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Induction of Somatic Embryogenesis by Salt Stress in Carrot

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It is well known that embryogenic cells can be induced by exogenously applied auxin and that their development into somatic embryos are inhibited by the same hormone.[1] A number of researchers have investigated morphological and biochemical aspects of somatic embryo development from embryogenic cells,[2-8] but only a few reports deal with an inductive process of embryogenic cells from somatic cells. The reason for this is mainly due to diverse actions of auxin on plant tissues, such as the promotion of adventitious root differentiation, callus formation and cell enlargement. To conduct biochemical and physiological studies on induction of embryogenesis from a somatic cell, it is necessary to develop a new tissue culture system which does not require any auxin treatment of explants. Recently, we[9,11] demonstrated that carrot somatic embryogenesis could be induced by treatment with high concentration of sucrose, mannitol or heavy metal ions such as Cd^{2+}, Zn^{2+}, Ni^{2+} and Co^{2+} in auxin-free medium and suggested that osmotic and other physiological stress might trigger the somatic embryogenesis. In this report, we describe the effects of salt stress on carrot somatic embryogenesis.

Seven- to ten-day-old seedlings of *Daucus carota* L. cv. US-Harumakigosun grown on vermiculite were surface-sterilized with 10% (v/v) sodium hypochlorite solution (available chlorite concentration of *ca.* 1%) for 15 min, then rinsed three times with sterilized distilled water. Five mm long segments of cotyledons, hypocotyls, roots and apical tips were cut out from the seedlings. These explants were cultured in plastic Petri-dishes (60×15 mm) containing 8 ml of Murashige and Skoog's[10] agar (0.8% Difco Bacto agar) medium with 0.1 M sucrose (hereafter referred to as MS medium) to which no plant growth regulator was added and NaCl was supplemented at a concentration of 0.1 M to 0.4 M. After 1 to 3 weeks in culture, the explants were transferred onto hormone-free MS medium without NaCl. Cultures were carried out under a 16 hr light/8 hr dark illumination (approx. 3,000 lux) at 25℃. Each treatment consisted of at least twenty replicates and was repeated three times.

When apical tips were used as explants, the first and second leaves protruded and elongated during 1-2 week culture on MS medium containing NaCl at a low concentration (0.1 M), but they did not form somatic embryos before and even after the transfer to hormone-free MS medium without NaCl. On the other hand, apical tips cultured with a high concentration (0.2-0.4 M) of NaCl or those cultured for 3 weeks with a low NaCl concentration (0.1 M), turned to red and formed somatic embryos on the surface of the explants and/or elongated leaves without visible callus formation after the transfer to hormone-free MS medium without NaCl (**Figs. 1, 2**). These embryos developed into young plantlets (**Fig. 3**).

When cotyledon, hypocotyl and root explants were cultured on MS medium with 0.1 M to 0.4 M NaCl, their color turned brown or white during the culture and no somatic embryos was observed before as well as after the transfer to hormone-free MS medium without NaCl.

Frequency of somatic embryo formation obtained with apical tip segments has been summarized in **Fig. 4**. Higher frequency with the shorter treatment has been observed at the higher concentration

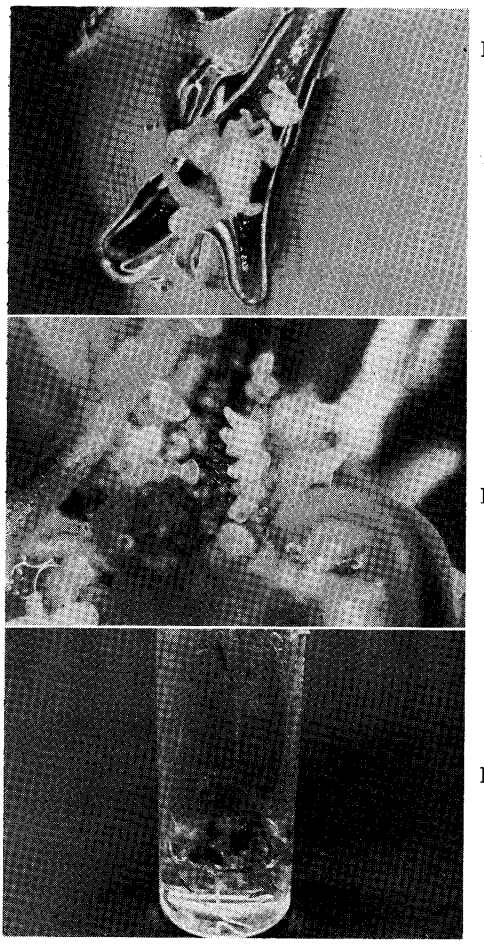

Fig. 1. Somatic embryos induced by salt stress in carrot. Apical tip segments were cultured for 3 weeks on hormone-free MS medium with 0.3 M NaCl, then transferred to hormone-free MS medium without NaCl. Somatic embryos were formed on the surface of the explants without visible callus formation. The photograph was taken 6 weeks after the transfer (×20).

