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SEM study of the morphology of leaves of four dessert banana cultivars (*Musa* spp. cv. 'Intan', 'Jari Buaya', 'Novaria' and 'Raja Udang Merah') in Malaysia

[Kajian SEM tentang morfologi daun daripada empat kultivar pisang (*Musa* sp. kultivar 'Intan', 'Jari Buaya', 'Novaria' dan 'Raja Udang Merah') di Malaysia]

W. Zakaria* and A. R. Razak**

Key words: banana, scanning electron microscopy, banana cultivars, morphology

Abstrak

Morfologi permukaan daun empat kultivar pisang, iaitu 'Intan', 'Novaria', 'Jari Buaya' dan 'Raja Udang Merah' telah dikaji menggunakan SEM. Kepadatan stomata antara kultivar dan permukaan daun didapati sangat berbeza. Permukaan bawah daun mempunyai 2–3 kali lebih banyak stomata/mm^2 berbanding permukaan atas, dengan 'Jari Buaya' mempunyai bilangan stomata yang terbanyak. Lapisan lilin di kedua-dua permukaan daun juga berbeza antara kultivar. Permukaan bawah daun pula mempunyai lapisan lilin yang lebih banyak dan tersusun. Kompleks stomata di kedua-dua permukaan daun didapati terletak di kawasan lekuk di antara sel-sel epidermis, dengan kedudukan liang stomata lebih tinggi berbanding dengan sel pengawalnya. Keputusan yang didapati dihubungkaitkan dengan pertalian air tumbuhan dan keberkesanan penggunaan racun kulat untuk mengawal penyakit daun.

Abstract

The leaf surface morphology of four banana cultivars namely, 'Intan', 'Novaria', 'Jari Buaya' and 'Raja Udang Merah' was studied using the SEM. Stomatal density differed among the cultivars and between the two leaf surfaces. The abaxial surface had 2–3 fold more stomata/mm^2 than the adaxial surface. 'Jari Buaya' had the highest stomatal density. Wax layer between the two leaf surfaces also differed. The abaxial surface showed more conspicuous and well-organized wax layer. The stomatal complex of both surfaces was located in a slight depression among the epidermal cells but with the stomatal aperture situated slightly higher than the position of the guard cells. The results are discussed in terms of plant water relations and the effectiveness of fungicide sprays to control leaf diseases.

*Dept. of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia
**Dept. of Plant Protection, Faculty of Agriculture, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia
Authors' full names: Zakaria bin Wahab and Abd. Rahman Abd. Razak

Introduction

Stomata play an important role in regulating water loss and gas exchange between the internal tissues of plants and the outside atmosphere. Approximately 99% of water absorbed by the roots is lost during transpiration through the stomata of leaves. Generally, the stomata are located on green parts of the plant particularly the leaves. Currently, information on the stomatal and leaf morphology of banana leaves are mainly based on the improved commercial Cavendish variety cultivated under near to optimum agronomic management in the subtropical regions (Stover and Simmonds 1987; Eckstein and Robinson 1995; Robinson 1996). The stomatal density of the commercial cultivars differed between the abaxial and adaxial surfaces and among different genomic groups. Generally, the stomatal density of the abaxial surface of subtropical banana is 140 stomata/mm^2, which is lower than the stomatal density of strawberry leaves, ranging from 280–330 or apple leaves of between 320–350, which are typical temperate crops (Blanke and Bower 1990).

The humid tropics is believed to be the centre of origin of banana. Many of the wild forms are still found growing at the fringe of the virgin forest. They are protected from prolonged direct sunlight by the higher leaf canopies of the dipterocarps and maintained in a natural damp and humid microenvironment. The domestication and commercial cultivation of some of the cultivars for the local or regional market have introduced a new growth environment for the crops. Cultivars such as ‘Mas’ and ‘Berangan’ have been commercially selected for monoculture, open field cultivation. Others such as the ‘Raja Udang Merah’ is grown in the backyard garden for specific purpose. However, information on the stomatal complex of the banana leaves grown under the natural environmental conditions of the hot, humid tropics is lacking. This paper reports on the scanning electron microscopy (SEM) study on the surface morphology of four dessert banana cultivars grown under the natural tropical environment.

