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Article

The Extension of Vase Life in Cut Gerbera Flowers through Pretreatment with Gibberellin A_3 in Combination with Calcium Chloride

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Abstract: The effect of pretreatment with gibberellin A_3 (GA_3) and calcium chloride ($CaCl_2$) on the vase life of cut gerbera 'Minou' was investigated. Cut gerbera flowers were treated with GA_3 and/or $CaCl_2$ for 24 h and then transferred to an antimicrobial solution. Pretreatment with GA_3 at 0, 29, 72, 144, and 289 µM delayed the opening of the tubular florets and promoted stem bending due to elongation of the flower stems, which shortened vase life. When cut gerbera was pretreated with 144 µM GA_3 in combination with $CaCl_2$ at 90, 180, 270, and 360 mM, stem elongation was suppressed with the increasing concentration of $CaCl_2$. Next, the effect of pretreatment with 144 µM GA_3, 270 mM $CaCl_2$, and their combination on the vase life of the cut gerbera was investigated. Combined treatment with GA_3 and $CaCl_2$ significantly extended vase life more than GA_3 alone and $CaCl_2$ alone. The vase life of the cut gerbera 'Banana', 'Kimsey', 'Pinta', 'Tim', and 'Vivid' was significantly extended by pretreatment with 144 µM GA_3 and 270 mM $CaCl_2$. It was concluded that combined treatment with GA_3 and $CaCl_2$ was effective in extending the vase life of the cut gerbera.

Keywords: calcium chloride; cut gerbera; flower stem bending; gibberellin A_3 (GA_3); petal longevity; tubular floret opening

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1. Introduction

Gerbera, belonging to the Asteraceae family, is one of the ten most important popular cut flowers in the world due to its wide diversity in colors and shapes [1]. Wilting petals and bending flower stems are signs that cut gerbera flowers are losing their ornamental value [2]. Wernett et al. [3] reported that the vase life is relatively long when cut flowers lose their ornamental value due to petal wilting, but it is relatively short when they lose their ornamental value due to stem bending. Therefore, the occurrence of flower stem bending is a major issue to consider in the vase life of cut gerbera.

Calcium inhibits stomatal opening [4] and suppresses transpiration in chrysanthemum [5]. Milani et al. [6] reported that the vase life of gerbera was extended as the amount of calcium fertilizer increased. In cut gerbera flowers, spraying, dipping, or injecting with calcium chloride ($CaCl_2$) solution suppresses the occurrence of flower stem bending and extends vase life [7]. Pulse and continuous treatments with $CaCl_2$ extend the vase life of cut gerberas [8,9].

Phytohormone gibberellin (GA) has various functions, including cell expansion and the promotion of seed germination and flowering [10]. Treatment with GA_3 delays leaf yellowing in cut *Alstroemeria* [11], lily [12], and *Narcissus* [13]. Exogenous GA_3 promotes c [14,15] and the flower opening of statice [16]. Treatment with GA_3 extends the vase life of cut carnation [17] and rose [14]. Emongor [18] reported that continuous treatment with GA_3 was effective in extending the vase life of cut gerbera, but this evaluation of vase life did not follow standard methods. Therefore, the vase life of cut gerbera appears to be inadequately characterized.

Preservatives that can extend the vase life of flowers by short-term treatment are useful for growers. Many cut flowers, including carnations and *Delphinium,* are treated with preservatives before shipping to extend their vase life [19,20]. In the present study, we investigated the effect of pretreatment with GA_3 and/or $CaCl_2$ on stem elongation, the opening of tubular florets, and the vase life of cut gerbera flowers.

2. Materials and Methods

2.1. Plant Materials

The cultivation of the gerbera (*Gerbera jamesonii* Bolus ex Hook. F.) plants and the experiments were conducted at the Shizuoka Prefectural Research Institute of Agriculture and Forestry (E 137°50′, N 34°43′). Gerbera 'Banana', 'Kimsey', 'Minou', 'Pinta', 'Tim', and 'Vivid', which are major cultivars in Japan, were grown in a soilless culture system in a greenhouse under natural daylight conditions as described in Umeda and Tonooka [21]. The Petal colors of 'Banana', 'Kimsey', 'Minou', 'Pinta', 'Tim', and 'Vivid', are yellow, pink, orange, red, yellow, and orange, respectively. Ventilation or heating was set to begin at 25 and 15 °C, respectively. The harvesting stage and handling of the gerbera flowers were as described in Tonooka et al. [2].

