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Morphological changes of the leaf surfaces of *Zea mays* induced by rayado fino virus infection

by

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Abstract: Naturally infected field-grown or greenhouse inoculated maize leaves were used to study the anatomical changes caused by rayado fino virus infection. Infected leaves showed the typical symptoms of the disease, characterized by the appearance of small chlorotic spots in the costal regions of the adaxial surface, which may extend to the intercostal zones with increasing severity of the disease. Examination of the chlorotic areas by scanning electron microscope reveals abnormal development and reduction in size of stomata and guard cells, or failure of formation of stomata. Reduction in size of the stomatal structures progresses towards the proximal end of the leaf blade and seems to be correlated with intercalary growth. Chlorotic areas appear almost glabrous since macrohairs, typical of the costal areas of healthy leaves, fail to develop. However, microhairs proliferate in other areas covering the veins. Epidermal, siliceous and bulliform cells show abnormal development in severely affected areas.

The rayado fino disease of maize is widely distributed in the American Continent, from southern United States in the north, to Uruguay in South America (Gámez, 1977). Rayado fino virus (REV), the causal virus, has small isometric particles approximately 30 nm in diameter, transmitted in a persistent manner by *Dalbulus maidis, D. elimatus* and *Graminella nigrifrons* (Gámez, 1969; Gámez *et al.*, 1977; Nault & Bradfute, 1977). Yield losses caused by the disease average 40-50% in individual plants of local cultivars, but may attain 100% in newly developed or foreign varieties (Gámez, 1977). Typical symptoms of the disease are characterized by the formation of small chlorotic spots or short stripes associated with the leaf veins. This paper describes the anatomy of the spots as well as the surrouding tissues in slightly and severely infected leaves as observed under the scanning electron microscope (SEM).

MATERIAL AND METHODS

Both healthy and diseased leaves were obtained from naturally infected, field-grown plants and greenhouse inoculated plants; also from Tico-V1 and Tico-H-4 cultivars. The isolate of RFV and the inoculation methods employed were the same as those described by Gámez (1969) in previous investigations. The

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identity of the virus was confirmed serologically by Ouchterlony gel diffusion tests.

For examination with the SEM the material was fixed in 4% gluteraldehyde buffered with 0.05 M sodium cacodylate (ph 7.0). After washing with water, the material was dehydrated through a graded series of alcohol-amyl acetate and stored in 100% amyl acetate. Samples were sonicated for 2 or 3 seconds and later dried by critical point method using CO_2, mounted on aluminum grids, gold-coated and examined in a Hitachi HHS-2R SEM at 20 Kv.

RESULTS

Reactions to the virus were similar in the two cultivars as well as in the naturally-infected, field-grown plants and greenhouse inoculated plants. Symptoms were first evident in RFV-infected leaves 10-15 days after inoculation. Chlorotic spots appeared in the costal regions of the adaxial surface near the proximal end of the developing blade, close to the leaf sheath. New spots continued to develop at the base of the leaf blade as a result of its intercalary growth. These small spots rapidly increased in size and frequently overlapped neighboring spots along the vein, resulting in stripes of variable length.

When examined under the SEM, the very young chlorotic spots on the adaxial leaf surface appeared almost glabrous since macrohairs, typical of the non-chlorotic areas (Fig. 1), fail to develop (Fig. 2). In the chlorotic areas lacking macrohairs, many microhairs develop along the rib margins (Fig. 3) so that veins are observed only with difficulty (Fig. 4). In some cases microhairs also are missing and veins and bulliform cells are easily distinguished (Fig. 5). The epidermis covering the ribs in the chlorotic areas shows a characteristic wrinkled pattern (Fig. 6). Bulliform cells are not externally modified (Fig. 6) except in leaf areas with very severe symptoms (Fig. 7). Abnormal development of the silica cells is evident on both the adaxial and abaxial surfaces of chlorotic spots (Figs. 8 and 9). Two different types of short silica cells are found in green areas: "dumb-bell shaped" and crenate or tetralobulate cells (Fig. 8). Only bilobulate silica cells, however, are seen in chlorotic areas (Fig. 9) and they are not in rows and show no specific pattern of distribution. Malformation of epidermal cells adjoining silica cells is also noticeable (Fig. 9).

