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Structure and Macromolecular Composition of the Seed Coat of the Musaceae

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Mature seed coats of representatives of all three genera of Musaceae were analysed for macromolecular composition with various mass spectrometric techniques and compared with scanning electron microscopy and light microscopy in combination with histochemical techniques. Mass spectrometric techniques are more sensitive and more specific in identifying macromolecular compounds than histochemical methods. The macromolecular ‘fingerprint’ of the seed coats of Musaceae showed unique components of aromatic phenols. The seed coat structure of all three genera is homogeneous within the Musaceae. It is characteristic at the family level and most complex within the Zingiberales. Very remarkable are the separation of the outer cell walls from the exotestal layer, exposing a secondary surface with silica crystals, and the relatively thick mesotesta which protects the seed, e.g. against the biting forces and passage through the digestive tracts of dispersing agents. Germination takes place with an operculum and is facilitated by a predetermined rupture layer in the micropylar collar. The Musaceae seed presents a good example of the solution of conflicting demands of protection and germination.

Key words: Musaceae, Musa, Ensete, Musella, seed coat, pyrolysis (gas chromatography) mass spectrometry, histochemistry, anatomy, macromolecules, silica, lignin, cellulose, vegetable polyphenols, operculum, germination.

INTRODUCTION

Hardly anything is known about the chemistry of seed coats. The biochemical and histochemical literature on seeds is almost fully focused on the composition of storage material in embryo and endosperm. The functional interpretation of the seed coat is an almost unexplored field of research, because of the lack of reliable data on the presence and distribution of macromolecular components. Currently it is possible to describe the anatomy in more detail with microscopical and histochemical techniques and to study the macromolecular composition of the testal layers of the mature seed with mass spectrometrical techniques.

In source pyrolysis mass spectrometry (PyMS) is a relative simple method to obtain information from plant tissue at the molecular level (Boon, 1989). The PyMS analysis provides a chemical ‘fingerprint’ of the seed coat. Chemical identification of individual pyrolysis products is performed by using pyrolysis gas chromatography (PyGC) combined with mass spectrometry (PyGCMS).

The order Zingiberales is a well-defined and coherent taxon. Within this order there are questions about the relation of seed coat structure and chemistry to germination. The Musaceae represents the most basal lineage in the order (Manchester and Kress, 1993). This basal position of the Musaceae, its special type of germination and its well-differentiated seed coat forms an interesting subject to study the relation of structure, macromolecular composition and functions of the testal layers, and moreover to produce a new data set relevant for macrosystematic research.

In most recent classifications the family of the Musaceae, to which the bananas belong, there are three genera: Musa L., with about 35 species, Ensete Bruce., with three species and a more obscure genus Musella (Fr.) C. Y. Wu with one species. The acceptance of the Musella as a distinct genus is only provisional (Manchester and Kress, 1993). A number of genera formerly mostly included in the Musaceae are nowadays placed in the separated families Strelitziaceae, Heliconiaceae and Lowiaceae (Nakai, 1941; Tomlinson, 1962; Cronquist, 1981; Dahlgren and Rasmussen, 1983). Each taxon possesses a comparable range of anatomical variation which overlaps to a minimum with other groups within the Zingiberales (Tomlinson, 1962). The family is tropical-subtropical Asian (Musa, Musella) and African (Ensete), but Musa-cultivars are nowadays cultivated throughout the tropical regions of the world.

Most edible cultivated clones are seedless whereas other plants, wild or cultivated, produce one to many seeds. Breeding programs are hindered by the low seed set, as well as by slow and non-uniform germination (Shepherd, 1954; Stotzky, Cox and Goose, 1962). The ovary of Musa is three locular, contains numerous ovules, and develops into a longish berry. The fruit of Musa contains a higher number of seeds than that of Ensete. The seeds rarely attain 1 cm in diameter, whereas the seeds of Ensete are mostly larger, up to 1.8 cm, although there may be some overlap in size. The shape of the seeds varies greatly, is often irregularly and sharply angular, either rarely globose or smooth. The variability in shape of the pale or dark brown to black seeds is due to compression between neighbouring seeds (Simmonds, 1960). The embryological literature has been summarized by Davis (1966), that on seed anatomy by...
Netolitzky (1926) and Takhtajan (1985). In earlier studies McGahan (1961a) described the anatomy of the seed of *Musa balbisiana* and Bouharmont (1963) the development of the ovule to mature seed in *Musa acuminate*.

The ovule is anatropous, bitegmic, and crassinucellar, with a multi-layered outer integument and a thin inner integument. The micropylar part of the seed coat develops into an operculum or seed lid. This operculum is surrounded by a characteristic micropylar collar. During germination the seed lid is displaced by the elongating radical-hypocotyl axis (McGahan, 1961b). The mature seed contains mainly endosperm, which is copious, starchy and mealy, with large, eccentric, compound starch grains, and some perisperm (Cronquist, 1981). The endosperm is initially nuclear, becoming cellular later, and extends during its development at the expense of the perisperm. The embryo remains relatively small, is situated under the operculum and is partly enclosed by the micropylar collar. In the ovular stage a trichomatous aril develops out of the funicle surrounding the seed coat (Netolitzky, 1926; Friedrich and Strauch, 1975). According to Friedrich and Strauch (1975) the aril becomes reduced and only some small vestiges can be found near the funicle on the mature seed.

