Effects of Gibberellin Application on Growth and Development of *Curcuma alismatifolia* Gagnep.

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Summary

Effects of gibberellin (GA_3) application on growth and development of *Curcuma alismatifolia* Gagnep. were investigated. Four levels of GA_3 at 0, 100, 300 and 500 mgL^{-1} were supplied by drenching (the soil) twice at 4 weeks after planting (shoot emergence) and 6 weeks after planting. The results showed that the application of GA_3 at 300 and 500 mgL^{-1} gave the best results in terms of plant height for 101 and 97.4 cm, respectively, but the treatments did not affect the number of leaves per plant and number of shoots per cluster. The GA_3 level at 500 mgL^{-1} gave the longest scape (spike stalk) for 87.1 cm which is longer than those in control plants with about 30 cm. The GA_3 application at 300 and 500 mgL^{-1} gave the longest number of days to flower for 76 and 73 days respectively, which were longer than control treatment for about 5-8 days. At flowering stage, the contents of nitrogen, potassium and phosphorous were analyzed in the aboveground and underground parts. The result showed that GA_3 application affected the concentration and content of nitrogen and potassium but not for phosphorus. The nitrogen content in underground part was lowest (96.8 mg $plant^{-1}$) with GA_3 application at 500 mgL^{-1}. Application of GA_3 at 100 mgL^{-1} increased potassium content in underground part. At 32 weeks after planting at harvest stage, GA_3 at the level of 300, 500 mgL^{-1} increased rhizome length.

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Key words : *Curcuma alismatifolia*., flowering, gibberellin, nutrient, rhizome

Curcuma alismatifolia Gagnep., a member of the ginger family (Zingiberaceae), is known as Siam tulip or Pathumma. The curcuma family is native to tropical and sub tropical areas (Apavatjrut *et al*., 1999). *Curcuma* species have colorful, long-lasting inflorescences with few pest problems. It has been used for cut flowers, pot plants and garden plants. *Curcuma alismatifolia* Gagnep. is the main species in international trade (Lekawatana and Pituck, 1998), although the number of the curcuma cut flowers exported from Thailand is still very low compared with orchid cut flowers.

Curcuma alismatifolia are herbaceous perennials, the inflorescence is showy upper bract or coma bract (purplish pink with brownish green tip), lower bract (green). True flowers (a delicate purple labellum with yellow medium stripe) hide in the axils of the bracts (Hagiladi *et al.,* 1997). Underground part consists of two types of storage organs, rhizomes and storage roots. The rhizome or stubbed rhizome is a site of bud formation, which produces leaves and inflorescences in next season (Hagiladi *et al.,* 1997). The stubbed rhizomes are the principle organ for N storage in this *Curcuma* species (Ruamrungsri *et al.,* 2001). The storage roots, which are morphologically changed from adventitious roots are know to be the major storage organ of carbohydrate (Ruamrungsri *et al.,* 2001).

Most of the *Curcuma* plants are planted in the rainy season in Thailand, so the products of flowers and rhizomes come to market at the same time resulting in the low price. In the peak season, some farmers lift the curcuma plants and leave them away in the field to spoil, because it is not profitable to harvest them. In addition, flowers and rhizomes could not be continuously supplied to market because farmer could not produce it for all year round. (Butrploy, 2000). Furthermore, rhizomes should be standardized and they should be healthy without diseases. Thus, it is essential to develop the method to produce *Curcuma alismatifolia* Gagnep. with high yield and good quality in off-season.