Materials and methods

Four cultivars of dessert banana, *Musa* spp. (AA group) ‘Intan’ and ‘Jari Buaya’ and *Musa* spp. (AAA group) ‘Novaria’ and ‘Raja Udang Merah’ (Stover and Simmonds 1987) were selected for the study based on the different levels of improvement of the cultivars for local cultivation. ‘Intan’ is an improved local ‘Berangan’ cultivar, selected through conventional selection by United Plantations Berhad. ‘Jari Buaya’ (JB) is a wild form identified for its tolerance to the major nematode pest of banana, *Radopholus similis.* ‘Novaria’ is a Cavendish-type banana bred by *in vitro* mutation breeding programme for resistance to *Fusarium* wilt disease. ‘Raja Udang Merah’ (RUM) is an indigenous cultivar, having quite similar taste and flavour to ‘Novaria’. RUM is not popular with the local population, however, it is cultivated for the purplish colour of the unripe fruits for special religious festival of the local community. No breeding work has been attempted on the cultivar apart from its cultivation as a backyard garden plant for the specific religious function.

Plantlets of ‘Intan’, JB and ‘Novaria’ were obtained from the tissue culture laboratory of United Plantations Berhad, Teluk Intan, Perak. They were raised in polythene bags of sterilised soil for six weeks in the nursery before field planting. Sword suckers were used as the planting material for RUM and planted direct to the field. Each plant was planted in a planting hole 3 m apart in the field. Fertilizers were applied at the rate and frequencies recommended by United Plantations Berhad. Water supply was dependent on the rain and no chemical treatments for control of insect pests and diseases were applied.

Leaf samples were collected at flower emergence from the mid region of leaf number 3 down the profile. Five rectangular pieces of 2 cm x 4 cm of leaf were cut at mid morning and fixed immediately in Bouin's fixative (Zakaria and Razak 1997). After 4 h under vacuum, the samples were washed in 1% cacodylate buffer, post fixed in 1% cacodylate buffered Osmium tetroxide for 2 h, dehydrated through graded series of ethanol to absolute ethanol and critical point dried in a Balzer CD 30 critical point drier. The leaf samples were mounted on stubs, sputter coated in gold and viewed in a JOEL 6400 SEM at an acceleration voltage of 15 kV.

For assessment of the stomatal density, five replicate samples of leaf pieces adjacent to those collected for the SEM work were taken to the laboratory. Imprints of the adaxial and abaxial surfaces of the leaves were prepared using nail varnish. Four imprints were prepared from each cultivar. Stomatal counts/mm^2 of leaf were performed using the stereo binocular microscope.

Results

Stomatal densities and aperture

The continuity of the surface topography of the leaf blade of all the four banana cultivars was interrupted at regular intervals by secondary veins. The weak line of tearing is located along the secondary veins arranged perpendicular to the long axis of the leaf. The secondary veins were approximately 4–6 cells wide, clearly demarcated by slightly raised, elongated and sausage-shaped epidermal cells *(Plate 1).* They were more pronounced on the abaxial than the adaxial surface of the leaf. Generally, the stomata were distributed between the secondary veins. No stomata were found along the secondary vein of these cultivars.

The four banana cultivars exhibit amphistomatous leaves with the stomatal complex resembling an anomocytic arrangement. The stomata were elliptical in shape and were present on both the adaxial and abaxial surfaces of the leaf *(Plate 2).* There was no set pattern in the arrangement of the stomata on both surfaces. Generally, the stomatal density per unit area was higher on the abaxial than the adaxial surface. The number of stomata per unit area on the adaxial surface was not significantly different (approximately 53 stomata/mm^2) among the four cultivars, but varied greatly on the abaxial surface *(Table 1).* 'Jari Buaya' had the highest stomatal density (234 stomata/mm^2), followed by 'Intan'

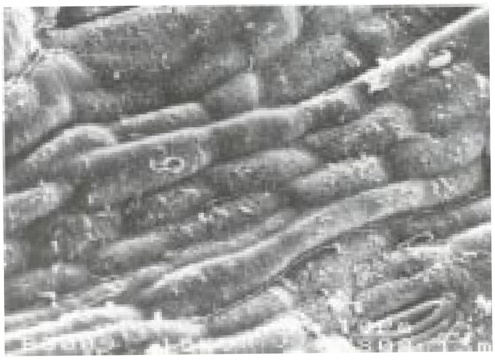

Plate 1. Secondary vein of 'Raja Udang Merah' with elongated, sausage-shaped epidermal cells. Stomata were absent along this vein (x 800)

