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EFFECT OF PULSE TREATMENT WITH EXOGENOUS CYTOKININS ON LONGEVITY AND ETHYLENE PRODUCTION IN CUT CARNATIONS (*Dianthus caryophyllus* L.)

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A B S T R A C T

The effect of 24-h pulse treatment with exogenous cytokinins BA and KIN on the longevity of cut carnations (*Dianthus caryophyllus* L.), cultivars 'Dolce Vita', 'Impala', 'Domingo', 'Tanga' and 'Charlotte', was investigated. The longevity of cut carnations can be improved by pulse treatment with KIN and BA at concentrations of 0.05 or 0.1 mM. The vase life of 'Dolce Vita', 'Impala', 'Domingo' and 'Tanga' was significantly extended by using 0.05 mM KIN or BA. Only 'Charlotte' did not positively respond to cytokinin treatment. KIN at concentrations of 0.05 and 0.1 mM also increased the flower diameter of cut carnations 'Charlotte' and 'Dolce Vita' as compared to distilled water. Pulse treatment with 0.05 mM KIN provoked a delay in the onset of climacteric ethylene production of 2 days, decreased the level of ethylene production in 'Dolce Vita' flowers and inhibited the activity of ACC oxidase in petals.

Key words: benzyladenine, carnations, ethylene production, kinetin, vase life

INTRODUCTION

The involvement of plant hormones in the regulation of senescence in plants is a well accepted concept. The gas ethylene, a naturally occurring plant hormone, is responsible for inducing many biochemical processes leading to programmed cell death, including an activation of senescence-related gene transcription (Woodson and Lawton, 1988; Lawton et al., 1990). Some flowers, such as carnations, are very sensitive to ethylene and react

to a few hours of exposure to one the atmosphere. Carnation petals exhibit a characteristic "inrolling" behaviour during senescence and in response to exogenous ethylene (Fujino et al., 1980). In carnation flowers ethylene is autocatalytically produced during senescence, leading to the enhancement of this process and shortening their vase life (Cook and Van Staden, 1988; Van Altvorst and Bovy, 1995). The final step in ethylene biosynthesis, the conversion of 1-aminocyclo-propane-carboxylic acid (ACC) to ethylene, is catalysed by the enzyme, ACC oxidase (Nijenhuis-De Vries et al., 1994). Treatment that blocks ethylene biosynthesis and action, such as pulsing with silver thiosulphate (STS), prolongs the vase life of cut carnations and reduces the sensitivity of the flowers to ethylene (Veen, 1979, Altman and Solomos, 1995).

Plant hormones other than ethylene are also involved in the regulation of senescence of cut flowers. Cytokinins have been well known for many years as growth regulators which markedly delay or reverse leaf yellowing and senescence in various species (Tjosvold et al., 1994; Han, 1995; Philosoph-Hadas et al., 1996; Skutnik et al., 1999). Also, the ageing of cut flowers is retarded by the external application of cytokinins, as demonstrated for carnations (Mor et al. 1983; Bosse and Van Staden, 1989; Van Staden et al., 1990b), roses (Łukaszewska and Barthe, 1995), tulips (Wawrzyńczak and Goszczyńska, 2000), anthurium, *Heliconia*, red and pink ginger (Paull and Chantrachit, part per million of this compound in 2001), irises (Wang and Baker, 1979), and gerbera (Van Meeteren and Van Gelder, 1980). The beneficial effect of the exogenous cytokinins on the quality of cut flowers has been reviewed (Halevy and Mayak, 1981; Goszczyńska et al. 1985). Application of these compounds to cut flowers has been shown to reduce water stress damage, improve water uptake and maintain petal turgidity, reduce respiration rates, as well as to inhibit ethylene production and reduce sensitivity to ethylene. Eisinger (1977) suggested that endogenous cytokinins are natural anti-senescence factors in carnation flowers. It has recently been shown that treatment of flowers with cytokinins can delay the senescence of cut carnations, however the results obtained have been variable. Boose and Van Staden (1989) demonstrated that the efficiency of these compounds depended on the mode of application as well as the type and concentration of a cytokinin used. Van Staden and coworkers (1989; 1990b) also reported that the antisenescent effect was achieved by the naturally occurring cytokinins, especially those the most abundant in the flowers. The synthetic cytokinins, kinetin (Mayak and Dilley, 1976) and benzyladenine (Van Staden et al., 1990a), also delayed wilting in carnations.

