INTRODUCTION

The Araceae are a family of herbaceous monocots with 106 genera and about 2000 species (Mayo et al., 1997). The vast majority of the genera occur in the New World tropics. Members of the family are highly diverse in life forms, leaf morphology and inflorescence characteristics. The family is best characterized by its distinctive inflorescence, a spadix with bisexual or unisexual (sometimes with sterile) regions and subtended by a solitary spathe on a long or very short peduncle. Arum alpinum is mainly distributed in Europe and central Asia and grows in temperate and warm temperate woodlands.

According to Weber et al. (1999) the outer pollen wall of Arum alpinum is formed by a thick, spongy endexine and lacks a sporopolleninous ektexine. Polysaccharidic spines are covering the endexine. Less information is available concerning the complete ontogeny—from pollen mother cell to mature pollen grain—of this unusual pollen wall (Paciń and Juniper, 1983).

In the present study, attention is focused on the development of the pollen grain wall, with special reference to callose and polysaccharidic spines.

MATERIALS AND METHODS

Transmission electron microscopy (TEM)

Anthers of Arum alpinum Schott & Kotschy (Araceae) were prefixed in 3 % glutaraldehyde in phosphate buffer (0.1 M, pH 7.5) for 8–10 h at room temperature. After rinsing in the same buffer the tissues were post-fixed in 2 % OsO₄ plus 0.8 % phosphate-buffered K₄[Fe(CN)₆] (2:1) overnight at 6°C and rinsed in distilled water. This was followed by dehydration in 2,2-dimethoxypropane and absolute acetone and embedding in Spurr’s epoxy resin (Spurr, 1969).

Ultra-thin sections (80–100 nm) were cut on a Reichert ultra-cut microtome (Leica Microsystems, Wetzlar, Germany) with an Ultra-Diamondknife (Diatome, Biel, Switzerland), mounted on formvar-coated gold slot grids. For localization of neutral polysaccharides (PAS reaction) sections were treated with 1 % periodic acid (PA) for 45 min, 0.2 % thiocarbohydrazide (THC) for 8–15 h, and 1 % silver proteinate (SP) for 30 min (Thiery, 1967). Without periodic acid oxidation the Thiery test was used to detect unsaturated lipids in osmium-fixed material (Rowley and Dahl, 1977). Staining was conventional either with uranyl acetate (U: 1 % methanolic solution, 45 min at room temperature) followed by lead citrate (Pb: 0.1 % solution, 5 min at room temperature) (Reynolds, 1963) or with a modified Thiery test (PA for 10 min, TCH for 15 min, SP for 10 min). All ultra-thin sections were examined in a Zeiss EM 900 TEM (Zeiss, Oberkochen, Germany) at 50 kV.

Scanning electron microscopy (SEM)

Anthers were dehydrated in 2,2-dimethoxypropane and critical-point dried in a Balzers CPD 010 (Balzbritter, 1998). The pollen grains were sputter coated with gold in a Balzers SCD 010 and observed in a Jeol T-300 at 10 kV.

Light microscopy (LM)

For detection of callose semi-thin sections were embedded in 0.05 % aniline-blue solution (Smith and McCully, 1978) and examined with an epifluorescence microscope (Leitz Ortholux II). The cellular condition of the pollen grain was detected by acetic-carmine staining (Gerlach, 1984).
Plant material

Plants of *Arum alpinum* growing in their natural habitat at Georgenberg, Maurer-Wald, Wien XXIII were collected once a week within the vegetative period from February to April.

**RESULTS**

Pollen mother cells to tetrads

Pollen mother cells (PMCs) are formed within the anthers as long as the flower buds are located underground. Young PMCs are isodiametric in shape and their cytoplasm shows a relatively simple ultrastructure (Fig. 1A). With the beginning of meiosis PMCs loose their isodiametric shape and become more and more spheroidal. No callose is deposited around PMCs or around microspores before and during meiosis (Fig. 1B and C). Karyokinesis is followed by cytokinesis with the formation of a cell plate, which grows centrifugally (Fig. 1D) and lacks callose too.

Cytokinesis results in the formation of a planar tetrad (Fig. 2A). Within the tetrad the four microspores are separated by a non-callosic space. This space is electron transparent after the modified Thiey test (Fig. 2A, asterisks) but appears structured after uranyl acetate–lead citrate staining (Fig. 2B, asterisk). Although the space appears callose-like, presence of callose could not be confirmed.

At that stage the wall of the microspores is exclusively formed by the endexine (Fig. 2A and B). No primexine was noticed prior to endexine formation.

The tapetum in *Arum alpinum* is of the amoeboid type. The walls of the tapetum cells start to degenerate at the late
PMC stage, completing degeneration at the tetrad stage. Lysis starts at the plasmodesmata areas of radial cell walls followed by tangential cell walls. The tapetal cells in the vicinity of the pollen mother cells dissolve last. Next the cytoplasm of the tapetum invades the non-callosic space separating the four microspores (Fig. 2C and D). Most striking is the distribution of the organelles within the tapetal cytoplasm. In the vicinity of the microspores, endoplasmic reticulum (ER) strands are exclusively arranged (Fig. 2E and F).
Microspores and wall formation

Shortly after tetrad breakdown the plasma membrane of the tapetal syncytium retracts from the pollen surface and conical spaces are formed (Fig. 3A). The retracting plasma membrane is closely connected with tapetal ER strands (Fig. 3A, arrows). Vesicles from the tapetum fuse with the conical spaces (Fig. 3B, asterisk). In this way the polysaccharidic spines are formed.

