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Callus Induction and Plant Regeneration from Rice Epicotyl

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Abstract

Of the three rice genotypes, namely LX 297, IR 64 (1-1-4) and V 19 most responsive genotype LX 297 was found to be best for callus induction (30.93%) in MS medium. For regeneration, calli were transferred to MS-based regeneration medium MSK_2. Highest regenerants (14.88%) were produced from calli induced on N_6 medium when transferred to MSK_2.

Introduction

Many agronomically valuable rice genotypes are recalcitrant to *in vitro* manipulation because of their poor callus production and regeneration ability. Hartke and Lorz (1989) tested 15 indica rice lines and found that only seven of them produced embryogenic calli while only four regenerated into plants. It has been a constant endeavor to identify suitable explants of rice to produce embryogenic calli under appropriate culture condition to maximise the callus yield with high plantlet regeneration.

Various explants such as root segments (Abe and Futsuhara 1984; Mandal et al. 2003), mature embryos (Abe and Futsuhara 1984, 1986; Wang et al. 1997; Seraj et al. 1997; Azria and Bhalla 2000), immature embryos (Seraj et al. 1997; Li and Liu 1982; Koetje et al. 1989), coleoptile (Oinam and Kothari 1995; Chand and Sahrawat 1997) and young inflorescences (Chen et al. 1985; Wang et al. 1987) have been used for regeneration of rice plant. But the available information on the use of epicotyl as explant is lacking or insufficient.

Therefore, the study was aimed at finding out the response of epicotyl tissue as explant and to determine its response to embryogenic calli regeneration.

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Materials and Methods

The experiment was undertaken in the Anther Culture and Genetic Engineering Laboratory of Philippines Rice Research Institute, Maligaya, Science City of Munoz, Nueva Ecija in the wet season, 2001.

Three rice genotypes, namely LX 297, IR 64 (1-1-4) and V 19 were used. Mature dehulled seeds of these genotypes were surface sterilized with 70% ethanol for 1 min and rinsed with distilled water and then sterilized with 50% Clorox for 2 × 30 min by vigorous shaking. Treated seeds were rinsed twice with distilled water and blot to dry them onto filter paper. For further study the surface sterilized seeds were inoculated in three basal media: MS, N6 and R. The epicotyls of aseptically germinated seedlings were cut from the proximal portion and were (approx. 1 cm in length) and were planted in MS (Murashige and Skoog 1962), N6 (Chu et al. 1975; Chu 1978; Li 1992) and R medium (Barba and Patena 1998). Media were solidified with 0.7% agar. The pH of all media was adjusted to 5.8 before autoclaving at 115^0C for 15 min. Media were poured into Magenta vessels and 15 epicotyl segments were cultured in each vessel for each replication per callus induction medium. Three vessels were used per replication.

The culture vessels were sealed using plastic tape and incubated at 27 ± 2^0C in total darkness for three weeks. Thereafter, epicotyl-derived calli were isolated and subcultured in the same medium for one week. Calli were then transferred to the regeneration medium MSK_2 (MS basal salt + 1.0 mg/l MS vitamins + 10.0 mg/l Fe-EDTA + 0.1 g/l myo-inositol + 30 g/l sucrose + 0.5 mg/l NAA + 2.0 mg/l Kn). Regeneration media were solidified with 0.3% agar and 0.2% phytagel. Regeneration of the type of calli produced that is embryogenic or non-embryo-genic, they were transferred to regeneration media. Cultures were incubated at 28 ± 1^0C for 16 h photoperiod (General electric cool white fluorescent tubes) for four weeks. Two - three cm long plantlets were transferred to hormone free MS for root induction. Experiments were carried out in a completely randomized design with three replications.

Data collected were subjected to analysis of variance and means were separated by LSD test as well as DMRT. IRRISTAT statistical program version 3.1 was used for analysis of data.

Results and Discussion

Embryogenic callus formation and plant regeneration from epicotyl segments are shown in Fig. 1. The production of calli (%) showed significant differences among the three genotypes (Table 1) which indicated that the genotypes accounted for the greatest variation in callus formation. Although variation between callus induction media and genotype × callus induction media found to be not significant it was observed that there was an effect of genotype on overall

callus induction media, for instance LX 297 produced more calli (23.07%) compared to V19 (6.5%) and IR64(1-1-4) (0.93%, Table 2). Khanna and Raina (1998) reported that genotype differentially affected callus induction and regeneration. When mean effect of callus induction media over all genotypes was tested, MS (15.40%) was found to be better than N6 (9.21%) and R (5.90%) medium.

