaction between NAA concentration and variety. Therefore further studies on optimum concentration of NAA and effect of different combinations of NAA, 2, 4-D and Kinetin for callus initiation is needed.

Key words: Anther culture, Callus, NAA, Indica Rice, Culture medium

Introduction

Anther culture is an important biotechnological tool in rice breeding. It produces genetically diverse haploid and homozygous diploids within short time period compared to the conventional breeding. Several new rice cultivars have been developed from rice anther culture in China, Taiwan, South Korea, Japan, USA, and India (Raina and Ifran, 1998). But the application of this technique to indica rice varieties is limited, because response of indica rice varieties to anther culture is less than japonica varieties (Bhojwani et al., 2003). There are several factors affecting the success of anther culture such as, the genotype of donor plant (Ranajan et al., 1998), composition of culture medium, pre treatment of buds or anthers, physiology of the donor plant, growth stage of microspores (Guha et al., 1970), Albinism, culture density, anther wall factor, effect of light and temperature.

Most of the in vitro morphogenic responses are genotype–dependent (Bhojwani and Razdan, 1996). Haploid plant production through anther culture has been limited or non existent in many plant species. Even within a species capability of haploid production, it varies from each other. In Zea mays, some cultivars are completely unresponsive in anther culture, while few produce haploid plantlets (Wan and Widholm,

1993). In Wheat (Triticum aestivum) many genotypes do not respond at all to anther culture and in only few genotypes green plants are regenerated at low frequency. In general, indica cultivars of rice exhibit poorer androgenic response than the japonica cultivars (Hu, 1985; Raina, 1997).

In Sri Lanka there has been tremendous improvement in rice production through conventional breeding. But creation of genetic variability and applications of advanced technologies to develop varieties resistance for biotic and abiotic stresses has been limited. Particularly anther culture is done in rarely for local rice varieties (Chandel et al., 1996).

The research was carried out to test eight local indica varieties for their ability to produce callus in two different culture mediums which have been prepared by modifying N6 medium with different concentrations and combinations of 2, 4 – D, Kinetin and NAA.

Materials and Methods

The tested varieties were Bg 358, Bg 379-2, Bw 361, Bw 363, Bw 364, Bw 267-3, Bw 272- 6b and At 306.

Table1. Selected Indica rice varieties with their characters

Variety	Some desirable characters
Bg 358	3 - 3 ½ months samba Variety, Resistant to BPH, BL and BLB, Moderately tolerant to iron toxicity, Recommended for general cultivation
Bg 379-2	4 – 4 ½ months variety, Have white pericarp, Resistant to BPH and BB
Bw 361	3 ½ months variety, Have red pericarp, Moderately resistance to GM, BPH, Recommended for dry and intermediate zones
Bw 363	3 ½ months variety, Have white pericarp, Moderately resistant to Blast, BPH and GM, Iron toxicity tolerance variety, Suitable for general cultivation
Bw 364	3 ½ months variety, Have red pericarp, iron toxicity tolerance variety, Moderately resistance to Blast, BLB,BPH and GM, Recommended for wet zone
Bw 267 – 3	3 ½ months variety, Have white pericarp and long slender grains, Resistance to Blast Iron toxicity and seed spotting, Recommended for wet zone
Bw 272 - 6b	3 months samba variety, Have red pericarp, Resist ant to Blast, Resistant to lodging, Iron toxicity tolerance, Recommended for to low country wet zone, Suitable for mineral, half bog and bog soils
At 306	3 months basmati variety, Long grains with high quality, Moderately resistant to BLB and GM, Resista nt to blast and BPH, Recommended for general cultivation

Twenty one days old, seedlings were planted in buckets containing sufficient paddy soil, taken from paddy cultivation fields. Four plants were reared in one bucket and each variety was reared in five buckets. The plants were maintained until flowering, with regular weeding, watering, and application of fertilizers at the recommended level.

Selection of explants and anther culture

Panicles were collected at the booting stage, before the heading when the distance between the flag leaf auricle and penultimate leaf collar was 7-12 cm by making clean cut below the node from which panicle arose. After removing the leaf blades panicles were wrapped together in Aluminum foil, placed in a polythene bags, and kept in an incubator at 10°C for 7-10 days for the cold pre treatment. Then they were sterilized with

wiping 70% ethanol and taken to laminar flow bench. The open panicles were sterilized with 20% Clorox for 15 minutes, followed by sterilization with 70% ethanol for another 2 minutes and then washed thoroughly with sterilized distilled water.

