Phyton (Austria)	Vol. 27	Fasc. 1	155-164	17. 7. 1987
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The Floral Nectaries of *Hibiscus rosa-sinensis* L. II. Plasmodesmatal Frequencies

By

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With 6 Figures

Received July 15, 1986

Key words: Floral nectaries, secretory hairs, plasmodesmatal frequencies; *Hibiscus rosa-sinensis*.

Summary

SAWIDIS Th., ELEFTHERIOU E. P. & TSEKOS I. 1987. The floral nectaries of *Hibiscus* rosa-sinensis L. II. Plasmodesmatal frequenties. – Phyton (Austria) 27 (1): 155–164, with 6 figures. – English with German summary.

Plasmodesmatal frequencies in all walls of the secretory hairs and the subglandular tissue of floral nectaries of *Hibiscus rosa-sinensis* L. have been estimated by means of electron microscopy. Results indicate that transversely oriented walls of the secretory hairs bear more plasmodesmata than longitudinally oriented ones. The greater numbers of plasmodesmata occur around the basal cell implying an important role for this cell in the collection and conveyance of pre-nectar from subglandular tissue towards the secretory hair. Great densities of plasmodesmata also occur in the walls of stalk cell, which provides a barrier to apoplastically carried substances but favours symplastically moving fluxes. The results are discussed in relation to findings in similar tissues of other plant species and are considered to support the view that pre-nectar follows a symplastic pathway from the phloem to secretory cells.

Zusammenfassung

SAWIDIS Th., ELEFTHERIOU E. P. & TSEKOS I. 1987. Die Floralnektarien von Hibiscus rosa-sinensis L. II. Plasmodesmen-Frequenzen. – Phyton (Austria) 27 (1): 155–164, mit 6 Abbildungen. – Englisch mit deutscher Zusammenfassung.

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Mittels des Elektronenmikroskops wurden die Häufigkeiten der Plasmodesmen in allen Wänden der Sekrethaare und des subglandulären Gewebes der Blüten-Nektarien von *Hibiscus rosa-sinensis* geschätzt. Die Ergebnisse zeigen, daß Querwände der Sekrethaare mehr Plasmodesmen besitzen als längsorientierte. Reichlich finden sich Plasmodesmen an der Basalzelle, was auf die wichtige Rolle dieser Zelle für die Sammlung und Weitertransport der Nektarvorstufen vom subglandulären Gewebe in das Sekrethaar hinweist. Groß ist die Plasmodesmendichte auch in den Wänden der Stielzellen, was eine Barriere für den apoplastischen Substanztransport bedeutet, den symplastischen hingegen unterstützt. Die Ergebnisse werden im Vergleich zu den an ähnlichen Geweben anderer Pflanzen erhaltenen diskutiert, sie werden als Stütze für die Ansicht angesehen, daß die Nektarvorstufen dem symplastischen Weg vom Phloem zu den Sekretzellen folgen.

Introduction

Nectaries are systems well suited for a variety of studies concerning particularly the elucidation of the structural specialization of plant cells for they combine an anatomical simplicity with an intensive activity. This anatomical simplicity provides an opportunity to assess the functional potential of each of the possible pathways of transport in the system, in particular the role of plasmodesmata (GUNNING & HUGHES 1976). There is strong evidence that plasmodesmata have a general function in cell-to-cell transport by providing a pathway of lower resistance than the plasmalemmas and the intervening wall that separate adjacent cells (see GUNNING & ROBARDS 1976, GUNNING & HUGHES 1976, WARMBRODT 1985, and the references cited therein).

Intercellular communication in plants through plasmodesmata has been reviewed and integrated in a volume by GUNNING & ROBARDS more than a decade ago. Plasmodesmata, being cytoplasmic strands connecting the protoplasts of neighbouring cells and encountered almost in all walls of living cells of higher plants, play an important role for short distance transport of solvents, solutes and the relay of stimuli (ROBARDS 1976, GUNNING & HUGHES 1976, FAHN 1982). In addition, accumulating evidence suggests that viruses move through plasmodesmata (GIBBS 1976). For these reasons plasmodesmata have attracted the attention of investigators in all disciplines of plant research. The extensive work of light microscopists has been supplemented and expanded by the advent of electron microscopy (see ROBARDS 1976).

