

Sweetpotato, SPVD resistance and molecular marker development for SPVD resistance

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**When you have rice don't forget
the sweetpotato**



International Potato Center



Annual Sweetpotato Production (FAO 2006):

about 131 Mio t (grown in 114 countries)

107 Mio t China (21 t / ha)
3.0 Mio t North and South America (15 t / ha)
4.2 Mio t West Africa
7.2 Mio t East Africa
1.2 Mio t Central Africa
0.5 Mio t Southern Africa

About 13 Mio t Africa (?) We know Uganda + Nigeria > 6 Mio t)

(average yields across Sub-Saharan 4.8 t / ha)

A very cheap sources of energy + quality with very different quality characteristics e.g. extreme high β -carotene, or extreme low sugar, or) due to the genetic make up of sweetpotato the negative or positive genetic load in sweetpotato must be extreme



What is sweetpotato genetically?

Each Clone is a highly heterozygous Hybrid

and

Sweetpotato is hexaploid

Consequence: Even at medium high gene frequency recessive genes are rarely observed in phenotypes, but they continuously turn up

e.g. biallelism $q = 0.25 \Rightarrow$ phenotype freq. ≈ 0.00024

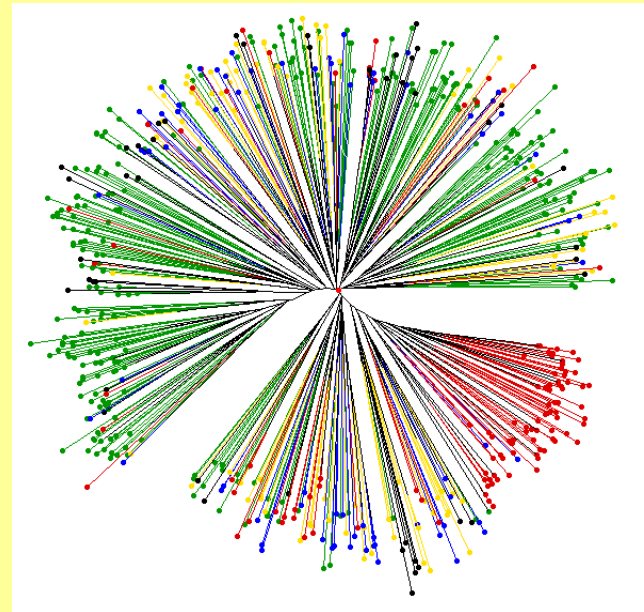
Genetic Diversity

Each region has large genetic diversity & very different groups

(back lines) - Primary diversity centre are Central and South America (black and green lines) - Secondary diversity centre in the Pacific, PNG, New Zealand and Austria (blue lines)

(we think 10 Mega Clusters – in total 46 significant Clusters)

Sweetpotato can be divided in many different groups / clusters / genepools



— Pacific, New Zealand, Australia
— Central America
— South America
— Africa
— Asia

Figure: Cluster analysis of 597 entries of sweetpotato based on 91 alleles generated by 20 SSR markers

Sweetpotato has many genepools

!!! A highly heterozygous hexaploid hybrid !!!

Perhaps due to the genetic made up of sweetpotato the crop has few pest and disease problems or many resistances:

1) Across regions sweetpotato virus disease (SPVD)

Combination of viruses: Mainly SP feathery mottle virus (SPFMV) and SP chlorotic stunt virus (SPCSV) [American OFSPs in East-Africa insufficient resistance - Pacific Coast of South America return to FVs “Jonathan” and “Huambachero” (also good Southern Africa, South West Central Asia;

Yield losses to SPVD had been about 30% in China (solved by healthy planting material)

Yield losses are 50 to 75% in Sub-Saharan Africa (not solved)

- No resistance until 2006 to the SPVD complex

2) Weevil damage in all drought prone regions across continents (Central and South America, SSA and SWCA) - storage roots deep in the soil, clearly tapering at top (Mozambique, Malawi); latex and other organic compounds in the storage root skin - varieties like “Santo Amaro” from Brazil or “New Kawogo” from Uganda
- No resistance until 2007 (??) => Bt sweetpotatoes

