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SURFACE STERILIZATION AND CALLUS INDUCTION POTENTIAL OF VARIOUS INDICA RICE (*Oryza sativa* L.) VARIETIES

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ABSTRACT

The present study was conducted to standardize the efficient and reliable surface sterilization and callus induction protocol for 22 rice (*Oryza sativa* L.) varieties developed by Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Maharashtra (India) using different combinations of various plant growth regulators. The dehusked mature rice seed was used as explant. Prior to callus induction, surface sterilization protocol was standardized using 70% ethanol and different concentrations of mercuric chloride ($HgCl_2$). The results revealed that surface sterilization treatment with 70% ethanol for 1 min, followed by 1.0% $HgCl_2$ for 10 min and then washing the seeds 4-5 times was most effective in establishing maximum aseptic cultures (86.67%). Callus induction potential differed significantly among the rice varieties for the concentrations of plant growth regulators in MS medium. Rice cv. 'Ratnagiri-711' followed by cv. 'Karjat-8' were most superior in callus induction and proliferation potential with the respective callus induction frequency of 87.56 and 87.0%, and callus weight of 269.37 and 235.20 mg after 45 days of inoculation. The supplementation of 2,4-D @ 1.0 to 2.0 mg L^{-1} along with 0.5 mg L^{-1} BAP in MS medium was found optimum for embryogenic callus formation. All the calli formed on MS medium supplemented with combinations of plant growth regulators were yellowish-white in colour but with different textures as compact, soft, friable that were embryogenic and mucilaginous that were non-embryogenic.

Keywords: 2,4-D, callus induction protocol, proliferation potential, rice, surface sterilization

INTRODUCTION

Rice (*Oryza sativa* L., 2n = 2x = 24) is the seed of grass species *Oryza sativa* L. belonging to the family graminae. World-wide, it is the most widely consumed staple food, especially in Asia (Anonymous, 2016). The efforts are being made to raise the rice productivity and its nutritional quality to meet the demands of increasing human population. Biotechnological approaches open the avenues to achieve such objectives through *in vitro* genetic manipulation for which the development and establishment of thorough efficient tissue culture techniques is a pre-requisite. More chances to incur genetic manipulations lie within the undifferentiated phase of tissue such as callus phase; which further by the renowned ability of the plant cell *viz.*, 'totipotency', can be regenerated into the whole intact plant of the desired genotype (Morrish *et al.*, 1987). The callus induction and proliferation potential are highly genotype-specific, thus there is need to assess the potential of each genotype and standardize the protocol for different genotypes (Abe and Futsuhara, 1986). The rice varieties

developed by Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Maharashtra (India) are from abode of the rice in Maharashtra i.e. the *Konkan* region and needed to be evaluated for their callusing ability. It is well-known that the decisive process in plant dedifferentiation or redifferentiation is regulated by plant growth regulators (Abe and Futsuhara, 1986). Hence their concentration and combination in a medium need to be standardized for optimum and friable callus which should be embryogenic in nature to give rise to the whole plant regeneration.

Successful callus culture of rice was first achieved by Fujiwara and Yatazama (1964) by culturing the nodes of young seedlings on Hellers medium with vitamins and 2,4-D (2 ppm) and later by Nishi *et al.* (1968) who successfully re-differentiated rice callus tissue from seeds into intact plantlet. Several other explants showing regeneration were also reported (Chen *et al*., 1985; Datta *et al*., 1990; Ramesh *et al*., 2009) but the mature seeds have the advantage of their year-round availability (Alam, 1994). In *in vitro* culture, aseptic conditions are to be maintained to keep away the microbial contaminants that compete for nutrients; therefore, surface sterilization of explants becomes indispensable. Various sterilizing agents are also toxic to the plant tissues. Mercuric chloride ($HgCl_2$), though a very strong sterilization agent, is also toxic to explant tissues when used for prolonged time period at high concentrations (Kataky and Handique, 2010); whereas the use of ethanol and Clorox in combination results in inadequate sterilization (Ullah *et al.*, 2007). Ethanol is a powerful sterilizing agent but extremely phytotoxic. Hence, its concentration in treatment and exposure time period needs to be wisely determined (Oyebanji *et al*., 2009). The present study was aimed at standardizing the surface sterilization protocol and explore the callus induction potential of different indica rice varieties.

MATERIALS AND METHODS

Callus induction studies were carried out during 2017 on 22 rice varieties developed by Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli (India). Prior to the callus induction, surface sterilization protocol was standardized using 70% ethanol and $HgCl_2$ (0.1, 0.5 and 1.0%) as surfactants for different exposure periods (Table 1). The mature dehusked rice seeds were used as explants. Seeds were soaked for 15-18 h in double distilled water and inoculated on callus induction medium. Observations on percent aseptic cultures were recorded. For callus induction, MS (Murashige and Skoog, 1962) medium was used as a basal medium with varying concentrations of auxins *viz*., 2,4-D, NAA and cytokinins [BAP and kinetin] (Raghawa and Nabors, 1984; Sripichitt and Cheewasestathum, 1994). The experiments were conducted in a factorial completely randomized design with each treatment replicated three times. The observations were recorded for days to callus induction and callus induction frequency (%). After 21-30 days of incubation, the established calli were sub-cultured on the same medium for proliferation. Observations on callus weight at 30 and 45 days of inoculation, increase in callus weight within the specified period and nature of calli were recorded. The experiments were conducted under well-defined aseptic culture conditions maintained at 26±2°C temperature, uniform light (1000-2000 lux) over a light and dark cycle of 16:8 h (DeFossard, 1986). The data was analyzed by using analysis of variance as per Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Surface sterilization

The results of surface sterilization treatments were almost similar in all the 22 rice varieties. Out of various treatments evaluated, the 70% alcohol treatment for 5 min showed maximum aseptic cultures but adversely affected the survival of explants. The 70% alcohol treatment for 1 min. followed by

Table 1: Effect of surface sterilizing agents on aseptic culture establishment

Treatments	Time (min)	Percent aseptic cultures (%)
Control (DDW washing)	10	00.00 (00.00)
Alcohol (70%)	5	97.78 (85.00)
$HgCl_2$ (0.1%)	10	28.89 (32.47)
$HgCl_2$ (0.5%)	5	42.22 (40.50)
$HgCl_2$ (1.0%)	1	17.78 (24.84)
Alcohol (70%) + $HgCl_2$ (0.1%)	1 + 10	68.89 (56.11)
Alcohol (70%) + $HgCl_2$ (0.5%)	1 + 10	86.67 (68.99)
Alcohol (70%) + $HgCl_2$ (1.0%)	1 + 5	82.22 (65.13)
Alcohol (70%) + $HgCl_2$ (1.0%)	1 + 10	95.55 (80.00)
Range		00.00-97.78 (0.0-85.00)
SE±		2.34
$CD_{0.01}$		9.54

