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Intercellular pectic protuberances in *Hymenaea stigonocarpa* (Fabaceae, Caesalpinioideae): Occurrence and functional aspects

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Abstract

A study of the anatomy and ultrastructural aspects of leaf mesophyll and floral nectaries of *Hymenaea stigonocarpa* Mart. ex Hayne revealed the presence of intercellular pectic protuberances (IPPs) linking adjacent cells in both the leaf palisade cells and the secretory parenchyma of the floral nectary. Samples of the middle third of the leaf blade and of floral nectaries in anthesis were collected, fixed, and processed using standard procedures for light, transmission, and scanning electron microscopies. The IPPs of palisade cells of the mesophyll and the secretory parenchyma cells of the floral nectary take the form of scalae or strands, respectively. No evidence of the specific synthesis of these structures was observed, and they are apparently formed by the separation of adjacent cells due to cell expansion, when intercellular spaces develop. The IPPs observed in *H. stigonocarpa* increase cellular contact and probably act in apoplastic transport. ***To cite this article: E.A.S. Paiva, S.R. Machado, C. R. Biologies 331 (2008).***

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1. Introduction

Intercellular pectic protuberances (IPPs) have been observed in many plant *taxa* and in numerous plant tissues [1–5]. Due to the diverse shapes assumed by these protuberances, they have received many designations in the literature, including: intercellular wall thickenings, pectic strands, pectic filaments, pectic warts, scalae, microprojections, bead-like projections, papilla-like structures, protuberances, intercellular protuberances, and intercellular pectic protuberances (see [6]). The development of these structures is usually associated with the formation of intercellular spaces during cell expansion [4], although the formation of IPPs has been reported even after the development of intercellular spaces – and in these cases they have been observed to form by secretions from the protoplast [7]. There is still much controversy surrounding the origin, chemical composition, and function of these intercellular structures [4,7,8].

According to Potgieter and van Wyk [4], any biological functions attributed to the IPPs are still speculative,

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and include their involvement in apoplastic transport, hydration of the cell wall, reserve storage, cellular adhesion, and defense against pathogens. Although very few studies have examined this subject in any detail, it is quite possible that their diverse morphological forms do in fact reflect distinct functions – but there is growing evidence that IPPs interconnecting adjacent cells facilitate apoplastic transport [2,9].

The presence of IPPs connecting palisade cells is a common characteristic in the leaves of most angiosperm species [2]. Among the Fabaceae, these structures have also been reported in the seeds (see [1]) and stomata of *Vicia faba* [10].

We report here the occurrence of IPPs linking parenchyma cells of the mesophyll and secretory cells of the floral nectary of *Hymenaea stigonocarpa* (a species typical of the cerrado vegetation of central Brazil) and propose hypotheses concerning their function.

2. Materials and methods

The middle third section of the leaflet blade of mature leaves and the secretory tissue of the floral nectary, at anthesis, were collected and fixed in a Karnovsky solution [11] for 24 h. To prepare the specimens for light-microscopy studies, the fixed specimens were processed and embedded in historesin (Leica Embedding Kit) following standard methods, and sectioned transversely and longitudinally at 2 to 4 μm. The sections were mounted on slides and stained with toluidine blue [12]. Ruthenium red was employed to detect pectic compounds [13].

For examination under transmission electron microscopy, samples were fixed in a Karnovsky solution (Karnovsky, 1965) for 24 h, post-fixed in osmium-tetroxide (1%, 0.1 M phosphate buffer, pH 7.2) for 2 h, dehydrated in an acetone series and embedded in araldite resin [14]. Ultra-thin sections were stained with uranyl acetate and lead citrate, and examined using a Philips CM 100 transmission electron microscope at 60 kV.

For observation by scanning electron microscopy, leaf sections were dehydrated in the presence of silica gel at 50 °C until attaining a constant weight, fractured, mounted on aluminum supports, and subsequently gold coated. Hypanthium samples were fixed in a Karnovsky solution [11] as described earlier, dehydrated in an ethanol series, submitted to critical point drying, and subsequently gold coated [15]. The specimens were examined using a Philips SEM-515 scanning electron microscope.

3. Results

3.1. Intercellular pectic protuberances in leaf mesophyll

The mature leaves of *H. stigonocarpa* have a homogeneous mesophyll formed of various layers of palisade cells that are connected by intercellular pectic protuberances (IPPs) located predominantly on the anticlinal cell walls (Fig. 1A–D), besides usual cell contact by anticlinal walls. These protuberances take the form of filaments with a scalariform pattern (sometimes branching at the extremities), forming a web on the plane parallel to the major axis of the cells they connect (Fig. 1B and C).

The IPPs in the leaf mesophyll have frequently small, uniform diameters (0.05 to 0.4 μm) along their entire length – except at their extremities where discrete dilations increase their contact with the cell wall (Fig. 1E). These IPPs have a very homogeneous structure and no channels through their interiors were observed. Some IPPs demonstrated dilated sections and ramifications (Fig. 1F and G).

IPPs are difficult to observe under light microscopy as their diameters are very close to the resolution limit of this type of equipment. However, these structures stained deeply with ruthenium red and could be observed in some situations.

3.2. Intercellular pectic protuberances in floral nectaries

The flowers of *H. stigonocarpa* have well-developed floral nectaries vascularized with phloem, and the secretory parenchyma occupies a large portion of the hypanthium. Secretion is abundant, with the nectar being secreted into the space between the hypanthium and the stipe.

Sections through the parenchyma secretory region demonstrated numerous IPPs linking adjacent cells (Fig. 2A–D). These protuberances took the form of rarely branched strands and were distributed apparently randomly, without forming any discernable network. As in the leaf parenchyma mesophyll, these IPPs had a uniform thickness (0.05 to 0.2 μm) and were dilated only at their extremities, where they connected to the plant cell walls (Fig. 2D and E). The dilations observed at the extremities of the IPPs in the hypanthium were larger than those seen in the mesophyll.

Neither the cells of the mesophyll nor the secretory parenchyma of the floral nectaries demonstrated plasmodesmata directed towards the IPPs, nor was any other