MARDI Res. J. 19(1) (1991): 17–25

Effects of 6-benzylaminopurine on inflorescence development and quality in four sympodial orchids

(Kesan 6-benzilaminopurina terhadap pertumbuhan dan kualiti perbungaan dalam empat jenis orkid simpodium)

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Key words: orchids, 6-benzylaminopurine, inflorescence development, quality

Abstrak

Kesan benzilaminopurina (BA) terhadap pembungaan dan tempoh simpan bunga telah dikaji terhadap empat orkid simpodium yang merangkumi *D.* Mary Mak, *D.* Madam Uraiwan, *D.* Jacquelyn Concert x Jester dan *O.* Gower Ramsey.

Penyemburan BA pada semua aras tidak mengakibatkan apa-apa kesan terhadap bilangan bunga sejambak dan tempoh dari kemunculan kudup hingga 50% atau 100% kembang. Terdapat gerak balas saling tindak yang ketara terhadap BA dan hibrid pada variabel-variabel yang lain. Bagi variabel panjang tangkai pula terdapat pengurangan sebanyak 15–29% pada 200 bpj untuk tiga hibrid, berbanding dengan kawalan, kecuali *D.* Madam Uraiwan. Peratusan tunas gugur sangat ketara bagi hibrid *Dendrobium* pada aras BA yang tinggi iaitu 400 atau 800 bpj. Walau bagaimanapun, kelihatan kesan songsang pada *O.* Gower Ramsey. Peratusan tunas gugur yang lebih tinggi terjadi pada pokok kawalan berbanding dengan pokok yang diberi perlakuan BA. Bunga-bunga cacat meningkat dengan ketara daripada 2% pada pokok kawalan kepada 28% pada 800 bpj BA bagi *D.* Mary Mak. Tempoh simpan jambak bunga masing-masing berkurangan daripada 33 dan 55 hari bagi kawalan kepada 24 dan 27 hari bagi perlakuan 200 bpj BA untuk *D.* Mary Mak dan *O.* Gower Ramsey.

Abstract

The effects of Benzylaminopurine (BA) on flowering and shelf life were studied on four sympodial orchid hybrids, *D.* Mary Mak, *D.* Madam Uraiwan, *D.* Jacquelyn Concert x Jester and *O.* Gower Ramsey.

BA at all levels, when compared with the control, did not affect the flower number per inflorescence and the duration from flower emergence to 50% or 100% bloom. There were significant interaction responses to BA and hybrid for the remaining variables. In spray length, reductions of 15–29% were observed at 200 ppm compared with the control for three hybrids except *D.* Madam Uraiwan. The percentage of bud drop was significantly higher in the *Dendrobium* hybrids at high BA levels of 400 or 800 ppm. However, in *O.* Gower Ramsey, the reverse effect was seen, with a higher percentage of bud drop in the control compared with the BA-treated plants. Deformed flowers increased significantly

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from 2% in the control to 28% at 800 ppm BA for *D.* Mary Mak. The shelf life of inflorescences was reduced from 33 and 55 days for the control to 24 and 27 days for BA at 200 ppm for *D.* Mary Mak and *O.* Gower Ramsey respectively.

Introduction

The use of 6-benzylaminopurine (BA) on orchid *Dendrobium*, has shown to increase inflorescence production ranging from 48% to 108% through the application of 200–800 ppm BA when compared with the control (Zaharah and Lee 1987). However, only two of the orchid hybrids experimented showed significant differences.

There have been contradictory reports regarding the effects of BA on orchid flower quality. Goh (1979) found that BA, injected at the nodes in concentrations of 10^{-3} and $10^{-4}M$, increased the length and number of flowers per inflorescence in *D.* Louisae. Lee and Koay (1984) however, found that the length and number of flowers per inflorescence of *D.* Louisae did not differ significantly among BA-treated plants and control, using concentrations of 1 000–2 000 ppm/plant. Zaharah and Majni (1987) reported that application of BA at 200–800 ppm/plant in five successive applications did not affect inflorescence length in four monopodial orchids.

