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Plant regeneration in different genotypes of *indica* rice

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Of 10 indica rice varieties assessed, Annada showed the best *in vitro* culture response in respect of callus induction, plantlet regeneration and number of plantlets/seed callus. Other high yielding varieties, viz. Kasturi, IR 72 and Karnal Local, also displayed appreciable performance. Callus induced and proliferated in dark and gave high plantlet regeneration and more plantlets/seed callus across genotypes. Among three shock treatments, viz. heat (37°C for overnight), cold (4°C for over night) and dehydration, dehydration significantly enhanced the plantlet regeneration and number of plantlets/seed callus. Of 3-dehydration treatments (6, 12 and 24 h duration), the maximum plantlet regeneration and number of plantlets/seed callus were observed in the calli dehydrated for 12 h. Of three tested media, viz. MS, LS, and N_6, MS emerged the best for callus induction, plantlet regeneration and number of plantlets/seed callus. In contrast, among the modified media formulations, modified N6 [5 m*M* $(NH_4)_2$ SO_4] produced maximum plantlets/seed callus across varieties. Increased (5 m*M*) $(NH_4)_2SO_4$ content in the medium was found to be better than the higher dose of KNO_3 or its normal level in the respective media formulations. Across genotypes, 2 mg L^{-1} 2,4-D in the callus induction medium (CIM) was the most suitable for callus induction. However, 2,4-D in combination with other growth regulators, such as 2 mg L^{-1} 2,4-D and 1 mg L^{-1} Kn, performed better. In regeneration medium (RM), 2 mg L^{-1} BAP with 1 mg L^{-1} Kn and 0.5 mg L^{-1} NAA, or 2 mg L^{-1} BAP with 0.5 mg L^{-1} NAA, were optimum for the maximum plantlet regeneration. Across varieties, coconut water (10%) was found to be the most effective to enhance the *in vitro* culture response as evident from prolific callus induction, increased plantlet regeneration and more plantlets/seed callus.

Keywords: Genotypes, rice**,** media, adjuvant, hormones
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Introduction

Generally, callus development in rice under *in vitro* culture system occurs on suitable media from mature and immature explants. However, somatic embryogenesis in rice has been reported from leaf tissues[1], inflorescences[2], mature and immature embryos[3] and protoplasts[4]. It is noted that *japonica* varieties are more responsive to *in vitro* culture and produce more plantlets than *indica* varieties[5]. Many factors, viz. genotype, explants, media composition, carbohydrate source, types of growth regulators used, gelling agents, *p*H and *in vitro* physical environment, are crucial in governing plantlet regeneration. Genotypes and explant sources are key factors in determining the success of rice plant regeneration in culture[6-8] .

In the present investigation, efforts have been made to identify suitable *indica* varieties and to optimize the physico-chemical environments for maximum callus induction and plantlet regeneration for routine use in developing rice transgenics and in related works.

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Materials and Methods

Genotype

To assess the influence of different genotypes in governing *in vitro* culture response and plantlet regeneration, initially 10 *indica* rice varieties, viz., Annada, Kasturi, Taichung Sen Yu, Bhabani, MTL 13, IR 72, Nanjing 630563, IR 43, RP 1442-22-3-5 and Karnal Local, obtained from International Rice Research Institute (IRRI), Philippines and Directorate of Rice Research (DRR), India, were used. One *in vitro* culture-responsive *japonica* variety Taipei 309 was used as check.

Somatic Embryogenesis and Plant Regeneration

Mature healthy seeds were dehusked manually and immersed in 5% aqueous Teepol solution. Seeds were then surface sterilized with 0.1% mercuric chloride for 10 min under laminar airflow (LAF) and washed thrice with sterile distilled water. In each

