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Growth Regulators Prolong Bract Longevity of Potted Bougainvillea

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Abstract. When bougainvilleas are subjected to indoor low-light conditions, flower bracts regularly abscise. This study elucidates the effects of plant growth regulators on bract longevity of potted bougainvillea. Potted 'Taipei Red' bougainvillea in four different bract development stages were treated with 1-MCP (1-methylcyclopropene), NAA (1-naphthaleneacetic acid), SNA (sodium salt of naphthaleneacetic acid), IBA (indolebutyric acid), BA (6-benzylaminopurine), KH_2PO_4 (potassium dihydrogen phosphate), Put (diamine putrescine), SA (salicylic acid), or STS (silver thiosulfate) and were moved to indoor low-light conditions after treatments. Experimental results indicate that 1-MCP, NAA, SNA, BA, Put, and SA prolonged bract longevity, and this effect increased as bract stage increased. The effect of STS was significant in early bract stages and decreased as bract stages increased. Additionally, 1-MCP, NAA, SNA, BA, Put, SA, and STS treatment significantly reduced endogenous ACC (1-aminocyclopropene-1-carboxylate) content and ACC oxidase activity, suggesting that the inhibition of ethylene production was achieved via physiological metabolism. However, treatment with IBA or KH_2PO_4 had no effect on the bract longevity at any stage. In the combined chemical treatments, NAA + STS or NAA + SA were effectively for prolonging bract longevity and contained less protein or chlorophyll degradation, decrease ACC oxidase or ethylene production than the control. In conclusion, we propose that combined chemical treatment significantly prolonged the bract longevity and more effectively than single chemical treatment at any stage.

Additional key words**:** ACC content, ACC oxidase, bract stage, ethylene

Introduction

Bougainvillea is a popular ornamental plant and a genus of flowering plants in the Nyctaginaceae family, which is native to South America. Previous studies have shown that bougainvillea flowering is enhanced by appropriate regulation of environmental factors, such as the photoperiod (Allard, 1935), light intensity (Hackett and Sachs, 1968), temperature (Hackett and Sachs, 1966; Norcini, 1993), and water (Henrard, 1976). Effective culture management includes pruning (Cherian et al., 2004) and the application of plant growth regulators, including chlormequa, daminozide (Hackett and Sachs, 1967), ancymidol (Criley, 1977) and dikegulac (Norcini et al., 1994). However, dark transport or indoor low-light conditions typically induce flower and leaf abscission or significantly decrease bract longevity (Custódia et al., 2001; Gago et al., 2001). Cameron and Reid (1981) and Chang and Chen (2001) applied STS to potted 'Taipei Red' bougainvillea to antagonize the ethylene-mediated reduction in bract longevity, thereby prolonging bract longevity. Therefore, how to prevent bougainvillea bract abscission is an important issue. Inhibitory effects of 1-MCP on ethylene biosynthesis with reduced activities of ACC synthase and ACC oxidase and their respective gene transcriptions have been reported for peaches (Mathooko et al., 2001), bananas (Pathak et al., 2003), and plums (Khan and Singh, 2007). Notably, 1-MCP has been widely applied to harvested ornamental plants to prolong flower longevity of rieger begonia, roses, kalanchoes (Serek et al., 1994), and geraniums (Cameron and Reid, 2001), and to inhibit ethylene action in carnations (Serek et al., 1995). The 1-MCP treatment also inhibited ACC synthase activity in open flowers and ACC oxidase activity in floral buds of dendrobium (Uthaichay et al., 2007). In a fruit tree study, the rooting of kiwi fruit (Ücler et al., 2004) and olives (Özelbaykal and Gezerel, 2005) was promoted by treatment with low level of auxin. Conversely, a high level of auxin triggered production of ethylene in potatoes and apples (Richard, 1949; Yuan and Carbaugh, 2007). Treatment with 0.11 mM NAA prevented

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apple fruit abscission and ripening (Cin et al., 2008; Yuan and Carbaugh, 2007). Treatment with NAA or 2,4-D also prevented flower abscission of *Cestrum elegans*. (Abebie et al., 2008). Auxins influence ACC synthase activity in the ethylene pathway; however, the degree to which its mRNA levels increase due to auxin application differed among different fruit trees (Coenen et al., 2003; Ishiki et al., 2000). Additionally, low levels of auxins, such as NAA, inhibited the abscission of flower buds and fruit, such as *Bougainvillea* spp. (Murphy 2002; Saifuddin et al., 2009) and suspended fruit drop for ripening apples (Yuang and Carbaugh, 2007).

