SOME PHYSIOLOGICAL AND MORPHOGENETIC ASPECTS OF FLOWERING OF SPIRODELA POLYRHIZA (L.) SCHLEIDEN

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SUMMARY

A flower of *Spirodela polyrhiza* of the clone used in the experiments arises in the left reproductive pocket of a frond at the proximal part of the meristematic area outside the part which gives rise to daughter fronds.

If the medium is unfavourable for vegetative multiplication of fronds, flowers can be produced by not completely exhausted older fronds and their still attached daughter fronds.

Sometimes the flowers or inflorescences produce 1 to 5 roots.

1. INTRODUCTION

Various *Lemnaceae* are used for investigations on the physiology of flowering in higher plants. These plants are suitable for such experiments because they multiply vegetatively and they are easy to grow in sterile media. So far the genus *Spirodela* was hardly ever used in such experiments because the genus rarely bears flowers in nature, and most attempts to cause flowering under laboratory conditions failed. Under the controlled conditions we used to grow *Spirodela* plants flowering fronds were found.

In this paper some preliminary investigations on physiological and morphogenetic aspects of flowering are given.

2. MATERIAL AND METHODS

The plants were cultivated in erlenmeyer flasks of 300 ml filled with 100 ml of the basic medium described in LACOR (1968) to which was added 20 mg casein hydrolysate. The media were sterilised for 90 min at 100 °C, inoculated with a colony of 2 to 3 visible fronds and placed under 20-hr light conditions (Philips TLF 55/40W, 20W m^{-2}) at a temperature of 26 °C ± 1 °. After 10 days some of the flasks were placed at 14 °C in continuous light (Philips TLF 55/40W, 15W m^{-2}). Fronds and flowers were fixed in FAA, dehydrated in alcohol-xylol series and embedded in paraffin of 60 °C melting point. The FAA used contained ethylalcohol (96 %) 50 ml, glacial acetic acid 5 ml, formaldehyde (40 %) 10 ml and water 35 ml. Sections of 10 μ thick were stained with Mayer's hemalum and fuchsin (0.5 % in 25 % alcohol).

3. OBSERVATIONS AND DISCUSSION

The plant body of *Spirodela polyrhiza* is called a frond. For detailed descriptions of a frond see HEGELMAIER (1868). A frond can be divided in a distal and a proximal part, which is the part nearest to the mother frond as long as it is attached to it (*fig. 1*). The proximal part of a frond consists of a nodal sector consisting of meristematic tissue, flanked by two unequal reproductive pockets, and a region containing the continuation of the stolon and a part of the reproductive pockets. A stolon connects the daughter frond to the mother frond. The nodal sector produces the roots. Also from the nodal sector, but in the reproductive pockets above the point where the roots are attached, the daughter fronds arise. At the same place in the reproductive pockets the flowers develop.

The internal organisation of a colony of fronds consisting of a mother frond (first generation) and two daughter fronds (second generation) is given in *fig. 1*. In unequal growth the right hand daughter frond, if the colony is regarded as lying lower-side-down with its proximal end pointing away from the observer, always protrudes first from the mother frond. This daughter frond arises in the larger reproductive pocket. According to RIMON & GALUN (1968) a daughter frond of *Spirodela oligorhiza* is already a carrier of at least two later generations of fronds at the time of protrusion from the mother frond.

The meristematic areas in the two reproductive pockets of a frond are of unequal size. In the smaller, left reproductive pocket, the meristematic area is much larger and extends much farther alongside the axis which connects the stolon to the nodal part of the frond than the meristematic area in the right pocket (*figs. 2* and *3*). Daughter fronds and turions arise from the part of the meristematic area nearest to the nodal point. The flowers, however, arise at the proximal part of the meristematic area (*figs. 2* and *3*).

In the clone of *Spirodela* used in these experiments the flowers arise – if the fronds have the same position as in fig. 1 – in the left pocket (*fig. 3*). Not in all genera of the *Lemnaceae* or even in each clone of one genus the flowers grow in the left reproductive pocket of the fronds; for example in a strain of *Lemna perpusilla* used by HILLMAN (1959) and in the clone of *Spirodela* used by MAHESHWARI (1958) the right side of the fronds bears the flowers.

