

## Research Note

# A simple and highly efficient protocol for somatic embryogenesis and plant regeneration from proembryonic mass suspension culture in 'Autumn Royal Seedless'

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**Introduction:** Although somatic embryos could be multiplied by recurrent subculture on fresh solid medium, in general, yield still remains low. In addition, somatic embryos are highly asynchronous in solid medium. Quality of somatic embryos predominantly affects regeneration frequency, which varies in each subculture on solid medium (BORNHOFF and HARST 2000). Furthermore, somatic embryos on solid medium showed exhibit dormancy, whereas in liquid medium they were not dormant and also showed higher plant regeneration efficiency (JAYASANKAR *et al.* 2003).

In this report we demonstrated a highly repeatable protocol for somatic embryogenesis and plant regeneration from proembryonic mass suspension culture of 'Autumn Royal Seedless' (*V. vinifera* L.). The protocol consists of two major steps: (1) multiplication of PEMs in liquid medium and (2) regeneration of PEMs in solid medium.

**Material and Methods:** Establishment of PEMs suspension culture: MSGGN liquid medium consisted of MS (MURASHIGE and SKOOG 1962) medium plus 20 g l<sup>-1</sup> maltose, 500 mg l<sup>-1</sup> glutamine, 5 ml l<sup>-1</sup> glycerol, and 1 mg l<sup>-1</sup> NOA, adjusted to pH 5.7. The liquid medium, Erlenmeyer flasks sealed with double-folded aluminum foil, and other equipments were sterilized by autoclaving at 121 °C and 1.1 kg cm<sup>-2</sup> pressure for 20 min. Fifty and 100 ml medium was then poured into 125 ml and 250 ml flasks, respectively. Approximately 500 mg of 'Autumn Royal Seedless' primary somatic embryos, induced in the same lab (XU *et al.* 2005), was initially incubated in 50 ml MSGGN medium. The contact area between the edge of aluminum foil and the neck of the flask was sealed with Parafilm. The flasks were placed on a rotary shaker at 120 rpm in 26 ± 2 °C and maintained in darkness.

**Subculture:** The cultures were subdivided at 3-week-intervals to fresh MSGGN by using the modified

procedure of JAYASANKAR *et al.* (1999). Briefly, flasks were transferred to aluminum flow hood for 5 min in order to allow PEMs to precipitate. Approximately 60 % of the supernatant was gently decanted. The suspension culture remaining was swirled and filtered through a 1 mm stainless steel sieve. The filtered PEMs were briefly rinsed with fresh MSGGN medium in order to bring passable PEMs through sieve pores. The large cluster of proembryonic masses (LPEMs, >1 mm diameter) were retained on the sieve, whereas the flow-through liquid culture containing small cluster of proembryonic masses (SPEMs, <1 mm diameter) was collected in a beaker. The liquid culture was swirled and re-filtered through a single layer of Kimwipe in a funnel. Approximately 200 and 400 mg of SPEMs were transferred by stainless steel spatula to 50 ml and 100 ml of fresh MSGGN flasks.

**Somatic embryogenesis and plant regeneration:** Approximately 50 mg of SPEMs in 6 clumps were placed in a 100 x 15 mm Petri dish filled with 20 ml FMSC medium (MS medium plus 30 g l<sup>-1</sup> sucrose with 3 g l<sup>-1</sup> activated charcoal, pH 5.8). The cultures were maintained at 26 ± 2 °C in darkness for somatic embryogenesis. After 4 weeks, the cultures were subdivided by the following procedure: somatic embryos (≥5 mm) were transferred to regeneration medium (see below) and 2 clumps were spread out evenly in a 100 x 25 mm Petri dishes containing 25 ml FMSC medium; this was transferred to fresh medium every 4 weeks to promote somatic embryo development. The somatic embryos (≥5 mm) were harvested individually and transferred to Magenta GA7 boxes containing 80 ml FMSC medium for plant regeneration. The boxes were maintained in a 16 h photoperiod. There were 9 somatic embryos per box.

**Plant establishment:** When plantlet length reached at least 7 cm and they had developed at least 5 leaves, they were transferred to potting soil mixture in 3 inches plastic cups, each of which was covered with another plastic cup on top.

**Results and Discussion:** Establishment of PEMs suspension culture: Whitely PEMs appeared in the suspension 2 weeks after the somatic embryos were incubated in MSGGN medium. The PEMs grew rapidly and suspension culture was established in two subcultures. At this time, the PEMs were yellowish and capable of rapid multiplication. The results demonstrated that the MSGGN medium with the culture techniques were highly efficient for producing proembryonic mass of 'Autumn Royal Seedless' (Figure, A). Sieving was very helpful to synchronize suspension culture by separating of SPEMs (Figure, B) and LPEMs during subculture.