As an ethylene action inhibitor, STS prolonged flower longevity, for ornamental plants, such as *Dendrobium* spp (Uthaichay et al., 2007), *Lathyrus latifolius* (Koike and Imanishi, 2009), and *Delphinium* spp. (Tanase et al., 2009). Polyamines, such as Put, Spd (triamine spermidine) and Spm (tetraamine spermine), are polycationic compounds with low molecular weights that exist in all living organisms (Liu et al., 2006). Polyamines are a new category of plant growth regulators and that are purportedly involved in ethylene biosynthase of ornamental plants, such as *Dianthus caryophyllus* L. (Lee et al., 1997), and *Helianthus annuus* (Alvarez et al., 2003). Notably, SA, a simple phenolic compound, is involved in regulation of many processes associated with plant growth and development, such as delayed fruit ripening, likely through inhibition of ethylene action (Zhang et al., 2003). A previous study demonstrated that cytokinin prevented flower bud abscission of bell peppers (Wien and Zhang, 1991). Additionally, GA_{4+7} + BA effectively increased flower longevity and *Tulipa gesneriana* quality (Kim and Miller, 2009). However, little information exists for how plant growth regulators affect bougainvillea bract longevity. Therefore, the effects of plant growth regulators on ACC content and ACC oxidase activity as well as ethylene production in bracts of potted bougainvillea are of considerable interest. The aims of the present research are twofold: (1) this study investigates whether pretreatment with plant growth regulators prolongs bract longevity and, (2) if so, this study determines how this treatment functions in terms of ethylene biosynthesis.

Materials and Methods

Plant Material

One-year-old seedlings of potted 'Taipei Red' bougainvillea were obtained from a commercial bougainvillea production garden and planted in 7-inch pots with medium (2900 mL) composed of field soil and peat at a ratio of 7:3. Experiment 1 used seedlings with an average height of 47.4 cm and 13.5 nodes on averages. Experiments 2 and 3 used seedlings with an average height of 45.7 cm with 13.8 nodes on average. The seedlings were watered when the surface medium was slightly dry. For fertilization, 3 g of osmocote was regularly supplied at monthly intervals.

1-MCP and Auxin Treatment

Bract development was divided into four stages based on bract diameter: 0.5-0.9 cm (stage 1), 1.0-1.4 cm (stage 2), 1.5-1.9 cm (stage 3) and 2.0 cm and over (stage 4), and the plants were labeled accordingly. Subsequently, the potted 'Taipei Red' bougainvillea seedlings were placed in a hermetically sealed fumigation box (L90 cm × W50 cm × H50 cm) at 25 ± 1℃ and then fumigated with powdered 1-MCP (EthylBloc, Biotechnologies for Horticulture, Inc., USA) at concentrations of 800 $nL{\cdot}L^{-1}$ for 4 h or each plant was sprayed with 0.13 mM NAA, 0.12 mM SNA, 0.12 mM IBA, or water (control treatment) (experiment 1). Each treatment had five potted plants, and each plant had all four bract stages. Bracts of four flowers in the same stage were considered to be a unit, and four units formed a replication.

Treatment with Plant Growth Regulators

Bract development was divided into four stages based on experiment 1. Each plant was sprayed with 0.04 mM BA, 14.7 mM KH_2PO_4, 0.13 mM NAA, 1 mM Put, 0.5 mM SA, 0.5 mM STS, or water (control) (experiment 2). Each treatment had five potted plants, and each plant had all four bract stages. Bracts of four flowers in the same stage were considered to be a unit, and four units formed a replication.

Combined Chemicals Treatment

Bract development was divided into four stages based on experiment 1. Each plant was sprayed with 0.13 mM NAA + 0.04 mM BA, 0.13 mM NAA + 1 mM Put, 0.13 mM NAA + 14.7 mM KH_2PO_4, 0.13 mM NAA + 0.5 mM SA, 0.13 mM NAA + 0.5 mM STS, or water (control) (experiment 3). Each treatment had five potted plants, and each plant had all four bract stages. Bracts of four flowers in the same stage were considered to be a unit, and four units formed a replication.

1-Aminocyclopropene-1-Carboxylate (ACC) Content

Four different stages of bract development were used. ACC content was assayed using the method described by Lizada and Yang (1979). The samples were then volume of 1 mL of the gas was extracted for analysis with a GC-14A gas chromatograph (Shimazdu, Japan).

1-Aminocyclopropene-1-Carboxylate (ACC) Oxidase

Four different stages of bract development or detach leaves were used. The ACC oxidase detection method was modified from a previously described method (Mekhedov and Kende, 1996; Ververdis and John, 1991). The samples were then volume of 1 mL of the gas was extracted for analysis with a GC-14A gas chromatograph (Shimazdu, Japan).

Ethylene Production

For each sample, bracts from the same stage or leaves were placed in a 40 mL sealed plastic jar at a temperature of 20-28℃ for 2 h under continuous illumination from cool-white fluorescent lamps providing 15-16 $\mu mol \cdot m^{-2} \cdot s^{-1}$. A 1.0 mL gas sample was injected into a Shimadzu GC-14A gas chromatograph with a flame ionization detector. The carrier gas was nitrogen and the standard gas was 1 $mg \cdot L^{-1}$ ethylene.