2.2. Chemical Treatment and Evaluation of Vase Life

Pretreatment with chemicals and the evaluation of vase life were conducted in an environmentally controlled chamber, which was kept at 23 °C with 70% relative humidity in a 12 h light period (6:00–18:00) with PPFD set to 10 µmol m^{-2} s^{-1}. The flowers were cut to a length of 40 cm and individually placed in test tubes (diameter of 40 mm; length of 130 mm) containing 100 mL of GA_3 (GA_3; Tokyo Kasei, Tokyo, Japan) at 0, 29, 72, 87, 144, and 289 µM and/or $CaCl_2$ at 0, 90, 180, 270, and 360 mM and kept for 24 h. The uptake of GA_3 and $CaCl_2$ by the flowers was calculated based on their concentrations and solution uptake (weight of solution uptaken per g fresh weight of cut flowers). After treatment, the cut flowers were transferred to 0.25 mL L^{-1} isothiazolinone antimicrobial compound, Kathon CG (Rohm and Haas Japan, Tokyo, Japan). Vase life was determined from the end of pretreatment to the time when one of the following symptoms was observed: bending of the flower stem exceeded 90°, abscission of one ray petal, breakage just below the flower head, or wilting of the petals. Ten flowers were used per treatment.

2.3. Relative Area of Unopened Tubular Florets

The diameter of the unopened tubular florets was measured daily. The unopened tubular floret area was calculated by multiplying the square of the radius by π. The relative area of unopened tubular florets was calculated as the ratio of the unopened tubular floret area at the start of pretreatment.

2.4. Measurement of Fresh Weight, Water Uptake, Transpiration, and Elongation of the Flower Stems

The fresh weight of the cut flowers, the amount of water uptake, and the length of the cut stems were measured daily. The relative fresh weight (RFW) and flower stem elongation were calculated as described in Tonooka et al. [2].

2.5. Measurement of EC and pH in $CaCl_2$ Solution

Electrical conductivity (EC) and pH were measured once by a LAQUA cond meter (DS-71; Horiba, Kyoto, Japan) and pH meter (F-22; Horiba), respectively.

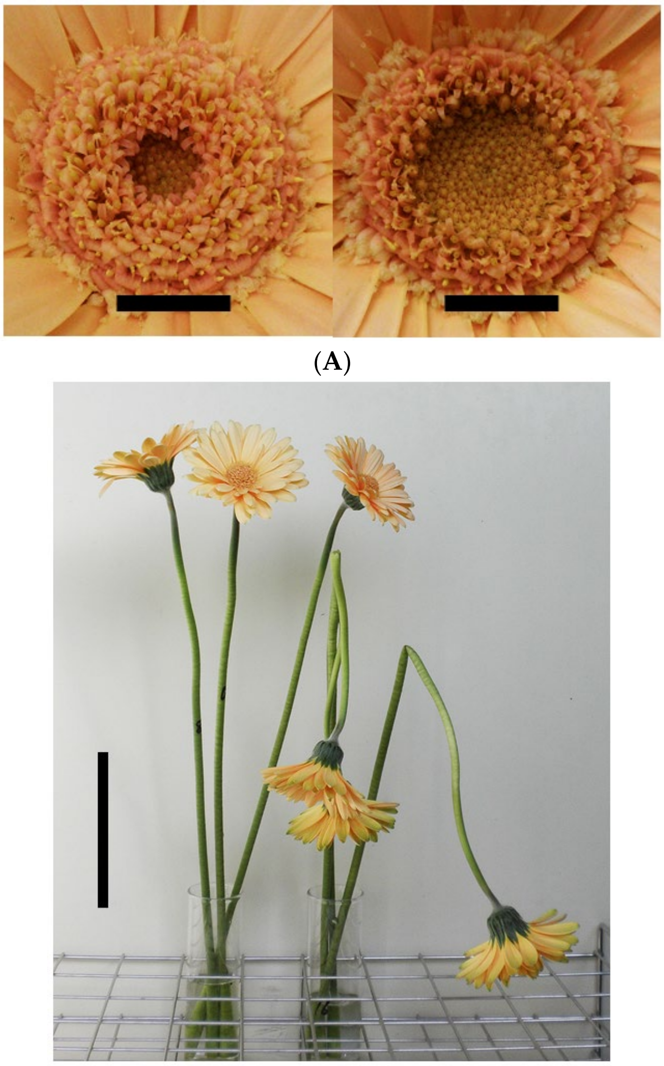
2.6. Statistical Analysis

Student's *t*-test, the Tukey–Kramer multiple range test, and two-way ANOVA were conducted using the BellCurve for Excel software (Social Survey Research Information, Tokyo, Japan). The results are expressed as the means of 10 replicates ± SE in the tables and figures. Unless otherwise stated, different letters within the column and at each time point indicate significant differences ($p < 0.05$) by the Tukey–Kramer multiple range test in the results shown in the tables and figures, respectively.

