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The promoted longevity of gerbera cut flowers using geranyl diphosphate and its analog

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Abstract: Terpenoids, the most abundant and structurally diverse class of plant secondary metabolites, play an important role in plants. With the purpose of obtaining new and better chemical preservatives, the present study investigated the effects of geranyl diphosphate (GDP), a precursor of terpenoids, and its analog on the vase life of cut flower gerbera. GDP and its analog were used in 5 different concentrations (0, 25, 50, 100, and 200 mg L^{-1}). Flowers were grouped into 9 different treatment groups: control (C), G25, G50, G100, G200, A25, A50, A100, and A200. The application of geranyl diphosphate and its analog resulted in increased solution uptake, fresh weight, soluble solids, membrane stability, and longevity of cut flowers and alleviated stem bending with the best observed results in the A100, A200, A50, and G100 treatment groups. According to the results, the present research indicated that GDP and its analog, and especially its analog, may promote the longevity of cut flowers via increased solution uptake, soluble solids, fresh weight, and membrane stability and alleviated stem bending.

Key words: Geranyl diphosphate, gerbera, monoterpenes, ornamental, postharvest, terpenoids

1. Introduction

Gerbera, a member of the Asteraceae family, is a valuable flower cultivated worldwide as a cut flower (Danaee et al. 2011). Terpenoids, the most abundant and structurally diverse class of plant secondary metabolites (Abrahim et al. 2000; Davis and Croteau 2000; Zinov'eva et al. 2001; Cheng et al. 2007; Van Schie et al. 2007; Schmidt et al. 2010), play an important role in different aspects of plant growth and development. These compounds have diverse functional roles in plants as many physiologically important substances such as vitamins, phytohormones, pheromones, photosynthetic pigments, electron carriers, and phytosterols are terpene-derived (McGarvey and Croteau 1995; Davis and Croteau 2000; Zinov'eva et al. 2001; Van Schie et al. 2007). It has been well documented that isoprenoids perform multiple ecological functions in plants, providing protection against herbivores and microbial diseases, attracting pollinators, and causing allelopathy (Abrahim et al. 2000; Zinov'eva et al. 2001). Isopentenyl pyrophosphate and dimethylallyl diphosphate, the terpene building blocks, may be produced via 2 pathways: in the cytosol by the mevalonic pathway and in the chloroplasts by the MEP pathway (Lichtenthaler 1999; Nogués et al. 2006; Van Schie et al. 2007). They are used by prenyltransferases to form geranyl diphosphate (GDP), farnesyl diphosphate, and geranylgeranyl diphosphate, the immediate precursors of monoterpenes, sesquiterpenes, and diterpenes, respectively (Cheng et al. 2007). GDP is considered a precursor of monoterpene (Van Schie et al. 2007). Monoterpene biosynthesis occurs in both compartments from GDP available in situ or translocated from its place of biosynthesis (Nogués et al. 2006). Monoterpene synthases, capable of generating acyclic, monocyclic, and bicyclic products, have been isolated from a variety of plants (Davis and Croteau 2000). It has been proposed that monoterpene cyclases are inhibited by analogs of GDP (Karp et al. 2007).

The longevity of cut flowers may be affected by different chemical preservatives in the vase solution (Prashanth et al. 2010). Effects of the application of plant growth regulators have been studied by many researchers. However, neither effects of GDP, one of the most important intermediate metabolites of terpenoid metabolism, nor those of its analog have been studied. With the purpose of obtaining new and better chemical preservatives, the present study was carried out to investigate the possible effects of GDP and its analog on the vase life of gerbera cut flowers.

