

# Promotion of Spike Elongation in Cut Snapdragons by Mannitol

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## Abstract

Mannitol was identified as one of the major carbohydrates in flowers, stems and leaves of snapdragon (*Antirrhinum majus* L.). To investigate the possible role of mannitol, we treated the cut flower spikes of snapdragon with mannitol at various concentrations. The effects of glucose, sucrose or sorbitol were also examined for comparison. The treatment with 10 to 500 mM mannitol markedly promoted flower bud development and spike elongation accompanied by an increase in the number of nodes. Although glucose or sucrose at 250 mM promoted the flower opening more than mannitol, these carbohydrates promoted the bud development and spike elongation only slightly. The treatment with mannitol increased the concentration of mannitol in terminal buds more than those of other carbohydrates. On the contrary, the treatments with glucose and sucrose markedly increased the concentrations of glucose, fructose and sucrose, but only slightly that of mannitol in these organs. These results show that mannitol in snapdragon, has a specific physiological action, which is observed neither with glucose, sucrose nor sorbitol.

## INTRODUCTION

Snapdragon is desirable for cut flowers because of its wide range of petal colors and fragrance. The snapdragon flower is sensitive to ethylene, and its vase life is relatively short (Larsen and Scholes, 1966; Nowak, 1981). Furthermore, most buds do not open fully and the color of their petals does not develop properly (Larsen and Scholes, 1966).

The addition of sugars such as sucrose to vase water is effective in promoting bud opening and extending vase life of many cut flowers (Halevy and Mayak, 1979). Furthermore, pigmentation was improved by the sugar treatment (Koyama and Uda, 1994; Ichimura and Hiraya, 1999). In snapdragon flowers, treatment with sucrose also extends their vase life (Larsen and Scholes, 1966; Ichimura and Hisamatsu, 1999).

Generally, soluble carbohydrates in plants are analyzed by HPLC. When we analyzed soluble carbohydrates in snapdragon, a mannitol-like compound was detected. In some plants, such as celery, mannitol can be translocated in the phloem (Bieleski, 1982). However, mannitol treatment causes deleterious effects on cut rose (Ichimura et al., 1999) and chrysanthemum (Kofranek and Halevy, 1972), but these flowers do not contain mannitol. We have expected that mannitol may have some available effect on cut snapdragon flowers which originally contains mannitol. The purpose of present study is to investigate effect of mannitol on the vase life of cut snapdragon spikes.

## MATERIALS AND METHODS

### Plant Material

Plants of snapdragon (*Antirrhinum majus* L.) cv. Yellow Butterfly were grown under natural day-length conditions in a greenhouse (15°C minimum and 25 °C maximum temperature).

### **Isolation and Structural Analysis of Mannitol**

Leaves (5 g) were immersed in 80% ethanol (50 ml), and then homogenized. The homogenate was centrifuged at 3,000 g for 10 min. The supernatant was concentrated in vacuo and fractionated using an HPLC system (Jasco, Tokyo). The mannitol-like compound was isolated using a Shodex NH column (5E, Showa denko, Tokyo) and Shodex SUGAR SP0810 column. The purified compound was lyophilized and analyzed by NMR. The spectrum of <sup>1</sup>H-NMR was measured in D<sub>2</sub>O at 500 MHz using a JEOL JNM-EX270 instrument (JEOL, Tokyo, Japan). Acetone was used as an internal standard.

### **Treatments with Soluble Carbohydrates**

Flower spikes in which oldest flower bud (3 cm in length) was expected to open the next day, were cut from the plants and recut to 30 cm. Three flower spikes were placed in a 500-ml glass vessel containing 500 ml of mannitol at various concentrations or glucose, sucrose or sorbitol at 250 mM. All solutions including the control were supplemented with 200 mg·l<sup>-1</sup> 8-hydroxyquinoline sulfate (HQS) to inhibit microbial proliferation. The spikes were kept at 23°C, 70% relative humidity, and 12-h light at 10 μmol·m<sup>-2</sup>·s<sup>-1</sup> from cool-white fluorescence lamps. The numbers of visible buds longer than 5 mm including open and wilted flowers were scored every 2 days. Under a stereoscopic microscope, terminal buds were dissected and the buds with bracts were scored as flower buds. Length of flower spike was measured every 5 days.

### **Determination of Soluble Carbohydrate Concentration**

Terminal buds, 5 mm long, were collected on the day of harvest and 10 days after harvest. The soluble carbohydrates were extracted from buds and their concentrations were determined as previously described (Ichimura et al., 1999).