3. Results

3.1. Effect of GA_3 Concentrations on the Vase Life of Cut Gerbera 'Minou'

The opening of tubular florets was delayed by pretreatment with GA_3 (Figure 1A). The relative area of unopened tubular florets in the control (0 µM) decreased with time (Figure 2A). In contrast, the relative area of unopened tubular florets in the flowers treated with GA_3 at 72, 144, and 289 µM GA_3 increased during the first 2 or 4 days and decreased thereafter. Stem elongation was promoted by GA_3 at all concentrations and the stem length exceeded 10 cm in flowers treated with 72, 144, and 289 µM GA_3 (Figure 2B).



(**A**)

(**B**)

Figure 1. Flower of cut gerbera 'Minou' pretreated with or without 144 µM GA_3. (**A**). Tubular florets 5 days after treatment. Left: Distilled water (control), Right: GA_3. The scale bars represent 1 cm. (**B**). Cut flowers 10 days after treatment. Left tube: Distilled water (control), Right tube: GA_3. The scale bar represents 10 cm.

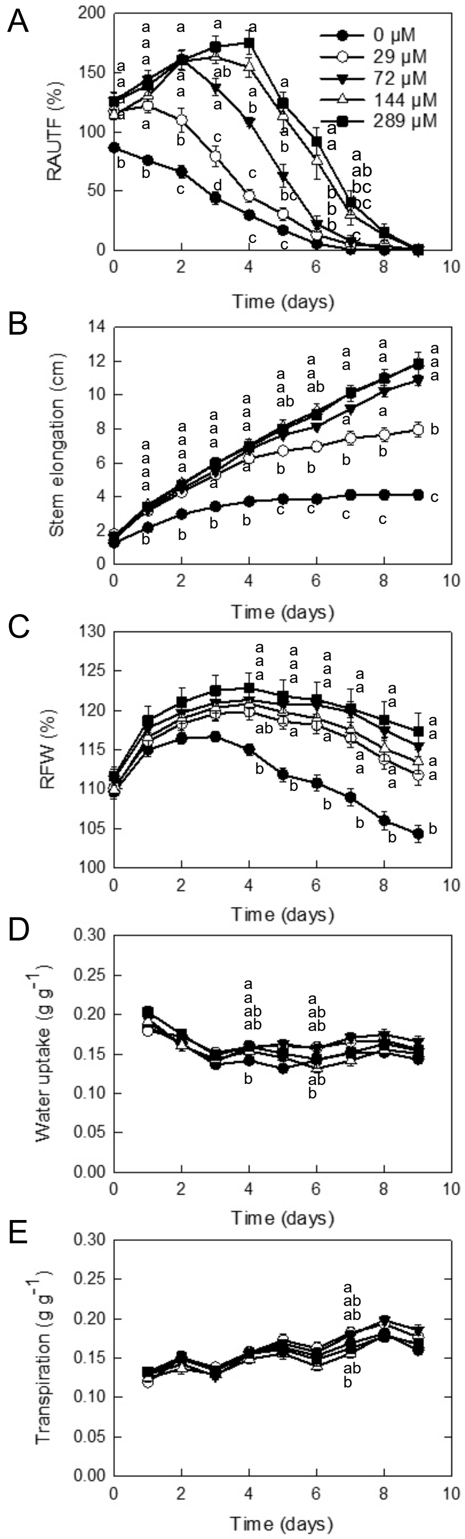


Figure 2. Effect of GA_3 concentration for pretreatment on the relative area of unopened tubular florets (RAUTF) (**A**), stem elongation (**B**), RFW (**C**), water uptake (**D**), and transpiration (**E**) of cut gerbera 'Minou'. Values are means of 10 replicates ± SE. Different letters at each time point indicate significant differences ($p < 0.05$) using Tukey–Kramer's multiple range test.

