

# Identification of molecular markers associated with sweet potato resistance to sweet potato virus disease in Kenya

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**Abstract** Sweet potato virus disease (SPVD), a result of the co-infection of whitefly transmitted *Sweet potato chlorotic stunt virus* (genus *Crinivirus*, family *Closteroviridae*) and the aphid transmitted *Sweet potato feathery mottle virus* (genus *Potyvirus*, family *Potyviridae*), is the most destructive disease of sweet potato in East Africa. A study was conducted to establish if genotypes identified as resistant or susceptible to SPVD in Kenya could be distinguished using molecular markers. A total of 47 unrelated sweet potato genotypes were selected from germplasm collections and classified into two phenotypic groups as resistant or susceptible to SPVD. Genotype selection was based on disease severity or days to symptom development in plants following graft inoculation.

Amplified fragment length polymorphism (AFLP) marker profiles were generated for each individual and used in association studies to identify markers suitable for classifying the two pre-defined phenotypic groups. Analysis of molecular variance showed significant ( $P < 0.002$ ) variation between the two groups using 206 polymorphic AFLP markers. Discriminant analysis and logistic regression statistical methods were used to select informative markers, and to develop models that would classify the two phenotypic groups. A training set of 30 genotypes consisting of 15 resistant and 15 susceptible were used to develop classification models. The remaining 17 genotypes were used as a test set. Four markers, which gave 100% correct classification of the training set and 94% correct classification of the test set, were selected by both statistical methods.

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## Introduction

Sweet potato virus disease (SPVD), a result of co-infection of whitefly transmitted *Sweet potato chlorotic stunt virus* (SPCSV, genus *Crinivirus*, family *Closteroviridae*) and the aphid-transmitted *Sweet potato feathery mottle virus* (SPFMV, genus *Potyvirus*, family *Potyviridae*), is the most destructive

disease of sweet potato world-wide and especially in East Africa (Geddes 1990; Gibson et al. 1997; Carey et al. 1999; Karyeija et al. 2000; Gutierrez et al. 2003). The disease is characterized by chlorosis, small, deformed leaves, and severe stunting and can reduce yields of infected plants by over 90% (Gibson et al. 1998; Gutierrez et al. 2003).

Attempts to controlling SPVD are through host-plant resistance backed up by farmers selecting disease-free planting stock (apparently free of known viruses). Studies in Uganda indicate no benefit of using pathogen-tested plants when compared with farm-derived plants of the same cultivars in the region (Gibson et al. 1997; Carey et al. 1999). Thus, plant resistance is important to SPVD control (Karyeija et al. 1998b; Carey et al. 1999). Hundreds of sweet potato cultivars (landraces) are grown in the region (Gichuki et al. 2003), which have been shared by farmers through generations. The landraces have large differences in susceptibility to SPVD, and good sources of resistance are present in local germplasm (Aritua et al. 1998a). The impact of SPVD has been reduced by the use of resistant cultivars and landraces (Aritua et al. 1998b; Karyeija et al. 1998a). However, resistance has been associated with relatively late maturing, low-yielding genotypes (Aritua et al. 1998b). Despite the short comings, unintended gains in the development of virus resistance has occurred as farmers and breeders have both selected for high yield, and/or mild symptoms in plantings that were exposed to natural virus infection.

Prior efforts of virus resistance breeding were focused on control of SPFMV given its universal distribution. However, many sweet potato cultivars are naturally resistant to SPFMV, showing no or only mild initial symptoms, from which they usually recover, and containing very low-virus titers (Esbenshade and Moyer 1982; Abad and Moyer 1992; Kokkinos and Clark 2006b). Many East African sweet potato cultivars are resistant to SPFMV (Gibson et al. 1998; Mwangi et al. 2002b). Resistance is broken when the varieties are co-infected with SPCSV (Karyeija et al. 1998a; Mwangi et al. 2002b). There have been efforts toward developing a transgenic sweet potato resistant to SPVD through resistance to SPFMV both in Kenya and other parts of the world (Okada et al. 2001; Wambugu 2003). However, it is becoming increasingly clear that the

