Development of an *In Vitro* Protocol for the Production of Cassava Doubled-Haploids and its Use in Breeding

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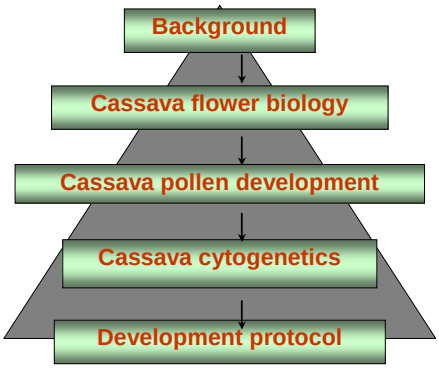
Introduction

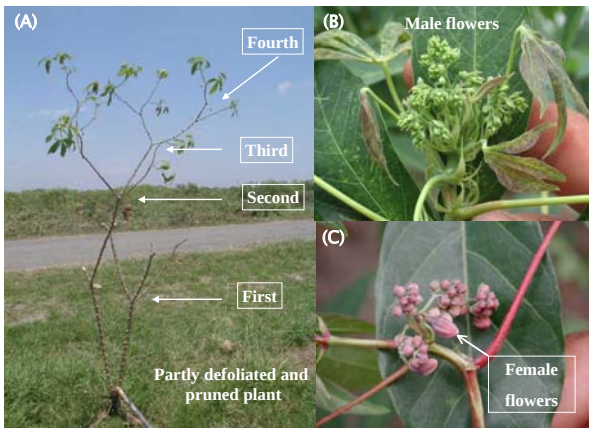
Introducing inbreeding in cassava genetic improvement has many advantages, among those are the identification of high-value recessive traits in early generations, the ease application of molecular tools in breeding, and the expedite generation of diversified improved breeding lines. But developing inbred lines through self-pollinations would require in cassava about 9-12 years. Rapid and complete homozygous can be reached by using *in vitro* haploid technology. Androgenesis, the process by which microspores develop to form haploid embryoids and subsequently regenerate into doubled haploids (DH, homozygous) plants has been applied successfully in plant breeding of various crops. This project aims to develop an *in vitro* protocol for the production of doubled-haploid from anther or microspore culture in cassava.

Materials and Methods

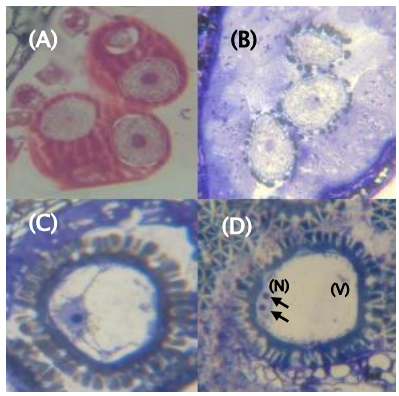


Results

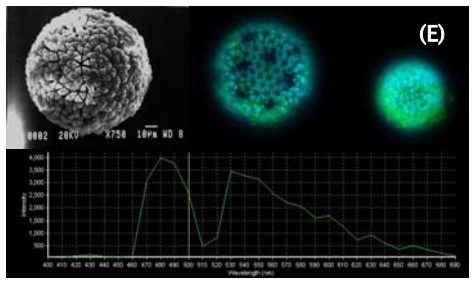
Cassava flower biology. (A) Various flowering cycles associated with branching. (B) Inflorescence with only male flowers. (C) Inflorescence with male and female flowers at the base

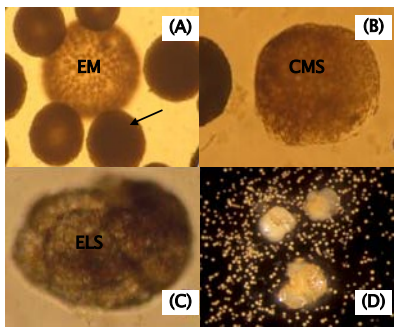


Pollen developmental stages from microspores (A) Tretrads, (B) early uni-nucleate, (C) late uni-nucleate, and (D) early binucleate stage. Vacuole (V), nucleus (N), nucleolus (arrow) and exine (E).



Structures towards androgenesis in cassava. (A) Enlarged microspore (EM) showing increased volume about 4 fold 1 week after culture with a lose exine pattern (arrow indicating un-induced microspores). (B) Multi-cellular structure (MCS) derived from microspore cultured *in vitro*. (C) Embryo-like structure (ELS). (D) Microcallus formation. (E) Mature pollen grain electron microscopy photograph (left), mature pollen grain dimorphism showing auto-fluorescence (middle and right).





Various parameters are being analyzed to increase the androgenesis response for the regeneration of doubled haploid plants using selected commercial clones and lines with increased tolerance to inbreeding depression generated from the breeding program.

Acknowledgements

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