

原著論文

## Extension of the vase life of cut roses by treatment with sucrose before and during simulated transport

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### Summary

We investigated the effects of treatment with sucrose plus CMI/MI (an isothiazolinonic germicide) in combination with aluminum sulfate (CMI/MI-AS) and abscisic acid (ABA) before and during simulated transport on the vase life of cut 'Rote Rose' rose flowers. First, cut roses were held in solution containing 1% to 3% sucrose plus CMI/MI-AS at 10°C for 72 h. The flowers were then transferred to distilled water (DW) and held at 23°C. Sucrose at 2% and 3% plus CMI/MI-AS significantly extended the vase life, although the effect was small. Next, flowers were held in solution containing 2% or 4% sucrose plus CMI/MI-AS, with or without ABA, at 10°C for 24 h to simulate storage, then at 15°C for 48 h to simulate transport. They were then transferred to DW and held at 23°C. The vase life was extended to as much as 4.5 times compared with treatment with DW. ABA did not significantly extend vase life, but did increase fresh weight (FW). Sucrose uptake was greater at 15°C than at 10°C when the sucrose concentration was 2%. The difference in the extension of vase life between the two experiments is apparently due to the amount of sucrose taken up.

**Key Words:** abscisic acid, aluminum sulphate, dry transport, isothiazolinonic germicide, rose, sucrose, vase life, wet transport.

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## Introduction

The vase life of cut roses is generally short (Ichimura et al., 1999). Large amounts of soluble carbohydrates are required for flower opening, yet roses are harvested at the bud stage, limiting the soluble carbohydrate content (Ichimura et al., 2003). Treatment with sucrose markedly extends the vase life of cut roses, suggesting that the short vase life is due to a shortage of soluble carbohydrates (Ichimura et al., 2003). It has also been attributed to vascular occlusion, which restricts the water supply to the flowers (Mayak et al., 1974). The hydraulic conductance of cut rose stems decreases with time after harvest, and this decrease is associated with bacterial proliferation (van Doorn et al., 1989; Ichimura et al., 2003). Treatment with antimicrobial compounds inhibits bacterial proliferation and suppresses the decrease in hydraulic conductance of cut roses (van Doorn et al., 1989; Ichimura et al., 2003). These findings support the view that vascular occlusion is due mainly to bacterial proliferation (Zagory and Reid, 1986; van Doorn et al., 1989).

Although cut flowers are usually transported dry, wet transport has become popular recently in Japan, because it maintains the freshness of cut flowers. Furthermore, the vase life of cut rose and *Gypsophila* flowers has been shown to be longer after wet transport than after dry transport (Hu et al., 1998; Miyamae et al., 2007).

To extend the vase life of cut roses, many preservatives have been developed. There are basically three types of preservatives: those designed for growers, for wet transport, and for consumers. Preservatives for growers are applied to the cut flowers for the short period before shipment. Such preservatives include RNA-Ag-tris (Ohkawa et al., 1999) and 2-hydroxy-3-ionene-chloride polymer (Ueyama and Ichimura, 1998). However, they are not widely used by Japanese growers, probably because of their low effectiveness and high cost. Owing to the expansion of wet transport, appropriate preservatives have been developed. Their main components are antimicrobial compounds (Ichimura, 2006). Preservatives for consumers include sugars and antimicrobial compounds that inhibit vascular occlusion (Ichimura, 2006).

Preservatives for consumers are the most effective of the three types in extending the vase life of cut roses (Ichimura et al., 2006). Indeed, continuous treatment with sucrose plus an 8-hydroxyquinoline compound, such as 8-hydroxyquinoline sulphate (HQS) or 8-hydroxyquinoline citrate, extends the vase life of cut roses (Marousky, 1969; Kaltaler and Steponkus, 1976; Ichimura et al., 1999). Furthermore, continuous treatment with a formulation known as GLCA, which is composed of glucose, CMI/MI (a mixture of isothiazolinonic germicides), citric acid, and aluminum sulphate (AS), markedly extended the vase life of cut roses (Ichimura et al., 2006). On the other hand, treatment with 0.1 M fructose plus HQS during simulated transport extended the vase life of cut 'Bridal Pink' roses by only 15% compared with treatment with HQS (Hu et al., 1998). Similarly, pulse treatment with GLCA only slightly extended the vase life of cut 'Rote Rose' flowers (Ichimura and Taguchi, 2006). Since applied sugars are rapidly consumed in cut flowers (Ichimura and Suto, 1999), their slight effectiveness can be attributed to insufficient uptake. The uptake of sugars by cut flowers can be increased by treatment at high concentrations. However, sucrose or glucose at high concentrations damages the leaves of cut roses (Markhart and Harper, 1995). Since water uptake by cut flowers is suppressed in the dark (Uda et al., 1995; Doi et al., 1999), the use of sugar solution in the dark may thus avoid leaf damage.