Protein Assay

Protein was extracted as described previously (Bradford, 1976) from samples of detached leaf segments (0.1 g) taken from the stem below the apical bud on 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, and 43 d of bract longevity.

Chlorophyll Determination

Chlorophyll was extracted by ethanol as described previously (Wintermans and De Mots, 1965), from samples of detached leaf segments (0.1 g) taken from the stem below the apical bud on 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, and 43 d of bract longevity.

Experimental Design and Statistical Analyses

After the treatments, the plants were placed indoors at 25 ± 1℃ with a relative humidity (RH) of 60-80% under 12-h illumination from cool-white fluorescent lamps providing a light intensity of 15-16 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Experiments 1-3 were designed using a completely randomized design and were conducted twice. During the experimental period, bract drop, leaf abscission, leaf chlorosis and bract longevity (number of days from the treatment to bract abscission) were recorded daily. The between-treatments ANOVA used CoStat 6.2 (CoHort Software, Monterey, CA, USA), and bar charts and regression maps were created using the SigmaPlot software (version 11.0, Systat software, Inc., Richmond, CA, USA).

Results

Bract Longevity after 1-MCP or Auxin Treatment

Four groups (stages 1-4) of potted 'Taipei Red' bougainvillea plants were treated with 800 $nL \cdot L^{-1}$ for 4 h or foliar sprayed with 0.13 mM NAA, 0.12 mM SNA, and 0.12 mM IBA to determine if these chemicals would prolong bract longevity. The plants were placed indoors under low-light after the treatments. The results showed that 4 h of fumigation with 800 $nL \cdot L^{-1}$ 1-MCP prolonged bract longevity compared to the control. The NAA and SNA treatments, but not the IBA treatment, were as effective as the 1-MCP treatment prolonging bract longevity (Table 1). Treatments with 1-MCP, NAA or SNA were effective for prolonging bract longevity in stages 3 and 4 but not in stages 1 or 2. The IBA and control treatments did not prolong bract longevity for any of the bract stages. However, treatment with 1-MCP prolonged bract longevity by 3.4, 4.2, 8.4, and 8.4 d during bract stages 1, 2, 3, and 4, respectively. Additionally, treatment with NAA prolonged bract longevity by 15.8, 23.2, 36.6, and 40.2 d during bract stages 1, 2, 3, and 4, respectively. Moreover, treatment with SNA also prolonged bract longevity by 13.2, 17.2, 21.6, and 23.2 d during bract stages 1, 2, 3, and 4, respectively (Table 1).

ACC Content and ACC Oxidase Activity of Potted 'Taipei Red' Bougainvillea

To identify the mechanisms involved in prolonging bract longevity by these chemical treatments, the activities of endogenous ACC content and ACC oxidase were determined.

Table 1. Effects of 1-MCP and auxin applications on bract longevity of different stages in potted bougainvillea.

Treatment	Bract longevity (d)				
	Stage 1	Stage 2	Stage 3	Stage 4	$LSD_{0.05}$
Control[z]	10.2 b A[y]	10.2 b A	9.0 d B	8.2 d B	0.9
800 $nL \cdot L^{-1}$ 1-MCP	13.6 b B	14.4 b B	17.4 c A	16.6 c A	1.5
0.12 mM IBA	11.4 b A	11.0 b A	11.8 cd A	11.2 d A	2.1
0.12 mM SNA	23.4 a A	27.4 a A	30.6 b A	31.4 b A	8.2
0.13 mM NAA	26.0 a B	33.4 a B	45.6 a A	48.4 a A	10.1
$LSD_{0.05}$	6.4	6.5	5.7	4.5	

[z]Each value represents the mean of five replicates.
[y]Mean separation within the same rows (uppercase) and columns (lowercase) by LSD test, $P < 0.05$.

