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CHANGES IN ENDOGENOUS ABA AND GA CONTENTS DURING FLORAL INDUCTION OF LEMNA AEQUINOCTIALIS

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SUMMARY

The contents of endogenous ABA° and GA_3 in *Lemna aequinoctialis* were assayed by means of gas chromatography. A comparison was made between non-induced plants, grown under continuous LD, and induced plants, which received 4 SD for flower induction. It appears that flowering of the SDP *Lemna aequinoctialis* is associated with a high ABA content and a low GA_3 content. These results are consistent with our previous results which showed that CCC, an inhibitor of GA_3 biosynthesis, induced flowering of the *Lemna* plant under non-inductive continuous light.

1. INTRODUCTION

According to KANDELER (1985) the correct name for the *Lemna* strain 6746 should be *Lemna aequinoctialis* since LANDOLT's revision of Lemnaceae (1980). This name has been adopted. The effects of external application of phytohormones, growth retardants and related substances on flowering of the SDP *Lemna aequinoctialis* have been summarized comprehensively in KANDELER's paper. Little attention, however, has been paid to the amounts of endogenous hormones during flower induction in this plant. The present study aims to elucidate the possible role of hormones in flower induction in *Lemna*.

2. MATERIALS AND METHODS

2.1. Culture of the plants

Floating plants of *Lemna aequinoctialis* strain 6746 were cultured under sterile conditions on Hutner medium, 80% strength, and under artificial illumination with fluorescent lamps; intensity of about 1250 lux on the plant level. The culture temperature was maintained at 28.2° C (ZHOU et al. 1983).

The control plants, cultured under 16 h LD remained vegetative and kept on multipying vegetatively. For flower induction plants were cultured under 8 h SD for 4 days te become induced (more than one critical cycle), followed

° Abbreviations: ABA, abscisic acid; GA_3, gibberelic acid; SD, Short-day; SDP, Short-day plant; LD, Long-day; LDP, Long-day plant.

by transfer to LD for further development. Flowers usually began to appear on the 8th day after the onset of the SD induction.

2.2. Extraction and derivatization of ABA and GA_3

Samples of fresh material were first blotted with filter paper to remove the attached water, then accurately weighed and quickly homogenized with acetone. The homogenate was transferred to iodine bottles and extracted three times with 100 ml acetone. The combined extract was filtered three times to remove the residue. The filtrate was condensed under reduced pressure at 50° C and directly used for thin-layer chromatography on silica gel GF 254, which was developed in a mixture trichloromethane:ethyl acetate:acetic acid (75:25:10, v/v/v). The zones corresponding to authentic ABA and GA_3 were removed and eluted with a solution of methyl alcohol:acetone (1:1, v/v). The eluant was dried with N_2 and methylated with diazomethane. The methylated ABA samples were subjected to gas chromatographic (GC) analysis (TAN 1985). GA_3 samples were subjected to GC analysis after acetylation with heptofluorobutyric anhydride.

2.3. GC analysis of ABA and GA_3

ABA was assayed as its methyl ester, Me-ABA, and GA_3 as its heptafluorobutyrate derivative, Me-GA_3-HFB.

GC analysis of Me-ABA was carried out on a GC instrument with a ^{63}Ni electron capture detector, model sp-2308 (made by Beijing Analytical Instrument Co.), using a glass column (2 m × 2.2 mm) packed with chromasorb W.AW.DMCS. 80–100 mesh coated with 1.2% SE-30 0.5% OV-225. Highly purified N_2 was used as carrier gas at a rate of 80 ml/min. The column temperature was maintained at 197° C and the inlet and detector temperature at 250° C.

GC analysis of Me-GA_3-HFB was carried out on a SC-7 capillary gas chromatograph with a modified electron capture, using a SE-30 SCOT glass capillary column (18 m × 0.35 mm). Highly purified N_2 gas was used as the carrier gas at a flow rate of 3 ml/min obtained by splitting (split ratio 20:1) the main flow rate of 80 ml/min. Column temperature was 210° C, temperature of inlet 250° C and of the detector 240° C.

3. RESULTS AND DISCUSSION

A peak identical to the standard sample of Me-ABA both in retention time and in shape was observed in all the plant samples tested. This peak is followed in retention by a trans-Me-ABA peak, which increases upon ultra-violet illumination. Together these features indicate the authenticity Me-ABA peak in the samples (*figs. 1* and *2*).

