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Somatic Embryogenesis in Root Derived Callus of *Indica* Rice

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Abstract

Prolific callus induction was established in *in vitro* grown young roots of two high yielding *indica* rice varieties *viz*. Quing Livan 1 and IET 13856. Roots from eight-day-old seedlings displayed better callus health, maximum callus induction and plantlet regeneration. Callus induction was completed within 20 - 25 days enmassing the entire root explant. Quing Livan 1 showed more callus induction and plantlet regeneration and took less time also for callus development. Primary calli were white, wet surfaced and mucila-geneous. Quing Livan 1 showed maximum green plant : embryo conversion ratio on regeneration medium consisting of MS with 2 mg/l BAP and 0.5 mg/l NAA. Embryogenic cell suspensions were induced from the calli within two and a half months. Early establishment of cultures from tender roots deems to be telescoping the time requirement for callus induction from immature and mature seed embryos in rice.

Introduction

Callus having more proembryos/embryos and high plantlet regeneration per cent is an essential prerequisite for developing an efficient tissue culture system. It has been a constant endeavor of rice tissue culturists to identify suitable explants to produce embryogenic calli under appropriate physical and chemical environments to maximise the callus yield with high plantlet regeneration.

Besides, mature and immature seeds, young inflorescences, rachilla, anthers and microspores; roots (Kawata and Ishihara 1968, Tran and Nguyen 1976, Abe and Futsuhara 1985, Toshinori and Futshuhara 1985, Kavikishor, 1986, Sticklen 1991) and coleoptiles (Oinam and Kothari 1995, Chand and Sahrawat 1997) were reported to be good sources of embryogenic calli. The

establishment of callus cultures from tender roots shorten the time requirement for callus induction in comparison to mature panicles (Shu and Wei 1980, Wu and Chen et al. 1985), rachilla (Visarada et al. 1996, Biswas et al. 2002), immature embryo (Koetje et al. 1989), leaf sheath (Wu and Li 1971, Bhattacharya and Sen 1980) and young inflorescence (Chen et al. 1985). In addition, plantlets regenerated from root derived calli displayed no morpho-logical abnormalities and were found to be fully fertile (Mukhopadhyay et al. 1997), indicating their ample scope in genetic transformation.

In this backdrop, efforts were made to explore the feasibility of using root explants from Quing Livan 1 and IET 13856 to induce embryogenic callus and to regenerate plantlets therefrom for early development of an efficient somaculture system in *indica* rice.

Materials and Methods

Seeds of two *indica* rice varieties viz. Quing Livan 1 and IET 13856 were obtained from Directorate of Rice Research, Hyderabad. They were grown in experimental net house and healthy mature seeds were used in this experiment. The seeds were dehusked, cleaned and dipped in 5% Teepol solution for 5 min with intermittent shaking and washed thoroughly with distilled water. The seeds were then surface sterilized for 10 min in freshly prepared 0.1% aqueous $HgCl_2$ solution with 2 - 3 drops of Tween 20 as wetting agent, washed in sterile double distilled water three times under laminar air flow (LAF). They were blot dried on sterile tissue paper and cultured onto half strength MS. The pH of the medium was adjusted to 5.8 and 0.8% agar was added prior to autoclaving to solidify the medium. The cultures were placed in dark for three days and then transferred to 16/8 h light (~130 µE m^{-2} s^{-1})/dark cycle in the culture room at 25 ± 2_C. Seedlings at the age of 4, 8 and 11 days with well developed root system were used in this experiment. Primary roots were extracted carefully, the tips removed and cut into small segments of ~ 8 - 10 mm length and sorted into different groups based on their position in the original root. Twenty five segments of roots were placed on callus induction medium (CIM) containing MS with 2 mg/l 2, 4-D, 3% sucrose and 0.8% agar. All the experiments were replicated five times. After transferring the root segments, the Petri dishes were sealed with parafilm and kept in dark at 25 $\pm$ 2_C in the culture room. After two weeks, calli with globular proembryos were carefully removed from the root surface and subcultured on freshly prepared callus maintenance medium (CMM) constituted of 2,4-D (1 mg/l). After two subcultures, globular, friable, dry surfaced embryogenic calli were selected under stereomicroscope and half of them were subcultured on EIM containing 4.6 µM abscisic acid (ABA), 0.1%

casein hydrolysate (CH), 0.3% sucrose and 0.8% agar and the remaining half was transferred directly onto regeneration medium (RM) containing MS with 2.0 mg /l BAP and 1.0 mg /l Kn. The cultures were kept under 16/8 h light (130 µE $m^{-2}s^{-1}$)/dark cycle at 25 ± 2_C. Within 15 - 20 days of culture, calli turned greenish and plantlets with root and shoots were developed within a month. They were grown in the culture tubes till they attained 6 - 8 cm height and finally transferred to experimental net house up to maturity. Individual panicles at the flowering stage were covered with butter papers bags to prevent any cross-pollination. The seeds from individual plants were collected separately, thrashed, cleaned and stored for rising the next generation.

