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Study and Effect of Different Tissue Culture Media on the Propagation of Rice by Anther Culture

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Abstract: ***The investigation was conducted to standardize culture media for callus induction and regeneration from anther derived callus of Sona Masuri (Oryza sativa subspindica), NLR-145 (Oryza sativa subspindica, Pusa-834(IET-11674) rice varieties. Before inoculation of the anthers, a cold pre-treatment of 5^0C for 8 days was given to the selected panicles.Anther culture is useful technique in rice breeding programms.as production of haploid plants speeds up the breeding cycle by fixing homozygosis in one generation.Rice is the world's single most important food crop and is the primary food for more than one third of the world's population. Production and consumption are concentrated in Asia where more than 90% of all rice is produced and consumed. MS basal medium supplement with 30 and 40 g/l sucrose, 8 g/l agar and different plant growth regulator were used .Rice was cultured in different ratio of BAP: IAA (10:10, 10:5, 5:10, and 5:5 µM) in combination. Development was not identical in all the treatments, cultured tissues were observed in treatments about a week after culture. Results showed that the treatments containing 10 µM BAP in combination with 10 µM IAA is suitable for callus induction. While the tissue formed in 5:5 µM BAP: IAA was found to be delicate, short. Also, the tissue formed in 5:10 BAP:IAA was delicate, short but was higher in density as compared to treatment contained 5:5 µM BAP:IAA***

Keywords: anther culture, haploid, oryza sativa, regeneration

1. Introduction

Rice is the world's single most important food crop and is the primary food for more than one third of the world's population. Production and consumption are concentrated in Asia where more than 90% of all rice is produced and consumed. Its importance can be estimated by the fact that the year 2004 was declared as International Year of Rice by the United Nations Food and Agriculture[12] Organization. In direct proportion to the predicted rise in the world's human population, rice consumption[6] and demand will increase over the next several decades [10]

On the other hand the society needs to increase rice yield further to meet the growing demand emanating from population growth. The United Nations (UNO, 1998) project that .Farmers will have to generate larger marketable surplus to feed the growing population.Thus no option remains but to bridge the gap between potential and actual yields., Although plant[7] breeding methods have considerably contributed to increasing the productivity of modern rice, the advanced

The nutrient medium not only provides nutrition to the microspores but also directs the pathway of embryo development(Romana Siddique-2016). It is critical to change the composition of the media or replenish them to keep the balance of micronutrients[16] and maintain the pH. The most commonly used basal media for anther culture are N6 medium (Chu 1978), (modified) MS medium (Murashige and Skoog 1962), Nitsch and Nitsch (1969) medium and B5 medium [8]but

A carbohydrate source is essential in anther culture because of its osmotic and nutritional effects [11]. Maltose has been shown to be superior source of carbohydrate than sucrose for androgenesis in several species, including cereals [9 and 10]. Nitrogen can be supplied to the culture medium in an inorganic or organic form. The inorganic nitrogen is usually introduced in the form of nitrate or ammonium ions while nitrogen in the organic form can be supplied as vitamins and amino acid supplements. The ratio of NO3-:NH4 + has been observed to be an important determinant for success of anther culture inindica rice [11]. The indica cultivars require even lower level of (NH4)+ ions. Organic nitrogen supplements such as casein hydro lysate (CH) which is a sources of calcium, several micronutrient, vitamins and amino acids added to the medium have been particularly beneficial for positive anther culture response [7] although a few reports suggest otherwise [6 and 11]. Micronutrients also play an important and sometimes crucial role in normal plant growth and development. Copper and Zinc are two important micronutrients influencing microspore embryogenesis [10]. The effect of plant growth regulators has been widely investigated in anther culture. The type and concentration of growth regulators as well as their interactive presence can be the deciding factors that would influence pollen embryogenesis [9].