Gibberellins (GAs) are a family of plant hormones controlling many aspects of plant growth and development including stem elongation, germination, and the transition from vegetative growth to flowering (Stephen *et al,*. 2005). Gibberellin has been applied to bulbs of duch iris, lily, and tulip (Hanks, 1979), and to *Liatris* corms (Moe and Berland, 1986) as a substitute for a cold treatment for forcing cultivation. Phytohormones also play dominant roles in the regulation of the growth and development of higher plants, for example, the sink-source relationship, plant yield and mineral nutrients. On the other hand, mineral nutrients, especially nitrogen has known to give the most prominent effect on GA levels (Marschner, 1986). Effects of gibberellin application on the growth and development of *Curcuma*

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alismatifolia Gagnep. have been reported rarely (Kuehny *et. al.*, 2002), and the effects of GA application on mineral nutrients (N, P, and K) in the upperground and underground parts have not been investigated so far. In this study, we investigated the effects of four levels of gibberellin application on the growth and development of curcuma and on the contents of mineral nutrients (N, P, and K) in both aboveground and underground parts of *C. alismatifolia* Gagnep., with the aim to produce flowers and rhizomes at off-season with high yield and good quality.

MATERIALS AND METHODS

A rhizome of *Curcuma alismatifolia* Gagnep. with four storage roots was planted at 24 June 2004 in a 6 x 12 (diameter x height) inch pot using soil sand and rice husk charcoal ratio 1:1:1 (by volume). Plants were supplied with the complete solution comprised of (in mgL^{-1}) N, 200; P, 50; K, 200; Mg, 25; Ca, 136; B, 0.216; Mn, 0.812; Zn, 0.262; Cu, 0.025; Mo, 0.0435; Fe, 0.405. Four levels solution (100 ml water) at 0, 100, 300 and 500 mgL^{-1} GA_3 were supplied to the soil twice at 4 weeks after planting (WAP) (shoot emergence) and 6 WAP by drenching. The experiment was a completely randomized design with four replications per treatment. Data collected included plant growth and development; plant height, number of leaves per plant, number of shoots per pot and number of days from planting to flowering. Flower quality and quantity collected consisted of scape (spike stalk) length, spike length, number of spikes per cluster and number of pink and green bracts. At flowering stage, plants were separated to two parts upperground and underground parts. Samples were washed with tap water and deionized water two times than dried and ground into powder. Dried sample were digested using a Kjeldahl digest solution (Ohyama *et al.* 1985 : 1991) to determined N concentration by a modified indophenol method, P concentration by the ammonium molybdate method (Davidescu and Davidescu, 1972). K concentration was determined by atomic absorption spectrophotometry using a $HClO_4$-HNO_3 digestion modified method (Mizukoshi *et al.* 1994). At rhizome harvesting stage on date, the plants were harvested and collected data included rhizome weight per cluster, rhizome width, rhizome length, number of storage roots per rhizome, storage roots length.

Fig. 1. Effects of GA_3 application on visual morphological changes at flowering stage of *Curcuma alismatifolia* Gagnep.
T1: untreated plants, T2: GA_3 100 mgL^{-1}, T3: GA_3 300 mgL^{-1}, T4: GA_3 500 mgL^{-1}.

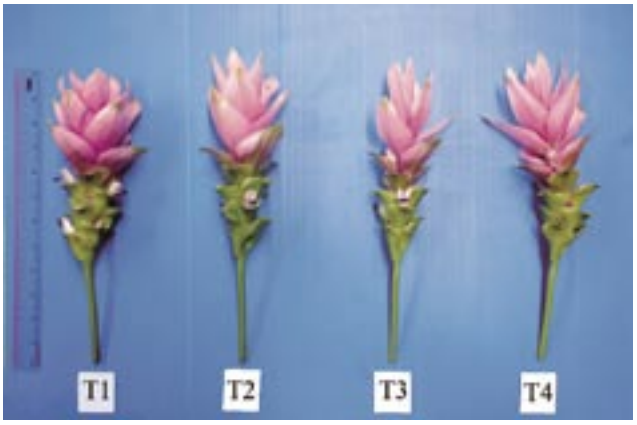

Fig. 2. Structure of inflorescence of *Curcuma alismatifolia* Gagnep. after treated with various GA_3 concentrations
T1: untreated plants, T2: GA_3 100 mgL^{-1}, T3: GA_3 300 mgL^{-1}, T4: GA_3 500 mgL^{-1}.

