Methodology for Inoculating Sweetpotato Virus Disease: Discovery of Tip Dieback, and Plant Recovery and Reversion in Different Clones

R. O. M. Mwanga, Department of Horticultural Science, North Carolina State University, 201 Kilgore Hall, Box 7609, Raleigh, NC 27695-7609, USA, and National Agricultural Research Organization (NARO), National Crops Resources Research Institute (NaCRRI), Box 7084, Kampala, Uganda; **G. C. Yencho**, Department of Horticulture, 214A Kilgore Hall, Box 7609, Raleigh, NC 27695-7609, USA; **R. W. Gibson**, Natural Resources Institute, University of Greenwich, Central Avenue, Chatham Maritime, Kent, ME4 4TB, United Kingdom; and **J. W. Moyer**, Department of Plant Pathology, North Carolina State University, 1414 Gardner Hall, Box 7616, Raleigh, NC 27695-7616, USA

Abstract

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Evaluating sweetpotato (*Ipomoea batatas*) genotypes for resistance to sweetpotato virus disease (SPVD) has been slow and inefficient. *Ipomoea setosa* plants, normally used as the source of scions for graft-infecting sweetpotatoes with viral diseases, are often severely stunted and their mortality is 10 to 30% when infected with SPVD, making them unsuitable as scions. Tanzania, a landrace of *I. batatas* widely grown in East Africa, was found to be a superior host for maintaining and increasing SPVD inoculum (scions) for mass grafting. Modifications to a cleft-grafting technique also increased survival of grafted

SPVD-affected scions from 5 to 100%. These modifications, coupled with an efficient SPVD scoring technique, allowed rapid screening of large sweetpotato populations for SPVD resistance. Plant recovery from SPVD is reported here as a component of SPVD resistance. Differences in recovery from SPVD were detected among progenies, indicating its genetic basis. Plant tip dieback, a hypersensitivity response, was observed only in families with cv. Wagabolige as a parent. These findings may open up new opportunities for improved understanding and control of this devastating disease.

Sweetpotato (*Ipomoea batatas* (L.) Lam.), with a mean annual production of 126 million tons between 1991 and 2009, is ranked among the top 10 most important food crops globally by the Food and Agriculture Organization of the United Nations (19). Increases in sweetpotato productivity have been constrained by many major factors, including sweetpotato virus disease (SPVD), drought, low yielding varieties, insects, and postharvest losses. SPVD is widespread (30,40,60) and causes up to 98% yield loss to individual plants (22,36,40). The disease is caused by the synergy between *Sweet potato feathery mottle virus* (SPFMV) and *Sweet potato chlorotic stunt virus* (SPCSV) (11,34,35,38,50). SPFMV is transmitted nonpersistently by aphids (42,50,59), while SPCSV is transmitted semipersistently by *Bemisia* spp. of whitefly (11,59). Development of cultivars with genetic resistance to SPVD is the most practical strategy to control this devastating disease.

Field screening of sweetpotato germplasm for resistance to SPVD has commonly been based on symptom severity and incidence of plants infected with SPVD following natural infection (5,10,29), although a root-grafting technique has been used for artificial inoculation (8,29,45,46,58). Field screening for resistance is problematic because vector populations fluctuate over seasons and years (3,6,29,52) and roots are available only after completing a first seedling generation. Large (50,000 to 100,000) populations of F1 seedlings require testing each season to identify agronomically superior genotypes with SPVD resistance (10,21,44). However, plants in the field may initially escape infection, taking 3 or 4

years to show SPVD symptoms, making field screening for resistance slow and inefficient. A major complicating factor for virus control in sweetpotato is that farmers propagate the crop vegetatively using vine cuttings, thus providing opportunity to perpetuate systemic viruses. In cassava, another tropical crop propagated vegetatively via cuttings, cultivars resistant to *African cassava mosaic virus* (ACMV) do not become systemically infected and virus titer remains low. Therefore, plants may recover (i.e., plants that initially had symptoms become asymptomatic) and/or cuttings taken from infected plants may revert and produce healthy plants (17,18,32). Recovery has been defined as the extent of the partial or complete loss of symptoms on originally infected plants, while reversion refers to lack of disease symptoms in cuttings obtained from infected plants that form new sprouts (17,21). Symptom severity is typically the initial selection criterion for SPVD resistance, but this rating scheme may not be ideal for genotypes with these other modes of resistance.

In this study, foliar graft inoculation procedures were developed to induce SPVD routinely, and serology and scoring methods were evaluated for screening large sweetpotato populations against SPVD. We characterized 36 sweetpotato families, 10 genotypes each, for resistance to SPVD, a family being a group of individuals from the same cross. Criteria used to characterize the families were mainly based on symptoms, but recovery from SPVD, not previously characterized in sweetpotato, was a particularly useful criterion. This work was used to: (i) improve methods of evaluation of sweetpotato genotypes for resistance to SPVD, and (ii) assess the use of recovery from SPVD as an additional selection criterion for resistance to SPVD.