Figures in parentheses indicate arcsine transformed value

$HgCl_2$ (1.0%) for 10 min had 95.55% aseptic cultures without any detrimental effect (Table 1). It is followed by the treatment comprising of 70% alcohol for 1 min. and $HgCl_2$ (0.5%) for 10 min. (86.67%). Other workers have reported several treatment combinations to achieve maximum aseptic cultures. Revathi and Pillai (2011) recommended the treatment of seeds with 70% ethanol for 1 min followed by 0.1% $HgCl_2$ for 15 min. Sananda *et al.* (2013) observed best results (95%) with the treatment of 0.1% $HgCl_2$ for 5 min but found detrimental effect of 1% $HgCl_2$ on calli growth. Ahmad *et al.* (2016) achieved best sterilization (97.14%) by using 0.2% $HgCl_2$ for 8 min without any significant effect on callus induction frequencies. Higher concentration of $HgCl_2$ used in the present study showed no detrimental effects on explants as it appears that mature seeds contain the tissue to be developed in culture within a layer that is surface sterilized. Further, thorough washing of seeds 4-5 times with double distilled water after treatment might have removed its traces.

Table 2: Media combinations used for callus induction showing different nature of callus

Treatments	Nature of callus
MS medium (control)	No callus
MS + 1.0 mg 2,4-D L^{-1} + 0.5 mg BAP L^{-1}	Yellowish-white, compact
MS + 1.5 mg 2,4-D L^{-1} + 0.5 mg BAP L^{-1}	Yellowish-white, compact
MS + 2.0 mg 2,4-D L^{-1} + 0.5 BAP mg L^{-1}	Yellowish-white, compact
MS + 2.5 mg 2,4-D L^{-1} + 0.5 mg BAP L^{-1}	Yellowish-white, compact
MS + 1.0 mg 2,4-D L^{-1} + 0.5 mg BAP L^{-1} + 0.1 mg NAA L^{-1}	Yellowish-white, more compact
MS + 1.5 mg 2,4-D L^{-1} + 0.5 mg BAP L^{-1} + 0.1 mg NAA L^{-1}	Yellowish-white, more compact
MS + 2.0 mg 2,4-D L^{-1} + 0.5 mg BAP L^{-1} + 0.1 mg NAA L^{-1}	Yellowish-white, more compact
MS + 2.5 mg 2,4-D L^{-1} +0.5 mg BAP L^{-1} + 0.1 mg NAA L^{-1}	Yellowish-white, soft, friable
MS + 2.0 mg 2,4-D L^{-1} + 0.5 mg KIN L^{-1} + 0.1 mg NAA L^{-1}	Yellowish-white, soft, friable
MS + 2.5 mg 2,4-D L^{-1} + 0.5 mg KIN L^{-1} + 0.1 mg NAA L^{-1}	Yellowish-white, soft, friable

MS - Murashige and Skoog medium; Plant growth regulators used: 2,4-D = 2,4-dichlorophenoxy acetic acid; BAP = 6-benzyl amino purine; NAA = naphthalene acetic acid; KIN = kinetin

Callus induction and proliferation

The callus induction potential of different rice varieties was studied in terms of days to callus induction, callus induction frequency (%), weight of callus at 30 and 45 days of inoculation along with the increase within the period and nature of callus.

Days to callus induction: Days to callus induction significantly varied among the varieties for different plant growth regulator (PGR) combinations supplemented to the MS medium (Table 3). Rice cv. 'Ratnagiri-711' induced callus significantly earlier (7.67 days) than the other varieties on MS medium supplemented with 1.5 mg 2,4-D L^{-1} + 0.5 mg BAP L^{-1} as well as on MS supplemented with 2.5 mg 2,4-D L^{-1} + 0.5 mg BAP L^{-1} + 0.1 mg NAA L^{-1}. Rice cv. 'Ratnagiri-4' took significantly more (35.33) days to induce callus on MS supplemented with 1.5 mg 2,4-D L^{-1} + 0.5 mg BAP L^{-1}. Further, each variety also differed significantly for different PGR combinations. The cv. 'Ratnagiri-711'required as maximum as 17.67 days to induce callus on MS supplemented with 1.0 mg 2,4-D L^{-1} + 0.5 mg

BAP L^{-1} + 0.1 mg NAA L^{-1}. This clearly indicated the presence of significant variety × PGR interaction. Similar differential responses were reported by Gowda *et al.* (2009) in indica rice varieties 'Rasi' and 'IR 20' inducing earlier callus in 14 and 16 days, respectively, on MS + 2 mg 2,4-D L^{-1} + 1 mg kinetin L^{-1} medium whereas the same varieties took 15 and 17 days, respectively, on MS medium containing 4 mg 2,4-D L^{-1}. Sah *et al.* (2014) reported callus induction after 9 days in a japonica rice cv. 'Kitaake' on MS medium containing 3.0 mg 2,4-D L^{-1} + 0.25 mg BAP L^{-1} + 0.6 g proline L^{-1}. Amrita *et al.* (2015) observed callus induction in just 6.5 days in cv. 'BR-14' at 2.0 mg 2,4-D L^{-1}. It seems to be the resultant of interactions among the variety × PGR concentrations indicating the dependency of callus induction potential on the genetic potentials of different genotypes that specify the different endogenous levels of growth hormones naturally present in them.

Callus induction frequency

The varieties and PGR combinations added to the MS medium differed significantly with respect to callus induction frequency (Table 4). Rice variety 'Ratnagiri-711' on MS medium supplemented with 1.5 mg 2,4-D L^{-1} + 0.5 mg BAP L^{-1} showed highest callus induction frequency of 87.56% which is in conformity with the findings of Shukla *et al*. (2014) in cv. 'Swarna Sub1' (80.0%). The cv. 'Karjat-8' on MS with 1.0 mg 2,4-D L^{-1} + 0.5 mg BAP L^{-1} (87.0%) and 'Ratnagiri-711' on MS with 2.0 mg 2,4-D L^{-1} + 0.5 mg kinetin L^{-1} + 0.1 mg NAA L^{-1} (86.93%) proved next best performing varieties.

