Pseudocercospora fijiensis mycelia-based infection system enhances investigational efficacy of *P. fijiensis*-banana pathosystem

Kumakech Alfred[1], Laban F. Turyagyenda[1], Richard Edema[2] & Patrick Okori [3]

[1] National Agricultural Research Organization (NARO), Ngetta Zonal Agricultural Research and Development Institute, Lira, Uganda

[2] Department of Agricultural Production, Makerere University, Kampala, Uganda

[3] Regional University Forum, Makerere University, Kampala, Uganda

Corresponding author: Kumakech Alfred, National Agricultural Research Organization, Lira, Uganda. kumalfred@gmail.com

Received: November 11, 2023 DOI: 10.14295/bjs.v3i2.485

Accepted: December 22, 2023 URL: https://doi.org/10.14295/bjs.v32.485

Abstract

Screening procedures for black Sigatoka have limitations. Thus, there is need for alternative screening procedure. A robust controlled-environment methodology for testing reaction of banana genotypes to *Pseudocercospora fijiensis* is, thus, still required. The objective of this study was, therefore, to assess the effect of *P. fijiensis* fragmented mycelia-based inoculum on black Sigatoka development in banana under screen house conditions with the view of developing a procedure for early assessment of resistance. Black Sigatoka severity increased significantly ($P < 0.05$) over time in all genotypes apart from Kayinja. Significant differences ($P < 0.05$) in severity were also recorded among the genotypes at 3, 4, 5 and 6 weeks after inoculation. All east African highland banana and plantain genotypes tested exhibited susceptible reaction, characterized by rapid progression of symptoms to necrotic lesions. Kayinja and M9 hybrids exhibited resistant reactions, characterized by small necrotic specks and chlorotic or brown blotches, respectively. The fragmented mycelia-based infection system classified the banana genotypes into resistant and susceptible clones, making it a reliable and efficient infection technique to assess black Sigatoka disease damage. The infection system is recommended for early screening for black Sigatoka resistance.

Keywords: *Psedocercospora fijiensis,* fragmented mycelia, banana genotypes, severity.

Sistema de infecção baseado em micélio de *Pseudocercospora fijiensis* aumenta a eficácia investigacional do patossistema *P. fijiensis*-banana

Resumo

Os procedimentos de rastreio da Sigatoka Negra têm limitações. Assim, há necessidade de procedimento de triagem alternativo. Uma metodologia robusta de ambiente controlado para testar a reação de genótipos de bananeira a *Pseudocercospora fijiensis* ainda é portanto, necessária. O objetivo deste estudo foi, portanto, avaliar o efeito do inóculo baseado em micélio fragmentado de *P. fijiensis* no desenvolvimento da Sigatoka negra em bananeira sob condições de telado, com o objetivo de desenvolver um procedimento para avaliação precoce de resistência. A severidade da Sigatoka Negra aumentou significativamente ($P < 0,05$) ao longo do tempo em todos os genótipos, exceto Kayinja. Diferenças significativas ($P < 0,05$) na severidade também foram registradas entre os genótipos às 3, 4, 5 e 6 semanas após a inoculação. Todos os genótipos de banana e banana das terras altas da África Oriental testados exibiram reação suscetível, caracterizada pela rápida progressão dos sintomas para lesões necróticas. Os híbridos Kayinja e M9 exibiram reações resistentes, caracterizadas por pequenas manchas necróticas e manchas cloróticas ou marrons, respectivamente. O sistema de infecção fragmentado baseado em micélios classificou os genótipos de bananeira em clones resistentes e suscetíveis, tornando-se uma técnica de infecção confiável e eficiente para avaliar os danos da Sigatoka negra. O sistema de infecção é recomendado para triagem precoce da resistência à Sigatoka negra.

Palavras-chave: *Psedocercospora fijiensis*, micélios fragmentados, genótipos de bananeira, severidade.

