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The ability of cultivars of sweetpotato in East Africa to 'revert' from *Sweet potato feathery mottle virus* infection

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ABSTRACT

Asymptomatic field plants are the normal source of the vine cuttings used as sweetpotato planting material in Africa. Previous and new tests of such East African material, mostly using the very sensitive method of graft inoculation to the indicator plant *Ipomoea setosa*, showed that a majority tested virus-negative. This was despite their never having undergone any science-based therapy. To investigate how this occurs, in a replicated greenhouse experiment, plants of susceptible cultivars from the USA and Peru and three resistant Ugandan cultivars were graft-inoculated with *Sweet potato feathery mottle virus* (SPFMV), the commonest virus infecting sweetpotato. When the grafts were established, cuttings were taken, rooted and proved to be infected. The health status of each of these new plants was then followed over a 10-week period using a quantitative polymerase chain reaction assay. Most of the plants of the Ugandan cultivars eventually tested SPFMV-negative whereas those of the USA and Peru seldom did. Furthermore, in subsequent graft-inoculations of scions from the tip, top, middle and base of the vine of every plant to *I. setosa* plants, again, most of the scions of the Ugandan cultivars tested SPFMV-negative whereas those of the USA and Peru seldom did. These tests demonstrate the phenomenon of reversion in the Ugandan cultivars and can explain how most unprotected Ugandan sweetpotato field plants tested SPFMV-negative.

1. Introduction

Crops of sweetpotato (*Ipomoea batatas* (L.) Lam.) are always planted using vine cuttings. At least in East Africa, farmers usually select asymptomatic plants as sources of planting material and may also rogue out (remove) diseased plants from young crops (Bashaasha et al., 1995; Gibson et al., 2000). This avoids establishing plants with conspicuous viral diseases – mostly those dually infected with *Sweet potato feathery mottle virus* (SPFMV: family *Potyviridae*; genus *Potyvirus*) and *Sweet potato chlorotic stunt virus* (SPCSV: family *Closteroviridae*; genus *Crinivirus*). The latter virus acts synergistically on SPFMV to cause sweet potato virus disease (SPVD) (Gibson et al., 1998). In contrast, plants infected solely with SPFMV, the commonest virus infecting sweetpotato, develop only slight or transitory symptoms (Karyeija et al., 1998). Such infected plants cannot easily be selected against and so should continue to be propagated. As further infections naturally occur under field conditions, virus incidence would be expected to increase until all plants are infected. SPFMV can spread rapidly, reaching 51–84% infection in a single growing cycle when planted near infected plots in Israel (Milgram et al., 1996). Elsewhere, total infection occurred within 5–10 weeks in the USA (Bryan, 2002) and 80% infection in Brazil (Pozzer et al., 1994).

Asymptomatic field plants of Ugandan landraces mostly tested virus-negative in serological and graft inoculation tests using the indicator plant *Ipomoea setosa* (Gibson et al., 1997). These plants had been exposed to virus infection during vegetative propagation in the field for tens if not hundreds of generations and had never undergone virus therapy. Subsequent surveys in East Africa of asymptomatic plants, which constitute the majority of plants in sweetpotato crops there, have given similar results:

- In Uganda, 'The [200] vigorous and healthy looking plants . . . . . did not develop symptoms in the screenhouse and did not react with any virus antisera' (Mukasa et al., 2003).
- In Tanzania, 38 (52%) of 73 asymptomatic field plants were seronegative in tests for SPFMV, SPCSV, *Sweet potato mild mottle virus* (SPMMV: family *Potyviridae*; genus *Ipomovirus*) and *Sweet potato chlorotic fleck virus* (SPCFV: family *Flexiviridae*; genus *Carlavirus*) (Tairo et al., 2004).
- In Kenya, 477 (75%) of 638 asymptomatic field plants were seronegative to SPFMV, SPCSV, SPMMV, SPCFV, *Cucumber mosaic virus* (CMV: family *Bromoviridae*; genus *Cucumovirus*), *Sweet potato latent virus* (SPLV: family *Potyviridae*; genus *Potyvirus*),

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Sweet potato mild speckling virus (SPMSV: family *Potyviridae*; genus *Potyvirus*), *Sweet potato caulimo-like virus* (SPCaLV: family *Caulimoviridae*; genus *Cavemovirus*) and C-6 virus, and indexed virus-negative in graft inoculation tests using the indicator plant *I. setosa* (Ateka et al., 2004).

