Simple Micropropagation by Indirect Somatic Embryogenesis in Sugarcane (*Saccharum officinarum* L.)

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ABSTRACT— ***The simple method for producing sugarcane somatic embryos was developed by reducing concentration of 2,4-D. Callus of sugarcane was maintained on MS medium supplemented by 3 ppm of 2,4-D (D3). The callus surface changed into white opaque colour (white nodule) when the concentration of 2,4-D was decreased to 1.5 ppm (D1.5). Thereafter, the somatic embryos developed from the white nodule. There are various developmental stage of sugarcane somatic embryos on D1.5 medium, namely globular, early scutellar, late scutellar, coleoptilar embryos. When the 2,4-D was omitted from the medium (D0), the coleoptilar somatic embryos germinated , formed shoot and root. The germinated somatic embryos were growing well forming plantlet. This simple method offers a convenient tool for micropropagation of sugarcane.***

Keywords— Sugarcane, 2,4-D, somatic embryo, plantlet

1. INTRODUCTION

Somatic embryogenesis is an amazing process because the bipolar structure possessing shoot and root resembling zygotic embryo (Mariani et al., 1998). Williams and Maheswaran (1986) reported that somatic embryogenesis could be induced through direct (without intervening callus) and indirect (callus mediated). Usually, only small amount of somatic embryos were obtained by direct somatic embryogenesis. In contrast, large amount of somatic embryos were obtained by indirect somatic embryogenesis, such as in oil palm (Purnaning, 200).

Sugarcane is one of the important plant in the world. The sugar derived from sugarcane could be used as an ingredient for medicine. Therefore, it is important to propagate the sugarcane biotechnologically, such as by somatic embryogenesis method.

In sugarcane, there are many reports on plant regeneration via indirect somatic embryogenesis (Rodriguez et al., 1995; Desai et al., 2006; Brisibe et al., 1993, 1994). However, they used complex medium and supplement so that the methods were not suitable for sugarcane commercialization.

In the present study, we report a simple method of indirect somatic embryogenesis in sugarcane. We used simple medium and simple method so that the price of plantlet production was low cost. Furthermore, this simple method will be useful when research on genetic transformation will be performed.

2. MATERIAL AND METHODS

Sugarcane (Saccharum officinarum L.) cultivar Ps 869 was employed in this study. The maintained callus was cultured on MS medium supplemented with 3 ppm of 2,4-D (D3). For somatic embryogenesis process, the callus was subcultured on MS medium supplemented with 1.5 ppm 2.4-D (D1.5) for 5 weeks. Thereafter, the somatic embryos were germinated on MS medium without 2,4-D (D0). The germinated somatic embryos were further grown on D0 medium so that plantlet can grow vigorously. The cultures were kept in the dark at 25^{o}C. After the somatic embryo germinated, they were placed in 16 hr photoperiod for plantlet development. The developmental stages of the somatic embryos were followed by stereo microscopy.

3. RESULT AND DISCUSSION

Callus of sugarcane on D3 medium was friable and yellowish. The callus surface changed into white opaque colour (white nodule) when the concentration of 2,4-D was decreased to 1.5 ppm (D1.5). In this condition, the callus has been developed into embryogenic callus (Fig. 1). Rodriguez et al. (1995) also described that embryogenic callus of sugarcane was white and nodular in appearance.