The use of BA as a preharvest treatment or postharvest dip has proved effective in prolonging the storage life of many leafy vegetables (Kaufman and Ringel 1961; Lipton and Ceponis 1962; Wittwer et al. 1962). Such treatments also improved the keeping quality of cut flowers such as carnation and chrysanthemum (MacLean and Dedolph 1962). Experiments by Heide and Oydvin (1969) with carnations indicated that a 2-min pretreatment with $10^{-3}M$ to $10^{-5}M$ BA increased its keeping quality significantly. However, immersion in $10^{-3}M$ BA for 12 h was detrimental. They concluded that prolonged treatment with BA could be harmful even when rather dilute solutions were employed. Similarly, Halevy and Wittwer (1965) found that BA treatment was ineffective or even detrimental with cut carnations.

This study further examines the effects of BA with respect to floral quality in four sympodial orchids and also assesses the detrimental effects of BA application over a period of 48 weeks.

Materials and methods

The four sympodial orchids studied were *Dendrobium* Mary Mak, *D.* Madam Uraiwan, *D.* Jaquelyn Concert x Jester and *Oncidium* Gower Ramsey. Three levels of BA at 200, 400 and 800 ppm and a control were tested. The 4 x 4 factorial combinations of hybrids and BA levels were tested in a randomised complete block design with three replications. Four pots were grouped together as an experimental unit. The plants were treated with BA for 5 successive days, and repeated for three cycles of 16 weeks each, beginning 12 February 1985. Other experimental details are as described by Zaharah and Lee (1987).

Measurement variables

The effects of BA on flower development were measured by tagging all developing inflorescences from flower emergence to bloom. Four measurements were taken i.e.

- length of inflorescence – this was measured from the base of the stalk with the stem to the tip of the inflorescence;
- flower number per inflorescence – this was a count on the number of flowers on each inflorescence;
- days to 50% bloom – the number of days from flower emergence to the opening of 50% of the flowers for each inflorescence; and
- days to 100% bloom – the number of days from flower emergence to full bloom for each inflorescence.

The influence of BA on the keeping quality of cut inflorescences was determined using inflorescences taken after the third spraying cycle. Flower sprays from the

different BA treatments were kept in separate containers filled with tap water from the laboratory, and the number of days to first flower drop and 50% flower drop were recorded.

Attribute variables

During the second spraying cycle, some inflorescences were observed to have deformities of the stalk and buds. As such, the number of such inflorescences in each BA treatment was recorded. In addition, the number of aborted flower buds (dropped off prematurely) was also recorded.

Statistical model

The three way classification with interaction was adopted for the analyses of the measurement variables. The equation of the model is given as

$Y_{ijkl} = m + R_i + V_j + B_k + (VB)_{jk} + (RVB)_{ijk} + E_{ijkl}$, Y_{ijkl} being the lth observation in the kth BA treatment of the jth hybrid in the ith replicate, where

m = the mean effect,
i = 1 to 3 replicates,
j = 1 to 4 hybrids,
k = 1 to 4 BA treatments,
l = 1 to n_{ijk},
n_{ijk} = the number of inflorescences in the (i, j, k)th cell, and
E_{ijkl} = the sampling error.

As the number of inflorescences produced varied in each cell, the analysis followed that of unbalanced data with unequal subclass number (Searle 1971). All effects and their interactions except for the replicate effects are assumed fixed and tests of significance for the BA x hybrid interactions were made with the replicate x BA x hybrid component of the variation. The partial sum of squares and its corresponding mean squares, equivalent to the Type III SS of the SAS Procedure of Generalised Linear Model (GLM), was used for the tests of significance.

The procedures of GLM, and the New Duncan's Multiple Range Test (DUNCAN) of the SAS Institute Inc. (1985) were used for measurement variables like length of inflorescence and number of days from flower emergence to 50% bloom. As the data were unbalanced with unequal numbers of observations at each BA level, the DUNCAN's procedure utilising the harmonic mean of the frequencies, was used in each hybrid.

Homogeneity tests of error variances (the errors referred to were those derived from the variations among inflorescences, the sample variation after removing the effects of replicate, BA treatment and their interaction in each hybrid) were conducted to determine whether the variances could be pooled for a combined analysis as shown by the given model.

For attribute data like the number of aborted buds and inflorescences with deformed flowers, the non-parametric Chi-Square test was used in a two-way contingency table to test for the response to BA treatment in each variety, taken separately. For this, the PROC FREQ of the SAS (Anon. 1985) was used.

It was necessary to present the results by variety as the response variables, like inflorescence length or flower number were different for the different hybrids.

