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Evidence for Physiological Vascular Occlusion in Stems of Cut Gerbera cv. Hongyan

R. Wang[1*], X. Zheng[1], and X. Xu[1]

ABSTRACT

The aim of this paper was to assess the effect of catechol (CH), as a peroxidase inhibitor, and 8-hydroxyquinoline citrate (HQC), as a bacterial inhibitor, on keeping the quality of *Gerbera Jamesonii* cv. Hongyan cut flowers, known to be limited by vascular occlusion. Blockage in the stem xylem vessels of cut gerbera was studied using light and scanning electron microscope. The results showed that some stem xylem vessels of the cut flowers were blocked during the vase period, and the blockage consisted of some amorphous or physiological deposition and rod-shaped bacteria located within the 5cm stem end of the cut flower. In addition, CH (1.0mM) or 8-HQC (0.45mM) decreased the blockage of stems and reduced the bacterial growth in the vase solution, but extended the vase life without statistical significance. The combination of CH (0.5 or 1.0 mM) and 8-HQC (0.45mM) decreased the blockage and inhibited the bacteria more than CH or 8-HQC alone, and extended the vase life significantly (P≤ 0.05).

Keywords: Cut flowers, Catechol, *Gerbera jamesonii,* Bacterium inhibitor, Vase life, Xylem blockage.

INTRODUCTION

Many works on wilting mechanism and preservation of cut flowers have been reported (Podd *et al.*, 2002; van Doorn and Vaslier, 2002; Damunupola *et al.*, 2010; Ahmad *et al,* 2013). The vase life of cut flowers is determined by the phenotype×postharvest conditions interaction (Fanourakis *et al.*, 2013). However, insufficient water uptake due to the xylem occlusion is one of the direct reasons for wilting during the vase period (Ieperen *et al.*, 2002).

Gerbera jamesonii and its hybrids are well known for their variable shapes and colors in the world (Solgi *et al.*, 2009; Liu *et al.*, 2009). But, the vase life of cut gerbera is often shortened by stem bending due to insufficient water uptake (van Meeteren, 1978). Stem end blockage is a major factor in the imbalance between water uptake and water loss from cut flowers (He *et al.*, 2009). Some studies suggested that the blockage of cut gerbera flower is caused by bacteria (microbes) (van Meeteren, 1978; Put, 1990; He *et al.*, 2009) or bacteria and decay products (Liu *et al.*, 2009). Bacterial inhibitors such as 8-HQC, silver nanoparticles or salicylic acid could extend vase life of cut flowers, whose vascular occlusion is caused by bacteria (Marousky, 1971; Loubaud and van Doorn, 2004; Solgi *et al.*, 2009; Mashhadian *et al,* 2012). HQC, as an antibacterial agent, may also promote flower longevity by acidifying the vase solution, or influencing the activity of some enzymes, or their chelating properties (Edrisi *et al*., 2012).

Physiological deposition such as lignin, suberin, mucilage, gums or tyloses (Loubaud and van Doorn, 2004, van Doorn and Cruz,

[1] School of Urban and Environmental Science, Jiangsu Normal University, Xuzhou, People's Republic of China.
[*] Corresponding author; e-mail: wangronghua73@sohu.com

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2000) and air emboli or cavitations (van Meeteren *et al.*, 2006) may also decrease the vase life of some cut flowers. The air emboli or cavitations may be not serious if cut flowers are put into water immediately after cutting (Ieperen *et al.*, 2002). Thus, physiological inhibitors such as CH and S-carvone extended the vase life of cut chrysanthemum or *Grevillea* flowers, whose stem blockage was due to physiological deposition (van Doorn and Vaslier, 2002; Loubaud and van Doorn, 2004; He *et al.*, 2006).

The blockage can be directly seen with the light microscopy technique, but this technique could not distinguish blockage caused by physiological deposition or bacteria (He *et al.*, 2009; Liu *et al.*, 2009), which can be directly observed with scanning electron microscopy (SEM). Most previous studies have reported the vascular occlusion of some cut flowers indirectly by deduction and not by observation with SEM. Only a few studies were performed using SEM (Clerkx *et al.*, 1988; Macnish *et al.*, 2008; He *et al.*, 2009). Further observation with SEM can contribute to determine whether physiological deposition or bacterial growth occurred in stem of cut flowers during vase period.