Materials and Methods

Sources of inoculum. Inoculum of SPFMV and SPCSV were obtained from a naturally SPVD-infected sweetpotato plant of the cultivar Tanzania (TAN) obtained from a field at the National Crops Resources Research Institute (NaCRRI), Uganda. Naturally infected refers to infection via insect transmission under field conditions. These infected cuttings were propagated in a screenhouse where they tested sero-negative by enzyme-linked immuno-

Corresponding author: R. O. M. Mwanga,
E-mail: r.mwanga@cgiar.org, rmwanga7@gmail.com

Current address for R. O. M. Mwanga: International Potato Center (CIP), Box 22274, Kampala, Uganda.

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sorbent assay (ELISA) using antisera from J. Vetten (Federal Research Center for Cultivated Plants, Braunschweig, Germany) and L. Salazar (International Potato Center [CIP], Lima, Peru) for other viruses commonly found in Uganda: *Sweet potato mild mottle virus*, *Sweet potato chlorotic fleck virus*, and *Sweet potato latent virus* (22). Aphids, *Myzus persicae* Sulz. (one aphid/seedling), and whiteflies, *B. tabaci* (50/seedling), were used to transmit SPFMV and SPCSV, respectively, from these SPVD-infected cuttings to *I. setosa* seedlings grown in insect-proof cages in a screenhouse (22). SPVD-infected TAN plants used in subsequent studies as sources of inoculum for SPVD were obtained by cleft grafting pathogen-tested plants of TAN with SPFMV- and SPCSV-infected *I. setosa* scions, and these TAN plants were propagated as stem cuttings. The details of double inoculation of sweetpotato with SPFMV and SPCSV to develop SPVD were described by Gibson et al. (22).

Top- and side-cleft wedge grafting studies. The efficiencies of graft inoculating sweetpotato with *I. setosa* or TAN scions infected with SPFMV + SPCSV were compared using the standard cleft grafting, side cleft grafting, and approach grafting illustrated by Green (27). Each graft was sealed with Parafilm, and the grafted plant was covered with a clear polyethylene bag previously sprayed inside with water to maintain high relative humidity, which promotes the grafting process. About 3-cm-long *I. setosa* or TAN scions were grafted onto rootstocks of the test sweetpotato genotypes. The different types of grafting, size of scion, and position of graft were compared for graft-infecting sweetpotato genotypes with SPVD. From preliminary evaluations based on survival of scions, top-wedge grafting was subsequently used. Plants with dead scions not showing SPVD symptoms were grafted again.

SPVD resistance evaluation in the screenhouse. Sweetpotato diallel progenies (360 genotypes in 36 full-sib families, each family derived from a single cross) of 10 parental clones varying in SPVD field resistance (susceptible to resistant) were evaluated for SPVD resistance in the screenhouse. The 10 previously described (44) parental clones selected under high SPVD pressure environment, were Wagabolige (WAG), Tanzania (TAN), Bikila Maliya (BMA), Kanziga 2 (KAZ), New Kawogo (NKA), Tororo 3 (TOR), Kanziga 1 (KAN), Bwanjule (BWA), Sowola (SOW), and Camote Tallo (CTA). Ten plants per genotype were graft inoculated using an SPVD-infected TAN scion in a screenhouse at 1.5 months after planting. The study was conducted during August to December 1998 and repeated during October 1999 to February 2000 using a randomized complete block design (RCBD). Genotypes were evaluated for SPVD resistance following a previously published procedure (44), using a 1 to 5 rating scale, where: 1 = no symptoms, 2 = mild symptoms (a few local lesions on a few leaves), 3 = moderate symptoms (mosaic symptoms on leaves), 4 = severe symptoms (mosaic with plant stunting), and 5 = very severe symptoms (severe mosaic with severe plant stunting, even necrosis).

Effect of SPVD on yield. The same 10 genotypes of each of 36 families (360 genotypes) that were SPVD graft-inoculated and evaluated for SPVD resistance in the screenhouse were planted at both an upland (Borehole) and a swampy site (Kisenyi) at NaCRRI in February 1999. Ten cuttings were planted per genotype at each of the two sites. The experiment was a split-split-split layout in three replications with family as the main plot, genotype as the subplot, and inoculation as the secondary subplot, with 10 plants per row on ridges 1 m apart and 10-cm centers. The secondary subplot consisted of one row of plants grafted-infected with SPVD and one row of healthy control plants. The healthy control plants were to be the plants naturally inoculated via insect transmission. SPVD severity was scored at monthly intervals after planting using the 1 to 5 rating scale. The plants were harvested at 3.5 months after planting. Total plant weight, shoot weight per plant, number of storage roots per plant, and root weight per plant of SPVD graft infected plants were expressed as percentages of healthy controls, and their standard deviations (SD) were computed.

