African Journal of Biotechnology Vol. 11(7), pp. 1592-1596, 24 January, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.2472
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Cytokinin treatment and flower quality in *Phalaenopsis* orchids: Comparing N-6-benzyladenine, kinetin and 2-isopentenyl adenine

Po-Hung Wu and Doris C. N. Chang*

Department of Horticulture, National Taiwan University, No. 1, Sec. 4, Roosevelt Rd., 10617, Taipei, Taiwan.

Accepted 27 October, 2011

We previously documented an N-6-benzyladenine (BA) protocol to increase spike and flower number in *Phalaenopsis* orchids. To increase options for growers, we tested two additional cytokinins, kinetin (Kin) and 2-iso-pentenyl adenine (2-iP), comparing them with BA. Two key commercial cultivars were used (*Phalaenopsis* Sogo Yukidian 'V3' and *Phalaenopsis* Tai Lin Redangel 'V31'). We counted spike number and length, flower number and diameter, time to anthesis and flower longevity. Whole plants were sprayed once with BA (50, 100 or 150 mg·L^{-1}), Kin (100, 200 or 300 mg·L^{-1}) or 2-iP (50, 100 or 150 mg·L^{-1}) either on day 1 of 26 /18°C treatment (Time 1) or when the first flower bud reached 0.2 cm in diameter (Time 2). Surprisingly, all three cytokinins increased flower diameter. With *Phalaenopsis* Sogo Yukidian 'V3' sprayed at Time 1, Kin (200 mg·L^{-1}) increased the number of spikes (1 to 1.5) and flowers (8.4 to 10.4 per plant). BA increased both spike and flower number. Also, at Time 2, BA (100 mg·L^{-1}) increased spike numbers. In the second cultivar, *Phalaenopsis* Tai Lin Redangel 'V31,' spike and flower number increased only with BA. Kin showed no effect in measured parameters, while 2-iP increased flower diameter and longevity. Although, BA was shown to be the most effective in increasing flower and spike number, this research suggests that non-BA cytokinins are useful for increasing flower quality in Phalaenopsis orchids.

Key words: 2-Iso-pentenyl adenine (2-iP), kinetin (Kin), spike number, flower count.

INTRODUCTION

Within the last decade, the global orchid market has grown rapidly with public awareness of orchids. Orchids have become one of the top-selling potted plants in Europe (US Department of Agriculture, 2010). *Phalaenopsis* orchids account for 80% of all orchids sold, and can only be sold on the retail market when in flower. Growers who produce the *Phalaenopsis* need to deliver high quality flowering plants to market during key sales periods, such as Thanksgiving, Christmas and Chinese New Year. The attributes considered valuable vary with the target market. Some markets favor a double-spike plant, while others prefer a single spike on a plant. Preference also varies for spike height. However, in all cases, the orchids plant must be flowering intensively, have many flowers and achieve high prices. Therefore, the main factors that could increase profitability of orchids cultivation is the improvement of flowering characteristics such as spike number, spike length, and number of flower, (Wang, 2004).

Timing of flowering is controlled through timing of spiking. Growers can modulate flowering time in each *Phalaenopsis* cultivars, based on reliably controlling the time of spiking when a potentially flowering stem emerges from a leaf axis. *Phalaenopsis* orchid grow at various light intensity, photoperiod or fertilizer condition, with only slight changes in spiking induction (Wang and Hsu, 1994; Lin and Lee, 1998). However, spike induction followed by temperature control is a reliable way to induce and control

*Corresponding author. E-mail: cymmvlsc@hotmail.com. Tel: +886-2-33664869. Fax: +886-2-23625542.

Abbreviations: Kin, Kinetin; **2-iP,** 2-iso-pentenyl adenine; **BA,** N-6-benzyladenine.

flowering time. For spike induction, vegetative plants are moved from a day temperature 30°C/night temperature 25°C to a lower temperature (day 26°C/night 18°C) and left under such conditions for 4 to 6 weeks (Lee and Lin, 1984; Blanchard and Runkle, 2006).

