ABSTRACT

Mwanga, O. M. Robert. Nature of Resistance and Response of Sweetpotato to Sweetpotato Virus Disease (Under the direction of G. Craig Yencho and James W. Moyer)

Sweetpotato virus disease (SPVD) is a devastating disease due to the dual infection and synergistic interaction of sweetpotato feathery mottle potyvirus (SPFMV) and sweetpotato chlorotic stunt crinivirus (SPCSV). This study was conducted to: 1) determine the inheritance of resistance to SPVD in sweetpotato; 2) estimate the nature of genetic variance; and 3) evaluate methods for screening large populations for resistance to SPVD. The genetic basis of resistance to SPVD was investigated in three studies. The first genetic study consisted of a randomized block design at two sites in Uganda, during 1998-2000, using 45 full-sib diallel (half) families of 10 parental clones varying in SPVD resistance. The second study also conducted in Uganda, examined progeny from 15 promising sweetpotato diallel families (1352 genotypes), while the third examined two of the most promising families (294 genotypes) from the same diallel at the International Potato Center (CIP), Lima, Peru. Genetic component analysis of the 45 diallel families showed significant general combining ability (GCA) and specific combining ability (SCA) effects for resistance to SPVD. GCA to SCA variance components ratios were large (0.51-0.87) and resistant parents exhibited high GCA, indicating that additive gene effects were predominant in the inheritance of resistance to SPVD and recovery. Use of a suitable sweetpotato genotype for increase of SPVD inoculum and modified cleft graft inoculation led to rapid progress in screening large populations for SPVD resistance. The distribution of SPVD scores in the promising families was skewed toward highly susceptible categories, in Uganda and Peru.

Inoculation of the two families at CIP with either SPCSV or SPFMV, and Mendelian segregation analysis for resistant versus susceptible categories for the two viruses suggest that resistance to SPCSV and SPFMV is conditioned by two, separate recessive genes. In the proposed model for inheritance, the two genes are unlinked and they are inherited in a hexasomic or tetradisomic manner. Based on amplified fragment length polymorphism (AFLP) and quantitative trait (QTL) loci analyses we identified two AFLP unlinked markers associated with loci conferring resistance to SPCSV and SPFMV in these progenies. We propose s*pcsv1* and s*pfmv1* to be the names of the genes.

Nature of Resistance and Response of Sweetpotato to Sweetpotato Virus Disease

By

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A dissertation submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the Degree of
Doctor of Philosophy

Department of Horticultural Science

Raleigh

2001

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DEDICATION

To the millions of people
for whom sweetpotato is
important for their survival
and to those who do the work
to improve the crop.

BIOGRAPHY

Robert O. M. Mwanga was borne on 24 May, 1954 at Budhabangula, Iganga, Uganda. He was brought up with his four bothers and three sisters by his parents, Joyce and Abuneri Mwanga, in Busota Village, Kamuli District. He obtained his Primary Leaving Certificate of Education (P.L.E) from Bugema Adventist Primary School in 1968, East African Certificate of Education (E.A.C.E.) from Jinja College in 1972 and, East African Advanced Certificate of Education (E.A.A.C.E) from Namilyango College in 1974. Robert graduated with an honors BS degree in Botany and Zoology from Makerere University, Kampala, Uganda, in 1978. He obtained his Master of Science in Agronomy and Plant Breeding from the University of the Philippines at Los Baños (UPLB), Laguna, in 1986.

He started the root crops program at Kawanda Agricultural Research Institute (KARI) in 1978, and at Namulonge Agricultural and Animal Production Research Institute (NAARI) in the National Agricultural Research Organization (NARO) in 1986 in Uganda. Robert was the Team Leader of the National Sweetpotato Research Program from 1989-1995, and he has bred and released seven sweetpotato varieties in Uganda.

He has attended short courses related to his profession at the International Insistitute of Tropical Agriculture (IITA), Ibadan, Nigeria; Ergerton University, Njoro, Kenya; International Agricultural Center (IAC), the Netherlands; and the Institute of Genetics and Plant Breeding, Warsaw Agricultural University, Poland. He was a research associate at IITA in 1983.

Robert was awarded a grant by the McKnight Foundation Collaborative Crop Research Program (CCRP) to pursue his Ph.D. at NCSU in 1996. His project was part of a multidisciplinary partnership involving NAARI in Uganda, NCSU, the US Vegetable

Laboratory (USV, USDA-ARS) and Clemson University, Charleston, South Carolina, and the International Potato Center (CIP). Robert, the principal investigator for the Ugandan research team, and Richard Manning, author of the book, "Food's Frontier: The Next Green Revolution", which was recently written to describe the projects funded by the CCRP, were interviewed in New York on National Public Radio (NPR) about this unique program. You can listen to or download the radio interview from the NPR Living On Earth Archives (October 20, 2000) website at (http://www.loe.org/archives/001020.htm).

Robert has made numerous presentations, mainly on sweetpotatoes at national, regional and international workshops and conferences. He was also a visiting scientist at the International Potato Center (CIP), Lima, Peru, in 2000 and is co-author of a number of papers in international journals.

ACKNOWLEDGEMENTS

My deep gratitude goes to Dr. Craig Yencho and Dr. James Moyer, Co-chair of my Graduate Committee for their advice and guidance throughout my study. It has been a rewarding experience working with them, especially during preparation of my dissertation. I would like to express my thanks to the other members of my Graduate Committee, Dr. Jonathan Schultheis and Dr. George Wilson who have done an excellent job guiding me to completion of my degree. I cannot forget Dr. Wanda Collins, former Chair of my Graduate Committee, for giving me a very good start in my course work and dissertation.

I wish to thank the McKnight Foundation for supporting my Ph.D. program and the partnership, and the National Agricultural Research Organization (NARO) for the joint support and for giving me study leave.

I am very grateful to Jim Cervantes for ever willing to help whenever I had software problems. Ken Pecota, Cindy Pierce, and Rachel McLaughlin; thank you for your encouragement and help.

Many thanks go to Dr. Dapeng Zhang, Dr. Lucho Salazar, and Fuentes Segundo for their guidance and for enabling me to conduct research successfully at the International Potato Center (CIP), Lima, Peru. I wish to thank their teams, virology and sweetpotato genetics, Hurtado Oscar and Geneveva, to mention but two, for the practical guidance they offered me.

The Namulonge sweetpotato research team and the NAARI Director, are highly appreciated for helping to maintain the experiments especially when I was at NCSU.

To my partners on the Sweetpotato McKnight Research Project: Drs. Janice Bohac Ted Carey, Richard Gibson, Nicole Smit; Members of the Advisory Committee, Prof.

Joseph Mukiibi, Drs. Theresa Sengooba, Peter Ewel; PRAPACE staff; and Dr. Bob Goodman and the Oversight Committee of the CCRP, thank you for your support.

Finally, to my wife, Faith, and my family, who have seen me through the whole journey, I can only say, God bless you, and thank and glorify God for bringing the Ph.D. program to a successful end.

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CHAPTER 1

Global Perspective of the Dissertation and Sweetpotato

Introduction

The sweetpotato, *Ipomoea batatas* L. (Lam.), is grown for its storage roots and vines. Its storage roots are used for human consumption, animal feed, and seed in temperate regions, while its vines are used as animal feed and seed mainly in the tropics. Sweetpotatoes are an important food crop in terms of area and production. They are grown in over 100 countries, with 78% of the global sweetpotato area located in developing countries in Asia and Africa. Roughly 73% of the global area and 84% of the global production of sweetpotato is concentrated in China, yet production is spread over many countries (International Potato Center 1999; Luisa and Hijmans 2000). Other significant concentrations of sweetpotato are in Asia (Vietnam, Indonesia, India and the Philippines) and the East African Highlands (Uganda, Rwanda, Burundi and Kenya) (Woolfe 1992; Luisa and Hijmans 2000).

In East Africa, sweetpotato plays an important role in the diet and food security of the population indicated by the high per capita consumption (e.g. 160 and 85 kg/cap/year for Rwanda and Uganda, respectively) (International Potato Center, 1999). However, sweetpotato yields in the region are very low (1.6-9.7 t/ha) compared to yields of over 20 t/ha (24, 26, 32 t/ha for Japan, the Cook Islands and Israel, respectively) (International Potato Center 1999; Huaccho and Hijmans 2000). Major constraints to increased sweetpotato productivity in East Africa include, sweetpotato weevils (*Cylas puncticollis* and *C. brunneus*), viruses (mainly sweetpotato virus disease), *Alternaria* stem blight, poor yielding varieties of low nutritive value (low or no ß-carotene), shortage of high quality planting materials, marketing problems, and limited range of processing and utilization options, leading to high postharvest losses, estimated between 30-35% (Friedman 1960; Jenkins 1982; Woolfe 1992).

Viruses, mainly sweetpotato virus disease (SPVD), constitute the biggest disease problem, followed by *Alternaria* stem blight in sweetpotato production in East Africa. SPVD causes up to 98% yield loss in sweetpotatoes (Hahn 1979; Gibson et al. 1998; Karyeija et al. 1998).

SPVD is due to the dual infection and synergistic interaction of sweetpotato feathery mottle potyvirus (SPFMV) and sweetpotato chlorotic stunt crinivirus (SPCSV) (Schaefers and Terry 1976; Karyeija et al. 2000). SPVD is characterized by a range of symptoms including, chlorotic mosaic, vein clearing, leaf distortion, strapping and crinkling of leaves, and a general stunting of plant growth. SPFMV is transmitted non-persistently by aphids (*Myzus persicae*, *Aphis gossypii* and *A. cracivora*) (Stubbs and McLean 1958), while SPCSV is transmitted semi-persistently by whitefly (*Bemesia tabaci*) (Schaefers and Terry 1976; Cohen et al. 1992). Development of cultivars with increased resistance to SPVD is considered to be the only effective control strategy.

Emphasis on developing SPVD resistance has focused mainly on the development of resistance to SPFMV, the most widespread of all known sweetpotato viruses. However, it is difficult to breed for resistance to SPVD by developing genotypes resistant to SPFMV and SPCSV separately because of the existence of different strains of SPFMV (Cadena-Hinojosa and Campbell 1981; Cali and Moyer 1981; Moyer 1986; Kennedy and Moyer 1982; Kreuze et al. 2000) and different serotypes of SPCSV (Hoyer et al. 1996; Wisler et al. 1998; Alicai et al. 1999b) in different regions of the world. Further, these resistances must then be combined in a common background to develop sweetpotatoes resistant to SPVD. For example, sweetpotato genotypes from Peru and Nigeria with high levels of resistance to SPFMV were susceptible to SPVD when they became infected with SPFMV and SPCSV in Uganda (Mwanga et al. 1991; Gibson et al. 1998; Karyeija et al. 2000). Likewise, Karyeija

et al. (2000) evaluated resistance to SPFMV in East African cultivars and found that resistance to SPFMV was overcome when the same SPFMV-infected plants were co-infected with SPCSV, which enhanced SPFMV titer and increased susceptibility to SPVD (Aritua et al. 1998b; Gibson et al. 1998; Karyeija et al. 2000).

Although there has been much emphasis on developing resistance to SPFMV in sweetpotato (CIP 1990; Karyeija et al. 1998, 2000), there have only been a couple of studies on the inheritance of resistance to SPVD. Hahn et al. (1981) estimated broad-sense heritability of resistance to SPVD to be 0.48-0.98 while Ngeve and Bouwkamp (1991) observed that storage root yields of two of eight tolerant sweetpotato genotypes evaluated over two years were not significantly reduced by SPVD. The **general objective** of study was to understand the genetic basis of resistance of SPVD. The **specific objectives** were to:

1) Determine the inheritance of resistance of SPVD;
2) Estimate the components of genetic variance (GCA and SCA);
3) Determine the type of gene action controlling resistance to SPVD;
4) Improve methods of evaluating sweetpotato genotypes for resistance to SPVD;
5) Assess the use of recovery from SPVD as an additional selection criterion for resistance to SPVD;
6) Elucidate the inheritance of resistance to SPCSV and SPFMV (the components of SPVD); and
7) conduct preliminary studies to identify molecular markers linked to genes conferring resistance to SPCSV and SPFMV.

Organization of the dissertation

The dissertation is organized into five chapters. Chapter 1 provides a general perspective of sweetpotato, and it covers information on importance of sweetpotato, growth conditions, constraints of production, botany, germplasm, origin and distribution, phylogeny, Mendelian genetics, quantitative genetics and breeding, molecular markers, pests, virus diseases worldwide and identification problems, and an overview of sweetpotatato viruses in Uganda. Chapter 2 addresses objectives 1-3; Chapter 3 addresses objectives 3 and 4; and Chapter 4 covers objectives 3, 6 and 7. Chapter 5 provides a summary of the major findings of the research, and identifies information gaps and suggests future research needed to fill those gaps.

Importance of sweetpotatoes

Sweetpotato *Ipomoea batatas* (L.) Lam, with a mean annual production of 132 million tons between 1991-2000, is ranked among the top ten most important food crops globally (Woolfe 1992; International Potato Center 1999; FAO 2000). Sweetpotatoes are grown in over 100 countries and over 98% of their production and consumption is in developing countries (Horton 1988, Gregory et al. 1990). For every calorie consumed sweetpotatoes provide over 90% of essential nutrients except for protein and niacin (Food Nutrition Board 1980; Watt and Merrill 1975). Orange-fleshed sweetpotatoes are particularly nutritious, ranking highest in nutrient content of all vegetables for vitamins A and C, folate, iron, copper, calcium, and fiber (Woolfe 1992).

Growth Conditions

Sweetpotatoes are a warm weather crop produced in the tropics, subtropics and

warm temperate regions. They are predominantly grown in latitudes 48°N to 40°S of the equator where they take 3-6 months to produce a crop (Bouwkamp 1984; Woolfe 1992). On the equator, sweetpotatoes are grown from sea level to 3000 m. Growth is best under high light intensity at 24°C, while temperatures above 35°C or below 12°C retard growth. Dry matter production increases with increasing soil temperatures between 20°-30°C and declines above 30°C. Sweetpotatoes grow best with an evenly distributed annual rainfall of 600-1600 mm, on well drained, sandy-loam soils of pH 5.6-6.6. Soils with high density or poor aeration retard storage root formation (Watanabe et al. 1968; Woolfe 1992).

Production Constraints

Constraints to sweetpotato production include, pests, mainly sweetpotato weevils (*Cylas puncticollis* and *C. brunneus* in Africa, and *C. formicarius* in the US and other parts of the globe) and viral diseases, especially sweetpotato virus disease (SPVD) due to infection and synergistic interation of sweetpoato feathery mottle virus (SPFMV) and sweetpotato chlorotic stunt virus (SPCSV). Other constraints of sweetpotato production include, a shortage of high quality planting materials, low yielding cultivars, short shelf-life, limited processing and utilization outlets, and marketing constraints.

Botany

Sweetpotato is a perennial dicot, but it is cultivated as an annual for vines and storage roots. Sweetpotato belongs to a single species, *Ipomoea batatas* (L.) Lam. The sweetpotato and closely related species are classified in the family Convolvulaceae (Morning glory), genus *Ipomoea*, subgenus, *Eriospermum*, section *Eriospermum* (formerly *Batatas*) and series *Batatas* (Austin 1988; Austin and Huaman 1996). Sweetpotatoes are sensitive with

photoperiod 11.5 hrs day length or less promoting flowering, while at 13.5 hrs day light, flowering ceases but storage root yield is not affected (Kays, 1985). Short days with low light intensity promote root development. Flowers are perfect and produce capsules with 1-4 seeds after pollination and seed set. Complex sporophytic self- and cross-incompatibility cause serious problems in breeding (Jones 1967b; Nakanishi and Kobayashi 1979).

Sweetpotato is hexaploid with 2n = 6x = 90 chromosomes, and although some plants morphologically similar to *I. batatas* with 2n = 4x = 60 have been described and named, they are considered synonyms of this species. Among the approximately 50 genera and more than 1000 species in the family Convolvulaceae, only *I. batatas* is of major economic importance as a food (Woolfe 1992). However, *I. aquatica* is also used as a raw salad or a cooked green vegetable or used as animal fodder in South East Asia. Storage root initiation varies from 21-35 days after planting (Austin et al. 1970; Bhattachary et al. 1985).

Germplasm

Sweetpotato exhibits great phenotypic and genotypic diversity an this is reflected by the color of skin or flesh of the root, the size and shape of roots, leaves and branches (Austin et al. 1970; Bhagsari and Brown 1986), the depth of rooting, and time to maturity, resistance to pests and diseases, and even the flavor and texture of cooked roots (Huaman 1992; Woolfe 1992; Austin and Huaman 1996).

The high level of genetic diversity of sweetpotato is reflected in the fact that over 8,000 accessions of sweetpotato are maintained at various gene banks worldwide (Kuo 1991; Nissilä et al. 1999). Chang (1992) speculated that this might represent only a fraction of the existing diversity. A total of 1157 wild accessions of series *Batatas* and 5,526 accessions of *I. batatas* are maintained at the International Potato Center (CIP), Lima, Peru (Huaman and

Zhang 1997). The United States Department of Agricultural Research Service (USDA-ARS) collection at Griffin, Georgia, has 759 *I. batatas* and 440 wild accessions (http:/www.ars-grin.gov/).

Origin and distribution

Current scientific evidence suggests that the sweetpotato is of American origin (Central or South America) where it was widely established by the time the first Europeans arrived. Sweetpotato may be one of the earliest domesticated plants (Yen, 1976). It is not clear whether sweetpotato reached Polynesia through human contact or by chance (e.g. washing ashore Woolfe, 1992; Yen, 1976; O'Brien, 1972). Sweetpotato could have been introduced into Polynesia before the 8th century AD, and named *kumara* (Yen, 19782). The evidence for prehistoric spread of sweetpotato include, the recovery of storage roots from archaeological sites in Hawaii, New Zealand, and Easter Island; the presence of prehistoric root storage facilities in temperate New Zealand; the fact that sweetpotato germplasm is less diverse outside of the Americas (Yen, 1974); and lexical parallels between a Quechua (the Inca language) name (*apichu*) for sweetpotato and the Polynesian *kumara* (Yen, 1982

Two main groups of sweetpotato, the *aje* (an Arawakan word) group (starchy and slightly sweet) and the batata (a Spanish word) group (starchy and very sweet) were known and are evidence of the widespread distribution of sweetpotato through the migration routes in the New World tropics before the discovery of America (Austin 1988). O'Brien (1972) showed linguistic and historic evidence indicating that sweetpotato had reached southern Peru and southern Mexico around 2000-2500 B.C. According to linguistic evidence there are three lines of dispersal of sweetpotato. The *kumara* line is prehistoric and is based on lexical parallels between the Quechua name and the Polynesian word, kumara.

This could explain the movement of sweetpotato by Peruvian or Polynesian voyagers from northern South America to eastern Polynesia around 400 AD. The *batata* line, which dates back to the first voyage of Columbus in 1492, resulted in the introduction of West Indian sweetpotatoes to western Mediterranean Europe. The Portuguese explorers had introduced sweetpotatoes from western Mediterranean Europe to Africa, India, South East Asia, Indonesia, the East Indies and South China by the 16th century, and Southern Japan by 1698. The *camote* (derived from *camotli* in the Mayan language Nahuatl) line was directly introduced from Mexico by Spanish trading galleons between Acapulco, and Manila, the Philippines, and Guam, in the 16th century (Yen, 1982). Progress in subsequent sweetpotato development was probably due to chance seedlings and vegetative propagation of somatic mutants (Yen, 1976).

Phylogeny

I. batatas is not known in the wild state and its ancestor is not known with certainty. Nishiyama (1963) considered 6x *I. trifida* (accession K123), a morphologically similar species with *I. batatas* with small, slightly swollen storage roots collected from Mexico, to be the potential wild ancestor of sweetpotato. However, Jones (1967a) suggested that K123 could be an *I. batatas* derivative growing in the wild, and that traits considered by Nishiyima as typical of wild plants in determining his classification, such as twining habit, are common in sweetpotato. He also showed that the F1 (K123 x *I. batatas*) hybrids produced plenty of seed, and that chromosome pairing in metaphase I of the hybrids was similar to the crosses between sweetpotatoes.

Sexual polyploidization through the production of unreduced gametes might have facilitated the evolution of *I. batatas* to the hexaploid level (Huaman and Zhang, 1997).

Allopolyploidy (Ting and Kehr 1953; Jones 1965b; Magoon et al. 1970; Nishiyama 1971; Austin 1988) and autopolyploidy (Nishiyama et al. 1975; Ukoskit and Thompson 1997; Shiotani 1988) were both proposed as a means to obtain *I. batatas.* In support of the alloploid hypothesis, unnreduced pollen in diploid *I. trifida* (Orjeda et al., 1990) and in some tetraploid and hexaploid *I. batatas* (Bohac et al. 1992), and 2n egg production in 3x *I. trifida* that generated 6x genotypes in their progenies (Freyre et al. 1991) have been reported. Nishiyama (1971) and Austin (1988) considered *I. trifida* to be the most closely related species to sweetpotato and suggested an alloploid origin. According to Nishiyama (1971), 2x *I. leucantha* gave rise to 4x *I. littoralis* and the cross between the two species (2*x* x 4*x*) gave rise to 3x *I. trifida* from which 6x *I. trifida* was derived . Selection and domestication of 6x *I. trifida* wild plants gave rise to 6x *I. batatas*.

Magoon et al. (1970) indicated that the three genomes of sweetpotato are partly homologous and two of the genomes show closer homology than the third. On the basis of numerical analysis of key morphological characters Austin (1988) proposed *I. triloba* and *I. trifida* to be the donors of the sweetpotato genome, and suggested that *I. tiliacea* may also have been involved in the origin of sweetpotato. From cytogenetical evidence, Shiotani and Kawase (1987), and Shiotani (1988) ruled out a genomic differentiation with respect to the genomic homology proposed by Magoon et al. (1970) and postulated the genome constitution of sweetpotato to be autohexaploid ($B_1B_1B_2B_2B_2B_2$, B_1 is homologous to B_2) with the B genome that exists in autotetraploids and diploids of the *I. trifida* complex. However, the degree of homology could not be estimated accurately.

Austin (1988) postulated that the center of origin of *I. batatas* was between the Yucatan Peninsula of Mexico and the mouth of the Orinoco River in Venezuela, where *I. trifida* and *I. triloba* might have crossed to produce the wild ancestor of *I. batatas*. Natives in

the area may have discovered the sweetpotato and brought it into cultivation. By 2500 BC, the cultigen had been spread by the Mayas and Incas into Central and South America and it was domesticated by the time the Europeans arrived. Carbon dating estimated the age of roots found in Chilca Canyon, Peru, to be around 8,000-10,000 BC, suggesting that sweetpotato may be among the world's earliest domesticated crops (Engel 1970; Yen 1974).

Germplasm characterization work by Yen (1982) and Austin (1983, 1988) indicates that the primary center of diversity of sweetpotatoes is in northwestern South America (Colombia, Ecuador and Peru) and parts of Central America (e.g. Mexico, Guatemala, Nicaragua) where a great diversity of sweetpotatoes, weeds and wild *Ipomoea* exist. Secondary centers of sweetpotato diversity outside of the Americans are in China, Southeast Asia, New Guinea and East Africa (Yen, 1982; Austin 1983, 1988). Zhang et al. (1999), using amplified fragment length polymorphism (AFLP) and simple sequence repeat (SSR) markers, observed greater molecular variability among the samples from Central America compared to the South American samples, suggesting that Central America may be a more likely center of diversity compared to South America.

Mendelian genetics

Sweetpotato is a hexaploid (2n = 6x = 90) (King and Bamford 1937; Jones 1965b; Magoon et al. 1970). Meiotic abnormalities include multivalent formation, translocations, and deletions (Oracion et al. 1990; Magoon et al. 1970). Cytological anomalies include various degrees of cross- and self-incompatibilty (Martin, 1965, 1968, 1970). Complex sporophytic self- and cross-incompatibility and an extremely complex hexaploid genome make sweetpotato research in cytogenetics difficult and genetic studies complicated. Poole (1955) studied phenotypic ratios in sweetpotato (rooting vs nonrooting, brown skinned

roots vs cream skinned roots, ridged vs smooth root surface, orange vs cream flesh color red vs green stem, flowering vs nonfloweing, smooth vs lobed leaf margins). Jones (1967b) published theoretical segregation ratios for qualitative traits and presented four hypotheses (hexasomic, tetradisomic, tetrasomic, disomic) of inheritance. Kumagai et al. (1990) tested the models and showed that the ß- amylase null trait in storage roots was controlled by one recessive gene, and that it was inherited in a hexasomic or tetradisomic manner, but not disomically or tetrasomically.

