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Institut für Botanik und Mikrobiologie der Technischen Universität München, München, Federal Republic of Germany

Welwitschia mirabilis: Fine Structure of the Germinating Seed I. Orientation

CHRIS H. BORNMAN, VALERIE BUTLER, and WILLIAM A. JENSEN[1])

With 10 figures

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Summary

In the germinating seed of the gnetalean plant *Welwitschia mirabilis* a collared zone of the embryo produces a rapidly-developing, positively geotropic, nonvascularized lateral protuberance. This structure, if it did act haustorially or absorptively as a feeder, would appear to have its closest parallel in morphology and function with other plants in the lepidophyte *Selaginella*. The lower, mucilaginous surface of the protuberance, which reaches its maximum length of approximately 5 mm six days after emergence of the radicle, remains closely cemented to the megagametophyte. The various regions of the gametophyte and the embryonic protuberance – especially their shared interface – that were studied cytochemically and ultrastructurally in order *inter alia* to clarify the feeder's function, are circumscribed.

Key words: Welwitschia, seed, gametophyte, feeder, germination.

Introduction

In comparison to the angiosperms, relatively few ultrastructural studies have been carried out on germinating gymnospermous seed (CHING, 1965, 1972; DURZAN et al., 1971; SIMOLA, 1974, 1976) and except for one study, investigations in the case of *Welwitschia mirabilis* (BORNMAN et al., 1976) have been restricted to the light microscope (HOOKER, 1863; BOWER, 1880, 1881; NAUDIN, 1882, SYKES, 1910; HILL and DE FRAINE, 1910; PEARSON, 1929; RODIN, 1953; MARTENS and WATERKEYN, 1964; BUTLER et al., 1973). Although a member of the Gymnospermae, *Welwitschia* displays certain morphological and physiological features that may be regarded as

[1]) Department of Instruction in Biology, University of California, Berkeley, California, USA.

angiospermous (BORNMAN, 1977). It is an endemic of the Namib Desert, occupying a singular position among vascular plants (BORNMAN, 1978), and apart from its grotesque habit, its ability to survive under conditions of severe environmental stress makes it a subject of considerable interest. To forge the link between reproduction and survival, those of its seeds that germinate have to do so readily and rapidly.

The germinating embryo produces a protuberant outgrowth that remains embedded in the seed. This emergence is non-vascularized and was termed *feeder* by BOWER in 1881, although he had no evidence that it functioned suctorially. It is known, for example from the work of WILKINS and WAIN (1975), that root cap cells are geoperceptive. This is of interest in *Welwitschia* as the ventral cells of the embryonic feeder appear to be of root cap origin, and the apparent positive geotropism of this protuberance is commented on in our study. We examined the seed in detail from the point of view of its structure, chemical composition and the resultant changes during germination. Part of our study is devoted to the interrelationship between the embryo – sporophyte – and its encompassing nutritive tissue – megagametophyte – which, incidentally, is erroneously referred to as endosperm throughout most of the literature (e. g. BOWER, 1881; PEARSON, 1929; MARTENS, 1971). The ultrastructure of the quiescent (dry) and hydrated gametophyte and embryo as well as that of their shared interface, including the degradation, mobilization and fate of the storage products, and the cytochemistry of these tissues, will be considered in subsequent papers. However, in the case of the embryo we shall only focus attention on the salient features of the collar and the feeder.

It seems advisable as a background to a fuller understanding of the ultrastructural and cytochemical changes that occur to comment briefly on the morphology and anatomy of the germinating *Welwitschia* seed. In this paper detailed reference is also made to procedures.