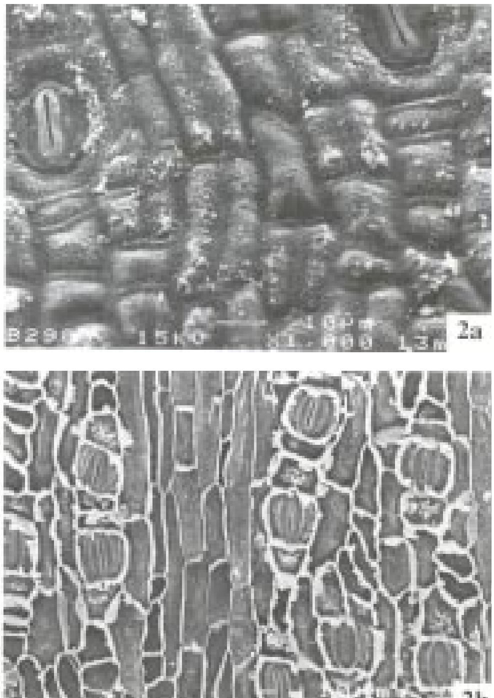

Plate 2. Stomatal density (a) adaxial surface of 'Raja Udang Merah' (x 1000), (b) abaxial surface of 'Novaria' (x 400) leaf

Table 1. Stomatal densities of the mid region of leaf 3 of four dessert banana cultivars at floral emergence

Cultivars	Group	Stomatal density/mm²±SD	
		Adaxial	Abaxial
Intan	AA	53 ± 5	167 ± 15
Jari Buaya	AA	55 ± 7	234 ± 25
Novaria	AAA	52 ± 3	138 ± 12
Raja Udang Merah	AAA	55 ± 5	143 ± 20

Table 2. Stomatal length on the adaxial and abaxial surfaces of leaf 3 of four dessert banana cultivars at floral emergence

Cultivars	Group	Stomatal length (µm±SD)	
		Adaxial	Abaxial
Intan	AA	14.1 ± 2.9	13.2 ± 2.8
Jari Buaya	AA	13.5 ± 1.9	14.8 ± 2.3
Novaria	AAA	26.2 ± 3.2	14.7 ± 2.9
Raja Udang Merah	AAA	18.1 ± 2.2	12.8 ± 2.6

(167/mm^2), RUM (143/mm^2) and 'Novaria' (138/mm^2). Similarly, the length of the stomatal aperture on the adaxial and abaxial surfaces differed among the four cultivars *(Table 2)*. Two of the cultivars, 'Novaria' and RUM, apparently had longer stomatal aperture on the adaxial surface than the abaxial surface. Stomatal aperture on the abaxial surface, however, were approximately similar for the four cultivars. Interestingly, the stomatal aperture on the adaxial surface is approximately 70% longer than the abaxial surface for 'Novaria' and RUM (AAA group) as compared to the other two cultivars.

Surface morphology

Adaxial surface SEM of the adaxial leaf surface of the four banana cultivars are presented in *Plate 3*. The adaxial surface of the leaf was covered by a thin layer of wax granules. The size and distribution of the granules differed among the cultivars. The wax granules of 'Intan', RUM and 'Novaria' appeared to be smaller, more uniform in size and evenly distributed on the leaf surface