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2. Materials and methods

Cut gerbera flowers, *Gerbera jamesonii* 'Stanza', were obtained from commercial growers. The red flowers were harvested just before sunrise at the mature stage. Experiments were performed in a postharvest room (22 ± 1 °C, 60 ± 5% relative humidity, and 12-h photoperiods). The 45-cm-long flowers were cut, weighed, and placed in 500-mL containers containing 350 mL of solution containing 3% sucrose, GDP (G) or its analog (A), and (E)-4-[2-diazo-3-trifluoropropionyloxyl-3-methyl-2-buten-l-yl-pyrophosphate] (Sigma) in 5 concentrations (0, 25, 50, 100, and 200 mg L^{-1}). Cut flowers were grouped into 9 different treatment groups with 3 replications and 5 flowers per replication: control (C), G25, G50, G100, G200, A25, A50, A100, and A200. Flowers in a solution containing 3% sucrose in distilled water were used as control samples.

The fresh weights of flowers were recorded every 3 days and expressed in grams. Solution uptake rates were estimated by measuring the vase solution remaining and were expressed as mL day^{-1} $stem^{-1}$. The amount of water evaporated in the solution uptake was not measured. Stem bending in gerbera was determined. Scape curvature was individually determined during vase life by measuring the angle of the scape by protractor with respect to its angle on day zero. Measurements were expressed in degrees.

The ion leakage percentage for estimation of membrane stability was estimated on the basis of the electrolyte leakage of petals using an electrical conductivity meter as described by Danaee et al. (2011). Briefly, the petal disks were rinsed in deionized water prior to incubation in 10 mL of deionized water for 3 h at room temperature. After incubation, the conductivity (EC1) of the bathing solution was measured with the conductivity meter. Petal disks were boiled with bathing solution for 15 min to kill tissue. After cooling to room temperature, the conductivity (EC2) of the bathing solution was measured again. Ion leakage was expressed as a percentage according to the formula given below.

The total amount of soluble solids of cut flower stems was measured by digital refractometer and expressed in degrees Brix. The vase life of cut flowers was completed when petals lost their turgidity. Vase life was determined from the number of days to senescence of the cut flowers.

Data were analyzed as a factorial experiment in a completely randomized design by analysis of variance using SPSS. Mean separation was performed with Duncan's multiple range test at $P < 0.05$.

3. Results

Treatments with GDP and its analog had significantly enhancing effects on solution uptake rates (Table 1; Figure 1). A100 and A200 resulted in higher solution uptake compared to other treatments. The decreasing rates of fresh weights were significantly alleviated with the application

Table 1. The effects of different concentrations of geranyl diphosphate and its analog on solution uptake rates of gerbera cut flowers (expressed as mL day^{-1} $stem^{-1}$) over 9 days.

Treatments Days	C	G25	G50	G100	G200	A25	A50	A10	A200
3	5.48 a*	5.67 a	6.43 a,b,c	6.39 a,b	6.19 a	6.55 a,b,c	6.31 a	7.44 c	7.332 b,c
6	2.1 a	2.49 a,b	2.65 b	3.67 d	3.104 b,c	3.656 c,d	4.30 d,e	4.69 e	5.044 e
9	1.36 a	2.12 b,c	1.86 a,b	3.34 d	1.67 ab	2.54 c	3.33 d	3.93 e	4.03 e

*Mean values (n = 3); values followed by different letters are significantly different at $P < 0.05$ according to Duncan's multiple range test.