Results and discussion

Test for homogeneity of variances among hybrids

Only two of the six measurement variables tested for homogeneity showed that the error variances estimated from the analysis done by hybrid were homogeneous *(Table 1)*. For one variable, the number of days from bud initiation to 100% flower bloom, all variances tested were significantly different, and comparisons of BA treatment were made using separate mean square errors (MSE) and degrees of freedom (DF) for each hybrid. For the remaining variables, either one or two hybrids had to be treated separately from the rest.

Where two or more hybrids were found

Table 1. Summary of homogeneity test of variances, with the number of hybrids tested homogeneous

Variable	Homogeneous set	Hybrids excluded
Length of inflorescence	3	*O.* Gower Ramsey
No. of flowers per inflorescence	4	None
No. of days from flower emergence to 50% bloom	2	*D.* Madam Uraiwan *O.* Gower Ramsey
No. of days from flower emergence to 100% bloom	0	All
No. of days from 100% bloom to 1st drop	4	None
No. of days from 100% bloom to 50% drop	3	*D.* Jaquelyn Concert x Jester

Table 2. Partial mean squares for quantitative variables analysed as a combined analyses in a 3-way classification with interactions

Source of variation	Stalk length		No. of flowers		50% bloom		1st bud drop		50% bud drop	
	DF	MS	DF	MS	DF	MS	DF	MS	DF	MS
Replicate	2	44	2	29	2	135	2	94	2	422
Hybrid	2	8 881	3	300	1	1	3	1 515	2	1 648
BA	3	848	3	50	3	68	3	964	3	1 411
Hybrid x BA	6	230	9	70	3	76	9	363	6	479
Rep. x hybrid x BA	22	183	30	75	14	100	26	99	18	135
Error	841	102	887	33	385	49	249	37	224	48
Hybrids excluded in analysis	*O.* Gower Ramsey		None		*O.* Gower Ramsey *D.* Madam Uraiwan		None		*D.* Jaquelyn Concert x Jester	

to have homogeneous variances for a particular variable, the data were pooled over hybrids for a combined analysis in each variable.

Response of measurement variables to BA

The application of BA did not affect the number of flowers per inflorescence and the number of days from initiation to 50% flower bloom. Here, the BA effects were made by comparing the mean squares for BA with that of the rep. x hybrid x BA, which, for both variables, were less than 1 *(Table 2)*. The analysis for number of days to 100% bloom, done separately by variety, as the variances were not homogeneous, also showed no significant differences between the BA levels. The mean number of days from flower emergence to 50% and 100% bloom is given in *Table 3*.

The length of inflorescence was significantly influenced by BA application. Generally, the lengths were longer in the control plants than that of the BA-treated plants. In three hybrids, *D.* Mary Mak, *D.* Jaquelyn Concert x Jester and *O.* Gower Ramsey, the application of 200 ppm BA significantly reduced the length by 15, 29 and 28% respectively compared with the control plants. This reduction may not be a disadvantage for a variety with a long stalk like *O.* Gower Ramsey. However, it may be disadvantageous for the shorter and intermediate *D.* Jaquelyn Concert x Jester and *D.* Mary Mak. No significant reduction in length was recorded in the *D.* Madam Uraiwan variety *(Table 4)*.

In the shelf life studies on cut

Table 3. Effects of BA on the mean number of days from flower emergence to 50% and 100% bloom for four orchid hybrids

BA level (ppm)	*D.* Mary Mak		*D.* Madam Uraiwan		*D.* Jaq. Concert x Jester		*O.* Gower Ramsey	
	50%	100%	50%	100%	50%	100%	50%	100%
Control	52 (43)	62 (52)	48 (58)	60 (96)	48 (8)	59 (10)	63 (10)	109 (11)
200	49 (97)	57 (104)	50 (98)	61 (120)	45 (16)	56 (18)	83 (11)	111 (18)
400	48 (81)	58 (95)	49 (52)	61 (82)	49 (28)	57 (33)	79 (15)	106 (19)
800	48 (126)	57 (139)	49 (59)	60 (121)	49 (32)	56 (34)	76 (14)	99 (19)
Harmonic mean of cell	74.3	85.9	62.9	102.0	15.7	18.6	12.2	15.9
MSE	100.0	171.4	99.8	46.5	109.1	45.6	100.0	478.0
Error DF	14	6	6	6	6	6	14	6