variety, 200 axenic seeds were cultured on callus induction medium (CIM), containing Murashige and Skoog (MS) medium[9] (*p*H 5.8) supplemented with 2 mg L^{-1} 2, 4-D and 0.5 mg L^{-1} Kn. The cultures were incubated in dark at 25±2°C. Ten-d-old calli were excised out from the scutellar surface of the germinating seedlings and placed onto callus maintenance medium (CMM) consisting of MS with half dose of 2,4-D of CIM (1 mg L^{-1} 2,4-D and 0.25 mg L^{-1} Kn). Calli from individual seeds were maintained separately. After two passages on CMM (15 d each), calli were separated into two groups. One set was shifted to embryogenic induction medium (EIM), made up of MS with 0.1% casein hydrolysate (CH), 1 mg L^{-1} Kn and 1 mg L^{-1} abscissic acid (ABA)[10], and other half was shifted onto regeneration medium (RM). The target was to select *in vitro* responsive high yielding varieties (HYVs) to develop sufficient somatic embryos. After 7 d of culture, white, dry surfaced friable calli containing somatic embryos were shifted onto RM consisting of MS with 2 mg L^{-1} BAP, 1 mg L^{-1} Kn and 0.5 mg L^{-1} NAA. The cultures on RM were kept under 16/8 h light/dark cycle (130 μE m^{-2} s^{-1}).

Vigorously rooted plants of 8-10 cm height, after taking out from culture tubes, were transplanted into soil in small plastic pots covered with polyethylene bags for 4-5 d to reduce evapo-transpiration loss. Established plantlets were subsequently shifted to soil in big cement pots and grown to maturity. Seeds from individual plants were harvested, threshed and kept separately for raising the next generation crop.

Photoperiod

For the purpose of callus induction, axenic seeds of the varieties were grouped into two sets. One set was kept under 16 h/8 h light/dark regime and other in dark. Ten-d-old calli regenerated from both the culture conditions were excised and subcultured only under 16 h/8 h light/dark period.

Abiotic Stress

The calli were pre-treated for dehydration (6 h/12 h/24 h), cold incubation (4°C overnight) or heat shock (37°C overnight). Before transferring onto RM, dehydration of calli was done under LAF, while heat and cold treatments were given in incubator. Pre-treated calli were shifted onto RM for plantlet regeneration.

Media

Dehuskd seeds were cultured on MS (19 m*M* KNO_3), modified MS (35 m*M* KNO_3), LS (19 m*M* KNO_3), modified LS (35 m*M* KNO_3), N_6 (28 m*M* KNO_3) and modified N_6 [5 m*M* $(NH_4)_2SO_4$] medium. Axenic seeds were shifted onto different CIM, viz. MS, LS and N_6 media and their modified formulations, as mentioned above, each supplemented with 2 mg L^{-1} 2,4-D and 0.5 mg L^{-1} Kn. Subcultured calli were shifted to RM consisting of basal media, as used for CIM, with 2 mg L^{-1} BAP, 1 mg L^{-1} Kn, and 0.5 mg L^{-1} NAA.

Phytohormone

For callus induction, axenic seeds were cultured on MS medium enriched with different growth regulators. Different concentrations (1.0, 2.0, 3.0 & 4.0 mg L^{-1}) of 2,4-D was tried singly. Kn/BAP and NAA (0.5 to 2.0 mg L^{-1}) separately or in combination were also used with 2 mg L^{-1} 2,4-D in CIM. Thus, 12 profiles of hormonal combinations were constituted to initiate and promote callus induction. After two subcultures on CMM, subcultured calli were shifted onto RM involving 9 different combinations of treatments consisting of BAP+NAA/Kn+NAA/BAP+Kn+NAA (0.5 to 2.0 mg L^{-1}).

Adjunvants

Axenic seeds were cultured onto CIM containing MS medium with 2 mg L^{-1} 2,4-D and different concentrations of adjuvants, viz. casein hydrolysate (CH) (100, 200 or 300 mg L^{-1}), yeast extract (YE) (100, 200 or 300 mg L^{-1}) and coconut water (CW) (5, 10 or 15%). Calli of individual seeds were maintained separately and shifted to RM.

Results and Discussion

Experiments were conducted to optimize somatic tissue culture protocol in rice with special emphasis in modulating physical factors, chemical composition of the medium and additives. Appropriate media manipulation for maximum callus induction, callus proliferation and healthy plantlet regeneration is immensely important to develop an efficient somaculture system. Steps involving somatic tissue culture in *indica* rice with mature seed embryo have been depicted in Fig. 1 (A-G).