Spikelets were cut at the base and collected the anthers. About 100-120 anthers were cultured in each Petri dish containing 15 ml of culture medium and incubated in dark at 25°C for 60 days. Callus initiation from anthers was tested on two culture media, which were derived by modifying the N6 medium (Chu et al., 1978) with different concentrations and combinations of 2, 4 – D, Kinetin and NAA.

Table 2. Different concentrations and combinations of growth regulators in callus induction medium (N6)

Medium	2,4-D (mg/l)	NAA (mg/l)	Kinetin (mg/l)
M1	2	2.5	0.5
M2	2	2	0.5

Results and Discussion

Different response with respect to callus induction was noticed among the ten cultivars. Callus induction frequencies were variable and dependent upon the genotypes used. Calli were formed in six out of ten varieties and those were Bg 379/2, Bw 361, Bw363,Bw 267/3, Bw 272-6b, and At 306. The callus formation ability was varying among the varieties. This could be due to its inheritance as a recessive trait conditioned by a single block of gene (Pinghe et al., 1998). The variety Bw 272-6b was significantly different from varieties Bw 361, Bw 363, Bw 267/3 and At 306. But there were no significant difference between Bw 272-6b and Bg 379/2, which have 7.0 and 2.667 mean callus formation frequencies respectively. Even the 9th week after culture varieties Bg 358 and Bw 364 were not formed any callus. There were two types of calli initiated from anthers of rice varieties, some were friable and shiny white while the others were hard and cream in colour.

