ONTOGENESIS OF THE POTAMOGETON FLOWER AND FRUITLET IN SEM RESEARCH

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ABSTRACT

The morphological development of fruitlets representing the four sections of the genus Potamogeton was investigated. Evident morphological differences in the course of the development of fruitlets between the species studied were found. They concern: a) size, shape and outline of carpels and fruitlets, b) individual rate of fruitlet development, c) angle arrangement of the fruitlets, and d) structure of the basal and the apical parts of the fruitlets. Aerocetal initiating of the floral primordia was found, as well as the fact that the fruitlet surfaces are very variable and thus useless for taxonomical purposes.

KEY WORDS: morphology, Potamogeton, fruitlet, surface, flower.

INTRODUCTION

Plants of the genus Potamogeton are water monocotyledons and angiosperms (Szafer et al. 1986; Preston 1995). Both in relation to water plants and other groups of plants, there is a noticeable lack of works on the problems of fruit development (Hejnówicz 1985). Learning about fruit development ought to be considered in relation to flower development. This is a natural, further developmental stage of the flower which ends with the formation of a fruit with a seed or seeds. There are a number of works on Potamogeton fruitlets, e.g., morphological keys on mature Potamogeton fruitlets (Mądzalski 1949, 1977; Jezsen 1955); also some studies on sub-fossil fruitlets (Aalto 1970, 1974; Cappers 1993) and fruitlet development (Toma 1998). The genus Potamogeton is used in some keys to identify seed or fruit (Martin, Barkley 1961; Kac 1965; Sopian 1994). Other studies are concerned with the general development of water plant families or that of Potamogeton inflorescence. The aim of this work was to study the order in which flower appendages arise, the morphological changes of the carpel and of the developing Potamogeton fruitlet surfaces as exemplified by the four sections of the genus Potamogeton: Potamogeton lucens L. representing the section of Potamogeton L., Potamogeton pusillus L. representing the section Grammitifoliis Fries., Potamogeton crispus L. representing the section Battrachoseres Irmsch., Potamogeton pectinatus L. representing the section Coleongeton Reichenb. The succession I accepted was that of Dandy (1980).

METHODS

Pondweeds were identified by keys of Szafer et al. (1986), Dostal (1989) and Hegi (1981). The fruitlets in the SEM research were fixed in glutaraldehyde and a cacodyl buffer for 24 hours at a temperature of 18-20°C. They were dehydrated in ethanol-acetone series (Ciecierwa 1989) and critical point in Pelo CDP2. The fruitlets were coated with a mixture of carbon/gold in a Jeol 60x Els vacuum evaporator and observed with a Nanolab 7 Opton scanning electron microscope.

The collected fruitlets come from individual plants of a particular species. Following morphological observation, four developmental stages were distinguished. In the first stage, the flowers are found under the surface of water and are thoroughly covered with undifferentiated tepals. In the second stage, the tepals are half-open and stamens begin to produce pollen. In the third stage, young fruitlets are present and tepals are degenerated. In the fourth stage, the fruitlets are mature and reach their maximum size.

In the micromorphological description of the surface the following concepts were used: the arrangement of epidermal cells, the primary sculpture and the secondary sculpture according to Boesewinkel and Bouman (1984). The primary sculpture of the surface describes the cell shape, the curvature of the outer periclinal and anticlinal walls, and the relief of the boundary which may be obscure or raised. The secondary sculpture of the surface describes the condition of the outer cell surface, for instance a smooth, reticulate or striate surface. The concept of secondary
sculpture is not homogeneous because it includes a diverse cuticular sculpture, micro-papillate and also secondary wall thickenings occur. These are arranged in a specific pattern on the inner side of the outer, or inner, periclinal walls (Boesewinkel, Bouman 1984).