Table 3: Effect of varieties and PGR supplementations on days to callus induction in rice

Variety/PGR combination*	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}	Mean
Ratnagiri-73	0.0	18.6	18.3	18.3	21.3	20.7	19.3	21.6	22.0	20.0	21.0	18.3
Karjat-184	0.0	19.3	18.6	17.3	19.3	19.7	18.3	18.6	16.7	18.0	17.7	16.7
Ratnagiri-24	0.0	20.6	20.3	20.0	21.7	21.3	21.0	22.0	22.0	21.0	21.3	19.2
Ratnagiri-711	0.0	8.0	7.7	8.3	10.7	17.7	15.3	8.7	7.7	14.3	9.7	9.8
Ratnagiri-1	0.0	20.6	22.3	23.3	24.7	21.7	22.0	25.0	24.3	23.7	24.3	21.1
Karjat-3	0.0	19.0	19.3	17.3	20.0	20.3	18.0	18.6	17.7	19.7	17.3	17.0
Karjat-4	0.0	19.6	19.6	18.3	20.3	20.7	19.0	19.3	17.7	19.0	18.7	17.5
Phondaghat-1	0.0	16.0	18.0	16.6	15.7	15.7	13.3	16.6	18.3	15.3	15.7	14.7
Karjat-7	0.0	27.6	25.3	29.0	29.3	28.3	27.7	24.3	28.0	26.7	29.7	25.1
Ratnagiri-5	0.0	25.3	24.6	27.0	25.7	25.7	26.7	27.0	26.7	26.3	26.7	23.8
Palghar-1	0.0	27.6	28.6	30.3	29.3	29.7	27.3	30.3	31.3	32.0	29.0	26.9
Palghar-2	0.0	24.3	24.6	28.0	27.3	27.7	25.3	28.0	29.0	29.3	26.7	24.6
Karjat-5	0.0	20.3	20.0	21.6	20.7	18.3	20.0	19.0	20.7	19.3	22.0	18.4
Karjat-6	0.0	21.6	21.3	22.3	22.7	19.7	22.0	20.3	21.7	20.3	22.7	19.5
Ratnagiri-4	0.0	31.6	33.6	34.3	31.0	31.3	31.3	31.0	33.7	34.7	35.3	29.8
Karjat-2	0.0	13.6	15.0	16.6	16.3	18.7	15.0	18.3	17.0	16.0	17.7	14.9
Ratnagiri-3	0.0	14.3	14.6	17.3	15.7	17.3	17.3	18.3	19.3	15.7	16.3	15.1
Ratnagiri-2	0.0	19.6	17.0	18.6	18.3	18.3	16.7	17.6	17.3	16.7	19.7	16.4
Karjat-8	0.0	13.3	14.3	13.6	19.3	15.3	18.3	18.6	24.7	23.3	17.0	16.2
Panvel-1	0.0	21.0	20.0	21.3	20.3	20.3	21.3	20.0	19.7	19.3	20.3	18.5
Panvel-2	0.0	14.6	14.0	16.3	17.3	17.0	18.7	17.3	16.7	13.7	16.3	14.7
Panvel-3	0.0	15.3	15.3	16.3	18.7	17.3	19.0	18.3	17.3	14.7	17.3	15.4
Range - Min.	0.0	8.0	7.7	8.33	10.7	15.3	13.3	8.7	7.7	13.7	9.7	9.8
- Max.	0.0	31.6	33.6	34.3	31.0	31.3	31.3	31.0	33.7	34.7	35.3	29.8
Mean	0.0	19.6	19.6	20.5	21.2	21.0	20.6	20.8	21.3	20.9	21.0	18.8
		Variety				Medium				Variety × Medium		
SE+		0.12				0.09				0.41		
$CD_{0.01}$		0.46				0.32				1.51		

*C_1 - MS medium (control); C_2 - MS + 1.0 mg 2,4-D L^{-1} + 0.5 mg BAP L^{-1}; C_3 - MS + 1.5 mg 2,4-D L^{-1} + 0.5 mg BAP L^{-1}; C_4 - MS + 2.0 mg 2,4-D L^{-1} + 0.5 mg BAP L^{-1}; C_5 - MS + 2.5 mg 2,4-D L^{-1} + 0.5 mg BAP L^{-1}; C_6 - MS + 1.0 mg 2,4-D L^{-1} + 0.5 mg BAP L^{-1} + 0.1 mg NAA L^{-1}; C_7 - MS + 1.5 mg 2,4-D L^{-1} + 0.5 mg BAP L^{-1} + 0.1 mg NAA L^{-1}; C_8 - MS + 2.0 mg 2,4-D L^{-1} + 0.5 mg BAP L^{-1} + 0.1 mg NAA L^{-1}; C_9 - MS + 2.5 mg 2,4-D L^{-1} +0.5 mg BAP L^{-1} + 0.1 mg NAA L^{-1}; C_{10} - MS + 2.0 mg 2,4-D L^{-1} + 0.5 mg KIN L^{-1} + 0.1 mg NAA L^{-1}; C_{11}- MS + 2.5 mg 2,4-D L^{-1} + 0.5 mg KIN L^{-1} + 0.1 mg NAA L^{-1}

Table 4: Effect of varieties and PGR supplementations on callus induction frequency (%) in rice

