Effects of Scape-injected 1-Aminocyclopropane-1-carboxylic Acid (ACC) on the Vase Life of 'Testarossa' Cut Gerberas

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ABSTRACT. 'Testarossa' gerbera (*Gerbera jamesonii* Bolus) scapes were injected with distilled water (control), or 0.3, 0.6, and 0.9 mM ACC at harvest, then held at 20 °C for 15 days in a preservative solution. PAL activity and ethylene production increased within 1 day proportionally to injected ACC. ACC injection reduced bending incidence, inhibited flower scape elongation, enhanced firmness of the flower scapes and increased vase life. Flower scapes treated with ACC reached full maturity 3 days before the end of vase life of the control, which bent before reaching full maturity.

Cut gerberas are affected by normal senescence, and vase life is normally limited by petal wilting (De Jong, 1978), premature senescence folding (De Jong, 1978), stem break (van Meeteren, 1978a), and stem bending (Abdel-Kader and Rogers, 1986), terms that are used to describe the sudden bending of the stem, similar to bent neck in roses (Marousky, 1972). Premature senescence has been related to growing conditions (Nowak and Rudnicki, 1990), genetic differences (Wilberg, 1973), morphological properties (Marousky, 1986), and water stress (van Meeteren 1978a, 1978b). Aging of a cut gerbera inflorescence in a vase at 23 °C caused a decline in water absorption and a sharp decrease in fresh mass 3 d before stem break occurred (van Meeteren, 1979, 1980). Moreover, van Meeteren (1978a) suggested that water uptake was strongly inhibited by growth of bacteria at the cut end. The zone of scape elongation, located three-fourths up the scape (Wilberg, 1973), is the weak point (of low lignin content) where gerbera scapes bend (Steinitz, 1982). Chemical analysis confirmed that the lower part of the scapes has greater lignification than the upper part (Marousky, 1986). Mencarelli et al. (1995) reported that lack of lignin in the tissue below the flower head is an indication of immaturity, as this part of the scape will still elongate after harvest. Thus, low bending frequency is found among flowers with firm stems (De Jong, 1978).

Phenylalanine ammonia lyase (PAL) is an important regulatory enzyme of secondary metabolism. PAL catalyzes the deamination of phenylalanine to trans cinnamic acid, which is used to synthesize many phenolic and lignin-like compounds associated with plant metabolism (Brown, 1990). The mechanical rigidity of lignin strengthens stem and vascular tissue, allowing upward growth and promoting water and mineral conduction through the xylem under negative pressure without collapse of the tissue (Taiz and Zeiger, 1991).

Ethylene has been shown to promote PAL activity (Brown,

1990; Hyodo et al., 1991; Prasad and Cline, 1987). Ethephon treatment of *Pharbitis nil* [or *Ipomea nil* (L.) Roth.] dramatically stimulated PAL activity and lignin accumulation (Prasad and Cline, 1987). Exposure of lettuce to ethylene resulted in higher peroxidase activity, much higher PAL activity browning intensity, and slightly higher and soluble phenolic content (Couture et al., 1993). Furthermore, Ievinsh and Romanovskaya (1991) suggested that ethephon and ACC accelerate PAL activity and lignification, resulting in shortening of the final length of the leaf blade. It was also demonstrated that preharvest ethephon treatment to gerbera scapes reduces scape length and increases lignin content compared to the control (Papadopoulou et al., 1996). While ACC promotes lignification when it is converted to ethylene, it also advances senescence if sprayed or provided through the vase solution to cut flowers (Reid and Wu, 1992).

The purpose of this study was to determine if scape-injected ACC could stimulate PAL activity and improve the vase life of 'Testarossa' gerbera.

Materials and Methods

PLANT MATERIAL. Flowers (capitulum and scape) of *Gerbera jamesonii* 'Testarossa' obtained from 1-year-old plants grown on cocopeat in open bags and fertigated with a standard nutrient solution for gerbera in a greenhouse were used. The gerberas were harvested (by pulling the stems from the crowns) in the morning (in April) at the commercial harvest stage, when two to three of the stamens disc florets were mature, and transferred to the laboratory. The flowers were screened for defects and assessed for marketable quality (scape length ≈ 45 cm, capitulum diameter ≈ 7 cm). The scapes were trimmed to a uniform length of 40 cm and held at 25 °C, 60% relative humidity, and a 16-h photoperiod (28 μmol·m⁻²·s⁻¹).

