The systematic relevance of fruit and seed anatomy and morphology of *Akania* (Akaniaceae)

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The fruit and seed anatomy and morphology of *Akania bidwillii*, the monotypic genus in the Akaniaceae, have been studied in an effort to clarify its systematic position. The loculicidal (1, 2-) 3-locular fruit with lignified fibrous 6-7-layered endocarp is clearly of capsular type. Seeds of *Akania* are relatively large, smelling of bitter almonds, abundantly albuminous, with straight dicotyledonous embryo. The seed coat of *Akania* is exo-mesotestal with fully obliterated tegmen in early stages; 1-layered exotesta represented by columellar thick-walled cells with numerous invaginations of inner cell walls; mesotesta 25-35-layered, composed of thick-walled, but not lignified mostly rounded sclereids filled with tannin-like substances, the innermost part of mesotesta is aerenchymatous, sometimes collapsed, and traversed by 6(8) postchalazal vascular bundles, 2-3 layers of endotesta composed of enlarged cuboid cells with heavily thickened walls. Evidence mainly from seed morphology and anatomy of seed coats emphasizes the anomaly of the traditional inclusion of Akaniaceae in the Sapindales, being quite distinct in spermomd form and origin from both Sapindaceae and Staphyleaceae in particular as well as from other families of the order, excepting somewhat anomalous exo-mesotestal Bretschneideraceae. Furthermore, seed anatomy does not confirm any relationships with Capparales. It is suggested that Akaniaceae together with Bretschneideraceae constitute a distinct relict side-branch of connaraceous-sapindaceous ancestry tracing back to primitive exo-mesotestal Rosales. On the basis of available data of seed coat anatomy it is appropriate to remove archaic Akaniaceae with Bretschneideraceae from more advanced Sapindales. Furthermore, with the addition of more data on carpology and seed anatomy of basal Rosidae the systematic position of the family should be redefined in terms of its primitiveness and the lack of close relationships with Sapindales.

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Akaniaceae is one of 15 myrosin-producing angiosperm families whose relationships are ill understood (Rodman, 1991a, b; Gadek et al., 1992; Rodman et al., 1993). *Akania*, the only genus of Akaniaceae, was described by Hooker (Bentham & Hooker, 1862) as a member of the Sapindaceae, but Baillon (1874) regarded it as a doubtful element of the whole order noticing sharp differences in floral structure. Later Mueller (1875) tried to connect *Akania* with his ‘Staphyleae’. Radlkofer (1890), emphasizing the lack of close relationships with Sapindaceae, agreed with its inclusion in Staphyleaceae, but nevertheless pointed out its anomalous position within the order in having alternate extipulate leaves, perigyny of the flower, no floral disc, diplostemonous androecium, two pendulous superposed epitropous ovules in each locule and seeds with abundant endosperm. In spite of these numerous differences, Solereder (1892) taxonomically formalized a new tribe Akanieae of Staphyleaceae, also including also *Tapiscia* and *Huertia*. In contrast, Pax (1893) excluded *Akania* from the family. Thus *Akania* was determined an ‘incertae sedis’ until Stapf’s (1912) creation of a new family Akaniaceae emphasizing thereby its differences with both Sapindaceae and Staphyleaceae. The segregation of *Akania* into a distinct family is accepted in most modern systems of angiosperms (Takhtajan, 1987; Dahlgren, 1989; Cronquist, 1992; Thorne, 1992), but in none of them (e.g. in Baillon’s *Histoire des Plantes* (1874)) is the anomaly of the systematic position of *Akania* in the Sapindales questioned, except for simple comments on the differences between Sapindaceae and Akaniaceae in the structures of the secondary xylem (Heimsch, 1942; Metcalfe & Chalk, 1950). Recently Rodman (1991a,b), Gadek et al. (1992) and Rodman et al. (1993) in trying to prove the monophyly of all glucosinolate-producing angiosperm families suggested on the base of cladistic and *rbcL* analyses that Akaniaceae together with Bretschneideraceae forms a basal clade in the highly advanced Capparales. The latter is characterized by a very specialized exotegmic seedcoat construction and paracarpous fruits. Tobe and Raven (1995) in a thorough study of the embryology of the genus concluded that Akaniaceae with closely allied Bretschneideraceae forms a basal clade in the highly advanced Capparales. They suggested its systematic position “as a separate family in or near Sapindales” having found akaniaceous seedcoat structure similar with sapindalean ones. However, the differences in some exomorphic characters noted by Radlkofer (1890) plus the occurrence of pachytestal seedcoat structure within typical Sapindales (Corner, 1976; Doweld, 1996) questioned such a close relationship. Here we attempt to elucidate the phylogenetic relationships of Akaniaceae using the carpological features of *Akania*.

