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Sodium selenite improves the vase life of *Eustoma grandiflorum* cut flowers

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Abstract: Previous studies indicated that the antioxidant and water balance capacity played important roles in improving the vase life of cut flowers. As a trace element, selenium (Se) has been proven to play an important role in extending the vase life of lily cut flowers. However, there is still no report on the effects of Se on the vase life of other cut flowers. In this study, we explored the role of inorganic selenium, named sodium selenite (Na_2SeO_3), in improving the vase life of *Eustoma grandiflorum* cut flowers. Compared with the control (distilled water) and the other Na_2SeO_3 concentrations, 4.0 mg/L Na_2SeO_3 significantly increased the superoxide dismutase, catalase, ascorbate peroxidase glutathione reductase, glutathione peroxidase and glutathione S-transferase activities, and decreased the malondialdehyde content and the electrolyte leakage. Meanwhile, compared with the control and the other of Na_2SeO_3 concentrations, 4.0 mg/L Na_2SeO_3 significantly increased the soluble sugar, proline and soluble protein contents, relative water content, average fresh weight change rate and average water balance value. The above results imply that Na_2SeO_3 showed an important role in strengthening the antioxidant capacity and maintaining the water balance. Besides, 4.0 mg/L Na_2SeO_3 significantly increased the flower diameter and the vase life. The above findings suggested that Na_2SeO_3 extended the vase life and the ornamental value of *E. grandiflorum* cut flowers by enhancing the antioxidant capacity and water balance, which provides new knowledge for the application of Na_2SeO_3 in improving the fresh keeping of *E. grandiflorum* cut flowers.

Keywords: Lisianthus; inorganic selenium; preservation; antioxidant enzyme; osmotic adjustment

The lisianthus (*Eustoma grandiflorum*) is a common cut flower in the world. However, *E. grandiflorum* cut flowers have a very short vase life, which seriously limits the commercial value. As reported, the vase life of cut flowers is closely related to the antioxidant capacity and water balance capacity (Lu et al. 2020). Thus, it is necessary to take measures to prolong the vase life of *E. grandiflorum* cut flowers by enhancing of the antioxidant capacity and water balance capacity. Many studies have reported that exogenous chemicals can be applied to improve the cut flowers' vase life by enhancing the antioxidant capacity and water balance (Shabanian et al. 2018; Zheng, Guo 2018). Selenium (Se) is a very important beneficial element, which has been reported to show beneficial effects on the growth, development and stress response of plants (Azizi et al. 2020; Zahedi et al. 2020). For cut flowers, it has been reported that Se could extend the *Lilium longiflorum* cut flower vase life through the enhancement of the antioxidant capacity and water balance capacity (Lu et al. 2020). However, the effect of Se on the vase life of *E. grandiflorum* cut flowers has still not been reported. Hence, investigating the effects of Se on the antioxidant capacity and water balance capacity of *E. grandiflorum* cut flowers will be very interesting.

Many reports have demonstrated that the cut flowers' vase life was closely related with their antioxidant capacity (Hou et al. 2018; Lu et al. 2020).

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Previous research studies have suggested that Se improved the antioxidant capacity of many plants via antioxidases (Pereira et al. 2018). The results of Lu et al. (2020) indicated that Se increased the enzymatic activities of the antioxidases in *L. longiflorum* cut flower petals, including superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR), which further enhanced the antioxidant capacity. Water balance plays a significant role in maintaining the cut flowers' vase life.

More and more research studies have indicated that the water balance capacity was closely related with the contents of the osmolytes, such as the soluble sugar (SS), proline (PRO) and soluble protein (SP). Lu et al. (2020) reported that Se strengthened the water balance capacity of the *L. longiflorum* cut flower through the increases in the contents of the osmolytes SS, PRO and SP, which further induced the increase in the relative water content (RWC) of the cut flower. However, the influence of Se on the antioxidant capacity and water balance capacity of *E. grandiflorum* cut flowers has still not been reported. Therefore, investigating the effects of Se on the enzymatic activities of the antioxidases and water balance of *E. grandiflorum* cut flowers will be very interesting.

The ornamental value of cut flowers was closely related with the flower opening degree and opening time. The flower opening degree can be indicated by the flower diameter and the flower opening time can be indicated by the vase life. It has been reported that Se could increase the vase life of snapdragon (*Antirrhinum majus* L.) and lily (*Lilium longiflorum* Thunb.) cut flowers (Tognon et al. 2016; Lu et al. 2020). However, there is still no report on the effect of Se on the flower diameter. Hence, it will be very interesting to explore the effect of Se on the flower diameter, which will provide more information for its application in the cut flower industry.

