Involvement of Ethylene and Gibberellin in the Development of Rhizomes and Rhizome-like Shoots in Oriental Cymbidium Hybrids

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Summary

Effects of plant growth regulators on the growth of rhizomes developed from defoliated pseudobulbs of *Cymbidium* Elfs Castle 'China Peak', as well as ethylene evolution and gibberellin activities in rhizomes were investigated for the utilization of rhizomes for propagation. Rhizomes, growing in the dark, were sprayed with a solution of 100 ppm aminoethoxyvinylglycine (AVG), uniconazole or 10 ppm gibberellic acid (GA₃), while the control rhizomes were treated with water and cultured in the dark. A group of water-treated rhizomes were exposed to light (60% of natural daylight). Light-treated rhizomes ceased to grow and but promoted leaf development. AVG decreased the number of nodes and length of internodes of rhizomes, but increased leaf length and number of roots. GA₃ promoted leaf growth but did not affect rhizome growth and rooting. Uniconazole reduced rhizome growth without affecting number of nodes and leaf growth, while it promoted rooting. The above treatments were conducted with Cym. 'Miss Taipei' to determine ethylene evolution and gibberellin (GA) substance content. Ethylene evolution from rhizomes 'Miss Taipei' was inhibited by light, AVG, and uniconazole treatments. Bioassays of the control rhizomes revealed that GA₃-, GA₄- and GA₇-like substances were found in fraction numbers of 6, 13, 10 on the TLC plates. GA₃-like substance increased in the light-treated rhizomes, but decreased significantly in the uniconazole treatment, whereas AVG had no effect on the total GA-like substance contents. Thus, it is suggested that growth characteristics of rhizomes in cymbidium plants may be controlled not only by GA activities but also by ethylene, and that it is possible to propagate mother stocks by controlling the levels of plant hormones in rhizomes.

Key Words: aminoethoxyvinylglycine, ethylene, gibberellin, rhizome, rhizome-like shoot.

Introduction

Cymbidium pseudobulbs, grown under dark conditions, increased lateral bud sprouting with age (Zheng and Matsui, 1998), while some defoliated lateral ones formed more rhizomes. Rhizomes are transformed to shoots with elongated internodes and scaly leaves (Shimasaki and Uemoto, 1984), that bear lateral buds which are micropropagated and for propagation by cutting or division (Paek and Kozai, 1998). Morphogenesis of sprouted buds and meristematic clones are controlled by plant growth regulators (Hasegawa, 1987; Kim and Kako, 1982; Kusumoto, 1978). Kim and Kako (1982) suggested that GA controlled shoot and root growth of protocorm-like bodies in vitro and that GA acted on organogenesis in the meristem tissue cultures of cymbidiums. Uniconazole inhibited elongation of pseudobulbs and leaves in cymbidiums (Goi et al., 1999)

but promoted root formation in chrysanthemum plants (Jung et al., 2000) with enhanced ethylene production (Zheng et al., 2004). On the contrary, Hasegawa et al. (1986) reported that ethephon enhanced rhizome formation with elongated internodes in darkness like 'super-growth' of deepwater rice (Cohen and Kende, 1987). AVG inhibited ethylene evolution in satsuma mandarin (Ogata et al., 2002) and chrysanthemums (Zheng et al., 2004). We found that growth characteristics could be altered by endogenous ethylene and GAs in AVG- and uniconazole-treated chrysanthemum plants. Conversion of cymbidium rhizomes to normal shoots might be controlled by endogenous ethylene and GAs. Furthermore, this event helps to promote utilization of old pseudobulbs and rhizomes to obtain mother stocks for meristematic cloning of cymbidium and to diminish risks of clonal mutation. Thus, it is important and useful to get stable mother stocks of cymbidium. The aim of this study is to verify if the growth responses of rhizomes to AVG, GA₃, and uniconazole under dark or light conditions are caused by changes in endogenous ethylene

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evolution or endogenous gibberellin contents that make them useful for propagation.

