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Research Article

Callus induction and regeneration in selected *indica* genotypes of rice (*Oryza sativa* L.)

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Abstract
Plumule of four rice varieties *viz*., TRY 2, TRY 3, ADT 53 and CO 52 were studied for callus induction and regeneration using different concentrations and combinations of plant growth regulators in MS medium. The combination of MS medium with 2,4-D (2.0 mg/l) + Kn (0.5 mg/l) showed maximum callus induction frequency in all four varieties ranged from 93.33 to 76.67 compared to other growth regulator combinations. Upon subculture of the callus, MS medium with NAA (0.5 mg/l) + BAP (2.0 mg/l) was found to be superior for greening of calli and regeneration of shoots, followed by MS + NAA (0.5 mg/l) + BAP (1.5 mg/l). Among the four varieties TRY 2 and TRY 3 showed positive responses for regeneration.

Key words: Rice, *In vitro* culture, Plumule culture, Callus induction and regeneration.

INTRODUCTION

Variations in plant genotypes can be created through different approaches like induced mutations, hybridization and recombination, *in vitro* methods of culturing plant cells to produce somaclonal variants and latest by genome editing technologies. *In vitro* techniques have different outcomes based on the objective of the study. Culturing of callus from explants for prolonged periods induces somaclonal variations (Leva *et al.,* 2012). These novel variations can be utilized to screen for abiotic and biotic stresses (Ranghoo-Sanmukhiya, 2021). In addition, the embryogenic calli are highly suitable for the genetic transformation and regeneration of transgenic plants. Thus, the proliferation of embryogenic calli and regeneration of calli is inevitable for the successful development of transformed plants. Embryogenic calli are induced from different types of explants *viz.,* matured seed (Aananthi *et al.,* 2010; Kumar and Ajinder, 2013; Utharasu and Anandakumar, 2014), leaf segments (Ramesh *et al.,* 2009), root tips (Mandal *et al.,* 2003), immature embryo (Nouri-Delawar *et al.,* 2001) and anthers (Rahman *et al.,* 2021). In general, induction of embryogenic calli from mature embryos and transformation of calli is effective. Year around availability of mature seeds for callus induction is the main advantage over other explants. Callus induction and regeneration potential are influenced by the type of explants, its genotype, growth media, hormone combinations and culture conditions. Many studies have been conducted to elucidate the standardization of callus induction and regeneration of different genotypes (Ge *et al*., 2006; Shanthi *et al*., 2010, Abiramasundari *et al.,* 2014).

The present study was aimed to find out the potentiality of selected rice genotypes for callus induction from plumules obtained from germinated seeds and to identify the suitable plant growth regulator combinations for callus induction and regeneration.

MATERIALS AND METHODS

Four genotypes of rice *viz*. TRY 2 (Short Duration - 115 days, Medium slender white rice), TRY 3 (Medium Duration - 135 days, Medium bold white grain), ADT 53 (Short Duration - 115 days, Medium slender white rice) and CO 52 (Medium Duration - 135 days, Medium slender white rice) were obtained from Department of Rice, Tamil Nadu Agricultural University, Coimbatore. The explant used for callus induction was plumule removed from germinated seeds of four rice genotypes

Matured disease-free and healthy seeds of rice genotypes were treated with 0.2% Carbendazim, 50 % WP (Teja of Agastya Pvt. Ltd.) for 12 hrs. and washed with sterile distilled water thrice to remove fungicidal residues. Seeds were dried to remove excess moisture. Dried seeds were manually dehusked without damaging the embryo. The dehusked seeds were immersed in 70% ethanol (v/v) for 3 min. followed by 0.1% mercuric chloride for 5 min. and washed with sterile distilled water once and then treated in 2% (v/v) sodium hypochlorite with few drops of tween-20 for 15 min. The treated seeds were thoroughly washed thrice with sterilised distilled water and allowed to dry for some time in aseptic conditions to remove excess moisture. Eight to ten days after inoculation of surface sterilized seeds in MS basal medium, the germinated seeds were taken out and the plumule was dissected out in a laminar airflow chamber using a scalpel and used as explant for callus induction.