Evaluation of inoculated materials by serology. To test whether there were differences in virus content between screen-

house and field grown plants, 14 genotypes from both environments (screenhouse and field) were tested serologically. Composite samples of 0.2 g were obtained from leaves representing a range of SPVD symptoms of each genotype. The samples were assayed for SPFMV using a double-antibody sandwich (DAS)-ELISA (9) and for SPCSV by triple-antibody sandwich (TAS)-ELISA (23) in microplates. Each plate had duplicate wells for the test sample, a positive control, pathogen-tested negative control (cultivar TAN), and a buffer control. Immuno-globulin (IgG) against SPCSV (SPCSV-KyCP) (dilution 1:500) for coating ELISA plates and monoclonal antibodies (MAb) for detecting SPCSV were donated by J. Vetten (Federal Research Center for Cultivated Plants, Braunschweig, Germany). IgG and MAb for SPFMV were supplied by L. Salazar (CIP, Lima, Peru). Goat anti-mouse alkaline phosphatase-conjugated antibody (Sigma Chemical Co. Ltd., Poole, UK) was used at a dilution of 1:1,000. The color reaction was obtained using nitrophenol phosphatase substrate, and absorbances were recorded at 405 nm (A_{405}) with a microplate reader (Labsystems Multiskan S, Middlesex, UK). Genotypes testing negative by ELISA were regrafted with SPVD-infected scions or were grafted on *I. setosa* seedlings and observed for symptoms. Test samples with absorbance values exceeding twice that of the healthy control samples were considered positive.

Evaluation of families for recovery from SPVD. To assess recovery from SPVD, 10 cuttings with SPVD symptoms of all the genotypes in the 36 families (360 genotypes) were planted in a field in single rows 30 cm apart on ridges spaced 1 m apart 2 months postinoculation (November 1999). The genotypes were randomized within each family, and the families were arranged in an RCBD with three replications. At 2 and 5 months after planting, the plants were scored on a per plot basis for SPVD using the 1 to 5 rating scale described above.

The index for recovery from SPVD (0 to 5, where 0 = no apparent recovery and 5 = apparent complete recovery) used in this study incorporates separate ratings for plant pigmentation, growth, and size scored in the field, and its computational procedure was published previously (44).

Statistical analysis. Analysis of variance (ANOVA) for SPVD score means was done using the generalized linear model, SAS-PROC ANOVA procedure (49) with families as the main plot, genotypes within families as the subplot, and inoculations (field and graft infection) as the secondary subplot. Percentages of infected over healthy control plants per plot were used in the ANOVA for the effect of SPVD on total plant weight, shoot weight, root weight, and number of roots per plant with families as main plot and genotypes within families as the subplot.

Results

Insect and graft transmission. *I. setosa* seedlings inoculated by aphids developed mild to severe mosaic or chlorotic spots and vein clearing symptoms on leaves typical of SPFMV at 2 to 6 weeks after inoculation. Apical symptomless leaves started appearing on some *I. setosa* plants 5 to 6 weeks after inoculation. *I. setosa* plants inoculated with SPCSV became stunted, their leaves became brittle, cupped downward or upward, and chlorotic although not necrotic. SPFMV was transmitted by 5 aphids and SPCSV by 50 whiteflies per *I. setosa* seedling. Whitefly mortality was high under the screenhouse conditions. *I. setosa* seedlings double grafted with SPFMV- and SPCSV-infected *I. setosa* scions became severely stunted with general chlorosis and necrosis of old leaves, leading to death in 10 to 30% of seedlings within 3 weeks. It was impractical to maintain SPVD in *I. setosa* because of the high plant mortality rate or to use it as scions for graft-inoculation experiments because of the severe stunting. Double (SPFMV and SPCSV components)-grafting pathogen (common sweetpotato viruses)-tested TAN plants with SPFMV- and SPCSV-infected scions of *I. setosa* were affected by SPVD. These SPVD-affected TAN plants were vegetatively multiplied and used as a source of inoculum. This procedure was also preferred to using *I. setosa* as inoculum because this latter species cannot easily be propagated vegetatively, requiring seeds to

Table 1. Sweetpotato virus disease (SPVD) symptoms in 36 families (360 genotypes), percent families, and genotypes showing SPVD symptoms, Namulonge, Uganda, 1998 to 2000

Symptoms	Families exhibiting symptom	% Families exhibiting symptom	Genotypes in all families exhibiting symptom	Comment
1. Vein clearing	1-36	100	346	Severity varies with clone
2. Chlorotic mosaic	1-36	100	244	May be chlorotic spots
3. Reduced leaf size	1-36	100	85	Sign of stunting
4. Crinkling (leaf distortion)	1-9, 11-17, 21, 27, 28, 36	56	47	More severe in divided leaf lamina and highly susceptible clones
5. Strapping (narrow leaf lobes)	3, 6, 13-17, 21, 35	25	16	Mostly in clones with highly divided lamina
6. Cupping (up or down and rolling of leaf margins)	1-4, 11, 13, 15-18, 24, 27-31, 34-36	53	26	Occurs with water stress in some genotypes
7. Puckering (raised lamina between veins)	1-6, 12, 14, 20, 21, 30, 31, 36	37	25	Common in highly susceptible clones with broad leaves
8. Bronze or purple mottling	1-9, 12-13, 15, 18-20, 23, 26, 28, 31, 33	56	38	In combination with chlorosis, may appear like herbicide damage or senescence
9. Tip dieback (hypersensitivity)	1-3, 6-8, 36	20	21	Occurred only in one parent, Wagabolige (WAG); families 1-3, 6-8 were progenies of WAG
10. Stunting	1-34, 36	98	86	Most evident in highly susceptible clones
11. Reduced shoot weight	1-36	100	97	Most evident in highly susceptible clones
12. Reduced number of storage roots	1-36	100	87	Most evident in highly susceptible clones
13. Reduced weight of storage roots	1-36	100	100	Most affected trait

