Final Performance Report on USDA Specific Cooperative Agreement # 58-6607-7-101

"Evaluation of sweetpotato germplasm for resistance to *Sweet potato leaf curl virus*"

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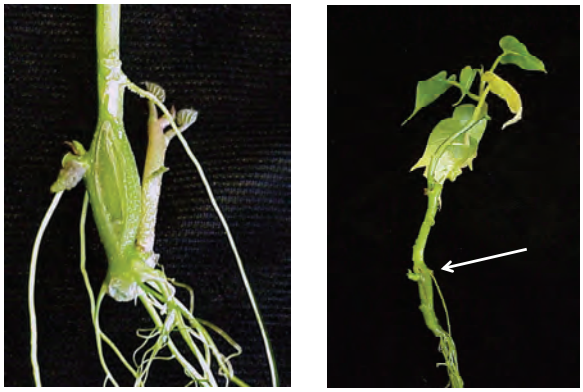
Seventy one sweetpotato accessions from the USDA Sweepotato Germplasm Repository were evaluated for resistance to *Sweet potato leaf curl virus* (SPLCV) to identify sources of resistance.

The initial proposal included a two-year work plan. The intent was to concentrate on tissue culture grafting and DNA extraction and analysis during the first year and field evaluation during the second year. In the end, the project took three years with some field data collected in 2008 and more extensive plantings established in 2009. A group of 71 accessions was selected for evaluation. Some were chosen because they were high-yielding, orange-fleshed lines that preliminary data suggested might be resistant to SPLCV, some were chosen from a group of lines from Kenya for which there are molecular marker data from Miano et al. (2008) on SPVD, and some were chosen from China because initial results showed Xushu-18 to be resistant to SPLCV and we anticipated that Chinese germplasm might have a greater proportion of resistant lines.

Accessions were received from the germplasm repository in several different shipments during late 2007 and were transferred to establish fresh tissue cultures at LSU. Accessions were increased in tissue culture to allow five plantlets for each accession to be micro-grafted with chips from Beauregard tissue cultures infected with isolate SWFT-1 of SPLCV (an example of a micro-grafted plantlet is shown at right). The first group of plantlets was allowed to grow out for 6 or 9 weeks to determine optimal sampling time.



Examples of micro-graft results: left = scion has remained green and succulent, but in some cases was only weakly attached to the stock, right = many times the bud on the scion began to grow out, suggesting a good graft.

Initial titer assays led us to select 6-7 weeks as the standard incubation time following micro-grafting, after which leaves were collected separately from each replicate plant and immediately frozen in liquid nitrogen and stored at -70 to -85°F until they were extracted. Frozen leaf tissue was ground to a fine powder in liquid nitrogen using a mortar and pestle and total DNA extracted using GenElute™ Plant Genomic DNA Miniprep Kits (Sigma-Aldrich, St. Louis, MO), according to the manufacturers' directions. The titer of SPLCV was determined by real-time PCR using the method of Kokkinos and Clark (2006). Additional real-time PCR assays for SPLCV titer were performed on a selected group of lines at several different times to assess variation that might be caused by environmental conditions: in Spring 2009 titers were determined on plants grown from infected tissue cultures in the greenhouse, at six weeks after transplanting the field plots in Summer 2009, and a second greenhouse comparison was done in Fall, 2009 between non-inoculated plants and plants graft inoculated in the greenhouse.

Once the plantlets were established in tissue culture, plantlets were also established in soil in the greenhouse. When grown out sufficiently in the greenhouse, new cuttings were transplanted for subsequent grafting and the original plant was maintained as a non-inoculated control. Twenty four of the accessions were not established in the greenhouse early enough to be graft inoculated in time to transplant to the field in 2008. Inoculated plants were grafted with Beauregard scions infected with isolate SWFT-1 of SPLCV. The plants were allowed to grow out and on May 14, 2008 cuttings were taken from the plants and transplanted into single five-plant plots each for SPLCV-inoculated and non-inoculated at the Burden Center in Baton Rouge. Inoculated and non-inoculated plots were established for forty accessions. The plots were observed throughout the season for development of leaf curl symptoms. Leaf curl symptoms were observed on 13 of 40 inoculated accessions (Table 2). Four high-yielding accessions were dug by hand on 17 Sept and the remaining accessions were dug on 16 Oct. Excessive rains from hurricanes Gustav and Ike caused some souring in Beauregard plots, but little souring was noted in the other plots. Total yields were recorded and are shown in the attached Table 2 and SPLCV-inoculated yields are also shown as a percentage of the non-inoculated control.

