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# Heat Stress Induction of Carrot Somatic Embryogenesis

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Since the independent discoveries by Reinert[1] and Steward *et al.*[2] that carrot somatic cells cultured *in vitro* were able to form somatic embryos, somatic embryogenesis has been extensively investigated as a model system to understand the mechanisms of zygotic embryogenesis, because morphological changes of somatic embryos are similar to those of zygotic ones. Especially, in carrot somatic embryogenesis which is the most established experimental system, many molecular biological analyses have been conducted on the developmental processes of somatic embryos[3]. It is well known that carrot somatic embryogenesis can be readily induced by transferring somatic tissues cultured on 2, 4-dichlorophenoxyacetic acid (2, 4-D)-containing medium to 2, 4-D-free medium. However, the induction system of somatic embryogenesis by 2, 4-D is unsuitable for analysis of prerequisite biochemical changes for induction of somatic embryogenesis, because 2, 4-D is a synthetic auxin which affects many other physiological phenomena. Therefore, to clarify how induction of embryogenic competence occurs in the somatic cells, it is necessary to develop a new induction system for somatic embryogenesis which does not require any phytohormone treatment. It has already been reported that carrot somatic embryogenesis can be induced by treatment of apical tip segments with high concentrations of sucrose[4,5], heavy metal ions such as $Cd^{2+}$ [6], or NaCl[7] in phytohormone-free medium. In these methods, after the segments are transferred to phytohormone-free medium without these substances, somatic embryos form directly on the surface of the elongating leaves without visible calls formation. In addition to the previous reports indicating that 2, 4-D is a suitable substance for induction of somatic embryogenesis, 2, 4-D is thought to be a stress agent, because 2, 4-D is known as a strong herbicide. It is possible to say that treatment with appropriate chemical stresses including 2, 4-D might trigger the induction of somatic embryogenesis and important changes for acquisition of embryogenic competence might occur during the stress treatments. In *Brassica* species, it has been reported that a short period of high temperature enhanced the frequency of haploid embryogenesis from immature microspores[8,9]. Moreover, the addition of mannitol at 300 mM strongly stimulated haploid embryogenesis from immature pollen grains in *Nicotiana* species[10]. Therefore, it is thought that adventitious embryogenesis including somatic and androgenetic ones might be induced by different kinds of stress treatment. In this report, we examined the effects of high temperature as one type of physical stress on carrot somatic embryogenesis.

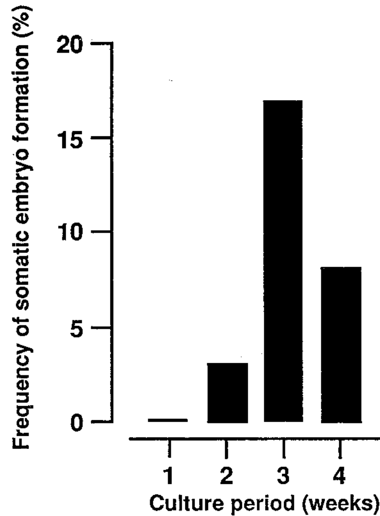


**Fig. 1** Effect of high temperature on the induction of somatic embryogenesis.
Apical tip segments were cultured at 25°C after pre-culturing at 37°C for 1-4 weeks. Frequency of somatic embryo formation (%) was calculated by the No. of segments producing somatic embryos / No. of treated segments.

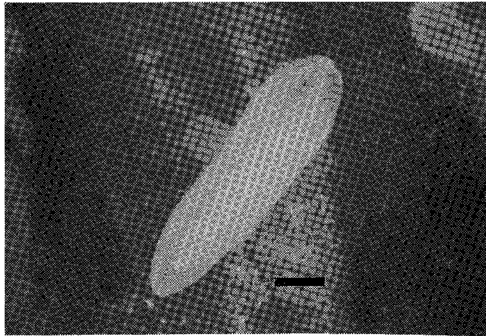

**Fig. 2** Somatic embryogenesis induced by the high temperature treatment.
Somatic embryos were formed on the surface of true leaves without callus formation. The photograph was taken 6 weeks after the transfer. Bar indicates 100 μm.