Stomata are severely affected in the chlorotic areas. The length of the stomata of healthy maize leaves ranges from 27 to 30 μm, as reported by Flores and Espinoza (1977); while the length of altered stomata, if formed, ranges from 4 to 8 μm. The progressive reduction in the length of the stomata seems correlated with the leaf blade extension produced by intercalary growth at the base. Altered morphology of ostioles and guard cells, or complete failure of their formation are conspicuous features of the chlorotic areas of infected leaves (Figs. 12, 13, 14). Under the light microscope the infected leaves also show severe internal changes (Flores and Gámez, 1978).

DISCUSSION

An extensive review of the macroscopic symptoms of "rayado fino" disease of maize (Williams *et al.*, 1976), reveals no information on the external morphological alterations occurring in the infected plant such as that found in our scanning electron microscope studies.

One of the most conspicuous modifications of the RFV infected tissues of the plant varieties examined is the absence of macrohairs and the presence of numerous young developing microhairs in some of the chlorotic spots. There was

no evidence of mechanical breaking of the trichomes during the processing of the samples, which suggests direct failure of the development of the macrohairs as a result of the disease.

The reduction in size and number of the stomata and their abnormal development in the chlorotic tissues may be correlated with changes in transpiration and respiration. Alteration of the normal pattern of cell distribution is another important pathological change observed in the leaves. Deviations in the normal pattern of cell morphogenesis in local areas of the leaf surface appear to be responsible for the noticeable growth abnormalities.

Preliminary studies of the histological and cytological effects of RFV infection revealed only very slight modifications of the mesophyll and phloem parenchyma of the maize tissues examined (Kitajima & Gámez, 1977). The observations described in the present paper were made of different genotypes than those used by Kitajima and Gámez (1977), and suggest the possibility of more severe internal changes in the plant genotypes here examined. Kitajima and Gámez (1977), did not determine whether the histological sections examined corresponded to the chlorotic spots or the surrounding green areas of the infected leaf.

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RESUMEN

Hojas de maíz naturalmente infectadas o inoculadas en el invernadero para estudiar los efectos anatómicos causados por el virus del rayado fino muestran pequeñas manchas cloróticas, típicas de la enfermedad, en las regiones costales de la superficie adaxial, que pueden extenderse a las zonas intercostales al aumentar la severidad de la infección. El examen de las áreas cloróticas al microscopio electrónico de rastreo revela desarrollo anómalo y reducción de los poros estomáticos y células guardianas. En ciertas áreas muy afectadas no se observan estomas. La reducción en tamaño de las estructuras estomáticas aumenta hacia la parte proximal de la lámina, y parece correlacionarse con el crecimiento basal de la lámina que produce el meristema intercalar. Las áreas cloróticas aparecen casi glabras, ya que no se desarrollan los macropelos característicos de las zonas costales en las áreas verdes. Sin embargo, ocurre proliferación de micropelos a lo largo de las venas, dificultando su visualización. Las células epidérmicas, silíceas y buliformes muestran desarrollo anómalo en áreas severamente afectadas.

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Fig. 1. Pubescent epidermis of the healthy adaxial leaf blade.

Fig. 2. Adaxial leaf epidermis of infected leaf blades.

Fig. 3. Prickle microhairs along the rib margins of infected leaf blades.

Fig. 4. Detail of Fig. 3. Vein area covered by prickle microhairs.

Fig. 5. Vein of a healthy leaf blade.

Fig. 6. Healthy bulliform cells.

