SHORT COMMUNICATION

Plant regeneration from callus culture of vetiver (*Vetiveria zizanioides* Nash)

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Abstract

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The present research aimed to establish cell suspension culture of vetiver (*Vetiveria zizanioides* Nash) from Surat Thani germplasm source and efficient plant regeneration from callus derived from such cultures. Cell suspension cultures were established from calli derived from inflorescence of vetiver. Optimum cell proliferation occurred in liquid N_6 medium supplemented with 10 μM 2,4-dichlorophenoxyacetic acid (2,4-D) and 10 mM proline. The cell suspension formed the highest small colonies when plated on solid MS medium containing 0.45 μM 2,4-D. After subsequent transfer to regeneration medium (MS free medium) 65% of plantlets were obtained.

Key words : regeneration, callus, vetiver, *Vetiveria zizanioides*

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บทคัดย่อ

สมพร ประเสริฐส่งสกุล

การเกิดต้นจากแคลลัสของหญ้าแฝก (*Vetiveria zizanioides* Nash)

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งานวิจัยนี้มีวัตถุประสงค์เพื่อทำให้เกิดเซลล์แขวนลอยและทำให้เกิดต้นจากแคลลัสที่ได้จากเซลล์แขวนลอยหญ้าแฝก (*Vetiveria zizanioides* Nash) แหล่งพันธุกรรมสุราษฎ์ธานี จากการชักนำให้เกิดเซลล์แขวนลอยจากแคลลัสที่ได้จากการเพาะเลี้ยงช่อดอกของหญ้าแฝก เซลล์แขวนลอยเกิดได้ดีในอาหารเหลวสูตร N_6 ที่ประกอบด้วยสารควบคุมการเจริญเติบโต 2,4-ไดคลอโรฟีนอกซีอะซีติคแอซิด (2,4-D) ความเข้มข้น 10 ไมโครโมลาร์ และเสริมด้วยโพรลีน ความเข้มข้น 10 มิลลิโมลาร์ เมื่อนำเซลล์แขวนลอยไปเลี้ยงในอาหารแข็งมูราชิกและสกุด (MS) ที่เติม 2,4-D ความเข้มข้น 0.45 ไมโครโมลาร์ ช่วยส่งเสริมการสร้างโคโลนีขนาดเล็ก จากนั้นเมื่อนำโคโลนีไปเลี้ยงต่อในอาหารสูตรเดิมที่ปราศจากสารควบคุมการเจริญเติบโตสามารถชักนำให้เกิดเป็นต้นแฝกที่สมบูรณ์ได้ 65%

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Vetiver (*Vetiveria zizanioides* Nash) is known as a miracle plant in Thailand. It has been widely used for soil and water conservation, oil production, thatching, as well as handicrafts. Vetiver, a densely tufted, glabrous perennial, is a shy breeder and considered sterile outside its natural habitat. It has no rhizome or stolons and is propagated by root division or slips (Sayamanonta *et al*., 1996).

Tissue culture techniques are used for the mass propagation of plant. Cell suspension cultures facilitate the isolation of protoplast, genetic transformation, *in vitro* selection, physiological and biochemical studies (Ozawa *et al*., 1996). Plant regeneration from tissue and cell culture depends on the embryogenic cell type. The development of a system of embryogenic suspension culture has been a focal point for improving the regeneration frequency in many plants. Plant regeneration from embryogenic suspension culture was suitable for the mass propagation of plants. Many authors (Choi *et al*., 1997; Patnaik *et al*., 1997; Redway *et al*., 1990) reported on regenerated plant from embryogenic suspension culture. Liu *et al*. (1998) succeeded in the development of an efficient system of embryogenic suspension cultures and plant regeneration in eight sweet potato cultivars. They indicated that the embryogenic suspension culture needed to be subcultured in a timely fashion. In *Acanthopanax koreanum* Nakai, a lack of growth regulators was necessary for their regeneration (Choi *et al*., 1997). Similarly, for regeneration responses of embryo-derived suspension cultured cells of *Angelica sinensis* (Oliv.) Diels., no plant growth regulators were needed for plant regeneration (Tsay and Huang, 1998). However, there is no report on suspension culture and plant regeneration from callus derived from cell suspension culture in vetiver. In vetiver, the regeneration of complete plantlet has been achieved from callus (Mucciarelli *et al*., 1993; Leupin *et al*., 2000).