Abscisic acid (ABA), a phytohormone, is involved in stomatal opening and closure (Mittelheuser and van Steveninck, 1969; Kriedemann et al., 1972). Treatment with ABA significantly extends the vase life of cut roses with leaves, but shortens it without leaves (Halevy et al., 1974). Hence, the effect of ABA is due to suppression of transpiration. ABA also suppresses damage to leaves caused by sucrose (Markhart and Harper, 1995; Pompadakis and Joyce, 2003). This effect is attributable to suppression of uptake of sucrose by inhibition of transpiration.

Here, we investigated the effect of treatment with sucrose plus CMI/MI-plus AS with or without ABA before

and during simulated transport on the vase life of cut roses.

## Materials and Methods

### *Plant Materials*

Roses (*Rosa hybrida* L.) cv. Rote Rose, harvested at normal harvest maturity (stage 2, as described by Ichimura and Ueyama, 1998) when the petals started to unfold, were obtained from a commercial grower in Ishioka, Ibaraki Prefecture, Japan, in 2006 and 2007. These flowers had not undergone any preservation treatment before we obtained them. After harvesting, the cut ends of the stems were immersed in tap water, and the flowers were stored at 5°C overnight. Next day, the flowers whose cut ends were placed in tap water were transported to the laboratory and used within 2 h for the experiments.

### *Chemicals*

The antimicrobial CMI/MI-AS solution contained 283 mg L<sup>-1</sup> 5-chloro-2-methyl-4-isothiazolin-3-one, 98 mg L<sup>-1</sup> 2-methyl-4-isothiazolin-3-one, and 5 g L<sup>-1</sup> aluminum sulphate as active ingredients. It was provided by Kumiai Chemical Industry (Tokyo, Japan).

### *Treatment with sucrose plus CMI/MI-AS under conditions simulating transport conditions (Exp. 1)*

Two cut flowers 50 cm long were placed in each of four 500-ml beakers containing 125 ml of test solution (eight flowers per treatment) or distilled water (DW). Each solution contained 0, 10, 15, 20 or 30 g L<sup>-1</sup> sucrose and 20 ml L<sup>-1</sup> CMI/MI-AS. The flowers were held at 10°C under 70% relative humidity (RH) in the dark for 72 h. They were then re-cut to 45 cm, and all but the upper three leaves were removed. The flowers were then placed in 500 mL DW, two to a beaker, and held at 23°C under 12-h photoperiod with 10 µmol m<sup>-2</sup> s<sup>-1</sup> irradiance from cool-white fluorescent lamps.

### *Treatment with sucrose plus CMI/MI-AS and ABA under conditions simulating storage and transport conditions (Exp. 2)*

Individual cut flowers 50 cm long were placed in test tubes containing 50 ml of test solution (eight flowers per treatment). The flowers were held in solutions of 0, 20, or 40 g L<sup>-1</sup> sucrose, 20 ml L<sup>-1</sup> CMI/MI-AS, and 0 or 10 µM ABA ((+)-2-cis, 4-trans-abscisic acid, Toray, Tokyo, Japan) at 10°C and 70% to 75% RH in the dark for 24 h to simulate brief storage before shipping, then at 15°C and 70% RH in the dark for 48 h to simulate wet transport. To simulate dry transport, eight flowers that had been held in DW for 24 h were placed in a corrugated cardboard box (29 cm × 73 cm × 11 cm) together with another 42 cut flowers 50 cm long. After simulated storage for 24 h followed by simulated transport for 48 h, the flowers were cut to 48 cm. They were then placed in 500 mL DW, two to a beaker, and held at 23°C, and 70% RH, under a 12-h photoperiod at 10 µmol m<sup>-2</sup> s<sup>-1</sup> irradiance from cool-white fluorescent lamps.

