

Susceptibility of sugarcane, plantation weeds and grain cereals to infection by *Sugarcane yellow leaf virus* and selection by sugarcane breeding in Hawaii

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Abstract Hawaiian commercial sugarcane cultivars (*Saccharum spp.*), noble canes (*S. officinarum*), robust canes (*S. robustum*) and wild relatives of sugarcane (*S. spontaneum* and *Erianthus arundinaceus*) were tested by tissue blot immunoassay to determine whether they were infected by *Sugarcane yellow leaf virus* (SCYLV). Two-thirds of the commercial hybrids and noble canes were infected and therefore classified as SCYLV-susceptible, in contrast to the wild cane relatives where less than one third of the varieties were infected. The pedigree list of commercial, registered cultivars showed that 80% of cultivars were SCYLV-susceptible and that also 75–90% of the progeny of resistant (female) parents were susceptible (male parents are mostly unknown because of polycross breeding). In contrast, a cross between a resistant *S. robustum* and a susceptible *S. officinarum* cultivar yielded 85% resistant progeny clones, which indicated that SCYLV-resistance is a dominant trait. It is concluded that the breeding program selected against SCYLV-resistance with the result that 80% of the newly bred cultivars were susceptible. Exceptional was the period between 1950

and 1970, in which 50% of the newly-bred clones were resistant. This is the period in which SCYLV had entered Hawaii. Weed grasses and cereal grasses which grew in or next to sugarcane fields were not infected by SCYLV. Thus SCYLV does not spread from infected sugarcane plants to adjacent grasses or cereals under field conditions, although cereal grasses can be infected experimentally.

Keywords Cereal grasses · *Erianthus arundinaceus* · Inheritance *Saccharum spec.* · SCYLV-resistance, susceptibility · Weed grasses · Yellow leaf

Abbreviations

SCYLV *Sugarcane yellow leaf virus*
TBIA tissue blot immunoassay
YL Yellow leaf

Introduction

The sugarcane disease Yellow leaf was first described in the 1990s (Schenck 1990; Comstock et al. 1994). A polerovirus, *Sugarcane yellow leaf virus* (SCYLV), was identified as a causal agent (Vega et al. 1997). Schenck and Lehrer (2000) tested for SCYLV in varieties in the cultivar collection of Hawaii Agriculture Research Center by tissue blot immunoassay (TBIA) and they found that more than 50% of *Saccharum officinarum* and *S. sinensis* contained

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SCYLV, whereas only 10–20% of *S. robustum* and *S. spontaneum* varieties were infected. The relatives of sugarcane, *Miscanthus* and *Erianthus*, were not infected. Since the breeding station is heavily infested by viruliferous aphids, the uninfected varieties were proposed to be SCYLV-resistant. Basically similar results were obtained by Comstock et al. (2001) when they screened the United States National Germplasm Repository for Sugarcane and Related Grasses in Miami, Florida. They detected a high percentage of infected varieties in *S. officinarum*, *S. sinense* and *S. robustum*, whereas most *S. barberi* and *S. spontaneum* were not infected.

Schenck and Lehrer (2000) placed viruliferous *Melanaphis sacchari* on cereal grass seedlings and 4 weeks later the plants were tested for SCYLV by TBIA. More than 90% of the inoculated wheat, oat and barley plants and 10% of rice and corn plants contained SCYLV. These results were surprising because the cereal grasses are less related to sugarcane than *Miscanthus* or *Erianthus* which were SCYLV-resistant. The question was if these grass relatives can serve as a reservoir for SCYLV when standing next to virus-free seed cane fields. In addition, *Erianthus* and *Miscanthus* are in the focus as biomass crops for non-fossil energy generation in tropical and moderate climate regions, where grain production is a major agricultural commodity. Possible pathogens of these energy crops and their host specificity are therefore of agricultural interest.

The objective of the following study was to:

- explore the host specificity of SCYLV under field conditions with respect to sugarcane cultivars, grass relatives of sugarcane and cereal grasses, and
- follow the pedigree of commercial sugarcane cultivars to reveal whether the breeding and selection program selected for or against SCYLV-resistance.