The objectives of this study were to establish cell suspension culture and report on plantlet regeneration from callus derived from embryogenic suspension culture of vetiver.

Materials and Methods

Initiation of cell suspension culture

Inflorescences of the vetiver (*Vetiveria zizanioides* Nash) from Surat Thani germplasm source were surface sterilized by soaking in 70% ethyl alcohol for 5 min. Then they were cut into small pieces and transferred to the solid Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) supplemented with 15 μM 2,4-D. After three weeks, calli were transferred to solid MS medium supplemented with 10 μM 2,4-D. Cultures

were maintained at 25±2ºC with a 16-h photoperiod. After culturing for 4 weeks, 2 grams of compact embryogenic calli were transferred to a 125 ml Erlenmeyer flask with 30 ml liquid N_6 medium containing 10 µM 2,4-D, 20 g/l sucrose (Chu *et al*., 1975) and several concentrations of proline (0, 5, 10, 15 and 20 mM). The pH of the medium was adjusted to 5.8. The cultures were maintained at 25±2ºC on a gyratory shaker at 110 rpm under 16 h of fluorescent light. Cell suspension growth was determined by packed cell volume (PCV) at 2-day intervals. Three replications were used for each experiment.

Plant regeneration from cell suspension culture

Six-day-old cells subcultured from three-month-old embryogenic cell suspension culture derived from compact callus of vetiver were used for regeneration. Single cells and small cell aggregate were obtained by filtering the resultant suspension through a sterile nylon net (100 µm pore size). The cells were plated onto solid medium by pour plate technique. Cells (1 ml) were pipetted directly onto a 5.6 cm Petri dish with different solid media (MS, ½ MS and N_6 media). The larger colonies developed from these aggregates after 14 days that were subcultured onto fresh medium of the same composition for further proliferation. The calli were placed on one layer of dry sterilized filter paper in a 9 cm Petri dish sealed with parafilm and incubated for 2 days. After dehydration, they were cultured on MS-free medium consisting of three replications with 150 explants and the experiment was repeated twice.

Results and Discussion

Initiation of cell suspension culture

Callus initiation occurred after culturing inflorescences on solid MS medium supplemented with 15 µM 2,4-D for three weeks. The small protuberances as initial calli appeared on the surface of florets. Calli started to rapidly divide when transferred to solid MS medium containing 10 µM 2,4-D. These results indicated that 2,4-D is critical for callus induction. Zheng and Konzak (1999) reported that 1.0-2.0 mg/l 2,4-D for 10-15 days culture followed by a reduced concentration of 2,4-D (0.2 mg/l) are both sufficient for optimal induction of calli from wheat anther. Lin *et al*. (2000) also reported that 2,4-D induced cells formation of *Alstroemeria*. Inflorescence-derived callus developed into two distinct types, which were observed by stereo microscope. The embryogenic callus was compact and yellow or whitish-yellow and non-embryogenic callus was friable and yellow. Cell suspension initiated from embryogenic callus was cultured in liquid N_6 medium supplement with 10 µM 2,4-D and different concentrations of proline (5-20 mM). Growth was measured by packed cell volume at 2-day intervals. Figure 1 shows the effects of proline in the medium. Cell growth in the presence of proline, was greater than those without proline in the medium. This is probably due to the fact that the cells can divide well caused by proline. A proline concentration of 10 mM was adequate for the optimal cell-suspension culture. Ella and Zapata (1993) reported that proline promoted secondary calli on the periphery of the mother callus in rice. O'Neill *et al*. (1996) indicated that proline also promoted shoot regeneration from stem explants of *Brassica napus*. The effects of proline may facilitate cell division and feration of secondary calli.

