ENDOGENOUS GROWTH PROMOTER AND INHIBITOR LEVELS IN PLEIONE FORMOSANA CORMS

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Abstract

Endogenous hormones of the corms of Pleione formosana Hayata were analyzed with HPLC at various stages of growth and development. The content of abscisic acid (ABA) decreased with corm growth, but increased rapidly during leaf abscission and at harvest. It decreased again during a natural chilling period. The GA and IAA contents increased rapidly during sprouting and reached to a peak when flower buds show color. The ratio of growth promoter/inhibitor dropped to a minimum of 0.35 at leaf abscission. The ratio increased during cold storage and reached to a maximum of 7.52 at the sprouting stage when flower buds show color. The highest GA content was found in August, the time of flower bud formation.

1. Introduction

Pleione formosana Hayata, known as Taiwan-Yee-Yeh-Lan (one-leaf-orchid), was found by Mori in 1909 and named by Hayata in 1911 (Chiang and Chen, 1968). It is an alpine orchid (Fig. 1A) which has an annually replaced corm and a single deciduous leaf (Fig. 1B). The sprouting and anthesis occur in warm spring under the natural conditions of 1500-2500m high mountains in Taiwan (Fig. 1C). In a phytotron at day/night temperature of 20/15°C, flower initiation of daughter corms occurs about 60 days after flower senescence and leaf has fully expanded, while the corms are growing actively (Fig. 1D). Flower bud are well differentiated and developed in the fall. Leaves abscise in mid-November, about one month after the first frost in the fall. The dormant corms are often harvested after leaf drop and stored at 5-10°C for 8-10 weeks before being grown as indoor flowering orchids (Lee, 1981; 1984; Lee and Liu, 1982; Liu, 1982). If corms are harvested before mid-October and stored at 23°C, flower-bud blasting will occur (Teng and Lee, 1985).

Kamerbeek et. al. (1970) indicated the some flower bulbs and corms have a dormancy analogous to that of seeds and buds. If we define an endogenous or true dormancy as a physiological condition which prevents normal growth, then Pleione formosana corms have a dormancy similar to tulip bulbs (Kamerbeek et. al. 1970; Rudnicki, 1974). It is generally known that auxin, gibberellins and cytokinins have stimulatory effect on plant growth, whereas abscisic acid plays a role in inhibiting plant growth. The onset and termination of dormancy may be regulated by the growth promoters and inhibitors. At the onset of dormancy, the balance is shifted in favor of the inhibitor component; at the termination of dormancy, it is shifted back in favor of the promoter components (Amen, 1968; Rudnicki, 1974).

In this study, endogenous growth promoters and inhibitors were extracted from the mother and daughter corms of Pleione formosana and

analyzed with High Performance Liquid Chromatography (HPLC). The purpose is to examine the changes of plant hormones during bulbing and flowering-bud formation in the summer, corm maturation in the autumn, cold storage in the winter, and sprouting and anthesis in spring.

2. Materials and methods

Corms of uniform size (8gm) were stored at 5°C from December 1983 to mid-March 1984 and then planted at Mei-feng (2100m altitude) in Taiwan. Samples of corms were taken every 3 to 5 weeks after flower senescence, every 2 weeks during the 8-week storage at 5°C, and at the stages of bud elongation and anthesis (Fig. 2). Samples, 40 to 50gm each, were taken from mother and daughter corms of 20 plants. Corm tissues were frozen in liquid nitrogen then transfered into 80% of methanol and stored at -20°C until hormones were analyzed with a method modified from Cheng (1976).

Frozen tissues were grounded and homogenized for 5 minutes with a vibrator. The homogenates were then filtered through two layers of Whatman No. 1 filter paper. The residues was rinsed with warm methanol (80°C) for 2 to 3 times and then all filtrates were added together. The filtrates were concentrated in vacuo, and then adjusted pH 8.0 with NH_3 solution. The filtrates were then run through a PVP column, and readjusted to pH 2.5 with concentrated H_3PO_4. The aquaeous phase of the filtrate was washed with ethyl acetate for 3 times. The ethyl acetate fraction was designated as basic fraction, and the aquaeous extracts were concentrated in vacuo at 40°C. After adding 0.1ml of methanol, the extracts were analyzed on thin layer chromatography (TLC). A descending chromatography was used with a solvent mixture of chloroform , ethyl acetate and acetic acid (60:40:5 v/v/v). Standards were prepared by dissolving ABA, GA_3 and IAA in methanol to make 1000ppm solutions. ABA and IAA spots which had the same Rf's as ABA and IAA standards under ultraviolet light were scraped off and dissolved in a mixture of methanol and ethyl acetate (9:1 v/v). A strip of plate containing GA spots were cut and soaked in a solution of H_2SO_4 and CH_3CH_2-OH (9:1 v/v) and then warmed with a dryer. GA spots were traced according to the Rf under ultraviolet light and rinsed off in acetone. The collected ABA, IAA and GA solutions were filtered through glass wools per 2 to 3 times. The filtrates were concentrated in a vacuo and then dissolved in methanol (0.5ml) and transfered into microfilter tubes. These concentrates were further concentrated in the vacuo before being injected into a High Performance Liquid Chromatography (HPLC) for the final analysis of ABA, IAA and GA contents.