HEGELMAIER (1871) and RIMON & GALUN (1968) suppose that at a certain

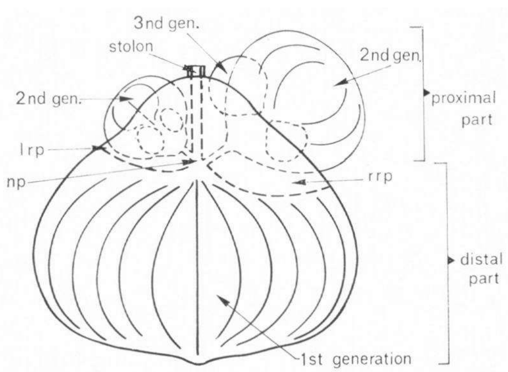


Fig. 1. Scheme of a colony of fronds; dorsal view. np, nodal point; rrp, right reproductive pocket; lrp, left reproductive pocket.

Fig. 2. A ventral view of the nodal sector of an old frond sectioned parallel to its surface, × 40. a, mother frond; b, flower; c, daughter fronds.

Fig. 3. A mother frond (a) with a daughter frond (c) and a flower (b) sectioned parallel to their surface, × 40. The spathe, ovary and ovules are clearly visible.

developmental stage an embryonic bud in a pocket is destined to become a frond or a flower. *Fig. 2* shows that in the left pocket (at the right side of the picture) the fronds arise in succession in a direction from proximal to distal. In the right pocket (left side of the picture) the fronds emerge in a direction from distal to proximal. In addition the flower in the left pocket develops outside the area from which the first daughter frond arises. Based on these observations we may conclude that not every embryonic bud of the meristematic area will differentiate into a frond or a flower, but only those that are situated at the proximal part of the meristematic area. Also KANDELER (1955, 1968) reports that the meristematic part of the pockets which gives rise to the flowers of *Lemna* cannot produce fronds or turions.

As far as is known the fronds of *Lemna* produce one flower in their lifetime (HILLMAN 1959, 1961). Also the fronds used in these experiments of the genus *Spirodela* produced only one flower. However, HEGELMAIER (1871) reported that sometimes a flowering frond could produce one more flower.

Both older and younger fronds can bear flowers. *Fig. 4* shows an older flowering mother frond with attached to it a flowering young daughter frond. This observation is in agreement with the observations of HEGELMAIER (1871) and LUDWIG (1934). According to these investigators as a rule a "Beispross" develops besides the "Blütenspross". Not seldom this "Beispross" should produce flowers.

The fronds flower at the time the medium is less favourable for vegetative multiplication since flowering occurs when turion production starts. At the time the first turions sink to the bottom the oldest fronds, which have been in the medium for the longest time, become yellow. It is these older fronds and the daughter fronds that are attached to them which can produce flowers. Of the connected daughter fronds the last produced by the old mother fronds mostly bear the flowers. Sometimes the old mother frond itself does not bear a flower but only those daughter fronds produced last.

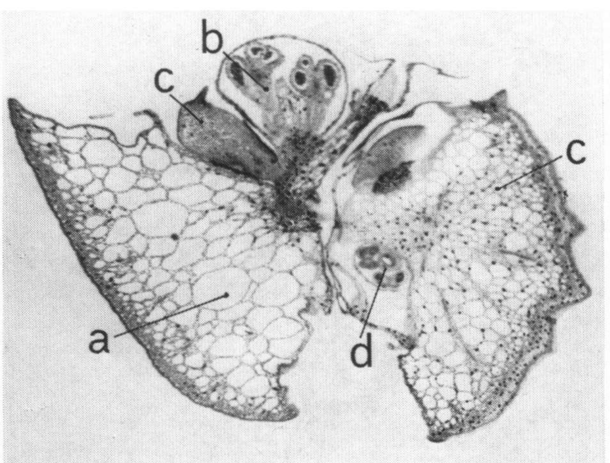


Fig. 4. A mother frond with a daughter frond sectioned parallel to their surface, × 30. a, mother frond; b, flower of the mother frond; c, daughter fronds; d, flower of the right daughter frond.

Fig. 2 shows a flowering old frond which has released several daughter fronds. This old mother frond still bears more young fronds still attached to it. We may therefore conclude that flowers are induced before the mother fronds are completely exhausted.

The fact that only colonies having their origin in an old frond flower, explains why only a small percentage of the fronds in an erlenmeyer flask flowers (LACOR 1968).

The old fronds probably produce a flowering hormone which may induce flowering in the old fronds and also after having been transported along the stolon, in the last produced still attached daughter fronds.

Not only the age of the mother fronds but also the quality of the medium is important for flowering. Also KANDELER (1955) and LANDOLT (1957) reported that crowded old cultures of some strains of *Lemna minor* and *Lemna gibba* flowered. If a flowering colony is inoculated into a fresh medium flowering stops. The ability to flower is not transmitted to the whole progeny.

