Acta Bot. Neerl. 47(3), September 1998, p. 299–312

The aril of *Chamaecytisus proliferus* (L.fil.) Link (Leguminosae): its structure, histochemistry and role in the dispersal and in water–seed interaction

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SUMMARY

The aril structure and histochemistry of *Chamaecytisus proliferus* seed were investigated by bright-field and fluorescent light microscopy, and scanning electron microscopy. The aril consisted of three different regions: a loose peripheral tissue composed of large cells; a median region composed of closely packed elongated cells lying parallel to the axis ridge-lobes; an inner region composed of thick-walled cells close together and orientated perpendicularly to the same axis. These structural features of the aril and the occurrence in it of many different substances detected by toluidine blue O, alcian blue at low pH (polysaccharide hydrophilic substances), by iodine-potassium iodide (starch), naphthol blue black (proteins) and fluorol yellow (lipids) suggested a multipurpose role of the aril regarding the main functions of the seed: germination and dispersal. In particular the nutrient composition of the aril, due to the presence of starch, proteins and lipids, might be related to dispersal by ants (myrmechory), whereas the aril cell walls, rich in polysaccharide substances, might be involved in germination acting as a site of apoplastic absorption of water.

Key-words: aril, *Chamaecytisus proliferus*, histochemistry, seed dispersal, structure, water–seed interaction.

INTRODUCTION

Next to pollen transport, seed dispersal is the single most important factor promoting gene flow in plants. Furthermore, the seed must be successful in dispersing and settling in an appropriate environment, in surviving adverse conditions and in the rapid optimization of favourable conditions (Van Staden *et al.* 1989). The adaptation of seeds of legumes to facilitate dispersal most commonly concerns the development of funicular or other extratestal structures designated as arils by Corner (1976). According to Corner aril is a general name for pulpy structures which grow from some parts of the ovule or funicle after fertilization and invest a part of or the entire seed. Sernander (1906; cited in Boesewinkel & Bouman 1984) introduced the term 'elaiosome' for all fleshy and edible parts of seeds dispersed by ants. According to Boesewinkel & Bouman (1984) the elaiosome attracts ants and becomes detached easily from the remainder of

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the seed, which is hard and inedible due to tissues made of thick-walled cells. The colour of the elaiosome is white or yellow and differs from that of the rest of the seed, which is generally darker. The elaiosome contains nutrients such as proteins, lipids, starch and vitamins. Beside the dispersal function, elaiosomes are known to have other functions related to seed adaptation: facilitation of dehydration and hydration of the seed, induction of seed dormancy, creation of reserve of water absorbed from the soil and its transfer to the rest of the seed during germination. Different classifications of seminal outgrowths are reported in relation to their anatomical, ecological and functional characteristics (Lisci *et al.* 1996). For *Chamaecytisus proliferus* (L. fil.) Link (Leguminosae) we have used the term aril in a general sense, as reported recently by Reghunath *et al.* (1993), in spite of its probable involvement in myrmechory (McKey 1989).

Chamaecytisus proliferus is a taxonomical complex of plants endemic to the dry vulcanic land of the Canary Islands (Francisco-Ortega *et al.* 1990). Many studies have been dedicated to the var. *palmensis* (Christ) Hansen & Sunding owing to its suitability to infertile soil (sand, gravel and laterite), associated with its high nutritional values and high digestibility. *C. proliferus* is an ideal component of subtropical ruminants' diets and could even act as a source of human food (Hawley 1984; Snook 1986). The marked physical dormancy of the seed sets a limit on the large scale use of *C. proliferus* in tropical pastures, and structural changes of its surface are needed to obtain a high percentage of germination (Reghunath *et al.* 1993).

The aim of our work was to investigate the structures involved in the seed–water interaction, the hilar region and especially the aril, in order to provide new information about the structural and histochemical features by bright-field and fluorescence light microscopy and scanning electron microscopy.

MATERIALS AND METHODS

Seeds of *C. proliferus* were obtained from the FAO seed bank in Rome and were stored at 4°C until examined. In order to test their hardness, the seeds were placed in Petri dishes on wet filter paper. The seeds, which did not appear to be swollen after 10 d of imbibition, were considered to be hard. Ninety-seven per cent of the seeds were found to be hard. To locate the site and the chemical nature of the barrier to water penetration, whole hard seeds were soaked in a crystal violet aqueous solution (0·5% w/v) according to Jannerette (1979). Soaked seed halves and hand-sectioned thin slices were observed by means of a stereoscopic and optical light microscope, respectively. To study the single cells of the aril we macerated them, treating small pieces of arils with 2% HCl for 40 min at 90°C, 2% HCl at room temperature for 10 min and 2% NH_3 at 90°C for 40 min (modified from Hamly 1932). After maceration the arils were shaken with a Vortex shaker and finely pressed using a glass rod. Finally, the cells in suspension were concentrated by centrifugation at 4000 r.p.m. for 5 min. For bright-field microscopy whole hard seeds were fixed in FAA (formalin 5:acetic acid 5:50% ethyl alcohol 90) for 24 h, dehydrated in a graded ethanol series and embedded in JB4 resin (Polyscience Inc., Warrington, PA, USA) in BEEM capsules (Brinn & Pickett 1979). The tissue blocks were sectioned at 5–7 μm on a Reichert Om U2 ultra-microtome equipped with a glass knife. The following histochemical reactions were carried out: (a) toluidine blue O (TBO) at pH 4·4 as a metachromatic stain to detect pectins (Feder & O'Brien 1968); at this pH, several different polyanions (polyphosphates, polycarboxilic acids and

