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The unique features and ultrastructure of the *Vinca minor* L. intine

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ABSTRACT

Cytochemical techniques, LM, SEM and TEM have been used to characterize the structure and nature of the *Vinca minor* intine. Mature pollen grains of angiosperms are usually characterized by a thick intine in the aperture areas, whilst in the interapertural areas the intine is thin. *V. minor* pollen is organized unusually and is an exception to this rule. It is 3-colporate, with a psilate surface and interapertural depressions in the center of each mesocolpia. The intine is thinner in the areas of apertures and interapertural depressions. The sporoderm has special paired thickenings, one on both sides of each aperture. These thickenings are formed by the intine and traversed by tubular protein-containing components. The intine thickenings with protein inclusions in the interapertural areas have been reported for *Vinca rosea* and were interpreted as the sites of pollen tube formation. Our experiments on germination of *V. minor* pollen have shown that pollen tubes are always formed in the apertural areas. These sites of pollen tubes formation are marked by β-glucans associated with the inner intine layer of apertures (Calcofluor White positive). The accumulation of protein components does not always indicate the germination zone.

1. Introduction

The angiosperm pollen wall usually consists of an outer exine layer and an inner intine layer (Erdtman, 1952; Knox and Heslop-Harrison, 1970; Heslop-Harrison, 1979, 1987; Pacini and Franchi, 1999). In most aperturate pollen the wall has one or more apertures—potential sites for the pollen tube emergence. Apertures are regions where the exine is fragmentary or absent, and the intine is usually thickened (Knox and Heslop-Harrison, 1970; Heslop-Harrison, 1979; Blackmore and Barnes, 1986; Heslop-Harrison and Heslop-Harrison, 1991). The intine is usually stratified into layers delimited structurally, chemically, and functionally. In species with aperturate pollen, three layers (outer, middle and inner) are usually well defined in the apertural areas, but they are compact in the interapertural areas, and the middle layer may be absent or much reduced (Heslop-Harrison, 1987). Structural variations of intine organization among genera are due to the fact that the intine tends to conform to the exine architecture, and to numerous additional morphological and physiological specializations associated with various secondary functions exist (Heslop-Harrison, 1987; Heslop-Harrison and Heslop-Harrison, 1991; Li et al., 1994; Suárez-Cervera et al., 2002).

An outer homogeneous intine layer is thin, and formed by pectic microfibrils (exintine). It is usually thickened in the apertural areas. At the time of pollen dispersal gel-forming pectins are dehydrated and so sealing the aperture (Heslop-Harrison, 1979). As the pollen grain hydrates before germination, the pectins undergo gelation and the swelling gel opens germination aperture. Gel-swelling forces are required for pollen tube escape and, thus, must be focused at the site of pollen tube emergence (Edlund et al., 2004). Then this layer dissolves in apertures during germination (Knox and Heslop-Harrison, 1970; Southworth, 1973; Heslop-Harrison, 1979, 1987; Pacini and Juniper, 1979; Kress and Stone, 1982; Heslop-Harrison and Heslop-Harrison, 1991; Nepi et al., 1995). A middle intine layer carries glycoproteins, enzymes and other proteins embedded in a microfibrillar pectic matrix (middle intine). In inaperturate pollen proteins are held in the middle layer of the intine over the whole pollen perimeter. In aperturate pollen they are concentrated in the apertural regions in tubules or vesicles. These tubules are derived from the plasmalemma and subsequently become cut off from the microspore cytoplasm by selective membrane fusions. The proteins contained in such tubules or vesicles are named gametophytic because they are produced by the gametophyte itself and released during the initial hydration period as the outer pectic layers disperse. These proteins are involved in early nutrition of the pollen tube, the stigmatic papillae cuticle dissolution and the pollen-stigma recognition process (Knox and Heslop-Harrison, 1970, 1971; Southworth, 1973; Pacini and Juniper, 1979; Heslop-Harrison and Heslop-Harrison, 1985, 1991; Heslop-Harrison, 1987; Hess, 1993; Nepi et al., 1995; Pacini and Franchi, 1999; Nepi and Franchi, 2000; Saad-Limam et al., 2005; Eliseu and Dinis, 2008). An inner microfibrillar layer contains mixed cellulosic β-1,4-glucan and β-l,3-glucan like callose and amorphous pectic components (Heslop-Harrison, 1979, 1987; Heslop-Harrison and Heslop-Harrison, 1982; Nepi et al., 1995).

