**Short Communication**

# Osmotic and Non-osmotic Induction of Somatic Embryogenesis by Sucrose at High Concentrations in *Daucus carota* L.

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## Abstract

In the detached cotyledons of carrot, *Daucus carota* L., somatic embryogenesis was induced by 2-week treatment with sucrose at 0.29-0.73 M both in the epidermal cells (type-1) and the callus-like cells generated at the cut end of the cotyledons (type-2). Type-2 embryos were produced predominantly, after the treatment with 0.29-0.44 M sucrose and type-1 embryos after treatment with 0.58 - 0.73 M sucrose. Sucrose and glucose were more effective than mannitol and sorbitol for the induction of somatic embryogenesis. Furthermore, both type-1 and type-2 embryos were induced by sucrose and glucose, but only type-2 embryos by mannitol and sorbitol. These results suggest that sucrose does not work as a mere osmoticum.

Somatic embryogenesis in carrot has been used as a model system to study the mechanisms of embryogenesis. Although plant growth regulators are generally used for the induction of somatic embryos, it was reported that somatic embryos were induced in carrot without plant growth regulators. Explants exposed to a high osmotic pressure (Kamada *et al.*, 1993), NaCl (Kiyosue *et al.*, 1989a), heavy metals (Kiyosue *et al.*, 1990), hypochrolite (Kiyosue *et al.*, 1989b) or high temperature (Kamada *et al.*, 1994) formed somatic embryos. Those authors concluded that somatic embryogenesis was induced by various kinds of stress. In addition, the same protein induced by those stress in apical segments were detected in embryogenic cells formed by the treatment of 2,4-dichloroacetic acid (Tachikawa *et al.*, 1998). Whereas sucrose is used as an osmoticum in these study (Kamada *et al.*, 1993, Tachikawa *et al.*, 1998), it is known that sucrose plays physiological roles in plant cells, e.g. carbon sources and a signal which control gene expression (Halford *et al.*, 1999). Therefore, we examine whether sucrose act as a mere osmoticum.

Seeds of carrot (*Daucus carota* cv. MS-harumakigosun, Kyowa Seed Co. Tokyo, Japan) were sterilized by usual method (2% NaClO, 20 min) and germinated on basal medium (MS medium (Murashige and Skoog, 1962) solidified with 2 g/l gellan gum, containing 20 g/l sucrose) under continuous darkness at 25 °C for 10 to 14 days. Tips of cotyledons (ca 8 mm long) were used as experimental materials. As experimental medium, sucrose, glucose, mannitol or sorbitol at 0.29 M, 0.44 M, 0.58 M or 0.73 M was added to the basal medium. To treat the explants by these sugars, they were cultured on the experimental media for 2 weeks. After the treatment, they were transferred to basal medium and cultured under continuous darkness at 25 °C. At intervals of 1 week, explants were observed under a stereo microscope (Olympus SZH-ILLB) and subcultured. Embryos were photographed by a CCD camera (Shimadzu CCD-Z1) attached to the stereo microscope. Each experiment was conducted with 20 explants for each treatment and repeated twice. The rate of embryogenesis (%) was evaluated as (No. of explants with somatic embryos formed / No. of total explants) × 100.

