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Effects of pre-harvested N-(2-chloro-4-pyridinyl)-N'-phenylurea (CPPU) spraying on the improvement of flower quality of *Dendrobium* Sonia 'Earsakul'

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Abstract

Improvement of flower quality is a major concern which plays a part in the enhancement of the marketability of the '*Dendrobium*' cut flower. In this study, both synthetic cytokinins: *N*-(2-chloro-4-pyridinyl)-*N'*-phenylurea (CPPU) and N-6-benzyladenine (BA), were foliar sprayed at rates of: 1, 5 or 10 mg L^{-1} and 100, 200 or 400 mg L^{-1}, respectively, on current pseudobulbs of the *Dendrobium* Sonia 'Earsakul' with 45-50 cm in length. The treatments were applied three times at fortnight intervals, prior to terminal bud initiation. The results revealed that an application of 10 mg L^{-1} CPPU significantly increased the numbers of inflorescence per pseudobulb (from 1.1 to 1.7 inflorescence), and the number of flower on an inflorescence was increased from 12.2 to 13.8 flowers. The length and the diameter of inflorescence, having 10 mg L^{-1} CPPU application, also increased from 49.4 cm to 55.1 cm, and 0.57 cm to 0.66 cm, respectively. In addition, the largest flower width and the highest fresh inflorescence weight were also obtained with application of 10 mg L^{-1} CPPU treatment. Despite the application of BA, at 400 mg L^{-1}, enhancing the highest amount of flower counts of inflorescence (at 14.8 flowers), 33.3% of those inflorescence obtained at least one deformed flower. Overall, the results suggest that CPPU spray has a higher potential to elevate flowers, along with the inflorescence qualities of *Dendrobium* Sonia 'Earsakul'. Furthermore, according to this study, CPPU has lower effects upon abnormal flower shapes, and their times of harvest.

Key words: *Dendrobium*, cytokinin; BA, inflorescence, pseudobulb, deformed flower

Introduction

Dendrobium Sonia 'Earsakul' is a famous hybrid for cut flower production, and can produce flowers all the year round, yielding high number of inflorescence. Thailand annually exports large volumes of this orchid, and quality of flower is the main criteria to be considered when exporting cut orchids. Grading standards of *Dendrobium* orchids generally evaluate from healthy inflorescence (pest and pathogen-free), which includes flower-shape, length of inflorescence, numbers of flowers per inflorescence, and vase life etc. In order to promote growth and the developmental processes of flowering plants, some plant growth regulators are usually applied. Cytokinin, a plant growth regulator, plays a role in: 1) the cell cycle process, regulating cell division (Zhang *et al.*, 2005), 2) the function for nutritional signal transduction (Takei *et al.*, 2001; Sakakibara, 2006), 3) the regulation of leaf senescence (Gan and Amasino, 1995; Wingler *et al.*, 1998), 4) the involvement of vegetative- to-reproductive phase transitions (Corbesier *et al.*, 2003), and 5) concerns pertaining to the development of reproductive organs (Bartrina *et al.*, 2011). In *Arabidopsis*, increased levels of cytokinins in leaf tissues, and shoot apical meristem, have been detected during early phase of flower transition (Corbesier *et al.*, 2003). Exogenous application of cytokinins has been reported to induce flower initiation, and increase flower numbers in several plant species. For example, in lychee (*Litchi chinensis*) dormant buds have been stimulated to promote flower bud initiation, after being treated with kinetin (Chen, 1991). In Protea cv. Carnival, the application of N-6-benzyladenine (BA) can also promote off-season flowering (Hoffman *et al.*, 2009). Furthermore, the application of BA has been found to increase a female-to-male floral ratio of jatropha, resulting in an increase in fruit and total seed yields (Pan and Xu, 2011).

In parallel, exogenous BA application has been found to enhance flower budding initiation, and increased spiking percentages in several orchid species, such as the genera of: *Phalaenopsis*, *Doritaenopsis* and *Dendrobium* (Blanchard and Runkle, 2008; Nambiar *et al.*, 2012; Wu and Chang, 2012). Increasing the spike percentages (from 58 to 98 %) has been reported in *Phalaenopsis* Luchia Pink '244', treated with 70 mg L^{-1} BA, on days 1 and 14, after the plants were incubated at low-temperatures (26/18 ^{o}C). However, some deformed inflorescence was detected (Wu and Chang, 2009). In *Dendrobium* Angel White, the application of 200 mg L^{-1} BA promoted early flowering, increased spiking percentages and improved flower quality, in extending length of inflorescence and the number of flowers per inflorescence (Nambiar *et al.*, 2012). In addition, BA plays a role in promoting *in vitro* flowering of *D.* Madame Thong-In, *D.* Chao Praya Smile and *D.* Sonia17 (Sim *et al.*, 2007; Hee *et al.*, 2007; Tee *et al.*, 2008).