Materials and Methods

Fresh, plump seeds from the mid-region of the megastrobilus were surface-sterilized in 1 % Cetavlon, denuded of the outer seedcoat and germinated at 25 ± 1 °C. For light microscopy the material was fixed in 10 % acrolein, dehydrated and embedded in either Tissuemat or glycol methacrylate (JENSEN, 1962; FEDER and O'BRIEN, 1968). Sections of material ranging from quiescent seeds to 8-day-old seedlings were stained with periodic acid-Schiff (PAS) reagent (JENSEN, 1962), mercuric bromphenol blue (PEARSE, 1961), toluidene blue (JACOBSEN et al., 1971) and Sudan IV. For electron microscopy diced tissue pieces were fixed in 6 % glutaraldehyde buffered with 0.05 M sodium cacodylate for 6h at 4 °C and post-fixed in similarly buffered 2 % OsO_4. pH of all buffered solutions was 7.2. During the graded acetone dehydration the tissues remained overnight in 70 % acetone containing 1 % uranyl nitrate. The material was embedded in Araldite-Epon and the sections stained on lead citrate (REYNOLDS, 1963).

Cytochemical Procedures

BERJAK's (1968) modified GOMORI (1952) method was used for localizing acid, phosphatase. Tissue pieces were fixed in acetate-buffered (pH = 7.2) 6 % glutaraldehyde, followed

by three washes in acetate buffers of descending pH, namely 7.2, 6.0 and 5.0. The tissues were then incubated in Gomori medium at 37 °C for 1h, washed in pH 5.0 acetate buffer for 10 min, 2 % acetic acid for 1 min and acetate (pH 7.2) for 5 min. Post-fixation was carried out in Luft's permanganate buffered with phosphate at pH 7.2 and dehydration with a graded alcohol series. As control, material was processed in a similar manner but with 0.42 % sodium fluoride added to the Gomori medium as an enzyme inhibitor. Sections were stained on lead citrate.

The silver hexamine test for carbohydrate was performed according to RAMBOURG (1967). Sections of conventionally prepared EM material were floated on 1 % periodic acid for 20 min. After three brief rinses, sections were stored overnight on distilled water. They were then stained on 3 % silver hexamine for 30 min at 60 °C, rinsed and restained for another 30 min. After a further rinsing the section were floated on 5 % sodium thiosulphate for 7 min. Controls were prepared by staining sections without previous hydrolysis on periodic acid.