Quantitative genetics and breeding

Adaptation of quantitative genetic principles to sweetpotato breeding have enabled the study of sweeetpotato traits which otherwise would not have been possible because of the hexploid nature of the crop. To date, quantitative genetic studies have been conducted to obtain heritability estimates for yield (Bacusmo et al. 1988), root traits (Jones et al. 1969, 1977, 1978), vine traits (Jones 1969) root quality factors, including dry matter, intercellular space, protein and baking quality (Jones 1977; Jones et al. 1978, 1987) and resistance to soil insects (Jones et al. 1979) and root-knot nematodes (Jones and Dukes 1980). Use of heritablity estimates in sweetpotato breeding is described by Jones (1986) and Jones et al. (1979).

Sweetpotato breeding started in the US in the 1920s, relying mainly on plant introductions from the tropics and favorable mutations (Edmond and Ammerman, 1971; Yen 1974). A major break-through in sweetpotato breeding came with the induction of sweetpotato flowering under long-day conditions (Miller 1939; Fugise et al. 1955; Hsia and Chen 1956; Wang 1964) which led to transition from mutation-based breeding to true sexual seed production. Jones (1965a) proposed a breeding procedure for sweetpotato based

on the recurrent selection procedures for improvement of maize and other crop species. This recommendation was based on the fact that the use of polycross and recurrent selection is an effective way to combine favorable genes and alleles in parental genotypes (Jones et al. 1986). The procedures are especially useful for long-term population improvement of traits with low heritability. Selection based on individual plants is unreliable because within-clone and within-plot variations are usually significant and many sweetpotato traits are influenced signicantly by genotype x environment interactions (Collins et al. 1987).

Molecular markers

Great potential exists for application of DNA markers for the improvement of crops but in sweetpotatoes the technologies are only being developed and have not had immediate practical application for breeding. To date, DNA markers have been used in sweetpotato in phylogenetics and gene pool evaluation (Jarret et al. 1992; Jarret and Bowen 1994; He, et al. 1995; Prakash et al. 1996; Buteler et al. 1999; Zhang et al. 1999; Huang and Sun 2000), genome characterization (Villordon and La Bonte, 1995; 1996), fingerprinting (Connolly et al. 1994); map-making strategies (Ukoskit and Thompson, 1997; Kriegner et al. 2001); and markers for root-knot nematode resistance (Ukoskit et al. 1997), and SPFMV and SPCSV resistance (Mwanga et al. 2001, Chapter 4).

Sweetpotato pests

Sweetpotato weevil (SPW), *Cylas* spp., is the most destructive pest of sweetpotato globally (Jansson and Raman, 1991). Although sweetpotato clones differ in their level of resistance to SPW (Hahn and Leuschner 1982; Mullen et al. 1985; Stathers et al. 1999), to

date, there are no genotypes, including landraces and bred clones that can withstand high weevil pressure. While entomopathogenic fungi were reported to be effective in an integrated pest management sweetpotato weevil program in Cuba (Stathers et al. 1999), the entomopathogenic fungi (*Beauveria bassiana* and *B. brogniartii*) in Uganda were ineffective in checking weevil populations (Smit 1997; Stathers et al. 1999)). The sex pheromone of *Cyclas formicarius* was effective in checking weevil populations in mass trapping trials in Taiwan (Hwang and Hung, 1991), India (Pillai et al., 1993), Vietnam (Braun and van de Fliert, 1997) and Cuba (Alcazar et al. 1997) but not in Japan (Yasuda, 1995) and Indonesia (Braun and van de Fliert, 1999) although large numbers weevils were trapped. Sex pheromones of *C. puncticollis* and *C. brunneus* were also ineffective in controlling weevil populations in mass trapping experiments in Uganda (Downham et al. 1998). Thus, sweetpotato weevil remains the biggest insect pest problem in East Africa.

Pathogens that infect sweetpotato include bacterial and fungal diseases such as bacterial wilt (*Pseudomonas solanacearum*), soil rot or pox (*Streptomyces ipomoea*), fusarium rot (*Fusarium solani*), fusarium wilt (*Fusarium oxysporum f.sp. batatas*) and scab (*Elsinoe batatas*) (Clark 1988; Clark and Moyer 1988). Resistant sweetpotato germplasm sources exist for the diseases mentioned above. Still, sources of resistance to most diseases in sweetpotato and wild relatives are not well studied, and resistance to many diseases has not been exploited commercially (Clark 1988).

Sweetpotato virus diseases

Virus diseases cause considerable damage worldwide. Development of virus-resistant cultivars has been the most effective means of reducing sweetpotato losses due to virus infection (Hahn et el. 1981). Some sweetpotato germplasm is resistant to sweetpotato

feathery mottle virus (SPFMV) (Huaman and Zhang 1997) but immunity has not been observed (Karyeija et al. 2000). At CIP, viruses were considered to be the major constraint to germplasm distribution and crop improvement in most developing regions of the world, and studies on virus identification and diagnostics receive major emphasis in CIP's plan of work (CIP 1990, Moyer et al. 1989).

Identification of sweetpotato viruses

About 20 sweetpotato viruses are known to infect sweetpotatoes (Table 1). However, SPFMV, SPCSV and sweetpotato mild mottle virus (SPMMV) have been well studied. Of the 20 viruses only 5 have been sequenced, namely, SPFMV, SPCSV, SPMMV, sweetpotato latent virus (SwPLV), sweetpotato leaf curl geminivirus (SPLCV), and sweetpotato G virus (NCBI, 2001). Because the majority of the viruses have not been sequenced, nor completely characterized, and no antiserum or any other reagents for diagnosis are available, confusion in the naming of some sweetpotato viruses exists (see question marks, e.g. SPLCV named as a geminivirus and badnavirus, SPCSV named as a crinivirus as well as potyvirus, Table 1). Currently, CIP provides diagnostic kits with all the necessary reagents for the nitrocellulose membrane enzyme linked immunosorbent assay (NCM-ELISA) for detecting the following viruses: SPFMV, SPCSV, SPMMV, SwPLV, sweetpotato chlorotic flecks virus (SPCFV), sweetpotato mild speckling virus (SPMSV), sweetpotato caulimo-like virus (SPCaLV), and C-6 (Carey et al. 1999). Some virus detection methods such as nucleic acid spot hybridization (NASH), PCR-based protocols and electron microscopy are available in advanced laboratories. Because two or more viruses of economic importance may occur in a sweetpotato growing region, breeding for multiple virus resistance is the only feasible approach to mitigating virus induced losses in sweetpotato.

Sweetpotato viruses in Uganda

Viruses are the most serious common disease of sweetpotato in Uganda (Aldrich 1963, Nyiira, 1982; Geddes 1990; Karyeija et al. 1998). The first record of sweetpotato viruses in Uganda was by Hansford in 1944, but virus identification and confirmation work has been conducted recently (Karyeija et al. 1998). Virus diseases known to affect sweetpotato in Uganda include: 1) SPFMV 2) SPCSV; 3) sweetpotato mild mottle virus (SPMMV); 4) sweetpotato chlorotic flecks virus (SPCFV); 5) sweetpotato latent virus (SwPLV); and 6) C-6 (Table 1). Wambugu (1991) reported cucumber mosaic virus (CMV) and sweetpotato caulimo-like virus (SPCaLV) occurring in Uganda, but there have been no confirmed reports (Carey et al. 1998; Gibson et al. 1998; Karyeija et al. 1998; Carey et al. 1999)

Sweetpotato feathery mottle virus (SPFMV)

SPFMV was first reported by Sheffield (1957) in East Africa (Kenya, Tanzania, and Uganda) as sweetpotato virus A. SPFMV is the most common virus infecting sweetpotato in Africa (Schaefers et al. 1976; Karyeija et al. 1998) and worldwide (Clark and Moyer 1988; Moyer and Salazar 1989; Brunt et al. 1996). Karyeija et al. (1998) reviewed extensively the significance of SPFMV in Africa. In East Africa, SPFMV is transmitted in a non-persistent, noncirculative manner by aphids (e.g. *Myzus persicae*, *Aphis gosssypii* and *A. cracivora*) that do not colonize sweetpotato (Aritua et al. 1998a, b). SPFMV causes mild or no symptoms in sweetpotato (Clark and Moyer 1988; Brunt et al 1996), and most East African cultivars do not exhibit symptoms on the foliage (Gibson et al. 1998). Plants of the sweetpotato cultivars, Tanzania and Wagabolige, when graft-inoculated with SPFMV alone showed no symptoms and had low virus titer (below detection level by ELISA). Howver, SPFMV

could still be detected by grafting on susceptible indicator plants such as *I. setsosa* (Gibson et al. 1998).

SPFMV symptoms in sweetpotato are usually mild and transient. Foliar symptoms include, vein clearing, vein feathering, and chlorotic spots, especially on older leaves. Symptoms on the roots include, external cracking, internal cork, and internal necrosis, depending on cultivar and isolate. In the indicator plants, *Ipomoea setosa* and *I. nil* foliar symptoms are similar but more severe and include, vein clearing, mosaic, leaf stunting and distortion. SPFMV has a narrow host range compared to typical potyviruses but some isolates infect *Chenopodium amaranticolor, C. quinoa*, or *Nicotiana benthamiana*, while others are apparently restricted to species of the genus *Ipomoea* (Moyer and Salazar 1989, Gibson et al. 1998, Karyeija et al. 1998).

Kreuze et al. (2000) identified two SPFMV serotypes in Uganda based on coat protein (CP) gene sequences. The two serotypes in East Africa form a separate cluster from the rest of the world and differ from the West African isolate (SPV-1) and from the United States russet crack strain (SPFMV-C). The two Ugandan SPFMV isolates can be transmitted simultaneously by a single aphid but they differ in their ability to infect sweetpotato previously infected with SPCSV (Karyeija et al. 2000). Two CIP clones (420020 and 420026) have extreme resistance to SPFM-C (Fuentes and Salazar, 1996). CIP 420020 was susceptible to both Ugandan SPFMV serotypes, whereas CIP 420026 expressed extreme resistance with no virus being detected by ELISA or back-grafting with *I.setosa*. However, extreme resistance of CIP 420026 to SPFMV broke down when co-infected with SPCSV (Karyeija et al. 2000), indicating the need to breed for multiple resistance. Karyeija et al. (1998), found *Ipomoea tenuirostris* to be a natural host of SPFMV in Uganda. SPFMV has economic impact as a component of sweetpotato virus disease

(SPVD) (Karyeija et al. 1998), the most important disease of the crop in Africa (Geddes 1990; Schaefers and Terry 1976; Hahn et al. 1981). The titer of SPFMV is increased in SPVD-infected plants, and SPFMV is readily acquired by aphid vectors from such plants (Schaefers and Terry, 1976; Gibson et al. 1998).

Sweetpoatato chlorotic stunt virus (SPCSV)

SPCSV has been referred to as SPVD-associated closterovirus in the USA (Winter et al. 1992), and sweetpotato sunken vein closterovirus (SPSVV) in Israel (Cohen et al. 1992; Hoyer et al. 1996). SPSVV isolated from sweetpotato in Israel is serologically similar to the West African closterovirus (Hoyer et al. 1996). SPCSV isolates from East Africa, referred to as S_{EA}, are serologically different from most other non-East African isolates that occur in West Africa (Nigeria, Gabon), Israel, America (Brazil, Argentina, USA) and Asia (Taiwan) (Hoyer et al. 1996). In Uganda, graft-inoculations SPCSV to cultivars Tanzania and Wagabolige showed that SPCSV alone causes a disease commonly observed and detected in naturally diseased field plants. Infected plants are stunted with yellowing or purpling of the foliage depending on the cultivar. SPCSV in Uganda reduced foliage production of infected cuttings by 75% and storage root yield by 87 % compared to healthy controls (Gibson et al. 1998). Indeed, the disease develops rapidly in the field of cultivar Tanzania (Gibson et al. 1998) and Alicai et al. (1999a) reported that the incidence of SPCSV increased in cultivar Tanzania during the peak period of whitefly numbers, then stabilized while SPVD increased with crop age. Cultivars resistant to SPVD in Nigeria were susceptible to SPVD in Uganda (Mwanga, et al. 1991). In Nigeria, SPCSV alone causes no symptoms in sweetpotato (Schaefers and Terry, 1976), suggesting that SPCSV in Uganda is more severe than the Nigerian serotypes (Gibson et al. 1998). Alicai et al. (1999b)

distinguished two serotypes (S_{EA1} and S_{E2}) in Uganda using a panel of monoclonal antibodies. The Ugandan SPCSV serotypes differ in their geographical distribution (Legg et al. 1998; Alicai, 1999a).

Sweetpotato mild mottle ipomovirus (SPMMV)

SPMMV is whitefly-borne (*Bemesia tabaci*) member of the family *Potyviridae* (Hollings et al. 1976). Sheffield (1957) identified two viruses, virus A, transmitted by aphids and virus B transmitted by whiteflies in East Africa. Virus A seems likely to have been SPFMV and virus B may have been what is currently known as SPMMV (Hollings et al. 1976) or SPCSV (Schaefers and Terry, 1976). SPMMV causes relatively mild symptoms in sweetpotato (Hollings et al. 1976). No yield loss assessment of SPMMV in sweetpotato has been done in Uganda. SPMMV was detected in sweetpotato samples from Uganda in the districts of Mbale, Mpigi, Masindi, Kabale (Carey et al. 1998), Tororo and Iganga (Gibson et al. 1998) at very low frequencies, and was not common (Gibson et al. 1997, 1998; Carey et al. 1998, 1999) contrary to other reports (Wambugu 1991; Wambugu et al. 1991).

Sweetpotato chlorotic flecks virus (SPCFV) and others

SPCFV is a possible member of the family *Potyviridae* (CIP 1992; Gibson et al. 1997;). SPCFV was detected in sweetpotato plants from Mbale, Mukono, Mpigi, Masindi, Rakai and Kasese (Gibson et al. 1997; Carey et al. 1998; 1999) and in the districts of Kabarole (Fort Portal), Tororo and Iganga (Gibson et al. 1998) in Uganda. SPMMV, SPCFV, C-6 potyvirus, and sweetpotato latent virus (SwPLV) are less common viruses in Uganda occurring sporadically and at much lower frequencies than SPFMV and SPCSV (Gibson et al. 1997; Carey et al. 1998). Although Wambugu (1991) reported cucumber

mosaic virus (CMV), sweetpotato caulimo-like virus (SPCaLV) and sweetpotato latent virus (SPLV) as common in Uganda, these viruses were not detected in later surveys (Carey et al. 1997, 1998; Gibson et al. 1997; 1998). Also, complexes (of up to six viruses) of SPFMV, SPLV, SPMMV, SPCSV, CMV and SPCV reported to occur at high frequencies in all sweetpotato crops in Namulonge and Mukono by Wambugu (1990) and Wambugu et al. (1991), were not detected in later surveys.

Sweetpotato virus disease (SPVD)

Surveys (Aritua et al. 1998b; Legg et al. 1998, Carey et al. 1998; Alicai et al. 1999a; Carey et al 1999; Gibson et al. 2000), yield loss assessment studies (Mukiibi 1977; Gibson et al. 1997; Aritua et al. 1998a, b) and transmission studies of SPCSV and SPFMV (Gibson et al. 1998, Karyeija et al. 1998, 2000) have shown that dual infection by SPCSV and SPFMV is the main cause of SPVD in Uganda. SPVD is the most destructive disease of sweetpotato in Africa (Geddes, 1990).

Symptoms of SPVD vary with plant genotype, but include stunted plants with small often distorted, narrow (strap-like) and crinkled leaves with a chlorotic mosaic and/or vein clearing; affected plants, generally appear pale (Gibson et al. 1998). In Uganda, root yield losses due to SPVD were estimated at 66% in severely diseased plants of cultivar Bitambi (Aldrich, 1963), 57% in cultivar, Kyebandula (but some control plants became diseased (Mukiibi, 1977) and 98% in cultivar, Tanzania, compared to negative control plants (Gibson et al. 1998). Mwanga et al. (2001, Chapter 2 in this dissertation) reported mean yield reduction due to SPVD of 60-98% in 36 sweetpotato families (360 genotypes). In Nigeria, root yield losses due to SPVD were 60% in cultivar, TIB 4, and 76-78% in TIS 1499 (Hahn, 1979), and in Cameroon in different yield trials root yield losses varied between 0-90% in

eight clones (Ngeve and Bouwkamp, 1991). Mean frequencies of SPVD-affected plants in crops in different parts of Uganda, varied between 0 and 30% (Karyeija et al. 1998), but a larger range was reported in Kenya (Sheffield, 1953; Wambugu, 1991). SPVD is most severe in western-central Uganda, especially in the districts of Kasese, Kabale, Kabarole, Masindi, Mpigi, Rakai, Luwero, Mukono, and Iganga in eastern Uganda (Gibson et al. 1996; 1998).

Average incidences (proportion of diseased plants in 100 sample plants in a diagonal line across each crop) of SPVD in farmer's fields are low (0-20%) in parts of Northern and Eastern Uganda (Aritua 1998a; Legg et al. 1998) but are often high (50-95%) in some fields in the districts of Busia in Eastern Uganda, and in Mpigi and Rukungiri in Southern Uganda (Aritua et al. 1998a; Gibson et al. 2000), and SPVD incidence and severity is closely associated with prevalence of whiteflies (Aritua et al. 1998a, b; Legg et al.1998).

Although there are varietal differences in resistance to SPVD (Carey et al. 1998, 1999; Aritua et al. 1998b; Mwanga et al. 2001), extreme resistance to the components of SPVD has been demonstrated only in the sweetpotato wild relatives, *I. wightii* to SPCSV, *I. hildebrandtii* to SPFMV, and both *I. cairica* and *I. involucra* to SPCSV and SPFMV in a dual infection (Karyeija et al. 2000).

Di Feo et al. (2000) reported another sweetpotato virus diesease complex, sweetpotato chlorotic dwarf disease in Argentina. The disease complex, termed chlorotic dwarf (CD) causes to up to 80% yield reduction. CD is due to the infection and synergism of three viruses, SPFMV, SPCSV, and sweetpotato mild speckling potyvirus (SPMSV). Wambugu (1990) and Wambugu et al. (1991) reported complexes of SPCSV, SPFMV, SPLV, SPMMV, and CMV in Uganda, but only SPVD (SPCSV and SPFMV) has been confirmed to occur in Uganda (Gibson et al. 1998; Karyeija et al. 1998; Legg et al. 1998).

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Table 1. Sweetpotato viruses and their distribution

Virus	Genus	Shape and size	Vector	Distribution	Reference
Sweetpotato feathery mottle virus (SPFMV)	Potyvirus	Flexuous 850 nm	Aphid	Worldwide	Moyer and Kennedy 1978; Brunt et al. 1996
Sweetpotato vein mosaic virus (SPVMV)	Potyvirus?	Flexuous 760 nm	Aphid	Argentina	Nome 1973; Brunt et al. 1996
Sweetpotato virus II (SPV-II)	Potyvirus	Flexuous 750 nm	Aphid	Taiwan	Moyer et al. 1989
Sweetpotato latent virus (SwPLV)	Potyvirus?	Flexuous 750 nm	Aphid?	Uganda, Kenya, Egypt, Asia (China, Taiwan, India, Philippines), Peru	Clark and Moyer 1988; Green et al. 1988; Brunt et al. 1996
Sweetpotato mild speckling virus (SPMSV)	Potyvirus	Flexuous 800 nm	Aphid	Argentina, Peru, Indonesia, Philippines	Brunt et al. 1996; Alvarez et al. 1997
Sweetpotato leaf speckling virus (SPLSV)	Luteovirus	Isometric 30 nm	Aphid	Peru, Cuba	Nakano et al.1992
Sweetpotato mild mottle virus (SPMMV)	Ipomovirus	Flexuous 950 nm	Whitefly	Africa, Indonesia, Philippines, India, Egypt, Peru	Hollings et al. 1976; Atkey and Brunt 1987
Sweetpotato yellow dwarf virus (SPYDV)	Ipomovirus?	Flexuous 750 nm	Whitefly	Taiwan	Chung et al. 1986

Table 1. Continued

Virus	Genus	Shape and size	Vector	Distribution	Reference
Sweetpotato leaf curl virus (SPLCV)	Badnavirus?	Bacilliform 130x30 nm	Whitefly	Taiwan, Japan, Egypt	Liao et al. 1979; Brunt et al. 1996
Sweetpotato leaf curl virus (SPLCV)	Geminivirus	Geminate	Whitefly	USA	Lotrakul et al. 1998
Ipomoea crinkle leaf curl virus (ICLCV)	Geminivirus?	Geminate	Whitefly	USA?	Cohen et al. 1997
Sweetpotato chlorotic stunt virus (SPCSV) (or SPSVV)	Crinivirus	Flexuous 850-950 nm	Whitefly	Africa (Nigeria, Uganda, Kenya, Zaire), Asia (Taiwan, China, Indonesia, Philippines), America (USA, Brazil, Argentina, Peru), Israel	Hoyer et al. 1996; Wisler et al. 1998
Sweet potato chlorotic stunt virus (SPCSV?)	Potyvirus	Flexuous 850-900 nm	Unknown	Caribbean Region, Kenya, Puerto Rico, Zimbabwe	Brown et al. 1988; Brunt and Brown 1988
Sweetpotato chlorotic flecks virus (SPCFV)	Potyvirus?	Flexuous 750-800 nm	Unknown	Peru, Japan, Brazil, China, Cuba, Panama, Colombia, Bolivia, Indonesia, Philippines, Uganda, Egypt, India	CIP 1992; Gibson et al. 1997; Carey et al. 1999
Sweetpotao virus G (SPVG)	Potyvirus	Flexuous	Unknown	China	Colinet et al. 1994
Sweetpotato caulimo-like virus (SPCaLV)	Caulimovirus	Isometric 50 nm	Unknown	Puerto Rico, Madeira, Salomon Islands, Australia, Papua New Guinea	Atkey and Brunt 1987
Sweetpotato ring spot virus (SPRSV)	Nepovirus?	Isometric 25 nm	Unknown	Papua New Guinea	Brown et al. 1988

Table 1. Continued

Virus	Genus	Shape and size	Vector	Distribution	Reference
Sweetpotato phytoreo	?	Isometric 70 nm	Unknown	Asia	Brown et al. 1988
Sweetpotato Ilar-like virus (Ilar-like) (Reo-like)	?	Isometric 30 nm	Unknown	Guatemala	Moyer and Foster 1986
C-6	Potyvirus?	Flexuous 750-800 nm	Unknown	Uganda, Indonesia, Philippines, Peru	Gibson et al. 1998 Di Feo et al. 2000
Cucumber mosaic virus (CMV)	Cucumovirus	Isometric 29 nm	Aphid	Widespread	Cohen et al. 1988
Tobacco mosaic virus (TMV)	Tobamovirus	Rigid rods 300 x 30 nm	Contact	Widespread	Brunt et al. 1996

CHAPTER 2

Diallel Analysis of Sweetpotatoes for Resistance to Sweetpotato Virus Disease

(In the format appropriate for submission to Euphytica)

For: Euphytica

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Diallel analysis of sweetpotatoes for resistance to sweetpotato virus disease

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Abstract

Sweetpotato virus disease (SPVD) is due to the dual infection and synergistic interaction of sweetpotato feathery mottle potyvirus (SPFMV) and sweetpotato chlorotic stunt crinivirus (SPCSV), and causes up to 98% yield loss in sweetpotato in East Africa. This study was conducted to determine the inheritance of resistance to SPVD in sweetpotato and to estimate the nature of genetic variance. Ten parental clones varying in resistance to SPVD were crossed in a half diallel mating design to generate 45 full-sib families. The families were graft-inoculated with SPVD and evaluated for resistance to SPVD in a randomized complete block design at two sites in Namulonge, Uganda during 1998-2000. In serological assays for SPFMV and SPCSV, resistance to symptom development and recovery from initial systemic SPVD symptoms, characterised resistant genotypes. Genetic component analysis showed significant effects for both general combining ability (GCA) and specific combining ability (SCA) for resistance to SPVD. GCA to SCA variance components ratios were large (0.51-0.87), hence GCA effects were more important than SCA effects. Resistant parents exhibited high GCA indicating that additive gene effects were predominant in the inheritance of resistance to SPVD and recovery. Narrow-sense heritability (31-41%) and broad-sense heritability (73-98%) were moderate to high, indicating that rapid genetic gains for SPVD resistance could be accomplished by mass selection breeding techniques. Two genotypes, New Kawogo and Sowola, had high negative GCA effects and had several families in specific crosses, which exhibited rapid recovery from SPVD, and are promising parents for enhancement of SPVD resistance and recovery.