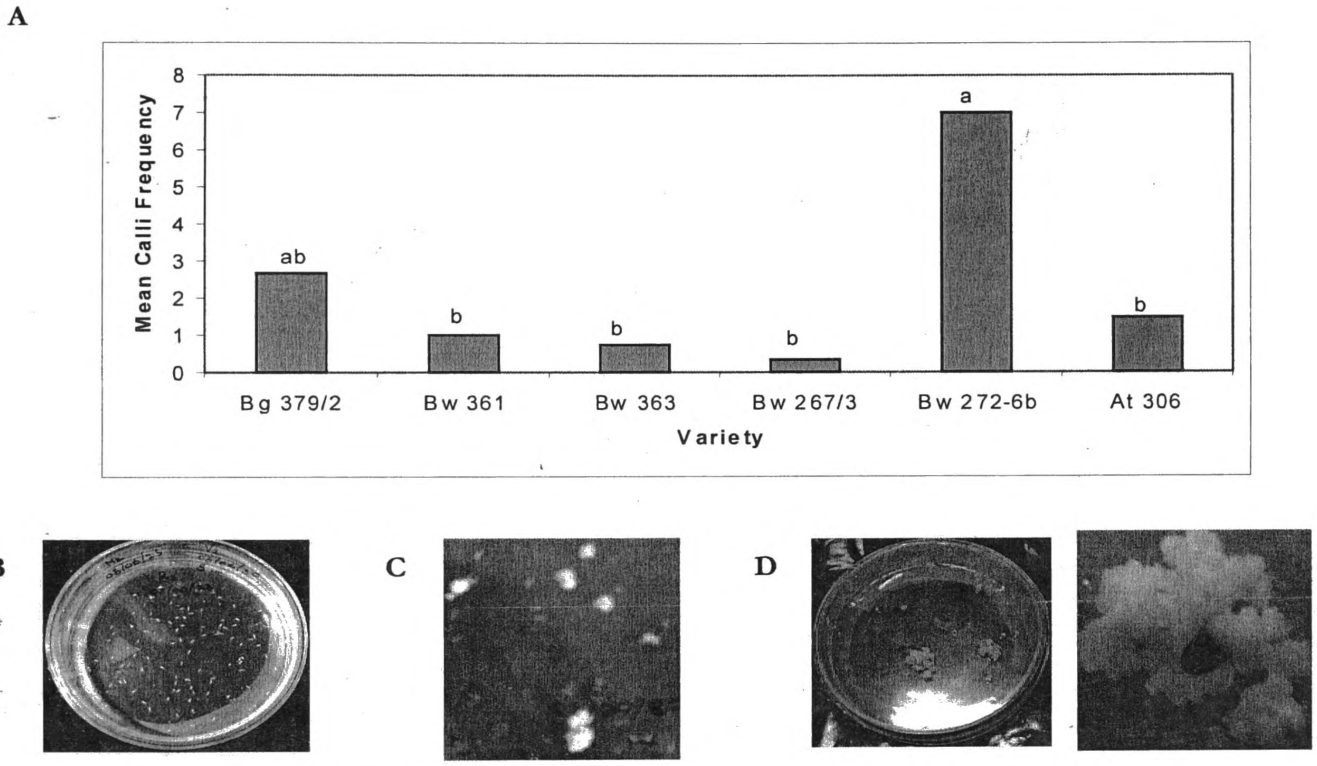

Figure 1. Callus formation from anthers

(A) Variation of callus formation ability among the varieties (B). Anthers at time of planting

(C) Initiation of callus form anthers (D). Fully grown callus 10 days after initiation.

According to Kumari et al (2006), the time required for callus initiation was different in different varieties. Calli were initiated within 6 to 8 weeks after anther planting in tested varieties. The varieties Bg 379/2 and Bw 272-6b were taken comparatively less time as 6 weeks for callus initiation, while varieties Bw 363 and Bw 267/3 were taken 8 weeks.

Table. 3. Variation of time in callus initiation

Variety	Time for callus initiation
Bg 379/2	6 Weeks
Bw 361	7 Weeks
Bw 363	8 Weeks
Bw267/3	8 Weeks
Bw 272-6b	6 Weeks
At 306	7 Weeks

Growth hormones play an important role in induction of callus from the anthers. In this study two concentrations and combinations of auxins and cytokinins were used to test the callus induction in rice anthers. It was observed that both medium had an affect on callus initiation, but there was no statistically significant difference between two mediums.

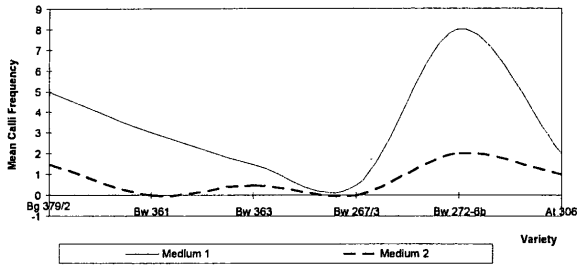

Figure 2. Variation of callus initiation between two mediums

Auxins and Cytokinin together were necessary for good callus formation (Niizeki and Oono, 1968). They interact in vitro to regulate the cell division and differentiation. Higher concentration of non – phenoxy auxins (IAA, NAA, IBA) alone often promote root initiation rather than shoots whereas phenoxy auxins (2, 4-D) promote callus growth and embryogenesis (Niizeki et al., 1968; Pande et al., 1996). However, the monocotyledons appear to be more Auxin dependent. The mean callus formation frequency of variety Bw 272-6b in medium 1 (2mg/l 2, 4-D, 0.5 mg/l Kinetin, 2.5 mg/l NAA) and medium 2 (2mg/l 2, 4-D, 0.5 mg/l Kinetin, 2.0 mg/l NAA) were 8.00 and 2.00 respectively. Though different modifications of N6 medium initiated different responses, there were no significant interaction between variety and medium.

Conclusion

Highly responsive genotypes were Bg 379/2 and Bw 272-6b with regarding the mean callus initiation frequency and time requirement for callus initiation. Bg 358 and Bw 364 are less responsive genotypes, because callus were not initiated even after 9th week after planting. All responsive varieties had higher callus initiation ability in medium with 2.5mg/l of NAA. Therefore the callus initiation ability of genotypes increased with the increasing of concentration of NAA in the medium. After ten days from callus initiation they could be transferred to MS medium (Murashige and Skoog, 1962) to regenerate haploid plantlets. Thus, anther culture technology can be used in rice improvement programme via creating genetic variants and producing double haploid with early expression of recessive genes and shortened breeding cycle.

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