Genotype

In majority of the *indica* rice varieties, mature seed embryos started sprouting after 3-4 d upon culturing. The junction between radicle and

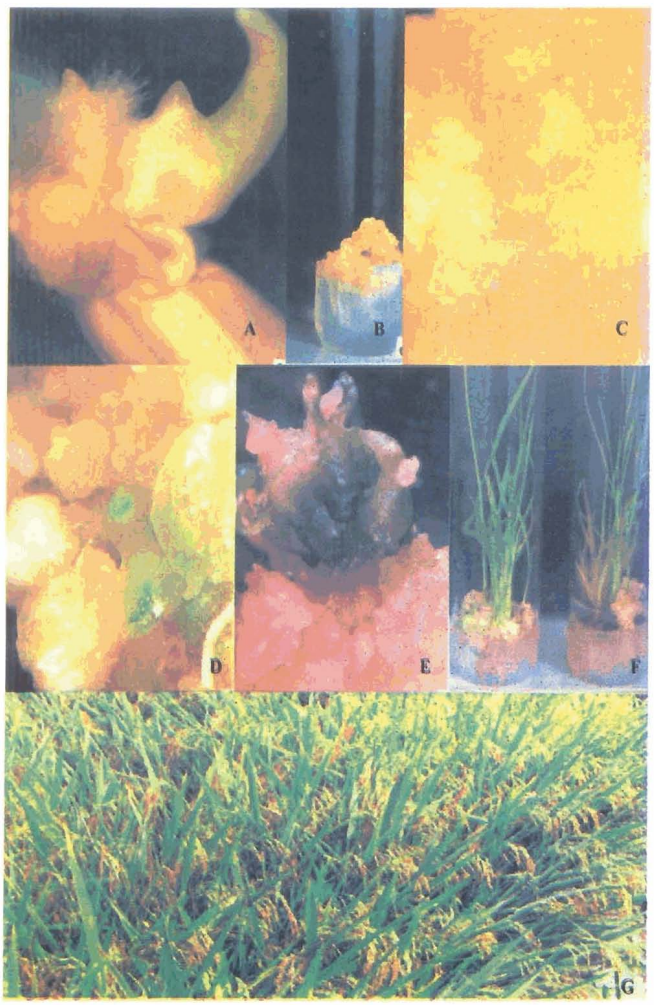

Fig. 1(a-g) — Somatic tissue culture in *indica* rice involving mature seed embryos: a) germinating seedling showing callus at the junction of radicle and mesocotyl; b) proliferating calli after 15 d of subculture on CIM; c) stereomicroscopic view of embryogenic calli; d) calli on regeneration medium showing green spots; e) emerging plantlets on regeneration medium; f) regenerating plantlets in culture tubes; and g) *in vitro* culture derived plantlets at maturity under glass house condition.

mesocotyl swelled up within 5-6 d and appreciable amount of calli developed after 10-12 d. Primary calli mass were white and globular comprising numerous proembryos and embryo initials. Stereomicroscopic observations showed that such calli were consisted of embryogenic cells (whitish, compact, dry surfaced, friable, cytoplasm rich) and non-embryogenic cells (yellowish, vacuolated, elongated, tubular) as observed earlier in wild rice[11].

Callus formation rate was reported to be a recessive character and controlled by a single block of gene[12]. This is a prime parameter to be judged critically while developing an efficient *in vitro* culture system[13]. Among the different combinations used for EIM in the present study, the maximum callus induction among the *indica* varieties was observed in Annada, followed by Kasturi; while IR 43 showed the minimum callus induction. However, the overall maximum callus induction was observed in the *japonica* variety Taipei 309 (Table 1). Most calli were yellowish white or bright white indicating the presence of embryogenic cells to a considerable extent. A very good callus health was observed in all the varieties. When the calli were directly transferred onto RM after two subcultures, the maximum plantlet regeneration among *indica* varieties was observed again in Annada, followed by Kasturi and Bhabani. Again, Taipei 309 showed the highest plantlet regeneration among all the varieties. Further, when calli were passaged through EIM, Annada among *indica* varieties showed the maximum plantlet regeneration, followed by Kasturi; while Taipei 309 showed the highest plantlet regeneration among all the varieties.

