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Polyglutamic acid as a vase life improver for cut lilies

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Abstract: The vase life of cut lilies is short. Improving the vase life is very important to keep this kind of cut flower fresh by using exogenous improvers. The aim of this study was to elucidate the effectiveness of polyglutamic acid (PGA) on the vase life of cut lilies, in order to introduce a new vase life improver for cut lilies. The results demonstrated that PGA significantly strengthened the antioxidant capacity by enhancing the antioxidant activities (superoxide dismutase, peroxidase, catalase, ascorbate peroxidase, glutathione peroxidase and glutathione S-transferase) and the antioxidant contents (total phenolics, total flavonoids, total anthocyanin and vitamin C), which further decreased the electrolyte leakage and the malondialdehyde and hydrogen peroxide contents. Meanwhile, the PGA significantly maintained the water balance by decreasing the water saturation deficit and increasing the relative water content and the soluble sugar and proline contents, as well as the average fresh weight change rate and average water balance value of the cut flower. Besides, the PGA significantly decreased the wilted flower numbers and increased the open flower numbers, flower diameter and the vase life of lily cut flower. The above findings provided useful information for the potential application of PGA as a new vase life improver for cut lilies.

Keywords: postharvest quality; biopolymer; aging; antioxidase; oxidative damage

The lily is an important cut flower in the world. Whereas the short vase life of this kind of cut flower negatively affects its ornamental and commercial value. An increasing number of studies have demonstrated that the vase life of cut flowers have shown a close relationship to the antioxidant capacity and water balance (Zhang et al. 2022). An increasing amount of evidence has also demonstrated that exogenous chemicals have improved the vase life of cut flowers by improving the antioxidant capacity and water balance, exogenous chemicals rare earth elements (REEs) and selenium (Se) (Zheng, Guo 2019; Lu et al. 2020; Zhang et al. 2022). Polyglutamic acid (PGA) is a kind of anionic natural polymer produced by microbial fermentation, which is easily biodegradable, non-toxic and environmentally friendly. Several studies have shown that PGA had beneficial influences on the plant growth, nutrient balance and stress response, as well as on food preservation (Chunhachart et al. 2014; Guo et al. 2019; Tao et al. 2020, 2021). For cut lilies, it has been reported that REEs and Se could improve their vase life (Zhang et al. 2022; Lu et al. 2020). However, whether PGA can improve the vase life of cut lilies is still unknown.

Some exogenous chemicals have been proven to be useful as vase life improvers for cut flowers by enhancing their antioxidant capacity (Zheng, Guo 2019; Zhang et al. 2022). For cut lilies, Zhang et al. (2022) found that the REE praseodymium (Pr) improved the antioxidant activities of peroxidase (POD), catalase (CAT) and the enzymes in the ascorbate-glutathione cycle in petals, which further enhanced the antioxidant capacity. Lu et al. (2020) found that Se enhanced the antioxidant capacity

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of cut lilies by improving the POD, ascorbate peroxidase (APX), superoxide dismutase (SOD) and CAT activities. For PGA, Guo et al. (2017) and Xu et al. (2020) demonstrated that PGA increased the antioxidant capacity of rape seedlings and wheat seedlings under stresses by enhancing the SOD, CAT and APX activities. However, whether PGA can improve the antioxidant capacity of cut lilies is unclear. Soluble sugar (SS), proline (PRO) and soluble protein (SP) are important osmolytes in maintaining the plant water balance. Some exogenous chemicals have been proved to be used as water balance improvers for cut lilies by increasing the above osmolyte contents, including Se and REEs (Lu et al. 2020; Zhang et al. 2022). For PGA, Ma et al. (2022) showed that PGA improved the water balance capacity of maize seedlings under drought stress by increasing the SS and PRO contents in leaves. Mu et al. (2021) also reported that PGA increased the SP and PRO contents in *Sesbania cannabina* leaves. However, whether PGA can improve the water balance capacity of cut lilies is also unknown.

The ornamental value of cut flowers mainly depends on the opening degree indicated by the flower diameter and the opening time indicated by the vase life and by the number of open and wilted flowers (Żurawik et al. 2019; Sun et al. 2022). Previous studies have demonstrated that exogenous chemicals prolonged the vase life of cut lilies (Lu et al. 2020; Zhang et al. 2022). However, whether PGA can improve the ornamental value of cut lilies is also unclear.

