Detection of a quantitative inherited resistance to SPCSV by crossing DLP3163 with OFSP clones

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Abstract

Sweetpotato is a tropical crop considered as the seventh most important crop of the world. However, Sweetpotato virus disease (SPVD), produced by the co-infection of the *Sweet potato clorotic stunt virus* (SPCSV) and *Sweet potato feathery mottle virus* (SPFMV), results in serious yield looses in most regions of the world. No resistance to SPCSV has been identified and proofed so far, but a clone DLP3163 (CIP 420269) from CIP germplasm collection was recently considered as a source of resistance to SPCSV in a previous screening. The objectives of this study were to confirm the resistance of DLP3163 and to determine if this resistance is inherited. In total 103 clones of the population "Jewel I" were crossed with DLP3163. All parents (N = 104) and the offsprings (N = 707) were propagated and grown under greenhouse conditions. Parents and offspring were grafted with scions of *Ipomoea setosa*. For each genotype three plants were used. Symptom expressions and ELISA test evaluations for both viruses were carried 1 month after grafting and repeated three times on each plant replication. Visual ELISA reactions were recorded. The mean across each repeated measurement and plant replication was calculated by genotypes. DLP3163 was not negative for SPCSV across all ELISA tests, but it showed a low mean and median for recorded ELISA scores. Significance tests revealed significant lower SPCSV scores in the offspring group compared to the parental group. The most striking result of this study was that some new genotypes were found, which were tested negative across all SPCSV ELISA tests. In conclusion, DLP3163 is not immune but exhibit a quantitative inherited resistance to SPCSV and we might have found several new clones with resistance to SPCSV.

Introduction

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). There are more than 20 viruses known to infect cultivated sweetpotato worldwide (Loebenstein et al, 2003), the whitefly-borne *Sweet potato chlorotic stunt virus* (SPCSV) is the main constrain in the crop because of synergism with other sweetpotato viruses, especially the aphid-borne *Sweet potato feathery mottle virus* (SPFMV). The SPCSV in co-infection with other sweetpotato viruses – mainly SPFMV – causes the sweetpotato virus disease (SPVD). SPVD is the most damaging disease of the crop in many regions of world, in particular in East Africa. This disease, first describe in East Africa, reduces severely the yield (up to 90%) of affected plants. Today SPCSV and SPFMV occur worldwide and their damage is considerable when they are infecting together. No clone has been reported so far that shows resistance to SPCSV. However, several clones have been identified that exhibit resistance to SPFMV, but this resistance breaks when a co-infection of SPCSV occurs. It is assumed that SPCSV breaks the sweetpotato resistance mechanism against viruses by RNA silencing.

SPVD induces severe mosaic, chlorosis, stunting and leaf reduction and deformation. These symptoms are typical for the disease and easily to be recognized. Grafting is the universal way to transmit viruses independently of the concentration of the virus in the tissues used as virus source. In this way, transmission (when inoculating) or infection (when detecting) of viruses is assured. NCM-ELISA is a sensible test and reliable in detecting SPCSV and SPFMV infected and susceptible genotypes, respectively. In sweetpotato plants affected with SPVD, titer of SPFMV increases several times making its detection quite easy. In 2006 the virology group at CIP identified a Peruvian landrace (DLP 3163), which had not been infected by SPCSV after inoculations and this clone was named "Resistan". This clone was the only SPCSV resistant clone found in a screening among 2000 germplasm clones and it was assumed that this SPCSV resistance is qualitative and recessive inherited with very low gene frequencies in sweetpotato populations. Provided that this assumption is true nearly no resistant clones can be found in the offspring after a one- way crosses with "Resistan" and only by backcross step or successful auto-fertilizations many resistance genotypes might be found.

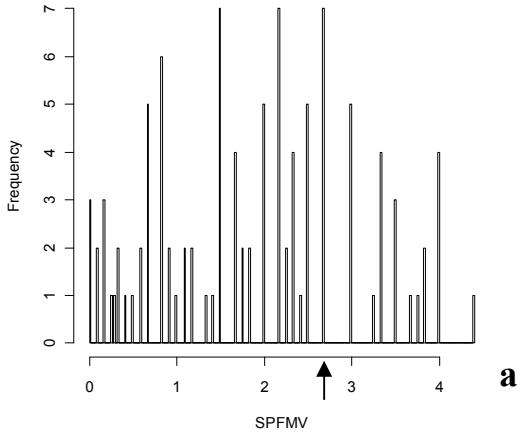
There were two objectives of this study. The first was to use "Resistan" (DLP3163) to develop a pre-breeding population, which carries the resistant allele at low to medium gene frequencies. This pre-breeding population is the prerequisite to develop a backcross population in which a clear segregation of resistant and susceptible clones can be expected. It should be noted that sweetpotato is hexaploid, so that recessive alleles must occur at higher frequencies before there is a chance to observe corresponding recessive homozygous genotypes. The second objective was to confirm the resistance of the "Resistan" clone and to determine if this resistance is inherited.

