

TISSUE CULTURE POTENTIAL OF TRADITIONAL INDICA RICE VARIETIES

(*Oryza sativa* L.) OF SRI LANKA

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ABSTRACT

High – frequency callus formation and plant regeneration, are essential pre-requisites for the application of biotechnology to plant breeding. Intervarietal variation in callus formation and plant regeneration from callus cultures were tested using mature seeds of five commercially cultivated improved indica cultivars along with 20 traditional Sri Lankan indica rice (*Oryza sativa* L.) varieties, for most of which *in vitro* techniques have not been tried out before. Two lines each of japonica and IRRI varieties were also tested for comparison. Both callogenesis and plant regeneration abilities were high in improved indica rice and comparable with the control varieties. There was a difference with respect to callusing, regeneration and calli necrosis, between the two age groups (3-3 1/2 m and 4-4 1/2 m) tested and the traditional varieties. Improved indicas and the control varieties showed high percentage of calli greening and shoot bud initiation at 2 weeks while traditional indicas required 4 weeks to reach maximum greening. Results indicated that traditional indica varieties possess *in vitro* plant regeneration ability, but with a varying degree of response. Based on the results of this study, rice varieties were classified according to their callusing and plant regeneration potential.

KEY WORDS: Callogenesis, Indica rice, *Oryza sativa*, Regeneration.

INTRODUCTION

Rice (*Oryza sativa* L.) is the most important cereal crop in Asia and approximately one tenth of the arable land in the world is occupied by rice. Immense progress has been made in rice research on the whole and significant improvements achieved in modern biotechnology. An important requisite for using biotechnology is the superior morphogenetic potential of plant cells i.e. high frequency callus formation and plant regeneration abilities. This is not a major problem for the japonica rice group, but for the indica type, lack of a well-established *in vitro* culture system is a bottleneck for further advancement of research. Previous studies have shown that the japonica types possess higher regeneration capability than indica type which is moderately or highly recalcitrant to callusing and plant regeneration (Chaleff, 1979; Zhao *et al.*, 1999). Tissue culture studies have therefore mostly concentrated on japonica rice and very limited work has been done on indicas. However, most countries where rice is the staple food consume indica rice and hence, understanding the genetic mechanism of *in vitro* regeneration and related cellular traits of this group is an important requisite for varietal improvement.

There are many traditional rice varieties possessing valuable traits, that could be incorporated into cultivated rice. Genetic transformation and somatic hybridization are some of

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the techniques that can aid this work. Callusing and plant regeneration ability are two essential genetic characters for using these techniques. Kawata *et al.* (1992) reported variation in protein patterns of rice protoclones, suggesting, that genes involved in the expression of these proteins are activated differentially during organized development. It was also reported by Yamagashi *et al.* (1998) that one region on chromosome 1 controls callusing from rice microspores and one region on chromosome 10 controls the green to albino regenerant ratio. Lange *et al.* (1998) reported that *in vitro* organogenesis and germination of immature embryo culture of wheat showed a complex gene action controlling both traits.

Within this context, a genetical mechanism on *in vitro* morphogenesis of plants is obvious. Hence, it is most probable that the recalcitrant behaviour of indica rice in tissue culture is controlled by one or more genes. The aim of this study was therefore to apply tissue culture technology to some of traditional rice varieties and identify the lines for which tissue culture technology can be applied.

MATERIALS AND METHODS

Dry mature seeds were de-husked and surface sterilized by immersing in 40% (v/v) Clorox solution for 15 min followed by 70% (v/v) ethanol for 5 min and washed 3-4 times with sterilized distilled water.

Mature seeds of 25 indica rice varieties belonging to traditional and improved indica were obtained from the gene bank, Plant Genetic Resources Center (PGRC), Peradeniya. Controls were 02 lines each of japonica and IRRI lines (Table 1).