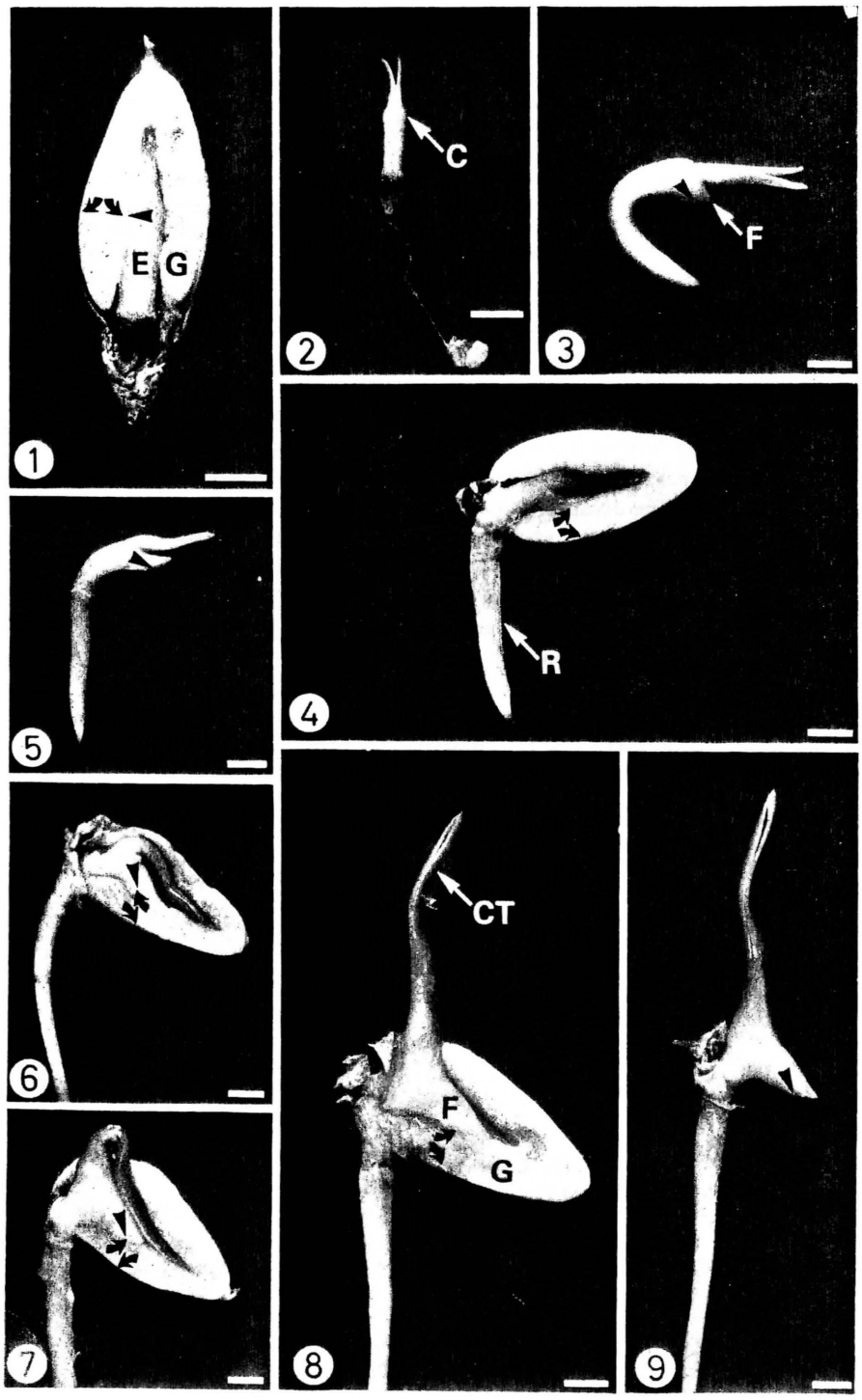
Biochemical Analyses

Embryos of unimbibed dry seed and of 2-day and 5-day treatments were removed and the gametophyte dried to constant weight at 100 °C. The decrease in gametophyte mass over the experimental period was calculated and the material ground for the determination of fats, free fatty acids, protein and free amino acids. Fats were quantitatively extracted with diethylether in a Soxhlet apparatus. Free amino acids in fat-extracted material were dissolved in 70 % ethanol and quantitatively separated from the protein fraction by ultrafiltration through an Amicon Diaflo conical filter. The material containing the protein fraction was quantitatively removed from the filter and hydrolyzed *in vacuo* with 6 N HCl for 24 h at 110 °C. The amino acids from both the free amino acid fraction and the protein hydrolysate were separated on a Beckman 120B amino acid analyzer using SPECKMAN's (1963) double column accelerated system. The South African Bureau of Standards carried out the determination of fatty acid content and composition of fat extracted by us from the gametophyte at each of the three stages.

Results

In the dry state a *Welwitschia* seed is ca. 7 mm long, 5 mm wide and 2–3 mm thick, and has a mass of about 120 mg. The embryo is shrunken and brittle and often separated from the surrounding gametophyte by a narrow space. When fully imbibed (after 7 h at 25 °C), the embryo becomes swollen and pressed against the now moist, spongy gametophyte (Fig. 1). Figure 2 shows the embryo in Fig. 1 after removal of the gametophyte tissue. It consists of a long radicle and a short hypocotyl separated by a uniform bulge, the collar (BOWER, 1881). The shoot apex is situated between two laterally compressed cotyledons. The tip of the radicle is enclosed in a loose-fitting coleorhiza-like cap of dead cells to which a coiled suspensor is attached.