Table 2. Field sweetpotato virus disease (SPVD) scores for natural inoculation and graft-inoculation for 36 families (10 genotypes/family, 10 plants/genotype)^a

Family	Natural inoculation				Graft-inoculation			
	Mean	SD	Min	Max	Mean	SD	Min	Max
1. WAG × TAN	1.8	1.0	1.0	4.0	4.1	0.7	2.0	5.0
2. WAG × BMA	1.9	1.0	1.0	4.0	4.4	0.6	3.0	5.0
3. WAG × KAZ	1.6	1.0	1.0	5.0	4.9	0.5	3.3	5.0
4. WAG × NKA	1.7	0.9	1.0	4.0	3.8	0.8	2.0	5.0
5. WAG × KAN	1.9	1.0	1.0	4.0	4.0	0.7	2.8	5.0
6. WAG × BWA	1.9	1.1	1.0	4.0	4.2	0.8	2.8	5.0
7. WAG × SOW	1.9	0.8	1.0	4.0	4.2	0.8	2.8	5.0
8. WAG × CTA	1.8	1.0	1.0	4.0	4.2	0.7	3.0	5.0
9. TAN × BMA	1.9	1.2	1.0	4.0	4.1	0.7	3.0	5.0
10. TAN × KAZ	1.8	1.1	1.0	4.0	3.9	0.6	2.8	5.0
11. TAN × NKA	1.6	1.0	1.0	5.0	3.9	0.6	2.8	5.0
12. TAN × TOR	1.9	1.0	1.0	4.0	4.2	0.7	3.0	5.0
13. TAN × KAN	2.3	1.2	1.0	4.0	3.8	0.8	2.0	5.0
14. TAN × BWA	2.2	1.1	1.0	4.0	4.0	0.7	2.0	5.0
15. TAN × SOW	1.9	1.0	1.0	4.0	4.0	0.8	3.0	5.0
16. TAN × CTA	2.8	1.2	1.0	5.0	4.2	0.7	2.5	5.0
17. BMA × KAZ	2.2	1.3	1.0	4.0	3.7	0.8	2.0	5.0
18. BMA × NKA	2.4	1.0	1.0	4.0	4.0	0.6	3.0	5.0
19. 1BMA × TOR	2.3	1.0	1.0	4.0	4.2	0.6	3.0	5.0
20. BMA × KAZ	1.8	1.1	1.0	4.0	4.0	0.8	2.0	5.0
21. BMA × CTA	2.2	0.9	1.0	4.0	4.0	0.7	2.8	5.0
22. KAZ × NKA	1.9	1.0	1.0	4.0	3.4	0.9	2.0	5.0
23. KAZ × KAN	2.5	1.3	1.0	5.0	4.1	0.7	2.8	5.0
24. KAZ × BWA	2.1	0.9	1.0	4.0	3.6	0.6	2.5	5.0
25. NKA × TOR	1.9	0.9	1.0	4.0	3.5	0.8	2.0	5.0
26. NKA × BWA	1.9	0.9	1.0	3.0	3.4	0.7	2.5	5.0
27. NKA × SOW	1.9	0.9	1.0	3.0	3.2	0.6	2.0	5.0
28. NKA × CTA	2.0	0.9	1.0	4.0	3.5	0.5	2.0	5.0
29. TOR × KAN	2.2	1.1	1.0	5.0	4.1	0.9	2.0	5.0
30. SOW × TOR	1.7	0.9	1.0	3.5	3.8	0.9	2.0	5.0
31. TOR × CTA	2.4	1.0	1.0	4.0	4.0	0.4	3.0	5.0
32. KAN × BWA	1.7	1.0	1.0	4.0	3.8	0.8	2.0	5.0
33. KAN × SOW	1.9	0.8	1.0	4.0	4.3	0.6	3.0	5.0
34. KAN × CTA	2.7	1.0	1.0	5.0	4.3	0.6	3.0	5.0
35. BWA × CTA	1.9	1.0	1.0	4.0	4.0	0.8	2.0	5.0
36. SOW × CTA	1.8	1.1	1.0	5.0	3.4	0.6	2.0	5.0
Mean	2.0	—	1.0	4.1	4.0	—	2.5	5.0

^a LSD (0.05) = 0.38 for comparing inoculation treatments within families or families within inoculation. Each main plot consisted of a family, subplot of genotypes, and secondary subplot of inoculations (natural and graft) at two sites in Namulonge, Uganda, 1999. Means ($n = 60$) and standard deviations (SD) are shown. Clones are represented by three letters: Wagabolige (WAG), Tanzania (TAN), Bikila Maliya (BMA), Kanziga 2 (KAZ), New Kawogo (NKA), Tororo 3 (TOR), Kanziga 1 (KAN), Bwanjule (BWA), Sowola (SOW), and Camote Tallo (CTA). SPVD scale is 1 to 5, where 1 = no visible symptoms and 5 = very severe symptoms.

be obtained, seedlings to be grown, and the seedlings to be inoculated.