Material and methods

Test for SCYLV

SCYLV was assayed by tissue blot immunoassay (Schenck et al. 1997; Fitch et al. 2001). Leaf midribs or in case of grasses whole leaves and stems were

freshly cut and immediately pressed onto nitrocellulose membranes (Biorad Transblot membrane) in triplicate. After a pretreatment with chloroform to extract chlorophyll, the membranes were blocked with TBS-buffer (50 mM Trizma base plus 50 mM NaCl pH 7.5 containing 2% dry milk), then incubated with rabbit antiserum raised against SCYLV (Scagliusi and Lockhart 2000). The blots were probed with goat anti-rabbit alkaline phosphatase-conjugated antibody followed by colour development using BCIP/NBT substrate (Sigma, St. Louis, USA). The tissue prints were analysed under the microscope. No grading of colour intensity was performed, i.e. whenever a positive reaction was observed in one of the vascular bundles, the plant was considered to be infected.

Collection of plant material from the field

Samples of sugarcane and sugarcane relatives were collected from the breeding station and the museum plot of the Hawaii Agriculture Research Center (formerly Hawaiian Sugarcane Planters' Association) at Maunawili, Oahu, Hawaii, which assembled clones of past and present commercial sugarcane cultivars and clones of recent breeding efforts which were awaiting field tests. It also includes some traditional Hawaiian *S. officinarum* clones and wild sugarcane relatives such as *S. spontaneum* and *Erianthus* (which was later renamed to *Miscanthus*). All sugarcane clones were exposed to natural SCYLV-transmitting aphid infestation in the field for their entire life, i. e. which was usually more than 10 years and at least 5 years for the most recently bred clones.

Weeds were also collected from the fields in the breeding station at Maunawili and in the Experiment Station at Kunia, Oahu, next to SCYLV-infected sugarcane.

Rice plants were collected from a small rice plot at the breeding station (Maunawili, Oahu), wheat plants from a plot in the Experiment station at Kunia, Oahu, and corn plants from a seed field at Kekaha, Kauai. The cereal plants were either blooming or bearing developing seeds. All the cereal plants grew next to SCYLV-infected sugarcane and had been exposed to SCYLV-vectors for their entire life of 4–6 months. The collection of samples was performed in the years 2000, 2001, 2004 and 2009.

Infection experiment

Rice (M 202), wheat (Bobwhite), barley (HA 4900), oat (Coker 227 and HA-5062) and corn plants (Supersweet #10) were sown in pots with commercial garden soil and germinated for 2 weeks in a greenhouse. Then they were placed outdoors onto a concrete shelf at the breeding station in random order mixed with 3-months old SCYLV-infected sugarcane plants (cv. H87-4094). Two months later, tissue prints from leaves and stems of the cereal plants were made and tested for SCYLV. The wheat and barley plants had just produced ears with developing seeds, the corn plants carried fully developed male flowers, the rice and oat plants had no ears yet. All plants had soon become heavily naturally infested by aphids.

Crossings of SCYLV-susceptible and -resistant cultivars

A cross of Mol 5829 (*S. robustum*) and LA Purple (*S. officinarum*) was made and the progeny (165 clones) was grown in the breeding station of the HARC Experiment Station at Maunawili (Ming et al. 1998). Furthermore, a few clones which resulted from crosses in the 1970s of noble cane with *S. spontaneum* or *Miscanthus* sp. were tested. All plants grew next to SCYLV-infected sugarcane cultivars for 5 years or longer.

The pedigree list of registered Hawaiian germ-plasm clones up to breeding year 1987 was provided by Dr. K.-K. Wu, HARC. The parental cultivars of the pedigree list which were still available at the breeding station were tested for SCYLV. All of them had been grown among infected sugarcane at the breeding station for more than 10 years.

Statistics

GLMM for binomial error structure with a logit-link function was used for calculating the differences among the different phases of cultivar breeding (Fig. 1). Analyses were carried out with the program R, version 2.10.0 (R Development Core Team 2009). Mixed-effects models were fitted with the package lme4 (Pinheiro and Bates 2000). The occurrence of infection with SCYLV per cultivar (response variable) was coded as 1 (infested) or 0 (not infested). The results of each five-year-period were included in the model as a

