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EFFECT OF DIFFERENT MEDIA AND SOLIDIFYING AGENTS ON CALLOGENESIS AND PLANT REGENERATION FROM DIFFERENT EXPLANTS OF RICE (*ORYZA SATIVA* L) VARIETIES SUPER BASMATI AND IRRI-6

HUMERA AFRASIAB* AND RABIA JAFAR

Department of Botany,
University of the Punjab, Lahore, Pakistan.
**Corresponding author E-mail: nida13pk@yahoo.com*

Abstract

In the present work, plant tissue culture conditions were optimized for *In vitro* germination from dehusked seeds of two varieties of rice (*Oryza sativa* L), Super Basmati and IRRI-6 on MS and LS medium solidified with agar and phytagel. MS medium solidified with agar was optimum for *In vitro* germination of Super Basmati and LS medium containing agar was best for germination of IRRI-6. For best callus induction and proliferation from mature embryos of both varieties and leaf bases of IRRI-6, MS medium supplemented with 2.0 mg/l 2, 4-D was efficient, while leaf bases of Super Basmati required a higher concentration of 2,4-D. For efficient regeneration from callus, MS medium supplemented with NAA 1.0 mg/l with BAP 3.0 mg/l proved better for Super Basmati and for IRRI-6, MS medium containing NAA 1.0 mg/l and BAP 5.0 mg/l showed better results. Light conditions proved better for callogenesis and regeneration from mature seed explants of both the varieties in MS medium supplemented with 2.0 mg/l 2, 4- D.

Introduction

Rice (*Oryza sativa* L.) is one of the leading food crops of the world and is a staple food for a large part of the world's population, making it the second-most consumed cereal grain after wheat (Anon., 2006). Rice provides more than one fifth of the calories consumed by population worldwide. Due to increasing world population, rate of rice eaters is increasing day by day and the number of rice consumers will probably be twofold by the year 2020 (Kabir *et al*., 2008).

Pakistan is well known for its Basmati rice, as well as for its non- basmati *indica* varieties. Pakistani Basmati rice is world famous and enjoys monopoly in the international market due to its quality characteristics, strong aroma, slender and long kernel, gelatinization, temperature and high degree of grain elongation on cooking. The famous basmati varieties include Super Basmati, Kernel Basmati and Basmati 385 and non-basmati varieties are IRRI-6, IRRI-9, KS-282, DR-82, DR-83 etc (Anon., 2008).

The area under rice production at the time of independence was 883 thousand hectares with an average production of 768 thousand tons. In 2007-08, an area of 2.515 million hectares was brought under rice crop with 2,205kg per hectare produce. This year, the area was increased to 2.594 million hectare showing a three percent increase in the area under the rice crop (Anon., 2008).

Although Basmati and non-Basmati rice varieties of Pakistan are economically important, however average yield is lower as compared with other countries. Besides, cultural practices, the main reason for low productivity in Pakistan is that, the local varieties are susceptible to different biotic and abiotic factors like insect pests, diseases,

drought and salinity. Therefore, varietal improvement of rice for resistance against insects, pathogens and other stresses including nutritional quality are necessary. Different approaches have been undertaken in Pakistan to tackle these problems through traditional breeding of selection and crossing and mutation breeding which has played an important role (Bashir, *et al*., 2007).

During the past few decades techniques of tissue culture, like anther culture, protoplast fusion, leaf culture, root culture and dehusked grain culture are being employed in rice breeding to exploit somaclonal variation for the creation of novel rice varieties (Dorosieve, 1996, Sikder *et al*., 2006). The higher percentage of regenerated plants obtained by the culture of somatic tissue shows desirable genetic changes such as disease resistance, salt tolerance and other physiological properties (Kucherenko, 1994). Several high yielding rice varieties were developed through the application of anther culture in China (Ying, 1983). In Pakistan Rachna Basmati is a useful example of genetic variation produced through tissue culture (Abbasi, 2000).

Although there are a few reports available on callus induction, regeneration and transgenic plant production in some Basmati varieties (Rashid *et al*., 1996, 2001, 2004), but still there is a strong need for studies on developing high frequency regeneration from commercial grown rice varieties of Pakistan.

By considering all these aspects, the following research work was intended to develop a reproducible and an efficient procedure for callus induction and plant regeneration for two varieties of rice (Super Basmati and IRRI-6).

Materials and Methods

Seeds of two varieties of rice (*Oryza sativa* L.) Super Basmati and IRRI-6 were obtained from Punjab Seed Corporation, Lytton Road, Lahore.

***In vitro* germination:** The dehusked seeds of both the varieties were inoculated for *in vitro* germination in MS (Murashige & Skoog 1962) and LS (Linsmaier & Skoog 1965) media solidified with agar and phytagel. The test tubes were then placed in culture room at 16 hour photoperiod with 2000lux light at 27±1ºC day and 25±1ºC night temperature.

Callogenesis: Callus was initiated from two explants, leaf bases and mature embryos in MS and LS medium supplemented with different concentrations of 2,4-D alone and in combination with BAP and Kinetin.

Regeneration: The well-proliferated callus produced from two rice varieties (Basmati and IRRI-6) from the best selected callus inducing media were transferred to regeneration media comprising MS medium supplemented with different concentrations of NAA and BAP, to study the regeneration of plants.