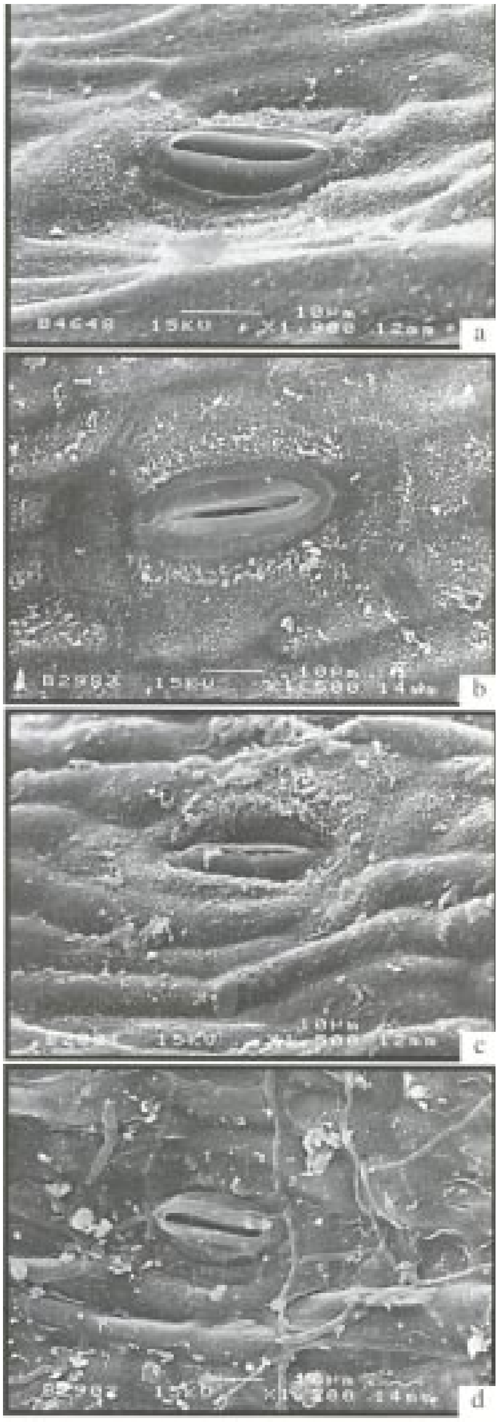

Plate 3. The adaxial surface, showing size and distribution of the wax granules and stomatal complex of (a) 'Intan' (x 1900), (b) RUM (x 1500) (c) 'Novaria' (x 1500) and (d) 'Jari Buaya' (x 1200)

than JB. For JB, the wax granules appear to congregate into larger units and were distributed at random on the surface. Ramification of fungal hyphae was observed on the leaf surface of JB but was absent on the other three cultivars *(Plate 3d)*. The stomatal complex was located in a slight depression on the adaxial surface and was bordered by elongated epidermal cells. The general appearance of the stomata in three of the cultivars were spindle-shaped while in RUM appeared as sausage-shaped with a slight constriction in the middle *(Plate 2a).*

Abaxial surface Wax deposits on the abaxial surface were more apparent than the adaxial surface *(Plate 4).* They appeared as flakes effusing from the intercellular walls of the epidermal cells. The flakes were apparently lacking in JB *(Plate 4a).* Wax deposits in JB were in the form of granules similar to the adaxial surface, except occurring in two sizes, small and large. The smaller granules were more uniformly distributed while the larger granules were unevenly scattered on the abaxial surface. Wax flakes in 'Intan' *(Plate 4b),* RUM *(Plate 4c)* and 'Novaria' *(Plate 4d)* appeared to be produced by the fusion of individual finger-like wax fibres into a continuous wall. The size of each fibre was approximately 0.5–1.0 µm thick by 1 µm wide, while the length varied. Thus the wax walls were more irregular in height. The identity of the individual wax fibre was more distinct in 'Novaria' and RUM where flakes of some of the broken fibres were found deposited on the epidermal cells within the wall boundary. In contrast, the wax fibres in 'Intan' formed a continuous wall and were more uniform in height, distinctly demarcating the boundary of the individual epidermal cell *(Plate 4b)*. Deposits of the broken pieces of the wax flakes were few on the surface of the epidermal cells enclosed by the wax wall.

The stomatal complex was located in a slight depression on the abaxial surface of the leaf with the stomatal aperture