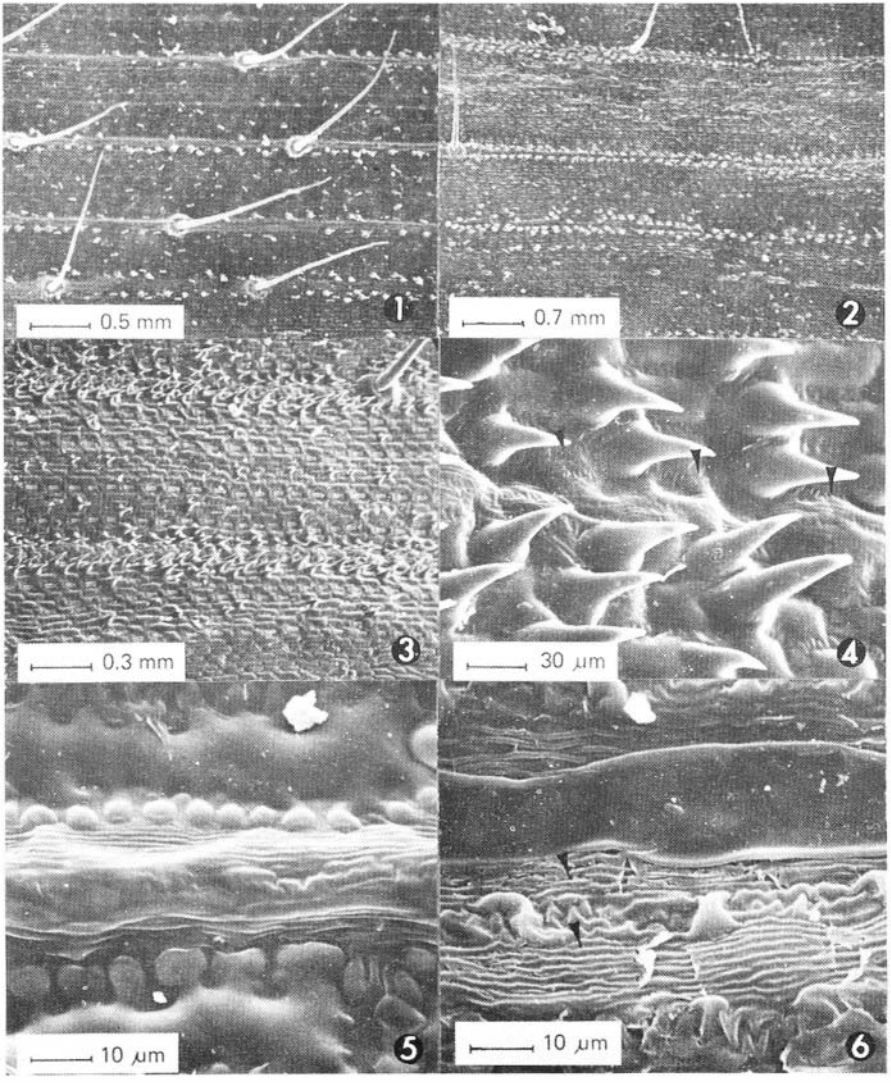
0.5 mm
1
0.7 mm
2
0.3 mm
3
30 µm
4
10 µm
5
10 µm
6

Fig. 7. Modified bulliform cells of an infected leaf.

Fig. 8. Healthy silica cells.

Fig. 9. Anomalous development of silica cell rows.

Fig. 10. Healthy stoma of the adaxial surface.

Fig. 11. Several affected stomata in the chlorotic areas.