In this research, we studied the effect of CH, as a physiological inhibitor, on the vase life of cut gerbera, and observed the stem vessel blockage with light microscope and SEM. The objectives of this study were to investigate: (1) how CH, as a physiological inhibitor, affects the vase life of cut gerbera flower, (2) what materials other than bacteria block the vascular stem of cut gerbera during vase period, and (3) what are the influences of the blockage on the vase life of cut gerbera flower?

MATERIALS AND METHODES

Plant Materials

Gerbera Jamesonii cv. Hongyan flowers were obtained from a commercial grower in Linyi, located at 35°03′N, in the south of Shandong province, China. The flowers were grown in a greenhouse and harvested in the morning on May, 2011. Cut flowers were covered with plastic films and transported within 4 hours to the laboratory in Jiangsu Normal University, where the flower stems were re-cut in distilled water to a length of 25 cm. Thirty-two flowers were selected for the vase life experiment and 3 flowers for fresh stem structure observation.

Experimental Designs and Treatments

The experiment was conducted in the laboratory at 18–22°C, 35–55% *RH*, and 15 μmol m^{-2} s^{-1} light intensity (cool white fluorescent tubes) under a daily light period of 12 hours. Vase solutions were freshly prepared at the beginning of the experiment and not renewed in the course of study.

The experiment was carried out in a factorial design. The vase solutions included distilled water, 0.45 mM 8-HQC with 0.06M sucrose, 0.5 and 1.0 mM CH with 0.45 mM 8-HQC and 0.06M sucrose (Table 1). A complementary experiment containing CH at 1.0 mM was conducted to verify the effect of CH alone on cut gerbera flowers. CH was obtained from a Chemical company (Alfa Aesar Chemical Co. Ltd. Tianjin, China). Sucrose was used as a food source or for water balance maintenance of vase flowers (Solgi *et al.*, 2009).

The cut flowers were immediately immersed individually into a 150 ml conical

Table 1. Prescription for preserving cut gerbera flowers.

Treatments	water	HQC	HQC and 0.5 mM CH	HQC and 1.0 mM CH
Components	Distilled water	0.45mM 8-HQC +0.06M sucrose	0.5mM catechol+ 0.45 mM 8-HQC +0.06M sucrose	1.0 mM catechol+ 0.45 mM 8-HQC +0.06M sucrose

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beaker containing 100 ml solution after re-cut. Every treatment included 3 replications for stem structure observation and 5 replications for the other experiments. Vase mouths were covered with small masses of absorbent cotton to minimize water evaporation and prevent contamination. The vase life, fresh weight (FW), relative fresh weight (RFW), and water balance (WB) of the flowers were assessed daily during the vase period.

Vase Life and Relative Fresh Weight

The vase life was judged to the end when the cut flowers were dropping or their petals were moderately wilting (Macnish *et al.*, 2008). The *FW* (g) of cut flowers was daily recorded and the *RFW* was calculated by the formula:

$$RFW\ (\%) = FW_t/FW_0 \times 100 \quad (1)$$

Where, *t*= Day 0, 1, 2, etc., respectively, FW_t is the fresh weight at day *t*, FW_0 is the fresh weight at day 0 (He *et al.*, 2006).

The *WB* was calculated by the formula:

$$WB = U - L \quad (2)$$

Where, *U* is water uptake and *L* is water loss of the cut flowers. And $U = SC_{t-1} - SC_t$, where, SC_t is total weight of the solution and conical beaker at *t*= Day 1, 2, 3, etc, respectively, and SC_{t-1} is the weight of the solution and conical beaker on the previous day; $L = SCF_{t-1} - SCF_t$, where, SCF_t is total weight of the same solution and conical beaker and flowers at *t*= Day 1, 2, 3, etc., respectively, and SCF_{t-1} is the weight of the same solution and conical beaker and flowers on the previous day.