In addition, the application of cytokinins has been reported to promote flowering (Bernier et al., 1993). Previous study on orchids has explored how cytokinins can enhance flower quality by spike induction. An earlier work was concerned with describing the effect of plant growth regulators, such as BA and GA on growth and flowering of *Phalaenopsis* (Ho and Yang, 1990; Lin, 1994). Later, researches focused on gibberellic acid (GA) (Chen et al., 1994, 1997; Wang, 1995) and N-6-benzyladenine (BA), as the one of the cheapest cytokinins (Kubota et al., 1997). Moreover, with the huge growth in global demand for orchids, researchers have looked at how to create cytokinin protocols to further regulate flowering in orchids (Blanchard and Runkle, 2008; Wu and Chang, 2009). Blanchard and Runkle (2008) treated *Phalaenopsis* and *Doritaenopsis* orchids with either BA or a BA+GA_3 mixture one week after transferring plants into a low air temperature (from a 29 to 23°C). Their main finding was that after BA treatment, the number of flower spikes increased. They also reported that earlier spiking caused early flowering.

Our previous work showed that combining low temperatures and the BA application can be used to control flowering (Wu and Chang, 2009). Thus, two *Phalaenopsis* cultivars showed increased in numbers of flower spikes and flower when plants were treated with a foliar application of BA (100 or 150 mg·L^{-1}) at the day after transfer to lower air temperatures. Plants were ready for market 14 to 16 weeks following transfer to a low air temperature. Wu and Chang (2009) stated that *Phalaenopsis* cultivars respond differently to treatment with BA, either favorably (increased the spike and flower number) or negatively (deformation of spikes). However, there is lack of research on the effect of cytokinins other than BA, used during flowering of commercial *Phalaenopsis* cultivars. Therefore, the objective of this study was to compare the effects of BA and other cytokinins on flowering of potted *Phalaenopsis*.

MATERIALS AND METHODS

Plants of the two important commercial *Phalaenopsis* cultivars exported from Taiwan, *Phalaenopsis* Sogo Yukidian 'V3' and *Phalaenopsis* Tai Lin Redangel 'V31', were used in this study. Mature plants (six leaves with a leaf spread of 25 to 30 cm, approximately 14 to 16 months after deflasking) used in this experiment were initially grown (from 10th March to 25th September, 2006) in 10.5 cm diameter pots (650 ml in volume) that were filled with Chilean sphagnum moss as the sole growing substrate. The greenhouse had average day/night air temperatures of 28/23°C, maximum photosynthetic photon flux (*PPF*) at 400 μmol·m^{-2}·s^{-1} at noon and natural photoperiod (latitude 23° N). They were fertilized weekly, alternating between 0.2 and 0.5 g·L^{-1} of a Peters water soluble fertilizer (20N-8.6P-16.6K; Scotts, Marysville, OH).

To initiate spiking, on the 25th of September 2006, mature plants were moved into a low air temperature (day 26°C/ night 18°C, 800 μmol·m^{-2}·s^{-1} *PPF*, maximum and natural photoperiod). Plants were sprayed weekly with 0.3 g·L^{-1} 10N–12.9P–16.6K water-soluble fertilizer (Peters), and sprayed biweekly with 1 g·L^{-1} 10N–12.9P–16.6K water-soluble fertilizer at 1500 HR. Furthermore, plants were treated with one of three cytokinins, at one of three concentrations, and at one of two times. Treatments were either applied after they were moved into the cool room (Time 1), or at the time when the first flower bud reached 0.2 cm in diameter (Time 2, approximately 2 to 3 months after Time 1). These times were chosen based on our previous unpublished trials, which indicated that these application times were the most effective on spike and flower numbers and least likely to lead to deformity of spikes. The following cytokinins used were BA, kinetin (Kin), or 2-Isopentenyl adenine (2-iP) (all from Sigma, St. Louis). Leaves were sprayed to drip with about 10 ml of a solution containing either BA (50, 100 or 150 mg·L^{-1}), Kin (100, 200 or 300 mg·L^{-1}), or 2-iP (50, 100 or 150 mg·L^{-1}) and 0.05% Tween 20 (by vol. Sigma) at dusk. The powders of BA, Kin, and 2-iP were each dissolved in 1 N NaOH and diluted for spraying.

The duration to anthesis, spike and flower number, spike length (from the base to the top of spike), flower diameter, and flower longevity (days from first flower opening to wilting) were recorded at 14th week. These measurements were continued until the first flower wilted. We used a randomized complete block design (RCBD) and least significant difference (LSD) test for comparing treatment effects. Each treatment was used on eight blocks of plants, each with eight plants. Eight plants, each block one plant, was randomly selected, giving eight replicates per treatment. There were three concentrations per cytokinin, three cytokinins and a control, or 1216 plants of each cultivar.