Effect of dark and light conditions: For callus induction, mature seeds as explants from both the varieties (Super Basmati and IRRI-6) were cultured in MS medium supplemented with growth regulators and incubated under light (35 µmole m^{-2} s^{-1}) and dark conditions (24 h dark) at 25±2ºC. The data was collected for callus induction percentage after 4-6 weeks of inoculation.

Results

***In vitro* germination response of two varieties of rice (*Oryza sativa* L.) on different media using different solidifying agents:** The data given in Table 1 shows that the germination response of Super Basmati was best (100%) in MS (A) medium as compared to IRRI-6 (77%). In MS (P) medium Super Basmati showed 96% germination response, while 68% germination was observed for IRRI-6.

In LS (A) medium the germination frequency of Super Basmati was observed to be 93.3% while that of IRRI-6 was 90%, while both the varieties showed similar %age germination (Super Basmati 85.7% and IRRI-6 84.6%) in LS medium solidified with phytagel. Better shoot length and number of roots were observed in IRRI-6 as compared to Super Basmati in which better root length was observed in both the media tried. It can be concluded from all the given data that MS medium solidified with agar is the best medium for *in vitro* germination studies for Super Basmati and LS medium containing agar is better for germination of IRRI-6.

Callus induction response of two varieties of rice (*Oryza sativa* L.), Super Basmati and IRRI-6, on different media

Effect of different concentrations of 2, 4-D alone, and in combination with BAP and Kinetin in MS medium on callus induction and proliferation in Super Basmati: In Super Basmati, mature embryos of Super Basmati showed highest callus induction response (63.33%) in MS medium supplemented with 2.0 mg/l 2, 4-D (Fig. 1a). In case of leaf bases, maximum response (50%) was observed in MS medium containing 5.0 mg/l 2,-4 D (Table 2). Calli obtained from leaf bases were somewhat friable and granular and well-proliferated while yellowish white, compact and granular calli were observed from mature embryos.

Among the four different concentrations of BAP tried in combination with 2.0 mg/l 2, 4-D, 40% callogenic response was exhibited by mature embryos on MS medium supplemented with 2.0 mg/l BAP. For leaf bases, 1.0 mg/l BAP + 2.0 mg/l 2, 4-D proved better, giving highest callus induction frequency (50%) as presented in Table 2. Calli produced from mature embryos was yellowish, mucilaginous and somewhat compact, while from the leaf bases were granular, light yellowish and friable.

In MS basal medium supplemented with different concentrations of Kinetin with 2.0 mg/l 2, 4-D, and the highest callus induction (53.33%) was noted for mature embryos of Super Basmati at 1.0 mg/ Kinetin, while 51.66% callogenic response was shown by the leaf bases in the same medium (Fig. 1b). The callus produced was mucilaginous and white in embryo explants and granular and proliferated in leaf bases.

Effect of different concentrations of 2, 4-D alone, and in combination with BAP and Kinetin in MS medium on callus induction and proliferation in IRRI-6: The data presented in the Table 3 shows that the best callus induction percentage (90%) was observed in MS medium supplemented with 2.0mg/l 2, 4-D by mature embryos, and maximum of 50% response was also shown by leaf explants in the same medium (Table 3). Callus produced from leaf bases was friable, yellowish and proliferating, while callus initiated from mature embryos showed granular structures.

Table 1. Study of *In vitro* germination response of two varieties of rice (*Oryza sativa* L.) Super Basmati and IRRI-6 on different media using different solidifying agents.

Media used	Percentage germination	Average no. of roots	Average no. of leaves	Average root length (cm)	Average shoot length (cm)
			Super Basmati		
*MS(A)	100	2.40 ± 0.11	0.96 ± 0.03	2.61 ± 0.42	5.15 ± 0.21
** MS(P)	96	1.06 ± 0.17	1.0 ± 00	4.12 ± 1.63	1.29 ± 0.47
***LS(A)	93.3	2.50 ± 0.17	1.0 ± 00	2.85 ± 0.67	6.12 ± 0.36
****LS(P)	85.7	2.00 ± 0.05	1.0 ± 00	1.96 ± 0.53	3.79 ± 0.41
			IRRI-6		
*MS(A)	77	2.56 ± 0.50	2.00 ±00	1.95 ± 0.28	5.16 ± 0.50
** MS(P)	68	1.30 ± 0.21	1.00 ±00	1.30 ± 0.66	5.12 ± 0.23
***LS(A)	90	3.23 ± 0.20	1.40 ± 0.20	2.93 ± 0.37	6.24 ± 1.12
****LS(P)	84.6	2.16 ± 0.32	0.86 ± 0.20	1.49 ± 0.26	4.68 ± 0.65

*MS (A): Murashige & Skoog (1962) medium with Agar.
**MS (P): Murashige & Skoog (1962) medium with phytagel.
***LS (A): Linsmaier & Skoog (1965) medium with Agar.
**** LS (P): Linsmaier & Skoog (1965) medium with phytagel.
±: the standard error applied on the data.

Table 2. Effect of different plant growth regulators on callogenic response of *Oryza sativa* variety Super Basmati in MS medium.

