RESEARCH ARTICLE

Gibberellin-induced flowering in sexually defective *Remusatia vivipara* (Araceae)

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ABSTRACT: *Remusatia vivipara* is an epiphyte of possibly ornamentally and medically important plant, but flowering is rare in fields. The present experiments were conducted to study the influences of different concentrations of gibberellic acid (GA3) and tuber sizes on the flower initiation, inflorescence characteristics and vegetative growth in *R. vivipara*. GA3 concentration as low as 25 mg L^{-1} could induce flowering. The results of a binary logistic regression analysis indicated that the flowering was significantly associated both with GA3 concentration and tuber size. However, comparing with the non-GA3 treated tubers, different GA3 concentrations did not significantly affect flowering. The result also showed no significant effect induced by GA3 treatments on the number of days to flower. In contrast, the Wald statistic revealed that both tuber size (2.51–3.00 cm) and tuber size (3.01–3.50 cm) made more significant contributions to the prediction of flowering. Tuber diameters above 3.01 cm with 100 mg L^{-1} GA3 treatment could bring all plants to flower. Results of canonical discriminant analysis and ANOVA tests indicated that there were no differences for all inflorescence characters (inflorescence length, male zone length, sterile zone length and female zone length) among different concentrations of GA3 tested. On the contrary, significant differences among the tuber diameter classes for all inflorescence characteristics measured were markedly evident. Generally, sizes of almost all inflorescence characteristics increased with increasing tuber sizes. When considering vegetative characters, significant differences in the fresh and dry weights of bulbil stolon were found between treated and untreated tubers. Although there was a trend of increase in weights with increasing GA3 concentrations, but this was not statistically significant. Our results for *R. vivipara* showed the induction of flowering by GA3 only influence of flower initiation, but no effects on inflorescence and vegetation characteristics. The present study also revealed that large tuber size made more significant contributions to the prediction of flowering, and the magnitude of inflorescence characteristics.
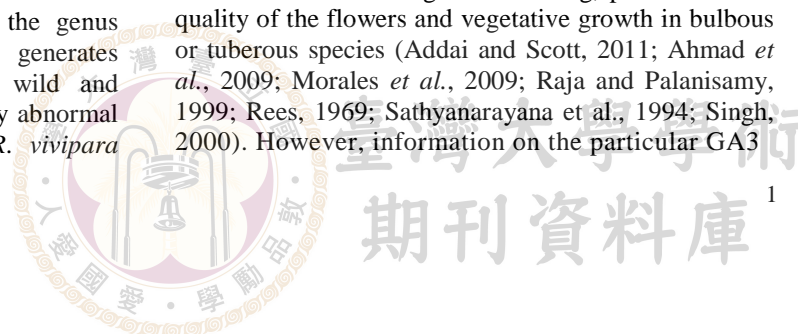
KEY WORDS: Bulbil stolon, Gibberellin acid, Inflorescence characteristics, *Remusatia,* Tuber size, Vegetative growth.

INTRODUCTION

The family Araceae consists of 105 genera and more than 3300 species in the tropics of America, Southeast Asia, tropical Africa, southern Africa, Madagascar and Australia (Mayo *et al.*, 1997). Several genera of Araceae with colorful and long-lasting spathes are highly valued plants (Henny, 1995). Adjustment of growth and flowering was showed particular concern for plants grown for cut flower production (Al-Khassawneh *et al.*, 2006). *Anthurium* and *Zantedeschia* are very attractive plants for cut flowers and pot plants (Treder, 2005). The natural flowering rate is quite low. For increasing flower yield, the tubers are usually treated with gibberellins before planting (Brooking and Cohen, 2002; Corr and Widmer, 1990; Dennis *et al.*, 1994).