Therefore, it will be important to clarify the roles of PGA in regulating the antioxidant activities, the antioxidant contents, the water balance, the flower diameter, the vase life and number of open and wilted flowers of cut lilies, which can provide knowledge for the application of PGA as a new vase life improver for cut lilies.

MATERIAL AND METHODS

In the present study, we investigated the effects of PGA on the vase life, the SOD, POD, CAT, APX, glutathione peroxidase (GPX) and glutathione S-transferase (GST) activities, electrolyte leakage (EL), the total phenolic (TP), total flavonoid (TF), total anthocyanin (TA) and Vc, SS, PRO, malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) contents, the relative water content (RWC), the water saturation deficit (WSD), the average fresh weight change rate (FWCR), the average water balance value (WBV), the flower diameter and the number of open and wilted flowers of cut lilies. The aim of this study was to elucidate the effectiveness of PGA on the vase life and the ornamental value of cut lilies. This study will introduce a new effective measure to improve the vase life and the ornamental value of lily cut flowers.

Plant material and treatment. Cut lilies *Lilium longiflorum* were used in the study. Flowers with three buds in similar size were selected and then their stems were all cut into lengths of 25 cm. The above cut flowers were then divided into five groups and respectively treated by distilled water as the control, and PGA with concentrations of 0.05%, 0.10%, 0.30% and 0.50%. All the treated flowers were placed in an artificial climate chamber under conditions: 26 °C in the day and 15 °C at night, 60% relative humidity, 300 μmol/m/s photosynthetic active radiation and a 12 hours photoperiod. Each treatment was repeated four times with two cut flowers in three buds per time. All the treatment solutions were replaced daily. After 3 days of treatment, the petals of the first opened flowers were sampled to measure the corresponding indicators. After 7 days of treatment, the open and wilted flower numbers were recorded. After all the cut flowers wilted, the vase life was recorded.

Antioxidant enzyme activities. The POD (Nickel, Cunningham 1969), CAT (Zhang et al. 2022), SOD (Beyer, Fridovich 1987) and APX (Nakano, Asada 1981) activities were determined by previous methods. The GPX and GST activities were analysed by using the method of Shan, Zhao (2015) and Bibi et al. (2014), respectively.

TP, TF, TA and Vc contents. TP content was analysed according to Swain and Hillis (1959) and is presented as mg of gallic acid equivalents per g of fresh petals. The TF content was analysed according to Parhat et al. (2013) and is presented as mg of rutin equivalents per g of fresh petals. The TA content was analysed according to Zhang et al. (2004) and is presented as mg of pelargonidin equivalents per g of fresh petals. The Vc content was measured by using the method of Shan et al. (2018).

MDA and H_2O_2 contents and EL. The MDA content was determined according to the method described by Hodges et al. (1999). The EL and H_2O_2

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content were measured according to Brennan and Frenkel (1977).

Contents of the osmolytes SS and PRO. The SS content was measured according to Smith et al. (1983) by using the anthrone-sulfuric acid method. The PRO content was determined according to Bates et al. (1973) by using the acidic-ninhydrin method.

RWC and WSD. The RWC and WSD were measured according to Barrs and Weatherley (1962). The RWC value was calculated by using the following equation: RWC = [(FW – DW)/(SW – DW)] × 100. The WSD value was calculated by using following equation: WSD = [(SW – FW)/(SW – DW)] × 100. In the above equations, FW, DW and SW indicate the fresh weight, dry weight and saturated weight, respectively.

Vase life. The vase life of the cut flowers was determined by recording the days from the cut flowers were placed in the flasks until all the flowers had wilted.

Flower diameter. The flower diameter was measured by digital callipers every day, with an accuracy of millimetres.

Average FWCR and average WBV. The fresh weight of the cut flowers was measured every 2 days. The value of the fresh weight change rate (FWCR) was calculated by using the following formula. FWCR = [(FW – initial FW)/initial FW] × 100%. From the start (day zero) to day 10 of treatment, the fresh weight change rate was measured every 2 days. The average FWCR was the mean value of the FWCR, which was measured 5 times from day zero to day 10 of treatment.

