WALL PROJECTIONS IN THE SPOROPHYTE AND GAMETOPHYTE OF *SPHAEROCARPOS*

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INTRODUCTION

The nutrition of the sporophyte in *Sphaerocarpos* and liverworts in general has long been controversial. It has not yet been determined whether the chloroplasts in the sporophyte capsule do, in fact, photosynthesize or whether the carbohydrates stored as starch are translocated to the sporophyte from the gametophyte which is known to be photosynthetic and to which the sporophyte remains attached throughout its development. Although movement of carbohydrates from the gametophyte to the sporophyte has not been shown, this has long been thought to occur.

It is the purpose of this report to describe the nature of the juncture between the sporophyte and gametophyte of *Sphaerocarpos donnellii* Aust., with particular emphasis on specialized wall protuberances found in this region. The possible significance of these protuberances in translocation and their relationship to similar structures found in other plants will be discussed.

MATERIALS AND METHODS

Young sporophytes with a small amount of attached gametophytic tissue were immersed in a 6% glutaraldehyde solution in 0.05 M phosphate buffer at pH 7.3 and fixed for 2 hr at room temperature. Fixation was followed by successive washing in phosphate buffer, after which the tissue was post-fixed in 2% osmium tetroxide in veronal-acetate buffer (8) at room temperature for 1½ hr.

The sporophytes were dehydrated in a graded acetone series and embedded in Epon 812 (6). Sections cut on a Porter-Blum MT-2 microtome were stained with uranyl magnesium acetate (2) for 10 min and lead citrate (10) for 5 min and were examined with an RCA EMU-3H electron microscope.

Material for light microscopy was fixed in FAA (formalin, acetic acid, and alcohol) dehydrated through alcohol and embedded in Tissuemat (Fisher Scientific Company). Sections were cut at 10 μ and stained for total carbohydrate with periodic acid–Schiff's (PAS) reagent and for nucleic acids with Azure B (4).

RESULTS

The general morphology of the sporophyte of *Sphaerocarpos* is shown in Fig. 1 in a median longitudinal section. The cell types to be discussed here are those of the sporophyte foot and of the gametophytic tissue immediately surrounding the foot. Cells of the foot are quite large, often measuring 40 μ in width and 60 μ in length. The cytoplasm is strongly basophilic and gives a positive reaction with Schiff's reagent. At the ultrastructural level many large, often elongate, plastids containing thylakoids and varying quantities of starch are visible (Fig. 2). Numerous mitochondria are distributed throughout the cytoplasm of the foot cells, many of them being in the area of the wall projections (Figs. 2 and 3). The

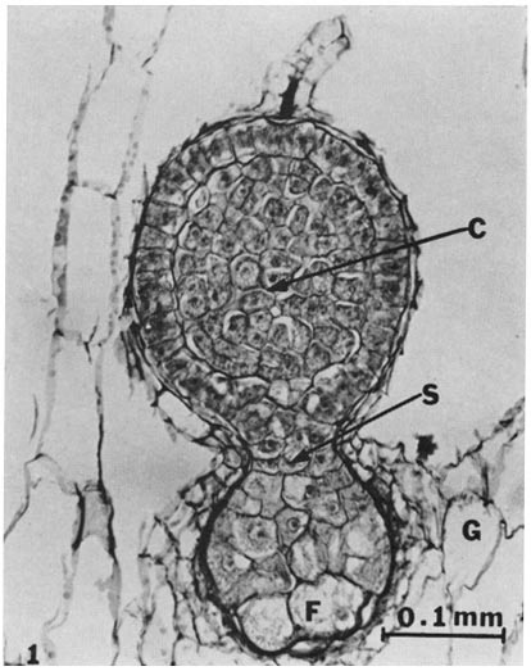

FIGURE 1 Light micrograph of a sporophyte of *Sphaerocarpos donnellii* showing the capsule (*C*), seta or stalk (*S*), foot (*F*), and gametophytic tissue (*G*). Periodic acid–Schiff's reagent. × 164.