Medium	Growth regulators concentrations	Embryos		Leaf bases	
		% Callus induction	Callus morphology	% Callus induction	Callus morphology
MS + 2,4-D	Control	00.00±0.00	No callus	00.00±0.00	No callus .
	1	56.66±5.91	Yellowish compact	16.33±3.85	White, friable,
	2	63.33±6.79	Off-whitish	23.33±4.35	Off-white
	3	43.33±6.78	Crystalline	23.33±3.85	Proliferated
	4	36.66±6.57	Yellowish-white	36.66±5.69	White & friable
	5	23.33±3.85	Transparent	50.00±4.18	Granular
	6	16.66±4.00	Compact	30.33±3.93	Mucilaginous
	7	10.00±00	Granular	23.00±2.67	Friable
	8	10.00±10.01	Friable	10.00±4.00	Mucilaginous
MS + 2,4-D (2.0 mg/l) + BAP	1	36.66±7.52	Yellowish mucilaginous, friable	50.00±2.00	Off-white, friable
	2	40.00±7.03	Compact	38.33±2.43	whitish
	3	31.66±9.16	Granular	28.33±1.52	Yellowish
	4	23.33±4.03	Whitish	15.00±4.59	Crystalline
MS + 2,4-D (2.0 mg/l)+ Kinetin	1	53.33±9.28	embryogenic mucilaginous	51.66±5.51	Granular,
	2	36.66±1.41	Non-embryogenic	31.66±3.34	Yellowish
	3	40.00±4.23	Mucilaginous	45.00±8.18	Friable
	4	26.66±3.93	White	21.66±5.12	Transparent

± represents standard error applied on the data

MS basal medium containing BAP in various concentrations along with 2.0 mg/l 2, 4-D showed that the concentration of BAP at 1.0 mg/l was more effective as compared to others, giving a maximum of 70% callus formation by mature embryos (Table 3). Developed calli were compact, whitish, and morphogenic, while with leaf base explants only 15% callus induction was observed at 3.0 mg/l BAP with mucilaginous and proliferating calli. Among all the concentrations tried, 2.0 and 3.0 mg/l Kinetin along with 2.0 mg/l 2, 4-D, gave 30% callus induction frequency with embryos as mentioned in Table 3. The leaf base explants at 2.0 mg/l of Kinetin gave best results (50%). Calli developed from mature embryos were yellowish and granular (Fig. 1c), while callus obtained from leaf base was yellowish, compact and mucilaginous (Fig. 1d).

Table 3. Effect of different plant growth regulators on callogenic response of different explants of *Oryza sativa* variety IRRI-6 in MS medium.

Medium	Growth regulators concentrations (mg/l)	Embryos		Leaf bases	
		% Callus induction	Callus morphology	% Callus induction	Callus morphology
MS + 2,4-D	Control	00.00±0.00	No callus	00.00±0.00	No callus
	1	50±3.85	Compact, yellowish, granular	20±5.40	Friable, yellowish-white
	2	90±2.16	Granular	50±4.81	Friable
	3	70±7.40	Off-whitish	30±1.17	Mucilaginous
	4	30±3.85	Mucilaginous	20±1.17	Proliferating
	5	20±.00	Crystalline	10±7.39	Granular
	6	10±1.68	Compact	10±5.40	Off-white
	7	10±.00	Compact	10±1.54	Friable
	8	10±.00	Proliferating	10±4.17	Friable
MS + 2,4-D (2.0 mg/l) + BAP	1	70±1.98	Whitish, embryogenic	10±10.36	Mucilaginous, proliferating
	2	50±1.44	Non-embryogenic	10±9.51	Friable
	3	10±5.58	Mucilaginous	15±1.24	Proliferating
	4	10±1.54	proliferating	10±5.68	Compact
MS + 2,4-D (2.0 mg/l)+ Kinetin	1	10±5.38	Yellowish, granular & mucilaginous	30±3.04	Yellowish, compact & mucilaginous
	2	30±1.54	Compact	50±4.81	Mucilaginous
	3	30±4.59	Whitish	10±6.20	Friable
	4	10±1.77	mucilaginous	10±5.35	Friable

± represents standard error applied on the data

Table 4. Effect of different plant growth regulators on callogenic response of different explants of *Oryza sativa* variety Super Basmati in LS medium.

Medium	Growth regulators concentrations	Embryos		Leaf bases	
		% Callus induction	Callus morphology	% Callus induction	Callus morphology
LS + 2,4-D	Control	00.00±0.00	No callus	00.00±0.00	No callus
	1	16.33±7.52	Granular friable.	36.66±2.70	Off-white, compact and mucilaginous.
	2	20.66±3.85	Compact	35.00±6.65	Proliferated
	3	23.33±3.84	Yellowish	30.00±6.32	Whitish
	4	63.33±3.52	Mucilaginous	30.00±6.32	Mucilaginous
	5	43.33±2.71	Granular	26.66±4.72	Mucilaginous
	6	36.66±7.51	Compact	23.33±3.93	Granular
	7	26.66±1.52	Morphogenic	23.33±6.57	Crystalline
	8	26.66±4.59	Whitish	15.00±4.81	Friable
LS + 2,4-D (2.0 mg/l) + BAP	1	45.00±7.03	Yellowish-white, non- Morphogenic, proliferated	15.00±10.08	Non-proliferated, Mucilaginous.
	2	40.00±1.94	Compact	40.00±4.03	Granular
	3	31.66±6.64	Granular	38.33±3.69	Proliferated
	4	18.33±6.39	Mucilaginous	20.00±5.00	Mucilaginous
LS + 2,4-D (2.0 mg/l)+ Kinetin	1	26.66±3.85	Brownish, Compact	50.00±1.72	Friable off- white
	2	50.00±3.77	Mucilaginous	48.33±4.90	Proliferated
	3	26.66±3.86	Mucilaginous	23.33±3.52	Friable
	4	23.33±3.54	Compact	10.00±00	Whitish

