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Effects of Silver Thiosulfate Complex (STS), Sucrose and their Combination on the Quality and Vase Life of Cut *Eustoma* Flowers

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Summary

Effects of pulse treatment with silver thiosulfate complex (STS), sucrose and their combination on the quality and vase life of cut *Eustoma grandiflorum* flowers were investigated. Cut *Eustoma* flowers with two open florets and four buds were treated with 0.2 mM STS, 4% sucrose and 0.2 mM STS combined with 4% sucrose and kept at 23℃, 70% relative humidity in the dark for 20 h. The vase life of cut flowers is considered to be from harvest to when less than two open florets are subtended with erect pedicels. Treatment with STS plus sucrose and sucrose alone extended vase life, advanced bud opening, and increased anthocyanin concentration in the colored parts of petals more than did STS alone. These results indicate that pulse treatments including sucrose are more effective than STS alone to improve the quality of cut, floret-bearing *Eustoma* flowers which are not highly sensitive to ethylene.

Key Words: anthocyanin, *Eustoma grandiflorum*, silver thiosulfate complex (STS), sucrose, vase life.

Introduction

Eustoma grandiflorum which has many morphological variations in flower color, size and shape, due to breeding, was introduced into Japan about 70 years ago. Physiological studies on flowering enabled year-round production and, thus, it has become a popular flower in Japan. The vase life of *Eustoma* flower is known to be relatively long (Halevy and Kofranek, 1984; Ichimura and Korenaga, 1998; Ichimura et al., 1998; Shimamura and Okabayashi, 1997), but there are cultivar variation (Motozu and Makihara, 2001; Shimizu and Ichimura, 2002). Because long vase-life flowers are desirable for personal use (Imanishi et al., 1992), postharvest treatment for cut *Eustoma* is considered to be important.

Ethylene was shown to be involved in the flower senescence in *Eustoma* (Ichimura et al., 1998), while STS, an ethylene action inhibitor was shown to be effective delaying flower senescence in many ethylene sensitive plants (Woltering and van Doorn, 1988). Carnation flowers senescence immediately after exposure to ethylene (Woltering and van Doorn, 1988), whereas STS delayed it dramatically (Uda, 1996). However, STS treatment did not extend vase life of florets strongly in *Eustoma* because its ethylene sensitivity was reported to be relatively low (Ichimura et al., 1998).

Since cut *Eustoma* flowers have many florets and buds on each flower stem, promotion of bud opening is as important as delay of senescence of each floret to extend vase life. Application of sugars to cut flowers promotes bud opening (Koyama and Uda, 1994; Mor et al., 1984) and pigmentation of petals by increasing anthocyanin concentration (Ichimura and Hiraya, 1999; Ichimura and Korenaga, 1998; Maekawa and Nakamura, 1978) while extending vase life (Halevy and Mayak, 1981). Sucrose treatment of *Eustoma* has been shown to extend the vase life (Cho et al., 2001; Halevy and Kofranek, 1984; Huang and Chen, 2002; Ichimura and Korenaga, 1998). However, extension of vase life by sucrose treatment is less than that by STS in some ethylene sensitive flowers, such as sweet pea (Ichimura and Hiraya, 1999) and *Oxypetalum* (Hiraya et al., 2002). In these flowers, combined treatment with STS plus sucrose extended their vase life more than did STS or sucrose alone.We investigated the effects of pulse treatments with STS, sucrose, and their combination on the quality and vase life of cut *Eustoma* flowers with many florets.

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Materials and Methods

Plant materials

Flowering stems of *Eustoma* 'Asuka-no-nami' that were growing in a glasshouse from November 2001 to June 2002 were harvested from 4 to 13 June. Some florets and buds were removed, leaving two open florets, one of which stigma had not yet matured, and four buds. Bud lengths at harvest were 4.5 ± 0.1, 3.7 ± 0.1, 3.0 ± 0.1, 2.3 ± 0.0 cm for No.1, 2, 3 and No.4, respectively. The flower stems were recut to 50 cm length from the

lowest opened floret at harvest.