In the current research, we explored the role of sodium selenite (Na_2SeO_3) in regulating the vase life, the enzymatic activities of SOD, POD, CAT, APX, GR, glutathione peroxidase (GSH-Px) and glutathione S-transferase (GST), the contents of SP, SS and PRO, malondialdehyde (MDA) content, electrolyte leakage (EL), RWC, average fresh weight change rate, average water balance value and the flower diameter of *E. grandiflorum* cut flowers. The aim of this research was to explain the positive effects of Na_2SeO_3 on the extension of the vase life and the improvement in the ornamental value of *Eustoma grandiflorum* cut flowers.

MATERIALS AND METHODS

Plant material and treatment. Cut flowers of white *E. grandiflorum* were purchased from Xinxiang flower market. We selected the cut flowers having five buds of a similar size for this study. The stems of all the cut flowers were cut into the same length (30 cm). Then, the cut flowers were divided into four groups and then placed into 500 mL vases containing 300 mL distilled water (Control), 2.0, 4.0 and 8.0 mg/L Na_2SeO_3. The above-treated cut flowers were placed into a climatic chamber (28 °C during the day and 15 °C during the night, at 60% relative humidity, with light for 12 hours every day under 300 μmol/m^2/s photosynthetic active radiation). Each treatment was repeated four times. For each time, two cut flower stems were used for each treatment and each cut flower stem had five buds. The Na_2SeO_3 solutions used for the different treatments were changed once a day. After 4 days of treatment, the petals of the first opened flowers were sampled and then used to measure the physiological and biochemical indicators. When 80% of the flowers under the different treatments were wilting, the vase life was recorded.

Activities of the antioxidases. Fresh petal samples (0.5 g) were homogenised in 10 mL of ice-cold 50 mM phosphate buffer (pH 7.0). The homogenates were then centrifugated at 12 000×g for 10 minutes. Then, the supernatant was used to measure the activities of the antioxidant enzymes. The activities of POD, CAT, SOD, APX and GR were analysed according to the methods in Lu et al. (2020). The POD activity was determined spectrophotometrically by measuring the oxidation of guaiacol to tetraguaiacol at 470 nm. The CAT activity was measured by recording the decrease in the absorbance at 240 nm over 3 minutes. The SOD activity was measured by monitoring the superoxide radical-induced nitro blue tetrazolium reduction (NBT) at 560 nm. The APX activity was measured by recording the decrease in the absorbance at 290 nm over 3 minutes. The GR activity was measured by recording the decrease in the absorbance at 340 nm over 3 minutes.

For the GSH-Px and GST, the crude enzyme extracts were prepared by using 0.5 g samples in a 5 mL 0.1 mol/L phosphate buffer (pH 7.5). The homogenate was centrifuged at 1 000×g for 10 minutes at 4 °C. Then, the supernatant was used to analyse the activities of the GSH-Px and GST according

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to the instruction manual of the GSH-Px and GR detection kits. The GSH-Px activity was assayed spectrophotometrically at 348 nm. The GST activity was assayed spectrophotometrically at 340 nm.

Malondialdehyde (MDA) content and electrolyte leakage (EL). The MDA content was assayed spectrophotometrically by measuring the absorbance at 450 nm, 532 nm and 600 nm according to the thiobarbituric acid (TBA) reaction as described by Hodges et al. (1999). The EL was measured by the method of Brennan and Frenkel (1977). Fresh samples of petal discs were immersed in water at 25 °C for 6 hours. Then the electrical conductivity (EC) was measured by a conductivity meter and recorded as EC_1. Samples of the petal discs were heated at 100 °C for 20 min. After cooling, the EC was measured and recorded as EC_2. The EL was calculated as $(EC_1/ EC_2) \times 100\%$.

Contents of the osmolytes soluble sugar (SS), proline (PRO) and soluble protein (SP). The SS content was estimated by using anthrone-sulfuric acid method and expressed as mg/g Fresh Weight (FW). The PRO content was estimated by the acidic-ninhydrin method and expressed as mg/g FW. The SP content was estimated by using the Bradford method and expressed as μg/g FW.

Relative water content (RWC). Firstly, fresh petals were weighed and recorded as the fresh weight. Then, the petals were immersed in water for 12 hours. After 12 hours, the above petals were weighed and recorded as the saturated weight (SW). Then the petals were placed in an oven at 105 °C for 5 min followed by 65 °C until reaching a constant weight. Finally, the petals were weighed and recorded as the dry weight (DW). The RWC was calculated by using the equation: $RWC = [(FW - DW)/(SW - DW)] \times 100\%$.