± represents standard error applied on the data

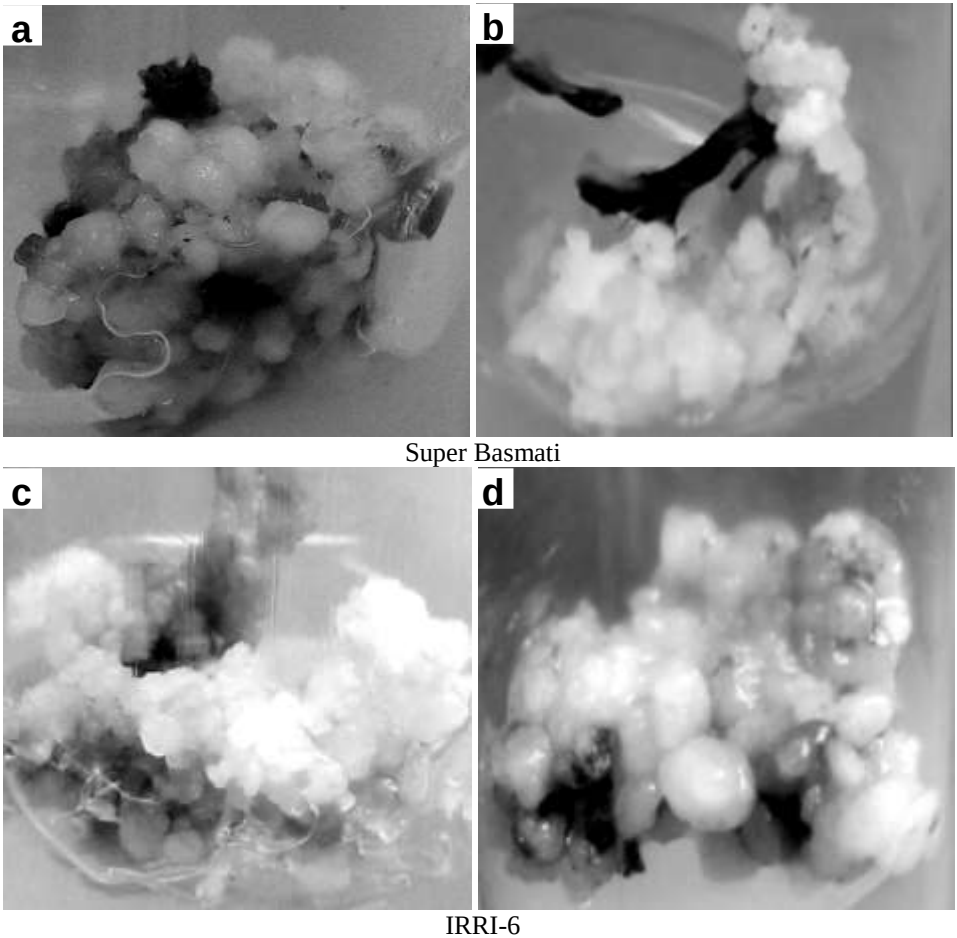


Fig. 1. **(a)** Best response for callus induction and proliferation was obtained from mature embryo explants in MS medium supplemented with 2.0 mg/l 2,4-D and from **(b)** leaf base explants in MS medium containing 2.0 mg/l 2, 4-D with 1.0 mg/l Kinetin in Super Basmati.**(c)** Maximum callus induction and proliferation was observed from mature embryo explants in MS medium supplemented with 2.0 mg/l 2,4-D and from **(d)** leaf base explants in MS medium containing 2.0 mg/l 2, 4-D with 2.0 mg/l Kinetin in IRRI-6.

Effect of different concentrations of 2, 4-D alone, and in combination with BAP and Kinetin in LS medium on the callus induction and proliferation in Super Basmati: According to the data given in Table 4, 4.0 mg/l 2, 4-D gave maximum callus induction frequency (63.33%) from mature embryos (Fig. 2a). In case of leaf base explants, best callus induction response was found in medium containing 1.0 mg/l 2, 4-D giving 36.66% callus response. Granular and friable calli were observed from mature embryos and off-white, compact and mucilaginous calli were observed for leaf base explants. The results of experiments showed that when combination of growth regulators were used, embryo explants showed maximum response (45%) to callus formation in LS medium supplemented with 1.0 mg/l BAP along with 2.0 mg/l 2,4-D (Table 4).