Plantlet regeneration is a genotype specific character. Callus induction and plantlet regeneration were found more in the *japonica* variety than the best *indica* variety. In the present study, it was

Table 1 — Effect of genotypes and embroygenic induction medium (EIM) on somatic embryogenesis and plantlet regeneration in selected *indica* rice varieties

Genotype	Callus induction (%)	Control		Calli enrouted through EIM	
		Plantlet regeneration (%)	No. plantlets/seed callus	Plantlet regeneration (%)	No. of plantlets/seed callus
Annada	95.4	75.4	6.7	93.3	8.2
Kasturi	92.4	70.1	5.9	90.0	8.0
Bhabani	82.1	71.0	4.5	77.7	7.1
Karnal Local	88.1	58.6	3.4	76.1	7.5
Taipei 309*	96.9	80.1	7.0	97.5	8.4

**Japonica* variety (check)

observed that the number of plantlets regeneration/seed callus was more when calli were enrouted through EIM rather than direct regeneration from seed callus, which was supported by earlier evidences[14]. In both the cases, the *japonica* variety performed better than the best *indica* performer, Annada. Further, preregeneration treatment with ABA and CH was reported to increase embryogenesis of callus and more plantlet regeneration[15].

In vitro culture response is the manifestation of interactions among physiological state of the explants, genotypes, culture environment and ecogeographic origin of the genotypes. Since the cultured plant cells are cytologically heterogenous owing to polysomatic nature of the initial explants, extent and nature of genetic changes that often take place under *in vitro* culture condition vary greatly[16,17]. Therefore, culture response in individual varieties was found to be optimum under a particular nutritional and physical milieu. Precisely, it seems that the process of dedifferentiation and redifferentiation involves activation and/or suppression of several metabolic pathways.

Among all the *indica* varieties, Annada excelled in respect of callus induction, plantlet regeneration as well as in number of plantlets/seed calli. Kasturi, Karnal, IR 72 were other *indica* varieties that performed substantially good and hold promise in future works in relation to genetic transformation. The afore-said four varieties also possess high yielding ability with considerable pest and disease resistance and are cultivated on large-scale by small and marginal farmers.

Photoperiod

Under both dark and light/dark (16 h/8 h) conditions, the maximum callus induction, plantlet regeneration and number of plantlets/seed callus were observed in Annada, followed by Kasturi. IR 72 and Karnal Local also performed substantially well in terms of above parameters. However, *japonica* variety Taipei 309 performed better than the *indica* varieties alike previous performances (Table 2).

Seed germination, callus induction, plantlet regeneration and number of plantlet regeneration per seed callus were more in dark as compared to light/dark regime in case of all the varieties tested, irrespective of their *indica* or *japonica* origin. This observation was supported earlier by scientific studies performed in rice[18] and *Jatropha curcas*[19]. Generally, callus proliferation is enhanced in dark and light tends to promote embryogenesis, shooting and greening of callus. Explants are frequently established under 500-1000 lux illuminations following a 16 h photoperiod. High light intensities (5000-10,000 lux), amply used to promote leaf development and enhanced photosynthetic turnover, often result in more plantlet regeneration. Some investigators have reported more dependence of photoperiodism on photosynthesis than for supply of carbohydrates. In spite of these factors, plantlet regeneration and number of plantlets/seed callus were reduced under light, which might be due to altered hormonal activity.

Table 2 — Effect of light and dark in influencing *in vitro* culture response in *indica* rice

Culture condition	Genotype	Callus induction (%)	Plantlet regeneration (%)	No. of plantlets/seed callus
Dark	Annada	96.00	100.00	8.60
	Kasturi	90.52	85.02	7.62
	IR72	87.62	83.30	5.09
	Karnal Local	85.22	80.04	6.56
	Taipei 309*	98.00	100.00	8.90
Light/dark (16 h/8 h)	Annada	85.55	78.57	7.10
	Kasturi	81.87	67.61	6.22
	IR72	76.47	60.00	4.40
	Karnal Local	80.00	60.23	5.42
	Taipei 309*	90.10	86.00	7.23

**Japonica* variety (check)

Abiotic Stress

Dehydration of callus on filter paper was reported to produce somatic embryos capable of regenerating vigorously growing plants[20], while partial dehydration of rice callus was reported to increase shoot regeneration 3-folds. Across physical treatments among *indica* genotypes, maximum number of calli were transformed into plantlet in terms of percentage. The highest number of plantlet regeneration from calli was observed in Annada when these were dehydrated for 12 h. Interestingly, the plantlet regeneration and number of plantlets/seed callus were found to be less in the *japonica* variety than in *indica* varieties (Table 3). This observation was supported by an earlier study where dehydration treatment was found to be more conducive in influencing parameters related to *in vitro* culture response and plantlet regeneration in *indica* varieties than in *japonica*[21]. It was also

