ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. 50, nr 1—2: 339—344
1981

Storage products and tissue interaction in the ovule of *Pinus silvestris* (L.)

F. M. ENGELS

Department of Plant Cytology and Morphology, Agricultural University, Arboretumlaan 4, Wageningen, The Netherlands

Abstract

The organel-sequence in ovular cells of *Pinus silvestris* was investigated by light- and electronmicroscopy during the post-pollination and pre-fertilization period. Changes in starch and lipid storage suppose starch to be a pool for lipid synthesis and a reserve for ovule development. The base nucellus plays an important role in the distribution of metabolites all over the ovular tissues. Lipid, starch and callose are of interest for the cells to protect them against low temperatures by means of isolation, anti-freeze and plug formation respectively.

INTRODUCTION

The qualitative and quantitative changes of enzymes, sugars, starch and lipids in the ovule of *Pine* species have been related to periodicity of the seasons during the three years of development of the ovule (K o-n a r, 1958; W i l l e m s e, L i n s k e n s, 1969; G e l i s s e n, 1971). An ultrastructural investigation of the ovular tissue was carried out during the pre-fertilization period (September 1978 — April 1979) and attention was paid to changes in starch, lipid and cell organelles in relation to a possible system of transport in the ovule and to seasonal influences.

MATERIAL AND METHODS

Ovules from cones of *Pinus silvestris* (L). are prepared and fixed during 10 hrs at room temperature (r.t.) in 0.1 M cacodylate-buffer (c.b.) p.H. 7.8 with 5% (v/v) glutaraldehyd. The ovules are then placed in c.b. with 1% (w/v) OsO_4 for 3-5 hrs at r.t. and afterwards embedded in Epon or ERL. Ultrathin longisections are afterstained with 1% (w/v) acqueous uranyl acetate and lead citrate successively and examined in a Philips 301 at 60 Kv. Monthly sections of 2-3 ovules were used for organel pattern analyses. Starch and lipid are detected by light microscopy by JKJ-staining of 1-3 µm Epon sections and nile brilliant blue of 9 µm freeze sections respectively. Callose formation in fresh deep

frozen ovule was demonstrated with 1% (w/v) aequeous Wasser-blue and Digestive juice cellase treatment (8000 pmm) in 0.1 M phosphate buffer.

RESULTS

Cell organel analyses from longisections of the ovule in the pre-fertilization period are presented schematically on Fig. 1. The organel pat-

