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Callus Induction and Plant Regeneration on Optimization of the Culture Conditions in Jow Haw Rice (*Oryza Sativa* L.)

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Abstract The objective of this study was to develop an efficient protocol for optimum callus induction and regeneration of Jow Haw (*Oryza sativa* L.). MS (Murashige and Skoog) and NB (Nitch and Nitch) media supplemented with 0.5 mg/l NAA (α-Naphthaleneacetic acid) and 0.5, 1, 2, 3 and 5 mg/l 2,4-D (2,4-Dichlorophenoxyacetic acid) in different concentrations were used for callus induction. The callus induction frequencies were 90-100% on NB medium containing 3 mg/l 2,4-D for 4 weeks. The maximum mean size of callus was 181.76 mm^3 and mean fresh weight of callus was 0.2552 g. For plant regeneration the callus were cultured on MS and NB media containing with 0.5 mg/l NAA and different concentrations of 1, 2 and 3 mg/l BAP (6-Benzylaminopurine). The highest regeneration frequency (100%) was grown on MS medium containing with 2 mg/l BAP in 5.2 g/l phytagel. Complete plantlets were regenerated in 6 weeks. When the plantlets regeneration were be strong. Then transferred to the sterile soil into pots

Keywords: Callus induction, Plant regeneration, *Oryza sativa* L., Jow Haw

Introduction

Rice is the most important food in the world. In asia where it includes half of the suitable for growing crops land used for agriculture in many countries. Now rice production needs increase to meet the predicted needs of increasing population. Therefore, is to increase productivity by advances in biotechnology. *In vitro* techniques constitute an important component of biotechnology and have the potential not only to improve the existing cultivars, but also for the synthesis of novel plants and early release of high-yielding plants resistant to various diseases, pets, stresses and temperature (Tariq *et al.*, 2008). The successful application of plant tissue culture techniques for crop improvement requires suitable plant regeneration methods. The aim of this

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study was to determine the most suitable concentrations of growth regulators for improvement in callus induction and plant regeneration and optimization of the culture conditions in Jow Haw rice.

Materials and methods

Surface sterilization

The dehusked seeds were surface-sterilized in sterile distilled water for 1 minute and then 70% ethanol for 1 minute., followed by 30 minutes shaking in 20% Sodium hypochlorite and finally rinsing three times in sterile distilled water. Seeds were dried on a sterile filter paper in a sterile petridish.

Callus induction

Sterilized seeds were cultured on MS medium (Murashige and Skoog, 1962) and NB medium (Nitsch and Nitsch, 1969) supplemented with 0.5, 1, 2, 3 and 5 mg/l 2,4-D (2,4-Dichlorophenoxyacetic acid), 1 mg/l NAA, 1 g/l L-proline, 30 g/l sucrose and 2.6 g/l phytagel. The pH of the media was adjusted to 5.8 prior to autoclaving. Seeds were maintained in the dark at 25±2 ºC for 4 weeks. Each treatment consisted of 40 seeds. After 4 weeks, the callus induction frequency were scored as percentages and calculated mean size and mean weight of callus formation.

Plant regeneration

Firstly, fresh callus were directly transferred to regeneration media and secondly, callus were desiccated by transferring on a sterile filter paper in a sterile petridish. The petridish were maintained under dark condition for 7 days. After desiccation, calli were transferred to regeneration media. The regeneration media containing with MS and NB media supplemented with 1, 2 and 3 mg/l BAP (6-Benzylaminopurine), 0.5 mg/l NAA (α-Naphthaleneacetic acid), 30 g/l sucrose, 2.6 and 5.2 g/l phytagel. Calli were maintained at 25±2 ºC under light condition for 6 weeks. Each treatment consisted of 12 embryogenic callus. In this experiment, callus from induction MS medium were transferred on regeneration MS medium. Similarly callus from induction NB medium were transferred on regeneration NB medium. After 6 weeks regeneration frequency were calculated as percentages.

Results and Discussion

Callus formation was appeared in 2 weeks of culturing. Callus were induced in all media. The percentages of callus induction frequency were 90 to 100% (Table 1). Figure 1 presents the effect of different 2,4-D concentrations (0.5, 1, 2, 3 and 5 mg/l). Non-embryogenic and embryogenic callus were observed in culture. Characteristic of callus were soft, friable, yellow in colour which found in non-embryogenic and in some cases have roots structure. But the embryogenic callus were compact and creamy in colour. Auxin is essential for indirect somatic embryogenesis and 2,4-D normally used to produce embryogenic calli at concentrations between 10^{-5} M and 10^{-7} M (Meneses, 2005). And the different concentrations in 2,4-D that needed for callus germination probably due to genetic variation in interval level of hormones which affected the callus germination (Verma *et al.*, 2011).