1. Introduction

Black Sigatoka is one of the main constraints to banana and plantain production in sub-Saharan Africa, impacting directly on yields (Batte et al., 2019; Arango et al., 2016). The disease, caused by the fungus *Pseudocercospora fijiensis* (Morelet) Deighton (teleomorph *Mycosphaerella fijiensis* Morelet), is the most important constraint to production of the east African highland banana in Uganda (Nowakunda *et al*., 2015). Black Sigatoka is endemic and found in nearly all banana-growing regions of Uganda (Kimunye *et al*., 2019), although the highland cool regions are less affected (Kimunye et al., 2019). The disease is most severe in humid and warm conditions below 1200 meters above sea level. Churchill (2011) reported banana bunch weight loss of up to 37% and decline in quality of fruits as a result of black Sigatoka infection. According to FAO (2012), black Sigatoka has a negative effect on the economics of banana production.

The tall stature of banana plants, presents challenges in screening for foliar disease reaction, further compounding efforts to manage the disease. Current controlled environment methods for black Sigatoka resistance screening in banana have limitations (Twizeyimana et al., 2007). Such limitations include: colonization of media by non-target fungi, and the difficulty associated with the maintenance of excised leaf discs in a non-senescent state. Additionally, black Sigatoka disease development on detached leaves is not consistent, and it is extremely difficult to produce sufficient amounts of conidia for artificial inoculation.

The use of mycelial suspensions as inocula, while promising, has been limited due to difficulty of quantification and standardization (Ojiambo et al., 2010; Twizeyimana et al., 2007). Methods for assaying lack Sigatoka are therefore lacking. As a result, black Sigatoka resistance screening is commonly done in the field in hot spots under differing environments, pathogen pressure, and very slow disease development (Yonow et al., 2019). Field evaluation is nonetheless, only useful for germplasm characterization but not sufficient to identify partial resistance components at different phases of black Sigatoka infection cycle (Yonow et al., 2019; Balint-Kurti et al., 2001).

A reliable controlled-environment procedure for assessing reaction of banana genotypes to *P. fijiensis* is, thus, still needed. The objective of this study was, therefore, to determine the effect of *Pseudocercospora fijiensis* fragmented mycelium on black Sigatoka development in banana under screen house conditions with the aim of developing a methodology for early evaluation of resistance reaction.

2. Materials and Methods

2.1 Plant materials

Tissue culture banana plantlets of five east African highland banana genotypes (Nfuuka, Mpologoma, Kibuzi, Mbwazirume and Musakala), three plantain genotypes (Sukali Ndiizi, Kisubi & Kayinja), two dessert genotypes (Gonja & Gros Michel) and one hybrid (M9) were used for screen house assays (Table 1). Gros Michel and Kayinja were included as susceptible clone and standard resistant clone for black Sigatoka. Two-month-oldseedlings were transplanted to individual pots containing pre-sterilized loam soil, and were grown in thescreen house for one month.

2.2 Preparation of Pseudocercospora fijiensis inoculum

The isolate Mak 01 of *P. fijiensis* was prepared following the procedure described by Kumakech et al. (2015). Mak 01 isolate was selected for its virulence. Twenty-five (25) malt extract agar (Oxoid, UK) plates were aseptically inoculated and incubated at 25 °C for 14 days. Mycelia were removed with a sterile scalpel. The mycelia were bulked and weighed in a sterile filter paper that was pre-weighed. Weighed mycelia was fragmented in a blender at full speed for 3 minutes, and a master mycelium suspension was prepared and diluted to a final concentration of 15 mg mL^{-1}.

2.3 Experimental design

Two screenhouse experiments were established following a randomised complete block design, with four replications. In both experiments, 3-month-old banana plantlets were inoculated with 1ml of 15 mg mL^{-1} mycelia. Inoculum was applied using a painter brush on the abaxial surfaces of the first and second fully unfolded leaves, until run-off. Twelve banana plantlets were inoculated for each genotype. Inoculated plantlets were incubated in a

humidity chamber at a temperature of 28-31°C and relative humidity of 90% for 48 h.

