PRECOCIOUS FLOWERING IN WILD KUMQUAT IS ONE WAY FOR BREEDING TECHNIQUES

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ABSTRACT

The delay in flowering caused by a long juvenile phase occurs in *Citrus* and its related genera. Reliable methods to shorten the juvenile period are required to overcome this major obstacle in citrus breeding programs and to accelerate the production of improved genotypes. Photoperiod and benzyladenine (BA) influenced flowering of *Fortunella hindsii* seedlings. More than 80 % of seedlings flowered 91 days after germination *F. hindsii* seedlings sprayed with 0.1 mg 1^{-1} BA and maintained at 25°C under 16 h photoperiod illuminated with fluorescent tubes at 52.9 □mol m^2 s^{-1} flowered within 106 days after germination, and the seedlings without BA treatment flowered within 130 days after germination. Percentage of seedling flowering in 0.1 mg 1^{-1} BA treatment was reached to 97 % when photoperiod was extended to 20 h. The highest percentage of flowering and fruiting was achieved in the seedlings sprayed with 0.1 mg 1^{-1} BA under 20 h photoperiod at 25°C. This paper describe the effects of BA, photoperiods and temperatures on subsequent flowering of *F. hindsii* seedlings.

Key words: Benzyladenine, Fortunella-hindsii, Flowering, photoperiod, temperature

INTRODUCTION

Juvenile period is a major obstacle to the breeding of woody plants. Juvenility is the period between seed germination and the time when a ability to flower is attained and maintained by a plant (Hackett 1985). The delay in flowering caused by a long juvenile phase occurs in Citrus and its related genera. Reliable methods to shorten the juvenile period are required to overcome this major obstacle in citrus breeding programs and to accelerate the production of improved genotypes.

Fortunella hindsii also know as Hong Kong Kumquat or Wild Kumquat, has several unique features among *Fortunella* genus. They are grown in gardens as an ornamental plant. They are also kept as houseplants and are used in bonsai. In the extreme environments its species know have ability to promote precocious flowering (Jumin. And Nito 1996a).

Precocious flowering of *Fortunella hindsii* occurred *in* vitro when the plants derived from protoplasts and branch internodes and epicotyls were cultured under 16 h photoperiod on half-strength MT medium supplemented with BA under 16 h photoperiod (Jumin and Nito 1996b, 1999). If flowering of seedlings could be induced and the flowers were similar in form and function to these mature tree, such a system would be promising for studies on flowering and juvenility and pollen could be made available earlier for breeding purposes. This paper describe: effects of BA, photoperiods and temperatures on subsequent flowering of *F. hindsii* seedlings.

MATERIALS AND METHODS

The seeds were placed in trays containing 75 % peat and 25 % sand (v/v) and kept under a greenhouse condition (20-250C) for 4 weeks. Seedlings were selected for uniformity in growth habit and size at 2-leaf stage and transplanted into 0.5 1 pots containing 50 % peat and 50 % sand.

Effect of BA on flowering

The seedlings at 2-leaf stage were maintained in a growth chamber at 25°C illuminated with fluorescent tubes at 52.9 mmol s^{-1} m^{-2} under 16 photoperiod. BA treatment was carried out immediately after the seedlings were transplanted to pots. The seedlings were foliage sprayed with either distilled water or 0.01, 0.1, 1.0 and 10.0 mg 1^{-1} BA once a week for 3 weeks. The concentrations of BA were chosen based upon a preliminary dose response trial on stock seedlings.

Effect of temperature and photoperiod on flowering

Another series of experiment were carried out to combination effects of 25°C or 30°C and 16 h or 20 h photoperiod. The seedlings at 2-leaf stage were foliage sprayed with 0.1 mg 1^{-1} BA once a week for 3 weeks and maintained in a growth chamber at 25°C or 30°C illuminated with fluorescent at 52.9 □mol $s^{-1}m^{-2}$ under 16 h or 20 h photoperiod. This experiment was undertaken to observe a second and third flowering of seedlings. All of the seedlings in this experiment were initially grown under a growth chamber condition for 14 months. The seedlings were hand watered every day. Seedlings were subsequently fertilized with a 0.1 % Hyponex solution 1 month after germination and thereafter once a month. Pest-control procedures were not adopted because they have been shown to influence flowering in sweet orange (Moss 1976).

Vegetative growth and flower formation were measured approximately 4 months after germination. Number of nodes on main stem was counted from the most basal node to the node nearest to the apex. For pollen viability test, pollens were suspended in 1.0 % (w/v) acetocarmine and a live pollens were counted under a microscope.

RESULTS DISCUSSION

BA-sprayed *F. hindsii* seedlings flowered 128 to 140 days after germination, before control seedlings did not flower (Table 1). BA at 10.0 mg l^{-1} resulted in a greater net increase in vegetative growth, but inhibited flower initiation. BA at 0.1 mg l^{-1} retarded the vegetative growth, but promoted the emergence of floral buds. This treatment was used in all subsequent experiment. The flowers occurred at the stem apex. The number of floral parts organs were the same as that of flowers derived from mature plants. Flower developed to fruit. Pollen viability from the seedlings grown under 20 h photoperiod at 25°C was over 80 % as assessed by an acetocarmine staining.