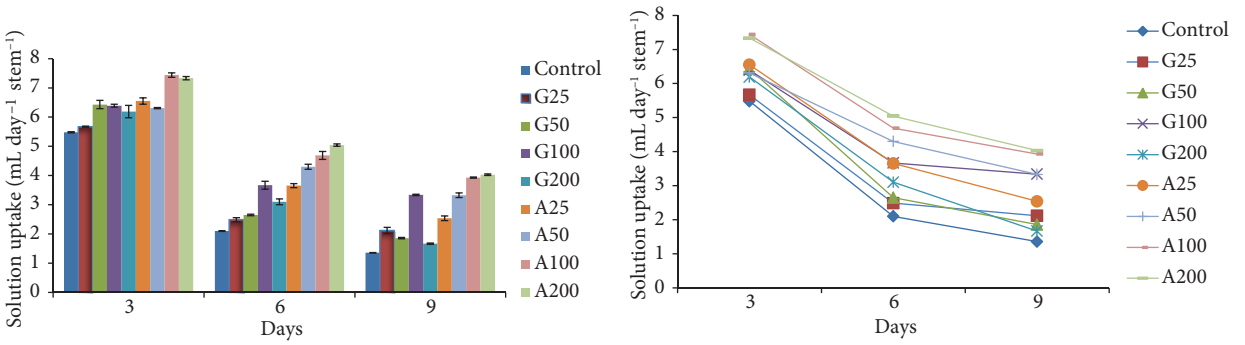

Figure 1. The effect of applied geranyl diphosphate and its analog on solution uptake rates of gerbera cut flowers. Vertical bars indicate the standard error of 3 replications.

of GDP/its analog, and the best results were obtained with A200, A100, A50 and G100, respectively (Table 2; Figure 2). Total soluble solids of the cut flowers significantly increased with the application of GDP/its analog (Table 3; Figure 3). The application of GDP/its analog resulted in significantly improved membrane stability (Table 4; Figure 4), and GDP/its analog significantly alleviated stem bending, as shown in Table 5 and Figure 5. The application of different concentrations of GDP/its analog promoted longevity of the cut flowers, as shown in Figure 6.

Table 2. The effects of different concentrations of geranyl diphosphate and its analog on fresh weights of gerbera cut flowers (expressed as grams) over 9 days.

Days	Treatments								
	C	G25	G50	G100	G200	A25	A50	A10	A200
0	30.64 a*	28.45 a	30.08 a	28.87 a	29.17 a	29.66 a	27.33 a	31.81 a	29.6 a
3	25.14 a	28.42 a	29.5 a	29.13 a	28.6 a	29.36 a	27.15 a	30.87 a	29.756 a
6	20.23 a	24.9 a,b	26.11 a,b	28 b	25.5 a,b	24.52 a,b	24.8 a,b	28.7 b	28.19 b
9	13.6 a	18.9 a,b,c	19.5 b,c	22.4 b,c	19.3 b,c	18.3 a,b	21.7 b,c	24.1 c	24.56 c

*Mean values (n = 3); values followed by different letters are significantly different at $P < 0.05$ according to Duncan's multiple range test.

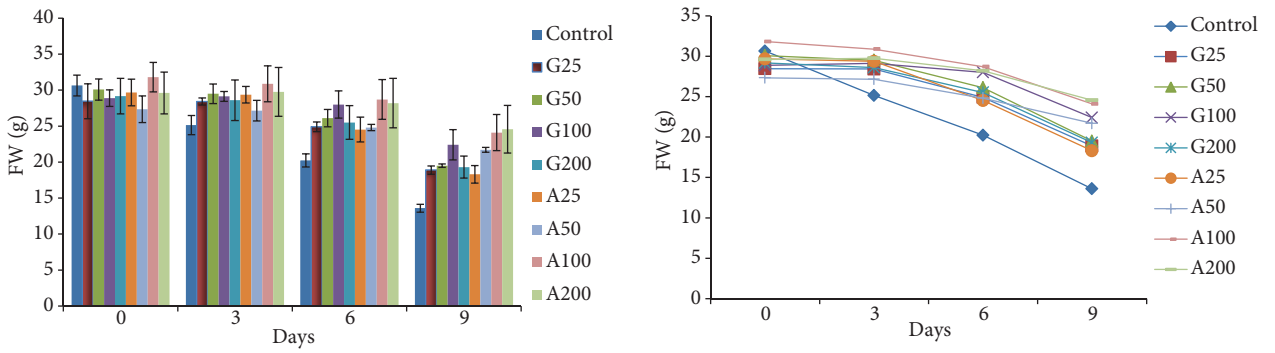

Figure 2. The effect of applied geranyl diphosphate and its analog on the fresh weight (FW) of gerbera cut flowers. Vertical bars indicate the standard error of 3 replications.

Table 3. The effects of different concentrations of geranyl diphosphate and its analog on total soluble solids of gerbera cut flowers (expressed as degrees Brix) over 9 days.