It is the most widespread species in the genus *Remusatia*. However, *R. vivipara* rarely generates flowers and produces no seeds in the wild and herbarium records. No seeds might cause by abnormal male meiosis in triploid population of *R. vivipara*

(Huang and Hsieh, 2014). Asexual reproduction by bulbils and tubers are perhaps the major reproductive strategy of *R. vivipara* (Huang and Hsieh, 2014; Li *et al.*, 2012). The newly recorded of *Remusatia yunnanensis* (H. Li & A. Hay) A. Hay in the habitat of *R. vivipara* in Taiwan (Huang *et al.*, 2013). The *R. vivipara* and *R. yunnanensis* were very similar in tuber, leaf, and bulbil. These two species could distinguish by inflorescences, but *R. vivipara* were seldom flowering (Huang and Hsieh, 2014). Previous studies had reported cold temperature and plant hormone (gibberellic acid) can induce flowering in the species of Araceae, such as *Zantedeschia* spp., and *Amorphophallus muelleri* (Brooking and Cohen, 2002; Treder, 2005; Zhao *et al.*, 2010). An increasing number of studies also reported that the initial tuber, bulb or rhizome size is one of the critical factors affecting the flowering, production and quality of the flowers and vegetative growth in bulbous or tuberous species (Addai and Scott, 2011; Ahmad *et al.*, 2009; Morales *et al.*, 2009; Raja and Palanisamy, 1999; Rees, 1969; Sathyanarayana et al., 1994; Singh, 2000). However, information on the particular GA3

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concentrations and tuber size that will produce good quality flowers and vegetation growth in R. vivipara has not been documented in the literature. The objectives of this study were to examine the effects of different concentrations of gibberellic acid (GA3) and tuber sizes on the flower initiation, inflorescence characteristics and vegetative growth in *R. vivipara*.

MATERIALS AND METHODS

Tubers of the R. vivipara were collected at two locations in winter 2011. The first habitat was a natural secondary forest in Nantou. The second habitat was the nursery in the Department of Forestry and Nature Conservation of the Chinese Culture University. A total of 245 tubers were stored in cold storage house at 5℃ for one month before GA3 treatments.

GA3 (90% purity; Sigma–Aldrich Inc., St. Louis, MO, USA) was dissolved in 95% (v/v) ethanol and then dissolved further with deionized water to make up 0, 25, 50, 75 and 100 mg L-1 GA3 solution. The tubers were fully immersed in each GA3 concentration treatment for 30 minutes. The GA3 treatments were repeated three times. At the end of each treatment, tubers were rinsed with tap water and drained on paper towels. The purpose of water rinsing was to remove GA3 that had not been absorbed by the tubers or the apical buds within the stated treatment times.

Nine tubers were used for each group of the treatment in the first period, 20 tubers were used for each group of the treatment in the second period , and 20 tubers were used for each group of the treatment in the third period. Tubers of the same treatment were planted together in a 27.5×23×8 cm pot using peat:pearlite (1:1 v/v) non-soil substrate. The pots were watered and placed at 18–22℃ and relative humidity 60–80% in a plant growth chamber under 11 hour photo-period of 3500 to 18000 lux intensity provided by fluorescent light. The pots near the light source were the maximum of light intensity (18000 lux), and pots away from the light source were the weakest of light intensity (3500 lux). The pots were turned clockwise daily exchange position.

The tubers were divided according to their diameters into five categories. The numbers of bulbil stolons (bulbiliferous shoots) in the first leaf were recorded at the time of opening of the first leaf. For GA3 treatment analyses, inflorescences were tagged to determine dates of spathe opening. The morphological characteristics of inflorescences after development were recorded as the lengths of inflorescences, male zones, sterile zones, female zones and peduncles. After flowering the vegetative characteristics collected for each tuber included dry weight of leaves, fresh and dry weights of bulbil stolons. Besides, inflorescence characteristics from eight individuals in the wild (wild

plant) were measured and included for comparisons. Statistical differences in morphological characteristics among GA3 treatments and among tuber-size classes were analyzed with a one-way ANOVA, followed by a Games-Howell post hoc test. Multivariate differences in inflorescence characteristics among GA3-treated plants and wild plants were analyzed with a Canonical Discriminant Analysis. The effects of GA3 treatments and tuber sizes on the plant flowering were analyzed using binary logistic regression with flowering status (flowering or not) as dependent variable and GA3 treatments and tuber size-classes as independent variables. The probability value associated with the Wald chi-square statistic was examined to determine the significance of model variable parameters. All statistical analyses were carried out using SPSS 11 (2001).