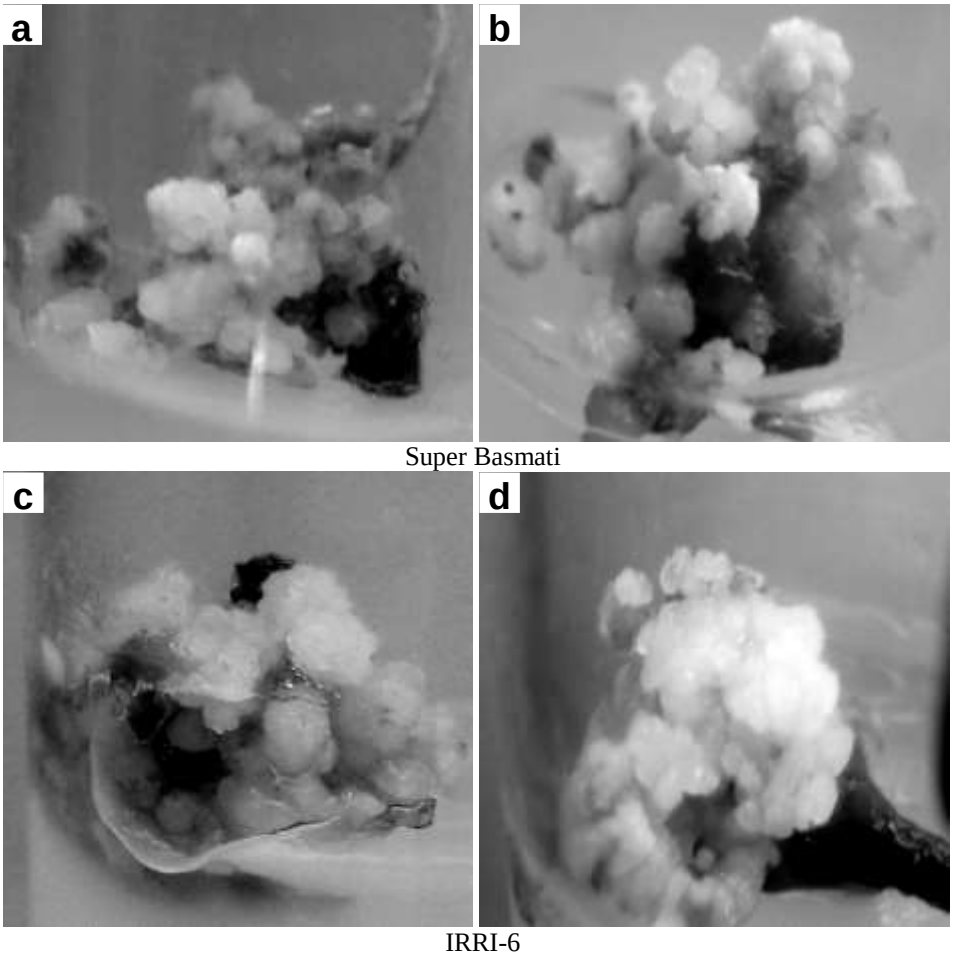


Fig. 2. **(a)** Maximum callus induction and proliferation was obtained from mature embryo explants in LS medium supplemented with 4.0 mg/l 2,4-D and from **(b)** leaf base explants in LS medium containing 2.0 mg/l 2, 4-D with 1.0 mg/l Kinetin in Super Basmati (**c)** Best callus induction and proliferation response was obtained from mature embryo explants in LS medium supplemented with 1.0 mg/l 2,4-D and from **(d)** leaf base explants in LS medium containing 3.0 mg/l 2, 4-D in IRRI-6.

Yellowish–white, non-morphogenic and proliferated calli were developed from mature embryos. Maximum of 40% callus induction frequency was observed for leaf bases in LS medium supplemented with 2.0 mg/l BAP with 2.0 mg/l 2, 4-D. Non-proliferated, mucilaginous calli were produced by leaf bases.

Maximum callus induction frequency (50%) was observed in LS medium containing 2.0 mg/l 2, 4-D with 2.0 mg/l Kinetin from mature embryos as presented in Table 4. Brownish, compact and morphogenic calli were observed from mature embryos. Leaf bases also showed 50% callus induction frequency but the concentration of Kinetin was lower i.e., at 1.0 mg/l along with 2.0 mg/l 2, 4-D. Friable, off-white calli were given by leaf bases (Fig. 2b , Table 4).

Effect of different concentrations of 2, 4-D alone, and in combination with BAP and Kinetin in LS medium on the callus induction and proliferation in IRRI-6: It is evident from the data given in the Table 5 that 1.0 mg/l 2, 4-D gave the maximum callus induction percentage (60%) from mature embryos (Fig. 2c). The maximum callus response (50%) from leaf bases was observed at 3.0 mg/l 2, 4-D (Fig. 2d). Calli were light yellow to off-white in color and were compact from embryo explants, while non-morphogenic and proliferating calli were observed in case of leaf bases.

The data given in Table 5 shows that LS basal medium supplemented with 3.0 mg/l BAP along with 2.0 mg/l 2, 4- D, gave a maximum of 30% callus induction in case of embryo explants. Leaf base explants gave 20% callusing frequency in LS medium supplemented with 2.0 mg/l BAP along with 2.0 mg/l 2, 4-D. Calli of embryos were whitish in color and were granular and non-morphogenic in appearance. The calli of leaf bases were somewhat mucilaginous and whitish in texture.

Data shows that callus response from mature embryos was 30% in LS basal medium supplemented with 1.0 mg/l Kinetin and 2.0 mg/l 2, 4-D (Table 5). Maximum callus production from leaf bases was also obtained in the same hormonal combination and the callogenesis response was 15% (Table 5). Crystalline and whitish calli were given by embryos, while mucilaginous and fragile and yellowish white calli were produced from leaf bases.