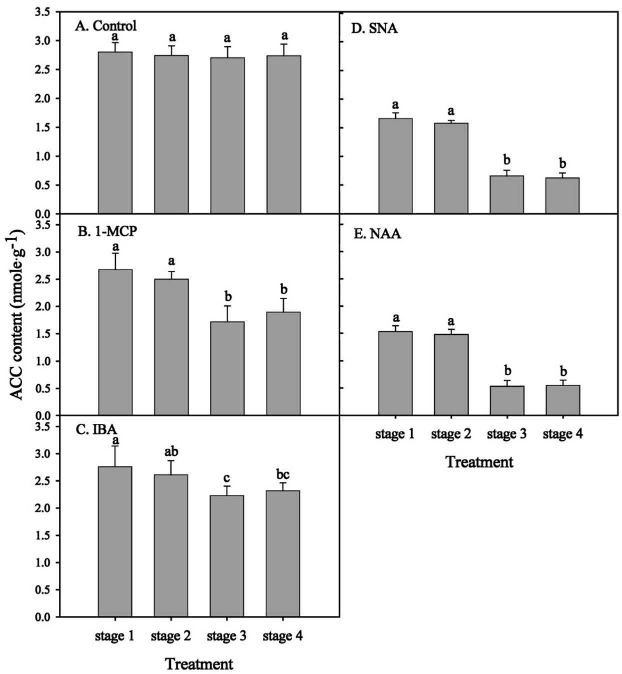

Fig. 1. Effects of different chemical applications of 800 nL·L^{-1} 1-MCP, 0.12 mM IBA, 0.12 mM SNA, and 0.13 mM NAA on ACC content of bracts at stage 1-4 by potted bougainvillea (5 days after treatment). The vertical bars represent the standard error (n = 5). Significant differences between treatments are designated by letters a, b, and c (LSD test, P < 0.05).

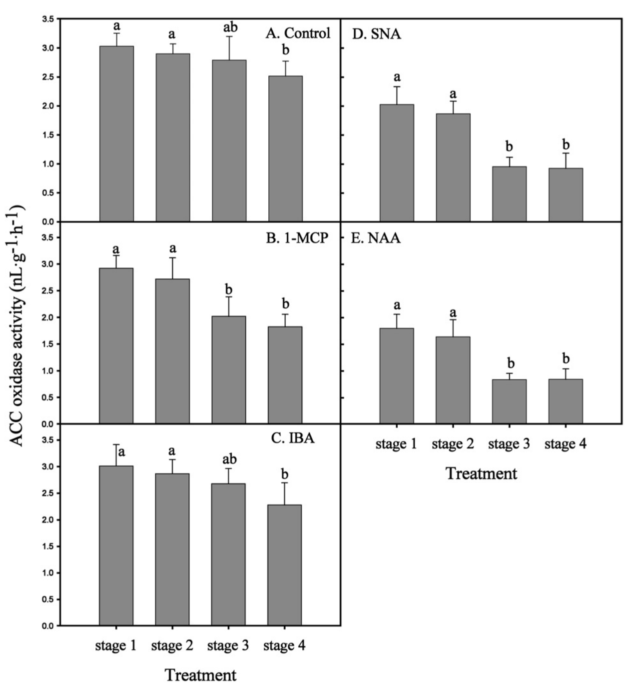

Fig. 2. Effects of different chemical applications of 800 nL·L^{-1} 1-MCP, 0.12 mM IBA, 0.12 mM SNA, and 0.13 mM NAA on ACC oxidase activity of bracts at stage 1-4 by potted bougainvillea (5 days after treatment). The vertical bars represent standard error (n = 5). Significant differences between treatments are designated by letters a, b, and c (LSD test, P < 0.05).

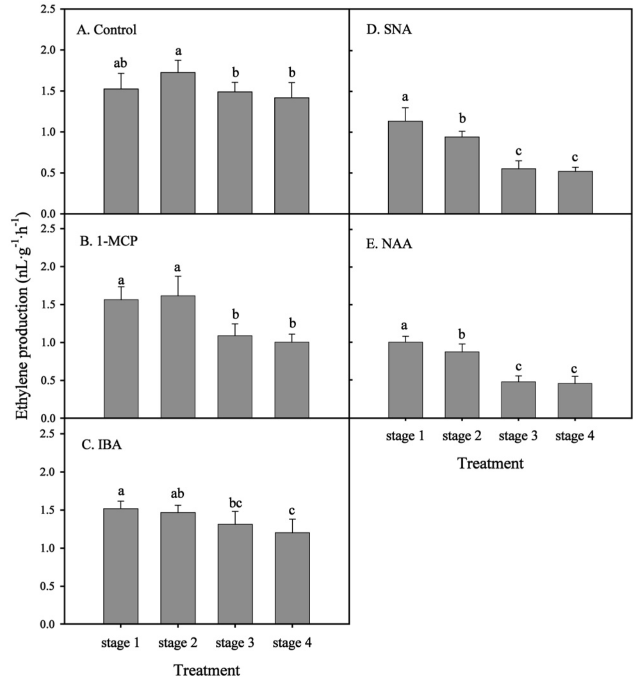

Fig. 3. Effects of different chemical applications of 800 nL·L^{-1} 1-MCP, 0.12 mM IBA, 0.12 mM SNA, and 0.13 mM NAA on ethylene production of bracts at stage 1-4 by potted bougainvillea (5 days after treatment). The vertical bars represent the standard error (n = 5). Significant differences between treatments are designated by letters a, b, and c (LSD test, P < 0.05).