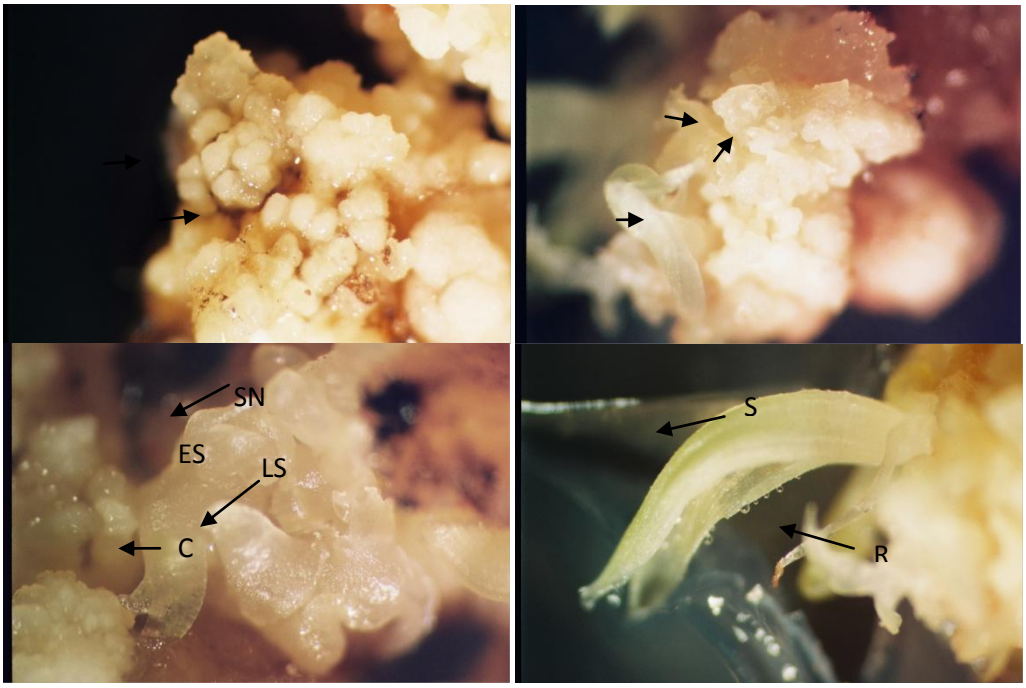

Fig. 1. Embryogenic callus of sugarcane after 3 weeks of culture on MS medium supplemented with 1.5 ppm 2,4-D (D1.5). (Note the white nodule (arrow). Fig. 2. Globular embryos of sugarcane (arrow) after 4 weeks of culture on D1.5 medium. Fig. 3. Early scutellar embryo (ES) with its scutellar notch (SN), Late scutellar embryo (LS) and coleoptilar embryo (C), after 5 weeks of culture on D1.5 medium. Fig. 4. Germinated embryo with shoot and root after 1 week of culture on MS medium without hormone (D0). (S =shoot, R=root).

Fig.5. Plantlet of sugarcane after 4 weeks of culture on MS medium without hormone.

After 5 weeks of culture, the somatic embryos developed from the white nodule of embryogenic callus. There are various sequential developmental stage of somatic embryos on D1.5 medium, namely globular (Fig. 2)., early scutellar, late scutellar, coleoptilar embryos (Fig.3). In early scutellar, scutellar notch was observed. Later in coleoptilar stage, the coleoptiles was coming out from this scutellar notch. Rodriguez et al. (1995) also observed the lateral scutellar notch in sugarcane somatic embryos. Brisibe et al. (1993) reported the developmental stage of sugarcane somatic embryos as follow: proembryogenic globule, globular, scutellar and coleoptilar stages. This typical somatic embryogenesis was also found in rice as reported by Mariani et al. (1998). They observed proembryo, globular, scutellar, coleoptilar embryos in rice somatic embryo.

Brisibe (1993) reported that there were protein, starch and lipid storage material in scutellar stage of sugarcane somatic embryo. Therefore, in this study, when the 2,4-D was omitted from the medium (D0), the coleoptilar somatic embryos germinated , formed shoot and root after one week of culture (Fig. 4). The germinated somatic embryos were growing well forming plantlet after 4 weeks of culture on D0 medium (Fig. 5).

In the present study, the theory of somatic embryogenesis, i.e. putting on high concentration of 2,4-D for induction and decreasing the 2,4-D for development followed by omitting the 2,4-D for germination of the somatic embryos was proved. This simple method offers a convenient tool for micropropagation of sugarcane.

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5. REFERENCES

- Brisibe, E.A., Nishioka, D., Miyake, H., Taniguchi, T., and Maeda, E. 1993. Developmental electron microscopy and histochemistry of somatic embryo differentiation in sugarcane. Plant Science. 89: 85-92
- Brisibe, E.A., Miyake, H., Taniguchi, T, Maeda, E. 1994. Regulation of somatic embryogenesis in long-term callus cultures of sugarcane (Saccharum officinarum L.). New Phytologist. 126 (2) : 301-307
- Desai, N.S., Joseph, D., Suprasanna, P., Bapat, V.A. 2006. Study of elemental variations during somatic embryogenesis in sugarcane using photon induced X-ray probe. Nuclear instruments and Methods in Physics Research B 252:299-302
- Mariani, T.S., Miyake, H. and Takeoka, Y. 1998. Changes in surface structure during direct somatic embryogenesis in rice scutellum observed by scanning electron microscopy. Plant Production Science. 1(3): 223-231
- Purnaning, A.S., 2006. Pengaruh penambahan glutamine pada tahap maturasi terhadap germinasi dan konversi plantlet embrio somatic kelapa sawit (Elaeis guineensis Jacq.). Scription. School of Life Sciences and Technology, Bandung Institute of Technology, Indonesia.
- Rodriguez, S., Mondejar, C., Ramos, M.E., Diaz, E., Maribona, R., Ancheta, O. 1995. Sugarcane somatic embryogenesis: a scanning electron microscopy study. Tissue& Cell. 28(2) : 149-154
- Williams, E.G. and Maheswaran, G. 1986. Somatic embryogenesis: Factors influencing coordinated behavior of cells as an embryogenic group. Ann. Bot. 57: 443-462.