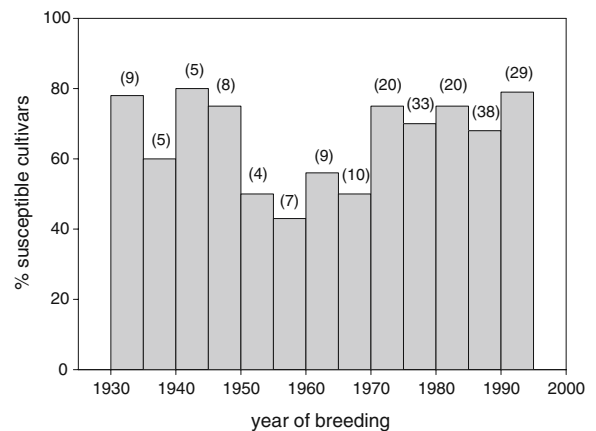


Fig. 1 Percentage of SCYLV-susceptible *Saccharum* sp. hybrids bred over the period of 1928 until 1995. The years were grouped in 5-years portions (the clones from 1928 were added to the period of 1930–1935). The numbers in brackets indicate the number of tested clones from the respective time segment. GLMMs for binomial error structure with logit-link function were used for calculating the differences among the 3 phases of cultivar breeding, phase 1 (1930–1950), phase 2 (1950–1970) and phase 3 (1970–1995). Phase 1 to phase 2: $\chi^2=3.54$, $P=0.06$, phase 3 to phase 2: $\chi^2=5.67$, $P=0.02$

random factor in order to correct for the same origin of the respective cultivars. P -values were calculated using likelihood ratio tests based on changes in deviance when each term was dropped from the full model.

Results

SCYLV-infection status of sugarcane clones and wild relatives of sugarcane at the Hawaiian breeding station

The plants of the HARC breeding collection were tested in 2000 and 2001 for SCYLV by TBIA and the results are listed in Table 1. Sixty to seventy percent of the sugarcane hybrids, noble canes (*S. officinarum*) and *S. sinense* were infected by SCYLV. In contrast only 27% of the *S. spontaneum* clones and 10% of the *S. robustum* and the *Erianthus* clones contained SCYLV. This result agrees basically with the analysis of Schenck and Lehrer (2000) which was performed 3 years earlier, except that they did not test such a large number of hybrids and that they did not notice SCYLV-infection in the *Erianthus* species. No cultivar names were mentioned in their report.

The SCYLV-infection state of cultivars was broken down according to their year of breeding (i. e.

Table 1 Presence of SCYLV in sugarcane and *Erianthus* cultivars at the HARC breeding station in 2001. Tissue prints in triplicate of at least 2 clones of each cultivar were tested for SCYLV. All cultivars were from the collection and museum site of the HARC breeding station at Maunawili, Oahu