- In Uganda, only nine (4.5%) of 200 asymptomatic plants were found to be infected with SPFMV and five (2.5%) with SPCSV in tests using a combination of antiserum against SPFMV, SPMMV, SPLV, SPMSV, SPCSV, SPCFV, SPCaLV and C-6 virus, graft inoculation to *I. setosa* and sap inoculation to a range of other indicator plants. Sap extracts from the original and the inoculated plants that tested virus-negative were also checked for virus particles in an electron microscope with negative results (Aritua et al., 2007).
- In Rwanda, 71 (69%) of 103 asymptomatic field plants were seronegative to SPFMV, SPCSV, SPMMV, SPCFV, SPCaLV, SwPLV and CMV (Njeru et al., 2008).

SPFMV can be unevenly distributed in sweetpotato (Nielsen and Pope, 1960; Green et al., 1988; Abad and Moyer, 1992). Aritua et al. (1998) showed that, over a six-month period, an increasing proportion of shoot tips taken from initially SPFMV-infected plants of East African landraces grown in a screenhouse in Uganda and all shoot tips from initially SPFMV-infected plants grown outside tested SPFMV-negative. Similar results have also been observed for plants infected with *Sweet potato leaf curl Uganda virus* (SPLCUV: Family *Geminiviridae*; genus *Begomovirus*) (Wasswa et al., 2011) and for SPVD in a few genotypes (Mwanga et al., 2013). The phenomenon whereby infected plants naturally become healthy is known as reversion. It has been reported in other crop species (Thresh et al., 1998) including tropical crops. For example, it is a component of resistance used in the control of *Sugarcane mosaic virus* (SCMV: family *Potyviridae*; genus *Potyvirus*) in sugarcane (Comstock and Gilbert, 1991) and of cassava mosaic geminiviruses (CMGs: family *Geminiviridae*; genus *Begomovirus*) in cassava (Fargette et al., 1994; Gibson and Otim-Nape, 1997). It can result in a natural equilibrium between infected and reverted plants becoming established in crops (Fargette et al., 1994) and Thresh et al. (1998) describe it as having 'great epidemiological significance'. The work described here brings together previous East African and new Ugandan results of virus testing asymptomatic field sweetpotato plants, all showing virus-negative plants to be prevalent. An experiment is described which explains these results by demonstrating a high frequency of reversion in initially SPFMV-infected sweetpotato plants of Ugandan cultivars (but not of non-African ones).

2. Materials and methods

Surveys of virus infection of sweetpotato crops in East Africa which included asymptomatic plants were identified (Gibson et al., 1997; Mukasa et al., 2003; Ateka et al., 2004; Tairo et al., 2004; Aritua et al., 2007; Njeru et al., 2008) and the numbers of asymptomatic plants which tested either virus negative or positive were collated (Table 1).