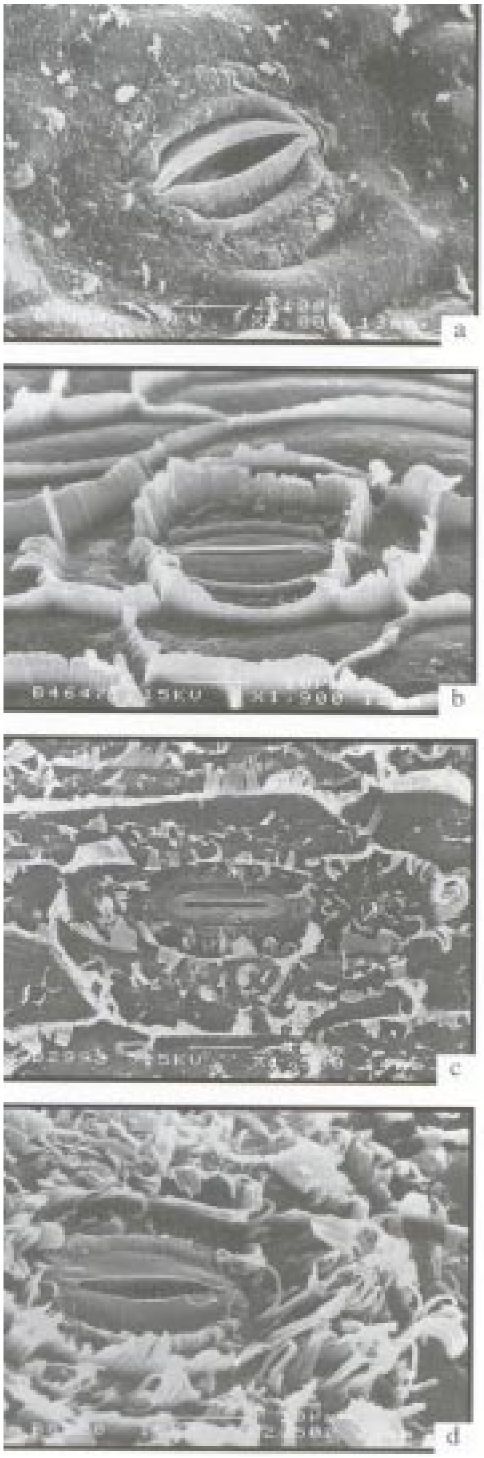

Plate 4. Wax deposits on the abaxial surface of (a) 'Jari Buaya' (x 2000), (b) 'Intan' (x 1900) (c) RUM (x 1500) and (d) 'Novaria' (x 2500)

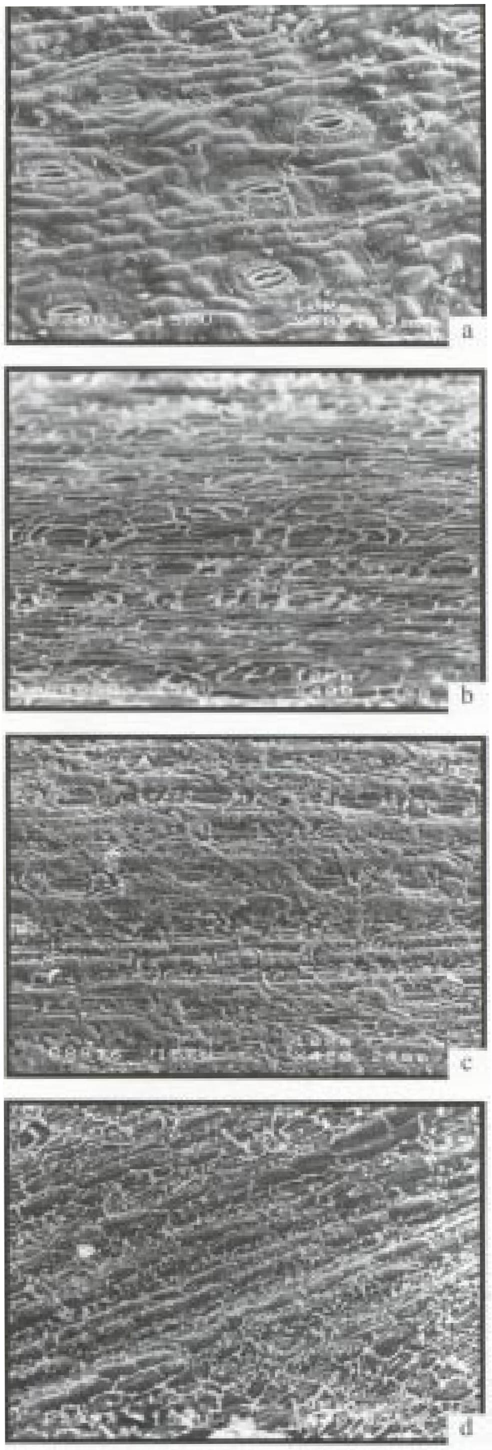

Plate 5. The stomatal complex on the abaxial surface of (a) 'Jari Buaya' (x 500), (b) 'Intan' (x 400), (c) 'Novaria' (x 400) and (d) RUM (x 400)