Species	Cultivars	% SCYLV-infected
<i>Saccharum spec. hybrids</i>	<p>SCYLV-positive (susceptible):</p> <p>H28-4291, H28-4399, H31-0624, H31-1389, H32-1063, H32-8560, H35-0585, H37-1933, H38-2915, H40-1184, H41-3340, H44-2364, H44-2772, H44-3098, H46-2404, H48-3717, H49-0005, H49-0104, H49-3533, H50-7209, H54-0807, H54-2759, H56-0278, H59-3775, H60-5627, H61-1820, H61-5433, H62-1526, H62-4671, H65-7052, H66-4927, H68-0388, H68-1158, H69-9092, H69-9103, H71-5813, H72-6418, H72-6418, H73-5959, H73-6110, H73-8505, H74-1715, H74-4527, H74-4527, H74-6001, H75-3661, H75-6096, H75-6104, H75-6212, H75-6278, H76-4713, H77-0682, H77-1028, H77-2545, H77-3676, H77-4643, H77-9288, H77-9381, H77-9545, H78-0292, H78-0698, H78-0878, H78-1207, H78-1379, H78-3567, H78-3606, H78-3967, H78-6799, H78-7234, H79-7808, H80-2339, H80-2931, H80-4053, H81-0648, H81-9091, H82-5959, H83-0815, H83-0959, H83-1575, H83-4308, H83-4501, H83-7206, H84-0837, H84-6094, H85-1515, H85-1978, H85-6697, H85-6782, H86-0357, H86-0810, H86-7076, H87-4094, H87-4288, H87-4319, H87-5707, H87-5773, H87-5802, H87-5840, H87-5901, H87-7218, H88-4223, H88-6124, H88-7553, H89-5814, H89-7088, H89-7127, H89-7241, H89-7325, H89-7378, H90-5518, H90-5771, H90-7344, H90-7350, H90-7453, H91-4004, H91-4301, H91-6187, H91-6217, H92-5798, H92-5927, H92-5954, H92-5979, H92-6923, H92-7358, H94-0111, H94-5642, H94-5948, H94-6113, H94-6125, H95-1406, H95-1420, H95-1429, H95-1441, H95-1447, H95-1448, H95-1450</p> <p>SCYLV-free (resistant):</p> <p>H28-2055, H32-6171, H39-5803, H39-7028, H41-1181, H47-4991, H50-2036, H51-8194, H53-0263, H57-5174, H59-5862, H60-3862, H60-3875, H61-0467, H61-1721, H62-1613, H65-8425, H66-6938, H67-5273, H67-5630, H69-6209, H69-8235, H71-6505, H72-1365, H73-3775, H73-7324, H75-8865, H76-5698, H77-0723, H77-1756, H77-4000, H77-9047, H77-9271, H78-4153, H78-7750, H79-2583, H80-4246, H83-4129, H84-6180, H85-4106, H85-6762, H85-7662, H86-1040, H86-1407, H86-3792, H87-1061, H87-4128, H87-4189, H87-5794, H88-4046, H89-7056, H89-7178, H90-4378, H90-4418, H91-4038, H95-1449, H95-1690, H95-1693, H95-1706, H95-1739</p>	70%
<i>Saccharum officinarum</i>	<p>SCYLV-positive (susceptible):</p> <p>27 MQ1124, 28 NG20, 247 B, Assam Red, Fiji, H109, IJ76-462, Jamaica Red, LA Purple, Lahaina, Luzon White, Mahaiula, Manteiga, Muntok Java, NC 74, off 8275, R568, Red Tip, Vae Vae Ula, Zopilota,</p> <p>SCYLV-free (resistant):</p> <p>51 NG44, 51 NG111, 57 NG24, 57 NG256, Badilla, Bastard Rayada, Caledonia Ribbon, H52, IJ76-558, NG77-098, NH70-33, Pawn</p>	61%
<i>Saccharum robustum</i>	<p>SCYLV-positive (susceptible):</p> <p>51 NG 63, Mol 5119,</p> <p>SCYLV-free (resistant):</p> <p>51 NG63, 57 NG208, 57 NG249, IJ76-543, IK76-004, Mol 5829, Mol 5916, Mol 6023, Mol 6059, Mol 6076, Mol 6077, Mol 6088, Mol 6118, NG77-122, Raitea 1, Tanange LA</p>	11%
<i>Saccharum sinensis</i>	<p>SCYLV-positive (susceptible):</p> <p>CH64/21, Chynia, Cayanna 10, Gungera, LU cane</p> <p>SCYLV-free (resistant):</p> <p>Hawaiian Uba, Maneria</p>	71%

<i>Saccharum spontaneum</i>	SCYLV-positive (susceptible): 28NG-101, Kaludai Bootham, Natal Uba, POJ 2364, Saipan 17, SES 14	27%
	SCYLV-free (resistant): Burma, Chunnee, D 1153, Glagah 1286, Glagah Mandalay, IA 3330, IN84-012, IND81-172, IS76-165, JW43, Moentai, Mol 4009, Mol 6031, SES 208, US 56-13-7 Thai spont., Yellow Caledonia	
<i>Erianthus arundinaceus</i> later renamed to <i>Miscanthus</i> <i>arundinaceus</i> . (elephant grass)	SCYLV-positive (susceptible): 93-9985, 83-9986, IJ 76-342, Timor Wild	13%
	SCYLV-free (resistant): 83-9985, IJ76-327, IJ76-342, IJ76-384, IJ76-390, IJ76-392, IJ76-397, IJ76-398, IJ 76-502, IJ76-503, IJ76-513, IK76-025, IK76-027, IK76-055, IN84-014, IN84-018, IN84-045, IND81-035, IND81-139, IS76-162, IS76-174, Mol 563, SES 336, <i>Erianthus</i> sect. <i>Ripidium</i> sp, <i>Erianthus</i> sp.	

crossing and germination from true seeds) and grouped in 5 year-intervals (Fig. 1). Seventy percent of newly bred clones since 1930 were infected, except in the years between 1950 and 1970, when significantly less namely 50% of newly bred clones were infected. Since all the clones were grown at the heavily SCYLV-infested breeding station, it can be assumed that all susceptible clones became infected during their life time and that those which were not infected are resistant.