Scapes of 60 flowers per treatment were injected by a syringe with 0 (water control), 0.3, 0.6, and 0.9 mM ACC (≈ 0.5 mL) at 10 cm below the capitulum and placed in 500-mL Erlenmeyer flasks containing 2.5% of sucrose and 125 mg·L⁻¹ hydroxyquinoline sulfate (HQS) at pH 4. The flowers were considered at full maturity (100%) when all six stamen disc florets were mature (open). Scape length and mass, PAL activity, internal ethylene, and firmness

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were measured every 3 d. The preservative solution was replaced after every sampling. At the end of vase life the segments 5 to 15 cm below the capitulum were weighed (fresh mass), dried at 60 °C for 48 h, and reweighed (dry mass).

SCAPE INTERNAL ETHYLENE CONCENTRATION AND FIRMNESS. Six flowers per treatment were taken from the preservative solution every 3 d and individually immersed in water, and an internal atmosphere sample (≈ 0.5 mL) was extracted from each scape (directly by a syringe) to measure ethylene in a gas chromatograph (5890 series II; Hewlett Packard S.A., Geneva, Switzerland) equipped with a fused silica column (10 m in length, 0.53 mm in diameter) at oven temperature of 85 °C. Scape firmness was measured at 5, 10, 15, and 20 cm below the capitulum on three flowers used for internal ethylene measurements using a penetrometer (John Chatillon & Sons Inc., N.C.) equipped with a flat pressure surface of 13 mm diameter; the applied pressure was recorded when the scape surface was crushed (Steinitz, 1982).

PAL ACTIVITY DETERMINATION. Scape segments 5 to 15 cm below the capitulum of three flowers (those used for internal ethylene measurements) with five segments per replication were homogenized at 0 °C (ice bath) using a microprocessor (Virtis co., New York) with 0.1 M borate buffer at pH 8.8, 2-mercaptoethanol, and 10% (w/w) of polyvinylpyrrolidone (PVP). The homogenate was

filtered through four layers of cheesecloth and the filtrate was centrifuged at 4 °C and 13000 g_n for 20 min. The supernatant was used for the enzyme assay, which was performed at 25 °C and pH 8.8 (Ritenour et al., 1995). Increase in absorbance at 290 nm against a control without phenylalanine was recorded over a period of 1 h with a spectrophotometer (HP 8452A; Hewlett Packard). The rate of appearance of trans-cinnamic acid was taken as a measure of the level of PAL activity, expressed as $\mu\text{mol}\cdot\text{g}^{-1}$ of trans-cinnamic acid on a fresh mass basis.

STATISTICAL ANALYSIS. The LSD test was used for to compare treatments during vase life and Duncan's test was used to compare treatments at the end of vase life, using the procedure within SAS statistical software (SAS Institute Inc., Cary, N.C.).

Fig. 1. Internal scape ethylene production of 'Testarossa' gerbera scapes injected at harvest with 0, 0.3, 0.6, and 0.9 mM ACC, then held at 20 °C for 16 d in a preservative solution.

Fig. 2. PAL activity of 'Testarossa' gerbera scapes injected at harvest with 0, 0.3, 0.6, and 0.9 mM ACC, then held at 20 °C for 16 d in a preservative solution.

Fig. 3. Firmness at 5 (A), 10 (B), 15 (C), or 20 (D) cm below the capitulum of 'Testarossa' gerbera scapes injected at harvest with 0, 0.3, 0.6, and 0.9 mM ACC, then held at 20 °C for 16 d in a preservative solution.

Results and Discussion

Internal ethylene concentrations of gerbera scapes at harvest were as low as $0.68 \mu\text{L}\cdot\text{L}^{-1}$ (Fig. 1) and, following injection with distilled water (control), increased to a maximum of $1.28 \mu\text{L}\cdot\text{L}^{-1}$ on day 3. Subsequently, ethylene decreased steadily over the duration of vase life. Scape internal ethylene following injection with ACC was elevated compared to control when measured after 1 d but was similar to the control after 3 d.

PAL activity increased $\approx 2\times$ from the initial level of $\approx 1.32 \text{ pmol}\cdot\text{g}^{-1}$ for the control scapes over 9 d of vase life (Fig. 2). PAL activity of ACC-injected scapes peaked on day 1 and correlated with the amount of ACC injected (Fig. 2). It has been clearly established that PAL is an inducible key regulatory enzyme of the phenylpropanoid biosynthesis pathway (Hanson and Havir, 1981); PAL activity increases more rapidly at elevated temperature (Ritenour et al., 1995). Treatment with ethylene induced PAL activity and russet spotting in lettuce tissue (Couture et al., 1993), and the longer treatment durations with ethylene induced higher maximum levels of PAL activity (López-Gález et al., 1996). Scapes treated with ACC developed deep red color stripes $\approx 25 \text{ cm}$ below the capitulum by the end of vase life, indicating stimulation of anthocyanin production (data not shown).