polysulphates) carry a negative charge reacting metachromatically reddish-blue with TBO (Bullock *et al.*1980); moreover, the green staining is a characteristic metachromasy for the phenolic substances as lignin (O'Brien & McCully 1981); (b) alcian blue 8 GX at pH 2·5 and at pH 0·5 to detect faintly acid polysaccharides (with presence of $-COOH$ groups) and highly acid polysaccharides (with presence of $-SO_4^{2-}$ groups), respectively (Lev & Spicer 1964); (c) ruthenium red for pectic substances (Jensen 1962); (d) safranin-fast green as a contrast stain (O'Brien & McCully 1981); (e) naphthol blue black to detect proteins (Ling-Lee *et al.* 1977); and (f) iodine-potassium iodide for starch (Gahan 1984). For fluorescence microscopy, freehand slices of unfixed hard seeds were treated with one of the following: (a) periodic acid-acriflavine SO_2 (F-PAS) for general localization of polysaccharides (Pearse 1980). Following this procedure the cellulose of the cell walls fluoresces green, while starch and other non-cellulosic polysaccharides emit orange-red fluorescence. However, when the cellulosic/non-cellulosic polysaccharide ratio in the cell wall is modified, the fluorescence changes from green to orange-red (Bruni & Modenesi 1983); (b) Nile red for cytoplasmatic neutral lipid localization using an H_2 (BP 390–490) filter block (Greenspan *et al.* 1985); (c) fluorol yellow 088 for localizing non-polar substances such as suberin lamellae and lipids using H_2 (BP 390–490) and A (BP 340–380) filter blocks (Brundrett *et al.* 1991). A Leitz Dialux fluorescence microscope equipped with an HBO 50 W mercury vapour lamp was used. For scanning electron microscope (SEM) investigation whole seeds and halves were dehydrated in a graded ethanol series prior to drying in a critical-point drying apparatus (Termoelectric Control Emscope CPD 750). They were then mounted on stubs, coated with gold (200–220 Å in thickness) using an Agar Aids Sputter Coater, and viewed with a Philips 515 SEM at an acceleration voltage of 20 kV. The macerated cells were mounted directly on stubs and coated with gold. Kodak Technical Pan film was used to record the images.

RESULTS

Structural features

The seeds of *C. proliferus* showed the outer epidermis of the testa consisting of a palisade of radially elongated cells which stood above a hypodermal layer of osteosclereids or hourglass cells with large intercellular spaces (Fig. 1a). The seeds of *C. proliferus* measured about 2×4 mm. The hilar region presented an oval structure with a median groove (Fig. 1b). In transverse section the hilar region consisted of a continuation of the palisade layer, externally associated with a counterpalisade. Below the fissure was the tracheid bar, pear-shaped in transverse section (Fig. 3b). Around the hilum stood a large horseshoe-shaped aril with a slit over the hilum and two outgrowths on its sides, covering much of the seed's apex (Fig. 1c). The aril had an irregular surface, smoother on the upper part and discontinuous on the lower part (Fig. 1d). In transverse section the aril revealed a spongy appearance and the horseshoe-shape clearly showed a ridge on the side opposite to the lobes (Fig. 2a). The morphology of the tissue identified three regions: an outer region (A) characterized by large cells with a wide lumen, forming a loose peripheral tissue (Fig. 2b); a middle region (B) made up of cells with slit-like lumen lying parallel to the axis ridge-lobes, separated by abundant intercellular substance (Fig. 2c,d); and an inner region (C) made up of close-packed cells perpendicular to the axis ridge-lobes, with a small circular lumen and thick walls (Fig. 2c,d). With a higher magnification, the cells in the B region, were found to have