From day zero to day 10 of treatment, the water loss and absorption were measured every 2 days. The water loss was expressed as the difference in the total weight of the cut flower, treatment solution and flask on two adjacent days. The water absorption was expressed as the difference in the total weight of the treatment solution and flask on two adjacent days. The water balance value (WBV) was expressed as the difference between the water absorption and water loss. The average WBV was the mean value of the WBV, which was measured 5 times from day zero to day 10 of treatment.

Statistical analysis. The data are presented as the mean of four replications. The software SPSS statistics (Version 25.0, Chicago, USA) was used to perform the statistical analyses. The assumptions of normality on the data sample were tested by the Shapiro-Wilk test. The homogeneity of variances for all the samples was tested by the Bartlett test. The means were compared by a one-way analysis of variance (ANOVA) and Tukey's test at a 5% level of significance.

RESULTS

Effects of PGA on the antioxidant enzyme activities. Compared with the control, the PGA markedly improved the SOD, POD, CAT, APX, GPX and GST activities in the petals of cut lilies (Table 1). Among the different concentrations, the 0.30% PGA showed better positive effects on the activities of the above enzymes. In comparison with the control, 0.30% PGA improved the SOD, POD, CAT, APX, GPX and GST activities by 124.2%, 190.0%, 150.0%, 164.0%, 137.4% and 250.0%, respectively. These results indicated that PGA enhanced the antioxidant capacity of cut lilies by improving the activities of the above antioxidant enzymes.

Effects of PGA on the antioxidant contents. In comparison with the control, the PGA markedly increased the TP, TF, TA and Vc contents (Ta-

Table 1. Effects of the polyglutamic acid (PGA) on the peroxidase (POD), catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione peroxidase (GPX) and glutathione S-transferase (GST) activities

Treatment	SOD (U/g FW)	CAT (U/g FW)	POD (U/g FW)	APX (U/g FW)	GPX (U/g FW)	GST (U/g FW)
Control	3.3 ± 0.12^{d}	2.0 ± 0.09^{d}	3.0 ± 0.13^{d}	2.5 ± 0.10^{d}	9.1 ± 0.33^{d}	1.0 ± 0.07^{d}
0.05% PGA	4.2 ± 0.22^{c}	2.8 ± 0.10^{c}	4.3 ± 0.18^{c}	3.0 ± 0.14^{c}	11.0 ± 0.29^{c}	1.5 ± 0.10^{c}
0.10% PGA	5.7 ± 0.18^{b}	3.9 ± 0.11^{b}	6.4 ± 0.31^{b}	4.5 ± 0.19^{b}	15.2 ± 0.55^{b}	2.3 ± 0.12^{b}
0.30% PGA	7.4 ± 0.39^{a}	5.0 ± 0.23^{a}	8.7 ± 0.37^{a}	6.6 ± 0.26^{a}	21.6 ± 0.97^{a}	3.5 ± 0.20^{a}
0.50% PGA	6.0 ± 0.24^{b}	3.5 ± 0.16^{b}	6.0 ± 0.25^{b}	4.0 ± 0.13^{b}	16.5 ± 0.64^{b}	2.0 ± 0.11^{b}

The data are the mean ± standard deviations (SD) (n = 4); different lowercase letters indicate a statistical difference among the treatments at $P < 0.05$

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Table 2. Effects of the polyglutamic acid (PGA) on the total phenolic (TP), total flavonoid (TF), total anthocyanin (TA) and vitamin C (Vc) contents

Treatment	TP (mg/g FW)	TF (mg/g FW)	TA (μg/g FW)	Vc (μmol/g FW)
Control	19.3 ± 1.24^{d}	29.6 ± 1.40^{d}	80.7 ± 3.39^{d}	1.0 ± 0.08^{d}
0.05% PGA	25.6 ± 1.17^{c}	36.0 ± 2.22^{c}	100.0 ± 6.71^{c}	1.5 ± 0.12^{c}
0.10% PGA	32.3 ± 1.73^{b}	41.9 ± 1.36^{b}	146.5 ± 9.96^{b}	2.2 ± 0.11^{b}
0.30% PGA	48.0 ± 2.30^{a}	56.0 ± 2.19^{a}	198.0 ± 12.45^{a}	3.6 ± 0.23^{a}
0.50% PGA	34.0 ± 1.52^{b}	43.0 ± 2.48^{b}	136.4 ± 9.22^{b}	2.0 ± 0.10^{b}

