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Identification of *in vitro* Regeneration Potential of Rice (*Oryza sativa* L.) Landraces

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ABSTRACT: An efficient callus induction and *in vitro* regeneration potential of ten rice landraces were analysed along with indica rice variety check ASD 16. The effect of plant growth regulator (2,4-D) on callus induction was studied to maximize the production of embryogenic calli in rice land races. This study found that all the rice land races exhibited high frequency of callus induction on Murashige and Skoog (MS) medium supplemented with 2,4-D 2.5 mg/l. In this concentration, higher callus induction frequency was exhibited by the land race Kallurundikar followed by the landraces Arupatham Kuruvai, Noorungan, Poongar Mattaikar, Kuliyadichan, Chithikaikar, Vellaichithiraikar, Kichalisamba and Nooripathu. The hundred percent regeneration potential was recorded by the land races Chithikaikar, Kallurundikar, Kuliyadichan, Mattaikar and Noorungan when MS media supplemented with BAP 3.0 mg/l + Kinetin 1.5 mg/l + NAA 3.5 mg/l + IAA 1.5 mg/l. Whereas, the landrace Vellaichithiraikar exhibited higher browning rate when compared to other land races. Hence, the optimized conditions for callus formation and regeneration may be used for micropropagation studies for the improvement of rice landraces.

Keywords: Landraces, Rice, Callus, Growth Regulator, *In vitro*, Shoot regeneration, Rooting.

INTRODUCTION

In any crop species, for developing transgenic varieties, germplasm recovery and gene editing requires tissue culture based genetic transformation system. However, the lack of an efficient tissue culture method for regeneration plantlets remain a main barrier for modification of wide range of plant species. Rice (*Oryza sativa* L.) is the second most widely cultivated crop in the world. Among the three subspecies (*indica*, *japonica* and *javanica*) of rice *indica* subspecies is the most widely cultivated crop in south and South East Asian countries. In India, after the green revolution the introduction of high yielding varieties has indirectly affected the cultivation of landraces in all crops (Umadevi *et al*., 2019). The traditional rice landraces have many health benefits and they can withstand the abiotic and biotic stress. The rice landraces have high nutritional values and the high stress tolerance and these traits can be utilized by employing molecular breeding techniques (Keerthivarman *et al*., 2019). The plant regeneration is the prime step and prerequisite before employing any genetic improvement program involving transgenic or gene editing in any crop species. Through tissue culture technique assessing regeneration potential is essential for improving rice cultivars through transgenic breeding (Sundaram *et al*., 2019; Sankepally and Singh 2016). Hence, the present study was conducted to identify the callus induction and the regeneration potential of rice landraces along with check variety ASD 16.

MATERIALS AND METHODS

The mature seeds of ten rice landraces which were obtained from Agricultural Research Station, Paramakudi along with check variety ASD16 were taken for this study. The dried seeds of rice landraces were manually dehusked without damaging the embryo (Fig. 1). The dehusked seeds were immersed in 70% ethanol (V/V) for 3 minutes followed by 0.1% mercuric chloride for 5 minutes and washed with sterile distilled water once and then treated in 2% (v/v) sodium hypochlorite with few drops of tween 20 for 15 minutes. The treated seeds were thoroughly washed the with sterilised distilled water and allowed to dry for sometime in aseptic conditions to remove excess moisture.

Fig. 1. Preparation of rice land race for callus induction.

Callus induction. The different concentrations of 2,4-D (2, 2.5, 3, 3.5, and 4 mg/l) supplemented in MS media was prepared for callus induction. The sterilized rice land race seeds were aseptically cultured on callus induction medium and further subculturing was performed on MS medium fortified with 2mg/l 2,4-D for callus proliferation. About 25 seeds were placed on a petridish and kept at 25±ºC under dark condition. The seeds were examined for callus induction frequency after 24 days of inoculation (Fig. 2 & 3).

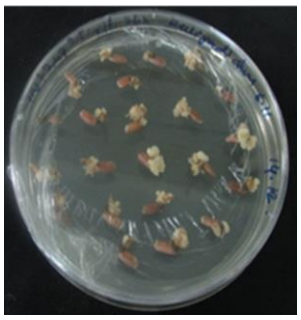

Fig. 2. Callus induction on MS medium supplemented with 2,4 –D.

Fig. 3. Scutellum – derived callus of rice land race.

Plant regeneration. The morphological analysis of embryogenic calli through scanning electron microscopy (SEM) confirmed the embryogenicity of the induced callus (Fig. 4). The embryogenic calli were transferred to shoot and root induction media contains growth hormones *viz.,* BAP, kinetin, NAA and IAA (Fig. 5).