MORPHOLOGICAL OBSERVATIONS

Micromorphology of young developmental flower stages

*P. lucens*. Stage 1: The flower meristem is of an oval and convex shape. The dimensions of the meristem are as follows: width 120 μm, length 220 μm. The width of the perianth primordium is 20 μm. The four tongues shape primordia of the perianth on the edges of the flower’s meristem are visible (Fig. 1a). Stage 2: The width of the perianth primordium is 30 μm, the width of the stamen primordium is 40 μm. There are four primordia of perianth and four primordia of stamen, which surround the differentiated place of the carpel primordium. Each stamen primordium has two pollen sacs (Fig. 1b). Stage 3: The width of primordium of the perianth is 40 μm, the width of primordium of the stamen is 40 μm, the width of primordium of the carpel is 40 μm. The primordia of the stamen narrow in the middle (Fig. 1c). Stage 4: The developing perianth covers stamens and carpels. The width of the carpel is 50 μm. Young carpels are chalice shaped. The carpels with crest-shaped stigma are turned to the flower axis. They are higher than the stamens. Beneath, there are four stamens, close to the carpels. The width of the carpel is 500 μm (Fig. 1d). The

Fig. 1. The young stages of *P. lucens* flower development. a – stage 1: PT – the primordium of perianth, M – the meristem of flower; b – stage 2: PT – the primordium of perianth, PA – the primordium of stamen, MG – the meristem of carpel; c – stage 3: PT – the primordium of perianth, PA – the primordium of stamen, PG – the primordium of carpel; d – stage 4: T – the perianth, A – the stamen, G – the young carpel.
flower development of *P. crispus* is very similar to that of *P. lucens*.

**Micromorphology of developmental fruitlet stages**

*P. lucens*. Stage 1: The flowers are in spikes. Each flower has four carpels, four stamens and four parts of perianth, which is not differentiated. The basal part of the carpel has a follicular shape and flattened stigma. The cell pattern of the carpel is irregular. The outer epidermal cells are of an irregular shape. The outer periclinal walls of outer epiderma cells are smooth, slightly convex; the anticlinal walls are not creased. The stigma is of an oval shape. There are round, small structures visible on the surface of the carpel. They are artefacts or unnatural cell secretions (Fig. 2a, b). Stage 2: The perianth is half-open. The cap-like shape of the stigma comes down in a crest formation to the basal part of the carpels. The carpels grow more intensive-

ly in length than in width. In the basal part of the carpel, the cells are elongated and parallel to the longitudinal axis of the carpel. The surface of the periclinal walls of the outer epiderma cells are smooth or longitudinally creased. The anticlinal walls are straight and somewhat convex (Fig. 2c, d). Stage 3: The perianth degenerates and falls off before the fruitlets achieve their final size. Young fruitlets grow more intensively along their width than lengthwise. The longitudinal axes of the fruitlets are arranged at an angle of 10°. The cell pattern of the fruitlet is invisible. The cells have a different shape in the apical part of the fruitlet. There is visible pollination by *Diatoma* on the fruitlet surface (Fig. 3a, b). Stage 4: The fruitlets achieve the final size and adhere to the basal part. The longitudinal axis of the fruitlets is at an angle of 45°. The fruitlets increase by 30% lengthwise and by 60% along the width. The cell pattern of the fruitlet is irregular and reticulate. The cells are of an

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Fig. 2. The SEM microphotography of *P. lucens*. A – the carpel at stage 1, b – the surface of the basal part of the carpel at stage 1, c – the carpel at stage 2, d – the surface of the basal part of carpel at stage 2.
irregular shape. The surface of the periclinal walls is smooth, the anticlinal walls are straight (Fig. 3c, d).

*P. pusillus.* Stage 1: The carpels are covered by four parts of the perianth, which is not differentiated. The carpels have a tubular shape with a widened stigma. The cell pattern of the carpel is invisible. The periclinal walls of the epiderma cells are broken down. Stage 2: The parts of perianth are half-open. The carpels are characterized by intensive growth in length and width. The stamens start the pollination. The carpels extend in the basal part and in the stigmas. The cell pattern of the carpel is invisible. The borders of the outside epidermal cells are not distinguished. The periclinal walls of the outside epidermal cells have longitudinal creases which are parallel to the longitudinal axis of the carpel. The stigma is of oval shape. Stage 3: The stamens are degenerating. The cell pattern of the fruitlet is invisible. The fruitlets are deformed as a result of dehydration. Stage 4: The fruitlets are arranged in fours and increase in width and in length by about 100%. The basal part of the fruitlets is expanded. The angle between the longitudinal axes of the fruitlets is 50°. The perianth still exists. The cell pattern of the fruitlets is gentle and reticulate. The cells of the outer epiderma are elongated parallel to the longitudinal axis of the fruitlet. The surface of the epidermal cells is smooth. The size and shape of the epidermal cells are variable.

