Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(1): 2683-2690
Received: 19-11-2017
Accepted: 20-12-2017

GB Sawant
Ph. D. scholar, College of Agriculture, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Ratnagiri, Maharashtra, India

SG Bhave
Director of Extension Education, College of Agriculture, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Ratnagiri, Maharashtra, India

SV Sawardekar
Incharge, Plant Biotechnology Centre, Dr. BSKKV, Dapoli, Maharashtra, India

SM Jadhav
Ph. D. scholar, College of Agriculture, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Ratnagiri, Maharashtra, India

Correspondence
GB Sawant
Ph. D. scholar, College of Agriculture, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Ratnagiri, Maharashtra, India

Regeneration potential of scutellum-derived calli from different *Indica* rice (*Oryza Sativa* L.) varieties

GB Sawant, SG Bhave, SV Sawardekar and SM Jadhav

Abstract
The present investigation was carried out to standardize efficient and reliable regeneration protocol in scutellum-derived rice calli from 22 rice varieties developed by Dr. B.S.K.K.V., Dapoli. All the varieties with all the media combinations and their interactions significantly affected the regeneration potential of the calli. The variety Ratnagiri-711 showed earliest shoot induction (11.67 days) among the all on medium MS +3.0 mg/l BAP +1.0 mg/l IAA showing highest shoot induction frequency (87.50%) and maximum number of shoots per callus (7.67). It rooted also earlier in 3.67 days after transferring the shoots to rooting medium 1/2 MS +0.5 mg/l IBA +0.1 mg/l BAP and shown highest root induction frequency (73.33%) with maximum roots per shoot (11.33). For hardening, potting mixture of soil, organic manure and cocopit in 1:1:1 proportion showed highest survival of *In Vitro* grown plantlets (73.33%). No morphogenesis was observed on only MS medium without growth regulators.

Keywords: regeneration, shoot and root induction, BAP, hardening

Introduction
Rice (2n=2x=24) is the staple food for more than one third of world's population belonging to the family Poaceae [1]. Therefore, it is of utmost importance to augment the productivity of rice to cope with the increased threat of population boom. The production and productivity of rice suffers significantly because of biotic and abiotic stresses. Recent advancement in biotechnology, such as transformation and *In Situ* and *In Vitro* hybridization has enhanced the introgression of new genes from different sources to the cultivated species [2]. Efficient plant regeneration from cultured cells and tissues constitutes the basis for producing transgenic crops. Plantlets regenerated through the embryogenic or organogenic pathway is well established in hundreds of plant species. Shoot meristems and embryo like structures are often observed in callus cultures, which eventually develop into whole plants. A basic regulatory mechanism underlying organized development involves a balance between auxins and cytokinins. Many factors that affect shoot regeneration involve genotype [3], exogenous and endogenous hormones [4, 5], carbon sources [6] and osmotic requirements [5]. Mostly, indica rice varieties are known to be recalcitrant for tissue culture practices. Hence the present study was aimed at studying regeneration potential of calli from different indica rice varieties and identifying best regenerable varieties.

Material and Methods
Regeneration studies were carried out on calli from 22 rice varieties developed by Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli (Table 3). The calli of different rice varieties produced on previously standardized callus induction and proliferation media were cut into 0.5 cm pieces and inoculated on shooting media, MS [7] supplemented with different concentrations of growth regulators (Table 1). Observations on days to shoot induction, shoot induction frequency (%) and number of shoots per callus bit were recorded.
After development of multiple shoots, the plantlets were separated and transferred to the root induction media having different concentrations of growth regulators (Table 2). Observations were recorded on days to root induction, root induction frequency (%) and number of roots per shoot. The plantlets were then transferred to different potting mixtures for hardening (Table 9) and percent survival rate was observed. The experiment was carried out in 3 replicates each having 5 culture bottles under well-defined culture conditions maintained at 26± 2 °C temperature, uniform light (1000-2000 lux) over a light and dark cycle of 16:8 hours. Statistical analysis was carried out using Factorial-Completely Randomized Design (F-CRD).

Table 1: Media combinations used for shoot induction with various levels of BAP

Tr. No.	Media combinations
S_1	MS medium (control)
S_2	MS+0.5 mg/l BAP +0.2 mg/l IAA
S_3	MS+0.5 mg/l BAP +0.5 mg/l IAA
S_4	MS+0.5 mg/l BAP +1.0 mg/l IAA
S_5	MS+1.0 mg/l BAP +0.5 mg/l IAA
S_6	MS+1.5 mg/l BAP +1.0 mg/l IAA
S_7	MS+2.0 mg/l BAP +1.0 mg/l IAA
S_8	MS+2.5 mg/l BAP +1.0 mg/l IAA
S_9	MS+3.0 mg/l BAP +1.0 mg/l IAA
S_{10}	MS+3.5 mg/l BAP +1.0 mg/l IAA
S_{11}	MS+4.0 mg/l BAP +1.0 mg/l IAA

Table 2: Media combinations used for root induction

Tr. No.	Media combinations
R_1	MS medium (control)
R_2	MS+ 0.5 mg/l NAA
R_3	MS+ 1.0 mg/l NAA
R_4	MS+ 1.5 mg/l NAA
R_5	MS+ 2.0 mg/l NAA
R_6	MS+ 3.0 mg/l NAA
R_7	MS+1.0 mg/l NAA + 2.0 mg/l BAP
R_8	1/2 MS+ 0.2 mg/l IBA+ 0.1 mg/l BAP
R_9	1/2 MS+ 0.2 mg/l IBA+ 0.2 mg/l BAP
R_{10}	1/2 MS+ 0.5 mg/l IBA+ 0.1 mg/l BAP
R_{11}	1/2 MS+ 0.5 mg/l IBA+ 0.2 mg/l BAP

Results and Discussion

In present investigation, the regeneration potential of calli from different varieties were studied in terms of days required for shooting, shoot induction frequency (%), number of shoots per callus bit days to root induction, root induction frequency (%) and number of roots per shoot.

Days to shooting

A significant difference was observed for all the varieties, media combinations and their interactions for days to shoot induction (Table 3). The variety RTN-711 on medium MS+3.0 mg/l BAP +1.0 mg/l IAA required the least (11.67) days to shoot induction. Whereas the maximum days (32.33) were taken by variety PLG- 1 on medium MS +1.5 mg/l BAP +1.0 mg/l IAA. The media without growth regulators as well as media containing BAP up to only 1.0 mg/L were not responded for shoot induction.

These results are in similarity with the findings of Sah *et al.* [8] for a japonica rice Cv. Kitaake who observed the earliest shoot regeneration in 18 days on medium containing 3.0 mg/L BAP and 0.2 mg/l NAA. Whereas Gowda *et al.* [9] reported earlier response for shoot induction (32 days) in variety IR 20 than in Rasi (34 days) on an average among the treatments. Callus from both IR 20 and Rasi did not show morphogenesis on MS basal medium. Amrita Mukherjee *et al.*[10] also reported such a differential response for days to shoot induction with the earliest shoot induction (22.25 days) in variety BRRI dhan 29 on medium MS + 6 mgL^{-1} Kn + 0.5 mgL^{-1} NAA. This suggests that different levels of endogenous hormones among different varieties play role in exhibiting differential morphogenetic response.