3. Results

3.1. Changes in ABA, GA and IAA contents in corms during growth and development

Trans- and cis ABA contents in daughter corms decreased during the growing season June to September, increased sharply during leaf senescence (late October) and reached a maximum just before leaf abscission which occured 5 days after an early frost (5 Nov.). The maximum trans- and cis ABA contents were 286 and 521ng/g FW, respectively. When the mature corms were left in the field in the highland of Mei-feng, to receive a natural chilling (4-16°C) from November to December, the ABA

content decreased rapidly to a low level. The ABA content of the mother corms was lower than that of daughter corms, and the former became undetactable by the end of October (Fig. 3). The GA content was high at the end of July when bulbing and flower bud formation occured. GA and IAA levels were low from late August to late December. IAA content did not decrease from August to December when the corms entered into the rest period. GA and IAA contents in the senescencing mother corms were very low and were undetectable after October (Fig. 4).

3.2. Changes in ABA, GA and IAA contents in cold storage and during forcing

When corms were stored at 5°C for chilling, ABA and GA contents decreased sharply and IAA decreased slightly. After the corms were moved from 5°C to 20°C for forcing, shoots elongated (Fig. 1C and Fig. 2) and GA and IAA increased dramatically and ABA increased slightly. GA and IAA contents reached maximum values of 614 and 668ng/g FW, respectively when flower bud showed color (Changed from green to pink), but the contents decreased sharply at the stage of anthesis (Fig. 5).

4. Discussion

The corms of Pleione formosana had a high level of ABA in autumn when leaf senescence and corm dormancy occured. ABA content decreased during chilling in the field or in cold storage, a phenomenon similar to seeds and buds (Amen, 1968; Wareing and Saunders, 1971). Our unpublished data indicated that the ABA content in leaves increased in late September when the leaves were still green, reached its maximum level in late October when the leaf chlorophyll content decreased to one-half, and became undectablly low in early November, just before leaf abscission. The ABA content in the daughter corms (enlarged internode just below the leaf) reached to its maximum level when that in the leaf dropped to its minimum.

The GA content decreased to the lowest level at the end of cold storage just prior to corm sprouting, a phenomenon similar to some other cold-requiring plant species (Wareing and Saunders, 1971; Lin et. al., 1975). It has been reported that GA and IAA are produced by a young flower organs, increase as flower buds grow, reach to the peak as flowers show color, and decrease to a low level at anthesis (Hans and Rees, 1975).

From these observations we postulate that the dormancy in Pleione corms may be controlled by a balance between endogenous inhibitors, such as ABA, and promoters, such as gibberellins, IAA, and cytokinins. Lewak (1979) has proposed a similar hypothesis on other plants. The ratio of (GA+IAA)/ABA in corms harvested on 10 November 1984, was 0.35; the ratio increased to 3.49 at the end of cold storage and reached to the highest of 7.52 at the shoots elongation stage when flower buds showed color. Hormonal changes in conjunction with specific metabolic inhibitors might mediate the activation and/or synthesis of hydrolytic enzymes (Amen, 1968) which in turn regulates and changes in carbohydrates (Liu and Lee, 1982; Teng and Lee, 1985) and affect subsequent flowering and growth.

Pleione formosana is a self-induction plant. Flower initiation occ-

urs after the leaf has fully expanded and the corm is actively growing. The expanding leaf has the highest GA content; and the GA content decreased later in the growing season (unpublished data). The highest GA content in daughter corms was found on 29 July, during the flower bud formation period (Fig. 1D). We can not explain well on this phenomenon , because this species is neither a cold induction plant nor a photoinduction plant (Lee,1981). Cytokinins might also play an important role in the growth and dormancy breaking of this species. It has been found that 100ppm of BA can substitute cold treatment and decrease percentage of flower-bud blasting (Teng, 1985). Cytokinins were not analyzed in this experiment due to technical problems.