The size of scion and the position of the graft were varied, but these methods still resulted in less than 5% (sample of 720 plants) disease transmission, essentially because the scions died. However, the following modifications resulted in increased transmission. Dipping the scions in water and covering the grafted plants minimized stress of the scions due to excessive transpiration and reduced callus formation at the cut surfaces in subsequent days. The grafted plants were then kept in shade for 4 to 7 days or until the leaves on the SPVD-infected scion or the stock started senescing. Covering grafted, pathogen-tested, symptomless TAN plants with polyethylene bags sprayed on the inside with water, and leaving the plants in shade for 4 to 7 days, increased survival of SPVD-infected TAN scions and transmission of SPVD from 2 to 5% to 97 to 100%. Regrafting symptomless plants (i.e., those that had dead scions) resulted in 100% SPVD transmission. This modified graft method was used to evaluate resistance to SPVD of sweetpotato diallel progenies in the screenhouse and field.

SPVD resistance evaluation in the screenhouse. Various combinations of SPVD symptoms were exhibited by the sweetpotato genotypes following graft inoculation (Table 1). Moderate symptoms included vein clearing, chlorotic mosaic, chlorotic spots, reduced leaf size, upward or downward rolling of leaf margins (cupping), and bronze or purple mottling. Severe symptoms additionally included narrowing of the leaf lamina (strapping), leaf distortion (crinkling), stunting, reduction in shoot weight and total plant weight, tip dieback, and death. Most of the genotypes (>95%) were highly susceptible to SPVD (Table 1), and they exhibited the combination of symptoms of severe SPVD. Of the 10 parental clones evaluated for SPVD resistance, only WAG suffered tip dieback when graft-inoculated with SPVD, and some of its progeny showed similar responses. Six percent (21 genotypes) of the 360 genotypes of the diallel progeny suffered tip dieback (Table 1). Tip dieback was expressed between 10 and 22 days after graft-inoculation, but in most genotypes the symptom appeared at 12 days after graft-inoculation. Tip dieback was characterized by gradual loss of the green color, browning, or blackening and gradual dying of the young shoots, starting at the tips, progressing to the larger branches, and spreading to the stem base. Tip dieback caused death of whole plants in genotype 7/3 (WAG × BWA), 34 days after graft-inoculation.

Graft-inoculation with SPVD revealed significant differences in SPVD severity between and within families, and between natural and graft infection. Minimum (2.0 to 3.3) and maximum (5.0) SPVD scores of graft-inoculated plants were greater than the minimum (1.0) and maximum (4.1) SPVD scores due to natural (field) inoculation. Furthermore, graft-inoculation resulted in more severe and rapid SPVD progress in all 36 families compared to field inoculation (Table 2). As expected, the mean of graft-infected SPVD severity scores (4.0) was greater than the mean (2.0) of naturally (field) infected SPVD severity scores.

Serology. Fourteen genotypes were sampled to compare SPFMV and SPCSV ELISA results from the screenhouse and field. SPCSV and SPFMV optical density (OD) values of leaf samples of screenhouse-grown plants were higher than those of field-raised plants (data not shown), indicating a higher titer present in the screenhouse-grown plants, but the OD values did not show a correlation with resistance.

Recovery from SPVD. The 36 full-sib families exhibited significant variation in their recovery from SPVD (Table 3). Families 8 (WAG × CTA) and 23 (KAZ × KAN) had the greatest mean recovery scores (0.8 and 0.9, respectively), with a range of 0.6 to 1.0 (SPVD recovery index, 0 = no recovery, 5 = maximum recovery [44]), indicating that the majority of progeny were highly susceptible to SPVD. Recovery in some genotypes progressed from near plant death (SPVD score = 5) to symptomless plant (SPVD score = 1). In some severely infected genotypes, SPVD symptoms were localized. In these, whole branches or individual shoots developed that were asymptomatic and had apparently reverted,

being free of detectable virus when assayed by ELISA or grafted onto *I. setosa*. Twenty-four families (66.7%) had very low recovery ability (mean recovery scores 0.1 to 0.4). In these genotypes, symptoms appeared to remain constant or the disease progressed over time, resulting in death.

Effect of SPVD on yield. There were significant differences among families, and genotypes within families, due to the effects of SPVD on plant weight, shoot weight, root weight, and number of roots per plant (Tables 4 and 5). Although SPVD was severe in all the families, the type of symptom expression varied greatly (Table 1) among families (Tables 2 to 4) and among genotypes within families, as demonstrated especially within families 3 and 27 (Table 5). Some genotypes were susceptible to SPVD with severe symptoms and poor storage root yields as in family WAG × SOW. However, a few genotypes (e.g., 34/7 in family NKA × SOW) were tolerant to the disease (Table 5), giving high root and shoot weights in spite of the moderate to severe symptoms expressed. The mean family plant weight of SPVD graft-inoculated plants ranged from 2.2 to 36.2% of healthy controls (Table 4). Family means for shoot weights of SPVD-inoculated plants ranged from 1.9 to 40.4% of healthy controls. The family means for the number of roots per plant of SPVD graft-inoculated plants ranged between 9.4 and 30.1% of healthy control plants. For all the traits studied, the family means (0.2 to 24.2% of healthy controls) of root weight per plant were reduced most by SPVD (Tables 1, 4, and 5). Progenies of the parent SOW, which has moderate field resistance