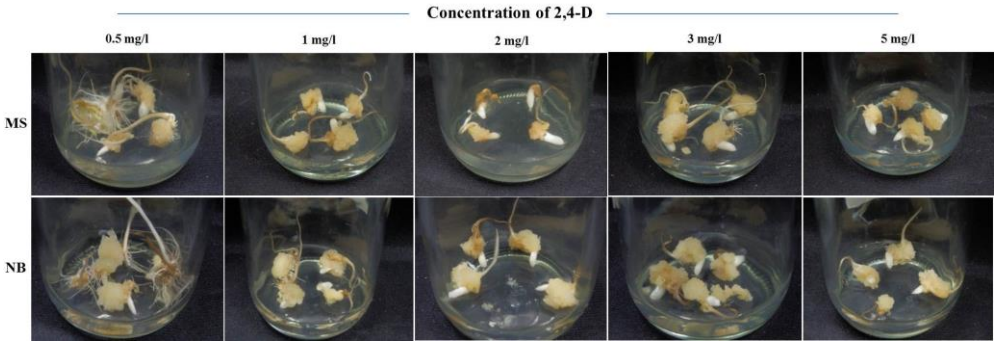

Figure 1. Callus from seeds of Jow Haw rice cultured on solid MS and NB media supplemented with 0.5, 1, 2, 3 and 5 mg/l of 2,4-D, 1 mg/l NAA, 1 g/l L-proline, 30 g/l sucrose and 2.6 g/l phytagel after 4 weeks.

Callus were induced in NB medium supplemented with 3 mg/l 2,4-D and 0.5 mg/l NAA was found most effective in callus induction. It gave the maximum mean size of callus was 181.76 mm^3 and mean fresh weight of callus was 0.2552 g is shown in Table 1. Callus induction on NB medium was found better than that on MS medium, similar to the reported of Tariq *et al.* (2008) that the overall frequency (%) of callus induction on Chu's N6 medium was found better than on MS medium. According to Rashid *et al.* (2004) and Rashid *et al.* (2001) presented that callus can be induced and grown on N6 medium was better than MS medium with callus induction frequency in all the four varieties of rice. It is, perhaps due to the reason that N6 medium contained

more nitrogen than MS medium (Rashid *et al.*, 2004). which NB medium was more nitrogen than MS medium, that similar to N6 medium.

For regeneration, fresh callus and desiccated callus were transferred to MS and NB media supplemented with 1, 2 and 3 mg/l BAP and 1 mg/l NAA. Green spot were appeared on callus within 2 weeks after transferring to regeneration media (Fig. 2A). Multiple shoot, shoot bud, and root were developed after 3-4 weeks (Fig. 2, B-F). Callus obtained at optimal concentration between cytokinins (BAP) and auxin (NAA) to induce shoot and root.

Table 1. Callus induction frequency (%), mean size and mean weight of callus formation from mature seed cultured on MS and NB media supplemented with different concentrations of 2,4-D for 4 weeks

Media	Concentration of 2,4-D (mg/l)	No. of seeds	callus induction frequency (%)	Mean size of callus (mm^3)	Mean weight of callus (g)
MS	0.5	40	100	126.84^{bc}	0.1660^{def}
	1	40	95	137.97^{bc}	0.1639^{def}
	2	40	100	108.82^{c}	0.1419^{f}
	3	40	100	161.22^{ab}	0.2000^{cde}
	5	40	100	134.38^{bc}	0.1578^{ef}
NB	0.5	40	90	132.14^{bc}	0.1785^{cdef}
	1	40	92.5	131.84^{bc}	0.2085^{cd}
	2	40	100	157.07^{ab}	0.2790^{a}
	3	40	100	181.76^{a}	0.2552^{ab}
	5	40	100	156.69^{ab}	0.2145^{bc}

Values followed by different letters indicating significant differences according to Duncans's Multiple Range Test ($p \leq 0.05$).

The part of cytokinins like BAP and NAA in plant regeneration has been presented in many reports. In this report, the percentages of regeneration frequency were 8.33 to 100% (Table 2). Shoot can be induced on MS medium was better than NB medium which MS medium supplemented with 1, 2 and 3 mg/l BAP and 0.5 mg/l NAA. In a recent report it has been referred to plant regeneration was achieved through embryogenic callus on MS medium supplemented with different concentrations of BAP and NAA (Karthikeyan *et al.*, 2009; Ramesh *et al.*, 2009). The highest frequency of shoot regeneration (100%) was observed on MS medium supplemented with 0.5 mg/l NAA and 1 and 2 mg/l BAP for fresh callus and desiccated callus, respectively (Table 2). Like in case of Kumar *et al.* (2013) the highest frequency of shoot regeneration (80.70%) was appeared on MS medium supplemented with 4 mg/l BAP and 0.2

mg/l NAA for Kitaake rice. However, this study shows that 2 mg/l BAP was the most suitable for plant regeneration due to callus in 2.6 g/l and 5.2 g/l phytagel were induced by 2 mg/l BAP which callus were cultured in 1 mg/l BAP can be regenerated just 5.2 mg/l phytagel.

Concentration of phytagel, when concentration of phytagel was increased excessive water is lost from explant and established a desiccation condition for explant which it provided for regeneration of plant (Manchanda and Gosal, 2012). Effect of concentration for phytagel was showed in Table 2. The frequency of regeneration in 5.2 g/l phytagel was higher than 2.6 g/l phytagel in regeneration medium. Similarly to Yinxia and Te-chato (2013) presented it was observed that phytagel at concentration of 0.3% supported the highest frequency of green spot forming calli at 100% and produced the highest number of regenerated shoots per callus at 7 shoots with a frequency of 61%.