Non-BA cytokinins also play role in the regulation of flower development in orchids. Application of 2-iso-pentenyl adenine (2-iP) (150 mg L^{-1}) and kinetin (300 mg L^{-1}) to whole plants, grown under low temperatures (26 ^{o}C/18 ^{o}C), increased flower diameters of *Phalaenopsis* Sogo Yudian 'V3'. Furthermore, kinetin (200 mg L^{-1}) also increased the number of inflorescence per plant of the *Phalaenopsis* Sogo Yudian 'V3' (Wu and Chang, 2009). CPPU

(Forchlorfenuron, (*N*-(2-chloro-4-pyridinyl)-*N'*-phenylurea) is a synthetic cytokinin. Only low concentrations of exogenous CPPU applications can affect the growth of plants, such as: CPPU promoted fruit sets of Japanese persimmon, muskmelon fruit (Sugiyama and Yamaki, 1995; Hayata *et al.*, 2000), and enlarge the sizes of fruit in kiwifruit, grapes and Japanese persimmon (Antognozzi *et al.*, 1993; Zabadal and Bukovac, 2006; Sugiyama and Yamaki, 1995). Although CPPU is widely used in fruit tree production, its effects upon the promotion of cut flower quality have never been reported, specifically for orchids. Therefore, the objective of this study was to investigate the effects of CPPU upon inflorescence and flower development of commercially cut orchids, namely *D.* Sonia 'Earsakul', in contrast to widely-used BA as cytokinin.

Materials and Methods

Two year old plants of *Dendrobium* Sonia 'Earsakul' were used as plant materials in this study. The plants were grown in coconut husks under 70% shade, watered once a day, and fertilized once a week. This is the general practice for orchid commercial production at the 'Jittrakarn orchids farm' in Karnchanaburi province, Thailand. Terminal flower buds of *D.* Sonia 'Earsakul' are generally developed from new developing current pseudobulbs (frontal bulbs), when they grow to approximately 50-60 cm in height. In this experiment, two synthetic cytokinins: BA, at 100, 200 or 400 mg L^{-1}, and CPPU, at 1, 5 or 10 mg L^{-1}, were foliarly sprayed to the current pseudobulbs (45-50 cm in height), exactly before flower initiation stage. Each plant was given an application of 70 mL BA or CPPU liquid solutions mixed with surfactant solution. The BA or CPPU applications were reapplied fortnight intervals for three times. The experiment was randomized in fifteen replications with seven treatments. A 'least significance difference' (LSD) test was used to compare the treatment effects. Thereafter, the BA and CPPU applied *D.* Sonia 'Earsakul' flowers were harvested, when each inflorescence had four fully-opened flowers. Data collection included growth and yield parameters: the number of inflorescence per pseudobulb, lengths and diameters of inflorescence, numbers of flowers per inflorescence and the fresh and dry weights of complete inflorescence. In addition, the percentages of inflorescence obtained, for at least one abnormal flower, and the numbers of days taken after BA or CPPU applications until harvesting, were also recorded. The experiment was carried out between January and April 2012.

Results

Inflorescence development of *D.* Sonia 'Earsakul': Application of 10 mg L^{-1} CPPU to *D.* Sonia 'Earsakul' before flower bud initiation resulted in significantly increased inflorescence numbers per pseudobulb, and the number of inflorescence increased from 1.1 to 1.7 inflorescence per bulb. However, BA concentration (100, 200 or 400 mg L^{-1}) had less effect upon the inflorescence number (Table 1). Interestingly, CPPU applications (1, 5 or 10 mg L^{-1}) encouraged and enhanced the inflorescence length which increased from 49.4 cm to 52.6-55.1 cm, whilst the BA application, at 100, 200 or 400 mg L^{-1}, had no effect upon the length of inflorescence (Table 1). Furthermore, the 10 mg L^{-1} CPPU application promoted the highest inflorescence diameters, and they significantly increased from 0.57 to 0.66 cm (Table 1). All BA treatments (100, 200 or 400 mg L^{-1}) produced high amounts of flowers per inflorescence, and there were from 14 to 14.8 flowers per inflorescence, while the non-treated control produced only 12.2 flowers. Although the BA treatments promoted number of flower per inflorescence, the size of the flowers was not significantly influenced. In contrast, the application of 10 mg L^{-1} CPPU not only increased number of flower, but also enlarged flower size. Flower width, with 10 mg L^{-1} CPPU treatment, significantly increased from 6.9 cm to 7.3 cm, compared to non-treated control samples (Table 1).