2.4 Data collection and analysis

Data on severity, incubation and latent periods were collected. A 1-5 scale developed by Fullerton and Olsen (1995) was used to evaluate stages of symptom development, where 1 = Light brown flecks 1-2 mm diameter on abaxial surface, 2 = A diffuse speckled blotch on adaxial surface, 3 = Grey, dark grey, or reddish discrete spots, 4 = Black or brown circular spots with dry centre of grey colour, possibly with a yellow halo on the upper leaf surface, 5 = Necrotic leaf. Maximum stage of symptom development and time data was used to grade the reactions of genotypes on a scale of 1-5, where 1 = symptom stage 1 or 2, 2 = symptom stage 3, 3 = symptoms stage 4 > 45 days, 4 = symptoms stage 4 < 45 days, 5 = symptoms stage 4 < 30 days. Area under disease progress curve (AUDPC) values were calculated from severity data (Madden et al., 2007) prior to statistical analysis. All data were subjected to analysis of variance (ANOVA) at 5% significance.

3. Results

3.1 Psedocercospora fijiensis incubation and latent periods

Results of the effect of fragmented mycelium inoculum on *P. fijiensis* incubation and latent periods is presented in (Table 1). The incubation period differed significantly ($P < 0.05$) among the tested genotypes. The mean incubation period varied between 22 and 43 days for susceptible and resistant genotypes, respectively. On average, small necrotic specks appeared on average at 23.2 days for susceptible cultivars and at 38.7 days for resistant cultivars. Symptoms for EAHBs generally appeared after 21 days. In EAHBs black or brown lesions developed on inoculated leaves. The lesions were surrounded with a yellow hallow, and dry grey centres in 30 days. Similar observation was recorded on Gros Michel, a reference susceptible standard for black Sigatoka. Furthermore, highly significant differences ($P < 0.05$) in latent periods were found in all banana cultivars. The latent period varied from 8.2 to 12.9 days in susceptible genotypes and 16.3 to 28.2 days for resistant genotypes.

Table 1. Incubation and latent periods of black Sigatoka on banana cultivars inoculated with 15 mg/ml fragmented mycelium suspension under screen house conditions.

Banana cultivar	Resistance category	Mean values (days)	
		Incubation period	Latent period
Mologoma	Susceptible	22.2c	9.4cd
Musakala	Susceptible	21.7c	9.4d
Nfuuka	Susceptible	22.2c	9.7cd
Mbwazirume	Suceptible	22.7c	9.7cd
Kibuzi	Susceptible	22.0c	9..7cd
Gonja	Susceptible	22.5c	10.3cd
Kayinja	Resistant	43.3a	28.2a
Sukali Ndiizi	Susceptible	29.6b	12.9c
Gros Michel	Susceptible	22.7c	8.2d
M9 hybrid	Resistant	34.1b	16.3b
P value		<.001	<.001

Note: [abc]Means followed by the same letter within the column are not significantly different ($P > 0.05$).

3.2 Black Sigatoka severity in banana genotypes

Results of disease severity are presented in (Table 2). Significant differences ($P < 0.05$) in severity were recorded among banana genotypes tested. Infact, high disease severity (AUDPC) was recorded on Gros Michel, Gonja, Mpologoma, Kibuzi, Mwazirume, Ndiizi, Nfuuka and Musakala. Gros Michel had the highest severity (217)

followed by Gonja (195), Kibuzi (158), Mbwazirume (129) Musakala (118) and Mpologoma (121) at six weeks. Nfuuka (95) and Ndiizi (96) were the only susceptible cultivars with low AUDPC values when compared to Gros Michel. On the other hand, M9 Hybrid had a very low AUDPC value (24). Kayinja was the only cultivar that did not develop black Sigatoka symptoms; it registered an AUDPC of zero. Overall, significant differences ($P < 0.05$) in severity were also recorded among the genotypes at 3, 4, 5 and 6 weeks after inoculation.

Table 2. Comparison of AUDPC mean values of banana cultivars inoculated with fragmented mycelia under screen house condition.

