

## EFFECT OF 8-HYDROXYQUINOLINE CITRATE, SUCROSE AND PEROXIDASE INHIBITORS ON VASE LIFE OF LISIANTHUS (*EUSTOMA GRANDIFLORUM* L.) CUT FLOWERS

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Received: February 11, 2013; Accepted: April 9, 2014

### ABSTRACT

Cut lisianthus flowers have a short vase life, possibly due to blockage of xylem vessels. The effect of 8-hydroxyquinoline citrate, sucrose and peroxidase inhibitors on delaying senescence and extending vase life of cut lisianthus flowers was tested. The peroxidase inhibitors used in this experiment were catechol (CH) (5, 10, 15 mM) and *p*-phenylenediamine (PD) (5, 10, 15 mM). All vase solutions contained 200 mg·dm<sup>-3</sup> 8-hydroxyquinoline citrate (8-HQC) and 3% sucrose. 10 mM CH treatment was the most effective for vase life extension (13.3 days), increasing water uptake, and delaying fresh weight loss. The vase solution containing 10 mM CH significantly increased superoxide dismutase (SOD) and decreased peroxidase (POD) activities. Similarly, 10 mM PD increased anthocyanin content more than the other treatments. Protein degradation was significantly delayed by application of 5 mM PD. The malondialdehyde (MDA) accumulation was reduced when CH at 5 mM and PD in 5 and 15 mM were added to the vase solution. Results indicated that peroxidase inhibitors in combination with 8-HQC and sucrose increase vase life of lisianthus by improving water uptake and delaying fresh weight loss.

**Key words:** lisianthus, peroxidase, postharvest, water uptake, protein, anthocyanin content, SOD and POD activity

### INTRODUCTION

Lisianthus (*Eustoma grandiflorum*) is native to the southern parts of the United States, where it inhabits mainly the moist meadows from Nebraska to Colorado and Texas (Ohkawa et al. 1991). Ichimura et al. (1998) reported that *E. grandiflorum* is sensitive to ethylene and has a short vase life. Short vase life is one of the most important problems in cut flowers production and its extension is a serious research challenge (Da Silva 2003; Kader 2003). Water uptake of cut stems kept in water, in that of *E. grandiflorum* cut flowers, decreases with time, possibly due to occlusions in xylem vessels (van Doorn 1996, van Doorn

& Cruz 2000). There are two mechanisms of water uptake blockages. One related to an air embolism (cavitation) or when small particles or bacteria and bacterial products cause physical blockages of xylem (van Doorn 1996). The second one is probably of a physiological origin. It starts at the stem base and moves up the stem (van Doorn & Vaslier 2002). Physiological plugging as a result of defensive metabolic responses to wounding (lignin production) has been reported for *Chrysanthemum* (van Doorn & Cruz 2000; van Doorn & Vaslier 2002) and *Bouvardia* (Vaslier & van Doorn 2003). Activities triggered by wounding plant tissue are mechanisms for defence and healing (Leon et al. 2001). Deposition of materials such as lignin and suberin in the xylem conduits, as a physiological response to wounding,

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was suggested some years ago (Dean & Kolatukudy 1976; Halevy & Mayak 1981; van Doorn 1996). Peroxidases (PODs, EC 1.11.1.7) are heme-containing enzymes omnipresent in plants. PODs are involved in many growth-related processes, including cell wall extension, lignin synthesis and auxin catabolism. Moreover, they are involved in stress-related processes such as wounding response and disease resistance (Moerschbacher 1992). POD is involved in lignin formation associated with wound healing in potato tuber tissue (Espelie et al. 1986). Both peroxidases and catechol oxidase were involved in physiological blockage of water uptake in *Bouvardia* flower stems (Vaslier & van Doorn 2003). Inhibition of wound-induced xylem occlusion by antioxidants has been reported to occur in *Chrysanthemum* both during dry and wet storage (van Doorn & Cruz 2000). Increasing vase life by *p*-phenylenediamine (PD), catechol (CH) and other inhibitors of oxidative enzymes has been reported in *Acacia holosericea* and *Chamelaucium uncinatum* (Çelikel et al. 2011). Application of POD inhibitors such as hydroquinone, PD, copper ions and inhibitors of catechol oxidase, such as tropolone and 2-3-dihydroxynaphthalene, delayed leaf wilting in bouvardia (Vaslier & van Doorn 2003). PODs link some hydroxyl groups to a range of substances, including extracellular phenols (Bernards et al. 1999). They are involved in the monolignol step in lignin biosynthesis (Sterjiades et al. 1993). As a first reaction to wounding in wheat roots, POD is released from cell surface into apoplast, where it performs both oxidative and peroxidative activity (Minibayeva et al. 2009). The aim of the research presented was to test the hypothesis that POD activities are involved in the occlusion of xylem vessels in lisianthus stems and to select the optimum concentrations of CH and PD for improving vase life and quality of cut lisianthus flowers.

