Phyton (Austria)	Vol. 17	Fasc. 1–4	151–158	18. 8. 1975

Leaf anatomy of *Catharanthus roseus (Apocynaceae)* infected with mycoplasm

By

J. A. INAMDAR, R. C. PATEL, M. GANGADHARA and A. BALAKRISHNA *)

With 2 Figures

Received 30 January 1975

Abstract

Pathological leaf anatomy of *Catharanthus roseus* is described. The affected leaves are pale green to pale yellow, membranous and considerably reduced in size as compared to normal ones. It has been noticed that the reduction in the general surface results in the reduction of the internal tissues. Anomocytic, anisocytic and paracytic stomata are noticed in both normal and affected lamina. The ontogeny of anomocytic stomata is perigenous, anisocytic and paracytic stomata is mesogenous. Stomatal abnormalities such as persistent stomatal initials, stoma split at one of the poles, single guard cells in relationship with a normal stoma, contiguous stomata, cytoplasmic connection, single guard cells are noticed in affected lamina. The frequency of stomata, stomatal index, size of guard cells, frequency of epidermal cells and size of epidermal cells are variable in normal and affected lamina.

Zusammenfassung

Es wird die pathologische Anatomie mycoplasma-infizierter Blätter von *Catharanthus roseus* (L.) G. DON beschrieben. Befallene Blätter sind bleichgrün bis hellgelb, hautartig und in der Größe stark reduziert, ihr Mesophyll ist schwach ausgebildet. In normalen wie befallenen Blättern kommen anomocytische, anisocytische und paracytische Stomata vor. Die anomocytischen Stomata entstehen perigen, die anisocytischen und paracytischen mesogen. In infizierten Blättern finden sich Stomata-Abnormitäten wie

*) J. A. INAMDAR, R. C. PATEL, M. GANGADHARA, A. BALAKRISHNA: Department of Botany, Sardar Patel University, Vallabh Vidyanagar (Gujarat, India).

nicht entwickelte Stomatainitialen. Klaffen der Schließzellen an einem Pole, einzelne Nebenzellen in Verbindung mit einer normalen Spaltöffnung, Zwillinge und Plasmabrücken zwischen benachbarten Schließzellen. Häufigkeit der Stomata, Stomata-Index, Größe der Nebenzellen und Größe der Epidermiszellen variieren sowohl in normalen wie in befallenen Blättern.

Introduction

According to METCALFE and CHALK (1950) stomata in the normal leaves of the species of *Vinca* are rubiaceous; vascular bundle in the midrib of *Apocyanum cannabinum* L. and probably other genera is typically bicollateral and petiole has crescentric bicollateral median vascular bundle. Accessory vascular bundles are recorded by SOLEREDER (1908) in *Vinca*.

Many members of the *Solanaceae* are affected by mosaic virus. Recently infection of mycoplasma has been reported in some plants. *Catharanthus roseus* (L.) G. DON is one of them. In *Catharanthus roseus* the infected leaves are very small in comparison with the normal leaves. Therefore, it was thought worthwhile to study the pathological leaf anatomy of *Catharanthus roseus*. The present paper deals with the anatomy of petiole, lamina and stomatal structure and ontogeny in *Catharanthus roseus* normal and affected by mycoplasma.

Material and Methods

The material for present investigation was collected from the University campus. Fine hand sections of petiole and lamina of normal and infected leaves were taken. The sections were double stained with safranin and fast green and mounted in glycerin.

Epidermal peels were taken from fresh as well as fixed material of normal and infected leaves. Camera-lucida drawings were made from epidermal peels stained with Delafields' haematoxylin and mounted in glycerin.

Mean values of 15 observations showing the size of the leaf, stomatal frequency, stomatal index, frequency of epidermal cells per mm^2, size of guard and epidermal cells in μm of normal and affected are charted in Table 1.