*P. crispus.* Stage 1: The flowers are covered by undifferentiated perianth. The carpels are higher than stamens. The carpels are chalice shaped. The surface of the carpel is creased and irregular. The outside epidermal cells are of different shape. The periclinal walls are smooth and slightly convex. The anticlinal walls are straight. The stigma is rounded with a narrow hole (Fig. 4a). Stage 2: The perianth is completely open and starts to degenerate. The apical part

Fig. 3. The SEM microphotography of *P. incens.* a – the fruitlet at stage 3, b – the surface of the basal part of the fruitlet at stage 3, c – the fruitlet at stage 4, d – the surface of the basal part of the fruitlet at stage 4.
of the carpel increases by 300%. The basal part of the fruitlets is slightly enlarged. The angle between the longitudinal axes of the fruitlets is 8°. The surface of carpel style has an irregular and reticulate cell pattern. The outer periclinal walls are convex and creased. The anticlinal walls are straight at the basal part of the carpel. The oval shaped stigma is elongated, with a narrow hole (Fig. 4b). Stage 3: The angle between the longitudinal axes of the fruitlets is 40°. There is a considerable elongation in the basal part of the fruitlets and small increase in length. The cell pattern of the carpel is invisible. The epidermal cells are slightly concave or convex, they have smooth periclinal walls in the basal part of the fruitlet. The rest of the stigma is oval (Fig. 4c). Stage 4: The fruitlets attain their final size. The angle of the fruitlet inclination is 80°. The fruitlets increase in length and in width by about 100%. The cell pattern of the fruitlet is irregular and reticulate. The epidermal cells are elongated parallel to the longitudinal axis in the apical part of the fruitlet. The epidermal cells of periclinal walls are smooth. The anticlinal walls are concave in the apical part of the fruitlet Fig. 4d).

P. pectinatus. Stage 1: The perianth is tetramerous. The shape of the carpels is similar to that of P. lucens. The basal part of the carpel is slightly follicled. The stigma is flattened. The cell pattern of the carpel is invisible. The outside epidermal cells are of irregular shape. The anticlinal walls of the outside epidermal cells are undulated. The stigma is of irregular shape with a narrowing. Stage 2: The perianth is half-opened. The carpels increase somewhat in the basal part, and by 100% in length. The cell pattern of the carpel is reticulate in the apical part of the carpel. The borders of the outside epidermal cells are difficult to distinguish. The periclinal walls are smooth in the apical part of the carpel, and are slightly concave in the basal part. Stage
3: The perianth degenerates and falls down. The basal part of the carpel increases about by 200% and the carpel length increases about by 100% as compared with stage 2. The angle between the fruitlets longitudinal axes is 12°. The cell pattern is irregular. There is visible pollution by *Diatoma* on the fruitlet surface. Stage 4: The carpels increase in length and in width by about 40%. The fruitlets adhere to the basal part. The angle between the longitudinal axes of fruitlets is 32°. The apical part of the fruitlets is increased, contrary to *P. lucens*. The cell pattern of the fruitlet is reticulate. The periclinal walls of the outside epidermal cells are slightly convex and smooth. The anticlinal walls are straight. The epidermal cells are of irregular shape.

RESULTS

Hypogynous flowers are arranged in spikes. Each of them has four carpels, four stamens and four squamous parts of the perianth, which is not differentiated into petals and sepals. The *P. lucens* inflorescence has 95 flowers on average. In other *Potamogeton* species, only a few to a dozen flowers are present on one spike. The flowers of the apical parts of the *P. lucens* spike show a spiral phyllotaxis at a 77.9° divergence angle, which change into a whorled phyllotaxis in the basal part of the spike. There are four flowers in one whorl. Clear morphological differences in the development process between the species studied were found. These differences concern the arrangement of floral appendages in the flower; the size, shape and outline of the carpels and fruitlets; the individual rate of fruitlet development; the angle values of spatial fruitlet arrangement; and the structure of the basal and apical parts of the fruitlets. The color of the carpels and fruitlets change from white in stage 1 and 2, to green – olive, and bright or dark brown in stage 3 and 4. The changes in the size and shape of the carpels and fruitlets are described in the morphological observations section. The longest are *P. crispus* fruitlets, the widest are *P. lucens* and *P. pectinatus* fruitlets, the narrowest are *P. pusillus* fruitlets. The important difference in morphology between *P. lucens* and *P. pectinatus* is that the first species is widest in the basal part, whereas the second specie is widest in the apical part of the fruitlets in stage 4. The differences between the outlines of the fruitlets have been shown (Fig. 5).

