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Optimization of hormonal combination for callus induction and regeneration in gamma irradiated rice

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SUMMARY

The present investigation was carried out with the rice varieties Ponni and White Ponni, treated with gamma rays at various doses producing the following eight treatments *viz*., PC, P10, WPC , W10,WP20, WP20D, WP40, WP40D and using anther as explant. Different growth hormone combinations were tried for optimizing the concentration of hormonal combination for enhancing the callus induction and regeneration. The results were studied through factorial completely randomized design. Among the combinations tested N6 + 2,4-D 2.50 mg/l + kin 1.00 mg/l was best for the anther callus induction. For P and WP the treatments and hormone highly significant for callus induction , while treatment –hormone interaction exhibited significance in callus formation only in P.The plant regeneration was maximum in the hormonal combination MS+. BAP1.00 + IAA 1.00 mg/l.High frequency of plant regeneration and in addition few albinos were also observed in WP10 and WP40. Significant difference was noticed in varying hormone levels, treatments and their interactions in regeneration of green plants.

Key words : Gamma rays, Anther explant, Growth hormone, Callus induction, Regeneration, Albino, Ponni, White ponni

Developments in plant tissue culture techniques offer possibilities of introducing variability into plants that could be utilized for crop improvement. Haploids with their unique genomic constitution, have potential for accelerating the production of homozygous new varieties. Plant growth hormones are the substances that when added in small quantity ,modify the growth of plants usually by stimulating the part of the natural growth system. Callus induction is the preliminary stage. The most commonly used growth hormone for callus induction in cereal tissue culture is 2,4-D(Abe and Futsuhara,1986 and Bregitzer *et al.*,1989). Many cereals express embryogenic competence in the presence of 2,4-D.Heyser *et al*. (1983) reported varying responses among rice varieties in producing competent culture with the use of 2,4-D.Though other auxins such as IAA, NAA, and PCPA of Benzolin are available .2,4-D either alone or in combination with any one of the above mentioned auxin were widely used by many scientists in callus culture.(Mandal and Bandyopadhyay,1996). Addition of cytokinins facilitated the callus initiation and maintanance (Mandal *et al.*,1998). A combination of auxins and cytokinins were found to be suitable for embryogenic callus initiation in cultivars of rice. A critical level of auxin and cytokinin was found to be essential for optimum levels of callus induction and plantlet regeneration.

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MATERIALS AND METHODS

The present investigation was carried out to find the optimum concentration of hormonal combination for enhacing callus induction in two rice varieties of Tamil Nadu. The gamma irradiated (10KR,20 KR,40KR) M3 generation anthers *viz*., P10, WP10, WP20, WP 20 dwarf, WP40 and WP40 dwarf and their parents, Ponni and White Ponni formed the base material for present study. All materials were obtained and the experiments carried out in the department of Plant breeding and genetics, Agricultural College and Research Institute, Madurai,

Details of the parents

Variety	Characters	Treatments gamma irradiation doses (KR)	Symbols used
Ponni	Normal	0	PC
		10	P10
White Ponni	Normal	0	WPC
		10	WP10
		20	WP20
		40	WP40
	Dwarf	20	WP20D
		40	WP40D

Tamil Nadu.

Anthers at intermediary stage between late uninucleate and early binucleate stage were collected when the distance between the base of the flag leaf and the auricle of the next leaf was six to ten centimeter as suggested by Mandal and Bandhyopadhyay(1996).

The spikelets were given cold pretreatment at ten degree celsius for 5-25 days in BOD incubator. The spikelets were surface sterilized using 70% ethanol for 2-3 minutes followed by 0.10 % $HgCl_2$ for 8-10 minutes. Then the spikelets were washed and anthers were inoculated in the test tubes having the callus induction media with a density of 50-60 anthers /tube in each treatment with three replication. The cultures were incubated in the dark at 25 plus or minus 2 degree C. All these operations were carried our under aseptic conditions in culture room .