Key words

general combining ability, heritability, *Ipomoea batatas*, sweetpotato chlorotic stunt virus,

sweetpotato feathery mottle virus

Introduction

Sweetpotato virus disease (SPVD) is a serious problem in East Africa (Hahn 1979; Gibson et al. 1998) and occurs worldwide (Schaefers & Terry 1976; Moyer & Kennedy 1978; Hahn et al. 1981; Wisler et al. 1998). The disease is due to the dual infection and synergistic interaction of sweetpotato feathery mottle potyvirus (SPFMV) and sweetpotato chlorotic stunt crinivirus (SPCSV) (Schaefers & Terry 1976; Karyeija et al. 2000). SPVD is characterized by a range of symptoms including, chlorotic mosaic, vein clearing, leaf distortion, strapping and crinkling of leaves, and a general stunting of plant growth. The impact of the disease is to reduce storage root yields by 56-98% (Hahn 1979; Ngeve & Bouwkamp 1991; Gibson et al. 1998; Karyeija et al. 1998b).

SPFMV is transmitted non-persistently by aphids (*Myzus persicae*, *Aphis gossypii* and *A. cracivora*) (Stubbs and McLean 1958), while SPCSV is transmitted semi-persistently by whitefly (*Bemesia tabaci*) (Schaefers & Terry 1976; Cohen et al. 1992). Emphasis on developing SPVD resistance has focused on the development of resistance to SPFMV, the most cosmopolitan of all known sweetpotato viruses. However, it is difficult to breed for resistance to SPVD by developing genotypes resistant to SPFMV because of the existence of different strains of SPFMV (Cali & Moyer 1981; Kreuze et al. 2000) and different serotypes of SPCSV (Hoyer et al. 1996; Wisler et al. 1998; Alicai et al. 1999) in different regions of the world. For example, sweetpotato genotypes from Peru and Nigeria with high levels of resistance to SPFMV were susceptible to SPVD in Uganda (Mwanga et al. 1991; Gibson et al. 1998; Karyeija et al. 1998b). Likewise, Karyeija et al. (2000) evaluated

resistance to SPFMV in East African cultivars and found that resistance to SPFMV was overcome when the same SPFMV-infected plants were co-infected with SPCSV, which enhances SPFMV titers and increased susceptibility for SPVD (Aritua et al. 1998; Gibson et al. 1998; Karyeija et al. 2000).

Although there has been much emphasis on developing resistance to SPFMV (Karyeija et al. 1998a, 2000), there have only been a few studies on the inheritance of resistance to SPVD. Hahn et al. (1981) estimated broad-sense heritability of resistance to SPVD to be 0.48-0.98, while Ngeve and Bouwkamp (1991) observed that storage root yields of two of eight tolerant sweetpotato genotypes evaluated over two years were not significantly reduced by SPVD. To date, extreme resistance to the components of SPVD has been demonstrated only in the sweetpotato wild relatives, *I. wightii* to SPCSV; *I. hildebrandtii* to SPFMV; and both *I. cairica* and *I. involucra* to SPCSV and SPFMV in a dual infection (Karyeija et al. 1998a).

Studies of inheritance of resistance to SPVD, or any trait in sweetpotato, is complicated by the fact that sweetpotato is a highly heterozygous hexaploid (x = 15, 2n = 90) with complex segregation ratios. Its complex genome makes research in cytogenetics difficult and genetic studies complicated. However, it is possible to partition genetical variation into components due to general combining ability (GCA) and specific combining ability (SCA) (Collins 1977; Bradshaw et al. 2000; Mihovilovich et al. 2000) and to thus obtain a relative estimate of the inheritance of a given trait. This study used sweetpotato diallel progenies to determine the inheritance of resistance of SPVD, estimate the components of genetic variance (GCA and SCA), and to determine the type of gene action controlling resistance to SPVD.

Materials and methods

Ten parental (p) sweetpotato clones with varying SPVD field resistance and other attributes (Table 1) were crossed in a modified diallel, disregarding reciprocals, using the model II, method 4 mating design of Griffing (1956) in 1996/97 at the Namulonge Agricultural and Animal Production Research Institute (NAARI) in Uganda. This design yielded a total of 45 families [p(p-1)/2]. The clones were previously tested for resistance to SPVD and selection was based on field resistance under natural SPVD inoculum pressure (Mwanga et al. 2001). Three experiments were conducted to evaluate the families for resistance to SPVD. The experiments consisted of a screenhouse, field and recovery tests. For each experiment, 10-15 plants per genotype of the 45 families (440 genotypes in total) were screened.

Screenhouse SPVD resistance evaluation

Ten to 15 plants per genotype of the 45 families were planted in sterilized soil in perforated 2-litre plastic buckets in a large screenhouse. The progeny and the crosses were arranged in a randomized complete block (RCB) design. Foliar fertilizer, Rapid Grow (9-18-9), and Rogor (Dimethoate) were applied according to the manufacture's specifications. The plants were watered every morning and evening. Approximately 1.5 months after planting, the plants were graft-inoculated with SPVD. The source of SPVD inoculum was maintained in the cultivar Tanzania (Gibson et al. 1998), and the infected plants were maintained and multiplied in a separate screenhouse. After inoculation, symptoms, percentage of infected plants, and SPVD severity were recorded for each genotype using a subjective 5 point

severity rating scale where: 1 = no symptoms; 2 = mild symptoms; 3 = moderate symptoms; 4 = severe symptoms; and 5 = very severe symptoms.

The screenhouse experiment was conducted between August and December 1998 and was repeated using the same clones for a second season from October 1999 to February 2000. To confirm the presence of SPFMV and SPCSV in each genotype, a sample of several leaves representing a range of SPVD symptoms exhibited by each genotype was collected from each pot. Approximately 0.2 g samples were used to assay for SPFMV using a double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) and SPCSV by triple antibody sandwich (TAS-ELISA) in microplates (Gibson et al. 1998). Absorbances were recorded after an overnight incubation period at 405 nm with a microplate reader (Labsystems Multiskan S, Middlesex, UK). Absorbance values twice the values of healthy control samples were regarded as virus infected. A total of 370 samples were assayed. Genotypes without leaves due to the severity of SPVD were excluded from the test.

Field SPVD resistance evaluation

Two sites, one situated upland (Borehole) and the other located in a low land area (Kisenyi) were used for the field studies at NAARI. Ten cuttings per genotype (440 genotypes) from SPVD graft-infected plants of the 45 families studied in the screenhouse were planted in two sites, February 1999. The genotypes were randomized within each family and the families were arranged in an RCB with 3 replications. Plants were planted in single rows on ridges spaced 1 m apart and 30 cm within row. Each plot consisted of 2 rows per genotype (one SPVD-infected and one healthy control). SPVD was scored 3 times on a whole plot

basis, at monthly intervals after inoculation, using the 1-5 rating scale described above.

SPVD recovery experiment

Because genotypes with the same SPVD severity score may differ in their ability to recover from SPVD, we conducted an experiment to determine the importance of recovery in SPVD resistance. In this experiment, 10 to 15 cuttings of all the genotypes in the 45 families with obvious SPVD symptoms were planted in the field in single rows on ridges spaced 1 m apart and 30 cm within a row in November 1999 (2 months postinoculation). The genotypes were randomized within each family and the families were arranged in an RCBD with 3 replications. Rapid Grow (9-18-9) and Dimethoate were applied according to the manufacture's recommendations. The plants were watered to avoid soil moisture stress.

While symptom expression at a point in time is one measure of sensitivity to SPVD, some cultivars are capable of recovering from the disease. To more precisely describe the ability of genotypes to recover from SPVD, we developed an index for SPVD recovery that incorporated separate ratings (1-5) for plant pigmentation, growth, and size. The rating scales were as follows: pigmentation, (1 = normal pigmentation, 5 = extreme discoloration); growth, (1= normal growth of stems, internodes and leaves, 5 = no growth, dead plant); and size (1 = normal size of stems, internodes and leaves, 5 = diameter and length of internodes of stem and leaf width and length greatly reduced, stunted). The plants were scored on a per plot basis for SPVD and recovery at 2 and 5 months after planting. To calculate recovery two models were evaluated. The first calculated recovery from SPVD as $SPVD_i$ - $SPVD_f$ (initial and final scores of SPVD, respectively). The second was computed based on the mean recovery score as $(G_i + P_i + S_i)/3 - (G_f + P_f + S_f)/3$, where i and f are the

initial and final times, respectively, of scores for growth (G), pigment (P) and size (S). Analyses using the two models gave similar results but results of the second model were less variable compared to the first model. GCA and SCA analysis for recovery using the second model gave higher R square values (90 vs 67), lower cv (20 vs 60) and detected more significant GCA and SCA values than analysis using the first model. The data of the second model for recovery was therefore used to do the analysis presented in this study.

Statistical analysis

The progeny means of full-sib families for each of three replications in the resistance evaluation and recovery experiments were used in the diallel analyses. GCA and SCA variance components were computed according to the random-effects model II, method 4 (only F_1s; parents and reciprocals excluded) described by Griffing (1956) using the DIALLEL-SAS program developed by Zhang and Kang (1997).

Results

Screenhouse and field resistance, and recovery evaluation

The ten parental sweetpotato clones were classified as resistant, moderately resistant or susceptible (Table 1) on the basis of field evaluations in Uganda under natural SPVD inoculum pressure. Graft-inoculation resulted in severe SPVD in both screenhouse and field experiments in all diallel progenies of the 10 parental sweetpotato clones. However, there were significant differences in resistance to SPVD among the families (Table 2). Parental

SPVD scores ranged between 2.3-5.0 in the screenhouse and field with SPVD mean scores 3.5-4.2. Progenies of the susceptible parents, BMA and KAN and the moderately resistant parents, TAN, KAZ, TOR, and BWA had higher mean SPVD scores (3.8-4.0), with a range of 3.5-4.4 in the screenhouse and field compared to the resistant parent, NKA which had a mean SPVD score of 3.5 and a range of 2.3-4.3. The resistant parent, WAG, the moderately resistant parent, SOW and the susceptible parent CTA, with a mean SPVD score of 3.7 each in the screenhouse, were all more susceptible to SPVD in the field.

Full-sib families exhibited significant variation for recovery from SPVD (Table 2). KAN and BMA each had the highest mean SPVD recovery scores (0.5), with a range of 0.2-0.9 followed by TAN, BWA, SOW and NKA with a recovery mean score of 0.4 and a range of 0.1-0.7. CTA had the lowest recovery mean score, 0.2 (range, 0.1-0.4). WAG, and KAZ had intermediate scores, 0.3 (range, 0.2-0.5).

The results of the DAS and TAS assays of the diallel progenies are in Table 3. Absorbance (405 nm) values (not presented) for SPFMV and/or SPCSV were high for all the families. Absorbances of leaf samples of screenhouse grown plants were higher than those of field raised plants (data not shown). In each family nearly all clones tested were positive for SPFMV and SPCSV, and none of the families had low SPCSV and SPFMV virus titers. In most families fewer clones tested positive for SPCSV than for SPFMV. Genotypes which tested negative for SPCSV and/or SPFMV were not necessarily from progenies of families with low SPVD mean scores. NKA was a parent for greater than half of the genotypes which tested negative for both SPCSV and SPFMV; but the cross, BKM x CTA, had the highest number of genotypes testing negative for both SPCSV and SPFMV. Still, all genotypes that tested negative could be infected by repeated graft inoculation or tested positive when grafted on *Ipomoea setosa*, indicating that those genotypes were not

immune.

Combining ability analysis of diallel progenies for resistance to SPVD in the screenhouse and field

The contribution of each parental clone to the full-sib family progenies was evaluated by calculating and comparing GCA effects. Analyses of variance showed significant differences among diallel progenies in the screenhouse and field resistance evaluations, and in the recovery experiment (Table 4). Differences among the families for resistance to SPVD were due to both GCA and SCA effects, although SCA effects were not significant in the screenhouse but were significant in the field (Tables 4 and 5).

The total genetic variance ($2V_g^2 + V_s^2$) among single-cross progeny is double the GCA component of variance ($2V_g^2$) plus the SCA component of variance (V_s^2). The relative importance of GCA and SCA in resistance to SPVD was assessed by estimating the two variance components and expressing them in the ratio, $2V_g^2/(2V_g^2 + V_s^2)$, according to Baker (1978). The GCA:SCA variance ratios were 0.87 in the screenhouse resistance evaluation, 0.59 in the field, and 0.51 in the recovery experiments suggesting that GCA effects were more important than SCA effects in predicting progeny performance for SPVD resistance. The importance of GCA was also evident based on the significant positive correlations calculated between the full-sib family means for each parent and GCA effects ($r = 0.98$, for screenhouse, $r = 0.99$ for field, and $r = 0.99$ for recovery experiment). Additive genetic variance ($V_a^2 = 0.154$) accounted for 77% of total genetic variance in the screenhouse study, while in the field and recovery experiments it accounted for 69% and 55%, respectively. The dominance component of genetic variance ($V_d^2 = 0.045$) was high

although non-significant in the screenhouse resistance evaluation, but significant in the field and recovery experiments.

The GCA and SCA values for the 10 parents are presented in Table 5. Positive effects indicate a contribution to greater susceptibility to SPVD, whereas negative effects indicate a contribution to greater resistance to SPVD. The susceptible parents BMA (GCA = 0.11 and 0.08 in the screenhouse and field, respectively), and KAN (GCA = 0.18 and 0.19 in the screenhouse and field, respectively) had significant GCA effects and contributed toward susceptibility in the screenhouse and field. Parents NKA (GCA = -0.15, -0.43) and SOW (GCA = -0.14, -0.07) had significant negative effects in the screenhouse and field and contributed toward resistance. Parents which contributed towards resistance, but were not consistent in performance in the screenhouse and field were KAZ (0.03, -0.08), BWA (<0.01, -0.03) and CTA (-0.26, 0.03). Surprisingly, the resistant parent WAG (-0.04, 0.29) did not contribute toward resistance in the screenhouse, and contributed to susceptibility in the field.

Based on GCA values, KAZ, NKA, BWA and SOW were the best combiners for SPVD resistance. However, some combinations of resistant x moderately resistant (e. g. NKA x TAN (SCA = -0.23), or moderately resistant x moderately resistant (e. g. SOW x KAZ (SCA = - 0.11), or moderately resistant x susceptible (e.g. BMA x BWA (SCA = -0.43), or susceptible x susceptible, (e.g. BMA x CTA (SCA = -0.25) produced progenies with significant desirable SCA. Contrary to our prediction, the cross NKA x WAG, which was a resistant x resistant cross, did not produce progenies with desirable SCA.

Combining ability analysis for recovery from SPVD

As observed in the screenhouse and field analysis, differences among the families for recovery were due to GCA and SCA effects (Table 4, 5). In the recovery analysis, positive GCA effects indicate a contribution to recovery, whereas negative GCA effects indicate a contribution toward no or low recovery. Parents TAN (GCA = 0.03), BMA (GCA = 0.11) and KAN (GCA = 0.19) were the best GCA parents for recovery. For all possible cross combinations of the best GCA parents for recovery, only TAN x BMA (SCA = 0.14) had a desirable SCA effect. In contrast the three highest SCA combinations were KAZ x KAN (SCA = 0.34), BMA x NKA (SCA = 0.23), and BWA x SOW (SCA = 0.2). Other genotypes with positive contributions to SPVD recovery are in Table 5. All other desirable positive SCA came from a combination with other parents.

Discussion

Graft-inoculation resulted in severe SPVD in both screenhouse and field experiments in all diallel progenies of the 10 parental sweetpotato clones but there were significant differences in resistance to SPVD among the families (Table 2). Parental SPVD scores ranged between 2.3-5.0 in the screenhouse and field with SPVD mean scores 3.5-4.2. These results indicate that selection for genotypes with moderate to high SPVD resistance would be practical. There were significant differences in response to SPVD among the diallel progenies in the screenhouse and field, and the relative ranking of the reaction of progeny families to SPVD changed when evaluated in the field and screenhouse (Table 2 and 4). For example, the lowest SPVD scores were recorded for NKA (3.6), WAG, SOW, and CTA (3.7 each) in the

screenhouse, while NKA (3.5), SOW and KAZ (3.8) had the lowest scores in the field. The consistent performance of progenies of the resistant (NKA) and moderately resistant (SOW) and susceptible parents (BMA, KAN) in the screenhouse and field, and the shift in the relative ranking of intermediate progenies contributed to the source of the family x location interaction (Table 4).

The screenhouse SPVD resistance evaluations were conducted over two seasons, whereas the field evaluations were conducted at two sites (Borehole and Kisenyi). This difference undoubtedly contributed to the interaction between the performance of diallel progenies in the field and screenhouse and resulted in a low correlation ($r = 0.312$) between the screenhouse and field SPVD scores. These results suggest that a combination of screenhouse and field screening is desirable to identify stable genotypes with SPVD resistance under different environments.

Absorbance (405 nm) values were high for all families for SPFMV and/or SPCSV (Table 3). These results suggest that virus titer increases in most genotypes when the two viruses co-infect the sweetpotato plant. These results concur with other reports of increased SPFMV titer due to synergism between SPFMV and SPCSV (Schaefers & Terry 1976; Gibson et al. 1998, Karyeija et al. 2000).

Most of the genotypes in the five families (WAG x BMA, TAN x SOW, NKA x TOR, NKA x BWA, and NKA x SOW) which had a single genotype negative for both viruses were resistant to SPFMV, showing mild or no symptoms when infected by SPFMV. However, coinfection with SPCSV and SPFMV produced severe symptoms in the majority of the genotypes. This suggests that resistance to SPFMV is either reduced, or not expressed in the presence of SPCSV and the virus titer does not remain low for the majority of genotypes, or that these two viruses interact synergistically to overcome resistance. Only

8 (2.2%) out of 370 genotypes tested negative for both SPCSV and SPFMV (Table 3). These genotypes were either: 1) difficult to infect; 2) able to suppress the rapid titer rise which occurs in other genotypes; or 3) have a mechanism of lowering titer during progress of the SPVD. The fact that some genotypes have the ability to recover from SPVD (Table 2) suggests that the third mechanism proposed may be important in resistance to SPVD. More genotypes tested positive for SPFMV (95.4%) than for SPCSV (80.3%). The reasons for this are unclear, but Winter et al. (1992), Cohen et al. (1992) and Karyeija et al. (2000) have suggested that during co-infection SPCSV titer does not increase as much as much as SPFMV, or it remains constant or even decreases slightly (Gibson et al. 1998).

Extreme resistance to SPFMV (Nakashima 1993) has been reported to break down when co-infection with SPCSV occurs (Winter et al. 1992; Gibson et al. 1998; Karyeija et al. 1998a). This phenomenon has also been reported by Cohen and Loebestein (1991) where resistance of sweetpotato to cucumber mosaic virus (CMV, genus *Cucucumovirus*) is also overcome when co-infection with SPCSV occurs. Karyeija et al. (2000) suggested that SPCSV may interfere with the systemic signaling of post-transcriptional gene silencing (PTGS) or supply a factor that helps the partially functional suppressors of PTGS produced by SPFMV or CMV. This system may result in a breakdown of resistance to SPFMV. Regardless of the mechanistic interactions occurring between SPFMV and SPCSV, our results indicate that it is desirable to incorporate multiple virus resistance into improved sweetpotato to achieve durable resistance against SPVD.

Our results suggest that ELISA tests are not precise enough to serve as a reliable measure to identify sweetpotato genotypes resistant to SPVD. This is consistent with a previous study (Abad & Moyer 1992). Although SPFMV was not detected by ELISA in all genotypes, infection was demonstrated following repeated graft inoculation by graft assays

on *I. setosa*. Resistance to SPCSV and SPFMV, the components of SPVD, breaks down in a dual infection resulting in confounding of resistance to SPCSV and SPFMV as the titer of one or both components increases. However, ELISA tests are useful for confirming the presence of the two viruses after visual SPVD symptoms have been recorded. In routine breeding for SPVD resistance, initial screenhouse screening for resistance to the disease using ELISA could be done to eliminate highly susceptible genotypes, as they give high absorbance values for SPCSV and/or SPFMV.

Recovery is an important component of resistance to SPVD. Recovery in some genotypes progressed from near plant death (SPVD score = 5) to symptomless plant (SPVD score = 1). In some genotypes the change was difficult to detect, while in others the disease progressed over time resulting in death. Some severely infected genotypes had the ability to localize the distribution of SPVD. In those, whole branches or individual shoots developed that were asymptomatic and free of detectable virus when assayed by ELISA or grafted on *Ipomoea setosa*. Recovery or reversion has also been observed in African cassava mosaic virus (ACMV)-resistant cassava varieties (Jennings 1957; Fargette et al. 1994). In the present study, resistant sweetpotato progenies of the parent SOW and the susceptible progenies of parental clones KAN and BMA exhibited rapid recovery. The most plausible explanation for this is that SPVD, like ACMV does not become fully systemic (Aritua et al. 1998; Gibson et al. 1998) and a proportion of uninfected cuttings can be obtained from previously infected plants. Various studies have shown that SPFMV exhibits uneven distribution in sweetpotato (Daines & Martin, 1964; Moyer et al. 1980; Cadena-Hinojosa & Campbell 1981; Frison & Ng 1981; Esbenshade & Moyer 982; Green et al. 1988; Abad & Moyer 1992). While low virus titer and irregular distribution are problematic in virus detection, sweetpotato genotypes with such levels might be expected to show more rapid

recovery compared to plants with fully systemic and high virus titers. The reversion or recovery phenomenon may be of great epidemiological significance in decreasing sustained losses, but it has received little attention in sweetpotato research. Indeed the ability of some sweetpotato cultivars to recover from SPVD may help to explain why some seemingly highly susceptible genotypes stay for long periods in cultivation. They may have rapid recovery enabling them to give reasonable yields, thus they are not dropped by subsistence farmers. 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 African cultivars.

The inconsistent GCA and SCA effects exhibited by progeny of some parents in the screenhouse and field tests contributed to the significant GCA and SCA x site interactions (Table 4). Parent WAG, rated as resistant, and parents TAN, KAZ, and TOR, considered moderately resistant under natural SPVD inoculum pressure, contributed susceptibility to their progenies. The differences in SPVD reaction may be attributed to different factors including: 1) aphid and whitefly inoculation vs graft-infection; 2) simultaneous plant inoculation of SPFMV and SPCSV vs inoculation of either virus singly depending on which one is inoculated first; or 3) differential reactions of genes in diallel progenies to SPVD under screenhouse and field environments.

Based on GCA values, KAZ, NKA, BWA and SOW were the best combiners for SPVD resistance. Crosses between these parents and moderately resistant, or resistant x susceptible, or susceptible x susceptible parents, all produced progenies with significant desirable SCA. Surprisingly, the cross NKA x WAG, which was a resistant x resistant cross, did not produce progenies with desirable SCA. Considering all the crosses, NKA x SOW, would be most preferable because both the parents involved were good general

combiners for SPVD resistance and their progeny performance was stable in the screenhouse and the field.