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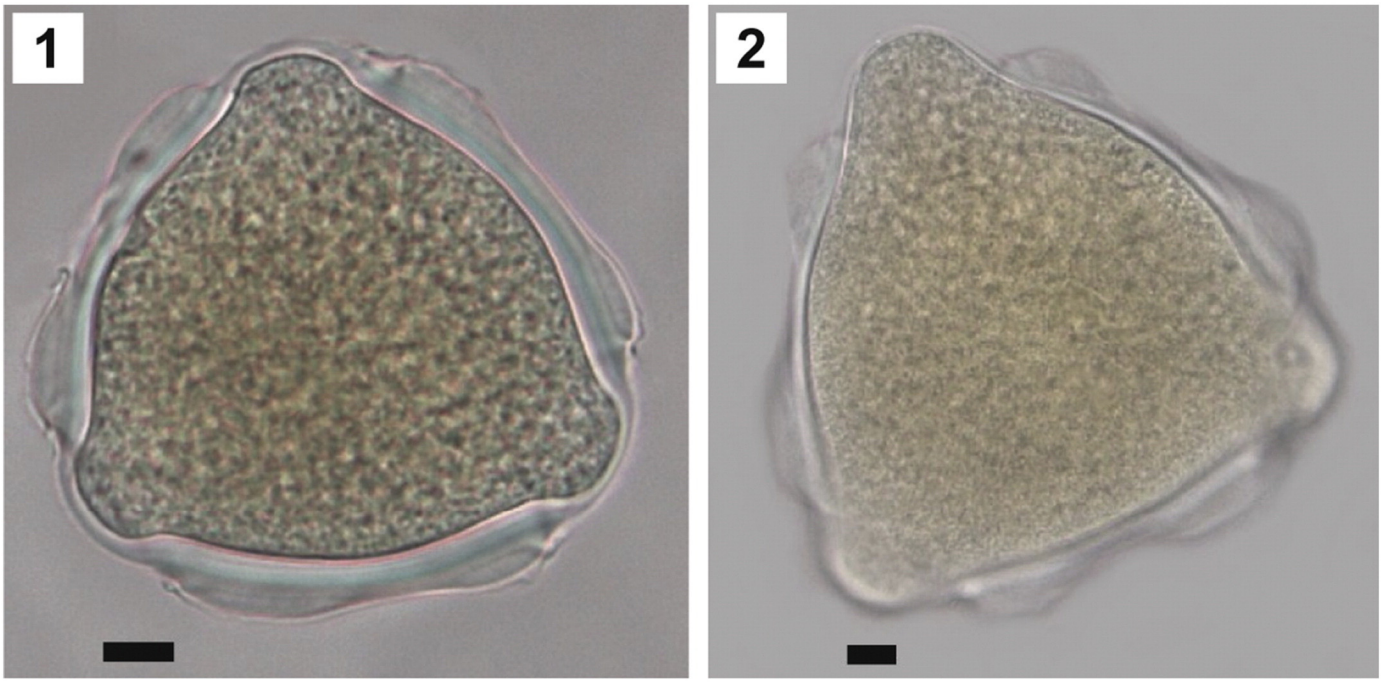

Plate I. Pollen grains of *Vinca minor* L. Light micrographs. 1, 2. Polar view. Scale bar 3 μm.