Table 1. Effects of GA_3 concentrations on growth and development of *C. alismatifolia*

GA_3 concentrations (mgL^{-1})	Plant height (cm)[1/]	Number of leaves per plant (leaf)[NS]	Number of shoot per cluster (shoot)[NS]	Number of days to flower (days)[1/]
0	51.8 c	3.00	3.25	68.1 b
100	76.3 b	3.00	3.00	69.8 b
300	101.0 a	3.00	2.75	76.0 a
500	97.4 a	3.00	2.50	73.0 a

[1/] Means with the same letter within column are not significant difference at $p<0.05$ by least significant difference.
[NS] no significant difference.

Table 2. Effects of GA_3 concentrations on quality and quantity of flowers

GA_3 concentrations (mgL^{-1})	Spike stalk length (cm)[1/]	Spike length (cm)[NS]	Number of pink bracts[1/]	Number of green bracts[NS]	Number of spikes per cluster[NS]
0	57.8 c	18.5	14.0 a	11.0	2.0
100	58.3 c	20.4	12.5 b	11.3	1.5
300	78.1 b	19.0	12.0 b	9.75	1.5
500	87.1 a	20.6	12.5 b	11.0	1.5

[1/] Means with the same letter within column are not significant difference at $p<0.05$ by least significant difference.
[NS] no significant difference.

Table 3. Effects of GA_3 concentrations on nutrient concentrations in plant organs

GA^3 concentrations (mgL^{-1})	Nutrient concentration (mg g DW^{-1})					
	Aboveground parts			Underground parts		
	N[1/]	P[NS]	K[1/]	N[NS]	P[NS]	K[NS]
0	20.6 a	8.9	58.4 b	26.9	19.2	43.4
100	21.7 a	8.5	60.3 b	29.0	19.1	43.7
300	20.0 a	9.2	58.4 b	29.2	21.7	42.3
500	15.6 b	9.1	63.2 a	24.0	22.7	45.1

[1/] Means with the same letter within column are not significant difference at $p<0.05$ by least significant difference.
[NS] no significant difference.

Table 4. Effects of GA_3 concentrations on nutrient contents in plant organs

GA^3 concentrations (mgL^{-1})	Nutrient contents (mg per plant)					
	Aboveground parts			Underground parts		
	N[NS]	P[NS]	K[1/]	N[1/]	P[NS]	K[1/]
0	161	71.8	468 b	148 a	101	227 b
100	194	80.1	576 a	178 a	110	245 a
300	167	85.7	538 a	154 a	105	181 c
500	139	82.0	570 a	96.8 b	94.6	188 c

[1/] Means with the same letter within column are not significant difference at $p<0.05$ by least significant difference.
[NS] no significant difference.

RESULTS

Plant growth and development

Rhizomes of *Curcuma alismatifolia* Gagnep. were planted on 24 June 2004. Plants were supplied with four levels of GA solution at 0, 100, 300 and 500 mgL^{-1} GA_3 twice at 4 WAP (shoot emergence) and 6 WAP by drenching. The results showed that plant heights increased rapidly from 4 to 8 WAP and it increased continuously until 12 WAP especially with the application of 300 and 500 mgL^{-1} GA_3. Furthermore, the GA_3 application delayed flowering date, which was about 5 to 7 days later than control plants at 300 and 500 mgL^{-1}, respectively. The GA_3 application did not affect on the number of leaves but leaves became longer and slimmer than control ones (**Fig. 1**). However, increasing the level of GA_3 application tended to decrease the number of shoot per cluster (**Table 1**) and supplied GA_3 at 500 mgL^{-1} had affected on curcuma plants which were easily overthrown by wind, due to thin and longer scapes.

Quality and quantity of flower

In this experiment *C. alismatifolia* flowered on September. The GA_3 application had affects on increasing inflorescence length particularly at 300 and 500 mgL^{-1} GA_3 were 78.1 and 87.1cm respectively (**Table 2**). In addition, we found that coma bracts or pink bracts had difference shape from control plants which were slimmer and more tapering than normal flower (**Fig. 2**).