Another experiment involving eight-day-old seedlings was conducted involving two varieties to identify the best regeneration media. The effect of subculture on EIM was also evaluated. Plantlets of 6 - 8 cm were transferred from RM to experimental net house and their seeds were collected. Efforts were also made to develop embryogenic cell suspension (ECS) involving friable root calli of both varieties following standard protocol (Mandal and Bandyopadhyay 1996).

Results and Discussion

Stereomicroscopic observations revealed that minute adventitious roots were developed first from the surface of the cultured roots, which produced calli at their bases and ultimately thick callus covered the entire root surface (Fig. 1). The calli were found to be mucilagenous at the beginning but after two subcultures they became friable with distinct white globular pro-embryos and embryos. Callus was found to appear at the basal region of the adventitious roots (Mukhopadhyay et al. 1997). This might be due to the variation in hormone levels in various parts of the root segment. Development of more adventitious roots in the proximal region as compared to the distal root tip was found to be similar to the observations of Mukhopadhyay et al. 1997. Quing Livan 1 showed better root callus development and plantlet regeneration. This variety also took less time for callus development (Table 1). Variability in callus forming ability, embryogenic callus induction per cent and plantlet regeneration were also observed earlier (Kawata and Ishihara 1968, Sticklen 1991, Abe and Futsuhara 1985, Kavikishor 1986, Wu and Li 1971, Tran and Nguyen 1976, Shu and Wei 1980, Bhattacharya and Sen 1980, Toshinori and Futshuhara 1985, Chen et al. 1985, Abdullah et al. 1986, Wu and Chen 1989, Koetje et al. 1989, Oinam and Kothari 1995, Visarada et al. 1998.

Table 1. Comparative assessment of morphogenetic response of calli developed from roots and mature somatic embryos in Quing Livan 1 and IET 13856.

Variety	Age of *in vitro* grown seedling (days)	Days taken for callus induction	Av. No. of calli induced/root segment (8 - 10 mm)	Callus health scale (1 - 9)*	Plantlet regeneration (%)			
					Direct regeneration		Subculture on EIM	
Quing	4	13.0b#	3.72c	2	39.20b		64.36b	
Livan 1	8	15.0ab	8.14b	1	43.70a	(69.50)	72.00a	(97.16)
	11	16.0ab	2.25cd	4	39.60b		60.50b	
IET	4	15.0ab	1.70d	4	9.10d		18.30c	
13856	8	17.0ab	15.06a	2	27.40c	(63.16)	50.40b	(94.25)
	11	21.0a	1.51d	6	6.30d		14.50c	

*1= excellent; 2 - 3 = very good; 4 - 5 = moderate; 6, 7, 8 = poor and 9 = very poor. # = values bearing same letter in the column are not significantly different at P = 0.05 of DMRT. Figures in parentheses indicate plantlet regeneration (%) in calli derived from mature somatic embryos.

Young roots from eight-day-old seedlings showed maximum adventitious root formation as well as callus induction. About 64.28% of the root explants produced only five calli from the distal portion, while a very few root segments