After treatment with or without 1-MCP and auxins for 5 d, bract discs of each stage were collected for ACC content and ACC oxidase activity analyses. Treatment with NAA and SNA, but not IBA, markedly reduced ACC content and ACC oxidase activity in all bract stage (Figs. 1 and 2). Treatment with 800 nL·L^{-1} 1-MCP modestly reduced ACC content and ACC oxidase activity in stage 3 and 4 bracts (Figs. 1B and 2B). Notably, IBA, an auxin, slightly reduced ACC content and ACC oxidase activity in stage 3 and 4 bracts (Figs. 1C and 2C).

Ethylene Production of Potted 'Taipei Red' Bougainvillea

The production of ethylene mirrored the endogenous ACC content and ACC oxidase activity. Treatment with NAA and SNA, but not IBA, markedly reduced ethylene production in bracts stage 3 and 4 (Fig. 3). Treatment with 800 nL·L^{-1} of 1-MCP modestly reduced ethylene production in stage 3 and 4 bracts. However, IBA was slightly reduced ethylene production in stage 3 and 4 bracts (Fig. 3).

Bract Longevity after Treatment with Plant Growth Regulators

Treating bougainvillea with single or combined chemicals prolonged bract longevity compared to that of the control

Table 2. Effects of plant growth regulators on bract longevity of different stages in potted bougainvillea.

Treatment	Bract longevity (d)				
	Stage 1	Stage 2	Stage 3	Stage 4	$LSD_{0.05}$
Control[z]	6.8 d B[y]	7.2 c B	8.6 e AB	9.2 e A	1.5
0.04 mM BA	18.8 c B	19.8 b AB	21.6 d A	20.6 d AB	1.8
14.7 mM KH_2PO_4	9.2 d A	9.6 c A	10.6 e A	11.4 e A	2.3
0.13 mM NAA	23.2 b C	29.4 a B	38.4 a A	40.0 a A	4.0
1 mM Put	22.2 b B	23.6 b B	31.2 b A	31.0 b A	4.4
0.5 mM SA	17.0 c B	20.6 b B	25.8 c A	26.8 c A	4.7
0.5 mM STS	29.4 a A	28.4 a A	23.6 cd AB	21.2 d B	4.9
$LSD_{0.05}$	3.3	3.8	3.3	3.7	

[z]Each value is the mean of five replicates.
[y]Mean separation within the same rows (uppercase) and columns (lowercase) by LSD test, $P < 0.05$.

Table 3. Effects of combined chemicals treatment on bract longevity of different stages in potted bougainvillea.

Treatment	Bract longevity (d)				
	Stage 1	Stage 2	Stage 3	Stage 4	$LSD_{0.05}$
Control[z]	6.8 e B[y]	7.2 d B	8.6 d AB	9.2 e A	1.5
0.13 mM NAA	23.2 c C	29.4 b B	38.4 ab A	40.0 ab A	4.1
0.13 mM NAA + 0.04 mM BA	28.8 b A	33.0 b A	34.8 b A	35.0 bc A	7.9
0.13 mM NAA + 14.7 mM KH_2PO_4	15.8 d B	17.6 c B	23.2 c A	25.4 d A	4.9
0.13 mM NAA + 1 mM Put	32.0 ab A	34.0 b A	35.4 b A	33.6 c A	7.7
0.13 mM NAA + 0.5 mM SA	35.0 a B	40.2 a A	41.4 a A	40.0 ab A	4.0
0.13 mM NAA + 0.5 mM STS	33.4 a B	41.8 a A	42.8 a A	43.6 a A	5.4
$LSD_{0.05}$	4.5	5.8	5.3	5.4	

[z]Each value is the mean of five replicates.
[y]Mean separation within the same rows (uppercase) and columns (lowercase) by LSD test, $P < 0.05$.

(water). In single chemical treatments, NAA was more effective in prolonging bract longevity than Put, SA, STS, and BA. However, NAA treatment prolonged bract longevity by 16.4, 22.2, 29.8, and 30.8 d during bract stages 1, 2, 3, and 4, respectively (Table 2). Additionally, treatment with KH_2PO_4 did not prolong bract longevity during any bract stage (Table 2). In combined chemical treatments, NAA + STS and NAA + SA were more effective in prolonging bract longevity than NAA + BA, NAA + Put, and NAA + KH_2PO_4. However, treatment with NAA + STS prolonged bract longevity by 26.6, 34.6, 34.2, and 34.4 d during bract stages 1, 2, 3, and 4, respectively (Table 3). Moreover, treatment with NAA + SA prolonged bract longevity by 28.2, 33.0, 32.8, and 30.8 d during bract stages 1, 2, 3, and 4, respectively (Table 3).

Effects of Plant Growth Regulators on ACC Oxidase Activity and Ethylene Production in Potted 'Taipei Red' Bougainvillea

Bougainvillea had less ACC oxidase and ethylene production than the control after treatment with single or combined chemicals, and ACC oxidase activity and ethylene production decreased as treatment duration increased. However, treatment with KH_2PO_4 did not affect ACC oxidase activity or ethylene production in any stage. Therefore, combined chemical treatments inhibited ACC oxidase activity and ethylene production more effectively than did single chemical treatments. No significant differences existed for endogenous enzyme between chemical treatments (Figs. 4 and 6).