Fig. 2. Somatic embryos formed directly on the surface of the first leaf grown from an apical tip segment. The apical tip segment was cultured under the same conditions as described in **Fig. 1** (×60).

Fig. 3. Young plantlets derived from somatic embryos which were induced by a 3-week treatment with 0.3 M NaCl. The photograph was taken 10 weeks after the transfer to hormone-free MS medium without NaCl (×1.5).

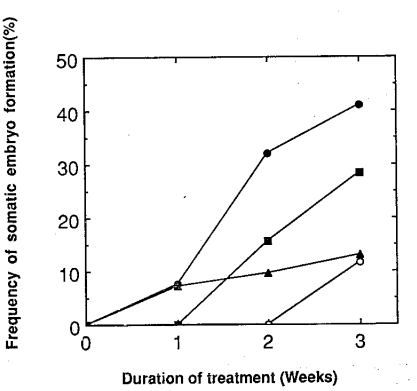

Fig. 4. Effects of NaCl on somatic embryo formation in apical tip segments of carrot. Apical tip segments were cultured on hormone-free MS medium with NaCl for 1 to 3 weeks and then transferred to hormone-free MS medium without NaCl. The concentrations of NaCl used were 0.1 M (○), 0.2 M (■), 0.3 M (●) and 0.4 M (▲). Frequency of somatic embryo formation (%) was calculated by applying the following formula;

$$\frac{\text{No. of segments produced somatic embryos}}{\text{No. of treated segments}} \times 100$$

of NaCl excepting at 0.4 M NaCl treatment, where many of the explants died (data not shown) rendering the frequency of somatic embryo formation a little low (*ca.* 10%).

As mentioned above, the treatments with NaCl at high concentrations induced somatic embyo formation on the apical tip segments of carrot. Recently, we found that the treatment of the same plant material (carrot apical tip segments) with several different heavy metal chlorides ($CdCl_2$, $CoCl_2$, $NiCl_2$ and $ZnCl_2$) induced somatic embryogenesis.[11] In that experiment, the concentrations of the heavy metal chlorides which were able to induce somatic embryo formation were quite low (0.25–1.0 mM) as compared to that of NaCl. It is likely that the stress caused by heavy metal ions initiated the process of somatic embryogenesis in carrot explants. On the other hand NaCl added to the media might have acted as an osmoticum and resulted in the induction of somatic embryogenesis. It should be noted that, in the case of sucrose treatment at high concentrations, both apical tips and cotyledonal segments formed somatic embryos on their surfaces,[9] while in the case of NaCl treatment, cotyledonal segments did not form somatic embryos. These differences in the response of cotyledonal segments with NaCl and sucrose treatments suggest that NaCl acts not only as an osmoticum but also exerts certain other physiological effects. NaCl at a high concentration may modify metabolic function of cells, such as maintenance of membrane potential, triggering indirectly the induction of somatic embryogenesis.

In addition to NaCl treatment, our recent findings on induction of somatic embryogenesis in carrot without auxin treatment[9,11] provide a new tool to investigate the mechanism of somatic embryogenesis.

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References

1) Thorpe, T. A., 1988. ISI Atlas of Science: Animal and Plant Sciences, p. 81-88.
2) Sung, Z. R., R. Okimoto, 1981. Proc. Natl. Acad. Sci. USA, **78**: 3683-3687.
3) Sung, Z. R., R. Okimoto, 1983. Proc. Natl. Acad. Sci. USA, **80**: 2661-2665.
4) Fujimura, T., A. Komamine, 1975. Plant Sci. Lett., **5**: 359-364.
5) Fujimura, T., A. Komamine, 1981. Z. Pflanzenphysiol., **102**: 293-298.
6) Kamada, H., H. Harada, 1979. Z. Pflanzenphysiol., **9**: 255-266.
7) Nomura, K., A. Komamine, 1986. Oxford Surveys of Plant Mol. Cell Biol., **3**: 456-466.
8) Satoh, S., H. Kamada, H. Harada, T. Fujii, 1986. Plant Physiol., **81**: 931-933.
9) Kamada, H., K. Ishikawa, H. Saga, H. Harada, 1989. Plant Cell Reports, (submitted).
10) Murashige, T., F. Skoog, 1962. Physiol. Plant., **15**: 473-497.
11) Kiyosue, T., K. Takano, S. Kawakami, H. Kamada, H. Harada, 1989. Plant Cell, Tissue Organ Cult., (submitted).

≪和文要約≫

塩ストレスによるニンジン不定胚の誘導

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ニンジン（*Daucus carota* L. cv. US 春蒔五寸）実生の頂芽を含む組織片を高濃度（0.1-0.4 M）の塩化ナトリウムを含む MS 培地で培養した後，これを含まない Murashige & Skoog 培地に移植・培養することで，植物ホルモンの添加無しに，体細胞から不定胚形成を行わせることに成功した．