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EFFECTS OF GIBBERELLIC ACID AND PHOTOPERIOD ON FLOWERING OF YOUNG SEEDLINGS OF ORANGE JESSAMINE (*Murraya paniculata* (L.) Jack)

(Pengaruh gibberellic acid dan photoperiod pada pembungaan seedling orange jessamine (*Murraya paniculata* (L.) Jack)

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ABSTRAK

Photoperiod dan gibberellic acid (GA3) berpengaruh terhadap pembungaan seedling orange jessamine (*Murraya paniculata* L. Jack). Perlakuan 3 kali semprot dengan GA3 pada konsentrasi 0.01 mg/l di bawah 16 jam photoperiod memacu pembungaan lebih awal dan meningkatkan persentase (83 %) pembungaan. Lebih dari 80 % pembungaan pada umur 91 hari saat tanam (61 hari setelah disemprot dengan GA3, sedangkan hanya 33.3 % dari kecambah pada perlakuan tanpa GA3 berbunga pada umur 136 hari setelah tanam. Konsentrasi GA3 yang tinggi walaupun dibawah 16 jam photoperiod akan menghambat pembungaan. Bila photoperiod 8 jam sampai selama 5 bulan dipelihara tidak satupun seedling berbunga. Analisis kesuburan serbuk sari (pollen) dengan acetocarmine staining yang dihasilkan seedling menunjukkan lebih dari 70 % pollennya subur. Bentuk, ukuran dan jumlah organ bunga pada seedling adalah sama dengan bentuk bunga tanaman normal. Pembungaan kedua muncul pada umur 2 bulan setelah berakhirnya pembungaan pertama bila seedling diperlakukan dengan 0.01 mM GA3 dan dipelihara di bawah 16 jam photoperiod.

Kata-kata Kunci : Floral bud, gibberellic acid, juvenility, *Murraya paniculata*, orange jessamine, precocious flowering

INTRODUCTION

Orange jessamine (*Murraya paniculata*) is a member of orange subfamily and is potential germplasm source for many trait desirable for citrus root stock improvement including resistance to pest and pathogen (Swingle and Reece 1967. On the other hand, the wood of genus *M. paniculata* is valued and the tree has potential as an ornamental due to its large white flowers and attractive red fruits (Sykes 1968). The general area of origin of Murraya is believed to southeastern Asia, including that from eastern Arabia east Philippines and from the Himalayas south to Indonesia or Australia. Within the large regions, northeastern India and northern Burma were believed to be the center of origin, it may be as important due to the diversity of species found there, and the system of rivers that could have provide dispersal to the south (Nito *et al.* 1997).

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

Flowering of woody plants is affected by photoperiod, temperature, plant growth regulators and by genetic factors (Zimmerman 1972; Jackson and Sweet 1972; Bernier *et al.* 1993). Gibberellic acid has been found to promote the precocious flowering in very young seedlings of certain coniferous species (Pharis *et al.* 1965; Pharis and Morf 1969; Pharis *et al.* 1970). Precocious flowering of *M. paniculata* occur *in vitro* when plants derived from protoplasts were cultured under 16 h photoperiod in growth chamber (Jumin and Nito 1995. 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 (Furr *et al.* 1947; Hield *et al.* 1967; Iwamasa and Oba 1975; Snowball *et al.* 1994a, 1994b) when pollens could be made available earlier, it could be used to breeding purposes. This paper describes the effects of photoperiod and gibberellic acid (GA_3) sprays on flowering seedling of *M. paniculata*.

MATERIALS AND METHODS

Germination of seeds

Seed of *M. paniculata* were obtained from the germplasm collection at Faculty of Agriculture,

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Saga University Japan. Seed were placed in trays containing 75 % peat and 25 % sand (v/v) and kept under greenhouse conditions (20-25°C for 30 days. Seedlings were selected for uniformity in growth habit and size at the 2-leaf stage and transplanted into 0.5 l pot containing 50 % peat and 50 % sand.

Effect of gibberellic acid on flowering

The seedlings at 2-leaf stage were maintained in a growth chamber at 25°C and illuminated with fluorescent tubes at 52.9 µmol $m^{-2}s^{-1}$ with 16 photoperiod. GA_3 treatment was carried out immediately after the seedlings were transplanted to pots.The seedlings were sprayed with either distilled water or 0.01, 0.1, 1.0 and 10.0 mgl^{-1} GA_3 once a week for 3 weeks. Concentration of GA_3 were chosen based upon preliminary dose response trial on stock seedlings. Another series of experiment is repeated sprays of 0.01 mgl^{-1} GA_3 were made and the seedlings were kept at 25°C under 16 h photoperiod at 52.9 µmol $m^{-2}s^{-1}$ light intensity.

