

Optimum Callus Production and Complete Plant Regeneration

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LETTER

The effect of plant phytohormone (PGR) on the regeneration frequency of Malaysian upland rice was investigated. Mature seeds were used as a starting material for callus induction experiment using various concentrations of two, 4-D and NAA. Optimal callus induction frequency at 90% was obtained on MS media containing 2, 4-D (3 mg L⁻¹) and NAA (2 mg L⁻¹) after 6 weeks while no significant difference was seen on tryptophan and glutamine parameters. Embryo genic callus was recorded as compact, globular and lightweight yellowish in color. The embryo genic callus morphology was further confirmed with scanning microscopy (SEM) analysis. For regeneration, induced callus were treated with various concentrations of Kin (0.5–1.5 mg L⁻¹), BAP, NAA and 0.5 mg L⁻¹ of TDZ. Rice may be a strategic crop within the Asian region especially Malaysia and it's essential to take care of a domestic production level for food security reasons. Upland rice is one among the special rice types grown on limited irrigation conditions. Malaysian up and rice normally is cultivated in Sabah and Sarawak by the rural communities for low-scale production. Upland rice yield in Malaysia is recorded ranging from 0.46 to 1.1 tons per hectare. Malaysia typically imported 1031.4 thousand metric tons of rice from different countries due to insufficient supply to feed its blooming population. For this reason, rice transformation using the biotechnology approach needs to be applied to address both biotic and abiotic factors that hinder the rice production globally. However, an effective and robust tissue culture protocol system in upland rice was limited and relatively low and varied among genotypes tested. Thus, the most viable option that is still feasible is through optimization of several parameters in rice genetic improvement program.

The purpose of this study aimed to ascertain high quality embryo genic callus from mature seeds using optimal concentration of 2, 4 dichlorophenoxy acetic acid (2, 4-D) and naphthalene acetic acid (NAA) and amino acid concentrations as well as their morphological variations under SEM. Keeping in view the above statements, an attempt was made to establish an improvement regeneration protocol for Malaysian upland rice cv. Panders.

Sterilized seeds were cultured on a basal callus induction medium consisting of MS salts and B5 vitamins fortified with 2,4-D (1–4 mg L⁻¹) and NAA (0–4 mg L⁻¹) either alone or in combination, 30 g L⁻¹ sucrose, 0.5 g L⁻¹ casein hydrolysate and 0.4% (w/v) gelrite. Subsequently, the influence of various concentrations (0, 25, 50, 100, 150 mg L⁻¹) of amino acids (tryptophan and glutamine) on callus induction performance was carried out to optimize the best induced callus media.

Embryo genic callus and non-embryo genic callus were fixed in 3% buffered glutaraldehyde (0.1 M phosphate buffer, pH 7.2) for 2 h at room temperature. Later, the calluses were dehydrated through a gradient ethanol series [30% (v/v), 50% (v/v), 70% (v/v), 80% (v/v), 90% (v/v)] and 95% (v/v) for 15 min each and 100% ethanol twice for 15 min. The samples were dried and coated with gold (JEOL 780174712) and viewed under scanning electron microscopy (JEOL JSM6390LV). Many studies revealed the presence of synthetic auxin, 2, 4-D was an important catalyst factor for successful rice callus induction but other researchers used 2, 4-D combined with BAP or NAA. Further, the use of 2, 4-D was observed as inevitable for micro propagation through calluses. However, our studies showed that the combination of 2, 4-D (3 mg L⁻¹) and NAA (2 mg L⁻¹) induced better callus induction frequency (90%). Our finding was also in contrast to previous reports on other Malaysian upland rice grown on MSB5 media consisting of 2, 4-D only. Nevertheless, our finding was also in agreement with who observed that 2, 4-D combined with BAP or NAA gave better response to callus induction but in wetland rice. Also suggested in their finding that a combination of auxin (NAA and 2, 4-D) was a better alternative rather than using the single auxin. Also reported supplementation of 2, 4-D alone or in combination into callus induction media enhanced callus induction. Since the same hormonal composition is not suitable for all rice varieties, the modifying media were diversified to overcome the genotypic influence for particular rice varieties. In fact, NAA function was reported to stimulate the frequency of embryogenesis in the initial culture stage of rice while Endress suggested 2, 4-D could promote DNA hyper methylation in a pre-embryonic phase which was responsible to preserve the cell in highly mitotic mode.

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