**MATERIAL AND METHODS**

Fruits and seeds of *Akania bidwillii* were obtained from the Australian National Herbarium (voucher: Jones 2948 at CANB) and additional fresh seeds were provided by North Coast Regional Botanic Garden, Coffs Harbour, Australia (voucher at Gaertnerian Institution, NICAR). Drawings were made from cross-sections prepared by the usual paraffin method. Scanning electron microscope observations were made with a HITACHI S-405A at 15 kV.
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OBSERVATIONS

**Fruit**

*Mature fruit.* (Fig. 1). This is 2.5–3 cm long and 1.5–2.5 cm wide, dull red, oblong-ovate, and more often of irregular shape owing to abortion of locules. It is formed from the trimerous syncarpous gynoecium (Fig. 2), with non-persistent thickened style with 3-lobed rounded stigma (Cronquist, 1992) and axile placentation of two superposed, pendulous ovules; it is loculicidally dehiscent. The vasculature of the capsule consists of 6 (3 × 2) main central vascular bundles and 8–10 (4–5 × 2) symmetrically arranged bundles usually in the pervading endocarp (Fig. 2) but sometimes penetrating the mesocarp.

**Pericarp** (Fig. 3) This is differentiated into a 3–4-layered exocarp, up to 30–35 layers of parenchymatous mesocarp and 6–7-layered lignified fibrous endocarp. Such a sclerendocarp-like construction of the whole pericarp is typical of capsular fruits (Weberbauer, 1898).

Epidermal cells of the parenchymatous exocarp are differentiated into specialized 1- or 2-celled thick-walled trichomes with large cavities filled with myrosinoid substances (Figs 3–5). In the parenchymatous slightly thick-walled mesocarp there are numerous solitary or often irregular clusters of macrosclereids with lignified, thickened and pitted walls and relatively large cavities filled with glucosinolate-like substances. There are also rows or mostly clusters of myrosiniferous cells in the inner part of mesocarp. In septal parenchyma there are specialized cells with orange contents located in the outer part of mesocarp (Fig. 3). The endocarp is composed of longitudinal fibres, heavily lignified and thick-walled, rather coarsely pitted and a single layer of slightly thick-walled, un lignified cells in the inner epidermis. As a rule, endocarpic fibres are absent in the regions of ovule attachment and fruit dehiscence (Fig. 2), and in such cases the endocarp is represented by its epidermal layer only.
Seed

*Mature seed* (Fig. 8a). This is relatively large, 10–15 mm long, 8–10 mm wide and 5–7 mm thick. It is rounded, ovoid, slightly oblong at the hilum with expanded micropylar rostrum and slightly differentiated antiraphe. The exarillate seeds are dirty yellow or brownish, smelling of bitter almonds. The hilum is basal and long, with a single vascular bundle expanding in the chalaza where it splits into six postchalazal bundles (sometimes eight, in case of further splitting of any two bundles) which extend through the outer integument up to the micropyle (Fig. 8b). Sometimes one of the six postchalazal bundles is differentiated into an antiraphe. The mature seeds are abundantly albuminous (Fig. 8 C–D) and oily. The symmetrical, straight embryo is large, with two flat cotyledons 7–10 times longer than the rest of the embryo. A micropyle is formed by both inner and outer integuments.

*Spermoderm.* The seed coat (Fig. 9) is formed from both integuments. It is exomesotestal, with fully obliterated tegmen in the early stages of seed development (Tobe & Raven, 1995), with a slightly rough surface and finely faceted seed sculpturing (Figs 6, 7).

The testa is clearly differentiated into three zones: outer (exotesta), middle (scleromesotesta) and inner (endotesta). The exotesta is composed of a single layer of columellar thick-walled cells filled with orange, granular and oily contents. Its walls have numerous intracellular invaginations. The multilayered (25–35 layers) meso-

![Figure 2. Cross-section of the capsule of *Akania bidwillii* with axile placentation. Scale bar = 3 mm. Abbreviations: cvb, central (3 × 2) vascular bundles; ovb, outlying vascular bundles; sp, seed; endep, fibrous endocarp; myrosiniferous cells intensively dotted (inner part), cells with orange substances diffusively dotted (outer part).]
testa is represented by thick-walled but not lignified macrosclereids with large cavities filled with tannin-like contents. Their form changes from rounded to longitudinal towards the centre of the seed. The aerenchymatous innermost part of the mesotesta is composed of tangentially oblong cells, with slightly thickened walls: its layers are sometimes obliterated. The postchalazal vascular bundles are located in only the aerenchymatous part of scleromesotesta. The endotesta is represented by 2–3 layers of slightly oblong, tangentially enlarged, cuboid cells with heavily thickened outer periclinal walls. They are filled with tannin-like substances.

