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A COMPARATIVE STUDY OF DEVELOPING AIR CHAMBERS IN FLAT AND GIBBOUS FRONDS OF LEMNA GIBBA L.

YAEL EFRAT*[1], ARNOLD H. PIETERSE* and FERRY BOUMAN**

*Koninklijk Instituut voor de Tropen, Afdeling Agrarisch Onderzoek, Amsterdam.
** Hugo de Vries Laboratorium, Universiteit van Amsterdam, Amsterdam.

SUMMARY

Cross-sections were made of developing gibbous and flat fronds of *Lemna gibba* G3 which were emerging from the reproductive pocket of the mother plant. In the gibbous fronds the cells of the developing air chamber walls and peripheral walls were in a more active stage of division than in the flat fronds. Consequently, it was demonstrated that gibbosity is initiated in a young developing frond mainly as a result of enhanced cell division and the subsequent increase in the number of cells.

1. INTRODUCTION

The duckweed species *Lemna gibba* exhibits a typical characteristic in which it differs from the other species in the genus *Lemna*, viz., its fronds are capable of bulging at the lower side and as a result of this thickening become hemispherical in appearance. It has not yet been properly ascertained which particular factors induce gibbosity in nature. However, the gibbous modification is generally considered to be an aestival form. In the laboratory it has been shown that flat fronds produce gibbous daughter fronds if cultured in the presence of certain chemicals, such as the chelating agent EDDHA, salicylic acid, and the ethylene releasing compound ethrel (PIETERSE et al. 1970a, b, PIETERSE 1975, 1976).

Gibbosity in *L. gibba* is caused by a vertical elongation of the one cell thick partition walls of the lowest layer of air chambers and a subsequent expansion of the peripheral wall on the lower side of the fronds (HEGELMAIER 1868). As a consequence, the air chambers which are surrounded by these tissues increase considerably in size. HEGELMAIER (1868) explained the elongation as the result of a relatively high air pressure within the air chambers and apparently considered a passive stretching of the walls to be responsible. PIETERSE (1975), however, demonstrated that the number of cells increases with gibbosity, and he consequently assumed that there is an active elongation of the partition walls attributable to cell division.

Full-grown flat fronds never become gibbous spontaneously (PIETERSE et al.

[1] Present address: Tel Aviv University, Department of Botany, Tel Aviv, Israël.

1970a), but are able to produce gibbous daughter fronds under inductive circumstances in vitro. It may, therefore, be expected that the increase in number of the cells in the air chamber walls takes place during the early development of the gibbous frond. In order to clarify the mechanism by which gibbosity is brought about, the development of air chambers in flat and gibbous fronds has been studied in detail by making cross sections during their early development.

2. MATERIALS AND METHODS

Fronds of *Lemna gibba* L. strain G3 were aseptically cultured in 100 ml Erlenmeyer flasks containing 50 ml of a nutrient medium supplemented with 1% sucrose. Gibbous modifications were grown on M-medium (HILLMAN 1961) containing 10 mg/l of the chelating agent EDDHA (ethylenediamine-di-o-hydroxyphenylacetic acid), and flat plants on $^1/_3$ strength Hutner's medium (HUTNER 1925). The plants were exposed to an illumination of 12,000 erg/cm^{-2}/s^{-1} under conditions of continuous light at a temperature of 25 $\pm$ 2°C.

Fronds containing a daughter frond which has just started to emerge from the reproductive pocket were fixed in a solution of Craf for 24 hrs., dehydrated by transferring to 100% ethanol, then to n-propanol, and finally to n-butanol. Subsequently the plant material was embedded at 0°C in glycolmethacrylate, and transferred to gelatin capsules (one plant per capsule) which were kept for two days at a temperature of 60°C (FEDER & O'BRIEN 1968). The fronds were sectioned in a transverse direction through a reproductive pocket and stained with PAS-Feulgen.

3. RESULT AND DISCUSSION

In his classical monograph of the Lemnaceae, HEGELMAIER (1868, pp. 43–54) described the morphogenesis of a young *Lemna* frond by means of vegetative multiplication in great detail. A young bud develops from a small protuberance on the mother frond and at a very young stage initiates primordia of the second generation of daughter fronds. These primordia are formed on approximately $^1/_3$ of the length of the young bud from the end which is designated as proximal since it is the portion closest to the mother frond. The primordia become completely enclosed in a reproductive pocket when (in *L. gibba*) the bud is approximately 0.9 mm long. The young bud differentiates into a plantlet through cell divisions progressing mainly in two directions in a horizontal plane thus forming the future frond disc. The largest part, the area between the reproductive pockets ('the node') and the distal end, is formed first. In contrast to the pattern of cell division, cell expansion progresses from the ends inwards towards the nodal area. When a frond is approximately 0.2 mm long, the formation of air chambers is initiated near the distal end. More recently the morphogenesis of fronds of *Spirodela oligorrhiza* (Kurz) Hegelmaier, which is very similar to that of *Lemna*, was described in detail by RIMON & GALUN (1968). These authors noted that once the newly formed tissues were 'pushed' away from the meristematic area of the frond, the cells cease to divide. They also emphasized the fact that the supply of new cells toward the distal end terminates before the supply of cells to the proximal end.

