ABSTRACT

SUSIYANTI. Insertion of phytase gene into genome of sugarcane cultivars, regeneration, expression and acclimatization. Under supervision of G.A. WATTIMENA, DWI ANDREAS SANTOSA, MEMEN SURAHMAN, AND AGUS PURWITO.

Sugarcane (Saccharum officinarum L.) is one of the important crops grown in marginal land in Indonesia. Phosphorus (P) is critical to the growth and development of plant in the marginal land. P is stored in plant as phytic acid (myo-inositolhexakisphosphate). Phytic acid is hydrolyzed by the activity of phytases to yield inositol and free phosphate. Genus Saccharum has low phytase activity, so it needs phytase gene insertion from other source. Genetic transformation of sugarcane holds promise to provide enough P during period of rapid cell division and growth of plant. The efficient insertion of phytase gene from other source requires plant transformation process through Agrobacterium tumefaciens. Plant transformation mediated by Agrobacterium tumefaciens, has become the most used method for the introduction foreign genes into plant cells and the sub sequens regeneration of transgenic plant. Successfulness of transformation process depends on regeneration, transformation, gene expression, and methode of acclimatization.

In vitro culture technique is needed to multiply clones of transgenic sugarcane. As the first step in many tissue culture experiment, it is necessary to induce callus formation from the primary explant before regenerating to be plantlets. In general sugarcane transformed lines show chlorophyll content and leaf colour such as albino, discoloration, lack of chlorophyll in the particular spot of leaves. The abnormal leaves showed low chlorophyll content. To increase the chlorophyll content of sugarcane transgenic line can be treated with putrescine. At the moment there is no data about the expression of phytase gene in form of phytase activity and its effect to phosphate and chlorophyll content in sugarcane. The transformed sugarcane plantlets should be able to acclimatize to field condition.

The appropriate callus induction from 3 clones of sugarcane was observed on medium containing 3 mg l⁻¹ 2.4 D. Shoot of sugarcane has able to induce by using modified MS medium + kinetin (0.1 mg l⁻¹) + BAP (0.5; 1.0 ; 1.5; 2.0 mg l⁻¹). Shoot started with the appearance of green dot on callus within a week on regeneration medium. The best shootlet medium of three clones of sugarcane was MS + kinetin (0.1 mg l⁻¹) + BAP (1.5 mg l⁻¹). The best rooting medium of three clones of sugarcane was MS + kinetin (0.1 mg l⁻¹) + IBA (1.0 mg l⁻¹).

The research showed that callus of putative transgenic line can survive under 150 mg l⁻¹ kanamycin medium. The efficiency regeneration of putative transgenic line cv. Triton, cv. PSJT 94-41, and cv. PA 175 were succesively 30 %; 25 %; and 30 %. Efficiency transformation of putative transgenic line were: cv. Triton = 21 %; cv. PSJT 94-41 = 15 %; and cv. PA 175 = 24 %. The analyzed of integrated phytase gene was proven by the appearance of 900 bp of PCR band.