Figure 3. Cross-section of the pericarp of Akania bidwillii, scale bar = 5 mm. Abbreviations: exocp, exocarp; mscp, mesocarp; endcp, endocarp; vb, vascular bundle; mscl, macrosclereids. Myrosiniferous cells intensively dotted (inner layers), cells with orange substances diffusively dotted (outer layers).
Relationships with Capparales/Moringales

Rodman (1991a,b), Gadek et al. (1992) and Rodman et al. (1993) using phenetic, cladistic and molecular analyses have advanced a hypothesis suggesting affinity of the Akaniaceae with the Capparales emphasizing its basal position within the order. The

Figures 4–7. SEMs of the fruit and seed of Akania bidwillii. Fig. 4. Surface of the pericarp with numerous epidermal trichomes. Scale bar = 40 μm. Fig. 5. The same, enlarged. Scale bar = 20 μm. Fig. 6. Seed sculpture. Scale bar = 80 μm. Fig. 7. The same, enlarged. Scale bar = 40 μm.
exo-mesotestal seed coat of *Akania* with unspecialized, entirely obliterated tegmen is related to multilayered outer integument. In contrast, in the capparalean, most exotegmic seed coats appear very simplified and reduced having a structural system based on the inner integument (mostly exotegmen and rarely the little-layered endotesta). The morphogenetic gap between akaniaceous exo-mesotestal spermoderma and highly advanced and specialized tegmic seed coat types of Capparales is so great that it may be bridged only by several revolutionary and highly improbable steps in the morphogenetic reorganization of the structural integument. In any case the exo-mesotestal seedcoats of *Akania* have nothing in common with endotestal-exotegmic (*Cadaba*) or mostly exotegmic spermoderma of Capparaceae (Rodionova, 1992a) which are also characterized by a mostly 2-layered simplified testa in contrast to the compoundly differentiated testa (25–35 layers) of *Akania*. The same is true for exotegmic Resedaceae (Czernia kowskaya, 1992), again with bilayered unspecialized testa, and also for the exotegmic Tovariaceae (Boesewinkel, 1990; Rodionova, 1992b). It would be unwise to relate the mostly endotestal (Rodionova, 1992b) or even sometimes endotestal-exotegmic (*Lunaria*: Corner, 1976) seeds and highly specialized paracarpous fruits of Brassicaceae (siliquas) to akaniaceous syncarpous primitive capsular fruit with exo-mesotestal seedcoats. At the same time it is out of the question to propose any relationships of the very specialized mesotestal Moringaceae with secondary multiplicative and compoundly differentiated testa (Komar & Rodionova, 1992). In addition, it is quite impossible to suggest

Figure 8. Mature seed of *Akania bidwillii*. Scale bar = 2 mm. A, general view. B, vascularization of the seed. C, longitudinal section of seed showing embryo (half removed) and abundant endosperm. D, lateral section of seed showing dicotyledonous embryo. Abbreviations: endsp, endosperm; cot, cotyledons; pvb, postchalazal vascular bundles; rvb, raphal vascular bundle.
relationship of *Akania* with Tropaeolaceae which is characterized by the very advanced pachychalazal exalbuminous seeds with thin-walled, simplified 10–15-layered spermoderma (Corner, 1976). Thus, from the stand point of seed anatomy only it is evident that any attempt to include Akaniaceae to Capparales is artificial and untenable in spite of sharing all eight coded synapomorphies (Gadek *et al.*, 1992).

Figure 9. Cross-section of the spermoderm of *Akania bidwillii*. Scale bar = 2 mm. Abbreviations: extt, exotesta; mstt, mesotesta; endtt, endotesta, iinuc, crushed inner integument and nucellus; endsp. endosperm.
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Relationships with Sapindales