3) Of minor importance Alternaria and Nematodes

A list of recognized viruses known to infect sweetpotato

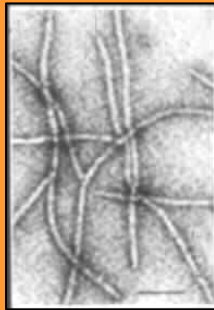
Virus group	Member virus(es)	Particle size (nm)	Natural transmission
Potyvirus	SPFMV	Flexuous, 850 nm.	Aphid
	SwPLV	Flexuous, 750-790 nm.	Aphid
	SPMSV	Flexuous, 800 nm.	Aphid
	SPVY	Flexuous, 850 nm.	Aphid
	IVMV	Flexuous	Aphid
	SPCSV?	Flexuous, 850-950 nm.	Unknown
	SPVG	Flexuous	Unknown
	SPVMV	Flexuous, 761 nm.	Aphid
Ipomovirus	SPMMV	Flexuous, 800-950 nm.	Whitefly
	SPYDV	Flexuous, 750 nm.	Whitefly
Crinivirus	SPCSV	Flexuous, 850-950 nm.	Whitefly
Cucumovirus	CMV	Isometric, 30 nm.	Aphid
Begomovirus	SPLCV	Geminate	Whitefly
Carlavirus	SPCFV	Flexuous, 750-800 nm.	Unknown
Nepovirus	SPRSV	Isometric, 28 nm.	Unknown
Caulimo-like	SPCaLV	Isometric, 50 nm.	Unknown
Iilar-like	TSV	Isometric, 30 nm.	Unknown
Polerovirus?	SPLSV	Isometric, 30 nm.	Aphid
Geminivirus?	ICLCV	Geminate	Whitefly
Tobamovirus	TMV	Rod, 300 nm.	None
Unknown	C-3	Flexuous?	Unknown
	C-6	Flexuous, 750-800 nm.	Unknown

The problem →

Sweetpotato virus disease (SPVD)



Aphids



← **SPFMV+ SPCSV** →
and / or Crinivirus
other
viruses



Whiteflies



Apparently healthy
SPFMV and / or other
viruses

SPVD
SPCSV + SPFMV and / or
Other viruses

In experiments:
Yield reduction up to 90%

In practice:
Yield reduction up to 75%

It is assumed that sweetpotato has a very good defense mechanism against viruses by recognizing the virus DNA and chopping it up, but

=> the defense mechanism of sweetpotato against viruses is broken by SPCSV, which opens the “door” for other viruses

In 2006 **CIP 420269 PER (Resistan)** was found after screening about 2000 germplasm clones, which **appeared to be resistant or immune to SPCSV** (L. Salazar unpublished) – we gave this clone the nickname Resistan

Frequency 0.0005 (1/2000) 95% conf. limits (-0.001 – 0.002)

Assuming recessive inheritance because it is very rare, biallelism and only 1 loci this would correspond to

=> An allele frequency $q \approx 0.28$ (0 – 0.35) for the loci in sweetpotato germplasm, so that the phenotype turns up to 95% in less than 5 clones in 2000 screened clones

SPCSV Resistance Test

Symptoms ✓ ELISA ✓ Real Time PCR

Field screening: 86 families (2190 genotypes)
NaCRRI-Uganda

Greenhouse screening: 104 parents + 718 offsprings
CIP-SanRamon

Virus source

Ipomoea setosa plants infected with SPVD [SPCSV (M2-47) + SPFMV (RC)], were used in graft-inoculation of SP clones for virus infection.

3 replications (plants) for each parental and offspring clones

3 repeated measurements for each plant (week 4, week 7, week 10)

Serological test

Polyclonal antibodies to SPCSV were used in the serological test NCM-ELISA to detect and confirm presence of both viruses.

Note: OFSP parents had passed 1 cycle of selection for SPVD (field tests)!!



SPCSV Resistance Test with ELISA

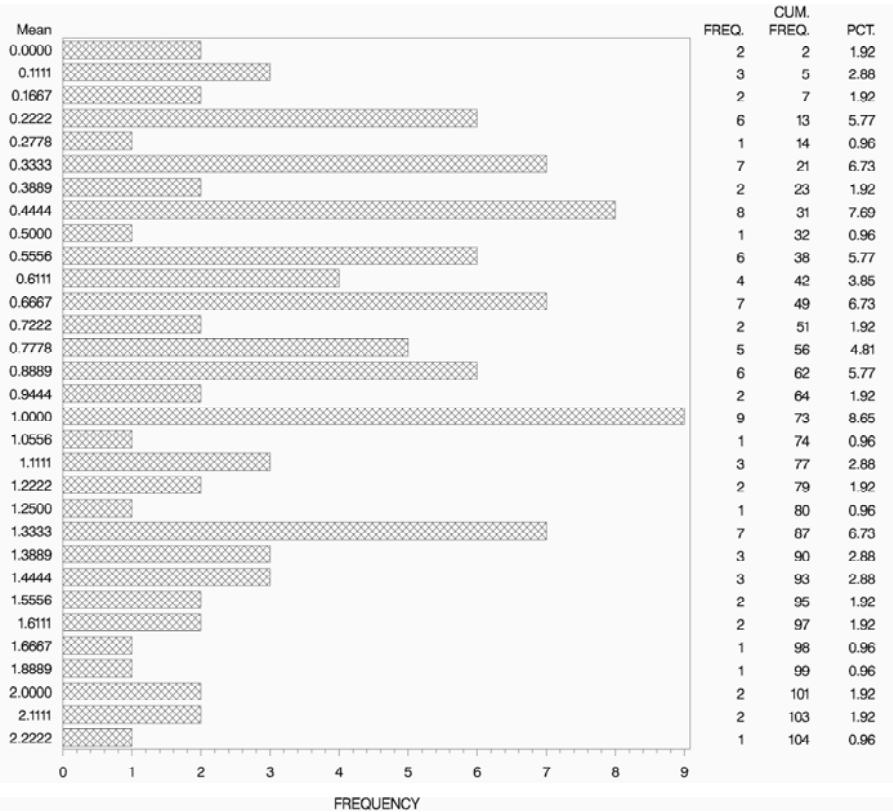
Table. Results for Resistan, PJ05.064, and PJ05.042 as an example of ELISA results for the parent (N=104) and offspring (N = 707) clones