The increase in ethylene and PAL activity of gerbera scapes injected with ACC was followed by a continuous increase in scape firmness 5 cm below the capitulum, while the control showed little if any increase (Fig. 3A). Firmness 10 cm below the capitulum, the region where the scape shows more bending, was increased when

the flowers were injected with 0.6 and 0.9 mM ACC on day 6 (Fig. 3B). At 15 cm below the capitulum, the control did not reveal any significant increase in firmness on day 9, while injected scapes showed a marked increase (Fig. 3C). At 20 cm below the capitulum, control flowers were observed to have a small increase in scape firmness, while the scapes injected with ACC had a larger increase on day 9 (Fig. 3D). The increase in scape firmness after 15 d of vase life could have partially been due to a wounding effect, since wounding has been shown to increase lignification in gerbera (Mencarelli et al., 1995) and asparagus (*Asparagus officinalis* L.) spears (Hennion et al., 1992).

There was no significant difference in the fresh mass between the treated and the control flower scapes during the first 3 d (Fig. 4A). However, after that, there was a slight difference between the treatment and the control. Fresh mass increased during the first 6 d, probably due to the increased water content and growth (van Meeteren, 1978a). Injection of postharvest scapes with ACC significantly decreased fresh mass of the segments 5 to 15 cm below the capitulum compared to the control at the end of the vase life (Table 1); however, dry mass increased as ACC concentrations increased. Similar results were found in gerbera flower scapes preharvest-treated with ethephon (Papadopoulou et al., 1996).

There was a $\approx 7\text{-cm}$ increase in the postharvest scape length of control flowers. However, scape elongation was reduced by 0.9 mM ACC (Fig. 4B). The younger cells, being in the upper part (Wilberg, 1973), had not yet developed a stable cell wall and still elongated after harvest (Steinitz, 1982). Elongation inhibition is one of the earliest reported actions of ethylene (Ievinsh and Romanovskaya, 1991) and has been associated with an alteration of cell wall mechanical properties via modification of the orientation of cellular microfibrils, cell wall pectic fraction, or cell wall cross linking (Prasad and Cline, 1987). Lignin formation has been demonstrated to correlate with elongation inhibition of *Pharbitis nil* stem treated with ethephon (Prasad and Cline, 1987) and of winter rye (*Secale cereale* L. 'Priekulu') seedlings treated with ACC and ethephon (Ievinsh and Romanovskaya, 1991).

Based on the number of mature of stamen disc florets, the control flowers started to bend after 4 d before reaching full maturity, while flowers treated with 0.9 mM ACC reached full maturity 6 to 9 d after harvest (Fig. 5A). This indicates that ACC advanced maturation (Abeles et al., 1992). Bending of control flowers started on day 3, reaching 80% bending in 9 d. However, only 33% to 46% of ACC-injected scapes bent after 9 to 10 d; the remaining flowers did not bend during the rest of the vase life (Fig. 5B). This was probably due to the increased lignification of the treated scapes under the increased PAL activity. Higher lignin accumulation was found in gerbera scapes preharvest-sprayed with 6 ppm ethephon (Papadopoulou et al., 1996).

The results of this work show that ACC increases PAL activity,

Fig. 4. Fresh mass (A) and scape length (B) of 'Testarossa' gerbera scapes injected at harvest with 0, 0.3, 0.6, and 0.9 mM ACC, then held at 20 °C for 16 d in a preservative solution.

Table 1. Dry and fresh mass of scape segments between 5 and 15 cm below the capitulum of 'Testarossa' gerberas, injected with 0, 0.3, 0.6, and 0.9 mM ACC at the end of vase life at 20 °C in a preservative solution.

ACC (mM)	Fresh mass (g)	Dry mass (g)	Dry mass (%)
0 (control)	28.09 a ^c	2.37 b	8.44 c
0.3	27.53 c	2.3 b	8.65 b
0.6	27.41 c	2.54 a	9.27 a
0.9	27.93 b	2.59 a	9.27 a

^cMeans in the same row followed with the same letter are not significantly different for Duncan's multiple range test ($P \leq 0.05$).

Fig. 5. Number of stamen disc florets (A) and bending incidence (B) of 'Testarossa' gerbera scapes injected at harvest with 0, 0.3, 0.6, and 0.9 mM ACC, then held at 20 °C for 16 d in a preservative solution.

leading to increased scape firmness and reduced bending, and enhances ethylene production, leading to advanced maturation of the stamen disc florets.

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