Shoot induction frequency (%)

In present investigation, shoot induction frequency varied considerably over a wide range (Table 4). The variety RTN-711 on medium MS + 3.0 mg/l BAP +1.0 mg/l IAA and variety KJT- 8 on medium MS + 3.5 mg/l BAP +1.0 mg/l IAA recorded same and the highest shoot induction frequency of 87.50 % significantly different from other combinations. Whereas the lowest shoot induction frequency (20.83 %) was shown by varieties PLG- 1, KJT- 4 and PNL- 3 on MS media containing 1.5 or 2.0 mg/L BAP with 1.0 mg/L IAA. No shooting (0.00 %) was observed on media without growth regulators as well as media containing BAP up to only 1.0 mg/L. Hoque *et al.*[11] also reported such differential response for best regeneration frequency (75 %) as in Pakhibiroin at the concentration of NAA 0.5 mg/l + BA 2 mg/l + Kinetin 0.5 mg/l; in Hati Baromashi and Kacha Biroin at NAA 0.5 mg/l + BA 3 mg/l + Kinetin 0.5 mg/l while in BRRI Dhan53 at, MS + NAA 1 mg/l + BA 2 mg/l + Kinetin 3 mg/l. This significant interaction effect between variety and media combinations with different concentrations of growth regulators were also reported by a number of other workers [9, 10, 12-20].

However different concentrations of plant growth regulators were found efficient by different scientists for obtaining high shoot induction frequency in different varieties. Among them the BAP reported by majority of the workers mostly at a concentration of 2 mg/L [11, 15, 21].

The best concentration of BAP found in present investigation i.e. 3 mg/L is in exact conformity with the findings of Krishnan *et al.* [22], Sah *et al.* [8] and Haque *et al.* [11]. Several other concentrations were also reported as best *viz.* 0.5 mg/L [16, 17], 2.5 mg/L [13, 23] and also some higher concentrations as 4 mg/L [9, 24] and 5 mg/L [20, 25].

Some other scientists observed better results on other plant growth regulators *viz.* TDZ [12]; Kinetin [10, 11, 14, 23, 26] and 2,4-D [15, 16, 18, 19]. However, shoot regeneration by using medium supplemented with kinetin, was very low or even failed in varieties IR 64 and MTL 250 [21].

Combinations of auxin and cytokinin along with the effect of basal salts played an important role for plant regeneration [27, 28]. Wang *et al.* [29] also reported that high concentration of cytokinin and low of auxin promoted plantlet regeneration. In present investigation, IAA at a concentration of 1.0 mg/L in addition to BAP gave best regeneration. Our results are in agreement with Sripichitt and Cheewasestatham [30] and Gowda *et al.* [9] who used 1 mg/L IAA with 4 mg/L BAP and Kinetin, respectively. The interaction of auxin and cytokinin is important for *In Vitro* organogenesis [31]. In the present study cost effective media combinations are obtained for shoot induction.

Number of shoots per callus bit

Number of shoots per callus bit was significantly influenced by various varieties, media treatments as well as interaction between Variety × Media treatments (Table 5). The highest number of shoots per callus (7.67) was observed for variety RTN-711 on medium MS+3.0 mg/l BAP +1.0 mg/l IAA followed by both varieties viz. KJT- 8 and KJT- 2 on medium MS+3.5 mg/l BAP +1.0 mg/l IAA (7.33). The least number of shoots per callus (2.00) was observed with the varieties RTN-2, PLG- 1 and RTN- 5 on medium MS+1.5 mg/l BAP +1.0 mg/l IAA. Similar results were also evidenced by Muhammad *et al.* [13], Mannan *et al.* [15], Amrita *et al.* [10] and Sankepally and Singh [20].

Regarding the growth regulators, BAP could be preferred more than Kinetin as per the results that the range of 4-8 plantlets per callus found on 2.5 mg/L BAP by Muhammad *et al.* [13] in variety Basmati-371 whereas the maximum as 5.65 shoots per callus found by Amrita *et al.* [10] in variety BRRI dhan-29 on medium with 6 mg/L Kinetin. In present experiment, the concentrations of plant growth regulators have shown the same trend of effects as that in shoot induction frequency and confirmed the genotype specificity.

Root induction

Adventitious roots as a cereal crop in rice can be induced by transferring the callus to medium containing different ratios of auxin and cytokinin. Similar to that of shoot regeneration, root organogenesis is also dependent on type and concentration of growth regulators, type of media, strength and nature of media, photoperiod, genotype, etc.

Wijisekara *et al.* [32] observed that roots were regenerated on regeneration medium and special rooting treatments were not required. Sometimes regenerating calli maintained on hormone free medium to facilitate root growth. Lee *et al.* [28] also observed rooting of the embryogenic calli-derived shoots of different *japonica* rice cultivars on a hormone-free MS medium. This may be due to effect of callus induction media where concentration of auxin could be more. But in the present study, a significant difference was observed among varieties, media combinations and interaction of Variety × Media combinations for days to root induction (Table 6). The variety RTN- 711 on medium 1/2 MS+ 0.5 mg/l IBA+ 0.1 mg/l BAP recorded the minimum days to root induction (3.67 days) and found to be the best interaction. While the variety KJT- 8 on medium MS+2.0 mg/l NAA) recorded the maximum days to root induction (16.67 days). Artadana *et al.* [25] observed the regeneration initiated by root formation within the first week followed by green spots formation in a red rice cultivar Barak Cenana.

The root induction frequency significantly differed for all the varieties and media combinations but not for the interaction effects of Variety × Media combinations (Table 7). Although the effect of Variety × Medium interactions was found non-significant regarding the root induction frequency, a wide variation was observed among them ranging from 13.33 % to 73.33 %. The highest root induction frequency (73.33 %) was recorded by the variety RTN- 711 on medium 1/2 MS+ 0.5 mg/l IBA+ 0.1 mg/l BAP. Media combinations without any growth regulators and also having NAA concentration only from 0.5 to 1.5 mg/L were found with 0.00 % root induction. Such a differential response was also found by Gowda *et al.* [9], Amrita *et al.* [10] and Upadhyaya *et al.* [19] for different varieties.

The exact similarity of optimum concentrations of plant growth regulators in media combination was observed with the report by Amrita *et al.* [10] who also found the highest root initiation (97.50%) on MS + 0.5 mg/L IBA in BRRI dhan-29. Gowda *et al.* [9] observed better rooting with IAA concentration of 1.0 and even low at 0.25 mg/L in varieties Rasi and IR 20, respectively. However, several workers reported that rooting could be obtained on regeneration media favouring both shooting and rooting due to presence of auxins yet at lower quantities and special rooting treatments were not required [8, 14, 23, 32]. In present investigation, though difference was observed, it was not a wide among interactions for root induction frequency but if duration and quantity of rooting concerned along with frequency, the role of special rooting treatments seems to be important. Besides this, most of the workers have reported the reduction in MS salts strength in rooting media [8, 14, 23, 32].