Vase life. The vase life of the cut flowers under each treatment was recorded as the number of days from when the cut flowers were placed in flasks to when 80% of all the flowers were wilting.

Flower diameter. From 0 day to 10 days of treatment, the diameter of each flower per treatment was measured every two days. The diameter of each flower was measured two times vertically with a digital calliper and the average of above vertically measured two flower diameters was taken as the diameter of each flower.

Average fresh weight change rate and average water balance value. For fresh weight change rate (FWCR), we weighed the total weight of the cut flowers, vase solution and flask (W_1), and the total weight of vase solution and flask (W_2). The fresh weight of the cut flowers was measured every 2 days and expressed as the difference between W_1 and W_2. The value of FWCR was calculated as [(FW – initial FW) ÷ initial FW] × 100%. The average fresh weight change rate (AFWCR) was the mean FWCR value measured five times from 0 day to 10 days of treatment.

For the average water balance value (WBV), we weighed W_1 and W_2. The water loss was measured every two days and expressed as the difference of W_1 in two adjacent days. The water absorption was also measured every two days and expressed as the difference of W_2 in two adjacent days. The WBV was calculated as the difference of the water absorption and water loss. The average water balance value (AWBV) was the mean WBV value measured five times from 0 day to 10 days of treatment.

Statistical analysis. The data are presented as the mean of four replications. To perform the analysis of variance, we tested the assumptions of normality on the data sample by the Kolmogorov-Smirnov test. We tested the heteroscedasticity on the data sample by both the Graphical test method and the Goldfeld-Quandt test. The means were compared by a one-way analysis of variance and Duncan's multiple range test at a 5% level of significance. The statistical analysis and correlation analysis were performed by using the software SPSS Statistics 25.0.

RESULTS AND DISCUSSION

Effects of Se on malondialdehyde (MDA) content and electrolyte leakage (EL). Compared with the control, Na_2SeO_3 significantly reduced the MDA content and the EL in the petals ($P < 0.05$, Table 1). Compared with other concentrations, 4.0 mg/L Na_2SeO_3 showed more significant effects on the alleviation of the lipid peroxidation ($P < 0.05$). Compared with control, 4.0 mg/L Na_2SeO_3 decreased the MDA content and the EL by 47.8% and 42.1%, respectively.

The vase life of the cut flower is closely related to the lipid peroxidation of the petals during the ageing process. It has been reported that the MDA and EL were two important indicators of lipid peroxidation (Lu et al. 2020). Previous study showed that Se alleviated the lipid peroxidation of snapdragon cut flowers, which further extended the vase life (Tognon et al. 2016). Lu et al. (2020) reported that Se also alle-

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Table 1. Effects of selenium (Se) on the malondialdehyde (MDA) content and electrolyte leakage (EL)

Treatment	MDA (nmol/g FW)	EL (%)
Control	6.7 ± 0.61[a]	16.4 ± 1.47[a]
2.0 mg/L Na_2SeO_3	5.6 ± 0.45[b]	14.0 ± 1.20[b]
4.0 mg/L Na_2SeO_3	3.5 ± 0.40[d]	9.5 ± 1.03[d]
8.0 mg/L Na_2SeO_3	4.8 ± 0.53[c]	12.2 ± 1.10[c]

Values represent the means of four replications, different letters indicate a statistical difference at $P < 0.05$

viated the lipid peroxidation of lily cut flowers, which also further extended the vase life. However, whether and how Se alleviates the lipid peroxidation of *E. grandiflorum* cut flowers has still not been reported. Our findings indicated that Na_2SeO_3 could also alleviate the lipid peroxidation of *E. grandiflorum* cut flowers by reducing the MDA content and the EL, which was consistent with the results on the snapdragon and lily cut flowers (Tognon et al. 2016; Lu et al. 2020).

Effects of Se on the activities of antioxidant enzymes. Compared with the control, the Se significantly increased the CAT, SOD, APX, GR, GSH-Px and GST activities in the petals of the *E. grandiflorum* cut flowers ($P < 0.05$, Table 2 and Figure 1), but had no significant effect on the POD activity ($P > 0.05$, Table 2). Compared with the other concentrations, 4.0 mg/L Na_2SeO_3 showed more significant effects on the activities of the above antioxidant enzymes except for POD ($P < 0.05$). Compared with the control, 4.0 mg/L Na_2SeO_3 increased the CAT, SOD, APX, GR, GSH-Px and GST activities by 50.0%, 40.3%, 43.1%, 75.3%, 63.2% and 141.7%, respectively.