## **RESULTS**

### **Identification of Mannitol**

A major peak other than glucose, fructose and sucrose was detected in HPLC elution profile. The compound was isolated using HPLC and subjected to structural analysis using <sup>1</sup>H-NMR. Since the retention time of this compound on HPLC was the same as that of authentic mannitol, we compared the <sup>1</sup>H-NMR spectrum of authentic mannitol with that of the isolated compound. Spectra were identical (data not shown). Therefore, this compound was identified as mannitol. Mannitol was detected in all the organs examined at relatively high concentrations (data not shown).

### **Effects of Mannitol at Various Concentrations on Flower Bud Development, Flower Opening and Spike Elongation**

Stems in the control and those treated with 1 mM mannitol elongated during the first 10 days, but not thereafter. Mannitol at 10 mM or higher concentrations promoted spike elongation, and the optimal concentration was 100 mM. At this concentration, spike elongation was accelerated from the 10<sup>th</sup> day. The promotion of spike elongation was accompanied by an increase in the number of buds (data not shown).

### **Effects of Various Carbohydrates on Bud Number, Flower Opening and Spike Elongation**

The treatment with glucose promoted flower opening (Fig. 1) and increased flower size as compared with control (data not shown). The petal color of open flowers in the spikes treated with glucose became yellow, which is the original color of this cultivar. However, the treatment with glucose caused browning of terminal buds one week after the start of treatment, after which the number of buds did not increase (Fig. 2). Spikes treated with glucose markedly elongated during the first 5 days, but the rate of elongation gradually decreased thereafter (Fig. 3). The effects of sucrose on the number of buds and spike length were almost the same as those of glucose. Treatment with sorbitol promoted

flower opening, but inhibited bud development and spike elongation. In contrast, spikes treated with mannitol elongated linearly until the 20<sup>th</sup> day, accompanied by a marked increase in the number of buds, and browning of terminal buds was not observed until about the 30<sup>th</sup> day. Buds at the middle part of the spikes aborted, but those on the upper part opened, although the flowers were smaller than those on the basal part of the spikes. The time to wilting of all open flowers was  $11.0 \pm 0.4$  days in the control, and  $22.7 \pm 0.8$ ,  $15.4 \pm 0.4$ ,  $18.2 \pm 0.6$ ,  $29.8 \pm 1.3$  days in the spikes treated with glucose, sucrose, sorbitol and mannitol, respectively (mean  $\pm$  standard errors).

Furthermore, we examined the total number of flower buds at the 30<sup>th</sup> day using a stereoscopic microscope. The total number of flower buds was the highest in the spikes treated with mannitol (data not shown), suggesting that mannitol promotes the differentiation of flower buds.

### **The Concentrations of Soluble Carbohydrates in the Terminal Buds**

Before the treatment, glucose was the most abundant carbohydrate, followed by mannitol. Treatment with mannitol increased the concentrations of mannitol, particularly at a high concentration (250 mM). Treatment with glucose increased the concentrations of glucose, fructose and sucrose. The treatment with sucrose and sorbitol extraordinarily increased the concentrations of sucrose and sorbitol, respectively (Table 1).

### **DISCUSSION**

In the present study, we found that mannitol is one of the major soluble carbohydrates in snapdragon. Distribution of mannitol in higher plants is limited to certain families such as the *Scrophulariaceae*, *Oleaceae*, *Rubiaceae* and *Umbelliferae* (Bieleski, 1982). Moore et al. (1997) also recently reported that mannitol is a major carbohydrate in snapdragon leaves.

In higher plants, the key enzyme for mannitol catabolism is mannitol dehydrogenase, which catalyzes the conversion of mannitol to mannose. The activity of this enzyme was very low in snapdragon (unpublished results). Sorbitol is hardly metabolized in plants except for some *Roseaceae* plants (Chong and Taper, 1972; Coffin et al., 1976). This is also the case in snapdragon (Table 1), and sorbitol promoted neither bud development nor spike elongation in snapdragon (Figs. 2 and 3). Mannitol is not readily metabolized in snapdragon as mentioned above, but promoted bud development and spike elongation much more than other carbohydrates (Table 1). In addition, browning of terminal buds in the spikes was markedly delayed by the treatment with mannitol (data not shown). Therefore, we propose that the promotion of bud development and spike elongation by mannitol is attributable to a specific action of mannitol, not to its difficulty in metabolism.