## MATERIAL AND METHODS

### Plant material and treatments

Stems of lisianthus (*E. grandiflorum* Mariachi. cv. Pink) ~50 cm long with fully open flowers were harvested and immediately transported to the Laboratory of Horticultural Sciences, Faculty of Agricultural Sciences, University of Guilan, Rasht, Iran.

In the laboratory, the stems were re-cut to 40 cm under deionised water. The cut flowers were placed in a 250 mL distilled water (control) or 250 mL of water supplemented with: (i) 200 mg·dm<sup>-3</sup> 8-hydroxyquinoline citrate (8-HQC); (ii) 3% sucrose, (iii) catechol (CH) (5, 10, 15 mM) + 8-HQC (200 mg·dm<sup>-3</sup>) + sucrose (3%); (iiii) *p*-phenyldiamine (PD) (5, 10, 15 mM) + 8-HQC (200 mg·dm<sup>-3</sup>) + sucrose (3%). After 5 h of the treatments, the flowers were placed in distilled water. CH was purchased from Sigma-Aldrich and PD from Merck. For PD and CH solutions, NaOH and H<sub>3</sub>PO<sub>4</sub> were used to adjust pH to 6.0 (Çelikel et al. 2011). Cut flowers were kept at 20 °C, 60% relative humidity and 12 h photoperiod with 15 µmol·m<sup>-2</sup>s<sup>-1</sup> irradiance from cool-white fluorescent lamp.

### Vase life, water uptake and relative fresh weight evaluation

Vase life was evaluated daily, and was judged to have ended when 50% or more of the flowers on an inflorescence were deemed unattractive (Cho et al. 2001). The weights of each vase, with and without cut stems, were recorded daily during the vase life evaluation period. Average daily water uptake (WU) was calculated using the following formula:  $WU (g \cdot stem^{-1} \cdot d^{-1}) = (S_{t-1} - S_t)$ ; where  $S_t$  is the weight of vase of solution (g) at  $t =$  days 1, 2, 3, etc., and  $S_{t-1}$  is the weight of vase solution (g) on the previous day (He et al. 2006). The fresh weights of cut stems were measured daily. The relative fresh weight (RFW) of cut stems was calculated using the following formula:  $RFW (\%) = (FW_t / FW_{t=0}) \times 100$ ; where  $FW_t$  is the fresh weight of stem (g) at  $t =$  days 0, 1, 2, etc., and  $FW_{t=0}$  is the fresh weight of stem (g) at  $t =$  day 0 (He et al. 2006).

### Determination of anthocyanins

Anthocyanins content in petals was measured based on the methods of Murr et al. (2008). Anthocyanins were extracted with methanol containing 1% HCl for 24 h at 4 °C. The absorbance of the extract was measured at 520 and 700 nm with a spectrophotometer model T80+ UV/VIS Spectrometer PG Instrument Ltd.

### Peroxidase activity

The POD (EC 1.11.1.7) activity in petals was measured based on the method of Hart et al. (1971). The enzyme was extracted with 50 mM potassium phosphate buffer (pH = 7) containing 0.5 mM EDTA and

2% polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 15 000 rpm for 15 min at 4 °C and the supernatant used for the assay. The reaction mixture contained crude enzyme, 225 mM H<sub>2</sub>O<sub>2</sub> and 45 mM guaiacol. POD activity was measured as the oxidation of guaiacol in the presence of H<sub>2</sub>O<sub>2</sub>. The absorbance (read at 470 nm) was measured and the POD activity of the extract was expressed as POD U·g<sup>-1</sup> FW.