Table 3. Summary of recovery score means, standard deviations (SD), minimum (Min), and maximum (Max) values for 36 families (10 genotypes/family, 10 plants/genotype) (scale 0 to 5, where 0 = no recovery, 5 = maximum) at Namulonge, Uganda, 1998 to 2000^a

	Family	Mean	SD	Min	Max
1.	WAG × TAN	0.3	0.09	0.2	0.4
2.	WAG × BMA	0.4	0.05	0.4	0.5
3.	WAG × KAZ	0.1	0.02	0.1	0.2
4.	WAG × NKA	0.5	0.05	0.4	0.5
5.	WAG × KAN	0.6	0.23	0.3	0.8
6.	WAG × BWA	0.3	0.04	0.2	0.3
7.	WAG × SOW	0.2	0.03	0.1	0.2
8.	WAG × CTA	0.8	0.19	0.6	1.0
9.	TAN × BMA	0.6	0.23	0.4	0.8
10.	TAN × KAZ	0.3	0.16	0.2	0.5
11.	TAN × NKA	0.2	0.13	0.1	0.4
12.	TAN × TOR	0.3	0.14	0.2	0.4
13.	TAN × KAN	0.6	0.07	0.5	0.7
14.	TAN × BWA	0.5	0.07	0.5	0.6
15.	TAN × SOW	0.4	0.05	0.4	0.5
16.	TAN × CTA	0.1	0.01	0.1	0.2
17.	BMA × KAZ	0.3	0.05	0.3	0.4
18.	BMA × NKA	0.7	0.03	0.6	0.7
19.	BMA × TOR	0.5	0.08	0.4	0.6
20.	BMA × KAZ	0.6	0.08	0.5	0.7
21.	BMA × CTA	0.2	0.04	0.2	0.3
22.	KAZ × NKA	0.3	0.04	0.2	0.3
23.	KAZ × KAN	0.9	0.05	0.8	0.9
24.	KAZ × BWA	0.3	0.05	0.2	0.3
25.	NKA × TOR	0.1	0.01	0.1	0.1
26.	NKA × BWA	0.4	0.02	0.3	0.4
27.	NKA × SOW	0.5	0.11	0.4	0.6
28.	NKA × CTA	0.1	0.01	0.1	0.1
29.	TOR × KAN	0.4	0.01	0.3	0.4
30.	SOW × TOR	0.4	0.03	0.3	0.4
31.	TOR × CTA	0.1	0.02	0.1	0.1
32.	KAN × BWA	0.4	0.03	0.3	0.4
33.	KAN × SOW	0.5	0.05	0.4	0.5
34.	KAN × CTA	0.4	0.03	0.3	0.4
35.	BWA × CTA	0.2	0.22	0.2	0.2
36.	SOW × CTA	0.1	0.01	0.2	0.2
	LSD (0.05)	0.13	—	—	—

^a Clones are represented by three letters: Wagabolige (WAG), Tanzania (TAN), Bikila Maliya (BMA), Kanziga 2 (KAZ), New Kawogo (NKA), Tororo 3 (TOR), Kanziga 1 (KAN), Bwanjule (BWA), Sowola (SOW), and Camote Tallo (CTA).

Table 4. Effect of sweetpotato virus disease on plant weight, shoot weight, root weight, and number of roots per plant (infected expressed as percentage of healthy)^a