Days	Treatment								
	C	G25	G50	G100	G200	A25	A50	A10	A200
0	4.3 a*	4.4 a	4.2 a	4.3 a	4.3 a	4.5 a	4.1 a	4.5 a	4.63 a
3	1.66 a	3.5 b,c	3.1 b,c	4.1 b,c	2.9 b	4.4 b,c	4.43 b,c	4.16 b,c	4.66 c
6	1.066 a	3.96 b,c,d	2.766 c	3.36 b,c,d	2.66 b	4.93 d,e	4.3 c,d,e	4.73 d,e	5.26 e
9	-	2.93 b,c	3 b,c	3.6 b,c,d	2.43 b	3.86 c,d	3.13 b,c	4.16 c,d	4.8 d

*Mean values (n = 3); values followed by different letters are significantly different at $P < 0.05$ according to Duncan's multiple range test.

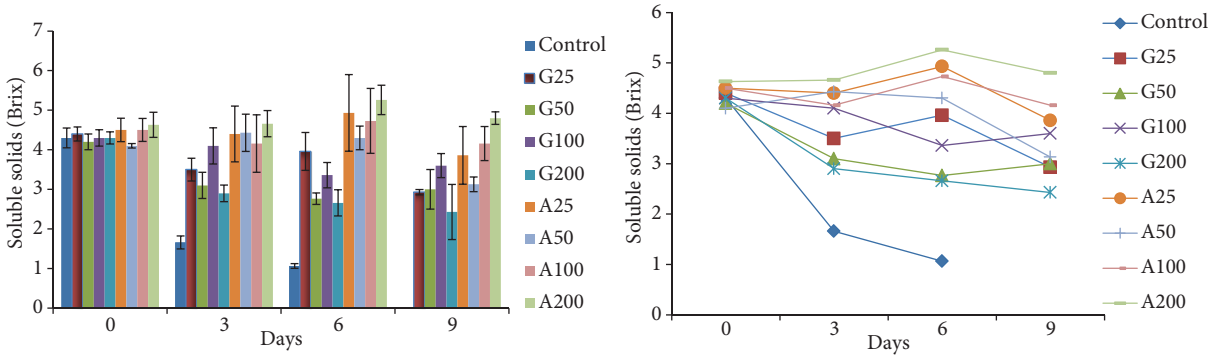

Figure 3. The effect of applied geranyl diphosphate and its analog on total soluble solids contents of gerbera cut flowers. Vertical bars indicate the standard error of 3 replications.

Table 4. The effects of different concentrations of geranyl diphosphate and its analog on ion leakage percentages of gerbera cut flowers (expressed as a percentage) over 9 days.

Days	Treatment								
	C	G25	G50	G100	G200	A25	A50	A10	A200
0	12.86 a*	12.51 a	13.5 a	13.8 a	12.51 a	13.55 a	12.17 a	12.96 a	13.6 a
3	40.2 d	35.4 b,c	33.8 b,c	31.9 b	30.026 c	32.11 b,c	32.62 b,c	20.21 a	20.4 a
6	59.41 c	35.44 a,b	34.94 a,b	33.3 a,b	41.12 c	35.72 a,b	33.2 a,b	28.28 a	25.5 a
9	85.1 e	53.1 d	44.6 b	43.9 b	51.12 c,d	45.5 b,c	43.6 b,c	36.6 a	33.8 a

*Mean values (n = 3); values followed by different letters are significantly different at $P < 0.05$ according to Duncan's multiple range test.