Effect of photoperiod on flowering

The seedlings at 2-leaf stage were foliage sprayed with 0.01 $mg.l^{-1}$ GA_3 once a week for 3 weeks and maintained in a growth chamber at 25°C and illuminated with fluorescent tubes at 52.9 $\mu mol.m^{-2}.s^{-1}$ with either 20, 16, 12, or 8 h photoperiod. The seedling was also exposed to a high light intensity at 74.9 $\mu mol.m^{-2}$ s^{-1} for 8 h photoperiod to distinguish photoperiod and photosynthetic effects.

All seedlings in this experiment were initially grown under growth chamber conditions for 10 months. The seedlings were hand watered every day. Seedlings were subsequently fertilized with a 0.1 % Hyponex solution 1 month after germination and once a month thereafter. Pest control procedures were not used because they have been shown to influence flowering in orange subfamily (Most 1976).

Measurements

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

Statistical analysis

Analysis of variance was used to statistically analyse the data. Significant differences between treatments were established with a paired Duncan's multiple range test. All experiments were replicated at least twelve times.

RESULTS AND DISCUSSIONS

Under a 16 h photoperiod and low level of GA_3 (0.01 mgl^{-1} with 3 sprays), floral buds formed approximately 71 days after germination or 41 days after GA_3 treatment (Table 1). Highest percentage of seedling flowering was also associated with either flowering (Table 1, 2 and 3). Over 80 % of seedlings flowered 91 days after germination (61 days after GA_3 application), whereas only 33.3 % of the seedlings without GA_3 flowered 136 days after germination. Plants in high level of GA_3 treatments have a rapid vegetative growth, but inhibited flower initiation. Pollen viability of GA_3-treated seedlings was over 70 % as assessed by acetocarmine staining. The flower shape, size and organs in seedlings were same as flowers of mature *M. paniculata* plants (Fig. 1). Fruit set and development was normal. Flower blossomed approximately 20 days after the floral bud emergence. Flower buds were observed at the original shoot terminal and shoot lateral. The emergence of floral bud occurred at the same time on the shoot terminal and shoots lateral. The lateral floral bud developed later than terminal floral bud.

Table 1. Effect of Gibberellic acid on vegetative growth and flowering of *M. paniculata* seedlings at 25°C under 16 h photoperiod for 5 months after germination (first flowering)

GA3 mg per l	Stem Length (cm)	No. of node per plant	No. of branch per plant	First emergence of floral bud (day)*	No. of floral bud per plant	First opening of flower (day)*	Percentage of seedling flowered (%)
0.0	6.3d	6.4cd	3.0b	119.7a	1.5b	135.7b	33.3a
0.01	5.1c	4.0ab	2.5b	71.0b	4.2c	910b	83.3b
0.1	7.2e	6.5cd	4.3c	89.7c	1.3b	141.3	25.0ac
1.0	7.7e	7.2d	4.0c	-	0.0a	-	0.0d
10.0	8.5f	8.2d	5.2d	-	0.0a	-	0.0d

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. Effect of spraying frequencies of 0.01 mgl^{-1} gibberellic acid on vegetative growth and flowering *M. paniculata* at 25°C under 16 h photoperiod for 5 months after germination

Frequency of spray	Stem Length (cm)	No. of node per plant	No. of branch per plant	First emergence of floral bud (day)*	No. of floral bud per plant	First opening of flower (day)*	Percentage of seedling flowered (%)
3 x	5.1a	4.0a	2.5a	71.0a	4.2a	91.0a	83.3a
4 x	6.2b	4.0ab	2.8a	119.0b	1.4b	140.0b	16.7b
6 x	7.7c	5.3	3.5ab	-	0.0c	-	0.0c
8 x	9.2d	5.7b	3.7ab	-	0.0a	-	0.0c

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 3. Effect of photoperiod on vegetative growth and flowering of *M. paniculata* seedlings sprayed with 0.01 mgl^{-1} gibberellic acid and kept in growth chamber at 25°C for 5 months after germination (first flowering)

Photoperiod (h)	Stem Length (cm)	No. of node per plant	No. of branch per plant	First emergence of floral bud (day)*	No. of floral bud per plant	First opening of flower (day)*	Percentage of seedling flowered (%)
8 h	4.5a	4.4a	1.5a	-	0.0	-	0.0
12	6.2b	6.3b	3.0b	130.0a	1.5b	136.0a	5.3a
16	5.1a	4.0ac	2.5ab	71.0b	4.2c	91.0b	83.3b
20	4.5a	3.0c	3.0b	123.5a	0.8a	139.0a	16.7c

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.