Banana cultivar	AUDPC mean values				
	Week 3	Week 4	Week 5	Week 6	LSD (5%)
Mpologoma	3.3b	19.6c	62.1d	121.2d	8.6
Musakala	3.4b	17.7c	58.7d	118.6d	8.1
Mbazirume	3.5b	20.2c	64.3d	129.5d	8.6
Kibuzi	2.6bc	22.7c	76.3c	158.3c	6.4
Nfuuka	4.6b	18.3c	45.9e	95.6e	7.5
Gonja	7.2b	34.4b	108.3b	195.1b	9.2
Kayinja	0.0c	0.0d	0.0g	0.0g	0.0
Ndiizi	0.0c	0.0d	25.4f	96.7e	9.7
Gross Michel	15.8a	53.1a	124.6.9a	217.9.1a	11.2
M9 hybrid	0.0c	0.0d	6.3g	24.5f	2.3
P value	<.001	<.001	<.001	<.001	

Note: [abc]Means followed by the same letter within the column are not significantly different ($P > 0.05$).

3.3 Resistance classification of genotypes

The results of the reaction of banana genotypes to the fragmented mycelium inoculum is presented in (Table 3). All east African highland banana and plantain genotypes tested exhibited susceptible reaction. A susceptible reaction was characterized by rapid progression of symptoms through the stages outlined under data collection and analysis, often with a yellow halo (symptom development stage 4). Two resistance reaction types were recognized, suggesting that different mechanisms of resistance were expressed in the resistant genotypes. In the first type (symptom development stage 1), symptoms were characterized by small necrotic specks which never developed further. Meanwhile, in the second type exhibited by M9 hybrid (symptom development stage 3), symptoms developed through the early stages to form diffuse, chlorotic or brown blotches, but stopped at stage 3 for an extended period, where necrosis only occurred as the leaf senesced.

Table 3. Reaction of ten banana cultivars to inoculation with fragmented mycelial suspensions of *Psedocercospora fijiensis* in the screenhouse.

Cultivar	Stage of symptom development (42 DAI)	Reaction
Mbwazirume	4	Susceptible
Bogoya	4	Susceptible
Kibuzi	4	Susceptible
Mpologoma	4	Susceptible
Nfuuka	4	Susceptible
Musakala	4	Susceptible
Gonja	4	Susceptible
Kayinja	1	Highly resistant
M9 hybrid	3	Moderately resistant
Ndiizi	4	Susceptible

Note: DAI = days after inoculation. 0-5 stage of BSD symptom development (Fullerton; Olsen, 1995).

4. Discussion

Significant differences in the response of banana cultivars to *P. fijiensis* infection was observed three weeks after inoculation. All the east African highland banana clones tested exhibited susceptible reaction. None of the EAHB clones tested, was in the reaction range of Kayinja (ABB), a resistant check to black Sigatoka (Paparu et al., 2007; Tushemereirwe et al., 2004). East African highland genotypes were however, all in the reaction range of Bogoya, a standard susceptible reference clone for black Sigatoka. Earlier, Tushemereirwe et al. (2004) reported that most cultivars in the AAA EAHB group were susceptible to black Sigatoka under natural infection in the field. In the current study, the reaction of EAHB was characterized by the presence of symptoms after the first 21 days after inoculation. Symptom development reached stage 4 in 30-31 days after inoculation. When compared to Bogoya, black Sigatoka development on the tested EAHB clones was faster for Kibuzi, and slower for Nfuuka. This result suggests that there is variation in the level of susceptibility of AAA EAHB cultivars. The differences in the reaction of banana is an indication of the ability of fragmented mycelium-based inoculum to categorize banana into resistant and susceptible genotypes.

On the whole, the infection system categorized the banana genotypes into resistant and susceptible clones, thus, providing a credible and useful infection technique to assay black Sigatoka disease severity. Inoculation of *in vitro* plants with mycelial inoculum was simple, fast and realistic for determining reaction of banana to *P. fijiensis*. The utilization of mycelia-based infection system in pathogenesis testing and black Sigatoka resistance screening will enhance the investigational efficacy of *P. fijiensis*-banana pathosystem.