Parent	Rep	SPCSV Repeated Obs.			SPFMV Repeated Obs.		
		1	2	3	1	2	3
Resistan	1	0	0	0	2	2	2
	2	1	0.5	0.5	3	2	2
	3	1	2	2	4	4	4
PJ05.064	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3	0	0	0	0	0	0
PJ05.042(†)	1	0	0	0	4	4	4
	2	0	0	0	2	4	4
	3	0	0	0	3	4	4

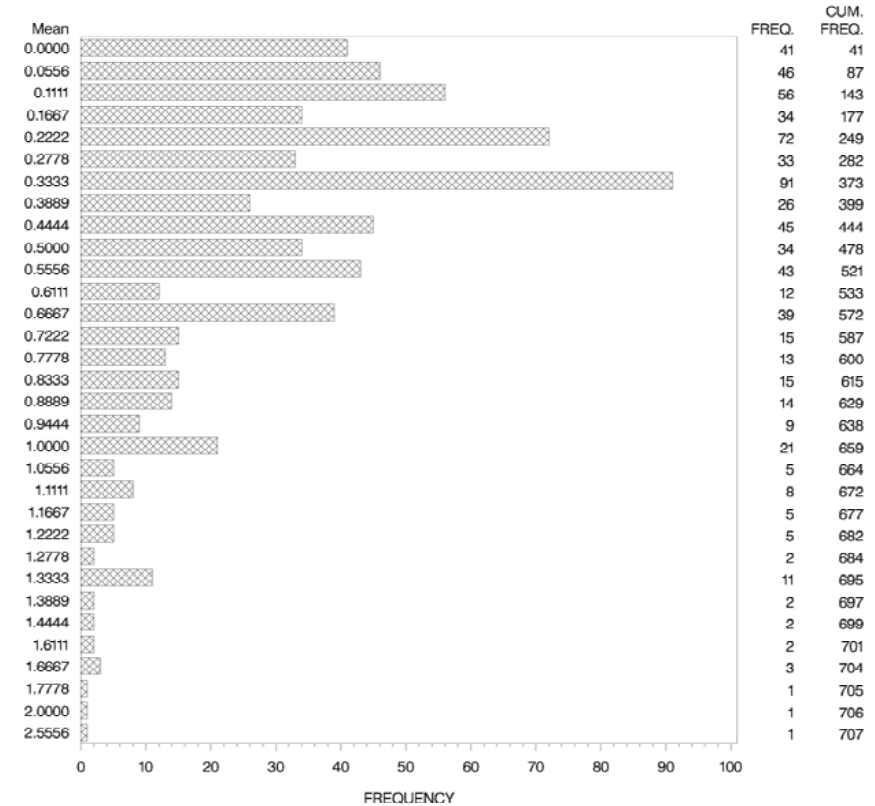
0 = no reaction, 0.5 = unclear no reaction, 1 = positive reaction, 2 = strong positive reaction, 3 = very strong positive reaction, 4 = extreme positive reaction, (†) doubtful resistance because of considerable SPFMV infection

Obviously DLP3163 is not immune, but there are more interesting clones
=> To make things easier we calculated at first the mean over repeated measurements and plant replications to obtain one value per clone and genotype, respectively.