In all treatments, the RFW of the cut flowers increased during the first 3 or 4 days and decreased thereafter. Decreases in RFW were suppressed by GA_3 treatment (Figure 2C). Water uptake and transpiration were not significantly affected by GA_3 treatment (Figure 2D,E).

In the control flowers, the vase life was terminated without stem bending (Table 1). In contrast, the vase life of the cut flowers pretreated with GA_3 was mainly terminated due to stem bending, which was pronounced at 144 and 289 μM GA_3 (Figure 1B). There was no significant difference in vase life between the control and 29 μM GA_3, but the vase life of the cut flowers was shortened significantly by GA_3 at 72, 144, and 289 μM (Table 1). However, the petal longevity of the flowers without stem bending was significantly longer in 72 μM GA_3 than in the control (0 μM GA_3).

Table 1. Effect of GA_3 concentration for pretreatment on the water uptake and vase life of cut gerbera 'Minou'.

GA_3	Solution Uptake	GA_3 Uptake		Vase Life		Ornamental Value Loss Symptoms (%)		Vase Life of Flowers Showing Symptoms Other than Bending	
(μM)	(g g^{-1})	(μg g^{-1})		(Days)		Bending	Other	(Days)	
0	0.20	0	a	16.1	a	0	100	16.1	b
29	0.21	2.1	a	15.4	a	80	20	16.5	b
72	0.22	5.4	b	12.5	b	70	30	19.7	a
144	0.20	10.1	c	9.1	b	100	0		
289	0.22	22.5	d	9.4	b	100	0		

Different letters within columns indicate significant differences ($p < 0.05$) using Tukey–Kramer's multiple range test.

3.2. Effect of $CaCl_2$ Concentrations Combined with GA_3 on the Vase Life of Cut Gerbera 'Minou'

EC and pH increased as the $CaCl_2$ concentration increased. The GA_3 concentration combined with $CaCl_2$ was set at 144 μM, which has a marked effect of delaying floret opening. The uptake of the pretreatment solution tended to decrease with increasing $CaCl_2$ concentration (Table 2).

Table 2. Effect of $CaCl_2$ concentration and 144 μM GA_3 for pretreatment on the water uptake and vase life of cut gerbera 'Minou'.

$CaCl_2$	EC	pH	Solution Uptake		$CaCl_2$ Uptake		Vase Life		Ornamental Value Loss Symptoms (%)	
(mM)	(S m^{-1})		(g g^{-1})		(mg g^{-1})		(Days)		Bending	Other
0	0.00	4.55	0.29	a	0	e	7.7	b	100	0
90	1.75	4.68	0.20	b	2.0	d	10.9	b	100	0
180	3.21	5.19	0.16	c	3.1	c	17.6	a	50	50
270	4.63	6.38	0.13	c	4.0	b	19.3	a	10	90
360	5.80	7.28	0.13	c	5.1	a	19.2	a	10	90

Different letters within columns indicate significant differences ($p < 0.05$) using Tukey–Kramer's multiple range test.

The relative area of unopened tubular florets increased during the first 1 or 2 days and decreased thereafter in all treatments. The decrease in the relative area of the unopened tubular florets was enhanced with increasing $CaCl_2$ concentration (Figure 3A).

The elongation of the flower stem was not affected by 90 mM $CaCl_2$ but was significantly inhibited by 180, 270, and 360 mM $CaCl_2$ pretreatment (Figure 3B).

At the end of pretreatment, the RFW of the cut flowers increased with decreasing $CaCl_2$ concentration (Figure 3C). The RFW of the flowers in the control gradually decreased with time. A decrease in RFW was suppressed by $CaCl_2$ pretreatment and the suppression tended to increase with increasing $CaCl_2$ concentration. The water uptake and transpiration of the flower stems were suppressed by $CaCl_2$ irrespective of concentration (Figure 3D,E).

Stem bending was suppressed with increasing $CaCl_2$ concentration (Table 2). Vase life was significantly longer in 180, 270, and 360 mM $CaCl_2$ than in the control and 90 mM $CaCl_2$ (Table 2).

