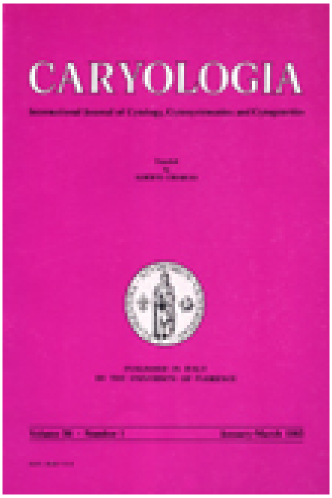

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Morphological Variations in Lemna Minor L. and Possible Relationships with Abscisic Acid

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MORPHOLOGICAL VARIATIONS IN *LEMNA MINOR* L. AND POSSIBLE RELATIONSHIPS WITH ABSCISIC ACID

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SUMMARY — Morphological, anatomical and ultrastructural differences have been observed in fronds of two months old cultures of *Lemna minor* L. These fronds are reduced in area and in thickness and never form colonies. They have been compared with normal and ABA treated fronds in order to determine if the above mentioned differences could be caused by ABA, which might have been released in the culture medium.

INTRODUCTION

The Lemnaceae often represent a suitable material for various studies due to their specific features, namely small size, relative structural simplicity and rapid growth. These plants can be grown axenically, simplifying work with organic compounds. Genetic variability can be eliminated using a single clone for all experiments since their reproduction is usually vegetative.

As far as growth-cycles and morphogenetic events, many Lemnaceae show several variations under particular conditions. *Spirodela polyrrhiza* produces special dormant structures, called turions (HEGELMAIER 1868; JACOBS 1947). These modified fronds, smaller and thicker than normal ones, have few air chambers and their cells show many starch grains. Other *Lemna* species produce similar structures but usually less modified than the preceding ones (HILLMAN 1961). Wellknown physiological events, strictly related to growth and development, are caused by ABA in many Lemnaceae (VAN OVERBEEK and MASON 1968; VAN OVERBEEK *et al.* 1968). In fact, ABA induces turion formation in *Spirodela polyrrhiza* (PERRY and BYRNE 1969; STEWART 1969) and affects fresh and dry weight and starch storage in *Lemna gibba* (TILLBERG 1975; TILLBERG *et al.*, 1979, 1980). In *Lemna minor*, ABA causes the reduction of area and thickness of fronds (NEWTON 1977), the increase of starch storage and changes in pigments content, without turion formation (MC LAREN and SMITH 1976).

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Two months old cultures of *Lemna minor* were grown axenically in Gorham II medium (GORHAM *et al.* 1964). Changes in morphology and size of the fronds and the absence of colonies were occasionally observed at different times of the year in these cultures. These single fronds with reduced area provide the opportunity to examine their morphology, anatomy, ultrastructure and pigments content in comparison with normally sized fronds, regularly forming colonies. The comparative study is also extended to clones grown in varying ABA concentrations in order to determine if the above mentioned differences are caused by ABA which might have been released in culture medium (SAKS *et al.* 1975, 1980).

MATERIAL AND METHODS

Lemna minor L. plants with an abnormal morphology, i.e., fronds with reduced area, never forming colonies (single fronds), made their appearance at the end of October in two months cultures, grown in sterile Gorham II medium (GORHAM *et al.* 1964) and maintained in a greenhouse; the cultures were therefore subject to the autumn decline of daylenght and mean temperature. Some of the single fronds were then transferred to fresh medium and kept in controlled environmental chambers at 25° C, under photoperiodic cycles of 10 h light (from fluorescent tubes having an intensity of 15,000 lux) and 14 h darkness.

Within a few days, numerous new fronds, showing typical species features, originated from the single fronds. Some of these new fronds were used as control; some were transferred to two series of Erlenmeyer flasks containing sterile Gorham II medium and ABA at final concentrations of 10^{-7} M and 10^{-5} M, in order to determine whether ABA induced the formation of single fronds. ABA treated plants are maintained in the same environmental conditions as control plants for a twenty days period.

The three types of whole fronds (single, control, ABA treated) were fixed in GA 3% 0.05 M phospate buffered; pH 6.8 for 48 h at 4° C and post-fixed in OsO_4 1% in the same buffer for 12 h at 4° C. During the fixation period, the fronds were kept completely submerged in the fixative by a stainless steel grid. After dehydratation in a graded series of ethyl alcohol, the samples were embedded in Araldite.

One μm thick sections, obtained with an LKB Ultrotome III, were examined under a Leitz Orthoplan light microscope.

Ultrathin sections, obtained with the same ultramicrotome, were stained with lead citrate (REYNOLDS 1963) and examined under a Hitachi HS-9 electron microscope.