In the present communication the distribution and frequency of plasmodesmata within the secretory hairs of nectaries of *Hibiscus rosa-sinensis* are described. This study, which represents the second in a series of investigations from this laboratory concerning the structure and function of nectaries (the first one refers to the development of secretory hairs, SAWIDIS & al. 1987), aims at contributing to the question of elucidating the mechanisms of intercellular transport.

Methods and Results

Active (secreting) floral nectaries of *H. rosa-sinensis* L. (one day preanthesis) have conventionally been prepared for electron microscopy. For the purpose of plasmodesmatal counting longitudinal and transverse sections to the secretory hairs were cut with a diamond knife on a Reichert-Jung Ultracut E ultramicrotome adjusting the ultra-feed system mechanism at 80 nm (0.08 μ m). Section thickness was critically judged and ascertained by the interference colour of the sections floating on the water trough (fairly gold to silver), according to the colour and thickness scale of PEACHEY (1958).



Fig. 1. Diagrammatic illustration of a secretory hair with cell nomenclature on the right, helpful for plasmadesmatal frequency estimations. Abbreviations on the left marking cell walls are explained in the text.

Floral nectaries of *H. rosa-sinensis* consist of numerous densely-packed secretory hairs occurring on the lower inner side of the calyx. Each hair is a multicellular structure built by several cell types, the general layout of which is diagrammatically illustrated by Fig. 1. Plasmodesmata occur between all neighbouring cells of secretory hairs, but in electron micrographs it seemed that there were differences in their frequency and distribution, especially between the upper and lower walls of a given cell when the latter is separating two different cell types (Figs. 2, 3, 4, 5, 6). In order to study comparatively the plasmodesmatal incidence, all walls shared by two contiguous cells have been given a discriptive name and allocated in 10 wall types, abbreviated as follows:

LS	=	Longitudinal wall of Secretory cell
CS	=	Cross wall of Secretory cell
LI	=	Longitudinal walls of Intermediate cells
CI	=	Cross walls of Intermediate cells
DS	=	Distal wall of Stalk cell
IS	=	Intermediate wall of Stalk cell
\mathbf{PS}	=	Proximal wall of Stalk cell
AB	=	Anticlinal wall of Basal cell
PB	H	Periclinal wall of Basal cell
ST	=	cell walls of Subglandular Tissue

The walls of the intermediate cells were classified in two groups depending on their orientation (cross, longitudinal), and were further collectively considered as two different wall types. In Fig. 1 walls represented by a broken line were not always encountered: it was estimated that the IS wall occurs in 40% of secretory hairs, while LS in only 0.5–1% of secretory cells (SAWIDIS & al. 1987).

Plasmodesmatal numbers were estimated from micrographs such as those of Figs. 2. 3. 4. 5. 6. but. in order to save time, photographic material and be able to take into account as many walls as possible, the greater part of this work was carried out by counting plasmodesmata directly on the microscope screen. Considering the diameter of the illuminated screen and the image magnification it is quite simple to measure the wall length under observation (Table 1). This method was used for longitudinally sectioned secretory hairs only, where walls are transversely and plasmodesmata longitudinally cut (Figs. 1, 2, 3, 4). For comparison, plasmodesmata have also been counted from micrographs from transversely sectioned secretory hairs, in which cell walls are tangentially cut; such sections provide face views of the cell walls in which plasmodesmata are transversely cut (Figs. 5, 6). However, the small proportion of walls present in such sections in favourable glancing views in addition to the difficulty of identifying each time the correct wall (see also ROBARDS 1976) contributed much in reducing the volume of sampling.

When counting the plasmodesmata from sections transverse to the cell walls (Figs. 1, 2, 3, 4) both partial and complete plasmodesmatal profiles present within the walls were taken into account. The results are listed in Table 1. The frequency of plasmodesmata (F) per square micrometre (μm^2) was calculated following the formula proposed by GUNNING (in ROBARDS 1976):

$$F = \frac{\text{count per } \mu \text{m of wall length}}{T + 1.5 \text{ R}}$$

where T is the section thickness $(0.08 \ \mu\text{m})$ and R the average radius of plasmodesmata. The latter, estimated to be $0.019 \ \mu\text{m}$ (19 nm), was determined by measuring the diameter (outer diameter including the plasmalemma lining the tube) of 100 plasmodesmata at their narrowest position from photographs printed at high magnification.

Results from tangentially sectioned cell walls were found slightly greater than those exhibited in Table 1 (by 1–3 plasmodesmata/ μ m²). The differences are attributed to errors of methods empolyed as well as to the small amount of sampling for these estimations.