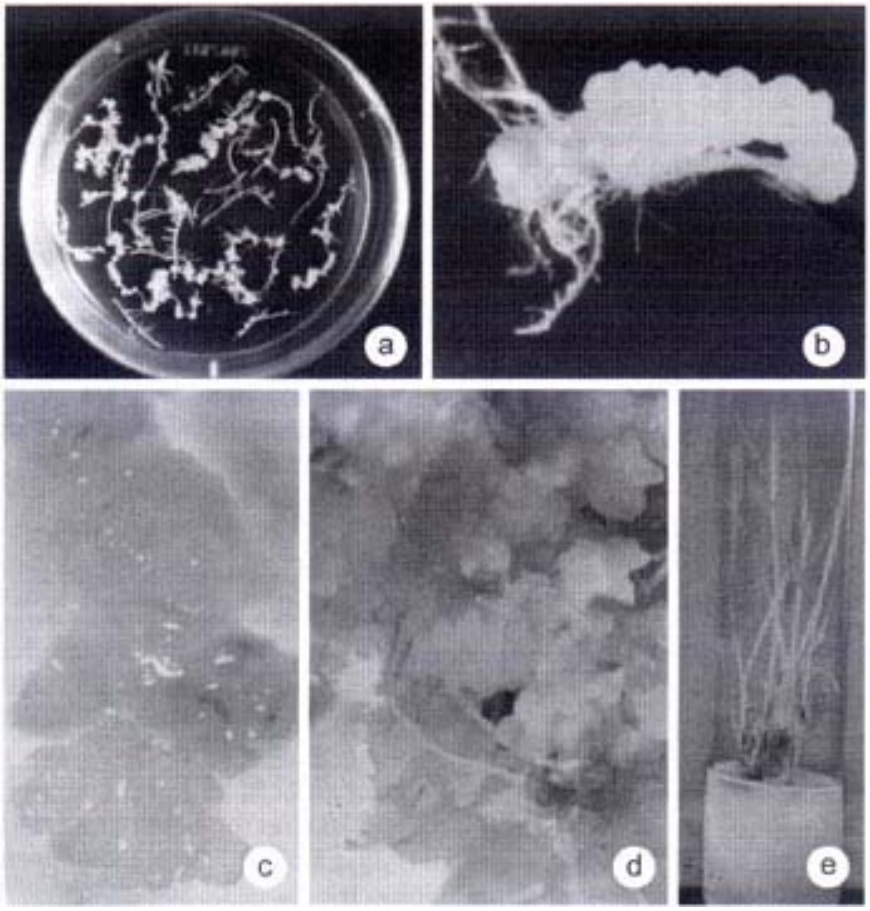

Fig.**1**. Callus induction, proliferation and plantlet regeneration from the roots of *in vitro* grown rice seedlings. (**a**). Prolific callus induction on the root surface of eight days old seedlings in Quing Livan 1. (**b**). Stereomicroscopic view of callus from eight days old seedlings showing formation of adventitious roots also. (**c**) Pro-embryos and embryos in embryogenic calli on the surface of root segments. (**d**) Green spots and emerging plantlets from root derived calli. (**e**) A few regenerating plantlets from root callus.

(5.35 - 8.92%) proximal to root tips produced more number of calli (11 - 20). However, in IET 13856, middle portion produced more calli. It was observed that those calli which were transferred onto RM after a subculture in EIM showed more plantlet regeneration (Fig. 1), being 92 and 69.3% in Quing Livan 1 and IET 13856, respectively (Table 1). Eight-day-old explants displayed consistently appreciable results in both varieties.

Table 2. Root callus induction and plantlet regeneration from eight-day-old seedlings in Quing Livan 1 and IET 13856.

Variety	Length of root segments plated on CIM (mm)	Av. No. of calli produced per segment	Treatment in RM** (mg)	No. of calli directly regenerated on RM	No. of calli regenerated after subcultured on EIM**	Plant/embryo conversion (%)	
						Calli not *en routed* EIM	Calli *en rout* EIM
Quing Livan 1	8 - 10	9.20	Control	200 (16.00)**cd#	256 (38.12)**d	28.17d	45.68e
			1.00 BAP	504 (27.50)bc	232 (87.93)b	58.20a	93.25a
			2.00 BAP + 0.5.00 NAA	256 (43.75)a	320 (92.00)a	61.62a	97.52a
			0.5.00 Kn + 0.5.00 NAA	260 (26.60)bc	284 (69.00)c	49.16b	85.42b
IET 13856	8 - 10	14.51	Control	139 (8.63)d	200 (14.00)c	21.10e	32.50f
			1.00 BAP	73 (12.32)cd	144 (18.75)c	42.00c	61.30c
			2.00 BAP + 0.5.00 NAA	56 (17.85)cd	70 (22.85)c	45.15bc	67.25c
			0.5.00 Kn + 0.5.00 NAA	55 (40.00)ab	75 (69.30)c	26.15d	53.25d

*RM and EIM are detailed in Materials and Methods. **Figure in parentheses indicates per cent regeneration of plantlets.
= values bearing same letter in the column are not significantly different (for the per cent values only) at p = 0.05 of DMRT.

The plantlet regeneration increases when the calli were subcultured on EIM by 40 and 49% in Quing Livan 1 and IET 13856, respectively involving mature somatic embryos. In contrast, increase in plantlet regeneration was 71 and 49.2%, respectively in case of root explants. It is discernible that subculturing on EIM could enhance plantlet regeneration in both types of calli derived from root explants and mature somatic embryos. It was further observed that embryogenic calli developed from mature seeds took two - three subcultures to develop good embryos while root calli directly developed into globular embryos as evident from stereomicroscopic observations.

The second experiment showed that irrespective of the hormonal combination, subculture on EIM boosted plantlet regeneration (Table 2). MS supplemented with 2 mg/l BAP and 0.5 mg/l NAA was found to be best for Quing Livan 1, while MS with 0.5 mg/l Kn and 0.5 mg/l NAA performed best for IET 13856. The plant/embryo conversion ratios also followed a similar trend wherein the increase was observed to be 58.2 and 48.9%, respectively under the best hormonal combination in both varieties. The plausible reason for this could be the presence of ABA, CH and Kn, in EIM, which might have enhanced the formation and maturation of somatic embryos. This is corollary to the earlier observations of Suprasanna et al. 1995. It is thus evident that by using EIM the plantlet regeneration could be improved substantially.

It is also mentionable that root derived callus from Quing Livan 1 produced very fine ECS within 2.5 months whereas IET 13856 took about 3.5 months for development of moderate ECS. All the plantlets regenerated from root derived calli were found to be fertile. This is similar to the observations of Mukhopadhyay et al. 1997. In essence it can be concluded that *in vitro* grown young roots may be a good source in fast generation of embryogenic calli and somatic embryos in rice even though the root derived calli does not perform at par with mature seed derived calli. However, availability of roots in plenty at the very beginning stages of seed germination definitely provides an additional advantage in initiating *in vitro* culture in rice.

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