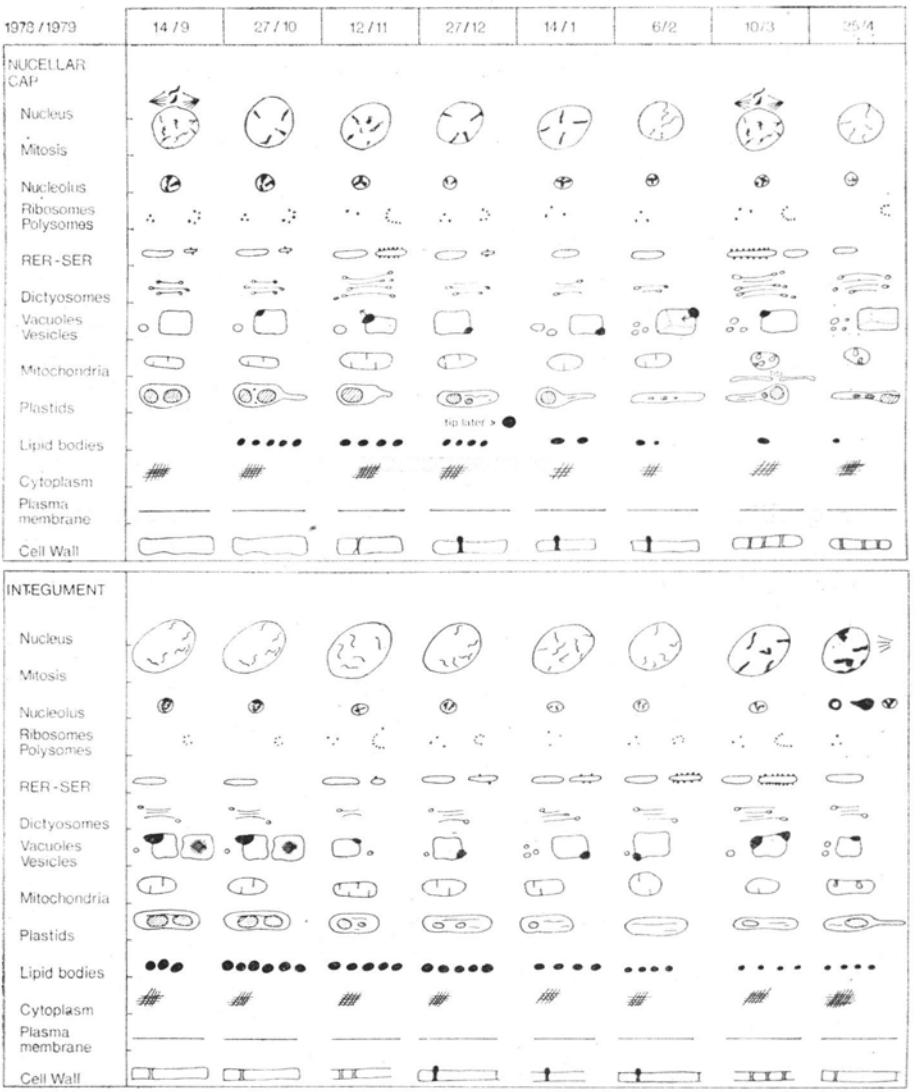

Fig. 1A, B. Schematic organel pattern of the cells of different tissues in the ovule

A — Nucellar cap: subdivided into tip and base nucellus; B — Integument.

terns show structural changes mainly its plastids, lipid bodies, vacuoles and Golgi apparatus in all tissue cells of the ovule.

In the base nucellus and integument (Fig. 2) starch accumulation in plastids is observed in August-September. During the next months this starch fades away and in February only some small starch grains are present (Fig. 1A, B). Lipid droplets appear in the same tissues when the starch-grains decrease in volume and number in September-October. Small lipid droplets appear within the membrane of small vacuoles,