SCYLV in grain and weed grasses

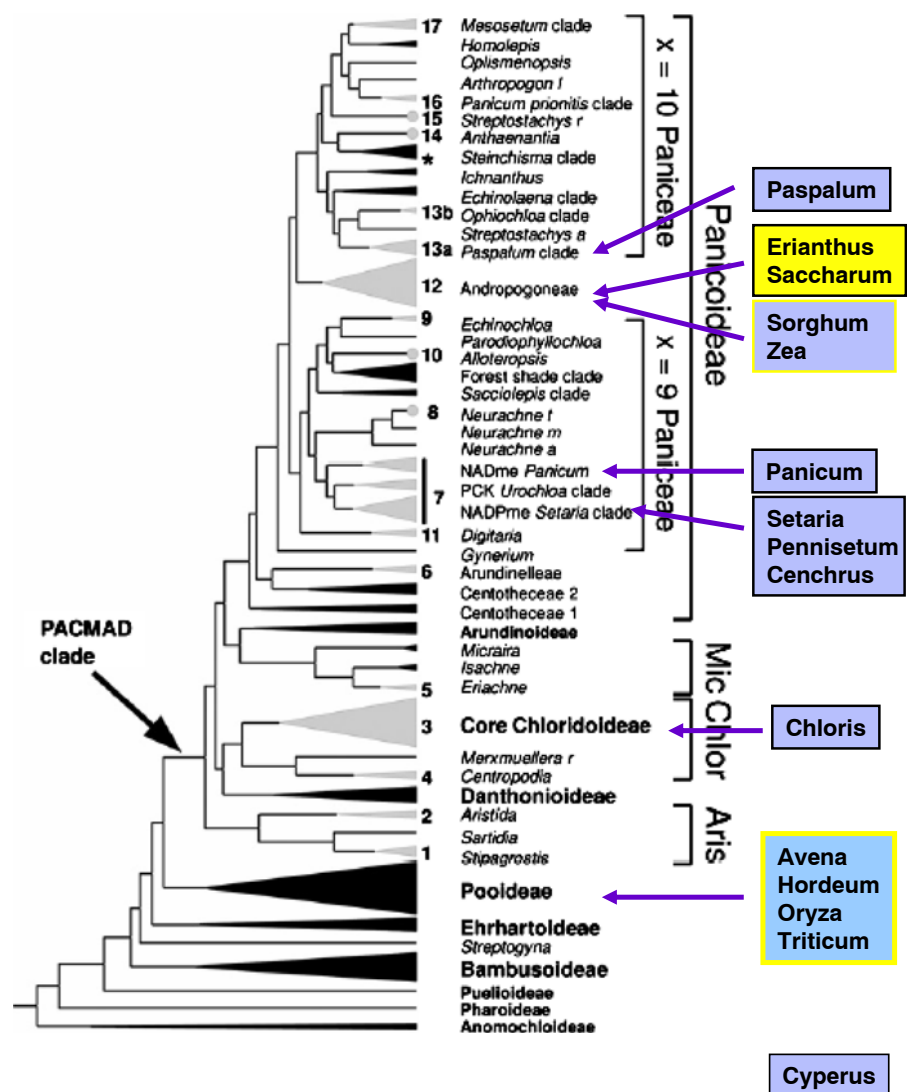
The borders of sugarcane fields are populated by perennial grass species (Peng 1984) which might possibly serve as reservoir for viruses. None of the tested weed grasses reacted positively for SCYLV in the TBIA test (Table 2), although several species belong to the two Paniceae tribes, which are sister tribes of the Andropogoneae together with *Saccharum* and *Erianthus* (Fig. 2).

Rice, wheat and corn plants were collected from fields which were next to SCYLV-infected sugarcane fields. No sample contained the virus (Table 3). Grain cereals were also grown in pots outdoors in the breeding station on a shelf together with infected sugarcane. The plants were soon heavily infested by aphids (*Histonera setarii* and *Rhopalosiphon maidis* were identified), however all tested cereal plants remained SCYLV-free (Table 3). The grain grasses, except *Zea*, belong to the tribe Pooideae, which is relatively distant to the Andropogoneae tribe (Fig. 2).

Table 2 SCYLV in the grass weeds at the border of sugarcane fields. The weed grasses were sampled from the sides of infected sugarcane plots at the Kunia field station and at the Maunawili breeding station

Species (trivial name)	Infected / number of tested clones
<i>Cenchrus echinatus</i> (sandbur)	0/2
<i>Chloris barbata</i> (swollen fingergrass)	0/2
<i>Cyperus rotundus</i> (purple nutsedge)	0/6
<i>Panicum maximum</i> (Guinea grass)	0/7
<i>Paspalum conjugatum</i> (Hilo grass)	0/2
<i>Pennisetum purpureum</i> (Bannagrass)	0/10
<i>Setaria glauca</i> (yellow fox tail)	0/5

