Developing Plantain for resistance to banana aphids by RNA interference

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Introduction

Plant pests and diseases are major threat to global food production. Banana aphid (*Pentalonia nigronervosa*) is a known vector that transmits banana bunchy top disease (BBTD) **(Fig 1)** causing up to 100 % yield loss to banana and plantain production. Improvement of farmer preferred plantain production vis-à-vis genetic transformation needs a robust plant cell suspension system and an advance biotechnological approach due to sterility of the crop (Strosse *et al.*, 2003). RNA interference (RNAi) is a potential host-plant resistant mechanism for controlling the spread and effect of BBTD.

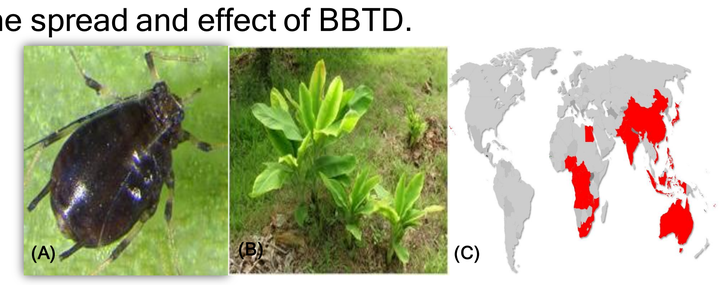


Fig 1: Banana aphid (A), BBTD infected plant (B), Geographical distribution of BBTD (C).

Materials and Methods

• RNAi construct (pNXT-35S-ACEhp) targeting acetylcholinesterase gene **(Fig 2)** was designed at QUT.

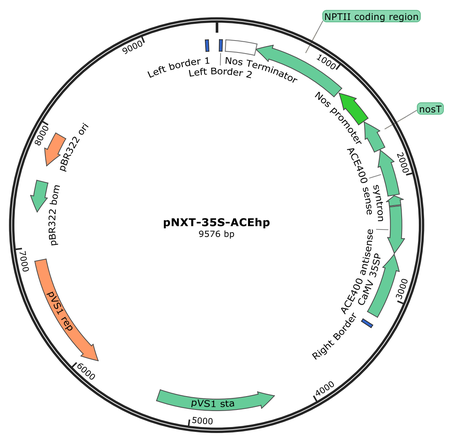


Fig 2: RNAi construct harboring acetylcholinesterase gene

•Three farmer preferred plantain cultivars, namely: Agbagba, Obinol'ewai and Orishele were collected as shoots from the field genebank at IITA, Nigeria.

• Shoots were screened for quarantine important viruses (e.g. Banana bunchy top virus, cucumber mosaic virus *etc.*) and clean plants were used for subsequent experiments.

• Clean lines were rapidly multiplied using temporary immersion bioreactor. Buds were excised to generate scalps and subsequent embryogenic callus and cell suspension were generated.

• *Agrobacterium* mediated transformation of acetylcholinesterase (ACE) gene in plantain cell was carried out as describe by Tripathi *et al.*, (2012)