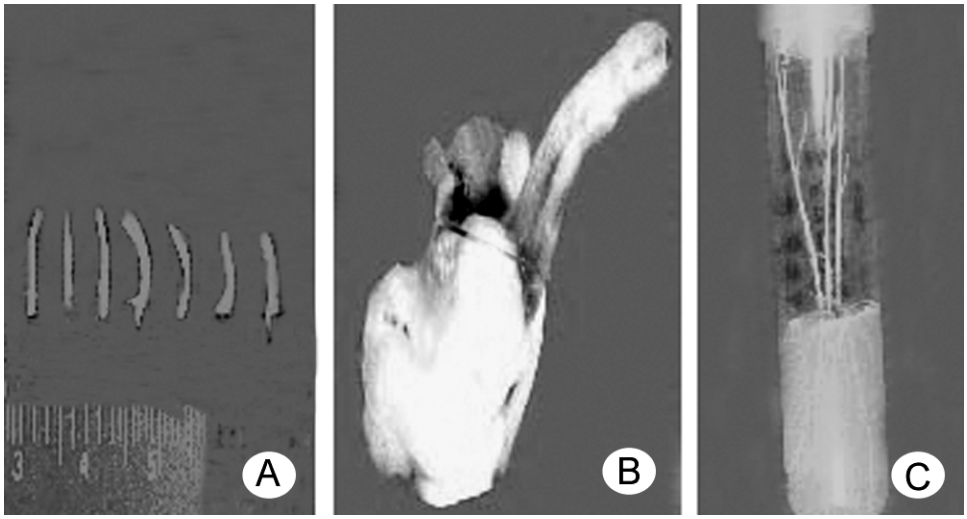

Fig. 1. Callus induction and plantlet regeneration from epicotyl segments of in vitro grown rice seedling. A. Excised epicotyl (proximal portion). B. Epicotyl-derived 21 days old embryogenic calli. C. Rooting of in vitro proliferated shoots.

Table 1. Analysis of variance for per cent calli produced from epicotyl segment of three rice genotypes in three callus induction media.

SD	df	SS	MS	F
Replication	2	881.39	440.69	4.79*
Genotype (G)	2	2387.76	1193.88	12.97**
CIM (C)	2	418.62	209.31	2.27 ns
G × C	4	172.76	43.19	< 1
Error	16	1472.82	92.05	-
Total	26	5333.35	-	-
CV (%)	94.3	-	-	-

**Significant at 1% level. *Significant at 5% level. ns = not significant. CIM = Callus induction medium.

Results also showed that the genotype LX 297 produced the highest percentage of callus in MS (30.93) followed by N6 (23.92) and lowest in R

medium (14.37). Genotype V19 showed 12.50% callus in MS followed by N6 (3.7%) and lowest in R medium (3.33%) while IR 64 (1-1-4) produced calli only in MS (2.78%) and there was no callus formation in N6 and R medium (Table 2). Macabale et al. (2001) used epicotyl segment as explant in their experiments. They reported that genotype LX 297 produced more calli (70.3%) than IR 64 (1-1-4) (66.1%) in R medium (Table 3). This result was supported by the present study which showed that genotype LX 297 was more responsive for callus production whether the medium is MS or R. In another study, Chand and Sahrawat (1997) reported that callus formation was more (72.0%) from coleoptile segments in MS containing 2, 4-D and Kn 0.5 mg/l compared to four-day-old-coleoptile segments.

Table 2. Genotype × callus induction media means for per cent epicotyl derived calli.

CIM (C)	Genotype (G)			
	V 19	LX 297	1R 64 (1-1-4)	C mean
N6	3.70	23.92	0.0	9.21
MS	15.50	30.93	2.78	15.40
R	3.33	14.37	0.0	5.90
G-mean	6.51	23.07	0.93	10.17

Table 3. Table showing mean square values and the probability of the significance following ANOVA of genotype LX 297 in regeneration medium from epicotyl-derived calli.

Source of variation	df	No. of regenerants (%)	No. of calli with green spot (%)	No. of calli with roots (%)	No. of calli with necrosis (%)
Replication	2	50.32 ns	522.1 *	30.9.54 ns	291.61 ns
CIM	2	190.10 ns	1161.6 **	83.95 ns	202.32 ns
Error	4	183.71	28.01	226.43	179.99
CV (%)	-	149.4	14.2	71.7	143.9

ANOVA was carried out for three callus induction media involving three replications.

In case of plant regeneration, only the responsive genotype (LX 297) was subjected to MS-based regeneration medium MSK_2. The results showed that variation of media significantly affected the per cent of green spots developed from calli. Variation in induction media did not affect per cent regenerants, per cent calli with roots or per cent calli with necrosis. Results indicated that LX 297 epicotyl-derived calli induced on N6 when transferred to MSK_2 regeneration medium, produced the highest regenerants (14.88%). On the other hand, when

calli induced on R medium was transferred to MSK_2 regeneration medium, they failed to produce any regenerants while 12.47% regenerants were obtained from MS-MSK_2 callus induction regeneration medium (Table 4).

In case of embryogenic calli, more green spotted (55.87%) were produced from MS-MSK_2 medium combination and less in R-MSK_2 media combination (16.67%). R-MSK_2 media which produced more (27.08 + 12.5 = 39.58%) non-embryogenic calli compared to MS-MSK_2 media combination which produced less non-embryogenic calli (18.21%). Embryogenic calli production was better in MS-MSK_2 compared to N6-MSK_2 media.

Table 4. Mean effect of callus induction regeneration media for regeneration ability of epicotyl derived-calli of rice genotype LX 297.

	No. of regenerants (%)	No. of calli with green spot (%)	No. of calli with roots (%)	No. of calli with necrosis (%)
N6-MSK_2	14.88	39.29	17.66	15.48
MS-MSK_2	12.47	55.87	18.21	0.0
R-MSK_2	0.0	16.67	27.08	12.5
SED	7.83	3.06	8.67	7.75
5% LSD	30.67	11.98	34.05	30.36

In case of effect of genotype and culture media on induction of green spoted embryogenic calli in MS-MSK_2 media was found better than N6-MSK_2.

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