Acknowledgements

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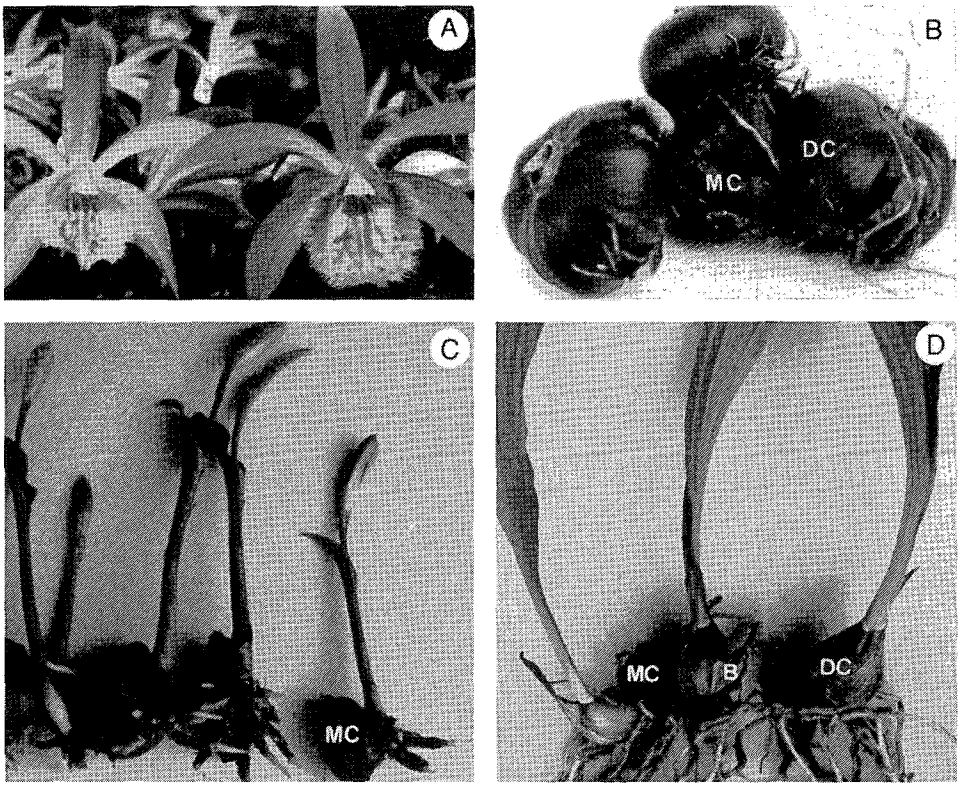

Fig 1. The life history of Pleione formosana. A, an alpine orchid looks like Cattleya; B, annually replaced corms with single deciduous leaf scar; C, sprouting after breaking dormancy; D, bulbing and flower-bud formation in summer.

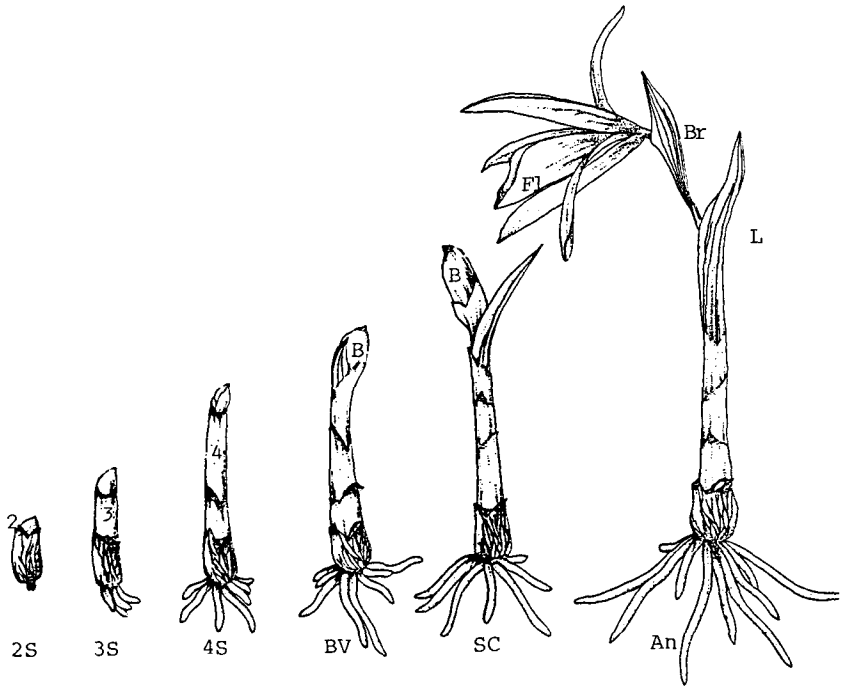

Fig 2. Stages of bud elongation and anthesis of Pleione formosana. 2S, bud protrudes scale 1 and 2 in dormant stage; 3S, bud protrudes scale 3 at sprouting; 4S, bud protrudes scale 4; BV, flower-bud visible; SC, flower-bud show color; An, anthesis; S, scale; L, leaf; Br, bract; B, flower-bud; Fl, flower.