The callus induction media used were MS basal with 2,4-D at four levels *viz*., 1.0 mg/l, 1.5 mg/l, 2.0 mg/l and 2.5 mg/l either alone or along with kinetin at three levels *viz.,* 0.0 mg/l, 0.5 mg/l and 1.0 mg/l. The explant inoculated test tubes were kept in the culture room in total darkness at 24ºC. Days to callus induction and callus initiation frequency was observed at 7-12 days, while the colour and texture of calli were recorded at 30 days after inoculation of explants. The calli obtained were subjected to stereo microscope visualization to study their morphological features. For each treatment, 20 explants were used. The same media was used for sub-culturing at 15 days.

The media used for regeneration were MS basal with NAA at two levels *viz.,* 0.5 mg/l and 1.0 mg/l either alone or along with BAP at five levels *viz*. 0.0 mg/l, 0.5 mg/l, 1.0 mg/l, 1.5 mg/l and 2.0 mg/l**.** Callus inoculated test tubes were incubated for 16h/8h (light/dark) at 24ºC. The callus weight, proliferation rate, regeneration response, colour and texture of calli were recorded at 30 days after inoculation. For each treatment, 20 calli were used. The percentage of shoot regeneration was calculated based on the number of calli showing shoot regeneration excluding the number of contaminated calli.

Per cent values of data were transformed with arcsine transformation and data analyzed using the STAR (Statistical Analysis for Agricultural Research) computer package. The level of significance (P value at 5% level) was determined using the standard ANOVA (Panse and Sukhatme, 1964).

RESULTS AND DISCUSSION

The response of four *indica* rice varieties in different callus induction media is shown in Table 1. Callus induction frequency varied from 13.33 to 93.33 per cent in TRY 2, 10.00 to 90.00 per cent in TRY 3, 13.30 to 83.33 per cent in ADT 53 and 10.00 to 76.67 per cent in CO 52 across the different combinations of auxin and cytokinin ratios. Among them, the maximum callus induction was recorded in basal MS media supplemented with 2,4-D (2.0 mg/l) + Kn (0.5 mg/l) and the variety TRY 2 had a higher response for callus induction. Shanthi *et al.* (2010) were induced TRY 2 callus in MS media supplemented with 2,4-D (2.0 mg/l) + Kn (0.5 mg/l) for *in vitro* salinity screening. In CO 51 rice variety, 81 to 94 per cent of callus induction rate was observed by Shweta *et al.* (2020) in NB media supplemented with 2,4-D (2.0 mg/l) + Kn (0.5 mg/l). Similar significant differences in callus induction among genotypes were also reported by Vennapusa *et al.* (2015) in AC 39020 and Upadhyaya *et al.* (2015) in three rice cultivars *viz.,* Sita, Rupali, Masuri. It was observed that the callus induction frequency increased with increasing concentration of 2,4-D and decreasing kinetin concentration.

The maximum callusing efficiency of selected rice varieties in the descending order was 93.33 per cent (TRY 2), 90.00 per cent (TRY 3), 83.33 per cent (ADT 53) and 76.67 per cent (CO 52). The proliferation was higher in all four varieties in the combination MS basal + 2,4-D (2.0 mg/l) + Kn(0.5mg/l) compared to others. Thus in the present study, for all the four varieties, MS Basal media supplemented with 2,4-D (2.0 mg/l) + Kn (0.5 mg/l) was found to be effective for both callus induction and proliferation. Abiramasundari *et al.* (2014) reported similar findings with ADT varieties of rice. Rima *et al.* (2020) found 2,4-D as the effective auxin source for callus induction and proliferation.

It was observed that the callus induction frequency increased with the increasing concentration of 2,4-D and decreasing kinetin concentration. Further increase in 2,4-D concentration from 2.0 mg/l, reduced the callus induction frequency which was also noticed by Jaseela *et al.* (2009). Decrease of 2,4-D concentration from 2.0 mg/l led to rhizogenesis in the callus induction medium itself as also observed by Shanthi *et al.*, (2010). In lower concentrations of 2,4-D, callus induction was poor and with higher concentration, necrosis occurred and the calli were not suitable for subculturing.

