

Potentiality of DNA- methylation sensitive amplification polymorphism (MSAP) technique in early detection of pest resistance in GM Banana crop

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Presentation Outline

- Pest resistance development in GM crops
- Monitoring of pest resistance
- Pest resistance GM banana varieties
- Application of DNA MSAP Technique and detection of Pest resistance
- Conclusion
- The way forward



Development of resistance pest against GM crops

- Modeling studies predicted that without the reliable pest resistance management strategies, target pests will develop resistance against pest tolerance GM crops.
 1. European corn borerworm in maize (SA and china)
 2. Pink Bollworm in Cotton, India (2009 – Ongoing)
 3. Corn Rootworm, United States (2011 – ongoing)



Monitoring of pest resistance

- The effectiveness of a pest resistance monitoring program highly depends on the Capacity of one or more techniques to correctly detect resistance at an **early stage of development**
- The desired detection technique is the one that is able diagnose resistance at very low level- preferably at 1% of pest population or below



Resistance detection techniques

2 Categories

- Bio-assays
- Biochemical assays
- Molecular techniques

Pest resistance GM banana varieties

- Researchers are now developing transgenic banana varieties which have the potential to resist pest infestations
- Uganda developing weevil and nematodes resistance varieties



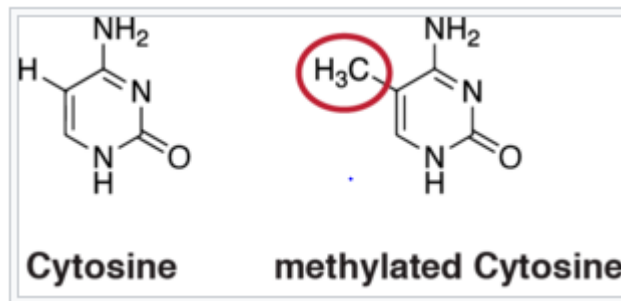
DNA MSAP Technique and detection of Pest resistance in GM Banana Crop

- Response against pest and diseases in plant is complex but include elements that's are modulated by DNA methylation
- Pest Stress can lead to demethylation of previously inactive (hypermethylated) genes allowing the plant to speedily adapt to the introduced stress
- It possible to use plants plant as the detection units using genome-wide DNA methylation profiles to diagnosis pest or pathogen related stress in plants and by extension pest resistance breakdown in GM crops