positioned on a raised level relative to the position of the two guard cells *(Plate 5).* Although the specimens were collected at the same time and processed using the same procedure, many of the stomata in JB *(Plate 5a)* remained open while that of 'Intan' *(Plate 5b)* were closed. The state of stomatal opening of 'Novaria' *(Plate 5c)* and RUM *(Plate 5d)* ranged from complete closure to partially open. The distribution of the stomata on the abaxial surface was similar to the pattern observed on the adaxial surface. Some of the stomata were found adjacent to one another while some were arranged parallel to one another. This was an exception to the otherwise random distribution of stomata in between the secondary veins of the leaf.

Discussion

Stomata occur in large numbers on the aerial parts of the plant and mainly on the green parts especially the leaves. They play an important role in regulating gas exchange and water loss from the plant to the outside environment. The stomatal density on the abaxial surface of the dessert banana leaves (138–234 stomata/mm^2) was within the lower range (between 135–469 stomata/mm^2) of those reported earlier (Stover and Simmonds, 1987). Three of the cultivars, 'Intan', JB and RUM had higher stomatal density (> 140 stomata/mm^2) than cultivars grown in the subtropical climate as indicated by Eckstein and Robinson (1995) but still very much lower than some of the temperate fruits such as avocado at 730 stomata/mm^2 or apple at 390 stomata/mm^2 (Blanke and Bower, 1990). 'Novaria', however, appeared to be within the stomatal density range of the subtropical banana. According to Stover and Simmonds (1987), stomatal density is inversely correlated to the ploidy level while the stomatal length is directly related to the ploidy of the plants. The results from this work conformed to this statement. 'Intan' and JB, the diploid (AA) group, had higher stomatal density than 'Novaria' and RUM, the triploid (AAA) group. 'Novaria' and

RUM, however, had longer stomatal aperture on the adaxial surface compared to the abaxial surface. On the abaxial surface, the length of the stomata in all four cultivars were almost similar. From this work, increased cell size with increased ploidy level (Robinson 1996) could not fully account for the differences observed in the stomatal density as well as between the abaxial and adaxial surfaces in these four dessert banana cultivars.

Differences in the amount of wax deposits between the abaxial and adaxial surfaces, and the architecture of the wax wall among the cultivars are of interest. The wax deposit on the subtropical banana leaves was reported to resemble long coiled cylindrical rods (Anon 1996) as opposed to the flakes and wall-like wax fibres in these cultivars. The differences could possibly suggest an adaptation of the four dessert banana cultivars to the warmer tropical environment. The height of the wax wall and the wax architecture would influence the boundary layer of air on the surface of the leaf. A thicker boundary layer would form a better buffer zone to reduce the rate of water loss through evaporation in the hot tropics. The position of the stomatal complex in a slight depression on the abaxial surface as opposed to a more raised position on the peel of banana fruit reported earlier (Zakaria and Razak 1997) could further suggest an adaptation to the tropical environment.

The four cultivars were selected for the study based on the state of improvement of the crop. ‘Novaria’ is an improved cultivar of the Cavendish group through mutation breeding for resistance to *Fusarium* wilt disease while ‘Intan’ was developed through conventional selection for local commercial production. In contrast, RUM is a domesticated cultivar grown in the backyard garden for special religious function, while JB is an indigenous wild form generally found at the fringe of the forest. The growth environment for JB is different from the other three cultivars. In the natural environment, JB receives restricted amount of direct sunlight due to shading from the higher canopy of the forest and with continuous supply of water and moist microenvironment supplied by the organic matter. The cultivar is not subjected to water stress as compared to an exposed plantation. Hence the significantly higher stomatal density on the abaxial surface of JB could be necessary to remove the excess water absorbed by the roots. JB was selected for its resistance to the banana nematode, *Radopholus similis.*

The presence of the wax layer could be significant in the disease management of the crop. It may restrict the selection of fungicides for foliar disease control to lipophilic based chemicals. Water based chemicals may not be retained on the surface for effective control of the disease. Similarly, there is also a possibility of the wax layer acting as a protectant against germination of fungal spores on the leaf surface. Surface lacking in wax layer, such as in JB, may encourage fungal colonization as seen in this study.

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