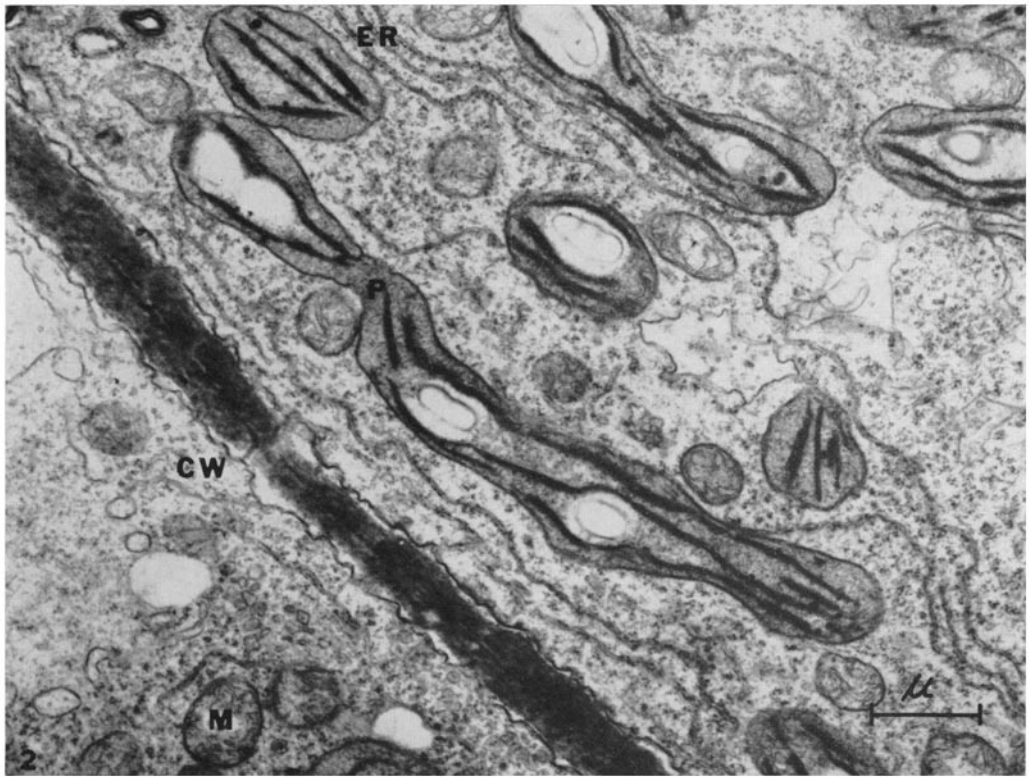

FIGURE 2 Electron micrograph showing two adjacent cells in the sporophyte foot. Plastids (*P*) are often elongate. The endoplasmic reticulum (*ER*) is arranged in parallel rows, and the cell wall (*CW*) lacks projections. Several mitochondria (*M*) can be seen. × 14,600.

cytoplasmic ground substance is filled with polyribosomes, and the rough endoplasmic reticulum is frequently arranged in parallel rows (Fig. 2). Dictyosomes containing three to five cisternae with associated vesicles are abundant (Fig. 3). Wall projections which react positively for carbohydrates are present on those walls of the foot cells immediately adjacent to the gametophyte (Fig. 3). The cell walls delimiting two contiguous foot cells lack such projections (Fig. 2). Depending on the plane of section, the fibrillar wall projections are seen either as short, finger-like protuberances or as a network-like proliferation of wall material. The projections are always bounded by the plasma membrane.

The gametophytic cells adjacent to the sporophyte foot have plastids with many plastoglobuli (Fig. 3) and fewer mitochondria per cell than do the foot cells. Projections are found on the walls of the gametophytic cells which are contiguous with the sporophyte foot. They are not present on the walls separating two adjacent gametophytic cells. Like those of the foot, the projections stain intensely after PAS treatment, are bounded by the plasma membrane, and have a fibrillar appearance. Wall proliferations observed in the gametophytic cells are reticulate (Fig. 3).