Black Sigatoka development in plantain was as fast as in Bogoya, a reference susceptible genotype. Tushemereirwe *et al*., (2004) reported a similar reaction under field conditions in Uganda. On the other hand, AUDPC value of Ndiizi (96.7) was significantly lower than that of Bogoya (217.9), but the final disease development was in the range of susceptible clones based on symptom grading according to Fullerton & Olsen (1995). This confirmed findings from previous field evaluations that reported a susceptible reaction of Ndiizi (Tushemereirwe *et al*., 2004). However, in the current study, Ndiizi had a long incubation period (approx. 30 days), with a rapid disease development after symptom appearance. The hybrid M9 (AAAA) and Kayinja (ABB) were the only genotypes that exhibited resistant reactions. The AUDPC for M9 (24.5) was however, significantly higher than that of Kayinja (0.0). The current study therefore, revealed that in some susceptible cultivars, black Sigatoka disease appearance after artificial inoculation can be delayed as was reported for Ndiizi. The implication of this finding is that incubation period alone cannot be used to determine the resistance reaction of banana genotypes to *P. fijiensis*. This, however, is contrary to the report of Alvarez et al. (2010), where incubation was used to evaluate resistance to black Sigatoka of plantain and banana genotypes under greenhouse conditions.

It is important to that that black Sigatoka reactions reported in the current study apply only to young banana plantlets in the screenhouse. The relationship between young and adult plant reactions to black Sigatoka for all the tested varieties is not well documented in literature. Nonetheless, disease reactions exhibited by young plants in this study matched the reaction in adult plants reported by Tushemereirwe et al. (2004). Inoculated 3-month-old plants exhibited symptoms that were typical of adult plants infected in the field. This finding demonstrated that black Sigatoka disease development on 3-month-old plantlets in the screen house is not different from banana plants in the field. Thus, the approach of using 3-month-old plants is useful for classifying reaction of banana to black Sigatoka in the screen house. This supports further, the use of fairly older plants (3 months) in the banana-*P. fijiensis* pathosystem experiments under screen house conditions.

5. Conclusions

Black Sigatoka infection levels caused by fragmented mycelia of *Pseudocercospora fijiensis* increased with the level of susceptibility of banana genotypes. Resistance to black Sigatoka in banana inoculated with fragmented mycelia can therefore be evaluated by quantification of disease severity using AUDPC. The use of fragmented mycelia provided an efficient and precise early evaluation procedure.

6. Acknowledgments

We thank Makerere University Agricultural Research Institute, Kabanyolo (MUARIK) and the National Agricultural Research Laboratories of the National Agricultural Research Organisation (NARO) for offering facilities required to conduct this study.

7. Auhors' Contributions

Kumakech Alfred: responsible for the ideas, formulation, evolution of the objectives of the research, carrying out experiments, data collection, statistical analysis and writing the original draft. *Laban. F. Turyagyenda*, *Richard Edema* & *Okori Patrick*: supervision and review of the research work.

8. Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

9. Ethics Approval

Not applicable.

10. References

Arango, I. R. E., Diaz-Trujillo, C., Dhillon, B., Aerts, A., Carlier, J., & Crane, C. F. (2016). Combating a global threat to a clonal crop: banana black Sigatoka pathogen *Pseudocercospora fijiensis* (synonym *Mycosphaerella fijiensis*) genomes reveal clues for disease control. *PLoS Genetics*, 12(8), 432-441. https://doi.org/10.1371/journal.pgen.1005876

Alvarez, E., & Cuellar, A. (2010). Evaluating the resistance of plantain and banana genotypes to Black Sigatoka (*Mycosphaerella fijiensis*) under greenhouse conditions. American Phytopathology Society Annual Meeting. Celebrated in August 7-11. Available in: http://www.apsnet.org/meetings/ Documents/2010_Meeting_Abstracts/ a10ma27.htm. Access in: December 22, 2023.