On different occasions, cuttings ~30 cm long were obtained from asymptomatic field plants of cvs Dimbuka, Ejumula, New Kawogo, NASPOT 1, NASPOT 10 and NASPOT 11. Dimbuka and New Kawogo are white-fleshed Ugandan landraces (Mwanga et al., 2001, 2009), Ejumula is an orange-fleshed Ugandan landrace (Mwanga et al., 2007), NASPOT 11 is a white-fleshed cultivar derived from cv. New Kawogo (Gibson et al., 2008) and NASPOT 1 is a white-fleshed cultivar bred from other Ugandan landraces (Mwanga et al., 2003). All were from farmers' fields near or within the National Crops Resources Research Institute (NaCCRI) near Kampala, Uganda. They were brought to the UK and each was planted in an 8 cm × 8 cm × 9.5 cm square pot of John Innes No. 2 soil-based compost (William Sinclair Horticulture Ltd., UK). They were grown in a quarantine greenhouse at NRI with natural daylight supplemented to a light period of 16 h each day. Once established (2–3 weeks), they were tested for virus infection, usually by wedge-grafting a scion (1–2 cm long) from each plant to the stem of a seedling of *I. setosa*. Symptoms were observed over a 4 week period. This is the standard indicator plant for sweetpotato viruses and the standard testing method (Moyer et al., 1989). Numbers of each cultivar tested are given in Table 2. In one test with 50 plants of NASPOT 11, 10 plants of *I. setosa* were also wedge-grafted as a control with scions obtained from a plant of the USA cv. Resisto (Jones et al., 1983) obtained in Uganda naturally infected with SPFMV (confirmed by graft inoculation to *I. setosa* seedlings and a polymerase chain reaction (PCR) assay – see later). In another test, 100 cuttings of NASPOT 11 and 10 control SPFMV-infected plants of cv. Resisto were wedge-grafted with scions of *I. setosa* seedlings; 82 and 2, respectively of the grafts survived and were observed for virus symptoms over the next 4 weeks (Table 2). Sample virus identities were confirmed by PCR.

In a replicated experiment at NRI again in the quarantine greenhouse (see above for detailed conditions of growing plants), two plants each of the USA cv. Beauregard (Rolston et al., 1987), of the Peruvian cv. Huachano (Kreuze et al., 2009) and of the Ugandan cvs New Kawogo, NASPOT 1 and NASPOT 11 were infected by wedge-grafting with scions of the SPFMV-infected cv. Resisto. Once each graft was established, the section of the original plant growing above it was excised, planted in a square pot (8 cm × 8 cm × 9.5 cm) of compost and allowed to re-establish (2 weeks). Infection status was determined for all the cuttings at the start of each replicate, either by graft-inoculation to *I. setosa* in replicate 1 or by quantitative (q)PCR in replicate 2. One plant of cv. New Kawogo in replicate 2 did not test positive and was eliminated. In both replicates, the remaining plants, all testing positive initially for SPFMV, were retested at weekly intervals using qPCR, each time sampling the current top mature leaf. Retesting started at week 1 for replicate 1 and at week 5 for replicate 2. After the 10th week, each plant vine was ~1 m long and these were also tested for SPFMV by grafting short sections from the tip, top, middle and base of each vine to individual *I. setosa* seedlings and observing for symptoms over 4 weeks.

PCR and qPCR used the same methods as Wasswa et al. (2011) and used the same primer pair for SPFMV detection as Kokkinos and Clark (2006). Total nucleic acid (TNA) was extracted using a cetyl trimethyl ammonium bromide (CTAB) method described originally by Lodhi et al. (1994) and later modified by Maruthi et al. (2002). QPCR was performed using a PE 7900 Sequence Detection System and using duplicated samples, a negative control (molecular grade water), a positive control and a housekeeping gene, cytochrome oxidase (Weller et al., 2000) on each plate.

3. Results

The results of previous East African surveys of asymptomatic sweetpotato plants (Table 1) and those of newly tested Ugandan field plants (Table 2) are shown. Overall, of 1844 asymptomatic field plants tested, 70% tested virus-negative and 77% tested SPFMV-negative. In the new data, most plants of cvs New Kawogo and NASPOT 11 tested virus-negative when grafted onto *I. setosa*. By contrast, few control plants of the known susceptible cv. Resisto infected with SPFMV tested virus-negative. Even when *I. setosa* was grafted onto plants of NASPOT 11, which might detect lingering infections in the old parts of the plant resulting from field inoculation (detailed in the last two lines of Table 2), 44% of plants still tested virus-negative. Other viruses infecting sweetpotato were detected even more rarely. Atypically for East African cultivars,

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