When apical tip segments were cultured at 35 or 37°C, true leaves elongated with the loss of chlorophyll during 1-2 weeks of culture. After the leave-elongating segments were transferred to 25°C under light conditions, the color of the leaves turned green. Somatic embryo formation were observed at the frequency of 3, 17 or 8% when the apical tip segments were cultured for 2, 3 or 4 weeks at 37°C and then transferred to 25°C, respectively (**Fig. 1**), but not on those which were cultured 1 week at 37°C followed by culture at 25°C. The number of somatic embryos formed per explant were few compared to those iuduced by heavy metal ions or high concentration of sucrose[4-7]. The somatic embryos were formed on the surface of the elongating true leaves without visible callus formation (**Fig. 2**). When the apical tip segments were cultured at 35°C for 1-4 weeks, followed by 1 week at 37°C, somatic embryos were not formed and the segments developed into young plantlets. Apical tip segments that were cultured at 40 or 42°C died within a few days of culture with the loss of chlorophyll. In order to clarify the duration of heat treatment, the apical tip segments were cultured at 40 or 42°C for 2, 4, 6, 8, 10, 15, 20, 30, 40, 72 h and then transferred to 25°C. However, no somatic embryos were formed in any case. Almost all of the apical tip

segments cultured at 40°C for 30, 40 and 72 h or at 42°C for 10, 15, 20, 30, 40 and 72 h, died, and the other remaining segments survived and developed into young plantlets. Among the treatments tested, treatment with culture at 37°C for 3 weeks was the most effective. On the other hand, no somatic embryos were formed on explants treated at 35, 40 or 42°C.

From the findings that almost all of the explants cultured at 35°C developed into plantlets and those cultured at 40°C and 42°C died, the temperature effective for induction of somatic embryogenesis is limited over a narrow range. It is known that pollen embryogenesis in *Brassica campestris* can be induced directly from microspores after high temperature treatment[8]. Moreover, it was demonstrated that 32. 5°C treatment for 3 days has strongly stimulated the induction of pollen embryogenesis in *Brassica napus*[9]. These results show that high temperature is one of the stresses effective for induction of adventitious embryogenesis including somatic and androgenetic ones. It has already been reported that carrot somatic embryogenesis could be induced by the treatment with NaCl, sucrose and heavy metals ($CdCl_2$ was the most effective)[4-7]. The frequencies of somatic embryo formation were *ca.* 40% (0. 3 M NaCl for 3 weeks), 80% (0. 7 M sucrose for 2 weeks) and 38% (0. 5 mM $CdCl_2$ for 2 weeks). In addition to the findings described above, it has been reported that treatments with 300 mM mannitol for 2 days just after isolation of immature pollen grains strongly stimulated haploid embryogenesis from the grains[10]. The frequency of somatic embryo formation was higher than that obtained in this study. Taking these facts into consideration, it is likely that certain stresses including chemical and physical stresses, such as high osmotic stress, heavy metal stress and high temperature stress, trigger the induction of adventitious somatic and androgenetic embryogenesis especially the acquisition of embryogenic competence. These differences in response to different stresses suggest that the induction mechanism of somatic embryogenesis is different depending on the kind of stress.

Including high temperature treatment, stress induction of carrot somatic embryogenesis without phytohormone treatment provides us a new tool to investigate the mechanism of somatic embryogenesis, especially the mechanism concerning with the acquisition of embryogenic competence.

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**《和文要約》**

# 熱ストレスによるニンジン体細胞不定胚誘導

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ニンジン実生の茎頂部をホルモン無添加のムラシゲ & スクーグ培地で37°C 2-4週間培養し，その後25°Cで培養したところ，伸長した本葉の上に不定胚が形成され，その頻度は3-17%であった．しかし，37°Cで1週間処理した場合には不定胚形成は見られなかった．また，35°Cで1-4週間培養した後に37°Cで1週間培養した場合にも，不定胚形成は見られなかった．さらに，40°Cあるいは42°Cで2-72時間培養し，25°Cに移しても不定胚形成は認められなかった．