Testing callogenetic potential

The sterilized seeds were cultured in 9 cm petri dishes (9 seeds / dish; 3 dishes / variety) containing agar-solidified Rc medium [Linsmaier & Skoog (1965) salts, thiamin hydrochloride (1.0 mg/l), sucrose (3% w/v), yeast extract (3.0 g/l), 2,4-D (2.0 mg/l) and BAP (0.2 mg/l); Rajapakse *et al.* (1991)] and incubated at $25 \pm 1^\circ\text{C}$ under dim fluorescent light ($5 \mu\text{Em}^{-2}\text{s}^{-1}$; 10h) for callus induction. Three weeks later, germination percentage of seeds, callus formation efficiency (CFE %), calli morphology (calli colour, texture) and fresh weight of callus (mg/callus) were recorded. The calli were separated from the mother tissue and sub-cultured in a freshly-prepared Rc medium and incubated as before.

Testing regeneration ability

Two weeks after subculture, calli were transferred to the regeneration medium, Rg [$\times 1/2$ MS (Murashige & Skoog, 1962) salts, myo-inositol (100 mg/l), thiamin HCl (0.5 mg/l), pyridoxin HCl (0.5 mg/l), nicotinic acid (5.0 mg/l), d-biotin (0.05 mg/l), glycine (2.0 mg/l), sucrose (1.5 %w/v), mannitol (1% w/v), BAP (6.0 mg/l) and NAA (2.0 mg/l)]. The pH of the medium was adjusted to 5.8 and agar (0.8% w/v) was incorporated prior to autoclaving at 121°C

for 15 min. Calli in Rg medium were incubated at $25 \pm 1^\circ\text{C}$ under bright fluorescent light ($30 \mu\text{Em}^{-2}\text{s}^{-1}$; 10h). At 2, 4 and 6 weeks after culture, green bud induction frequency (GIF %) and number of shoots per calli were recorded.

GIF (%) = number of calli with shoot initiation $\times$ 100 / number of calli cultured in Rg medium

The shoots were separated and cultured in jars containing MS medium with sucrose (3% w/v) and without any growth regulator to induce rooting. Plantlets were acclimatized and successfully transferred to the glasshouse.

Table 1. Rice varieties tested.

Rice group	Age group (months)	Variety name
Traditional Sri Lankan indica	3	Demas (V1)
		Dahanala (V2)
	3 - 3 1/2	Weda heenati (V3)
		Heenati 310 (V4)
		Hetada (V5)
		Herath banda (V6)
		Kalu heenati (V7)
	3 1/2	Handiran (V8)
		Wanduruwee (V9)
		Heenkuruwee (V10)
	4	Sudumadael (V11)
		Pokkali 809 (V12)
	4 - 4 1/2	Thatu wee (V13)
		Moragolla wee (V14)
		Murungakayan 302 (V15)
	4 -	Gires (V16)
		Moddai karuppan (V17)
		Yakada wee (V18)
	4 1/2 - 5	Suduru samba (V19)
		Sudu illankayan (V20)
Improved Sri Lankan indica	3 - 3 1/2	Bg 94-1 (V21)

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	4 - 4 1/2	Bg 34-8 (V22)
		Bg 400-1 (V23)
		H4 (V24)
		MI-273 (m) (V25)
Japonica	3 1/2 - 4	Vasikoku (V26)
		Sinaba (V27)
IRRI lines	3 1/2	IR-54 (V28)
		IR-40 (V29)

RESULTS AND DISCUSSION

Callogenetic potential of indica rice

Callus formation was observed in all tested varieties. Calli initiated from the scutellum area, between the endosperm and slightly elongated coleoptile. The calli were mostly white or light yellow in color, shiny and compact. Calli formation ability was comparatively high (76.2% - 100%) in the 3-3 1/2 m varieties than in the 4-4 1/2-5m varieties (53.6% - 92.8%) (Table 2). Fresh weight of calli was also generally higher in the 3-3 1/2 m varieties. However, var. Suduillankayan (4 1/2-5m) and Sudumadael (4 m), produced large calli, 90.8 and 93.0 mg/callus respectively. However, it was clearly observed that callus forming ability of traditional indica rice was either similar to or better than that of the improved or control varieties. Calli fresh weight was also higher in the indica varieties. These results suggest that traditional varieties are not recalcitrant with respect to callus formation ability but variations occur between varieties.