Bacterial Counts

Bacterial counts in the vase solutions were examined using the plate count method (Liu *et al.*, 2007) on the 5th day. The vases were agitated to stir their solution. A 0.5ml sample solution was taken separately from three vases and serially diluted 10^0–10^{-5} folds in sterile distilled water for 5 times. The 0.2 ml of the diluted sample solution was pipetted onto a plate count agar (beef extract peptone medium). The beef extract peptone medium consisted of beef extract (0.3 g), peptone (1 g), sodium chloride (0.5 g), agar (2 g), and distilled water (100 ml), and its pH value was 7.2. The bacteria on the agar were incubated for 24 hours at 37°C and the bacterial colonies were enumerated.

Light Microscopy and Scanning Electron Microscopy

To observe the blockage of the fresh stem on day 0 and the stems of different treatments on the 5th day, a 2 cm segment (denoted the basal 0–2cm segment) and a 1 cm segment (denoted the basal 5–6 cm segment) were excised and fixed in formalin-acetic acid (FAA). For the light microscopy observation, the specimens in the FAA were rinsed with distilled water, and, then, for paraffin sectioning through a gradual concentration series of Ethyl alcohol, as a dehydration agent, and Dimethylbenzene, as a transparence agent, and with Safranin T and fast green as double staining agents (Li, 1996). Photographs were taken by the light microscope with YM310 DV. For the SEM observation, the specimens in the FAA were rinsed in distilled water, and dehydrated in a gradual series of 70, 80, 95, and 100% (v/v) Tert-butyl alcohol for 10 minutes every step and 100% Tert-butyl alcohol 3 times (Gu, 2006). The dehydrated specimens were frozen and dried in a Hitachi ES-2030 freeze dryer (Hitachi Ltd., Tokyo, Japan), then sputter-coated with gold in a Hitachi E-1010 ion sputter (Hitachi Ltd., Tokyo, Japan). The transverse section of the stem base was observed under the Hitachi S-3400N SEM (Hitachi Ltd., Tokyo, Japan).

Statistical Analyses

The data were analyzed by relative analysis of variance (ANOVA) with the

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SPSS 17.0 software, and LSD tests at P= 0.05 level. The experiments involved 5 replications for every treatment.

RESULTS

Vase Life

At the end of vase life, the stems in distilled water and in HQC alone bent over at 15-20 cm from stem bottom (Figure 1-B), and in 0.5 or 1.0 mM CH solution with HQC, the stem broke at points 10 cm from the bottom and the stem base turned brown (Figure 1-C). The petals of cut gerbera flowers in different treatments did not wilt.

Compared to the control (distilled water), the vase solution of 0.5 or 1.0 mM CH with HQC extended the vase life of flowers significantly, and the longest vase life was obtained at 1.0 mM (Table 2). HQC alone extended the vase life, but not significantly (Table 2), and CH alone at 1.0 mM had no effect on the vase life (data not shown).

Relative Fresh Weight and Water Balance

The treatments of 0.5 and 1.0 mM CH with HQC both delayed loss of the *RFW* during the vase period, and 1.0 mM CH best maintained the *RFW* (Figure 2-A) and the *WB* (Figure 2-B).

Bacterial Numbers

Compared to the control (distilled water), different treatments decreased bacterial numbers of vase solutions significantly (Table 2). CH (0.5 or 1.0 mM) with HQC reduced bacterial numbers more than HQC alone did.

Stem Blockages

Observation with the light microscopy showed that the xylem vessels at the 0–2 cm stem base in water were blocked by some