Plasmodesmata have not been found in the cell walls between the sporophyte and gametophyte, nor would one expect to find them if one considers the ontogenetic relationship between the sporophyte and gametophyte. Numerous clustered vesicles which appear to be enclosed by a double membrane occur in the space between adjacent sporophyte and gametophyte cell walls (Fig. 4).

DISCUSSION

Several recent reports have described the occurrence of cell wall protuberances and extensions in

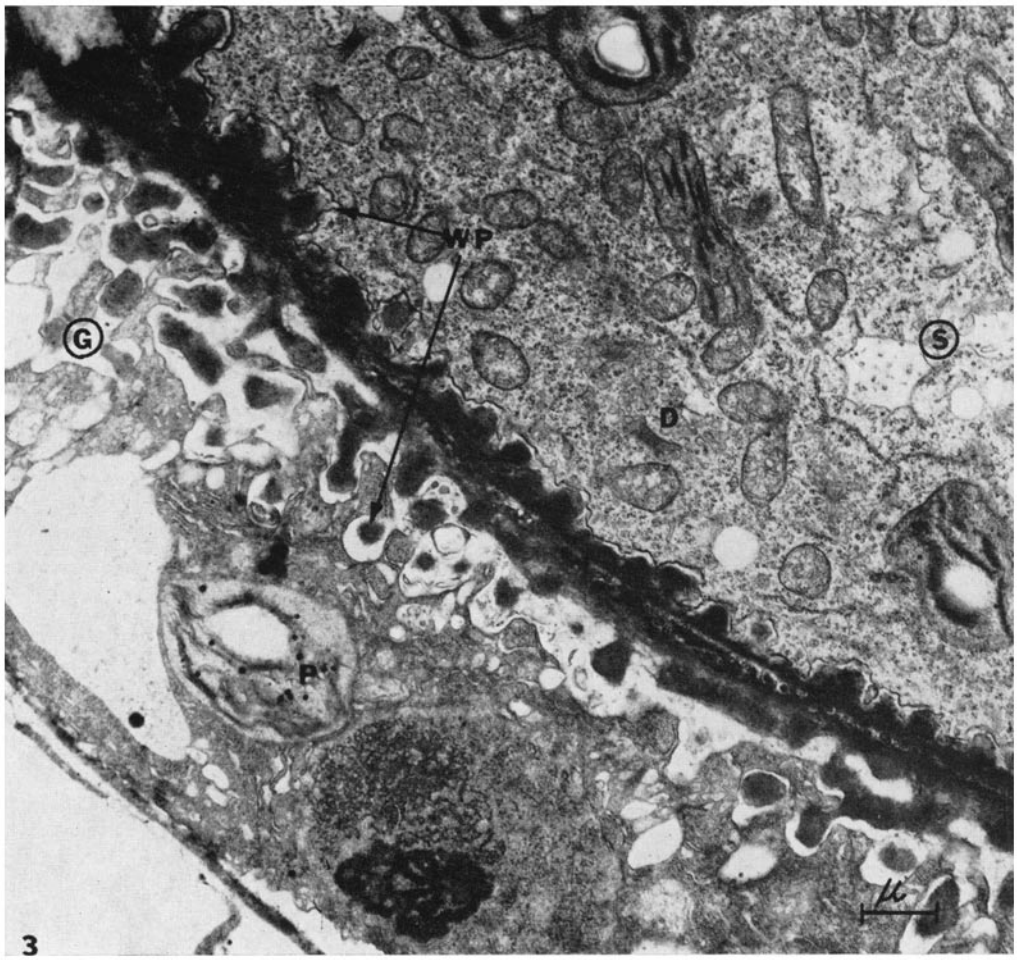

FIGURE 3 An electron micrograph of the cells located at the juncture of sporophyte (*S*) and gametophyte (G). The wall projections (*WP*) occur along the walls of adjacent sporophytic foot cell and gametophytic cell. Dictyosome (*D*) is indicated in the foot cell. Plastid (*P*) is shown in the gametophytic cell. × 9,500.

the suspensor of *Phaeseolus* (1), the transfer cells of *Pisum* and *Lupinus* (3), cotton synergids (5), the sporophyte foot of the moss, *Polytrichum* (7), and the digestive gland of Venus's flytrap (9). Wall protuberances characteristically have been observed in cells presumed to have a role in absorption. Gunning et al. (3) calculated that the projections in *Pisum* transfer cells increase the absorptive surface of the plasma membrane by tenfold. The presence of the projections in cells be can considered as indirect evidence that they are involved in absorption.