Parents TAN, BMA and KAN had the best GCA for recovery from SPVD. Among all possible cross combinations of the best GCA combiners for recovery, only TAN x BMA (SCA = 0.14) had a desirable SCA effect. All other desirable positive SCA came from a combination with other parents (Table 5). Based on positive SCA effects, crosses in which NKA was one of the parents should yield the best families for recovery type of resistance to SPVD and reduced SPVD severity because NKA is also a good combiner for SPVD resistance.

Crosses between the best GCA combiners (TAN, BMA, KAN) did not result in progeny with highest SCA for most of the combinations (e. g. TAN x KAN (SCA = 0.03), BMA x KAN (SCA = -0.02). This was not expected and suggests that crosses between parents with high rates of recovery from SPVD may not result in increase in the expression of recovery in their progenies. The reason for this is unclear, but sweetpotato is a highly heterozygous hexaploid, and it is possible that on crossing parents selected for desirable traits more favorable combinations of genes are broken down than reassembled in the offspring. In crosses between poor combiners or between good and poor combiners which produced desirable specific effects, it is possible that dominance gene effects resulting from non-allelic interactions from the poor combining parent increase the expression of recovery in the progenies. This reaction suggests that parents exhibiting rapid recovery mostly carry additive genes, whereas the non-recovering parents have non-additive genes.

Narrow-sense heritability (h^2) was moderate (31 and 41% in the recovery and field experiments, respectively) to high (60%) in the screenhouse, and broad-sense (H) heritability was high (73-98%) in all experiments. Hahn et al. (1981) reported similar

estimates (48-98%) for H for SPVD based on variance components similar to the present study. These studies suggest that rapid genetic gains should be possible with mass selection based on the phenotype of the parent. In addition, mass selection could be combined with other techniques designed to utilize the non-additive variance component. Intermating compatible superior genotypes from the full-sib families would lead to further accumulation of additive genes for resistance to SPVD and recovery.

Although additive gene effects were predominant in the inheritance of resistance to SPVD and recovery, the dominance component of genetic variance was also significant. If epistatic effects are present, what is expressed as the dominant variance component consists of the epistatic effects plus dominance variance. There is a need to determine the magnitude of epistatic effects and their effect on heritabilities of SPVD resistance and other traits for exploitation in a sweetpotato breeding program. The conditions, which favor recovery from SPVD in sweetpotato, are not well known. There is a great need to identify the major conditions which favor recovery from SPVD in East Africa. The conditions can be optimized for screening rapid recovering sweetpotato genotypes to be crossed with superior genotypes with desirable traits.

The studies reported here indicate that resistance to SPVD can be transferred into improved cultivars by utilizing parents that possess high levels of resistance to SPVD and the ability to recover from SPVD after infection. Future work on resistance to SPVD should include: 1) the introgression of genes for extreme resistance to SPCSV and SPFMV from wild relatives of sweetpotato (*I. cairica* and *I. involucra*) into improved sweetpotato cultivars; 2) refinement of methodologies to rapidly identify genotypes with fast recovery from SPVD, and the definition and optimization of the conditions for screening fast recovering genotypes; 3) determination of epistatic effects and their significance in SPVD

resistance and recovery and whether these effects can be exploited for rapid gains in breeding for resistance to SPVD; and 4) determination of the mechanisms of resistance to SPVD.

Acknowledgements

We thank Joe Vetten (Federal Biological Research Center for Agriculture, Braunschweig, Germany) for IgG, SPCSV-KY; Lucho Salazar (CIP, Lima, Peru) for IgG and MAb for SPFMV; Jonathan Schultheis, George Wilson, Randy Gardner (NCSU) for critical reviews and suggestions; Richard Gibson (University of Greenwich, UK), Ted Carey (Kansas State University, USA), Janice Bohac (US Vegetable Lab, South Carolina), and Wanda Collins (CIP, Lima) for suggestions in designing the experiments; Jim Cervantes (NCSU) and Dapeng Zhang (CIP, Lima) for ideas on statistical analysis; and Agnes Alajo, Benjamin Kigozi, V. Aritua and Gard Turyamureeba for assistance in running the experiments at Namulonge in Uganda. This research was part of the Collaborative Crop Research Program and was jointly funded by the McKnight Foundation, Minnesota, USA, and the National Agricultural Research Organization (NARO), Uganda.

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Table 1. Attributes of sweetpotato parental genotypes used in the diallel cross for determining inheritance of resistance to sweetpotato virus disease

Parent	Attribute							
	Resistance to[b]				Dry matter content (%)	Maturity[c]	Origin of parent	Year released/ germplasm (GM)
	SPVD	SPCSV	SPFMV	*Alternaria*				
Wagabolige (WAG)	R	R	R	S	33	L	Uganda (U)(Eastern)	1995
Tanzania (TAN)	MR	MR	R	R	32	E	U (Eastern)	1995
Bikila Maliya (BMA)	S	S	R	MR	30	E	U (Western)	GM
Kanziga 2 (KAZ)	MR	MR	R	MR	30	L	U (Eastern)	GM
New Kawogo (NKA)	R	R	R	S	32	L	U (Central)	1995
Tororo 3 (TOR)	MR	MR	R	MR	30	L	U (Eastern)	1995
Kanziga 1(KAN)	S	S	R	MR	29	E	U (Eastern)	GM
Bwanjule (BWA)	MR	MR	R	MR	30	L	U (Western)	1995
Sowola (SOW)	MR	MR	R	R	34	E	U (bred clone)	1995
Camote Tallo (CTA)[a]	S	-	HR	MR	31	E	CIP (Peru)	GM

[a] CTA Camote Tallo is International Potato Center (CIP) clone No. 420026
[b] SPVD = sweetpotato virus disease, SPCSV = sweetptato chlorotic stunt virus, SPFMV = sweetpotato feathery mottle virus, R = resistant, S = susceptible, MR = moderately resistant, HR = highly resistant. Classifications of the relative resistance were based on field evaluation in Uganda
[c] E = early (3-4 months), L = late (>4 months)

Table 2. Summary of diallel progeny means, standard deviations (SD), and minimum (min), and maximum (max) values observed for the 10 parental clones, and analysis of variance (ANOVA) table for screenhouse and field sweetpotato virus disease (SPVD) scores (SPVD scale, 1-5; 1 = no visible symptoms, 5 = most severe symptoms; recovery scale, 0 = no recovery, 5 = maximum recovery), Namulonge, Uganda, 1998-2000

Summary of diallel progeny means for the 10 parental sweetpotato clones

Parents	SPVD (screenhouse)				SPVD (field)				Recovery			
	Score	SD	Min	Max	Score	SD	Min	Max	Score	SD	Min	Max
1. WAG	3.7	0.2	3.4	4.0	4.2	0.7	2.7	5.0	0.3	0.1	0.2	0.6
2. TAN	3.9	0.6	3.6	4.2	3.9	0.3	3.4	4.3	0.4	0.2	0.1	0.7
3. BMA	3.9	0.8	3.5	4.3	4.0	0.3	3.3	4.2	0.5	0.2	0.2	0.7
4. KAZ	3.8	0.6	3.5	4.1	3.8	0.3	3.5	4.4	0.3	0.2	0.2	0.9
5. NKA	3.6	0.9	3.5	3.8	3.5	0.7	2.3	4.3	0.4	0.2	0.1	0.7
6. TOR	3.9	0.4	3.7	4.1	4.0	0.3	3.3	4.0	0.3	0.1	0.1	0.5
7. KAN	4.0	0.6	3.6	4.3	4.0	0.3	3.5	4.2	0.5	0.2	0.4	0.9
8. BWA	3.8	0.4	3.6	4.0	3.9	0.2	3.4	4.3	0.4	0.1	0.2	0.5
9. SOW	3.7	0.7	3.3	4.0	3.8	0.4	3.5	4.2	0.4	0.2	0.1	0.5
10. CTA	3.7	0.5	3.3	3.8	4.0	0.7	2.6	5.0	0.2	0.1	0.1	0.4
LSD (P =0.05)	0.18				0.09				0.13			

ANOVA for screenhouse (SCN) and field (FLD) SPVD scores, SCN and FLD are treated as locations

Source of variation	df	Mean Squares	F-value
Replications	2	0.053	0.38^{ns}
Families (F)	44	0.537	3.84^{**}
Locations	1	1.557	11.12^{**}
F x L	44	0.296	2.11^{**}
Error	448	0.140	

$^{*, **}$ Significant at the 0.05 and 0.01 probability level, respectively, ^{ns} not significant

Table 3**.** Diallel families and their mean, minimum, maximum, sweetpotato virus disease (SPVD) scores (scale, 1 - 5; 1 = no visible symptoms, 5 = most severe symptoms), and number of genotypes in each family tested for sweetpotato chlorotic stunt crinivirus (SPCSV) and sweetpotato feathery mottle potyvirus (SPFMV). Samples with absorbance values greater than 2x the negative control were considered positives

Family	SPVD score			Number of genotypes tested	Positive		Negative for SPCSV and SPFMV
	Mean	Min	Max		SPCSV	SPFMV	
1. WAG x TAN	4.1	3.4	5.0	10	8	10	0
2. WAG x BMA	4.0	3.3	5.0	10	6	7	1
3. WAG x KAZ	4.1	3.7	5.0	10	7	9	0
4. WAG x NKA	3.9	2.6	4.3	10	6	10	0
5. WAG x TOR	4.0	2.8	4.7	8	7	8	0
6. WAG x KAN	4.1	3.5	5.0	10	6	10	0
7. WAG x BWA	4.0	3.1	5.0	10	7	10	0
8. WAG x SOW	4.1	2.8	5.0	10	6	10	0
9. WAG x CTA	3.7	2.8	4.3	10	8	10	0
10. TAN x BMA	4.0	3.7	5.0	10	8	10	0
11. TAN x KAZ	4.4	3.7	5.0	9	9	9	0
12.TAN x NKA	4.0	3.2	4.3	10	9	10	0
13. TAN x TOR	4.3	3.7	5.0	10	8	10	0
14. TAN x KAN	4.2	4.0	4.7	10	10	10	0
15. TAN x BWA	4.2	3.9	4.7	10	10	8	0
16. TAN x SOW	3.7	3.2	4.3	10	9	9	1
17. TAN x CTA	4.2	3.5	4.7	10	10	10	0
18. BMA x KAZ	4.1	3.8	5.0	10	7	10	0
19. BMA x NKA	3.9	3.3	4.3	10	4	10	0
20. BMA x TOR	4.2	3.8	5.0	10	7	9	0
21. BMA x KAN	4.0	3.9	5.0	10	9	10	0
22. BMA x BWA	3.5	3.3	5.0	8	7	8	0
24. BMA x CTA	4.5	3.4	5.0	10	9	7	3
25. KAZ x NKA	4.5	3.5	5.0	10	7	10	0
27. KAZ x KAN	4.0	3.6	5.0	9	7	9	0
29. KAZ x SOW	3.9	3.2	5.0	10	9	9	0
31. NKA x TOR	3.9	3.6	5.0	10	9	9	1
33. NKA x BWA	3.9	3.3	4.3	10	9	9	1
34. NKA x SOW	3.8	2.9	4.7	10	9	9	1
35. NKA x CTA	3.8	3.3	5.0	10	10	10	0
36. TOR x KAN	4.1	3.6	4.7	10	7	10	0
38. SOW x TOR	3.8	3.4	4.5	10	10	9	0
39. TOR x CTA	4.1	3.7	4.3	10	8	9	0
40. KAN x BWA	4.1	3.7	5.0	9	7	8	0
41. KAN x SOW	4.1	3.9	5.0	10	7	10	0
42. KAN x CTA	3.9	3.8	5.0	8	5	8	0
44. SOW x CTA	4.2	3.6	5.0	10	8	10	0
45. SOW x CTA	3.9	3.2	5.0	10	8	10	0

Table 4. Analysis of variance for combining ability of sweetpotato virus disease (SPVD) scores in screenhouse and field, and recovery scores in field, Namulonge, Uganda, 1998-2000

Source of variation	df	Mean squares	F-value	GCA/SCA ratio[a]	H[b]	h[c]
Screenhouse experiment						
Seasons (S)	1	1.41	20.14**			
Reps/seasons	4	0.24	3.43*			
Families (F)	44	0.3	4.29**			
GCA	9	1.03	14.71**			
SCA	35	0.11	1.57ns	0.87	0.73	0.60
F x S	44	0.17	2.43**			
GCA x S	9	0.25	3.57**			
Error	176	0.07				
Field experiment						
Sites (S)	1	31.01	3101.00**			
Reps/sites	4	0.01	1.00ns			
Families (F)	44	0.54	54.00**			
GCA	9	1.65	165.00**			
SCA	35	0.25	25.00**	0.59	0.98	0.41
F x S	44	0.17	17.00**			
GCA x S	9	0.33	33.00**			
SCA x S	35	0.12	12.00**			
Error	176	0.01				
Recovery experiment						
Replications	2	0.06	8.57**			
Families	44	0.09	12.86**			
GCA	9	0.23	32.86**			
SCA	35	0.05	7.14**	0.51	0.93	0.31
Error	88	0.007				

*, ** Significant at the 0.05 and 0.01 probability level, respectively; ns not significant;
[a] GCA/SCA ratio = $2V_g^2/(2V_g^2 + V_s^2)$ ratio (Baker 1978), where V_g^2 and V_s^2 refer to general (GCA) and specific (SCA) combining ability variance components;
[b] H = broad sense heritability;
[c] h^2 = narrow sense heritability

Table 5. General combining ability (GCA) effects (in bold) and specific combining ability (SCA) effects (off diagonal) for resistance to sweetpotato virus disease (SPVD) in screenhouse and field, and recovery from SPVD experiments, Namulonge, Uganda, 1998-2000

Screenhouse experiment (standard error, GCA = 0.037; SCA = 0.098)

Parents	1	2	3	4	5	6	7	8	9	10
1. WAG	**-0.04^{ns}**	0.13^{ns}	0.05^{ns}	0.30**	-0.08^{ns}	-0.10^{ns}	-0.01^{ns}	-0.18^{ns}	0.06^{ns}	-0.09^{ns}
2. TAN		**0.10****	-0.05^{ns}	-0.07^{ns}	-0.10^{ns}	-0.03^{ns}	0.11^{ns}	-0.07^{ns}	-0.16^{ns}	0.10^{ns}
3. BMA			**0.17****	0.12^{ns}	-0.05^{ns}	0.05^{ns}	0.11^{ns}	-0.01^{ns}	0.04^{ns}	-0.25*
4. KAZ				**0.03^{ns}**	0.16^{ns}	-0.20*	-0.13^{ns}	-0.08^{ns}	-0.18^{ns}	0.14^{ns}
5. NKA					**-0.15****	0.10^{ns}	-0.17^{ns}	0.02^{ns}	0.09^{ns}	0.07^{ns}
6. TOR						**0.11****	0.05^{ns}	0.06^{ns}	-0.02^{ns}	0.14^{ns}
7. KAN							**0.18****	-0.05^{ns}	0.19^{ns}	-0.08^{ns}
8. BWA								<**0.01^{ns}**	0.10^{ns}	0.06^{ns}
9. SOW									**-0.14****	-0.09^{ns}
10. CTA										**-0.26****

Field experiment (standard error, GCA = 0.013; SCA = 0.034)

Parents	1	2	3	4	5	6	7	8	9	10
1. WAG	**0.29****	-0.15**	0.14**	0.23**	0.07*	-0.11**	-0.26**	0.07*	0.06^{ns}	-0.04^{ns}
2. TAN		**0.03***	-0.06^{ns}	0.04^{ns}	-0.23**	0.23**	-0.25****	0.07*	0.15**	0.19**
3. BMA			**0.08****	-0.18**	0.45**	0.13**	-0.05^{ns}	-0.43**	-0.02**	-0.01^{ns}
4. KAZ				**-0.08****	-0.02^{ns}	0.01^{ns}	0.06^{ns}	-0.02^{ns}	-0.11**	-0.04^{ns}
5. NKA					**-0.43****	-0.09*	-0.16*	0.10**	-0.15**	-0.01^{ns}
6. TOR						**0.07****	0.04^{ns}	-0.02^{ns}	-0.12^{ns}	-0.03^{ns}
7. KAN							**0.11****	-0.05^{ns}	0.38**	0.30**
8. BWA								**-0.03***	0.22**	0.10**
9. SOW									**-0.07****	-0.43**
10. CTA										**0.03***

Recovery experiment (standard error, GCA = 0.016; SCA = 0.042)

Parents	1	2	3	4	5	6	7	8	9	10
1. WAG	**-0.05****	-0.09^{ns}	-0.01^{ns}	-0.13**	0.16**	0.04^{ns}	0.08^{ns}	-0.03^{ns}	-0.12**	0.11*
2. TAN		**0.03***	0.14**	-0.04^{ns}	-0.15**	-0.01^{ns}	0.03^{ns}	0.16**	0.02^{ns}	-0.08^{ns}
3. BMA			**0.11****	-0.10*	0.23**	0.04^{ns}	-0.02^{ns}	-0.23**	0.02^{ns}	-0.07^{ns}
4. KAZ				**-0.04***	-0.06^{ns}	0.06^{ns}	0.34**	-0.03^{ns}	-0.15**	0.06^{ns}
5. NKA					**-0.01^{ns}**	-0.18**	-0.08^{ns}	0.06^{ns}	0.10*	-0.08^{ns}
6. TOR						**-0.05****	-0.12**	0.03^{ns}	0.08^{ns}	0.05^{ns}
7. KAN							**0.19****	-0.14**	-0.10*	0.04^{ns}
8. BWA								**-0.02^{ns}**	0.20**	0.06^{ns}
9. SOW									**-0.01^{ns}**	-0.09^{ns}
10. CTA										**-0.17****

$^{*, **}$ Significant at the 0.05 and 0.01 probability level, respectively; ^{ns} not significant.

CHAPTER 3

Phenotypic Differences of Resistance to Sweetpotato Virus Disease

(In the format appropriate for submission to Plant Disease)

Phenotypic Differences of Resistance to Sweetpotato Virus Disease

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ABSTRACT

Evaluating sweetpotatoes for resistance to sweetpotato virus disease (SPVD) has been slow and inefficient. SPVD-infected *Ipomoea setosa* plants, normally used as the source of scions for graft infecting sweetpotatoes with viral diseases, were severely stunted and plant mortality was 10-30%, making them unsuitable for studying SPVD. We found that the cultivar, Tanzania, a cultivated landrace of *I. batatas*, was a superior host for maintaining and increasing SPVD inoculum (scions) for mass grafting. Further, the modified cleft-grafting technique, described increased survival of grafted SPVD symptomatic scions from 5 to 100%. This study demonstrates that use of a suitable sweetpotato genotype for maintenance and increase of SPVD inoculum and, appropriate cleft-graft inoculation

modifications, coupled with efficient SPVD scoring techniques, can be used to facilitate rapid screening of large sweetpotato populations for SPVD resistance. Criteria to characterize genotypes for SPVD resistance based on symptoms indicated significant variation among families and among genotypes within families. Recovery from SPVD was a component of SPVD resistance. There were significant genotypic differences detected for recovery from SPVD among 36 sweetpotato families evaluated for SPVD resistance, indicating that recovery contributes to SPVD resistance. This finding may open up new opportunities for control of this devastating disease.

Additional keywords: *Bemesia tabaci***,** *Myzus persicae*, sweetpoto chlorotic stunt virus, sweetpotato potatao feathery mottle virus

Sweetpotato (*Ipomoea batatas* (L.) Lam.), with a mean annual production of 132 million tons between 1991-2000, is ranked among the top ten most important food crops globally by the Food and Agriculture Organization of the United Nations (17). Increases in sweetpotato productivity have been constrained by many major factors including, sweetpotato virus disease (SPVD), low yielding varieties, insects, and postharvest losses. SPVD is widespread (25, 30, 52) and causes up to 98% yield loss (19, 29). The disease is caused by the dual infection and synergistic interaction of sweetpotato feathery mottle virus (SPFMV) and sweetpotato chlorotic stunt virus (SPCSV) (10, 30, 43). SPFMV is transmitted non-persistently by aphids (47), while SPCSV is transmitted semi-persistently by the whitefly (*Bemesia tabaci*) (10, 43). Development of cultivars with genetic resistance to SPVD is the only feasible strategy to control this devastating disease.

Field screening of sweetpotato germplasm for resistance to SPVD has been based on

phenotypic expression of symptom severity and incidence of plants infected with SPVD following natural infection (4, 9, 25). This is problematic because transmission efficiency of SPVD by vectors may varies with environmental conditions (25, 45), and vector population size fluctuates over seasons and years (3, 5, 25). Where inoculum pressure is high, as in the Lake Victoria basin of East Africa, susceptible cultivars exhibit severe symptoms in one to two seasons. Large (50,000-100,000) populations of F_1 seedlings are required each season to identify agronomically superior genotypes with SPVD resistance (9, 20). However, plant escapes initially rated as resistant and/or moderately resistant genotypes, may take three or four years to show SPVD symptoms, which makes field screening for SPVD slow and inefficient. Symptom severity is the initial selection criterion for SPVD resistance. SPVD severity may not be the ideal selection criterion because some genotypes have the potential to rapidly recover from the effects of SPVD. This phenomenon has also been observed in cassava where cultivars resistant to African cassava virus (ACMV), have also characteristics other than resistance. For example, in some genotypes ACMV does not become fully systemic, and virus titer remains low or the spread within and among plantings is relatively slow and some cuttings may revert to healthy plants or recover (15, 16, 28).

In this study, insect, mechanical, and graft inoculation procedures were used to induce SPVD, and serology and scoring methods were evaluated for screening large sweetpotato populations against SPVD. To do this we characterized 36 sweetpotato families (360 genotypes) for resistance to SPVD. 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: 1) improve methods of evaluation of sweetpotato genotypes for resistance to SPVD and; 2) assess the use of recovery from SPVD as an additional selection criterion for resistance to SPVD.

MATERIALS AND METHODS

To avoid escapes which may occur under field screening and to ensure the presence of SPVD inoculum; mechanical, insect and graft inoculation techniques were evaluated to determine the most efficient method of inoculating and screening large sweetpotato populations for SPVD resistance. The most efficient method was subsequently used to inoculate the 36 families (360 genotypes) with SPVD and to rate the families for resistance to the disease in the screenhouse and field and for recovery from SPVD.

Sources of inoculum. Inoculum of SPFMV and SPCSV was obtained from SPVD-infected sweetpotato plants of the cultivar, Tanzania (TAN), which were collected from experimental fields and propagated by cuttings in a screenhouse at the Namulonge Agricultural and Animal Production Research Institute (NAARI), Uganda. To obtain *I. setosa* plants infected with SPFMV, a colony of aphids, *Myzus persicae* Sulz., was established on cabbage and apterous, fasted (2 hr) aphids were allowed to feed on SPVD-infected TAN plants for 2 min before being transferred to five *I. setosa* seedlings (one aphid/seedling, overnight). The seedlings were grown in insect-proof cages in a screenhouse. To obtain *I. setosa* plants infected with SPCSV, whiteflies collected from severely SPVD infected TAN field plants at NAARI were transferred to the healthy *I. setosa* seedlings (50 whiteflies/seedling) for overnight inoculation access feeding. The same SPVD-diseased TAN plants from the field propagated in the screenhouse tested negative for sweetpotato mild mottle virus, sweetpotato chlorotic fleck virus and sweetpotato latent virus (19). To obtain SPVD infected TAN plants, five pathogen-tested plants of TAN were cleft grafted with SPFMV- and SPCSV-infected *I. setosa* scions and these were propagated by stem cuttings in separate screenhouses. These graft-inoculated plants were used as sources of

inoculum for SPVD.