Fresh and dry weights of inflorescence: Fresh weights of the inflorescence significantly increased from 26.4 g to 29.9-31.5 g after the pseudobulbs were treated with 200-400 mg L^{-1} BA, or 5-10 mg L^{-1} CPPU, respectively (Fig. 1). In addition, the maximum dry weight of single inflorescence was obtained from pseudobulbs treated with 10 mg L^{-1} CPPU. The inflorescence dry weight increased from 2.8 to 3.4 g compared to non-treated control (Fig. 2).

Percentages of inflorescence obtained from deformed flowers: Foliar applications of BA concentrations (100, 200 and 400 mg L^{-1}) for three times caused deformation of flower morphology. In abnormal flowers, there were additional lip-like organs present in the basal parts of columns (a fusion unit of orchids; male and female reproductive organs), or at the position of flower pollen caps (Fig. 4). The highest percentage of inflorescence obtained in such deformed flowers was found in the 400 mg L^{-1} BA treatment (33.3%), followed by the 200 mg L^{-1} BA treatment (26.7%) (Fig. 2). However, there were no deformed flowers in all CPPU treatments, and non-treated control specimen (Fig. 2).

Table 1. Effects of different BA or CPPU concentrations on flower characteristics of *Dendrobium* Sonia 'Earsakul'

Cytokinins		Inflorescence			Flowers	
Treatment	Concentration (mg L^{-1})	Number per pseudobulb	Length (cm)	Diameter (cm)	Number per inflorescence	Width (cm)
Control	0	1.1±0.1^{c}	49.4±1.4^{b}	0.57±0.02^{b}	12.2±0.3^{c}	6.9±0.1^{b}
BA	100	1.0±0.0 ^{c}	49.0±0.9^{b}	0.59±0.03^{b}	14.1±0.5^{a}	6.9±0.1^{b}
	200	1.2±0.1^{bc}	49.1±1.3^{b}	0.61±0.02^{ab}	14.0±0.7^{a}	6.8±0.1^{b}
	400	1.3±0.1^{abc}	49.1±1.0^{b}	0.62±0.01^{ab}	14.8±0.5^{a}	6.8±0.2^{b}
CPPU	1	1.3±0.1^{abc}	52.6±0.7^{a}	0.60±0.02^{ab}	12.4±0.2^{c}	7.0±0.1^{ab}
	5	1.4±0.2^{ab}	53.1±0.8^{a}	0.62±0.02^{ab}	12.7±0.4^{bc}	7.0±0.1^{ab}
	10	1.7±0.1^{a}	55.1±1.2^{a}	0.66±0.02^{a}	13.8±0.2^{ab}	7.3±0.1^{a}

Values are means± standard error.
Mean separation within columns by least significant difference test at $P \leq 0.05$