Lu et al. (2020) reported that Se alleviated the lipid peroxidation of lily cut flowers by enhancing the POD, CAT, SOD, APX and GR activities in the petals. Our results indicated that Se enhanced the CAT, SOD, APX and GR activities in the petals of the *E. grandiflorum* cut flowers, which was consistent with the findings of Lu et al. (2020)

Table 2. Effects of selenium (Se) on the peroxidase (POD), catalase (CAT) and superoxide dismutase (SOD) activities

Treatment	POD (U/g FW)	CAT (U/g FW)	SOD (U/g FW)
Control	129.0 ± 10.35[a]	18.0 ± 1.66[c]	167.8 ± 11.94[d]
2.0 mg/L Na_2SeO_3	136.7 ± 13.11[a]	21.2 ± 1.83[b]	190.0 ± 14.67[c]
4.0 mg/L Na_2SeO_3	131.0 ± 14.74[a]	27.0 ± 3.15[a]	235.5 ± 16.82[a]
8.0 mg/L Na_2SeO_3	141.3 ± 12.86[a]	23.5 ± 2.09[b]	212.7 ± 15.30[b]

Values represent the means of four replications
Different letters indicate a statistical difference at $P < 0.05$

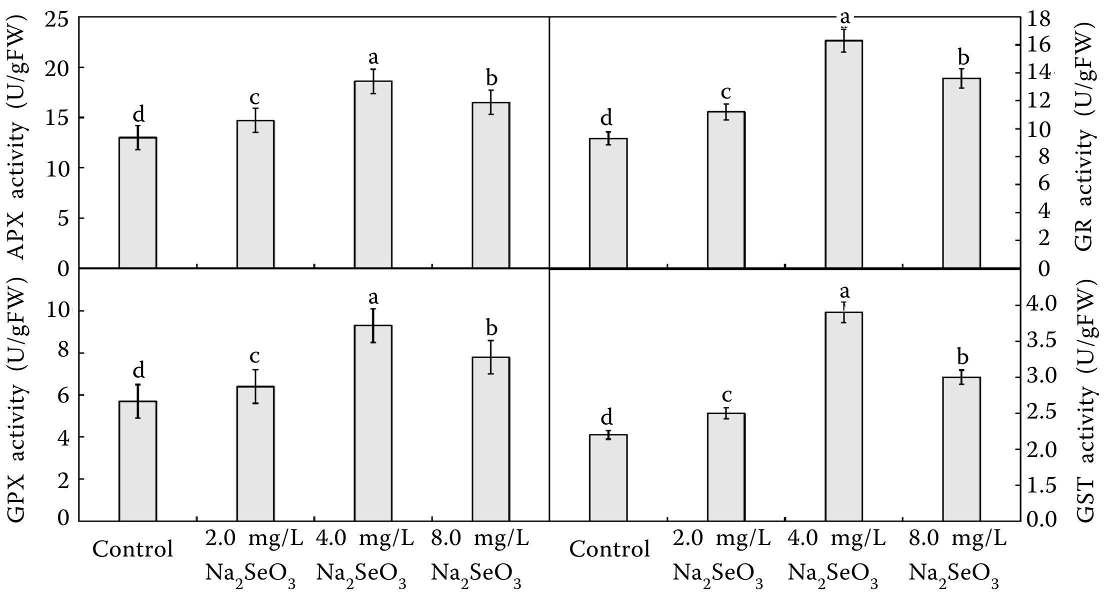

Figure 1. Effects of selenium (Se) on the ascorbate peroxidase (APX), glutathione reductase (GR), glutathione peroxidase (GSH-Px) and glutathione S-transferase (GST) activities
Values represent the means of four replications, different letters indicate a statistical difference at $P < 0.05$

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on *L. longiflorum* cut flowers. However, our results showed that Se had no significant effect on the POD activity in the petals of the *E. grandiflorum* cut flowers, which was not consistent with the findings of Lu et al. (2020) on *L. longiflorum* cut flowers. This difference is probably due to difference in the plant species. Moreover, the current results indicated that Se increased the GSH-Px and GST activities in the petals of the *E. grandiflorum* cut flowers. Therefore, Na_2SeO_3 could alleviate the lipid peroxidation of *E. grandiflorum* cut flowers by enhancing most antioxidant enzymes of the antioxidant system, which extended the vase life. Through the correlation analysis between the MDA content, the EL and the activities of the above antioxidant enzymes, we found that the lipid peroxidation of the petals indicated by the MDA and EL had significant negative correlations with the activities of the antioxidant enzymes CAT, SOD, APX, GR, GSH-Px and GST (Table 3). These results further indicated that Se could alleviate the lipid peroxidation of petals by enhancing the activities of the above antioxidant enzymes.