Family ^b	Plant weight (%)		Shoot weight (%)		Root weight (%)		Roots/plant (%)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1. WAG × TAN	15.8	21.7	19.3	23.8	6.7	22.1	8.3	20.3
2. WAG × BMA	11.3	16.8	13.7	18.7	3.6	15.6	8.1	19.9
3. WAG × KAZ	2.2	5.8	1.9	4.2	0.2	0.5	11.1	24.3
4. WAG × NKA	12.3	17.7	14.3	21.8	3.0	13.4	14.6	24.0
5. WAG × KAN	10.1	12.0	10.8	13.2	2.3	5.6	20.4	29.7
6. WAG × BWA	7.8	12.2	7.3	12.3	0.6	2.7	7.1	16.1
7. WAG × SOW	13.4	20.2	12.8	17.8	13.9	26.7	16.9	23.1
8. WAG × CTA	11.0	18.1	13.3	23.5	5.4	13.6	15.1	24.3
9. TAN × BMA	13.7	12.8	15.3	12.9	11.5	25.8	10.6	19.4
10. TAN × KAZ	29.8	30.0	26.8	31.7	19.1	27.7	22.6	28.7
11. TAN × NKA	27.7	22.7	25.2	24.1	16.7	19.5	25.9	39.4
12. TAN × TOR	8.9	11.8	10.0	13.0	2.1	9.0	9.0	17.2
13. TAN × KAN	25.2	27.2	33.7	26.8	19.6	30.7	12.0	21.3
14. TAN × BWA	30.9	25.4	30.0	26.5	16.6	20.6	9.1	17.9
15. TAN × SOW	22.4	20.3	27.0	23.6	11.8	16.7	23.4	27.4
16. TAN × CTA	19.7	22.7	22.4	28.4	12.4	20.2	20.8	32.2
17. BMA × KAZ	29.8	21.3	34.5	26.4	11.1	15.4	29.1	29.2
18. BMA × NKA	26.0	20.7	28.2	22.7	2.6	4.2	17.8	25.1
19. BMA × TOR	18.4	19.7	20.0	20.4	3.5	8.4	7.6	13.8
20. BMA × KAZ	16.5	16.2	16.9	18.7	9.5	23.0	22.6	29.2
21. BMA × CTA	27.4	26.6	29.7	30.5	15.5	21.2	31.8	30.8
22. KAZ × NKA	26.3	20.3	34.7	28.9	15.1	19.4	37.3	34.0
23. KAZ × KAN	20.4	23.4	25.5	33.4	19.4	25.4	35.4	38.0
24. KAZ × BWA	30.9	28.5	16.7	14.4	14.6	24.1	24.1	31.9
25. NKA × TOR	25.0	22.3	29.6	25.8	3.0	12.5	2.4	9.1
26. NKA × BWA	34.1	32.5	37.1	32.7	15.7	25.5	18.9	30.0
27. NKA × SOW	35.2	22.8	37.0	27.9	17.2	20.1	32.6	34.5
28. NKA × CTA	33.6	18.2	40.1	26.2	14.7	14.5	23.3	32.9
29. TOR × KAN	19.6	22.3	20.3	21.5	10.1	27.7	18.3	29.4
30. SOW × TOR	22.4	20.9	23.2	22.6	16.4	27.1	17.3	30.4
31. TOR × CTA	36.2	26.2	40.4	26.3	24.2	31.5	28.0	33.6
32. KAN × BWA	27.3	24.9	24.2	26.5	15.0	26.7	27.1	28.9
33. KAN × SOW	16.9	24.8	15.9	23.1	11.7	27.4	13.7	26.9
34. KAN × CTA	18.9	16.7	19.6	18.9	7.1	15.0	18.0	27.2
35. BWA × CTA	21.8	20.6	23.3	23.7	9.3	15.3	30.6	32.5
36. SOW × CTA	25.7	16.1	31.9	28.4	20.9	25.6	33.7	34.0
LSD (0.05)	2.18	–	2.15	–	1.85	–	6.31	–

^a Mean and standard deviation (SD) values are shown for each family at two sites in Namulonge, Uganda, 1999. There were 36 families (10 genotypes/family, 10 plants/genotype) with family as main plot, genotype as subplot, and inoculations (natural and graft) as secondary subplot.

^b Clones are represented by three letters: Wagabolige (WAG), Tanzania (TAN), Bikila Maliya (BMA), Kanziga 2 (KAZ), New Kawogo (NKA), Tororo 3 (TOR), Kanziga 1 (KAN), Bwanjule (BWA), Sowola (SOW), and Camote Tallo (CTA).

to SPVD, and KAN, a highly susceptible parent under the same conditions, had higher root weight, number of roots per plant, and shoot weight per plant compared to other families. Progenies of NKA, a parent with high field resistance to SPVD, had high plant weight, shoot weight, and number of roots per plant but low root weight per plant when inoculated. Progenies of parent WAG, which has high field resistance to SPVD, had the lowest number of roots per plant, root weight per plant, shoot weight, and plant weight when inoculated.

Discussion

Various inoculation techniques (2,7,14,20,25,26,31,36,37,39,40,42,43,45,47,48,51) have been used to ensure infection and increase the number of genotypes that can be efficiently evaluated, but most techniques still have major shortcomings. In the present study, high whitefly mortality made it impractical to mass rear aphids and whiteflies for screening large sweetpotato seedling populations for resistance to SPVD under Ugandan conditions. The use of TAN SPVD-symptomatic scions for graft inoculation circumvented the problem of high plant mortality of SPVD-inoculated *I. setosa* and saved on time, labor, and space. Graft modifications, namely, dipping the scions in water, covering the grafted plants with moist, clear polyethylene bags, and keeping the plants in shade for 4 to 7 days, reduced excessive transpiration, reduced callus formation, and improved grafting success. Attempts of the plants to heal the cut surfaces at the grafts under ambient conditions led to excess

callus formation. However, our graft procedure modifications possibly provided optimal relative humidity for wound healing and reduced stress due to excessive transpiration, leading to efficient SPVD transmission in the grafted plants. The procedures used in our experiments have been replicated and repeated over seasons using the same virus isolates with graft inoculation, which is not possible if *I. setosa* seedlings are inoculated because SPVD kills them. Further, TAN SPVD-affected plants can supply more scions for longer periods than *I. setosa* SPVD infected plants. TAN plants with storage roots can withstand water stress conditions for a longer time than *I. setosa* seedlings, and the storage roots of the TAN plants, absent in *I. setosa*, can sprout if the shoot dies.