### *Evaluation of vase life*

The FW and water uptake of cut flowers were measured daily. Relative FW was expressed as a percentage

of initial FW. After cutting at the end of simulated transport, relative FW was corrected using the following equation:

$$\text{Relative FW (\%)} = (\text{FW on each day}/\text{initial FW}) \cdot (\text{FW before cutting}/\text{FW after cutting})$$

Maximum flower diameter was recorded. The vase life was defined as the period from the end of chemical treatments to the time when the petals wilted or showed severe bluing.

#### *Determination of sucrose uptake by cut flowers*

In both Exp. 1 and Exp. 2, the volume of sucrose solution taken up by cut flowers from 24 h to 48 h after the start of treatment was determined as the difference in solution volume, then corrected by subtracting the evaporation of water from a test tube without cut flowers. The amount of sucrose uptake was calculated from the volume taken up and the sucrose concentration. Sucrose uptake is proportional to solution uptake (Ichimura et al., 2006).

## Results

#### *Effect of sucrose plus CMI/MI-AS during simulated transport on vase life (Exp. 1)*

The FW of cut flowers in all treatments increased during simulated transport (“Treatment” in Fig. 1). Subsequently, in DW and CMI/MI-AS, it increased slightly on the first day of vase storage, then decreased sharply thereafter. In contrast, treatment with 1% to 3% sucrose plus CMI/MI-AS increased FW and maintained it at a relatively high level for up to 5 days. Flowers in DW did not open fully, but those in sucrose plus CMI/MI-AS opened almost completely (data not shown).

Treatment with CMI/MI-AS extended the vase life slightly compared with treatment with DW. Treatment with 2% and 3% sucrose plus CMI/MI-AS significantly extended the vase life (Table 1). Uptake of sucrose increased with sucrose concentration (Table 2).

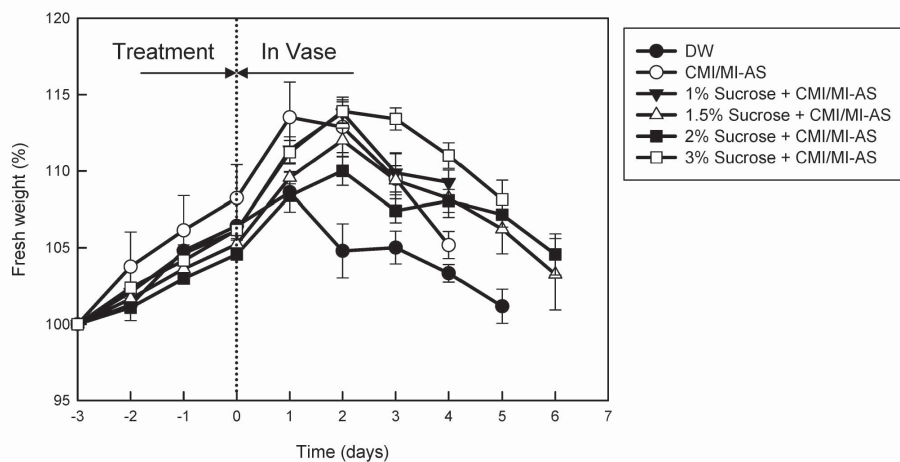


Fig. 1. Changes in the fresh weight of cut roses. Flowers were treated in various solutions at 10°C for 72 h to simulate transport and then held in DW at 23°C. Values represent the means of 4 replications  $\pm$  SE.

Table 1. Effects of various chemical treatments on the vase life of cut roses

Treatment	Vase life <sup>z</sup> (days)
DW	2.6 ± 0.5a
CMI/MI-AS	3.3 ± 0.1ab
1% Sucrose + CMI/MI-AS	4.0 ± 0.4ab
1.5% Sucrose + CMI/MI-AS	4.1 ± 0.5ab
2% Sucrose + CMI/MI-AS	4.5 ± 0.6ab
3% Sucrose + CMI/MI-AS	5.1 ± 0.1b

Cut roses were treated in various solutions at 10°C for 72 h and then held in DW at 23°C.

<sup>z</sup>Values represent means of 4 replications ± SE. Values followed by the same letter do not differ significantly (P<0.05, Tukey-Kramer multiple range test).