Since sporophyte nutrition has been thought to occur via absorption of metabolites from the gametophyte, it is not surprising that wall projections are present in the sporophyte foot cells. It was unexpected, however, to find that wall projections also extend into the gametophytic tissue adjacent to the foot cells. The presence of wall projections on both sides of the juncture of sporophyte and gametophyte would serve to increase even more the area available for absorption and translocation.

Associated with the wall projections of the foot cells of *Sphaerocarpos*, as well as with the filiform apparatus of the cotton synergids (5), are large numbers of mitochondria. Mitochondrial number and distribution apparently reflect the energy

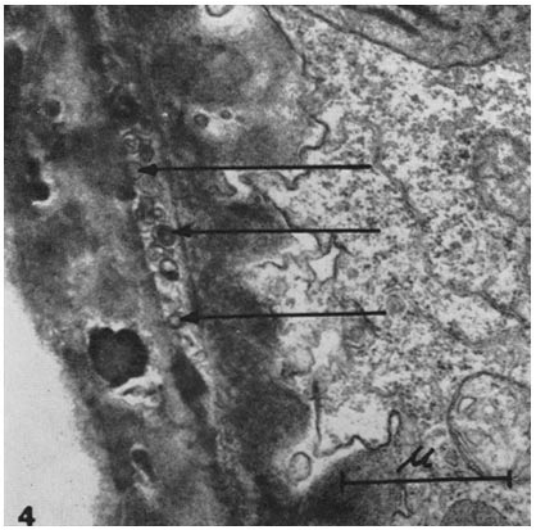

FIGURE 4 Electron micrograph of clustered vesicles (indicated by arrows) between the cell walls. × 22,400.

required for absorption and movement of nutrients across the cell membranes. Well differentiated plastids containing starch and extensive grana are found in the cells of the foot. Whether these plastids are photosynthetic is not known. However, experiments conducted on sporophytes excised at the same stage of development as those described here and grown in the light indicate that normal sporophyte development will not proceed unless glucose is supplied exogenously in the culture medium.

Structures similar to the vesicles observed at the juncture of the sporophyte and gametophyte occur in the intercellular spaces of the sporophyte capsule during its maturation. In the capsule, these vesicles originate as an accumulation of Golgi secretory vesicles into a multivesicular body which then empties its contents at the cell wall. However, the origin of the vesicles found at the sporophyte-gametophyte juncture is still unknown.

The wall projections in *Sphaerocarpos* are similar to others that have been reported in that they give a positive reaction to the PAS test, and are, therefore, a real extension of carbohydrate wall material. Unlike the wall projections in the digestive gland of Venus's flytrap (9), which occasionally appear darker than the cell wall proper, and the projections in the sporophyte foot of *Polytrichum* (7), which accumulate electron-opaque material, the wall projections in *Sphaerocarpos* appear to be similar in structure and composition to the cell wall proper.

It has been noted in *Pisum* transfer cells (3), as well as in the digestive gland of Venus's flytrap (9), that a space separates the plasma membrane from the wall projections. This space may result from fixation damage (3), or may be a zone involved in the secretory process itself (9). It is interesting to note that in *Sphaerocarpos* this "interfacial zone" is found in both gametophytic and sporophytic cells, but it is always a wider zone in the gametophytic cells (Fig. 3).

Wall projections previously described occur only in cells into which materials are thought to be absorbed. *Sphaerocarpos* differs in that projections occur not only in those cells into which absorption occurs, but also in the cells which are involved in translocation. The presence of wall elaborations on contiguous cell walls increases the total amount of membrane surface area available for active transport, thereby efficiently increasing the potential rate of solute movement between plant cells which may lack plasmodesmata.

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