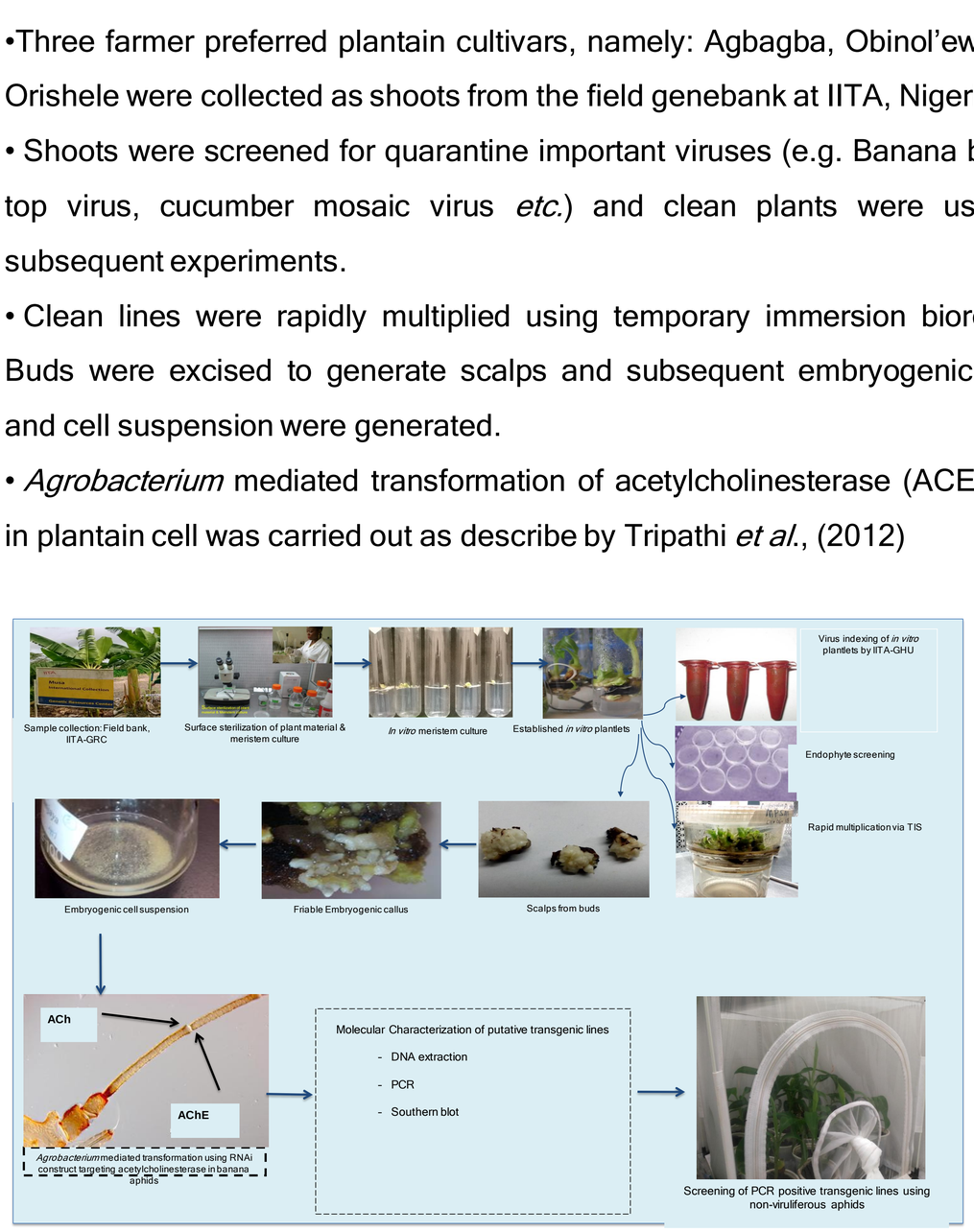
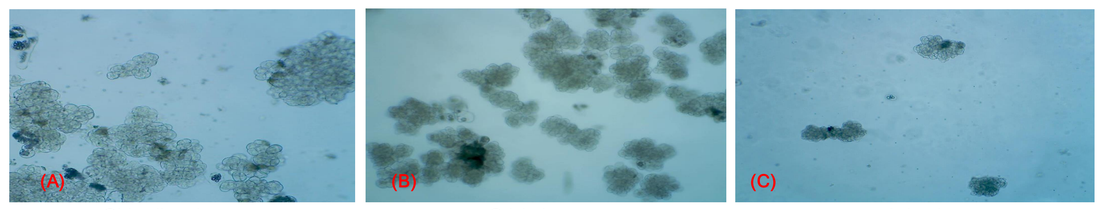


Fig 3: Embryogenic cell suspension generation, *Agrobacterium* mediated transformation, molecular characterization and screening of transgenic plants