It can be concluded from the above experiments that out of all the media and different hormones tried for maximum callus induction and proliferation, MS and LS medium with 2.0 mg/l 2,4-D was best for mature embryo explants and MS with 2.0 mg/l 2,4-D in combination with 1.0 mg/l Kinetin was best for leaf base explants of Super Basmati. In case of IRRI-6, MS medium supplemented 2.0 mg/l 2,4-D was best for callus induction from mature embryo explants and LS medium with 3.0 mg/l 2,4-D was best for leaf base explants. Best callogenic response was obtained from mature embryo explants of IRRI-6.

Effect of light condition on callus induction and proliferation: Highest callus induction frequency was exhibited by the mature seed explants of Super Basmati in light conditions (Table 6). Callus induction was initiated after 7 days of inoculation. The average callus induction frequency calculated was 64.66% under light conditions for Super Basmati. Calli were compact, greenish yellow, proliferating and embryogenic. Same experiment under the same conditions was conducted for other cultivar IRRI-6. The average callusing % age was 36.33% and the average days of callus initiation was approximately 10. Mucilaginous, off-white, compact and granular calli were observed.

Effect of dark conditions on callus induction and proliferation: Under dark conditions the callus induction frequency in both the varieties was low (Table 6). In Super Basmati the average days for the callus induction were 13 with 43% callus induction under dark conditions. Morphologically calli were embryogenic, greenish-yellow, compact and smooth. For IRRI-6 under dark conditions, the average callus induction was 27% and the average numbers of days for callus induction were 15. So in light conditions better results were shown by Super Basmati for callus induction as compared to IRRI-6. Calli were yellowish and compact.

Table 5. Effect of different plant growth regulators on callogenic response of different explants of *Oryza sativa* cultivar IRRI-6 in LS medium.

Medium	Growth regulators concentrations	Embryos		Leaf bases	
		% Callus induction	Callus morphology	% Callus induction	Callus morphology
LS + 2,4-D	Control	00.00±0.00	No callus	00.00±0.00	No callus
	1	60±6.51	Light yellow compact	30±3.85	Friable & mucilaginous
	2	50±2.33	Granular	30±6.51	Granular
	3	30±6.98	Whitish	50±2.19	Off-white
	4	15±4.67	Compact	20±1.54	Compact
	5	20±1.54	Yellowish	10±.00	Proliferating
	6	10±.00	Mucilaginous	10±4.81	Friable
	7	10±.00	White	10±1.54	Proliferating
	8	10±.00	Proliferating	10±.00	Mucilaginous
LS + 2,4-D (2.0 mg/l) + BAP	1	20±1.54	White, Compact	10±1.54	white, mucilaginous Non Proliferated
	2	10±1.18	Granular	20±7.40	Compact
	3	30±3.85	Mucilaginous	10±.68	Proliferating
	4	15±3.06	Off-white	10±8.50	Friable
LS + 2,4-D (2.0 mg/l)+ Kinetin	1	30±1.18	Whitish, Crystalline	15±6.13	Friable, mucilaginous
	2	20±1.33	Yellowish	10±6.13	Proliferating
	3	15±1.33	Granular	10±8.09	Off-whitish
	4	10±.00	Mucilaginous	10±8.51	Friable

± represents standard error applied on the data

Regeneration frequency of the two varieties of rice (Super Basmati and IRRI-6): Best calli obtained from both the varieties (Super Basmati and IRRI-6) were transferred to regeneration medium to study organogenic response (Table 7). Data was collected after 7 weeks of inoculation. Best organogenesis (80% shoots and 100% roots) was obtained from embryo derived calli of Super Basmati in MS medium supplemented with NAA 1.0 mg/l and BAP 3.0 mg/l (Fig. 3a, 3b). While in case of IRRI-6, 100% shoot regeneration and 80% root formation was obtained with NAA 1.0 mg/l and BAP 5.0 mg/l (Fig. 3c, 3d).

Discussion

In tissue culture work different gelling agents are being used for solidifying culture medium. Agar is the most commonly used gelling agent. It is a complex polysaccharide obtained from some species of algae. During fabrication it is subjected to varying degree of purification. However mineral and organic impurities remain in it (Romberger & Tabor, 1971). The most popular alternative to agar is Phytagel or Gelrite (Gellan gum). It is a complex extra-cellular polysaccharide produced by *Pseudomonas elodea*. Gelrite contains less free minerals and impurities than agar (Pasqualetto *et al*., 1986, 1988). In media solidified with agar the pH often drops as the culture ages; in media solidified with Gelrite the pH tends to be more stable (William *et al*., 1990).

In the present study, comparison was made between Murashige & Skoog, (1962) and Linsmaier & Skoog, (1965) media solidified with agar and phytagel to get best *In vitro* germination response from two rice (*Oryza sativa* L.) varieties, Super Basmati and IRRI-6. MS-agar was best for germination of Super Basmati giving 100% germination while LS–agar medium proved better for IRRI-6 giving 90% germination rate. Average number

of roots, leaves, root length and shoot length was better in LS (agar) medium in both the cultivars. Several other workers have also used MS-agar medium for germination of rice seeds under *In vitro* conditions (Khanum *et al*., 1997, Khanna & Raina, 1998, Noor *et al*., 2005, Manickavelu *et al*., 2006).

In the present work for callogenesis, MS medium supplemented with 2, 4-D in different concentrations was tried and maximum callus induction was achieved at 2.0 mg/l from mature embryos in both the varieties and leaf bases of IRRI-6, while leaf bases of Super Basmati required a higher concentration of 2,4- D. In LS medium, the explants of Super Basmati required a higher concentration of 2, 4-D as compared to IRRI-6 for callus induction.