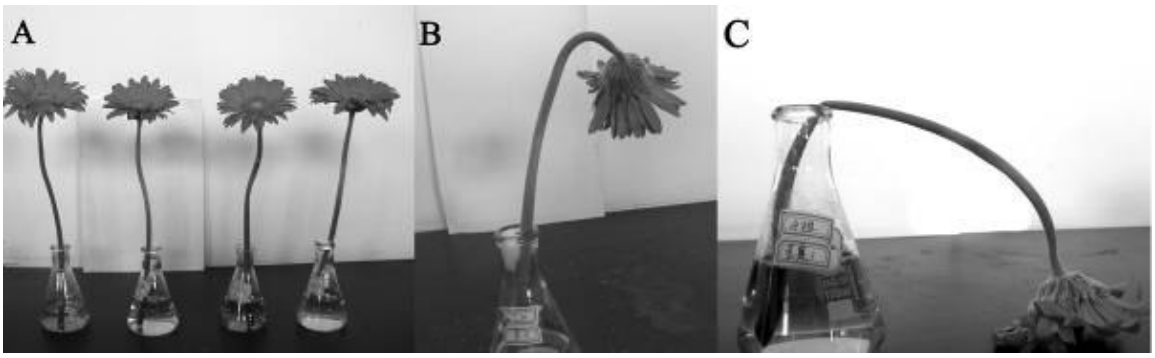

Figure 1. Effect of different vase solution on the vase life of gerbera flowers. (**A**) Flowers in different treatments at the first day; (**B**) Flowers in distilled water on the 5th day, (**C**) Flowers in HQC and 1.0 mM CH on the 8th day.

Table 2. Vase life and bacterial counts of gerbera cut flowers in different treatments.[a]

Treatments	water	HQC	HQC and 0.5 mM CH	HQC and 1.0 mM CH
Vase life (d)	5.2±0.4^{c}	6.2±1.2^{bc}	7.2±1.7^{ab}	8.8±1.2^{a}
Bacterial counts (cfu ml^{-1})	5.80×10^{6a}	2.00×10^{6b}	2.97×10^{5c}	2.65×10^{5c}

[a] Data are means of 5 replications. Significant differences at P< 0.05 level are shown by a different letter (a, b, and c).

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material dyed into dark green (Figure 3-A). HQC or CH (0.5 mM or 1.0 mM) with HQC decreased the blockage (Figure 3, B-D). No blockage was observed in the fresh stem. Since SEM image shows the results more clearly, the data with light microscopy are not shown.

Observation with SEM showed that the xylem vessel of fresh stems had no occlusion (Figure 4-A). On the 5th day of the vase life, some mixture of amorphous matter and rod-shaped bacteria was observed in the xylem vessels of stems in distilled water (Figure 4, C–F), but few in 1.0 mM CH with HQC treatment (Figure 4-B).

In the basal 5–6 cm segment, no blockage was observed in all treatments, which were the same as that of the fresh stem, therefore, the data are not shown.

DISCUSSION

The vascular occlusion of gerbera stems has been considered to be mainly due to microbial proliferation (van Meeteren, 1978; Put, 1990; He *et al.*, 2009, Liu *et al.*, 2009). In the present study, besides the rod-shaped bacteria, some amorphous matter was observed in the vascular vessels of gerbera stems. This amorphous matter might be mucilage or gums due to wound reaction rather than lignin or suberin or tyloses, because lignin or suberin could be dyed red by Safranine, and tyloses are membrane-bound invaginations (Tagne *et al.*, 2002) or the membrane-bound cell bodies into vessels. It was during the vase period that the amorphous matter was produced, because no blockage was observed in the vessels of fresh stems. This result suggested

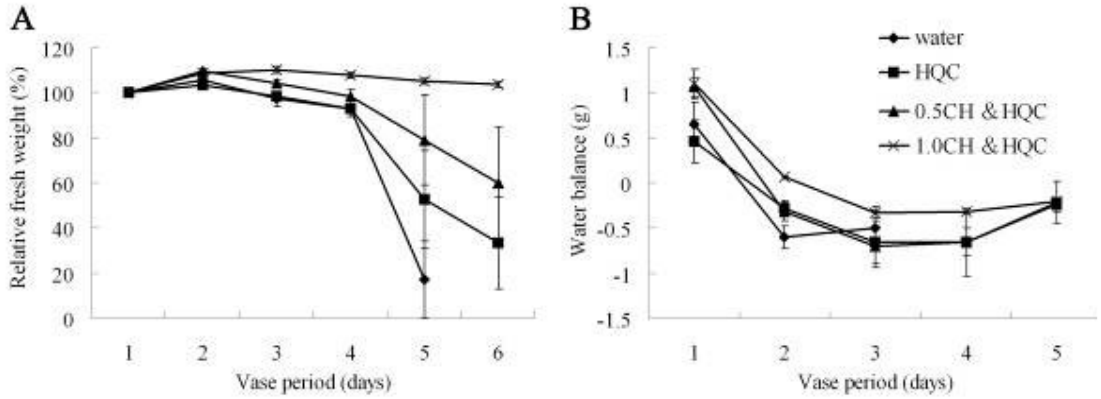

Figure 2. Changes of relative fresh weight (**A**) and water balance (**B**) of cut gerbera flowers in different treatments. Data are means of five replications±S.E.