The callus induction medium included N_6 supplemented with different concentrations of 2,4-D and Kinetin in combination with sugar.

The treatments were as follows

– N_6 + 2,4-D 2.00 mg/l + kin 0.50 mg/l
– N_6 + 2,4-D 2.00 mg/l + kin 1.00 mg/l
– N_6 + 2,4-D 2.50 mg/l + kin 0.50 mg/l
– N_6 + 2,4-D 2.50 mg/l + kin 1.00 mg/l
– N_6 + 2,4-D 3.00 mg/l + kin 0.50 mg/l
– N_6 + 2,4-D 3.00 mg/l + kin 1.00 mg/l

After 45 days, the number of calli produced was noted and the percentage of callus induction was calculated as follows.

$$\text{Callus induction percentage} = \frac{\text{No.of calli produced from anthers}}{\text{No. of anthers placed}} \times 100$$

Embryogenic calli (smooth, white and knobby appearance) and non embryogenic calli (yellow translucent and wet) were assorted from the calli mass before subculture.

Approximately two weeks old embryogenic calli were subcultured in MS medium supplemented with different levels of BAP, NAA and kinetin

Regeneration:

The callus tissues derived from anthers were used for regeneration studies. The callus tissues weighing approximately 50 mg were transferred to regeneration medium.

The treatments are as follows

– MS + IAA1.00 + Kin 1.00 mg/l + Sucrose 30 g/l
– MS + IAA2.00 + Kin 3.00 mg/l + Sucrose 30 g/l
– MS + BAP1.00+NAA0.10 mg/l +Sucrose 30 g/l
– MS + BAP1.00+ IAA 1.00 mg/l + Sucrose 30 g/l

The cultures were kept under continuous light (3000 lux intensity) at 25 plus or minus 2^0 C and the percentage of plant regeneration was worked out as follows.

$$\text{Plant regeneration percentage} = \frac{\text{No. of plants produced}}{\text{No.of calli placed}} \times 100$$

Statiscal analysis:

The effect of growth regulators 2,4-D alone or in combination with kinetin on callus induction (% of callus induction) and the effect of BAP, IAA and NAA on shoot formation (% or shooting) was studied by subjecting the data in to factorial completely randomized design .Each treatment was replicated three times. The arc sin values being reliable were used throughout the study.

RESULTS AND DISCUSSION

The results obtained from the present investigation are presented below:

Callus induction:

Callus initiation was stimulated at all levels of growth regulators in all treatments. A wide variation in per cent of callus induction 4.31 to 27.11 was observed in the treatments studied (Table 1). The comparison among the growth regulators revealed that the hormonal combination of 2,4-D 2.50 mg/l + kin 1.00 mg/l has pronounced the effect on callus induction of all treatments.

In P higher callus induction range of 23.97 to 25.78 per cent was observed in 10 KR treatment ,which was lower than the highest callus response in WP 10 KR treatment with the range of 24.53 to 27.11 per cent. The least response were of 4.31 to 6.98 per cent has been observed in WPC. For P and WP the treatment and hormone highly significant for callus induction ,while treatment –hormone interaction exhibited significance in callus formation only in P.

The present investigation was carried out to find the optimum concentration of hormonal combination for enhancing the callus formation. N_6 medium supplemented with 2,4-D 2.50 and Kin 1.00 (mg/l) exhibited the highest frequency of non embryogenic callus was more than that of the embryogenic callus in the media supplemented with 2,4-D alone .The results are in agreement with the observations of Chaliha *et al.*(1993) and Hemalatha (1995).

