



Callus Induction and Regeneration in Commercial Rice Cultivars of Tamil Nadu

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Abstract – Ten commercial rice cultivars belonging to subspecies *indica* were compared for its capacity in callus formation and regeneration. Among the different media composition, it was found that the dark condition with optimum level of 2, 4 D (2mg/l) + Kin (0.5mg/l) was the best to get better and early callus induction frequency followed by 1.5 and 2.5 mg/2,4 D/l. It was found that the dark condition with optimum level of 2, 4, D (2mg/l) + Kin (0.5mg/l) was the best to get better callus induction frequency. Among the different varieties tested the varieties viz., ADT 37, ADT 43 and ADT (R) 47 responded better for callus induction than the others ADT 44, ADT (R) 48, TNAU Rice ADT 49 and ADT (R)45. The genotype TNAU Rice ADT 49 had very low per cent of callus induction frequency. Among the rice genotypes ADT 43 only produces shoot in MS + 1.5mg/l BAP+1.5 mg/l Kin+0.5 mg/l NAA media composition. The varieties ADT (R) 46, ADT (R) 47, ADT (R) 48 and ADT 37 have produced only the green spots in the above combination.

Keywords – Callus, Matured Embryo, Rice, Regeneration, Somaclone.

I. INTRODUCTION

Food security in the world is challenged by increasing food demand and threatened by declining water availability and other natural resources. Rice, the staple food for more than half of the world population need to scale up its productivity by breaking the yield plateau in the irrigated areas which contribute to 60 per cent of rice production. The world population is predicted to reach approximately to 8 billion by 2030 (www.fao.org) and there is a need to further increase in rice production by 40 per cent. This scenario can necessarily be contemplated to India. In India, rice is grown in an area of 44.6 million hectares with a production of 109.5 million tonnes with an average productivity of 2.62 tonnes/ha and is predicted have to improve more than 30 per cent by 2030.

The untapped genetic variation existing in wild relatives and land races in crop plants could be exploited gainfully for the development of agronomically superior cultivars. Introgression of novel alleles from wild relatives of crop plants into cultivated varieties (Kovach, *et al.*, 2007) have

clearly demonstrated that certain alleles and their combinations potentially make dramatic changes in trait expression when moved to a suitable background by overcoming the genetic bottlenecks which restricted the introgression to cultivars through conventional breeding.

Rice improvement using biotechnology such as somaclonal variation, *in vitro* selection, production of doubled haploid lines from anther culture and genetic transformation might be an alternative way for achieving desirable rice varieties. It is more easier compared to conventional plant breeding (Yamada, 1986).

In India, *indica* rice is predominantly grown for its fine grain nature and other phenotypic advantages; however most varieties have part of *japonica* type contributions during the course of breeding. Somaclonal variation is the one of the most important and easiest method to create new variations in rice. Plant regeneration through tissue culture is comparatively easy for *japonica* rice than the *indica* rice. Successful callus induction from rice seed has been reported by several researchers (Gonzalez 2000; Navraj *et al.* 1999; Marrassi 1996; Valdez *et al.* 1997; Xie *et al.* 1995) in some varieties. An improved method for callogenesis was reported by Rashid *et al.* 2000. He also recorded that rice seeds have more potential for callogenesis as compared to node or tip. But each and every variety responded differently in different hormonal combinations for callus induction as well as regeneration. Hence there is a need to study and standardize the protocol of regeneration and media composition. The present study aimed to identify a suitable medium for callus induction and the ability of plant regeneration in different rice varieties through somatic embryogenesis.

MATERIALS AND METHODS

This experiment was carried out in tissue culture laboratory of Tamil Nadu Rice Research Institute, Aduthurai during the year 2012-13. The mature embryos of ten rice varieties were used for the study are mentioned in detail (Table 1). The varieties taken for study are highly diverse with *japonica* parental contributions.

Table 1: Details of varieties taken for study

S.No.	Variety	Parentage	Duration (Days)	Salient features
1.	ADT 36	Triveni / IR 20	105-110	Medium slender rice, resistant to blast and stem borer, suitable for Sornavari /Kar /Kuruvai season
2.	ADT 37	BG 2801-2 / PTB 33	105 - 110	Non lodging plant type, short plant stature, more number of seeds per panicle, suitable for Sornavari /Kar /Kuruvai/ Navarai season
3.	ADT 42	AD 9246/ADT 29	115	Long slender rice, suitable for Sornavari /Kar /Kuruvai.