Materials and methods

In total 103 clones of the population "Jewel I" (breeding code PJ05.__) were crossed with DLP3163. All parents (N = 104) and the offsprings (N = 707) were propagated and synchronically grown under greenhouse conditions at the experimental station San Ramón. Parental and offspring clones were grafted with scions of *Ipomoea setosa* or sweetpotato plants infected with SPVD (SPCSV and SPFMV). For each genotype three plants were used. Symptoms expression and ELISA test evaluation for both viruses were carried 1 month after grafting and repeated three times on each plant replication. Visual ELISA reactions were recorded as 0 = no reaction, 0.5 = unclear positive reaction (threshold), 1 = positive reaction, 2 = strong positive reaction, 3 = very strong positive reaction, 4 = extreme positive reaction. The mean across each repeated measurement and plant replication was calculated by genotypes. The female parents and off-spring group was compared by the T-test and the Wilcoxon test.

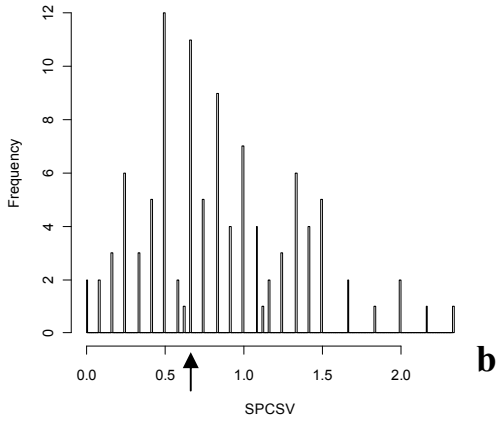
Results

The ELISA titer means (mainly represented as the mean of 9 titers that came from 3 repetitions by 3 observations) for both SPVD viruses were calculated. Among the orange fleshed female parents the titers for both viruses nearly had normal or "bell" shaped distribution (with mean and median around 1.9 and 0.8 for SPFMV and SPCSV respectively). Unfortunately, the DLP3163 clone, formerly considered as resistant, resulted susceptible with a mean of 2.83 and 0.78 for SPFMV and SPCSV, respectively. However, the offspring ELISA titers were considerably lower for SPFMV and SPCSV compared to female parents and male parent (Fig. 1).



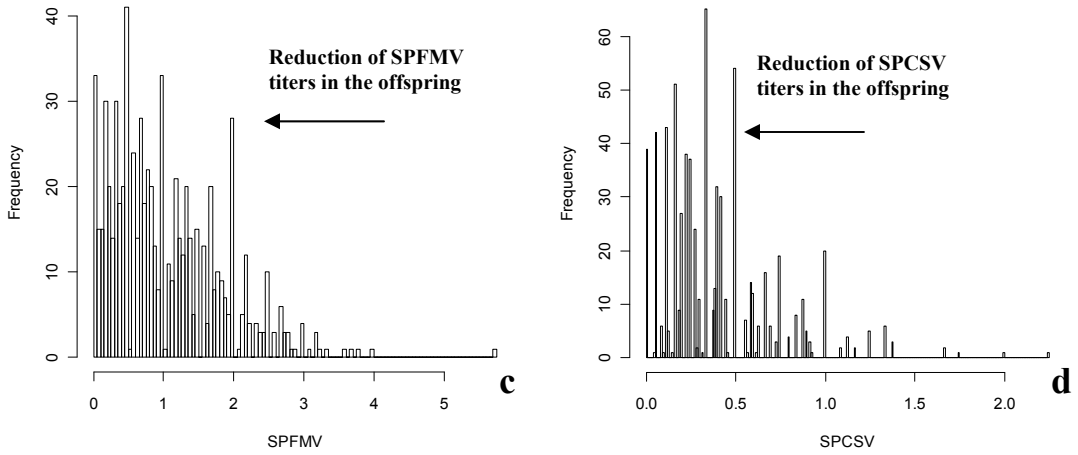
a

Average of ELISA titers for SPFMV in female parents (PJ05.__ clones)



b

Average of ELISA titers for SPCSV in female parents (PJ05.__ clones)



Average of ELISA titers for SPFMV in the offspring (PJ05.__ x DLP3163)

Average of ELISA titers for SPCSV) in the offspring (PJ05.__ x DLP3163)

Figure 1. Distribution and of ELISA titers for SPVD viruses among parental (a,b) and offspring material (c,d); [SPFMV among female parents (a), SPCSV among the female parents (b), SPFMV in the offspring (PJ05.__ x DLP3163) (c), SPCSV in the offspring (PJ05.__ x DLP3163) (d); arrows in (a) and (b) present the mean for SPFMV (2.83) and SPCSV (0.78) in the male parent (DLP3163)].