Table 2. Callogenetic potential of indica rice at 3 weeks of culture.

Variety	Germination (%)	CFE ^a (%)	Calli fresh weight (mg/calli) (Mean $\pm$ SE)	Calli morphology ^b
Traditional indica				
3-3 1/2 months				
Demas	100	95.5	67.1 $\pm$ 2.2	LY,S,C
Dahanala	100	96.4	67.0 $\pm$ 2.1	W-LY,S,F
Weda heenati	100	100.0	43.3 $\pm$ 6.4	W,S,C
Heenati 310	91	91.3	41.9 $\pm$ 5.8	W-LY,S,N
Hetada	95	80.9	31.8 $\pm$ 8.4	W-LY,S,C
Herath banda	95	76.2	37.5 $\pm$ 7.3	W-LY,S,C
Kalu heenati	100	80.0	70.0 $\pm$ 0.7	W-LY,S
Handiran	100	82.1	43.0 $\pm$ 2.5	LY,S,C
Wanduru wee	100	85.7	52.9 $\pm$ 7.2	W-LY,S,C
Heenkuruwee	100	78.3	41.7 $\pm$ 11.5	W,S
4-5 months				
Sudumada el	96	90.9	93.0 $\pm$ 1.2	W-LY,S,F
Pokkali	96	69.6	44.4 $\pm$ 2.4	W-LY,S,C
Thatu wee	100	53.6	41.3 $\pm$ 2.2	W-LY,S
Moragolla wee	100	65.2	22.0 $\pm$ 1.8	W-LY,S
Murungakayan 302	100	85.7	39.4 $\pm$ 6.1	LY-DY,S,C
Gires	96	70.8	30.6 $\pm$ 8.5	LY,S,C
Moddai karuppan	96	64.3	16.1 $\pm$ 2.2	W-LY,S,C
Yakada wee	100	75.0	24.3 $\pm$ 1.5	LY,S,C
Suduru samba	100	76.2	17.3 $\pm$ 3.2	LY,S
Suduillankayan	100	92.8	90.8 $\pm$ 2.6	W-LY,S,C
Improved indica				
Bg 94-1	95	79.2	33.3 $\pm$ 4.1	W-LY,S,C
Bg 34-8	100	87.5	43.6 $\pm$ 1.2	LY,S,F
Bg 400-1	100	100.0	30.8 $\pm$ 3.7	LY,S
H4	100	100.0	37.1 $\pm$ 2.1	LY-DY,S
MI-273 (m)	89	82.1	56.1 $\pm$ 1.1	W-LY,S
Japonica				
Vasikoku	100	96.4	53.7 $\pm$ 3.1	LY,S,C
Sinaba	100	68.2	34.7 $\pm$ 2.2	W-LY,S
IRRI lines				
IR-54	95	84.2	40.6 $\pm$ 4.2	LY,S,C,N
IR-40	100	100.0	20.0 $\pm$ 3.6	W-LY,S,N

^a: CFE : (number of seeds which formed calli / number seeds germinated) x 100

^b: Code for calli morphology : Colour : LY – light yellow, DY – dark yellow, W – white
Texture : C – compact, F – friable, S – shiny, N – nodular

Regeneration potential of rice callus

Calli green bud formation, which is an early sign of regeneration, was checked initially to identify rice varieties with possible regeneration ability. Results show, that 4-4 1/2-5m traditional rice varieties have a higher possibility of calli greening than 3-3 1/2m traditional varieties. In this respect, 4 - 4 1/2m varieties are similar to improved indica, japonica and IRRI rice groups (Figure 1A).

High rate of calli greening occurred within two weeks and remained so until the 6th week in the improved indica rice types (Figure 1B). In traditional indicas of both age groups however, maximum greening was observed at a later stage i.e. around the 4th week, and by the 6th week, the number of cultures with green buds reduced due to high level of browning. Calli necrosis, which is indicated by calli browning, started around the 5th week. In traditional varieties, the effect of calli necrosis was obvious than in the other types, but there was a wide variation. Shoots regenerated from green calli during the 4th week in all rice groups. These shoots produced plantlets in the growth medium.