The flower consists of 1 pistil and 2 stamens enclosed in a spathe. Both stamens are implanted on one side of the pistil. In not completely developed flowers the stamens are often of unequal size. In some flowers 3 stamens were all found to be of unequal size. By growing longer, the stolon, which connects the flower to the meristematic part of the frond by which it was produced, the flower can protrude from the reproductive pocket.

MAHESHWARI (1958) has already described the development of the embryo sac and the endosperm. However, he only gave drawings of the ovule, and the alliance of the *Lemnaceae* with other families of plants has been a matter of dispute for many years; therefore, some pictures of the ovule are given in this paper. An ovary contains two ovules (*fig. 3*). *Fig. 5* presents the metaphase plate and spindle of the megaspore mother cell.

An older ovule in *fig. 6* shows the nucellar cap at the apex of the embryo sac. The nucellar cap consists of nucellar tissue which has not been consumed during enlargement of the megaspore mother cell (MAHESHWARI & KAPIL 1963a).

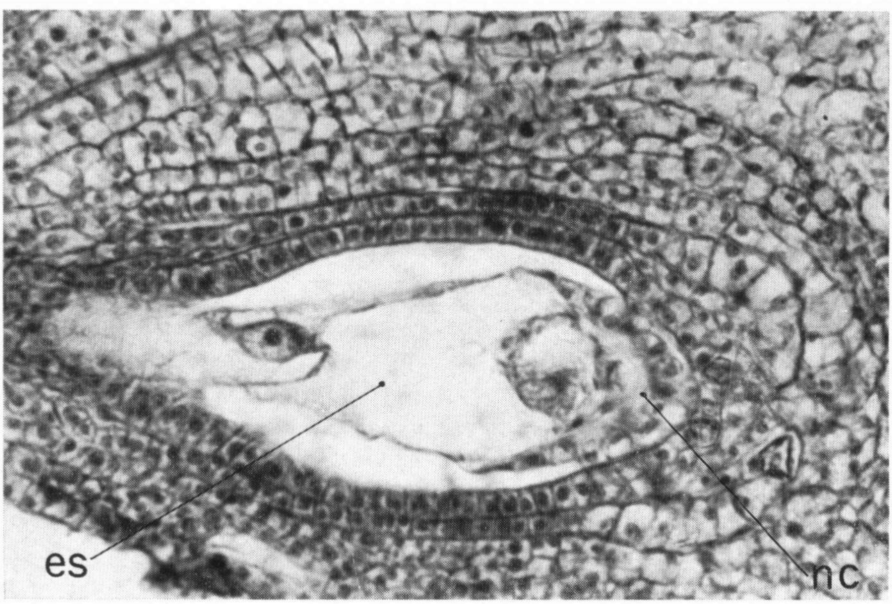
Fig. 7 shows the cellular endosperm. This cellular development of the endosperm contradicts any affinity of the *Lemnaceae* with the *Helobiales* in which free-nuclear divisions occur during development of the endosperm (MAHESHWARI 1956 and MAHESHWARI & KAPIL 1963b).

The question whether the floral complex of the *Lemnaceae* represents a single flower or an inflorescence has often been discussed. In 1868 HEGELMAIER described the complex of sexual organs as an inflorescence but in a later paper (1871) he was convinced that it is only one flower, consisting of an open "Deckblatt" enclosing 2 stamens and 1 pistil. LUDWIG (1934) and MAHESHWARI (1963), however, describe the "flower" of the *Lemnaceae* as an inflorescence consisting of 2 male flowers and 1 female flower enclosed in a thin membranous sac mostly called spathe. According to DAUBS (1965) again the flower may be regarded as bisexual and the spathe as a reduced perianth.

In cultures that had been placed at 14 °C for 20 days after the fronds had been grown for 10 days at 26 °C, sometimes one or two flowers were found which

Fig. 5. This section shows an ovule in which the metaphase plate and spindle of the megaspore mother cell are visible, × 1000.

Fig. 6. This section shows an ovule with the nucellar cap (nc) at the apex of the embryo sac (es), × 400.