Observations

I. Morphological features:

Leaf: The normal leaves are shining green while the infected leaves are pale green to pale yellow and membranous. The affected leaves occur on very slender lateral branches. The normal leaves measure 6.5 cm × 2.5 cm while the affected leaves measure 1.7 cm × 0.5 cm (see Table 1).

II. Anatomical features·

A. Petiole: The petiole in transection of both normal as well as affected leaf is differentiated into epidermis, collenchymatous hypodermis and parenchymatous ground tissue with a single typically bicollateral vascular bundle. (Figs. C & D). But there is a marked difference in the size of the normal and affected petiole (Figs. C & D). The size is also reduced. The phloem consists of discrete patches and not a continuous layer alround xylem. In the normal the collenchymatous hypodermis comprises 3—4 layers while in the affected petiole it comprises 1—2 layers.

B. Lamina: There is also a marked difference in the transectional size of normal (Figs. E, F) and affected (Figs. G, H) lamina. As in the petiole, the vascular bundle is poorly developed and typically bicollateral (Fig. H). The general reduction in the size of lamina results in the reduction of

Table 1

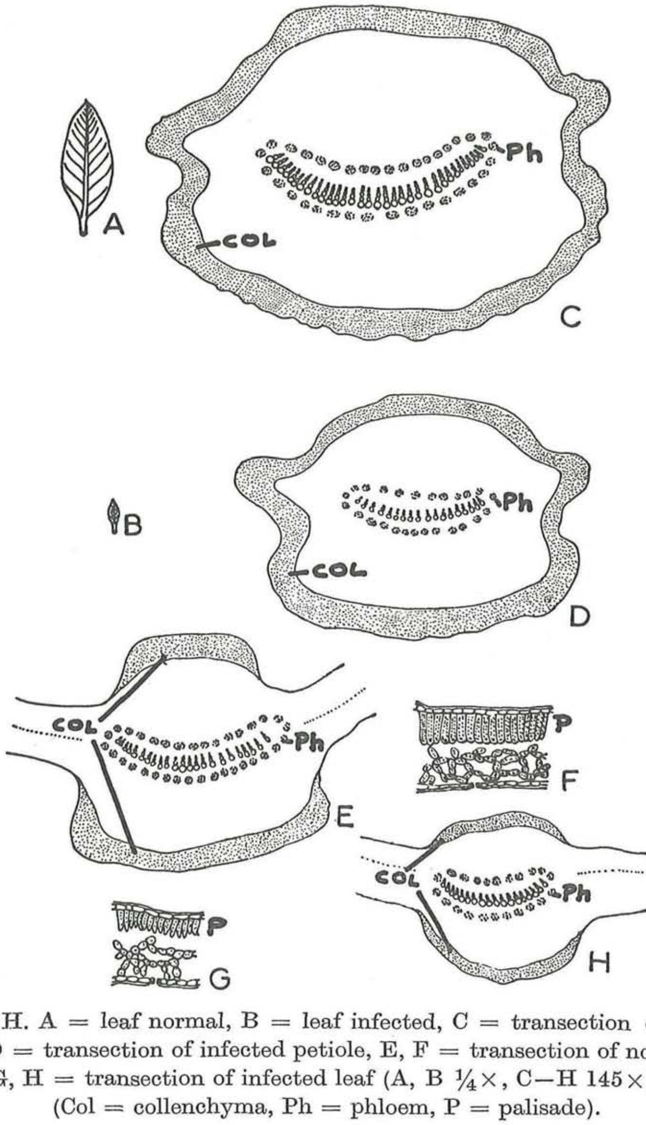
Showing the size of leaf, stomatal frequency, stomatal index, frequency of epidermal cells, size of guard and epidermal cells