In the present study cross sections were made of daughter fronds which had just started to emerge from the reproductive pocket of a gibbous as well as of a flat mother frond. The cross sections are shown in *fig. 1a* and *2a*. The length of both the young gibbous frond and the young flat one was about 1.7 mm and they represent a stage in which most of the distal portion had already been formed. As the sections are made through the distal end and one of the reproductive pockets, i.e. the sections are not median, the proximal ends are not visible in the figures. In the reproductive pockets a developing frond of the third generation is already discernable. It is clear from the figures that near the 'nodal' area the cells are in a more active stage of division (as recognisable by their dense arrangement and small size) in the gibbous than in the flat daughter frond. This accounts for divisions in a direction perpendicular to the plane through the frond disc in those tissues which give rise to the one cell thick partition walls of the air chambers. Anticlinal divisions in the upper and lower peripheral walls of the gibbous frond are also visible at this stage of development. In the portions which are enlarged in *fig. 1b* and *2b*, containing the frond of the third generation, it should be noticed that the air chambers develop as

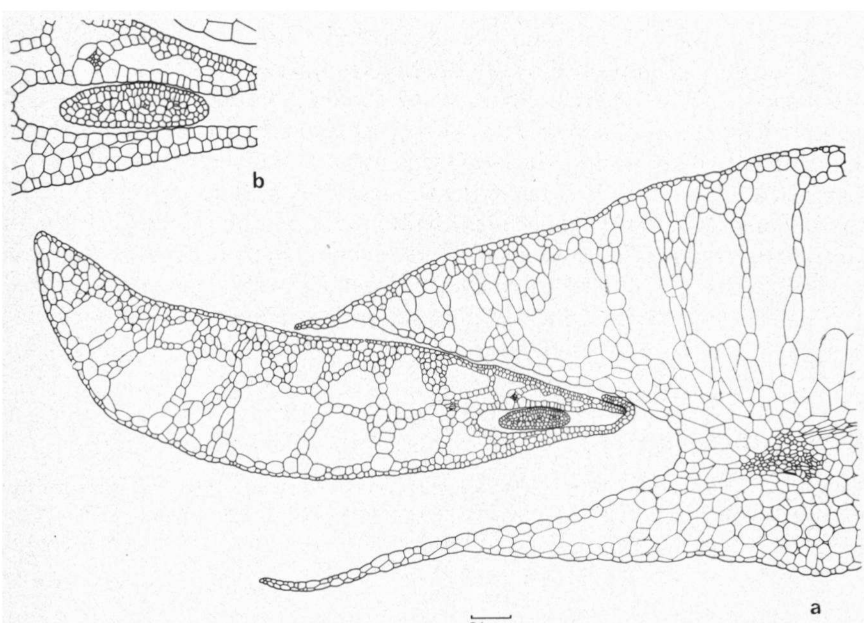

Fig. 1. (a) Cross section of a young flat *Lemna gibba* frond just emerging from the mother frond. (b) enlarged portion from (a) containing a young frond of the third generation.

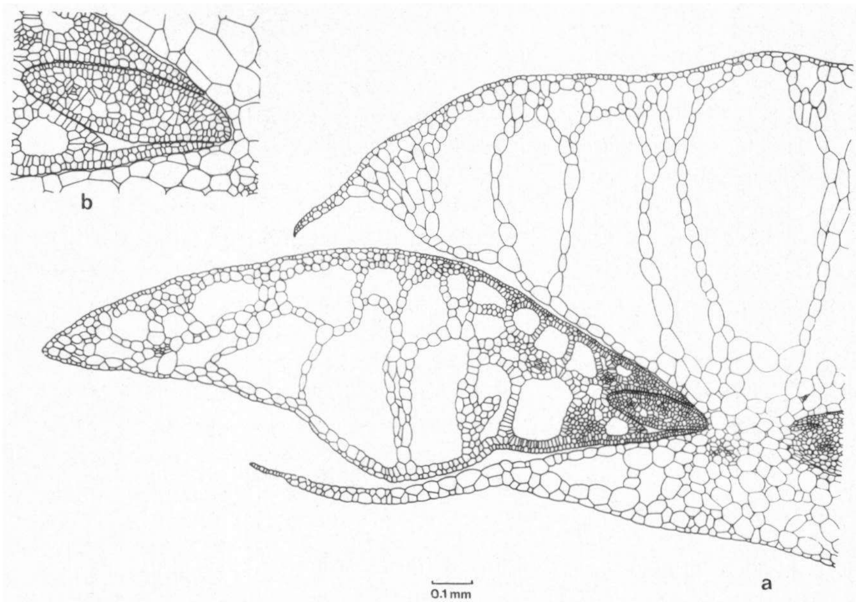

Fig. 2. (a) Cross section of a young gibbous *Lemna gibba* frond just emerging from the mother frond. Note the increased cell divisions in the developing tissues. (b) enlarged portion from (a) containing a young frond of the third generation. Note the initiation of air chambers.

a result of an increase in volume of intercellular spaces. It appears that this enlargement is accomplished by cell divisions in the surrounding cells.

The sections illustrate that gibbosity is initiated in young developing fronds mainly as the result of enhanced cell divisions and the subsequent increase in cell numbers. This finding is in agreement with the recent speculation of PIETERSE (1975), which was based on the fact that in fully grown plants the number of cells in the expanded air chamber walls of gibbous fronds is significantly higher than in the walls of flat fronds. Consequently the earlier suggestion of HEGELMAIER (1868) who explained the swelling of the fronds as the result of a passive stretching of the walls by gas pressure, appears to be definitely refuted.

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