The aim of the present work was to determine the effect of kinetin and benzyladenine in different concentrations, used as a pulse treatment, on the longevity of 5 carnation cultivars. For 'Dolce Vita' pulsed with 0.05 mM

KIN, ethylene production and activity of ACC oxidase during the flower vase life were also determined.

MATERIAL AND METHODS

The experiments were carried out on cut carnations (*Dianthus caryophyllus* L.) of cultivars 'Charlotte', 'Dolce Vita', 'Domingo', 'Impala', and 'Tanga'. The flowers were obtained from a local grower in the autumn and winter (from September to March). They were harvested at the paint brush stage and transported dry to the laboratory.

Treatments of cut flowers. Prior to treatment, carnation stems were trimmed to a length of 40 cm and the lowermost leaves were discarded. The flowers were pretreated for 24 hours in solutions of cytokinins. The conditioning solutions contained benzyladenine (BA) and kinetin (KIN) at concentrations of 0.01, 0.05, 0.1, 0.5 mM. They were prepared in distilled water, which was also used for the non-treated flowers as a control. After 24 hours of treatment the stems were recut and then the flowers were placed into distilled water. The treatments and vase-life observations were carried out in a climate-controlled room at 20°C, about 60% RH and a 16 hour day at 10 μmol $m^{-2}s^{-1}$ provided by fluorescent lamps.

The effects of treatments were evaluated by recording the longevity and diameter of flowers after full opening. The flower longevity was recorded every day. Vase life of carnations was terminated when petal inrolling or wilting occurred. Flower diameter of 'Charlotte' and 'Dolce Vita' cultivars was measured on day 6th of the flower vase life. In experiments, 3 replicates each of 5 flowers were used per treatment. The results were tested by an analysis of variance and the Duncan's t-test at P=0.05.

Ethylene measurements. Experiment was conducted with the carnation cultivar 'Dolce Vita'. After 24 hours of treatment with 0.05 mM KIN, the stems were recut to a length of 5 cm and flowers were weighed. They were individually placed in vials containing distilled water and enclosed in 1 l glass jars. After incubation for 2 h at 20°C, 1 ml gas samples were withdrawn from the head space then injected into a flame ionization gas chromatograph (Hewlett Packard 5890). The amount of ethylene was calculated by comparison to its standard concentrations. Ethylene produced by flowers was measured daily. After each determination, the jars were aerated. Results are presented as nanoliters of ethylene produced per 1 gram of fresh weight of flowers per hour (nl $g^{-1}h^{-1}$) and are the mean (±SD) of six flowers.

Determination of ACC oxidase activity. ACC oxidase activity was determined according to Nijenhuis-de Vries et al. (1994) with some modifications. The petals of the outer whorls of flowers were frozen at –70°C

and ground with 10% polyvinylpolypyrrolidone (PVPP). The ground petals were homogenized in 2 ml g^{-1} tissue of 0.1 M Tris buffer (pH 8.0) containing 30 mM sodium ascorbate, 0.1 mM $FeSO_4$, 30% glycerol, 0.5 mM 1-aminocyclo-propane-1-carboxylic acid (ACC), 5 mM dithiothreitol, and 0.1% Triton X-100. The homogenate was centrifuged at 28000 g for 30 min at 4°C, and the supernatant was filtered. The extract (0.2 ml) was incubated in a 10-ml vials with 0.8 ml of the reaction mixture containing 0.1 mM Tricine (pH 6.5), 10 mM ACC, 30 mM sodium ascorbate, 50 μM $FeSO_4$ and 30 mM $NaHCO_3$. Vials were sealed and kept at 23°C for 1 hour. The ethylene concentration in the headspace was measured by gas chromatography. ACC oxidase activity was expressed as nanoliters of ethylene released per gram of fresh weight per hour (nl $g^{-1}h^{-1}$). Data are given as means (±SD) of six measurements.