Effects of Plant Growth Regulators on Protein Degradation and Chlorophyll Degradation in Potted 'Taipei Red' Bougainvillea

Bougainvillea contained less protein degradation and chlorophyll degradation than the control after treatment with single or combined chemicals. Protein degradation or chlorophyll degradation increased as treatment duration increased. No significant differences for endogenous enzyme existed between chemical treatments (Figs. 5 and 7).

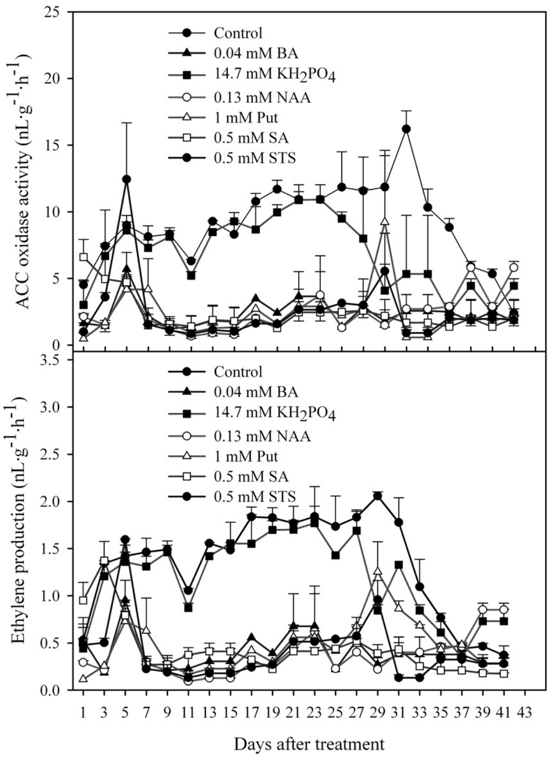

Fig. 4. Effects of plant growth regulators on endogenous ACC oxidase activity or ethylene production of detached bougainvillea leaves. The vertical bars represent the standard error (n = 5) (LSD test, P < 0.05).

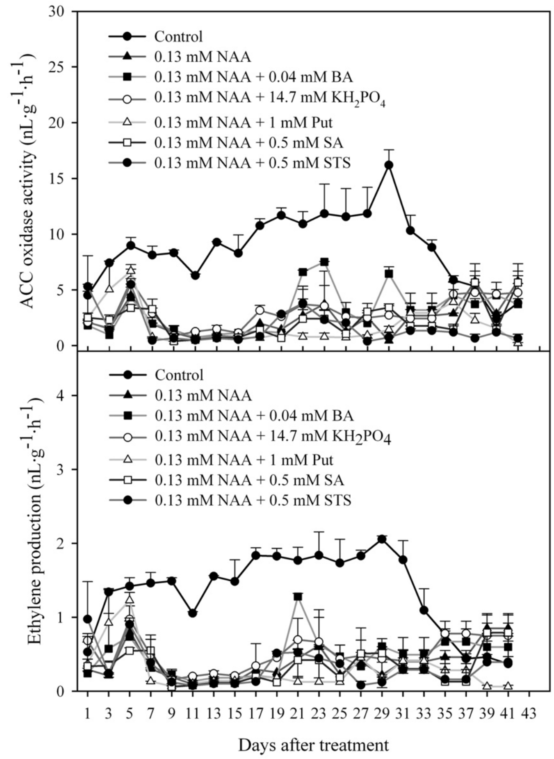

Fig. 6. Effects of combined chemicals treatment on endogenous ACC oxidase activity or ethylene production of detached bougainvillea leaves. The vertical bars represent the standard error (n = 5) (LSD test, P < 0.05).

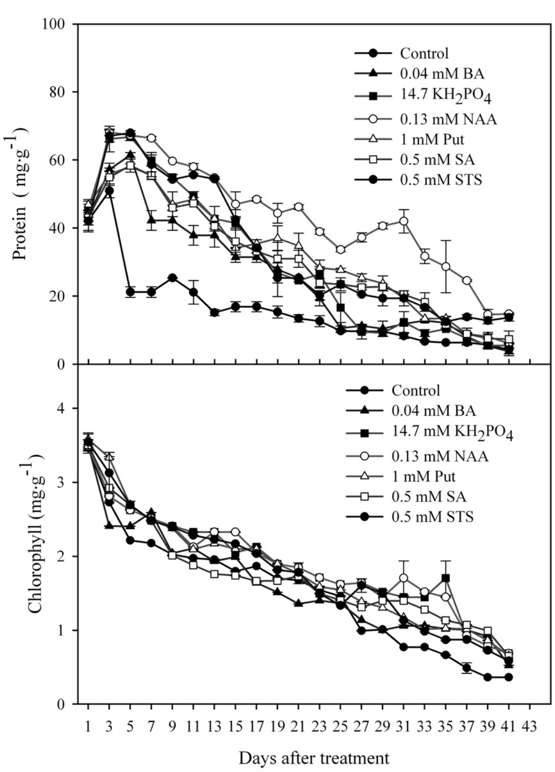

Fig. 5. Effects of plant growth regulators on protein activity or chlorophyll content of detached bougainvillea leaves. The vertical bars represent the standard error (n = 5) (LSD test, P < 0.05).