#### Superoxide dismutase (SOD) activity

The SOD (EC 1.15.1.1) activity in petals was measured based on the method of Giannopoliti and Ries (1977); 0.5 g of petals was ground in liquid nitrogen. The enzyme was extracted with 50 mM phosphate buffer (pH = 7.0) containing 0.1 mM EDTA. The homogenate was centrifuged at 15 000 rpm for 15 min at 4 °C and the supernatant used for the assay. The reaction mixture contained crude enzyme, 0.12 mM riboflavin, 13 mM L-methionine, 75 μM nitroblue tetrazolium (NBT). The SOD activity in extract, visualised as reduction of nitroblue tetrazolium, was determined spectrophotometrically at 560 nm and expressed as SOD U·g<sup>-1</sup> FW.

#### Assay of malondialdehyde (MDA) content

The MDA content in petals was measured based on the methods of Heath and Packer (1968). Equal volume of 0.5% thiobarbituric acid (TBA) in 20% trichloroacetic acid solution was added to petal extract and the sample was incubated at 95 °C for 30 min. The reaction was stopped by putting the reaction tubes in an ice bucket. The samples were then centrifuged at 10 000 rpm for 30 min. The supernatant was removed and read at 532 nm. The value for nonspecific absorbance at 600 nm was read and subtracted from this at 532 nm. The MDA content of the extract was expressed as nmol·g<sup>-1</sup> FW.

#### Determination of protein content

The protein content in petals was determined based on the methods of the Bradford (1976) by measuring the optical density at 595 nm using bovine serum albumin as a standard. The protein content of the extract was expressed as protein mg·g<sup>-1</sup> FW.

#### Statistical analysis

This experiment was conducted in a split-plot design with four replications. Three stems were used for each replication. Duncan's test has been applied

to evaluate significance of differences between means using SAS 9.1 software.

## RESULTS

### Effect of POD inhibitors on vase life, water uptake and relative fresh weight

All concentrations of CH and 5 and 15 mM of PD in combination with 8-HQC and sucrose significantly ( $p < 0.01$ ) prolonged the vase life of cut lisianthus flowers in comparison to the control (Fig. 1).

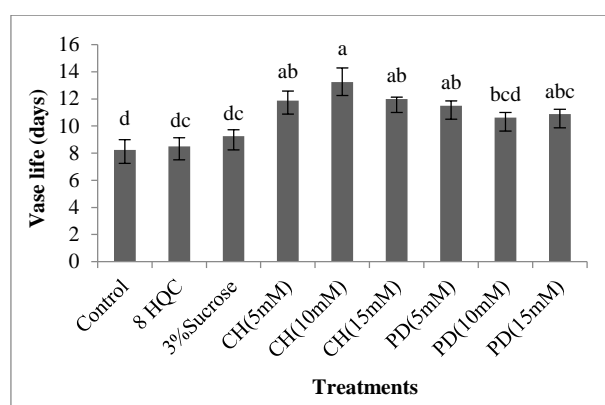


Fig. 1. Effect of pulse treatments on vase life of *E. grandiflorum* cut flowers. Means in each column with the same letter are not significantly different at 1% level of probability based on Duncan test.

The use of 10 mM CH resulted in the greatest extension of vase life (13.3 days), whereas vase life of cut flowers held in distilled water was 8.3 days (Fig. 1). In addition, water uptake and relative fresh weight significantly increased due to CH presence in vase solution ( $p < 0.01$ ) (Figs. 2A & 3A). The addition of PD to vase solution had lesser influence on vase life, water uptake and relative fresh weight than CH treatment (Figs. 1, 2B & 3B). The 200 mg·dm<sup>-3</sup> 8-HQC and 3% sucrose added to vase solution separately had no influence on vase life (Fig. 1). Sucrose at 3% present in vase solution increased water uptake only in the first 5 days (Fig. 2). Both 8-HQC and sucrose added to vase solution separately increased fresh weight of shoots in comparison to distilled water as the control beginning from the 4<sup>th</sup> day (Fig. 3).

