Rev. Fac. Agron. (LUZ). 2006, 23: 291-296

Stomatal density in plantains materials (*Musa* AAB, AAAB and ABB) susceptible and resistant to Black Sigatoka (*Mycosphaerella fijiensis*, Morelet)

Y. Hernández[1], F. Portillo[1], M. Portillo[1], C. Navarro[1], M. Rodríguez[1] y J. Velazco[1]

[1]Universidad Nacional Experimental Sur del Lago "Jesús María Semprum" (UNESUR)

Abstract

A study on stomatal density and influence on the probability of fungi penetration was carried out in order to generate basic information on the possible relation between partial resistance and fungi attack causing Black Sigatoka. More than 70% of the penetration occurs through stomates. Different plantain varieties from the Maracaibo Lake's south zone were chosen: FHIA 20, fhia 21 AND Topocho Cachaco as resistant; Horn and Africa as susceptible clones. Leaf sample were taken from the fifth position in flowering plants. Stomates were observed under a microscope (40X) and counted with the help of a New Bauer camera. Stomatal density was given per mm^2 for both upper and leaf surfaces. In terms of stomatal density for the lower surface, mean values were higher for Hartón and Africa clones (337 and 342, respectively), followed by Topocho, FHIA 21 and FHIA 20 (208, 196 and 151 respectively). Hartón presented the maximum value on the upper surface (58), while Topocho showed the minimum (32). The minimum value for stomates on the lower surface was for FHIA20 (75) and the maximum (550) for Hartón.

Key words: stomatal density, plantain, resistance, black Sigatoka.

Introduction

Black sigatoka is known as one of the most serious diseases that attack plantain crops, and it is even more virulent than Yellow sigatoka, being necessary its control for obtaining an acceptable production with a good commercial value (9). The characteristic of higher virulence of the disease is manifested by a high sporulative capacity with short reproductive cycles and an elevated severity on the affected tissue (leafs), which has taken the producers to implement more continuous programs and a higher use of agro-chemicals. It is evident that the more applications of products done, the highest the negative incidence will be

Received March 17, 2003 ● Accepted October 10, 2005
Author email: yvo333@hotmail.com

in the production expenses, also higher environmental pollution and it might induce the fungus resistance to these biocides (8). Black sigatoka is caused by an ascomycete fungus Mycosphaerella *fijensis* Morelet (11 and 12). The first symptom of the disease, generally speaking, is that it appears on the bundle of the limb as longitudinal dots with a pale yellow color or dark brown on the underside of the limb from 1 to 2 mm length, that increase their size forming necrotic lesions with yellow traces and clear gray centers, causing a reduction in the production by the violent reduction of the foliar area, and therefore of the photosynthetic capacity of plants (9).

Conidium and ascospore are the mean propagation of the disease and its dissemination in the plantation is mainly through the wind, being very high the sporulation rate of the fungus if climatic conditions are favorable. Once the fungus arrives in the plant, the penetration of it inside the tissues occurs in more than 70% through the stomata of leafs. It has been proved through observations done in a microscope that just after the fungus penetrates through the stomata, chemical reactions of hypersensitivity are produced, and that would avoid the expansion of the pathogen to the vegetal tissue. In many cases, the pathogen surpasses rapidly this chemical resistance and therefore it does not last (1). Some researches done in evaluations about the morphological and physiological characteristics of the stomata in leafs, showed that the resistance to Black sigatoka is mainly due to non stoma mechanisms (14). However, the stomatic density is of the structural mechanism that acts as a physical barrier without allowing the penetration of the pathogen in the plant (6). Also, the stomata number might indicate the efficiency of determined vegetal specie in the respiration process and photosynthesis that contribute among other functions, in the formation of chemical substances able to stop the progression of the pathogen in the leaf (1). The introduction of plantain with a resistance to Black sigatoka in classic improvement programs, are based in the use of the resistance found in wild species of musa and obtained hybrids (4). In banana, the anatomic analysis showed that the stomatic density in the surface of leafs is inversely proportional to the ploidy, since polyploidy causes an increment in the cells' size. This difference is not significant among the diploid and tetraploid materials of Musa (2). The basic knowledge provided on this research will allow relating which are some of the specific component of the partial resistance that may reduce significantly the development rate of the disease in the field, also providing little information about the stomatic density of susceptible and tolerant clones in the area.