DNA- MSAP working principles

- Detect Change of DNA methylation at the CCGG sites



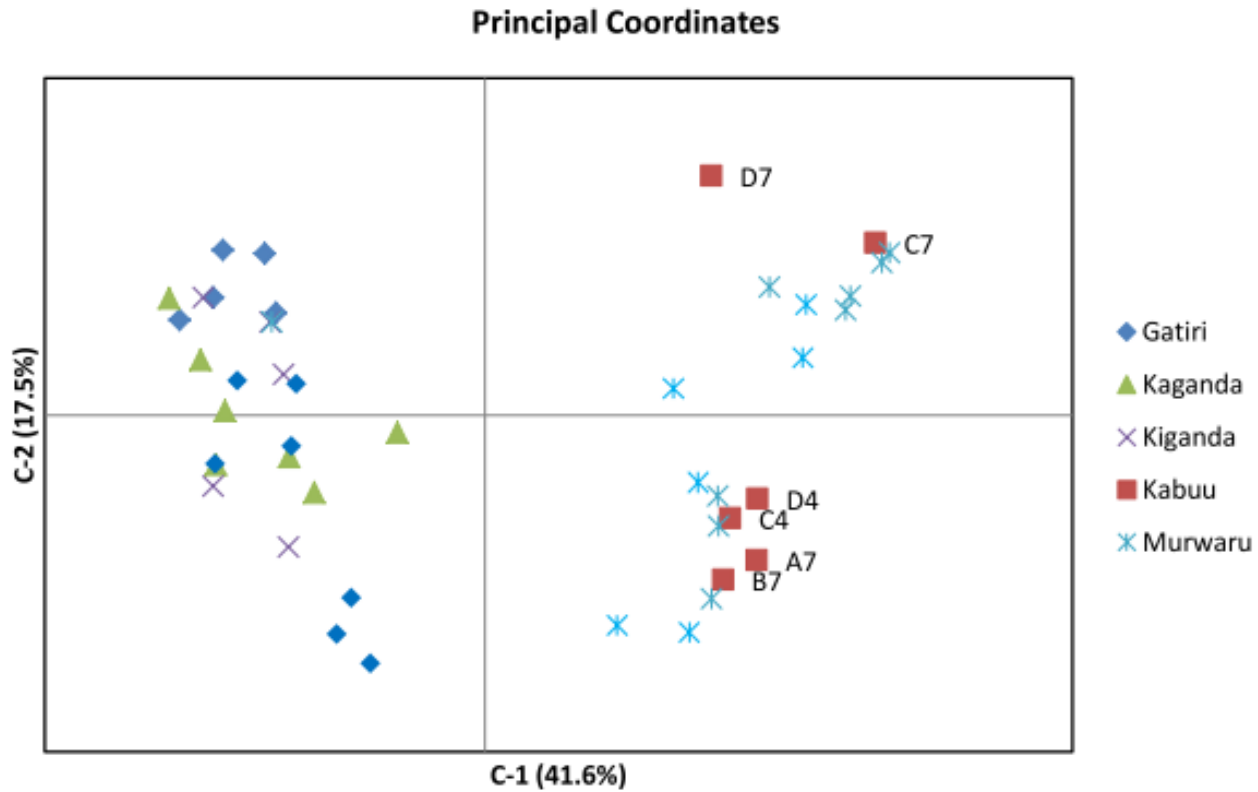
- Use of Restriction enzymes

The study to test MSAP capacity

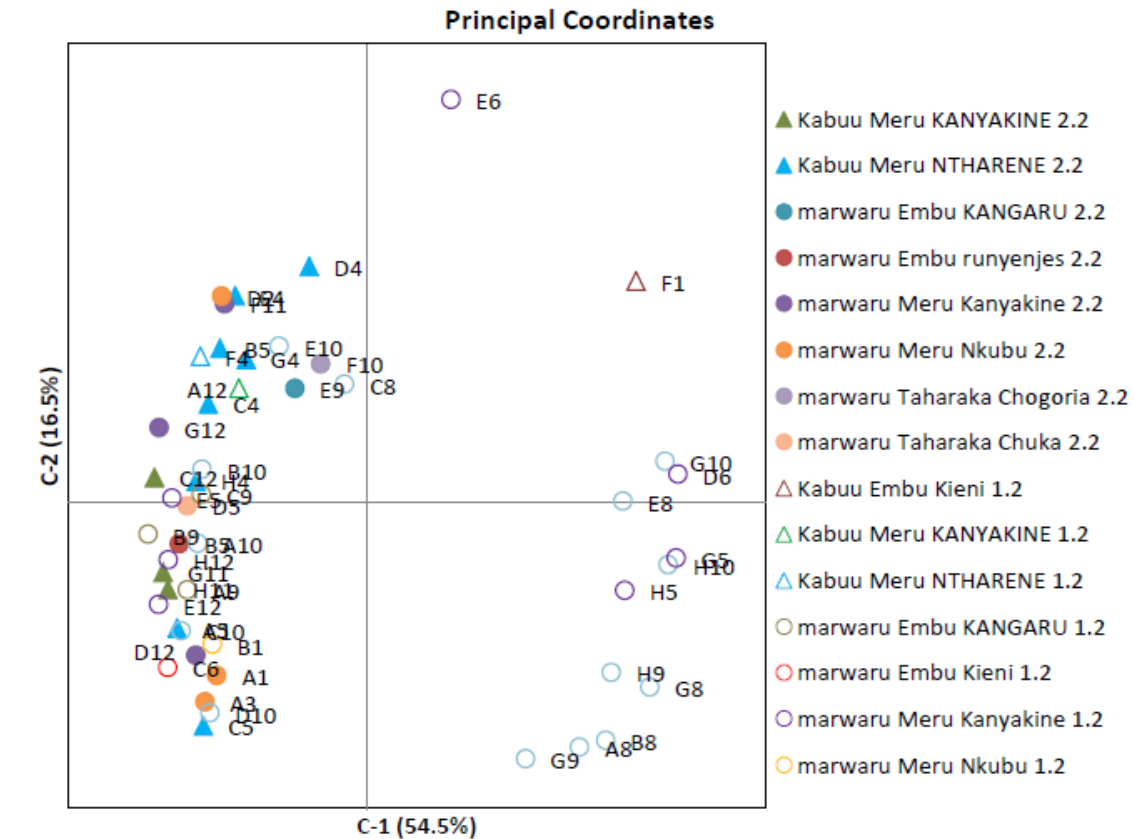
- Flag leaves from young conventional (non-GM) Banana plants were used in this study.
- Amplified fragment length polymorphisms (AFPL) protocol was conducted to determine the genetic variability within and among the local cultivars (Gatiri, kaganda and kiganda cultivars and kabuu and murwaru cultivars)
- MSAP protocol was used in an attempt to separate infested and non-infested samples under the two genetic groups as established by the AFPL protocol.