Table 2. Sucrose uptake by cut roses

Treatment	Sucrose uptake <sup>z</sup>	
	(mg flower <sup>-1</sup> day <sup>-1</sup> )	(mg g <sup>-1</sup> FW day <sup>-1</sup> )
1% Sucrose + CMI/MI-AS	33.5 ± 5.7	0.75 ± 0.07
1.5% Sucrose + CMI/MI-AS	39.4 ± 1.1	0.85 ± 0.02
2% Sucrose + CMI/MI-AS	65.5 ± 6.7	1.58 ± 0.23
3% Sucrose + CMI/MI-AS	78.0 ± 7.6	1.60 ± 0.16

Cut roses were treated in various solutions at 10°C for 72 h, then the amount of sucrose taken up from 24 to 48 h after the start of treatment was determined.

<sup>z</sup>Values represent means of 4 replications ± SE.

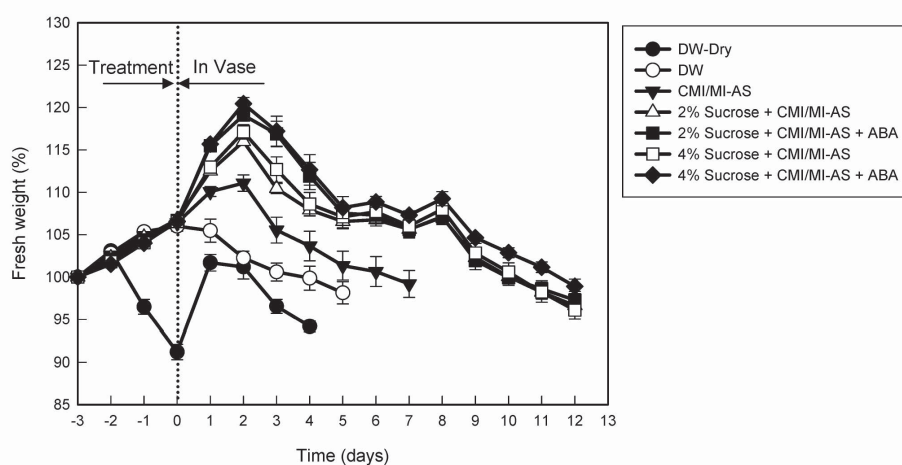


Fig. 2. Changes in the fresh weight of cut roses held under conditions simulating storage and transport. Flowers were treated in various solutions at 10°C for 24 h to simulate storage then at 15°C for 48 h to simulate transport, and then held in DW at 23°C. Values represent the means of 4 replications ± SE.

### *Effect of sucrose plus CMI/MI-AS and ABA during simulated storage and transport on vase life (Exp. 2)*

The FW of cut flowers in all treatments except dry transport somewhat increased during simulated storage and transport (Fig. 2). That of dry-transported flowers increased during the first day in the vase, then decreased thereafter. That of flowers treated with DW did not increase in the vase. Treatment with 2% and 4% sucrose plus CMI/MI-AS increased the maximum fresh weight more than treatment with CMI/MI-AS alone. This increase was enhanced slightly by ABA. Treatment with sucrose plus CMI/MI-AS maintained a higher FW for longer than treatment with CMI/MI-AS alone.

Vase life was shortest in dry-transported and DW-treated flowers. Treatment with CMI/MI-AS extended vase life slightly and that with 2% or 4% sucrose plus CMI/MI-AS extended it significantly. This extension of vase life was slightly enhanced by ABA in 2% sucrose. Flower diameter was significantly increased by treatment with 4% sucrose plus CMI/MI-AS, with or without ABA, and by treatment with 2% sucrose plus CMI/MI-AS with ABA, compared with that in DW (Table 3). Figure 3 shows photographs taken on day 7. Flowers in the DW + dry-treatment were severely wilted and bent (A). Those in the DW treatment were wilted and blued (B). Similarly, flowers in the CMI/MI-AS treatment showed some bluing and wilting (C). In contrast, flowers in 2% and 4% sucrose plus CMI/MI-AS did not show bluing (D-F).

Sucrose uptake was higher in solution containing 4% sucrose than in solution containing 2% sucrose. ABA tended to decrease sucrose uptake (Table 4).