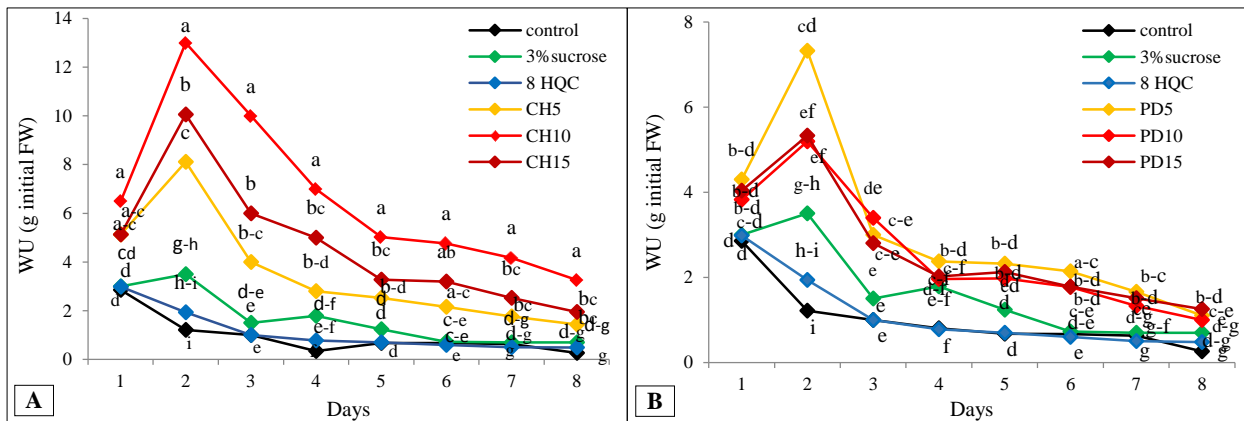


Fig. 2. Effect of CH concentrations, 3% sucrose, 8-HQC (A) and PD concentrations, 3% sucrose, 8-HQC (B) on water uptake (WU) of *E. grandiflorum* cut flowers. Means with the same letter are not significantly different within treatments at 1% level of probability based on Duncan test.

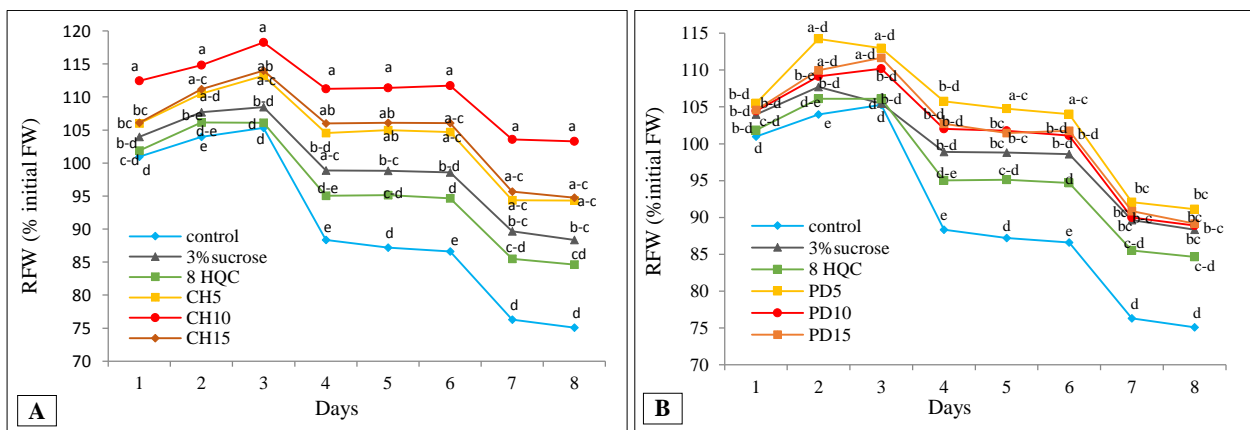


Fig. 3. Effect of CH concentrations, 3% sucrose, 8-HQC (A) and PD concentrations, 3% sucrose, 8-HQC (B) on relative fresh weight of *E. grandiflorum* cut flowers. Means with the same letter are not significantly different within treatments at 1% level of probability based on Duncan test.

### POD and SOD activity

CH at 10 and 15 mM, and PD at 5, 10 and 15 mM diminished significantly POD activity (Table 1). Minimum POD activity was recorded with 10 mM CH, 10 and 15 mM PD. CH at concentrations 5 and 10 mM significantly increased SOD activity in comparison to the control (Table 1). No significant effects of  $200 \text{ mg} \cdot \text{dm}^{-3}$  8-HQC or 3% sucrose, included in vase solution separately, on SOD and POD activity were observed (Table 1).

