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Efficient Plant Regeneration via Organogenesis in Winter Squash (*Cucurbita maxima* Duch.)

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Objectives

In this study, we have established an efficient regeneration protocol from cotyledonary explants of winter squash (*C. maxima* Duch.) as a preliminary investigation of genetic transformation in *C. maxima*. This is the first report on successful regeneration from cotyledonary explants via adventitious shoot organonegenesis in winter squash (*C. maxima* Duch.).

Materials and Methods

1. Plant materials and regeneration

The winter squash cultivar, Juktoja (Nongwoo Seed, Suwon, Korea) was used in all experiments. The effects of shoot induction from excised different segment of winter squash in germination days of seeding were investigated. Different segment of winter squash seedlings at various stages of germination ranging from 4 to 9 days were cultured on MS medium containing different BA concentration. Cotyledonary, hypocotyl and root explants of winter squash were excised from in vitro grown seedlings after germination in darkness. For adventitious shoot induction, explants were cultured on MS medium supplemented with 1, 2, 5 or 10 mg/L BA. Elongated shoots were then rooted in MS medium without growth regulator.

2. Flow cytometric analysis

Nuclei were prepared from the leaves of regenerated plants. Each leaf was chopped with a razor blade in nuclear extraction buffer (Partec, Munster, Germany). The samples were filtered through a 30 μm nylon filter and nuclei in the filtrate were stained with 4,6-diamidino-2-phenylindole (DAPI; Partec). Flow cytometric analysis was performed with a Ploidity Analyzer (Partec).

Results and Discussion

Using cotyledonary explants excised from seedlings after in vitro germination, an efficient plant regeneration via organogenesis was established for Korean (*Cucurbita maxima* Duch.) cultivars. To establish optimal conditions for adventitious shoot induction, a variety of explants were prepared from seedlings of different ages, and cultured using media with different concentrations of growth regulators. For cultivar, plant regeneration was optimal when the proximal parts of cotyledons from 4-day old seedlings were cultured on induction medium composed of Murashige and Skoogs (MS) medium with 1 mg/L 6-benzylaminopurine (BA). After 3 weeks of culture in the induction medium, 82% of explants from Korean cultivar regenerated shoots. The adventitious shoots were subcultured on elongation medium composed of MS medium with 0.1 mg/L BA, and the elongated shoots were successfully rooted in MS medium without growth regulator for 2 weeks. Flow cytometric analysis revealed that most of the regenerated plants were diploid.

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