Seeds of *Ensete* can be distinguished from those of *Musa* by a rimmed hilar depression that is nearly as wide as the seed itself. Seeds of *Musella* resemble those of *Musa*, being distinguished from *Ensete* by the lack of a wide hilar depression and rim (Manchester and Kress, 1993). Most species of *Musa* have a rough verrucate seed coat, whereas the seed coat of *Musella* and *Ensete* is smooth.

In this study data from scanning electron microscopy (SEM), light microscopy (LM), histochemistry and mass spectrometry are compared, to acquire insight into the functions and chemical composition of specialized testal layers.

**MATERIALS AND METHODS**

**Plant material**


**Germination**

Seeds of *Musa velutina* and *Musa roacea* were soaked in water for 24 h, and sown in petri dishes on wet filter paper and in soil under greenhouse conditions.

**Sample preparation for mass spectrometry**

Samples of seed coats of Musaceae were collected and homogenized in a few drops of water in a small glass mortar with a glass pestle. The homogenized seed coat was extracted with a hexane/dichloromethane mixture (1:1 v/v), after 3 h it was centrifuged, the pellet was suspended in deionized water, dried and extracted with an ethanol/aceton mixture (1:1 v/v) for another 3 h, and finally washed several times in deionized water. The homogenized and extracted seed coat material was suspended in a few drops of water.

**Transmethylation**

For methylation of phenolic hydroxyl and carboxyl groups, a small droplet of 2-5% (w/v) tetramethylammonium (TMAH) was added to the wet sample on the wire of the direct insertion probe prior to PyMS and PyGCMS experiments.

**Silylation**

An off-line pyrolysate was prepared by placing a few drops of an aqueous suspension of extracted seed coat on a wire with a Curie point of 510°C. The wire was dried under rotation in vacuum. After drying the wire was placed in a glass liner and the glass liner was flushed for a minute with argon, directly followed by pyrolysis at atmospheric pressure. The procedure was repeated several times with a new wire in the same glass liner and the final condensate that formed on the inner wall of the glass liner was collected.

Silylation of the off-line pyrolysate was performed by adding 70 µl of BSTFA/TMSCL [N.O.bis(TMS) trifluoroamide/trimethylchlorosilane] in 235 µl pyridine to the sample in the dark. After 24 h the pyridine was evaporated by flushing with nitrogen and the reaction product was resuspended in dichloromethane and analysed with GCMS.

**Acid transesterification**

For hydrolysis a few milligrams of the extracted seed coat were placed in a tube with a screw top and 12 µl sulphuric acid (approx. 2 ml) was added. The tube was flushed with nitrogen before sealing and the sample was incubated at room temperature for 3 h. The acid was diluted to 1 µl and the sample was further hydrolysed at 100°C overnight. The sample was then centrifuged and the residue was hydrolysed again with 0.25 µl sulphuric acid. The tube was flushed with nitrogen and incubated at 100°C overnight. The solid residue was separated from the liquid hydrolysate by centrifugation and washed several times with deionized water and dried overnight over phosphorus pentoxide in a vacuum dessicator at 50°C (Pastorava, Oudemans and Boon, 1993).

Ethyl esters were prepared by using approx. 1 µg of the extracted sample in a plastic tube with a screw top with a 20 µl H₂SO₄/ethanol solution (1:25 µl H₂SO₄ in 1 ml ethanol). The tube was sealed with a Teflon tape and incubated at 100°C for 4 h.

**Direct temperature resolved or pyrolysis mass spectrometry**

A small drop of a suspension of the unextracted or homogenized and extracted seed coat was placed on a
Fig. 1. A, Mature seed of Musa balbisiana (× 10). B, Musa sp. showing, partly removed, the thin brittle remains of the aril (a), the outer epidermis (oe) of the outer integument and crystalline silica bodies (si). Irregular radial divisions arise at the outer layer of the mesotesta (arrowhead), which gives the seed a rough appearance in some species (× 90). C–E, Musa balbisiana; The mesotesta (me) has developed into 20–25 layers of sclerotic cells. C, The cells of the four to five uppermost layers are more thickened and less elongated (× 140). D, Backscatter image showing the silica bodies as a thin layer on top of the mesotesta (× 135). E, Sclerotic cells with pits at the lower part of the mesotesta (× 1010). F, The inner epidermis of the testa develops into an endotesta (en) consisting of U-shaped thickened cells. The tegmen (te) consists of two layers of tangentially elongated cells. A thick cuticle bounds the inner surface of the tegmen and has a tegmic and nucellar origin (Musa balbisiana) (× 610). G, In the mature seeds the perisperm (pe) is completely compressed and only the cell walls remain (Musa mannii) (× 1380). H, The tegmic cells are filled with a spongy like structure (Musa balbisiana) (× 2690).
Fig. 2. For legend see facing page.
Pt/Rh (9:1) wire of the insertion probe for in-source pyrolysis mass spectrometry (PyMS) on a JEOL DX-303 double focusing mass spectrometer. The wire was inserted into the evacuated ion source, after drying the sample. The ion source temperature was 180 °C. The wire was resistively heated at 16 °C s⁻¹ to a final temperature of 800 °C. Compounds, formed upon desorption pyrolysis, were ionised by 16 eV electron impact (EI) or ammonia chemical-ionization (CI) PyMS. The scan cycle time was 1 s and the selected mass range was m/z 20–1000.