problem is not SPFMV but SPCSV which synergizes with different unrelated viruses (Cohen and Loebenstein 1991; Di Feo et al. 2000; Kokkinos and Clark 2006b; Mukasa et al. 2006), and resistance to SPFMV may not hold in the presence of SPCSV. Although SPCSV can cause yield losses on its own, little effort seems to be directed toward developing varieties resistant to SPCSV. Selection for resistance to SPVD is based on symptom development after field exposure in disease prone areas and by graft-inoculations (Hahn et al. 1981; Mihovilovich et al. 2000; Mwangi et al. 2002b), processes requiring considerable time and resources. Attention is focused on DNA markers to accelerate breeding through early selection. While studying inheritance of resistance to SPCSV, SPFMV, and SPVD in sweet potato, Mwangi et al. (2002a) constructed a preliminary linkage map of sweet potato, and identified markers linked to SPCSV and SPFMV resistance. They found an Amplified fragment length polymorphism (AFLP) marker (*spcsv1*) which explained 70% of the variation in resistance to SPCSV and one RAPD marker (*spfmv1*) which explained 72% of the variation in SPFMV resistance. Mwangi et al. (2002a) could not determine if the two genes present in a common background would suppress SPVD effectively.

Discriminant analysis and logistic regression represent novel approaches in marker-assisted selection (Cruz-Castillo et al. 1994; Ebdon et al. 1998; Capdevielle et al. 2000; Fahima et al. 2002; Aluko 2003; Mcharo 2005). Genotypes possessing trait extremes, e.g., highly resistant versus highly susceptible are used exclusively, and those with intermediate reactions ignored. Discriminant and logistic regression analysis are especially useful in dealing with populations of unrelated clones such as landrace genotypes since quantitative trait loci (QTL) analysis is not suited for such populations with no progeny-parent combinations (Mcharo 2005).

The objective of this study was to identify and classify unrelated sweet potato genotypes selected from East African germplasm collections into phenotypic groups as resistant and susceptible to SPVD, and to identify the most important AFLP markers contributing to variation among the phenotypic groups using discriminant and logistic regression analysis.

## Materials and methods

### Selection of SPVD-resistant and susceptible sweet potato genotypes

The germplasm pool surveyed consisted of over 400 genotypes collected from the main sweet potato growing regions of Kenya (Western, South-western, Eastern, and Central Provinces), neighboring and non-contiguous countries and maintained in situ. The collections are located at the University of Nairobi, Kabete Field Station Farm in Central Kenya and at the Kakamega Research Station in Western Kenya. The plants were assessed for symptoms caused by virus diseases twice in a period of 2 years. SPVD severity in each genotype was assessed using a subjective five-point severity rating scale where: 1 = no symptoms observed; 2 = mild symptoms consisting mainly of chlorotic and/or purple spots; 3 = moderate symptoms which included chlorotic spots, vein clearing, interveinal chlorosis, mottling, and mosaics; 4 = plants were not stunted, but had severe symptoms of vein clearing, interveinal chlorosis, chlorotic spots, mottling, mosaics, and general chlorosis; and 5 = very severe symptoms, which included severe chlorosis, small-deformed leaves (shoestring), and severe plant stunting.

A total of 92 genotypes with SPVD severity rating of 1 or 2 were selected as resistant and used for challenge inoculations. Two apical cuttings from each genotype were planted in a greenhouse in Kenya and allowed to grow for 2 weeks. The plants were then graft-inoculated with a scion from a sweet potato plant (cultivar 'Marera I'), which had previously been confirmed to be infected with both SPFMV and SPCSV (East African serotype) from a Kenyan field using nitrocellulose membrane enzyme linked immunosorbent assay (NCM-ELISA) (CIP 2001). The scions were multiplied and maintained in the greenhouse. Three susceptible cultivars from tissue culture, which had tested negative to different viruses using NCM-ELISA were also grafted with Marera I to ensure it induced SPVD. Inoculated plants were monitored for symptom development for 2 months. Genotypes were again selected as resistant or susceptible based on symptom severity and time (in days) to symptom development. A total of 28 genotypes were selected as resistant to SPVD.

Twenty out of the 92 SPVD graft inoculated clones were randomly selected and used to determine

efficiency of inoculation by confirming the presence of SPFMV and SPCSV. The genotypes were grafted onto *I. setosa* indicator plants and NCM ELISA done as described in the text.