Embryogenic callus induction is dependent on the interaction between the genotypes and culture conditions. Plant growth regulators play a central role in plant tissue culture, in which a high auxin/cytokinin ratio usually is used for initiation of the embryogenic callus, while a low ratio is used for regeneration of plantlets. (Ge *et al*., 2006). Although the exact functional mechanism of plant growth regulators in tissue culture remains unclear, it is suggested that they function by mediating the signal transduction cascade that leads to programming of embryogenic genes (Dudits *et al*., 1995).

In most cases, 2,4-D as a strong synthetic auxin was sufficient to initiate and sustain embryogenic callus growth in rice and has been used as the only growth regulator in callus induction medium (Seraj *et al*., 1997, Khanna & Raina, 1998, Lee *et al*., 2002, Ozawa *et al*., 2003, Lin & Zhang, 2005). Many workers obtained high frequency callus production from different explants of *indica* rice when cultured on MS based medium supplemented with 2, 4-D at 2.0 mg/l (Pandey *et al*., 1994; Rashid *et al*., 2000; Noor *et al*., 2005; Henke *et al*., 2006; Kabir *et al*., 2008) while others reported a higher or lower concentration of 2,4-D for callus induction (Khan *et al*., 2000; Azria & Bhalla 2000; Al-Khayri & Al-Bahrany, 2000).

Several auxins 2, 4-D, NAA and IAA, combined with a kind of cytokinin at specified concentrations were found to be optimum for callus induction in many rice cultivars. It has been reviewed that hormonal metabolisms operated in an integrated manner and that several, potential, mutual functional interacting points exist between different hormones (Coenen & Lomax, 1997; Gasper *et al*., 2000).

In the present study, among the auxin-cytokinin combination (2, 4–D + BAP and 2, 4-D + Kinetin), BAP at lower concentration (1.0-2.0 mg/l) with 2, 4-D (2.0 mg/l) showed better callogenic response in the two media from both the explants in both the cultivars. Similarly in 2, 4-D + Kinetin combination, lower concentration of Kinetin (1.0-2.0 mg/l) was favourable for callus induction in all cases.

A requirement for a specific combination of auxin and cytokinin for callogenesis has also been reported by other workers. Raman *et al*., (1994), Al-Khayri & Al-Bahrany (2000), Shahnewaz *et al*., (2004) and Ilahi *et al*., (2005) used MS medium supplemented with 2, 4-D and Kinetin for callusing while Khan *et al*., (1999) and Khaleda & Al-Forkan (2006) reported that a combination of 2,4-D with BAP proved better for callus induction from different explants of rice.

In the present work, both the varieties were good for callus induction; however IRRI-6 has more potential for callogenesis. The significant difference between two varieties for callogenesis under that the same nutritional condition indicates that callus induction efficiency is genotype specific. These findings are similar with the reports of Khanna & Raina, (1998), Abbasi *et al*., (2000) and Nasreen & Mohammad (2000).

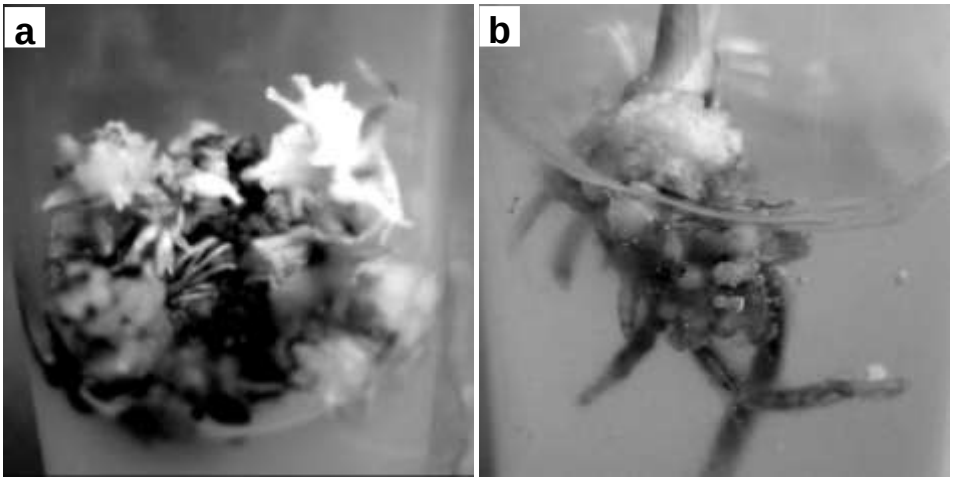
Table 6. Effect of light and dark conditions on callogenesis in two rice varieties (Super Basmati and IRRI-6.) using mature seeds as explants.