The auxin IBA was found better than NAA for maximum roots per shoot in present experiment (Table 8) and also as per the reports by Gowda *et al.* [9] and Amrita Mukherjee *et al.* [10]. Whereas the media having 2.0 mg/L NAA and 1.0 mg/L Kinetin was reported as optimum by Libin *et al.* [26] in Sarawak rice variety Biris.

Table 3: Effect of varieties and media combinations on days to shoot induction in rice

Variety / Medium		S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}	S_{11}	Mean
V_1	RTN-73	0.00	0.00	0.00	0.00	0.00	24.00	23.00	18.33	14.33	17.00	21.67	10.76
V_2	KJT-184	0.00	0.00	0.00	0.00	0.00	23.67	22.67	20.00	17.00	21.00	24.67	11.73
V_3	RTN-24	0.00	0.00	0.00	0.00	0.00	25.00	22.33	19.00	16.67	18.67	24.33	11.45
V_4	RTN-711	0.00	0.00	0.00	0.00	0.00	13.33	14.00	13.00	11.67	12.00	14.33	7.12
V_5	RTN-1	0.00	0.00	0.00	0.00	0.00	24.67	23.00	20.33	16.67	19.67	22.67	11.55
V_6	KJT-3	0.00	0.00	0.00	0.00	0.00	21.67	18.67	14.00	16.00	20.00	22.33	10.24
V_7	KJT-4	0.00	0.00	0.00	0.00	0.00	24.67	23.33	18.67	15.67	21.33	26.00	11.79
V_8	PND-1	0.00	0.00	0.00	0.00	0.00	22.33	20.33	17.00	13.00	17.00	23.33	10.27
V_9	KJT-7	0.00	0.00	0.00	0.00	0.00	23.67	21.67	15.33	19.67	24.33	26.33	11.91
V_{10}	RTN-5	0.00	0.00	0.00	0.00	0.00	22.67	20.33	17.00	15.00	18.00	24.33	10.67
V_{11}	PLG-1	0.00	0.00	0.00	0.00	0.00	32.33	31.67	28.67	24.00	21.67	26.00	14.94
V_{12}	PLG-2	0.00	0.00	0.00	0.00	0.00	27.67	26.67	27.00	24.33	21.33	25.67	13.88
V_{13}	KJT-5	0.00	0.00	0.00	0.00	0.00	31.00	26.67	26.33	23.67	18.00	22.67	13.48
V_{14}	KJT-6	0.00	0.00	0.00	0.00	0.00	27.33	28.67	27.67	26.00	22.67	25.33	14.33
V_{15}	RTN-4	0.00	0.00	0.00	0.00	0.00	26.67	25.33	24.33	20.00	23.00	27.67	13.36
V_{16}	KJT-2	0.00	0.00	0.00	0.00	0.00	29.67	28.67	26.33	24.67	22.00	26.00	14.30
V_{17}	RTN-3	0.00	0.00	0.00	0.00	0.00	31.33	30.33	29.67	25.67	23.67	28.33	15.36
V_{18}	RTN-2	0.00	0.00	0.00	0.00	0.00	28.33	27.00	25.33	22.33	20.33	25.00	13.48
V_{19}	KJT-8	0.00	0.00	0.00	0.00	0.00	25.67	24.00	24.00	21.67	21.00	27.33	13.06
V_{20}	PNL-1	0.00	0.00	0.00	0.00	0.00	28.67	28.00	25.33	23.33	21.00	25.67	13.82
V_{21}	PNL-2	0.00	0.00	0.00	0.00	0.00	27.33	24.67	23.00	20.00	22.67	27.00	13.15
V_{22}	PNL-3	0.00	0.00	0.00	0.00	0.00	31.00	30.67	28.67	27.00	24.67	21.67	14.88
Range	Min	0.00	0.00	0.00	0.00	0.00	13.33	14.00	13.00	11.67	12.00	14.33	7.12
	Max	0.00	0.00	0.00	0.00	0.00	32.33	31.67	29.67	27.00	24.67	28.33	15.36
Mean		0.00	0.00	0.00	0.00	0.00	26.03	24.62	22.23	19.92	20.50	24.47	12.52

	Variety	Medium	Variety × Medium
SE m	0.16	0.11	0.53
CD at 1 %	0.58	0.41	1.94
Significant at 1 %	Sig	Sig	Sig

Table 4: Effect of varieties and media combinations on shoot induction frequency (%) in rice

