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Somatic embryogenesis, synthetic seed preparation and plant regeneration in *Apium graveolens* var. *Dulce pers.*

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Summary On the MS medium supplemented with 2 ppm 2,4-D,calli were induced after 4-6 weeks from the petioles of an American celery plant (*Apium graveolens* var. *Dulce pers.* cv. *Florida*). Suspension culture was started from the calli in a hormone-free liquid MS medium on a gyratory shaker at 110 rpm,and kept at 26℃. To stimulate cell division and dedifferentiation,the subcultures were conducted for 7 days each on the same medium. The liquid suspension containing single cells,cell aggregates,and somatic embryos in different stages were screened 2－3 weeks later and 1.0－1.5mm somatic embryos were obtained. These embryos were encapsulated with sodium alginate by dropping-bead method and solidified with 0.1mol $CaCl_2$. These synthetic seeds germinated and developed well into seedlings in the sterilized vermiculite substrate.

Key words *Apium graveolens* var. *Dulce pers.*, cell culture, somatic embryogenesis,synthetic seeds

Introduction

The production of synthetic or artificial seeds is one of the high bio-technological research fields being explored in the world. It is considered that the production and practice of synthetic seeds will lead to a new green revolution in conventional crop breeding and seed production. So far,the synthetic seeds have been obtained in certain crops,including carrot(Li Xiuqing, 1987; Kitto et al., 1985a; Rendenbaugh, 1984), orange (Kitto et al., 1985b), lucerne(Demarly,1985),rice(Ni Dexiang,1987) etc. It is commonly accepted that the vegetable crops will be one of the important application fields of the future synthetic seed technology. This study was conducted to determine the environmental conditions for somatic embryogenesis and regeneration from synthetic seeds as well as the methods of making synthetic seeds of occidental celery (*Apium graveolens* var. *Dulce pers.* cv. *Florida*).

Materials and methods

Experimental materials

Petiole explants were elutriated with fresh water for 2 hr,then sterilized in 70% ethanol for 30 sec,and then treated in 6% (W/V) calcium hypochlorite solution for 50 min at room temperature,and finally elutriated with sterilized water 3 times.

Dedifferentiation culture

The MS medium supplemented with 2,4-D 2mg/L,sucrose 20g/L,agar 6g/L(pH 5.8)was autoclaved at 121℃ for 20min. The petiole was cut into squares of about 5 mm each,which were placed on the medium MS and then kept in a growth chamber at 26℃ with about 2000 lux light intensity. The photoperiod was 12 hr/day.

Somatic embryogenesis

Cell suspension was obtained from friable calli derived from petiolar discs. The liquid hormone-free MS medium was used for suspension culture. The suspension was agitated continuously at 110 rpm on a gyratory shaker at 26℃. To stimulate cell division and the dedifferentiation, the cell suspension was subcultured on the same nutrient medium which was renewed every 7 days.

Selection of somatic embryos

To obtain celery embryos of 1.0-1.5mm long suitable for making synthetic seeds, the suspended embryos were filtered through nylon or metal screens in different mesh sizes.

Preparation of synthetic seeds

The somatic embryos were encapsulated with 1.5% sodium alginate containing 1/4 MS basal medium and then solidified with 0.1mol $CaCl_2$ by the dropping-bead method.

Germination experiment of synthetic seeds

The synthetic seeds were sown on 3 different sterilized media: (1)vermiculite supplemented with distilled water; (2)vermiculite supplemented with the macroelements of the 1/4 MS medium; or (3) vermiculite supplemented with nutrient soils for bedding plants(1:1).

The synthetic seeds were then kept in the growth chamber at 25℃ during the day and 21℃ during the night. The photoperiod was 16 hr/day. The germination rate was calculated after 3 weeks.

Results

Dedifferentiation culture

4—6 weeks later, the light yellow calli appeared on the edges of the petiolar squares. These calli were then subcultured on the MS medium supplemented with 0.5ppm of 2,4-D for rapid propagation. Then microscopic bead-like projectures were observed on the surface of calli.

Suspension culture

In the initial stage of the suspension culture, single cells, large clumps of cells and globular embryos were seen under microscope in the liquid suspension. After the first subculture, a large quantity of embryos of different stages, i.e. of the globular, heart, torpedo and stick stages, were formed. Two weeks later, most of the somatic embryos were 0.5-1.0mm long, and some of them even developed into cotyledons and radicles. Besides, some single cell and cell aggregates still could be seen in the liquid suspension. This indicated that celery somatic embryogenesis is asynchronous.

Encapsulation and preparation of synthetic seeds

The somatic embryos with strong vitality were suitable for encapsulation when they were 0.5-1.8mm long and could be screened through nylon or metal sieves in different mesh sizes. They also could be stably suspended in the gel of 1.5% sodium alginate and solidified quickly after treated by 0.1 mol $CaCl_2$ for 20 minutes to form bead-shape and translucent synthetic celery seeds.

Germination of the synthetic seeds on different growth beds

Study results indicated that their germination rate varied from 2.2% to 75% in different treatments. The synthetic seeds sown on the vermiculite substrate supplemented with macroelements of the 1/4 MS medium germinated at the highest rate of 75.0%(60/80), 50%(50/100) of seeds germinated after being sown on vermiculite substrate containing nutrient soils for bedding plants. And in the vermiculite substrate supplemented with distilled water the germination rate was only 2.2% (2/92). Therefore,in three treatments above the vermiculite substrate supplemented with the macroelements of 1/4 MS medium seems to be the optimum medium for the germination of the synthetic celery seeds.

Discussion

It is believed that the research on synthetic celery seeds has a great potential in practical use (Rendenbaugh et al. ,1987) because single plants in the population of any excellent celery variety may vary in productivity. Hence,in the conventional production of celery,the choice of raised seedlings is essential in order to ensure plant uniformity for high yield. The suitable characteristics of the maternal plant will be passed onto its progenies according to the principle of asexual propagation,it is to say,the synthetic celery seeds from somatic cells can grow into uniform plants without any manual choice. And this would be very important for celery production.

Considerable progress has been made in the research on large-scale embryogenesis, screening of synchronously-developed embryos,and encapsulation and solidification of the embryos. Moreover,plantlets were obtained from the synthetic celery seeds and grew well on seeds beds protected by windbreaks in winter of 1988 and spring of 1989. A total of 200 plants were tested and variation showing a much earlier bolting stage was observed on only one plant.

Its final goal is industrialized production of high-quality seeds for agricultural crops and trees. Yet,there exist a lot of technical difficulties. Embryogenesis,for example,for many important crops has not yet been successful,the variation of synthetic seeds,the extension of storaging period,and the protection of the seeds from microbial infection after sowing in the field,all are problems remaining to be solved.

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