Nineteen other genotypes with severe SPVD symptoms in the germplasm collection plots (rated at a scale of 4 or 5) were selected as susceptible, giving a total of 47 genotypes (Table 1). Cuttings from 38 of the selected genotypes were sent to Plant Germplasm Quarantine Office of USDA-ARS, Beltsville, MD for virus indexing and therapy. The remaining nine genotypes were obtained from the USDA/ARS repository, Griffin, GA.

### DNA extraction

DNA extraction out of the nine clones obtained from the USDA/ARS repository was done using the GeneElute Plant Genomic DNA Kit (Sigma-Aldrich Inc., St. Louis, MO, USA) as described by Mcharo et al. (2004). The 38 clones sent to the Plant Germplasm Quarantine Office of USDA-ARS were planted in a greenhouse and total DNA extracted from leaves 1 month later using a cetyltrimethylammoniumbromide (CTAB) extraction method (Li et al. 2004). DNA was further purified using GeneElute DNA binding columns (Sigma-Aldrich Inc.).

### AFLP analysis

Amplified fragment length polymorphism analysis was conducted as previously described (Mcharo et al. 2005). The DNA samples were amplified in a three-step process using GeneAmp PCR system 9600 thermocycler (Perkin Elmer, Fulerton, CA, USA). Reagents for AFLP™ were obtained from Invitrogen™ (AFLP starter primer kit, Cat No. 10483-014) and LI-COR Inc. (Lincoln, NE, USA, Cat. No. 420032). About 100 ng/μl of total genomic DNA was digested using an EcoRI/MseI restricted enzyme mix in 5× reaction buffer at 37°C for 3 h. The enzymes were inactivated by incubating the mix at 70°C for 10 min. Double stranded adaptors were then ligated to the restricted DNA fragments resulting in template DNA which was used for pre-amplification.

Diluted template DNA (1.5 μl) was added to 10 μl pre-amp primer mix, 1.25 U Taq DNA polymerase and 1.25 μl RedTaq™ PCR reaction buffer 10x with MgCl<sub>2</sub> (Sigma-Aldrich™) to make a 13 μl reaction

**Table 1** Sweet potato clones used for marker selection and country of origin

Number	Clone	SPVD reaction group <sup>a</sup>	Country of origin	Number	Clone	SPVD reaction group <sup>a</sup>	Country of origin
(a) Training group							
1	Nyathi Odiewa	Resistant	Kenya	16	Beauregard	Susceptible	USA
2	Osombo Nyuol	Resistant	Kenya	17	Bungoma	Susceptible	Kenya
3	Unknown-1	Resistant	Kenya	18	Mar Ooko	Susceptible	Kenya
4	Unknown-2	Resistant	Kenya	19	Namaswakhe	Susceptible	Kenya
5	Agriculture	Resistant	Kenya	20	Spoungi	Susceptible	Tanzania
6	Nyar Busia	Resistant	Kenya	21	K37	Susceptible	Kenya
7	Mwavuli	Resistant	Kenya	22	K46	Susceptible	Kenya
8	Maria Angola	Resistant	Peru	23	K207	Susceptible	Kenya
9	Nyar Koyugi Mumbo	Resistant	Kenya	24	SPK 013	Susceptible	Kenya
10	Butso Butso I	Resistant	Kenya	25	Cheglina Mowar	Susceptible	Kenya
11	Tanzania <sup>b</sup>	Resistant	Uganda	26	Marera I <sup>b</sup>	Susceptible	Kenya
12	Unknown-3	Resistant	Kenya	27	Nyaboro	Susceptible	Kenya
13	Ondieki Chilo	Resistant	Kenya	28	Kemb 10	Susceptible	Kenya
14	Nyandere	Resistant	Kenya	29	Salyboro	Susceptible	Peru/CIP <sup>c</sup>
15	Jayalo	Resistant	Kenya	30	W220	Susceptible	USA
(b) Validation (test) group							
31	K118	Resistant	Kenya	41	Nemanete	Susceptible	Peru
32	Polista	Resistant	Tanzania	42	Mugande <sup>b</sup>	Susceptible	Rwanda
33	NASPOT I	Resistant	Uganda	43	Merenge	Susceptible	PNG <sup>d</sup>
34	Rateng	Resistant	Kenya	44	Naveto	Susceptible	PNG <sup>d</sup>
35	Ondijo	Resistant	Kenya	45	Webuye	Resistant	Kenya
36	Mary Oketch	Resistant	Kenya	46	Nduma	Resistant	Kenya
37	Nyanduwo	Resistant	Kenya	47	Nyar Buhola	Resistant	Kenya
38	Nyandegge	Resistant	Kenya				
39	Tur Ninde	Resistant	Kenya				
40	Unknown-4	Resistant	Kenya				