In the present investigation, TRY 2 and TRY 3 showed callus initiation in 7 to 10 days, ADT 53 in 8 to 12 days and CO 52 in 10 to 12 days. Bano *et al.* (2005) and

Table 1. Response of plumule of four rice genotypes for callus induction in MS + combinations of 2,4-D and kn

S. No.	Variety	PGR combn.: MS +			Response for callus initiation			Nature of callus, 30 days after inoculation		
		Treat No.	2,4-D (mg/l)	Kn (mg/)	Callus induction frequency (%)		Days taken for callus initiation	Proliferation rate	Colour of callus	Texture of callus
1.	TRY 2	T1	1.0	0.0	26.67efg	(31.0)	7-10	Low	Pale yellow	Friable dry
2.		T2	1.0	0.5	20.00fg	(26.7)	7-10	Low	Pale yellow	Friable dry
3.		T3	1.0	1.0	13.33g	(21.1)	7-10	Very Low	Brownish yellow	Friable dry
4.		T4	1.5	0.0	53.33cde	(46.9)	7-10	Medium	Pale yellow	Friable dry
5.		T5	1.5	0.5	60.00bcd	(50.9)	7-10	Medium	Pale yellow	Friable dry
6.		T6	1.5	1.0	43.33def	(41.1)	7-10	Low	Creamy yellow	Friable dry
7.		T7	2.0	0.0	80.00ab	(63.9)	7-10	High	Creamy yellow	Friable dry
8.		T8	2.0	0.5	93.33a	(77.7)	7-10	High	Pale yellow	Friable dry
9.		T9	2.0	1.0	70.00bcd	(57.0)	7-10	Low	Pale yellow	Friable dry
10.		T10	2.5	0.0	76.67bc	(61.2)	7-10	High	Creamy yellow	Compact dry
11.		T11	2.5	0.5	83.33ab	(66.1)	7-10	High	Brownish yellow	Compact dry
12.		T12	2.5	1.0	26.67efg	(31.0)	7-10	Low	Dark yellow	Compact dry
				Mean: 53.89		CD 12.31				
13.	TRY 3	T1	1.0	0.0	31.67ef	(34.2)	7-10	Low	Pale yellow	Friable dry
14.		T2	1.0	0.5	23.33fg	(28.8)	7-10	Low	Pale yellow	Friable dry
15.		T3	1.0	1.0	11.67g	(19.9)	7-10	Very Low	Brownish yellow	Friable dry
16.		T4	1.5	0.0	56.67c	(48.9)	7-10	Medium	Pale yellow	Friable dry
17.		T5	1.5	0.5	53.33cd	(46.9)	7-10	Medium	Pale yellow	Friable dry
18.		T6	1.5	1.0	41.67ade	(40.2)	7-10	Low	Creamy yellow	Friable dry
19.		T7	2.0	0.0	83.33ab	(66.1)	7-10	High	Creamy yellow	Friable dry
20.		T8	2.0	0.5	90.00a	(71.6)	7-10	High	Pale yellow	Friable dry
21.		T9	2.0	1.0	53.33cd	(46.9)	7-10	Low	Pale yellow	Friable dry
22.		T10	2.5	0.0	76.67b	(61.2)	7-10	High	Creamy yellow	Compact dry
23.		T11	2.5	0.5	83.33ab	(66.1)	7-10	High	Brownish yellow	Compact dry
24.		T12	2.5	1.0	36.67def	(37.2)	7-10	Low	Dark yellow	Compact dry
				Mean: 53.47		CD: 9.32				
25.	ADT 53	T1	1.0	0.0	16.67d	(23.8)	8-12	Low	Pale yellow	Compact dry
26.		T2	1.0	0.5	16.67d	(23.8)	8-12	Low	Pale yellow	Compact dry
27.		T3	1.0	1.0	13.33d	(21.1)	8-12	Very Low	Brownish yellow	Compact dry
28.		T4	1.5	0.0	43.33bc	(41.0)	8-12	Medium	Pale yellow	Compact dry
29.		T5	1.5	0.5	40.00c	(39.1)	8-12	Medium	Pale yellow	Compact dry
30.		T6	1.5	1.0	33.33cd	(35.2)	8-12	Low	Creamy yellow	Compact dry
31.		T7	2.0	0.0	66.67ab	(54.8)	8-12	High	Creamy yellow	Compact dry
32.		T8	2.0	0.5	83.33a	(66.1)	8-12	High	Pale yellow	Compact dry
33.		T9	2.0	1.0	40.00c	(39.2)	8-12	Low	Pale yellow	Compact dry
34.		T10	2.5	0.0	70.00a	(57.0)	8-12	High	Creamy yellow	Compact dry
35.		T11	2.5	0.5	76.67a	(61.2)	8-12	High	Brownish yellow	Compact dry
36.		T12	2.5	1.0	30.00cd	(33.0)	8-12	Low	Dark yellow	Compact dry
				Mean : 44.17		CD: 11.67				
37.	CO 52	T1	1.0	0.0	20.00cde	(26.6)	10-12	Low	Pale yellow	Compact wet
38.		T2	1.0	0.5	16.67de	(23.9)	10-12	Low	Pale yellow	Compact wet
39.		T3	1.0	1.0	10.00e	(18.4)	10-12	Very Low	Brownish yellow	Compact wet
40.		T4	1.5	0.0	36.67c	(37.2)	10-12	Medium	Pale yellow	Compact wet
41.		T5	1.5	0.5	40.00bc	(39.1)	10-12	Medium	Pale yellow	Compact wet
42.		T6	1.5	1.0	26.67cd	(31.0)	10-12	Low	Creamy yellow	Compact wet
43.		T7	2.0	0.0	63.33a	(52.8)	10-12	High	Creamy yellow	Compact wet
44.		T8	2.0	0.5	76.67a	(61.2)	10-12	High	Pale yellow	Compact wet
45.		T9	2.0	1.0	36.67c	(37.1)	10-12	Low	Pale yellow	Compact wet
46.		T10	2.5	0.0	60.00ab	(50.9)	10-12	High	Creamy yellow	Compact wet
47.		T11	2.5	0.5	66.67a	(54.8)	10-12	High	Brownish yellow	Compact wet
48.		T12	2.5	1.0	23.33cde	(28.8)	10-12	Low	Dark yellow	Compact wet
				Mean: 39.72		CD: 10.42				