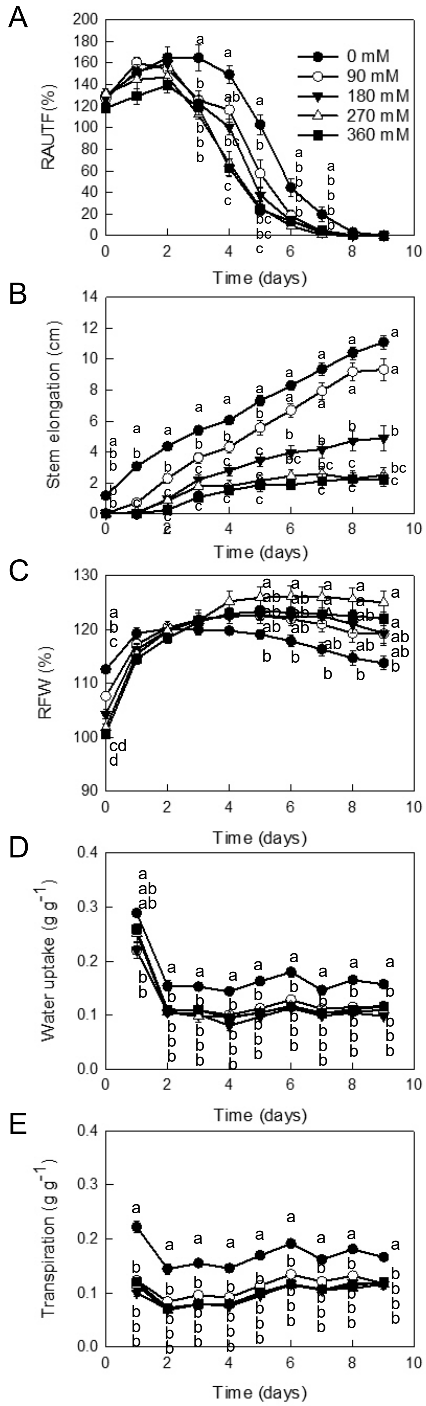


Figure 3. Effect of $CaCl_2$ concentration and 144 μM GA_3 for pretreatment on the relative area of unopened tubular florets (RAUTF) (**A**), stem elongation (**B**), RFW (**C**), water uptake (**D**), and transpiration (**E**) of cut gerbera 'Minou'. Values are means of 10 replicates ± SE. Different letters at each time point indicate significant differences ($p < 0.05$) using Tukey–Kramer's multiple range test.

3.3. Effect of GA_3, $CaCl_2$, and the Combination on the Vase Life of Gerbera 'Minou'

The uptake of pretreatment solution decreased in solution including $CaCl_2$ (Table 3). In the control and $CaCl_2$-treated flowers, the relative area of unopened tubular florets decreased gradually with time (Figure 4A). A decrease in the relative area of unopened tubular florets was suppressed in treatments containing GA_3. Elongation of flower stems was suppressed by $CaCl_2$ treatment but was promoted by GA_3 treatment (Figure 4B). The stem elongation induced by GA_3 was suppressed in combination with $CaCl_2$.

Table 3. Effects of pretreatment with GA_3 and $CaCl_2$ on the water uptake and vase life of cut gerbera 'Minou'. Different letters within columns indicate significant differences (p < 0.05) using Tukey–Kramer's multiple range test.

$CaCl_2$	GA_3	Solution Uptake		$CaCl_2$ Uptake		GA_3 Uptake		Vase Life		Ornamental Value Loss Symptoms (%)	
(mM)	(μM)	($g\ g^{-1}$)		($mg\ g^{-1}$)		($\mu g\ g^{-1}$)		(Days)		Bending	Other
0	0	0.29	a	0	b	0	c	15.8	b	0	100
270	0	0.14	b	4.2	a	0	c	14.8	b	0	100
0	144	0.32	a	0	b	16.0	a	9.0	c	90	10
270	144	0.15	b	4.5	a	7.5	b	18.6	a	30	70

Different letters within columns indicate significant differences (p < 0.05) using Tukey–Kramer's multiple range test.