RESULTS

The effects of GA3 concentrations and tuber sizes on flowering

The GA3 treatment resulted in 107 inflorescences induced from 245 tubers as compared to that only one inflorescence from 49 non GA3-treated tubers of R. vivipara. As GA3 concentration was increased, there was an increase in the proportion of initiated flowers (Figure 1). GA3 application could significantly enhance flowering percentage individually from 36.7% minimum to 63.9% maximum in R. vivipara. The minimum GA3 concentration used is 25 mg L-1 .
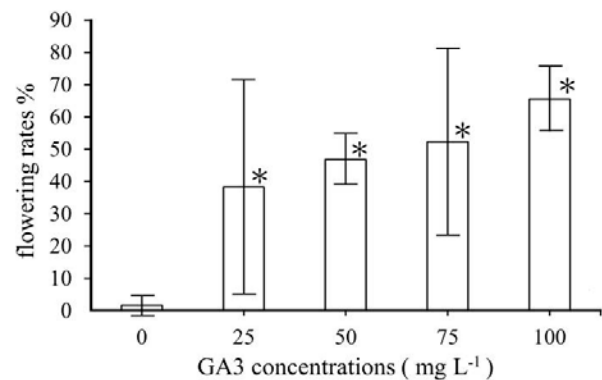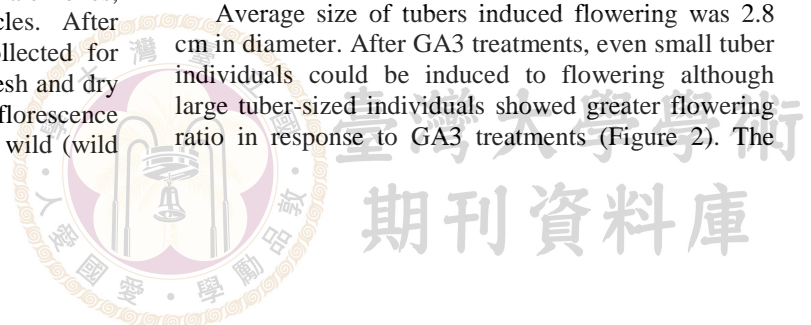

Fig. 1. The effects of different GA3 concentrations on the induction of flowering in *Remusatia vivipara*. * indicates that the mean values are significantly different at 0.05 probability level between GA3-treated and untreated tubers.

Average size of tubers induced flowering was 2.8 cm in diameter. After GA3 treatments, even small tuber individuals could be induced to flowering although large tuber-sized individuals showed greater flowering ratio in response to GA3 treatments (Figure 2). The

optimal tuber diameters with GA3 treatments to induce flowers were between 3.01 cm to 3.50 cm. Tuber diameters above 3.01 cm with 100 mg L^{-1} GA3 treatment could bring all plants to flower. The results of a binary logistic regression of flowering indicated that the probability of a plant fowering is signifcantly associated both with GA3 concentration and tuber sizes ($\chi2$ = 82.2, p < .0001 with df = 8). The model explained 38.4% (Nagelkerke's R2) of the variance in flowering. Prediction success overall was 70.2% (71.8% for flowering and 68.2% for non-flowering tubers). The Wald criterion revealed that both (2.51–3.00 cm) and (3.01–3.50 cm) tuber size-classes made more significant contributions to the prediction of flowering (p < 0.01). However, for inflorescence initiation different GA3 concentrations did not contribute (p = 0.133) to the model if the control category (non-GA3 treated) was excluded from the analysis.
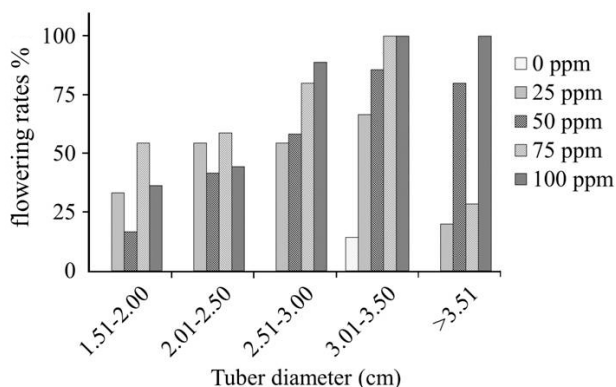

Fig. 2. The influences of GA3 treatments and tuber sizes on the percentage of flowering plants.