Curie-point pyrolysis gas chromatography mass spectrometry

Curie-point pyrolysis gas chromatography mass spectrometry under EI conditions was conducted on a Finnigan INCOX 50 quadrupole mass spectrometer connected to a HP 5890 series II gas chromatograph. About 5 μg of a suspension of a sample was applied to a ferromagnetic wire with a Curie-point temperature of 610 °C. The pyrolysis products were flushed towards a 25 m CPSILS-5 CB fused silica capillary column (i.d. 0.32 mm; film thickness 1.2 μm) using He as carrier gas (flow 30 cm s⁻¹). The GC oven was kept at 30 °C during pyrolysis and was subsequently programmed to 320 °C at a rate of 6 °C min⁻¹. The compounds were ionised at 70 eV electron impact energy. The scan cycle time was 0.5 s with a selected mass range of m/z 20–500.

On column injection gas chromatography mass spectrometry

On column injection gas chromatography mass spectrometry (GCMS) was performed using a HRGC MEGA 2 series Fisons instruments gas chromatograph which was connected to a Jeol DX-303 double focusing mass spectrometer. The column used was a 25 m SIMDIST (i.d. 0.32 mm; film thickness 0.15 μm). Helium was used as carrier gas (flow 30 cm s⁻¹). The GC oven was kept at 30 °C and subsequently heated to 375 °C at a rate of 6 °C min⁻¹. Compounds were ionized at 70 eV electron impact energy, at a source temperature of 180 °C. The scan cycle time was 0.8 s over a mass range of m/z 20–500.

Scanning electron microscopy

Seeds were sectioned transversely and fixed in FAA (formalin, ethanol, acetic acid), dehydrated in acetone, incubated in tetramethylsilane and air dried (Dey et al., 1989). The seeds were coated with a thin layer of gold/palladium and examined with an ISI-DS130 Dual Stage electron microscope (9 kV) fitted with an ISI-Robinson Detector RBSE 130R (19 kV) for backscatter imaging.

Mature seeds of Musa balbisiana, Musa paradisiaca, Musa textilis and Musa velutina were examined for the presence and localization of silica in the seed coat. The seeds were sectioned transversely, fixed on aluminium stubs cemented with carbon cement and air dried under low relative humidity at room temperature. The specimens were carbon coated and examined in a Cambridge Stereoscan 150 (at 20 kV) fitted with a backscatter detector (KE Developments, Cambridge, UK) and a LINK energy-dispersive X-ray analyser [Department of Molecular Cell Biology (EMSA) of the University of Utrecht, The Netherlands]. The presence of silica was detected with the help of the diagrams of the X-ray spectra. The specific peak (keV, 1.74) for silica in the spectrum was isolated and the spectrum pulses were simultaneous with the scanning of the sample carried back so that an element distribution image of silica could be created (X-ray mapping).

Light microscopy and histochemistry

Hand-cut sections were stained for vegetable polyphenols (‘tannins’) with ferric chloride, vanillin/HCl and the nitroso reaction, for lipids with Magdala red, Nile blue sulphate method, for cuticle (neutral fats and fatty acids) with Sudan IV, for ‘cutin’ with azure B method and auramine O method, for suberin with phosphate buffer method and cyaan method, for lignin with sodium hypochlorite/HCl, chlorogluconic/HCl and lignin pink, for callose with soda method, Corallin and aniline blue, for pectin with ruthenium red, and for cellulose with chlor-zine-iodide (Gahan, 1984; Krisnamurthy, 1988). Sections for staining with ruthenium red and for the cyanin/potassium method were also pretreated with Eau de Javelle (potassium hypochlorite).

Samples for semi-thin sectioning were fixed in FAA or Craf III, or were softened in 10% ammonia (1 h at 50 °C) or a mix of glycerol and Aerosol OT in water (Schmid and Turner, 1977; Setoguchi, Tobe and Ohba, 1992), dehydrated in n-butyl alcohol series and embedded in glycerol methacrylate polymer (Feder and O’Brien, 1968).