4.	ADT 43	IR 50/I.W.Ponni	105-110	Resistant to green hoppers, suitable for Sornavari /Kar /Kuruvai. This variety is growing larger area in Tamil Nadu.
5.	ADT 44	IR 56 /IR 142-99	145-150	Resistant to green hopper, blast and leaf spot. Moderately resistant to leaf folder. Suitable for samba season.
6.	ADT (R) 45	IR 50/ADT 37	110 - 115 days	Medium slender, tolerant to drought, resistant to gall midge, GLH and sheath rot. Suitable for Sornavari /Kar /Kuruvai.
7.	ADT (R) 46	ADT 38/CO 45	135	Long slender grains. Resistant to stem borer, and leaf folder at field condition. Moderately resistant to rice tungro virus. Suitable for Samba/ Thaladi/ Pisanam
8.	ADT (R) 47	ADT 43/ Jeeraga samba	118	More tillers with long compact panicles, Medium slender white rice, Tolerant to drought, Moderately tolerant to stem-borer, leaf hopper, WBPH and sheath blight. High protein content (9.47 %). Suitable for Sornavari /Kar /Kuruvai.
9.	ADT (R) 48	IET 11412/IR 64	95-100	Long slender grains. Tolerant to stem borer, Green hopper and gall midge under field condition. Suitable for Sornavari/ Kar.
10.	TNAU Rice ADT 49	CR 1009 / Jeeraga samba	130-135	Semi-dwarf, erect habit and non lodging. Moderately resistant to blast, sheath rot, sheath blight and resistant to RTD under artificial conditions; brown spot under field conditions. Suitable for Samba, Thaladi and Pisanam.

The nutrient medium chosen for the study was Murashige and Skoog (MS) medium (Murashige and Skoog 1962) supplemented with different concentration of growth regulators. The pH of the medium is 5.5-5.8 and this was maintained by 0.1N NaOH and finally 30 grams of sucrose (3%) was added as carbohydrate source and mixed well then 8 grams of agar (0.8%) was added to prepare semi solid medium and sterilization was done at 1.01kg/cm pressure at 121°C for 20 minutes then medium was pored into sterilized petri plates and packed with klin film under laminar flow chamber. The medium was allowed to cool at room temperature and stored at 10°C.

Explant

The explant used for this callus induction study was mature embryo. The mature embryo was experimented as explants because of the several advantages *viz.*, the availability of seeds (explants) throughout the year and the ease of storing compared to other explants (Lee *et al.*, 2002) and ability to produce high embryogenic calli (Shanthi *et al.*, 2010). Mature embryos were dehusked and surface sterilized with 70 % alcohol for 30 seconds followed by 15 % of common bleach for 20 minutes then finally rinsed several times with the sterile distilled water before inoculation into the callus induction media.

Callus induction and regeneration

The matured embryos were placed on corresponding callus induction medium with half of the embryo in contact with the medium. Fifteen seeds were plated in each petri plate contained the MS media with different hormonal combinations *viz.*, 1.5 mg/l 2,4,D + 0.5 mg/l Kin; 2mg/l 2,4,D + 0.5 mg/l Kin; 2 mg/l 2,4,D and 2.5mg/l 2,4,D. The cultures were incubated by covering with dark cloths and the temperature was maintained at 28±1°C for 4 weeks to induce callus (Singh *et al.*, 2005). The percentage of callus induction was calculated by taking into account the number of seeds giving callus 20-30 day after inoculation.

The different concentrations of growth regulators used for regeneration in MS media were 1mg/l Kin + 1mg/l BAP + 0.1 mg/l NAA; 1mg/l Kin + 0.1mg/l BAP + 0.5 mg/l IAA; 2.0 mg/l Kin + 0.1 mg/l NAA; 1mg/l Kin + 2mg/l BAP + 0.5 mg/l NAA; 2 mg/l Kin + 3 mg/l BAP + 1 mg/l NAA and 1.5 mg/l Kin + 1.5 mg/l BAP + 0.5 mg/l NAA. A total of 4-6 embryogenic calli were placed on the conical flask containing media for regeneration. The data obtained with percent values were subjected to arc sin transformation and analysed using the AGRES (Agricultural statistics) computer package. Level of significance (P value) was determined using the standard Analysis of Variance (AVOVA) (Panse and Sukhatme, 1964). Differences among mean values were assessed by LSD (Least Significant Difference) test

III. RESULTS AND DISCUSSION

In vitro culture could become an efficient tool for rice breeding especially for the genetic manipulation. In the present experiment, the callus was induced from the matured seeds of ten commercial rice genotypes. The observations on the effect of placement/ orientation of explants in the medium, callus initiation time, media favouring high callus induction, response of genotypes towards different media composition and its regenerative potential were studied.