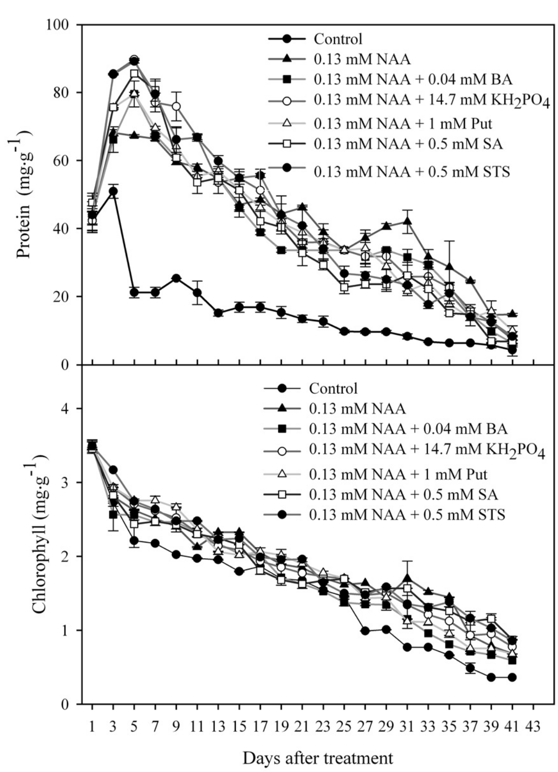

Fig. 7. Effects of combined chemicals treatment on protein activity or chlorophyll content of detached bougainvillea leaves. The vertical bars represent standard error (n = 5) (LSD test, P < 0.05).

Discussion

In the experiment, 1-MCP prolonged the longevity of stage 3 and 4 bracts of potted 'Taipei Red' bougainvillea and significantly reduced production of ACC content, ACC oxidase, and ethylene (Figs. 1, 2, and 3). These experimental results are similar to those obtained for *Pelargonium peltatum* (Cameron and Reid, 2001), parsley (Ella et al., 2003), and *Prunus salicina* Lindl. cv. Tegan Blue (Khan and Singh, 2007).

The effects of three auxins, NAA, SNA, and IBA, on bract longevity of potted 'Taipei Red' bougainvillea were investigated. Experimental results indicate that 0.13 mM NAA and 0.12 mM SNA prolonged bract longevity (Table 1). The experimental results with NAA are similar to those obtained for roses (Halevy and Kofranek, 1976), peppers (Wien and Turner, 1989), and bougainvillea (Chang and Chen, 2001; Hackett et al., 1972). However, NAA and SNA prolonged bract longevity more effectively (40.2 d) than did 1-MCP (8.4 d) in bract stage 4. Leaf drop and chlorosis were not investigated after treatment with NAA or SNA (data not shown). The auxins inhibit chloroplast degradation, leaf drop, and chlorosis has been demonstrated (Philosoph-Hadas et al., 1996).

Auxins influence ethylene production by inducing ACC synthase activity (Kondo et al., 2009). An interaction between ethylene and indole-3-acetic acid (IAA) as antagonists of the expression of genes encoding ACC oxidase has been reported (Rasori et al., 2003). Additionally, application of auxins to mung beans indicated that expression of the VR-ACC oxidase 1 and VR-ACC synthase 1 genes was via positive and negative feedback control by ethylene, respectively (Kim et al., 2001). Auxins applied to kiwi fruit inhibited ethylene action by reducing ACC oxidase activity or its transcriptional level (Fabbroni et al., 2007). In this study, NAA and SNA treatments were significantly reduced endogenous ACC content and ACC oxidase activity (Figs. 1, 2, and 3). ACC content (the precursor of ethylene, which is catalyzed by the ACC synthase, which have not been characterized previously, may be related to the auxin concentration used. For example, a high concentration of auxins triggered ethylene production in potatoes (Richard, 1949), apples (Li and Yuan, 2008; Yuan and Carbaugh, 2007), and dropwort (Eu and Lee, 2009). Conversely, a low concentration of auxins inhibited abscission of flower buds and fruit (Batjer and Marth, 1945; Murphy, 2002). These experimental results indicate that auxins are bi-directionally involved in regulating flowering longevity for some plants. Additionally, these auxins may be related to flower bud maturity. Ethylene production by mature apple (*Malus pumula* Mill.) and pear (*Pyruis corn* muiiinis L.) fruits was inhibited by NAA, and ethylene production by immature fruits was increased by NAA (Abeles and Rubinstein, 1964). Moreover, NAA application as a bloom thinning spray increased the rate of ethylene production at 3 d after treatment and decreased at 5 d after treatment of apples and NAA was not significantly different from that of the control at experiment end (McArtney, 2002). The NAA results obtained by this study are similar to those obtained by Cin et al. (2008). Therefore, treatment with auxins (not IBA treatment), increased ethylene production during the bract stages 1 and 2 and decreased ethylene production during bract stages 3 and 4.