Sl. No.			Normal	Affected
1.	Size of leaf including	Length	6.5 cm	1.7 cm
	petiole	Breadth	2.5 cm	0.5 cm
2.	Stomatal frequency	Upper	397	316
	per mm^2	Lower	442	336
3.	Stomatal index	Upper	23	23
	per mm^2	Lower	30	19
4.	Frequency of epi-	Upper	1322	1035
	dermal cells per mm^2	Lower	1014	1440
5.	Size of guard cells	Upper	22×9	18×4
	in μm	Lower	23×8	19×4
6.	Size of epidermal	Upper	31×20	38×25
	cells in μm	Lower	41×19	22×13

internal tissues (Figs. E—H). In the normal lamina the palisade and spongy tissues are well developed (Fig. F), while in the affected they are poorly developed (Fig. G). Palisade tissue in the normal lamina is compact and regularly arranged while in the affected it is not compact and irregularly arranged. Normal palisade cells are full of chloroplasts while those of affected contain few chloroplasts.

C. Mature epidermis: The normal and affected leaves are amphistomatic. The epidermal cells are polygonal, either isodiametric, or elongated with thick, either straight, arched or slightly sinous anticlinal walls. There is no difference in the epidermal cells of both upper and lower surface of normal and affected leaves. There is no definite pattern of arrangement of epidermal cells. The epidermal cells contain chloroplasts. The frequency of epidermal cells per mm^2 and size of epidermal cells in the normal and affected lamina are given in Table 1. There is a considerable difference in the frequency of epidermal cells and size of epidermal cells of normal and affected leaves.

D. Mature stomata: The stomata are distributed uniformly in between the veins, sometimes over the finer veins but not over the main veins, either close to or widely separated from each other. The mature stomata are anomocytic, anisocytic and paracytic (Figs. I—K). The stomatal fre-



Figs. A—H. A = leaf normal, B = leaf infected, C = transection of normal petiole, D = transection of infected petiole, E, F = transection of normal leaf, G, H = transection of infected leaf (A, B ¼×, C—H 145×).
(Col = collenchyma, Ph = phloem, P = palisade).

quency, stomatal index per mm^2 and size of guard cells in μm are given in Table 1. The stomatal frequency, stomatal index per mm^2 and size of guard cells are quite variable (see Table 1).

E. Development of stomata (normal): In a young leaf epidermis consists of polygonal, isodiametric, or elongated and uninucleate cells. A triangular meristemoid is cut off on one side of the epidermal cell. Meristemoid can be easily distinguished by its smaller size, prominent nucleus and dense staining properties (Fig. I).

Abnormalities are rare in the normal lamina. The development of different types of normal stomata is as under:

(a). Anomocytic stoma: The meristemoid directly functions as guard mother cell without cutting off any subsidiary cells, divides by a straight wall to give rise to a pair of guard cells (Fig. I).

(b). Paracytic stoma: The triangular meristemoid divides unequally to produce two cells. The larger differentiates as a subsidiary cell while the smaller enlarges and divides again on the other side to give rise to a second subsidiary cell and a central guard mother cell. (Fig. I). The middle guard mother cell, then, divides by a straight wall to form two equal guard cells.