Table 3. Effect of various chemical treatments under conditions simulating storage and transport on the vase life of cut roses

Treatment	Vase life <sup>z</sup> (days)	Flower diameter <sup>z</sup> (mm)
DW-Dry	2.0 ± 0.4a	87.6 ± 10.8a
DW	2.1 ± 0.1a	86.8 ± 6.4a
CMI/MI-AS	3.5 ± 0.4a	100.3 ± 3.2abc
2% Sucrose + CMI/MI-AS	7.4 ± 0.5b	108.4 ± 2.0abc
2% Sucrose + CMI/MI-AS +ABA	8.8 ± 0.3bc	113.1 ± 1.9c
4% Sucrose + CMI/MI-AS	9.4 ± 0.1c	111.3 ± 0.5bc
4% Sucrose + CMI/MI-AS + ABA	9.3 ± 0.3c	116.5 ± 3.5c

Cut roses were treated in various solutions at 10°C for 24 h and at 15°C for 48 h and then held in DW at 23°C.

<sup>z</sup>Values represent means of 4 replications ± SE. Values within a column followed by the same letter do not differ significantly ( $P < 0.05$ , Tukey-Kramer multiple range test).

Table 4. Sucrose uptake by cut roses

Treatment	Sucrose uptake <sup>z</sup>	
	(mg flower <sup>-1</sup> day <sup>-1</sup> )	(mg g <sup>-1</sup> FW day <sup>-1</sup> )
2% Sucrose + CMI/MI-AS	127.3 ± 15.5	6.75 ± 0.98
2% Sucrose + CMI/MI-AS +ABA	112.3 ± 7.6	5.55 ± 0.46
4% Sucrose + CMI/MI-AS	194.0 ± 15.9	9.68 ± 0.44
4% Sucrose + CMI/MI-AS +ABA	163.0 ± 8.2	8.58 ± 0.39

Cut roses were treated in various solutions at 10°C for 24 h and at 15°C for 48 h, then the amount of sucrose taken up from 24 to 48 h after the start of treatment was determined.

<sup>z</sup>Values represent means of 8 replications ± SE.

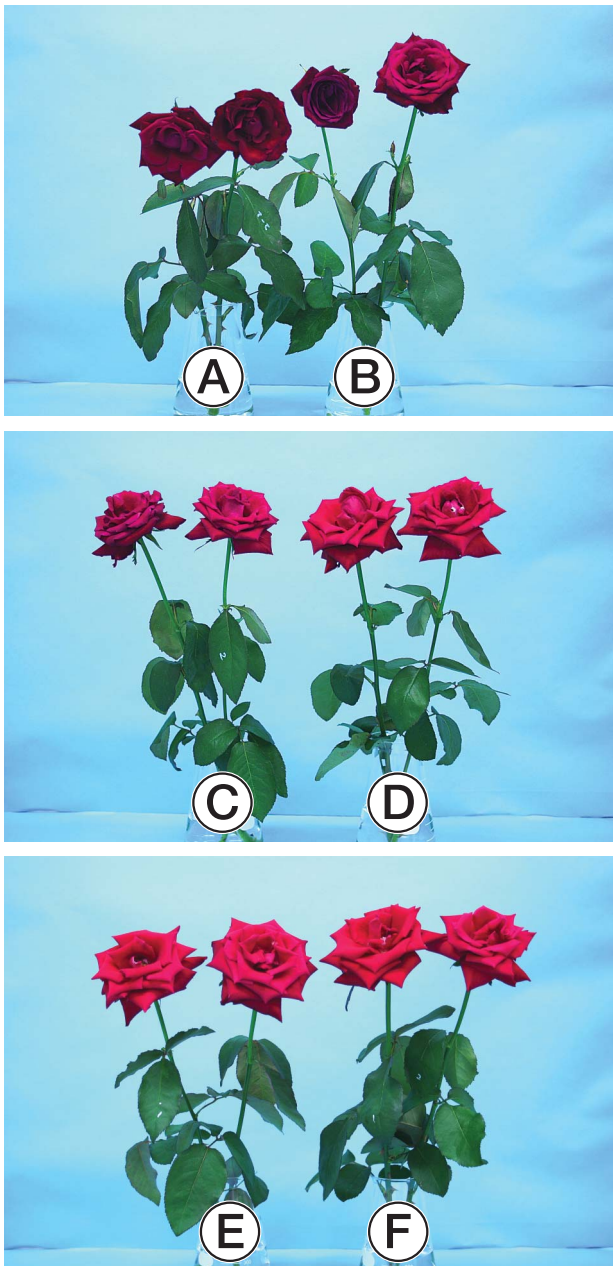


Fig. 3. Effects of various treatments on the vase life of cut roses: A, DW-dry transport; B, DW; C, CMI/MI-AS; D, 2% sucrose + CMI/MI-AS; E, 2% sucrose + CMI/MI-AS + ABA; F, 4% sucrose + CMI/MI-AS. The photograph was taken 7 days after transfer of the flowers to 23°C.