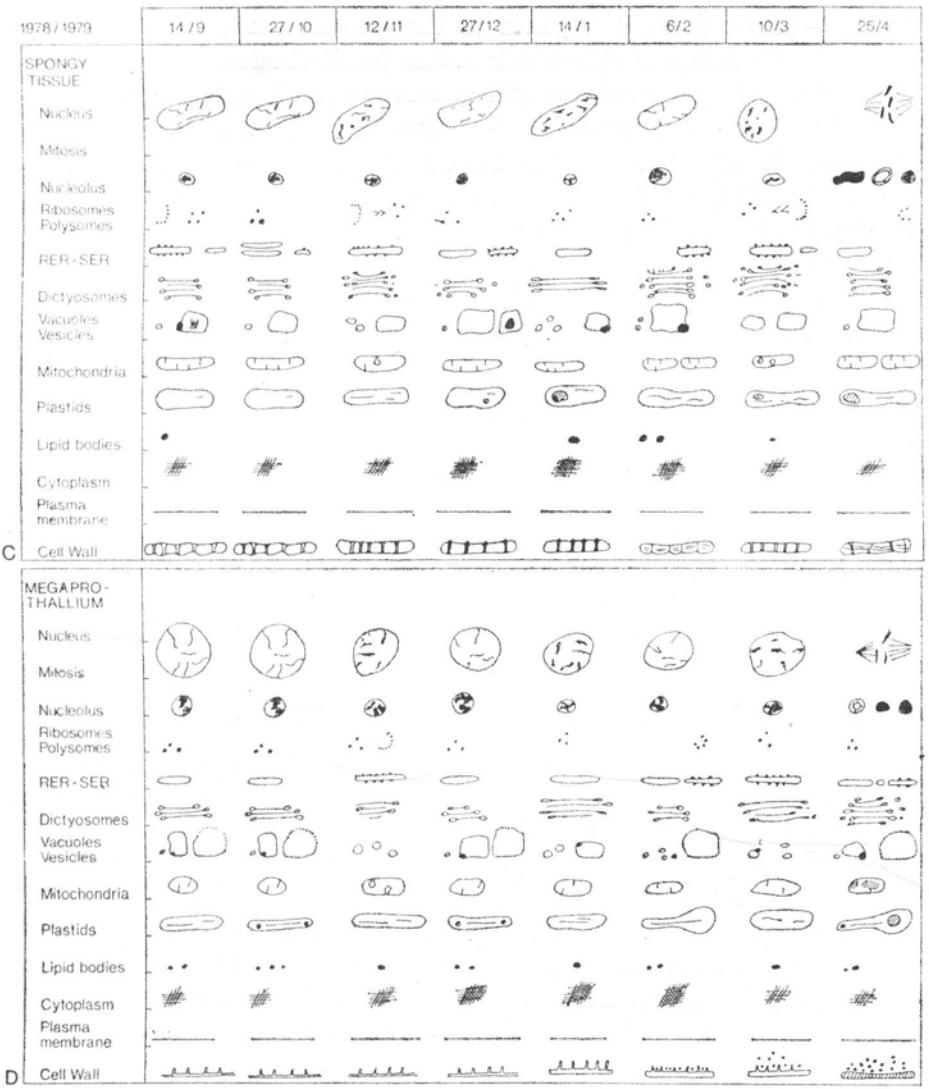

Fig. 1C, D. Schematic organel pattern of the cells of different tissues in the ovule

C — Spongy tissue; D — Megaprothalium (= female gametophyte)

when they reach a diameter between 1-3 μm, they migrate from the membrane towards the plasma membrane. A layer of these droplets is formed during October in all cells of the base nucellus and the integument. Most of these lipid-vacuole complexes then disappear. In the next months a slow diminishing of the lipid is observed. This again starts by localization of the lipid droplets at vacuole membranes.

In the tip nucellus, spongy tissue and megaprothallium starch and lipid storage goes on similarly as described before, but it was found to be delayed in time for 1-2 months (Fig. 1A, C, D). Lipid droplets in the tip nucellus did not migrate to the plasma membrane. Staining of starch by JKJ revealed blue-black grains in all tissues.

A second round of intense starch storage in plastids starts in March in the base nucellus, immediately followed by a break down in April (Fig. 1A). In all other ovular tissues starch increased only in April (Fig. 1B-D). The starch grains are now coloured red-brown after JKJ staining, suggestive of amylopectine. In all ovular cell walls, except the megaprothallium, callose plugs on the plasmodesmata were found in December-February. Callose plugs could be induced and enlarged by storage of the ovule at —18°C for 48 hrs. In March callose plugs were disappearing.

The Golgi apparatus in the spongy tissue and megaprothallium changed in time in the pre-fertilization period. Numerous cisternae and vesicles were found in November, February, March and April. The cell organelles, not mentioned so far, changed only slightly in their ultrastructure and indicative for a relative quiescent period was the slaw down of mitotic activity (Fig. 1A-D).

DISCUSSION

The storage of starch in the ovule of *Pinus silvestris* occurs bifasic, first in August-September and then in March-April. In both cases the base nucellus preceeds the other ovule tissues in accumulation and disappearance of starch. The lipids accumulate one to three months later than starch and this was always found to coincide with a break-down of starch grains. These data have been outlined in Fig. 2A-B and the possible starch and lipid succession in the tissues are indicated by arrows. In this scheme the starch primarily will function, partly as a pool for lipid synthesis and storage and secondly for the further development of the tissues of the ovule prior to fertilization.