RESULTS

Mature seed coat anatomy and histochemistry

In mature seed coats the cells are difficult to interpret with light microscopy due to the extreme hardness of the tissue. This has hampered the observations on the mature seed coat of Musa balbisiana with partly released epidermis and remains of the aril (x 165). J, Backscatter image of released epidermal wall of Musa balbisiana and silica bodies which are situated on the inner periclinal wall (x 300). K–L, Backscatter and bright dot mapping view on the inner side of the separated epidermal wall, showing the broken tips of the silica bodies and the silica encrusted walls and membrane of the exotesta of Musa balbisiana (K, L × 630).
coats of Musaceae. Softening of the seeds facilitates sectioning of the material, but it releases some of the components in the seed coat (e.g. silica). Most stains used were negative or aspecific.

The seed coat anatomy of all three genera in Musaceae was practically homogeneous therefore the structure of the *Musa* sp. only is described here.

During the development of the seed the aril jellifies and dries out. In the mature seed (Fig. 1A) the remains of the aril adhere closely to the seed coat (Fig. 1B). The aril surrounds the ripe seed partly as a thin brittle layer and, if not released, swells up during germination.

The cells of the outer epidermis of the outer integument are radially stretched and vary in length (65–190 µm). The epidermis is covered with a thin cuticle which stains positively with sudan IV, azure B and auramine O. This indicates 'cutin' and fatty acids in the cuticle. Each epidermal cell contains a multi-angular crystalline silica body, which is situated against the inner periclinal wall (Figs 1D, 2A, G–J). The walls of the epidermal cells remain relatively thin and are encrusted with a net-like silica structure. The outer periclinal and radial walls easily separate from the seed coat (Fig. 2G, J). During the release, the tips of the silica bodies may break off and stick to the membrane (Fig. 2B–C, J–L). The main part of the silica body remains attached to the seed coat after release of the epidermal wall layer (Fig. 2A, D–F).

The middle layers of the outer integument have developed into 20–25 layers (180–350 µm) of sclerotic cells (Fig. 1C–E). These vary greatly in shape (length up to 300 µm; width about 10 µm), size and orientation. The secondary cell wall contains phenolic compounds in the S2 layer, which gave a very weak response with the nitroso reaction and the vanillin/HCl test. These compounds increase in relative amount from the inner to the outer side of the mesotesta. The phenolic compounds are more concentrated in the four to five uppermost cell layers of which the cell walls are more thickened and the cells are less elongated. The histochemical tests on lignin were mostly negative, only in very thin sections a light pink colouration could be seen in the S1 layer of the secondary wall, using lignin pink.

In some species locally irregular periclinal divisions lead to protrusions on the outer layer of the mesotesta, which are the cause of a rough surface on the seed of most *Musa* species (Fig. 1A–B). The innermost layer of the outer integument develops into an endotesta consisting of U-shaped thickened cells (Fig. 1F). Testa and tegmen are not separated by a distinct cuticular layer. The tegmen consists of two layers of longitudinally elongated cells (Fig. 1F–G). The inner wall of the outer layer and the inner and outer

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**Fig. 3.** Schematic drawing of a longitudinal section of the *Musa* seed coat (×30). ab, abscission layer; cd, chalazal disk; em, embryo; end, endosperm; hi, hilum; ii, inner integument; mc, micropylar collar; mi, micropyle; nu, nucellar tissue or perisperm; oi, outer integument; op, operculum; pt, parenchymatic tissue between chalaza and nucellus; se, silicified exotesta.

**Fig. 4.** A. An abscission layer (ab) has developed through the outer integument and delimitates the operculum (op) (*Musa balbisiana*) (×40). B. The tegmen and nucellus (nu) rupture just beneath the abscission layer (*Musa sp.*) (×100). C–D. The operculum consists of three distinct layers. The raphal zone is followed by a parenchymatic tannin zone and a sclerenchymatic zone (*Musa sp.*) (C ×60, D ×30). E. The silicified vascular bundle ends in the chalazal disk (cd) consisting of thin walled cells (*Musa paradisiaca*) (×55). F. The powdery endosperm (end) forms a thick layer along the inner side of the seed (*Musa balbisiana*) (×705).
Fig. 4. For legend see facing page.
walls of the innermost layer are thickened. The tegmic cells are filled with a spongy-like structure of unknown composition (Fig. 1H). A thick cuticle, which strongly stains with Sudan IV and Nile blue, is grafted to the inner surface of the tegmen. This cuticle partly originates from the inner layer of the inner integument and partly has a nucellar origin (Fig. 1F–G).

In the mature seed the perisperm is completely compressed and only the cell walls remain (Fig. 1G). The powdery endosperm (Fig. 4F) of the mature seed forms a thick layer along the inner side of the seed and leaves a cavity in the middle of the seed (Fig. 3).