Variety / Medium		S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}	S_{11}	Mean
V_1	RTN-73	0.00	0.00	0.00	0.00	0.00	33.33	45.83	41.67	79.17	54.17	33.33	26.14
		(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(34.49)	(42.59)	(40.00)	(63.10)	(47.59)	(35.17)	(23.90)
V_2	KJT-184	0.00	0.00	0.00	0.00	0.00	25.00	29.17	58.33	75.00	62.50	33.33	25.76
		(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(29.49)	(32.59)	(49.83)	(60.00)	(52.41)	(35.17)	(23.59)
V_3	RTN-24	0.00	0.00	0.00	0.00	0.00	25.00	58.33	54.17	70.83	62.50	45.83	28.79
		(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(29.49)	(49.83)	(47.59)	(57.41)	(52.41)	(42.59)	(25.39)
V_4	RTN-711	0.00	0.00	0.00	0.00	0.00	41.67	54.17	58.33	87.50	70.83	54.17	33.33
		(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(40.17)	(47.41)	(49.83)	(69.30)	(57.41)	(47.41)	(28.32)
V_5	RTN-1	0.00	0.00	0.00	0.00	0.00	33.33	58.33	66.67	79.17	50.00	33.33	29.17
		(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(35.17)	(49.83)	(54.83)	(63.10)	(45.00)	(35.00)	(25.72)
V_6	KJT-3	0.00	0.00	0.00	0.00	0.00	29.17	54.17	58.33	83.33	62.50	45.83	30.30
		(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(32.59)	(47.41)	(49.83)	(66.20)	(52.41)	(42.41)	(26.44)
V_7	KJT-4	0.00	0.00	0.00	0.00	0.00	20.83	41.67	41.67	66.67	54.17	25.00	22.73
		(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(26.90)	(40.00)	(39.31)	(55.69)	(47.41)	(30.00)	(21.76)
V_8	PND-1	0.00	0.00	0.00	0.00	0.00	37.50	54.17	79.17	83.33	54.17	33.33	31.06
		(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(36.90)	(47.41)	(63.10)	(66.20)	(47.41)	(35.00)	(26.91)
V_9	KJT-7	0.00	0.00	0.00	0.00	0.00	37.50	62.50	70.83	62.50	45.83	37.50	28.79
		(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(37.59)	(53.10)	(58.10)	(52.24)	(42.59)	(37.59)	(25.56)
V_{10}	RTN-5	0.00	0.00	0.00	0.00	0.00	37.50	41.67	41.67	79.17	62.50	33.33	26.89
		(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(37.76)	(40.17)	(40.00)	(63.10)	(53.10)	(35.00)	(24.47)
V_{11}	PLG-1	0.00	0.00	0.00	0.00	0.00	20.83	20.83	33.33	45.83	66.67	37.50	20.45
		(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(26.39)	(26.90)	(35.17)	(42.59)	(54.83)	(37.59)	(20.32)
V_{12}	PLG-2	0.00	0.00	0.00	0.00	0.00	25.00	33.33	37.50	58.33	79.17	37.50	24.62
		(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(29.49)	(35.17)	(37.76)	(49.83)	(63.61)	(37.59)	(23.04)
V_{13}	KJT-5	0.00	0.00	0.00	0.00	0.00	33.33	37.50	50.00	66.67	75.00	45.83	28.03
		(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(35.00)	(37.59)	(45.00)	(55.00)	(60.00)	(42.59)	(25.02)
V_{14}	KJT-6	0.00	0.00	0.00	0.00	0.00	25.00	33.33	45.83	54.17	79.17	37.50	25.00
		(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(29.49)	(34.49)	(42.59)	(47.41)	(63.61)	(37.59)	(23.20)
V_{15}	RTN-4	0.00	0.00	0.00	0.00	0.00	29.17	33.33	41.67	70.83	50.00	33.33	23.48
		(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(32.08)	(34.31)	(40.17)	(57.41)	(45.00)	(35.00)	(22.18)
V_{16}	KJT-2	0.00	0.00	0.00	0.00	0.00	25.00	33.33	37.50	50.00	83.33	50.00	25.38
		(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(30.00)	(35.17)	(37.59)	(45.00)	(66.20)	(45.00)	(23.54)
V_{17}	RTN-3	0.00	0.00	0.00	0.00	0.00	29.17	29.17	45.83	41.67	83.33	54.17	25.76
		(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(32.59)	(32.59)	(42.59)	(40.00)	(66.20)	(47.41)	(23.76)
V_{18}	RTN-2	0.00	0.00	0.00	0.00	0.00	29.17	45.83	50.00	66.67	70.83	54.17	28.79
		(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(31.90)	(42.59)	(45.00)	(55.00)	(58.10)	(47.41)	(25.45)
V_{19}	KJT-8	0.00	0.00	0.00	0.00	0.00	37.50	45.83	54.17	66.67	87.50	66.67	32.58
		(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(37.59)	(42.59)	(47.41)	(54.83)	(69.30)	(54.83)	(27.87)
V_{20}	PNL-1	0.00	0.00	0.00	0.00	0.00	33.33	41.67	50.00	66.67	70.83	54.17	28.79
		(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(35.17)	(40.17)	(45.00)	(54.83)	(57.41)	(47.41)	(25.45)
V_{21}	PNL-2	0.00	0.00	0.00	0.00	0.00	37.50	41.67	41.67	79.17	70.83	45.83	28.79
		(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(37.59)	(40.00)	(40.17)	(63.61)	(57.41)	(42.59)	(25.58)
V_{22}	PNL-3	0.00	0.00	0.00	0.00	0.00	20.83	41.67	54.17	58.33	83.33	45.83	27.65
		(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(26.39)	(40.00)	(47.41)	(50.00)	(66.20)	(42.59)	(24.78)
Range	Min	0.00	0.00	0.00	0.00	0.00	20.83	20.83	33.33	41.67	45.83	25.00	20.45
		(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(26.39)	(26.90)	(35.17)	(40.00)	(42.59)	(30.00)	(20.32)
	Max	0.00	0.00	0.00	0.00	0.00	41.67	62.50	79.17	87.50	87.50	66.67	33.33
		(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(40.17)	(53.10)	(63.10)	(69.30)	(69.30)	(54.83)	(28.32)
Mean		0.00	0.00	0.00	0.00	0.00	30.30	42.61	50.57	67.80	67.23	42.61	27.38
		(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(32.92)	(40.54)	(45.38)	(55.99)	(55.71)	(40.59)	(24.65)

(Figures in parentheses indicate arcsine transformed value)

	Variety	Medium	Variety × Medium
SE m	1.57	1.11	5.19
CD at 1 %	5.73	4.05	18.99
Significant at 1 %	Sig	Sig	Sig

Table 5: Effect of varieties and media combinations on number of shoots per callus bit in rice

Variety / Medium		S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}	S_{11}	Mean
V_1	RTN-73	0.00	0.00	0.00	0.00	0.00	2.67	3.00	4.33	5.33	4.00	2.33	1.97
V_2	KJT-184	0.00	0.00	0.00	0.00	0.00	2.67	3.00	5.00	6.33	4.33	3.00	2.21
V_3	RTN-24	0.00	0.00	0.00	0.00	0.00	2.67	4.33	5.33	5.67	4.00	3.67	2.33
V_4	RTN-711	0.00	0.00	0.00	0.00	0.00	3.33	4.33	5.00	7.67	6.33	4.33	2.82
V_5	RTN-1	0.00	0.00	0.00	0.00	0.00	2.33	2.67	3.33	5.00	3.67	3.33	1.85
V_6	KJT-3	0.00	0.00	0.00	0.00	0.00	2.33	3.67	5.00	6.00	5.00	3.33	2.30
V_7	KJT-4	0.00	0.00	0.00	0.00	0.00	2.67	3.67	5.33	6.00	4.67	2.67	2.27

V_8	PND-1	0.00	0.00	0.00	0.00	0.00	2.33	3.00	5.00	6.00	5.33	3.33	2.27
V_9	KJT-7	0.00	0.00	0.00	0.00	0.00	3.33	4.33	5.33	6.67	5.33	4.67	2.70
V_{10}	RTN-5	0.00	0.00	0.00	0.00	0.00	2.00	2.67	4.00	5.33	3.33	2.67	1.82
V_{11}	PLG-1	0.00	0.00	0.00	0.00	0.00	2.00	2.67	3.00	3.00	3.33	2.67	1.52
V_{12}	PLG-2	0.00	0.00	0.00	0.00	0.00	2.33	3.00	3.00	4.33	5.67	4.33	2.06
V_{13}	KJT-5	0.00	0.00	0.00	0.00	0.00	3.33	4.00	5.00	5.33	6.00	5.67	2.67
V_{14}	KJT-6	0.00	0.00	0.00	0.00	0.00	2.67	3.00	4.00	6.00	6.33	4.67	2.42
V_{15}	RTN-4	0.00	0.00	0.00	0.00	0.00	2.67	4.00	5.00	6.67	5.67	3.00	2.45
V_{16}	KJT-2	0.00	0.00	0.00	0.00	0.00	2.33	3.33	4.33	4.67	7.00	5.00	2.42
V_{17}	RTN-3	0.00	0.00	0.00	0.00	0.00	2.33	2.33	3.67	4.00	4.67	3.00	1.82
V_{18}	RTN-2	0.00	0.00	0.00	0.00	0.00	2.00	2.33	4.00	4.67	5.67	5.00	2.15
V_{19}	KJT-8	0.00	0.00	0.00	0.00	0.00	3.00	4.00	4.33	5.33	7.33	6.33	2.76
V_{20}	PNL-1	0.00	0.00	0.00	0.00	0.00	2.67	3.00	5.00	6.00	6.33	4.33	2.48
V_{21}	PNL-2	0.00	0.00	0.00	0.00	0.00	3.00	4.67	5.67	6.67	5.33	3.67	2.64
V_{22}	PNL-3	0.00	0.00	0.00	0.00	0.00	3.00	4.00	4.67	6.67	5.00	2.67	2.36
Range	Min	0.00	0.00	0.00	0.00	0.00	2.00	2.33	3.00	3.00	3.33	2.33	1.52
	Max	0.00	0.00	0.00	0.00	0.00	3.33	4.67	5.67	7.67	7.33	6.33	2.82
Mean		0.00	0.00	0.00	0.00	0.00	2.62	3.41	4.52	5.61	5.20	3.80	2.29