Materials and Methods

AVG, GA₃, uniconazole and light treatments

Isolated two year-old pseudobulbs of *Cymbidium* Elfs Castle 'China Peak' were cultured in sphagnum moss beds covered with a single layer of aluminum foil and black plastic film in late June, 2002. One month later 30 rhizomes (2–3 cm in length) that sprouted from lateral buds of pseudobulbs were sprayed with a 100 ppm solution of AVG (Abott Chem. Co., Tokyo), uniconazole (Sumitomo Chem. Co., Tokyo), or a 10 ppm solution of GA₃ (Kasei Co., Tokyo). Then, they were grown for two months in a dark room kept at 28°C. Rhizomes sprayed with water (control) were also cultured in the dark. Some water-treated rhizomes were exposed to light. Rhizome length, internode length, and node number, leaf length, and root number were measured two months after treatments. Root length was determined as the average of three longest roots.

Ethylene measurement

Manually defoliated three-years old pseudobulbs (2–3 cm long) of *Cym.* 'Miss Taipei' that grown in dark were treated with AVG or uniconazole at the same concentration as above. Control rhizomes were grown in the dark or in the light as above. Rhizomes excised at the base of pseudbulbs were analyzed for ethylene evolution and GA contents 2 months after treatments. Three samples (4 g FW) without pseudobulbs of each treatment were enclosed in three 33 mL airtight vials, and incubated at 25°C for 2 h. Then, one-ml headspace gas sample was withdrawn and injected into a gas chromatograph (GC-14A, Shimadzu Co. Ltd., Kyoto), equipped with a flame ionization detector and a 2 m-stainless steel column (3 mm inner diameter) packed with Unibeads 2S (GL Science Inc., Tokyo). Column and injector temperatures were 80 and 120°C, respectively.

Extraction, purification, chromatography and bioassay of gibberellins

Rhizome samples (10 g dry weight) were ground and

extracted three times with 50 mL of 80% (v/v) methanol. The extract was evaporated to the aqueous phase and then adjusted to pH 2.5 with HCl. The aqueous phase was further extracted with diethyl ether and the ether fraction evaporated to dryness (Nishijima and Katsura, 1988). The residue was dissolved into acetone for thin layer chromatography (TLC) (Eliezer and Galily, 1974). An aliquot of the acetone solution was strip-loaded onto 20 × 20 cm TLC plates of silica gel 60 (Darmstadt, Co., Germany). The plates were developed with a mixture of chloroform: ethyl acetate: acetic acid (60:40:5, v/v/v) (Eliezer and Galily, 1974). Fifteen equal zones of the chromatogram were scraped from the plates and eluted 3 times with water-saturated ethyl acetate. The eluates were evaporated to dryness and dissolved again in 0.1 mL of 50% acetone for bioassay with seven rice 'Koshihikari' seedlings. Bioassay was carried out by the method of microdrop application as described by Nishijima and Katsura (1989). GA₁, GA₃, GA₄ and GA₇ at 1.000 pmol were tested with the same methods of TLC and bioassayed. GA₃ was purchased from Nacalai Tesque, Inc. Kyoto; GAs (GA₁, GA₄ and GA₇) came from Sigma-Aldrich Chemie GmbH P.O. Steinheim, Germany. The total level of endogenous GA-like activities for a TLC plate that exceeded the water control was expressed as GA₃ equivalents, and calculated by using a standard curve obtained from the bioassay response of the rice seedlings.

Results

Rhizome growth

Rhizomes of *Cym.* Elfs Castle grew to 10.5 cm in length in the dark after 2 months, whereas those grown in the light were only 2.4 cm (Table 1).

AVG and uniconazole reduced rhizome length, but GA₃ did not affect rhizome growth. Leaf growth was unaffected in uniconazole-treated plants, but it was promoted in the AVG- and GA-treated plants grown in the light compared to the controls. Leaves in AVG- and GA₃-treated rhizomes, especially in the former, lacked chlorophyll, while those grown in the light were green. Light- and AVG-treatments reduced the number of nodes. Whereas the number of roots increased by light and uniconazole treatments. Internode length was

Table 1. Effects of AVG, GA₃, uniconazole and light on growth of rhizomes of *Cymbidium* Elfs Castle 'China Peak' grown in the dark.