The observed difference in ELISA titers between parents and offsprings for both SPVD viruses was highly significant, according to the T-test and Wilcoxon rank sum test (table 1). These results demonstrate an inheritable quantitative or for horizontal resistance to both SPVD viruses in the clone DLP3163. On the basis of SPVD ELISA titers four groups were formed among parents and offspring clones (group 1: resistant to SPCSV and SPFMV, group 2: resistant to SPCSV and susceptible to SPFMV, group 3: susceptible to SPCSV and resistant to SPFMV, and group 4: susceptible to SPCSV and SPFMV) for further real time PCR virus detection and molecular marker studies. The first group consisted of 10 clones (Table 2); remaining groups are not presented. It appears that new material has been found which is much more attractive for SPCSV resistance studies compared to the clones DLP 3163. The new material is currently crossed in a diallel and it turned out that PJ05.064 is self-compatible and so far 34 seeds form auto-fertilizations have been developed.

Table 1. Comparison of SPCV and SPFMV infection between the parental and the offspring population by T-test (A) and Wilcoxon Rank Sum Test (B)

	ELISA score Mean (A)		ELISA score Median (B)	
Population	SPCSV	SPFMV	SPCSV	SPFMV
Parents	0.841	1.966	0.778	2.111
Offspring	0.436***	1.091***	0.333***	1.000***

*** significant lower than the parental group p <0.0001

Table 2. ELISA titer means for SPFMV and SPCSV in group 1 (ELISA titer means zero or close to zero) compared to the DLP3163 clone; PJ05. designate clones from the CIP breeding population Jewel 1 and VJ08. designate offspring clones between PJ05. clones and DLP3163

	Female parent	Male parent	Breeder code	SPFMV ELISA titer mean	SPCSV ELISA titer mean	SPFMV number of repeated measurements	SPCSV number of repeated measurements
1	-----	-----	PJ05.064	0	0	6	6
2	PJ05.154	DLP3163	VJ08.618	0.111	0	9	9
3	PJ05.072	DLP3163	VJ08.054	0	0.042	12	12
4	-----	-----	PJ05.019	0	0.083	6	6
5	PJ05.023	DLP3163	VJ08.286	0	0.111	9	9
6	PJ05.064	DLP3163	VJ08.684	0	0.111	10	9
7	PJ05.115	DLP3163	VJ08.330	0	0.111	9	9
8	PJ05.405	DLP3163	VJ08.277	0	0.111	9	9
9	PJ05.072	DLP3163	VJ08.049	0.111	0.055	9	9
10	PJ05.127	DLP3163	VJ08.401	0.111	0.055	9	9
Check	-----	-----	**DLP3163**	**2.833**	**0.777**	6	6

Discussion

SPVD is a disease that causes serious looses in the production of the seventh most important food crop of the world. There is a great interest in making sweetpotato clones resistant to both SPVD viruses. Basically, there are two ways to achieve this goal: one is by plant breeding and the other is by RNA silencing. Plant breeding needs at least one resistant clone. The DLP 3163, which were selected as resistant by the virology group in 2006 at the International Potato Center was found to be susceptible in the same year by Milton Untiveros and Heidi Gamarra (*personal communication*). Our results confirm the susceptibility of the clone DLP 3163 for both SPVD viruses, but it shows inheritable quantitative genetic components for resistance to both viruses. However, it appears that we have found new material, namely PJ05.064, VJ08.618, VJ08.054, PJ05.019, VJ08.286, VJ08.684, VJ08.330, VJ08.277, VJ08.049 and VJ08.401, which might be muchbetter suited for SPVD resistance studies compared to the clone The DLP 3163. Since the clone PJ05.064 is self-compatible we expect to develop abundant seed for dissemination to test the resistance in different regions of the world. Furthermore, it is of interest that our developed material is genetically close to the OFSP population "Jewel" and it might be possible to considerable increase the frequency of SPVD resistance in OFSP breeding populations. OFSP varieties with SPVD resistance would have an extreme advantage in dissemination this variety type in different regions of the world.

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