Mechanical inoculation. A combination of grafting and mechanical (2, 6) or aphid inoculation must be used to induce SPVD in *I. setosa* seedlings or sweetpotato because SPCSV is not mechanically transmitted (10). To test the suitability of mechanical inoculation for use on a large scale (1000s of genotypes), thirty seedlings of *I. setosa* were mechanically inoculated with SPFMV. SPFMV inoculum for inoculating *I. setosa* seedlings was obtained by grinding SPFMV-infected *I. setosa* leaves (2-3 g) in 2 ml of potassium phosphate buffer (0.005M potassium phosphate, 0.2% sodium sulfite, pH 7.2) with a mortar and pestle. To inoculate the seedlings, cotyledons of 25, 1- or 2-leaf stage *I. setosa* seedlings were dusted with carborundum (600-mesh) to abrade the epidermis. A sterile cotton swab dipped in the buffer containing the SPFMV inoculum was rubbed gently on the dusted cotyledons. The seedlings were covered with polythene bags previously sprinkled with moisture and placed in shade under benches in the screenhouse for two to three days, and then transferred to bench tops for symptom expression. Three to four weeks after inoculation, seedlings showing SPFMV symptoms (vein clearing, mosaic or chlorotic leaf spots, leaf deformation and twisting) were grafted with *I. setosa* scions exhibiting SPCSV symptoms (curling up or down of leaf margins and/or chlorosis) to induce SPVD.

Top- and side-cleft grafting (wedge method) studies. Because the previous methods described were considered to be complicated for large sweetpotato populations we conducted preliminary experiments to detect the most efficient inoculation methods. Ten to 20 cm potted TAN plants were used to evaluate the efficiency of graft inoculation in transmitting SPVD. Modifications of top and side grafting, varying size of scion and stock or position of graft, and grafting a healthy scion on a diseased stock were done, but these methods resulted in less than 5% (sample of 720 plants) disease transmission.

The wedge graft method of inserting a shaved node with petiole and leaf attached from the source into a node area of the stock plant was adopted with the following modifications. The SPVD infected scion was immersed in tap water immediately after cutting and before grafting on the TAN test plant. The grafted plant was then covered with a polythene bag, previously sprinkled with water, to include a few nodes and leaves below the graft area. Dipping the scion in water and covering the grafted plant minimized stress of the scion due to excessive transpiration and reduced callus formation at the cut surfaces in subsequent days. The whole plant was left in shade for 4-7 days or until the leaves on the scion and/or the enclosed leaves on the stock plant started senescing. Some scions died within 4-5 days after transmitting the disease while others died without transmitting the disease. Shoots not showing SPVD symptoms which had dead scions were regrafted. Multiple grafts were done on plants with multiple shoots. SPVD culture was subsequently maintained and increased in the cultivar, TAN. The plants were observed for symptom development and were scored for SPVD.

To compare the efficiency of *I. sotosa* seedlings double grafted with SPFMV- and SPCSV and SPVD-infected TAN as sources of SPVD inoculum, per cent survival of scions and transmission rates of the two sources of SPVD inoculum were compared.

SPVD resistance evaluation in the screenhouse. Sweetpotato diallel progenies (360 genotypes in 36 families) of 10 parental clones varying in SPVD field resistance (susceptible to resistant) were evaluated for SPVD resistance in the screenhouse. Ten to 15 plants per genotype were graft inoculated in a screenhouse at 1.5 months after planting. The study was conducted in August to December 1998 and repeated in October 1999 to February 2000, using a randomized complete block (RCB) design and experimental protocols described by Mwanga et al. (37). Genotypes were rated for SPVD resistance on a

subjective 1-5 rating scale (1 = no symptoms, 5 = very severe symptoms) (Fig. 1., Appendix 1).

Effect of SPVD on yield. The same 360 genotypes evaluated for SPVD resistance in the screenhouse were planted at an upland (Borehole) and lowland (swampy) site (Kisenyi) at NAARI in February 1999. Ten cuttings per genotype (360 genotypes) from SPVD graft-infected plants of the same 36 families studied in the screenhouse were planted at the two sites. The experiment was a split-split-split lay out. The main plot consisted of family, sub-plot plot genotype, and sub-sub-plot the inoculations (healthy and graft inoculated). The 36 families were randomized within each replicate, the 10 genotypes per family were randomized within each family, and the inoculations, healthy (field) and inoculated vine cuttings, were randomized within each sub-sub plot. Each sub-sub-plot had a single genotype of two rows (one SPVD-infected and one healthy control) in 3 replications. The rows (ridges) were spaced 1 m apart. Each row had 10 plants, 30 cm apart. SPVD severity was scored at monthly intervals after planting using the 1-5 rating scale. The plants were harvested at 3.5 months after planting. Total plant weight, shoot weight per plant, number of roots per plant, and root weight per plant of SPVD graft infected plants were expressed as percentage of healthy controls and their standard deviations were computed based on plot means.

Evaluation of inoculated materials by serology. To test whether there were differences in virus content between screenhouse and field grown plants, 14 genotypes from both environments (screenhouse and field) were evaluated by serology (ELISA). 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 enzyme-linked immunosorbent assay (DAS-ELISA) described by Cadena-Hinojosa and

Campbell (8) and SPCSV by triple antibody sandwich (TAS-ELISA) in microplates (19). Each plate had duplicate wells for the test sample, a positive control, pathogen-tested negative control (cultivar, TAN) and a buffer control. Immunoglobulin (IgG), SPCSV-KyCP (dilution 1:500) for coating ELISA plates, and monoclonal antibodies (MAb) for detecting SPCSV were donated by Dr. J. Vetten of the Federal Biological Research Center for Agriculture, Braunschweig, Germany. IgG and MAb for SPFMV were supplied by Dr. Lucho Salazar of the International Potato Center (CIP), Lima, Peru. Goat-antimouse alkaline phosphatase-conjugated antibody (Sigma Chemical Co. Ltd, Poole, UK) was used at a dilution of 1:1000 and the color reaction was catalyzed using nitrophenol phosphatase substrate. Plates were incubated overnight and absorbances were recorded at 405 nm (A_{405}) with a microplate reader (Labsystems Multiskan S, Middlesex, UK). Genotypes testing negative in ELISA tests 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. Because genotypes with the same SPVD severity score may differ in their ability to recover from SPVD, we conducted an experiment to assess the effects of recovery on SPVD incidence and its importance in SPVD resistance. In this experiment, 10 cuttings of all the genotypes in the 36 families (360 genotypes) with obvious SPVD symptoms were planted in the field in single rows on ridges spaced 1 m apart and 30 cm within a row in November 1999 (2 months post-inoculation). The genotypes were randomized within each family and the families were arranged in an RCBD with 3 replications. At 2 and 5 months after planting the plants were scored on a per plot basis for SPVD using the 1-5 rating scale described above. An index for recovery from SPVD used in this study incorporates separate ratings (1-5; 1 = minimum recovery, 5 =

maximum recovery) for plant pigmentation, growth, and size (37).

Statistical Analysis. Analysis of variance (ANOVA) for SPVD score means was done using the general linear model, SAS-PROC ANOVA procedure (42) with families as the main plot, genotypes within families as the sub-plot, and inoculations (field and graft infection) as the sub-sub-plot. Percentages of infected over the 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 sub-plot.

RESULTS

Insect and mechanical transmission. *I. setosa* seedlings inoculated by aphids developed mild to severe, mosaic, chlorotic spots and vein clearing symptoms, and deformed (twisted) leaves due to SPFMV within 2 to 6 weeks after infection. A few new apical symptomless leaves started appearing on some *I. setosa* plants 5-6 weeks weeks after inoculation. On average 5 aphids for SPFMV and 50 whiteflies for SPCSV per *I. setosa* seedling were required for successful transmission of the viruses to occur. Whitefly mortality was high under the screenhouse conditions. *I. setosa* seedlings double grafted with SPFMV- and SPCSV-infected *I. setosa* scions resulted in severe stunting, general chlorosis, and necrosis of old infected leaves, leading to death in about 10-30% of seedlings within three weeks. It was inefficient to maintain the SPVD in *I. setosa* and use it as a source of SPVD symptomatic scions for graft-inoculation experiments because of the severe stunting and high plant mortality rate.

Covering graft-inoculated plants with moistened polythene bags, and leaving them in shade for 4 to 7 days increased survival of the scions and transmission of SPVD from 2-5% to 97-100%. Regrafting symptomless plants, which had dead scions, resulted in 100%

SPVD transmission. The modified graft method was used to evaluate resistance to SPVD of sweetpotato diallel progenies in the screenhouse and field. This evaluation was not previously practical for large sweetpotato populations with insect inoculation (aphid and whitefly) or mechanical inoculation.

Graft infection and SPVD resistance screenhouse screening. Double grafting pathogen-tested TAN plants with SPFMV- and SPCSV-infected scions of *I. setosa* resulted in SPVD-infection. The SPVD graft-infected TAN plants were used as source of inoculum and were multiplied to supply SPVD-infected scions for screening sweetpotato diallel progenies for resistance to SPVD. Graft-inoculation adopted with the modifications of this study was efficient in transmitting SPVD to large numbers of genotypes in replicated experiments, and the experiments were repeated over two seasons. This procedure was prferred to the standard procedure because using *I. setosa* as a SPVD symptomatic scion required germinating two batches of *I. setosa* seed, where one set was mechanically inoculated with SPFMV and the second grafted with SPCSV. SPCSV symptomatic seedlings must then be grafted with SPFMV symptomatic *I. setosa* scions to induce SPVD. Clearly the use of TAN SPVD symptomatic scions for graft inoculation was more efficient and rapid than insect or mechanical inoculation.

Various combinations of SPVD symptoms were exhibited by the sweetpotato genotypes depending on the degree of susceptibility (Table 1). Moderate symptoms included, vein clearing, chlorotic mosaic, chlorotic spots, reduced leaf size, up or down rolling of leaf margins (cupping), and bronze or purple mottling. Severe symptoms included, narrowing of leaf lamina (strapping), leaf distortion (crinkling), stunting, reduction in shoot weight and total plant weight, tip die-back, and death.

Most of the genotypes (>95%) were highly susceptible to SPVD (Table 1) and they

exhibited a combination of symptoms of severe SPVD (crinkling, strapping, puckering, tip die-back, stunting and severe plant weight reduction). Of the 10 parental clones evaluated for SPVD resistance only WAG suffered tip die-back when graft-inoculated with SPVD and this response was, in turn, transmitted to its progeny. Six per cent (22 genotypes) of the 360 genotypes of the diallel progeny suffered tip die-back (Table 1).

Graft-inoculation of SPVD resulted in significant differences in SPVD severity among families and genotypes within families, and between inoculations (field vs graft-infection). Grand minimum (2.0-3.3) and maximum (5.0) SPVD scores of grafted-inoculated plants were higher than the grand minimum (1.0) and maximum (4.1) SPVD scores due to natural (field) infection. Further, graft-infection resulted in more severe and rapid SPVD progress in all the 36 families compared to field infection (Tables 2 and 3). The mean of graft-infected SPVD severity scores (4.0) was higher than the mean (2.0) of natural (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 (Table 3), indicating a higher titer present in the screenhouse grown plants.

Recovery from SPVD. The 36 full-sib families exhibited significant variation for recovery from SPVD (Table 4). Families 8 (WAG x CTA) and 23 (KAZ x KAN) had the highest mean recovery scores (0.8 and 0.9, respectively), with a range of 0.6-1.0. Twenty-four families (66.7%) had very low recovery ability (mean recovery scores, 0.1-0.4). Recovery in some genotypes progressed from near plant death (SPVD score = 5) to symptomless plant (SPVD score = 1). In some genotypes the change was difficult to detect, while in others the disease progressed over time resulting in death. Some severely infected

genotypes had the ability to localize the distribution of SPVD. In those, whole branches or individual shoots developed that were asymptomatic and free of detectable virus when assayed by ELISA or grafted on *I. setosa*.

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 5-7). Although SPVD was severe in all the families, there was great variation in the type of symptom expression (Table 1) among families (Tables 2-6) and among genotypes within families, as demonstrated within families 3 and 27 (Table 7). The mean family plant weight of SPVD graft-infected plants ranged from 2.2% to 36.2% of healthy controls (Table 6). 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 (0.2-24.2% of healthy controls) of root weight per plant were reduced most by SPVD (Tables 1, 6, 7). Progenies of the parent SOW, which has moderate field resistance 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. Progenies of parent WAG, which has high field resistance to SPVD had the lowest number of roots per plant, root weight paper plant, shoot weight and plant weight.

DISCUSSION

Among the SPVD symptoms used to characterize the 36 sweetpotato families (360

genotypes) graft inoculated with SPVD, tip die-back was associated with only progenies of the parent, Wagabolige (WAG). Tip die-back is possibly a hypersensitive reaction to SPVD infection and is probably controlled by one or two major genes. In soybean, hypersensitivity resistance to cowpea chlorotic mottle virus (CCMV) is conditioned by a single dominant gene (21).

Various inoculation techniques, including insect inoculation (19, 43), and Nusbaum's (21, 37, 40) core-graft inoculation technique have been used to ensure infection and to speed up SPVD progress in test genotypes (24, 47). Transmission of SPFMV by aphids (7, 12, 19, 34, 38, 46, 47, 50) and SPCSV by whiteflies (10, 26, 29, 30, 53, 51) in sweetpotato has been demonstrated on a small scale. Attempts by Hahn et al. (24) to mass rear aphids and whiteflies for rapid screening of large populations of sweetpotato seedlings for resistance to SPVD were unsuccessful. Similarly, 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.

Similarly, using SPVD-infected *I. setosa* or *I. nil* as SPVD inoculum source works well if local conditions favor fast increase and rapid utilization of the inoculum as at the International Potato Center, Lima, Peru. The system, however, is not efficient where SPVD-infected *I. setosa* or *I. nil* become extremely stunted with high plant mortality rates as in the present study.

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. Experiments can be replicated and repeated over seasons using the same genotypes with graft inoculation which is not possible if the cotyledons of sweetpotato seedlings are mechanically inoculated. Further, TAN SPVD infected plants can supply more scions for

longer periods than *I. setosa* SPVD infected plants. TAN SPVD infected 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 can sprout if the shoot dies, which is not possible with *I. setosa*. Cleft (top) grafting has been used to infect sweetpotato with SPFMV (6, 12, 20, 24, 27, 33, 35, 38, 41).

The comparison of screenhouse vs field OD values (Table 3) showed that some genotypes perform differently in the two environments, indicating that such genotypes may be unstable and field screening for SPVD resistance needs to be complemented with screenhouse screening. 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 (Tables 2 and 3). Under natural infection, some genotypes may escape infection by viruliferous vectors of SPFMV and/or SPCSV (25).

Vector populations may vary with location (5, 46) and/or seasons (3, 5, 25) resulting in reduced transmission of SPVD when the vector populations are low (25). The cleft graft modifications ensure SPVD transmission and overcome the difficulty of absence or fluctuation of vector populations. The cleft graft modifications make it possible to inoculate sweetpotato and to screen efficiently for SPVD all year round under tropical conditions.

Although TAS- and DAS-ELISA readily detected SPCSV and SPFMV, respectively, virus titer in the leaves did not always correlate with symptom severity of SPVD-infected plants. Mathews et al. (32) found ELISA reliable for detection of citrus tristeza closterovirus (CTV) in sweet orange trees in California, but unreliable during the summer months when trees had significant loss of virus titer. Lapidot et al. (31) found no correlation between cucumber mosaic virus (CMV) titer in pepper and the degree of tolerance to virus

determined by visual symptoms or field performance. In contrast, Karyeija et al. (30) using a single cultivar, TAN, and RNA dot-blot analysis, a more sensitive method than DAS-ELISA, found that the amount of SPFMV RNA in TAN varies between plants and correlated positively with symptom severity. Where virus symptoms correlate consistently with virus titer, quantitative ELISA has been used successfully to supplement selection of virus resistant genotypes based on symptoms [e.g. wheat resistant to wheat streak mosaic virus (WSMV) (44), barley resistant to barley yellow dwarf virus (BYDV)(23), and curcubits resistant to zucchini yellow mosaic virus (40)].

Our results suggest that quantitative ELISA is not precise enough to be used reliably to identify sweetpotato genotypes resistant to SPVD. Resistance to SPFMV and SPCSV, the components of SPVD breaks down in a dual infection. The virus titer of SPFMV increases dramatically due to synergism between SPFMV and SPCSV (30, 43) resulting in confounding of resistance to the SPVD components. However, ELISA tests are useful as a supplement to selection for SPVD resistance based on visual symptoms to confirm the presence of SPFMV and SPCSV. ELISA can be used to eliminate highly susceptible genotypes in screenhouse and field screening as they have high absorbance values for SPFMV and/or SPCSV.

Screening for resistance to SPVD by breeders is commonly done by visual selection. The method is simple and rapid, and it enables selection if contrasts between plants are marked. Compared to ELISA, visual scoring of SPVD severity was more reliable, consistent, and could be repeated several times during the growing season for hundreds of genotypes which was not possible with ELISA.

Recovery is an important component of resistance to SPVD. Recovery or reversion has also been observed in African cassava mosaic virus (ACMV)-resistant cassava varieties

(15,16, 28). In the present study, resistant sweetpotato progenies of the parent SOW and the susceptible progenies of parental clones KAN and BMA exhibited rapid recovery. The most plausible explanation for this is that SPVD, like ACMV does not become fully systemic (4, 19) and a proportion of uninfected cuttings can be obtained from previously infected plants. Various studies have shown that SPFMV exhibits uneven distribution in sweetpotato (1, 8, 11, 14, 18, 24). While low virus titer and irregular distribution are problematic in virus detection, sweetpotato genotypes with such levels might be expected to show more rapid recovery compared to genotypes with fully systemic and high virus titers. The reversion or recovery phenomenon may be of great epidemiological significance in decreasing sustained losses, but it has received little attention in sweetpotato research. Indeed the ability of some sweetpotato cultivars to recover from SPVD may help to explain why some seemingly highly susceptible genotypes stay for long periods in cultivation. They may have rapid recovery enabling them to give reasonable yields, thus they are not dropped by subsistence farmers. Although no data was collected on the effect of recovery on sweetpotato storage yields, studies in cassava indicate that resistant cultivars with reversion (recovery) have the potential to control ACMV, since the cultivars suffer lower yield losses when infected and are less likely to become infected, even after many years of successive crop growth (15, 16).

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 (48). Dougherty et al. (13) have proposed a cytoplasmic host system that would recognize specific RNA sequences and eliminate them to account for recovery. 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.

Depending on prevailing local environmental conditions, a combination of screenhouse, field and serological techniques should be used to breed for SPVD resistant genotypes. For example, as we found in Uganda, grafting procedures may need modification, *I. setosa* or *I. nil* might be substituted by a suitable susceptible sweetpotato clone as a source of SPVD inoculum (scion) if *I. setosa* or *I. nil* have high mortality when inoculated with SPVD.

Under Ugandan and similar conditions graft-inoculation and evaluation of large numbers of sweetpotato seedlings based on an SPVD scoring scale is more reliable and more efficient than insect transmission and mechanical inoculation. Using standard check genotypes, large sweetpotato populations can be graft-inoculated, evaluated and characterized for resistance to SPVD more rapidly than when screened under natural field inoculum pressure.

Graft inoculation modifications in this study, and the resulting symptoms used as criteria for defining resistant genotypes, enabled screening of 27,000 sweetpotato plants for resistance to SPVD in two seasons in 1998/1999. Graft inoculation was efficient in transmitting SPVD and was reliable for inoculating large numbers of sweetpotato genotypes. However, this requires large quantities of the inoculum (cultivar, TAN in Uganda's case), screenhouse space, pest control and is still labor intensive although with much less requirements compared to using SPVD infected *I. setosa* scions for inoculum. It remains a challenge to come up with a more rapid, efficient and less labor intensive way of inoculating sweetpotato with SPVD. Additionally, symptom expression used as the criteria

for characterizing genotypes for resistance to SPVD may be influenced by environmental factors. There is need to develop a rapid and simple method or assay for identification of SPVD and SPVD resistance. More studies are also needed to understand the role of recovery in alleviating yield losses under different SPVD inoculum pressures after successive crop growth cycles, and the contribution of recovery to SPVD resistance.

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Table 1. Sweetpotato virus disease (SPVD) symptoms exhibited by 36 families (360 genotypes) graft-infected by SPVD. Percent genotypes infected and type of sympotom are shown. Namulonge, Uganda, 1998-2000.

Symptoms	Families exhibiting symptom	Percent		Comment
		Families exhibiting symptom	Genotypes in all families exhibiting symptom	
1. Vein clearing	1-36	100	93	Severity varies with clone
2. Chlorotic mosaic	1-36	100	34	May include chlorotic spots
3. Reduced leaf size	1-36	100	23	Greater under water stress
4. Crinkling (leaf distortion)	1-8, 10-16, 20, 23, 29, 31, 35, 36	69	17	More severe in divided lamina and highly susceptible clones
5. Strapping (narrow leaf lobes)	3, 5, 12-16, 20, 28, 34-36	27	5	Mostly in clones with highly divided lamina
6. Cupping (up or down rolling of leaf margins)	1-4, 10, 12, 14-17, 19, 21, 23-25, 27-29,34	67	8	Occurs with water stress in some genotypes
7. Puckering (raised lamina between veins)	1-6, 11, 13, 19, 20, 25, 29	47	7	Common in highly susceptible clones with broad leaves
8. Bronze or purple mottling	1-8, 11, 12, 14, 17-19, 25, 26	58	11	In combination with chlorosis, may appear like herbicide damage or senescence
9. Tip die-back (hypersensitivity?)	1-3, 5-8, 29, 33, 34	22	6	Occurred only in one parent, Wagabolige (WAG); families, 1-3, 5-7, were progenies of WAG
10. Stunting	1-27, 29-36	98	10	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
14. Reduced weight of storage roots	1-36	100	100	Most affected trait

Table 2. Field sweetpotato virus disease (SPVD) scores for natural infection and graft-inoculation. Means (n = 60) and standard deviations (SD) are shown. SPVD scale, 1-5 (1 = no visible symptoms; 5 = very severe symptoms). Two sites at Namulonge, Uganda, 1999. There were 36 families, ten genotypes/family, 10 plants/genotype. Lay out, each main plot consisted of a family, sub-plot of genotypes, and sub-sub-plot of inoculations (natural and graft).

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

Table 2. Continued

Family	Natural infection				Graft-infection			
	Mean	SD	Min	Max	Mean	SD	Min	Max
31. TOR x CTA	2.4	1.0	1.0	4.0	4.0	0.4	3.0	5.0
32. KAN x BWA	1.7	1.0	1.0	4.0	3.8	0.8	2.0	5.0
33. KAN x SOW	1.9	0.8	1.0	4.0	4.3	0.6	3.0	5.0
34. KAN x CTA	2.7	1.0	1.0	5.0	4.3	0.6	3.0	5.0
35. BWA x CTA	1.9	1.0	1.0	4.0	4.0	0.8	2.0	5.0
36. SOW x 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

LSD (0.05) = 0.38 for comparing inoculation treatments within families or families within inoculation

Table 3. Analysis of variance (ANOVA) table for sweetpotato virus disease (SPVD) scores and optical density (OD) readings for sweetpotato chlorotic stunt virus (SPCSV) and sweetpotato feathery mottle virus (SPFMV). Two sites at Namulonge, Uganda, 1999. There were 36 families, ten genotypes/family, 10 plants/genotype. Lay out, each main plot consisted of a family, sub-plot of genotypes, and sub-sub-plot of inoculations (natural and graft).

ANOVA for SPVD scores

Source	df	Mean square	F-value	P>F
Site	1	0.81	0.37	0.5772
Rep x Site	4	2.20	5.00	0.0005
Family	35	7.18	8.35	<0.0001
Family x Site	35	3.23	3.76	<0.0001
Family x Rep (Site)	140	0.86	1.95	<0.0001
Genotype (Family)	324	1.50	3.66	<0.0001
Genotype (Family) x Site	324	0.87	2.12	<0.0001
Genotype (Family) x Rep (Site)	1296	0.41	0.93	0.8781
Inoculation	1	3899.75	8863.07	<0.0001
Inoculation x Site	1	513.02	582.98	<0.0001
Inoculation x Family	35	5.38	2.82	<0.0001
Inoculation x Family x Site	35	2.49	5.66	<0.0001
Inoculation x Genotype (Family)	324	0.90	2.05	0.9950
Inoculation x Site x Genotype (Family)	324	0.88	2.00	<0.001
Error	1440	0.44		

SPCSV OD values for screenhouse and field OD readings (Location = screenhouse vs field OD values)

Source	df	Mean square	F-value	P>F
Genotype	14	0.465	10.11	<0.0001
Location	1	1.186	25.78	<0.0002
Genotype x Location	14	0.456	2.00	0.0497
Error	30	0.023		

SPFMV OD values for screenhouse field OD readings(Location = screenhouse vs field OD values)

Source	df	Mean square	F-value	P>F
Genotypes	14	0.312	2.12	<0.0856
Location	1	4.053	27.57	<0.0001
Genotype x Location	14	0.147	29.40	<0.0001
Error	30	0.005		

Table 4. Summary of recovery score means, standard deviations and minimum and maximum values for the 36 families (scale, 0 = no recovery, 5 = maximum recovery). Each family had 10 genotypes (10 plants/genotype). Namulonge, Uganda, 1998-2000.