Balint-Kurti, P. J., May, G. D., & Churchill, A. C. L. (2001). Development of a transformation system for *Mycosphaerella* pathogens of banana: a tool for the study of host/pathogen interactions. *FEMS Microbiology Letters*, 195(1), 9-15. https://doi.org/10.1111/j.1574-6968.2001.tb10490.x

Batte, M., Swennen, R., Uwimana, B., Akech, V., Brown, A., Tumuhimbise, R., Hovmalm, H. P., Geleta, M., & Ortiz, R. (2019). Crossbreeding East African Highland Bananas: Lessons Learnt Relevant to the Botany of the Crop after 21 Years of Genetic Enhancement. *Frontier in Plant Science*, 81(10), 1-9. https://doi.org/10.3389/fpls.2019.00081

Churchill, A. C. L. (2011). *Mycosphaerella fijiensis*, the black leaf streak pathogen of banana: progress towards understanding pathogen biology and detection, disease development, and the challenges of control. *Molecular Plant Pathology*, 12(4), 307-328.https://doi.org/10.1111/j.1364-3703.2010.00672.x

Fao. (2012). A holistic integrated management approach to control black sigatoka disease of banana caused by *Mycosphaerella fijiensis.* Technical Manual. Available in: https://www.fao.org/fileadmin/templates/agphome/documents/caribbeanspci/IntApproachBSDManagement LVP.pdf. Access in: December 22, 2023.

Fullerton, R. A., & Olsen, T. L. (1995). Pathogenic variability in *Mycosphaerella fijiensis* Morelet, the cause of Black Sigatoka in banana and plantain. *New Zealand Journal of Crop and Horticultural Science*, 23(1), 39-48. https://doi.org/10.1080/01140671.1995.9513866

Kimunye, J. N., Were, E., Mussa, F., Mahuku, G., Tazuba, A., Jomanga, K., Viljoen, A., Swenne, R., & Muthoni, F. K. (2019). Distribution of *Pseudocercospora* species causing Sigatoka leaf diseases of banana in Uganda and Tanzania. *Plant Pathology*, 69 (1), 50-59. https://doi.org/10.1111/ppa.13105

Madden, L. V., Hughes, G., & vanden Bosch, F. (2007). The study of plant disease epidemics. American Phytopathological Society. St. Paul Minnesota, USA.

Nowakunda, K., Barekye, A., Ssali, R.T., Namaganda, J., Tushemereirwe, W. K., Nabulya, G., Erima, R., Akankwasa, K., Hilman, E., Batte, M., & Karamurat, D. (2015). 'Kiwangaazi' (syn 'KABANA 6H') Black Sigatoka, Nematode and Banana Weevil Tolerant 'Matooke' Hybrid Banana Released in Uganda. *Horticulture Science*, 50 (4), 621-623. http://hortsci.ashspublications.org/content/50/4/621.full.pdf

Ojiambo, P. S., Twizeyimanal, M., Tenkouano, A., & Bandyopadhyay, R. (2010). Rapid and mass screening of banana and plantain for resistance to Black sigatoka disease using detached leaf and in vitro plantlets. *In*: Mass screening techniques for selecting crops resistant to diseases (p. 91-100). Vielnla: International Atomic Energy Agency.

Paparu, P., Dubois, T., Coiney, D., & Viljoen, A. (2007). Defence-related gene expression in susceptible and tolerant bananas (*Musa* spp.) following inoculation with non-pathogenic *Fusarium oxysporum* endophytes and challenge with *Radopholus similis*. *Physiological and Molecular Plant Pathathology*, 71(4-6), 149-157. https://doi.org/10.1016/j.pmpp.2007.12.001

Tushemereirwe, W. K., Kangire, A., Kubiriba., J., & Nakyanjzi, M., & Gold, S. (2004). Field reaction of banana cultivars to Black Sigatoka in Uganda. *African Crop Science Journal*, 12(1), 19-26.

Twizeyimana, M., Ojiambo, P. S., Tenkouano, A., Ikotun, T., & Bandyopadhyay, R. (2007). Rapid screening of *Musa* species for resistance to black leaf streak using *in vitro* plantlets in tubes and detached leaves. *Plant Disease*, 91(3), 308-314. https://doi.org/10.1094/PDIS-91-3-0308

Yonow, T., Villegas, J. R., Abadie, C., Darnell, R. E., Noboru, O., & Kriticos, D. J. (2019). Black Sigatoka in bananas: Ecoclimatic suitability and disease pressure assessments. *PLoS One*, 14(8), 21-35. https://doi.org/10.1371/journal.pone.0220601

Funding

Not applicable.

Intitutional Review Board Statement

Not applicable.

Informed Consent Statement

Not applicable.