Fig. 1a shows the somatic embryos observed on the explants at 4 weeks after the end of sucrose treatment at 0.58 M. We classified the embryos formed on the cotyledons into two groups, based on the sites where they were formed, one group consisting of the embryos formed on the surface of the cotyledons (type-1) and the other of ones formed on the cut end of the cotyledons (type-2). As shown in **Fig. 2**, both type-1 and type-2 embryos were produced after the end of treatment, and rate of embryogenesis increased with time. But the type-1 embryos developed later than the type-2 embryos. **Fig. 3** shows the rate of the formation of type-1 and type-2 embryos on the explants treated with sucrose at various concentrations. The rate of type-
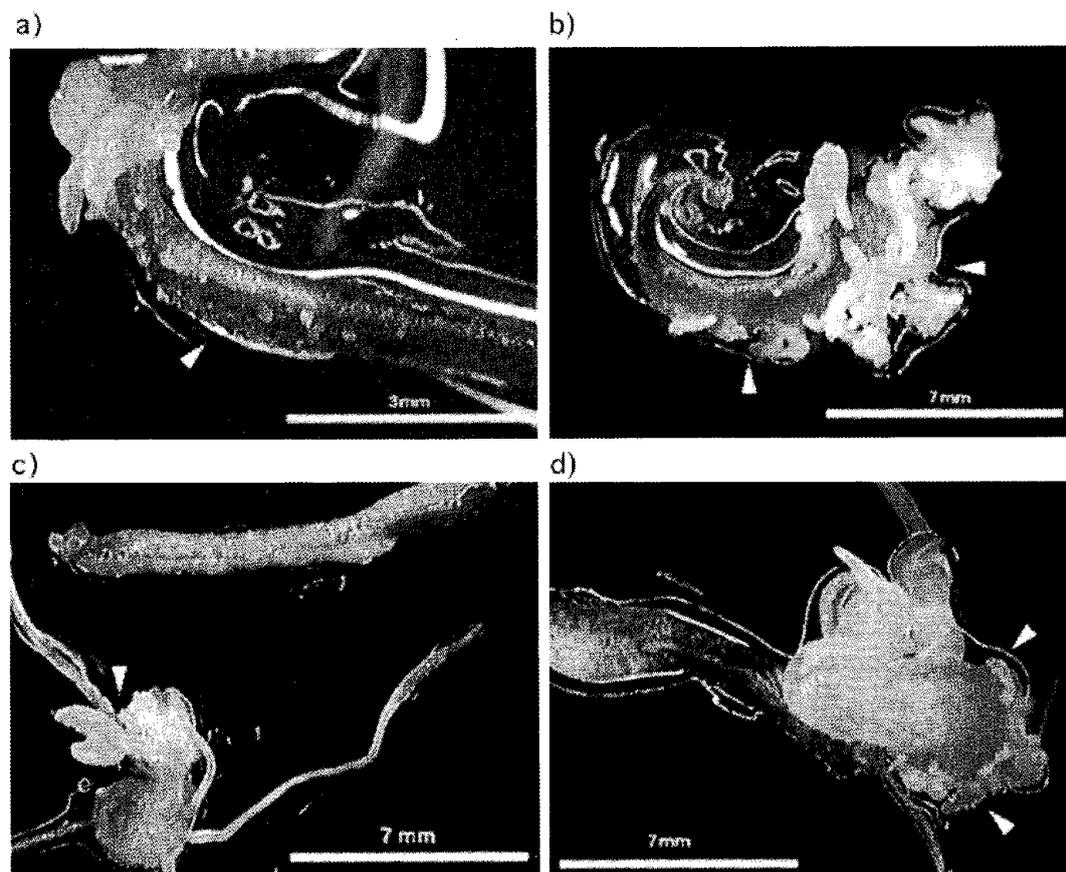
**Fig. 1**  Somatic embryogenesis induced by the sugar treatment

Type - 1 embryos formed on surface of cotyledon treated by a) 0.58M sucrose or b) 0.58M glucose for 2 weeks. On the cut end of cotyledon that sometimes formed callus-like tissue, type - 2 embryos were induced by c) 0.29M mannitol or 0.29M sorbitol treatment for 2 weeks. In c) and d), callus-like tissue separated naturally from cotyledon.

Arrow head: Somatic embryos, C: Callus like tissue

2 embryogenesis was the highest at 0.29 M sucrose, and decreased as the concentrations of sucrose increased. On the other hand, the rate of type-1 embryogenesis was low after the treatment with 0.29 M or 0.44 M sucrose, and the highest after the treatment with 0.58 M sucrose. At 0.58-0.73 M, the type-1 embryos were predominantly induced, and at 0.29 M-0.44 M, type-2 embryos were predominantly induced. These findings suggest that the type -1 and type-2 embryos are induced through the different processes, respectively.

The effects of other sugars on somatic embryogenesis are shown in **Fig. 4**. Sucrose and glucose were more effective than mannitol and sorbitol for the induction of embryogenesis. It is also clear that sucrose induced somatic embryos at lower concentrations than glucose, and that sucrose induced a higher rate of embryogenesis than glucose. The explants treated with sucrose or glucose produced both type-1 and type-2 embryos (**Fig. 1a, b**), whereas those cultured on mannitol or sorbitol supplemented medium produced only type-2 embryos (**Fig. 1c, d**). Thus, the rate of embryogenesis

and the site where embryos were induced varied with the kinds of sugar used.

In general, it is considered that sucrose at a high concentrations acts as an osmoticum. In the present experiment, however, the rate of embryogenesis varied with the kind of sugar even at the same molarity, and the optimum concentration for the induction of embryogenesis also varied with the kind of sugar. These findings indicate that sucrose does not work as a mere osmoticum.

Considering sucrose and glucose are the key substances in carbon metabolism in plant cells, whereas mannitol and sorbitol are not, the somatic embryogenesis may be induced though disturbances of carbon metabolism caused by a high concentration of sucrose or glucose in cotyledonary cells.

Our results suggest that it is possible to be plural causes in somatic embryogenesis induced by sucrose in carrot cotyledons. Before we obtain the conclusion, it is necessary to investigate how to work sucrose at high concentrations in cellular and molecular level.