<sup>a</sup> Genotypes were grouped as resistant or susceptible based on disease severity or days to symptom development in plants following graft inoculation using scions infected with SPFMV and SPCSV (resulting in SPVD) obtained from a Kenyan field

<sup>b</sup> Cultivar ‘Marera I’ was classified as resistant when two or three markers selected using STEPDISC procedure or logistic regression (SAS 2001) were used during cross-validation, while cultivar ‘Tanzania’ was classified as susceptible when a two-marker model was used. ‘Mugande’ was misclassified using the four-marker model

<sup>c</sup> International Potato Center

<sup>d</sup> Papua New Guinea

volume. The pre-amplification conditions were 20 cycles each of 94°C for 30 s, 56°C for 60 s, 72°C for 60 s and a final hold at 4°C for 2 h.

The reaction volume for selective amplification consisted of 3.5 µl pre-amplified diluted DNA, 0.4 µl of EcoRI (fluorescently labeled) primer (AAG), 4.4 µl of *MseI* (unlabeled) primer, 2.0 µl RedTaq™ PCR reaction buffer 10x with MgCl<sub>2</sub> (Sigma-Aldrich™), 1.4 µl MgCl<sub>2</sub> (Sigma-Aldrich™), 1 U Taq DNA polymerase (Invitrogen™, Carlsbad, CA, USA), dNTPs

(200 µM), and 6.38 µl double distilled or AFLP grade water. Four selective primer pairs identified by Fajardo et al. (2002) and also used by Mcharo (2005) were used for selective amplification (CAG, CTA, CTG, and CTT). Blue stop solution (5.0 µl) (LI-COR) was added onto each amplified DNA sample. The amplified DNA sample was denatured at 94°C for 4 min, covered in aluminium foil and placed in a freezer at –20°C for 10 min to prevent annealing of complementary fragments, before loading 0.8 µl of

the DNA sample onto a 25-cm acrylamide gel. PCR amplification fragments were separated by 6.5% acrylamide gel electrophoresis using LI-COR Global IR<sup>2</sup> sequencer (LI-COR) for 2 h 45 min. The AFLP fragments were automatically detected and recorded during electrophoresis using the LI-COR SAGA<sup>MX</sup> v 3.1.0 software. The markers were named starting with the three letters coding for the primer followed by the molecular weight of the marker in base pairs.

### Statistical analysis

Analysis of molecular variance (AMOVA) on the genotypes with the AFLP marker profiles was used to test genotypic variability based on molecular marker information using WINAMOVA 1.55 software (Excoffier et al. 1992; Huff et al. 1993). Genetic distances for the AMOVA analysis were estimated using the Euclidean metric distance of Excoffier et al. (1992).

Discriminant analysis as previously described by Mcharo et al. (2004) was used to select informative markers that are linked to SPVD resistance in the two populations. The 47 clones were divided into two populations, the first one consisting of 28 clones classified as resistant to SPVD and another 19 clones classified as susceptible to SPVD. A training sample consisting of 15 resistant and 15 susceptible clones from the original 47 was used for the development of a phenotypic group prediction model. The training sample proportions were equal (15 resistant and 15 susceptible) for both the susceptible and resistant group, making the prior probabilities of group membership equal at 0.5. A second group consisting of the remaining 4 susceptible and 13 resistant clones was used as a test population to validate the model.