Among the antioxidases, the percentage of Se increasing the GST activity was higher than that of the other enzymes, which suggested that GST played a significant role in the regulation of the vase life by the Se. However, the specific mechanism Se has in regulating the GST activity is still unclear. Hence, it will be interesting to investigate the mechanism of Se in regulating the GST activity, which will give a more theoretical basis for Se applications in extending the vase life of cut flowers.

Effects of Se on relative water content (RWC), the contents of osmolytes, average fresh weight change rate and average water balance value. Compared with the control, Se significantly improved the RWC and the osmolytes SP, SS and PRO contents of the petals ($P < 0.05$, Figure 2). Compared with the other concentrations, 4.0 mg/L Na_2SeO_3 showed more significant effects on the contents of the above three osmolytes ($P < 0.05$), which further increased the RWC. Compared with the control, 4.0 mg/L Na_2SeO_3 improved the RWC and the SP, SS and PRO contents by 11.7%, 92.8%, 79.1% and 139.1%, respectively. Compared with the control, Se significantly improved the average fresh weight change rate and average water balance value of the *E. grandiflorum* cut flowers ($P < 0.05$, Figure 3). Compared with the other concentrations, 4.0 mg/L Na_2SeO_3 showed more significant effects on the average fresh weight change rate and average water balance value of the *E. grandiflorum* cut flowers ($P < 0.05$). Compared with the control, 4.0 mg/L Na_2SeO_3 improved the average fresh weight change rate and average water balance value by 72.7% and 62.4%, respectively. The above results further indicated that Se showed an important role in maintaining the water balance of the *E. grandiflorum* cut flowers. Through the correlation analysis between the RWC, average water balance value and the contents of the osmotic adjustment substances in the petals, we found that the water balance indicated by the RWC and average water balance value had significant positive correlations with the contents of the osmotic adjustment substances SP, SS and PRO (Table 4). These results further indicated that Se could maintain the water balance of cut flowers by increasing the contents of the above osmotic adjustment substances.

The water balance of the cut flower has a close relationship to the vase life. Many studies have shown that osmolytes SP, SS and PRO played important roles in maintaining the water balance of cut flowers (Shan, Zhao 2015; Lu et al. 2020). The results of Lu et al. (2020) indicated that Se increased the SP, SS and PRO contents, which further enhanced the RWC and extended the vase life of *L. longiflorum* cut flowers. We found that Se increased the contents of the above osmolytes, which further enhanced the RWC and extended the vase life of the *E. grandiflorum* cut flowers. Therefore, Se

Table 3. Correlation analysis between the malondialdehyde (MDA) content, electrolyte leakage (EL) and the activities of antioxidant enzymes peroxidase (POD), catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR), glutathione peroxidase (GSH-Px) and glutathione S-transferase (GST) in the petals

Indicator	POD	CAT	SOD	APX	GR	GPX	GST
MDA	–0.183	–0.998**	–0.996**	–0.998**	–0.996**	–0.987*	–0.981*
EL	–0.199	–1.000**	–0.997**	–0.998**	–0.996**	–0.987*	–0.979*

*The correlation was significant at a level of 0.05 (double-tailed)