Variety/PGR combinations*	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}	Mean
Ratnagiri-73	0.00	41.06	46.05	54.77	78.12	23.35	16.57	21.20	68.54	51.42	60.42	41.95
	(0.00)	(39.85)	(42.73)	(47.74)	(62.12)	(28.89)	(23.99)	(27.36)	(55.89)	(45.81)	(51.02)	(38.67)
Karjat-184	0.00	57.86	50.78	41.14	81.80	32.74	24.73	17.30	73.54	44.96	63.60	44.40
	(0.00)	(49.52)	(45.45)	(39.89)	(64.75)	(34.89)	(29.79)	(24.56)	(59.06)	(42.11)	(52.90)	(40.26)
Ratnagiri-24	0.00	39.00	58.26	69.45	50.97	50.38	78.72	49.77	42.03	84.03	56.22	52.62
	(0.00)	(38.64)	(49.76)	(56.45)	(45.56)	(45.22)	(62.53)	(44.87)	(40.41)	(66.47)	(48.58)	(45.32)
Ratnagiri-711	0.00	78.47	87.56	80.82	75.98	75.17	76.67	71.74	76.30	86.93	34.13	67.62
	(0.00)	(62.35)	(69.35)	(64.04)	(60.65)	(60.11)	(61.13)	(57.90)	(60.89)	(68.81)	(35.71)	(54.63)
Ratnagiri-1	0.00	32.12	51.25	65.87	45.86	46.93	72.59	44.81	33.11	74.55	52.10	47.20
	(0.00)	(34.51)	(45.71)	(54.26)	(42.62)	(43.24)	(58.44)	(42.02)	(35.12)	(59.71)	(46.20)	(41.99)
Karjat-3	0.00	62.25	51.27	85.65	34.62	63.92	60.27	51.36	79.26	70.53	85.64	58.62
	(0.00)	(52.09)	(45.73)	(67.74)	(36.04)	(53.08)	(50.93)	(45.78)	(62.91)	(57.13)	(67.76)	(49.02)
Karjat-4	0.00	57.75	47.01	78.66	31.46	58.54	54.01	45.24	75.34	65.27	81.42	54.06
	(0.00)	(49.46)	(43.29)	(62.49)	(34.11)	(49.92)	(47.30)	(42.27)	(60.24)	(53.91)	(64.47)	(46.13)
Phondaghat-1	0.00	68.38	50.56	74.43	18.92	76.34	42.62	75.94	56.26	61.66	50.27	52.31
	(0.00)	(55.78)	(45.32)	(59.63)	(25.77)	(60.90)	(40.76)	(60.63)	(48.60)	(51.74)	(45.15)	(44.93)
Karjat-7	0.00	67.56	50.93	56.43	41.79	57.17	41.47	78.72	36.61	64.55	23.53	47.16
	(0.00)	(55.28)	(45.53)	(48.70)	(40.27)	(49.12)	(40.09)	(62.53)	(37.23)	(53.46)	(29.02)	(41.93)
Ratnagiri-5	0.00	27.55	46.79	61.35	42.85	41.14	67.47	42.43	29.66	68.73	47.16	43.19
	(0.00)	(31.65)	(43.16)	(51.57)	(40.88)	(39.90)	(55.23)	(40.65)	(32.99)	(56.00)	(43.37)	(39.58)
Palghar-1	0.00	46.58	28.39	14.30	8.64	45.68	50.75	36.15	21.29	12.19	37.27	27.39
	(0.00)	(43.04)	(32.20)	(22.22)	(17.01)	(42.52)	(45.43)	(36.96)	(27.48)	(20.42)	(37.62)	(29.54)
Palghar-2	0.00	50.74	33.03	18.73	14.52	50.58	55.28	39.04	26.04	15.01	40.35	31.21
	(0.00)	(45.42)	(35.08)	(25.64)	(22.39)	(45.33)	(48.03)	(38.67)	(30.68)	(22.79)	(39.44)	(32.13)
Karjat-5	0.00	33.33	50.72	35.45	50.84	80.75	21.71	60.88	36.04	77.48	43.82	44.64
	(0.00)	(35.26)	(45.41)	(36.54)	(45.48)	(63.98)	(27.77)	(51.28)	(36.90)	(61.67)	(41.45)	(40.52)
Karjat-6	0.00	29.89	47.21	32.45	46.06	76.23	19.63	56.98	31.09	73.34	40.10	41.18
	(0.00)	(33.13)	(43.40)	(34.72)	(42.74)	(60.82)	(26.29)	(49.01)	(33.89)	(58.92)	(39.29)	(38.38)
Ratnagiri-4	0.00	50.11	58.10	14.42	41.03	36.08	36.48	28.10	14.42	29.00	28.17	30.54
	(0.00)	(45.06)	(49.66)	(22.32)	(39.83)	(36.91)	(37.16)	(32.01)	(22.31)	(32.58)	(32.06)	(31.81)
Karjat-2	0.00	42.44	21.83	47.03	64.44	14.63	64.76	15.27	34.84	43.03	78.97	38.84
	(0.00)	(40.65)	(27.85)	(43.30)	(53.39)	(22.48)	(53.58)	(22.99)	(36.17)	(40.99)	(62.71)	(36.74)
Ratnagiri-3	0.00	59.49	54.60	36.22	84.68	64.12	78.53	56.95	56.58	56.85	71.27	56.30
	(0.00)	(50.47)	(47.64)	(37.00)	(66.99)	(53.20)	(62.40)	(49.00)	(48.78)	(48.94)	(57.59)	(47.45)
Ratnagiri-2	0.00	18.94	51.32	37.52	24.03	33.77	29.03	19.00	9.84	33.81	28.17	25.95
	(0.00)	(25.80)	(45.75)	(37.77)	(29.35)	(35.53)	(32.60)	(25.84)	(18.28)	(35.55)	(32.05)	(28.96)
Karjat-8	0.00	87.00	66.77	26.21	36.98	77.55	65.21	78.01	67.50	85.72	83.03	61.27
	(0.00)	(68.88)	(54.80)	(30.79)	(37.45)	(61.73)	(53.87)	(62.06)	(55.25)	(67.80)	(65.70)	(50.76)
Panvel-1	0.00	15.19	43.27	28.83	71.10	42.24	42.25	57.24	28.32	71.74	28.95	39.01
	(0.00)	(22.93)	(41.13)	(32.47)	(57.48)	(40.53)	(40.54)	(49.16)	(32.15)	(57.89)	(32.55)	(36.99)
Panvel-2	0.00	28.66	43.78	28.58	74.55	50.84	56.71	57.06	36.74	81.07	40.47	45.31
	(0.00)	(32.37)	(41.42)	(32.32)	(59.71)	(45.48)	(48.85)	(49.06)	(37.31)	(64.21)	(39.51)	(40.93)
Panvel-3	0.00	35.91	55.31	81.80	63.46	31.23	47.15	74.88	67.51	77.81	60.85	54.17
	(0.00)	(36.81)	(48.05)	(64.78)	(52.82)	(33.96)	(43.36)	(59.93)	(55.26)	(61.90)	(51.27)	(46.19)
Range	0.00	15.19	21.83	14.30	8.64	14.63	16.57	15.27	9.84	12.19	23.53	25.95
- Min	(0.00)	(22.93)	(27.85)	(22.22)	(17.01)	(22.48)	(23.99)	(22.99)	(18.28)	(20.42)	(29.02)	(28.96)
- Max	0.00	87.00	87.56	85.65	84.68	80.75	78.72	78.72	79.26	86.93	85.64	67.62
	(0.00)	(68.88)	(69.35)	(67.74)	(66.99)	(63.98)	(62.53)	(62.53)	(62.91)	(68.81)	(67.76)	(54.63)
Mean	0.00	46.83	49.76	48.64	49.21	51.33	50.12	49.00	45.49	60.44	51.63	45.68
	(0.00)	(43.13)	(44.93)	(44.20)	(44.43)	(45.81)	(45.00)	(44.30)	(42.17)	(51.31)	(46.15)	(41.04)