At the micropylar side a typical micropylar collar is formed by a local ingrowth of the outer integument into the inner integument and the nucellus (Figs 3 and 4A). An abscission layer continuous with the micropylar collar has developed through the outer integument and envelops the operculum (Fig. 4A). The operculum (width 1–1.5 mm) is mainly formed by the exostomal part at the outer integument and hilar/raphal tissue (Fig. 4D). The operculum consists of three distinct zones (Fig. 4B–D). The raphal top zone is followed by a parenchymatic ‘tannin’ zone and a sclerenchymatic zone. The hilum and micropyle are situated at the top of the operculum (Fig. 3). The silicified vascular bundle is widely branched through the top zone of the operculum, running through the raphal mesotesta and ending in the chalazal disk (Figs 3 and 4E). During germination the operculum is easily pushed out by the embryonic root, due to the abscission layer. Removing the operculum does not result in faster or higher percentage of germination.

**Table 1. List of a number of compound classes and the pyrolysis mass spectrometric and PyGCMS techniques by which they can detected**

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**Fig. 5.** Pyrolysis (EI) mass spectrum of the non-extracted seed coat of *Musa velutina* showing hydroxymethoxybenzyl cation (m/z 137) as major fragment ion. ○, guaiacyl lignin; ●, syringyl lignin; ▲, polysaccharides; +, lipids; ★: Musaceae characteristics; f, ferulic acid; pc, p-coumaric acid; t, vegetable polyphenol markers.
Fig. 6. Lipid fraction of pyrolysis (EI) mass spectrum of the total seed coat of *Musa velutina*. +, di- and triglycerides; \( \checkmark \), waxes.

tegmen and nucellus rupture also just beneath the abscission layer in the outer integument (Fig. 4B–C). The epidermal cells at the nucellar apex are radially elongated and fill the bottom of the micropylar channel (Fig. 3).

The inner and outer integument are discontinuous at the chalazal side. At this place a central disk has developed consisting of thin-walled cells (Fig. 4E). In the developing seed these cells are filled with small reddish brown vacuoles which fuse later on to a single vacuole. According to White (1928), McGahan (1961a) and Grootjen (1981, unpubl. res.) those cells are filled with a gelatinous substance. The contents become brittle in the mature seed. On top of the
syringyl lignin, indicated by 272, 328, 340, 358 of guaiacyl lignin. A low abundance of furthermore the spectrum yields peaks at 194, 196, 208 and 210, is present in the mass spectrum. 164, 166, 178, 180 and pyrolysis products of dimers at (e.g. and as shown in Fig. 6, diglyceridic fragments of triglycerides (e.g. monomeric pyrolysis products at extracted seed coat sample of Musaceae can be seen as the tmah-(ammonia)ci-gms (not shown), indicating the acids is confirmed with py-tmah-ei-gcms and py-marks of several compound classes as guaiacyl lignin (g), syringyl lignin (s), diglycerides (dg) and waxes (wx). chalazal disk, between the insertion of the inner integument, a parenchymatic tissue is situated. Figure 5 shows the overall spectrum of the non-extracted seed coat of Musa. This spectrum contains markers of a volatile fraction and a non-extractable part of the polymer system. All the three genera of the Musaceae gave the same characteristic chemical ‘fingerprint’ of their seed coat. A lipid fraction of fatty acids (m/z 256, c16:0; 262, c18:0[M-H2O]; 264, c18:1[M-H2O]; 280, c18:2; 282, c18:1; 284, c18:0), and as shown in Fig. 6, diglyceridic fragments of triglycerides (e.g. m/z 550, c16:16 [M-H2O]; 576, c16:18 [M-H2O]; 602, c16:18 [M-H2O]), triglycerides (e.g. m/z 830, c16:16:16 [M-H2O]; 857, c16:18:16 [M-H2O]; 882, c18:16:16 [M-H2O]) and wax esters (m/z 592+n.28), are thermally desorbed in an early stage of the temperature ramp (Fig. 7), whereas the biopolymeric materials are pyrolysed at higher temperatures. The presence of c16 and c18 hydroxy fatty acids is confirmed with py-tmah-ei-gcms and py-tmah-(ammonia)ci-gms (not shown), indicating the presence of cutin (boon, unpubl. res.). Evidence for lignin in the mass spectrum of the non-extracted seed coat sample of Musaceae can be seen as the monomeric pyrolysis products at m/z 124, 138, 150, 152, 164, 166, 178, 180 and pyrolysis products of dimers at m/z 272, 328, 340, 358 of guaiacyl lignin. A low abundance of syringyl lignin, indicated by m/z 154, 167, 168, 180, 182, 194, 196, 208 and 210, is present in the mass spectrum. Furthermore the spectrum yields peaks at m/z 137, 200, 338, 356, 374 which are prominent for the Musaceae seed coats. The lignin pyrolysis products and the prominent peaks for the Musaceae are not extractable and are part of the polymer system of the Musaceae seed coat, as shown in Fig. 8. The presence of these compounds is also confirmed by PyGCMS as shown in Fig. 9 and Table 2. The spectra of the extracted and non-extracted seed coat show also pyrolysis products indicating the presence of polysaccharides as shown by m/z 43, 57, 60, 73, 126 and 144 for hexose sugars and m/z 58, 85 and 114 for pentose sugars (pouwels, eijkel and boon, 1989). The presence of polysaccharides is confirmed by ammonia chemical ionization. Figure 10 shows the pyrolysis ammonia chemical ionization mass spectrum of the extracted seed coat of Musa. This spectrum contains mass peaks for ammoniated hexose sugar monomers (m/z 134, 144, 162, 180, 222) which are prominent for cellulose and ammoniated pentose sugar monomers (m/z 132, 150) and dimers (m/z 264, 282), which are prominent for hemi-cellulose (pouwels and boon, 1990). The abundance of dimeric and trimeric sugars is low due to the presence of anorganic matter as indicated by the relative high m/z 134 (scheijen and boon, 1989). The ammonia CI data also confirm the presence of guaiacyl (m/z 163) and syringyl (m/z 193) lignin (hage, mulder and boon, 1993; hage, weeding and boon, 1995). In the mass spectrum (Figs 5, 8) of the extracted and non-extracted seed coat samples mass peaks are observed for phenolic compounds (m/z 94, 110, 124), which amongst others can indicate the presence of vegetable polyphenols. These compounds are also components of the polymeric system and appear at high temperatures in the mass chromatogram as shown in Fig. 7. The presence of these compounds is confirmed with Py (EI) GCMS. Monocotyledonous plants usually have relatively high m/z 120 and 150 for p-coumaric acid and ferulic acids either present ester bound to the polysaccharide fraction or ether bound to the true lignin structure (boon, 1989). Also the extracted seed coat of the monocotyledonous Musaceae contains relative high amounts of these phenolic acids. The Py-El-MS (Fig. 8) shows peaks indicating ester bound (m/z 120) and ether bound (m/z 164) p-coumaric acid, and ester bound (m/z 150) and ether bound (m/z 194) ferulic acid. Also the ammonia CI spectrum indicates the presence of p-coumaric acid and ferulic acid as indicated by the pseudomolecular ions m/z 121 (vinylphenol) and m/z 151 (vinylguaiaicol). The PyGCMS of the TMAH treated samples of the seed coat confirm the presence of these compounds as shown in Fig. 11. In this figure the mass chromatograms of the masses m/z 192 and m/z 222 indicate p-coumaric acid and ferulic acid, respectively (mulder, hage and boon, 1992). The Musaceae seed coat contains a number of characteristic compounds. The Py-El-MS shows a peak at m/z 194, which is still present after extraction. This peak is identified with Py-El-GCMS as a caustic secondary metabolite zingirone. However, the most remarkable peaks are aromatic compounds which all have the fragment ion m/z 137 (hydroxymethoxybenzyl cation). Additional mass spectrometric techniques did not provide sufficient information to identify these compounds.