There are clear interspecies differences in the size of carpels and fruitlets (Table 1). The apical part of the fruitlets is deflected to the outside so that the angle between the longitudinal axes increase in the course of the development: *P. lucens* from 0° to 45°, *P. pusillus* from 0° to 50°, *P. crispus* from 0° to 80° and *P. pectinatus* from 0° to 32° (Fig. 6).

The arrangement of epidermal cells is various and not always visible. The surfaces of the apical and basal parts of the fruitlets and stigma surfaces show significant variability in the size and shape of the outer epidermal cells. The secondary sculpture does not occur in the outer epidermal cells of the carpels and fruitlets. The differentiation of the individual parts of a flower is acropetal, first primordia of perianth are initiated, next stamens and, in the last stage, carpels. The young carpels are chalice shaped and their later width is limited by common development in one

Fig. 5. The comparison of outlines of the *Potamogeton* fruitlets in four stages. a – *P. lucens*, b – *P. pusillus*, c – *P. crispus*, d – *P. pectinatus*.

![Diagram](image)

Fig. 6. The comparison of angles between the *Potamogeton* fruitlets. 1 – *P. lucens*, 2 – *P. pusillus*, 3 – *P. crispus*, 4 – *P. pectinatus*, 5 – the complementary angle to 360°.

flower. This is the cause of the radial flattening of the mature fruitlets. The stamens and carpels in the young flower are completely covered by perianth which increases
TABLE 1. Comparison of Potamogeton fruitlets dimensions.

<table>
<thead>
<tr>
<th>Species</th>
<th>Development stage</th>
<th>The width of the carpet and fruitlet in µm</th>
<th>The length of the carpet and fruitlet in µm</th>
<th>The length of outer epidermal cells in apical and basal part of fruitlet in µm</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. lucens</td>
<td>1</td>
<td>430</td>
<td>1000</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>800</td>
<td>2700</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2300</td>
<td>4000</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>3200</td>
<td>5600</td>
<td>40; 70</td>
</tr>
<tr>
<td>P. pusillus</td>
<td>1</td>
<td>100</td>
<td>220</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>220</td>
<td>500</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>600</td>
<td>1300</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1600</td>
<td>2900</td>
<td>60; 80</td>
</tr>
<tr>
<td>P. crispus</td>
<td>1</td>
<td>425</td>
<td>900</td>
<td>12; 20</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>700</td>
<td>2400</td>
<td>50; 20</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1750</td>
<td>3700</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2700</td>
<td>5800</td>
<td>63</td>
</tr>
<tr>
<td>P. pectinatus</td>
<td>1</td>
<td>230</td>
<td>500</td>
<td>10; 30</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>500</td>
<td>1450</td>
<td>35; 15</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1400</td>
<td>2900</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>2600</td>
<td>4300</td>
<td>40; 50</td>
</tr>
</tbody>
</table>

in width and length. The immature carpels have an extended base, follicular shape and flattened stigma. In P. lucens the carpels show a characteristic cap-shaped stigma which has the simple structure of a crest coming down to the basal part. In the rest of the species studied the stigmas have an oval shape. In the stage preceding perianth opening, and immediately after, the carpels grow more intensively in width than in length. The perianth degenerates and falls off before the fruitlets achieve the final size. The fruitlets are gathered in furs and adhere to the basal part.

The SEM technology adopted illustrates well the morphological changes in the developing flowers and fruitlets. It enables the recognition of the size and shape of the outer epidermal cells. The photographic documentation of the cell surfaces demonstrates that the arrangement of epidermal cells and primary sculpture cannot be diagnostic features in distinguishing Potamogeton species.