	Variety	Medium	Variety × Medium
SE+	0.24	0.17	0.80
$CD_{0.01}$	0.88	0.62	2.93

The figures in parentheses indicate arcsine transformed value; * See Table 3 for PRG combinations C_1 to C_{11} used

The lowest callus induction frequency was noticed in cv. 'Palghar-1' on MS supplemented with 2.5 mg 2,4-D L^{-1} + 0.5 mg BAP L^{-1} (8.64%). Ahmad *et al.* (2016) observed maximum callus induction frequency in cv. 'Dilrosh-97' (93.3%), 'Fakhre Malakand' (90.6%) and 'JP-5' (91.6%) on MS supplemented with 2.5, 4.0 and 3.5 mg 2, 4-D L^{-1}, respectively. A number of other workers also reported such a large significance of variety × PGR concentration interactions for callus induction frequency (Gowda *et al*., 2009; Anita *et al*., 2012; Sankepally and Singh, 2016).

Panjaitan *et al*. (2009) reported that 2,4-D concentrations play a key role in callus induction as it promotes somatic embryogenesis and plays an important role in cell division in rice. In present study also the use of low concentrations of 2,4-D (1.0-2.0 mg L^{-1}) gave high callus induction frequency. Better callus induction at lower concentrations of 2,4-D as 1-2 mg L^{-1} have previously been reported (Revathi and Pillai, 2011; Mannan *et al*., 2013; Sananda *et al*., 2013; Shukla *et al*., 2014; Amrita *et al*., 2015; Poeaim *et al*., 2016). Verma *et al.* (2011) noted that at higher concentration of 2,4-D callus induction in rice varieties decreased which is in due conformity with present findings. Although 2,4-D alone is capable of inducing callus formation in rice embryos, the addition of some organic substances such as casein hydrolysate, auxin (NAA, IAA) to the callus induction medium containing 2,4-D enhances the efficiency of callus formation (Sripichitt and Cheewasestathum, 1994). Mannan *et al.* (2013) reported high callus induction frequency (95.83%) in a short period (5-7 days) by using 2.0 mg 2,4-D L^{-1} in combination with a cytokinin. In present experiment also the addition of small quantities of auxin NAA and cytokinins as BAP and kinetin in addition to 2,4-D induced earlier and higher callus formation.

Weight of callus: The callus weight noted 30 and 45 days after inoculation revealed the same trend of increase in weight during the period of 15 days but with a slight deviation (Tables 5 and 6). The rice cv. 'Ratnagiri-711' raised on MS medium supplemented with 1.5 mg 2,4-D L^{-1} + 0.5 mg BAP L^{-1} recorded maximum callus weight at 30 days (108.70 mg) and 45 days (269.37 mg) with increase of 160.67 mg within 15 days and found the treatment being significantly superior over other treatments. The variety 'Karjat-8' on MS medium supplemented with 1.0 mg 2,4-D L^{-1} + 0.5 mg BAP L^{-1} surpassed the 2nd superior interaction of cv. 'Ratnagiri-711' with MS supplemented with 2.5 mg 2,4-D L^{-1} + 0.5 mg BAP L^{-1} + 0.1 mg NAA L^{-1} within 15 days by depicting 137.97 mg increase in callus weight from 30 to 45 days of inoculation. The cv. 'Ratnagiri-2' showed minimum increase in callus weight (4.50 mg) on MS medium + 2.5 mg 2,4-D L^{-1} + 0.5 mg kinetin L^{-1} + 0.1 mg NAA L^{-1}. It indicates that optimum PGR concentrations for callus induction also favours callus proliferation. The significance of variety × PGR interactions was again retraced which is evident from Fig. 1. The results are in line with Amrita *et al.* (2015), Poeaim *et al.* (2016) and Poraha *et al*. (2016). Alike callus induction, the variation in callus proliferation at different concentrations of 2,4-D may be attributed to the genetic variation present in endogenous level of hormones in different genotypes (Verma *et al.*, 2011). Sananda *et al.* (2013) reported that the increase in concentration of 2,4-D beyond the limit ($\geq$ 4 mg L^{-1}) may inhibit the proliferation of callus causing necrosis due to toxicity depending upon the varietal characters.

Nature of callus

Characteristics of callus *viz.*, colour, texture or physical appearance play an important role in regeneration ability making it embryogenic or non-embryogenic. Calli of all the 22 varieties of rice formed on the different PGR combinations were yellowish-white in colour (Table 2). Similar results of insignificant callus colour variation among different varieties were reported by Carsono and Yoshida (2006), Ullah *et al.* (2007) and Mannan *et al.* (2013).

The texture of calli showed significant variation on different PGR combinations (Fig. 1). The combinations of MS medium supplemented with 1.0, 1.5 or 2.0 mg L^{-1} 2,4-D in addition to 0.5 mg L^{-1} BAP had copious, compact and regenerable calli. This is in agreement with Libin *et al.* (2012) and Shukla *et al*. (2014). The addition of 0.1 mg NAA L^{-1} to the same combinations resulted in hard and compact calli which were non-regenerative and showed excessive rooting. However, when the concentration of 2,4-D was increased to 2.0 to 2.5 mg L^{-1} while keeping 0.1 mg NAA L^{-1} and 0.5 mg

Table 5: Callus weight (mg) at 30 days of inoculation in different rice varieties with different PGR supplementations