Fig. 2 Phylogeny of grasses (adapted from Christin et al. 2009) with the positions of the tested weed and cereal grasses. In yellow are SCYLV-susceptible genera, in blue resistant genera; *Cyperus* stands outside because it does not belong to the Pooideae. In yellow rim with blue background are the cereal grasses which were not infected in the natural environments, but appeared susceptible when inoculated with viruliferous aphids (Schenck and Lehrer 2000). The PACMAD clade includes all grass genera with C₄-photosynthesis



Pedigree of SCYLV-susceptible and -resistant cultivars

The Hawaiian sugarcane industry uses SCYLV-susceptible and -resistant cultivars in their plantations. The SCYLV-susceptibility of these cultivars was unknown at the time when they were bred and introduced to the plantations. The pedigree of those Hawaiian cultivars, for which a genealogy of SCYLV-susceptibility is recorded, is shown in Fig. 3. The crossings were mostly accomplished by so-called melting pot procedure (polycross), in which tassels of several cultivars were assembled in a tent to allow cross-pollination. Consequently, only the female parent of the true seeds is known, not the male

parent. In addition, some parent lines were no longer available for SCYLV-tests. Despite this incomplete picture, it is obvious that susceptible cultivars were derived from susceptible parents (e.g. H59-3775 and H49-0005) and from crosses between a susceptible and a resistant cultivar (e.g. H37-1933 and H41-3340). The parentage of resistant cultivars is very incomplete, in some cases a susceptible, in others a resistant cultivar is the female parent.

The pedigree scheme contains 56 parent cultivars which were tested for SCYLV. Two-thirds of these lines were SCYLV-infected (Table 4). Thirty progeny cultivars had at least one defined parent which could be tested for SCYLV. Crosses of a resistant female with a susceptible or an unknown male yielded 90%

Table 3 SCYLV in cereal grasses. The experiment samples were collected from potted plants which were kept outdoors next to each other and to SCYLV-infected sugarcane. The field samples were collected from fields in Kunia, Oahu, Maunawili, Oahu and Kekaha, Kauai, in each case next to infected sugarcane fields

Cereal species (trivial name, cultivar)	SCYLV-positives / number of samples
<i>Avena sativa</i> , oat	
outdoors with infected sugarcane (cv. Coker 227 and HA-5062)	0/9
<i>Hordeum vulgare</i> , barley	
outdoors with infected sugarcane (cv. HA 4900)	0/10
<i>Oryza sativa</i> , rice	
outdoors with infected sugarcane (M 202)	0/18
in a field (unknown cultivar)	0/5
<i>Triticum aestivum</i> , wheat	
outdoors with infected sugarcane (cv. Bobwhite)	0/14
in a field (unknown cultivar)	0/15
<i>Zea mays</i> , corn	
outdoors with infected sugarcane (cv. Supersweet #10)	0/15
in a field (unknown cultivar)	0/11

of susceptible progeny cultivars, only 10% resistant cultivars (Table 4). When the female was susceptible, 75% of progeny cultivars were susceptible.

SCYLV-susceptibility of a defined cross between *S. officinarum* and *S. robustum*

A controlled cross was made in 1990 between a SCYLV-resistant *S. robustum* (cv. Mol 5829) and a SCYLV-susceptible *S. officinarum* (cv. LA Purple) to obtain genetic markers for sugar storage and stress tolerance (Zhu et al. 1997; Ming et al. 1998). A F1-progeny of 165 clones was produced and tested for SCYLV. The largest part namely 85% of the progeny was SCYLV-free and only 15% showed SCYLV (Table 5) (whereby their virus titre was low as judged from the weak reaction in TBIA). Part of the progeny was tested again later by inoculating individual plants with viruliferous aphids and 57 plants turned out to be resistant and 28 plants susceptible (R. Ming, University of Illinois, USA, personal communication). Obviously SCYLV-resistance is a dominant trait. A qualitatively similar picture emerged from the few progeny clones from crosses between a susceptible