This layer is usually thickened in apertural areas and is the progenitor of the pollen tube wall (Suarez-Cervera et al., 1995).

Intine layers can be distinguished by staining ability (Larson, 1964). The outer layer often tests positive for pectic polysaccharides (ruthenium red positive), especially in the apertural region. The inner layer stains for β-glucans (calcofluor white positive) most strongly in apertural regions (Hughes and MacCully, 1975; Kress and Stone, 1982; Hughes; Keijzer, 1987).

The tubular/vesicular protein inclusions were found throughout the angiosperms within the apertural intine and sometimes they are even present in the interapertural intine (Knox and Heslop-Harrison, 1970; Kress and Stone, 1982; Kress, 1986; Hess, 1993). These proteins provide loosening of the intine fabric itself during hydration preparatory to the pollen tube emergence (Kress and Stone, 1982; Heslop-Harrison and Heslop-Harrison, 1991). Pressure of expanding intine breaks the exine and in inaperturate pollen ruptures it. It has been shown that pollen tube emergence in inaperturate *Heliconia* pollen is strictly associated with channeled intine layer and thus should be considered as cryptoaperturate (Kress and Stone, 1982; Kress, 1986). In aperturate pollen it is unnecessary to rupture the exine during germination, so, the primary function of the tubular intine is to expand the aperture. Thus, the tubular intine would indicate, together with the thickened inner intine layer, germination zones, i.e., apertures.

Structurally different intines have been noted in a number of groups (Suarez-Cervera et al., 1995; Eliseu and Dinis, 2008; Halbritter et al., 2010). Cousin (1979) investigated *Vinca rosea* pollen and concluded that it is characterized by a thick intine containing cytoplasmic channels in the interapertural areas, whereas in the apertural areas the intine is thin. The presence of tubular components in the intine thickening was the reason for Cousin (1979) to suggest the interapertural areas as the sites of the pollen tube emergence. Nevertheless, neither cytochemical studies nor investigation of the germination process of the *Vinca* pollen have been done. We present the first comprehensive study of pollen ultrastructure, cytochemical properties and germination experiments in a poorly known sporoderm type of *Vinca minor* L. This study aims to understand the nature of the aperture, the structure and function of the intine to get closer to germination process.

2. Material and methods

Pollen grains of *V. minor* have been examined using light (LM), scanning (SEM), and transmission (TEM) electron microscopy as well as by cytochemical methods. Fresh anthers were collected in the Botanical Garden of Moscow State University (MW).

2.1. Light microscopy

For light microscopy, pollen has been acetolysed (Erdtman, 1960) and mounted in glycerine jelly. Ten measurements of pollen grains were made. Measurements are mean values (with ranges in parentheses).

2.2. Electron microscopy

For scanning electron microscopy, pollen samples have been dehydrated through ethanol and acetone series, and critical-point-dried using a Hitachi HCP-2 critical point dryer. Dry specimens have been coated with gold and palladium using an Eiko IB-3 ion-coater (Tokyo, Japan) and observed under a JSM-6380LA SEM (JEOL, Tokyo, Japan) and a CamScan 4 DV (CamScan, UK).

For transmission electron microscopy, fresh material has been fixed in 2.5% glutaraldehyde buffered with phosphate buffer and postfixed with 1% OsO_4. Following dehydration in ethanol and acetone series, the material has been embedded in Epon. A Leica UC-5 ultramicrotome with a diamond knife has been used for making ultrathin sections. Sections have been stained with uranyl acetate and examined with a Jeol 1011 B microscope. The work has been performed at User Facilities Center of M. V. Lomonosov Moscow State University.

2.3. Cytochemical methods

Cytochemical methods have been used to examine the distribution of pectins and cellulose and callose in the walls of *V. minor* pollen grains. Pollen grains were stained with Calcofluor white—fluorochrome that binds with β-glucans contained in the intine layer (Hughes and