Regeneration:

Varying levels of hormones were used for plant regeneration (Table 2). The regeneration range in P was

Table 1 : Anther callus induction in different hormonal combinations

2,4-D+ kin(mg/l)	PC	P10	WPC	WP10	WP20	WP20D	WP40	WP40D
2.00 +0.50	18.79	23.97	4.31	24.53	7.87	12.71	15.11	18.23
	(25.68)	(29.30)	(11.98)	(29.68)	(16.29)	(20.88)	(22.87)	(25.26)
2.00+1.00	19.25	24.21	4.52	25.17	8.07	13.20	15.81	18.89
	(26.02)	(29.46)	(12.27)	(30.10)	(16.50)	(21.30)	(22.42)	(25.75)
2.50+0.50	19.52	24.52	4.89	25.91	8.33	14.13	16.20	19.27
	(26.21)	(29.67)	(12.77)	(30.59)	(16.77)	(22.07)	(23.72)	(26.03)
2.50+1.00	20.70	25.78	6.98	27.11	10.51	16.90	18.07	23.62
	(27.05)	(30.50)	(15.31)	(31.26)	(18.91)	(24.26)	(25.15)	(29.07)
3.00+0.50	20.20	25.22	5.81	26.30	9.73	15.20	17.30	21.21
	(26.70)	(30.14)	(13.94)	(30.84	(18.17)	(22.93)	(24.57)	(27.41)
3.00+1.00	19.57	24.97	5.22	26.12	8.72	14.90	!6.52	20.34
	(26.25)	(29.97)	(13.20)	(30.72	(17.17)	(22.70)	(23.97)	(26.79)
		S.E. ±		C.D. (P=0.05)		S.E. ±		C.D. (P=0.05)
Variety		0.02899		0.08465**		0.09534		0.269**
Hormone		0.05023		0.147*		0.09534		0.269**
V x H		0.071		0.207*		0.234		0.658

* and ** indicate significant of values at P=0.05 and 0.01, respectively
Values within the paranthesis are arc sin transformed.

Table 2 : Regeneration of plantlets on different hormone combinations

Hormones(mg/l)	PC	P10	WPC	WP10	WP20	WP20D	WP40	WP40D
IAA1.00 + Kin 1.00	6.93	9.31	4.91	10.52	11.32	10.71	11.30	7.11
	(15.26)	(17.76)	(12.80)	(18.92)	(19.65)	(19.09)	(19.63)	(15.46)
IAA2.00 + Kin 3.00	6.21	8.72	4.21	9.71	10.52	10.10	10.81	6.22
	(14.42)	(17.17)	(11.83)	(18.15)	(18.92)	(18.52)	(19.19)	(14.44)
BAP1.00 + NAA0.10	7.33	10.81	5.31	12.82	13.51	11.33	12.90	7.82
	(15.70)	(19.19)	(13.32)	(20.97)	(21.56)	(19.66)	(21.04)	(16.23)
BAP1.00 + IAA 0.10	5.82	7.93	3.93	9.21	10.30	9.73	9.70	5.73
	(13.95)	(16.35)	(11.43)	(17.66)	(18.71)	(18.17)	(18.14)	(13.84)
	S.E.±		C.D. (P=0.05)		S.E.±		C.D. (P=0.05)	
Hormones	0.04677		0.140**		0.04500		0.128**	
Variety	0.03307		0.09914**		0.05510		0.157**	
H x V	0.0614		0.198**		0.0110		0.313**	

* and ** indicate significant of values at P=0.05 and 0.01, respectively
Values within the paranthesis are arc sin transformed.

5.82 to 10.81 % .In case of WP the highest frequency was seen in the treatment WP20 (10.30 to 13.51 %) followed by WP 40 (9.70 to 12.90 %).The results showed that the hormonal combination of BAP(1.00 mg/l) and IAA (0.10 mg/l) served best for plant regeneration. Significant difference was noticed in varying hormone levels, treatments and their interaction in regeneration of plants.

Generally callus grow quickly during the first 20 to 25 days after being transferred to regeneration medium, then slows down with a change of colour from yellow to brown or dark yellow in the later days. This type of callus was regenerable to green plant. In cases where the callus kept growing quickly and maintained a light or bright yellow colour on the regeneration medium, only a few albino plants were produced.

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