**The correlation was significant at a level of 0.01 (double-tailed)

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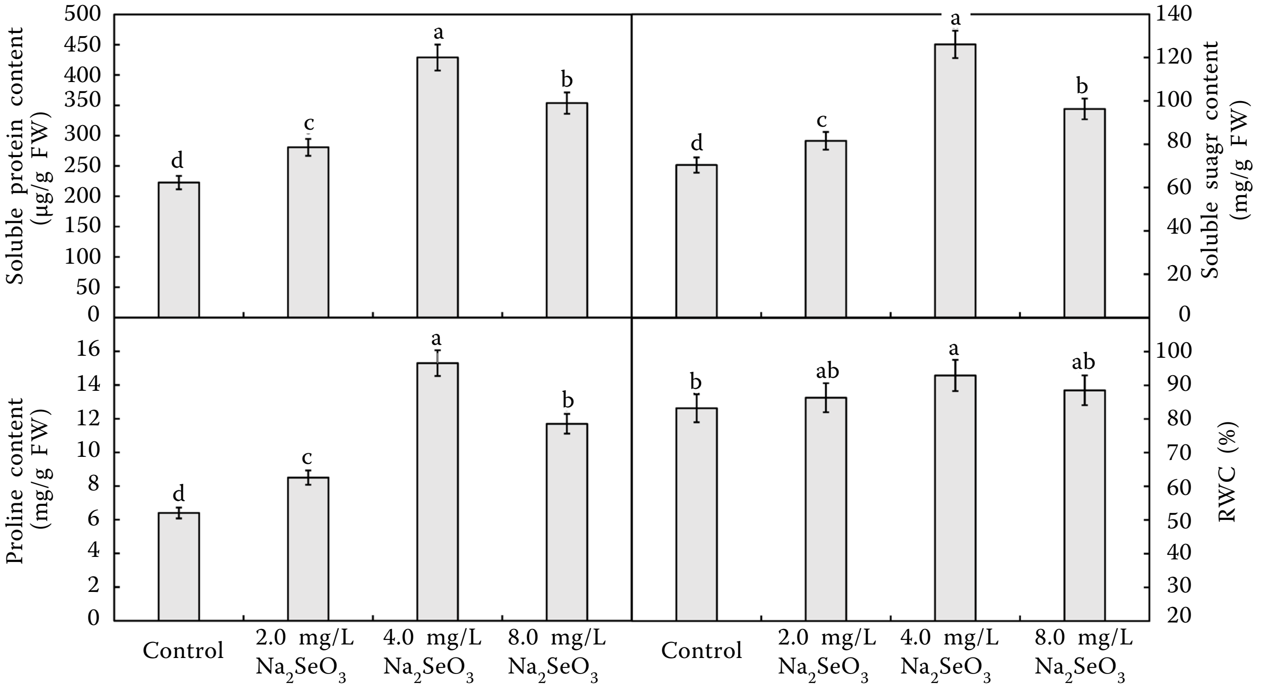

Figure 2. Effects of selenium (Se) on the relative water content (RWC) and the contents of osmotic adjustment substances

Values represent the means of four replications, different letters indicate a statistical difference at $P < 0.05$

showed the same effects on the RWC and the contents of the osmolytes in the petals of the *E. grandiflorum* cut flowers as that of *L. longiflorum* cut flowers (Shan, Zhao 2015; Lu et al. 2020). Generally, PRO is a stress marker. The current results showed that the PRO content was higher in the

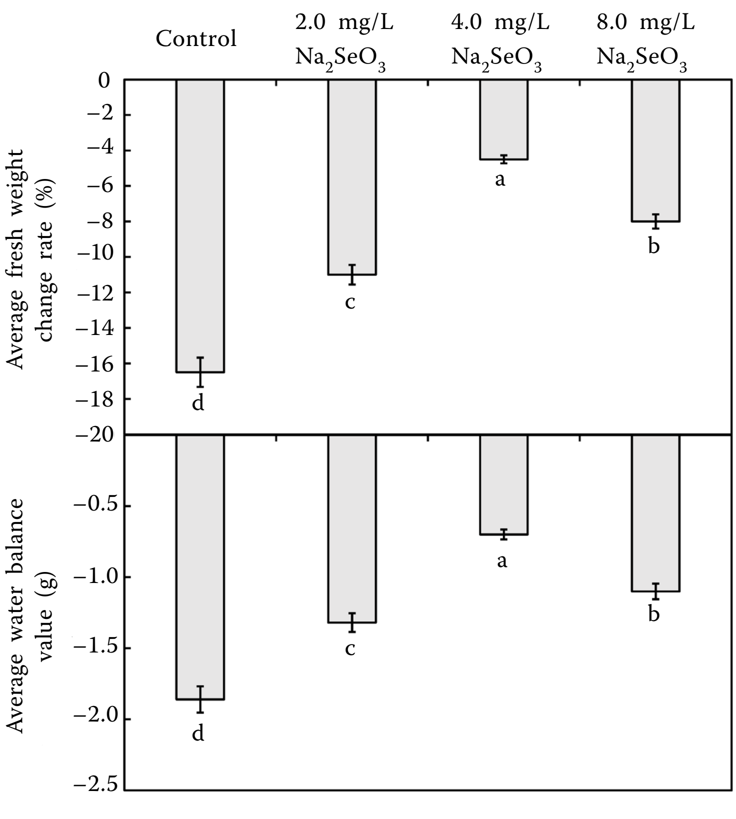

Figure 3. Effects of selenium (Se) on the average fresh weight change rate and the average water balance value