The highest number of floral buds was obtained in seedlings with decline in vegetative growth. These were also widely observed in woody plants (Bernier *et al.* 1981; Heller *et al.* 1994; De Baerdemaeker *et al.* 1994). However, a decline in vegetative growth was also observed in seedlings which were exposed to 8 h photoperiod, but no flower formation even after spraying with 0.01 mgl^{-1} GA_3. This result has been postulated that under inductive 16 h photoperiod, GA_3 triggers the flower formation that precedes flowering.

Flower initiation of *M. paniculata* under long days (16 h) can be considerably modified be the GA_3. These results are in general agreement with previous finding, GA_3 promoted flower initiation in conifer (Pharis *et al.* 1965; Pharis and Morf 1967; Pharis *et al.* 1969; Pharis *et al.* 1970). The promotion of flower initiation by GA_3 was also found in *Cordyline* (Agaveceae) and Araceae which are photoperiodically neutral (Fisher 1985). A single spray with GA3-induced profuse flowering 10 to 14 weeks after treatment in several ornamental aroids, such as *Aglaonema, Diffenbachia, Spathiphyllum, Carladium* and *Zantedeschia* (Henny 1988). Exogenous GA_3 has been shown to promote the switch from vegetative growth to flowering in *Arabidopsis thaliana* (Wilson *et al.*1992). As shown in *M. paniculata*, GA_3 was found to be suitable to initiate flowering and to promote the earlier flowering. Effect of GA_3 in several plant systems suggested that promotion of vegetative might also be expected in *M. paniculata*. As demonstrated, however, only a narrow level (concentration + spray frequency) of GA_3 was found to be promoted flower initiation. The narrow effect of GA_3 in respect of flowering is common: for example in *Gypsophila paniculata* (Shlomo et al. 1985), onion (Naami et al. 1980) and easter cactus (Boyle et al. 1994). Several reasons for a narrow effect of GA_3 can be envisage, including practical considerations such as the failure of applied chemical to penetrate, or non-persistence, or inactivation of the applied chemical, as well as interaction with other endogenous hormones which could involve blocking of GA_3 action (Hanks 1982; Lang 1987).

Flowering is considered as a complex process regulated by environment and internal plant factors (Bernier *et al.* 1981; Hackett 1985; Reid *et al.* 1991). Under 16 h photoperiod, GA_3-treated seedlings are continuously produced flowers. Second flowering occurred 2 months after the completion of first flowering. Although 16 h photoperiod with GA_3 could induce second flowering, there was a positive correlation between 0.01 mgl^{-1} GA_3 and 16 h photoperiod to induce second flowering. When 0.01 mgl^{-1} GA_3-treated seedlings were grown under 16 h photoperiod more percentage of second flowering were observed (Table 4). A 16 h photoperiod not only promoted the induction of floral buds, but also promoted development of flower and subsequently to produce fruit (Fig.2). Even though a few flowers were produced from seedling less than 20 h photoperiod, but they failed to produce fruits. GA_3 treatment has no effect on flowering under short day even after exposed to high light intensity at 74.9 $\mu mol\ m^{-2}\ s^{-1}$ (data not shown), indicating that very long day (> 16 h) is a limiting factor for flowering of *M. paniculata*. *In vitro*

study suggested that precocious flowering had been shown to be generally dependent upon an inductive photoperiod (Coleman and Thorpe 1978; Scorza 1982; Scorza and Janick 1980). The precocious flowering has been observed on progeny seedlings of citrus and its relatives (Furr *et al.* 1947; Hield *et al.* 1967; Iwamasa and Oba 1975; Snowball *et al.* 1994) and on plantlets of *M. paniculata* derived from protoplasts (Jumin and Nito 1995).

Table 4. Second flowering of seedlings of *M. paniculata*. Data recorded 2 months after completion of first flowering

GA3 (mg/l) x spray	No. of floral bud per seedling	No. of flower opened per seedling	Percentage of seedling flowered (%)
16 h photoperiod			
0.0 x 3	2.3a	1.0a	44.5a
0.01 x 3	5.7b	1.7b	88.9b
0.1 x 3	1.2ac	0.7a	13.9c
1.0 x 3	0.0c	0.0c	0.0c
0.01 x 4	1.0ac	0.3ac	5.6c
20 h photoperiod			
0.0 x 3	0.0c	0.0c	0.0c
0.01 x 3	1.5ac	0.8a	11.1c

Means within a column followed by the same letter are not significantly different at P= 0.05.

In conclusion, this study shows that GA_3 speeded up and enhanced flower initiation, the conditions required for full induction of flower was 16 h photoperiod. Exposure of the seedlings to short day (8 h) or more than 16 h resulted in a decline in their flowering capability. The flowering of *M. paniculata* was influenced by combination of GA_3 and photoperiod. The shorter juvenile period might be useful in breeding programs to introduce the characteristic of earlier flowering and so allow faster generation turnover (Hansche and Beres 1982).

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