Table 3 — Effect of cold and heat shocks in affecting *in vitro* culture response of *indica* and *japonica* rice

Genotype	Culture condition	Plantlet regeneration (%)	No. of plantlets/seed callus
Annada (96.73)#	6 h dehydration	90.54	7.62
	12 h dehydration	98.02*	9.21*
	24 h dehydration	83.03	8.21
	37°C incubation (overnight)	14.28	2.52
	4°C incubation (overnight	48.57	3.53
	Control	90.00	7.07
Kasturi (94.68)#	6 h dehydration	74.28	7.62
	12 h dehydration	96.78*	8.00
	24 h dehydration	81.42	8.21*
	37°C incubation (overnight)	30.00	2.52
	4°C incubation (overnight	40.04	3.53
	Control	73.52	7.54
	Control	71.50	6.08
Karnal Local (89.44)#	6 h dehydration	70.03	5.20
	12 h dehydration	90.00*	7.57*
	24 h dehydration	72.04	5.56
	37°C incubation (overnight)	20.02	2.06
	4°C incubation (overnight	35.03	3.30
	Control	69.00	6.05
Taipei 309 (*japonica* check) (98.45)#	6 h dehydration	90.57	7.80
	12 h dehydration	95.82*	7.84*
	24 h dehydration	80.02	4.08
	37°C incubation (overnight)	30.05	3.21
	4°C incubation (overnight	44.43	3.82
	Control	90.08	7.62

#% callus induction
*Best performance of genotypes with regard to *in vitro* culture responses

reported that, across genotypes, mean plantlet regeneration and mean numbers of plantlets/seed callus were maximum in case of dehydration for 12 h. Observations showed that pre-plantlet regeneration shock with dehydration was far more encouraging than with cold and heat shocks.

Across varieties, MS or modified MS was found to be most suitable among all the media for *in vitro* culture. As discussed earlier, phytohormonal profile used for callus induction was 2 mg L^{-1} 2,4-D and 0.5 mg L^{-1} Kn. Keeping the hormonal profile constant, Annada was found to be the best among *indica* varieties in respect of callus induction on modified MS, followed by Kasturi. The *japonica* variety, however, performed little better without much significant difference in callus induction under the same hormonal regime. Among all the rice varieties tested, increased NO_3 or its replacement with NH_4 in MS, LS and N_6 medium enhanced the callus induction. Although callus induction was more in medium supplemented with KNO_3, callus health was poor.

The hormonal regime 2 mg L^{-1} BAP, 1 mg L^{-1} Kn and 0.5 mg L^{-1} NAA along with the media or their modified form was tested for the purpose of higher plantlet regeneration and to get maximum number of plants per callus. In this profile, Annada showed the maximum plantlet regeneration on modified N_6, followed by Kasturi. Number of plantlets/seed callus was observed to be the maximum in Annada on MS, followed by Kasturi on modified N_6. In this context, the *japonica* variety performed better in terms of callus induction, plantlet regeneration and number of plantlets/seed callus but all in different media (Table 4).

In the present study, it was observed that plantlet regeneration was reduced across varieties in presence of high KNO_3 (35 m*M*), whereas increased $(NH_4)_2SO_4$ (5 m*M*) in N_6 medium enhanced the plantlet regeneration, which was supported by earlier studies[22]. Differential *in vitro* cell and tissue culture response in rice is chiefly attributed to the type of reduced nitrogen used. In case of finger millet also, high NH_4 was reported to enhance the germination of somatic embryos and regeneration of plantlets from callus[23].