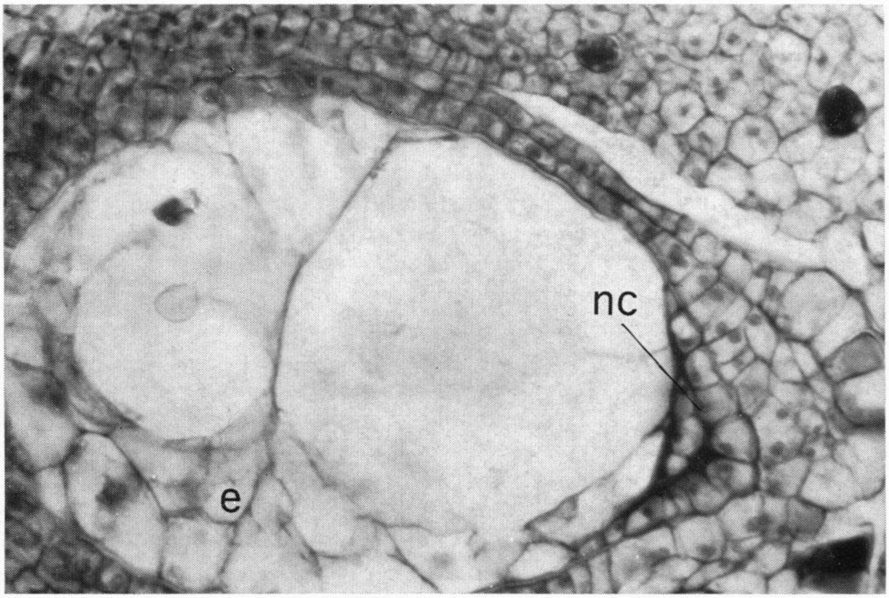
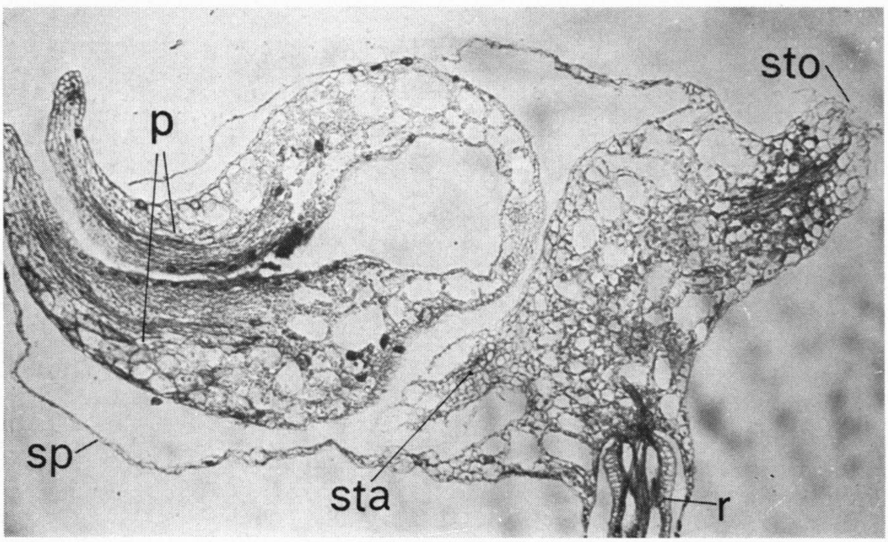


Fig. 7. This section shows an ovule with cellular endosperm (e) and the nucellar cap (nc) at the apex of the embryo sac, × 400.

Fig. 8. A longitudinal section of a flower. At the right upper side a part of the stolon (sto) and at the right lower side of the picture a part of a root (r) with a root sheath are visible, × 100. sp, spathe; p, pistil.



were bigger than normal flowers in cultures of 26 °C. Probably the induction of these flowers had taken place already in the culture period at 26 °C. If this is true it is possible to determine exactly the time the induction of flowers takes place, by culturing the fronds at 26 °C and 14 °C for different periods of time.

The flowers cultured at 14 °C had completely emerged from their mother fronds because the stolon increased in size. Of these probably unfertilized flowers the style protruded from the spathe and the stigma had broken the water surface. The stamens were always completely enclosed by the spathe. From the meristematic basal part of these flowers 1 to 5 roots were produced (*fig. 8*). At first the roots are covered by the epidermis, but when they elongate they pierce the epidermis, which remains as a small sheath around the base of each root. Also in cultures at 26 °C these big flowers were sometimes found, but then the stamens protruded from the spathes. Probably these rooting flowers arise if the medium long enough remains favourable for growth.

The structure of the rooted flowers is more or less comparable with the structure of a frond. These flowers also have a nodal sector connected to their mother fronds with a stolon which produces roots at the ventral side and above the roots the pistil and stamens. From the central point of the nodal sector vascular bundles run to pistil, stamens and roots.

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