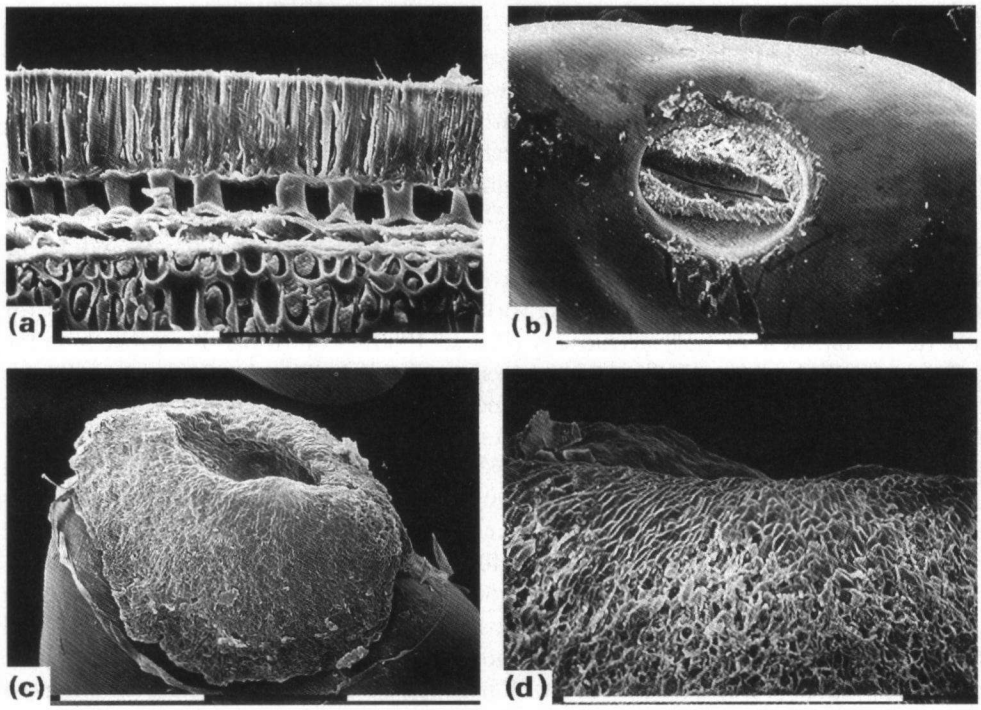

Fig. 1. SEM micrographs of a *Chamaecytisus proliferus* seed. (a) Anticlinal transection of seed coat. Scale bar = 0·1 mm. (b) Hilar region with the aril removed. Scale bar = 1 mm. (c) The aril covering the hilar region. Scale bar = 0·1 mm. (d) Detail of the aril showing an irregular surface, smoother on the upper part than on the lower part. Scale bar = 1 mm.

thickenings in the walls perpendicular to the cell's largest axis (Fig. 2e). In cross-section the thickenings were triangular (inset, Fig. 2e). Further ultrastructural information about the different components of the aril has been obtained through the study of macerated tissues. The single cells were mainly elongated, remarkably twisted and with wrinkled surfaces (Fig. 2f).

Water entry

When *C. proliferus* seeds were soaked in 0·5% aqueous crystal violet solution, the aril became stained after a few hours (2–4 h) of imbibition. The crystal violet solution stopped at the seed surface. Only the outer tangential walls of the extrahilar palisade cells showed typical violet staining, while the underlying light line appeared unstained. In the hilar region the counterpalisade, the hilar palisade (Fig. 3a) and the boundary zone between the tracheid bar and the adjacent stellate cell parenchyma remained unstained, also after 1 day of soaking.

Histochemical features

Extrahilar palisade. When *C. proliferus* seed-coat sections were stained with TBO at pH 4·4, the whole extrahilar palisade layer became stained metachromatically (reddish-blue) in the following parts: outer and inner tangential cell walls, radial walls for the