Figures in parenthesis are arc sine transformed values

Table 2. Response of callus induced in the Callus induction medium 2,4-D (2.0 mg/l) + Kn (0.5 mg/l) from plumule of four rice varieties during subculture in MS medium with different combinations of NAA and BAP

S. No.	Variety	Growth regulator combination in subculture medium: MS +		Callus wt.(mg) at 30 days	Nature of callus and response for regeneration 30 days after subculture		
		NAA(mg/l)	BAP(mg/l)		Proliferation rate, Colour and Texture of callus	Regeneration response	Per cent of shoot regeneration
1.	TRY 2	0.5	0.0	55	Very Low, Brownish yellow, Friable dry	-	-
2.		0.5	0.5	53	Very Low, Brownish yellow, Friable dry	-	-
3.		0.5	1.0	52	Very Low, Pale yellow, Friable dry	-	-
4.		0.5	1.5	92	High, Light green, Friable dry	Caulogenesis	5.5
5.		0.5	2.0	94	High, Light green, Friable dry	Caulogenesis	10.0
6.		1.0	0.0	56	Very Low, Brownish yellow, Friable dry	-	-
7.		1.0	0.5	48	Very Low, Brownish yellow, Friable dry	Rhizogenesis	15.0
8.		1.0	1.0	70	Low, Dark yellow, Friable dry	Rhizogenesis	21.0
9.		1.0	1.5	79	Medium, light green, Friable dry	-	-
10.		1.0	2.0	80	Medium, light green, Friable dry	-	-
11.	TRY 3	0.5	0.0	49	Very Low, Brownish yellow, Friable dry	-	-
12.		0.5	0.5	50	Very Low, Brownish yellow, Friable dry	-	-
13.		0.5	1.0	53	Very Low, Pale yellow, Friable dry	-	-
14.		0.5	1.5	88	High, Light green, Friable dry	Caulogenesis	5.0
15.		0.5	2.0	85	High, Light green, Friable dry	Caulogenesis	5.2
16.		1.0	0.0	49	Very Low, Brownish yellow, Friable dry	-	-
17.		1.0	0.5	48	Very Low , Brownish yellow, Friable dry	Rhizogenesis	15.7
18.		1.0	1.0	66	Low, Dark yellow, Friable dry	Rhizogenesis	10.5
19.		1.0	1.5	76	Medium, light green, Friable dry	-	-
20.		1.0	2.0	74	Medium, light green, Friable dry	-	-

Kumari *et al.* (2016) reported callus after 7 - 12 days of inoculation from the cut regions of plumules. Tripathy (2021) observed callus induction after nine days in the Khandairi rice cultivar. Ilahi *et al.* (2005) reported callus formation from the scutellar portion of rice. Callus formation was observed in germ pore of seed by Rima *et al.* (2020).