The data are the mean ± standard deviations (SD) (n = 4); different lowercase letters indicate a statistical difference among the treatments at $P < 0.05$

ble 2). Among the different concentrations, 0.30% PGA showed better positive effects on the contents of the above antioxidants. Compared with the control, the 0.30% PGA increased the TP, TF, TA and Vc contents by 148.7%, 89.2%, 145.4% and 260.0%, respectively. These results indicated that PGA also enhanced the antioxidant capacity of cut lilies by improving the contents of the above antioxidants.

Effects of PGA on the MDA, EL and H_2O_2. In comparison with the control, the PGA markedly reduced the EL and the MDA and H_2O_2 contents (Table 3). Among the different concentrations, the 0.30% PGA also showed better positive effects in alleviating the lipid peroxidation. Compared with the control, 0.30% PGA decreased the EL and the MDA and H_2O_2 contents by 34.7%, 51.6% and 45.5%, respectively. These results once again indicated that PGA enhanced the antioxidant capacity of cut lilies.

Effects of PGA on the osmolyte content, RWC and WSD. Compared with the control, the PGA markedly improved the osmolytes level and the RWC, and decreased the WSD of the petals (Table 4). Compared with the control, the 0.05%, 0.10%, 0.30% and 0.50% PGA respectively improved the SS content by 31.04%, 71.58%, 116.71% and 56.58%, and respectively improved the PRO content by 35.68%, 78.41%, 155.50% and 80.61%. Meanwhile, the 0.05%, 0.10%, 0.30% and 0.50% PGA respectively increased the RWC by 4.68%, 8.38%, 15.41% and 7.15%. However, the 0.05%, 0.10%, 0.30% and 0.50% PGA respectively decreased the WSD by 20.10%, 35.97%, 66.13% and 30.68%. Among the different concentrations, the 0.30% PGA showed better positive effects on the levels of the above osmolytes, which further increased the RWC and decreased the WSD. These results indicated that the PGA enhanced the water balance capacity of cut lilies through the osmotic adjustment.

Effects of PGA on the average FWCR and average WBV. Compared with the control, the PGA markedly improved the average FWCR and average WBV of lily cut flower (Table 5). Compared with the control, the 0.05%, 0.10%, 0.30% and 0.50% PGA respectively improved the average FWCR by 22.22%, 58.88%, 92.77% and 55.55%, and respectively improved the average WBV by 21.11%, 48.88%, 82.77% and 46.11%. Among the different concentrations, the 0.30% PGA showed better positive effects on the average FWCR and average WBV of lily cut flower. These results further implied that PGA enhanced the water balance capacity of cut lilies.

Table 3. Effects of the polyglutamic acid (PGA) on the electrolyte leakage (EL) and the malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) contents

Treatment	MDA (nmol/g FW)	EL (%)	H_2O_2 (μmol/g FW)
Control	9.3 ± 0.34^{a}	19.0 ± 1.02^{a}	7.7 ± 0.34^{a}
0.05% PGA	8.0 ± 0.32^{b}	17.0 ± 0.88^{b}	6.5 ± 0.47^{b}
0.10% PGA	6.0 ± 0.26^{c}	14.6 ± 0.65^{c}	5.6 ± 0.32^{c}
0.30% PGA	4.5 ± 0.25^{d}	12.4 ± 0.74^{d}	4.2 ± 0.39^{d}
0.50% PGA	5.7 ± 0.37^{c}	14.1 ± 0.80^{c}	5.2 ± 0.25^{c}

The data are the mean ± standard deviations (SD) (n = 4); different lowercase letters indicate a statistical difference among the treatments at $P < 0.05$

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Table 4. Effects of the polyglutamic acid (PGA) on the relative water content (RWC), water saturation deficit (WSD) and of osmolyte soluble sugar (SS) and proline (PRO) contents