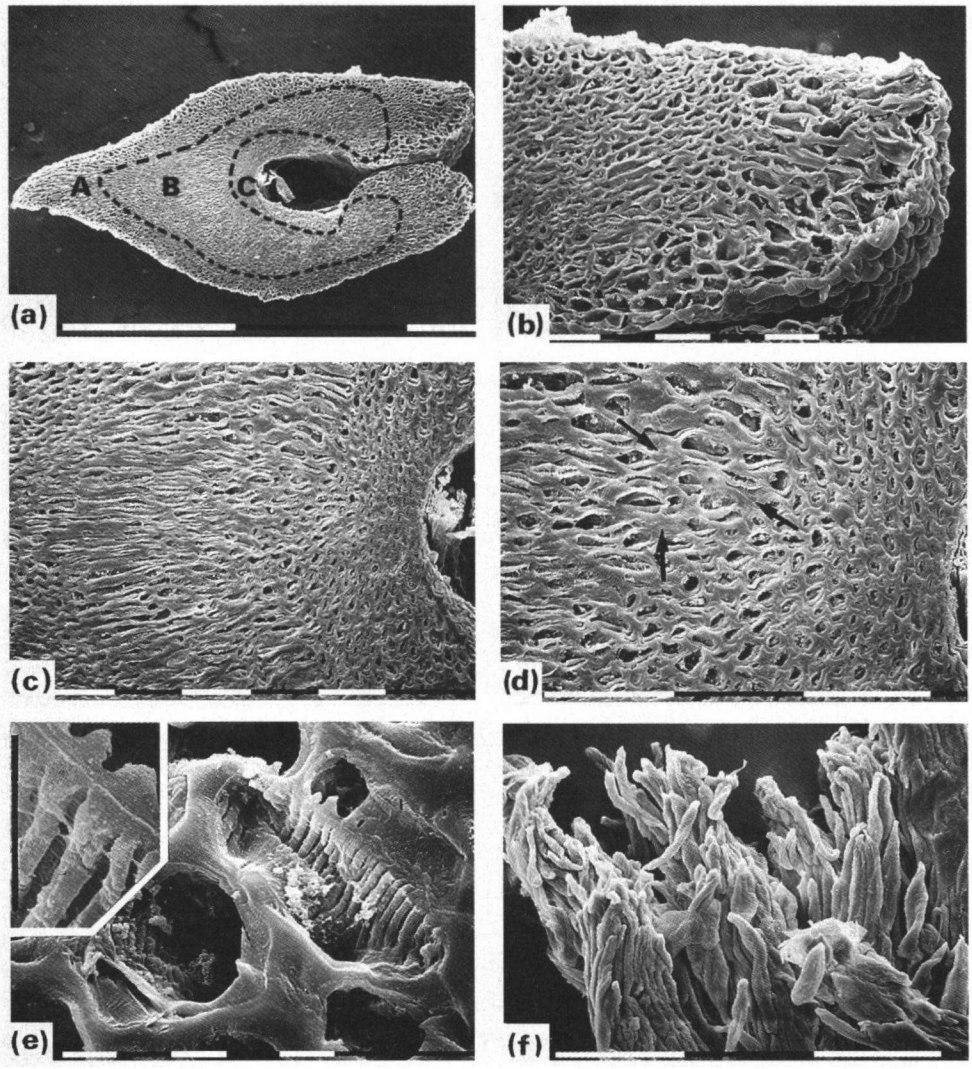

Fig. 2. SEM micrographs of the aril of a *Chamaecytisus proliferus* seed. (a) Periclinal transection, showing the horseshoe-shape of the aril. Note the morphology of the three regions: A, large cells with a wide lumen forming a loose peripheral tissue; B, cells with slit-like lumen lying parallel to the axis ridge-lobes; C, closely packed cells perpendicular to the axis ridge-lobes, with small circular lumen and thick walls. Scale bar = 1 mm. (b) Detail of region A. Bars = 0·1 mm. (c) Detail of regions B and C. Scale bar = 0·1 mm. (d) Detail of c showing abundant intercellular substance in region B (arrows). Scale bar = 0·1 mm. (e) Region B cells at high magnification showing cell wall thickenings. Scale bar = 10 µm. Inset: note the triangular section of the thickenings. Scale bar = 10 µm. (f) Macerated aril cells. Scale bar = 0·1 mm.

lower three-quarters of their length. Remarkably, the light line remained completely unstained (not shown). After treatment with alcian blue at pH 2·5 and pH 0·5, the palisade layer did not react. In fluorescence microscopy the extrahilar palisade cells were autofluorescent, in particular the light line fluoresced strongly yellow-green (Fig. 4b).