Some other soluble carbohydrates, such as galactose and mannose, have been reported to affect the growth and development in higher plants (Gross 1985; Watkins and Frenkel, 1987). However, the carbohydrates used in these studies were absent in the plant or the concentration of these carbohydrates applied was much higher than that in the plant. In this study, mannitol was effective at physiological concentrations on the growth and development of spikes and buds in snapdragons.

Treatment with mannitol extended the vase life of cut snapdragon more than those with glucose and sucrose. However, flower size of spikes treated with mannitol was much smaller than that with glucose and sucrose. In addition, mannitol was appeared on the surface when mannitol was treated. We are investigating the effect of mannitol in combination with glucose or sucrose on cut snapdragon flowers.

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## **Table**

Table 1. Soluble carbohydrate concentrations of terminal buds in cut spikes treated with various carbohydrate for 10 days.

Treatment	Concentration (mg g <sup>-1</sup> FW)				
	Glucose	Fructose	Sucrose	Mannitol	Sorbitol
Initial	7.7	3.5	4.2	6.7	0.0
Control	0.1	0.2	1.9	1.1	0.0
10mM Mannitol	0.1	0.4	2.7	1.8	0.0
100mM Mannitol	5.3	4.1	3.9	37.4	0.0
250mM Mannitol	9.0	6.3	5.0	51.4	0.0
250mM Glucose	16.1	8.3	30.0	5.7	0.0
250mM Sucrose	55.4	53.0	191.0	5.3	0.0
250mM Sorbitol	6.2	6.2	2.3	1.7	114.3

**Figures**

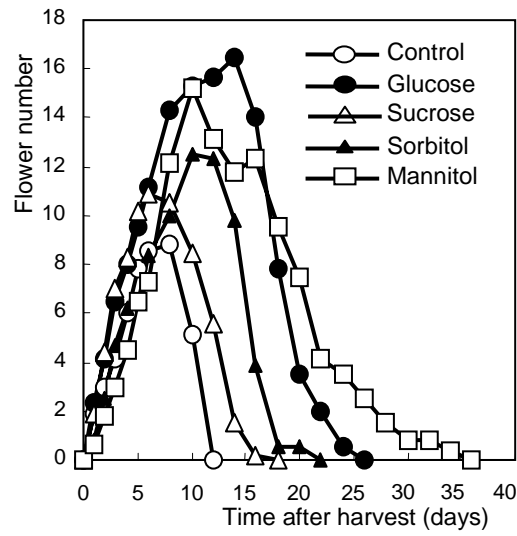


Fig. 1. Effects of various carbohydrates on the number of open flowers.

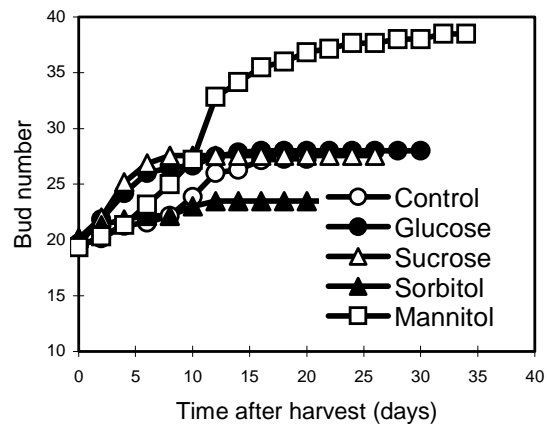


Fig. 2. Effects of various carbohydrates on bud number.

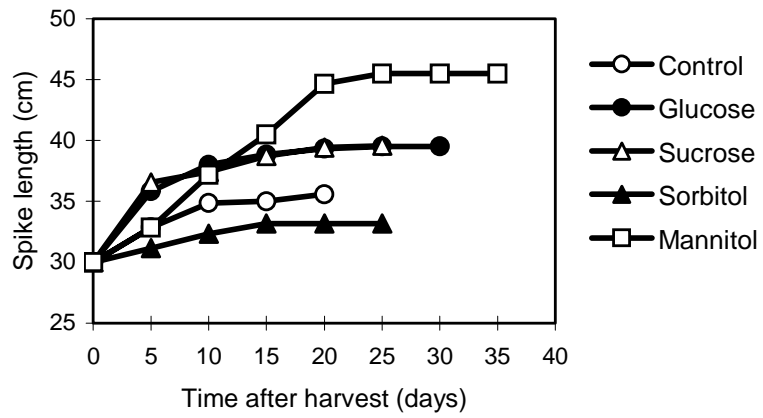


Fig. 3. Effect of various carbohydrate on spike length.