RESULTS AND DISCUSSION

It is generally accepted that cytokinins in higher plants are synthesized mainly in the root system and transported via the transpiration stream to the shoot, where they regulate development and senescence. Endogenous level of cytokinins in carnation petals decreases with ageing (Van Staden and Dimalla, 1980). Also, Mayak and Halevy (1970) observed that the endogenous level of cytokinins in the petals of rose decreased as the flowers aged, and their level was lower in a short-lived cultivar than in a long-lived one. Treatment of flowers with exogenous BA and KIN may compensate for the reduction in supply of the endogenous compounds. When applied in 24-hour treatments both cytokinins (BA and KIN) did not increase the vase life of 'Charlotte' flowers as compared to the control (Tab. 1). There was a reduction in the vase life at a high BA concentration (0.5 mM). Although the longevity of 'Charlotte' flowers pulsed with BA or KIN was not extended, the flower diameter was increased when carnations were placed in 0.05 and 0.1 mM solutions of KIN (Tab. 1). A similar response to KIN was observed in 'Dolce Vita' cultivar, where treatments with 0.05 and 0.1 mM solutions significantly increased the flower diameter (Tab. 2). Also, the flower longevity was extended when 'Dolce Vita' was pulsed with 0.05 mM BA or KIN. The maximum vase life increase (by 45% over the control) was obtained when 0.05 mM KIN was used for pulse treatment (Tab. 2). There are many reports that cytokinins delay the senescence of cut carnation flowers (Eisinger, 1977; Cook et al., 1985; Mor et al., 1983; Bosse and Van Staden, 1989), although the results were inconsistent. In some cases cytokinins have been shown to stimulate petal senescence (Eisinger, 1977; Van Staden and Joughin, 1988). Van Staden and Joughin (1988) indicated that, according to the concentration used, cytokinins

Table 1. Effect of pulse treatment with benzyladenine (BA) and kinetin (KIN) on longevity and flower diameter of 'Charlotte' cut carnations

Treatment	Vase life [days]	Vase life [%]*	Flower diameter [mm]
Water (control)	9.4 bcd	100	83.9 a
BA 0.01 mM	10.7 d*	114	85.3 abc
BA 0.05 mM	10.3 cd	110	86.8 abc
BA 0.1 mM	8.9 abc	95	85.5 abc
BA 0.5 mM	7.6 a	81	84.8 ab
KIN 0.01 mM	9.2 bcd	98	86.0 abc
KIN 0.05 mM	10.5 d	112	88.7 c
KIN 0.1 mM	9.9 cd	105	88.0 bc
KIN 0.5 mM	8.1 ab	86	87.3 abc

*Values are percentages of the control

Table 2. Effect of pulse treatment with benzyladenine (BA) and kinetin (KIN) on longevity and flower diameter of 'Dolce Vita' cut carnations

Treatment	Vase life [days]	Vase life [%]*	Flower diameter [mm]
Water (control)	6.4 a*	100	76.5 a
BA 0.05 mM	8.1 b	123	78.8 ab
BA 0.1 mM	7.1 ab	107	78.5 ab
KIN 0.05 mM	9.6 c	145	80.0 b
KIN 0.1 mM	7.5 ab	114	79.0 b

*Explanations see Table 1

accelerated or retarded the senescence of cut carnations. The application of KIN and BA at relatively high levels accelerated flower senescence (Bosse and Van Staden, 1989; Woodson and Brandt, 1991). It was reported that continuous treatment of cut carnation flowers with benzyladenine at concentrations >1.0 μM induced premature senescence (Woodson and Brandt, 1991). Similarly, in our experiment pulse treatment of 'Charlotte' carnations with a higher concentration of BA (0.5 mM) reduced the flower longevity as compared to the control (Tab. 1). Woodson and Brandt (1991) suggested that this cytokinin-induced senescence is modified through an interaction with the gynoecium that leads to ACC accumulation and premature ethylene production in both the gynoecium and the petals. The cytokinins have also been shown to delay the senescence of carnations. Cook et al. (1985) reported that continuous treatment of cut carnations with benzylaminopurine and other cytokinins delays flower senescence. Pulse treatments of carnations with BA have resulted only in a marginal increase in the vase life of flowers (Boose and Van Staden, 1989; Van Staden et al., 1990a). Van Staden and Joughin (1988) reported that BA was ineffective in delaying senescence

when pulsed into the flower via the stem. This may be due to the distance over which BA has to move to reach the petals of a whole flower (Upfold and van Staden, 1992). Authors have reported that BA movement up the stem of a cut carnation is a function of both stem length and holding time. The inclusion of cytokinins in the vase solution in general delayed petal wilting when applied to cut carnations with short (5-10 cm) stems (Eisinger, 1977), but has little effect in flowers with a stem length as used in commercial practice. Our results with carnations 'Dolce Vita', 'Tanga', 'Domingo' and 'Impala' showed that pulse treatment with BA or KIN at an appropriate concentration can significantly prolong the longevity of flowers with long (40 cm) stems (Tab. 2, Fig. 1 and 2). Bosse and Van Staden (1989) have suggested that the efficiency of applied cytokinins may be related to their type used, concentration and mode of application and the physiological state of the flowers used for the experiments.