Family	Mean	SD	Min	Max
1. WAG x TAN	0.3	0.09	0.2	0.4
2. WAG x BMA	0.4	0.05	0.4	0.5
3. WAG x KAZ	0.1	0.02	0.1	0.2
4. WAG x NKA	0.5	0.05	0.4	0.5
5. WAG x KAN	0.6	0.23	0.3	0.8
6. WAG x BWA	0.3	0.04	0.2	0.3
7. WAG x SOW	0.2	0.03	0.1	0.2
8. WAG x CTA	0.8	0.19	0.6	1.0
9. TAN x BMA	0.6	0.23	0.4	0.8
10. TAN x KAZ	0.3	0.16	0.2	0.5
11.TAN x NKA	0.2	0.13	0.1	0.4
12. TAN x TOR	0.3	0.14	0.2	0.4
13. TAN x KAN	0.6	0.07	0.5	0.7
14. TAN x BWA	0.5	0.07	0.5	0.6
15. TAN x SOW	0.4	0.05	4.0	0.5
16. TAN x CTA	0.1	0.01	0.1	0.2
17. BMA x KAZ	0.3	0.05	0.3	0.4
18. BMA x NKA	0.7	0.03	0.6	0.7
19. BMA x TOR	0.5	0.08	0.4	0.6
20. BMA x KAZ	0.6	0.08	0.5	0.7
21. BMA x CTA	0.2	0.04	0.2	0.3
22. KAZ x NKA	0.3	0.04	0.2	0.3
23. KAZ x KAN	0.9	0.05	0.8	0.9
24. KAZ x BWA	0.3	0.05	0.2	0.3
25. NKA x TOR	0.1	0.01	0.1	0.1
26. NKA x BWA	0.4	0.02	0.3	0.4
27. NKA x SOW	0.5	0.11	0.4	0.6
28. NKA x CTA	0.1	0.01	0.1	0.1
29. TOR x KAN	0.4	0.01	0.3	0.4
30. SOW x TOR	0.4	0.03	0.3	0.4
31. TOR x CTA	0.1	0.02	0.1	0.1
32. KAN x BWA	0.4	0.03	0.3	0.4
33. KAN x SOW	0.5	0.05	0.4	0.5
34. KAN x CTA	0.4	0.03	0.3	0.4
35. BWA x CTA	0.2	0.22	0.2	0.2
36. SOW x CTA	0.1	0.01	0.2	0.2
LSD (0.05)	0.13	-	-	-

Table 5. Analysis of variance table for effect of sweetpotato virus disease on plant weight , shoot weight, root weight and number of roots/plant(infected expressed as percentage of healthy). Two sites at Namulonge, Uganda, 1999. There were 36 families, ten genotypes/family,10 plants/genotype. Lay out, each main plot consisted of a family, sub-plot of genotypes, and sub-sub-plot of inoculations (natural and graft).

Plant weight

Source	df	Mean square	F-value	P>F
Site	1	161.99	10.53	0.0832
Rep	2	6.22	0.4	0.7119
Rep x Site	2	15.38	0.06	0.9394
Family	35	4459.2	124.45	<0.0001
Family x Site	35	1494.2	41.7	<0.0001
Family x Rep x Site	140	35.83	0.15	1.0000
Genotypes x Family	324	1579.03	6.42	<1.0000
Error	1620	245.971		

Shoot weight

Source	df	Mean square	F-value	P>F
Site	1	2309.54	4.18	0.1775
Rep	2	536.73	0.97	0.507
Rep x Site	2	552.05	0.4	0.6673
Family	35	560.35	5.72	<0.0001
Family x Site	35	2969.07	2.18	<0.0001
Family x Rep x Site	140	10.68	0.76	0.9795
Genotypes (Family)	324	2896.82	2.12	<0.0001
Error	1620	1364.58		

Root weight

Source	df	Mean square	F-value	P>F
Site	1	5011.07	53.12	0.0183
Rep	2	93.11	0.99	0.5033
Rep x Site	2	94.33	0.17	0.843
Family	35	5002.89	9.06	<0.0001
Family x Site	35	3548.99	12.18	<0.0001
Family x Rep x Site	140	410.711	0.74	0.9876
Genotypes (Family)	324	1705.22	3.09	<0.0001
Error	1620	552.26		

Table 5. Continued

Number of roots/plant				
Source	df	Mean square	F-value	P>F
Site	1	11254.1	1384.26	<0.0001
Rep	2	0.38	0.05	0.9548
Rep x Site	2	8.13	0.04	0.965
Family	35	2548.75	161.52	<0.0001
Family x Site	35	1152.55	73.04	<0.0001
Family x Rep x Site	140	15.78	0.07	1.0000
Genotypes (Family)	324	1458.39	6.38	<0.0001
Error	1620	228.41		

Table 6. Effect of sweetpotato virus disease on plant weight, shoot weight, root weight and number of roots per plant (infected expressed as percentage of healthy). Mean and standard deviation (SD) values are shown for each family. Two sites at Namulonge, Uganda, 1999. There were 36 families, ten genotypes/family, 10 plants/genotype. Lay out, each main plot consisted of a family, sub-plot of genotypes, and sub-sub-plot of inoculations (natural and graft).

Family	Plant weight (%)		Shoot weight (%)		Root weight (%)		Number of roots/plant (%)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1. WAG x TAN	15.8	21.7	19.3	23.8	6.7	22.1	8.3	20.3
2. WAG x BMA	11.3	16.8	13.7	18.7	3.6	15.6	8.1	19.9
3. WAG x KAZ	2.2	5.8	1.9	4.2	0.2	0.5	11.1	24.3
4. WAG x NKA	12.3	17.7	14.3	21.8	3.0	13.4	14.6	24.0
5. WAG x KAN	10.1	12.0	10.8	13.2	2.3	5.6	20.4	29.7
6. WAG x BWA	7.8	12.2	7.3	12.3	0.6	2.71	7.1	16.1
7. WAG x SOW	13.4	20.2	12.8	17.8	13.9	26.7	16.9	23.1
8. WAG x CTA	11.0	18.1	13.3	23.5	5.4	13.6	15.1	24.3
9. TAN x BMA	13.7	12.8	15.3	12.9	11.5	25.8	10.6	19.4
10. TAN x KAZ	29.8	30.0	26.8	31.7	19.1	27.7	22.6	28.7
11.TAN x NKA	27.7	22.7	25.2	24.1	16.7	19.5	25.9	39.4
12. TAN x TOR	8.9	11.8	10.0	13.0	2.1	9.0	9.0	17.2
13. TAN x KAN	25.2	27.2	33.7	26.8	19.6	30.7	12	21.3
14. TAN x BWA	30.9	25.4	30.0	26.5	16.6	20.6	9.1	17.9
15. TAN x SOW	22.4	20.3	27.0	23.6	11.8	16.7	23.4	27.4
16. TAN x CTA	19.7	22.7	22.4	28.4	12.4	20.2	20.8	32.2
17. BMA x KAZ	29.8	21.3	34.5	26.4	11.1	15.4	29.1	29.2
18. BMA x NKA	26.0	20.7	28.2	22.7	2.6	4.2	17.8	25.1
19. BMA x TOR	18.4	19.7	20.0	20.4	3.5	8.4	7.6	13.8
20. BMA x KAZ	16.5	16.2	16.9	18.7	9.5	23.0	22.6	29.2
21. BMA x CTA	27.4	26.6	29.7	30.5	15.5	21.2	31.8	30.8
22. KAZ x NKA	26.3	20.3	34.7	28.9	15.1	19.4	37.3	34.0
23. KAZ x KAN	20.4	23.4	25.5	33.4	19.4	25.4	35.4	38.0
24. KAZ x BWA	30.9	28.5	16.7	14.4	14.6	24.1	24.1	31.9
25. NKA x TOR	25.0	22.3	29.6	25.8	3.0	12.5	2.4	9.1
26. NKA x BWA	34.1	32.5	37.1	32.7	15.7	25.5	18.9	30.0
27. NKA x SOW	35.2	22.8	37.0	27.9	17.2	20.1	32.6	34.5
28. NKA x CTA	33.6	18.2	40.1	26.2	14.7	14.5	23.3	32.9
29. TOR x KAN	19.6	22.3	20.3	21.5	10.1	27.7	18.3	29.4

Table 6. Continued

Family	Plant weight (%)		Shoot weight (%)		Root weight (%)		Number of roots/plant (%)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
30. SOW x TOR	22.4	20.9	23.2	22.6	16.4	27.1	17.3	30.4
31. TOR x CTA	36.2	26.2	40.4	26.3	24.2	31.5	28.0	33.6
32. KAN x BWA	27.3	24.9	24.2	26.5	15.0	26.7	27.1	28.9
33. KAN x SOW	16.9	24.8	15.9	23.1	11.7	27.4	13.7	26.9
34. KAN x CTA	18.9	16.7	19.6	18.9	7.1	15.0	18.0	27.2
35. BWA x CTA	21.8	20.6	23.3	23.7	9.3	15.3	30.6	32.5
36. SOW x 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	-

Table 7. Effect of sweetpotato virus disease (SPVD) on two families with varying degrees of resistance and recovery from SPVD (SPVD rating; 1 = no visible symptoms, 5 = most severe symptoms. Recovery rating scale; 1 = no recovery, 5 = maximum recovery). Namulonge, Uganda, 1998-2000.

Family	Genotype	Mean SPVD score		Percent				Recovery
		Natural infection	Graft infection	Plant weight	Shoot weight	Root weight	No. roots/ plant	
3. WAG x KAZ	3/1	1.4	5.0	1.4	1.4	0.0	2.6	0.2
	3/2	1.7	4.8	0.1	0.1	0.0	0.0	0.1
	3/3	1.2	5.0	0.4	0.4	0.0	4.0	0.0
	3/4	1.7	4.8	0.1	0.1	0.0	10.0	0.0
	3/5	1.5	4.9	1.9	1.8	0.0	6.7	0.0
	3/6	1.6	5.0	0.9	0.1	0.0	0.0	0.0
	3/7	2.2	4.9	0.1	0.1	0.0	17.5	0.0
	3/8	1.6	4.6	1.9	2.0	0.3	0.1	0.2
	3/9	1.7	5.0	1.8	3.0	1.7	42.3	0.5
	3/10	1.5	5.0	13.4	9.9	0.0	27.5	0.0
	Mean	1.6	4.9	2.2	1.9	0.2	11.1	0.1
27. NKA x SOW	34/1	2.0	3.6	64.5	71.3	32.9	66.2	0.5
	34/2	2.0	3.5	32.0	27.5	13.8	14.7	0.4
	34/3	1.6	2.7	24.7	27.9	0.2	12.1	0.1
	34/4	2.2	3.6	10.4	13.1	3.5	34.8	1.1
	34/5	1.5	3.0	31.2	28.2	25.0	0.5	1.4
	34/6	1.6	3.7	37.8	13.9	20.9	33.5	0.5
	34/7	2.1	3.2	48.0	59.9	31.1	52.8	0.0
	34/8	2.5	2.7	41.4	60.8	23.2	50.6	0.2
	34/9	1.7	3.2	28.8	33.0	21.4	49.4	0.1
	34/10	1.7	3.3	33.1	34.4	0.1	11.4	0.7
	Mean	1.9	3.2	35.2	37.0	17.2	32.6	0.5

CHAPTER 4

Resistance to Sweetpotato Chlorotic Stunt Virus and Sweetpotato Feathery Mottle Virus is Mediated by Two Separate Recessive Genes in Sweetpotato

(In the format appropriate for submission to the Journal of the American Society for Horticultural Science)

Resistance to Sweetpotato Chlorotic Stunt Virus and Sweetpotato Feathery Mottle Virus is Mediated by Two Separate Recessive Genes in Sweetpotato

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This research was part of the Collaborative Crop Research Program and was jointly funded by the McKnight Foundation, Minnesota, USA, and the National Agricultural Research Organization (NARO), Uganda.

ADDITIONAL INDEX WORDS : *Ipomoea batatas*, linkage analysis, sweetpotato virus disease

ABSTRACT. The genetic basis of resistance to sweetpotato virus ideas (SPVD) was investigated in 15 promising sweetpotato diallel families (1352 genotypes) in Uganda and in two large families of the same diallel at the International Potato Center (CIP),

Lima, Peru. Graft inoculation of the 15 families with SPVD resulted in severe SPVD in all the families in Uganda. The distribution of SPVD scores was skewed toward highly susceptible categories (SPVD scores 4 and 5), eliminating almost all the resistant genotypes (scores 1 and 2). Graft inoculation with SPVD of two promising diallel families (Tanzania x Bikilamaliya and Tanzania x Wagabolige) from Uganda produced severe SPVD in all progenies (294 genotypes) at CIP, Lima. Individual inoculation of the two families with SPCSV and SPFMV at CIP, and Mendelian segregation analysis for resistant vs susceptible categories for the two viruses based on enzyme-linked immunosorbent assay (ELISA) results suggest that resistance to SPCV and SPFMV is conditioned by two, separate major recessive genes. In the proposed model for inheritance, the two genes are inherited in a hexasomic or tetradisomic manner. Two amplified fragment length polymorphic (AFLP) markers, one associated with the locus conferring resistance to SPCSV, and the second linked to the locus conferring resistance to SPFMV were identified in these progenies. The marker linked to resistance to SPCSV explained 70% of total variation in SPCSV resistance, while the marker linked to resistance to SPFMV explained 66% of the total variation in SPFMV resistance. We propose s*pcsv1* and s*pfmv1* to be the names of the two genes. The AFLP and QTL analyses verify our hypothesis based on quantitative genetic analyses of single gene mediation of SPCSV and SPFMV resistance. Our results also suggest that, in the presence of these viruses, additional genes mediate oligogenic or multigenic horizontal (quantitative) effects in the progenies studied.

Sweetpotato (*Ipomoea batatas* (L.) Lam.), with a mean annual production of 132

million tons between 1991-2000, is ranked among the top ten most important food crops globally (FAO, 2000). Sweetpotatoes are grown in over 100 countries and over 98% of total production is in developing countries (Horton, 1988; Gregory et al., 1990). Sweetpotatoes are a good source of carbohydrates, and both sweetpotato storage roots and tops are nutritious foods (Food Nutrition Board, 1980; Watt and Merrill, 1975; Woolfe, 1992). Orange fleshed sweetpotatoes are particularly nutritious, ranking highest in nutrient content of all vegetables for vitamins A and C, folate, iron, copper, calcium, and fiber (Woolfe, 1992).

Sweetpotato has enormous potential to contribute to the alleviation of widespread food shortages and malnutrition common to many developing countries in Sub-Saharan Africa. However, sweetpotato virus disease (SPVD) is a major constraint to increases in sweetpotato productivity in this region. SPVD is widespread (Schaefers and Terry 1976; Hahn et al. 1981; Wisler et al., 1998) and causes up to 98% yield loss (Gibson et al. 1998; Karyeija et al., 1998). The disease is caused by the dual infection and synergistic interaction of sweetpotato feathery mottle potyvirus (SPFMV) and chlorotic stunt crinivirus (SPCSV) (Schaefers and Terry 1976; Cohen et al. 1992; Hoyer et al. 1996; Karyeija et al., 2000). SPFMV is transmitted in a non-persistent, noncirculative manner by aphids (*Myzus persicae*, *Aphis gossypii* and *A. cracivora*) (Stubbs and McLean, 1958), while SPCSV is transmitted semi-persistently by the whitefly (*Bemesia tabaci*) (Schaefers and Terry 1976; Cohen et al., 1992).

Although SPVD is devastating, only a couple of studies of its inheritance have been conducted (Hahn et al. 1981; Ngeve and Bouwkamp, 1991). Genetic analysis of inheritance of SPVD and other traits in sweetpotato has been problematic because sweetpotato is a highly heterozygous hexaploid (x = 15, 2n = 90) (Ting and Kehr, 1953; Jones, 1967;

Magoon et al. 1970; Ukoskit and Thompson, 1997) with complex segregation ratios (Poole, 1955; Jones 1967; Kumagai et al., 1990). Its complex genome and, complex sporophytic self- and cross-incompatibility (Martin 1965, 1970; Jones 1967; Nishiyama et al. 1975; Nakanishi and Kobayashi 1979), and failure of many genotypes to bloom and set seed (Poole 1955; Jones 1967; Magoon et al., 1970) further exacerbate difficulties conducting genetic studies. In most crop species, backcross, testcross, or F_2 populations are usually used to study the segregation of gametes from a heterozygous individual. However, the regular use of backcross or testcross populations for genetic analysis of sweetpotato is not practical due to the factors mentioned above and the strong inbreeding depression observed in backcrosses (Nissila et al., 1999). As a consequence, F_1 progeny resulting from crosses between clones which result in high levels of segregation are used to study the inheritance of traits in sweetpotatoes.

Mwanga et al. (2001) studied the genetic basis of resistance to SPVD in 45 full-sib sweetpotato families in a diallel cross in Uganda. Genetic component analysis revealed highly significant general combining ability (GCA) and specific combining ability (SCA) effects for resistance to SPVD. The GCA to SCA variance ratios were high (0.51-0.87) suggesting that GCA effects were more important than SCA effects in predicting progeny performance for SPVD resistance (Mwanga et al., 2001).

Much attention has been given to the potential applications of DNA markers to improve plant breeding. These technologies open two new ways of improving sweetpotato for virus resistance. First, they provide a basis for accelerated breeding through early selection of traits, and second they may enable more efficient selection of parents for subsequent crosses. DNA based markers such as randomly amplified polymorphic DNA (RAPD), restriction fragment length polymorphisms (RFLP), microsatellites or simple

sequence length polymorphisms (SSLP) and amplified fragment length polymorphisms (AFLP) have been used to detect molecular markers associated with simply inherited and complex traits (quantitative trait loci, QTL), of cabbage (Voorips, 1997), oilseed rape (Pongam et al. 1998), potato (De Jong et al. 1997; Barone et al. 1990; Jacobs et al. 1995; Bendahmane et al. 1997; Bradshaw, 1998), and many other polyploid crops (Jin et al. (1998), and forest trees such as *Populus tremula* (Cervera et al., 1996), European larch (*Larix decidua* Mill.) (Arcade et al., 2000), and pines (*Pinus radiata* D. Don., *P. sylvestris*)(Cato et al. 1999; Lerceteau et al., 2000). The work reported here attempts to combine classical quantitative genetics with DNA-based screening methodologies. Specifically, we report on: 1) quantitative genetic studies focused on examining the inheritance of resistance to SPCSV, SPFMV and SPVD; and 2) the identification of AFLP markers linked to genes conferring resistance to SPCSV and SPFMV.

Materials and Methods

In a previous study, Mwanga (et al. 2001) crossed ten parental (p) sweetpotato clones with varying SPVD resistance in a modified diallel, disregarding reciprocals, using the model II, method 4 mating design of Griffing (1956). The work was conducted during 1996/97 at the Namulonge Agricultural and Animal Production Research Institute (NAARI) in Uganda. The mating design yielded a total of 45 families [(p(p-1)/2]. Details of plant materials, screenhouse and field experimental designs were described by Mwanga et al. (2001). The two experiments described here were conducted to further quantify the genetic basis of resistance to SPVD. The first experiment consisted of a test of 15 promising diallel progenies for resistance to SPVD at NAARI. The second consisted of a

test for resistance to SPCSV and SPFMV, the component viruses of SPVD, and it was conducted at the International Potato Center (CIP), Lima, Peru.

EXPERIMENT 1) - INHERITANCE OF RESISTANCE TO SPVD IN PROMISING DIALLEL PROGENIES. To generate information on possible modes of inheritance (Jones, 1967; Kumagai et al. 1990) of the resistance to SPVD, 15 promising F_1 families from the 45 family diallel cross studied in 1998-2000 (Mwanga et al. 2001) were selected for further study. Each progeny had a large number of individuals, up to 130 genotypes per family (1352 genotypes in total). The plants were grown in 2-liter perforated plastic buckets in the screenhouse using previously described methods (Mwanga et al. 2001). Three plants per genotype were graft-inoculated with SPVD at 1.5 months after planting. SPVD severity was scored at 1.5, 2.5 and 3.5 months after inoculation using a 1-5 scale, where 1 = symptomless and 5 = most severe symptoms (vein clearing, chlorosis, puckering, stunting, tip-die back) (Mwanga et al. 2001). The experiment was conducted during August to December 1998 and repeated during October 1999 to February 2000.

EXPERIMENT 2) - RESISTANCE TO SPCSV, SPFMV AND SPVD IN TWO PROMISING FAMILIES. To examine resistance to SPCSV and SPFMV individually and in dual graft-infection, two crosses between African landrace sweetpotatoes with resistance to SPVD were generated. Family 1 consisted of a cross between Tanzania (TAN) x Bikilamaliya (BMA) with 108 genotypes, while family 2 consisted of a cross between TAN x Wagabolige (WAG) with 96 genotypes. Seed from each cross were sent to CIP, Lima, Peru, and were grown in screenhouses there between May and December 2000. Each of the genotypes in the two families was propagated to furnish 9 plants for three 15.2-cm plastic pots (3 plants per pot).

To generate inoculum of SPFMV and SPCSV the following protocols were

followed. Inoculum of the russet crack strain of SPFMV (RC-SPFMV) was obtained from Dr. Lucho Salazar at CIP and used to infect *Ipomoea setosa* or *I. nil* seedlings using technology described by Cadena-Hinojosa and Campbell (1981). Cotyledons of 1- or 2-leaf stage *I. setosa* seedlings were mechanically inoculated with SPFMV. The seedlings were covered with moistened polythene bags for 3 days after inoculation and then the bags were removed to allow symptoms of SPFMV to develop. Using this procedure, roughly 400 *I. nil* seedlings were mechanically inoculated to supply SPFMV-symptomatic scions for the experiments conducted on the two families at CIP. Like SPFMV, inoculum of SPCSV was also maintained in *I. setosa* (setosa 4) at CIP by Dr. Salazar. However, because SPCSV cannot be transmitted mechanically (Cohen et al., 1992), inoculum was increased by graft inoculating approximately 600 *I. setosa* seedlings with SPCSV-infected scions of *I. setosa.* To induce SPVD, *I. setosa* seedlings with established SPCSV scions were grafted with SPFMV-symptomatic *I. setosa* or *I. nil* scions about one week after they were inoculated with SPCSV.

The sweetpotato plants in the three pots per genotype (3 plants per pot) were treated as follows: 1) each plant in one pot was graft inoculated with a SPFMV-symptomatic (vein clearing, chlorotic spots, purple rings, leaf deformation) *I. setosa* or *I. nil* scion; 2) each plant in the second pot was grafted with a SPCSV-symptomatic *I. setosa* scion; and 3) each plant in the third pot was grafted with SPFMV-symptomatic *I.nil* or *I. setosa* scion and SPCSV-symptomatic *I. setosa* scion to induce SPVD.