The texture of callus was friable and dry in TRY 2 and TRY 3, compact and dry in ADT 53 and compact and wet in CO 52 after 30 days of inoculation. The combination 2,4-D (2.0 mg/l) + Kn (0.5 mg/l) that gave the highest frequency of callus induction showed pale yellow colour callus in TRY 2 (**Fig. 1A**) and TRY 3 (**Fig. 1B**), yellowish white in ADT 53 (**Fig 1C**) and brownish yellow in CO 52 (**Fig. 1D**). The growth regulators 2,4-D and Kn in equal concentrations produced brownish yellow to dark coloured callus with root formation exhibiting a low level of callus initiation frequency. Among four varieties, TRY 2 and TRY 3 produced prominent sized friable calli, pale yellow in colour. In general, the colour of callus ranged from cream yellow to brownish yellow. Rima *et al.* (2020) reported that the colour variations are genotype dependent.

Callus induction response to different combinations of growth regulators in terms of fresh weight varied from one variety to another (**Table 1**). The size of the calli at MS Basal media + 2,4-D@ 1.0 to 2.0 mg/l was considerable while at a higher concentration of 2,4-D (> 2.0 mg/l), size reduction was noticed. Such size differences in different concentrations were also reported by Chung, (1975) and Abe and Futsufara, (1984).

The calli induced from plumule were cultured in different combinations of growth regulators and the results are furnished in **Table 2**. Caulogenesis was successful in the MS medium with NAA (0.5 mg/l) + BAP (2.0 mg/l). Ahmad *et al.* (2016) reported that Panderas, a Malaysian rice variety produced a higher regeneration rate in MS media with NAA (0.5 mg/l) + BAP (2.0 mg/l) (**Fig 2A and 2B)**. The same concentration of hormonal combination NAA and BAP was also successful for Tripathy (2021) in the Chittimuthyalu cultivar of rice at 30% sucrose concentration. The maximum green columnar projections were observed in regeneration media with NAA (0.5 mg/l) and BAP (2.0 mg/l) by Biswas and Mandal (2007).