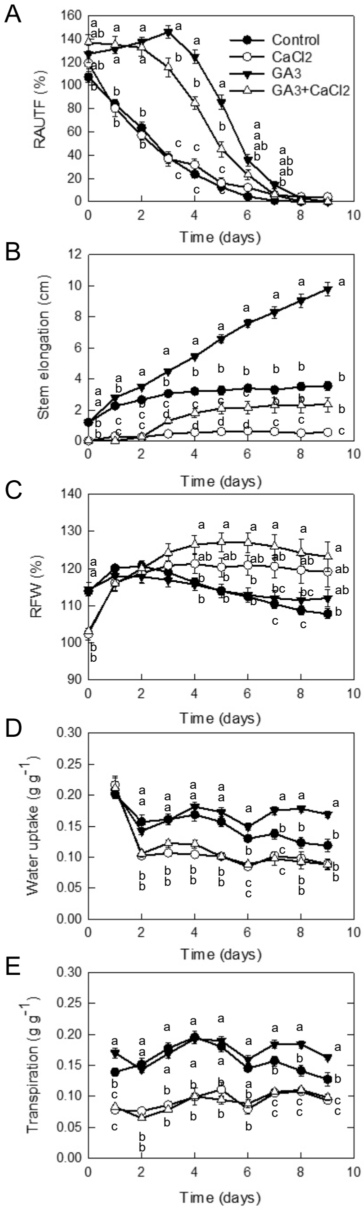


Figure 4. Effect of pretreatment with 144 μM GA_3 and 270 mM $CaCl_2$ on the relative area of the unopened tubular florets (RAUTF) (**A**), stem elongation (**B**), RFW (**C**), water uptake (**D**), and transpiration (**E**) of cut gerbera 'Minou'. Values are means of 10 replicates ± SE. Different letters at each time point indicate significant differences (p < 0.05) using Tukey–Kramer's multiple range test.

GA_3 treatment had a slight effect on the trend of RFW, but $CaCl_2$ treatment suppressed this decrease (Figure 4C). Treatment with $CaCl_2$ suppressed water uptake and transpiration (Figure 4D,E), while treatment with GA_3 increased water uptake and transpiration at a later stage of vase life.

GA_3 treatment increased the occurrence of stem bending and markedly shortened vase life (Table 3). $CaCl_2$ treatment did not significantly extend vase life, but combined treatment with GA_3 and $CaCl_2$ significantly extended vase life. Stem bending in the combined treatment with GA_3 and $CaCl_2$ was 30%. The vase life of flowers showing stem bending in GA_3 and $CaCl_2$ treatment was 15.3 days, which was shorter than that showing other symptoms.

3.4. *Effect of Combined Treatment with GA_3 and $CaCl_2$ on the Vase Life of the Five Gerbera Cultivars*

The relative area of unopened tubular florets decreased gradually with time. The relative area of unopened tubular florets at some time points was significantly greater in combined treatment with GA_3 and $CaCl_2$ than in the control in the five cultivars (Figure 5A).