Materials and methods

The research was carried out in the South area of Maracaibo´s lake, which belongs to a wet tropical forest with a height of 54 meters on the sea level, annual accumulated precipitation of 1331.8 millimeters, average annual temperature of 28.06ºC and relative humidity of 82.7%; winds with velocities from 4.7 Km per hour for almost the entire year (10). For the research, plantain materials were searched with a proved susceptibility and with resistance to Black sigatoka, finding on the respective order Africa and Hartón clones (AAB) (5), as susceptible and FHIA 21 (AAAB), FHIA 20 (AAAB) (3) and the «Cachaco» or «Topocho» clone (ABB) as resistant to the disease (7). Foliar samples of the plantain for Hartón, Africa, FHIA 21 and FHIA 20 materials, were collected from the Germplasm bank of musaceas of the International Center of Plantain (CIPLAT) and the «Cachaco» clone taken from the National Institute of Agriculture Research (INIA), 41 kilometers, Vigía, Chama station (Lat N:8°43´27":Long W:71°44´33"). For that, 8 plants in flowering or fruiting phase were selected, excepted the FHIA 20 material where only samples of 2 unique young plats from 3 to 4 leafs were recollected, collecting samples of approximately 25 cm^2 of the mean area of the limb of each side of the fifth leaf of each plant, counted from the apex to the base. Later, the foliar samples were identified and preserved with a chemical fixer FAA (Formol 75%, Ethanol 10% and Glacial Acetic Acid 15%). Subsequently, cuts with elimination of the epidermis were done, in the bundle and in the underside with new razors. Each cut was put in the center of the area recorded in a Newbauer camera, than adding using a dropper a little less than a drop of water to the cut and in the grating of the camera, to avoid the fast dehydration of the tissue (epidermis), covering the sample with a slide and observing it through an optic microscope (ocular increment 10X and objective 40X). In the observations, stomata were only counted, those of the bundle and of the underside that entered at random on each of the 16 squares of 0.0625 mm^2 that formed the central square of 1 mm^2 of the camera, trying to move the sample inside the microscope with the purpose of not having the same view, until reaching a total of 100 squares or minimum replications per sample of each side of the leaf, and coming from the different evaluated clones. The number of stomata observed was calculated by square millimeter (e/mm^2) and the tabulated values were analyzed (ANOVA) under the statistical software SAS for Windows, to calculate the mean, maximum and minimum values and correlations in the stomata present in both the bundle and in the underside.

Results and discussion

For the stomata number in the underside (table 1), the highest averaged values were for clones Hartón and Africa 218 and 215.7 e/mm^2 respectively, followed by "Cachaco", FHIA21 and FHIA20, 133.4 - 126 – 96.8 e/mm^2 respectively, to almost the half of the stomata found

Table 1. Average values of the stomatic density of Hartón (H), FHIA21 (F21), FHIA 20 (F20) África (A) and Topocho Cachaco (CA) clones

Variable	Meane /mm²	Standard deviation	Minimum valuee/mm²	Maximum valuee/mm²
HH	33.056	15.04	0	64
HE	218.18	40.368	104	352
F21H	30.096	13.504	0	60
F21E	126.1	24.176	72	192
AH	25.6	15.264	0	64
AE	215.71	37.42	112	304
F20H	25.648	13.36	0	64
F20E	96.89	15.84	48	128
CAH	20.352	11.04	0	48
CAE	133.4	19.23	64	176

H: bundle E: underside e/mm^2 : stomata per square millimeter

for clones Hartón and Africa. The minimum value of stomata in the underside was found in FHIA 20 and 21 with 72 and 48 e/mm^2 and a maximum level of 352 e/mm^2 Hartón materials. In these results, in spite of not finding significant differences in the underside among the evaluated clones, it was observed that the stomatic density is lower in resistant materials, contrary to the susceptible clones with higher stomatic density. The lather agrees to the reported by Trujillo *et al.* (1997), but in this case the relation between the stomatic density and the resistance to yellow Sigatoka was evaluated (13). For the bundle, the results did not show significant differences among all the used materials with a maximum averaged value correspondent to Hartón (33.05 e/mm^2), followed by FHIA21, África y FHIA20 (30.09 – 25.66 – 25.64 e/mm^2 respectively, and a minimum value for the "cachaco" clone (20.35 e/mm^2). In other researches done about the stomatic density in the bundle, was found that Topocho, Dominico Topocho and black topocho clones, densities of 32. 37 and 48.1 e/mm^2 and in «plátano vega» values of 68.1 e/mm^2 (3). It was only found a significant and positive correlation ($P<0.05$) between the stomata number of the bundle and the underside for Hartón clone. Just as what was reported for other Musa clones, all plantain clones presented a higher stomatic density in the underside than in the bundle (13). Considering the ploidy level and the stomatic density among the Musa materials studied in this research, it was not observed a good relation among both characteristics, since doing both comparisons between the resistant materials, topocho cachacho triploid and FHIA 20 and 21 tetraploids, all presented low stomatic density values. These results agree to those reported in other researches, where triploid susceptible clones (Brasilero and Pineo Gigante) and resistant (CIEN BTA-03), presented low stomatic density values (13). Likewise, studying the resistance to Black Sigatoka in relation to the ploidy level, it has been found that tetraploids clones of Musa FHIA 20 and 21 have presented a high resistance to the pathogen, but in tetraploids of FHIA 16, 04 and 05 showed susceptibility from medium to high to the disease respectively (3).