These results showed that traditional varieties express wide variability in their response to *in vitro* regeneration. Figure 1A shows that GIF % of traditional indica ranged from 0 – 100 % at the 2nd week. During the 4th week, GIF values were higher, i.e. 50%< in all improved indicas, controls and most traditional varieties. However, by the 6th week, GIF % of traditional varieties scattered from 0-100%, while in improved indica and controls, the values remained higher (Figure 1A & 1B). Based on the results, the tested indica varieties were categorized according to their callogenetic and shoot regeneration abilities (Table 3).

Varieties Suduillankayan (V20), Yakada wee (V18), Moragolla wee (V14), Pokkali (V12), Murungakayan 302 (V15) and Sudumada el (V11) had high values for green bud formation (75%<) at the 4th week. All these varieties belong to the 4-4 1/2-5m age group (Table 3). On the other hand, a 3m aged variety, Weda heenati (V3), had 100% callusing ability, but none of the calli turned green and interestingly, nor browning even at the 6th week. Another 3m aged variety, Heenkuru wee (V10) had only a brief period of green callus that lasted only during the 4th week and quickly turned necrotic

Table 3. Classification of rice varieties according to callusing and regeneration potential.

Range	Callus formation at 3 rd week		Shoot bud initiation at 4 th week and regeneration ability	
	3 - 3 1/2 m	4 -4 1/2 - 5 m	3 - 3 1/2 m	4-4 1/2- 5 m
100-75%	Heenkuru wee Wanduru wee Handiran Kaluheenati Herath banda Hetada Heenati 310 Weda heenati Dahanala Demas Bg 94-1, Bg 34-8, (IR-54), (IR-40)	Suduillanakayan Suduru samba Yakada wee Murungakayan 302 Sudumada el H ₄ , Bg 400-1, MI-273 (m), (Vasikoku)	Bg 94-1 ^a , Bg 34-8 ^a , (IR-54) ^a , (IR-40) ^a , (Sinaba) ^a	Suduillanakayan ^a Yakada wee ^a Murungakayan 302 ^a Sudumada el ^a Pokkali ^a Moragolla wee ^a H ₄ ^a , Bg 400-1 ^a , MI-273 (m), Vasikoku)
74-50%	(Sinaba)	Moddai karuppan, Gires, Moragolla wee, Thatu wee, Pokkali	Herath banda, Demas, Wanduru wee, Handiran, Dahanala, Kaluheenati	Suduru samba, Gires Thatu wee Moddai karuppan
< 50%			Heenkuru wee Hetada Heenati 310 Weda heenati	

^a : Formation of shoots (2 – 4 shoots per calli at 5 – 6 weeks in regeneration medium)

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CONCLUSION

Results show that 4 - 4 1/2 - 5m traditional and all the tested improved indicas of both age groups responded in a similar manner as the japonica and IRRI lines in this culture system. It should also be noted that these improved indica varieties, have either an IRRI or japonica parent in their pedigree i.e. Bg 94-1 [IR262/Ld 66 (Ld 66= H-501/Deo-Geo-Woo-gen)], Bg 400-1 (Ob 678//IR 20/H₄), Bg 34-8 (IR 246///Pachchaperumal/Mas//H-501), H₄ (Murungakayan 302/Mas). Hence it can be argued that their regeneration ability has been derived from those parents.

However, the traditional indicas do not have a similar parentage, but were yet able to produce calli and regenerate shoots in a manner comparable to japonicas and IRRI lines. Hence, it can be presumed that these traditional indicas also possess gene(s) responsible for these traits but with a wide variability. Therefore, the traditional varieties need to be screened for totipotency and highly responsive varieties identified for the successful application of biotechnological methods to these varieties. It will also be beneficial to study the genetic base of these traditional indicas and identify any molecular markers linked with the callogenetic and regeneration potentials of these varieties.

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Figure 1. Regeneration responses of rice varieties at 2nd, 4th and 6th week after culture