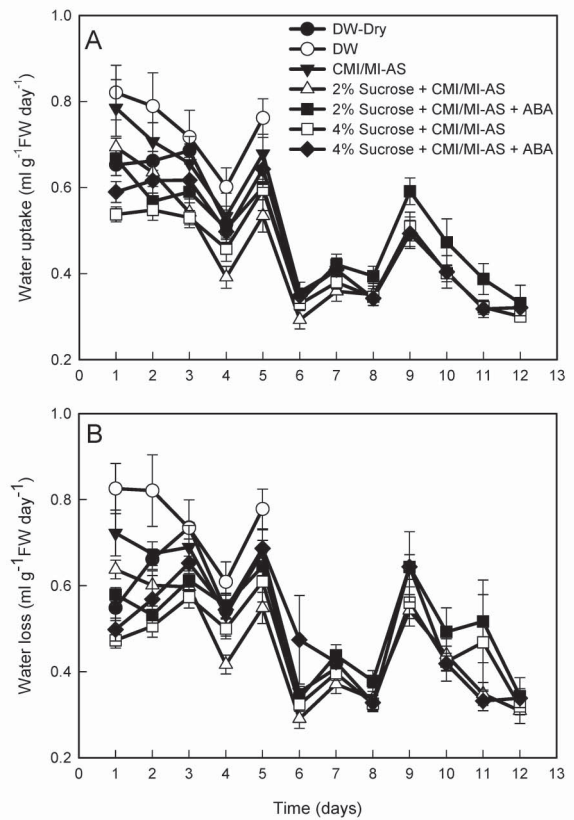


Fig. 4. Changes in the (A) uptake and (B) loss of water by cut roses treated with various solutions under conditions simulating storage and transport. Flowers were treated in various solutions at 10°C for 24 h and at 15°C for 48 h and then held in DW at 23°C. Values represent the means of 4 replications  $\pm$  SE.

Water uptake during vase life tended to decrease with time. Sucrose tended to reduce water uptake during the first 5 days, but ABA did not (Fig. 4A). Trends of water loss were similar to those of water uptake (Fig. 4B).

## Discussion

The vase life of cut rose and *Gypsophila* flowers was shorter in dry transport than in wet transport (Hu et al., 1998; Miyamae et al., 2007). Yet there was no significant difference in the present study (Table 3), although dry transport suppressed the increase in FW (Fig. 2). As the increase in FW is due to petal growth (Ichimura et al., 1999), these findings suggest that water stress caused by dry transport suppresses petal growth.

In wet transport, antimicrobial compounds are used to inhibit bacterial proliferation (Ichimura, 2006), which shortens the postharvest life of cut flowers (Zagory and Reid, 1986; van Doorn et al., 1989). Treatment with HQS during simulated transport slightly extended the vase life of cut roses compared with DW treatment (Hu et al., 1998). However, HQS shortens the vase life of some rose cultivars (Ichimura et al., 2006). In contrast, continuous treatment with CMI/MI extended the vase life of cut roses (Ichimura et al., 2006). Furthermore, treatment with glucose, CMI/MI, and AS extended vase life more than treatment with glucose and CMI/MI (Ichimura et al., 2006). In the present study, the vase life of cut roses treated with CMI/MI-AS tended to be longer than that of roses treated with DW (Tables 1 and 3), indicating the suitability of CMI/MI-AS for wet transport of cut roses.