Number of days to flower

One untreated plant of R. vivipara produced a single inflorescence by 88 days. The mean numbers of days to inflorescence emergence after treatments with four GA3 concentrations ranged from 87.5 to 97.6. The earliest (63 days) and latest (129 days) flowerings were observed both at GA3 concentration 100 mg L-1. No statistically significant differences were observed among the four GA3 concentrations according to Scheffé's test (P = 0.08).

The effects of GA3 concentrations on inflorescence characteristics

Results of canonical discriminant analysis for inflorescence characteristics of 105 GA3-treated samples and eight wild plants indicated that the first

discriminant function accounted for most of the variance (75.6%), and was statistically significant (Wilk's lambda = 0.664; x2 = 43.87, P = 0.002). The plot of all samples in the space defined by the first two discriminant functions provided a clear discrimination between GA3-treated plants and wild plants along the first axis (Figure 3). However, a great overlap of samples under different GA3 concentrations appeared on the first and second canonical axes, showing no difference in inflorescence characteristics. The tests of equality of group means indicated a highly significant difference (p = 0.001) of means between GA3-treated plants and wild plants for female zone length of the inflorescence. ANOVA tests also exhibited a significant difference ANOVA tests also exhibited a significant difference between GA3-treated plants and wild plants for female zone length of the inflorescence (Table 1, P = 0.001), but there was no difference for each of the five inflorescence characters between different concentrations of GA3 tested (Table 1). When the data of inflorescence characters under four GA3 treatments were grouped together and compared with those of the wild plants, significant differences were found for all inflorescnce characters.
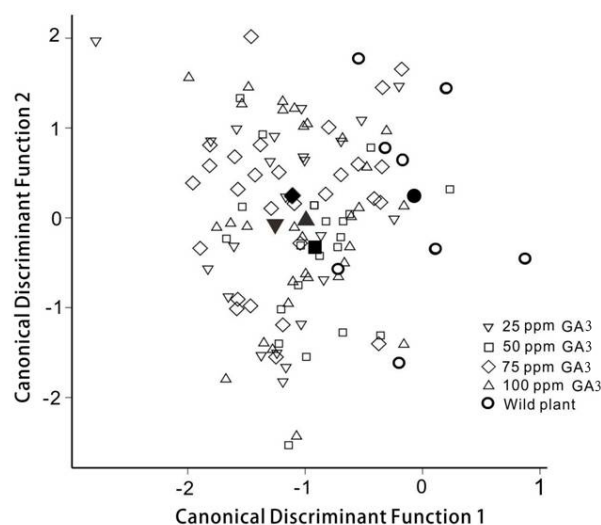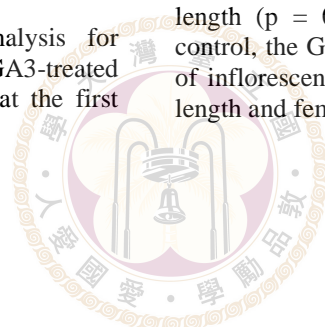

Fig. 3. Scatterplot of the first two canonical discriminant functions of the analysis of inflorescence characteristics under GA3 treatments and from wild plants. Open symbols are samples for each of the four GA3 treatments and wild plants; solid symbols represent group centroids.

The most important character for discriminating groups was female zone length (p < 0.01), followed by sterile zone length (p = 0.006), spathe length (p = 0.008), inflorescence length (p = 0.021) and male zone length (p = 0.028). Relative to the single untreated control, the GA3-treated tubes have lower mean values of inflorescence length, male zone length, sterile zone length and female zone length.

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Table 1. The effects of GA3 treatments on the inflorescence characteristics of *Remusatia vivipara* and the comparison of inflorescence characteristics between GA3-treated plants and wild plants.

GA3 concentration (mg L^{-1})	Mean value ± SD (cm)					N
	Inflorescence	Spathe length	Male zone	Sterile zone	Female zone	
0 (control)	16.00	8.50	1.50	2.00	1.00	1
25	12.97±5.35a	8.36±3.35a	1.16±0.43a	1.18±0.52a	0.86±0.36a	23
50	12.68±5.76a	8.25±3.46a	1.17±0.41a	1.10±0.44a	0.94±0.35a	22
75	13.33±6.14a	8.58±3.61a	1.27±0.44a	1.23±0.50a	0.93±0.40a	28
100	13.08±5.59a	8.03±3.32a	1.22±0.48a	1.18±0.46a	0.94±0.38a	32
Wild plants	17.76±2.23a	11.55±1.03a	1.56±0.18a	1.66±0.44a	1.51±0.27b	8
Significance	NS	NS	NS	NS	***	
All GA3 treatments	13.04±5.65a	8.29±3.39a	1.21±0.44a	1.18±0.48a	0.92±0.37a	105
Wild plants	17.76±2.23b	11.55±1.03b	1.56±0.18b	1.66±0.44b	1.51±0.27b	8
Significance	*	**	*	**	***	