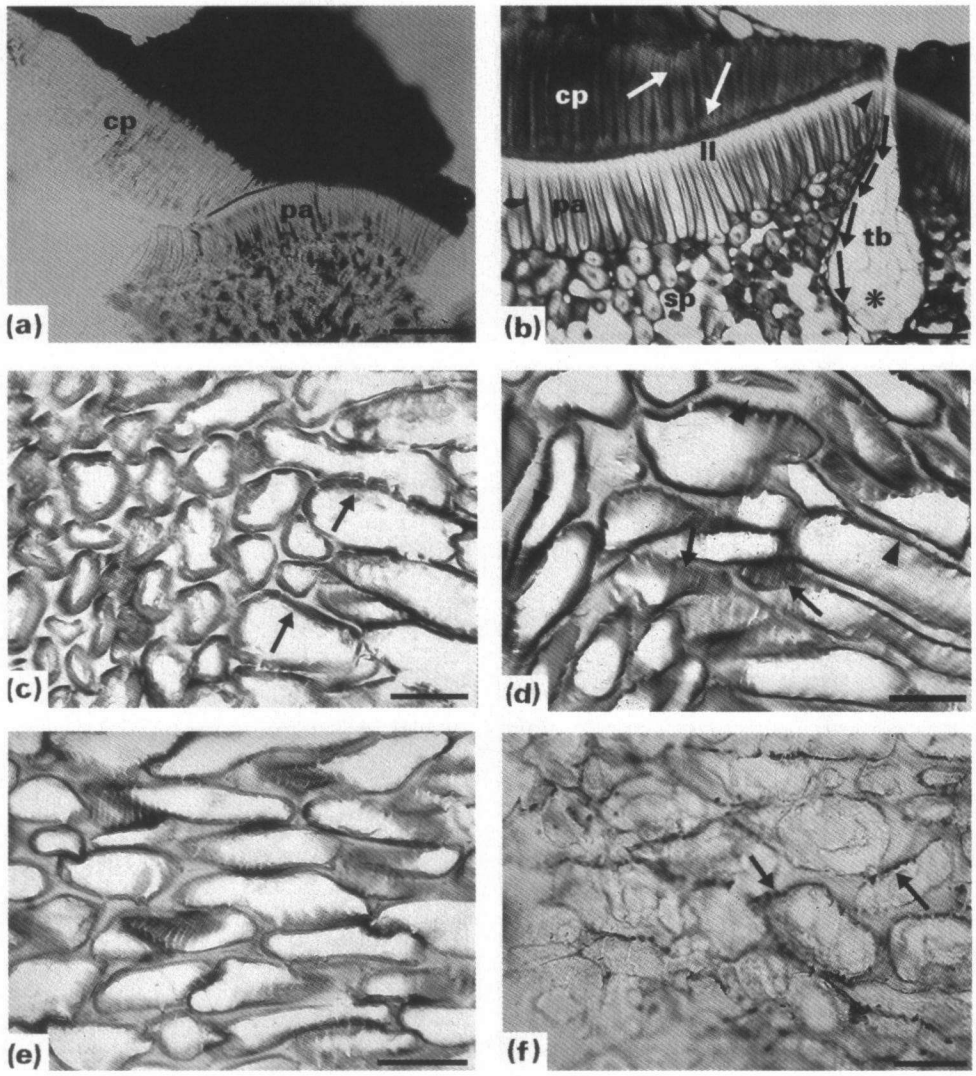

Fig. 3. Bright-field micrographs of a *Chamaecytisus proliferus* seed. (a) Free-hand anticlinal transection of a part of the hilar region. Note: the dark area (aril) is stained with crystal violet. The stain stops at the outer tangential walls of the counterpalisade cells and of the palisade cells layer. Scale bar = 65 μm. (b) Cross-section through the hilar region. TBO pH 4·4. The asterisk indicates the metachromatic green staining of the tracheid bar. Note boundary zone metachromatically reddish-blue stained (black arrows) and the unstained light line bending down at the height of the hilar groove (black arrowhead). The counterpalisade shows unstained outer and inner subterminal zones (white arrows). Scale bar = 60 μm. (c–f) Periclinal transection of the aril. Scale bar = 30 μm. (c) TBO pH 4·4. Region C. Note the metachromatic reddish-blue staining of the cell walls and their thickenings (arrows). (d) TBO pH 4·4. Region B. Note the metachromatic reddish-blue staining of the cell walls and their thickenings (arrows) while the intercellular substance shows an ortochromatic light-blue staining (arrowheads). (e) Ruthenium red. Region B. Note the tissue reacting extensively; in particular the cell wall thickenings reacted positively but the intercellular substance also reacted revealing the presence of pectin in their composition. (f) Alcian blue at pH 2·5. Region B. Note the distinct thickenings (arrows), see inset Fig. 2e. cp = counterpalisade; pa = palisade; ll = light line; tb = tracheid bar; sp = stellate parenchyma.