Pulse treatment

The flower stems were placed in distilled water (control), 0.2 mM STS, with or without 4% sucrose, and supplemented with 200 mg·L^{-1} 8-hydroxyquinoline sulfate. The flowers were kept at 23℃, 70% relative humidity, and in the dark for 20 h. At the end of the dark period, the flower stems were transferred to vessels containing distilled water and kept at 23℃, 70% relative humidity, under 10 μmol·m^{-2}·s^{-1} light intensity and a 12-h photoperiod.

Evaluation of vase life of cut flowers, longevity of each florets and pedicel bending

All florets were observed daily to note when the pedicels became extremely bent. The display life of open florets whose petals fully unfolded was evaluated from harvest to when petals lost turgor or pedicels became bent; the evaluation was continued until the last florets wilted. The vase life of cut flowers was determined as the interval from harvest to when the number of open florets with erect pedicels was less than two. Eight cut flowers were used per treatment.

Measurement of anthocyanin concentration

Violet color parts of picotee petals were collected from one floret at anthesis, diced, and immersed in 5 mL of methanolic: 1% HCl at 4℃ in the dark for 1 day. The supernatant was decanted and the residue washed twice with 2.5 mL of the same solution. The supernatants were combined and brought to 10 mL. The absorbance of the solution was measured at 530 nm with a spectrophotometer.

Measurement of sugar concentration

Petals (0.3 g), except for violet color parts that were used for the measurement of anthocyanin, were immersed in 5 mL of 80% ethanol at 75℃ for 20 min. After cooling, 50 μL of a 2.5% (v/v) sorbitol solution was added to the mixture as the internal standard, and the sample was homogenized. After centrifugation, the residue was re-extracted with 3 mL of 80% ethanol twice. The three supernatants were combined and concentrated to near dryness in vacuo below 40℃. After residue was re-dissolved in 1mL of distilled water, solution was passed through a Sep-Pak C-18 cartridge (Millipore, Milford) that was rinsed with 2 ml of distilled water; the eluate was evaporated to dryness in vacuo below 40℃. The residue was re-dissolved in 1 mL of distilled water which was passed through a cellulose acetate filter (0.45 μm, ADVANTEC). Constituents in the aliquots of known volume were separated by a high performance liquid chromatography system equipped with a refractive index detector (Jasco, Tokyo) and column of Shodex SUGAR SP0810 (Showa Denko, Tokyo). The column was kept at 80℃ and eluted with water at a flow rate of 0.8 mL·min^{-1}. Peak identity was confirmed by using authentic sugars. Quantitation was achieved by using peak area calculations, related to regression curves of standards.

Results

Effect of STS, sucrose and their combination on the vase life of cut flowers

The vase life of cut flowers was extended by STS plus sucrose and sucrose alone more effectively than STS alone or the water control. There was no significant difference in the vase life between sucrose alone and STS plus sucrose. The reciprocity between STS and sucrose in the vase life of cut flowers was not significant; sucrose was more effective in extending the vase life than STS, based on the two-way ANOVA (Table 1).

Effect of STS, sucrose and their combination on bud opening

Percentages of flowering in No. 1, 2, and 3 buds increased with STS alone but not in No. 4 buds, compared to the control during the vase life of cut flowers. STS plus sucrose and sucrose alone promoted 100% flowering in No. 1, 2, and 3 buds and a high percentage of flowering in No. 4 buds (Table 2).

Effect of STS, sucrose and their combination on the bending of pedicels

The rates of pedicel bending in the control and STS alone were higher than sucrose alone and STS plus sucrose. The time for bending pedicel was delayed by all treatments, compared to the control (Table 3).

Effect of STS, sucrose and their combination on the anthocyanin and sugar concentrations

Anthocyanin concentrations of color parts in the

Table 1. Effect of pulse treatments with STS, sucrose and their combination on the vase life of cut flowers.