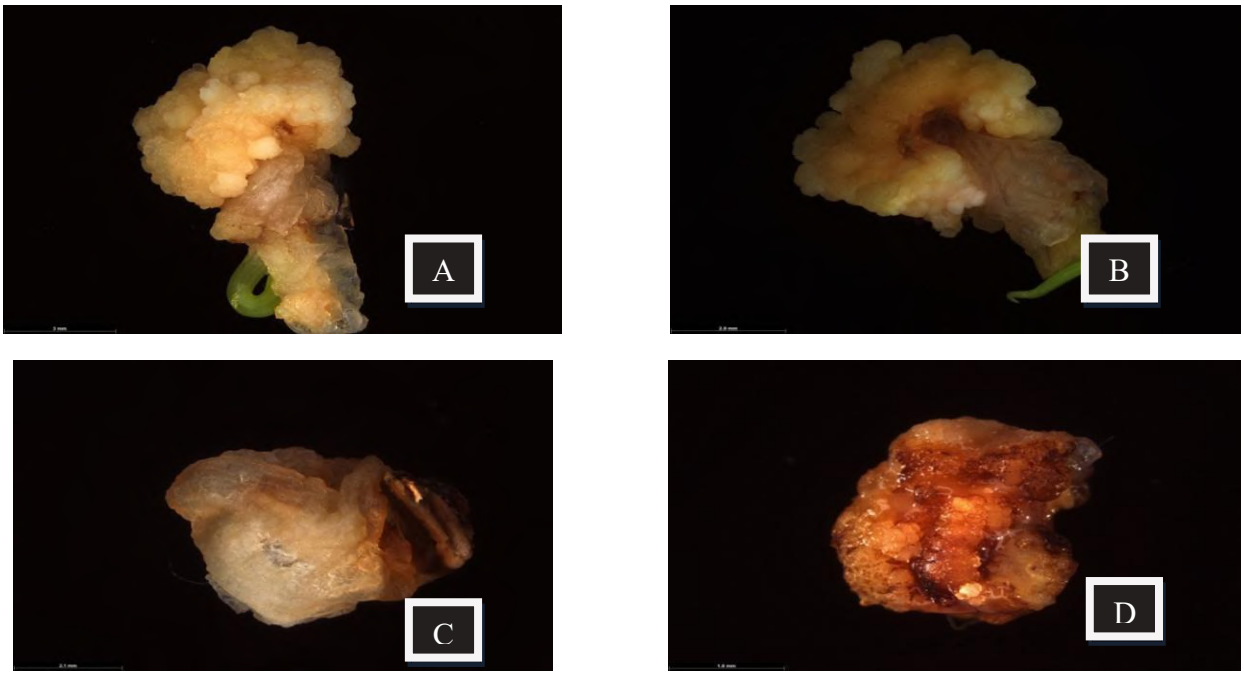

Fig. 1. Nature of callus induced from 2,4-D (2.0 mg/l) + Kn (0.5 mg/l) of four rice varieties

1A: TRY 2 – Pale yellow in colour, friable and dry, 1B: TRY 3 – Pale yellow in colour, friable and dry , 1C: ADT 53 - Pale yellow in colour, compact and dry, 1D: CO 52 – Pale yellow in colour, friable and wet .

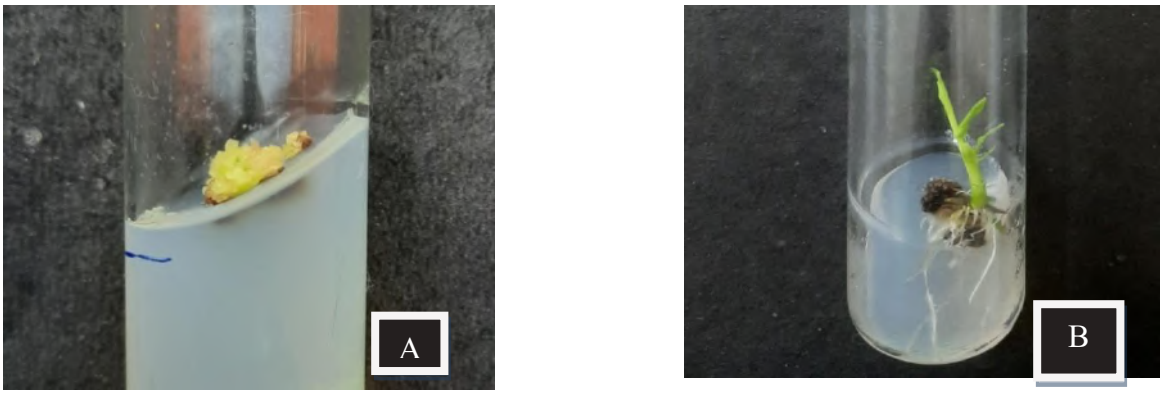

Fig. 2. Greening of callus in TRY 2 in regeneration media MS + NAA (0.5 mg/l) + BAP (2.0 mg/l)

2A: TRY 2 – Light green in colour, friable and dry nature of callus,
2B: TRY 2 – caulogenesis from TRY 2 callus in regeneration media MS + NAA (0.5 mg/l) + BAP (2.0 mg/l).

Media containing more than 2.0 mg/l of NAA resulted in browning of callus. Gasper (1996) reported that a high concentration of NAA inhibited the role of cytokinin and affected the shoot induction frequency. Shanthi *et al.* (2010) reported that TRY 2 rice variety had 63 per cent of regeneration frequency in MS + Kn ($5mM^{-1}$) + BAP ($5mM^{-1}$) + NAA ($0.5mM^{-1}$). The occurrence of green regions in callus was observed in media supplemented with lower levels of NAA and higher levels of BAP. High concentrations of auxin lead to the development of root

primordium. Karthikeyan *et al.* (2009) reported that ADT 43 showed higher regeneration in NAA (1.5 mg/l) + BAP (1.0mg/l). Abiramasundari *et al.* (2014) reported that different ADT varieties of rice showed green spots in MS + BAP (1.5 mg/l) + Kn (1.5 mg/l) + NAA (0.5 mg/l).

To conclude, all four rice varieties showed the maximum level of callus induction in MS media supplemented with 2,4-D (2.0 mg/l) + Kn (0.5 mg/l). Calli of TRY 2 and TRY 3 showed regeneration of shoots in MS media supplemented with NAA (0.5 mg/l) and BAP (2.0 mg/l). These two responding varieties may be used for the isolation of somaclones for further improvement of these varieties.

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