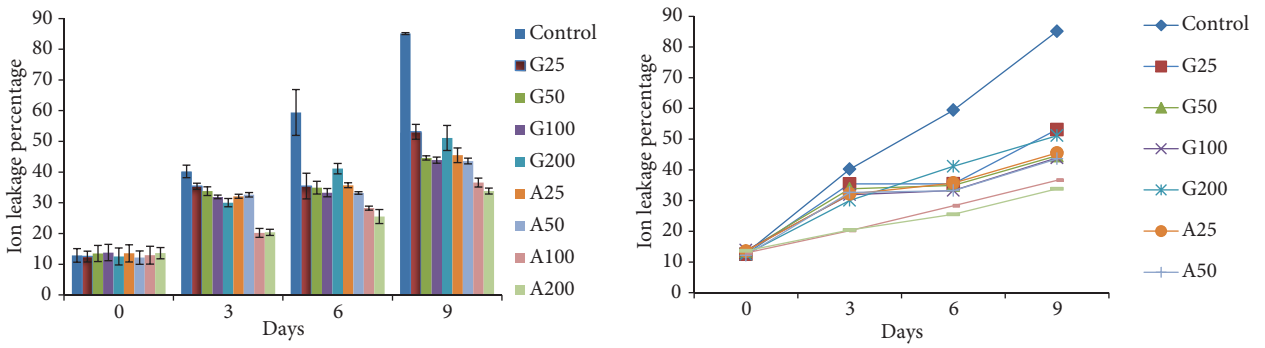

Figure 4. The effect of applied geranyl diphosphate and its analog on ion leakage percentages of gerbera cut flowers. Vertical bars indicate the standard error of 3 replications.

Table 5. The effects of different concentrations of geranyl diphosphate and its analog on stem bending of gerbera cut flowers (expressed in degrees) over 9 days.

Days	Treatment								
	C	G25	G50	G100	G200	A25	A50	A10	A200
0	12.55 a*	12.55 a	12.66 a	12.76 a	11.33 a	11.00 a	12.55 a	11.00 a	10.66 a
3	42.44 b	16.55 a	18.30 a	18.31 a	18.66 a	15.83 a	12.99 a	12.77 a	12.83 a
6	73.33 b	23.55 a	29.94 a	18.05 a	20.3 a	25.66 a	14.6 a	14.6 a	14.3 a
9	90.00 d	39.66 b,c	38.77 b,c	33.7 a,b,c	44.44 c	31.6 a,b	16.1 a	16.0 a	17.0 a

*Mean values (n = 3); values followed by different letters are significantly different at $P < 0.05$ according to Duncan's multiple range test.

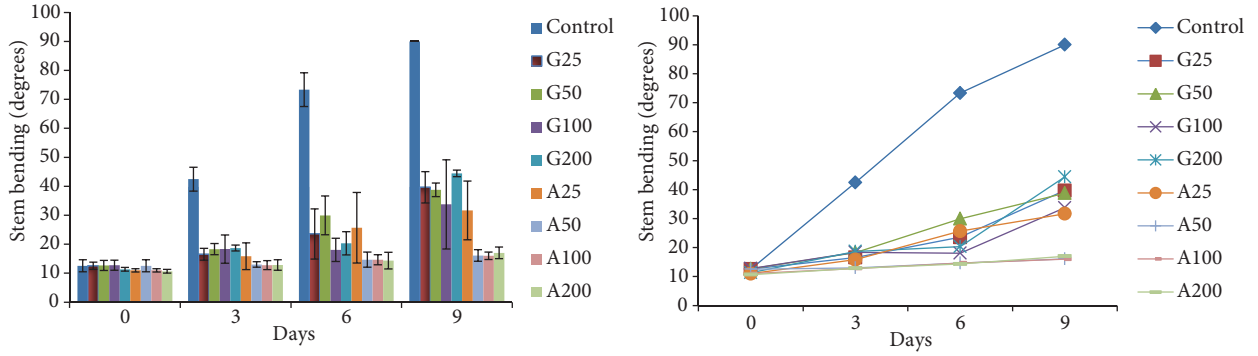

Figure 5. The effect of applied geranyl diphosphate and its analog on stem bending of gerbera cut flowers. Vertical bars indicate the standard error of 3 replications.

