CHARACTERIZATION OF TRANSGENIC SWEET POTATO (*Ipomoea batatas* L.) LINES AND THE RESPONSE OF TRANSFORMANTS TO VIRUS INOCULATION

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ABSTRACT

A survey was conducted to detect viruses and virus complexes that infect sweet potato in the major growing areas in Kenya. Four viruses namely sweet potato feathery mottle virus (SPFMV), sweet potato chlorotic stunt virus (SPCSV), sweet potato mild mottle virus (SPMMV) and sweet potato chlorotic fleck virus (SPCFV) were detected using nitrocellulose membrane enzyme-linked immunosorbent assay (NCM-ELISA). Five different virus complexes were detected of which SPFMV + SPCSV was the most common and the most widespread, being detected in 52% and 11% of the symptomatic and asymptomatic plants, respectively. Seventy nine percent and 17% of the symptomatic and asymptomatic plants, respectively, tested positive for at least one of the viruses under investigation.

Putatively transformed sweet potato lines were screened to identify true transgenic lines. Seven hundred and eight sweet potato lines of which 312, 172 and 224 were transformed with the coat protein (CP), replicase and inverted repeat of the CP genes, respectively, were characterized through kanamycin painting, graft-inoculation with SPVD-infected scions and PCR. Leaves of 597 (84.32%) lines remained green following treatment with 1% (w/v) kanamycin whereas those of 111 (15.68%) lines turned yellow. Of the 597 kanamycin-resistant lines inoculated with SPVD, only 20 (3%) lines were protected against the disease. In PCR, amplified DNA fragments of 450 bp were realised in 7 transgenic lines using specific primers to the coat protein, replicase and inverted repeat of the CP genes. This 450 bp fragment was the size of the CP, replicase and inverted repeat of the CP that had been inserted in the sweet potato using *Agrobacterium* as a vector. No amplification occurred with the DNA from non-transformed control plants and in 13 transformed plants.

Transgenic sweet potato lines expressing the CP, replicase and inverted repeat of the CP gene of SPFMV was evaluated for their reaction to SPFMV, SPCSV and SPVD inoculation under glasshouse conditions. Disease severity in transgenic lines was assessed by monitoring symptom development and enzyme-linked immunosorbent assays (ELISA). Ten transgenic sweet potato lines remained symptomless. SPFMV and SPCSV coulod not be detected in the 10 symptomless lines by nitrocellulose membrane (NCM) ELISA two months after inoculation. Results from triple antibody

sandwich-ELISA demonstrated that virus accumulation was suppressed in 6 transgenic lines compared to the non-transgenic control plants two months after inoculation. Of the 6 transgenic lines protected against sweet potato virus disease, 4 and 3 lines were expressing the CP and the replicase genes, respectively. Thus the 6 transgenic lines can be used for coat protein- or replicase-mediated resistance to the virus.