	Variety	Medium	Variety × Medium
SEm	0.10	0.07	0.32
CD at 1 %	0.35	0.25	1.15
Significant at 1 %	Sig	Sig	Sig

Table 6: Effect of varieties and media combinations on days to root induction in rice

Variety / Medium		R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	R_{10}	R_{11}	Mean
V_1	RTN-73	0.00	0.00	0.00	0.00	10.00	9.00	7.67	6.67	6.33	5.33	6.33	4.67
V_2	KJT-184	0.00	0.00	0.00	0.00	12.00	11.67	10.67	9.00	8.33	5.33	7.00	5.82
V_3	RTN-24	0.00	0.00	0.00	0.00	10.67	10.00	9.33	6.67	5.67	4.67	5.00	4.73
V_4	RTN-711	0.00	0.00	0.00	0.00	10.00	8.67	6.67	6.00	4.33	3.67	4.33	3.97
V_5	RTN-1	0.00	0.00	0.00	0.00	10.00	10.33	8.33	8.33	7.33	5.00	5.67	5.00
V_6	KJT-3	0.00	0.00	0.00	0.00	10.67	8.33	7.00	5.00	4.67	4.00	4.33	4.00
V_7	KJT-4	0.00	0.00	0.00	0.00	10.67	10.67	10.33	8.00	7.00	5.33	7.00	5.36
V_8	PND-1	0.00	0.00	0.00	0.00	10.33	10.33	9.67	7.67	7.00	4.67	5.33	5.00
V_9	KJT-7	0.00	0.00	0.00	0.00	10.00	9.00	8.00	6.33	4.67	4.00	5.33	4.30
V_{10}	RTN-5	0.00	0.00	0.00	0.00	10.33	10.33	9.67	8.00	8.00	5.67	8.33	5.48
V_{11}	PLG-1	0.00	0.00	0.00	0.00	13.67	13.33	13.33	11.33	10.00	8.67	9.67	7.27
V_{12}	PLG-2	0.00	0.00	0.00	0.00	11.33	11.00	10.67	9.33	8.00	7.00	8.00	5.94
V_{13}	KJT-5	0.00	0.00	0.00	0.00	13.00	12.67	11.00	9.33	8.33	7.67	9.00	6.45
V_{14}	KJT-6	0.00	0.00	0.00	0.00	13.67	13.00	11.67	10.00	8.33	6.67	8.00	6.48
V_{15}	RTN-4	0.00	0.00	0.00	0.00	12.67	13.00	12.33	10.33	9.00	7.67	9.33	6.76
V_{16}	KJT-2	0.00	0.00	0.00	0.00	15.33	13.67	11.67	9.00	8.67	7.67	8.67	6.79
V_{17}	RTN-3	0.00	0.00	0.00	0.00	16.00	14.33	13.33	11.00	8.67	8.33	10.33	7.45
V_{18}	RTN-2	0.00	0.00	0.00	0.00	16.33	16.00	15.33	11.33	9.33	9.00	9.67	7.91
V_{19}	KJT-8	0.00	0.00	0.00	0.00	16.67	15.67	14.67	11.67	9.00	7.33	10.33	7.76
V_{20}	PNL-1	0.00	0.00	0.00	0.00	15.67	13.67	12.67	11.00	10.67	9.33	10.67	7.61
V_{21}	PNL-2	0.00	0.00	0.00	0.00	15.67	14.67	13.00	11.33	10.00	8.00	9.00	7.42
V_{22}	PNL-3	0.00	0.00	0.00	0.00	16.00	15.00	12.67	11.67	10.33	8.67	9.67	7.64
Range	Min	0.00	0.00	0.00	0.00	10.00	8.33	6.67	5.00	4.33	3.67	4.33	3.97
	Max	0.00	0.00	0.00	0.00	16.67	16.00	15.33	11.67	10.67	9.33	10.67	7.91
Mean		0.00	0.00	0.00	0.00	12.76	12.02	10.89	9.05	7.89	6.53	7.77	6.08

	Variety	Medium	Variety × Medium
SEm	0.13	0.09	0.43
CD at 1 %	0.48	0.34	1.58
Significant at 1 %	Sig	Sig	Sig

Table 7: Effect of varieties and media combinations on root induction frequency (%) in rice

Variety / Medium		R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	R_{10}	R_{11}	Mean
V_1	RTN-73	0.00	0.00	0.00	0.00	13.33	20.00	23.33	26.67	40.00	50.00	43.33	19.70
		(0.00)	(0.00)	(0.00)	(0.00)	(21.14)	(26.07)	(28.78)	(31.00)	(39.15)	(45.00)	(41.15)	(21.12)
V_2	KJT-184	0.00	0.00	0.00	0.00	13.33	16.67	20.00	26.67	36.67	50.00	43.33	18.79
		(0.00)	(0.00)	(0.00)	(0.00)	(21.14)	(23.86)	(26.57)	(31.00)	(37.22)	(45.00)	(41.15)	(20.54)
V_3	RTN-24	0.00	0.00	0.00	0.00	13.33	16.67	26.67	33.33	40.00	53.33	40.00	20.30
		(0.00)	(0.00)	(0.00)	(0.00)	(21.14)	(23.86)	(31.00)	(35.22)	(39.15)	(46.92)	(39.15)	(21.49)