The results indicated that the embryos facing upside down were produced good callus and its regeneration ability was also higher than the embryos placed horizontally. This was also confirmed by Hodges, 1989 and Agarwal *et al.*, 2006.

The analysis of variance showed that the effect of genotype, medium and genotype x medium interactions on callus induction was found to be highly significant (Table 2). It showed that the materials used in this study and hormonal combinations and its interactions were significantly different from each other. Shanthi *et al.*

(2010) was also reported that the effect of genotypes, medium and genotype x medium interaction was highly significant.

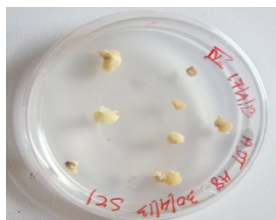


Fig.1. Callus induction from matured embryo

The days to callus initiation varied depending upon the growth regulators and the variety. In all the media compositions, the genotype ADT (R) 47 showed early callus initiation (average of four days) followed by ADT 37, ADT 43 and ADT (R) 48 (average of 4.5 days)(Table 3). The days to callus initiation showed significant difference between the medium and the genotypes. These results proved that each genotype responded differently in different combination of growth regulators. Hence there is a need to standardize the growth hormonal combination for each and every genotype. Similar observation of genotype dependent callusing efficiency was also confirmed by Rasheed *et al.*, 2005. The difference in callus initiation was also reported by Puspam and Sree Rangasamy (2000); Shanthi *et al.* (2010).

The results of this study indicated that increasing the 2,4 D beyond 2.5 mg/l is not desirable for callus induction. The lower level of 2,4 D concentration *i.e* below one mg/l increase the shoot and root growth of the embryo and no callus induction was noticed. The results proved that the presence of 2,4 D in the culture medium is vital for rice callus induction from mature seeds This is also confirmed by (Lee *et al.*, 2002). This observation supplements the result that a high auxin/ cytokinin ratio is necessary for starting embryogenic callus formation compared to a low ratio for the regeneration of plantlets (Ge *et al.*, 2006). Among the different media composition, It was found that the dark condition with optimum level of 2,4 D (2mg/l) + Kin (0.5mg/l) was the best to get better and early callus induction frequency. Contrastingly, the medium MS + 2,4 D (2.5 mg/l) showed late and less callus initiation. Increasing the 2,4 D in the media also increased the days to callus initiation (Table 3). Instead of using 2, 4 D alone, addition of Kin 0.5 to (1mg/l) along with 2,4 D recorded significant effect on callus formation. These results were in agreement with that of Rashid *et al.* (2001). Zhang (2005) and Selvaraj *et al.* (2007) also showed 2, 4 D in combination with kinetin was more effective in producing embryogenic callus.

The maximum callus induction frequency was observed in the MS + 2,4 D (2 mg/l) + Kin (0.5mg/l) (59.97 per cent) irrespective of all the genotypes except ADT 44, ADT (R) 48, TNAU Rice ADT 49 and ADT (R) 45 followed by MS + 2,4 D (2 mg/l) (33.54 per cent) and MS+ 2,4 D (1.5 mg/l) + Kin (0.5 mg/l) (31.32 per cent). Increasing the 2,4 D concentration, the callus induction frequency also reduced and beyond 2.5 mg/ l there was very low per cent callus induction. Among the ten

varieties studied ADT 37 (64.44 per cent) performed better followed by ADT(R) 47 (57.77 per cent) and ADT 43 (47.77) (Table 4).

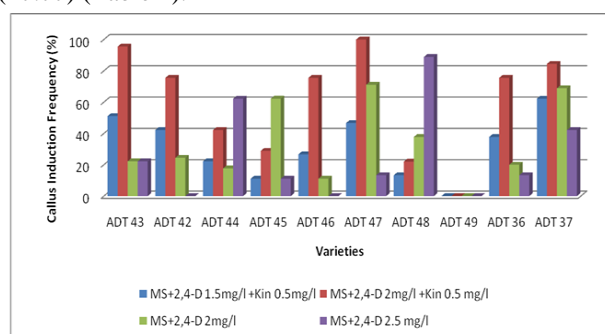


Fig.2. Callus Induction Frequency

The results indicated that, among the different varieties tested the varieties *viz.*, ADT 37, ADT 43 and ADT (R) 47 responded better for callus induction than the others ADT 44, ADT (R)48, TNAU Rice ADT 49 and ADT (R)45. The genotype TNAU Rice ADT 49 had very low per cent of callus induction frequency. Such a poor response of TNAU Rice ADT 49 may be attributed with the explanation that the pedigree of this variety *i.e* the parents CR 1009 (derivative of Punkaj x Jagannath) and Jeeraga samba (a land race) are *indica* types which is generally a poor respondent for tissue culture.