![Figure 7](image-url)

**Figure 7.** Total ion current and mass chromatograms of the total seed coat of *Musa velutina.* The ions of the mass chromatogram comprise markers of several compound classes as guaiacyl lignin (G), syringyl lignin (S), diglycerides (Dg) and waxes (Wx).
DISCUSSION

The main structure of the seed coats of the Musaceae taxa that have been examined are similar. As mentioned before, vestiges of the aril are still present on the mature seed, although it is easily released, especially when the aril and exotesta cells are dried out and compressed. Musaceae have fleshy fruits and are predominantly zoochorously dispersed. It seems that the aril has lost its function during evolution and probably the fruit has taken over the nutritive function for the dispersal of the seeds by animals (Friedrich and Strauch, 1975).

Very characteristic in Musaceae is the separation of the outer cell wall layer from the silicified exotesta. The silica on
the exposed surface of the mature seed coat of the Musaceae may function as a mechanical barrier against environmental forces, pathogens, chewing insects, other predators, and passing alimentary tracts of dispersers. In combination with vegetable polyphenols, which are located more to the inner side of the seed coat, in the mesotesta and in the tegmen, it can be a sufficient barrier against fungi. In other monocotyledons with silicified seed coats more often the silica is located in the endotesta. In Commelinaceae the siliciferous endotestal layer splits along its radial walls as a result of which the exo- and mesotestal layers of the seed become separated. Netolitzky (1926) mentioned the occurrence of siliciferous endotestas in Commelinaceae, Zingiberaceae and of a siliciferous exotesta in Musaceae, and suggested a protection of embryo and endosperm as one of the main functions.

The silica layer may help to prevent the seed coat from oppression and shrinking during germination. The role of silica in the cell wall seems to be analogous to that of lignin as a compression resisting element, i.e. more rigid replacement for the water between the microfibrils and other carbohydrate components of the wall of non-lignified cells (Raven, 1983). Furthermore, the silica can also restrict water loss by hydration of the silica gel (Kaufman et al., 1981; Yoshida, Ohnishi and Kitagishi, 1962).

