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ORIGINAL ARTICLE

Improvement of efficient *in vitro* regeneration potential of mature callus induced from Malaysian upland rice seed (*Oryza sativa* cv. Panderas)

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Abstract A new and rapid protocol for optimum callus production and complete plant regeneration has been assessed in Malaysian upland rice (*Oryza sativa*) cv. Panderas. The effect of plant growth regulator (PGR) on the regeneration frequency of Malaysian upland rice (cv. Panderas) was investigated. Mature seeds were used as a starting material for callus induction experiment using various concentrations of 2,4-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. Embryogenic callus was recorded as compact, globular and light yellowish in color. The embryogenic callus morphology was further confirmed with scanning electron microscopy (SEM) analysis. For regeneration, induced calli were treated with various concentrations of Kin (0.5–1.5 mg L^{-1}), BAP, NAA and 0.5 mg L^{-1} of TDZ. The result showed that the maximum regeneration frequency (100%) was

Abbreviations: 2,4-D, 2,4-dichlorophenoxyacetic acid; NAA, naphthaleneacetic acid; Kin, kinetin; MS, Murashige and Skoog; BAP, benzylaminopurine; TDZ, thidiazuron.

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achieved on MS medium containing BAP (0.5 mg L^{-1}), Kin (1.5 mg L^{-1}), NAA (0.5 mg L^{-1}) and TDZ (0.5 mg L^{-1}) within four weeks. Developed shoots were successfully rooted on half strength MS free hormone medium and later transferred into a pot containing soil for acclimatization. This cutting-edge finding is unique over the other existing publishable data due to the good regeneration response by producing a large number of shoots.

1. Introduction

Rice is a strategic crop in the Asian region especially Malaysia and it is essential to maintain a domestic production level for food security reasons. Upland rice is one of the special rice types grown on limited irrigation conditions. Malaysian upland 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 tonnes per hectare (Sohrabi et al., 2013). Efforts were made by Sohrabi et al. (2012) to study about 50 Malaysian upland rice varieties for genetic diversity, showing that various upland rice cultivars need to be improvised for crop improvement purposes. Research on upland rice improvement has gained little attention due to the unstable grain yields. Furthermore, the low yield of upland rice grain is commonly because of the poor agronomy management practices and lack of the application of nutrient management in the upland rice cultivation (Hanafi et al., 2009). In addition, a lot of strategies need to be devised to improve upland rice productivity via the conventional breeding approach along with recent achievements in genetic improvement to balance the world consumption of rice. Although Malaysian agricultural mostly depended on wetland rice varieties such as MR219 and MR220 it is still insufficient to support the demand of domestic consumption. To date, over a half of the population of this world would totally depend on rice as a staple food and that demand for rice production is still increasing everyday. Malaysia typically imported 1031.4 thousand metric tonnes of rice from different countries due to insufficient supply to feed its blooming population (DOA, 2011). 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 (Geng et al., 2008). Thus, the most viable option that is still feasibleis through optimization of several parameters in rice genetic improvement program. Numerous previous reports in line with the goals have been published such as genotype, type of explant used, desiccation treatment, carbon sources and plant growth regulators (PGRs) in medium (Ali et al., 2004; Saharan et al., 2004; Lin and Zhang, 2005; Ikram-Ul-Haq et al., 2009; Feng et al., 2011). Apart from modification in plant growth regulators used, amino acids such as tryptophan and sorbitol also showed positive effects on rice callus induction (Chowdhry et al., 1993; Shahsavari et al., 2010), while glutamine has been recommended by the researcher in rice tissue culture (Ge et al., 2006; Shahsavari, 2011). The selection of embryogenic callus induction was critical before any regeneration study took place. Numerous studies on SEM analysis of good quality of rice callus were performed (Bevitori et al., 2014; Narciso and Hatorri, 2010; Vega et al., 2009) as visual observation may misjudge the callus morphology and appearance. Therefore, SEM was used to identify the embryogenic rice callus appearance in this study as a pre-requisite for the successful application of the plant regeneration approach. Shahsavari (2010) found that application of various parameters resulted in 31–68% regeneration frequency in the Selasi cultivar of Malaysian upland rice.

Until now, there are several reports in upland rice micropropagation such as those reported by Geng et al. (2008), Shahsavari et al. (2010), Shahsavari (2010) and Zhao et al. (2011). Several reports have shown that the exogenous application of plant growth regulators such as kinetin (Kin), benzylaminopurine (BAP), and naphthalene acetic acid (NAA) with addition of thidiazuron (TDZ) could improve regeneration frequency in upland rice (Ge et al., 2006; Zhao et al., 2011). Other than that, sorbitol or maltose has been shown to have a promotive effect on regeneration of upland rice cultivar (Feng et al., 2011; Geng et al., 2008; Shahsavari et al., 2010). However, TDZ has been shown to improve regeneration of Handao 297 Chinese upland rice cultivar upto 81.2% (Zhao et al., 2011). Dey et al. (2012) also concluded that the addition of TDZ into the regeneration medium significantly enhanced the proliferation of multiple shoots using the shoot apex in rice (*Oryza sativa*).In this present study, the modified regeneration medium was applied to regulate the initiation of multiple shoots from each scutellum derived calli while the other carbon source and gelling agent were standardized to promote fast growth. To our best knowledge, this protocol for high frequency plant regeneration is still lacking in the other Malaysian upland rice cultivars using embryogenic callus cultures, hence the novelty of this study.

The purpose of this study aimed to ascertain high quality embryogenic calli from mature seeds using optimal concentration of 2,4 dichlorophenoxyacetic acid (2,4-D) and naphthaleneacetic 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 genotype (*Oryza sativa*) cv. Panderas.

2. Materials and methods

2.1. Establishment of aseptic explants

Manually dehusked seeds of upland rice, Panderas cultivar were obtained from Panderas village, Pahang, Malaysia. The seeds were surface sterilized with 70% alcohol (v/v) for one minute and followed by immersion of 100% commercial bleach plus a drop of Tween-20 (Sigma–Aldrich, USA) for