Among the four combination, MS + 2,4 D 2 mg/l + Kin 0.5 mg/l responded well in most of the genotypes. The genotype ADT (R) 47 produces 100 per cent calli in this combination followed by ADT 43 (95.55 per cent). ADT (R) 47 variety is a progeny derived from ADT 43 x Jeeragasamba. Similarly ADT 43 has the parentage of IR 50 x I.W. Ponni. Though all the parents are categorized in *indica* group, these varieties have responded better for callus induction and frequency contradicting the inference for poor response for TNAU Rice ADT 49 variety. However I.W.Ponni is a variety which possess a better regeneration potential as reported by Sridevi *et al.*, 2005 could have contributed for the callus induction ability to ADT 43 and ADT (R) 47 variety. The callus induction frequency of other genotypes are ADT (R) 48 (88.88 per cent), ADT 37 (84.44 per cent), ADT 42 (75.55) ADT 36 and ADT (R) 46 (75.33 per cent) ADT (R) 45 (62.22 per cent), ADT 44 (62.21). The genotype TNAU Rice ADT 49 did not give any response in this combination.

Among the ten genotypes ADT (R) 48 weighed upto 2 g of callus in MS+2,4 D (2.5 ml) combination after subculture followed by ADT 37 which was recorded upto 1 g of callus in MS +2,4 D (2 ml) + Kin (0.5 ml) and ADT 43 weighed 0.463 g in some combination (Table 6). The produced calli of convenient size were then transferred on MS medium supplemented with different combinations of 2,4 D showed marked differences for morphogenesis of plated calli. Some of the calli formed only roots without shoots, but others produced both roots and shoots. All these types of morphogenesis were regarded as organogenesis. Results indicated that calli derived from all the combinations showed reasonable potentiality for organogenesis (80% - 90%). Among the rice genotypes

ADT 43 only produces shoot in MS + 1.5mg/l BAP+1.5 mg/l Kin+0.5 mg/l NAA media composition. The varieties ADT (R) 46, ADT (R) 47, ADT (R) 48, ADT 37 have produced only the green spots in the above combination. The frequency of green spots are ADT (R) 46 (50 per cent) ADT 37 (75 per cent) ADT (R)47(75 per cent) ADT (R) 48 (30 per cent) ADT 43 (67 per cent).

The green islets were found only when 30 days old callus was transferred to the regeneration medium. Significant effect was found between the medium, variety and interaction of medium variety. Similar interaction pattern was also reported by Agarwal et al., 2006.

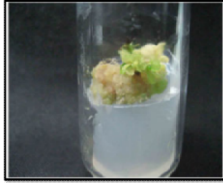


Fig.3. Green islets from callus

Although relatively high frequency of callus was observed in this study as compared to the report of other workers, the overall regeneration was quite low. It was observed that green plant regeneration ability of planted calli depended on the genotypes and the callus induction media. Islam et al (2005) also confirmed these results in their experiments.

The low responding variety TNAU Rice ADT 49 needs to try with some other growth hormones for the callus induction. The better responsive rice genotypes like ADT 37, ADT (R) 47 and ADT 43 and the medium MS+ 2,4,D (2mg/l) + Kin (0.5mg/l) would offer great promise for the induction of higher level of desirable somaclones and quality of callus for various means of genetic transformation and other studies for improving this world's staple food crop.

Table 2: Analysis of Variance

Source	Df	MS	F
Total	119	898.71	38.76**
Treatment	39	2694.68	116.24**
G	9	3776.35	162.90**
C	3	7069.98	304.97**
GC	27	1847.97	79.71**

** Significant at 1% level

Table 3: Number of days to callus induction

Genotype	C 1	C2	C3	C4	Mean
ADT 36	7	5	8	N.R	5
ADT 37	5	3	4	6	4.5
ADT 42	5	4	5	7	5.25
ADT 43	4	4	5	5	4.5
ADT 44	8	6	7	N.R	5.25
ADT (R) 45	8	6	5	N.R	4.75
ADT (R) 46	7	5	8	N.R	5
ADT (R) 47	4	3	4	5	4
ADT (R) 48	6	4	5	3	4.5
TNAU Rice ADT49	N.R	N.R	N.R	N.R	-
Mean	5.4	4	5.1	2.6	4.2

N.R. – No response

Table 4: The visual observations on callus induction in different media after 15-25 days

