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Development of an Efficient Virus Transmission Technique to Screen Cassava Genotypes for Resistance to Cassava Mosaic Disease

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Abstract

The most important disease affecting cassava (*Manihot esculenta* Crantz) production in Africa is Cassava mosaic disease (CMD), caused by several whitefly–transmitted begomoviruses. Advancement in cassava breeding for virus resistance is hampered because screening for resistance to CMD is tedious, relying on natural infection conditions and on virus types at a given time and location. Therefore, developing an efficient inoculation technique with defined viruses at an early stage in breeding for resistance would provide a major improvement to the resistance development in cassava. All major begomoviruses in African cassava genotypes were collected, typed by sequence analysis and maintained as reference in cassava cultivars. For inoculation of begomoviruses into cassava, a graft inoculation approach, the biolistic inoculation of total DNA from virus infected plants and a biolistic delivery of cloned viral DNA A and DNA B genomic components were attempted. Graft inoculation technique was effective in inducing cassava with defined viruses, but only in successfully grafted plants. With ACMV (isolate number 84, from Democratic Republic of Congo-DRC), disease symptoms were observed around 3 weeks after grafting with resistant plants eventually showing symptoms after 10 weeks, however, with very low severity levels. Three cassava clones, TME 3, TME 4 and 91/02324 recovered from CMD and developed into symptomless plants. With EACMV-UG2 [Ke], clones 96/1087, 96/1089A and TME 4 mostly developed into symptomless plants with only very mild symptoms occasionally found on few leaves above the graft insertion. All leaf samples from grafted lines tested positive for virus infections in PCR and ELISA, however, virus detection in cassava that had recovered from infections failed. Biolistic inoculation (BI) of total DNA extracted from diseased cassava plants resulted in infected plants showing symptoms between 10 and 12 days after inoculation. Severe infections were induced in ISU by shooting DNA from ACMV-[DRC] and EACMV-UG2 [Ke] infected plants, indicating a synergistic interaction of the two virus species. Plants of TME 3 and TME 4 inoculated with mixed virus infections still recovered after a period of symptom expression. The biolistic delivery of cloned viral DNA was carried out, but not sufficiently effective to reach higher number of infected cassava plants compared to BI of DNA extracts from diseased plants. Infections were induced by EACMV-UG2 [Ke] clones (Ugandan variant) in the highly susceptible cassava breeding lines Isunikankiyan and 96/1039. The effectiveness of BI of DNA extracts over other transmission techniques in screening cassava genotypes is discussed.

Keywords: Biolistic inoculation, cassava genotypes, cassava mosaic begomoviruses, cloned virus, DNA extracts, graft inoculation

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