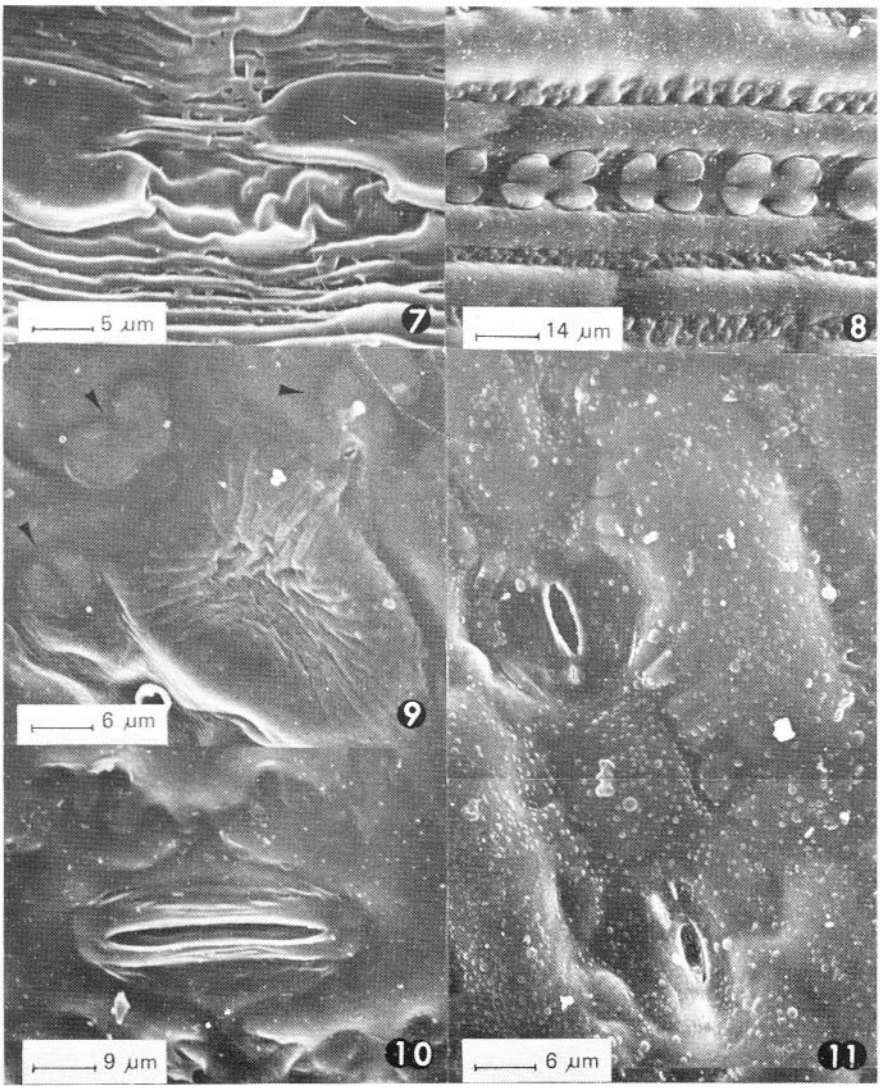
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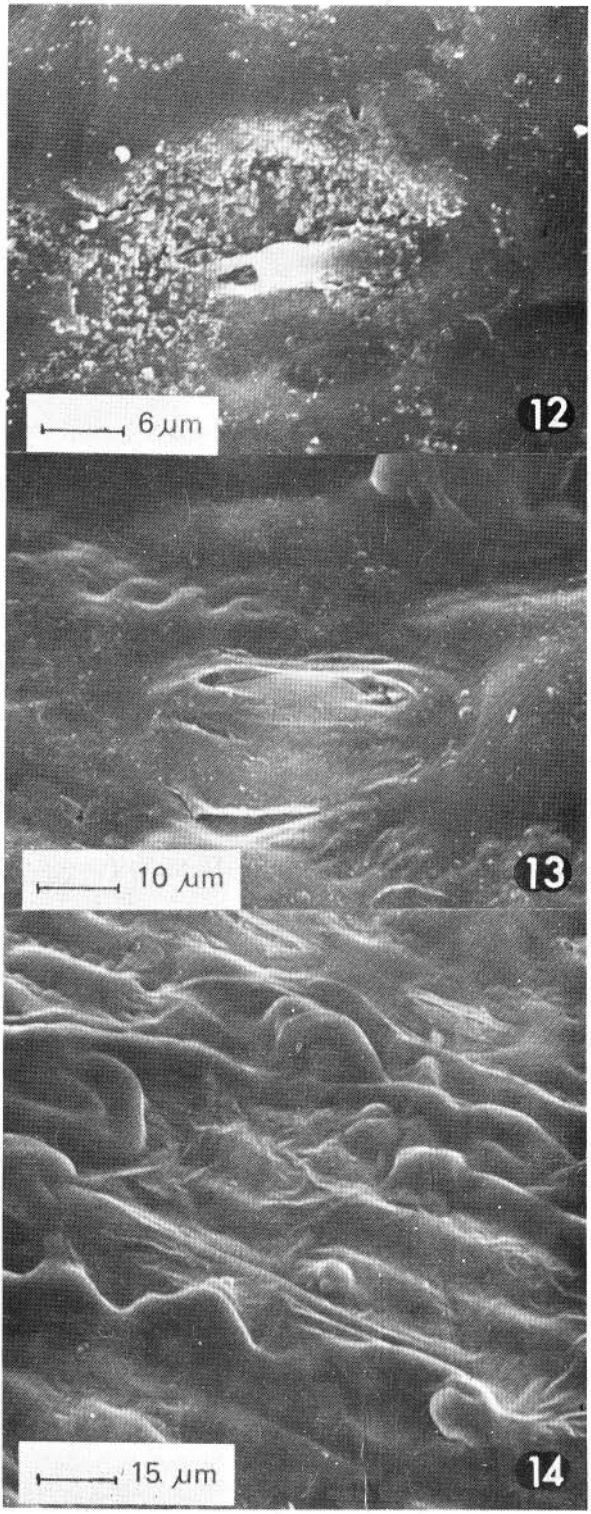
Fig. 12. Anomalous stomatal pore.

Fig. 13. Obliterated stomatal pore.

Fig. 14. Surface of leaf infected at a very early stage.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