Frond sizes were measured in the section pictures, 300 ×, at the greatest cross-sectional width and thickness. The final sizes were the mean size of ten section pictures examined.

After determination of frond fresh weight, pigment extraction was carried out by the McKINNEY method (1941). Optical density was determined at 663, 645 and 480 nm by a Beckman DB-GT spectrophotometer.

The data represent the average of five separate determinations.

RESULTS

Morphology and anatomy.

Lemna plants from about two months old cultures show small roundish fronds of 1.7 mm mean cross diameter. They are single or at the most have a small daughter frond, but never form colonies, as usually is the case (Fig. 1). These fronds present no roots.

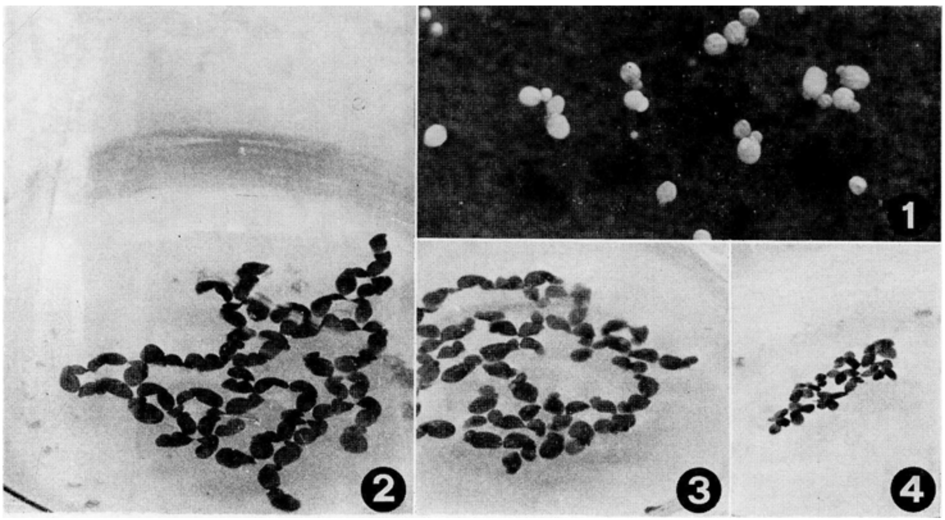

Fig. 1. — *Lemma minor* L.; roundish fronds, never forming colonies, observed in two months old cultures; × 2.
Figs. 2-4. — *Lemna minor* L.; 20 days old cultures; × 1. Fig. 2. - Control cultures. Fig. 3. - Cultures grown in presence of 10^{-7} M ABA. Fig. 4. - Cultures grown in presence of 10^{-5} M ABA.

In 1 μm thick cross section, the irregularly lenticular fronds exihibit small cells in the upper epidermis and larger cells in the lower one. The mesophyll shows large nearly isodiametric air chambers, irregularly distributed (Fig. 5 a). The maximum thickness of the fronds is 0.253 mm on the average.

Control plants display the usual morphology with almost elliptical form, typical species sizes, 2-4 fronds colonies (Fig. 2), and normally developed roots. In cross section, the planoconvex lens shaped fronds are flattened. Upper and lower epidermises are similar to those of the single fronds. The mesophyll present a well developed aerenchima, provided with almost isodiametric intercellular spaces, often they are regularly displaced in two layers (Fig. 5 b). The greatest mean width and thickness are 2.23 mm and 0.168 mm, respectively.

10^{-7} M and 10^{-5} M ABA treated plants are similar to control plants with regard to type and form of the colonies: only frond and root sizes (Figs. 3 and 4, respectively) are different from control ones. In cross section, 10^{-7} M ABA