Plant regeneration from cell suspension culture

Even though liquid medium can regenerate a large number of plantlets, plantlet regeneration from cell suspension culture has been limited in some species, swing to many factors such as aeration rate, agitation speed and gas composition affecting the regeneration process. In this present work, no somatic embryo was observed in liquid medium. Therefore, the solid medium was used. Soft friable calli proliferated in cell suspension culture within 4 weeks. The size of colonies in MS medium was larger than is other media (Table 1). Based on this study, the solid MS medium supplemented with 0.45 µM 2,4-D was optimal for induction of small colonies from cell suspension. These calli were similar to the callus used to initiate the cell suspension. They were dehydrated

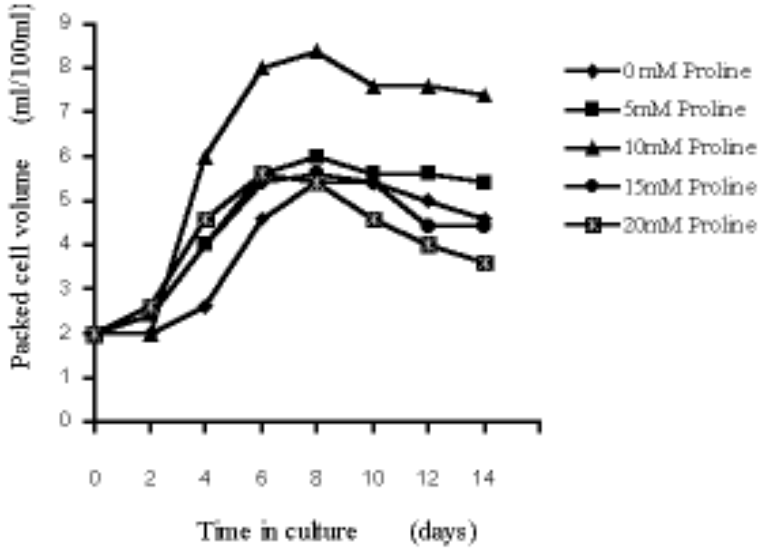

Figure 1. Growth response of suspension-cultured cells of *Vetiveria zizanioides* Nash Surat Thani germplasm cultured at different proline concentrations in the liquid N_6 media supplemented with 10 µM 2,4-D.

Table 1. Effect of media and growth regulators on colony formation from cell suspension culture of vetiver for 4 weeks.

Media and growth regulators	Cell size of colonies (cm)
MS basal medium	< 0.1
MS + 0.45 µM 2,4-D	0.25-0.45
MS + 0.45 µM 2,4-D + 0.5 µM kinetin	0.10-0.25
1/2 MS basal medium	< 0.1
1/2 MS + 0.45 µM 2,4-D	0.10-0.15
1/2 MS + 0.45 µM 2,4-D + 0.5 µM kinetin	0.10-0.20
N_6 basal medium	< 0.1
N_6 + 0.45 µM 2,4-D	0.10-0.15
N_6 + 0.45 µM 2,4-D + 0.5 µM kinetin	0.10-0.15

and transferred to the plant regeneration medium (MS free medium) 65% of plantlets were obtained (Figure 2). Tsukahara and Hirosawa (1992) indicated that dehydration process was necessary to enhance plantlet regeneration in rice.

According to this finding, the system of plant regeneration from embryogenic cell suspension culture of vetiver opens new vistas for improvement of plant regeneration in the Gramineae. Further studies need to be carried out in the field.

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A.

B.

Figure 2. Plant regeneration from suspension-derived callus of *Vetiveria zizanioides* Nash Surat Thani germplasm. A. Suspension-derived callus cultured on MS medium supplemented with 0.45 µM 2,4-D for 2 months, B. Regenerated plants cultured on MS free medium for 1 month.

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