Coincident with the formation of the spines, Ubisch-body like structures develop within the loculus at the inner anther wall (Fig. 3C). The pattern is determined by invaginations of the tapetal plasma membrane. The mode of formation, their shape, and chemical composition are identical to that of the spines. At the late microspore stage the wall is composed of a thick endexine with polysaccharidic spines and the formation of the intine starts (Fig. 4A and B). Later, the pollen grains enlarge and the surrounding tapetum dissolves.

Mature pollen grain

The pollen wall of mature pollen grains is composed of a continuous intine and a continuous endexine overspread with spines. The endexine is approximately as thick as the intine and has a spongy appearance (Fig. 4C). With tapetum breakdown an additional polysaccharidic layer appears and covers the whole pollen surface including the spines (Fig. 4C, arrow; and D). In hydrated condition, mature pollen grains are spheroidal without defined apertures (Fig. 4E). Pollen maturation completes in a three-celled pollen grain (Fig. 4F).
DISCUSSION

The pollen grains of *Arum alpinum* are characterized by a very special pollen wall-type. The outermost pollen wall layer is formed by a thick endexine, which is covered by polysaccharidic spines (Weber *et al.*, 1999). This special type of pollen wall is the result of a unique development, which differs in several aspects from conventional pollen-wall development: no callose, no primexine and no ektexine.

**FIG. 4.** Images taken using TEM (A–C), SEM (D and E) or LM (F). (A) Cross-section of pollen grain showing the beginning of intine formation. The asterisk indicates protein-crystal, U + Pb. (B) Detail of (A). The arrow indicates the intine (I). U + Pb. (C) Mature pollen wall with polysaccharidic spines (asterisks) and an additional polysaccharidic layer (arrow). Modified Thiéry-test. (D) Spiny pollen wall. (E) Hydrated pollen grain. (F) Pollen showing vegetative nucleus and two sperm nuclei (cells). Acetic-carmine staining. Abbreviations: E, endexine; I, intine; N, nucleus; Sp, spines; V, vacuole.
Pollen-wall formation in *Arum alpinum* does not follow the usual angiosperm pattern. The most striking feature is the lack of callose at any stage of microspore ontogeny. These results contrast with the observations in *Arum italicum* (Pacini and Juniper, 1983), where a thin callose layer during the tetrad stage is reported. In the tetrad of *Arum alpinum* a thin layer between the microspores is visible, too. This layer looks like callose, depending on staining methods. It appears structured after U + Pb-staining, electron transparent after the modified Thiéry test, and shows no fluorescence after aniline-blue staining.

There are three major consequences caused by the lack of callose: formation of the pollen grain wall; microspore separation during the late PMC and tetrad stage; and microspore release from tetrad.

Vijayaraghavan and Shukla (1977) report that the absence of callose in *Pergularia daemia* (Asclepiadaceae) results in a poor exine deposition, but does not affect pollen fertility. The exine is irregularly thin and sparsely deposited. In some aquatic plants callose absence results in a modification of the exine. The seagrasses *Amphibolis griffithii* and *Amphibolis antarctica* (McConchie et al., 1982; Pettitt et al., 1984) are two of only a few species of angiosperms with filiform pollen apparently lacking an exine layer.

An exception that proves the rule is *Pandanus odoratissimus*. In *Pandanus* the absence of callose at any stage of microsporogenesis does not affect pollen-wall formation. The result is a perfectly formed wall, including ektexine, endexine and intine (Periasami and Amalathas, 1991).

In *Arum alpinum* we do not confirm the presence of callose. The outermost pollen wall layer of the mature pollen grain is formed by a thick endexine. An ektexine is missing but without affecting fertility.

Microspore separation within the pollen mother cells and tetrad as well as microspore release from the tetrad is conventionally associated with the presence of callose (Blackmore and Crane, 1988; Prakash et al., 1992; Rhee and Somerville, 1998). Lack of callose requires another mode of separation. In *Arum alpinum* the non-callosic space takes over microspore separation and the tapetum is responsible for microspore release into the loculus by invading the tetrad. The amoeboid type of the tapetum is in accordance with other Araceae (Pacini and Juniper, 1983; Pacini et al., 1985; Grayum, 1991; Weber et al., 1998).

Another remarkable pattern in *Arum alpinum* is the presence of polysaccharidic wall ornamentations, in the form of spines. These spines are formed at the late microspore stage and do not resist acetolysis treatment. They are formed by the tapetum in the same way as reported for *Saurornatum* (Weber et al., 1998). So far, polysaccharidic spines are unique to the Araceae (Pacini and Juniper, 1983; Weber et al., 1999).

**LITERATURE CITED**