Auxins also retard senescence and abscission resulting from de novo synthesis of cellulase mediated by ethylene. Abebie et al. (2008) indicated that flower bud drop of *Cestrum elegans* delayed by NAA was attributed to acropetal transportation of NAA to the abscission zone; this zone was then insensitive to ethylene. Interestingly, not all auxins inhibited abscission or prolonged bract longevity of potted 'Taipei Red' bougainvillea. For example, 0.12 mM IBA did not prolong bract longevity and slightly reduced endogenous ACC content, ACC oxidase activity, and ethylene production during bract stages 3 and 4. Moreover, high IBA levels (0.24 mM and 0.48 mM) were also ineffective (data not shown). Notably, IBA likely differs from NAA and SNA in its effectiveness and chemical structure; that is, IBA is commonly used to trigger rooting of kiwi and olive trees (Ercisli et al., 2003; Özelbaykal and Gezerel, 2005) and *Tectona grandis* (Husen and Pal, 2007), but it is rarely applied to prevent bract or fruit drop. Harbage and Stimart (1996) determined that IBA treatment of apples increased ethylene production. This may also explain why not all auxins inhibited ethylene biosynthesis or prolonged bougainvillea bract longevity. Therefore, whether the application of auxins prolongs bract longevity, and whether ethylene production is caused by auxins warrant further research.

In experiments 2 and 3, treatment of bougainvillea with single or combined chemicals prolonged bract longevity and longevity increased as the bract stage increased. The chemical treatments decreased ACC oxidase activity, ethylene production, protein degradation and chlorophyll degradation. Treatment with KH_2PO_4 did not prolong bract longevity in any bract stage. Thus, KH_2PO_4 likely inhibits shoot growth, promotes shoot maturity, and improves flower bud formation; therefore, bract longevity was not significantly prolonged (Kumar and Reddy, 2008). However, Put, SA, and BA prolonged bract longevity and this longevity increased as the bract stage increased. These experimental results are similar those obtained previously for sunflowers, cucumbers, carnations, and kiwi fruit (Alvarez et al., 2003; Liu et al., 2006; Mor et al., 1983; Zhang et al., 2003). Treatment with single chemicals reduced protein degradation and chlorophyll degradation, and decreased ACC oxidase activity and ethylene production more than the control. Polyamines are reportedly effective anti-senescence agents and retard chlorophyll loss, membrane deterioration and increase RNase and protease activities, all of which help slow the senescence process (Evans and Malmberg, 1989). Notably, SA was shown to have various adverse effects on ethylene production; thus is, it either inhibited ethylene production in pear cell suspension or kiwi fruit ripening (Leslie and Romani, 1986; Zhang et al., 2003). Cytokinin prevented flower bud abscission and blocked conversion of externally supplied ACC to ethylene (Mor et al., 1983; Wien and Zhang, 1991). These experimental results are similar to those obtained by this study. Notably, STS inhibited ethylene production, thereby significantly prolonging the longevity of the bracts during bract stages 1 and 2. These observations are consistent with those of Chang and Chen (2001) that bougainvillea may be sensitive to ethylene in the early bract stages. The ethylene production rate of bracts was significantly higher in the early stages than in later stages of development. The STS on bracts in early stages may be due to bract abscission, mainly caused by increased ethylene production. Because abscission in late stages may be caused mainly by factor(s) other than ethylene, the prolonging effect of STS decreased when it was applied in the late stages.

A single chemical with NAA was more effective in prolonging bract longevity than other chemical treatments; that is, NAA was the main agent and combination with different growth regulators prolonged bract longevity. Treatment with single chemicals prolonged bract longevity and longevity increased as bract stage increased. Single chemical treatment was significant different between bract longevity and bract stages. Experimental results show that treatment with combined chemicals prolonged bract longevity in early stages. Treatment with combined chemicals was not significantly different from bract longevity and bract stage and was more effective than treatment with a single chemical. Therefore, NAA + STS and NAA + SA effectively prolonged bract longevity and reduced protein degradation and chlorophyll degradation, and decreased ACC oxidase activity and ethylene production more than the control (water).

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