Phytohormone

Earlier[24] as well as in the present study, it was observed that callus induction was hindered in the absence of 2,4-D when Kn, NAA and BAP were used singly or in combination. Maximum callus induction across varieties was observed in Annada (98.94%) when MS medium was supplemented with 2 mg L^{-1} 2,4-D and 0.5 mg L^{-1} Kn in the CIM. In Kasturi, maximum (93.68%) callus was induced with two hormonal combinations, viz. 3 mg L^{-1} 2,4-D or 2 mg L^{-1} 2,4-D with 1 mg L^{-1} NAA. Among *indica* cultivars, Annada showed cent per cent plantlet regeneration, followed by Kasturi (93.32%) when callus was induced on 1 mg L^{-1} 2,4-D (Table 5). Annada also showed maximum plantlets/seed callus across all the varieties from those calli which were induced on 2 mg L^{-1} 2,4-D, 0.5 mg L^{-1} BAP and 1 mg L^{-1} NAA. Hormonal treatments across varieties showed better performance (60.14-95.26%) in respect of callus induction on 3 mg L^{-1} 2,4-D. Plantlet regeneration across varieties was maximum on 2 mg L^{-1} 2,4-D with a range of 80.60-100%. Furthermore, number of plantlets/seed callus was found to be performed better on CIM consisting of 2 mg L^{-1} 2,4-D among the varieties tested. Effect of different hormones on callus induction was genotype specific[25] and their levels in the medium bear indirect effect on plantlet regeneration[26]. In wild rice, 1.5-2 mg L^{-1} 2,4-D was reported to be the optimum concentration for production of maximum embryogenic callus[27]. It was further reported that 2,4-D (2 & 3 mg L^{-1}) enhanced the callus induction but calli did not differentiate into green plantlets in rice[28]. When 2,4-D used singly in CIM, better performance was displayed in respect of callus induction in rice[29] and wheat[30].

Overall, 3 mg L^{-1} 2,4-D was found to be the optimal dose in callus induction for all the varieties tested in the present study. However, callus induced on 2 mg L^{-1} 2,4-D showed maximum plantlets/seed callus. So, 2-3 mg L^{-1} 2,4-D could be recommended for efficient somaculture involving mature somatic embryos for the set of varieties used in the present study.

Table 4 — Effect of different media in facilitating somatic tissue culture in *indica* and *japonica* rice

Genotype	Medium	Callus induction (%)	Plantlet regeneration (%)	Av. No. of plantlets/ seed callus
Annada (HYV indica)	MS	94.50	85.02	8.32*
	MS (Modified)	96.59	45.40	2.81
	LS	93.50	84.61	6.07
	LS (Modified)	94.10	42.82	3.05
	N_6	92.30	85.00	7.40
	N_6 (Modified)	95.21	93.34	8.22*
Kasturi (mild scented *indica)*	MS	90.50	80.09	7.22
	MS (Modified)	94.50	54.56	3.12
	LS	72.74	66.67	4.80
	LS (Modified)	88.71	22.21	3.05
	N_6	80.00	72.00	6.70
	N_6 (Modified)	85.41	88.32	8.23*
Taipei 309 (*Japonica* check	MS	96.50	88.01	9.00*
	MS (Modified)	97.00	52.04	4.23
	LS	64.00	65.00	5.31
	LS (Modified)	78.06	46.02	4.50
	N_6	92.50	80.03	7.84
	N_6 (Modified)	95.50	94.04	8.60*

*Medium induces maximum *in vitro* culture response

Hormones in Regeneration Medium

Nine combinations consisting various concentrations of NAA, BAP and Kn were tried with MS

Table 5 — Effect of diverse growth regulators in callus induction and plantlet regeneration medium to govern *in vitro* culture response in *indica* and *japonica* rice (only best two performance given)

Genotype	Callus induction (%)	Plantlet regeneration (%)	No. of plantlets/seed callus
Annada	98.94 (e)*; 95.26 (c)	100.00 (a)*; 95.08 (b)	10.00(f)* ;9.02(b)
Kasturi	93.68 (e)*; 93.68* (h)*	93.32 (a)*; 87.70 (l)	10.00 (k)*; 9.65(l)
IR 72	81.92 (g)*; 71.42 (h)	85.04 (i)*;83.04 (a)	6.80(i)*; 6.75(f)
Karnal Local	89.50 (c)*; 82.94 (h)	85.06 (f)*; 85.02 (a)	7.08(h)*; 6.67(f)
Taipei 309 (*japonica* check)	98.00 (b)*; 90.00 (c)	100.00 (b)*; 98.02 (i)	10.14(b)*; 8.82(h)