Serological assays for SPCSV and SPFMV. All mother plants of the two families TAN x BMA (108 genotypes) and TAN x WAG (96 genotypes) were tested for SPFMV, SPCSV, sweetpotato mild mottle virus (SPMMV), sweetpotato latent virus (SwPLV), sweetpotato chlorotic flecks virus (SPCFV), sweetpotato mild speckling virus (SPMSV), C-6 virus, and sweetpotato caulimovirus (SPCaLV) using nitrocellulose membrane enzyme-

linked immunosorbent assay (NCM-ELISA) kits developed by CIP and North Carolina State University, Raleigh, NC (CIP, 19990). Three leaves (one per plant) were sampled per genotype. In NCM-ELISA, a sample was considered as positive when a bluish-purple color developed on the test spot, and control spots from virus-free sweetpotato retained the color of the membrane without changing after 60 min of reaction with the substrate. Plants testing negative were propagated to furnish cuttings for experimental plants of each genotype. In the progeny tests only SPFMV and SPCSV were tested. SPFMV was detected using NCM-ELISA, while SPCSV was detected using TAS-ELISA as previously described (Mwanga et al. 2001). Optical density (OD) values twice or more the value of control samples were considered as positive in TAS-ELISA. Shoots from plants testing negative in ELISA assays 3 to 4 weeks after inoculation were grafted on *I. setosa* plants to confirm negative readings. The indicator plants were observed for symptom development for 2 months. Presence of virus in *I. setosa* scions of symptomless graft inoculated plants was confirmed and tested by ELISA.

QUANTITATIVE BASIS OF RESISTANCE TO SPVD - SWEETPOTATO INHERITANCE PREDICTED BY EXPECTED THEORETICAL RATIOS. The genetic basis (polygenic vs major gene) of SPVD resistance was hypothesized from observed frequency distributions of 15 promising families in Uganda. Inheritance of SPVD resistance was determined by separating the diallel progenies into resistant and susceptible categories based on the results of ELISA tests for SPCSV and SPFMV. None of the genotypes was immune to either SPCSV or SPFMV as demonstrated by infection following repeated graft inoculation by graft assays on *I.setosa* in genotypes that were negative in ELISA tests. However, genotypes that tested negative in multiple ELISA tests (3 tests with 2-3 replications per test) were considered resistant. Expected resistant: susceptible ratios

for different genetic models of inheritance in Table 1 (Jones 1967; Kumagai et al., 1990) were tested using a chi-squaregoodness-of-fit test.

QUANTITATIVE BASIS OF RESISTANCE TO SPVD – LINKAGE OF AFLP MARKERS WITH VIRUS RESISTANCE. The F_1-mapping population used to construct the AFLP map for this work was originally produced from a two-way pseudotest cross of TAN x WAG in Uganda. Both parents of this cross are resistant to SPVD but WAG is more resistant to SPVD than TAN under field SPVD inoculum pressure (Mwanga et al. 2001). The true seed from this cross was sent to CIP, where the AFLP mapping and SPCSV, SPFMV and SPVD phenotyping were conducted. Details of methods for DNA extraction, AFLP reactions, selection of primer combinations, AFLP marker nomenclature, marker scoring, estimation of recombination fractions, linkage mapping, and genome coverage and segregation ratios were described by Kriegner et al. (2001). AFLP markers present in one parent and absent in the other were tested for 1:1 segregation ratio (present: absence) in the progeny by a goodness-of-fit chi-square test (alpha, 0.05). This single-dose fragment (SDF) mapping strategy, described by Wu et al. (1992), has been widely used for developing genetic maps of polyploid species, such as sugarcane (Al Janabi et al. 1993; Da Silva et al. 1995; Ripol et al., 1999), potato (Li et al., 1998), sour cherry (*Prunus cerasus* L.) (Wang et al., 1998), and alfalfa (Yu and Paulus, 1993). The SDF mapping strategy is based on a simplex segregation pattern. In polyploids and outbreeding tree species, a popular mapping strategy employs the analysis of progeny of a cross between two unrelated heterozygous parents, with the cross being referred to as a two-way pseudotestcross (Grattapaglia and Sederoff, 1994). Linkage analysis of SDFs in coupling phase with markers placed on the mapping population results in two separate maps for each parent based on the male and female sources of markers.

The parental clones TAN and WAG were screened with 82 EcoRI/MseI primer combinations. The combinations resulting in a high number of polymorphic bands for each parental line and a high number (50-100) of amplified DNA fragments per reaction were selected to generate the AFLP MAP. To generate the mapping population, the fragments were scored for the presence/absence of AFLP markers. For the female parent (TAN) a total of 704 fragments were scored, but only 199 segregated in a 1:1 ratio, thus only these were used the linkage analysis. Each of these bands was given a locus name corresponding to the selective base and a number or letter specific for the individual fragment amplified. For the male parent (WAG) 577 AFLP polymorphic bands were identified. However, these were excluded from the analysis because the number of markers segregating in a 1:1 ratio was quite low.

Analysis of linkage relationships among markers and map construction was performed using MAPMAKER/EXP/version 3.0 software (Lander et al., 1987). A maximum detectable recombination fraction of r = 0.25 with a minimum of odds (LOD) score of 4.0 were used to group loci. This procedure placed the AFLP markers derived from segregation in TAN into 53 linkage groups (Fig. 1). This incomplete linkage map spanned 2050 cM with an average of 3 AFLP markers per linkage group and an average length of 38 cM per group.

The linkage map constructed from 199 markers and phenotypic data were entered into QGENE (Nelson, 1997) to conduct the QTL analyses. Phenotypic data for SPCSV, SPFMV, and SPVD (SPCSV and SPFMV) scores were coded as presence (= 1) for positive or absence (= 0) for negative results based on the results of ELISA tests described above. The relation between phenotype (virus resistance) and marker genotype was investigated using the single-point ANOVA and multiple-regression analyses with two markers in the

model by the QGENE program (Nelson, 1997).

Results

EXPERIMENT 1) - INHERITANCE OF RESISTANCE TO SPVD IN PROMISING DIALLEL PROGENIES. Graft inoculation of the 15 families in Uganda resulted in severe SPVD, eliminating class 1 (symptomless, highly resistant) in all the clones (Table 2). Only 4 families, WAG x BMA, WAG x KAN, WAG x SOW, and TAN x BMA, had a SPVD severity rating of 2. Highly susceptible genotypes occurred at high frequencies (e.g. 85% for class 4 and 5) among the families and highly resistant genotypes at much lower frequencies (<1%, class 2). In all the families, the observed distribution of resistant to susceptible genotypes was skewed towards the highly susceptible categories (classes 4 and 5).

EXPERIMENT 2) - RESISTANCE TO SPCSV, SPFMV AND SPVD IN TWO PROMISING FAMILIES. The results of the SPCSV and SPFMV resistance evaluation are presented in Table 3. Based on serological tests the TAN x BMA progenies were classified as 89 resistant: 109 susceptible to SPCSV, and 39 resistant: 159 susceptible to SPFMV. The TAN x WAG progenies were classified by ELISA as 15 resistant: 81 susceptible to SPCSV, and 45 resistant: 51 susceptible to SPFMV. Segregation analysis of resistance to SPCSV showed that the ratios of resistant to susceptible progenies in family TAN x BMA fit a 1:1 Mendelian ratio (chi-square = 2.02, p = 0.156), while the TAN x WAG family observed segregation ratios (OSRs) fit 4:1 and 5:1 ratios, respectively (chi-square = 3.26, p = 0.071 and chi-square = 0.08, p = 0.784). For inheritance of resistance to SPFMV, the OSR in family TAN x WAG fit a 1:1 ratio (chi-square = 0.38, p = 0.541),

whereas in family TAN x BMA, the OSRs fit 3:1, 4:1 and 5:1 ratios (chi-square = 2.97, P = 0.084; chi-square = 0.01, p = 0.092; chi-square = 1.31, p = 0.253), respectively.

Graft inoculating the two families (TAN x BMA and TAN x WAG) with SPCSV and SPFMV to induce SPVD at CIP resulted in severe SPVD in all the genotypes except two. Repeated graft inoculations of these two genotypes resulted in severe SPVD in all the genotypes similar to results obtained in Uganda.

QTL ANALYSIS OF RESISTANCE TO SPCSV AND SPFMV IN TAN x WAG. Table 4 shows the results of single-marker linear regression analysis for association of AFLP markers with virus resistance in the TAN x WAG population. Based on single point regression analyses, two markers, e41m33.a, and e38m36.u located on linkage groups 22 and 35, respectively, were highly significant (<0.0001) for resistance to SPCSV, and marker, S13.1130 located on linkage group 6 was highly significant (p<0.0001) for resistance to SPFMV. A multiple regression model incorporating markers, e41m33.a and e38m36.u, explained 72% of the variation in resistance to SPCSV, while markers S13.1130 and e33m59.a explained 71% of the resistance to SPFMV, respectively (Table 5). Markers e39m36.j (p = 0.0032) on linkage group 47 linked to SPCSV resistance and e40m36.d (p = 0.0158) on linkage group 43 linked to SPFMV resistance (Table 4) were minor QTLs that need to be confirmed in future investigations. Addition of the second locus in the multiple regression model did not significantly increase the percent variation explained in resistance to SPCSV or SPFMV, indicating the importance of e41m33.a and S13.1130.

QTL analysis results of resistance to dual infection of the progenies by SPCSV and SPFMV (SPVD) were not significant, and the correlation between AFLP markers and SPVD resistance was low (Table 4).

Discussion

Cytological studies suggest that the sweetpotato genome has three subgenomes ($B_1B_1B_2B_2B_2B_2B_2B_2$) (Shiotani and Kawase, 1987) and that two of them are very similar (Jones 1965; Ting and Kehr 1953; Shiotani and Kawase, 1987). Chromosome pairing in sweetpotato is as bivalents with very few quadrivalents. Assuming no double reduction, predominantly hexasomic ratios would indicate that all three genomes are similar, whereas predominantly disomic ratios would suggest that the three genomes are distinct. Prevalence of tetrasomic or tetradisomic ratios would support the hypothesis of two similar and one different genome (Jones, 1967). Because of a lack of precise information on the genomic constitution and the presence of up to three genomes which may be similar or different, four possible models (with dosage effects) of inheritance (hexasomic, tetradisomic, tetrasomic, and disomic) of one or more genes for sweetpotato were proposed (Table 1) by Jones (1967). For our studies, we excluded testing for segregation ratios for hypothesis 4 and quadruplex inheritance, because they require very large populations for verification, and our populations were small (96 genotypes for TAN x BMA, 198 genotypes for TAN x WAG).

Graft inoculation of progenies with SPVD resulted in severe SPVD in almost all clones of the 15 promising diallel families (1352 genotypes) in Uganda. The distribution of SPVD scores in the populations was skewed towards the highly susceptible category (score 5) and very few highly resistant genotypes were observed (score 1 and 2). Similarly, when TAN x BMA and TAN x WAG were graft inoculated with SPCSV and SPFMV to induce SPVD at CIP, severe SPVD was expressed in most of the 294 genotypes tested. Our chi-square analyses of the observed vs expected phenotypic data for the TAN x BMA and TAN x WAG families for resistance to SPCSV and SPFMV suggest that these two viruses are

controlled by two, separate major genes. The expected genetic segregation ratios used for the chi-square analyses were based on the assumption of random chromosome segregation, since the occurrence of primary bivalent pairing with limited quadravalent at meiosis allows the assumption of double reduction (Jones, 1967).

The segregation ratios observed in the two families (TAN x BMA and TAN x WAG) in the present study are similar to other traits (e.g. flowering vs non-flowering, red stems vs green stems, storage roots vs no storage roots) studied by Poole (1955), and presence vs absence of ß-amylase activity in storage roots observed by Kumagai et al. (1990). Although Jones' (1967) models for major gene inheritance were based on testcrosses, tests conducted by Poole (1955) and Kumagai et al. (1990) included testcrosses and other types of crosses (e.g. open pollination and single crosses) similar to the crosses used in the current study. Therefore, interpreting the results obtained in this study by Jones'(1967) inheritance models (Table 1) is valid, pending validation by testcrosses and further molecular investigations.

According to the four models of inheritance in sweetpotato (hexasomic, tetradisomic, tetrasomic, disomic) proposed by Jones (1967) and elucidated by Kumagai et al. (1990), SPCSV resistance in this study follows a simplex hexasomic and simplex tetradisomic/tetrasomic model in family TAN x BMA, and duplex hexasomic and tetradisomic/tetrasomic in family TAN x WAG. The inheritance of SPFMV exhibited simplex hexasomic and simplex tetradisomic/tetrasomic pattern in family TAN x WAG while it followed duplex hexasomic and tetradisomic/tetrasomic inheritance pattern in family TAN x BMA (Tables 1 and 3).

Dominance effects for SPCSV and SPFMV resistance genes were ruled out because all resistant genotypes would have at least one dominant allele. That would mean that at

least 50% of all progenies derived from the resistant parent would be resistant regardless of the model of inheritance (disomic, tetrasomic, tetradisomic, hexasomic). The frequency of genotypes resistant to SPCSV in TAN x BMA was 45.0% and to SPFMV was 19.7%, while the frequency of genotypes resistant to SPCV in TAN x WAG was 15.6% and to SPFMV was 40.6%.

The exact allelic frequencies or dosage effects could not be estimated in this study. The allelic composition and allelic diversity of a particular genotype may be revealed by molecular techniques and studies on segregation of identified major genes. Although our data suggest that the frequency of resistant SPFMV and SPCSV alleles is high, the resistant phenotypes are not as common due to the polysomic nature of sweetpotato and the traits being recessive.

There are many examples of virus resistance being conditioned by major recessive genes in plants. Resistance to bean yellow mosaic virus (BYMV) in the bean cultivar, Great Northern 1140, is conditioned by a single recessive gene (Provvidenti and Schroeder, 1973). In barley, resistance to barley yellow dwarf virus (BYDV) may be conditioned by dominant or recessive genes depending on environmental conditions (Jones and Catherall, 1970). In pepper, *Capscicum chinense* (PI 159236), resistance to a pepper strain of potato virus Y (PVY pathotype 1-2) is controlled by a major recessive gene (Boiteux et al., 1996). Fraser (1988) gives more examples of recessive resistance genes for plant viruses. Some recessive genes may completely suppress virus multiplication conferring immunity (Provvidenti and Schroeder, 1973), while others (Catherall et al., 1970) may confer resistance in a similar manner to those for resistance to SPCSV and SPFMV by reducing symptom severity and virus multiplication, but maintaining virus levels which can still be detected.

Studying resistance to SPCSV and SPFMV in the same plant is very difficult. It has

been previously demonstrated that the resistance to SPFMV and SPCSV breaks down when the two viruses co-infect the same plant resulting in severe SPVD symptoms (Schaefers and Terry 1976; Karyeija et al. 2000; Mwanga et al., 2001). The loss of resistance is expressed as occurrence of SPFMV symptoms and high absorbance values, indicating high SPFMV and/or SPCSV titers in the plant, eliminating the resistant category. For SPFMV, only highly susceptible cultivars suffer significant yield losses (Clark and Moyer, 1988). Economic loss due to SPFMV is mostly associated with external cracking and internal corkiness, which render storage roots unmarketable. In contrast, yield losses due to SPCSV are quite high with up to 87% loss in storage root yield being reported in screenhouse experiments (Gibson et al., 1998).

Based on our preliminary AFLP mapping studies, SPCSV resistance was associated with AFLP markers e41m33.a on linkage group 22 and e38m36.u on linkage group 35. Marker e41m33.a explained 66% of the variation in SPCSV resistance in single-marker regression analysis. When the two markers were evaluated in a multiple regression model, they explained 72% of the total variation in SPCSV resistance (Table 5). Because marker e41m33.a explained such a high percentage of the variation in SPCSV resistance we speculate that this marker is linked to a major recessive gene for resistance to SPCSV. We propose the name, s*pcsv1* (sweetpotato chlorotic stunt virus 1), for the gene mediating SPCSV resistance.

SPFMV resistance was associated with two AFLP markers, S13.1130 and e33m.59a on chromosome groups 6 and 37, respectively. Marker S13.1130 explained 70% of the resistance to SPFMV and the two markers (S13.1130 and e33m.59a) accounted for 71% of the total variation to SPFMV resistance when they were combined in a multiple regression model. As with SPCSV, because marker S13.1130 explained such a high percentage of

resistance to SPFMV, it is also likely associated with another major recessive gene. Thus we propose the name s*pfmv1* (sweetpotato feathery mottle virus 1) for the gene mediating resistance for SPFMV.

The phenotypic segregation ratios 1:1, 3:1, 4:1, and 5:1 obtained for resistance to SPCSV and SPFMV (Table 3) in TAN x WAG and TAN x BMA support the hypothesis that resistance to these viruses is governed by two separate major genes. The additional QTLs associated with SPCSV and SPFMV resistance suggest that there may also be one or more minor genes associated with SPCSV and SPFMV resistance. Similar results have been reported by Nandi et al. (1997) who localized QTLs linked to a major gene for submergence tolerance in rice, and Pongam et al. (1998) who identified QTLs linked to a single gene that governs avirulence for blackleg disease caused by *Leptoshaeria maculans* in rape seed. AFLP markers linked to a dominant gene (N6) that confers strain-specific hypersensitive resistance against potato virus X (PVX) was also reported by De Jong et al. (1997).

These studies represent the first quantitative and molecular genetic investigations of the inheritance of resistance to SPCSV, SPFMV and SPVD. Results from this work should provide impetus for more investigations using DNA markers for genetic studies of these important viruses and lead to improvements in breeding for SPVD resistance in sweetpotato. However, additional tests will be necessary to: 1) confirm that SPCSV and SPFMV are indeed governed by two major genes; 2) determine if the two genes present in a common background will suppress SPVD effectively; 3) establish if there are other genes conditioning resistance to SPVD and determine their nature, and 4) determine the exact allelic composition and allelic diversity of the genes conditioning SPVD resistance using molecular techniques and inheritance studies.

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Table 1. Expected phenotypic ratios (resistant: susceptible) in testcrosses involving virus resistance genes with dosage effects according to four cytological hypotheses, assuming sweetpotato genomic constitution is $R_1R_1R_2R_2R_2R_2$ (R = dominant, r = recessive). Tetradisomic and tetrasomic inheritance exhibit similar phenotypic ratios (Jones, 1967). Segregation ratios verified by Kumagai et al. 1990.

Gene dose	Hypothesis 1		Hypotheses 2 and 3		Hypothesis 4	
			Hypothesis 2	Hypothesis 3		
	Autohexaploid (hexasomic) non preferential pairing , $R_1 = R_2$		Tetradisomic Strict preferential pairing for R_1, R_2; genes on R_1 have disomic inheritance, genes on R_2 tetrasomic	Tetrasomic Alleles for resistance located on R_2 genome	Allohexaploid (disomic) Allele for resistance located on R_1	
Simplex	Rrrrrr	1:1	Rrrr rr	1:1	Rr rr rr	1:1
			Rrrr Rr	1:1		
Duplex	RRrrrr	4:1	RRrr rr	5:1	Rr Rr rr	3:1
			Rrrr Rr	3:1	RR rr rr	1:0
			Rrrr RR	1:0		
Triplex	RRRrrrr	19:1	RRRr rr	1:0	Rr Rr Rr	7:1
			RRrr Rr	11:1	RR Rr rr	1:0
			Rrrr RR	1:0		
Quadruplex	RRRRrr	1:0	RRRR rr	1:0	RR Rr Rr	1:0

Table 2. Frequency distribution of sweetpotato virus disease (SPVD) severity scores among promising families generated in the diallel cross (SPVD rating, 1 = no symptoms; 5 = very severe symptoms). Tests were conducted at Namulonge, Uganda, 1999-2000.

Family	SPVD rating and number of genotypes per class					
	1	2	3	4	5	Total
WAG x TAN	0	0	18	37	40	95
WAG x BMA	0	2	18	28	52	100
WAG x KAN	0	3	10	25	60	98
WAG x BWA	0	0	18	25	56	99
WAG x SOW	0	1	24	42	45	112
TAN x BMA	0	1	8	36	39	84
TAN x TOR	0	0	12	53	64	129
TAN x BWA	0	0	16	54	32	102
BMA x KAZ	0	0	7	32	43	82
BMA x TOR	0	0	10	29	51	90
KAZ x KAN	0	0	5	23	60	88
KAZ x BWA	0	0	21	40	41	102
NKA x TOR	0	0	6	20	32	58
NKA x CTA	0	0	9	13	35	57
KAN x CTA	0	0	9	15	32	56
Total No. of genotypes/class	0	7	191	472	682	1352

Table 3. Segregation of two sweetpotato families for resistance to sweetpotato chlorotic stunt virus (SPCSV) and sweetpotato feathery mottle virus (SPFMV), International Potato Center (CIP), Lima, Peru, 2000.

		No of genotypes		Hypothesis I		Hypothesis II and III	
		Elisa test		Ratio	Chi-square	Ratio	Chi-square
Family	Virus	Negative	Positive	tested		tested	
TAN x BMA	SPCSV	89	109	1:1	2.02	1:1	2.02
						3.1	34.35**
	SPFMV	39	159	1:1	72.73**	1:1	72.73**
				4:1	0.01	3:1	2.97
						5:1	1.31
						11:1	33.47**
TAN x WAG	SPCSV	15	81	1:1	45.38**	1:1	45.38**
				4:1	3.26	3:1	4.50*
						5:1	0.08
						11:1	6.68**
	SPFMV	45	51	1:1	0.38	1:1	0.38
						3:1	12.25**

*, ** Significant deviation from ratio tested at P = 0.05 and P = 0.01 levels, respectively

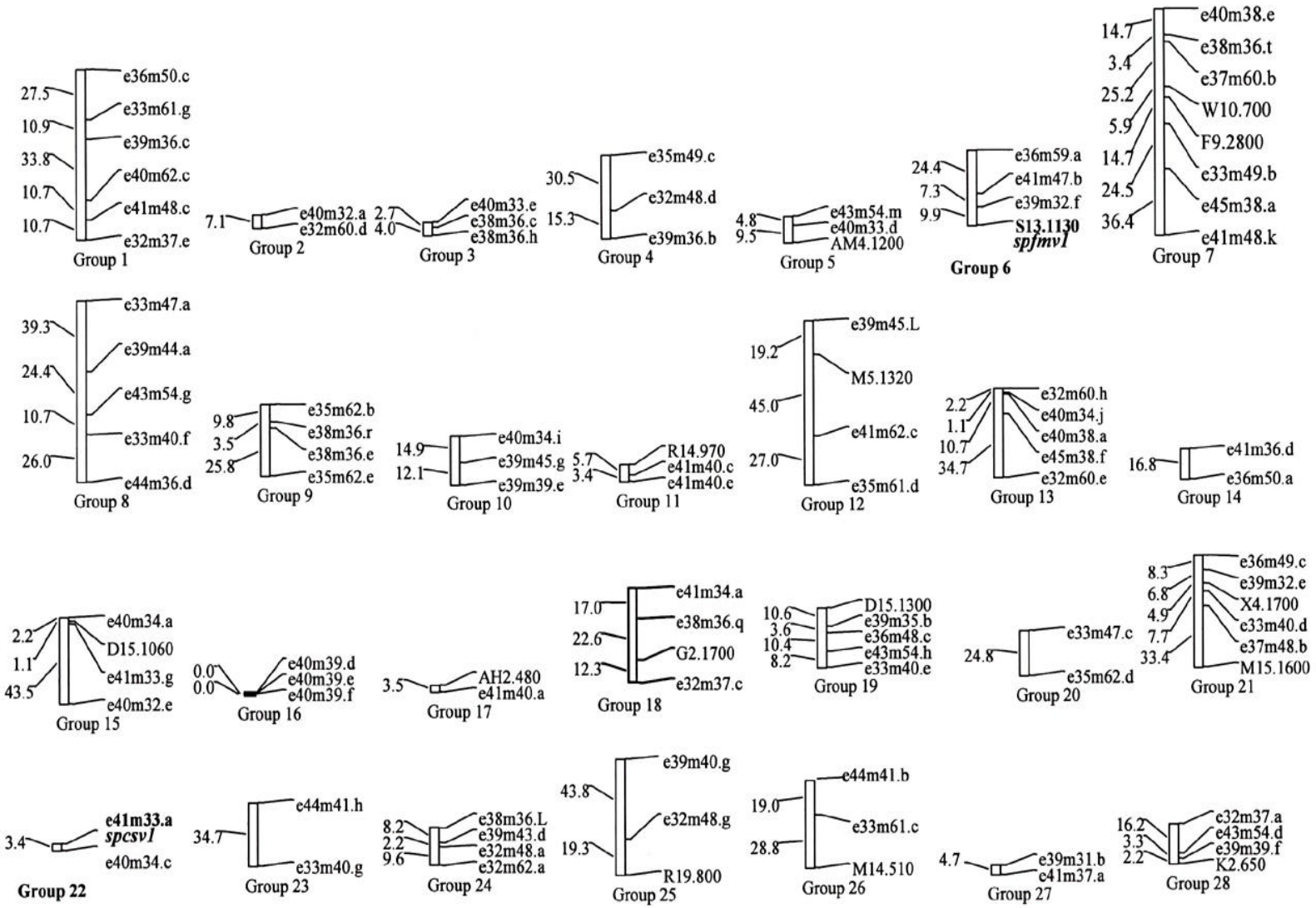
Table 4. Selected AFLP markers, for sweetpotato chlorotic stunt virus (SPCSV), sweetpotato feathery mottle virus (SPFMV), and sweetpotato virus disease (SPVD) and associated linkage groups for Tanzania, their F-, R square and p-values.