Values represent the means of four replications, different letters indicate a statistical difference at $P < 0.05$

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Table 4. Correlation analysis between the relative water content (RWC), average water balance value and the contents of osmotic adjustment substances in the petals

Indicator	Soluble protein	Soluble sugar	Proline
RWC	0.993**	0.991**	0.992**
Average water balance value	0.979*	0.950*	0.969*

*The correlation was significant at a level of 0.05 (double-tailed)

**The correlation was significant at a level of 0.01 (double-tailed)

Se treatment, which indicated that the cut flowers may have suffered some kind of stress under the Se treatment. However, our results clearly showed that all the Se treatments significantly increased the RWC and decreased the EL and MDA content in the petals, which indicated that the cut flowers treated by Se did not suffer some kind of stress. Therefore, the increase in the PRO content under the Se treatment was a favourable factor for the preservation of the cut flowers, which has been reported by many studies (Shan, Zhao 2015; Zheng, Guo 2019; Lu et al. 2020). Among the osmolytes, the percentage of the PRO content increased by the Se was higher than that of the other osmolytes, which suggested that the PRO played a significant role in the regulation of the vase life by the Se. However, the specific mechanism of Se in regulating the PRO content is still unclear. Hence, it will also be interesting to investigate the mechanism of Se in regulating the PRO content, which will give a more theoretical basis for Se applications in extending the vase life of cut flowers.

Effect of Se on vase life and flower diameter. Compared with the control, Na_2SeO_3 significantly improved the vase life (P < 0.05, Figure 4). Compared with the other concentrations, 4.0 mg/L Na_2SeO_3 showed a more significant effect on improving the vase life (P < 0.05). Compared with the control, 4.0 mg/L Na_2SeO_3 improved the vase life by 48.2%. Compared with the control, Na_2SeO_3 significantly increased the flower diameter (P < 0.05, Figure 5). As shown in Table 6, all the flower diameters of the different treatments showed a similar trend. From 0 day to 6 days, the average flower diameter of the different treatments gradually increased and reached the maximum flower diameter on day six. Then the flower diameter of the different treatments gradually decreased. Compared with the other concentrations, 4.0 mg/L Na_2SeO_3 showed more significant effects on the flower diameter. Compared with the control, 4.0 mg/L Na_2SeO_3 increased the flower diameter by 41.1%, 31.7%, 32.8%, 67.3% and 151.5% at 2 days, 4 days, 6 days, 8 days and 10 days of treatment, respectively. Through the correlation analysis between the vase life and the MDA, EL, RWC and average water balance value, we found that vase life had significant positive correlations with the RWC and the average water balance value, and had sig-

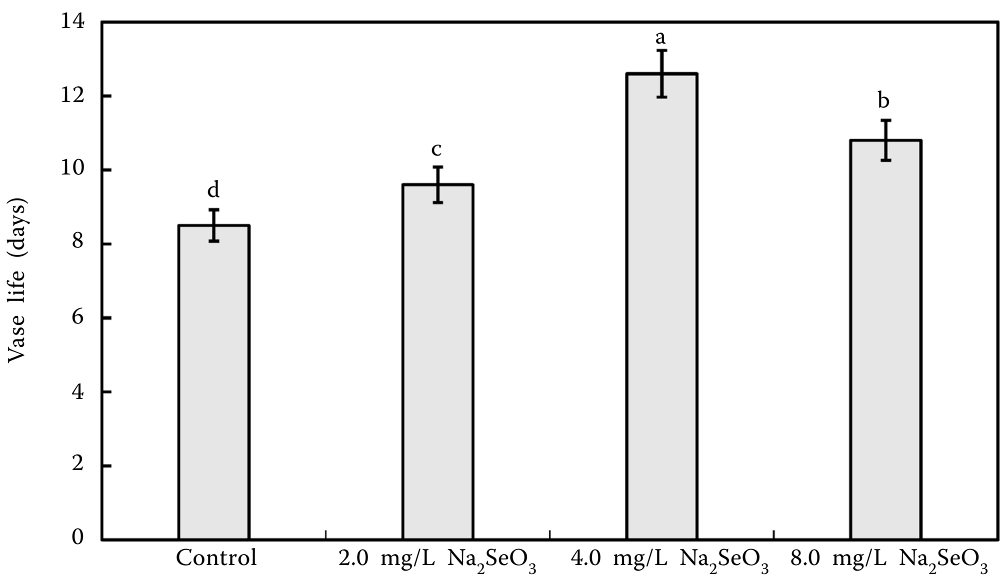

Figure 4. Effects of selenium (Se) on the vase life of cut flowers

Values represent the means of four replications, different letters indicate a statistical difference at P < 0.05