Nutrient concentration and nutrient content in plant organ at flowering stage

In flowering stage, underground organs (new rhizomes, storage roots and fibrous roots) and aboveground organs (leaves, flowers and scapes) were analyzed in the term of nutrient concentration and content. Application of GA_3 at highest level (500 mgL^{-1}) affected on the nitrogen concentration, which decreased to 15.6 mg g DW^{-1} compared with control plants 20.6 mg g DW^{-1}. In opposite, the potassium concentration increased to 63.2 mg g DW^{-1} in upper ground parts compared with control plants 58.4 mg g DW^{-1} (**Table 3**).

The GA_3 application gave the effects on nutrient contents (mg per plant) (**Table 4**). In this experiment GA_3 application at all concentration increased potassium contents in

Table 5. Effects of GA_3 concentration on rhizome quality and quantity

GA_3 concentrations (mgL^{-1})	Rhizome weight per cluster (g)^{NS}	The first new-rhizome weight (g)^{NS}	Rhizome width (cm)^{NS}	Rhizome length (cm)$^{1/}$	Number of storage roots per rhizome^{NS}	Storage roots length (cm)^{NS}
0	84.8	43.6	2.29	2.50 b	5.67	11.1
100	70.0	48.1	2.52	2.55 b	6.00	10.6
300	83.8	55.5	2.83	3.24 a	7.33	10.5
500	67.0	53.2	3.06	3.44 a	6.00	11.6

$^{1/}$ Means with the same letter within column are not significant difference at $p<0.05$ by least significant difference.
^{NS} no significant difference.

aboveground part organs. In underground part organs GA_3 application at 500 mgL^{-1} gave the lowest nitrogen contents was 96.8 mg per plant. Moreover, GA_3 at 300 and 500 mgL^{-1} gave low potassium contents were 181 and 188 mg per plant, respectively (**Table 4**).

Quality and quantity of rhizome

At 32 WAP the aboveground organs withered and dried. Rhizomes were harvested then cleaned and the size and weight were measured. The results showed that the rhizome length became longer when GA_3 was applied at 300 and 500 mgL^{-1} (3.24 and 3.44 cm, respectively). Increasing the level of GA_3 concentration tended to decrease the rhizome weight per cluster but increase the first new rhizome width (**Table 5**).

DISCUSSION

Plant growth and development

Application of GA_3 produced phenotypic changes of *Curcuma alismatifolia* Gagnep. such as leaves, inflorescence stalks, and pink bracts as compared with these organs in untreated control plants. In *Catharanthus roseus*, GA application had effects on visual morphological changes in leaves and internodes in flowering plants such as increasing in plant height and leaf length (Srivastava and Srivatava, 2007). Stimulation of plant stem elongation by gibberellins is the basis for this hormone's discovery and the effect was used for a biological assay for GAs. The GAs promote stem elongation through stimulation of both cell elongation and cell division (Huttly and Phillips, 1995). Otherwise, this experiment resulted that plant had delayed flowering date when GA_3 was applied at the level of 300 and 500 mgL^{-1}. It was approximately 5-8 days later than the control plants. This result was similar to the report by Kuenny et al. (2002) that the rhizomes were soaked in a solution containing GA_{4+7} 400 mgL^{-1} delayed flowering of *C. alismatifolia*. In most species, the transition to floral development is stimulated by GAs (Sun and Gubler, 2004). Ben-Tal and Erner (1999) reported that GAs had effects on flowering date in many plant species either becoming earlier in some plant or being delayed flowering in other plant. In some climatic areas with marked seasonal changes, bulbs have to develop mechanisms to survive under adverse low temperature, and they require vernalization which GAs are used as a substitute for low-temperature-requirment genera like tulip and *Muscari* (De Hertogh and Le Nard, 1993). *Curcuma alismatifolia* was sub tropical plant and have not vernalization trait, they grow on rainy season (Poobuapueon, 1992). Rhizome dormant in winter when the weather conditions are dry and short day length on September to February. In our results, the application of GA_3 did not promote flowering, but GA_3 application delayed flowering. In this experiment we found that using GA_3 at 500 mgL^{-1} was easily fallen down and broken by wind. Cell elongation by the effect of GAs was concerned with increasing elastic cell wall and decreasing of osmotic potential solution in cell (Kaweeta, 2003). GAs promote cell elongation by induction of enzymes that promote cell wall loosening and expansion such as xyloglucan endotransglycosylase/hydrolase (XET or XTH), expansins, and pectic methylesterase (PME) (Stephen *et al.,* 2005). Water percolate through cell rapidly therefore cell expand, plant keep a lot of water to become fragile (Jarassamrit, 1994).