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Cell wall type*)	Number of walls used	Total wall length (µm)	Number of plasmodesmata counted	Plasmo- desmata per μm of wall length	Plasmodes- mata per µm²
LS	3	26.3	11	0.42	3.8
CS	40	517.3	538	1.04	9.6
LI	60	485.8	379	0.78	7.2
CI	80	1246.7	1396	1.12	10.3
DS	40	715.1	920	1.28	11.8
IS	22	371.4	802	2.16	19.9
\mathbf{PS}	40	510.6	1161	2.27	20.9
AB	40	366.9	563	1.53	14.1
PB	40	407.8	686	1.68	15.5
ST	60	593.5	685	1.15	10.6

Table 1

Plasmodesmatal frequency in cell walls of secretory hairs estimated from transverse sections to the cell walls

*) Cell wall abbreviations given in the text.

While the walls of stalk, intermediate and secretory cells contain plasmodesmata randomly distributed over the entire wall extent (Figs. 2, 3, 4, 5), the basal cells and the subglandular tissue bear plasmodesmata grouped into primary pit fields; the wall in the pit fields area remains

considerably thinner than the rest which does not bear plasmodesmata at all (Fig. 6). Results cited in Table 1 refer to findings over the whole wall area. Plasmodesmata within the pit fields alone of the PB wall (Fig. 6) were found to occur at a frequency of $45/\mu m^2$.

Discussion

Results listed in Table 1 tend to be allocated in four groups. The smaller frequencies corresponding to the longitudinally oriented walls (LS, LI) constitute the first group. The second group contains the crossly oriented walls including the distal wall of the stalk cell (CS, CI, DS); calculations for subglandular tissue (ST) also fall within this group. Results for the basal cell (AB, PB) constitute the third group, while the fourth one contains the highest estimations corresponding to the intermediate and proximal walls of the stalk cell (IS, PS). The above data indicate that different walls of the same cell have different plasmodesmatal frequencies. This unequal distribution of plasmodesmata has also been observed in other cell types of various plant species, such as Nicotiana tabacum (LIVINGSTON 1935), Zea mays (JUNIPER & BARLOW 1969, JUNIPER & FRENCH 1970). Hordeum vulgare (ROBARDS & al. 1973), Cucurbita pepo (ROBARDS 1975), Sphagnum palustre (SCHNEPF & SYCH 1983), and Gossypium hirsutum (ELEFTHERIOU & HALL 1983). The increased densities of plasmodesmata at particular wall types has been considered as indicative of a promotion of the symplastic flow at a preferential direction (GUNNING & ROBARDS 1976). In H. rosa-sinensis the greater densities of plasmodesmata, occurring in the cross walls and being oriented parallel to the longitudinal axis, could be considered as favouring a flow towards the secretory hair tip.

ROBARDS (1976) has provided a list of plasmodesmatal dimensions and frequencies in several plants and various cell types. Calculations for H. rosa-sinensis nectaries fall within the broad range of plasmodesmatal frequencies found in other plant species, but presently we will focus on

Figs. 2, 3, 4. Cross section of the distal (Fig. 2), intermediate (Fig. 3) and proximal (Fig. 4) walls of a stalk cell (DS, IS, PS, respectively). The distal wall is traversed by relatively few plasmodesmata, while intermediate and proximal walls apparently bear more plasmodesmata (Index bar = $1 \mu m$).

- Fig. 5. Tangential section through a cross wall of intermediate cell (CI) revealing an even distribution of plasmodesmata over the whole wall area (Index bar = 0.5μ m).
- Fig. 6. Tangential section through pit fields of basal walls (the PB wall) showing a high concentration of plasmodesmata within the fields and an entire absence in the rest wall (Index bar = $1 \mu m$)

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discussing our results with respect to the relatively few reports on plasmodesmatal estimations concerning nectaries available in the literature.

Subglandular tissue of *H. rosa-sinensis* is structurally, and presumably functionally, analogous to that of cotton foliar nectaries (WERGIN & al. 1975). In both plants nectaries are subtended by a well vascularized subglandular tissue, which extends from the bases of the secretory hairs (in cotton, called papillae) to the phloem cells. Cells of the subglandular tissue are interconnected by plasmodesmata grouped in pit fields. The average number of plasmodesmata within pit fields in *H. rosa-sinensis* ($45/\mu$ m²) is somewhat greater than that found in subglandular tissue of cotton $40/\mu$ m²) (WERGIN & al. 1975), but smaller than estimations for the periclinal walls of basal cells (equivalent to our PB wall) of cotton secretory papillae ($50.2/\mu$ m²) (ELEFTHERIOU & HALL 1983).