RESULTS AND DISCUSSION

Effects of BA on flowering of the Phalaenopsis orchids

Flower spikes were increased in many treatments at Time 1. Spraying 50 to 150 mg·L^{-1} BA on whole plants on the first day of low temperature treatment increased the number of flower spikes per plant, as well as total number of flower (Table 1). However, the highest concentrations also significantly decreased flower diameter as compared to the control (from 11.2 to 10.4). This was probably due to competition in nutrient access for flower development after increasing in number of flowers. The effects of BA treatments were similar to that in our previous report (Wu and Chang, 2009). In *Phalaenopsis* Sogo Yukidian 'V3', the 100 mg·L^{-1} of BA at Time 2 (when the diameter of first buds were approximately 2 mm), significantly increased the total number of flower from 8.4 to 10.5 per plant (Table 1), and flower longevity from 126 to 133. However, the flower diameter was not significantly increased at the middle concentration. On the other hand, BA sprayed at Time 1 on *Phalaenopsis* Tai Lin Redangel 'V31' was only effective at high concentration, 150 mg·L^{-1}, to increase number of spike and flower longevity (Table 2).

Effects of 2-iP and Kin on flowering of the Phalaenopsis orchids

In the case of *Phalaenopsis* Sogo Yukidian 'V3', Kin was

Table 1. Flower characteristics of *Phalaenopsis* Sogo Yukidian 'V3' after applying cytokinins N-6-benzyladenine (BA), kinetin (KIN), or 2- isopentenyl adenine (2-iP). Cytokinins were applied either on the first day of low temperature treatment (Time 1) or when the first flower bud reached 0.2 cm in diameter (Time 2) [z].

Cytokinin		Spike				Flower				Timing			
Treatment	Concentration (mg·L^{-1}) [y]	Number (no./plant)		length (cm) [y]		Number (no./plant)		Diameter (cm) [y]		Flower longevity (days)		anthesis (days)	
Time		1	2	1	2	1	2	1	2	1	2	1	2
Control	0	1.0^{d}	1.0^{a}	71.1^{ab}	71.1^{bc}	8.4^{b}	8.4^{bc}	11.2^{bcd}	11.2^{b}	126^{a}	126^{b}	107^{ab}	107de
BA	50	1.6^{ab}	1.0^{a}	63.9^{abc}	76.5^{a}	11.5^{a}	9.3^{b}	11.1^{cde}	11.9^{a}	130^{a}	127^{ab}	106^{b}	113^{ab}
	100	1.6^{ab}	1.3^{b}	63.5^{bc}	72.5^{ab}	11.1^{a}	10.5^{a}	10.6^{de}	11.8^{ab}	127^{a}	133^{a}	105^{b}	115^{a}
	150	2.0^{a}	1.1^{ab}	57.1^{c}	76.3^{a}	12.1^{a}	10.5^{a}	10.4^{e}	11.9^{a}	127^{a}	128^{ab}	106^{b}	113^{ab}
Kin	100	1.1^{cd}	1.0^{a}	67.9^{ab}	72.5^{ab}	8.9b	8.8^{bc}	11.9^{ab}	12.1^{a}	126^{a}	127^{ab}	108^{ab}	110^{cd}
	200	1.5^{bc}	1.0^{a}	63.3^{bc}	75.5^{ab}	10.4^{ab}	8.6^{bc}	11.6^{abc}	12.3^{a}	128^{a}	130^{ab}	104^{bc}	108de
	300	1.3^{bcd}	1.0^{a}	71.9^{ab}	67.0^{c}	10.0^{ab}	8.0^{c}	12.0^{a}	12.1^{a}	130^{a}	131^{ab}	110^{a}	107^{e}
2-iP	50	1.3^{bcd}	1.1^{ab}	73.1^{a}	71.1^{bc}	10.5^{ab}	9.0^{bc}	11.7^{abc}	12.2^{a}	129^{a}	132^{ab}	105^{b}	109^{cde}
	100	1.1^{cd}	1.1^{ab}	67.0^{ab}	71.8^{abc}	8.5^{b}	9.3^{b}	11.6^{abc}	12.0^{a}	130^{a}	130^{ab}	106^{ab}	111^{bc}
	150	1.0^{d}	1.0^{a}	71.6^{ab}	74.9^{ab}	8.5^{b}	8.3^{bc}	12.1^{a}	12.3^{a}	133^{a}	130^{ab}	100^{c}	109^{cde}

[z]Randomized complete block design (RCBD) with eight replicates, eight plants each, and mean separation within columns at $P \leq 0.05$. [y]1 mg·L^{-1} = 1 ppm. 1 cm = 0.3937 inch

only effective in increasing of the number of spike and number of flower at Time 1 with 200 mg·L^{-1}. No significant effect was found with 2-iP. We see a potential in developing Kin protocols in *Phalaenopsis* orchids culture, but because of the high price of 2-iP, it is unlikely to be commercially viable. Flower number showed economically important differences that in this analysis, did not show statistical differences (Table 1). The control group has an average flower number of 8.4, while the Kin treatment group (Time 1) has an average flower number of 10.5. Commercially, the values of these two groups of plants are considerably different.