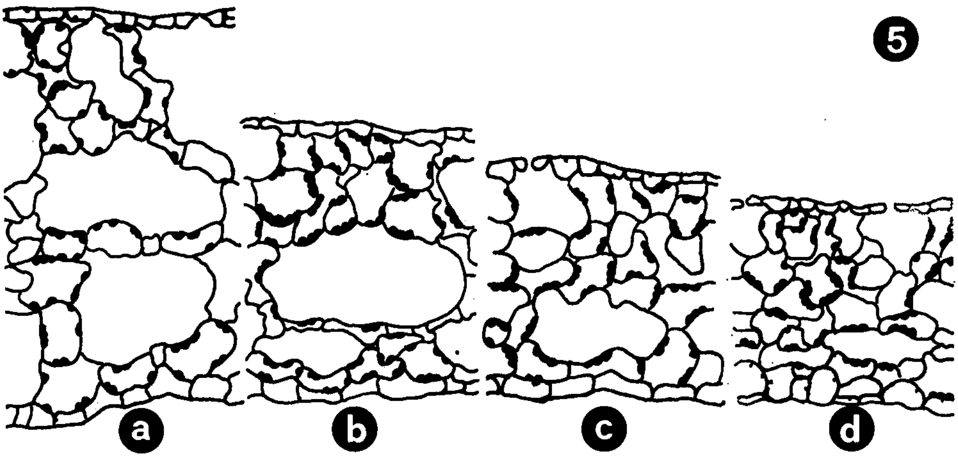

Fig. 5. — *Lemna minor* L.; cross sections of fronds; × 220.
Fig. 5a — Two months old fronds. Fig. 5b — Control fronds. Fig. 5c — 10^{-7} M ABA treated fronds. Fig. 5d — 10^{-5} M ABA treated fronds.

treated plants are flattened, with rather developed air chambers, generally located towards the lower side of the frond (Fig. 5 c). The greatest mean width and thickness are 2.08 mm and 0.156 mm, respectively. In cross section, 10^{-5} M ABA treated fronds are flattened with almost parallel epidermises. The air chambers are reduced and often located near the lower epidermis (Fig. 5 d). The greatest mean width and thickness are 1.87 mm and 0.141 mm, respectively. No notable differences are shown with regard to mean cell sizes in all three types.

Ultrastructure.

Mesophyll cells of single fronds contain scanty, scattered, small plastids, with few thylakoid membranes and small grana, forming a reduced lamellar system. The major part of the stroma shows large, electron dense globules, that sometimes appear to be released by the plastid itself (Fig. 6); similar globules have also been observed in the cytoplasm (Fig. 7). Very small or no starch grains are present in these plastids.

The mesophyll cells of control fronds have larger plastids, with a better developed lamellar system and smaller electron dense globules in the stroma. No starch grains have been observed (Fig. 8).

Finally, ABA treated plant cells contain plastids provided with large starch grains and tiny osmiophilic globules. The lamellar system and plastid size are similar to those of the control plants. In particular, Figure 9 shows a typical plastid of 10^{-5} M ABA treated plant cell; similar to these plastids are those of 10^{-7} M ABA treated plant cells.

The pigment content is different in the four types of fronds. The amount of chlorophyll is largest in control fronds and gradually decreases in 10^{-7} M ABA treated fronds, in single and in 10^{-5} M ABA treated plants. The largest carotenoid amount is found in single fronds, whereas the lowest one is found in 10^{-7} M ABA treated fronds (see Table 1).

TABLE 1 — *Pigment level in single, control, 10^{-7} M and 10^{-5} M ABA treated plants (µg/g f.w.).*

Pigment	Single	Control	10^{-7} M ABA	10^{-5} M ABA
Chlorophyll a	254	462	301	232
Chlorophyll b	102	128	88	104
Tot. chlorophyll	356	590	389	336
Carotenoids	106	70	76	88

DISCUSSION

The reduced size of single fronds, compared with control ones, is due to a smaller cell number, rather than to limited cell extension: indeed, single frond cells are, sometimes, larger than ABA treated and control ones. The larger thickness appears to be due to irregular and different arrangement of air chambers, which are conspicuous in comparison with control fronds. At present, these morphological and anatomical changes are difficult to explain: it is possible that these changes are the consequence of senescence-rejuvenation cycles (ASHBY 1950; ASHBY and WANGERMANN 1949, 1951). In fact, plastid ultrastructure and pigment contents seem to suggest a senescence process. This might be argued from the altered structures of the small plastids, as well as the considerable reduction of the lamellar system and the presence of large, electron dense globules. These globules could be related to the greater carotenoid amounts of single plant cells and to the occurrence of similar globules in the cytoplasm. With regard to size and morphology, these structures resemble oleosomes which are present in senescent leaf cells of several plants (PARKER and MURPHY 1981). Furthermore, a lower chlorophyll amount occurs in these fronds in comparison with control ones.