The senescence of carnation flowers is mediated by ethylene and associated with a climacteric-like burst of ethylene production (Halevy and Mayak, 1981). The treatment of cut carnations with ethylene inhibitors delays the climacteric rise in ethylene production and delays flower senescence (Veen, 1979, Altman and Solomos, 1995). The anti-senescent action of cytokinins in the carnation is thought to be through the blocking of ethylene biosynthesis (Eisinger, 1977; Mor et al., 1983; Cook et al., 1985) and action (Cook et al., 1985). In studies on the effect of cytokinins on ethylene biosynthesis in detached petals Mor et al. (1983) showed that pretreatment with BA, kinetin or zeatin blocked the conversion of externally supplied ACC to ethylene. BA also prevented the accumulation of endogenous ACC and inhibited ethylene production in ethylene-treated petals. However, these effects were only evident when ethylene was applied to preclimacteric flowers, suggesting that cytokinins do not directly inhibit the biosynthesis of ethylene, but rather delay the onset of the climacteric. Cook et al. (1985) reported that a continuous treatment of cut carnations with BA and other cytokinins inhibited both ethylene production and action, and delayed flower senescence. Also Bosse and Van Staden (1989) showed that dihydrozeatin was able to reduce and delay the production of endogenous ethylene and was effective in extending carnation flower longevity. Since the cultivar 'Dolce Vita' showed the greatest response to a kinetin treatment, i.e. 45% increase in vase life (Tab. 2), the effect of 0.05 mM KIN on ethylene production during the flower senescence was examined (Fig. 3). Cut carnations produced small amounts of ethylene just after harvest. In untreated flowers placed in distilled water, the peak of ethylene generation was observed a few days after harvest, just before petals began to wilt (Fig. 3). This autocatalytic production is the result of a feedback mechanism in which the produced

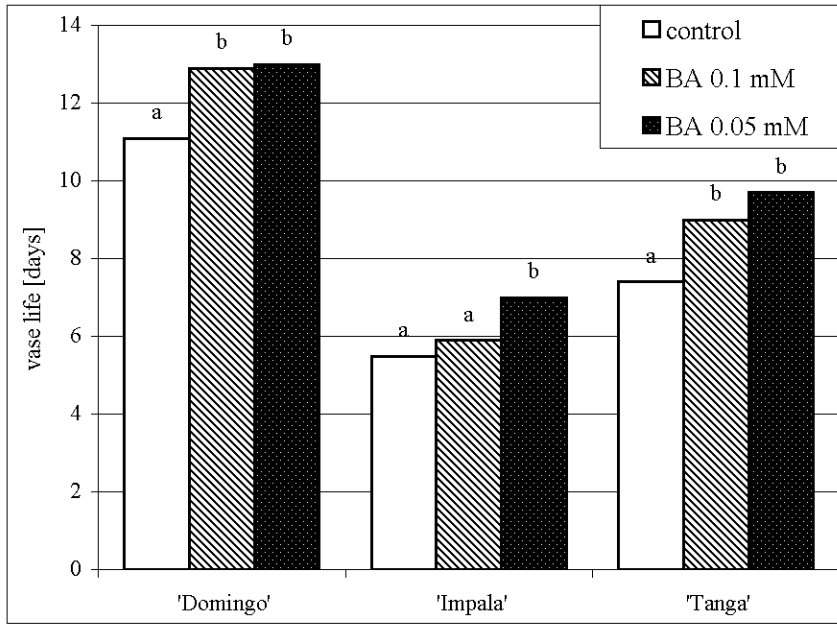

Figure 1. Effect of pulse treatment with benzyladenine on longevity of cut carnations ('Domingo', 'Impala', and 'Tanga' cultivars)

