

Original Research Article

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Effect of Different Concentration of Growth Regulators on *in vitro* Culture of Rice

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ABSTRACT

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A laboratory experiment was conducted in the laboratory of Biotechnology at Dayanand College, Hisar in which seeds of rice cv. MR219 were used for regeneration of whole plants. Seeds were soaked overnight in sterilized water for 24 hours to soften and imbibe the seed for recovery of embryos. Seed were dehusked with the forceps and collected in the Petri dish or washed for 3-4 times with sterilized distilled water and again washed for one minute in distilled water. After one minute seeds transfer in 75% ethanol for one minute and again sterilized in 30% sodium hypo chloride for 20 minutes. Finally thoroughly washed in sterile water for at least three times under the aseptic conditions and used as the source of material for explants. Mainly, MS (Murashige and Skoog) media was used in the tissue culture Operation. The pH was adjusted to 5.8. Best results of root generations were obtained by using MS media with IBA. The best results of shoot regeneration were obtained by using MS media with BAP and IAA.

Introduction

Rice is the staple food of many countries throughout the world. Thus, it is very important to ensure the constant availability of this crop. However, conventional propagation methods have several disadvantages such as the unavailability of large-scale true-to-type planting materials and vulnerability to environmental changes. In

in vitro regeneration has an advantage over conventional propagation method in producing disease free plants at high multiplication rate. The Malaysian indica rice MR219 is currently the most popular rice variety grown in Malaysia since its release in 2001. A few advantageous characteristics of this indica rice variety include short maturation period of only 105-111 days, fairly tall but strong culms and resistance to blast

and bacterial leaf blight. Regeneration studies have been established for rice using various sources of explants including mature seeds, shoot segments, embryos and axillary shoot segments. In previous studies, rice was regenerated for genetic transformation purposes using explants such as, Shoot apical meristem (SAM), coleoptile, mature embryo or shoot and root tips. However, to date, there are no reports of an ideal protocol that bypasses the intermediate callus phase for the in vitro regeneration of indica rice, Meristem tissue as an explant has the ability to regenerate shoots without the intermediate callus development in the ideal concentration and combination of PGRs. Shoot formation requires sub culturing processes that are laborious and time consuming. Meristem tissue ensures genetic stability and minimizes somaclonal variations by avoiding adventitious organogenesis. Also, juvenile tissue is advantageous because it contains most actively dividing cells; it is highly responsive in tissue culture medium and has high regenerative ability compared to tissue obtained from mature sources. Hence, the present study uses SAM obtained from juvenile stage (Plumule of 4 - day old germinating rice seedling) as an explant for plant regeneration. Apart for the type of explant, PGRs also determine the effectiveness of an in - vitro regeneration protocol. The type and concentration of PGRs shows significant variability in in vitro regeneration of various species and cultivars. According to the culture medium and PGRs determine the optimum response of an explant. It is especially vital to regenerate indica rice genotypes since these varieties are principal food source for most tropical regions in the world.

Tissue culture techniques have become necessary for the production of the transgenic rice plants (Peng *et al.*, 1992), hybrids (Mariam *et al.*, 1996) and for the recovery of germplasm when seed availability is limited.

These methods are based on adventitious shoot culture (Shahsavari, 2011) and somatic embryogenesis (Amarasinghe, 2009) which resulted into genetic variations in rice cultivars (Mannan *et al.*, 2013). Using exogenous 2, 4-D treatment and yeast extract callus induction of stem nodes as explant was carried out by Furushashi and Yantazawa (1964). This was further extended by (Yamada *et al.*, 1967) using IAA and 2, 4-D with cytokinins. The most effective growth regulator of callus induction in rice is 2, 4-D without cytokinin treatment. Micropropagation protocols have been established for rice cultivars using as explants like as mature seeds (Ullah *et al.*, 2007), shoot segments (Verma *et al.*, 2011), embryos (Ali *et al.*, 2004, Evangelista *et al.*, 2009), anthers (Khatun *et al.*, 2010) which produced regenerated plantlets. Fifteen rice cultivars of rice were screened for induction of callus as well as subsequent regeneration of plantlets through plant tissue culture (Hertke and Lo'rz, 1989). Similarly, 500 cultivars of rice were comprehensively studied for the induction of callus as well as regeneration of plantlets by Kamia *et al.*, (1988). It was also reported that an individual genotype of rice plays a significant role in the induction of callus and regeneration of plantlets (Hoque and Mansfield, 2004, Islam *et al.*, 2005).

