Short Communication

Selection of Embryogenic Callus of Carrot by Drying Treatment

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Embryogenic and nonembryogenic calli of carrot were visually selected and cultured individually for 3 weeks on MURASHIGE and SKOOG'S (MS) agar medium supplemented with 5×10^{-6} M 2,4-dichlorophenoxyacetic acid (2,4-D). They were dried to a moisture level of about 3% for 6 hours and recultured on MS medium without 2,4-D. All the dried embryogenic calli survived and formed many embryos or plantlets. The dried nonembryogenic calli could not survive except for very small parts which appeared to be embryogenic calli. These results showed the possibility of selection of embryogenic calli by the application of the drying method.

KEY WORDS: *Daucus carota*, embryogenic callus selection, drying.

Introduction

One of the most important problems in plant biotechnology is the redifferentiation of plantlets from callus. Therefore, many researchers have carried out studies on the induction of callus with a high morphogenetic potential such as the embryogenic callus. They analyzed the kind, concentration, and combination of organic and inorganic substances in the medium. However, few studies have been carried out on the selection of embryogenic calli. The development of such a selection method is therefore extremely important for the regeneration of plant from callus for plant biotechnology applications such as gene manipulation, cell fusion and mass propagation, because only a limited number of plants can be regenerated from callus. In this report a method for the selection of embryogenic calli in carrot is described.

Material and Methods

Calli were induced from hypocotyls of the domestic carrot, *Daucus carota* L. cv. Ogata Goshun Ninjin on MS (MURASHIGE and SKOOG, 1962) agar medium containing 5×10^{-6} M 2,4-D. The pH of the medium was adjusted to 5.7 before the addition of agar (9g/l). The cultures were maintained at 27°C under the illumination for 12 hours/day (about 600lux) by cool white fluorescent tubes. Induced calli were subcultured every 4 weeks in the same medium for about 3 months. Actively growing calli were used for the experiments 2 to 3 weeks after the subculture. Embryogenic and nonembryogenic calli were visually selected by their color carefully, and cultured individually during 3 weeks. The embryogenic calli were whitish yellow, while the nonembryogenic ones were translucent and pale yellow.

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Embryogenic and nonembryogenic calli with a fresh weight of approximately 10mg were put on a filter paper disc (Toyo Roshi, No. 2) in a 15 × 15mm petri dish, respectively. They were placed in a larger (60 × 60mm) and tall cylindrical vessel containing 15g of activated silica gel. The petri dish, silica gel and filter paper were sterilized by heating at 160°C for 1 hour before use. The calli were dried to equilibrium for 6 hours at 27°C in the vessel and stored under these conditions for 17 hours by sealing the petri dish with parafilm (American Can Co.). The water content of the dried calli was calculated from the difference in the weight of the calli before and after drying for 24 hours at 80°C.

All the calli were transferred to the MS agar medium without 2,4-D and cultured for 60 days under the conditions mentioned above. The growth of the calli and the differentiation of embryos and plantlets were examined under a stereoscope during the culture period.

Results and Discussion

The color of the dried embryogenic calli was yellow, while that of the dried nonembryogenic calli was dark gray. Growth and embryo differentiation after drying are shown in Table 1. The fresh and the dried embryogenic calli formed a large number of embryos or plantlets in all the parts of the calli after 21 days of culture (Table 1 and Fig. 1, A and B). Only 5 fresh nonembryogenic calli formed embryos or plantlets after 21 days of culture (Table 1 and Fig. 2, A and B), although all the calli grew actively. On the other hand, almost all the nonembryogenic calli failed to grow and died by drying. Only 3 dried nonembryogenic calli formed embryos, after 21 days of culture (Table 1 and Fig. 2, C and D). Furthermore, they formed embryos beside or below the dark gray dead calli on the medium.