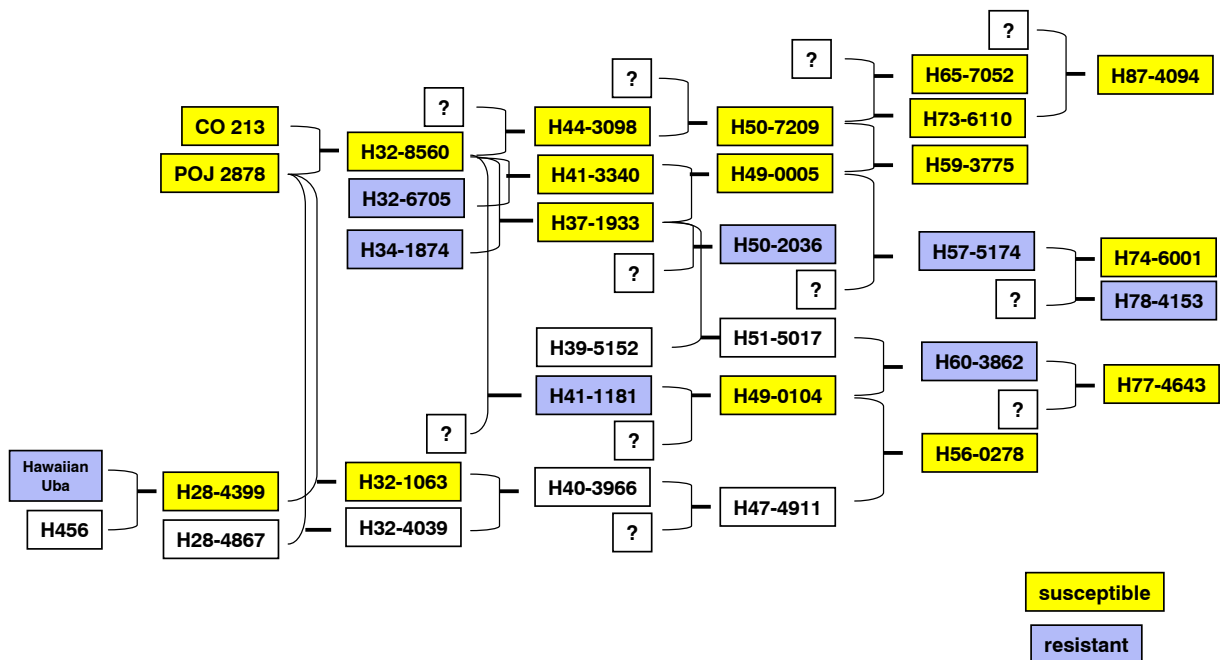


Fig. 3 Pedigree of registered germplasm lines of Hawaiian sugarcane cultivars of which at least some parentage was available in 2001. In yellow are SCYLV-susceptible varieties,

in blue resistant varieties. Varieties without colouring were unavailable for testing. Question marks indicate that the male parent is unknown

Table 4 Pedigree of registered Hawaiian clones and germplasm (until 1995). The pedigree of cultivars was registered by HSPA until 1995. The cultivars which were still available were

analysed for SCYLV by TBIA in 1999–2001. The female parent is always known, the male parent mostly unknown because of the melting pot crossing

	Number of cultivars	SCYLV-infected	SCYLV-free
Cultivars of the pedigree list which were tested for SCYLV	56	38	18
Cross: resistant×susceptible	4	4	0
Cross: resistant×unknown	6	5	1
Cross: susceptible×susceptible	5	5	0
Cross: susceptible×unknown	19	13	6

noble cane and a resistant *S. spontaneum* or *Miscanthus* sp. (Table 5).

Discussion

The present study determined the infection state of cultivars which were assembled in the HARC breeding station and planted in narrow rows at short distances. The station is heavily infested by viruliferous aphids with the consequence that virus-free susceptible varieties are infected within 2–3 years (Lehrer et al. 2007). The sugarcane clones are ratooned once a year and they re-sprout from the root system. A SCYLV-infection which had once occurred in the stool will remain from then on in the stool and therefore one can firmly assume that cultivars, which never showed SCYLV by TBIA, are resistant to SCYLV, whereas infected cultivars can be defined as SCYLV-susceptible. The survey on SCYLV-infection in the *Saccharum* clones of the Hawaiian breeding station extended the results from Schenck and Lehrer (2000). The results are also basically similar to those of Comstock et al. (2001) for clones in the Miami Germplasm Repository, in which many clones from Hawaii had been re-introduced shortly beforehand to