Therefore, the present study was undertaken to regenerate whole Malaysian indica rice cv. MR219 plants from SAM, in optimized PGRs without the intermediate callus phase. Also, in this study we evaluate the effects of different PGRs towards the in vitro regeneration of MR219 rice from SAM.

Materials and Methods

Plant material (explants)

Seeds of rice were used for regeneration of whole plant. Rice outer husk is dehusked and collect all the clean seeds in petridish.

Sterilization of glassware's media and other instruments

Glass wares were thoroughly washed with detergent or tap water and dried in hot air oven at 140°C. Forceps were dipped in alcohol and wrapped by aluminium foil and place it into the oven for sterilization. The working surface of laminar air flow was thoroughly cleaned with alcohol before starting the operation.

Sterilization of explant

Seed were dehusked with the forceps and collect all seed in the Petri dish or washed for 3-4 times sterilized distilled water and again wash for 1min in distilled water. After one min. seeds transfer in 75% ethanol for 1min. and again sterilized in 30% sodium hypochloride for 20min. Finally thoroughly wash in sterile water for at least three times under the aseptic conditions and used as the source of material for explants.

Preparation of stock solutions

At the time of media preparation, it is practically not possible to weigh each of the ingredients needed in the medium. Hence, for the sake of convenience, concentrated stock solutions of basal MS medium containing different ingredients were prepared (Table 1) in double distilled water and stored in glass bottles in refrigerator at 5°C temperature.

Preparation of culture media

After addition of various kinds of adjuvant (after bringing stock solutions to room temperature) to the MS basal medium, the pH of medium was adjusted to 5.8 using 0.1N HCl. The final volume adjusted and 8 gm/l agar-agar was added to the medium and then hormones in respective treatments were added to the media and then dispensed in suitable container i.e. culture bottles (Table 2).

Autoclaving was done using horizontal steam sterilizer at 121°C and 1.5 lbs/cm² pressure for 20 min. After sterilization, the medium was allowed to cool down then medium were poured in culture bottles and allowed to solidify at room temperature and stored in dust proof room for at least 3 days before use to check for any contamination.

Inoculation

Surface sterilized seeds were inoculated in media under aseptic environment provided by Laminar Air Flow. Surface of the chamber was washed with 70% ethanol before starting operation. The cotton bud of conical flask was removed which contained MS media. The seeds of explant were picked from petridish with the help of sterilized forceps and inoculate the explant seed into the conical flask which contained MS media (Plate 1). The neck of conical flask was sterilized on spirit lamp and then put the cotton bud on neck of the conical flask. To minimize chance of infection the instruments were dipped in disinfectant after every operation.

Shoot regeneration

After 7 days, the embryos and the embryonic axis were isolated aseptically from preconditioned explants and were culture on MS medium containing different concentration of BA and kinetin with or without NAA. All culture media were supplemented with 3% sucrose, 8ml / 1 agar. Explants were subculture to the same medium after 4 and 7 weeks on the same medium devoid of activated after 10 weeks of culture.

Rooting

After the culture, the regenerated shoots were rooted in MS medium containing different concentration of IBA (indole - 3 - butyric acids). After 4 weeks of rooting the rooting plantlets were transferred to plasmodesmata.