### MDA accumulation, anthocyanin and protein content

The flowers treated with 5 mM of CH or PD and with 15 mM of PD in combination with 8-HQC and

sucrose accumulated significantly ( $p < 0.01$ ) less MDA in comparison to the control (Table 1). The CH in the vase solution decreased MDA content to lesser extent than PD did. Anthocyanin content in the petals of lisianthus increased significantly ( $p < 0.01$ ) in all the treatments with CH and PD (Table 1). The application of PD to vase solution in all concentrations, in combination with 8-HQC and sucrose, prevented protein degradation in comparison to the control (Table 1), whereas CH did not influence this parameter. No significant effects of  $200 \text{ mg} \cdot \text{dm}^{-3}$  8-HQC and 3% sucrose added separately to vase solution were observed on MDA accumulation, anthocyanin and protein content (Table 1).

Table 1. Effect of pulse treatments of 8-HQC, sucrose, CH and PD on POD and SOD activity, and content of anthocyanin, protein and MDA in *E. grandiflorum* cut flowers

**Treatments	POD (U·g <sup>-1</sup> FW)	SOD (U·g <sup>-1</sup> FW)	MDA (nmol·g <sup>-1</sup> FW)	Anthocyanin (mg·g <sup>-1</sup> FW)	Protein (mg·g <sup>-1</sup> FW)
Control	0.023 <sup>a</sup>	197.92 <sup>bc</sup>	36.22 <sup>a,*</sup>	76.71 <sup>d</sup>	143.4 <sup>f</sup>
8-HQC (200 mg·dm <sup>-3</sup> )	0.020 <sup>ab</sup>	202.4 <sup>bc</sup>	34.78 <sup>ab</sup>	94.44 <sup>cd</sup>	148.83 <sup>ef</sup>
Sucrose (3%)	0.019 <sup>ab</sup>	205.63 <sup>bc</sup>	33.39 <sup>abc</sup>	80 <sup>d</sup>	150 <sup>ef</sup>
CH (5 mM)	0.015 <sup>bcd</sup>	271.62 <sup>a</sup>	29.64 <sup>cde</sup>	139.43 <sup>bc</sup>	159.75 <sup>cdef</sup>
CH (10 mM)	0.007 <sup>e</sup>	274.88 <sup>a</sup>	30.04 <sup>bcd</sup>	159.37 <sup>bc</sup>	156.12 <sup>cdef</sup>
CH (15 mM)	0.0119 <sup>cde</sup>	253.3 <sup>ab</sup>	32.19 <sup>abc</sup>	175.09 <sup>bc</sup>	158.58 <sup>cdef</sup>
PD (5 mM)	0.013 <sup>cde</sup>	253.29 <sup>ab</sup>	24.22 <sup>f</sup>	140.15 <sup>bc</sup>	185.6 <sup>a</sup>
PD (10 mM)	0.0082 <sup>de</sup>	243.92 <sup>ab</sup>	34.03 <sup>abc</sup>	195.58 <sup>a</sup>	180.85 <sup>ab</sup>
PD (15 mM)	0.0099 <sup>de</sup>	252.47 <sup>ab</sup>	24.86 <sup>f</sup>	125.91 <sup>bc</sup>	174.54 <sup>abc</sup>

\*\*All of CH and PD concentrations were combined with 8-HQC (200 mg·dm<sup>-3</sup>) and sucrose (3%).

\*Means in each column with the same letter are not significantly different at 1% level of probability based on Duncan test.

## DISCUSSION

Prolonging vase life of cut flowers has been an object of various studies (e.g. Da Silva 2003; Kader 2003). In the study presented, POD inhibitors in combination with 8-HQC and sucrose extended the vase life of lisianthus cut flowers. Several compounds, for instance CH and PD, are known to inhibit the activity of POD (Çelikel et al. 2011). These inhibitors delayed also senescence of lisianthus cut flowers, increased RFW, WU and vase life. These findings indicated that POD enzymes are involved in vessels occlusion. Similar effects of other inhibitors of oxidative enzymes have previously been reported for *Chrysanthemum* (van Doorn & Vaslier 2002); *Bouvardia* (Vaslier & van Doorn 2003); *A. holosericea* and *C. uncinatum* (Çelikel et al. 2011). All treatments that prolonged vase life also increased water uptake and relative fresh weight. This is possibly because other physicochemical processes related to water balance and senescence (Halevy & Mayak 1981) can mediate vase life. Also MDA accumulation in cut lisianthus flowers treated with POD inhibitors was lower than in the control. Presumably, the decrease in MDA level was due to MDA oxidation and the reduction of the level of the unsaturated fatty acid precursors (Beuge & Aust 1978).