Similarly, with the capillary GC analysis a peak identical in retention time and shape to the standard Me-GA_3-HFB was found in all samples, indicating the reliability of the analytical method (*figs. 3* and *4*).

The experimental results with regard to the changes in endogenous ABA and GA_3 during flower induction are presented in *figs. 5* and *6*. As expected, the

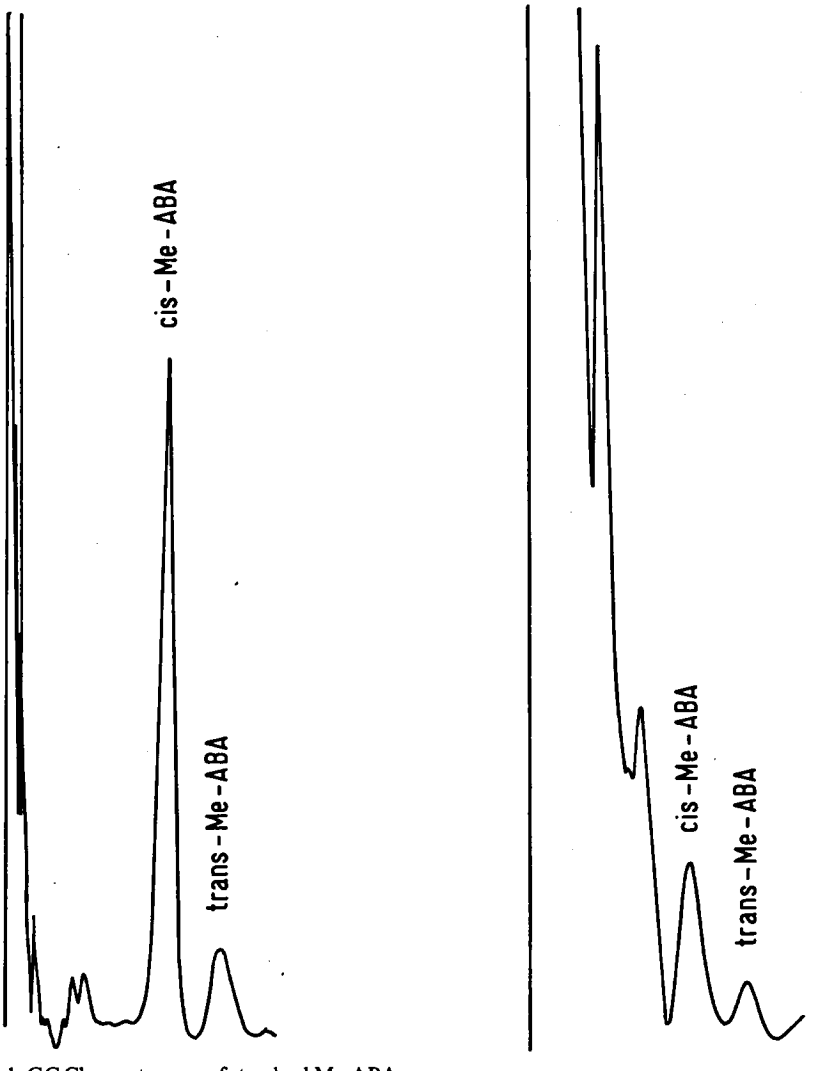

Fig. 1. GC Chromatogram of standard Me-ABA.
Fig. 2. GC Chromatogram of plant samples of *Lemna aequinoctialis* 6746.

SDP *Lemna aequinoctialis* did not flower under LD (*figs. 5* and *6*). The first flowers appear at 8 days after the onset of the SD induction. The ABA content of plants growing under LD remained rather constant at 35–55 ng/g fresh wt., whereas under SD the ABA content rose abruptly on the very first day after transfer to 138 ng/g fresh wt., about three times the control. Following this abrupt increase, the ABA content returned to a normal value of 51 ng., after which it remained more or less constant at this level. The GA_3 content changed in the opposite direction, i.e. stayed at a constant high level in the plant kept