Results and Discussion

Effect of media on shoot regeneration

Present investigation showed that the number of days required for shoot initiation was influenced due to different levels of BAP and constant IAA (Table 3 and Plate 2). Shoot regeneration was recorded in explants in all generation media, but 100% response shoot per explants were significantly different on both explants, BAP. IAA is responsible for the formation of shoots. The same results were observed by Dahanayaka *et al.*, (2012) the highest shoot induction was recorded on MS basal medium with 2.5 mg / 12, 4 - D and

0.1 mg / 1 BAP for selected Sri Lankan traditional rice varieties.

Effect of media on rooting

Results of root growth in media supplemented with IBA presented in Table 4. Root formation was recorded in explants in different generation gives different results. IBA effect the rooting in different generations. The quantity of IBA gives different results. The root number was influenced due to different levels of BA in combination with constant NAA was also studied by Bakshi *et al.*, (2020) in Bamboo.

Table.1 Preparation of stock solution

Sr. No.	Stock solution	Compound	Amount Per liter	Total Volume
1.	Major Salt	NH ₄ NO ₃ Cacl ₂ KNO ₃ MgSO ₄ KH ₂ PO ₄	16.5gm 4.4gm 19gm 3.7gm 1.7gm	500ml (20X)
2.	Minor Salt 1	H ₃ PO ₄ MnSO ₄ .4H ₂ O ZnSO ₄ .7H ₂ O KI Na ₂ MoO ₄ .2H ₂ O	0.62gm 2.230gm 0.860gm 0.083gm 0.25gm	100ml (200X)
3.	Minor Salt 2	CuSO ₄ .5H ₂ O CaCL ₂ .C6H ₂ O	0.025gm 0.025gm	200ml (200X)
4.	Chelating Agents	Na ₂ EDTA FeSO ₄ 7H ₂ O	0.373gm 0.278gm	200ml (200X)
5.	Vitamins	Myoniositol Nicotinic Acid Thymine HCL Glycine Pyridoxine HCL	10000mg/L 50mg/L 10mg/L 200mg/L 50mg/L	100mg/100ml Freshly prepared 100X 100X 100X 100X

Table.2 Preparation of culture media from stock solution

Sr. No.	Stock Solution	Total volume of stock solution(ml)	Volume for 11/t of media
1.	Major salt	500	50ml/l
2.	Minor Salt 1	100	5.0ml/l
3.	Minor salt 2	200	5.0ml/l
4.	Chelating agent	200	5.0ml/l
5.	Vitamins		
(a)	Myoinositol	100	10g/1000ml Freshly prepared
(b)	Nicotinic acid	25mg/ml	0.5ml/l
(c)	Thymine	25mg/25ml	0.1ml/l
(d)	Glycine	25mg/l	2.0ml/l
(e)	Pyridoxine	25gm/5ml	0.5ml/l

Agar= 0.5gm/l, Sucrose= 30gm/l,

Table.3 Effect of different concentration of growth regulators on shoot regeneration

Media	BAP	NAA	Kinetin	IAA	Result	Days
MS 1.	25µl	---	---	150µl	Forth day Contamination occurs	1 st three days shoot arise & than contamination occurs.
MS 2.	---	10µl	20µl	---	20% response	1 st two days shoot arise & than 3 rd day contamination occurs.
MS 3.	50µl	---	---	---	60% response	1 st four days shoot arise & than growth stop. No contamination occurs
MS 4.	50µl	---	---	10µl	30% response	1 st four days shoot arise & than growth stop. No contamination occurs
MS 5.	60µl	10µl	---	---	80% response	1 st six days shoot arise.

Table.4 Effect of different concentration of growth regulators on shoot regeneration

Sr.No.	Media	IBA(mg/L)	Results
1.	MS(1.1)	1.5	2.5 cm (fast growth)
2.	MS(1.2)	2.0	2.0 cm (slow growth)

Plate.1 Inoculation of seed



Plate.2 Formation of shoot



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