Starch and lipid storage in the ovular tissues in not random. A gradient was found within the tissues and a succession of starch and lipid within one tissue and over the ovule during the pre-fertilization period and especially during the winter was noted. All together this rather

suggests a process of development that is still going on than a decrease in all cell activities affected by low temperatures, as found in the wood phloem of *Populus* (Den Outer, 1973) as well as in many conifers (Wanner, 1958; Willemse, Linskens, 1969; Gelissen, 1971).

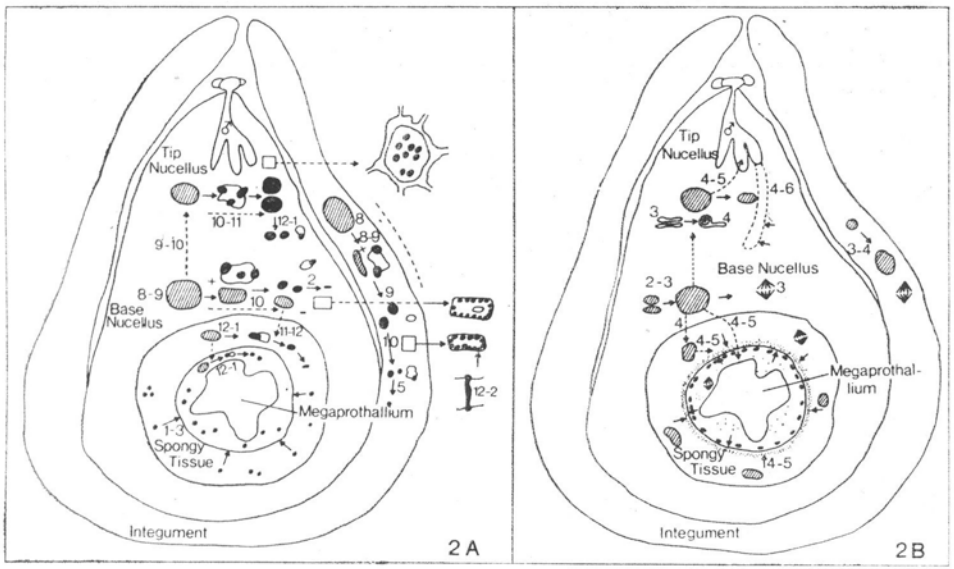

Fig. 2A, B. Schematic drawing from longisections of the ovule (× 70)
The numerical notations refers to the month in the year. The arrows point to a possible direction in transport and relation; stripped areas -- starch; dark areas — lipid; light areas with dark points — lipid synthesis or break down

Vascularisation of the ovule of the *Pine* is abscent (Singh, 1978) so transport of metabolites through the ovule must be from cell to cell. The base nucellus accumulates the starch and it is from this tissue that starch is distributed over the ovular tissues. The bended position of the ovule onto the sporophyl realizes a close contact with the underlying vascular system of the sporophyl with the base nucellus. These findings suppose the base nucellus to be a distribution centre of ovular metabolites.

The lipid present as a monolayered dropled coat around the cells in the ovule and the starch still present during winter and possibly converted into sugars as found *in Populus* (Sautler, 1967) when temperatures fall down to 5°C, do protect the cells during this period. Lipid serves as an isolator and the sugars as anti-freeze. Under such extreme conditions as artificially introduced at —18° C, cell isolation can be extended by cut off of the cells by callose plugs into the plasmodesmata.

REFERENCES

Gelissen A., 1971. Ann. Univers. et Arers. pp. 265-272.
Konar R. N., 1958. Phytomorphology 8: 168-173.
Outer R. W. den, 1973. Z. Pflanzenphysiol. 70: 266-269.
Sautler J. J., 1967. Z. Pflanzenphysiol: 56: 340-352.
Singh H., 1978. Ed. Embryology of Gymnosperms. Borntraeger, Berl., Stuttg.
Wanner H., 1958. Handb. d. Pflanzenphysiol. VI: 868-870.
Willemse M. T. M., Linskens H. F., 1969. Rev. Cytol. et Biol. vég. 32: 121-128.