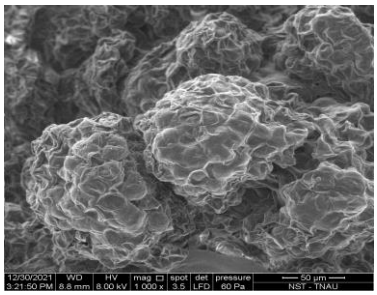

Fig. 4. SEM image of embryogenic calli.

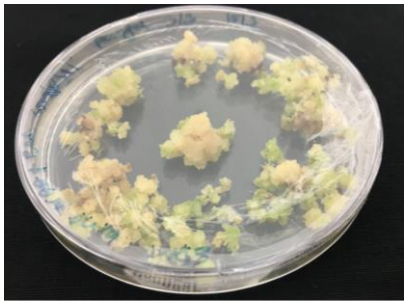

Fig. 5. Inoculation of embryogenic calli for shoot induction.

The callus induction frequency (%) and plant regeneration frequency (%) were measured using the following formula.

$$\text{Callus induction frequency (\%)} = \frac{\text{Number of explants producing callus}}{\text{Total number of explants cultured}} X\ 100$$

$$\text{Embryogenic Calli Frequency} = \frac{\text{Number of embryogenic calli}}{\text{Number of primary calli}} \times 100$$

$$\text{Plant regeneration frequency (\%)} = \frac{\text{Number of callus producing shoots}}{\text{Total number of calli incubated}} \text{X } 100$$

$$\text{Browning rate (\%)} = \frac{\text{Number of brown calli}}{\text{Total number of embryogenic calli}}$$

RESULTS AND DISCUSSION

The experiment was conducted in Completely Randomized Block Design (CRD) with three replications. About ten seeds were used for callus induction in each replication per treatment and five embroyogenic calli for plant regeneration. The data were statistically analysed by SAS package version 9.1. The maximum callus induction frequency was observed in the landrace Poongar (80.95%) followed by Norungan (76.67%), Aruvathamkuruvai (67.62%), Kallurundaikar (67.62%) and mattaikar (65.83%). The lowest callus induction frequency was recorded by the rice land race Kichilisamba (48.99%). Among the five different 2,4-D concentrations, the concentration 2,4-D 2.5mg/l expressed higher callus induction for all the rice land races followed by the concentration 2,4-D 3.5mg/l (Table 1). Similar findings were obtained by Shweta *et al*. (2020) found that 2,4-D as an effective auxin source for callus induction and proliferation The highest percentage of embryogenic calli was observed in the rice landrace Kuliyadichan (83.54%), Aruvaham kuruvai (83.42%) and Kallurundaikar (71.45%). The maximum embryogenic frequency was recorded in 2,4-D concentration of 2.5mg/l followed by 3mg/l and 2.0mg/l (Table 2).

Table 1: Mean comparison 2, 4 D concentration of calli induction frequency (%) of different rice landraces.

Sr. No.	Rice landraces	2.0 mg/l	2.5 mg/l	3.0 mg/l	3.5 mg/l	4.0 mg/l
1.	Chithiraikar	54.76	71.43	64.28	47.62	42.86
2.	Kichalisamba	47.83	60.87	52.17	46.38	42.03
3.	Kallurundikar	59.52	83.33	71.43	57.14	54.76
4.	Kuliyadichan	54.76	64.29	59.52	47.62	42.86
5.	ArupathamKuruvai	61.90	78.57	73.81	59.52	52.38
6.	Mattaikar	60.42	66.67	62.50	54.17	50.00
7.	Norungan	78.57	85.71	80.96	73.81	61.90
8.	Nootripathu	58.34	68.75	60.42	54.17	52.08
9.	Poongar	57.14	92.86	71.43	42.86	28.57
10.	Vellaichithiraikar	50.00	61.90	52.38	47.62	42.86
11.	ASD 16(control)	73.81	65.08	50.79	34.92	26.98
	Mean	59.73	72.68	63.61	51.44	45.21
	SE	2.78	3.25	2.98	3.06	3.20
	CV	0.15	0.15	0.16	0.20	0.23

Table 2: Mean comparison of embryogenic frequency (%) of different landraces.

Sr. No.	Rice landraces	2.0 mg/l	2.5 mg/l	3.0 mg/l	3.5 mg/l	4.0 mg/l
1.	Chithiraikar	69.57	80.00	74.07	65.00	61.11
2.	Kichalisamba	54.55	59.53	58.33	50.00	48.28
3.	Kallurundikar	76.00	82.86	80.00	75.00	69.56
4.	Kuliyadichan	65.22	70.37	68.00	60.00	55.56
5.	ArupathamKuruvai	84.62	91.89	87.10	80.00	72.73
6.	Mattaikar	72.41	75.00	73.33	65.38	62.50
7.	Norungan	72.73	86.11	79.41	70.97	69.23
8.	Nootripathu	60.71	66.67	65.52	57.69	52.00
9.	Poongar	78.79	94.87	82.35	70.97	72.41
10.	Vellaichithiraikar	57.14	61.54	59.09	50.00	44.45
11.	ASD 16(control)	64.52	78.05	71.88	50.00	47.06
	Mean	68.75	76.99	72.64	63.18	59.54
	SE	2.81	3.53	2.81	3.18	3.20
	CV	0.14	0.15	0.13	0.17	0.18

Table 3: Mean comparison of Browning rate (%) of different land races.

