GERMINATION AND APPRESSORIA FORMATION BY *Colletotrichum musae* IN FRUIT PEEL EXUDATES OF DIFFERENT BANANA VARIETIES

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Introduction

Anthracnose caused by *Colletotrichum musae* is a postharvest disease of banana causing significant economic losses. Eventhough, a clear variation of the intensity of anthracnose development has been identified among different banana varieties grown in Sri Lanka (De Costa et al. unpublished data), the exact reasons for this varietal variation have not been elucidated. Germination of spores and subsequent appressorium formation are two crucial steps of the infection process towards a successful infection of anthracnose causing fungi. Influence of banana fruit peel exudates on spore germination and appressorium formation of *C. musae* has been reported by Swinburne (1976) and Harper and Swinburne (1979). Therefore, it could be hypothesized that a variation in properties of fruit peel exudates (FPEs) could be responsible for variation in the intensity of anthracnose development in different banana varieties. Furthermore, pH changes in fruit peel during the ripening process could influence the infection of anthracnose pathogen, as has been shown for other tropical fruits such as avocado (Yakoby et al., 2000). As very little information is available on the change of pH among different Sri Lankan banana varieties during the process of ripening and also on the effects of FPEs of those varieties on the infection process of *C. musae,* the present study was conducted with the objectives of determining: (a) the pH changes of FPEs of different banana varieties during ripening and (b) effects of those FPEs on germination and appressoria formation of *C. musae* spores. Findings of this study would help elucidate the basis of varietal differences of banana anthracnose development and facilitate the design of effective management measures.

Materials and methods

Six varieties, including cooking-type and dessert-type banana grown in Sri Lanka were collected from a wholesale market. Based on our previous investigations on susceptibility to anthracnose at postharvest stage, two varieties each were collected from three different susceptibility groups of banana as following: Alukehel (ABB) and Mondan (ABB), representing the highly resistant, Embul (AAB) and Seeni (AAB) representing the moderately resistant and Embon (AAA) and Kolikuttu (AAB) representing highly susceptible groups were collected when the fruits were at the maturity index (m.i.) of 1 (all green stage). FPEs were collected daily, starting from m.i. 2 (light green stage) onwards by placing a 250 μl volume of sterilized distilled water on the peel and incubating overnight in a humid chamber until they reached m.i. 7 (full yellow stage). The collected exudates (i.e. 10 ml of peel exudates from each variety x maturity stage combination with five replicates) were filter sterilized using a 0.2 μm filter and stored at 4°C. pH of FPEs of different varieties was measured daily starting from m.i. 2. One ml of the exudates of different banana varieties collected from fruits having m.i. 2 and 7 was placed on cavity slides and incubated in a humid chamber at 28°C with a known volume of *C. musae* spore suspension. Spore germination (SG) and appressoria formation (AF) were quantified at x 400 magnification on a unit area using 30 - 50 spores at 2, 8 and 24 hours after incubation. To determine if pH has an effect on spore germination and appressoria formation, the pH value of FPEs was adjusted to the pH of the exudate giving the highest spore germination and appressoria formation (i.e. Kolikuttu) using malate-hydroxide buffer. Data were analyzed by

ANOVA and mean separation was done by LSD.

Results

pH of FPEs decreased significantly ($p<0.001$) during ripening in all banana varieties except Seenikehel, whose FPE showed a significant ($p<0.001$) increase (Table 1). It can be noted that the FPE pH of the two highly-resistant varieties, Alukehel and Mondan did not decrease below 6.0 while that of the two highly-susceptible varieties, Embon and Kolikuttu, decreased below 6.0 at ripening (i.e. 5.76 and 5.26 respectively). The lowest pH at the ripened stage was shown by Embul (i.e. 4.84).

Percentage spore germination (SG%) and appressorium formation (AF%) varied significantly (<0.0001) between varieties and maturity stage (Table 1). In addition, the variety x maturity stageinteraction on both SG% and AF% were significant ($p<0.001$). All varieties showed significant increases in SG% and AF% with ripening. Kolikuttu and Embon, the two varieties which were initially identified as anthracnose-susceptible, showed significantly greater SG% and AF% at the ripened stage. *Even at the unripe stage, Ambon showed a significantly greater SG%* than the rest. Alukehel, which was initially identified as highly-resistant to anthracnose, showed the lowest SG% and AF%.