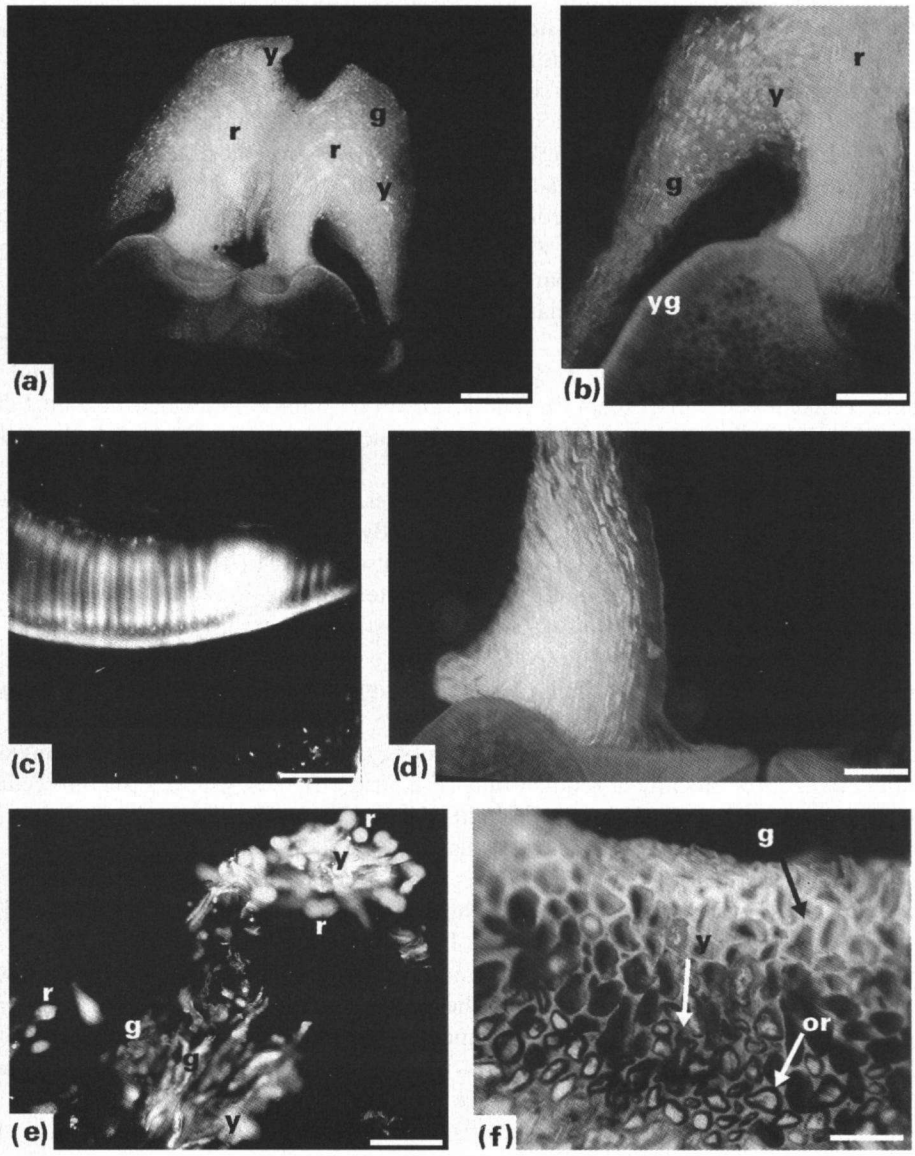

Fig. 4. Fluorescence micrographs of the aril and hilar region of *Chamaecytisus proliferus*. (a)–(d) Anticlinal free-hand transections. (a) and (b) Autofluorescence, H_2 filter (BP 390–490). Note the intense red-red/yellow autofluorescence of the aril inner zone and the green-green/yellow autofluorescence in the outer zone and light line. (a) Scale bar = 340 μm, (b) scale bar = 145 μm. (c) Fluorol yellow. Counterpalisade. Note the subterminal counterpalisade zones and the light line fluorescing strongly in yellow, H_2 filter. Scale bar = 60 μm. (d) Fluorol yellow. The rich lipidic composition of the aril cells is highlighted, A filter (BP 340–380). Scale bar = 170 μm. (e) Macerated cells. Autofluorescence, H_2 filter. Note the three types of fluorescence: red, green and yellow. Scale bar = 340 μm. (f) Periclinal transection of a small portion of aril. F-PAS. Note the fluorescence of the cell walls turning from green to orange-red while the intercellular substance remains yellow. Scale bar = 60 μm. y = yellow, r = red, g = green, or = orange-red, gy = green-yellow.

Hilar region. Some results of histochemical tests are summarized in Table 1. The principal results for the hilar region were the following: with TBO at pH 4·4 in the hilar palisade the same parts as in the extrahilar palisade became metachromatic (reddish-blue). The light line remained unstained and was highly visible in this region as well. In particular, at the height of the hilar groove, the light line bent down gradually, and the upper portions (above the light line) of the palisade layer cells reacted evenly and intensely in reddish-blue. The counterpalisade showed an intense metachromatical staining for its whole length, excluding the cell's outer and inner subterminal zones. The tracheid bar stained green and the boundary zone between the tracheid bar and the adjacent stellate parenchyma stained metachromatically reddish-blue (Fig. 3b). Alcian blue at pH 2·5 showed affinity for palisade cell walls, above all for the outer tangential ones, and showed affinity for the boundary zone and the counterpalisade cell walls, except the mentioned subterminal zones which were unstained with TBO. With alcian blue at pH 0·5, only the outer tangential walls and the boundary zone reacted (not shown).

The autofluorescence of the hilar palisade layer (Fig. 4a,b) varied in luminescence basically similar to the extrahilar palisade layer. By means of the fluorochrome fluorol yellow the lipidic components in the counterpalisade and in the palisade layer were displayed. Figure 4c shows the subterminal counterpalisade zones and the light line in the underlying palisade layer fluorescing particularly strongly in yellow.

Aril. Some results of the histochemical tests in bright-field microscopy are summarized in Table 2. The principal results for the aril were the following: with TBO at pH 4·4 the cell walls and their thickenings reacted positively, the first in a remarkably strong way, staining metachromatically in reddish-blue (Fig. 3c,d, arrows), whereas the intercellular substance showed an orthochromatic light blue staining (Fig. 3d, arrow heads). The remarkable difference between the cell walls and the intercellular substance pointed out the structure analysed in the ultrastructural test. In particular in the C region, where the intercellular substance is rare and the cells are thick-walled, the tissue stained uniformly reddish-blue whereas in regions B and A the metachromatic stain of the cell walls was alternated with the orthochromatic light blue staining of the intercellular substance. Ruthenium red pointed out the pectin composition of the cell walls and their thickenings. The intercellular substance also reacted to ruthenium red, although more faintly (Fig. 3e). After treatment with alcian blue at pH 2·5 the thickenings above all reacted positively (Fig. 3f). These were the only portions showing affinity for the basic stain alcian blue pH 0·5 (not shown). Using contrast stain such as safranin-fast green it was possible to identify differences in the composition of the aril cells. The cell walls stained blue-green whereas the cell content stained red. Further studies on the cell contents by means of naphthol blue black and iodine-potassium iodide indicated the presence of a large quantity of proteins and starch granules respectively (not shown).