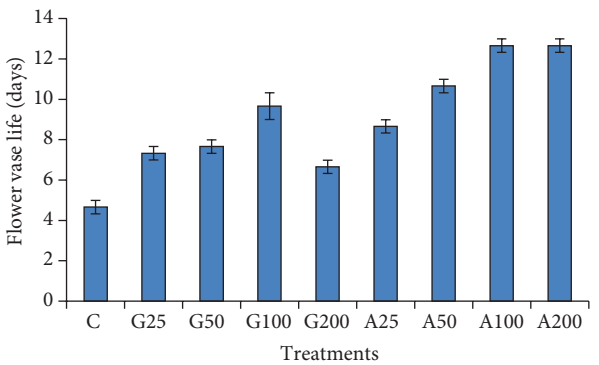

Figure 6. The effect of applied geranyl diphosphate and its analog on the longevity of gerbera cut flowers. Vertical bars indicate the standard error of 3 replications.

4. Discussion

The application of GDP and its analog resulted in increased solution uptake, fresh weight, soluble solids, longevity of cut flowers, and membrane stability and decreased stem bending, with the best observed results in the A100, A200, A50, and G100 treatment groups, respectively. GDP is considered a precursor of monoterpene. However, Van Schie et al. (2007) stated that GDP is linked to the gibberellin (GA) biosynthesis pathway. Therefore, GDP could be used to produce monoterpenes and GA. Thus, it seems that monoterpenes and probably GA were responsible for GDP-promoted longevity. As monoterpene cyclases were inhibited by the GDP analog (Karp et al. 2007), it could be used for the production of other kinds of terpenoids such as gibberellic acid, sterols, and other antimicrobial terpenoids, with every kind of these isoprenoid compounds involved in different aspects of metabolism. Therefore, we propose that the analog-enhanced production of different kinds of terpenoids, including sesquiterpenes (abscisic acid and antibiotic terpenes), diterpenes (GA and phytoalexin), and triterpenes (sterols, as a membrane component), may promote the longevity of cut flowers. Monoterpenes are quantitatively the most important compounds and have a strong influence on plant protection against abiotic and biotic stresses (Nogués et al. 2006). Many specific terpenoid compounds serve in communication and defense (McGarvey and Croteau 1995). In addition, certain diterpenes and sesquiterpenes are phytoalexins implicated in the direct defense of plants against microbial pathogens (Cheng et al. 2007). The metabolism of terpenoids in plants may be impaired during stress conditions (Zinov'eva et al. 2001).

GDP and its analog, especially its analog, had a stimulating effect on the total soluble solids. The increase in soluble solids could promote longevity. It seems that the terpenoids elevated by GDP and its analog, especially GA, were responsible for increased soluble solids via modification of the source–sink metabolism. Senescence of cut flowers is controlled by hormones and correlated with the carbohydrate status of the petals (Singh et al. 2008). Cut flower longevity is also correlated with the carbohydrate content (van Doorn 2004; Singh et al. 2008). Gibberellins may induce special physiological responses and alter the source–sink metabolism via sink formation (Iqbal et al. 2011). These hormones could stimulate phloem loading (Iqbal et al. 2011). By improving the sink strength, they are the key regulators of plant metabolism and photosynthate translocation (Iqbal et al. 2011). The application of GA resulted in decreased accumulation of active oxygen species through GA-increased activities of antioxidant enzymes and alleviated lipid peroxidation in cucumber (Li et al. 2011). Gibberellic acids stimulate sugar transport (Moreno et al. 2011).

The application of GDP and its analog resulted in higher solution uptake; however, the analog treatments, especially A100 and A200, were more effective. Decreased microbial infection induced by GDP, and especially by its analog, could lead to increased solution uptake. Using SEM observation, He et al. (2009) stated that the vascular

blockage resulted from bacteria. It is well recognized that terpenoids are responsible for plant resistance to pathogenic microorganisms (McGarvey and Croteau 1995; Abrahim et al. 2000; Zinov'eva et al. 2001; Cheng et al. 2007). Monoterpenes function as chemical barriers and inhibit parasite penetration, producing a preinfective effect (Zinov'eva et al. 2001). There is a positive correlation between vase life and solution uptake in cut flowers (Nazari Deljou et al. 2011).