Distribution and Frequencies of SPCSV ELISA scores in parental material (A) and offspring material (B) => recombination with Resistan appears to result in low SPVD infections (significant at 0.001 with T-test and Wilcoxon test)



A



B

New Promising Material for SPCSV resistance in addition to Resistan

Material which never was tested positive or doubtful negative for SPCSV and SPFMV

- 1) PJ05.064 parent selected from population Jewel I
- 2) Offspring clones VJ08.054 (cross Resistan x PJ05.072), VJ08.078 (cross Resistan x PJ05.074), VJ08.173 (cross Resistan x PJ05.090), VJ08.210 (cross Resistan x PJ05.325)

This material is tested now again by real time PCR including all parental material and offspring material

- 3) Resistan x 44 OFSP “Zap” Crossing with parents of OFSP “Pop. Zapallo 1” to have a population to validate molecular markers (in total 704 offspring + 44 parents) evaluated as described for the material Resistan x 103 OFSP “Jewel”
- 4) Crosses and families, respectively, from PJ05.064[†], VJ08.054, VJ08.078, VJ08.173, and VJ08.210 (10 families, 559 seeds, [†] 43 auto fertilization seed)
=> field tests in Uganda

Marker development

1) More SSR markers

- Using unexploited set of EST available 48 new SSR loci were identified.
- already available 64 SSR marker loci for sweetpotato.

2) Resistance groups formed from PJ parents & offspring

(- -) 0 in SPCSV and SPFMV ELISA scores

A) - - (5 clones)

B) - + (23 clones) inc. doubt full clones ↑ SPFMV

C) + - (31 clones)

D) - - (763 clones)

individual DNA → CIP (AFLP) & Australia (DArT)

3) Extreme groups formed from PJ parents & offspring

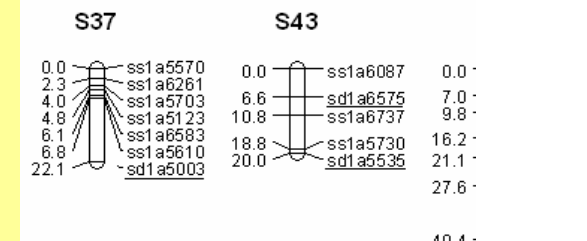
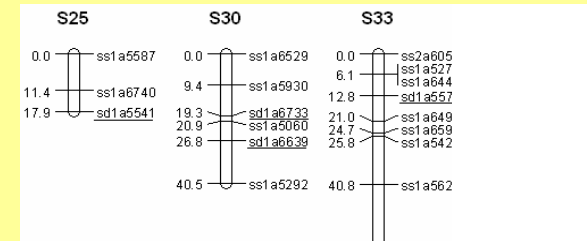
(-) 0 or very low in SPCSV ELISA scores

A) - 10 clones

B) + 298 clones

individual DNA → CIP (AFLP)

Markers



AFLP

DArT

Population PJ05.____ x Resistan + parents for AFLP marker screening

number	SPFMV	SPCSV
10	-	-
39	+	-
28	-	+
747	+	+

Form the AFLP markers screened so far 4 markers have been selected

E33M49.187	Chi-Square	11.82	Prt.	0.0006
E35M36.293	Chi-Square	6.16	Prt.	0.0130
E35M36.267	Chi-Square	22.35	Prt.	<.0001
E33M59.163	Chi-Square	11.04	Prt.	0.0009

10 clones

298 clones out of 747
(Highest ELISA titers)

For the combination 1st E33M49.187, 2nd E33M59.163, 3rd E35M36.293, 4th E35M36.267

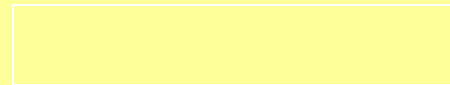
		E33M49.187	E35M36.293	E35M36.267
Resistant	no band (0)	1	0	3
	band (1)	9	5	2
	missin values	0	5	5
Susceptible	no band (0)	186	155	17
	band (1)	106	123	268
	missin values	6	20	13

**2 wrong classifications of
10 resistant clones**
**8 wrong classifications of
298 susceptible clones**

Note resistance group still too small for higher power of significance tests

Conclusions

- 1) SPCSV resistance is not a very rare event, in our parental and offspring material it occurs with a phenotypic frequency of 0.0385 95% conf. limits (0.002 – 0.075) in case of biallelism $q \approx 0.49$ 95% conf. limits (0.35 – 0.65)
- 2) SPCSV is inherited but at least to a certain extend quantitative
- 3) AFLP markers can be easily found which are associated with SPCSV resistance (moreover it appears that sweetpotato is full of DArT markers)
note 95% of the work so far has been invested in phenotyping, which is extremely labourious and time consuming
- 4) A larger group of SPCSV resistant clones might become available from a diallel cross with resistant clones PJ05.064, VJ08.054, VJ08.078, VJ08.173, VJ08.210 of which PJ05.064 is self compatible – this will increase the power of significance tests for molecular markers



Thank-you for your Attention