These results indicate that nonembryogenic calli could not survive after drying, and that the invisible small embryogenic calli in the nonembryogenic ones were able to survive and formed embryos. It is also possible to consider that the embryogenic calli survived in the dead nonembryogenic calli after drying, but did not grow due to the adverse effect of substances which the dead nonembryogenic callus produced during the incubation (HU and WANG 1983.) Regenerated embryos or plantlets from dried calli grew normally (Fig. 3, A and B) as reported earlier (KAIMORI and TAKAHASHI 1989).

Table 1. Growth and embryo differentiation of embryogenic and nonembryogenic carrot calli after drying

Treatment	Kind of calli	No. of calli tested	No. of calli which grew or regenerated embryos and plantlets							
			8 days		14 days		21 days		60 days	
			Growth	Embryo differ. ¹⁾	Growth	Embryo differ. ¹⁾	Growth	Embryo differ. ¹⁾	Growth	Embryo differ. ¹⁾
No drying	E ²⁾	20	20	7	20	20	20	20	20	20
No drying	NE ³⁾	20	20	1	20	2	20	5	20	8
Drying	E ²⁾	20	20	6	20	20	20	20	20	20
Drying	NE ³⁾	20	0	0	0	0	3	3	3	3

¹⁾ Embryo differentiation

²⁾ Embryogenic calli

³⁾ Nonembryogenic calli

We examined the possibility of selecting embryogenic calli in mitsuba, *Cryptotaenia japonica* H., by applying a method similar to that for carrot. Half of the embryogenic calli which were subcultured for about 8 months survived and regenerated many embryos and plantlets after being subjected to drying, whereas none of the nonembryogenic calli survived (unpublished data).

Embryogenic calli can be selected by their appearance such as color and shape or hardness (VASIL and VASIL 1984), and by procedures such as sieving and centrifugation which are commonly used in suspension culture (see the review by FUJIMURA and KOMAMINE 1984). Em-

Fig. 1. Embryos and plantlets differentiated from fresh embryogenic callus (A) and dried embryogenic callus (B) during 21 days of culture.

Fig. 2. Embryos and plantlets differentiated from fresh nonembryogenic callus (A, B) and dried nonembryogenic callus (C, D) during 21 days of culture.

Fig. 3. Plantlet differentiation from dried embryogenic (A) and nonembryogenic (B) calli during 60 days of culture.

bryogenic calli can be easily selected by their appearance if they are large. However, if they are too small, or grow more slowly than nonembryogenic calli (VASIL and VASIL 1984), selection based on the appearance or hardness is difficult.

The present study suggests that an embryogenic callus embedded in a nonembryogenic callus could be selected by the drying method. Suspension culture should be examined to select small pieces of embryogenic callus, and to eliminate the adverse effect of the dead nonembryogenic callus (HU and WANG 1983). We are currently subjecting to tissue cultures of several other plants to increase the embryo differentiation potential of callus by the application of the drying method.

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乾燥によるニンジン・エンブリオジェニック・カルスの選抜

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人参カルスを MURASHIGE と SKOOG の寒天培地で約 3 ヶ月間継代培養し, エンブリオジェニック・カルス (embryogenic calli, EC) とノンエンブリオジェニック・カルス (nonembryogenic calli, NEC) を可視的に選抜した。3 週間培養した後, それぞれのカルスを, 6 時間で約 3 % の乾燥平衡状態まで乾燥させた。乾燥処理及び乾燥無処理のすべての EC は, 多数の不定胚や幼植物体を分化させた (Table 1, Fig. 1, Fig. 3 A)。乾燥無処理のすべての NEC は活発に成長した。これらの約半数のカルスは, 多数の不定胚や幼植物体を分化させた。乾燥処理をした NEC は, ほとんどが枯死したが, EC と思われる一部は生存し, 幼植物体を分化させた (Table 1, Fig. 2, Fig. 3 B)。これらの結果は, 乾燥によって EC を選抜できる可能性を示している。