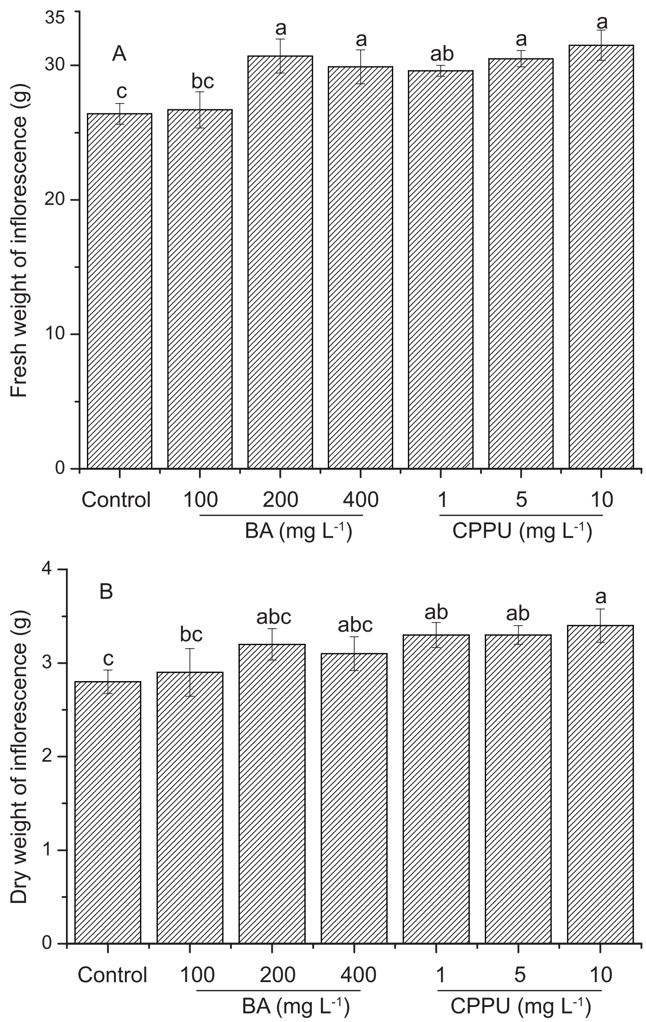

Fig.1. Fresh weight (A) and dry weight (B) of inflorescence of *D.* Sonia 'Earsakul' after applying different BA or CPPU concentrations. Values are means± standard error. Different letters indicate that the values are significantly different at the P<0.05 level.

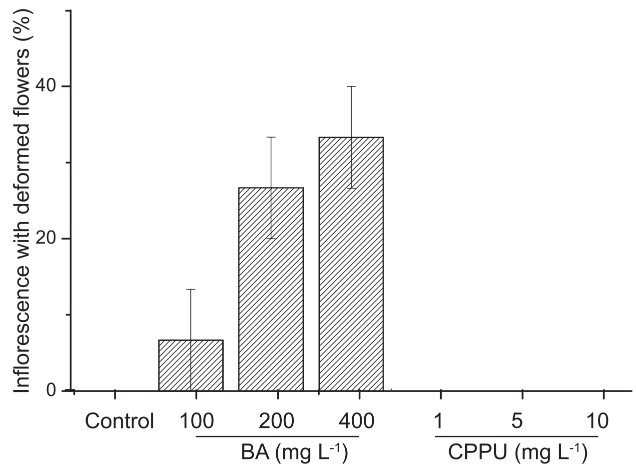

Fig. 2. Percentage of inflorescence withdeformed flowers of the BA treatments. Values are means± standard error. There were no deformed flowers in all CPPU treatments, and non-treated control.

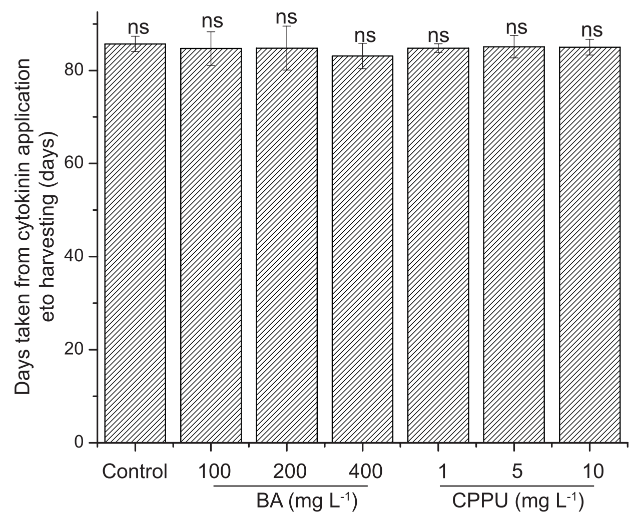

Fig. 3. Days taken from BA or CPPU applications before flower bud initiation to harvesting of *D.* Sonia 'Earsakul'. Values are means± standard error. ns indicates not significant.

Days taken from cytokinin application before flower bud initiation to harvesting of *D.* Sonia 'Earsakul': In commercial production, inflorescence of *D. Sonia* 'Earsakul' are harvested when obtaining 4-5 open flowers per inflorescence. It was found that the applications of different BA or CPPU concentrations had less effect upon harvesting time. It took 83.1 to 85.1 days after BA or CPPU application to harvest flowers, and there was no significant change as compared to the non-treated control samples, which took 85.7 days to harvest (Fig. 3).