Treatment with sugars extends the vase life of cut flowers, but sucrose treatment often damages the leaves of some, including rose (Markhart and Harper, 1995; Pompadakis and Joyce, 2003), *Eustoma grandiflorum* (Shimizu-Yumoto and Ichimura, 2007), and *Tweedia caerulea* (Hiraya et al., 2002). The degree of damage varies among rose cultivars (unpublished results). Glucose at higher than 1% damaged leaves of cut 'Rote Rose' flowers held at 23°C in the light (Ichimura et al., 2006), yet sucrose at 4% did not visibly damage leaves held at 23°C in the dark. Damage to leaves was associated with amount of sucrose uptake in cut *E. grandiflorum* (Shimizu-Yumoto and Ichimura, 2007). Water uptake is much greater in the light than in the dark (Uda et al., 1995; Doi et al., 1999). Thus, avoidance of damage caused by sucrose solution is due to suppression of transpiration in the dark.

The vase life of cut roses was extended slightly by treatment with fructose or glucose during simulated transport (Hu et al., 1998; Ichimura et al., 2006). To improve the outcome, we investigated sucrose plus CMI/MI-AS treatment before and during simulated transport. Such treatment significantly extended vase life (Tables 1 and 3). The extension of vase life was greater in experiment 2 than in experiment 1 at 2% sucrose. This result is explained by the much greater uptake of sucrose in experiment 2 (Tables 2 and 4). The difference between experiments 1 and 2 appears to be caused by different temperatures and vapor pressure deficits during treatment, because water uptake by cut roses is affected by vapor pressure deficit (Doi et al., 2000) and temperature (Ichimura et al., 1999). In experiment 1, RH was not controlled, but was higher than 90%, and thus suppressed solution uptake.

Cell expansion associated with flower opening requires water influx (Evans and Reid, 1988; Bielecki, 1993). When cut roses are wet-transported at 20°C, flowers open during transport (Hu et al., 1998). In the present study, the flowers reached developmental stage 3 (Ichimura and Ueyama, 1998) at the end of simulated transport. Petals do not reflex at this stage, and thus this stage may be acceptable to consumers for purchase.

The addition of ABA did not significantly extend vase life (Table 3). ABA inhibits transpiration from leaves (Mittelheuser and van Steveninck, 1969; Kriedemann et al., 1972) and extends the vase life of cut roses with leaves (Halevy et al., 1974). However, we did not observe inhibition of transpiration by ABA during vase life (Fig. 4), although sucrose uptake was suppressed by ABA (Table 4). Thus, the effectiveness of ABA is not attributable to the inhibition of transpiration. Petals are sink organs (Ho and Nichols, 1977; Paulin and Jamain, 1982), and ABA promotes the translocation of sugars to sink organs in some plants (Saftner and Wyse, 1984; Archbold, 1988;



Oofosu-Anim and Yamaki, 1994). Thus, ABA may stimulate sucrose translocation to petals, increasing FW.

In conclusion, treatment with sucrose plus CMI/MI-AS before and during transport under appropriate conditions markedly extended the vase life of cut roses. Sucrose treatment in the dark did not visibly damage leaves. These findings suggest that sucrose plus CMI/MI-AS will be widely useful for preserving cut roses.

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## 出荷前およびバケツト輸送中のスクロース処理による バラ切り花の品質保持期間延長

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### 和文摘要

出荷前およびバケツト輸送中のスクロース、イソチアゾリン系抗菌剤 (CMI/MI)、硫酸アルミニウム (AS) および ABA を組み合わせた薬剤処理がバラ切り花の品質保持期間延長にどの程度効果があるか調べた。1～3%のスクロースとイソチアゾリン系抗菌剤および硫酸アルミニウム (CMI/MI-AS) を組み合わせた処方を 10℃で 72 時間処理した後、蒸留水に移し、23℃で保持した。その結果、2% および 3% スクロース処理により品質保持期間を有意に延長したが、その効果は大きくなかった。そこで、2 および 4% スクロースと CMI/MI-AS に ABA で組み合わせた薬剤処方を 10℃で 24 時間、さらに 15℃で 48 時間処理した後、蒸留水に移し、23℃で保持した。スクロース濃度 2% および 4% としたとき、品質保持期間を蒸留水処理区のそれぞれ約 3.5 倍および 4.5 倍に延長した。スクロースと CMI/MI-AS に ABA を組み合わせることにより品質保持期間は有意には延長しなかった。スクロース濃度が 2% の場合、処理中に吸収したスクロース量は 15℃で処理したほうが 10℃での処理よりも多かった。このスクロース吸収量の違いが品質保持効果の差を生じさせたと考えられた。