GDP and the applied analog of GDP led to less stem bending of cut flowers, and the analog treatments, especially at concentrations of 100 and 200 mg L^{-1}, were most effective. Decreased microbial infection, induced by GDP and especially its analog, could lead to increased solution uptake and alleviate stem bending. In addition, GA-induced sugar transport could result in inhibited premature senescence. Cut gerbera longevity is often limited by bending of the flower stalk, a premature senescence (Prashanth et al. 2010). The stem biochemical status is directly involved in stem bending (Ferrante and Serra 2009). Significantly higher water content and reduced stem bending and flower senescence resulted from applied exogenous GA (Emongor 2004). Stem bending may be due to lower water potential and changes in physiological and biochemical components in the cut flower (Prashanth et al. 2010).

GDP, and especially its analog, were effective treatments for maintaining membrane stability. Increased monoterpenes and sterols induced by the applied treatments caused improved membrane stability. Various studies have demonstrated that the membrane stability index decreases rapidly with the age of the cut flower (Ezhilmanthi et al. 2007; Singh et al. 2008; Danaee et al. 2011). GA_3-restricted oxidation of polyunsaturated fatty acids may lead to reduced lipid peroxidation (Singh et al. 2008). Plant sterols, terpene-derived components of membranes, regulate fluidity and permeability (Zunino and Zygadlo 2005). Monoterpenes, as lipophilic compounds, may alter fluidity and the physical arrangement of membrane phospholipids (Zunino and Zygadlo 2005).

Overall, the changes induced by GDP and its analog promoted longevity in gerbera cut flowers, and the greatest longevity was detected with A100, A200, A50, and G100, respectively. According to our results, increased longevity of cut flowers using GDP as a preservative could result from the production of monoterpenes, which are antimicrobial compounds. GDP at 200 mg L^{-1}, the highest amount used, did not result in the greatest longevity. This result may be due to the toxic effects of some terpenoids, especially at high levels. Monoterpenes are toxic towards vascular plants (Zunino and Zygadlo 2005). The interaction of monoterpenes with mitochondrial respiration may be responsible for their inhibitory activity in plants (Abrahim et al. 2000). The results obtained from the present study indicate that the GDP analog, especially at concentrations of 100 and 200 mg L^{-1}, is more effective than GDP at increasing the longevity of gerbera cut flowers and had considerable effects.

From the present research we conclude that GDP and its analog, especially its analog, could promote the longevity of cut flowers via increased solution uptake (probably due to the antimicrobial properties of some terpenoids), soluble solids (with modification of the source–sink metabolism and induction of hydrolytic enzymes), fresh weight, and membrane stability (due to effects of terpenoids, especially GA, and monoterpenes and sterols on membrane lipid composition, fluidity, and permeability) and alleviated stem bending (via decreased microbial infection and increased uptake, soluble solids, and membrane stability).

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References

Abrahim D, Braguini WL, Kelmer-Bracht A, Ishii-Iwamoto E (2000) Effects of four monoterpenes on germination, primary root growth, and mitochondrial respiration of maize. J Chem Ecol 26: 611–624.

Cheng AX, Lou YG, Mao YB, Lu S, Wang L, Chen XY (2007) Plant terpenoids: biosynthesis and ecological functions. J Integr Plant Biol 49: 179–186.

Danaee E, Mostofi Y, Moradi P (2011) Effect of GA_3 and BA on postharvest quality and vase life of gerbera (*Gerbera jamesonii*. cv. Good Timing) cut flowers. Hortic Environ Biotechnol 52: 140–144.

Davis EM, Croteau R (2000) Cyclization Enzymes in the Biosynthesis of Monoterpenes, Sesquiterpenes, and Diterpenes. Topics in Current Chemistry 209. Springer-Verlag, Berlin.