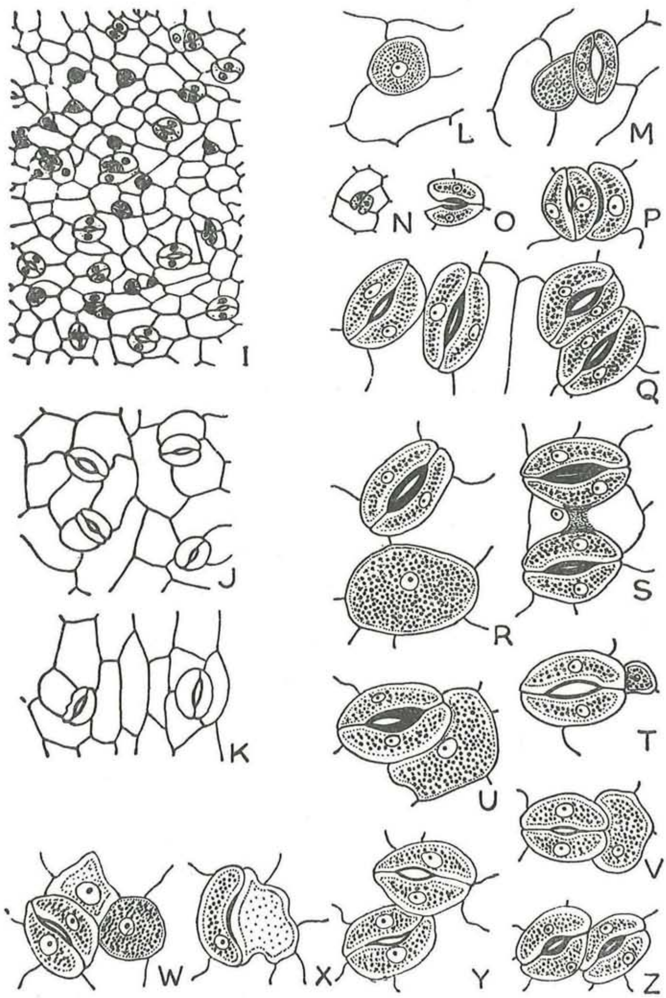
(c). Anisocytic stoma: The triangular meristemoid divides in a spiral manner by slightly curved walls perpendicular to the preceding ones to produce the unequal subsidiary cells and a central cell (Fig. I). The central cell functions as a guard mother cell, divides by a straight wall to produce a pair of guard cells (Fig. I). Here, the meristemoid behaves like an apical cell with three cutting faces.

F. Abnormalities caused due to infection: Anomocytic, anisocytic and paracytic stomata found in the normal lamina also occur in the infected lamina, and these develop in the same way as the normal stomata. However, many abnormalities occur in the affected lamina which are not observed in the normal lamina. Therefore, it is thought that the abnormalities are caused due to mycoplasmal infection.

The abnormalities are as under:

(a). Persistent stomatal initial: Here the meristemoid doesnot divide at all. It enlarges and increases in size, chloroplasts appear and becomes densely cytoplasmic. The persistent stomatal initial ultimately becomes filled with abundant chloroplasts. It has the same staining capacity as the guard cells of a stoma (Figs. L, M, R, W). It occurs either solitary (Figs. L, R) or in relationship with a normal stoma (Figs. M, T—W). The persistent stomatal initials are either spherical, dome-shaped, oval or subsidiary cell-like in shape. When the persistent stomatal initial occurs in relationship with the normal stomata, occurs by the side or at the pole of the guard cell.

(b). Stoma split at one of the poles: Here, a cleft develops between the two guard cells right from the initiation (Fig. N), cuticular thickening develops in the region of a stomatal pore (Fig. O).



Figs. I—Z. I = epidermal peels showing development of normal stomata, J, K = normal stomata, L—Z = abnormal stomata: L = persistent stomatal initial, M = persistent stomatal initial in relationship with normal stoma, N = developing stoma, open at one end, O stoma open at one end, P = one and a half stomata, Q = contiguous stomata, R = persistent stomatal initial nearby normal stomata, S = cytoplasmic connection between nearby stomata, T = normal stoma with a developing persistent stomatal initial at one end, U—W = persistent stomatal initials in a relationship with normal stomata, X = single guard cell without a pore, note other guard cell epidermal-like, Y—Z = contiguous stomata (I—K 145×, L—Z 550×).

(c). Single guard cell in relationship with a normal stoma: A single guard cell with pore occur parallel to and in relationship with the normal stoma (Fig. P).

(d). Contiguous stomata: Variously oriented contiguous stomata are noticed (Figs. Q, Y, Z). The contiguous stomata may be either juxtapposed or obliquely oriented or at right angles to each other.

(e). Cytoplasmic connection: Cytoplasmic connection between nearby stomata connecting the protoplasts of opposite guard cells is observed (Fig. S).

(f). Single guard cell: The guard mother cell divides to form two equal guard cells. One of these two guard cells becomes bean-shaped and differentiates into a single guard cell without a pore and the other into an ordinary epidermal cell-like instead of giving rise to a normal stoma with an intervening pore (Fig. X).