Effect	Media + growth regulator	Varieties					
		Super Basmati			IRRI-6		
		% callus induction	Average days for callus induction	Callus morphology	% Callus induction	Average days for callus induction	Callus morphology
Light	MS+2,4-D 2.0 mg/l	64.66	7	Compact greenish yellow	36.66	10	Mucilaginous off-white
Dark	MS+2,4-D 2.0 mg/l	43.00	13	White & compact	27.00	15	Friable, granular, non-embryogenic

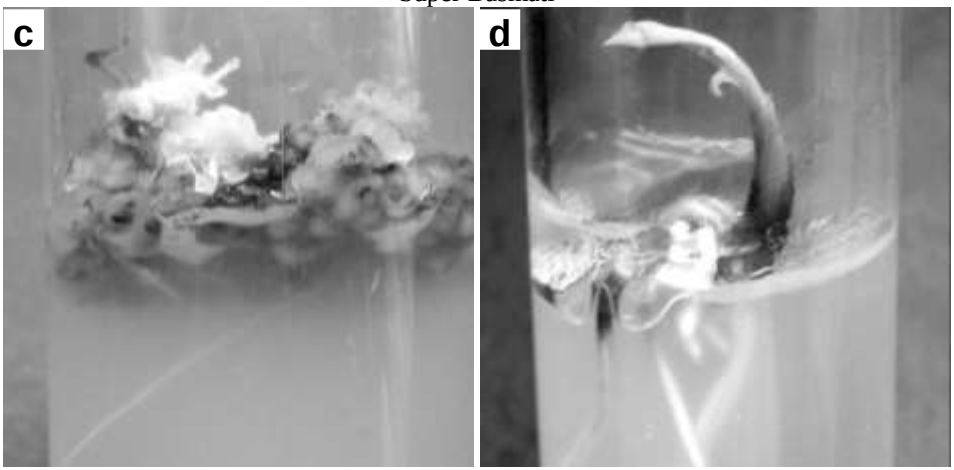
(10 test tubes were used per experiment in triplet).

Table 7. Study of the regeneration percentage frequency of the two varieties of rice (Super Basmati and IRRI-6) in MS medium supplemented with NAA and BAP.

MS Media	Super Basmati		IRRI-6	
No. of treatments	% Shoot regeneration	% Root regeneration	% Shoot regeneration	% Root regeneration
Basal medium (Control)	0	0	0	0
NAA 1.0 mg/l + BAP 1.0 mg/l	20.00	40.00	30.00	40.00
NAA 1.0 mg/l + BAP 2.0 mg/l	40.00	60.00	50.00	50.00
NAA 1.0 mg/l + BAP 3.0 mg/l	80.00	100.00	50.00	60.00
NAA 1.0 mg/l + BAP 4.0 mg/l	60.00	60.00	60.00	80.00
NAA 1.0 mg/l + BAP 5.0 mg/l	40.00	90.00	100.00	80.00



Super Basmati



IRRI-6

Fig. 3. Shoot induction **(a)** and root formation **(b)** from morphogenic calli of Super Basmati in MS medium supplemented with NAA 1.0 mg/l and BAP 3.0 mg/l. **(c)** Shoot induction and root formation **(d)** from morphogenic calli of IRRI-6 in MS medium supplemented with NAA 1.0 mg/l and BAP 5.0 mg/l.

Organogenic capacity of callus tissue depends upon the plant species, type of explant from which the callus was derived, age of callus tissue and composition of the nutritional medium. Another important factor is the natural level of various growth regulators (Ilahi *et al*., 2005). Genotype and media composition and their interaction largely affect callus induction and subsequent plant regeneration.

In the present study, MS medium supplemented with NAA 1.0mg/l and BAP 3.0 mg/l proved better for Super Basmati and for IRRI-6 MS medium containing NAA 1.0 mg/l and BAP 5.0 mg/l showed better results. Rashid *et al*., (2000, 2004) obtained shoot regeneration from calli of different varieties of *indica* rice from MS medium containing 1.0 mg/l NAA and 5.0 mg/l BAP. Azria & Bhalla (2000) also reported that both NAA

and BAP are required for shoot initiation. Pons *et al*., (2000) reported that BAP yielded more shoots than Kinetin in all varieties while in case of using auxin NAA and IAA, it depended on the varieties. Noor *et al*., (2005) found that with NAA 1.0 mg/l and BAP 2.5 mg/l Super Basmati showed 90% frequency of plant regeneration, while Basmati 385 gave 83% regeneration frequency on MS medium supplemented with NAA 1.0 mg/l and BAP 5.0 mg/l. Similar results were also observed by Sikder *et al*., (2006) that MS medium supplemented with 0.05 mg/l NAA and 5.0 mg/l BAP gave highest regeneration frequency.

Effect of light and dark conditions on the induction and proliferation from mature seed explants of both the cultivars in MS medium supplemented with 2,4-D 2.0 mg/l was also studied. The results showed that Super Basmati gave better callogenic response under both light and dark conditions as compared to IRRI-6. The calli upon sub-culturing in the same media showed 70% regeneration frequency in case of Super Basmati and 50% regeneration in IRRI-6.

These results are in accordance with the Khanna & Raina (1998), Kunnanuvatchaidach *et al*., (1995) and Seraj *et al*., (1997) who also obtained high frequency callus induction under light conditions using mature seeds of *indica* rice in MS basal media supplemented with auxin such as 2,4-D. Rashid *et al*., (2001) also reported 70% callus induction frequency of Super Basmati on MS medium containing 2.0 mg/l 2, 4-D under light conditions.

Conclusion

A reproducible and an efficient procedure has been developed for callus induction and plant regeneration from different explants of rice varieties (Super Basmati and IRRI-6) using different media and hormone combinations. Out of the two media tried for callus induction and plant regeneration, MS-agar medium was found to be suitable for regeneration for Super Basmati and LS (agar) for IRRI-6 from dehusked seeds. For callus induction MS medium supplemented with different hormones was more suitable than LS medium.

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