Treatment	Length (cm)			Number	
	Rhizome	Leaf	Root	Internode	Root
Control	10.5 a ²	0.6 c	1.8 b	8.7 a	0.3 c
AVG	6.7 b	6.5 a	2.1 a	7.5 b	1.0 b
GA ₃	10.9 a	3.2 b	1.3 c	8.8 a	0.5 c
Uniconazole	5.5 b	0.9 c	1.7 b	8.7 a	2.5 a
Light	2.4 c	2.6 b	2.1 a	7.3 b	2.2 a

² Different letters indicate significant differences at the 5% level by Fisher's PLSD test.

reduced markedly in AVG, light, and uniconazole treatments (Fig. 1). GA₃ increased length of 5th and 6th internodes from the base.

Ethylene evolution and gibberellin-like substance content

Rhizome growth of *Cym.* 'Miss Taipei' in the darkness was inhibited by AVG, uniconazole, and light treatments (Table 2).

Ethylene evolution from rhizomes was $4.8 \text{ nL} \cdot \text{g}^{-1} \text{FW} \cdot \text{h}^{-1}$ in the dark (the control plants), while it was $2.6 \text{ nL} \cdot \text{g}^{-1} \text{FW} \cdot \text{h}^{-1}$ in the light. Ethylene evolution was suppressed by AVG and uniconazole treatments.

Total GA-like substance contents were decreased by the uniconazole treatment but increased by the light treatment. GA content was unaffected by AVG treatment. GA-like substances were found in the fractions 4, 6, 10 and 13 in the control plants (Fig. 2). Authentic GA₁, GA₃, GA₄, and GA₇ were detected in the fractions 3, 6, 13, and 10, respectively. But GA₁-like substance was not detected in the control plants. GA₇-like substance content was reduced by AVG treatment, but total contents of GAs were not reduced as compared to the control. GA₃-like substance content was increased by the light treatment. GA₃- and GA₇-like substances

Fig. 1. Effect of AVG, GA₃, uniconazole and light treatments on internode length of rhizomes in *Cymbidium* 'China Peak'.

Table 2. Effects of AVG, uniconazole and light on rhizome growth, ethylene evolution and gibberellin content of *Cymbidium* 'Miss Taipei'.

Treatment	Rhizome length	Ethylene evolution ($\text{nL} \cdot \text{g}^{-1} \text{FW} \cdot \text{h}^{-1}$)	GA-like substance content ^z ($\text{ng} \cdot \text{g}^{-1} \text{FW}$)
Control	7.20 a ^y	4.83 a	89.6 b
AVG	55.64 b	3.25 b	84.7 b
Uniconazole	5.71 b	3.02 b	57.2 c
Light	5.66 b	2.60 b	110.1 a

^z Sum of active fractions in the bioassay with rice seedlings expressed as GA₃ equivalents.

^y Different letters indicate significant differences at 5% level by Fisher's PLSD test.

were markedly decreased by the uniconazole treatment, and this resulted in a big decrease in the total GA-like substance content.