Treatment	SS (μg/g FW)	PRO (μg/g FW)	RWC (%)	WSD (%)
Control	630.0 ± 13.45^{d}	22.7 ± 1.52^{d}	81.1 ± 0.75^{d}	18.9 ± 0.75^{a}
0.05% PGA	825.6 ± 22.60^{c}	30.8 ± 1.28^{c}	84.9 ± 1.07^{c}	15.1 ± 1.07^{b}
0.10% PGA	1 081.0 ± 30.79^{b}	40.5 ± 2.54^{b}	87.9 ± 0.90^{b}	12.1 ± 0.90^{c}
0.30% PGA	1 365.3 ± 38.21^{a}	58.0 ± 2.97^{a}	93.6 ± 0.88^{a}	6.4 ± 0.88^{d}
0.50% PGA	986.5 ± 20.48^{b}	41.0 ± 3.33^{b}	86.9 ± 0.71^{b}	13.1 ± 0.71^{c}

The data are the mean ± standard deviations (SD) (n = 4); different lowercase letters indicate a statistical difference among the treatments at $P < 0.05$

Effects of PGA on the vase life and the ornamental value. Compared with the control, the PGA markedly reduced the wilted flower numbers and increased the open flower numbers, the maximum flower diameter, the days to reach the maximum flower diameter and the vase life (Table 6). Compared with the control, the 0.05%, 0.10%, 0.30% and 0.50% PGA respectively increased the number of open flowers by 15.38%, 43.58%, 74.35% and 33.33%, and respectively decreased the number of wilted flowers by 14.63%, 41.46%, 70.73% and 31.70%. Meanwhile, the 0.05%, 0.10%, 0.30% and 0.50% PGA respectively increased the maximum flower diameter by 6.62%, 12.02%, 23.44% and 11.00%, and respectively delayed the days to reach the maximum flower diameter by 14.28%, 32.85%, 47.14% and 28.57%. Besides, the 0.05%, 0.10%, 0.30% and 0.50% PGA prolonged the vase life by 13.33%, 27.77%, 44.44% and 22.22%, respectively. Among the different concentrations, the 0.30% PGA showed better positive effects on the vase life and the ornamental value. These results further indicated that PGA improved the vase life and the ornamental value of cut lilies.

Table 5. Effects of the polyglutamic acid (PGA) on the average fresh weight change rate (FWCR) and average water balance value (WBV)

Treatment	Average FWCR (%)	Average WBV (g)
Control	−18.0 ± 1.06^{d}	−17.3 ± 1.35^{d}
0.05% PGA	−14.0 ± 1.20^{c}	−13.5 ± 1.10^{c}
0.10% PGA	−7.4 ± 0.58^{b}	−8.5 ± 0.66^{b}
0.30% PGA	−1.3 ± 0.14^{a}	−2.4 ± 0.19^{a}
0.50% PGA	−8.0 ± 0.77^{b}	−9.0 ± 0.83^{b}

The data are the mean ± standard deviations (SD) (n=4); different lowercase letters indicate a statistical difference among the treatments at $P < 0.05$

DISCUSSION

The vase life of cut flowers has a close relationship with the lipid peroxidation of petals. Zhang et al. (2022) and Lu et al. (2020) found that Pr and Se, respectively, decreased the lipid peroxidation, which further extended the vase life of cut lilies. Previous studies found that PGA decreased the lipid peroxidation indicated by the MDA of *Sesbania cannabina* and rape seedlings (Lei et al. 2015; Mu et al. 2021). For this study, the findings indicated that PGA also decreased the lipid peroxidation of cut lilies by reducing the EL and the MDA and H_2O_2 contents, which was in agreement with previous results about the effects of Pr and Se on cut lilies (Lu et al. 2020; Zhang et al. 2022).