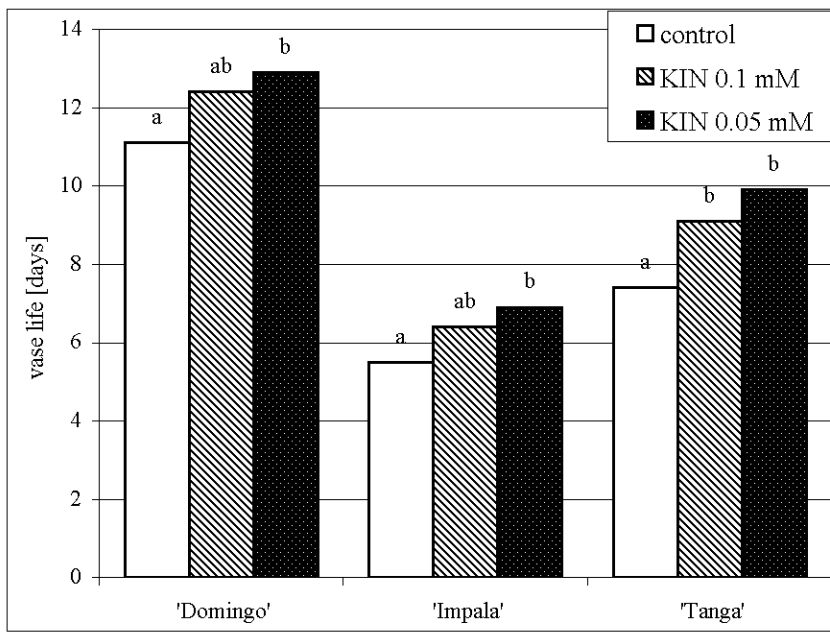

Figure 2. Effect of pulse treatment with kinetin (KIN) on longevity of cut carnations ('Domingo', 'Impala', and 'Tanga' cultivars)

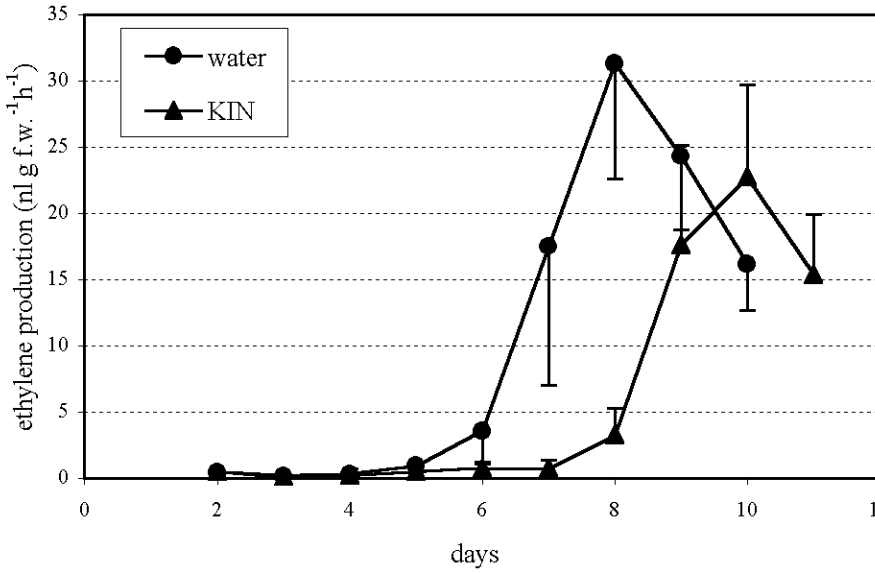

Figure 3. Effect of pulse treatments with 0.05 mM KIN on ethylene production in cut 'Dolce Vita' flowers

ethylene, through the interaction with a binding site, stimulates the activity of the enzymes involved in its biosynthesis. The produced ethylene initiates the biochemical changes leading to petal senescence (reviewed by Van Altvorst and Bovy, 1995). Compared to the control, 0.05 mM solution of KIN reduced the level of ethylene production, as well as delayed the time at which this occurred (Fig. 3). Similarly, in the experiments of Piskornik (1987) and Eisinger (1977) treatment of carnation flowers with kinetin inhibited ethylene production.

The influence of pulse treatment with KIN at a concentration of 0.05 mM on the activity of ACC oxidase in the carnation petals of 'Dolce Vita' flowers is shown in Table 3. The results demonstrate that there was a low activity of ACC oxidase in the petals of young carnations. The application of KIN markedly inhibited the activity of ACC oxidase in petals of wilting flowers when compared to the control. Thereafter, in wilted flowers, the activity of the enzyme declined to a level of 24.66 nl ethylene per g f.w. h in the control and 21.88 in the kinetin-treated flowers. Also, Mor et al. (1983) found that BA and KIN blocked the conversion of exogenous ACC to ethylene in carnation petals.