Discussion

A clear variation of spore germination and appressoria formation was observed in peel exudates of different banana varieties at both maturity stages. Germtube growth and appressorium formation of *Colletotrichum* spp. is principally determined by the physical and chemical environmental cues originating from host tissues (Dean, 1997). However, for *C. musae,* spore germination and appressorium formation are influenced primarily by chemical signals such as ethylene and chlorogenic acid rather than physical factors (Swinburne, 1976; Harper and Swinburne, 1979; Dean, 1997). In the present study, Embul, Seenikehel and Alukehel, which represented the highly- and moderately-resistant groups, reported the lowest SG% and AF% in their ripened peel exudates. Interestingly, Mondan, which is also categorized as highly-resistant, showed a relatively higher SG% at the ripened stage, , but a relatively lower AF%. The results indicate that AF is the most important event in anthracnose development of banana cultivars tested. Therefore, it could be hypothesized that the level of chemical cues originating in the micro-environment of the fruit peel, which favour the SG and AF, increase with ripening in anthracnose-susceptible banana varieties. Host surfaces release a variety of chemical signals such as sugars, phenolics and volatile metabolites which influence spore germination and appressoria formation of fungi (Dean, 1997). In general, the antifungal concentrations of phenolic compounds present in unripe fruits decrease with the ripening and while the concentrations of sugars and amino acids, which generally favour fungal growth, increase (Aydin and Kadioglu, 2001). Therefore, further investigations are needed to elucidate the variation of chemical signals in the ripening phase of anthracnose-resistant and susceptible banana varieties and their effect on the initial steps of *C. musae infection.*

It is possible that the reduction of fruit peel pH during ripening could have been responsible for increased susceptibility of a given variety to anthracnose. For example, Kolikuttu and Embon, which showed the highest susceptibility also showed significant reductions in FPE pH. In contrast, FPE pH did not decrease below 6.0 with ripening in the resistant variety Alukehel. However, results of the present study also show that FPE pH is not the only factor determining the susceptibility to anthracnose. Firstly, significant increases SG% and AF% observed in some varieties despite the FPE pH being maintained above 6.0 (e.g. Mondan). Furthermore, SG% and AF% did not increase substantially in Embul despite the FPE pH decreasing even below 5.0. Secondly, adjustment of FPE pH of all varieties to that of Kolikuttu did not increase SG% and AF%

(data not shown). Therefore, FPE pH may not be a significant factor influencing higher spore germination and appressoria formation.

Conclusions

Variable expression of chemical signals originating in the banana fruit peel during the ripening process may determine the resistance or susceptibility of different banana varieties to anthracnose through their influence of the infection cycle. Anthracnose resistance in different varieties may be operating at different stages of the infection cycle, namely either at spore germination or appressorium formation stage.

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References

Table 1. pH of peel exudates of different banana varieties at ripe and unripe stages and percentage spore germination and appressoria formation after 24 hours of incubation in the FPEs at the two maturity stages.

Variety	pH of peel exudates		Spore germination (%)		Appressoria Formation (%)	
	Unripe (m.i.=2)	Ripe (m.i.=7)	Unripe (m.i.=2)	Ripe (m.i.=7)	Unripe (m.i.=2)	Ripe (m.i.=7)
Kolikuttu	6.38^{d}	5.76^{d}	5.68^{b}	35.54^{a}	1.15^{ab}	28.66^{a}
Embon	6.39^{cd}	5.26^{e}	15.49^{a}	30.86^{ab}	3.17^{a}	22.77^{a}
Embul	6.25^{e}	4.84^{f}	4.53^{b}	10.54^{cd}	2.19^{ab}	5.27^{b}
Seenikehel	6.45^{c}	6.91^{a}	2.03^{b}	9.46^{cd}	0.00^{b}	2.31^{b}
Mondan	6.76^{a}	6.10^{c}	5.02^{b}	20.86^{bc}	0.81^{ab}	8.26^{b}
Alukehel	6.60^{b}	6.22^{b}	2.82^{b}	5.54^{d}	0.00^{b}	2.70^{b}
Mean	6.48^{A}	5.85^{B}	5.93^{B}	18.80^{A}	1.22^{B}	11.66^{A}

Note: Each value is a mean of five replicates. Values with same lower-case letter along a column are not significantly different at p=0.05. Mean comparisons between unripe and ripe stages are given in upper-case letters.