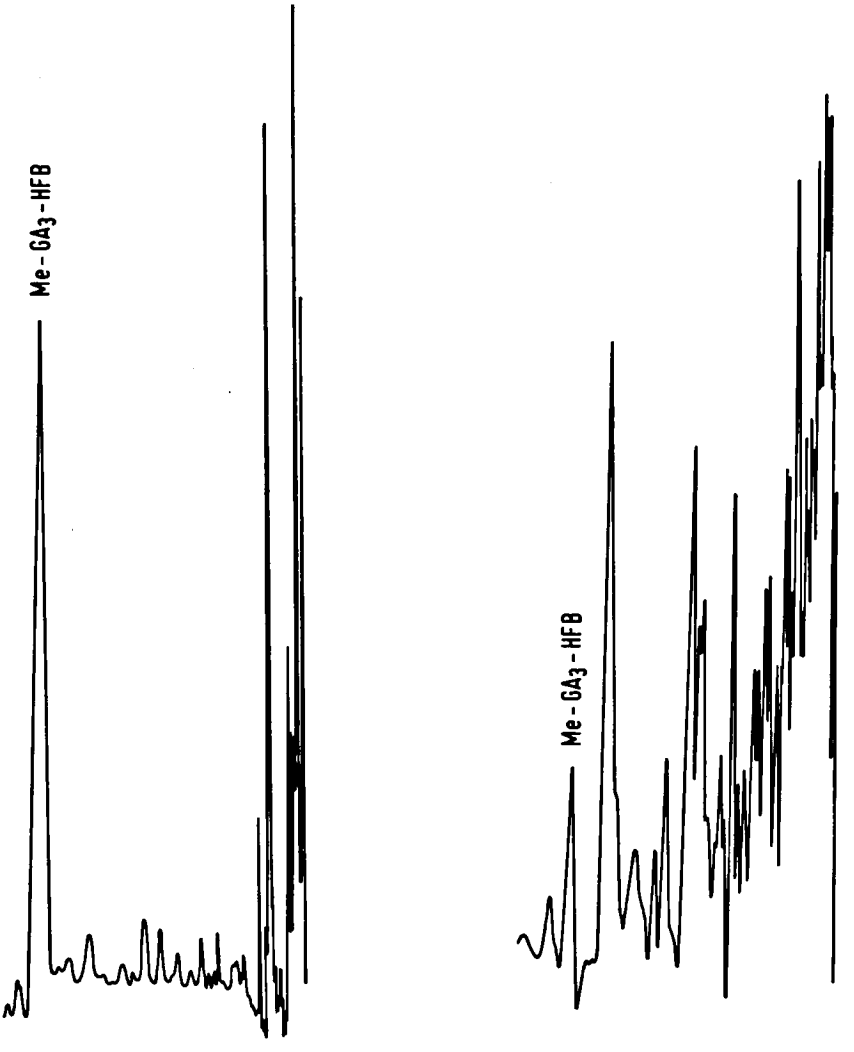

Fig. 3. GC Chromatogram of standard Me-GA_3-HFB.
Fig. 4. GC Chromatogram of Me-GA_3-HFB in plant samples.

under LD, but decreased gradually from the first day of SD induction till the end of the SD and then increased anew after the plants had again been transferred to LD.

The above results indicate that flower induction in the SDP *Lemna aequinoctialis* 6746 requires a high level of ABA, or is at least associated with an abrupt rise of the ABA content. This is consistent with the results of KANDELER & HÜGEL (1973) which showed that exogenous application of ABA and CCC lead to flowering of *Lemna* plant under non-inductive continuous illumination. It

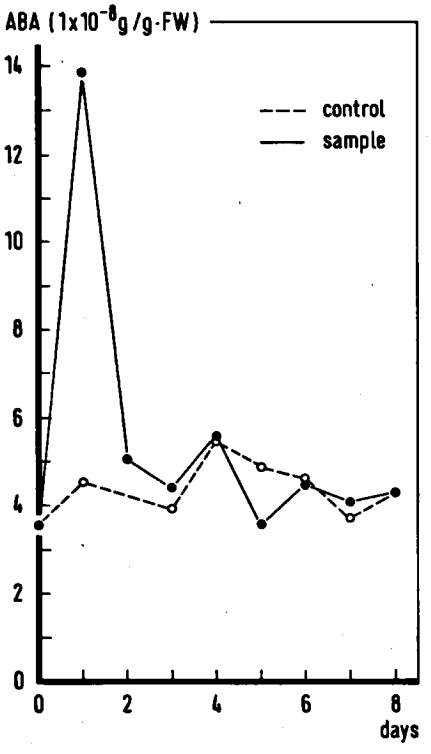

Fig. 5. Change of endogenous ABA during the 8 day experiment.