Means followed by the same letter within a column are not different from each other according to Games-Howell test. NS indicates not significant at the 0.05 probability level; *, **, *** significant at 0.05, 0.01 and 0.001 probability level, respectively. Means followed by the same letter within a column are not different from each other according to Games-Howell test.

The effects of tuber sizes on inflorescence characteristics

Results of canonical discriminant analysis for inflorescence characteristics of 113 samples (including eight wild plants) indicated that the first discriminant function accounted for most of the variance (77.3%), and was highly statistically significant (Wilk's lambda = 0.44; x2 = 87.66, p < 0.001).
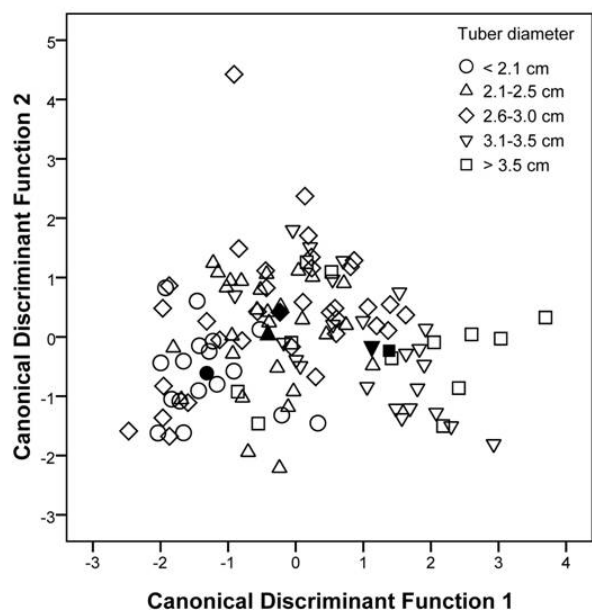

Fig. 4. Scatterplot of the first two canonical discriminant functions of the analysis of inflorescence characteristics under different tuber diameters. Open symbols are samples for each of the five tuber diameter class; solid symbols represent group centroids.

The plot of all samples in the space defined by the first two discriminant functions provided a clear discrimination between tuber diameter classes along the

first axis (Figure 4). Results for tests of Equality of Group Means revealed that all characteristics were highly significantly different (p < 0.001) between tuber diameter classes. The most important characteristics for discriminating groups based on F value were inflorescence length, followed by male zone length, female zone length, spathe length and sterile zone length.

ANOVA tests also exhibited a significant difference between tuber diameter classes for all inflorescence characters (Table 2, p < 0.001). In general, sizes of all characteristics increased with increasing tuber sizes.

Table 2. The effects of different tuber sizes on the inflorescence characteristics of *Remusatia vivipara*.

Tuber diameter class	Mean value ± SD (cm)					N
	Inflore-scence length	Spathe length	Male zone length	Sterile zone length	Female zone length	
(< 2.0cm)	7.00 ± 2.95a	4.62 ± 1.68a	0.81 ± 0.23a	0.72 ± 0.27a	0.58 ± 0.19a	17
(2.1–2.5cm)	12.49 ± 3.85b	8.12 ± 2.51b	1.08 ± 0.28b	1.20 ± 0.42b	0.89 ± 0.25b	28
(2.6–3.0cm)	13.01 ± 5.74b	8.53 ± 3.67b	1.24 ± 0.42b	1.21 ± 0.46b	0.88 ± 0.35b	33
(3.1–3.5cm)	17.49 ± 3.38c	10.73 ± 2.10c	1.52 ± 0.20c	1.47 ± 0.42b	1.27 ± 0.35c	23
(> 3.6 cm)	17.58 ± 5.68c	10.76 ± 3.08c	1.67 ± 0.61c	1.44 ± 0.60b	1.31 ± 0.47c	12
Significance	***	***	***	***	***	

Means followed by the same letter within a column are not different from each other according to Games-Howell test. *** indicates that F-value is significant at 0.001 probability level.