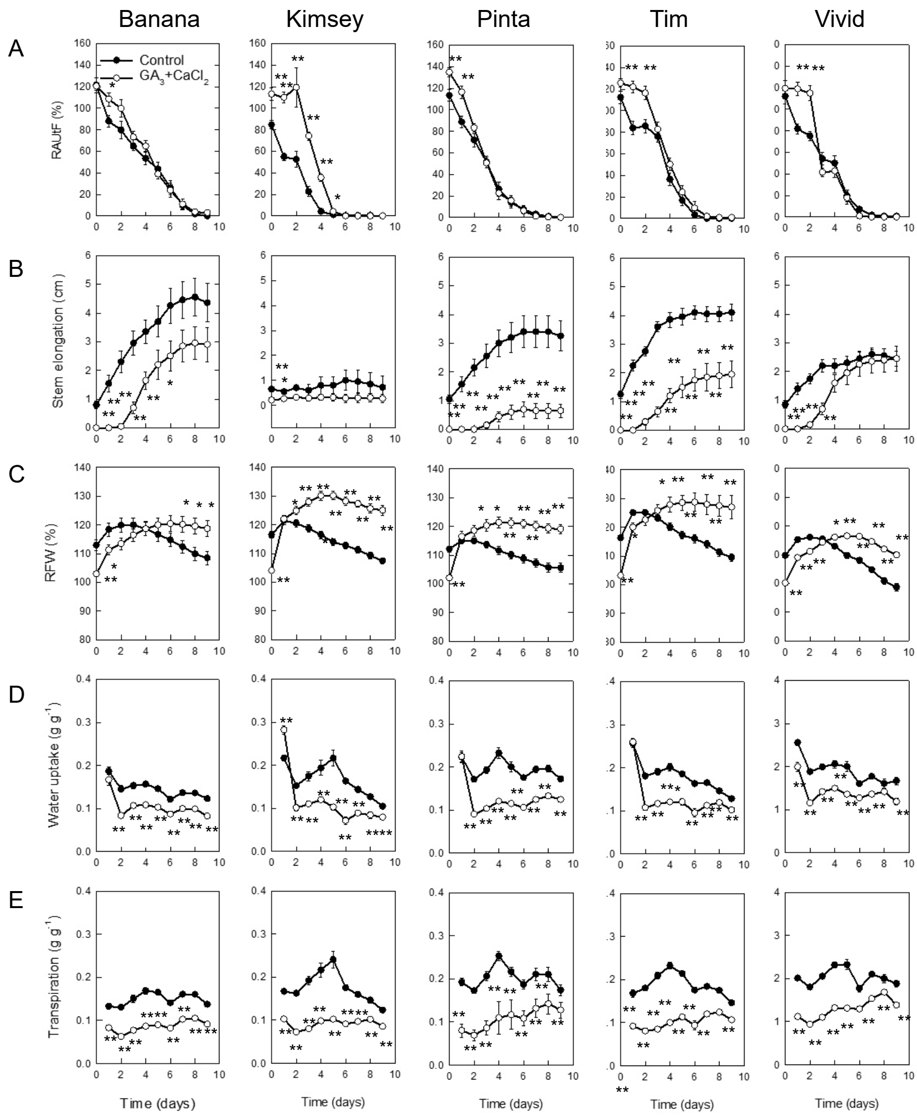


Figure 5. Effect of pretreatment with 144 μM GA_3 and 270 mM $CaCl_2$ on the relative area of unopened tubular florets (RAUTF) (**A**), stem elongation (**B**), RFW (**C**), water uptake (**D**), and transpiration (**E**) of cut gerbera 'Banana', 'Kimsey', 'Pinta', 'Tim', and 'Vivid'. The values are means of 10 replicates ± SE. ** and * indicate significant differences at $p < 0.01$ and $p < 0.05$, respectively, by estimated the t-test.

The degree of stem elongation varied with the five cultivars. The stem length in the five cultivars was significantly shorter in the GA_3 and $CaCl_2$ treatment than in the control at some time points (Figure 5B).

For the five cultivars, the RFW of the cut flowers in the control increased during the first 1 or 2 day and decreased thereafter (Figure 5C). The treatment with GA_3 and $CaCl_2$ suppressed decreases in the RFW of the flowers: it continued to increase over 5 days. In the five cultivars, the water uptake and transpiration of the cut flowers were significantly suppressed by GA_3 and $CaCl_2$ treatment (Figure 5D,E).

In the five cultivars, the vase life of the cut flowers was significantly extended by GA_3 and $CaCl_2$ treatment (Table 4, Figure 6).

Table 4. Effects of pretreatment with 144 µM GA_3 and 270 mM $CaCl_2$ on the water uptake and vase life of cut gerbera 'Banana', 'Kimsey', 'Pinta', 'Tim', and 'Vivid'.

Cultivar	**Treatment**	**Solution Uptake (g g^{-1})**		**$CaCl_2$ Uptake (mg g^{-1})**		**GA_3 Uptake (µ g^{-1})**		**Vase Life (Days)**		**Ornamental Value Loss Symptoms (%)**	
										Bending	**Other**
Banana	Control	0.26		0		0		14.0		0	100
	$GA_3 + CaCl_2$	0.13	** [1]	3.8	**	6.3	**	17.3	**	0	100
Kimsey	Control	0.33		0		0		15.5		0	100
	$GA_3 + CaCl_2$	0.14	**	4.3	**	7.1	**	19.7	**	0	100
Pinta	Control	0.28		0		0		15.9		0	100
	$GA_3 + CaCl_2$	0.12	**	3.5	**	5.8	**	20.3	**	0	100
Tim	Control	0.33		0		0		13.9		0	100
	$GA_3 + CaCl_2$	0.14	**	4.2	**	7.0	**	16.7	**	0	100
Vivid	Control	0.29		0		0		9.9		0	100
	$GA_3 + CaCl_2$	0.14	**	4.0	**	6.8	**	14.3	**	10	90
Two-way ANOVA [2]	Cultivar (C)	**		**		**		**			
	Treatment (T)	**		**		**		**			
	C × T	*		**		**		NS			

[1] ** indicates significant differences at $p < 0.01$ estimated by the t-test. [2] **, *, and NS indicate significant differences at $p < 0.01$, $p < 0.05$, and no significant differences, respectively, estimated by two-way ANOVA.