Treatment	Vase life (days)[z]
Control	5.8 a[y]
STS	9.1 b
Sucrose	12.3 c
STS+Sucrose	12.9 c
STS	P=0.0116[x]
Sucrose	P<0.0001
STS × Sucrose	P=0.0738

Values are means of 8 replications.

[z] Vase life of cut flowers was determined as the interval from harvest to when the number of open florets with erect pedicels was less than two.

[y] Mean separation within colum at P=0.05 by Fisher's PLSD test.

[x] Analysis by two-way ANOVA.

Table 2. Effect of pulse treatments with STS, sucrose and their combination on the rate of bud opening during the vase life of cut flowers.

Treatment	Rate of bud opening[z] (%)			
	No. 1	No. 2	No. 3	No. 4
Control	75	38	0	13
STS	88	75	71	13
Sucrose	100	100	100	75
STS+Sucrose	100	100	100	75

[z] Bud length at harvest was 4.5 ± 0.1, 3.7 ± 0.1, 3.0 ± 0.1 and 2.3 ± 0.0 cm for No. 1, 2, 3 and 4, respectively.

Table 3. Effect of pulse treatments with STS, sucrose and their combination on the rate and time for bending pedicels until last florets wilted.

Treatment	Rate of pedicel bending (%)[z]	Time for bending pedicel (days)
Control	56.3	5.7 a[y]
STS	62.5	9.8 b
Sucrose	29.2	11.0 c
STS+Sucrose	27.1	10.2 bc

[z] Number of florets whose pedicles bent /number of florets including buds and open florets at harvest for each treatment.
[y] Values are means of 8 replications. Mean separation within colum at P=0.05 by Fisher's PLSD test.

Table 4. Effect of pulse treatments with STS, sucrose and their combination on anthocyanin and sugar concentrations in petals of florets at anthesis.

Treatmnet	Anthocyanin concentration (OD530 nm · gFW^{-1})	Sugar concentration (mg · gFW^{-1})			
		Sucrose	Glucose	Fructose	Total
Control	3.8 a[z]	1.2 a	1.5 a	0.4 a	3.1 a
STS	3.8 a	1.5 a	1.5 a	0.4 a	3.4 a
Sucrose	6.9 b	7.0 b	9.9 b	0.2 a	17.1 b
STS+Sucrose	7.3 b	8.6 b	12.0 b	0.4 a	20.9 b

Values are means of 4 replications. Bud length at harvest was 4.4 ± 0.1cm.
[z] Mean separation within colum at P=0.05 by Fisher's PLSD test.

petals increased significantly in the sucrose and STS plus sucrose treatments. However, STS alone did not increase anthocyanin concentration, compared to the control (Table 4). Total sugar concentration, especially that of glucose and sucrose of petals was increased significantly by the addition of sucrose and STS plus sucrose. However, total sugar concentration was not increased by STS treatment compared to the control (Table 4).

Discussion

In this study, we investigated the effect of pulse treatment with STS plus sucrose on the quality and vase life of cut *Eustoma* flowers with two open florets and four buds. STS plus sucrose and sucrose alone treatments were effective in extending the vase life of cut flowers compared to STS alone, but there was no significant difference in the vase life between treatments that contain sucrose (Table 1). The effect of sucrose on the vase life was greater than that of STS; the reciprocity between STS and sucrose in the vase life of cut flowers was not significant by two-way ANOVA (Table 1), which suggests that sucrose independently and strongly affected the vase life of cut flowers. Ethylene production in *Eustoma* flowers was reduced by sucrose pulse treatment (Huang, 2002) and STS treatment (Ichimura et al., 1998). Thus, extension of vase life by sucrose treatment may be attributed to a reduction of ethylene production. Ethylene sensitivity of *Eustoma* flowers is not as high as carnations (Ichimra et al., 1998; Wortaling and van Doorn, 1988). In cut flowers, such as *Eustoma* with many florets that have low sensitivity to ethylene, pulse treatments with sucrose are supposed to be more effective than STS alone to improve the quality of cut flowers.