Genotype	C1	C2	C3	C4
ADT 36	Non embryogenic white callus	Compact yellowish white callus	Very small white callus	Yellowish brown callus
ADT 37	Compact, yellowish white callus induction	Embryogenic, Creamy white, friable callus	Soft, non Embryogenic yellowish callus	Soft, non Embryogenic yellowish Callus
ADT 42	Soft,non Embryogenic yellowish Callus	Embryogenic, Creamy white, friable callus	Very small white callus	Yellowish brown callus
ADT 43	Compact, yellowish white	Embryogenic,	Soft, non Embryogenic	Yellowish brown

	callus induction	Creamy white, friable callus	yellowish Callus	callus
ADT 44	Very small white callus	Very small white callus	Embrogenic compact callus	No response
ADT (R) 45	Soft, non Embryogenic yellowish Callus	Very small white callus	Embryogenic, Creamy white, friable callus	Very small white callus
ADT (R) 46	Very small white callus	Excellent compact callus	Very small white callus	No response
ADT (R) 47	Compact, yellowish white callus induction	Embryogenic, Creamy white, friable callus	Soft, non Embryogenic yellowish callus	Yellowish, very small size of callus
ADT (R) 48	Soft, non Embryogenic yellowish Callus	Soft, Embryogenic yellowish Callus	Soft, non Embryogenic yellowish callus	Embryogenic, Creamy white, friable callus
TNAU Rice ADT49	No response	No response	No response	No response

Table 4: Callus induction frequency

Genotype	MS+2,4-D 1.5mg/l+Kin 0.5mg/l	MS+2,4-D 2mg/l +Kin 0.5 mg/l	MS+2,4-D 2mg/l	MS+2,4-D 2.5 mg/l	Mean
ADT 43	51.10f (45.6)	95.53ab (79.7)	22.22ijk (28.0)	22.21ijk (27.8)	47.77 (45.3)
ADT 42	42.21gh (40.4)	75.55d (60.4)	24.44ijk (29.5)	0.00n (0.7)	35.55 (32.8)
ADT 44	22.21ijk (27.8)	42.21gh (40.4)	17.66klm (24.5)	62.21e (52.1)	36.07 (36.2)
ADT 45	11.10m (19.2)	28.88i (32.3)	62.22e (52.0)	11.10m (19.2)	28.07 (30.7)
ADT 46	26.66ij (31.0)	75.55d (60.4)	11.10m (19.2)	0.00n (0.7)	28.33 (30.7)
ADT 47	46.66fg (43.0)	100.00a (89.2)	71.10d (57.5)	13.33lm (21.40)	57.77 (52.8)
ADT 48	13.33lm (21.4)	21.99ijk (27.7)	37.77h (37.9)	88.88bc (71.1)	40.49 (39.5)
ADT 49	0.00n (0.7)	0.00n (0.7)	0.00n (0.7)	0.00n (0.7)	0.00 (0.7)
ADT 36	37.77h (37.9)	75.55d (60.40)	20.00ijk (26.5)	13.33lm (21.4)	36.66 (36.5)
ADT 37	62.21e (52.1)	84.44c (67.3)	68.88de (56.1)	42.21gh (40.4)	64.44 (54.0)
Mean	31.32 (31.9)	59.97 (51.8)	33.54 (33.2)	25.33 (25.9)	37.54 (35.6)

	SED	CD (0.05)	CD (0.01)
G	1.96	3.91	5.19
C	1.24	2.47	3.28
GC	3.93	7.82	10.38

* Values in parenthesis indicate the transformed arc sine values

* The mean having the same letter following is not significantly different at 0.01 probability level by Least Significant Difference Test (LSD)

Table 6: Weight of the callus 18-25 days

Variety	Media	Weight of the callus		
		18 days	20 days	25 days
ADT 37	MS+2,4 D 2 mg/l +Kin 0.5 mg/l	0.207 g	0.758 g	1 g
ADT 42	MS+2,4 D 2 mg/l +Kin 0.5 mg/l	0.070 g	0.187 g	0.430 g
ADT 43	MS+2,4 D 2 mg/l +Kin 0.5 mg/l	0.080g	0.192 g	0.464 g
ADT (R) 45	MS+ 2,4 D 2 mg/l	0.025 g	0.090 g	0.208 g
ADT (R) 46	MS+2,4 D 2 mg/l +Kin 0.5 mg/l	0.057 g	0.184 g	0.306 g
ADT (R) 47	MS+2,4 D 2 mg/l +Kin 0.5 mg/l	0.060 g	0.095 g	0.285 g
ADT (R) 48	MS+2,4 D 2.5 mg/l	0.272 g	0.437 g	2.005 g

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