Sr. No.	Rice landraces	RGM 1	RGM 2	RGM 3	RGM 4	RGM 5
1.	Chithiraikar	1.33	1.33	0.00	1.67	1.67
2.	Kichalisamba	1.33	2.33	0.67	2.00	1.67
3.	Kallurundikar	1.00	1.00	0.00	1.00	2.00
4.	Kuliyadichan	1.00	1.00	0.00	1.00	1.00
5.	ArupathamKuruvai	1.33	1.67	0.33	1.33	1.33
6.	Mattaikar	1.33	1.33	0.00	1.67	2.00
7.	Norungan	2.00	1.67	0.00	2.67	2.33
8.	Nootripathu	1.33	1.00	0.67	1.67	2.00
9.	Poongar	1.33	1.67	0.67	1.67	2.33
10.	Vellaichithiraikar	2.33	3.33	1.67	2.00	2.00
11.	ASD 16(control)	2.33	3.67	0.33	1.67	0.33
	Mean	1.51	1.82	0.39	1.67	1.70
	SE	0.14	0.28	0.15	0.14	0.18
	CV	0.32	0.51	1.30	0.28	0.36

The highest browning rate was obtained in the rice land race Vellai chithiraikar (43.79%) followed by Norungan (23.28%) and Chithiraikar (23.18%). The lowest was recorded by Kuliyadichan (13.34%) (Table 3).

In the present investigation cent percent plant regeneration exhibited by the four rice land races *viz.*, Norungan, Mattikar, Kuliyadichan and Kallurundaikar in the RGM 3 regeneration media (BAP 3.0MG/L + Kinetin 1.5mg/l + NAA 3.5mg/l + IAA 1.5mg/l) followed by RGM2 and RGM1 regeneration media (Table 4, Fig. 6, 7). Bared on above experimental results, it could be concluded that the yield improvement through biotechnological techniques is feasible by scutellum derived callus and it regeneration in rice land races.

Table 4: Mean comparison of regeneration frequency (%) of different land races.

Sr. No.	Rice landraces	RGM 1	RGM 2	RGM 3	RGM 4	RGM 5
1.	Chithiraikar	68.75	70.83	80.00	76.92	72.73
2.	Kichalisamba	33.33	36.00	52.38	43.75	42.86
3.	Kallurundikar	73.68	75.86	88.24	83.33	81.25
4.	Kuliyadichan	46.67	52.63	70.59	66.67	60.00
5.	ArupathamKuruvai	77.27	76.47	96.30	90.00	81.25
6.	Mattaikar	61.91	62.50	77.27	70.59	66.67
7.	Norungan	70.83	74.19	92.59	86.36	83.33
8.	Nootripathu	41.18	45.45	57.90	53.33	46.16
9.	Poongar	69.23	75.68	89.29	86.36	80.95
10.	Vellaichithiraikar	41.67	43.75	61.54	60.00	50.00
11.	ASD 16(control)	65.00	68.75	82.61	81.82	75.00
	Mean	59.05	62.01	77.16	72.65	67.29
	SE	4.63	8.20	4.46	4.57	4.59
	CV	0.26	0.24	0.19	0.21	0.23

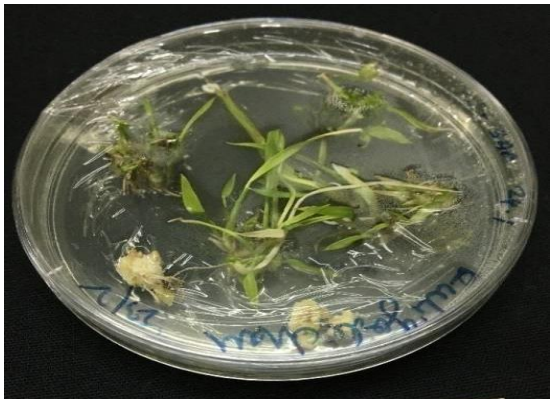

Fig. 6. Shoot regeneration from embryogenic calli.

Fig. 7. Shoot and root whole plant regeneration from embryogenic calli.

CONCLUSIONS

Based on above experimental results, it could be concluded that the yield improvement through biotechnological techniques is feasible by scutellum derived callus and it regeneration in rice land races.

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Conflict of Interest. None.

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