replace clones which were lost in a hurricane. The present survey included all commercial canes which had been bred in the periods from 1928 to 1995. Seventy percent of the commercial sugarcane hybrids were SCYLV-susceptible, i.e. a similar percentage as found in noble canes. The hybrids had been obtained by crossing in *S. robustum* clones into noble canes to yield progeny with improved stalk firmness. Considering that *S. robustum* clones are mostly SCYLV-resistant, a higher percentage of resistant hybrids would be expected. The defined cross of a *S. robustum* with a *S. officinarum* yielded 85% resistant clones in the F1-progeny, a clear indication that SCYLV-resistance is a dominant trait. Comstock et al. (2001) reported that a cross at Canal Point (Florida) between a SCYLV-susceptible *S. officinarum* with a resistant *S. spontaneum* yielded “a high proportion of progeny that have remained SCYLV-free for over 10 years”. When still only 30% of the selected commercial hybrid lines in Hawaii and in Florida were resistant, it means that the selection steps in the breeding program were (unknowingly) selecting against SCYLV-resistance. Obviously the traits desired by sugarcane breeders co-migrate with traits which allow SCYLV to proliferate in the plant to high virus titres. Recent tests by RT-PCR and real-time RT-

Table 5 SCYLV-susceptibility of defined crosses. The cross of *S. robustum* (Mol 5829) and *S. officinarum* (LA Purple) was made in 1990 and the progeny was tested for SCYLV by TBIA.

The crosses between noble cane and *S. spontaneum* or *Miscanthus* were made in 1970s. Only few, interesting plants of these 2 crosses were kept in the collection

Cross	SCYLV-susceptible progeny	SCYLV-resistant progeny
Mol 5829 (resistant) × LA Purple (susceptible)	25	140
Noble cane x <i>S. spontaneum</i>	1	2
Noble cane x <i>Miscanthus</i> sp.	0	3

PCR showed that the resistant cultivars contain SCYLV, however at an at least 100-fold lower virus titre than the susceptible cultivars (Zhu et al. 2010).

SCYLV had been introduced into Hawaii in the time period of 1950–1970 (Komor et al. 2010). Since SCYLV is harmful to growth and yield of susceptible cultivars (Grisham et al. 2002; Lehrer et al. 2009) a trend to more resistant commercial cultivars from that time on was expected. Indeed, the percentage of newly-bred resistant varieties increased to 50% in that time period, but from 1970 on it returned to the previous percentage. Possibly traits in susceptible lines, which compensated the adverse virus effect, have surfaced in the selection process after 1970. Nowadays, the susceptible cultivars are indistinguishable from the resistant cultivars with respect to growth and yield (Lehrer et al. 2009).

SCYLV appeared confined to the genus *Saccharum* and *Erianthus* of the Andropogonaceae, other closely related sister tribes of the Paniceae family to which several grass weeds of sugarcane fields belong to, are virus-free. Guinea grass and Bannagrass especially, which were tested in higher numbers, are the most abundant sugarcane weeds and they are SCYLV-free. An experimental inoculation of cereal grasses with viruliferous *Melanaphis sacchari* had infected the cereals with SCYLV (Schenck and Lehrer 2000). In contrast, wheat, rice and corn in fields next to infected sugarcane fields did not acquire SCYLV during their growth. The same was true for wheat, rice, corn, barley and oat in pots outdoors in the breeding station together with pots of infected sugarcane. Although the cereals were infested by aphids, including *Histonera setarii* and *Rhopalosiphon maidis*, the first is not reported as a SCYLV-vector, and the latter is a weak vector on sugarcane (Schenck and Lehrer 2000). *Melanaphis sacchari*, the most efficient SCYLV-vector, was not noticed on the cereals, although it is around in the breeding station during all seasons. Previous experiments with a *Melanaphis*-infested sugarcane plant next to a virus-free “bait” sugarcane showed that the virus-free plant was already SCYLV-infected after 4 weeks. The explanation for the discrepancy with previous results (Schenck and Lehrer 2000) lies most likely in the host specificity of *M. sacchari*, which does not colonize the cereal grasses unless forced on by a researcher. The cereals in the field were exposed for their entire life time till sampling, which was 4–6 months. This time is short

compared with the incubation times for sugarcane in the breeding station, however, the life cycle of the cereals is not much longer either and this is one factor besides the host-specificity of aphids and the host specificity of the virus which decreases the infection chance of cereals in the field. The absence of SCYLV-transmission to cereals under field conditions indicates that:

- rice, which grows next to *Erianthus* and *S. spontaneum* in countries such as Thailand (Sugimoto et al. 2002), where SCYLV is already present (Lehrer et al. 2008), will not become SCYLV-infected;
- cultivation of *Erianthus* as bio-energy crop in Europe and USA next to grain farming is acceptable from the viewpoint of SCYLV.

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