The best concentrations of BA and KIN (0.05 and 0.1 mM) were further examined by an application to 'Domingo', 'Impala' and 'Tanga' cultivars (Fig. 1 and 2). Benzyladenine and kinetin enhanced the flower longevity of tested cultivars when compared to the control. Pulse

Table 3. Activity of ACC oxidase from petals of 'Dolce Vita' flowers

Treatment	ACC oxidase activity [nl ethylene g f.w.$^{-1}$h^{-1}]		
	4 days after harvest	wilting	wilted
Water (control)	1.65 ± 2.00	74.20 ± 12.76	24.66 ± 4.42
KIN 0.05 mM	1.98 ± 2.37	33.88 ± 4.61	21.88 ± 3.67

treatment with BA at a concentration of 0.05 mM significantly prolonged the vase life of the three tested cultivars (Fig. 1). The effect of pulse treatment at 0.1 mM BA on the longevity of 'Domingo' and 'Tanga' cultivars was similar to that obtained with 0.05 mM BA. Likewise, the treatment of 'Impala' flowers with 0.1 mM BA was without any significant benefit. Flowers of all tested cultivars placed in 0.05 mM KIN for 24 hours showed a significant increase in their longevity in comparison to those in distilled water, although the treatment with a higher KIN concentration (0.1 mM) had no significant benefit for 'Impala' and 'Domingo' (Fig. 2).

Based on the results obtained, we can conclude that the longevity of cut carnations can be improved by pulse treatment with synthetic cytokinins, benzyladenine and kinetin. A significant increase in the vase life following BA and KIN treatments occurred for most studied cultivars ('Dolce Vita', 'Domingo', 'Impala' and 'Tanga'). They showed differences in response to cytokinin treatment from 17% ('Domingo') to 45% ('Dolce Vita') vase life increase. There was little or no response to cytokinin on 'Charlotte' flowers. The data suggested that cultivar may also be a factor affecting the efficiency of cytokinins in delaying flower senescence. Similarly, in the experiment of Paull and Chantrachit (2001) several anthurium cultivars showed different response to BA treatment: from 20% reduction to a 2.5 fold increase in vase life. Such differences in cultivar responsiveness to cytokinins can be related to the capacity determining the absorption of the applied compound or to different natural tissue levels of cytokinins. The longer vase life of 'Dolce Vita' carnations treated with 0.05 mM KIN was associated with a delay in ethylene evolution and a decrease of its production. The application of KIN to cut flowers also inhibited the activity of ACC oxidase in petals.

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WPŁYW KONDYCJONOWANIA W EGZOGENNYCH CYTOKININACH NA TRWAŁOŚĆ I PRODUKCJĘ ETYLENU PRZEZ CIĘTE GOŹDZIKI (*Dianthus caryophyllus* L.)

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S T R E S Z C Z E N I E

Badano wpływ 24-godzinnego traktowania egzogennymi cytokininami, BA i KIN, na trwałość ciętych goździków (*Dianthus caryophyllus* L.) odmian 'Dolce Vita', 'Impala', 'Domingo', 'Tanga' i 'Charlotte'. Trwałość ciętych goździków można przedłużyć kondycjonując je w KIN i BA o stężeniu 0,05 lub 0,1 mM. Znaczne przedłużenie trwałości kwiatów 'Dolce Vita', 'Impala', 'Domingo' i 'Tanga' uzyskano stosując 0,05 mM KIN lub BA. Jedynie 'Charlotte' nie reagowała pozytywnie na traktowanie cytokininami. KIN w stężeniu 0,05 i 0,1 mM zwiększała także, w porównaniu do wody destylowanej, średnicę kwiatów goździków 'Charlotte' i 'Dolce Vita'. Kondycjonowanie goździków 'Dolce Vita' w 0,05 mM KIN powodowało opóźnienie klimakterycznego wzrostu produkcji etylenu o 2 dni, zmniejszało poziom produkcji etylenu w kwiatach i hamowało aktywność oksydazy ACC w płatkach.

Słowa kluczowe: benzyloadenina, goździki, produkcja etylenu, kinetyna, trwałość kwiatów