OPTIMIZATION OF TISSUE CULTURE CONDITIONS FOR ANDROGENESIS OF AT405 AND BG360 RICE (*ORYZA SATIVA*) VARIETIES

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INTRODUCTION

Doubled haploid (DH) lines derived from rice anther culture are considered as study population and can be employed in rice breeding (Maeda *et al.*, 1988). At405 and Bg360 *indica* type rice varieties were selected for their contrasting grain quality characters to produce study population. Androgensis (anther culture performance) of *indica* type varieties is considerably low and highly depends on genotype (Roy and Mandel, 2005; Maeda *et al.* 1988). Hence, the study was designed to optimize androgenesis of the above two rice varieties.

MATERIALS AND METHODS

Androgenesis conditions such as pre-treatments, hormone combinations and media were optimized as shown in Table 01 and 02. Panicles at correct booting stage were pre-treated to convert from gametic stage to sporophytic stage and cultures were kept at 25 °C in dark to induce calli.

Table 1: Treatment combinations used in the study.*

	Pre-treatment					
	5°C for 3 -5 days followed by 7 days at 7°C			25°C for ½ hr		
Media types	N6 medium + 10% 10% Silver Nitrate	Letini's medium + 10% Silver Nitrate	Letini's medium + Myo-inositol + Casein Hydrolssate	N6 medium + 10% 10% Silver Nitrate	Letini's medium + 10% Silver Nitrate	Letini's medium + Myo-inositol + Casein Hydrolssate
Petri plant of (4 cm Φ)	H1, H2, H3, H4, H5	H1, H2, H3, H4, H5	H1, H2, H3, H4, H5	H1, H2, H3, H4, H5	H1, H2, H3, H4, H5	H1, H2, H3, H4, H5
Petri plate of (6 cm Φ)	H1, H2, H3, H4, H5	H1, H2, H3, H4, H5	H1, H2, H3, H4, H5	H1, H2, H3, H4, H5	H1, H2, H3, H4, H5	H1, H2, H3, H4, H5

***This table should be considered for both varieties separately. Φ - Diameter**

Table 2: Hormone combinations referred

Treatment	Hormone combination		
	2,4-D mg/L	**Kinetin mg/L**	**NAA mg/L**
H1	2.0	1.0	-
H2	-	2.0	1.0
H3	2.0	1.0	2.0
H4	1.0	-	2.0
H5	0.5	0.5	1.5

Source: Kumari *et al.*, 2006; Javed *et al.*, 2007

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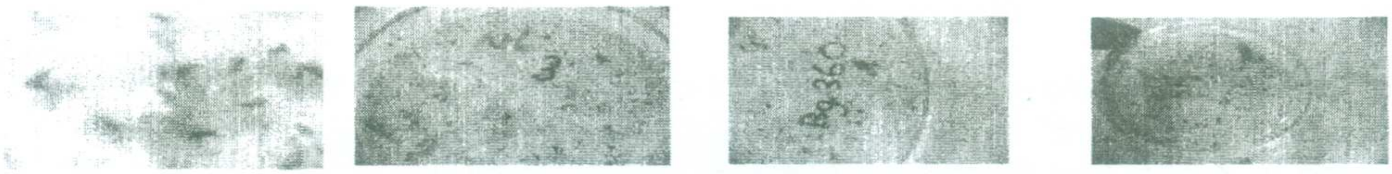
RESULTS AND DISCUSSION

Ten percent calli could be observed in both varieties till 12th week of anther establishment since 8th week. Further, 25% and 6% of androgenesis was recorded in At405 and Bg360 varieties respectively (Figure 1). Calli could be observed in Chu's N6 medium (Kinetin 0.5 mg/L 2, 4-D, 0.5 mg/L and 1.5 mg/L NAA) supplemented with 10% $AgNO_3$ for both varieties while Letini's medium (Kinetin 2.0 mg/L and 1 mg/L NAA) supplemented with 10% $AgNO_3$ for At405 and Letini's medium (2, 4-D 2.0 mg/L and Kinetin 1.0 mg/L) supplemented with 100 mg/L myo-inositol and 500 mg/L casein hydrolysate for Bg360 rice variety.

Green plant regeneration of At405 could be observed two weeks after androgenesis in Letini's medium with 10% $AgNO_3$ (4 cm Φ petri plate). Shoot initiation was observed subsequent to root initiation (Figure 2). High levels of cytokinins favour the shoot formation whereas roots are stimulated by high levels of auxins with low levels of cytokinins (Maeda *et al.*, 1988). Further, high anther density induces calli due to competition for resources (Bhojwani *et al.*, 1997). The possible reasons for the observed results could be the hormone combination and stress environment created by anther density and it is in agreement with Bhojwani *et al.* (1997) and Maeda *et al,* (1988).

Panicles pre-treated at 25 °C for ½ hour followed by 7 days at 10 °C could yield calli as alternative temperature could be developed stress on anthers and favoured androgenesis as described by Bhojwani *et al.* (1997) and Javed *et al.* (2007). However, 7 °C to 10 °C for 7 to 10 days is considered optimum pre-treatment for rice anther culture and can be varied with genotype (Maeda *et al.*, 1988; Kumari *et al.*, 2006) as it is the ultimate factor affects on anther culture performance (Bhojwani *et al.*1997; Roy and Mandel, 2005; Javed *et al.* 2007).

Calli were recorded in both At405 and Bg360 cultured on N6 medium with 10% $AgNO_3$. It is said that C_2H_2 produced in cultures suppresses the androgenensis and $AgNO_3$ inhibits C_2H_2 to promote callus formation (Javed *et al.* 2007). Maltose, a reduced sugar as a carbon source is better than sucrose to favour androgenensis. At the culture environment sucrose breaks down in to fructose and glucose whereas maltose in to two glucose molecules. Further, fructose inhibits the callusing and there is no negative effect by glucose for androgenesis (Javed *et al.* 2007).



(a) (b) (c) (d)

Figure 1- Callus initiation of At405 and Bg360 rice varieties In different media

(a) At405 - Letini's medium supplemented with 10% $AgNO_3$ (NAA – 1 mg/L ;Kinetin – 2 mg/L) (b) At405- N6 medium (NAA- 1.5 mg/L, 2,4-D – 0.5 mg/L, Kinetin – 0.5 mg/L) (c) Bg360 - N6 medium supplemented with 10% $AgNO_3$ (NAA-1.5 mg/L, kinetin - 0.5 mg/L and 2,4-D – 0.5 mg/L) (d) Bg360 - Letini's medium supplement with 100 mg/L Myo-inositol and 500 mg/L Casein Hydrosylate (2,4-D – 2 mg/L and Kinetin – 1 mg/L).

Figure 2- Regenerated plantlet of At405 at Letini's medium supplemented with 10% $AgNO_3$ (NAA – 1 mg/L; Kinetin –1 mg/L)

CONCLUSION

Letini's medium supplemented with 10% $AgNO_3$, 2 mg/L kinetin, 1 mg/L NAA could be employed in calli induction and green plant regeneration of At405 rice variety. Further, Chu's N6 medium with 10% $AgNO_3$ can be employed in androgenensis of At405 and Bg360 rice varieties. Panicles can be pre-treated at 25 °C for ½ hour followed 7 days at 10°C to acquire better anther culture performance in At405 and Bg360 rice varieties and can also be tried for other verities to optimize conditions for androgenesis.

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