2. Materials and Methods

Preparation and Sterilization of Plant Materials and Culture Media

Healthy seeds of *Oryza sativa* L.(family Poaceae) of three varieties viz. Sona Masuri-5204, NLR-145,pusa-834 (IET-11674) also known as Masuri were collected from Agricultural Blok Office, phirangipuram ,Guntur district (Andhrapradesh) .collected seeds were authenticated from Assistant Director of Agriculture.

Plant material was collected from their natural habitat and cultured to prepare the needed explants. In order to sterilize, plant materials were washed [11]with many drops of dish washing liquid under tap water for about 30 min and then immersed in ethanol. After this stage, plant materials were transferred to 1% sodium hypo chlorite solution in a sealed

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bottle under sterile condition[15], gently agitated for 20 min and then rinsed three times with sterile distilled water. Plant materials were transferred on sterile filter papers and cut to culture. MS basal media supplemented with 30 and 40 g/l sucrose, 8 g/l agar and different plant growth regulator was prepared, then autoclaved after adjusting pH.

Culture media

Plant material cultured in Petri dish containing 25 ml MS basal medium supplemented with 30 and 40 g/l sucrose, 8 g/l agar and plant growth regulators.BAP (6-Benzylaminopurine) IAA (Indole-3 acetic acid) and each in different concentration and in combination (5 and 10 µM) were used as cytokinin and auxine sources, respectively. All possible combinations[13] among these levels considered were used as treatments so 2hormonal treatments were made. Petri dishes were incubated at 25 ± 1ºC in dark. Fresh weights of formed calli were estimated and compared in the third week after culture.

The green regenerated plants were transferred to MS medium in the absence of phytohormones for root formation. Completely regenerated plants were kept for hardening and then cultivated in the laboratory for further observation and evaluation.

3. Results and Discussion

Development was not identical in all the treatments, cultured tissues were observed in treatments about a week after culture. The treatments contained different ratio of BAP:IAA (10:10, 10: 5, 5: 10, 5: 5 µM) in combination were used (Table 1). Results showed that the treatments containing 10 µM BAP in combination with 10 µM IAA is most suitable for the growth. Proliferation rate in these treatments was more than others as well (Figure 1). Minimum growth was observed in the treatments containing BAPor IAA in less concentration. About three weeks after culturing the explants little growth was observed. Generally regeneration performance from petioles was low (approximately 20%). The regenerated shoots were transferred to MS basal medium[3and4] without any hormones in photoperiod of 16 h light/8 h darkness to more growth. After ten days, green-long shoots were transferred to induction medium. The formed tissue in 5:5 µM BAP:IAA was found to be delicate, shorter than others. Also, the tissue formed in 5:10 BAP:IAA was delicate, short but was higher in density. Generally, 5: 5 µM was found not suitable treatment to induction in compare with the other treatments. In different concentrations and combinations of PBA and IAA different responses were observed. High concentration ratio of BAP and IAA directly enhance the induction following tissue formation; since these plantlets was both long and dense.

Different treatment ratio of BAP and IAA

S. No	treatment	Medium callus fresh Waight (gm /petridish)
1	10 µM BAP + 10 µM IAA	1.77
2	10 µM BAP + 5µM IAA	1.66
3	5µM BAP+ 10 µM IAA	1.56
4	5µM BAP+ 5µM IAA	0.46

Figure: Plant regeneration in laboratory

4. Conclusion

Various hormonal treatments and explant sources have been used for induction of cultured tissue growth different plants. Some researchers have used onlysauxins [7, 8] whereas some have used the combinations of auxins and cytokinins together [9, 10]. We used different combinations of BAP and IAA for this purpose. It was found that the presence of BAP not only was necessary to induction from explants of plant but improves proliferation as well. Suitable combination of BAP and IAA also improved tissue formation in culture. Induction of culture of this plant was suitable when BAP with IAA used in higher level. Generally, it can be deduced that in combinations of these hormones tissue induction is suitable when IAA is used in equal level of BAP .The results of this experiment revealed that in response to tissue culture approaches, such that different explants or hormonal treatments were suitable to different aims for each type.

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