Concerning fluorescence microscopy, the following results were obtained: in longitudinal sections the inner zone of the aril showed a strong red/red-yellow autofluorescence, whereas the outer zone fluoresced strongly in green/green-yellow (Fig. 4a, b). The above zones corresponded to regions B+C (inner zone) and A (outer zone), respectively, considered in the ultrastructural test. The intense autofluorescence of the aril made it impossible to identify the thickenings. More information at cell level was obtained studying the macerated cells. Observing them in fluorescent light with an H_2 filter the three types of autofluorescence (red, green and yellow), found in longitudinal

Table 1. Histochemical reactions in the cells of the hilar region of the *C. proliferus* seed

	Palisade			Counterpalisade				
	Radial walls	Outer tangential walls	Inner tangential walls	Cell walls	Subterminal zones	Tracheid bar	Boundary zone	Light line
TBO Different polyanions	+	++	+	+++	+/0	++ green	++	0
Alcian blue pH 2·5 Faintly acid polysaccharides	+	++	+	+	0	0	++	0
Alcian blue pH 0·5 Hightly acid polysaccharides	0	+	0	0	0	0	+	0

Reactions were as follows: +++, very intense; ++, intense; +, good; 0, no colour.

Table 2. Histochemical reactions in the *C. proliferus* aril

	Cell walls	Thickenings	Intercellular substance	Cell content
TBO Different polyanions	+ + +	+ +	+ light blue	0
Ruthenium red Pectins	+ + +	+ +	0/+	0
Alcian blue pH 2·5 Faintly acid polysaccharides	+	+ +	0/+	0
Alcian blue pH 0·5 Hightly acid polysaccharides	0	+	0	0
Safranin-fast green Contrast stain	+ + blue-green	+ +/+ blue-green	+ blue-green	+ + red
Auto-fluorescence	+ + + green-yellow	⊗	+ + + green-yellow	+ + + red/yellow
F-PAS Polysaccharides	+ + + orange-red/green	+ + orange-red/green	+ + + yellow-orange	0/+ yellow
Fluorol yellow Lipids				+ + +
Nile red Lipids				+ + +

Reactions were as follows: + + +, very intense; + +, intense; +, good; 0, no colour.
⊗ The intense autofluorescence of the aril made the identification of the thickenings impossible.

section, were emphasized. The cells contained globular structures showing red fluorescence or the content appeared diffusely yellow fluorescent while the cell walls fluoresced green-green yellow (Fig. 4e). The rich lipidic composition of the aril was highlighted by means of fluorochrome fluorol yellow (Fig. 4d) and Nile red in the study of the macerated cells (not shown). The F-PAS reaction revealed a considerably heterogeneous polysaccharidic composition throughout the whole aril, with fluorescence varying from yellow or green to orange-red (Fig. 4f).

DISCUSSION

The seeds of *C. proliferus* had many anatomical features in common with other species of Leguminosae. They were classified by Corner (1976) as exotestal seeds because of the structure of the testa. The seeds were medium-sized and their hilar region corresponded to previous descriptions of other papilionoid species (Lersten & Gunn 1982). Our investigation showed that in *C. proliferus* seed the hilar region and, above all, the aril, had complex compositions and structures. The counterpalisade had a double nature, in that it had affinity both for stains such as TBO and alcian blue at pH 2·5 and for the lipid soluble fluorochrome fluorol yellow, which caused the cell walls and the cell contents, respectively, to react. In particular, the hydrophobic substances of lipidic

nature identified in the counterpalisade were organized into two layers at the extremities of the cells forming a double barrier to water entry. The light line, which was not stained by TBO, but fluoresced particularly strongly with fluorol yellow, could be thought of as another barrier to water penetration.The ultrastructural study allowed us to distinguish between three morphologically and functionally different regions: a loose peripheral region, consisting of large cells with wide lumina (A), a median region consisting of closely packed elongated cells lying parallel to the axis ridge-lobes with a slit-like lumina and copious thickenings (B) and a inner region of small thick-walled cells, close to one another and perpendicular to the same axis (C). The structural data were confirmed in optical microscopy by means of autofluorescence analysis and TBO staining. The autofluorescence highlighted a different behaviour of the aril in the previously mentioned tissues: the peripheral region fluoresced strongly in green/green-yellow, while the median and inner regions showed a strong red/red-yellow autofluorescence. The TBO stain pointed out the intense metachromasy of region C due to the thickness of the closely packed cell walls.The sponge-like tissue of the aril turned out to be the only part permeable to water, as shown by the test with crystal violet which penetrated it after a few hours. Water was able to enter thanks to the presence of polysaccharide hydrophilic compounds detected by ruthenium red, TBO and alcian blue at pH 2·5. Furthermore, the fact that the cell wall thickenings stained with alcian blue at also pH 0·5 revealed the presence of highly acid polysaccharides, showing a very high affinity for water. These data related to the water–seed interaction of *C. proliferus* aril agree with the findings by Bianchini & Pacini (1996) for *Ricinus communis* and by Lisci *et al.* (1996) for some angiosperm species. The former authors demonstrated that the elaiosome (caruncle) of *R. communis* enabled seeds to germinate in conditions that were too dry for seeds without elaiosomes. Lisci *et al.* (1996) suggested that the pectin wall thickenings, similar to those shown by us in *C. proliferus* aril, were an essential requirement for creating a water reserve. The regional differentiation of the aril of *C. proliferus* might also be interpreted as an anatomical adaptation for accumulating water. Owing to the high ratio of cell walls (including their thickenings) to the surface area, the middle region (B) would be the major site of apoplastic absorption of water. The features of the inner region (C), made of cells with narrow lumina, very thick walls and showing cells with a high polarization in the direction of the counterpalisade cells, led us to suppose that this region has the purpose of directing the flux of water towards the hilar valve and simultaneously that it acts as a mechanical reinforcement. In particular, the intense staining with TBO and alcian blue at pH 2·5 plus the good staining with alcian blue at pH 0·5 of the boundary zone led us to suppose that the water, having overcome the barriers preventing entry (owing to structural changes in the seed coat) moves through this narrow zone between the tracheid bar and the adjacent stellate parenchyma further into the seed in a manner similar to that described previously for *Lupinus angustifolius* (Serrato-Valenti *et al.* 1989). Furthermore, our hypothesis about the double role of the inner region (C) is supported by the data regarding the *Strelitzia reginae* aril, made up of orange hair-like formations starting from an outgrowth zone (Serrato-Valenti *et al.* 1991). We suggested that the *S. reginae* aril threads might play a role of some importance as water absorbing organs. In addition the thickenings, which are clover leaf-like in *S. reginae* aril, together with the outgrowth zone, might provide a higher degree of rigidity.