Conclusions

Plantain clones FHIA 20, FHIA 21 and topocho "Pelipita", characterized as materials resistant to Black Sigatoka presented a much lower stomatic density than Hartón and Africa clones or materials non resistant to the disease.

The stomatic density under some bioclimatic and crop conditions may be one of the important factors of negative regulation to the entrance of the fungus inside the plant.

Literature cited

1. Beveraggi, A., M.F. Zapater and X. Mourichon.1995. Análisis de la resistencia de los bananas a la Sigatoka negra causada por *Mycospharella fijensis* (*Cercospora fijensis*).CIRAD-CA. Montpellier (FRA). Extraído de Referencias bibliográficas Musa Doc- 1999, sobre bananos y plátanos de las Memorias XIII Reunión ACORBAT, Guayaquil, Ecuador.1998.6-11.

2. Borges, O.L. 1992. Tamaño y densidad de estomas en clones cultivados y especies silvestres de Musa. *Agronomía Tropical*. Volumen XXI. Número 2. p 139 - 143.

3. Coto, J., E. Rosales, P. Rowe and J.M. Rivera. 1994. Reacción a Sigatoka Negra y comportamiento agronómico de plátanos híbridos (AAAB) sometidos a desmanes. Fundación Hondureña de Investigaciones Agrícolas (FHIA). Memorias de la XI reunión de ACORBAT. San José de Costa Rica. p399.

4. Craenen, K and R. Ortiz. 1997. Efecto del gen bs1 en respuesta a la Sigatoka negra en híbridos de plátano. Theorical and Applied Genetics (USA). 95 (3), 497-505.

5. Haddad, O. and O. Borges. 1992. Identificación de clones de bananos (cambures y plátanos) en Venezuela. Centro de Investigaciones Agronómicas, Sección Fitotecnia, Maracay. Agronomía Tropical 21(4):277-286.

6. Hermoso, L., H. Lindorf and E. de García. 1997. Anatomía Foliar del Variante Somaclonal (CIEN BTA 03) *Musa* sp, resistente a la Sigatoka Amarilla. *Anales de Botánica Agrícola*. 4:63-67.

7. Jiménez, F and N. Vásquez.1996. Estudio de la densidad y distribución estomática en Musáceas de diferentes ploidía. CATIE, Turrialba. Extraído de Referencias bibliográficas Musa Doc- 1999, sobre bananos y plátanos de las Memorias XIII Reunión ACORBAT, Guayaquil, Ecuador.1998.p 4.

8. Madrigal, A and W. Ruess. 1998. CGA245704 un nuevo activador de plantas para aumentar la

resistencia natural del cultivo del banano contra Sigatoka negra (*Mycosphaerella fijensis)*. Extraído de Referencias bibliográficas sobre bananos y plátanos de las Memorias XIII Reunión ACORBAT, Guayaquil, Ecuador.p.266-274.

9. Martínez, G., R. Pargas and D. Muños. 1997. "La Sigatoka negra y su avance en el territorio Venezolano: implicaciones socioeconómicas". Consultado en: http://www.fonaiap.gov.ve/publica/divulga/fd59/sigatok.html

10. Ministerio del Ambiente y los Recursos Naturales Renovables (MARNR). 1996. Datos Climatológicos, Estación Santa Bárbara, años 1968-1996. El Vigía, Edo. Mérida, Venezuela.

11. Nava, C. 1997. El Plátano, su cultivo en Venezuela. Ediciones Astro Data. Maracaibo. 122p.

12. Pineda, J., A. Carrasco, R. Cardona and R. Cooz. 1997. Presencia de la Sigatoka negra (*Mycosphaerella fijiensis*) en las principales zonas plataneras de Venezuela. Bioagro 9(2): 52-60. 1997

13. Trujillo, I., L. Hermoso and E. de García. 1997. Caracterización estructural de clones de banano: resistentes y no resistentes a la Sigatoka Amarilla. *Anales de Botánica Agrícola.* 4:59-62.

14. Vuylsteke, D.R., I. Ekanayake and R. Ortiz. 1998. Conductancia estomática de las hojas y morfología de los estomas del material genético de *Musa. Euphytica*. (NDL) Vol. 99(3), 211-229.