Cell browning has been a major obstacle in establishing a suspension culture for grape (JAYASANKAR *et al.* 1999, BORNHOFF and HARST 2000). In this study, browning of neither PEMs nor liquid medium was observed during the initial and subsequent cultures.

**Somatic embryogenesis:** Two weeks after being transferred to the FMSC medium, the SPEMs developed into somatic embryo stages including globular, heart-shape, torpedo, and early cotyledon. The somatic embryos

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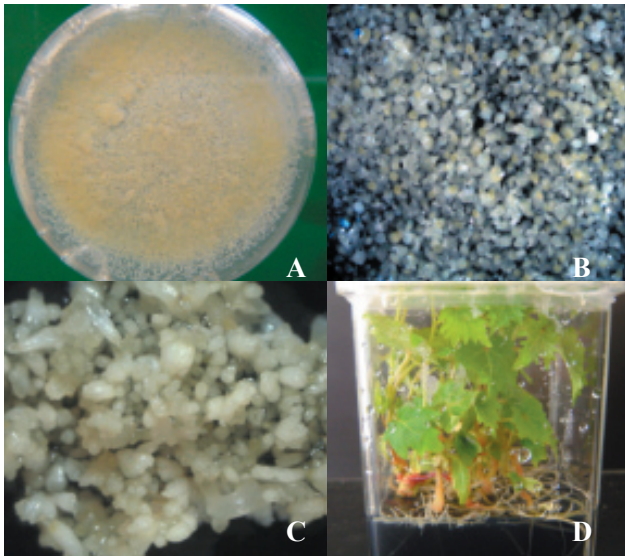


Figure: PEMS suspension culture in MSGGN medium after a 3-week-culture period in 125 ml flask (A). SPEMs separated by sieving (B). Somatic embryos developed asynchronously on FMSC medium in for 4 week (C). Regenerated plants in a Magenta box (D).

appeared asynchronously (JAYASANKAR *et al.* 1999), some somatic embryos precociously elongating to 1-2 cm within 4 weeks before the first subculture while the length of the majority was about 0.5-2 mm (Figure, C). More than 1,000 somatic embryos were obtained from approximately 50 mg of SPEMs within 14-16 weeks. LPEMs generated far less and highly asynchronous somatic embryos within the same time.

**Plant regeneration:** The somatic embryos of 'Autumn Royal Seedless' germinated in about 2 weeks after transferring to Magenta boxes in FMSC medium. The

germinated somatic embryos were converted into plantlets with roots, shoots and leaves in another week. Somatic embryo germination and conversion rate varied depending upon quality (MOTOIKE *et al.* 2001). In this study, cotyledons were found to be the most important factor for somatic embryo conversion. Somatic embryos germinated into perfect cotyledons (unfolded, expanded and green) were converted into plantlets much faster than those with poor cotyledons (folded, unexpanded, and yellowish with some green at the tip).

In this study, approximately 50 mg of SPEM generated more than 1,000 somatic embryos and more than 95 % of the germinated somatic embryos converted into normal plants (Figure, D), which is highly efficient. Dormancy of somatic embryos was not observed. The regenerated plants were successfully established in pots within 6 weeks.

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- BORNHOFF, B.; HARST, M.; 2000: Establishment of embryo suspension culture of grapevines (*Vitis* L.). *Vitis* **39**, 27-29.
- JAYASANKAR, S.; GRAY, D. J.; LITZ, R. E.; 1999: High-efficiency somatic embryogenesis and plant regeneration from suspension cultures of grapevine. *Plant Cell Rep.* **18**, 533-537.
- MOTOIKE, S. Y.; SKIRVIN, R. M.; NORTON, M. A.; OTTERBACHER, A. G.; 2001: Somatic embryogenesis and long term maintenance of embryogenic lines from fox grapes. *Plant Cell Tiss. Org. Cult.* **66**, 121-131.
- MURASHIGE, T.; SKOOG, F.; 1962: A revised medium for rapid growth and bioassay with tobacco tissue cultures. *Physiol. Plant.* **15**, 473-497.
- XU, X.; LU, J.; REN, Z.; 2005: Callus induction and somatic embryogenesis in muscadine and seedless bunch grapes (*Vitis*) from immature ovule culture. *Proc. Fla. State Hort. Soc.* **118**, (in press).

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