Soon after germination is initiated the collar forms a protuberance or lateral process on the lower surface of the embryo. This process was called feeder by BOWER (1881) who ascribed to it an absorptive function. By the time the radicle reaches a length of 10 mm (2 days after germination) the feeder is already conspicuous (Fig. 3, arrow). Growth of the feeder is rapid. Figure 4 shows the relative sizes of root, plumule and feeder 3 days after germination. The wedge-like shape of the feeder is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shown by Fig. 5. As the feeder continues its growth the hypocotyl lengthens and as shown in Fig. 6 (4 days after germination), is just beginning to arch upwards prior to its looped emergence from the seed. The close contact between feeder and gametophyte is depicted in Fig. 7 (5 days after germination). Force is required to separate them as they tend to become firmly cemented together. The hooked hypocotyl has now emerged and the cotyledons are in the process of emergence. At the 6-day-stage the cotyledons are completely withdrawn from the seed (Fig. 8). The mature feeder (Fig. 9, excised seedling of Fig. 8) is about 5 mm long with a convex lower surface, a flatter upper surface and a curved edge. The feeder has been variously described as wedgeshaped (BOWER, 1881), footlike or spadelike (COULTER and CHAMBERLAIN, 1910).

Discussion

The feeder invariably develops from the lower surface of the collar irrespective of the side on which the laterally compressed seed is placed. Even when seeds are placed on edge the feeder forms on the lower surface. If seeds are germinated on a vertical rotating wheel the feeder grows from any position on the collar. When they are germinated vertically with the blunt end of the seed projecting upwards the feeder grows downward from the collar in the form of a frill. This suggests that the feeder responds positively to geotropic stimulus.

Morphologically the feeder is regarded as an emergence, resulting from elongation and division of cortical tissue in the collar region (BOWER, 1881; MARTENS, 1971). It is non-vascularised, consisting of elongated thin-walled cells. The four vascular bundles of the hypocotyl enlarge as they traverse the collar zone, especially the two nearest the feeder which curve outward toward it but do not enter it (Fig. 10). The curvature seems to be the result of cell division in this area. Serial transverse and longitudinal sections reveal a slight increase in amount of phloem in all four bundles in the collar zone, but xylem increase is dramatic and is greatest in the region opposite the feeder (BUTLER, 1970). If the feeder in fact is an absorptive organ it might be responsible not only for uptake of nutritive material but also of water.

Figs 1–9: Seedling development. – Figs 1, 4, 6–8. Seedcoat and portion of gametophyte removed. – Figs 2–3, 5, 9. Seedcoat and gametophyte removed. – Fig 1. Mature embryo (E) in fully imbibed gametophyte (G). – Fig 2. Embryo of Fig 1 after removal of gametophyte. A uniform swelling or collar (C) separates cylindrical radicle and hypocotyl which bears two cotyledons. – Fig. 3. Excised embryo after 2 days germination. A feeder (F) has begun forming laterally from the collar. – Fig 4. Embryo 3 days after germination. R, radicle. – Fig 5. Excised embryo of Fig 4. Note wedge-like shape of feeder. – Fig 6. Embryo 4 days after germination. Hypocotyl has lengthened and is beginning to arch. Fig 7. Seedling 5 days after germination showing looped emergence of plumule. – Fig 8. Seedling 6 days after germination. Plumule is erect. CT, cotyledon. – Fig 9. Seedling of Fig 8 after removal of gametophyte. Feeder is mature. Curved arrows and arrowheads indicate regions of gametophyte and feeder isolated for ultrastructural studies (see Discussion). Bars represent 2 mm.