V_4	RTN-711	0.00	0.00	0.00	0.00	26.67	26.67	36.67	50.00	60.00	73.33	66.67	30.91
		(0.00)	(0.00)	(0.00)	(0.00)	(31.00)	(31.00)	(37.22)	(45.00)	(50.77)	(59.00)	(54.78)	(28.07)
V_5	RTN-1	0.00	0.00	0.00	0.00	16.67	23.33	26.67	36.67	43.33	56.67	50.00	23.03
		(0.00)	(0.00)	(0.00)	(0.00)	(23.86)	(28.29)	(31.00)	(37.22)	(41.07)	(48.85)	(44.92)	(23.20)
V_6	KJT-3	0.00	0.00	0.00	0.00	20.00	20.00	33.33	50.00	53.33	63.33	56.67	26.97
		(0.00)	(0.00)	(0.00)	(0.00)	(26.07)	(26.07)	(35.22)	(45.00)	(46.92)	(52.78)	(48.85)	(25.54)
V_7	KJT-4	0.00	0.00	0.00	0.00	16.67	26.67	30.00	40.00	46.67	56.67	43.33	23.64
		(0.00)	(0.00)	(0.00)	(0.00)	(23.86)	(30.79)	(33.00)	(39.15)	(43.08)	(48.85)	(41.07)	(23.62)
V_8	PND-1	0.00	0.00	0.00	0.00	16.67	20.00	26.67	33.33	50.00	60.00	53.33	23.64
		(0.00)	(0.00)	(0.00)	(0.00)	(23.86)	(26.57)	(31.00)	(35.22)	(45.00)	(50.77)	(46.92)	(23.57)
V_9	KJT-7	0.00	0.00	0.00	0.00	13.33	26.67	33.33	43.33	46.67	60.00	53.33	25.15
		(0.00)	(0.00)	(0.00)	(0.00)	(21.14)	(30.79)	(35.22)	(41.15)	(43.08)	(50.85)	(46.92)	(24.47)
V_{10}	RTN-5	0.00	0.00	0.00	0.00	16.67	20.00	30.00	40.00	50.00	53.33	43.33	23.03
		(0.00)	(0.00)	(0.00)	(0.00)	(23.86)	(26.57)	(33.00)	(39.15)	(45.00)	(46.92)	(41.07)	(23.23)
V_{11}	PLG-1	0.00	0.00	0.00	0.00	13.33	16.67	26.67	30.00	36.67	43.33	33.33	18.18
		(0.00)	(0.00)	(0.00)	(0.00)	(21.14)	(23.86)	(31.00)	(33.00)	(37.22)	(41.15)	(35.22)	(20.24)
V_{12}	PLG-2	0.00	0.00	0.00	0.00	13.33	16.67	20.00	30.00	40.00	46.67	40.00	18.79
		(0.00)	(0.00)	(0.00)	(0.00)	(21.14)	(23.86)	(26.07)	(33.00)	(39.23)	(43.08)	(39.15)	(20.50)
V_{13}	KJT-5	0.00	0.00	0.00	0.00	13.33	23.33	30.00	30.00	40.00	53.33	43.33	21.21
		(0.00)	(0.00)	(0.00)	(0.00)	(21.14)	(28.78)	(33.00)	(33.21)	(39.15)	(46.92)	(41.15)	(22.12)
V_{14}	KJT-6	0.00	0.00	0.00	0.00	13.33	23.33	26.67	30.00	43.33	53.33	46.67	21.52
		(0.00)	(0.00)	(0.00)	(0.00)	(21.14)	(28.78)	(31.00)	(33.00)	(41.15)	(46.92)	(43.08)	(22.28)
V_{15}	RTN-4	0.00	0.00	0.00	0.00	16.67	23.33	36.67	40.00	50.00	60.00	46.67	24.85
		(0.00)	(0.00)	(0.00)	(0.00)	(23.36)	(28.78)	(37.22)	(39.23)	(45.00)	(50.77)	(43.08)	(24.31)
V_{16}	KJT-2	0.00	0.00	0.00	0.00	16.67	20.00	26.67	36.67	46.67	50.00	43.33	21.82
		(0.00)	(0.00)	(0.00)	(0.00)	(23.86)	(26.07)	(31.00)	(37.22)	(43.08)	(45.00)	(41.07)	(22.48)
V_{17}	RTN-3	0.00	0.00	0.00	0.00	16.67	23.33	26.67	36.67	40.00	50.00	46.67	21.82
		(0.00)	(0.00)	(0.00)	(0.00)	(23.86)	(28.08)	(31.00)	(37.22)	(39.15)	(45.00)	(42.99)	(22.48)
V_{18}	RTN-2	0.00	0.00	0.00	0.00	13.33	23.33	30.00	33.33	46.67	53.33	50.00	22.73
		(0.00)	(0.00)	(0.00)	(0.00)	(21.14)	(28.78)	(33.21)	(35.22)	(43.08)	(46.92)	(45.00)	(23.03)
V_{19}	KJT-8	0.00	0.00	0.00	0.00	30.00	33.33	36.67	43.33	56.67	70.00	66.67	30.61
		(0.00)	(0.00)	(0.00)	(0.00)	(33.21)	(35.22)	(37.22)	(41.07)	(48.85)	(57.00)	(54.78)	(27.94)
V_{20}	PNL-1	0.00	0.00	0.00	0.00	20.00	26.67	36.67	36.67	40.00	56.67	46.67	23.94
		(0.00)	(0.00)	(0.00)	(0.00)	(26.57)	(30.79)	(37.22)	(36.93)	(39.15)	(48.85)	(43.08)	(23.87)
V_{21}	PNL-2	0.00	0.00	0.00	0.00	20.00	30.00	33.33	30.00	53.33	63.33	60.00	26.36
		(0.00)	(0.00)	(0.00)	(0.00)	(26.57)	(33.00)	(35.22)	(33.00)	(46.92)	(52.78)	(50.85)	(25.30)
V_{22}	PNL-3	0.00	0.00	0.00	0.00	16.67	20.00	23.33	30.00	40.00	46.67	36.67	19.39
		(0.00)	(0.00)	(0.00)	(0.00)	(23.86)	(26.07)	(28.78)	(33.21)	(39.15)	(43.08)	(37.14)	(21.03)
Range	Min	0.00	0.00	0.00	0.00	13.33	16.67	20.00	26.67	36.67	43.33	33.33	18.18
		(0.00)	(0.00)	(0.00)	(0.00)	(21.14)	(23.86)	(26.07)	(31.00)	(37.22)	(41.15)	(35.22)	(20.24)
	Max	0.00	0.00	0.00	0.00	30.00	33.33	36.67	50.00	60.00	73.33	66.67	30.91
		(0.00)	(0.00)	(0.00)	(0.00)	(33.21)	(35.22)	(37.22)	(45.00)	(50.77)	(59.00)	(54.78)	(28.07)
Mean		0.00	0.00	0.00	0.00	16.82	22.58	29.09	35.76	45.45	55.61	47.88	23.02
		(0.00)	(0.00)	(0.00)	(0.00)	(23.82)	(28.00)	(32.45)	(36.57)	(42.34)	(48.29)	(43.75)	(23.20)

(Figures in parentheses indicate arcsine transformed value)

	Variety	Medium	Variety × Medium
SEm	1.07	0.76	3.56
CD at 1 %	3.93	2.78	13.02
Significant at 1 %	Sig	Sig	Non Sig

Table 8: Effect of varieties and media combinations on number of roots per shoot in rice