One of the symptoms of senescence is degradation of protein and anthocyanins. Lisianthus flowers treated with POD inhibitors maintained protein and anthocyanin contents. Petals from PD-treated

flowers contained more anthocyanins than the petals from flowers treated with CH, possibly due to the same precursor of phenylenediamine and anthocyanin (Gould et al. 2009).

Colour fading and discoloration of petals is an important factor in determining the decorative value of cut flowers and, in many cases, it is the main reason for termination of vase life. The improvement of petal colour is at least partially due to the increase of anthocyanin content. Total POD activity was substantially lower and SOD activity was higher in flowers treated with peroxidase inhibitors. SOD and POD as antioxidants delay senescence and prolong vase life. SOD provides the first line of defence against the toxic effects of elevated levels of reactive oxygen species (Bowler et al. 1992). Petals of flowers treated with PD had more anthocyanins than the petals of CH-treated flowers, maybe due to the presence of the same precursor of phenylenediamine and anthocyanin (Gould et al. 2009).

These findings suggest a role of POD in physiological plugging of lisianthus stems. POD inhibitors probably delayed lignin production in xylem, which increased water uptake and relative fresh weight, finally prolonging vase life of lisianthus cut flowers. Occlusion in chrysanthemum was delayed by inhibitors of POD, catechol oxidase and phenol oxidase. The treatment with compounds that inhibit POD but stimulate phenoloxidase had the same effect (van Doorn & Vaslier 2002). Plants react to wounding by activating defence systems that can, at

least partially, repair damaged tissues and/or prevent pathogen infection. It is well proven that production of reactive oxygen species is one of the responses to wounding (Orozco-Cárdenas et al. 2001). Peroxidase inhibitors delayed leaf wilting in chrysanthemum, which indicates that they can limit the development of occlusion (van Doorn & Vaslier 2002). The results of other scientists suggested a role for POD inhibitors in preventing wound-induced xylem occlusion by delaying the production of phenolic compounds such as lignin and suberin (van Doorn & Vaslier 2002; Vaslier & van Doorn 2003; Çelikel et al. 2011). Wound-induced enzyme activities are generally considered to contribute to phenol and suberin deposition (Heredia & Cisneros-Zevallos 2009). Phenolic metabolism in response to wounding is likely associated with ethylene signaling (Heredia & Cisneros-Zevallos 2009). On the basis of the results of our experiment, it is possible to conclude that CH and PD, as POD inhibitors, in combination with 8-HQC and sucrose increase the vase life of lisianthus flowers by increasing water uptake and transport by xylem vessels. These treatments improved the physiological performance of the cut flowers by delaying anthocyanin and protein degradation and increasing SOD activity, which as an antioxidant, is very vital for longevity of vase life. SODs are methalo enzymes that protect the cells by converting  $O_2^-$  to  $O_2$  and  $H_2O_2$ .  $H_2O_2$  is less dangerous than  $O_2^-$  (Arora et al. 2002). PODs exist in numerous isoenzymatic forms and are separated into anionic (acidic) and cationic (basic) types according to their isoelectric point. Anionic PODs are involved in the processes of lignification (Mäder & Füssl 1982; Imberty et al. 1985; Chen et al. 2002) and suberisation (Espelie & Kolattukudy 1985). Nevertheless, cationic PODs are also involved in the biosynthesis of lignin and suberin (Quiroga et al. 2000). Peroxidase inhibitors that we have used block activities of isoenzymatic forms that are involved in the biosynthesis of lignin and suberin.

Our experiment demonstrated that CH and PD in combination with 8-HQC and sucrose delay flower senescence of lisianthus. However, more investigation is necessary to determine more precisely the effects of POD inhibitors on quality parameters of cut *E. grandiflorum* flowers.

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