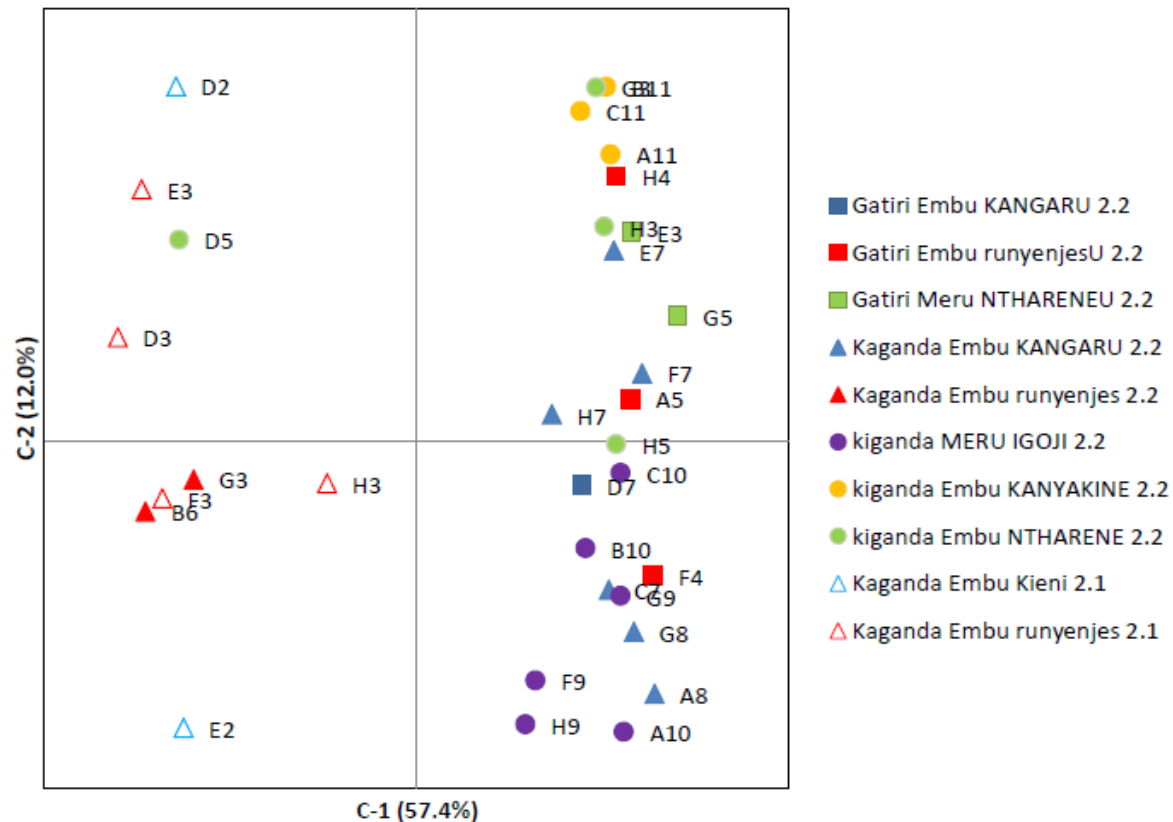
Results from AFLP



Weevil infested (empty shapes) vs non infested plants (solid shapes)



Nematodes infested vs healthy plants



Conclusion

- The MSAP protocol was able to distinctly discriminate majority of plant stressed by nematode and weevil infestations.
- These findings therefore suggest that MSAP Technique has the potential of diagnosing early pest resistance development in a GM banana crop using DNA methylation profiles.



Way forward

- Further investigations are now required to identify epiloci that are specific to the two infestations from the DNA profiles that were generated.
- This will allow for the design of simple PCR assays that are more effective, reproducible between laboratories and less costly.
- More broadly, the study demonstrates the potential value of epigenetic profiling as a diagnostic strategy in crop plants for the early detection of cryptic infestation and the emergence of pest resistance to transgene-induced immunity of a GM banana and other crops.