The thick cuticle at the innermost side of the seed coat may function as a last barrier to water diffusion in combination with outer layers. The tightly packed, thick walled cells of the mesotesta are probably impermeable for water by the presence of water repellent substances, such as lignin, deposited in the polymeric network of the cell walls. Also the unknown phenolic compounds may contribute to a tight polymeric network. The vegetable polyphenolic substance in the tegmen probably hampers the diffusion of oxygen through the seed coat (Guan, 1991). In addition, the other possible openings at the micropylar side and the chalazal side (chalazal disk) of the seed coat are blocked.

The germination of *Musa* is variable and relatively difficult under artificial conditions. Stotzky et al. (1962) scarified the lateral side of *Musa balbisiana* seeds, which raised the germination percentage up to 80% and shortened the time to germinate from 3–6 weeks to 6–10 d. Probably water entry and gas exchange are made possible by the interruption of the silica layer, cuticle, and mesotesta.

The hard seed coat of *Musa* leads to conflicting demands of protection and germination. Hard seed coats offer an efficient protection during maturation, dispersal and dormancy; however, it hampers germination, because the embryo needs strong forces to rupture the seed coat. Germination lids with predetermined rupture layers facilitate germination. In Musaceae germination takes place by squeezing out the operculum as a result of the elongating radical-hypocotyl axis, without rupturing the seed coat (McGahan, 1961b). Removal of the operculum of the
### Table 2. PyGCMS data on Musa velutina seed coat. Identified and unknown peaks from a chromatogram after extraction. From every peak the molecular ion is given (M⁺); PS, Polysaccharide; L, Lignin; Lp, Lipid; *, aromatic phenols of unknown structure with fragment ion m/z 137

<table>
<thead>
<tr>
<th>Peak no.</th>
<th>Compound</th>
<th>Origin</th>
<th>Scan</th>
<th>M⁺</th>
<th>Remarks</th>
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<td></td>
<td>120</td>
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<tr>
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<td>PS</td>
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<td>72</td>
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<tr>
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<td>2,3-butanedione</td>
<td>PS</td>
<td>226</td>
<td>86</td>
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<tr>
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<td>PS</td>
<td>252</td>
<td>82</td>
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<tr>
<td>6</td>
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<td>11</td>
<td>but-3-en-2-one</td>
<td>PS</td>
<td>491</td>
<td>84</td>
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<tr>
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<td>96</td>
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<td>L</td>
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<td>methyl 3-methyl cinnamate</td>
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<tr>
<td>57</td>
<td>phthalate</td>
<td></td>
<td>2750</td>
<td>(149)</td>
<td></td>
</tr>
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</table>
Musaceae seeds permitted water to reach the embryo, but did not result in a higher percentage of germination. This indicates that water entering the seed is unable to hydrate the embryo. So Musaceae also have an embryo imposed dormancy.

The mass spectrometric ‘fingerprint’ of the macromolecular composition of Musaceae shows some unique and characteristic mass peaks. Although no structure can be assigned, these mass peaks seem to be specific for Musaceae, and will be very useful as an additional data set for macrosystematic research of Angiosperm families (Graven, unpubl. res.). Further studies are in progress to obtain more information about the chemical structure of these unknown Musaceae components.

With the phloroglucinol/HCl test or other tests no lignin components could be detected, although the test with lignin pink gave the indication of the presence of lignin in the S1 layer of the secondary wall of the mesotesta. These findings are supported by the results of McGahan (1961a), who also obtained negative results for lignin when routine tests for lignin were used. Also Stover and Simmonds (1987) described the seed coat as not lignified. The PyMS fingerprint of Musaceae indicated the presence of guaiacyl and syringyl lignin components. This observation was confirmed with Py (EI) GCMS.

Harris and Hartley (1976) stated that cell walls containing ferulic acid bound to polysaccharides give a negative phloroglucinol/HCl test for lignin. The macromolecular data of Musaceae show that the seed coat indeed contains ferulic acid, but is also lignified. A combination of several components in the cell wall is probably the reason why most stains are negative.

Monocotyledonous plants usually have relatively high m/z 120 and 150 from p-coumaric and ferulic acids, either present ester bound to the polysaccharide fractions or ether bound in the true lignin structure (Boon, 1989). Ferulic acid bound to the primary wall has been recorded in nearly half of the monocotyledon families and in about 10 families of the dicotyledons only (Harris and Hartley, 1980).

Many studies have shown that phenolic acids are able to affect germination, but usually via the germination rate (time needed for germination) rather than via the total seed...
germination (Kuiters, 1990). Seeds of Musa velutina and Musa rosacea germinated relatively easily in 3–8 weeks. Within the Musa seed coat the phenolic acids seem to be genuine structural components of the cell walls, not affecting germination.