Results



Obino l'ewai Orishele Agbagba

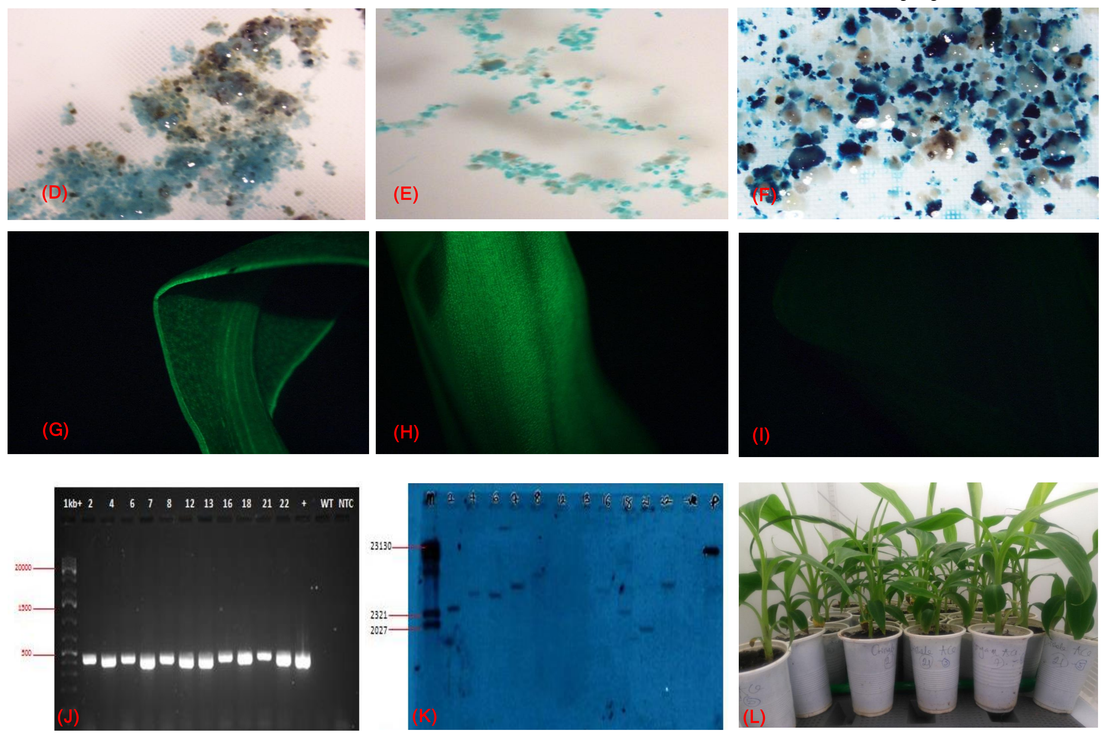


Fig 4: Embryogenic cell suspension (A-C), *gusA* assay (construct pCAMBIA 2301) (D-F), Green fluorescent protein expression (construct pCAMBIA 2300-gfp) (G-H), Control (I), molecular characterization and screening of plantain cultivars (J-L)

- Embryogenic cell suspension was successfully generated for all the three plantain cultivars.
- Over 79 transgenic plants were screened by PCR, 64 were confirmed to be positive, Selected lines were subjected to Southern blot analysis and 20 lines were confirmed positive with varying copy numbers, indicating integration of acetylcholinesterase gene in transgenic lines.
- Varying expression levels were observed for 8 selected southern blot positive lines using RT-PCR.
- These lines have been challenged with banana aphids and screening process is in progress.

Conclusions

- Embryogenic cell suspension was successfully developed for farmer preferred plantain cultivars.
- These were transformed using pCAMBIA 2300, pCAMBIA 2301-gfp and pNXT-35S-ACEhp constructs. Cells generated were successfully transformed, several lines were generated and validated by molecular characterization.
- Transgenic lines are currently being screened under confined condition using pure lines of non-viruliferous aphids. Selected resistant lines would be subjected to confined field trials.

References

Tripathi J.N., Muwonge A., Tripathi L. (2012) Efficient regeneration and transformation of plantain cv, "Gonja manjaya" (Musa spp. AAB) using embryogenic cell suspension. *In vitro cell.Dev.Biol.*

Strosse, H., Domergue, R., Panis, B., Escalant, J., & Côte, F. (2003). Banana and plantain embryogenic cell suspensions. *INIBAP Technical Guidelines*.

Acknowledgement

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