The ecological importance of seed hardness and consequently of dormancy is well known. It is a common occurrence in plant families found in arid and semiarid regions

(Kiegel 1995). It is largely adopted by Leguminosae Papilionoideae allowing dispersal in time rather than in space, when the probability of finding a suitable environment locally or temporally is greater than that of finding one at a greater distance or immediately (Harper 1982, as cited by Van Staden *et al.* 1989). Obviously, the realization of this strategy requires structures that allow the water to flow towards the environment during seed maturation and, on the other hand, prevent water entry when conditions are not favourable for germination. In some members of Papilionoideae, Hyde (1954) described the valve-like action of the hilum that plays a role in water loss increasing the duration of the seed impermeable condition. Also, Rangaswamy & Nandakumar (1985) demonstrated for *Rhynchosia minima* that the micropyle and the hilum functioned as hygroscopic valves. Van Staden *et al.* (1989), investigating a possible evolution of the legume seed, suggested that the valve-like hilar region represent an apomorphism within the Leguminosae. This permitted precisely controlled desiccation and a dimorphic/polymorphic dormancy suited to exploitation of very arid seasonal and moist temperate areas alike. The hydrophilic aril of *C. proliferus* directly connected to the hilar region could therefore provide a large dehydrating surface, which would be useful during seed maturation. On the other hand, in favourable environmental conditions for germination and after structural modifications of the integument due to microbial attack, abrasion by soil particles or temperature fluctuation (Egley 1989; Bewley & Black 1994) have occurred, the aril could act as a water reserve, absorbing water from the soil and transferring it to the rest of the seed. This would allow the seed to germinate in dry environment, as reported previously for the elaiosome of some other angiosperms (Lisci *et al.* 1996).

In addition, regarding the dispersal function, in the *C. proliferus* aril the abundance of lipid substances, together with other nutrients such as proteins and starch, revealed a probable adaptation to myrmechory. Van der Pijl (1982, quoted by Bianchini & Pacini 1996) points out that these substances in the aril play no role in germination but seem designed to attract and reward ants. Myrmechory is a common strategy in the Leguminosae. McKey (1989) reports that among the angiosperms legumes are probably rivaled only by *Euphorbiaceae* in the diversity of their ecological interactions with ants and the number of species involved. Legumes with seeds suitable for myrmechory occur in all three subfamilies, but are most frequent in the Papilionoideae. Within this last subfamily, this type of seeds occurs in different tribes including *Genisteae*. McKey's list of *Genisteae* comprises seven genera of plants with elaiosome bearing seeds including *Chamaecytisus*. According to the author, the higher frequency of elaiosome-like arils in the papilionoideae in comparison to the other two subfamilies was explained by the dry sites where these plants grow, since dispersal by ants offers the greatest selective advantage for different reasons: guarantee of seed dispersal at a lower nutrient cost than that of fleshy fruits attractive to birds, burial protects seeds against high temperatures on the soil surface during fires, burial reduces mortality due to seed predation on bare surfaces, possibility of germination in a nutrient rich microhabitat. Brew *et al.* (1989), in a study on the biochemical basis of dispersal of *Acacia myrtiflora* and *Tetratheca sfenocarpa* seeds by ants, reported a heterogeneous lipidic composition in the elaiosomes. The authors ascribed different functions to the different classes of lipids: while the nutrient composition of the elaiosome might provide the underlying selective advantage for ants in seed dispersal, specific compounds such as 1,2 diolein might manipulate their behaviour and maximize seed dispersal.

Further studies are in progress to examine closely the numerous aspects of the

problem. In particular, we feel that the nature and the features of the intercellular substance of the aril, as well as the quantification of the water-related functions (dehydration, hydration and water reserve) require additional detailed research.

ACKNOWLEDGEMENT

The authors would like to thank Dr Francesca Cambiaggi for her helpful suggestions and support.

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