Variety / Medium		R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	R_{10}	R_{11}	Mean
V_1	RTN-73	0.00	0.00	0.00	0.00	1.33	2.33	3.00	3.33	4.33	5.67	5.00	2.27
V_2	KJT-184	0.00	0.00	0.00	0.00	2.00	2.33	2.67	3.67	4.00	5.33	4.33	2.21
V_3	RTN-24	0.00	0.00	0.00	0.00	2.67	3.33	4.67	5.00	5.67	7.33	6.67	3.21
V_4	RTN-711	0.00	0.00	0.00	0.00	4.00	5.00	6.67	8.33	9.67	11.33	10.67	5.06
V_5	RTN-1	0.00	0.00	0.00	0.00	2.67	3.33	4.33	5.67	7.00	8.33	7.00	3.48
V_6	KJT-3	0.00	0.00	0.00	0.00	2.67	3.33	6.33	8.33	9.33	10.33	9.67	4.55
V_7	KJT-4	0.00	0.00	0.00	0.00	2.33	3.67	4.67	5.33	7.33	8.33	8.00	3.61

V_8	PND-1	0.00	0.00	0.00	0.00	3.00	3.67	5.00	6.67	8.33	9.33	9.00	4.09
V_9	KJT-7	0.00	0.00	0.00	0.00	2.00	4.00	6.00	6.67	8.00	9.33	8.67	4.06
V_{10}	RTN-5	0.00	0.00	0.00	0.00	2.00	3.00	4.67	5.67	6.67	7.00	6.33	3.21
V_{11}	PLG-1	0.00	0.00	0.00	0.00	1.33	2.33	2.67	3.00	4.00	4.33	3.67	1.94
V_{12}	PLG-2	0.00	0.00	0.00	0.00	2.00	2.67	3.67	4.33	5.33	6.00	5.00	2.64
V_{13}	KJT-5	0.00	0.00	0.00	0.00	2.00	3.33	3.33	4.67	5.33	7.33	6.00	2.91
V_{14}	KJT-6	0.00	0.00	0.00	0.00	2.00	2.67	3.67	4.33	5.33	7.67	6.67	2.94
V_{15}	RTN-4	0.00	0.00	0.00	0.00	2.33	3.33	5.00	7.33	8.33	9.33	8.67	4.03
V_{16}	KJT-2	0.00	0.00	0.00	0.00	2.00	2.33	3.00	3.67	4.00	6.33	5.00	2.39
V_{17}	RTN-3	0.00	0.00	0.00	0.00	2.00	2.33	3.00	3.67	4.33	6.33	5.00	2.42
V_{18}	RTN-2	0.00	0.00	0.00	0.00	2.33	3.67	4.00	5.00	6.00	7.00	6.33	3.12
V_{19}	KJT-8	0.00	0.00	0.00	0.00	3.00	5.00	6.67	8.00	9.00	11.00	10.33	4.82
V_{20}	PNL-1	0.00	0.00	0.00	0.00	2.67	3.67	4.00	5.33	8.00	8.67	7.33	3.61
V_{21}	PNL-2	0.00	0.00	0.00	0.00	2.67	4.00	5.33	7.00	8.67	10.33	9.33	4.30
V_{22}	PNL-3	0.00	0.00	0.00	0.00	2.00	2.33	2.67	3.67	5.00	5.33	4.33	2.30
Range	Min	0.00	0.00	0.00	0.00	1.33	2.33	2.67	3.00	4.00	4.33	3.67	1.94
	Max	0.00	0.00	0.00	0.00	4.00	5.00	6.67	8.33	9.67	11.33	10.67	5.06
Mean		0.00	0.00	0.00	0.00	2.32	3.26	4.32	5.39	6.53	7.82	6.95	3.33

	Variety	Medium	Variety × Medium
SEm	0.09	0.06	0.29
CD at 1 %	0.32	0.22	1.05
Significant at 1 %	Sig	Sig	Sig

Hardening and plant establishment

The type of potting mixtures greatly affected the survival rate of *In Vitro* grown plantlets. The highest number of plantlets (73.33 %) survived in a potting mixture containing soil, organic manure and cocopit in 1:1:1 i.e. in equal proportion (Table 9). It was followed by the plantlet survivability of 53.33 % in potting mixture of soil and vermiculite in 3:1 proportion. The potting mixture containing soil and FYM in 3:1 proportion showed 46.67 % plantlets survivability whereas none of the plantlet was survived potted on only soil or only FYM.

Table 9: Hardening and establishment of *in vitro* plantlets in different potting mixtures

Sr. No	Potting mixture	Number of plants shifted	Number of plants survived	Survivability (%)
T_1	Soil	15	0	0.00
T_2	FYM	15	0	0.00
T_3	Vermiculite	15	1	6.67
T_4	Soil + FYM (3:1)	15	7	46.67
T_5	Soil + Vermiculite (3:1)	15	8	53.33
T_6	Soil + Organic manure + cocopit (1:1:1)	15	11	73.33

Basak *et al.* [33] used sterile soil, sand and cow dung in a 1:2:1 ratio in plastic as well as earthen pots and observed highest survival rates of 96% in plastic pots for variety BRRI dhan-29. Different potting mixtures were used by various workers. El-Shawaf *et al.* [14] and Joiya *et al.* [34] used peat moss and soil (1:1) and watered the plantlets using half strength of Hoagland solution as needed. Puhan and Siddiq [35] used a potting mixture of non-sterile sand: soil: compost (1:3:1) for transferring the plants.

The all needed to plantlets to survive is compactness, aeration and nutrients which could be served with soil, cocopit and organic manure in 1:1:1 proportion hence providing favourable conditions to grow normally.

Conclusion

From the present investigation, it can be concluded that the *In Vitro* regenerability is a resultant of varietal characters interacting with different growth regulator combinations emphasizing the need to develop genotype-specific regeneration protocols. The variety Ratnagiri- 711 and Karjat-8 were found with the best regeneration ability. From the present work, efficient regeneration protocols for these two varieties were developed which could further be conveniently used for producing transgenic plants.

References

1. Anonymous. FAOSTAT. www.faostat.fao.org, 2016.
2. Sikder MBH, Sen PK, Mamun AA, Ali MR, Rahman, SM. *In Vitro* Regeneration of Aromatic Rice (*Oryza sativa* L.). Int. J Agri. Biol. 2006; 8(6):759-760.
3. Huang WL, Tsung YC, Liu LF. Osmotic stress promotes shoot regeneration in immature embryo-derived callus of rice (*Oryza sativa* L.). J Agric Assoc Chin, 2002; 3:76–86.
4. Jime´nez VM. Involvement of plant hormone and plant growth regulators on *in vitro* somatic embryogenesis. Plant Growth Regul. 2005; 47:91-110.
5. Huang WL, CH Lee, Chen YR. Levels of endogenous abscisic acid and indole-3-acetic acid influence shoot organogenesis in callus cultures of rice subjected to osmotic stress, Plant Cell Tissue Organ Cult. 2012; 108:257-263.
6. Huang WL, Wang YC, Lee PD, Liu LF. The regenerability of rice callus is closely related to starch metabolism. Taiwan J Agric Chem Food Sci. 2006; 44:100-107.
7. Murashige T, Skoog F. A revised medium for rapid growth and bioassay of tobacco tissue culture. Physiol. Plant. 1962; 15:473-497.