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Table 5. Correlation analysis between the vase life and physiological indicators representing membrane lipid peroxidation and water balance in the petals

Indicator	MDA	EL	RWC	Average water balance value
Vase life	0.997**	0.996**	0.998**	0.976*
Average flower diameter	0.980*	0.981*	0.976*	0.998**

MDA – malondialdehyde; EL – electrolyte leakage; RWC – relative water content

*The correlation was significant at a level of 0.05 (double-tailed)

**The correlation was significant at a level of 0.01 (double-tailed)

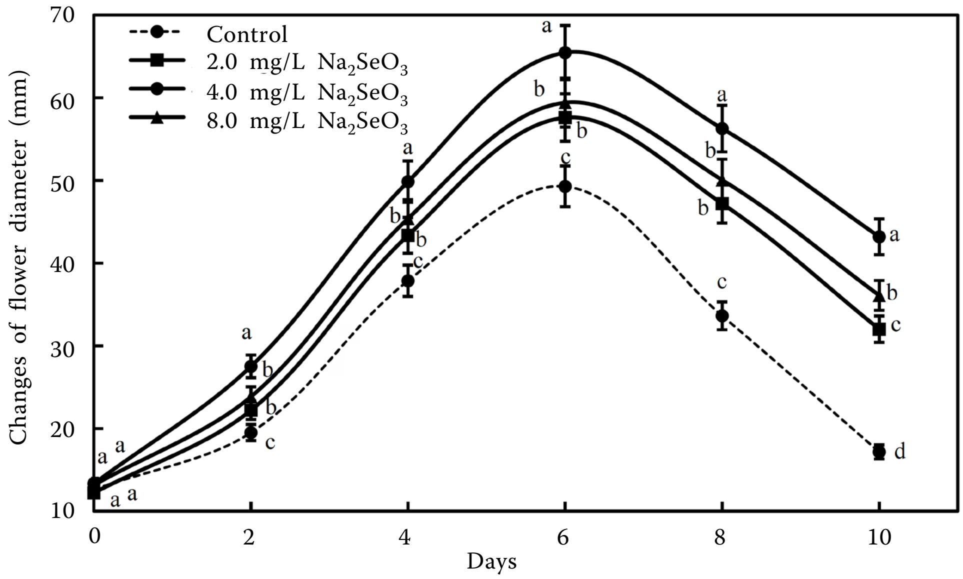

Figure 5. Effects of selenium (Se) on the changes in the flower diameter (mm)

Values represent the means of four replications, different letters indicate a statistical difference at $P < 0.05$

nificant negative correlations with the MDA and EL (Table 5). These results further indicated that Se could improve the vase life of cut flowers by decreasing the lipid peroxidation and maintaining the water balance of the petals.

The degree of flower opening, which was indicated by the flower diameter, is an important indicator to measure the ornamental value of cut flowers. However, there is still no report for the effects of Se on the degree of flower opening of cut flowers (Tognon et al. 2016; Lu et al. 2020). Our findings indicated that Se significantly increased the degree of the flower opening of the *E. grandiflorum* cut flowers by improving the flower diameter from 2 day to 10 days. Moreover, Se significantly increased the maximum value of the flower diameter at 6 days of treatment. Through the correlation analysis between the average flower diameter and the MDA, EL, RWC and average water balance value, we found that the average flower diameter had significant positive correlations with the RWC and the average water balance value, and had significant negative correlations with the MDA and EL (Table 5). These results indicated that Se could also improve the ornamental value of cut flowers by decreasing the lipid peroxidation while maintaining the water balance of the petals.

CONCLUSION

Our results clearly indicated that different Se concentrations showed significant effects on the of antioxidase activities, water balance and flower opening degree, which further improved the vase life and the ornamental value of *E. grandiflorum* cut flowers. Among different concentrations, 4.0 mg/L Na_2SeO_3 showed more significant effects on the above physiological and biochemical indicators, which suggested that 4.0 mg/L Na_2SeO_3 can be applied in the industry of *E. grandiflorum* cut flowers to improve the vase life and the ornamental value.

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