Emongor VE (2004) Effect of gibberellic acid on postharvest quality and vase life of gerbera cut flowers (*Gerbera jamesonii*). J Agron 3: 191–195.

Ezhilmanthi K, Singh VP, Arora A, Sairam RK (2007) Effect of 5-sulfosalicylic acid on antioxidant activity in relation to vase life of *Gladiolus* cut flowers. J Plant Growth Regul 51: 99–108.

Ferrante A, Serra G (2009) Lignin content and stem bending incidence on cut gerbera flowers. Acta Hortic 847: 377–384.

He S, Xiao D, Liu J, He S, Tu S, Lu P (2009) Anatomical structure observation of stem blockage in cut gerbera flowers. Acta Hortic Sin 36: 1077–1082.

Iqbal N, Nazar R, Khan MIR, Masood A, Khan NA (2011) Role of gibberellins in regulation of source-sink relations under optimal and limiting environmental conditions. Curr Sci 100: 998–1007.

Karp F, Zhao Y, Santhamma B, Assink B, Coates RM, Croteau RB (2007) Inhibition of monoterpene cyclases by inert analogues of geranyl diphosphate and linalyl diphosphate. Arch Biochem Biophys 468: 140–146.

Li Q, Li C, Yu X, Shi Q (2011) Gibberellin A_3 pretreatment increased antioxidative capacity of cucumber radicles and hypocotyls under suboptimal temperature. Afr J Agric Res 6: 4091–4098.

Lichtenthaler HK (1999) The 1-deoxy-D-xylulose-5-phosphate pathway of isoprenoid biosynthesis in plants. Annu Rev Plant Physiol Plant Mol Biol 50: 47–65.

McGarvey DJ, Croteau R (1995) Terpenoid metabolism. Plant Cell 7: 1015–1026.

Moreno D, Berli FJ, Piccoli PN, Bottini R (2011) Gibberellins and abscisic acid promote carbon allocation in roots and berries of grapevines. J Plant Growth Regul 30: 220–228.

Nazari Deljou MJ, Khalighi A, Arab M, Karamian R (2011) Postharvest evaluation of vase life, stem bending and screening of cultivars of cut gerbera (*Gerbera jamesonii* Bolus ex. Hook f.) flowers. Afr J Biotechnol 10: 560–566.

Nogués I, Brilli F, Loreto F (2006) Dimethylallyl diphosphate and geranyl diphosphate pools of plant species characterized by different isoprenoid emissions. Plant Physiol 141: 721–730.

Prashanth P, Chandra Sekhar R, Chandra Sekhar Reddy K (2010) Influence of floral preservatives on scape bending, biochemical changes and post harvest vase life of cut gerbera (*Gerbera jamesonii* bolus ex. Hook.). Asian J Hortic 5: 1–6.

Schmidt A, Wächtler B, Temp U, Krekling T, Séguin A, Gershenzon J (2010) A bifunctional geranyl and geranylgeranyl diphosphate synthase is involved in terpene oleoresin formation in *Picea abies*. Plant Physiol 152: 639–655.

Singh A, Kumar J, Kumar P (2008) Effects of plant growth regulators and sucrose on post harvest physiology, membrane stability and vase life of cut spikes of gladiolus. Plant Growth Regul 55: 221–229.

Van Doorn WG (2004) Is petal senescence due to sugar starvation? Plant Physiol 134: 35–42.

Van Schie CCN, Ament K, Schmidt A, Lange T, Haring MA, Schuurink RC (2007) Geranyl diphosphate synthase is required for biosynthesis of gibberellins. Plant J 52: 752–762.

Zinov'eva SV, Udalova ZV, Vasil'eva IS, Vanyushkin SA, Paseshnichenko VA (2001) Role of isoprenoids in plant adaptation to biogenic stress induced by parasitic nematodes. Appl Biochem Microbiol 37: 456–462.

Zunino MAP, Zygadlo JA (2005) Changes in the composition of phospholipid fatty acids and sterols of maize root in response to monoterpenes. J Chem Ecol 31: 1268–1283.