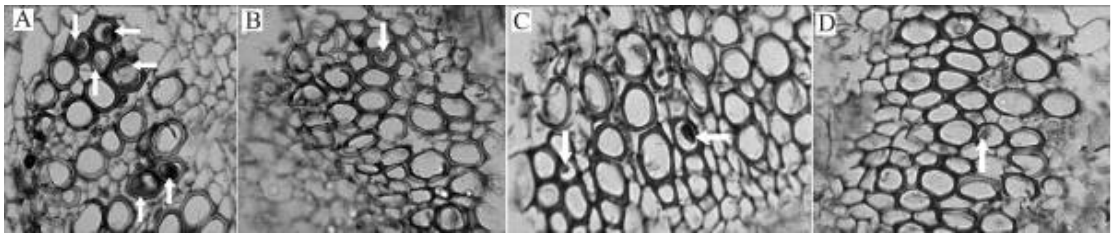

Figure 3. Vessel blockages in the stem end of cut flowers on the 5th day under light microscopy (240×). (**A**) The control (in distilled water); (**B**) HQC; (**C**) HQC and 0.5 mM CH, (**D**) HQC and 1.0 mM CH. Blockages are indicated with white arrowheads.

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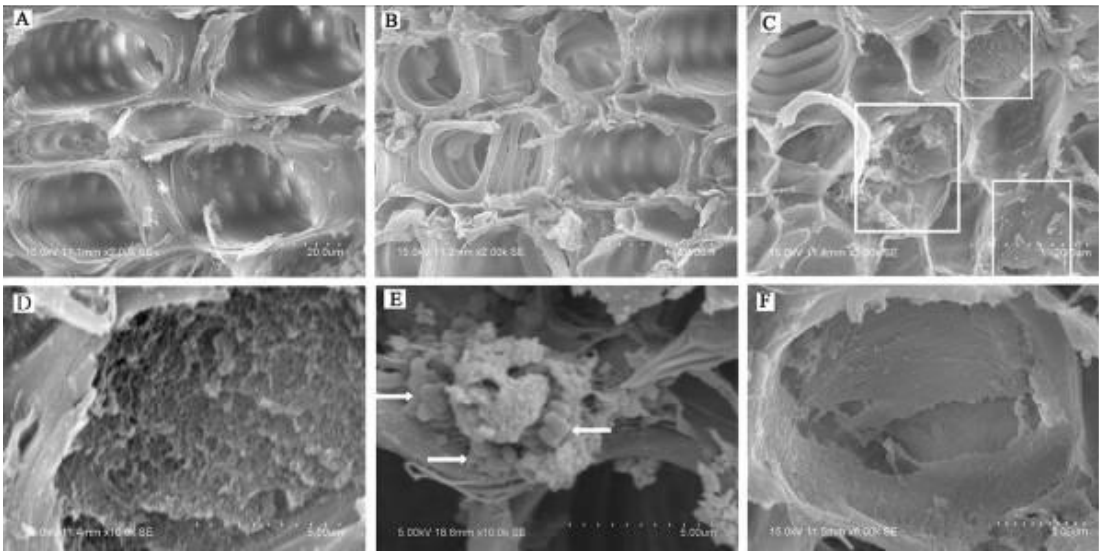

Figure 4. Vessel blockages in the stem end of cut flowers under SEM. (**A**) The fresh stem immediately after cutting; (**B**) The stem in HQC and 1.0 mM CH treatment on the 5th day, (**C**–**F**) The stem in distilled water on the 5th day. The white boxes show blockages in xylem vessels. White arrowheads indicate rod-shaped bacteria.

that the vascular occlusion of gerbera stems was apparently due to physiological process involving oxidation. Tagne *et al.* (2002) observed that the deposition occurred in maize xylem vessels after fungal invasion and considered the depositions were gums and gels, which were part of the host defense response. In this paper, the depositions may be a response to wound, and could be prevented by combining CH with HQC.