The use of histochemical methods for the detection of lipids shows the limitation of this approach (Gahan, 1984). There is no specific functional group in lipids for attachement of staining material. If a lipid is attached to other macromolecular components or even not attached but covered by other macromolecules, the result of a staining will be negative. The presence of the insoluble cutin polymer in the cuticle is not specifically detectable with histochemical methods. The PyMS and PyGCMS data still contained C_{16} and C_{18} fatty acids after extraction of lipids, which can indicate the presence of insoluble polymeric cutin. The
evidence for the presence of cutin hydroxy fatty acids was given by using methylated samples of the extracted seed coat using Py(EI)GCMS and Py(NH$_3$-CI)GCMS.

Waxes are also associated with the polymeric material in the cuticle to provide an effective diffusion barrier (Kolattukudy, Espelie and Soliday, 1981). This may explain the relatively high contribution of wax esters in the mass spectrum of the Musa seed coat. The cuticle between tegmen and the remains of the nucellus of the Musaceae have several functions as a last barrier against water loss, ion diffusion and several pathogens.

The mass spectrometric techniques are sensitive and specific methods in identifying macromolecular compounds. It is not always possible to locate the different compounds in the differentiated tissues of the seed coat, unless these can be separated from each other and analysed separately. The isolation of the different seed coat sections by hand is still the limiting factor. Histochemical methods are mainly useful in localising certain compounds. However, it has been shown that especially in mature seed coats the stains are not always successful in attaching a specific compound. Many pyrolysis products were identified using PyGCMS, confirming the general findings of the PyMS spectra.

Caustic products are widely distributed within the family Zingiberaceae. Members of the family Zingiberaceae have attracted continuous phytochemical interest due to their considerable importance as natural spices or as medicinal plants (Pandji et al., 1993). In literature a lot of rhizomes and seeds are described as tasting caustic (Hegnauer, 1962–1990). Taxa of Zingiberaceae have been studied for insecticidal activity (Grainge and Ahmed, 1988; Pandji et al., 1993). The identification of zingirone in the Musa seed coat confirms the relationship of the Musaceae with Zingiberaceae. Zingirone was first described in the rhizome of Zingiber officinale. The presence of products as zingirone in the seed coat of Musa is another defence line against specific chewing insects and other predators.

The thermal stability of condensed vegetable polyphenols is high. The occurrence of the components 1,2-dihydroxybenzene (m/z 110) and methyl-1,2-dihydroxybenzene (m/z 124) indicates the presence of proanthocyanidins. Lignins have been reported to contain only negligible amounts of catechol (Galletti and Reeves, 1992). And so it is reasonable to use catechol as a diagnostic fragment for condensed vegetable polyphenols (Galletti and Reeves, 1992). Vegetable polyphenols are extremely complex compounds. They may be highly specific in at least some and probably most of their possible interactions, via an enormous potential for stereo specificity (Zucker, 1983).

Within the seed vegetable polyphenols have several mixed functions, involving a defence mechanism against living plant enemies, a delay in decomposition when the seed is a component of the seed bank, and influencing dormancy in seeds.

The real cause of dormancy of Cornus seeds is due to a high level of vegetable polyphenols in the seed coat restricting the embryonal development (Guan, 1990). Proanthocyanidins are present in most families of the monocotyledons. According to Dahlgren, Clifford and Yeo (1985) cells with vegetable polyphenols of the Musaceae may contain proanthocyanidins. Ellis, Foo and Porter (1983) have described the occurrence of proanthocyanidins.

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**Fig. 11.** Py (El) GCMS partial mass chromatogram of the TMAH treated samples of the extracted seed coat of *Musa velutina* for m/z 192 (p-coumaric acid) and m/z 222 (ferulic acid).
in the fruit skin of \textit{Musa sapientum}. Within the line of this study no specific information on vegetable polyphenolic structures has been found.

Many monocotyledons have anatomically relatively simple seed coats. Endotestal scars as found in Dioscoreales, Zingiberales, Commelinaceae, and others may be original for monocotyledons. The seed coat structure of the Musaceae is characteristic at the family level and the most complex one within the Zingiberales. Very remarkable are the micropylar collars, the relative thick mesotesta, the unique macro-molecular ‘fingerprint’ with the typical Musaceae phenolic compounds of still unknown composition, and the breaking of the cell walls in the exostelial layer resulting in a surface with silica crystals. More often the endotesta is silicified, as in Commelinaceae, Strelitziaceae, Rapateaceae, and Marantaceae. In Commelinaceae also a silicified surface remains due to a breaking of the endotesta. The Zingiberales is one of the most indisputable natural suprafamilial groups (Dahlgren \textit{et al}., 1985). The seed coat structure of the Musaceae is characteristic and the most complex one of the Zingiberales, whereas that of the Cannaceae is considered as the most derived one within this order. The rather complex seed coat is characteristic for this family and seems to be unique if compared to other families of the Zingiberales and to other monocotyledons.

**ACKNOWLEDGEMENTS**

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**LITERATURE CITED**