Hormonal profile: (a) MS+1 mg L^{-1} 2-4 D; (b) MS+2 mg L^{-1} 2-4 D; (c) MS+3 mg L^{-1} 2-4D; (d) MS+4 mg L^{-1} 2-4 D; (e) MS+2 mg L^{-1} 2-4 D+0.5 mg L^{-1} Kn; (f) MS+2 mg L^{-1} 2-4 D+1 mg L^{-1} Kn;(g) MS+2 mg L^{-1} 2-4 D+0.5 mg L^{-1} NAA; (h) MS+2 mg L^{-1} 2-4 D+1 mg L^{-1} NAA; (i) MS+2 mg L^{-1} 2-4 D+0.5 mg L^{-1} Kn+0.5 mg L^{-1} NAA; (j) MS+2 mg L^{-1} 2-4 D+0.5 mg L^{-1} Kn+1 mg L^{-1} NAA; (k) MS+2 mg L^{-1} 2-4 D+0.5 mg L^{-1} BAP+0.5 mg L^{-1} NAA; (l) MS+2, mg L^{-1} 2-4 D+0.5 mg L^{-1} BAP+1 mg L^{-1} NAA.
*indicates best performance of genotypes with regard to *in vitro* culture response

medium as RM across rice varieties. Annada showed the maximum plantlet regeneration (96.7%) and plantlets/seed callus (10.06) on RM containing 2 mg L^{-1} BAP+0.5 mg L^{-1} NAA across the treatments. Cent per cent plantlet regeneration and a maximum 11.32 plantlets/seed callus was observed in Kasturi on RM containing 2 mg L^{-1} BAP+1 mg L^{-1} Kn+0.5 mg L^{-1} NAA. In IR 72 and Karnal Local, however, hormonal profile involved in the maximum plantlet regeneration and highest number of plantlets/seed callus were different. In the *japonica* variety, 2 mg L^{-1} BAP+1 mg^{-1} Kn+0.5 mg L^{-1} NAA showed cent per cent plantlet regeneration with a maximum 10.51 plantlets/seed callus. Across varieties, plantlet regeneration and plantlets/seed callus was found to be better on MS with 2 mg L^{-1} BAP, 1 mg L^{-1} Kn and 0.5 mg L^{-1} NAA. Among the 9 different combinations of hormonal profiles tried, best three combinations of RM in each variety has been highlighted in Table 6.

In cereals, high plantlet regeneration and development of more plantlets/seed callus largely depend upon medium and the type and quantum of growth regulators used. From earlier study, it was observed that, of the two cytokinins (BAP & Kn), BAP in combination with auxin (0.5 mg L^{-1} NAA) produced more plantlets than Kn among the rice varieties[31]. It was also reported that MS medium with 1.5 to 2 mg L^{-1} BAP was suitable for higher plantlet regeneration in rice[32]. However, in the present study, 2 mg L^{-1} BAP+1 mg L^{-1} Kn+0.5 mg L^{-1} NAA and 2 mg L^{-1} BAP+0.5 mg Ll^{-1} NAA emerged as the best hormonal combination, which may be used to develop efficient somatic tissue culture in rice for high plantlet regeneration.

Adjuvants

In the present study, all the varieties showed maximum plantlet regeneration and maximum number of plantlets/seed callus when 10% CW was used in CIM and RM. As for as varieties are concerned, the maximum callus induction, plantlet regeneration and plantlets/seed callus were observed in Annada, followed by Kasturi, Karnal Local and IR 72 on MS medium with 2 mg L^{-1} 2,4-D and RM containing 2 mg L^{-1} BAP, 1 mg L^{-1} Kn and 0.5 mg L^{-1} NAA. Across varieties, the maximum callus induction (85.0-97.5%) was observed on MS+2 mg L^{-1} 2,4-D with 10% CW. Plantlet regeneration was maximum on RM containing MS+2 mg L^{-1} BAP+1 mg L^{-1} Kn+0.5 mg L^{-1} NAA with 10% CW. The same profile also produced highest plantlets/seed callus (Table 7).

