Progress towards genetic transformation of Ugandan sweetpotato cultivars for weevil resistance

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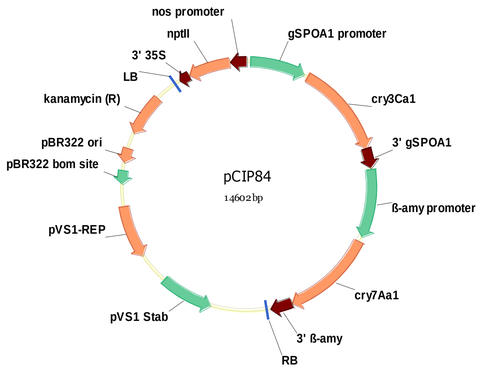
Summary

Sweetpotato weevil (*Cylas puncticollis* and *C. brunneus*) causes yield loss of over 28% between wet and dry seasons in Uganda. The improvement of weevil resistance in sweetpotato using classical breeding has been limited because no sources of resistance have been identified in the crop germplasm. In 2007, CIP and its collaborators identified three *Bacillus thuringiensis* (*Bt)* Cry proteins (Cry7Aa1, Cry3Ca1, ET33-34) active against the two African weevil species with an LC_{50} below 1ppm. However sweetpotato, especially African cultivars, is still considered recalcitrant to both regeneration and transformation. We report progress made towards optimising regeneration and transformation protocols and the identification of suitable Ugandan sweetpotato cultivars.

Methodology

Cultivars, Kyebandula and Bwanjule, were investigated for their organogenesis response to medium supplemented with different levels of thidiazuron (0.5, 2.0 and 4.0 µM) and 0.25 µM α-Naphthalene acetic acid on MS (1962) media. In order to compliment this organogeneic protocol, twenty Ugandan sweetpotato cultivars were screened for their response to embryogenesis. After investigating various explants, meristems were selected as ideal explants and 2,4-Dichlorophenoxyacetic acid (0.2 mg/L) was selected as the best auxin.

The optimised organogenesis regeneration protocol was used to regenerate plants from explants co-cultured with *Agrobacterium* strain EHA 105. This *Agrobacterium* harbours the pCIP84 plasmid construct which contains *cry7Aa1, cry3Ca1* and *nptII* genes in its transferred DNA (T-DNA).



Schematic representation of T-DNA region of pCIP84 vector plasmid

Results

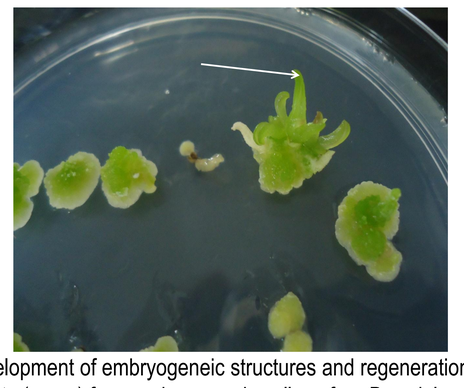
Regeneration through Organogenesis:

B ud induction frequency for stem explants was 66.7 and 58.9%, for cv Kyebandula and cv Bwanjule, respectively, when TDZ (4.0 µM) was supplemented alone. However, the bud induction frequency dropped to 58.8 and 48.9%, for cv Kyebandula and cv Bwanjule, respectively, when TDZ (4.0 µM) was supplemented together with NAA. Interestingly, shoot regeneration frequency improved from 12.1 to 22.6% for cv Kyebandula,and from 21.6 to 42.9% for cv Bwanjule, when NAA was added to the TDZ-based medium.

Adventitious shoot bud regeneration and plant development in *Ipomoea batatas*

Regeneration through somatic embryogenesis

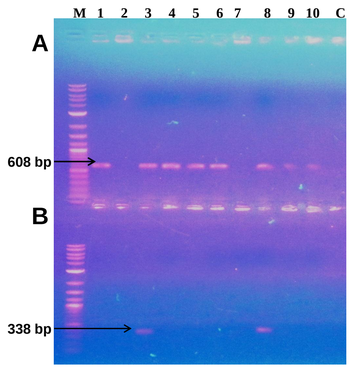
Seven de novo shoots were recently regenerated from 2 embryogenic calli proliferating from meristsm explants.



Development of embryogeneic structures and regeneration of shoots (arrow) from embryogeneic callus of cv. Bwanjule

Genetic transformation with *cry7Aa1, cry3Ca1*

PCR for *cry7Aa1* gene gave an early indication of transformation for 10 randomly selected plants among the regenerated 18 plants. Six of these plants showed that they were transformed with *cry7Aa1* gene representing a 2% transformation efficiency



Molecular analysis of putatively transgenic plants.
(A) PCR analysis for cry7Aa1. Lane C: Untransformed plant as negative control. Lane M: DNA marker. Lanes 1, 3-6, 8-10: Cry 7Aa1-positive plants. Lane 2 and 7: Cry 7Aa1-negative plants; (B) PCR analysis for virD2 gene. Lane 3 and 8: virD2-positve plants. Lanes 1, 2, 4-7, 9, 10: virD2-negative plants.

Conclusions

This research has demonstrated that it is possible to transform African cultivars that were thought to be recalcitrant. Although the first succes was with organogenesis, the recent progress towards embryogenesis is more valuable in terms of application to genetic transformation. Somatic embryogenesis has shown to be more cultivar-specifc also requires a long time to regenerate plants, with frequent medium changes. However, somatic embryogenesis remains the preferred method as the regenerated plants originate from single cells avoiding the possibility of chimeric plants for vegetatively propagated crops., like sweetpotato.

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