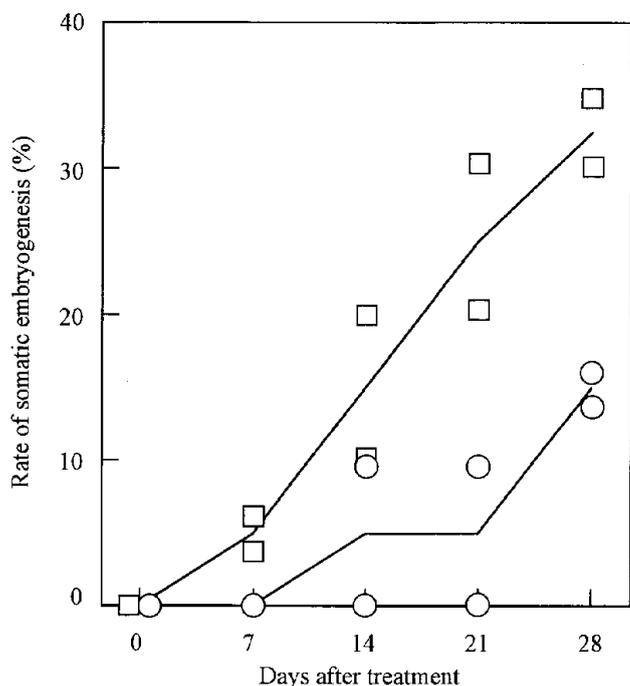We wish to thank J. Kaihara for critically reading

**Fig. 2** Rate of embryogenesis 4 weeks after the end of the sucrose treatment at 0.44 M

Twenty cotyledonal segments were tested for each of two replicates. The rate of embryogenesis (%) was evaluated as (No. of explants with somatic embryos (type-1 or type-2) formed/ No. of total explants) × 100. Circles: rate of type -1 embryos, Squares : rate of type-2 embryos.
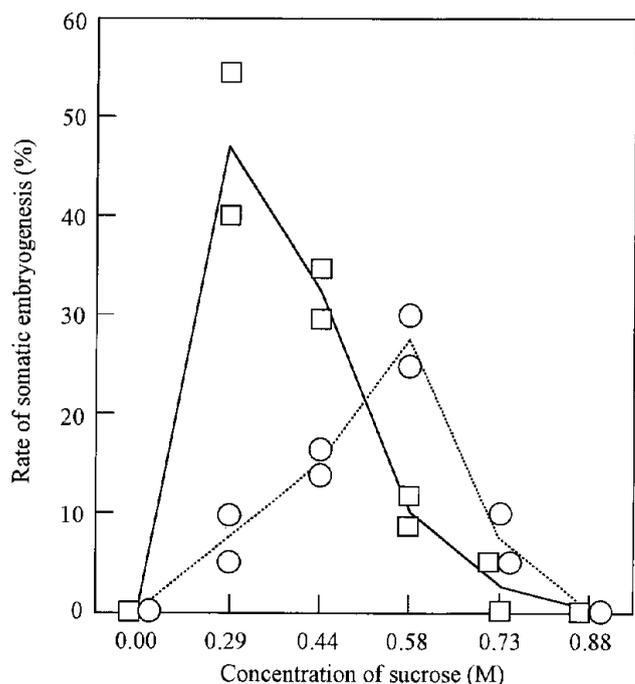


**Fig. 3** Rate of embryogenesis induced by various concentrations of sucrose

This treatment consisted of approximately 20 segments and was repeated two times. The rate of embryogenesis (%) was evaluated as (No. of explants with somatic embryos (type-1 or type-2) formed/No. of total explants) × 100. Embryogenesis rate at 28 days after the end of the sucrose treatment. Circles (dot line): type-1 embryos, Squares (solid line): type-2 embryos.
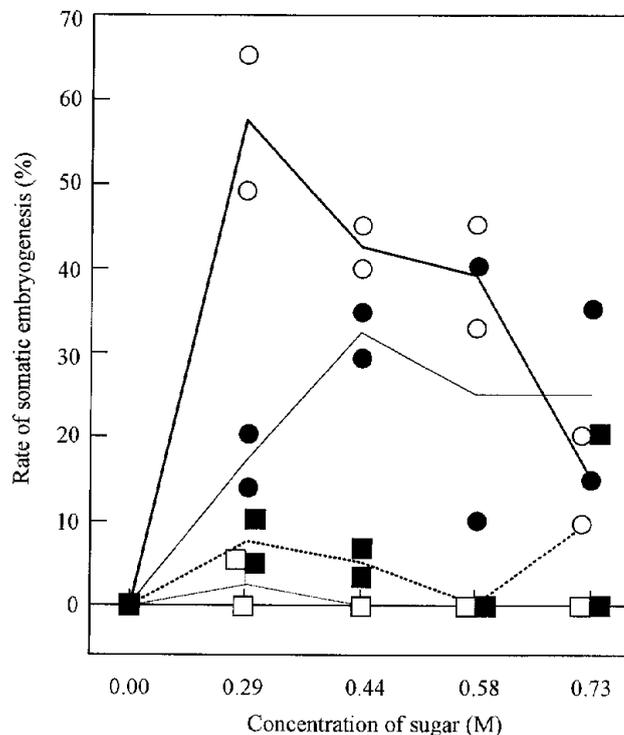


**Fig. 4** Rate of embryogenesis induced by various kinds of sugars

This treatment consisted of approximately 20 segments and was repeated two times. The rate of embryogenesis (%) was evaluated as (No. of explants with somatic embryos formed/No. of total explants) × 100. Embryogenesis rate at 28 days after the end of the treatment. Open circles (thick solid line): Sucrose, Closed circles (thin solid line): Glucose, Open squares (thick dot line): Mannitol, Closed squares (thin dot line): Sorbitol.

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