SPCSV					
Marker	Linkage group	Number of genotypes (n)	F-value	R^2	P-value
e41m33.a	22	94	175.94	0.66	<0.0001
e40m34.c	22	94	103.75	0.53	<0.0001
e38m36.u	35	94	21.09	0.19	<0.0001
e36m49.a	35	94	14.04	0.13	0.0003
e39m40.c	35	94	9.30	0.09	0.0030
e39m36.j	47	94	9.15	0.09	0.0032
e35m49.d	47	94	7.85	0.08	0.0062
R9.650	35	94	6.56	0.07	0.0121
e39m36.a	47	94	6.56	0.07	0.0121
SPFMV					
Marker	Linkage group	Number of genotypes (n)	F-value	R^2	P-value
S13.1130	6	94	212.64	0.70	<0.0001
e41m47.b	6	94	35.37	0.28	<0.0001
e36m59.a	6	94	10.26	0.10	0.0019
e33m59.a	37	94	8.34	0.08	0.0048
e41m33.a	22	94	7.44	0.07	0.0076
e38m36.u	35	94	6.71	0.07	0.0111
e40m36.d	43	94	6.05	0.06	0.0158
e36m49.a	35	94	6.05	0.06	0.0158
e44m41.g	33	94	5.84	0.06	0.0176
SPVD					
Marker	Linkage group	Number of genotypes (n)	F-value	R^2	P-value
e35m49.c	4	94	4.48	0.05	0.0371
e32m48.d	4	94	4.48	0.05	0.0371
e32m48.a	24	94	4.09	0.04	0.0459
Y20.650	29	94	4.09	0.04	0.0459
e43m54.m	5	94	4.09	0.04	0.0459
e39m43.d	24	94	4.09	0.04	0.0459
e40m38.d	36	94	3.91	0.04	0.0509

Table 5. Results of Kendell's tau and multiple regression (R^2) analysis results with two AFLP markers in the model for markers linked with sweetpotato chlorotic stunt virus (SPCSV) and sweetpotato feathery mottle virus (SPFMV) resistance. Values in parentheses are for the full multiple regression model with 94 genotypes and 199 AFLP markers included in the genetic map for Tanzania (the female parent).

Trait	AFLP marker	Linkage group	Kendell's tau statistic		R square		
			b-value	p-value	R^2	F-value	p-value
SPCSV							
	e41m33.a	22	0.81	<0.0001	0.72 (0.66)	108.41 (175.94)	<0.0001 (<0.0001)
	e38m36.u	35	0.43	<0.0001	(0.19)	(21.1)	0.0002 (<0.0001)
SPFMV							
	s13.1130	6	0.84	<0.0001	0.71 (0.70)	113.96 (212.64)	<0.0001 (<0.0001)
	e33m59.a	37	0.29	<0.0001	(0.08)	(8.34)	0.0236 (0.0048)

Fig. 1. Sweetpotato maternal (Tanzania) linkage map based on amplified fragment length polymorphism (AFLP) markers. Map distances (on left) are in cM. Each marker locus represents the individual fragments amplified by the same primer combination. Markers associated with resistance to sweetpotato chlorotic stunt virus (SPCSV) on linkage groups 22 and 35, and sweetpotato feathery mottle virus (SPFMV) on linkage groups 6 and 37 are in bold.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10.9
33.8
10.7
10.7
e36m50.c
e33m61.g
e39m36.c
e40m62.c
e41m48.c
e32m37.e
Group 1
7.1
e40m32.a
e32m60.d
Group 2
2.7
4.0
e40m33.e
e38m36.c
e38m36.h
Group 3
30.5
15.3
e35m49.c
e32m48.d
e39m36.b
Group 4
4.8
9.5
e43m54.m
e40m33.d
AM4.1200
Group 5
24.4
7.3
9.9
e36m59.a
e41m47.b
e39m32.f
S13.1130
spfmv1
Group 6
14.7
3.4
25.2
5.9
14.7
24.5
36.4
e40m38.e
e38m36.t
e37m60.b
W10.700
F9.2800
e33m49.b
e45m38.a
e41m48.k
Group 7
39.3
24.4
10.7
26.0
e33m47.a
e39m44.a
e43m54.g
e33m40.f
e44m36.d
Group 8
9.8
3.5
25.8
e35m62.b
e38m36.r
e38m36.e
e35m62.e
Group 9
14.9
12.1
e40m34.i
e39m45.g
e39m39.e
Group 10
5.7
3.4
R14.970
e41m40.c
e41m40.c
Group 11
19.2
45.0
27.0
e39m45.L
M5.1320
e41m62.c
e35m61.d
Group 12
2.2
1.1
10.7
34.7
e32m60.h
e40m34.j
e40m38.a
e45m38.f
e32m60.e
Group 13
16.8
e41m36.d
e36m50.a
Group 14
2.2
1.1
43.5
e40m34.a
D15.1060
e41m33.g
e40m32.e
Group 15
0.0
0.0
e40m39.d
e40m39.e
e40m39.f
Group 16
3.5
AH2.480
e41m40.a
Group 17
17.0
22.6
12.3
e41m34.a
e38m36.q
G2.1700
e32m37.c
Group 18
10.6
3.6
10.4
8.2
D15.1300
e39m35.b
e36m48.c
e43m54.h
e33m40.e
Group 19
24.8
e33m47.c
e35m62.d
Group 20
8.3
6.8
4.9
7.7
33.4
e36m49.c
e39m32.e
X4.1700
e33m40.d
e37m48.b
M15.1600
Group 21
3.4
e41m33.a
spcsv1
e40m34.c
Group 22
34.7
e44m41.h
e33m40.g
Group 23
8.2
2.2
9.6
e38m36.L
e39m43.d
e32m48.a
e32m62.a
Group 24
43.8
19.3
e39m40.g
e32m48.g
R19.800
Group 25
19.0
28.8
e44m41.b
e33m61.c
M14.510
Group 26
4.7
e39m31.b
e41m37.a
Group 27
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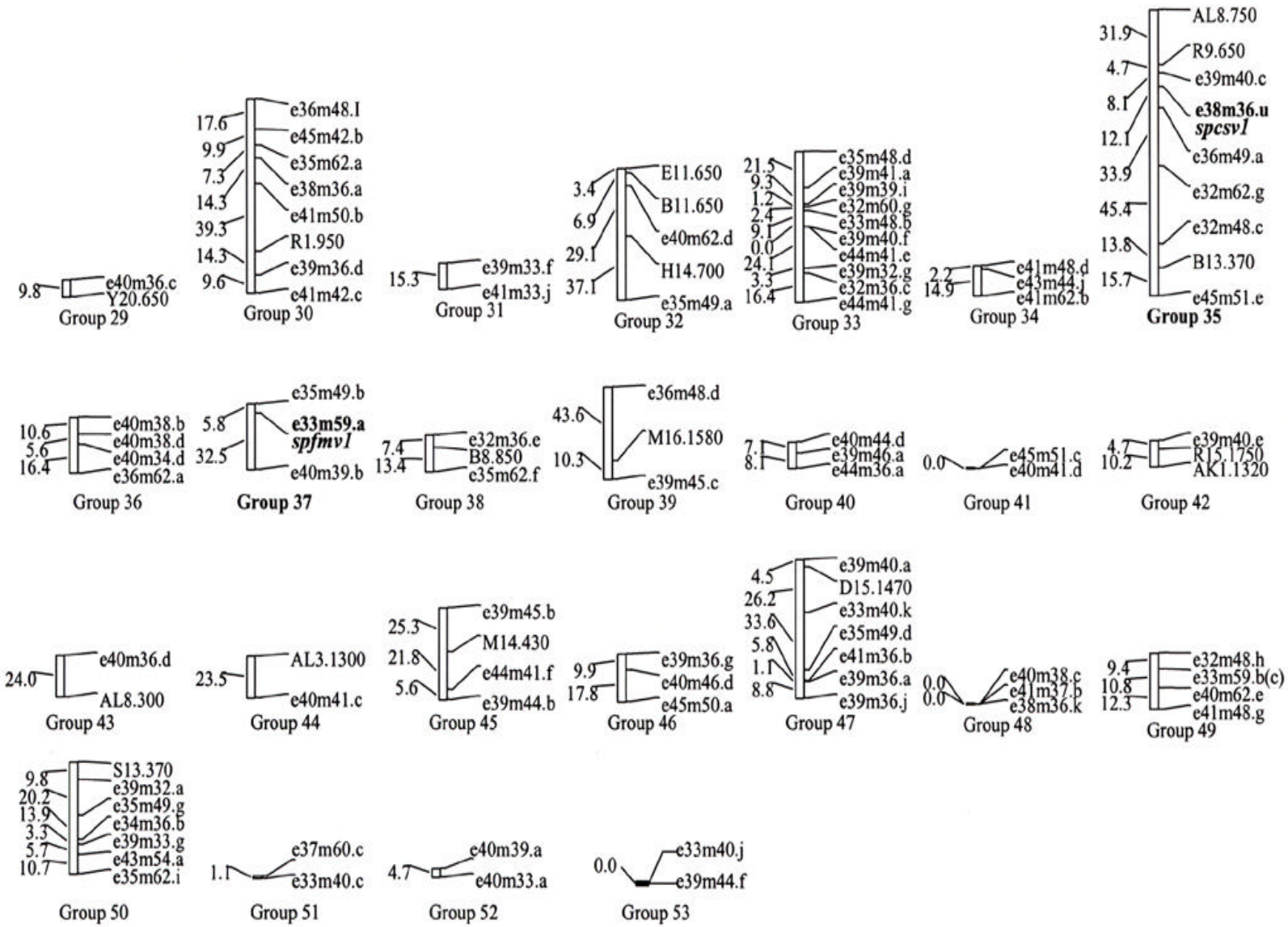
3.3
2.2
e32m37.a
e43m54.d
e39m39.f
K2.650
Group 28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Group 29
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e41m50.b
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e39m36.d
e41m42.c
Group 30
15.3
e39m33.f
e41m33.j
Group 31
3.4
6.9
29.1
37.1
E11.650
B11.650
e40m62.d
H14.700
e35m49.a
Group 32
21.5
9.3
1.2
2.4
9.1
0.0
24.1
3.3
16.4
e35m48.d
e39m41.a
e39m39.i
e32m60.g
e33m48.b
e39m40.f
e44m41.e
e39m32.g
e32m36.c
e44m41.g
Group 33
2.2
14.9
e41m48.d
e43m44.j
e41m62.b
Group 34
31.9
4.7
8.1
12.1
33.9
45.4
13.8
15.7
AL8.750
R9.650
e39m40.c
e38m36.u
spcsv1
e36m49.a
e32m62.g
e32m48.c
B13.370
e45m51.e
Group 35
10.6
5.6
16.4
e40m38.b
e40m38.d
e40m34.d
e36m62.a
Group 36
5.8
32.5
e35m49.b
e33m59.a
spfmv1
e40m39.b
Group 37
7.4
13.4
e32m36.e
B8.850
e35m62.f
Group 38
43.6
10.3
e36m48.d
M16.1580
e39m45.c
Group 39
7.1
8.1
e40m44.d
e39m46.a
e44m36.a
Group 40
0.0
e45m51.c
e40m41.d
Group 41
4.7
10.2
e39m40.e
R15.1750
AK1.1320
Group 42
24.0
e40m36.d
AL8.300
Group 43
23.5
AL3.1300
e40m41.c
Group 44
25.3
21.8
5.6
e39m45.b
M14.430
e44m41.f
e39m44.b
Group 45
9.9
17.8
e39m36.g
e40m46.d
e45m50.a
Group 46
4.5
26.2
33.6
5.8
1.1
8.8
e39m40.a
D15.1470
e33m40.k
e35m49.d
e41m36.b
e39m36.a
e39m36.j
Group 47
0.0
0.0
e40m38.c
e41m37.b
e38m36.k
Group 48
9.4
10.8
12.3
e32m48.h
e33m59.b(c)
e40m62.e
e41m48.g
Group 49
9.8
20.2
13.9
3.3
5.7
10.7
S13.370
e39m32.a
e35m49.g
e34m36.b
e39m33.g
e43m54.a
e35m62.i
Group 50
1.1
e37m60.c
e33m40.c
Group 51
4.7
e40m39.a
e40m33.a
Group 52
0.0
e33m40.j
e39m44.f
Group 53

CHAPTER 5

Summary: Nature of resistance and response of sweetpotato to sweetpotato virus disease

Summary: Nature of resistance and response of sweetpotato to sweetpotato virus disease

Sweetpotato virus disease (SPVD), due to the synergistic interaction of sweetpotato feathery mottle potyvirus (SPFMV) and sweetpotato chlorotic stunt crinivirus (SPCSV), causes up to 98% yield loss in sweetpotato in East Africa. Evaluating sweetpotatoes for resistance to SPVD is slow and inefficient. Currently, development of cultivars with increased resistance is considered to be the only effective control strategy. This study was conducted to determine the inheritance of resistance to SPVD in sweetpotato and to estimate the nature of genetic variance. Ten parental clones varying in resistance to SPVD were crossed in a half diallel mating design to generate 45 full-sib families. The families were graft-inoculated with SPVD and evaluated for resistance to SPVD in a randomized complete block design at two sites in Namulonge, Uganda during 1998-2000. Serological assays and the indicator plant, *Ipomoea setosa*, were used to detect SPFMV and SPCSV. Methods for screening large sweetpotato populations (5,000-100,000) for resistance to SPVD were evaluated. The genetic basis of resistance to sweetpotato virus disease (SPVD) was investigated in 15 promising sweetpotato diallel families (1352 genotypes) in Uganda, and two families of the same diallel at the International Potato Center (CIP), Lima, Peru.

SPVD-infected *I. setosa* plants, normally used as the source of scions for graft infecting sweetpotatoes with viral diseases, were severely stunted and plant mortality was 10-30%. However, we found that the cultivated landrace of *I. batatas*, Tanzania, was a superior host for maintaining and increasing SPVD inoculum (scions) for mass grafting. Further, modified cleft grafting, increased survival of grafted SPVD symptomatic scions from 5 to 100%.

Graft inoculation of the 15 families with SPVD resulted in severe SPVD in all the

families in Uganda. The distribution of SPVD scores was skewed toward highly susceptible categories (SPVD scores 4 and 5), eliminating almost all the resistant categories (scores 1 and 2). Graft inoculation with SPVD of two families (Tanzania x Bikilamaliya and Tanzania x Wagabolige) of the same diallel made in Uganda with SPVD produced severe SPVD in all progenies (294 genotypes) at CIP, Lima. The inoculation results from Uganda and Peru demonstrate that SPVD resistance does not exhibit a normal distribution pattern.

Resistance to symptom development and recovery from initial systemic symptoms, both of importance to production, characterised resistant genotypes. Genetic component analysis showed significant effects for both general combining ability (GCA) and specific combining ability (SCA) for resistance to SPVD. GCA effects were more important than SCA effects as suggested by the large ratios (0.51-0.87) of GCA to SCA variance components.

Resistant parents exhibited high GCA indicating that additive gene effects were predominant in the inheritance of resistance to SPVD and recovery. Narrow-sense heritability was moderate (31-41%) and broad-sense heritability was high (73-98%) indicating that rapid genetic gains for SPVD resistance can be accomplished by mass selection breeding techniques. Two genotypes, New Kawogo (NKA) and Sowola (SOW), had high negative GCA effects and should be promising parents for enhancement of SPVD resistance. NKA yielded several families in specific crosses, which exhibited rapid recovery from SPVD, indicating that it is a good source for recovery and reducing SPVD severity.

Current methods for screening large sweetpotato populations for resistance to SPVD can be improved. This study demonstrates that under conditions in Uganda, use of a suitable sweetpotato genotype for maintenance and increase of SPVD inoculum, appropriate cleft graft inoculation modifications, and SPVD scoring techniques, can lead to rapid

progress in screening large sweetpotato populations for SPVD resistance. Criteria to characterize genotypes for SPVD resistance based on symptoms indicated significant variation among families and among genotypes within families. Recovery was a very important component of SPVD resistance. There were genotypic differences for recovery from SPVD among 36 sweetpotato families evaluated for SPVD resistance, indicating the potential for control of SPVD and contribution to SPVD resistance by sweetpotato genotypes with rapid recovery.

Inoculation of the two families with SPCSV and SPFMV, and Mendelian segregation analysis for resistant vs susceptible categories for the two viruses based on enzyme-linked immunosorbent assay (ELISA) results suggest that resistance to SPCSV and SPFMV is conditioned by two, separate major recessive genes. In the proposed model for inheritance, the two genes are inherited in a hexasomic or tetradisomic manner. We have identified two amplified fragment polymorphic length polymorphism (AFLP) markers, one associated with the locus conferring resistance to SPCSV, and the second linked to the locus conferring resistance to SPFMV in these progenies. The gene for resistance to SPCSV explained 70% of total variation in SPCV resistance while the gene for resistance to SPFMV explained 66% of the total variation in SPFMV resistance. We propose s*pcsv1* and s*pfmv1* to be the names of the two genes. The AFLP and QTL analyses verify the presence of a single gene mediating resistance and also suggests that additional genes mediate oligogenic or multigenic horizontal (quantitative) resistance to both viruses.

The studies reported here indicate that resistance to SPVD can be transferred into improved cultivars by utilizing parents that possess high levels of resistance to SPVD and the ability to recover from SPVD after infection. Future work on resistance to SPVD should include: 1) the introgression of genes for extreme resistance to SPCSV and SPFMV

from wild relatives of sweetpotato (*Ipomoea cairica* and *I. involucra*) into improved sweetpotato cultivars; 2) refinement of methodologies to rapidly identify genotypes with fast recovery from SPVD, and the definition and optimization of the conditions for screening fast recovering genotypes; 3) determination of epistatic effects and their significance in SPVD resistance and recovery and whether these effects can be exploited for rapid gains in breeding for resistance to SPVD; and 4) determination of the mechanisms of resistance to SPVD.

Graft inoculation modifications in this study and the resulting symptoms used as criteria for defining resistant genotypes enabled screening of 27,000 sweetpotato plants for resistance to SPVD in two seasons in 1998/1999. Graft inoculation was efficient in transmitting SPVD and was reliable for inoculating large numbers of sweetpotato genotypes but requires large quantities of the inoculum (cultivar, TAN in Uganda's case), screenhouse space, pest control and is still labor intensive although with much less requirements compared to using SPVD infected *I. setosa* scions for inoculum. It remains a challenge to come up with a more rapid, efficient and less labor intensive way of inoculating sweetpotato with SPVD. Symptom expression used as the criteria for characterizing genotypes for resistance to SPVD may be influenced by environmental factors. There is need to develop a rapid and simple method or assay for identification of SPVD and SPVD resistance. More studies are also needed to understand the role of recovery in alleviating yield losses under different SPVD inoculum pressures after successive crop growth cycles, and the contribution of plant recovery to SPVD resistance.

AFLP markers and QTL studies have indicated that genetic factors have major effects on SPCSV and SPFMV. Results from these investigations should provide impetus for more investigations using DNA markers for genetic studies and breeding for SPVD

resistance. The genetic basis of the resistance to SPVD has been investigated and the results suggest that resistance to SPVD is conditioned by two major genes, one for SPCSV and the other for SPFMV. However, additional tests will be necessary: 1) to confirm that SPCSV and SPFMV are governed by two major genes; 2) to establish whether there are other genes involved in the resistance of SPVD resistance and determine their nature 3) to determine the exact allelic composition and allelic diversity of the genes conditioning SPVD resistance using molecular techniques and inheritance studies. The generated information will reveal the type of genotypes to use as best parents and the most effective mating design to combine the best parents.

APPENDIX

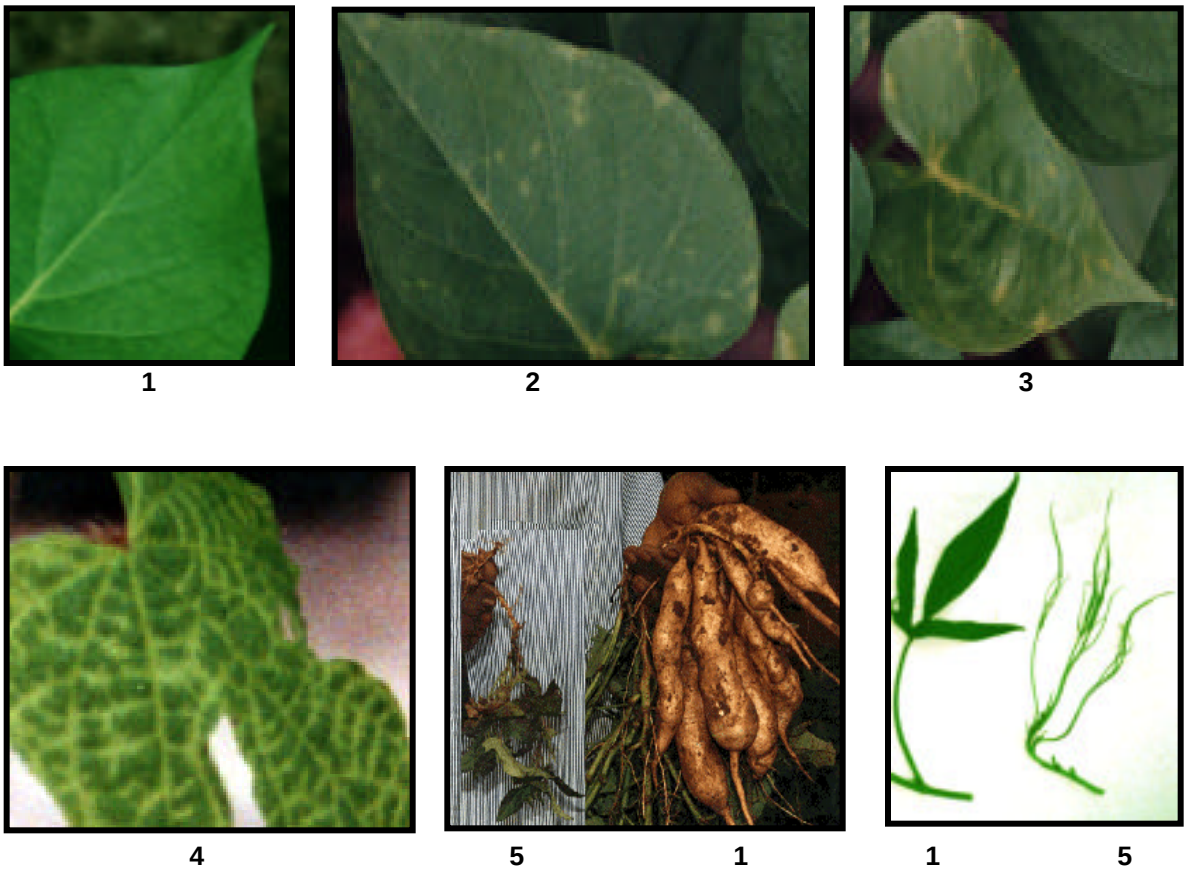

Fig. 1. Sweetpotato virus disease (SPVD) severity rating scale (1 = no symptoms; 2 = mild symptoms; 3 = moderate symptoms; 4 = severe symptoms; 5 = very severe symptoms).