For lily cut flowers, a great deal of evidence has indicated that Se and the REEs alleviated the lipid peroxidation by improving the POD, CAT and APX activities (Zheng, Guo 2019; Lu et al. 2020). For PGA, Guo et al. (2017) and Xu et al. (2020) demonstrated that PGA increased the antioxidant capacity of rape seedlings and wheat seedlings under stresses by increasing the SOD, CAT and APX activities. For this study, our results indicated that PGA also increased the SOD, POD, CAT and APX activities in cut lilies' petals, which was in agreement with the effects of Se and REEs on cut flowers and the effects of PGA on rape seedlings (Guo et al. 2017; Zheng, Guo 2019; Lu et al. 2020; Xu et al. 2020). Besides, we found that PGA increased the GPX and GST activities in the petals of cut lilies. Previous studies have shown that exogenous chemicals, such as REEs and Se, could alleviate the lipid peroxidation of cut lilies by increasing the Vc content (Lu et al. 2020; Zhang et al. 2022). The current study also showed that PGA increased the Vc content in petals of cut lilies. Moreover, the current findings indicated that PGA

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Table 6. Effects of the PGA on the number of open and wilted flowers, maximum flower diameter, days to reach the maximum flower diameter and the vase life

Treatment	Numbers of open flowers	Numbers of wilted flowers	Maximum flower diameter (mm)	Days to reach the maximum flower diameter (days)	Vase life (days)
Control	2.9 ± 0.20[d]	3.1 ± 0.20[a]	130.00 ± 5.33[c]	7.0 ± 0.44[d]	9.0 ± 0.32[d]
0.05% PGA	3.9 ± 0.56[c]	2.1 ± 0.56[b]	141.26 ± 4.42[b]	8.0 ± 0.35[c]	10.2 ± 0.22[c]
0.10% PGA	4.7 ± 0.19[b]	1.3 ± 0.19[c]	150.44 ± 6.60[b]	9.3 ± 0.32[b]	11.5 ± 0.40[b]
0.30% PGA	5.5 ± 0.10[a]	0.5 ± 0.10[d]	169.85 ± 7.57[a]	10.3 ± 0.50[a]	13.0 ± 0.65[a]
0.50% PGA	4.4 ± 0.25[b]	1.6 ± 0.25[c]	148.71 ± 7.69[b]	9.0 ± 0.48[b]	11.0 ± 0.33[b]

The data are the mean ± standard deviations (SD) (n = 4); different lowercase letters indicate a statistical difference among the treatments at $P < 0.05$

increased the TP, TF and TA contents in the petals, which further prolonged the vase life. Therefore, PGA alleviated the lipid peroxidation of lily cut flowers by improving the activity of the antioxidant enzyme system, including antioxidant enzymes and non-enzymatic antioxidants.

For cut flowers, it has been documented that osmolytes have shown to have significant roles in maintaining the water balance (Zheng, Guo 2019; Zhang et al. 2022). An increasing amount of evidence has indicated that Se and REEs have increased the contents of osmolytes, which have further improved the RWC and prolonged the vase life of lily cut flowers (Lu et al. 2020; Zhang et al. 2022). Mu et al. (2021) reported that PGA increased the SP and PRO contents in *Sesbania cannabina* leaves. Lei et al. (2015) also showed that PGA increased the PRO content of rape seedlings. Whereas, whether PGA affects the contents of osmolytes in cut lilies' petals is still unknown. For the current study, we firstly demonstrated that PGA improved the SS and PRO contents in petals, which further improved the RWC. Besides, the current study indicated that PGA significantly decreased the WSD and increased the values of the average FWCR and average WBV, which further proved the positive effect of PGA on the water balance of cut lilies. The above findings clearly indicated that PGA showed that it has an important role in maintaining the water balance of cut lilies.

The flower diameter is an important indicator to evaluate the ornamental value of cut flowers. In our study, the findings indicated that PGA improved the flower opening degree of cut lilies by increasing the maximum flower diameter and delaying the days to reach the maximum flower diameter. Besides, PGA improved the open flower numbers and reduced the wilted flower numbers. Meanwhile, we found that PGA delayed the vase life. These findings indicated that PGA also improved the ornamental value of cut lilies.

CONCLUSION

Our study indicated that PGA extended the vase life and improved the ornamental value of cut lilies by enhancing the antioxidant and water balance capacities, especially for 0.30% PGA. Thus, our results provide useful information for the application of PGA in improving the vase life and the ornamental value of cut lilies.

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