**DISCUSSION**

The fruitlets investigated come from tetramerous flowers. There are rarely trimerous or pentamerous flowers. The flowers are tri-, tetra-, penta-, and octamerous in P. lucens (Charlton, Posluszny 1991). The number of flowers and fruitlets is variable in Potamogeton spikes. In P. lucens, there are 15-72 flowers on the one spike, in P. crispus 2-10 flowers, in P. pusillus up to 5 flowers, and in P. pectinatus 4-8 flowers (Casper, Krausch 1980). In connection with the above fact, the number of fruitlets on the one spike is variable too. For instance in P. lucens there are 90 fruitlets (Charlton, Posluszny 1991) or 95 fruitlets on the one spike (Toma 1998).

The development of the individual appendages of the P. lucens and P. crispus flowers follow the same order as in P. zosteriformis (Posluszny 1981). Potamogeton carpel growth is slow during blooming. The more intensive growth of the Potamogeton carpel in width, before and after blooming, is similar to that of the cherry fruit (Tukey, Young 1939).

Aquatic vascular plants and seeds usually have little or no surface sculpturing (Barthlott 1981). Microstructural features of the seed on the surface of terrestrial plants have a diagnostic value (Barthlott 1984). The Potamogeton fruitlets do not have the specific sculpturing and characteristic epidermal pieces as they do in some terrestrial plants, for instance the papillates occurring on the surface of Saxifraga seeds (Kuźniewska 1980) or the particular cells arrangements on the surface of Nigella damascena seeds (Boesewinkel, Bouman 1984).

When collecting material for study, one ought to remember that Potamogeton fruitlets must be fixed immediately after being pulled out from water, without being allowed to become dry, not even partially so, otherwise the fruitlet surface will deform. The surfaces of epidermal cells disintegrate and make it impossible to document correctly. Potamogeton fruitlets show a different degree of sensitivity to dehydration. In the young stage of the fruitlets, sensitivity to dehydration is higher than at the later stage. The most tolerant to dehydration among the Potamogeton species studied is P. crispus; the least tolerant is P. pusillus. The carpels and fruitlets surfaces were difficult to observe because the walls of the outer epidermal cells disintegrated. Also the surfaces were covered by Dictoma and sometimes by an unidentified substance.

The fruitlets grow with an individual rate of development. This concerns the situation when each fruitlet from one flower, can present a different developmental stage. The fruitlets in identical morphological stages can have a different cell size of the outer epiderma. The epiderma cells of small, fruitleted species are big. For instance in P. pusillus, the epiderma cells are 80 µm in length (Table 1). However, in other species with bigger fruitlets, the epiderma cells are up to 50 µm in length. This confirms the thesis on the individual rate of fruitlet growth, which depends on external factors (light, temperature, pH, pollution, oxidation) and internal factors (local inflow diversity of nutritive substances and growth hormones, gene expression and mechanical tension between individual layers of pericarp and embryo cells). The contribution of environmental and genetic factors to the variation is a notable feature of many Potamogeton species (Preston 1995). The variation in the species Potamogeton has not been the subject of many studies and requires further investigation.

**LITERATURE CITED**


POWSTAWANIE KWIAIUTU I OWOCKA POTAMOGETON W BADANIACH SEM

STRESZCZENIE

Badano rozwój morfologiczny owoców reprezentujących cztery sekcje rodzaju Potamogeton L. Stwierdzono wyraźne różnice morfologiczne w czasie przebiegu rozwoju owoców pomiędzy badanymi gatunkami. Dotyczą one wielkości, kształtu i zarysu słupków i owoców, indywidualnego tempa rozwoju w obrębie gatunku, kąta ułożenia owoców w stosunku do siebie, budowy ich części bazalnej i apikalnej. Stwierdzono akropetalne inicjowanie związków poszczególnych części kwiatu oraz bardzo zmienną strukturę powierzchni owoców, a zatem jej nieprzydatność do celów taksonomicznych.

SŁOWA KLUCZOWE: morfologia, Potamogeton, owocek, powierzchnia, kwiat.