The same phenomenon is seen in spike number. The control group had an average spike number of 1.0, while the Kin treatment group (Time 1) has an average spike number of 1.1, 1.3 and 1.5. Treating whole plants with 200 mg·L^{-1} Kin also increased the flower spikes per plant. Similar effects were found in spraying with 50 mg·L^{-1} 2-iP. While 200 mg·L^{-1} Kin applications increased the flower count from an average of 8.4 on control plants to 10.4, the result was not statistically significantly. Flower diameter was enlarged by treatment with 300 mg·L^{-1} Kin or 150 mg·L^{-1} 2-iP. Moreover, 2-iP is significantly more expensive to use. No results here justify additional research using 2-iP. We expect, however, that Kin will be useful in commercial protocols if experimented with at higher concentrations (>200 mg·L^{-1}). Furthermore, other examined parameters showed no change in flower diameter at Time 2, or in flower longevity at Time 1. Only two favorable effects were found with 2-iP, both at the highest concentration and at Time 1. At Time 2, the greatest increase in flower diameter was around 1 cm, both with Kin and 2-iP. All three BA concentrations and the 100 mg·L^{-1} 2-iP delayed flowering. In *Phalaenopsis* Tai Lin Redangel 'V31', Kin and 2-iP treatments had no effect on increasing the number of flowers or spikes (Table 2) when applied at Time 1, but flower diameter increased with the highest concentration of 2-iP applied at Time 2. The results were overall less striking with this cultivar.

Conclusion

This research documents the effects of three various cytokinins concentrations and application times on two commercial cultivars. Results indicate that *Phalaenopsis* Sogo Yukidian 'V3' was

Table 2. Flower characteristics of *Phalaenopsis* Tai Lin Redangel 'V31' after applying cytokinins N-6-benzyladenine (BA), kinetin (KIN) or 2- isopentenyl adenine (2-iP). Cytokinins were applied either on the first day of low temperature treatment (Time 1) or when the first flower bud reached 0.2 cm in diameter (Time 2) [z].

Plant growth regulators		Spike				Flower				Timing			
Treatment	Concentration ($mg \cdot L^{-1}$) [y]	Number (no./plant)		length (cm) [y]		Number (no./plant)		Diameter (cm) [y]		Flower longevity (days)		Anthesis (days)	
Time		1	2	1	2	1	2	1	2	1	2	1	2
Control	0	1.0^{b}	1.0^{b}	61.4^{a}	61.4^{bc}	8.3^{b}	8.3^{bcd}	9.8^{ab}	9.8^{bc}	100^{b}	100^{abc}	109^{ab}	109^{c}
BA	50	1.0^{b}	1.0^{b}	60.5^{a}	61.4^{bc}	8.1^{b}	8.4^{bcd}	9.6^{ab}	9.6^{bc}	111^{ab}	114^{a}	110^{a}	111^{ab}
	100	1.3^{a}	1.1^{a}	62.8^{a}	62.9^{bc}	9.3^{ab}	8.9^{ab}	9.6^{ab}	9.5^{c}	116^{a}	99^{abc}	108^{ab}	112^{a}
	150	1.4^{a}	1.0^{b}	61.4^{a}	68.9^{a}	9.5^{a}	9.6^{a}	9.4^{b}	9.9^{abc}	111^{ab}	84^{c}	106^{b}	110^{bc}
Kin	100	1.0^{b}	1.0^{b}	62.6^{a}	60.9^{bc}	8.4^{ab}	7.6^{d}	9.8^{ab}	9.9^{abc}	110ab	110^{ab}	107^{b}	111^{ab}
	200	1.0^{b}	1.0^{b}	62.9^{a}	60.6^{bc}	8.1^{b}	7.9^{cd}	9.8^{ab}	10.0^{ab}	112ab	98^{bc}	109^{ab}	110^{ab}c
	300	1.0^{b}	1.0^{b}	65.0^{a}	59.1^{c}	8.6^{ab}	8.0^{bcd}	9.9^{a}	9.8^{bc}	106ab	97^{bc}	109^{ab}	110^{bc}
2-iP	50	1.0^{b}	1.0^{b}	61.9^{a}	63.5^{b}	8.4^{ab}	$8..6^{bc}$	9.9^{a}	9.8^{bc}	111^{ab}	101^{ab}	108^{ab}	110^{bc}
	100	1.0^{b}	1.0^{b}	61.9^{a}	60.5^{bc}	8.4^{ab}	7.9^{cd}	9.8^{ab}	10.0^{ab}	117^{a}	114^{a}	106^{b}	110^{bc}
	150	1.0^{b}	1.0^{b}	66.3^{a}	59.4^{bc}	8.8^{ab}	8.3^{bcd}	9.9^{a}	10.2^{a}	116^{a}	102^{ab}	107^{ab}	108^{c}