There have been many studies on bending of peduncels in cut roses that is called bent-neck or weeping. Van Doorn (1997) attributed this condition to the blockage of water transport by air bubble or bacteria in the xylem, whereas Zieslin et al. (1978) reported that bent-neck is a result of the heavy load imposed by the flower bud on the peduncel and thus, is related to the mechanical strength of the neck. In cut *Eustoma* flowers, continuous treatment with sucrose greatly reduced bending of pedicels while increasing their rigidity (Cho et al., 2002). In our study, STS treatment did not reduce the rate of pedicel bending, but it delayed its onset compared to the control (Table 3). This positive effect of STS remains to be elucidated.

Cut *Eustoma* flowers have many florets and buds on each flower stem. STS did not increase the rate of floret opening (Table 2) nor anthocyanin concentration of petals (Table 4), but pulse treatment with sucrose alone and STS plus sucrose did more than STS alone (Table 2, 4). Similar results associated high sugar concentration in cut flowers, e.g. on bud opening were reported pre-

viously by Mor et al. (1984), Koyama and Uda (1994) and that on anthocyanin concentration by Ichimura and Hiraya (1999) and Ichimura and Korenaga (1998).

Sugars serve as a substrate for glycoside residue of anthocyanin, but also for flavonoid biosynthesis via the shikimic acid and phenylpropanoid pathways. Furthermore, sugars induce gene expression involved in anthocyanin biosynthesis (Tukaya et al., 1991). In cut *Eustoma* flowers the transcript levels of chalcone synthase, chalcone isomerase, and dihydroflavonol 4-reductanse which are involved in anthocyanin biosynthesis, were enhanced by sucrose treatment (Kawabata et al., 1999).

In this study, STS plus sucrose and sucrose alone extended the vase life of cut flowers, hastened the rate of bud opening, and increased anthocyanin concentration in the colored parts of petals more than STS alone. These results suggest that pulse treatments, including sucrose, are more effective than did STS alone to improve the quality of cut *Eustoma* flowers. Under practical conditions, the vase life of *Eustoma* flowers will be affected more by pollination or exogenous ethylene than it will be this experimental condition. However, it is still unclear whether sucrose has same effect as STS which inhibits flower senescence induced by pollination (Ichimura and Goto, 2000) and exogenous ethylene (Veen and van Geijn, 1978). Moreover, combined treatment with surfactant and STS extended the vase life of cut spray carnations more than did STS alone (Uda, 1996). Surfactant is effective in improving water relations of cut flowers, especially those having many branches and many florets. Thus, it may be possible that STS plus sucrose treatments in combination with some surfactant could extend the vase life of cut *Eustoma* flowers more than sucrose could alone.

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チオ硫酸銀錯塩(STS), スクロースおよび両者を組み合わせた処理がトルコギキョウ切り花の収穫後の品質と花持ちに及ぼす影響

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摘 要

トルコギキョウにおいてチオ硫酸銀錯塩(STS), スクロース, STSとスクロースを組み合わせた前処理が切り花の品質と花持ちに及ぼす影響について調査を行った. 切り花を小花が2輪開花し4輪つぼみの状態に調整し, 0.2 mM STS, 4%スクロース, および両者を組み合わせた溶液を23℃, 相対湿度70%, 暗黒条件下で20時間吸水処理した. 切り花の花持ちは2花以上の小花が観賞価値を保持している期間とした. その結果, STSとスクロースを組み合わせた処理およびスクロース単用処理では対照およびSTS単用に比べて花持ち延長効果が高かった. また, スクロース単用およびSTSとスクロースを組み合わせた処理では, つぼみの開花率と花弁の覆輪部分のアントシアニン濃度がSTS単用に比べて著しく増加した. これらの結果から, エチレン感受性があまり高くなく, 小花を多数つけるトルコギキョウでは, スクロースを含む溶液による前処理はSTS単独処理に比べて切り花の品質向上に有効であると考えられた.