Discussion

Terrestrial cymbidium species form rhizomes after seed germination. Some of their hybrids are also reported to yield rhizomes in aseptic culture of the meristems (Kusumoto, 1980). Shimazaki and Uemoto (1984) regarded rhizomes of the temperate cymbidiums, *Cym. kanran* and *Cym. georingii* as transformed stems of tropical ones. In the dark defoliated pseudobulbs develop rhizomes or rhizome-like shoots with elongated internodes, scale-like leaves and a hook on the shoot apex. The most distinctive effect of AVG was to develop leaf blades that under light inhibited internode growth while promoting leaf elongation in the dark as well as in the light. These findings indicate that rhizome growth and leaf blade development are associated with endogenous plant hormone levels. Similarly, Cohen and Kende (1987) demonstrated that ethylene elongates stems of deepwater rice, whereas, Smalle and Van Der Straeten (1997) found that the gas induced hooked tips. Ethephon-induced rhizome formation was also reported for *Cym. Georingii* in the dark (Hasegawa et al., 1986), but they found that GA has almost no effect on rhizome formation in Japanese cymbidium species. In our study, GA₃ also did not affect rhizome elongation and number of nodes, but it promoted leaf length in darkness. This is contrary to the promotive effect of GA₃ on growth of protocorm-like bodies in vitro (Kusumoto, 1978). We attribute this to the excessive formation of GA induced by the GA₃ treatment which had no effect on rhizome growth. The result that uniconazole treatment inhibited rhizome growth in this experiment is similar to that in protocorm-like bodies of cymbidium (Goi et al., 1999) which indicated that uniconazole reduced ethylene evolution as did AVG and light (Table 2).

Active GAs such as GA₁ and GA₃ are effective on cell elongation of *Agrostemma* (Zeevaart, 1985) and rice plant (Matsukura et al., 1998), inhibition that of shoot elongation by a plant retardant was completely overcome by simultaneous application of GA₁ and GA₃ (Zeevaart, 1985). In this experiment a GA level in fraction 6, presumably to the enhanced GA₃, increase by

Fig. 2. Gibberellin-like activities in TLC fractions from *Cymbidium* 'Miss Taipei' rhizomes treated with AVG, GA₃, uniconazole or light. The ethyl acetate fraction equivalent to 10 g dry weight was assayed with rice seedlings 'Koshihikari'. The positions of authentic GA₁, GA₃, GA₄ and GA₇ on TLC chromatograms are also shown. Broken lines indicate the 95% confidence interval of the control in terms of *t* test (*n*=14).

light treatment, but it inhibited ethylene evolution and internode elongation in the rhizome, while promoting shoot and leaf development. Hence it seems that both GAs and ethylene levels are responsible for rhizome and shoot growth. Our findings on the interaction of AVG, uniconazole, and GA indicate that rhizome growth and leaf development are associated with endogenous ethylene and GA activities. Lastly, our data on the responses of rhizomes grown in the dark to phytohormones show the possibility of propagating mother stocks of mother stocks of cymbidiums by controlling their levels of plant hormones.

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シンビジウムの根茎および根茎様シュートの発育におけるエチレンとジベレリンの関与

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摘 要

東洋ランの *Cymbidium* Elfs Castle ‘China Peak’ の偽球茎から発育した根茎の繁殖への応用を目的として、植物生長調節物質処理における根茎の反応と根茎からのエチレン発生および内生ジベレリン活性を調査した。暗黒で発育した2~3 cmの根茎に100 ppmのアミノエトキシビニルグリシン(AVG)、ウニコナゾールまたは10 ppmジベレリン(GA₃)を散布し、さらに暗黒条件あるいは明条件(60%遮光下)で栽培した結果、根茎の生長は光によって抑制されたが、葉の生長は促進された。AVGの散布では根茎の長さや節間長は減少したが、葉長と根数は増大した。GA₃散布により葉長は増大したが、根数には影響が見られなかった。ウニコナゾール処理では根数は増加したが、葉長には影響が認められな

かった。エチレン生成量とジベレリン様物質含量の測定には東洋ランの *Cym.* ‘Miss Taipei’ の根茎を用い、*Cym.* Elfs Castle ‘China Peak’ と同様の処理を行った。エチレン生成は対照区と比べ、すべての処理区で根茎からのエチレン生成が抑制された。対照区では、GA₃、GA₄およびGA₇様物質が検出された。GA₃様物質含量は明条件では増加したが、ウニコナゾール散布区では著しく減少した。一方、全GA様物質含量に対するAVG散布の影響は認められなかった。これらの結果から、根茎の生長と発育にはジベレリンおよびエチレンの両者が関係していることが示唆され、根茎中の植物ホルモンレベルを制御することによって、繁殖用母株を確保できる可能性が示された。