From the original array of AFLP generated molecular markers, the most informative markers were selected using STEPDISC procedure, and logistic regression using PROC LOGISTIC procedure (SAS 2001). The forward selection option of STEPDISC was used to select markers to be used in the classification model. The forward selection process commences with no markers in the model. Entry-significance levels of  $P \leq .01$  of the chi-square score for entering an effect or marker into the model to achieve at least 95% prediction accuracy was imposed to choose the most discriminating markers (SAS 1999). The selected markers were then used in a

nonparametric discriminant analysis ( $k = 1$ ), DISCRIM option (SAS 2001), to construct and validate a class prediction function and to predict membership, resistant or susceptible, of the test. A nonparametric method; the k-nearest neighbor method (Rosenblatt 1956) was used to estimate the group-specific densities that produce a classification criterion because the data was categorical in nature and could not allow assumption of normal distribution. The genotypes comprising the training and test groups were mostly from Kenya, but included genotypes from seven other countries. We assume that genotypes used in the present study consisted of unrelated clones (mainly landraces) and therefore did not have any population structure. The performance of the discriminant criterion was evaluated by posterior probability error rate and group-specific error count estimates during cross-validation. The error estimator gives the proportion of misclassified observations in each group. Total error, from which the percent correct classification is derived, is the weighted mean error estimates of the two phenotypic groups.

PROC LOGISTIC (SAS 2001) was used to perform logistic regression to select markers that accounted for the phenotype variation, with the forward selection option used for marker selection. The phenotype was a binary outcome as either resistant or susceptible to SPVD. Significance level to include a marker was set at  $P = 0.01$ . The Akaike Information Criterion (AIC) model fit statistics for logistic regression were computed as described in SAS (1999) and by Mcharo (2005), and used to compare models with different variables. The model with the lowest AIC value achieves the best fit.

## Results

### Germplasm screening

All 92 sweet potato genotypes which initially had mild or no symptoms in the collection plots developed a range of symptoms at different times when inoculated with scions infected with SPVD. Symptoms observed included vein clearing, interveinal chlorosis, chlorotic spots, mottling, mosaics, general chlorosis, rugosity, and stunting. Some genotypes developed typical SPVD symptoms consisting of vein clearing, interveinal chlorosis, chlorotic spots,

mottling, and/or general chlorosis within 1 week, others took as long as 1 month, while some genotypes did not develop the typical severe symptoms characteristic of SPVD. The 20 clones taken from the 92 and grafted on to *I. setosa* were confirmed to be infected with both SPFMV and SPCSV using NCM-ELISA, validating the protocol used. Resistant genotypes were selected as those that showed mild chlorotic spots and/or flecks, mild vein clearing, or those that took longer to show SPVD symptoms. A total of 28 genotypes were grouped as resistant and 19 as susceptible (Table 1).

#### AMOVA, discriminant and logistic regression analysis

A high level of polymorphism was observed using AFLP markers. A total of 350 markers were generated using the four primer combinations, 206 of which were polymorphic. The number of polymorphic markers generated from each primer combination ranged from 45 to 61 with an average of 51 markers. The analysis of two groups (resistant and susceptible) using AMOVA and all 206 polymorphic markers showed a significant ( $P < 0.002$ ) variation between the two phenotypic groups of the 47-clone population.

The STEPDISC procedure identified nine markers that met the entry-significance level ( $P \leq 0.01$ ) to achieve at least 95% prediction accuracy (Table 2). The Wilks' lambda and  $Pr < \lambda$  were used to determine how powerful the selected markers are. No

more markers could be selected even when the entry significance levels were changed to  $P \leq 0.05$ . During evaluation by cross-validation to test the predictive power of the selected markers or model, only four markers selected by STEPDISC procedure were required to achieve 100% correct classification of the 30 genotypes in the training set. Three markers achieved a 96.7% correct classification rate. Genotype 'Marera I' was misclassified as resistant when two or three markers selected by STEPDISC procedure were used. When only two markers were included in the classification model, cultivar 'Tanzania' which was initially grouped as resistant, was classified as susceptible, reducing the classification accuracy to 93.3% using two predictor markers.

Four markers, identical to those selected by the STEPDISC procedure, were also selected by logistic regression (Table 3). However, the order of importance of marker selection differed between the two procedures. The four markers also gave 100% correct classification of the training population. Increasing the number of markers increased the AIC-value, indicating that a model with the four markers could be the most desirable. The rate of correct classification of genotypes into resistant and susceptible groups achieved by the various models using logistic regression is shown in Table 4.