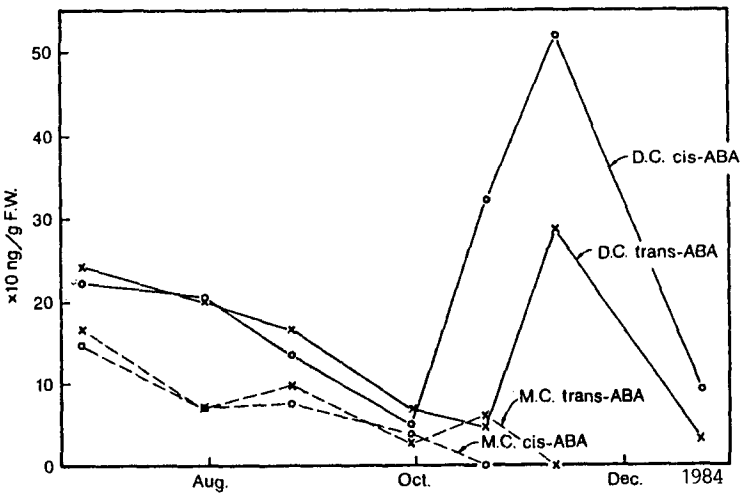

Fig 3. Changes in the endogenous ABA content in mother and daughter corms of Pleione formosana grown at Meifeng.

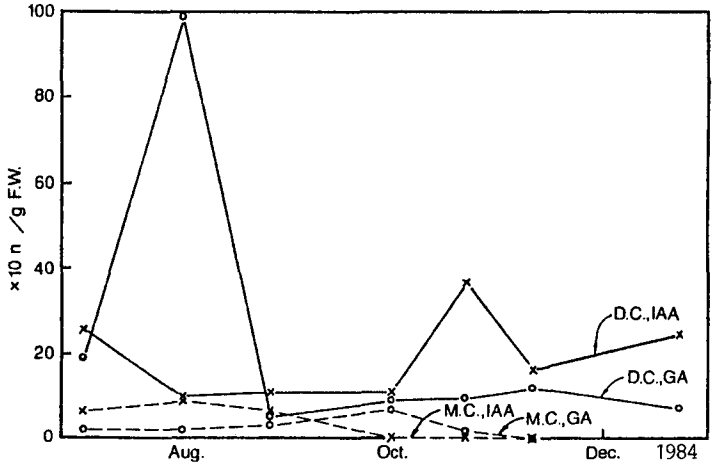

Fig 4. Changes in the endogenous GA and IAA contents in mother and daughter corms of Pleione formosana grown at Meifeng.

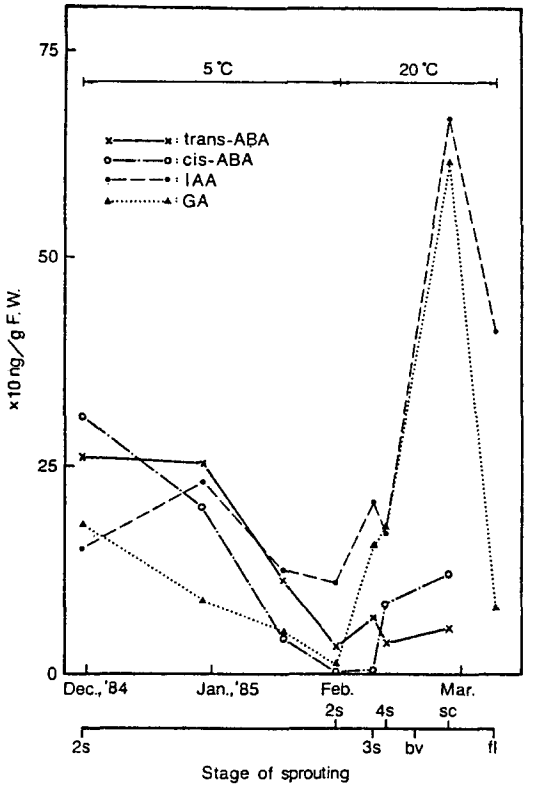

Fig 5. Changes in the endogenous GA, IAA and ABA contents in daughter corms of Pleione formosana during dormancy and flowering periods.