[z]Randomized complete block design (RCBD) with eight replicates, eight plants each and mean separation within columns at $P \leq 0.05$. [y]1 $mg \cdot L^{-1}$ = 1 ppm. 1 cm = 0.3937 inch.

far more responsive to the treatments than *Phalaenopsis* Tai Lin Redangel 'V31'. Cytokinin treatment on the first day of cold treatment (Time 1) appeared most advantageous, although, increased flower count was found when treated at Time 2. BA had the greatest potential for increase flower count on *Phalaenopsis* orchids. This study also reveals that the application of different cytokinin on *Phalaenopsis* plants may varied with be tested before large scale applications.

ACKNOWLEDGEMENT

We thank Ox Orchids for providing the raw trial data upon which this research was based, as well as the plant material and greenhouse space for this study.

REFERENCES

Bernier GA, Havelange C, Houssa A, Petitjean, Lejeune P (1993). Physiological signals that induce flowering. Plant Cell. 5: 1147-1155.

Blanchard MG, Runkle ES (2006). Temperature during the day, but not during the night, controls flowering of Phalaenopsis orchids. J. Exp. Bot. 57: 4043-4049.

Blanchard MG, Runkle ES (2008). Benzyladenine promotes flowering in *Doritaenopsis* and *Phalaenopsis* orchids. J. Plant Growth Regul. 27: 141-150.

Chen WS, Chang HW, Chen WH, Liu YS (1997) Gibberellic acid and cytokinin affect *Phalaenopsis* flower morphology at high temperature. HortScience, 32: 1069-1073.

Chen WS, Liu HY, Liu ZH, Yang L, Chen WH (1994). Gibberellin and temperature influence carbohydrate content and flowering in *Phalaenopsis*. Physiol. Plant. 90:391-395.

Ho FW, Yang L (1990). Effects of plant growth regulators on the growth and flowering of *Phalaenopsis*. Ann. Rept. Taiwan Sugar Res. Inst. 1989-1990: 17-18.

Kubota S, Hamotsu H, Kazuo I, Masaju K (1997). Effect of light condition and GA_3 application on development of axillary buds during low temperature treatment in *Phalaenopsis*. Jpn. Jan. Soc. Hort. Sci. 66: 581-585.

Lee N, Lin GM (1984). Effect of temperature on growth and flowering of *Phalaenopsis* white hybrid. J. Chinese Soc. Hort. Sci. 30: 223-231.

Lin YR (1994). Effect of light, temperature and plant growth regulators on flowering of *Phalaenopsis* spp. Graduate Institute of Horticulture, National Taiwan University, Taipei, Taiwan. Master's thesis.

Lin YR, Lee N (1998). Light requirement of *Phalaenopsis* prior to and after cool-temperature forcing. J. Chinese Soc. Hort. Sci. 44: 463-478.

US Department of Agriculture (2010). Floriculture crops summary. Agricultural Statistics Board, Washington, DC.

Wang YT (1995) Gibberellic acid on *Phalaenopsis*. Am. Orchid Soc. Bul. 8: 745.

Wang YT (2004) Flourishing market for potted orchids. Flower Tech. 7:

2-5.
Wang YT, Hsu TY (1994). Flowering and growth of *Phalaenopsis* orchids following growth retardant applications. HortScience, 29: 285-288.
Wu PH, Chang DCN (2009). The use of N-6-benzyladenine to regulate flowering of *Phalaenopsis* orchids. HortTechnology, 19: 200-203.