Of three adjuvants used, viz., CW, CH and YE, in the present study, CW was found to be best for use in CIM and RM, while CH showed poor performance. Probably, CH singly does not

Table 6—Effect of regeneration media in influencing somatic tissue culture in *indica* and *japonica* rice

Genotype	Hormone used in RM	Plantlet regeneration (%)	No. of plantlet/seed callus
Annada	**2 mg L^{-1} BAP+0.5 mg L^{-1} NAA**	**96.70**	10.06
	1 mg L^{-1} BAP+1 mg L^{-1} Kn+0.5 mg L^{-1} NAA	90.02	9.00
	2 mg L^{-1} BAP+ 1 mg L^{-1} Kn+0.5 mg L^{-1} NAA	90.01	9.52
Kasturi	1 mg L^{-1} BAP+1 mg L^{-1} Kn+0.5 mg L^{-1} NAA	90.02	7.30
	2 mg L^{-1} BAP+ 1 mg L^{-1} Kn+0.5 mg L^{-1} NAA	**100.00**	11.32
	2 mg L^{-1} Kn+1 mg L^{-1} BAP+0.5 mg L^{-1} NAA	90.60	8.05
IR 72	1 mg L^{-1} BAP+0.5 mg L^{-1} NAA	82.82	7.62
	2 mg L^{-1} BAP+ 1 mg L^{-1} Kn+0.5 mg L^{-1} NAA	**86.68**	7.45
	2 mg L^{-1} Kn+1 mg L^{-1} BAP+0.5 mg L^{-1} NAA	85.26	6.10
Karnal Local	2 mg L^{-1} BAP+0.5 mg L^{-1} NAA	88.52	7.30
	2 mg L^{-1} Kn+0.5 mg L^{-1} NAA	**96.50**	6.54
	2 mg L^{-1} BAP+ 1 mg L^{-1} Kn+0.5 mg L^{-1} NAA	80.02	6.04
Taipei 309 (japonica *check*)	1 mg L^{-1} BAP+0.5 mg L^{-1} NAA	80.04	8.04
	2 mg L^{-1} BAP+0.5 mg L^{-1} NAA	96.62	7.52
	2 mg L^{-1} BAP+ 1 mg L^{-1} Kn+0.5 mg L^{-1} NAA	**100.00**	10.51

Best three combinations for each variety are presented

Table 7 — Effect of coconut water (10%) in callus induction (MS+2 mg L^{-1} 2,4-D) and regeneration media (MS+2 mg L^{-1} BAP+1 mg L^{-1} Kn+0.5 mg L^{-1} NAA) in influencing somatic tissue culture response in *indica* rice

Genotype	Callus induction (%)	Plantlet regeneration (%)	No. of plantlets/ seed callus
Annada	95.85	92.52	8.65
Kasturi	96.50	90.02	8.32
IR 72	85.00	88.02	7.80
Karnal Local	94.02	87.05	8.10
Taipei 309*	97.50	94.05	9.82

**Japonica* variety (check)

substantially influence the somatic embryogenesis. However, when CH used in combination with ABA, embryo formation[10] and plantlet regeneration[15] were enhanced. It was reported that green plantlet regeneration was enhanced by using diverse supplements like complex growth substances, viz. CH and/or YE, in addition to 2,4-D[7]. Other supplements like tryptophan,[13,33] proline[24,34] and polyamines, such as spermidine[35], were used with varying degree of success. Elaborate experiments involving more adjuvants singly and in combination with more varieties would be logical to draw a generalized conclusion for *indica* varieties in respect of callus induction, plantlet regeneration and to number of plantlets/seed callus.

In short, it may be concluded that, callus proliferation, plantlet regeneration and number of plantlets/seed callus were more in dark. Further, subcultured calli dehydrated for 12 h before shifting onto RM showed maximum plantlet regeneration and more number of plantlets/seed callus. Among the media tested, MS was the best medium in respect of callus induction, plantlet regeneration and number of plantlets/seed callus. But, in case of modified media, N_6 (modified) showed maximum plantlets/seed callus. It was also observed that high $(NH_4)_2 SO_4$ content in the medium was promising than standard or increased dose of KNO_3 as present in original media formulations. On the other hand, 2 mg L^{-1} 2, 4-D was found to be most suitable for callus formation. However, RM supplemented with 2 mg L^{-1} BAP, 1 mg L^{-1} Kn and 0.5 mg L^{-1} NAA was most encouraging for plantlet regeneration. As adjuvant, 10% CW was found to be most effective for abundant callus induction, plantlet regeneration and number of plantlets/ seed callus.

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