One test clone (Mugande) could not be classified correctly out of the 17 genotypes when four markers were used, giving a 94.12% correct classification efficiency. Increasing the marker number above four could not classify the misclassified clone correctly.

**Table 2** STEPDISC selection for AFLP DNA markers in sweet potato associated with resistance to SPVD

Marker <sup>a</sup>	Entry step	Partial $R^{2b}$	Wilks' Lambda <sup>c</sup>	Pr < lambda
cag202	1	0.55	0.45	<0.0001
cta110	2	0.33	0.31	<0.0001
cta168	3	0.36	0.19	<0.0001
cta334	4	0.33	0.13	<0.0001
cta136	5	0.43	0.07	<0.0001
ctg621	6	0.33	0.05	<0.0001
cta195	7	0.29	0.04	<0.0001
cta076	8	0.63	0.01	<0.0001
cag246	9	1.00	0.00	<0.0001

<sup>a</sup> Markers are named starting with the three letters coding for the primer followed by the molecular weight of the marker in base pairs

<sup>b</sup> Partial  $R^2$  is the marginal variability accounted for by a variable when all others are already included in the model

<sup>c</sup> Wilks' lambda is the likelihood ratio measure of a marker's contribution to the discriminatory power of the model

**Table 3** Logistic regression selection for AFLP DNA markers associated with SPVD resistance in sweet potato

Marker entry step	Marker <sup>a</sup>	Estimate ( $\beta$ )	$\chi^2$ score	Pr > $\chi^{2b}$
0	Intercept	10.02		
1	Cag202	−17.76	16.43	<0.0001
2	Cta168	−18.04	13.00	0.0003
3	Cta110	−17.30	10.58	0.0011
4	Cta334	16.95	15.00	0.0001

<sup>a</sup> Markers are named starting with the three letters coding for the primer followed by the molecular weight of the marker in base pairs

<sup>b</sup>  $\chi^2$  score is the largest significant score for marker not in model to be included in the model

**Table 4** Rate of correct classification of 30 training clones of sweet potato into SPVD resistant (or tolerant) and susceptible groups and the AIC model fit statistic for logistic regression

Number of predictor markers	Probability level entry	Resistant group error rate	Susceptible group error rate	Total error rate	Akaike information criterion
1	<0.01	0.07	0.20	0.13	26.90
2	<0.01	0	0.20	0.10	21.84
3	<0.01	0	0.07	0.03	15.35
4	<0.01	0	0	0	10.01
5	0.95	0	0	0	12.01
6	0.95	0	0	0	14.01
7	0.95	0	0	0	16.01

## Discussion

In this study, we report on the first systematic effort to evaluate sweet potato genotypes grown in Kenya for their reaction to SPVD and identify a core collection of resistant and susceptible genotypes. Using discriminant and logistic regression analysis, we were able to select molecular markers associated with SPVD resistance that could be useful to sweet potato breeders.

When the 92 symptomless, field-grown sweet potato genotypes were graft-inoculated, over half of the genotypes developed severe symptoms indicating that field resistance was not durable and could have been due to escape, resistance to insect vectors or that the susceptible scion provided a continuous source of virus that overcame resistance. Graft inoculation represents the most potent, high dosage, continuous supply of virus inoculum to a plant (Wroth and Jones 1992; Njeru et al. 1995). Resistance is therefore overcome by graft inoculations even in sweet potato genotypes showing high-field resistance to SPVD (Mwanga et al. 2002b, 2003). Despite the strong challenge imposed on the genotypes by graft

inoculation, 28 genotypes showed mild chlorotic spots and/or flecks, mild vein clearing, or delayed symptom development, and were thus selected as resistant. Delayed symptom development can be an important part of resistance (Dasgupta et al. 2003), especially if it is associated with reduced virus replication and translocation. Reduction in yields may be moderate in such genotypes. It would be desirable to know the titer of viruses in the genotypes with varying levels of SPVD resistance. Standard ELISA assays are not reliable for this purpose because sweet potato contains large, variable quantities of interfering substances such as phenols, latex, and polysaccharides (Esbenshade and Moyer 1982; Abad and Moyer 1992). The use of real-time quantitative PCR (Kokkinos and Clark 2006a) will be helpful in determining the concentration levels of viruses between different genotypes and provide a better understanding of virus titer characteristics associated with resistance.