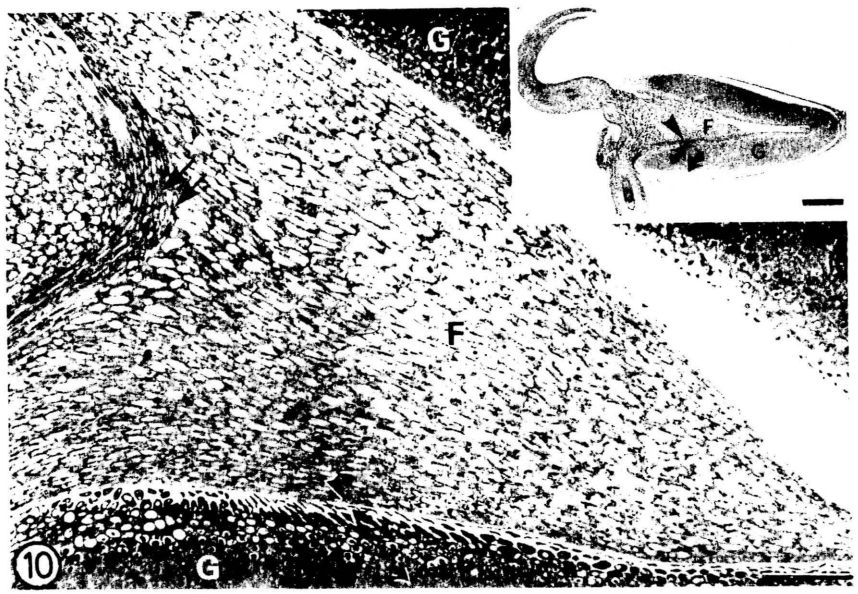

Fig 10: Light micrograph of LS of 6-day-old feeder showing its non-vascularised nature and the close contact between its ventral surface and the gametophyte. Arrows and arrowheads indicate gametophyte (G) and feeder (F) interface cells studied. Hypocotylary vascular bundles nearest the feeder curve toward its base (double arrow) but do not enter it. Inset: low magnification of LS through 6-day-old seedling. Bars represent 0.5 mm (Fig 10) and 2 mm (Inset).

During early germination (Figs. 3–7) the plumule separates the upper surface of the feeder from the gametophytic tissue. When the cotyledons have emerged (Fig. 8), a narrow space is left between upper feeder and gametophyte (Fig. 10 and inset). Presumably most absorption is via the lower mucilaginous surface of the feeder which remains in close contact with the gametophyte (Fig. 8, 10). In the seeds of other higher plants, vascularised reduced cotyledons (e. g. Palmae, Gramineae, *Cycas, Ginkgo*) or haustoria which develop from the suspensor (e. g. *Tropaeolum*) do act as absorptive organs (BOWER, 1881). A haustorial-like structure similar to the feeder of *Welwitschia* occurs in some members of the Cucurbitaceae (FLAHAULT, 1877). Although morphologically it is equivalent to the feeder of *Welwitschia*, it is strictly speaking not an absorptive organ as the seeds are exalbuminous. FLAHAULT attributes to it the mechanical function of aiding the escape of the cotyledons from the testa. He also describes a similar structure in *Mirabilis jalapa* which produces albuminous seed. However, even here, he attributes to it a mechanical function only. The feeder of *Welwitschia* could very effectively fulfill a mechanical role in aiding cotyledon escape, as the feeder and gametophyte become firmly welded together providing the plumule with a base during its withdrawal from the seed. However, if the feeder of

Welwitschia is also an absorptive organ, the closest parallel in morphology and function with other plants would appear to be found in *Selaginella*, a member of the Lepidophyta; the non-vascularised foot of *Selaginella martensii*, for example, is also produced by lateral extension and division of cortical tissue of the hypocotyledonary stem. Those regions of the quiescent and germinating seeds on which the respective ultrastructural and cytochemical investigations were carried out – and to be reported on in subsequent papers – are indicated on the figures as follows: outer or deeply-situated cells and interface cells of the gametophyte – days 0 and 1 (Fig. 1) and days 3–6 (Figs. 4, 6–8, 10) by curved arrows; and cells of the collar and developing feeder – days 0 and 1 (Fig. 1), day 2 (Fig. 3), and days 3–6 (Figs. 5–7, 9–10) by arrowheads. Day 0 refers to the dry or unimbibed or quiescent state and the numerals 1–6 represent the respective days after imbibition or hydration, and which, incidentally, coincide with the emergence of the radicle.

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Prof. CHRIS H. BORNMAN, Department of Plant Physiology, University of Lund, Fack, S-220 07 Lund, Sweden.