No blockage was observed in the basal 5-6 cm stem, which suggested that blockage of the gerbera flowers was located within the stem base of 5cm. This result was similar to the findings on chrysanthemum (van Doorn and Vaslier, 2002), *Bouvardia×Domestica* cv. Van Zijverden (Vaslier and van Doorn, 2003), *Grevillea* (He *et al.*, 2006) and gerbera (He *et al.*, 2009).

CH combined with HQC decreased bacterial numbers of vase solutions and reduced blockage (Figure 3-D) more than HQC alone did, which implied that CH as a peroxidase inhibitor (Srivastava and van Huystee, 1977; Wang *et al.*, 1991; van Doorn and Vaslier, 2002) suppressed the microbial proliferation, or increased the effect of HQC as bacterial inhibitor. Maddox *et al.* (2010) presented that CH was effective in inhibiting a pathogenic bacterium (*Xylella fastidiosa*). Alternatively, Loubaud and van Doorn (2004) considered that 8-HQC, as a bacterial inhibitor, suppressed the plant-induced xylem occlusion. Therefore, CH or HQC could not only be referred to as a physiological inhibitor, but also an antibacterial agent in the preservation of cut flower. The mechanism of CH or HQC acting on the vase life of cut flowers still needs more studies.

Water loss of cut gerbera flowers was aggravated by lower air humidity in the test room than recommended (Fanourakis *et al.*, 2013), therefore, in this study, the vase life of cut gerbera in distilled water was shorter than the findings of Solgi *et al.* (2009).

CONCLUSIONS

CH at 1.0 mM combined with HQC extended the vase life of the gerbera flowers significantly, while CH or HQC alone had a weak effect. Both CH with HQC and HQC alone reduced bacteria counts of the vase solution and decreased the occlusion of the cut flower stems. The occlusion occurred within 5 cm of the stem base, and consisted of not only bacteria, but also amorphous mucilage or

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gums involving wound-induced physiological processes of the cut flowers.

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شواهدی برای گرفتگی (انسداد) آوندی در ساقه ژربرای بریده کالتیوارHongyan

ر. وانگ، ز. ژنگ، و ز. زو

چکیده

ازآنجا که گرفتگی آوندها عمر گلدانی ژربرای بریده را محدود میکند، هدف پژوهش حاضر ارزیابی اثر catechol (CH)به عنوان بازدارنده پراکسیداز و 8-hydroxyquinoline citrate (HQC)به عنوان بازدارنده باکتری در حفظ کیفیت ژربرای بریده*Jamesonii cv. Hongyan* بود. برای مطالعه گرفتگی در آوند چوبی ساقه ژربرا از میکروسکوپ نوری و میکروسکوپ الکترونی استفاده شد. نتایج نشان داد بعضی آوندهای ساقه گل بریده در طی زمان در گلدان دچار گرفتگی می شدند و این گرفتگی ناشی از رسوب های آمورفوس(بی شکل) یا فیزیولوژیک در آوند یا رشد باکتری های لوله ای شکل در ۵ سانتی متری انتهای ساقه بریده ژربرا بود. بر اساس این نتایج، کاربرد CH (1.0mM) یا 8-HQC (0.45mM) باعث کاهش گرفتگی(انسداد) ساقه و کم شدن رشد باکتری ها در محلول داخل گلدان شد و عمر گلدانی ژربرا را طولانی کرد هرچند که این افزایش عمر از نظر آماری معنی دار نبود. اما، کاربرد ترکیب CH (1.0mM)و-8 HQC (0.45mM)گرفتگی آوند ها را کاهش داد و اثر بازدارنده آن روی رشد باکتری ها بیشتر از کار برد هر یک از مواد به تنهایی بود.همچنین، ترکیب مزبور عمر گلدانی ژربرا را به طور معنی دار($P\leq0.05$) افزایش داد.

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