Variety/PGR combinations*	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}	Mean
Ratnagiri-73	0.00	22.13	23.83	47.10	34.87	15.57	21.47	33.63	36.40	43.33	17.37	26.88
Karjat-184	0.00	14.70	26.57	37.57	35.10	13.37	11.70	26.33	21.87	18.50	19.90	20.51
Ratnagiri-24	0.00	13.67	22.50	30.10	7.10	12.63	12.70	18.00	8.80	7.33	16.17	13.55
Ratnagiri-711	0.00	47.10	108.70	53.73	47.43	43.87	48.17	34.87	103.63	50.27	39.77	52.50
Ratnagiri-1	0.00	11.13	12.80	24.53	6.20	12.57	8.70	18.47	8.03	7.20	14.47	11.28
Karjat-3	0.00	85.43	31.83	18.33	7.17	47.63	37.60	46.47	7.67	7.57	16.27	27.82
Karjat-4	0.00	66.83	31.93	19.40	5.77	46.23	30.60	44.13	7.10	6.70	17.47	25.11
Phondaghat-1	0.00	47.83	31.50	20.73	24.20	62.57	88.23	26.47	26.17	37.53	26.90	35.65
Karjat-7	0.00	77.97	25.73	12.07	10.33	17.87	13.93	49.40	9.37	41.13	3.40	23.75
Ratnagiri-5	0.00	10.63	20.90	23.40	4.50	9.73	7.53	13.97	6.87	6.70	13.43	10.70
Palghar-1	0.00	12.77	13.47	0.00	5.57	10.90	22.70	0.00	0.00	0.00	6.33	6.52
Palghar-2	0.00	13.67	22.23	13.30	7.63	11.47	27.47	13.43	0.00	4.10	7.40	10.97
Karjat-5	0.00	26.50	26.63	17.93	9.87	28.93	23.57	14.50	13.80	26.40	13.70	18.35
Karjat-6	0.00	23.40	27.37	17.40	8.40	26.60	22.70	14.50	10.67	26.60	9.87	17.05
Ratnagiri-4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Karjat-2	0.00	31.73	7.43	7.27	32.50	10.80	47.83	5.63	44.23	12.90	27.87	20.75
Ratnagiri-3	0.00	25.33	84.43	24.07	33.47	23.73	61.17	7.27	9.30	47.07	12.20	29.82
Ratnagiri-2	0.00	49.17	23.93	44.50	10.47	33.77	54.00	48.33	7.73	10.47	7.67	26.37
Karjat-8	0.00	97.23	46.23	77.93	36.83	46.87	33.27	35.70	35.77	95.43	45.80	50.10
Panvel-1	0.00	31.40	24.53	13.10	10.50	33.47	24.67	24.97	38.87	22.77	48.27	24.78
Panvel-2	0.00	36.90	54.77	47.00	57.50	38.53	37.43	35.67	32.97	67.00	54.07	41.98
Panvel-3	0.00	36.57	32.40	16.60	13.00	36.57	25.87	24.00	38.17	25.33	48.57	27.01
Range - Min	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
- Max	0.00	97.23	108.70	77.93	57.50	62.57	88.23	49.40	103.63	95.43	54.07	52.50
Mean	0.00	35.55	31.81	25.73	18.56	26.53	30.06	24.35	21.25	25.65	21.22	23.70

	Variety	Medium	Variety × Medium
SE+	0.22	0.16	0.73
$CD_{0.01}$	0.81	0.57	2.68

*See Table 3 for PRG combinations C_1 to C_{11} used

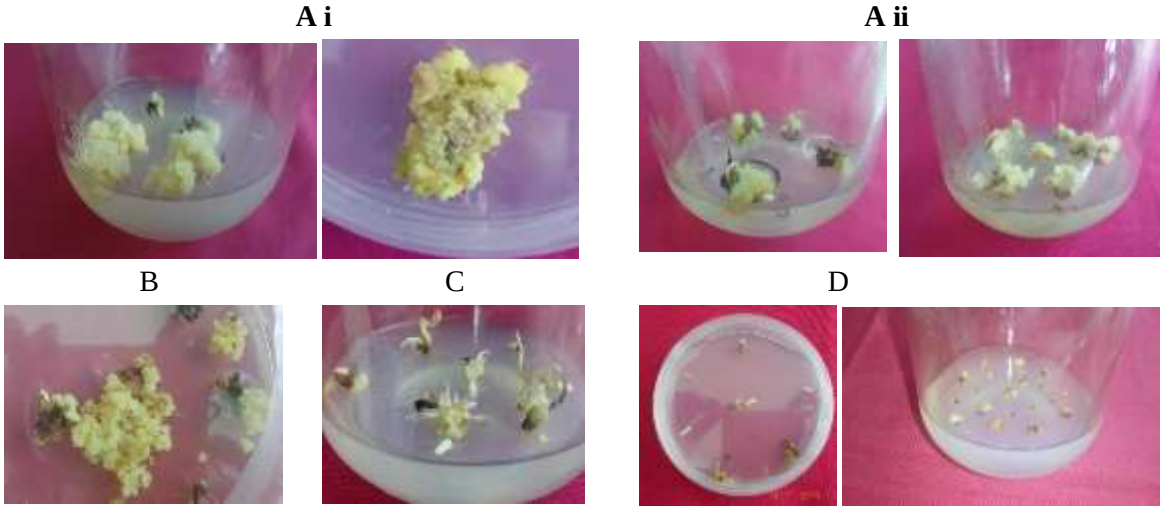

Fig. 1: Genotype-specificity in rice callus formation and nature of calli with different PGR supplementations A) Compact and embryogenic callus: i. cv. 'Ratnagiri-711' on MS + 1.5 mg 2,4-D L^{-1} + 0.5 mg BAP L^{-1}; ii. cv. 'Karjat-8' on MS + 1.0 mg 2,4-D L^{-1} + 0.5 mg BAP L^{-1}; **B) Loose, friable and embryogenic callus: c**v. **'**Karjat-7' on MS + 2.5 mg 2,4-D L^{-1} + 0.5 mg BAP L^{-1} + 0.1 mg NAA L^{-1}; **C) More compact, non-embryogenic callus (excessive rooting)** cv. 'Karjat-7' on MS + 1.5 mg 2,4-D L^{-1} + 0.5 mg BAP L^{-1} + 0.1 mg NAA L^{-1}; **D) Poor callus formation: cv. '**Palghar-1' on MS + 1.5 mg 2,4-D L^{-1} + 0.5 mg BAP L^{-1} + 0.1 mg NAA L^{-1}

Table 6: Callus weight (mg) at 45 days of inoculation in rice varieties raised on MS medium supplemented with different PGR combinations