An increase of photoperiod length increased the percentage of flowering of *F. hindsii* seedlings. When the photoperiod was extended to 16 h or 20 h the percentage of flowering increased to 81.7 % or 88.3 % (Table 2). Photoperiod length also affected both first emergence of floral buds and first opening of flowers. The early floral bud emergence and flower opening obtained in the seedlings which were exposed to 20 h photoperiod. The highest percentage of flowering (97 %) was achieved in the seedlings which were maintained at 25°C or 30°C. For development of flowers, however, 25°C was found to more suitable than 30°C. It was indicated by a greater number of fruits produced at 25°C in comparison with that at 30°C.

Table 1. Effects of BA concentration on vegetative growth and flowering of *F. hindsii* seedlings at 25°C under 16 h photoperiod for 6 months after germination (first flowering)

BA (mgl-1)	Stem length (cm)	No. of nodes per plant	No. of branches per plant	First emergence of floral bud (day*)	No. of floral bud per plant	First opening of flower (day*)	Flowered seedling (%)	No. of fruits per plant
0.0	8.8a[Z]	4.8a	1.0a	130.7a	0.5a	160.3a	20.0a	1.0ab
0.01	8.7a	6.2b	1.0a	110.8b	2.0b	130.0b	43.3b	1.0ab
0.1	8.2a	5.0a	1.0a	106.2b	2.0b	128.2b	80.0c	1.5a
1.0	105b	9.0c	2.0b	119.0d	1.0a	140.0c	50.0d	1.0b
10.0	12.6c	12.3d	3.0c	-	0c	-	0e	0b

[Z]Means within a column followed by the same letter are not significantly different at $p = 0.05$
(-) = The proportion of parameter was not observed in the 5 months.
* after germination

Table 2.. Effects of photoperiod and temperature on vegetative growth and flowering of *F. hindsii* seedlings sprayed with 0.1 mgl-1 BA and kept in a growth chamber for 6 months after germination (first flowering)

Photoperiod (h) Temperature °C	Stem length (cm)	No. of nodes per plant	No. of branches per plant	First emergence of floral bud (day*)	No. of floral bud per plant	First opening of flower (day*)	Flowered seedling (%)	No. of fruits per plant
16+25	8.3aZ	4.8ab	1.0a	109a	1.0a	129.0a	80.0a	1.5b
16+30	9.4b	7.5c	1.0a	121.8b	2.0b	141.2b	81.2a	1.0ab
20+25	7.2c	4.5a	2.0b	97.0c	2.5b	120.4a	97.2b	2.0b
20+30	8.2a	5.5b	1.0a	97.0c	1.0a	119.1c	97.0b	0.2a

(-) = The proportion of parameter was not observed in the 5 months.
* after germination

The highest number of floral buds was obtained in the seedlings with a decline of vegetative growth as was observed in a treatment of 0.1 mg l^{-1} BA. This kind of antagonism between vegetative growth and flowering is widely observed in woody plants (Bernier et al. 1981; Heller et al. 1994; De Baerdemaeker et al. 1994). A decline of vegetative growth was also observed in seedlings which were exposed to 8 h photoperiod, but no flower formation occurred even after a spray with 0.1 mg l^{-1} BA. Our results indicated that under inductive 16 h to 20 h photoperiods, BA triggered flower initiation that precedes flowering. BA exerts a major influence on flower initiation of *F. hindsii.* In previous studies, cytokinins seemed to be requisite for flower initiation *in* vitro in *F. hindsii* (Jumin and Nito 1996b), *Murraya paniculata* (Jumin and Nito

1996a, grapevine (Srinivasan and Mullin 1978), *Passiflora suberosa* (Scorza and Janick 1980) and bamboo (Nadgauda et al. 1990). In the present study, cytokinin enhanced flower formation of *F. hindsii* plants.

The earliest floral bud emergence and flower opening were obtained in the seedlings which were exposed to 20 h photoperiod. Lang (1987) mentioned that, after passing a juvenile phase, the plants may require a period of photoinductive condition, to produce a sufficient amount of a hormone-like (florigen), or flower-inducing substances, which causes flower formation quite soon. There is no indication, however, that a signal of second factor was involved in the attainment of the floral state. Flowering could be delayed by an early transfer of plants from inductive photoperiod to non inductive photoperiod (Bernier et al. 1981; Lang 1987; Davenport 1990; Heller et al. 1994).

BA treatment had no effect on a flowering under short day, and flowering response was not influenced by high light intensity (data not shown). It indicates that day length plays an important role for flowering of *F. hindsii.* Inability of long day plants to regenerate flower buds suggested that the factor(s) which stabilize the flowering state were absent in the plants exposed to short days (Bridgen and Veilleux 1985; Lang 1987; Rajeevan and Lang 1987; Compton and Veilleux 1992).

A positive effect of 20 h photoperiod at 25°C was observed in an initiation of second or third flowering. When 0.1 mg 1^{-1} BA-treated seedlings were grown under 20 h photoperiod at 25°C, the percentage of second and third flowering increased from 85 % to 90 % or 100 %. A similar result was also observed in the seedlings grown under 20 h photoperiod at 30°C, but, number of fruits per seedling decreased in this case. The effect of temperature under long day is dual. Higher temperature of 30°C promoted initiation of flowers at high frequency, but the development of flowers into fruits was better when lower temperature of 25°C was given under 20 h photoperiod.

In conclusion, the present showed that BA speeded up and enhanced flower initiation, and that the best condition required for full induction of flowering was a combination of 25°C and 20 h photoperiod. An exposure of the seedlings to short day (< 16 h) resulted in a decline of their flowering capability. The flowering of *F. hindsii* was influenced by a combination of BA, photoperiod and temperature. The understanding gained from this research may provide information for acceleration of a generation turnover with in a citrus breeding program.

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