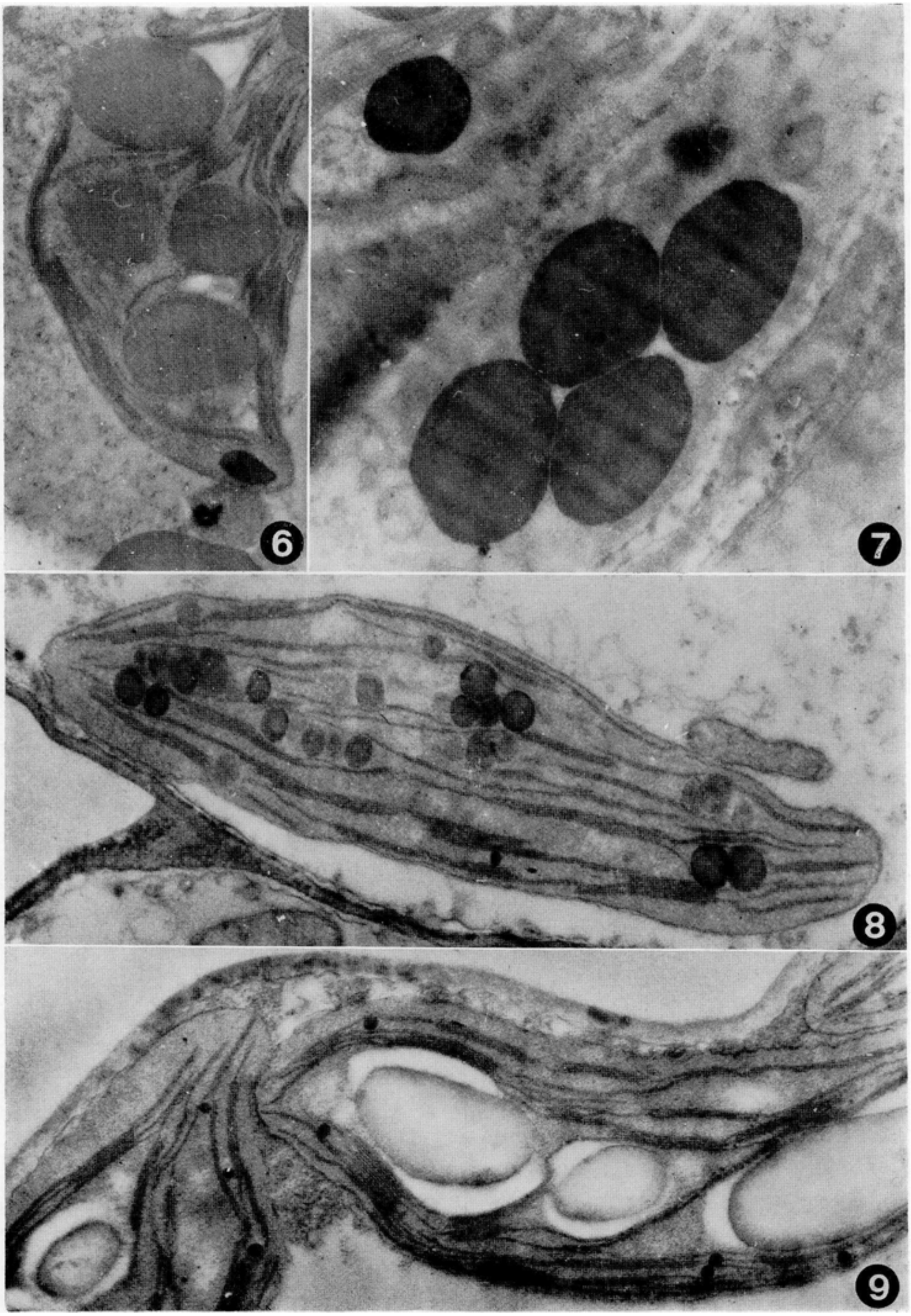

Fig. 6. — *Lemna minor* L.; mesophyll chloroplast of two months old frond cells; × 20,000.
Fig. 7. — *Lemna minor* L.; electron dense globules observed in the cytoplasm of two old frond cells; × 22,000.
Fig. 8. — *Lemna minor* L., mesophyll chloroplast of control frond cells; × 21,000.
Fig. 9. — *Lemna minor* L.; mesophyll chloroplast of 10^{-5} M ABA treated frond cells; × 21,000. Similar to these plastids are those of 10^{-7} M ABA treated plant cells.

In our opinion, ABA concentration might well affect only the frond size; in fact, whereas 10^{-5} M ABA induces a remarkable area reduction (see NEWTON 1977), 10^{-7} M ABA concentration appears to be rather less effective on the basis of present experiments. On the other hand, both concentrations used appear to exert similar effects on plastid ultrastructure, starch storage (Mc LAREN and SMITH 1976) and pigment content.

Our results appear to rule out the possibility that production of small, single fronds in aging cultures of *Lemna* be due to ABA released into the culture medium, as reported by SAKS *et al.* (1975, 1980) for *Spirodela polyrrhiza*.

Moreover, it is difficult to interpret these forms as turions, since turion features such as starch storage and reduction or absence of air chambers are quite different. These forms are able to resume typical species features as soon as environmental conditions become more suitable, on the presence of fresh medium. Investigations under way indicate that the unusual morphology of the fronds, described above, bears some resemblance and might be related to certain types of nutritional deficiency (magnesium inter alia), although they do not seem to support an involvement of nitrate or phosphate deficiency, as was referred by HILLMAN (1961).

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