Effects of GA3 concentrations and tuber sizes on vegetative growth

When considering vegetative characters, the dry weight of leaves did not show any difference between treated and untreated tubers and also among four GA3 concentrations (Table 3). However, significant differences (p < 0.05) in the fresh and dry weights of bulbil stolons were found between treated and untreated tubers. There was a trend of increase in mean weight
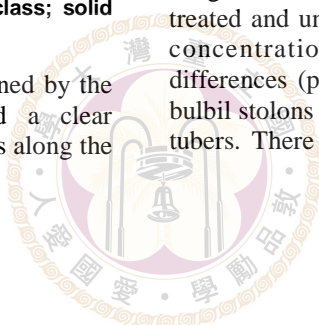
Table 3. The effects of GA3 treatments on the vegetative growth of *Remusatia vivipara*.

GA3 concentration (mg L^{-1})	Mean value ± SD (g)		
	Dry weight of Leaves	Fresh weight of bulbil stolons	Dry weight of bulbil stolons
0 (control)	0.37 ± 0.32a (49)	1.42 ± 2.09a (31)	0.49 ± 0.80a (31)
25	0.39 ± 0.31a (23)	2.87 ± 2.62b (28)	1.20 ± 1.15b (28)
50	0.47 ± 0.27a (18)	3.18 ± 3.99b (32)	1.21 ± 1.40b (32)
75	0.53 ± 0.36a (16)	3.87 ± 5.01b (28)	1.62 ± 1.97b (28)
100	0.45 ± 0.36a (25)	4.77 ± 5.28b (28)	1.75 ± 1.94b (28)
Significance	NS	*	*

Means followed by the same letter within a column are not different from each other according to Games-Howell test. NS indicates not significant at the 0.05 probability level; * significant at the 0.05 probability level. The numbers in parentheses are the sample sizes.

with increasing GA3 concentration for both fresh and dry bulbil stolons, although no significant differences were found among the four GA3 concentrations (Table 3). On the other hand, the ANOVA results showed that the tuber size significantly affected the growth of leaves and bulbil stolons. The weights of both leaves and bulbil stolons increased considerably with increasing tuber size (Table 4).

Table 4. The effects of different tuber sizes on the vegetative growth of *Remusatia vivipara*.

Tuber diameter class	Mean value ± SD (g)		
	Dry weight of Leaves	Fresh weight of bulbil stolons	Dry weight of bulbil stolons
1 (< 2.0 cm)	0.29 ± 0.23a (27)	1.81 ± 1.68a (27)	0.75 ± 0.69a (27)
2 (2.1–2.5 cm)	0.41 ± 0.35ab (35)	2.14 ± 1.87a (34)	1.06 ± 1.26ab (34)
3 (2.6–3.0 cm)	0.40 ± 0.22ab (26)	2.39 ± 3.01ab (41)	1.01 ± 1.29ab (41)
4 (3.1–3.5 cm)	0.52 ± 0.27b (21)	2.99 ± 4.26ab (23)	1.05 ± 1.52ab (23)
5 (> 3.6 cm)	0.73 ± 0.92b (23)	5.48 ± 5.16c (24)	1.95 ± 1.68b (24)
Significance	*	***	*

Means followed by the same letter within a column are not different from each other according to Games-Howell test. NS indicates not significant at the 0.05 probability level; * significant at the 0.05 probability level. The numbers in parentheses are the sample sizes.

DISCUSSION

Gibberellin acid has been reported to stimulate flower initiation in *Aglaonema, Caladium, Dieffenbachia, Spathiphyllum*, *Zantedeschia* and other genera of the Araceae (Harbaugh and Wilfret, 1979; Brooking and Cohen, 2002; Funnell *et al.*, 1992; Henny, 1988; Henny and Hamilton, 1992). Our results for *R. vivipara* also showed the induction of flowering by GA3 (Figure 1). Many studies (Brooking and Cohen, 2002; Cardoso *et al.*, 2010; Chen *et al.*, 2003) reported that increased gibberellin dose could increase in the proportion of initiated flowers, but in this study, the ratio

of inflorescence initiation was not significantly influenced by the GA3 at concentrations above 25 mg L^{-1} (Figure 1 & Figure 2). Therefore we recommend a single application of GA3 at 25 mg L^{-1} to promote inflorescence initiation.