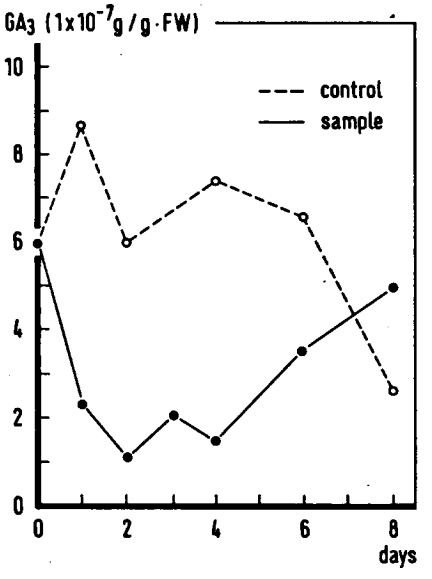

Fig. 6. Change of endogenous GA_3 during the 8 day experiment.

has been shown by WAREING & EL-ANTABLY (1981) that there was a rise in an ABA-like substance after 9 SD treatments when the axillary buds of *Ribes nigrum* transformed into floral buds. Similarly, they reported that in the SDP strawberry the ABA content also rose upon transfer to SD.

WAREING & EL-ANTABLY (1981) postulated a theory to explain how the rise of ABA-like substance leads to the flowering of SDP. Considering that in *Lemna aequinoctialis* 6746, the minimum number of inductive cycles is only 1 (HILLMAN 1959), it is interesting to note that after the drastic rise on the first day of induction, the ABA content fell immediately to normal. In our experiment 4 SD were given in order to obtain a higher percentage of flowering.

As for the content of GA_3 the present study demonstrated an immediate decline of GA_3 in the first SD. The GA_3 content remained low during the 4-day induction period and rose again upon transfer to LD. This result is according to expectation. It is common phenomenon that when SDP's are transferred to SD, their endogenous GA_3 content decreases before histomorphological changes occur (BERNIER et al. 1981). That is to say, in these plants, flowering is associated with a low GA_3 level. Additional evidence for the association of flowering with

a low endogenous level of GA_3 will be reported by WANG & TSAO (1986) who found that CCC, an inhibitor of GA_3 biosynthesis, when added to the culture medium enable *Lemna aequinoctialis* 6746 to flower under non-inductive continuous light.

Together, the data indicate that a high level of ABA and a low level of GA_3 are necessary for flower induction of the SDP *Lemna aequinoctialis* 6746.

REFERENCES

BERNIER, G., J.-M. KINET & R. M. SACHS (1981): *The physiology of flowering. Vol. II, Section III – Chemical regulation.* CRC Press, Boca Raton, Florida, U.S.A.; pp. 93–182.

HILLMAN, W. S. (1959): Experimental control of flowering in Lemna. I. General methods. Photoperiodism in L. perpusilla 6746. *Am. J. Bot.* **46**: 466–473.

KANDELER, R. & B. HÜGEL (1973): Blütenbildung bei Lemna paucicostata 6746 durch kombinierte Anwendung von Abscisinsäure und CCC. *Plant Cell Physiol.* **14**: 515–520.

— (1985): Lemnaceae. In: A. H. HALEVY (ed.): *Handbook of Flowering.* Vol. III. CRC Press, Boca Raton, Florida, U.S.A.

LANDOLT, E. (ed.) (1980): Biosystematic investigations in the family of duckweeds (Lemnaceae). Vol. I. *Veröff. Geobot. Inst. Eidg. Techn. Hochsch. Stift. Rübel. Zürich.* Heft **70**.

TAN, Z. Y. (1985): Changes of ABA and GA Contents During the Release of Dormancy in Winter Buds of Populus tomentosa Carr. *Acta Botanica Sinica* **27**: 381–386.

WANG, W. & T. H. TSAO (1966): CCC treatment makes L. aequinoctialis flower under long day. (In press).

WAREING, P. F. & H. M. M. EL-ANTABLY (1981): Cited after G. BERNIER, J.-M. KINET & R. M. SACHS: *The physiology of flowering. Vol. II, Section II: Basic mechanisms of flower initiation.* CRC Press, Boca Raton, Florida, U.S.A.

ZHOU, Y. Y., H. W. ZHONG & T. H. TSAO (1983): The experiment of the photo-periodism induction in L. aequinoctialis 6746. *Plant Physiol. Communications* **3**: 59–61. (In Chinese).