Discussion

In this study, we applied cytokinin-like compound CPPU to improve the cut flower quality of *D.* Sonia 'Earsakul' for the first time, in contrast to widely-used BA. The results showed that exogenous applications of CPPU (especially at 10 mg L^{-1}) to the current pseudobulbs, before flower bud initiation, significantly enhanced the numbers of inflorescence per plant, flower counts based upon inflorescence, and the length of inflorescence and sizes of flower in *Dendrobium* orchids. Increasing numbers of inflorescence per plant may be a result of the roles of cytokinin in promoting cell differentiation during the development of floral primodia. We found that pseudobulbs which had 10 mg L^{-1} CPPU treatment, mostly produced double inflorescences at the terminal, while other treatments generally produced a single inflorescence. Effects of BA application, in order to promote flower meristem activity and increased numbers of flower bud initiation, have been previously reported in other orchid species: *Dendrobium* Jaquelyn Thomas 'Uniwai Pricess' (Sakai *et al.*, 2000), *Dendrobium* Angel White (Nambiar *et al.*, 2012), *Doritaenopsis* and *Phalaenopsis* orchids (Blanchard and Runkle, 2008). In this study, we found that BA application could promote high numbers of flower counts of inflorescence, yet had fewer promotional effects upon stimulating numbers of inflorescence per plant, length of inflorescence, and size of flower.

Increased numbers of flower count in inflorescence were found in both BA (100-400 mg L^{-1}) and CPPU (10 mg L^{-1}) treatments. In the *Arabidopsis* ckx3 ckx5 double mutant, inflorescence carried higher numbers of flowers than the wild-type, because the plants had generated higher numbers of inflorescence meristematic cells during flower development. The content of biologically active and inactive trans-zeatin-type cytokinins in this mutant was also

Fig. 4. Flower morphology of *D.* Sonia 'Earsakul' treated with BA 0, 200 and 400 mg L^{-1} in Figure A, B and C, respectively. Normal flowers have only a lip (A). In abnormal flowers several additional lips are generated from the basal part of orchid columns (B, C). Scale = 1 cm.

dramatically higher than the wild-type (Bartrina *et al.*, 2011). Therefore, there is a relationship between increased endogenous cytokinin levels and the production of higher numbers of inflorescence meristematic cells. The exogenous cytokinin application has been reported to promote increased endogenous cytokinin levels (Letham, 1994; Blanchard and Runkle, 2008).

Length of inflorescence is a main criteria for the grading of cut orchids. CPPU at 10 mg L^{-1} had a dramatic effect upon extending the length of inflorescence, in which the CPPU treated-inflorescence could be classified as extra-long graded flowers (more than 55 cm in length). The application of appropriate BA concentrations (150 or 200 mg L^{-1}) was previously reported to increase the length of inflorescence in *D.* Angel White, when the plants were sprayed weekly in the first month, and every two weeks in the subsequent months for a total of six months (Nambiar *et al.*, 2012). However, foliar applications of BA or CPPU in this study were only performed at fortnight intervals for three times. We found that the BA application had less promotional effects upon extending lengths of inflorescence. Different responses to exogenous BA applications to *Dendrobium* species may be due to different durations and frequencies of applications, genetic backgrounds and stages of the plants.

Although applications of BA at 100, 200 or 400 mg L^{-1} had increased numbers of flowers per inflorescence, the flower shapes were deformed. The deformed flowers had additional lips generated from the basal part of the column, or pollen cap (Fig. 4). Abnormal flowers may be due to high dosages of BA cytokinin applications. The development of abnormally-formed flowers due to high concentrations of BA applications was formerly found in *D.* Jaquelyn Thomas 'Uniwai Pricess' (Sakai *et al.*, 2000) and in *Phalaenopsis* orchids (Wu and Chang, 2009).

Cytokinins also play a role in regulating the size of flower organs (Bartrina *et al*., 2011). Increasing endogenous cytokinins causes prolonged periods of cell division during flower development, and results in increased sizes and cell numbers of flower organs in the CKX3 CKX5 mutants of *Arabidopsis* (Bartrina *et al.*, 2011). We hypothesize that the exogenous application of CPPU in this study may enhance endogenous cytokinin levels and lead to enlarged sizes of flower. Therefore, endogenous cytokinin levels and inflorescence meristem activity after exogenous CPPU application should be studied further. The effect of cytokinin CPPU has been proven to increase fruit size and fresh weight in several fruit crops: kiwifruit (Patterson *et al.*, 1993), and berries (Zabadal and Bukovac, 2006).

In this study, we found CPPU as potential compound for promoting fresh weight of *Dendrobium* inflorescence which can increase fresh weight of inflorescence ultimately the total product volume for sale.

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