Variety/PGR combinations*	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}	Mean
Ratnagiri-73	0.00	40.63	54.07	104.93	84.60	32.67	38.43	72.63	79.37	99.10	68.27	61.34
Karjat-184	0.00	32.63	56.80	72.10	77.77	25.83	29.97	53.13	78.10	71.57	75.10	52.09
Ratnagiri-24	0.00	31.03	48.17	64.77	13.80	28.53	22.73	36.23	19.87	16.40	35.77	28.85
Ratnagiri-711	0.00	106.07	269.37	135.63	77.73	93.63	102.27	79.70	230.53	127.13	87.33	119.04
Ratnagiri-1	0.00	24.43	43.40	52.87	11.93	23.40	18.53	32.13	15.93	14.43	33.80	24.62
Karjat-3	0.00	194.59	80.17	43.37	12.90	105.93	75.53	103.77	16.63	15.10	39.43	62.49
Karjat-4	0.00	163.97	75.77	42.13	11.40	95.93	68.93	92.60	15.57	14.50	35.20	56.00
Phondaghat-1	0.00	131.70	80.03	47.27	52.70	156.43	195.17	56.50	55.53	74.93	55.77	82.37
Karjat-7	0.00	181.27	53.10	37.43	28.80	86.23	36.67	158.07	22.47	85.13	15.87	64.09
Ratnagiri-5	0.00	22.07	41.77	47.60	11.20	22.50	16.67	26.27	15.00	14.10	30.83	22.55
Palghar-1	0.00	25.87	36.87	28.70	13.47	23.60	43.43	26.00	14.03	30.03	14.73	23.34
Palghar-2	0.00	30.47	44.70	29.70	15.23	26.73	47.77	31.93	17.43	35.67	17.83	27.04
Karjat-5	0.00	55.33	56.23	42.00	24.40	58.87	48.47	36.27	25.17	55.37	27.63	39.07
Karjat-6	0.00	47.37	53.80	38.93	17.97	53.60	43.30	32.40	22.30	51.27	22.50	34.86
Ratnagiri-4	0.00	17.20	25.87	15.57	8.87	19.87	10.10	14.40	9.77	24.77	9.53	14.18
Karjat-2	0.00	69.53	18.10	13.17	70.20	16.13	118.30	12.47	91.73	35.80	60.00	45.95
Ratnagiri-3	0.00	53.70	189.23	54.50	85.83	46.77	171.73	14.83	18.20	123.63	30.43	71.72
Ratnagiri-2	0.00	142.20	48.93	96.80	20.20	65.00	140.17	90.87	12.87	18.40	12.17	58.87
Karjat-8	0.00	235.20	112.10	178.80	84.03	101.57	63.40	67.87	84.63	209.67	111.80	113.55
Panvel-1	0.00	63.90	52.63	33.00	19.60	61.97	47.97	45.13	87.03	44.03	102.67	50.72
Panvel-2	0.00	73.33	149.13	113.63	170.50	87.73	79.30	73.53	61.30	164.57	157.23	102.75
Panvel-3	0.00	68.43	57.43	35.77	24.97	68.67	52.73	46.90	84.43	49.03	102.30	53.70
Range - Min	0.00	17.20	18.10	13.17	8.87	16.13	10.10	12.47	9.77	14.10	9.53	14.18
- Max	0.00	235.20	269.37	178.80	170.50	156.43	195.17	158.07	230.53	209.67	157.23	119.04
Mean	0.00	82.31	74.89	60.39	42.64	59.16	66.89	54.71	49.00	62.48	52.10	54.96

	Variety	Medium	Variety × Medium
SE+	0.95	0.67	3.15
$CD_{0.01}$	3.48	2.46	11.53

*See Table 3 for PRG combinations C_1 to C_{11} used

BAP L^{-1} constant; the soft, friable and regenerative calli were formed indicating the necessity of proper balance in auxin and cytokinin for callus to be regenerative. Further, on replacing 0.5 mg BAP L^{-1} by 0.5 mg kinetin L^{-1}, in addition to the increase in 2,4-D concentrations by 2.0 to 2.5 mg L^{-1}, somewhat mucilaginous calli, lesser in regenerative capacity were obtained. Similar findings with respect to types of calli textures were reported by Visarada *et al.* (2002). Sankepally and Singh (2016) observed differential response from different rice varieties regarding the nature of callus in terms of colour and texture on the same PGR combinations as evident from the excellent creamish coloured embryoids in 'Sambha mahsuri' whereas a soft, friable, brown coloured callus in 'PR-116' on MS supplemented with 3.0 mg 2,4-D L^{-1}. This again could be attributed to the diverse parental and genetic backgrounds of the two varieties.

Conclusion: In present study the most effective surface sterilization of dehusked mature rice seeds was achieved by treating seeds with 70% ethanol for 1 min followed by $HgCl_2$ (1.0%) for 10 min and removing the traces of $HgCl_2$ by 4-5 times washing with double distilled water, thus establishing maximum aseptic cultures without any detrimental effect on explant. *In vitro* callus induction ability of different rice varieties varied significantly with plant growth regulator combinations due to the genotypic characters specifying the different internal level of hormones. The rice cv. 'Ratnagiri-711' and 'Karjat-8' were superior in callusing ability with excellent yellowish-white embryogenic callus. The 2,4-D concentration @ 1.0-2.0 mg L^{-1} in combination with 0.5 mg BAP L^{-1} was most effective for embryogenic callus induction and proliferation.

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REFERENCES

Abe, T. and Futsuhara, Y. 1986. Genotypic variability for callus formation and plant regeneration in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics*, **72**: 3-10.

Ahmad, M., Nangyal, H., Imran, M. and Ullah, F. 2016. Optimization of protocol for surface sterilization and callus induction for three rice varieties. *American-Eurasian Journal of Agricultural and Environmental Sciences*, **16**(2): 357-361.

Alam, M.F. 1994. *Protoplast Culture and Transformation in Rice (Oryza sativa L.).* Ph.D. (Genetics) Thesis. Faculty of the Graduate School, University of the Philippines, Los Banos, Philippines.

Amrita Mukherjee, Islam Mohd., R., Nasiruddin, K.M. and Banerjee, Purba 2015. Callus initiation and plantlet regeneration ability of some rice genotypes. *International Journal of Scientific and Technology Research*, **4**(10): 354-361.

Anita, Roy, Aich, S.S. and Mukherjee, S. 2012. Differential responses to indirect organogenesis in rice cultivars. *International Journal of Scientific and Research Publications*, **2**(9): 1-5.

Anonymous. 2016. FAOSTAT. [*http://www.fao.org/faostat/en/#data/QC/visualize*].

Carsono, N. and Yoshida, T. 2006. Identification of callus induction potential of 15 Indonesian rice genotypes. *Plant Production Science*, **9**(1): 65-70.