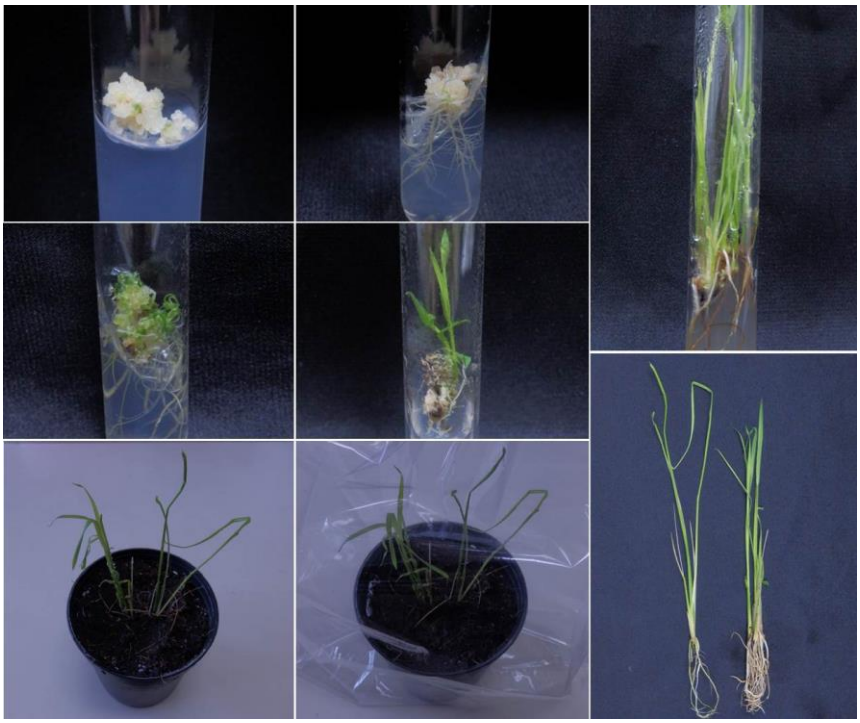

Figure 2. Green spots on the surface of callus after transferring to regeneration media for 2 weeks (A). Shoot bud and root were developed after 3-4 weeks (B-C). Multiple shoot was developed on MS medium supplemented with 2 mg/l BAP, 0.5 mg/l NAA and 5.2 g/l phytagel for 4 weeks (D-E). Shoot and root regeneration of callus for 6 weeks (F). Plantlets of Jow Haw rice from callus were transferred into the plastic pots (G-H)

Desiccated callus also advantaged for plant regeneration. Dehydration of calli for 24 h before transfer to regeneration medium was found highly stimulated plant regeneration for japonica rice (Tang *et al.,* 2000). Saharan *et al.* (2004) reported that shoot regeneration frequency increased from 1.2 to 5.6 fold after 48 h of rice callus desiccations. However, in this study desiccations of callus for 7 days produced shoot regeneration. The result showed that the maximum of percentage of desiccated callus frequency were 100% like Sompornpailin and Chutipaijit (2012) presented the green spots and shoot bud frequencies of desiccated calli were significantly more than those of non-desiccated calli (18.33 and 6.67%). Partial desiccated callus could be reabsorbed water and nutrients when desiccated callus was transferred to the regeneration medium (Chand and Sahrawat, 2001). However, in this study fresh callus and desiccated callus exhibited non-significant differences on plantlets regeneration frequency. Then regenerated plantlets were be strong and transferred to the sterile soil into pots (Fig. 2H).

Table 2. The shoot regeneration frequency cultured on MS and NB media supplemented with various concentration of BAP, phytagel and characteristic of callus (fresh and desiccated) for 6 weeks

Media	**Concentration of BA (mg/l)**	**Concentration of phytagel (g/l)**	**Fresh callus**		**Desiccated callus**	
			No.of callus	regeneration freq. (%)	No.of callus	regeneration freq. (%)
	1	2.6	12	0.00	12	0.00
		5.2	12	25.00	12	100.00
MS	2	2.6	12	91.67	12	8.33
		5.2	12	100.00	12	0.00
	3	2.6	12	16.67	12	0.00
		5.2	12	58.33	12	33.33
	1	2.6	12	0.00	12	16.67
		5.2	12	0.00	12	0.00
NB	2	2.6	12	0.00	12	75.00
		5.2	12	50.00	12	91.67
	3	2.6	12	0.00	12	0.00
		5.2	12	91.67	12	8.33

Conclusions

Callus induction frequency can be found in NB medium was better than MS medium. The maximum of mean size and mean weight of callus were found in 3 mg/l 2,4-D and 1 mg/l NAA. Regeneration rate for Jow Haw rice was found maximum on MS medium supplemented with 2 mg/l BAP, 0.5 mg/l

NAA and 5.2 g/l phytagel. In this study will be helpful for better improvement of rice.

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