Nutrient concentration and nutrient content

In flowering stage, aboveground parts were important sink organs. The GA_3 application had affected on nitrogen and potassium concentration in the upperground parts. GA_3 at 500 mgL^{-1}, had lowest nitrogen concentration. It was found that gibberellins decreased nitrogen concentration in aboveground organs. However, it did not affect on nitrogen content (mg $plant^{-1}$) because of the GA_3 concentration at 500 mgL^{-1} gave more dry weight than other treatment thus total nitrogen contents, therefore, the N content was not significantly different.

Potassium concentration and content in the upperground part organs were increased when applied GA_3 500 mgL^{-1}. Guaedia and Bentloch (1980) studied the effects of potassium and gibberellin on height, sugar and potassium concentration in sunflower, and reported that GA and potassium was synergistic interaction on the stem elongation. The enhancement of stem elongation by GA was also dependent on the K^+ supply. In the plants with a low K^+ supply, GA-stimulated growth was correlated with a marked increase in K^+ concentration in the elongation zone (Guaedia and Bentloch, 1980).

In flowering stage, underground part organs did not have sink function yet. Using GA_3 at high level supported to increase biomass (dry weight) of the upperground parts which may be concern with changes in nitrogen and

potassium allocated from underground to upperground part organs. Therefore, its effect on decreasing total nitrogen and potassium content in underground part organs.

In this experiment it was shown that GA_3 application was supplied by drenching at shoot emergence (4 WAP) and 2 weeks increase growth in term of cell elongation such as leaves, inflorescences and inflorescence stalks. Moreover, applied GA_3 could delay the flowering date even though short time delaying. However, it also affected on negative way in case of using at 500 mgL^{-1} therefore, suitable concentrations and timing of GA_3 application should be investigated furthermore in addition to the method of applying GAs.

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クルクマ アリスマティフォリア Gagnep. の生長と分化に対するジベレリン施用の影響

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要　約

クルクマ アリスマティフォリア Gagnep. の生長と分化に及ぼす、ジベレリン（GA_3）の施用効果を調べた。球根植込み4週間後（出芽）と6週間後にGA_3水溶液（0, 100, 300 または 500mg L^{-1}）を土壌に各ポット当たり100mL 添加した。GA_3　300 または 500mg L^{-1}区では、草丈が顕著に増加し、それぞれ、101cm, 97.4cm に達した。しかしながら、株当たり葉数、株当たりシュート数には、影響を与えなかった。GA_3　500mg L^{-1}区では、花茎長が最高となり87.1cm に達し対照区の花茎長より、約30cm 長かった。しかし、GA_3　500mg L^{-1}区では、花茎が折れやすくなった。GA_3　300 または 500mg L^{-1}区では、開花に到達する日数（到花日数）が、対照区よりも5から8日長くなった。開花期における地上部、地下部各器官の、窒素、カリウム、リン濃度と株当たり含有量を測定した。GA_3施用は、窒素とカリウム濃度と含有量に影響を与えたが、リン濃度には、影響しなかった。GA_3　500mg L^{-1}区では、地下部の株当たり窒素含有量は、96.8mg と対照（148mg）より低下した。GA_3　100mg L^{-1}区において、地下部のカリウム含有量が増加した。また、GA_3　300 または 500mg L^{-1}区では、塊茎長が増加した。

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