Figure 6. Gerbera 'Vivid' flowers at 13 days after the start of treatment. (**Left**): Flowers pretreated with 144 µM GA_3 and 270 mM $CaCl_2$. (**Right**): Control (distilled water). The scale bar represents 1 cm.

4. Discussion

Pretreatment with GA_3 alone promoted the stem elongation of the cut gerbera, resulting in stem bending. Similarly, the promotion of stem elongation by GA_3 treatment has been reported in cut tulip [22,23]. In many plants, including Arabidopsis [24], maize [25], rice [26], and tomato [27], cell elongation is promoted by exogenous GA_3. Therefore, the stem elongation of cut gerbera induced by GA_3 may be dependent on cell elongation. In contrast, Emongor [18] reported that GA_3 treatment did not cause stem bending in cut gerbera 'Ida Red', which differed from our results. This difference may be attributed to the different GA_3 concentrations used in the experiments. Although higher GA_3 concentrations promote stem bending, the highest concentration in their study was 22 μM, which is lower than the GA_3 concentration in our study.

Treatment with $CaCl_2$ alone and combined treatment with GA_3 and $CaCl_2$ suppressed stem elongation. $CaCl_2$ is known to induce stomatal closure and suppress transpiration [4,5]. In our study, water uptake and transpiration were markedly decreased by $CaCl_2$ treatment. This suppression was caused by the inhibition of transpiration. Water uptake is necessary for cell expansion associated with stem elongation [28,29]. Therefore, we propose that the inhibition of stem elongation by $CaCl_2$ is possibly due to the suppression of water uptake, which is caused by the suppression of transpiration.

Treatment with GA_3 alone promoted stem elongation and shortened the vase life of the cut gerbera. Since vase life was terminated by stem bending, the shortening of vase life is attributed to stem elongation. However, combined treatment with GA_3 and $CaCl_2$ extended vase life significantly more than treatment with $CaCl_2$ alone. This finding suggests that GA_3 can extend the longevity of gerbera petals. This explanation is supported by the finding that GA_3 treatments significantly extended the vase life of cut gerbera showing symptoms other than bending. The extension of petal longevity by GA_3 has also been reported in carnations [17] and roses [14].

Gibberellins are known to be involved in floral development in plants [30]. Exogenous GA_3 promotes floral development in many plant species, including petunia [31] and Japanese radish [32]. In cut gerbera, an increase in the relative area of unopened tubular florets was accompanied by an increase in the number of visible tubular florets, suggesting that exogenous GA_3 promotes the development of tubular florets. Also, GA_3 treatment delayed the opening of the tubular florets. Therefore, the freshness of cut gerbera appears to be maintained by exogenous GA_3.

To extend the vase life of cut gerbera flowers, the effect of pretreatment with antimicrobial compounds has been studied, but vase life was not significantly extended [9]. In contrast, pretreatment with GA_3 and $CaCl_2$ significantly extended the vase life of six gerbera cultivars. Therefore, this treatment appears to be useful to extend the vase life of cut gerbera. However, further studies may be needed to demonstrate the practicality of this treatment, including using different varieties and cut flowers produced under different environmental conditions. In our study, an antimicrobial solution was used as vase water because bacterial proliferation shortens the vase life of cut gerbera [33,34]. In general, consumers use tap water or preservatives consisting of sugar and antimicrobial compounds for cut flowers. The vase life of cut gerbera 'Kimsey' is extended by glucose and antimicrobial compounds. Further study is necessary to clarify whether pretreatment with GA_3 and $CaCl_2$ combined with preservative treatment is more effective than GA_3 and $CaCl_2$ pretreatment in extending the vase life of cut gerbera.

5. Conclusions

Pretreatment of cut gerbera with GA_3 caused stem bending due to its marked elongation, which shortened vase life. However, GA_3 treatment delayed the opening of the tubular florets and delayed the senescence of ray petals. Stem elongation caused by GA_3 treatment was suppressed by combined treatment with $CaCl_2$. Combined treatment with GA_3 and $CaCl_2$ extended the vase life of the cut gerbera more than pretreatment with GA_3 alone and $CaCl_2$ alone. Combined treatment with GA_3 and $CaCl_2$ significantly extended the vase life of the other five cultivars. We conclude that pretreatment with GA_3 and $CaCl_2$ is useful for extending the vase life of cut gerbera.

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