Note: Values in brackets below each mean represent the number of inflorescences from which the mean was derived

Table 4. Effects of BA treatment on inflorescence length for four orchid hybrids

	Inflorescence length (cm)			
BA level (ppm)	*D.* Mary Mak	*D.* Madam Uraiwan	*D.* Jaq. Concert x Jester	*O.* Gower Ramsey
Control	50.6a (49)	36.1a (88)	38.4a (10)	119.9a (10)
200	43.0b (99)	35.8a (120)	27.3b (18)	89.6b (16)
400	42.3b (94)	34.3a (76)	32.7ab (32)	107.3ab (17)
800	40.9b (136)	32.3a (121)	29.3ab (34)	89.4b (15)
Harmonic mean of cell	82.5	97.3	18.5	13.9
MSE	183.2	183.2	183.2	817.7
DF	22	22	22	6

Note: Values in brackets below each mean represent the number of inflorescences from which the mean was derived

inflorescences, results for the number of days to first flower drop and 50% flower drop showed a similar trend. Results showed a significant interaction effect in response between hybrids and BA application. Inflorescences taken from non-treated plants had a significantly longer shelf life compared with those taken from BA-treated plants of *D.* Mary Mak and *O.* Gower Ramsey. BA application reduced the number of days from full bloom to 50% flower drop by 8 days in *D.* Mary Mak and 26 days in *O.* Gower Ramsey. In the case of *D.* Jaquelyn Concert x Jester, although the observed differences between the control and the treated plants showed the same trend, with the low numbers of observations, the results were not significant. For *D.* Madam Uraiwan, there was no significant difference between the control and the BA-treated plants *(Table 5).*

Table 5. Effects of BA on the number of days from full bloom to 50% flower drop for four orchid hybrids

BA level (ppm)	No. of days from full bloom to 50% flower drop			
	D. Mary Mak	*D.* Madam Uraiwan	*D.* Jaq. Concert x Jester	*O.* Gower Ramsey
Control	32.8a (10)	21.4a (8)	31.4a (5)	55.1a (7)
200	23.8b (43)	21.9a (18)	21.3a (6)	27.1b (8)
400	24.4b (36)	24.2a (12)	22.9a (8)	35.2b (12)
800	24.5b (56)	19.5a (34)	23.6a (18)	24.3b (12)
Harmonic mean of cell	23.7	13.6	7.3	9.2
MSE	134.76	134.76	73.44	134.76
DF	18	18	6	18

Note: Values in brackets below each mean represent the number of inflorescences from which the mean was derived

Plate 1. Occurrence of aborted bud

Response of attribute variables to BA

Chi-Square tests conducted individually to determine the effects of BA on the number of aborted buds *(Plate 1)* and the number of flowers for each hybrid showed an overall significant effect of BA *(Table 6)*. However, the responses differed from hybrid to hybrid. For the three *Dendrobium* hybrids, BA increased the percentage of aborted buds, while in the *Oncidium*, the effect appeared to be reversed. In *O.* Gower Ramsey, the control gave a significantly higher percentage of aborted buds (75.13%) compared with the BA-treated plants (average of 47.7%). A separate Chi-Square test among the BA levels (with two degrees of freedom) showed that the percentages of aborted buds were not significantly different in this hybrid. The response to BA also varied within the individual *Dendrobium* hybrid. For *D.* Mary Mak, only applications of 400 and 800 ppm BA gave significantly higher percentage of aborted buds compared with the control. For *D.* Madam Uraiwan, only the highest level of BA (800 ppm) gave significantly higher percentage of aborted buds, with the two lower levels of BA not significantly different from the control. For *D.* Jaquelyn Concert x Jester, the BA levels of 200 and 800 ppm gave significantly higher percentage of aborted buds, with the 400 ppm level not significantly different from the control.