8. Sah SK, Ajinder Kaur, Sandhu JS. High Frequency Embryogenic Callus Induction and Whole Plant Regeneration in Japonica Rice Cv. Kitaake, J Rice Res. 2014; 2:2
9. Gowda HR, Krishna V, Kumara Swamy GK, Ramachandra R, Gowda TKS. Standardization of Regeneration Protocol for *Indica* Rice Varieties Rasi and IR-20 Mysore J Agric, Sci., 2009; 43(3):412-419.
10. Amrita Mukherjee, Mohd R, Islam Dr. Nasiruddin KM, Purba Banerjee. Callus Initiation And Plantlet Regeneration Ability Of Some Rice Genotypes, International Journal Of Scientific & Technology Research. 2015; 4(10).
11. Hoque KM A, Azdi ZA, Prodhan SH. Development of Callus Initiation and Regeneration System of Different Indigenous indica Rice Varieties. Journal of Biology. 2013; 1(2):46-51
12. Wenzhong T, Rance I, Sivamani E, Fauquet C, Beachy RN. Improvement of plant regeneration frequency *in vitro* in indica rice. Chinese Journal of Genetics. 1994; 21(3).
13. Muhammad Tariq, Gowher Ali, Fazal Hadi, Shakeel Ahmad, Nasir Ali, Aftab Ali Shah. Callus Induction and *in vitro* Plant Regeneration of Rice (*Oryza sativa* L.) Under Various Conditions. Pakistan Journal of Biological Sciences. 2008; 11(2):255-259.
14. El-Shawaf I, Hasan AW, Bekhit M, Hasanein ES, Salim Z, Bauwe H. Callus induction and plant regeneration of five Egyptian rice genotypes as affected by medium constituents, Arab J Biotech. 2011; 14(2):279-290.
15. Anita, Roy, Aich SS, Mukherjee S. Differential Reponses to Indirect Organogenesis in Rice Cultivars. International Journal of Scientific and Research Publications. 2012; 2(9):1-5.
16. Mannan MA, Sarker TC, Mst Akhter T, KABIR AH, Alam MF. Indirect plant regeneration in aromatic rice (*Oryza sativa* L.) var. ‘Kalijira’ and ‘Chinigura’. Acta agriculturae Slovenica. 2013; 101(2):231-238.
17. Roly ZY, Islam Md M, Shaekh Md PE, Arman Md SI, Shahik S, Das D, *et al*. *In vitro* callus induction and regeneration potentiality of aromatic rice (*Oryza sativa* L.) cultivars in differential growth regulators. Int J Appl Sci Biotechnol. 2014; 2(2):160-167.
18. Silva GJ, da RS, dos Santos TM, Souza ND, Machado JA, Peters AC, de Oliveira. Somatic embryogenesis and plant regeneration in Brazilian rice genotypes, AJCS. 2015; 9(11):1126-1130.
19. Upadhyaya G, Sen M, Roy A. *In vitro* callus induction and plant regeneration of rice (*Oryza sativa* L.) var. ‘Sita’, ‘Rupali’ and ‘Swarna Masuri’, Asian Journal of Plant Science and Research. 2015; 5(5):24-27.
20. Sankepally SSR, Singh B. Optimization of regeneration using differential growth regulators in indica rice cultivars Biotech (PROTOCOLS AND METHODS). 2016; 6:19.
21. Mai TTX, Lien NT, Hoa TTC, Angenon G. Development of an efficient *in vitro* plant regeneration protocol for indica rice varieties (*Oryza sativa* L.) in the Mekong Delta of Vietnam. Can Tho University Journal of Science. 2017; 5:141-149.
22. Krishnan SR, Priya AM, Ramesh M. Rapid regeneration and ploidy stability of ‘cv IR36’ indica rice (*Oryza Sativa*. L) confers efficient protocol for *in vitro* callus organogenesis and Agrobacterium tumefaciens mediated transformation, Botanical Studies. 2013; 54:47.
23. Prasad BD, Kumar P, Sahni S, Kumar V, Kumari S, Kumar P, Pal AK. An improved protocol for *Agrobacterium*-mediated genetic transformation and regeneration of Indica rice (*oryza sativa* l. var. Rajendra kasturi). Journal of Cell and Tissue Research. 2016; 16(2):5597-5606.
24. Amaranatha RV, Vemanna RS, Rajashekar RBH, Babitha KC, Kiranmai K, Nareshkumar A, Sudhakar C. An Efficient Callus Induction and Regeneration Protocol for a Drought Tolerant Rice Indica Genotype AC39020, Journal of Plant Science. 2015; 3(5):248-254
25. Artadana IBM, Suhono GBF, Hardjo PH, Purwanto MG M, Wang YB, Supaibulwatana K. Plant regeneration induced from mature Embryo-derived callus of Balinese red rice (*Oryza sativa* Cv. Barak Cenana), Bali Med J. 2017; 3(3):S12-S17.
26. Libin A, King PJH, Ong KH, Chubo JK, Sipen P. Callus induction and plant regeneration of Sarawak rice (*Oryza sativa* L.) variety Biris, African Journal of Agricultural Research. 2012; 7(30):4260-4265.
27. Prodhan SH, Nagamiya K, Komamine A, Hirai Y. Regeneration response of *indica* and *japonica* rice in different media. Bangladesh J Pl. Breed. Genet. 2001; 14(2):1-6.
28. Lee K, Jeon H, Kim M. Optimization of a mature embryo-based *in vitro* culture system for high frequency somatic embryogenic callus induction and plant regeneration from japonica rice cultivars. Plant Cell, Tissue and Organ Culture. 2002; 71(3):237-244.
29. Wang MS, Zapata FJ, De Castro DC. Plant regeneration through somatic embryogenesis from mature seed and young inflorescence of wild rice (*Oryza perennis* Moench). Plant Cell Rep. 1987; 6:294-296.
30. Sripichitt P, Cheewasestathum P. Plant regeneration from embryo derived callus of aromatic rice (*Oryza sativa* L.) variety Khao Dawk Mali 105. Kasetsart J (Nat. Sci.). 1994; 28:27-37.
31. Fan M, Liu Z, Zhou L, Lin T, Liu Y, Luo L. Effects of Plant Growth Regulators and Saccharide on *In vitro* Plant and Tuberous Root Regeneration of Cassava (*Manihot esculenta* Crantz). J Plant Growth Regul. 2011; 30:11-19.
32. Wijesekera TP, Iqbal MCM, Bandara DC. Plant regeneration *In Vitro* by organogenesis on callus induced from mature embryos of three rice varieties (*Oryza sativa* L. ssp. *Indica*), Tropical Agricultural Research. 2007; 19:25-35.
33. Basak Alam SMA, Sultana S, Prodhan MA, Dey RC, Hassan L. Studies on callus induction and plant regeneration potentialities of indica rice varieties. Intl. J BioRes. 2008; 4(4):128-134.
34. Joyia FA, Khan MS. Reproducible and expedient rice regeneration system using *in vitro* grown plants, African Journal of Biotechnology. 2012; 11(1):138-144.
35. Puhan PA, Siddiq E. Protocol optimization and evaluation of rice varieties response to *in vitro* regeneration. Advances in Bioscience and Biotechnology, 2013; 4:647-653.