Chen, T.H., Lam, L. and Chen, S.C. 1985. Somatic embryogenesis and plant regeneration from cultured young inflorescences of *Oryza sativa* L. (rice). *Plant Cell Tissue and Organ Culture*, **4**: 41-54.

Datta, K., Datta, I. and Potrykus. 1990. Embryogenesis and plant regeneration from microspores of both indica and japonica rice. *Plant Cell Reports*, **9**: 253-356.

DeFossard, R.A. 1986. Principles of plant tissue culture. pp. 1-13. **In**: *Tissue Culture as a Plant Production System for Horticultural Crops.* (ed. Zimmerman R.H., Griesbach R.J., Hammerschlag F.A., Lawson R.H.), Martinus Nijhoff Publ., Dordrecht, Netherlands.

Fujiwara, A. and Yatazama, M. 1964. Tissue culture in rice improvement: Status and potential. *Advances in Agronomy*, **41**: 339-297.

Gomez, K.A. and Gomez, A.A. 1984. *Statistical Procedure for Agricultural Research*. (2nd edn.). John Wiley and Sons, New York, USA.

Gowda, H.R., Krishna, V., Kumara Swamy, G.K., Ramachandra, R. and Gowda, T.K.S. 2009. Standardization of regeneration protocol for *indica* rice varieties Rasi and IR-20. *Mysore Journal of Agricultural Sciences*, **43**(3): 412-419.

Kataky, A. and Handique, P.J. 2010. Standardization of sterilization techniques prior to *in vitro* propagation of *Andrographis paniculata. Asian Journal of Science and Technology*, **6**: 119-122.

Libin, A., King, P.J.H., Ong, K.H., Chubo, J.K. and Sipen, P. 2012. Callus induction and plant regeneration of Sarawak rice (*Oryza sativa* L.) variety Biris. *African Journal of Agricultural Research*, **7**(30): 4260-4265.

Mannan, M.A., Sarker, T.C., Akhter, M.T., Kabir, A.H. and Alam, M.F. 2013. Indirect plant regeneration in aromatic rice (*Oryza sativa* L.) var. 'Kalijira' and 'Chinigura'. *Acta Agriculturae Slovenica*, **101**(2): 231-238.

Morrish, F., Vasil, V. and Vasil, I.K. 1987. Developmental morphogenesis and genetic manipulation in tissue and cell cultures of the gramineae. *Advances in Genetics*, **24**: 432-499.

Murashige, T., and Skoog, F. 1962. A revised medium for rapid growth and bioassay of tobacco tissue culture. *Physiologia Plantarum*, **15**: 473-497.

Nishi, T., Yamada, Y. and Takahashi, E. 1968. Organ differentiation and plant regeneration in rice callus. *Nature*, **219**: 508-509.

Oyebanji, O.B., Nweke, O., Odebunmi, O., Galadima, N.B., Idris, M.S., Nnodi, U.N., Afolabi, A.S. and Ogbadu, G.H. 2009. Simple, effective and economical explant-surface sterilization protocol for cowpea, rice and sorghum seeds. *African Journal of Biotechnology*, **8**: 5395-5399.

Panjaitan, S.B., Abdullah, S.N.A., Aziz, M.A., Meon, S. and Omar, O. 2009. Somatic embryogenesis from scutellar embryo of *Oryza sativa* L. var. MR219. *Pertanika Journal of Tropical Agricultural Science*, **32**: 185-194.

Poeaim, A., Poeaim, S., Poraha, R., Pongjaroenkit, S. and Pongthongkam, P. 2016. Optimization for callus induction and plant regeneration from mature seeds of Thai rice variety: Nam Roo (*Oryza sativa* L.) *Bioengineering and Bioscience*, **4**(5): 95-99.

Poraha, R., Poeaim, A., Pongjaroenkit, S. and Pongthongkam, P. 2016. Callus induction and plant regeneration on optimization of the culture conditions in Jow Haw rice (*Oryza sativa* L.). *International Journal of Agricultural Technology*, **12**(2): 241-248.

Raghawa Ram, N.V. and Nabors, M.W. 1984. Cytokinin mediated long term, high frequency plant regeneration in rice tissue cultures. *Zeitschriftfur Pflanzenphysiologie*, **113**: 315-323.

Ramesh, M., Murugiah, V. and Gupta, A.K. 2009. Efficient *in vitro* plant regeneration via leaf base segments of indica rice (*Oryza sativa* L.). *Indian Journal of Experimental Biology*, **47**: 68-74.

Revathi, S. and Pillai, M.A. 2011. *In vitro* callus induction in rice (*Oryza sativa* L.) *Research in Plant Biology*, **1**(5): 13-15.

Sah, S.K., Ajinder Kaur and Sandhu, J.S. 2014. High frequency embryogenic callus induction and whole plant regeneration in Japonica rice cv. Kitaake. *Journal of Rice Research*, **2**(2): 1-5.

Sananda Mondal, Singh, R.P. and Bose, Bandana. 2013. Standardization of culture medium for somatic embryogenesis of rice var. MTU 7029. *International Journal of Bio-resource and Stress Management*, **4**(4): 500-505.

Sankepally, S.S.R. and Singh, B. 2016. Optimization of regeneration using differential growth regulators in indica rice cultivars. *Biotech (Protocols and Methods)*, **6**: 1-19.

Shukla, R., Dube, A. and Koshy, E.P. 2014. Production of high quality embryogenic callus of rice. *Bioscan*, **9**(3): 1077-1080.

Sripichitt, P. and Cheewasestathum, P. 1994. Plant regeneration from embryo derived callus of aromatic rice (*Oryza sativa* L.) variety Khao Dawk Mali 105. *Kasetsart Journal (Natural Science)*, **28**: 27-37.

Ullah, H., Ullah, I., Jadoon, S.A. and Rashid, H. 2007. Tissue culture techniques for callus induction in rice. *Sarhad Journal of Agriculture*, **23**(1): 81-86.

Verma, D., Joshi, R., Shukla, A. and Kumar, P. 2011. Protocol for *in vitro* somatic embryogenesis and regeneration of rice (*Oryza sativa* L.). *Indian Journal of Experimental Biology*, **49**: 958-963.

Visarada, K.B.R.S., Sailaja, M. and Sarma, N.P. 2002. Effect of callus induction media on morphology of embryogenic calli in rice genotypes. *Biologia Plantarum*, **45**: 495-502.