The ontogeny of anomocytic stomata is perigenous (PANT 1965) while that of anisocytic and paracytic stomata is mesogenous (PANT 1965) as the subsidiary cells and guard cells originate from the same mother cell.

Discussion

Pathological leaf anatomy of *Catharanthus roseus* form the subject of the present discussion. Pathological anatomy of virus infected members of *Solanaceae* has been studied by BORGES and DAVID-FERREIRA (1968), ESAU (1967) and PATEL and SHAH (1972). ESAU and HOEFERT (1971) studied the cytology of beet yellows virus infection in *Tetragonia expansa*. RILLO *et al.* (1972) have made an anatomical study of coconut leaves from healthy trees and those affected by cadang-cadang. PATEL and SHAH (1972) pointed out that the branches are shorter, leaves are very small, thin and pale green in virus affected brinjal than normal. Number of cortical layers, vascular tissue and collenchyma in diseased internode vary from those of normal internode. The mycoplasmal affected plants of *Catharanthus roseus* show morphological expressions such as slender stem, short internodes and membranous pale green to pale yellow leaves. Considerable difference in the size of normal and affected organs has been noticed. Reduction in external size has also a bearing on the internal structure. The internal tissues of the affected organs are feebly developed.

Stomatal abnormalities have been induced artificially by growth regulators by some workers (see INAMDAR 1970). As far as the authors are aware, there is not even a single report on the stomatal abnormalities caused by mycoplasmal infection. GERTZ (1919) reported and illustrated persistent stomatal initials associated with galls of fungal and insect etiology. Stomatal abnormalities such as persistent stomatal initial, stomata split at one of the poles, single guard cells in relationship with a normal stoma, contiguous stomata, cytoplasmic connection between nearby stomata and single guard cells are observed in the affected lamina. It has also been noticed that the infection has effect on the stomata, stomatal frequency, stomatal index, size of guard and epidermal cells and frequency of epidermal cells.

Acknowledgements

We are thankful to Dr. M. V. DESAI, Professor of Pathology, Agriculture University, Anand, for identifying mycoplasmal infection. M. GANGADHARA thanks University Grants Commission for financial assistance.

Literature cited

*BORGES M. de L. V. and DAVID-FERREIRA J. F. 1968. Comparitive study of cell structure in *Datura metel* L. healthy and infected with potato virus. Y. Rev. Biol. **6**: 421–437.

*ESAU K. 1967. Anatomy of plant virus infections. Ann. Rev. Phytopathol. **5**: 45–76.

— and HOEFERT L. L. 1971. Cytology of Beet Yellows virus infection in *Tetragonia*. Protoplasma, **72**: 255–273.

*GERTZ O. 1919. Studier of ver klyfoppningarnas morphologimed Sarskild hansyn till deras pathologiska utbildingsformer. Acta. Univ. Lund N. F. **15**: 3–84.

INAMDAR J. A. 1970. Action of growth regulators on the development of stomata of *Abelmoschus esculentus* MOENCH. Flora **159**: 497–502.

METCALFE C. R. and CHALK L. 1950. Anatomy of dicotyledons, Vol. 2, Oxford.

PANT D. D. 1965. On the ontogeny of stomata and other homologous structures. Pl. Sci. Ser. Allahabad. **1**: 1–24.

PATEL J. D. and SHAH J. J. 1972. Pathological anatomy of Brinjal affected by "Little leaf virus". J. Indian bot. Soc. **5I**: 18–22.

RILLO E. P., PABLEO G. O. and PRICE W. C. 1972. An anatomical study of coconut leaves from healthy trees and those affected by cadang-cadang. Bull. Torrey. Bot. Club. **99**(6): 271–277.

SOLEREDER H. 1908. Systematic anatomy of dicotyledons. Vol. 1 & 2. Oxford.

---

* Not seen in original.