Returning to the traditional position of *Akania* into Sapindales we find that the distinctive exo-mesotestal spermoderm of Akaniaceae with entirely crushed tegmen is not similar to most of the families of the order except for exo-mesotestal Bretschneideraceae, whose systematic position within Sapindales is regarded as doubtful (Dahlgren, 1983). The resemblance of exo-mesotestal seed coats of *Akania* and exo-mesotestal representatives of Staphyleaceae (*Staphylea, Turpinia* p.p.) is superficial because they possess differences in the structure of the seed vascular system. The simple, rather primitive type of seed vascularization of *Akania* is readily distinguished from the distinctive vasculature of staphyleaceous seed coats where several bundles directly vascularize the outer integument (not forming chalazal plexus) (Le Monnier, 1872). Such a type of seed vasculature is a regressive derivative of an advanced pachytestinal seed construction (Doweld, 1996). Thus, Staphyleaceae (as well as closely related Aceraceae with a very simplified structure of seed coat (Magen, 1912)) in spite of some likeness in the histological differentiation of spermoderm with Akaniaceae are not closely related to the latter. The same is true for the highly advanced exotegmic Tapiaceae (Corner, 1976) which is absolutely anomalous within Sapindales (Doweld, 1996). Melianthaceae having a specialized exotestal spermoderm (Guerin, 1901; Corner, 1976) are too highly advanced to be related with *Akania*. Sapindaceae and the closely related Hippocastanaceae possess a very specialized pachytestal seed type based on exo-mesotestal spermoderm with a specialized exotegmic construction of the tegmen (Doweld, 1996). Within the tegmen of archaic Koelreuteriae, some Dodonaeae (Sapindaceae – Dodonaceoideae) are sometimes recorded rudiments of an ancestral exotegmic structure (*Alectryon* has even exotegmic fibres (Corner, 1976)). Such a tegmen construction has never been noted within the *Akania* with its unspecialized, entirely obliterated tegmen nor within *Bretschneidera* with undifferentiated tegmic construction. It is indicative of a morphogenetic distance between the true sapindacean line and *Akania*-Bretschneidera. The latter forms are heterobathmic in that *Akania* retains some archaic features of a seed structure. These include copious endosperm (almost lacking in *Bretschneidera*), smaller embryo, a marked compoundly differentiated palisade of thickened columellar exotestal cells with numerous invaginations (superseded by a smoother inner surface in *Bretschneidera*). In contrast to *Bretschneidera*, *Akania* loses a distinct inner integument at a very early stage of seed development (advanced feature). However a little morphogenetic distance between the more primitive Akaniaceae and more advanced Bretschneideraceae does not upset the integrity of this evolutionary line. At the same time the available evidence from seedcoat anatomy suggests that it is more appropriate to segregate both the Akaniaceae and Bretschneideraceae from the Sapindales while noting a lack of close relationships between other families in this heterogeneous alliance.

Systematic position

The traditional inclusion of Akaniaceae in the Sapindales (Takhtajan, 1987; Dahlgren, 1989; Cronquist, 1992; Thorne, 1992) is not supported by seedcoat anatomy although Tobe and Raven (1995) thought it possible to affiliate *Akania* to a specialized sapindalean phylum. However, it seems more plausible to separate the
Akaniaceae and the more advanced Bretschneideraceae from the order as it is not yet possible to see the origin of akaniaceous seedcoats in any typical representatives of Sapindales. In addition, it should be noted that similar exo-mesotestal (and additionally pachytestal) spermoderm of Sapindaceae and Hippocastanaceae with recorded rudiments of exotegmic construction of the inner integument (Guerin, 1901; Corner, 1976) are directly related to Connnaraceae which is characterized by testal-exotegmic seedcoats with arillate follicles (Corner, 1976; Takhtajan, 1987). The similar exo-mesotestal spermoderm of Staphyleaceae has quite distinct, complicated vasculature derived from a pachytestal seed type (Doweld, 1996) which is in strong contrast to the simpler primitive vascular system of akaniaceous seeds. The lack of any resemblance to the rest of the families of the Sapindales suggests that Akaniaceae and Bretschneideraceae (Tobe & Raven, 1995) constitute a single distinct evolutionary line diverging early from a common ancestor with Connarales/Sapindales with convergent evolution of the seedcoat.

At the same time, using seedcoat structure, Akania may not be considered as a basal clade for Capparales as was proposed by Rodman (1991a, b), Gadek et al. (1992) and Rodman et al. (1993). The exo-mesotestal spermoderm of Akaniaceae with a tegmen completely obliterated in early stages of a seed development is far from being close to most endotestal (Capparaceae, Brassicaceae) or exotegmic (Tovariaceae, Resedaceae, etc) seeds of Capparales. The morphogenetic hiatus is so great that it is out of the question to propose any relationships between these clades. It is also impossible to support with proximity of Akania (‘cladistically close’) to the malvalean clade as has been recently suggested (Rodman et al., 1993). The seed coats of Malvales are uniformly exotegmic with highly simplified and reduced testa (up to 5–10 most parenchymatous layers) sometimes even destroyed in indehiscent fruits (Corner, 1976). The contrast with the akaniaceous 25–35-layered exo-mesotestal spermoderm with completely crushed tegmen is more than obvious.

In summary, on the basis of available seed anatomical data the Akaniaceae and its newly established ally Bretschneideraceae (Tobe & Raven, 1995) are considered an independent relict clade, an early diverging line from connaraceous-sapindaceous ancestry which may probably be traced back to the primitive exo-mesotestal Rosales. Further studies on carpology and seed anatomy of other basal rosidaceous orders should throw light on the origin of this distinct, archaic group.

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REFERENCES

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