High gibberellin doses inducing more rapid floral initiation than lower doses was observed by Brooking and Cohen (2002) on the tubers of Zantedeschia "Black Magic". On the contrary, the present study showed similar flower initiation times on R. vivipara in ca. 93 days. This result is in agreement with the finding of Pandey *et al.* (2001), who suggested that the treatment of GA3 could be effectively in inducing uniform sprouting and flowering in rhizomes of *Podophyllum hexandrum*.

Previous studies indicated that the size of tubers or bulbs is very important to the plants in terms of vegetative growth and flower production (Addai and Scott, 2011; Ahmad *et al.*, 2009; Rees, 1969). In this study, the binary logistic regression revealed that larges tuber size made more significant contributions to the prediction of flowering. The results of canonical discriminant analysis and ANOVA (Table 2) showed that inflorescence characteristics (inflorescence length, male zone length, female zone length, spathe length and sterile zone length) and vegetative growth (fresh and dry weights of bulbil stolons) mostly increased with increasing tuber size (Table 2 & Table 3). A similar trend was observed by Burton (1966) in potatoes, Rees (1969) in tulips, De Munk and Schipper (1993) in *Iris*, Addai and Scott (2011) in hyacinth and lily. In general, bulb or tuber weight was an important indicator for flowering in these species, and as the size of the bulbs or tubers increased, both flower quality and vegetative growth also increased. The increase in magnitude of these characteristics may be attributed to the amount of reserves stored in the bulb prior to planting (Addai and Scott, 2011). Han *et al.* (1991) reported that the growth and flower quality was independent of the mother corm size in *Brodiaea* at the time of planting but it was the size of the apical meristem that determined the quality of the flowers produced. Addai and Scott (2011) mentioned that above 50 g bulb size either vegetative growth or flower quality was not significantly different from those of the smaller size bulbs. Our results also showed that the lengths of inflorescence, spathe, male zone, sterile zone and female zone of the tubers above the 3.6 cm size were similar to those of the smaller size tubers (3.1–3.5 cm). Above this threshold size, growth and development might be determined by the size of the apical meristem(Addai and Scott, 2011). Further studies are needed to assess the role of apical meristem for *R. vivipara*.

Many studies have been made to evaluate the use of GA3 on the inflorescence or floral quality of ornamental and flowering species (Cardoso *et al.*, 2012; Vieira *et al.*,

2011; Zalewska and Antkowiak, 2013). The results of the studies on the effectiveness of GA3 on flowers are contradictory. However, some reports indicated that GA3 could increase the length of the floret on tuberose flowers and elicit cell elongation (Hassanpour Asil *et al.*, 2011; Macnish *et al.*, 2010). The present study showed that GA3 applications at different concentrations did not affect the characteristics of inflorescences, although a clear discrimination between GA3-treated plants and wild plants was found along the first axis of canonical discriminant analysis (Figure 3) and also revealed by ANOVA (Table 1). Further analysis indicated that the larger mean values of inflorescence characteristics was mainly attributed to the larger tuber size of wild plants.
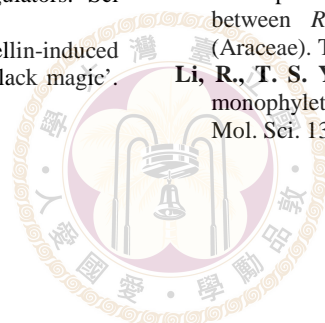
CONCLUSION

Our results for *R. vivipara* showed the induction of flowering by GA3 at and above 25 mg L^{-1}. However, no significant differences were observed among different GA3 concentrations in terms of inflorescence characteristics and vegetative growth. To produce *R. vivipara* as an ornamental plant, it is recommended to drench plants with 25 mg L^{-1} GA3. The present study also revealed that large tuber size made more significant contributions to the prediction of flowering, and the magnitude of inflorescence characteristics (inflorescence length, male zone length, female zone length, spathe length and sterile zone length) and vegetative growth (fresh and dry bulbil stolons) mostly increased with increasing tuber size.

ACKNOWLEDGMENTS

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