When considering plasmodesmatal frequencies in the PB wall with respect to the whole wall area (not within the pit field alone) the findings in the present study $(15.5/\mu m^2)$ are in good agreement with that of the corresponding wall of cotton $(14.1/\mu m^2)$. In the anticlinal walls of basal cells (AB), however, comparison between the two plants reveals a marked difference: while in *H. rosa-sinensis* the results $(14.1/\mu m^2)$ do not deviate significantly from the PB wall (Table 1), in the equivalent cotton wall plasmodesmatal frequencies were found to be as low as $1.6/\mu m^2$ (ELEFTHERIOU & HALL 1983).

In the distal wall of stalk cells (DS) plasmodesmatal frequencies have also been estimated in *Abutilon* trichomes (GUNNING & HUGHES 1976) and cotton papillae (ELEFTHERIOU & HALL 1983). Results for *H. rosa-sinensis* $(11.8/\mu m^2)$ are in good agreement with *Abutilon* (12.6/ μm^2), but differ slightly from cotton (15.4/ μm^2). Estimations for proximal walls (PS) of *H. rosasinensis* (20.9/ μm^2) exceed considerably those for cotton (12.9/ μm^2), while in *Abutilon* no estimations are available for this wall.

According to GUNNING & HUGHES (1976) there are three possible routes by which pre-nectar might pass beyond the stalk cell towards the apex of the secretory hairs: 1. via plasmodesmata, 2. across the successive plasmalemmas and the intervening walls of the stalk cell, and 3. by the apoplastic route around the stalk cell protoplast. Lateral walls of stalk cells were structurally and experimentally shown to be inaccessible to both solutes and solvents; thus, the apoplastic route, if open at all, contributes but slightly to the overall volume flow (GUNNING & HUGHES 1976). Of the remaining two routes the same authors substantiate how the plasmodesmatal pathway provides a much more feasible route for fluxes. Plasmodesmatal densities calculated for stalk cell in H. rosa-sinensis favour conclusions being in good agreement with those reached earlier for Abutilon nectary trichomes. The relatively great number of plasmodesmatal frequencies is essential if cells would be able to cope with the high flux requirements. Plasmodesmata encountered in sufficient frequencies and size provide an

effective symplastic route through which solutes can diffuse along concentration gradients.

In *H. rosa-sinensis* a 40% of stalk cells are periclinally divided. Two or more stalk cells were also "occasionally" seen in *Abutilon* nectary hairs (FINDLAY & MERCER 1971 a). A question arises whether there is any difference between divided and non-divided cells in their accessibility by the symplastically moving substances because the additional wall (the IS wall) might be considered as providing a further barrier. The considerably high plasmodesmatal frequency within the IS wall, however, stands against that hypothesis, and rather favours the conclusion that IS wall does not impede symplastic fluxes.

The greater numbers of plasmodesmata in H. rosa-sinensis occur in the walls around the basal cell (Table 1). While the primary function of stalk cell seems to be the provision of an apoplastic barrier for pre-nectar (FINDLAY & MERCER 1971 a, b, GUNNING & HUGHES 1976), the basal cell presumably plays a critical role in collecting the pre-nectar from the subglandular tissue and canalizing it towards the secretory hair. Its high densities of plasmodesmata might be essential in fulfilling such a scope.

Intermediate cells create a long distance for pre-nectar to go through, but the presence of many plasmodesmata, particularly in the longitudinal direction, can provide a symplastic pathway of low resistance, essential for bulk flow rates.

Acknowledgements

The authors wish to thank Mrs. KIRATZIDOU-DIMOPULU for skillful secretarial assistance, Mr. A. ZOUMBOS for drawing the illustration of Fig. 1, and the "Stiftung Volkswagenwerk" and "The Alexander von Humboldt-Stiftung" for support.

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Digitale Literatur/Digital Literature

Zeitschrift/Journal: Phyton, Annales Rei Botanicae, Horn

Jahr/Year: 1987

Band/Volume: 27_1

Autor(en)/Author(s): Sawidis Thomas, , Tsekos Ioannes

Artikel/Article: <u>The Floral Nectaries of Hibiscus rosa-sinensis L. II.</u> <u>Plasmodesmal Frequencies. 155-164</u>