The genotypes were graft inoculated with scions infected with SPFMV and SPCSV (East African serotype) from a Kenyan field. However, there are different strains of SPFMV (Cali and Moyer 1981;

Kreuze et al. 2000) and different serotypes of SPCSV (Alicai et al. 1999; Hoyer et al. 1996; Vetten et al. 1996) in different regions of the world. The different strains and serotypes have biological significance in as much as resistance to SPVD is concerned. Genotypes described as resistant to SPFMV in Peru were susceptible in East Africa (Gibson et al. 1998; Karyeija et al. 1998b). Genotypes resistant to SPVD in Nigeria where the West African serotype of SPCSV predominates were susceptible to SPVD in Uganda where the East African serotype is predominant (Alicai et al. 1999). The reaction of genotypes used in this study may therefore be limited to strains and serotypes common in East Africa.

The aim of our study was to identify a combination of molecular markers that could be used to assign individuals to resistant and susceptible groups, and to verify the predictive power of the selected markers or model. The analysis of molecular variance was significant, indicating statistical differences between the two pre-defined phenotypic groups, and thus we could determine which markers discriminate between the two groups. Application of discriminant and logistic regression analysis to a molecular marker data set enables one to determine which markers discriminate between groups and then use the information to predict group membership. Four markers, which gave 100% correct classification of the two groups in the training population, were identified by both discriminant and logistic regression. While comparing discriminant analysis and logistic regression, Mcharo (2005) reported that the marker variables selected by the two techniques differed, and that logistic regression was more accurate. In our case, the two methods selected the same four critical markers, suggesting that the markers selected have a strong association with the phenotypic traits. There is a likelihood that the markers identified in this study are linked to the genes responsible for resistance and susceptibility to SPVD. Capdevielle (2001), while investigating the linkage between marker assisted classification and differential response to rice sheath blight disease, noted that identified markers are associated with QTLs responsible for expression of this trait. Accuracy was slightly compromised when classification models were based on two or three markers. These results are consistent with previous studies (Mcharo 2005).

In the present study, 17 genotypes were assigned a resistance/susceptibility rating based on a four-marker

model. Only one genotype (Mugande), which was initially grouped as susceptible was not classified correctly and was instead grouped as resistant. It is possible that the classification criteria may misclassify genotypes at low frequency. Second, the nature of the resistance is yet to be established, i.e., whether it is due to tolerance or true resistance, due to the effect of different strains of viruses, or the presence of other viruses. Previous reports showed *Sweet potato mild mottle virus* (SPMMV), *Cucumber mosaic virus* (CMV), and *Sweet potato mild speckling virus* (SPMSV) may enhance symptom development of other viruses (Cohen and Loebenstein 1991; Di Feo et al. 2000; Mukasa et al. 2006). The presence of other viruses could result in reduced or delayed symptom development and remission (C. Clark, unpublished data). Any combination of these factors may result in phenotypic misclassification. Still our classification method was 94% accurate.

Mwanga et al. (2002a) reported that resistance to SPCSV and SPFMV was mediated by two major (but separate) recessive genes. These results were based on QTL analysis of progeny derived from a cross between two SPVD resistant genotypes. They further observed that some QTL might be associated with resistance to both viruses; however, no genes were identified for SPVD resistance given the paucity of SPVD resistant progeny in the population. Mcharo et al. (2004) noted that the power of discriminant and logistic regression analysis is that one does not need a parent-progeny population, unlike using QTL analysis, which needs closely related individuals. However, the genes controlling resistance to SPVD associated with markers identified in this study may be working in a similar manner to those identified by Mwanga et al. (2002a).

The fact that only one genotype from the test group could not be correctly classified when using the four-marker model means that the identified markers are strongly associated with the phenotypic traits and have potential in selection of putative SPVD resistant genotypes in East Africa. Our understanding of host-plant resistance can also be furthered by understanding the behavior of the two viruses in core collection genotypes.

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