Although natural SPVD inoculum pressure caused moderate to severe infection in uninoculated plants, graft-inoculation was more efficient and more rapid in inducing severe disease pressure at an early stage in plant development (Table 2). Under natural infection, some genotypes may escape infection by viruliferous vectors of SPFMV and/or SPCSV (28). Vector populations may vary with location (6) and/or seasons (3,6,30) resulting in reduced transmission of SPVD when the vector populations are low (30). The cleft graft modifications ensured SPVD transmission and overcame the difficulty of absence or fluctuation of vector populations. The cleft graft modifications made it possible to inoculate sweetpotato and to screen efficiently for SPVD symptoms year-round under tropical conditions. Among the SPVD symptoms used to characterize the 36 sweetpotato families (360 genotypes) graft-inoculated

Table 5. Effect of sweetpotato virus disease (SPVD) on two families differing in resistance and recovery from SPVD^a

Family	Genotype	Mean SPVD score		Recovery	Percent (inoculated/healthy)			
		Natural inoculation	Graft inoculation		Plant weight	Shoot weight	Root weight	Roots/plant
3. WAG × KAZ	3/1	1.4	5.0	0.2	1.4	1.4	0.0	2.6
	3/2	1.7	4.8	0.1	0.1	0.1	0.0	0.0
	3/3	1.2	5.0	0.0	0.4	0.4	0.0	4.0
	3/4	1.7	4.8	0.0	0.1	0.1	0.0	10.0
	3/5	1.5	4.9	0.0	1.9	1.8	0.0	6.7
	3/6	1.6	5.0	0.0	0.9	0.1	0.0	0.0
	3/7	2.2	4.9	0.0	0.1	0.1	0.0	17.5
	3/8	1.6	4.6	0.2	1.9	2.0	0.3	0.1
	3/9	1.7	5.0	0.5	1.8	3.0	1.7	42.3
	3/10	1.5	5.0	0.0	13.4	9.9	0.0	27.5
27. NKA × SOW	Mean	1.6	4.9	0.1	2.2	1.9	0.2	11.1
	34/1	2.0	3.6	0.5	64.5	71.3	32.9	66.2
	34/2	2.0	3.5	0.4	32.0	27.5	13.8	14.7
	34/3	1.6	2.7	0.1	24.7	27.9	0.2	12.1
	34/4	2.2	3.6	1.1	10.4	13.1	3.5	34.8
	34/5	1.5	3.0	1.4	31.2	28.2	25.0	0.5
	34/6	1.6	3.7	0.5	37.8	13.9	20.9	33.5
	34/7	2.1	3.2	0.0	48.0	59.9	31.1	52.8
	34/8	2.5	2.7	0.2	41.4	60.8	23.2	50.6
	34/9	1.7	3.2	0.1	28.8	33.0	21.4	49.4
	34/10	1.7	3.3	0.7	33.1	34.4	0.1	11.4
	Mean	1.9	3.2	0.5	35.2	37.0	17.2	32.6

^a SPVD rating: 1 = no visible symptoms, 5 = most severe symptoms. The index for recovery from SPVD incorporates separate ratings (0 to 5, where 0 = no apparent recovery, 5 = apparent complete recovery) for plant pigmentation, growth, and size, Namulonge, Uganda, 1998 to 2000.

with SPVD, tip dieback has been described for the first time, and this etiology appears to be associated with only progenies of the cv. WAG. Although tip dieback is clearly disadvantageous to the individual plant, it may improve crop health indirectly by helping farmers not to propagate infected material, especially when it occurs in combination with moderate to high SPVD resistance, as is the case with the parent cv. WAG. Tip dieback is possibly a hypersensitive reaction to SPVD infection and may therefore be controlled by a single major gene. In soybean, hypersensitivity resistance to *Cowpea chlorotic mottle virus* (CCMV) is conditioned by a single dominant gene (24). In potato (33) and tobacco (12), single dominant genes govern strong localized hypersensitive reactions. Contrary to earlier reports that failed to show recovery with SPVD (5), recovery is identified in Uganda as a component of resistance to SPVD (44). Recovery from SPVD was first reported by Terry (56,57) and Hahn et al. (29) in West Africa, and virus elimination was proposed as a mechanism to reverse infection in sweetpotato by Gibson et al. (23). Recovery or reversion has also been observed in ACMV-resistant cassava varieties (17,18,32). Various studies have shown that SPFMV exhibits uneven distribution in sweetpotato (1,9,13–16,20,28) and, as in ACMV-resistant cassava (17,18,32), the most plausible explanation for this is that SPVD also may not remain fully systemic in certain sweetpotato genotypes. As a result, a proportion of uninfected cuttings could be obtained from previously SPVD-infected plants. The recovery and reversion phenomena are recognized to be of great epidemiological significance in decreasing sustained losses to ACMV in cassava (17,18), but they have received little attention in sweetpotato research.

Various mechanisms have been advanced to explain the recovery phenomenon in different crops. Induction of resistance against *Tobacco etch virus* (TEV), exhibited as a recovery phenotype, was correlated with a decrease in RNA (and protein) steady-state levels without a concomitant decrease in transgene transcription (55). Dougherty et al. (15) have proposed a cytoplasmic host system that would recognize specific RNA sequences and eliminate them to account for recovery. However, Kreuze et al. (36) suggested RNA silencing (RS) as a surveillance system against invading viruses in resistant cultivars; susceptibility is associated with the infecting virus suppressing this mechanism. Regardless of the mechanism of recovery from SPVD, selecting for rapid recovery phenotypes and

incorporating fast recovery into superior sweetpotato clones may well be a way to increase the level of resistance to SPVD in superior cultivars.

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