Although the number of flowers produced per stalk were not significantly affected by BA levels, the number of aborted buds may seriously affect the

Table 6. Effects of BA on the number of aborted buds for four orchid hybrids

BA level (ppm)	*D.* Mary Mak		*D.* Madam Uraiwan		*D.* Jaq. Concert x Jester		*O.* Gower Ramsey	
	No. of AB	No. of UB	No. of AB	No. of UB	No. of AB	No. of UB	No. of AB	No. of UB
Control	37 (3.69)	966	117 (7.07)	1 539	34 (19.10)	144	441 (75.13)	146
200	69 (3.55)	1 876	148 (6.52)	2 122	137 (40.77)	199	217 (48.98)	226
400	103 (6.07)	1 594	104 (6.60)	1 472	139 (24.22)	435	316 (47.73)	346
800	139 (6.03)	2 168	368 (15.68)	1 952	185 (33.70)	364	267 (46.60)	306
Total	348	6 604	737	7 085	495	1 142	1 241	1 024
Chi-Square	21.4		160.6		41.1		132.9	
DF	3		3		3		3	
Prob.	<0.0001		<0.0001		<0.0001		<0.0001	

Note: AB = aborted buds
UB = Unaborted buds
Values in brackets indicate the percentage of aborted buds to the total no. of flowers in each BA level and hybrid

marketability of the spray. For *D*. Mary Mak with an average of 16.1 flowers per spray, a 6.03% abortion (as for 800 ppm BA) would give, on the average, one aborted bud per inflorescence compared with one aborted bud per two stalks in the control.

The application of BA produced a significantly higher incidence of deformed inflorescences *(Plate 2)* in one hybrid, *D*. Mary Mak *(Table 7)*. An average of 22% of the inflorescences produced by the BA-treated plants were deformed compared with 2% in the control.

In the other two *Dendrobium* hybrids, the data were too sparse and the Chi-Square test was not computed. However, there are indications that applications of BA as a whole tended to give rise to a higher incidence of deformed inflorescences. No deformity however, was observed in inflorescences of *O*. Gower Ramsey plants.

Conclusion

The application of BA did not have any significant effect on the number of flowers per inflorescence and the number of days from 50% bloom or full bloom in all stalks of inflorescences in the four hybrids tested. Stalk lengths of *D*. Mary Mak, *D*. Jaquelyn Concert x Jester and *O*. Gower Ramsey were significantly reduced with BA application of 200 ppm, while no significant differences were observed between the control and treated plants of *D*. Madam Uraiwan.

Cut inflorescences placed in water showed that inflorescences from BA-treated plants had a shorter shelf life compared with those of the control in hybrid *D*. Mary Mak and *O*. Gower Ramsey. The time from full

Plate 2. Deformed inflorescence

Table 7. Effects of BA on the number of inflorescences with deformed flowers or stalks for three orchid hybrids

BA levels (ppm)	*D*. Mary Mak		*D*. Madam Uraiwan		*D*. Jaq. Concert x Jester	
	Deformed	Perfect	Deformed	Perfect	Deformed	Perfect
Control	1 (2.38)	41	0 (0.0)	59	0 (0.0)	9
200	23 (19.33)	96	2 (2.02)	97	3 (10.71)	25
400	16 (16.49)	81	3 (5.88)	48	4 (10.53)	34
800	49 (27.84)	127	6 (8.96)	61	1 (2.63)	37
Chi-Square	15.33	*	*			
DF	3					
Prob.	0.002					

Note: *Chi-Square test was not computed as the no. of deformed flowers was low

bloom to 50% flower drop shortened by about 8 days for *D.* Mary Mak and 26 days for *O.* Gower Ramsey in the BA-treated plants.

The effects of BA on the aborted buds were consistent in all three *Dendrobium* hybrids. A higher percentage of aborted buds were seen in the treated plants, especially at the higher BA levels of 400–800 ppm. For the *Oncidium*, there was a reversal in trend, with BA levels reducing the percentage of aborted buds compared with the control.

Deformity of stalks, flowers and buds occurred in one hybrid, *D.* Mary Mak, where 22% of the inflorescences from BA-treated plants were deformed compared with 2% in the control. There were also indications that the two other *Dendrobium* hybrids may be similarly affected, although the sample size was too small to permit any meaningful test. In the *Oncidium*, no deformity was detected in all inflorescences produced.

Therefore it appears that the effects of BA on flower quality varied from hybrid to hybrid. As such, it is necessary to determine the appropriate concentration of BA for use on a commercial hybrid before embarking on a large scale. In hybrids where BA enhanced flowering without deleterious effects on flower quality, the use of BA to promote flowering would likely be commercially viable.

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Accepted for publication on 12 September 1990