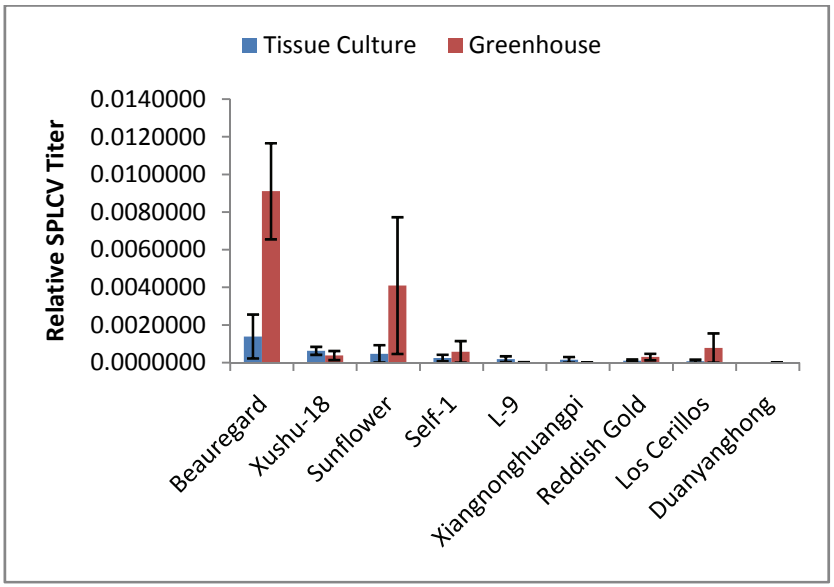
We have noted in the past that leaf curl symptoms can be especially pronounced on sprouts in plant beds, therefore roots from the 2008 plots were cured and stored and were bedded on March 23, 2009 to observe leaf curl symptom development on the sprouts. Leaf curl severity was evaluated in May-June, 2009.

In 2009, tissue culture plantlets from each line that had been micro-grafted and tested positive for SPLCV in PCR assays were established in the greenhouse during the winter and increased for use in field plots. At the same time, non-inoculated control plantlets were increased in the same way. These plants were transplanted to a field at the Burden Center on 27 April for further increase. The yield plots were transplanted on 27 May with three replicate five-plant plots per line per treatment in a randomized complete block design. The plots were laid out with the lines randomized but the non-inoculated control was planted immediately in front of the SPLCV inoculated treatment with a single hill of G0 O'Henry planted as a marker hill between the two treatments. Faster yielding lines were

planted in a separate block from the majority of the slower yielding lines. The faster yielding lines (Beauregard, Bienville, Covington, Hernandez, Georgia Jet, Siale, Xushu-18, Yizi-138, Zhenghong 3, and 60) were harvested on 4 September (105 days). Beginning in mid-September there were frequent rains which kept the land saturated until early November and prevented harvesting the slower yielding lines. When the slower yielding lines were finally harvested on 6 November (163 days), a small portion of the field remained too wet and had to be abandoned (it has had standing water constantly from mid-September to late January). Thus, for 2009 yield tests 1 or 2 replications were lost for several lines.

Results

Based on preliminary yield and SPLCV titer results, we selected Bienville (not currently in the germplasm collection) as a susceptible standard, Beauregard (PI-566613) as an intermediate type, and Xushu-18 (PI-595873) as a resistant standard. A summary of all SPLCV titer results is presented in Table 1. Most of the lines had a mean SPLCV titer in tissue culture plantlets equal to or less than the resistant standard Xushu-18 (Figure 1). When micro-grafted tissue culture plantlets were established in the greenhouse and grown out for six weeks, although SPLCV titers were higher in the greenhouse plants, there was a high correlation (coefficient = 0.898, Pr = 0.001) between mean SPLCV titers in the tissue culture plantlets and in the greenhouse-grown plants:

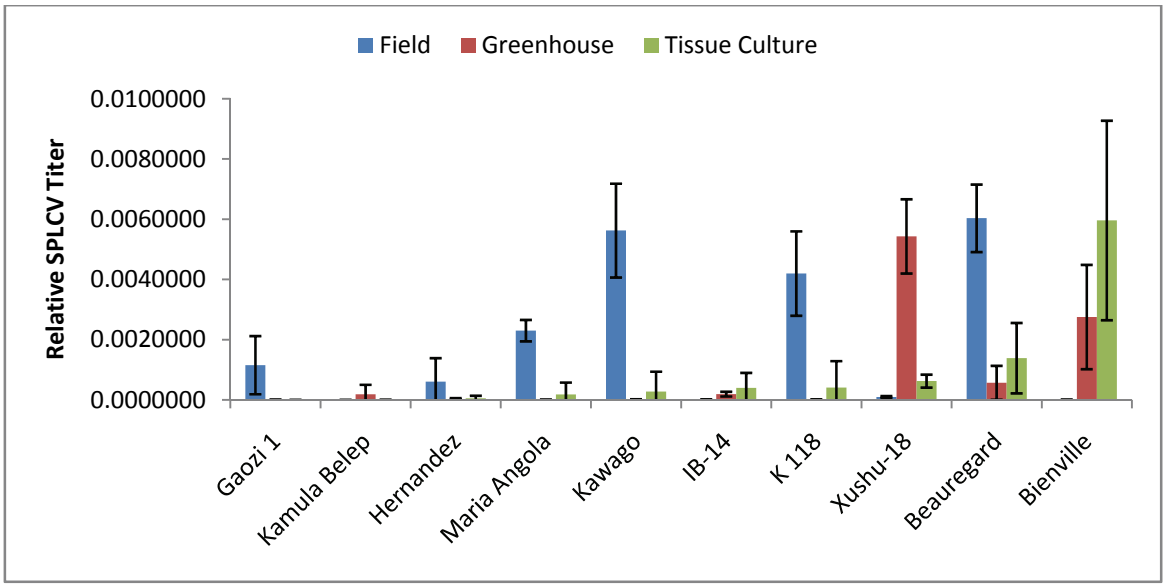


For some lines, such as Bienville, there was high plant to plant and sampling date to sampling date variability in SPLCV titer determinations (Figure 2). One possible source of the variation could be variation in micro-grafting. Generally, the scion (a piece of stem from a tissue culture plantlet infected with isolate SWFT-1) used to inoculate the test plantlets remained green and succulent throughout the six week incubation period, and in many cases shoots grew out from scions that had an axillary bud. However, when the plantlets were sampled, it appeared that some of these scions had formed relatively weak attachment to the stock plant. The high humidity in the culture vessels

may allow the scions to survive for extended times without necessarily forming a good graft union. The fact that very few test plants tested negative for SPLCV suggests that in the vast majority of cases the graft union was sufficient for virus transmission, but a limiting factor to this approach is the difficulty in assessing whether the graft was successful and the possibility that variation in graft union formation may affect the timing of infection and amount of inoculum introduced to the test plant initially.

In addition to comparing SPLCV titers between plantlets in tissue culture and after the same plants were grown out in the greenhouse, SPLCV titers were also determined on plants in field plots at six weeks after planting and on plants grown in the greenhouse at 6 weeks after standard graft inoculation in the greenhouse. There were two apparent anomalies in these evaluations: the Bienville plants grown in the field were not infected, possibly due to mislabeling of transplants, and the Xushu-18 plants in the greenhouse had titers much higher than for any other test of Xushu-18. We plan to repeat greenhouse tests with Bienville and Xushu-18. When those two lines were excluded from the analysis, the correlation for mean relative SPLCV titer between field samples and tissue culture samples was 0.681 (Pr. = 0.063), for greenhouse samples and tissue culture samples was 0.854 (Pr. = 0.07), and for field and greenhouse samples was 0.309 (Pr. = 0.457). These data are presented in both tabular and graphic form below:

Line	Field mean	Field SD	Ghse mean	Ghse SD	TC mean	TC SD
Gaozi 1	0.0011558	0.0009650	0.0000035	0.0000058	0.0000001	0.0000001
Kamula Belep	0.0000002	0.0000002	0.0001869	0.0003175	0.0000004	0.0000006
Hernandez	0.0006074	0.0007809	0.0000314	0.0000303	0.0000514	0.0000890
Maria Angola	0.0023019	0.0003564	0.0000050	0.0000045	0.0001820	0.0003970
Kawago	0.0056253	0.0015581	0.0000118	0.0000112	0.0002790	0.0006610
IB-14	0.0000052	0.0000032	0.0001958	0.0000784	0.0003970	0.0005035
K 118	0.0041990	0.0014010	0.0000069	0.0000041	0.0004118	0.0008787
Xushu-18	0.0000953	0.0000349	0.0054329	0.0012341	0.0006262	0.0002145
Beauregard	0.0060337	0.0011205	0.0005728	0.0005630	0.0013894	0.0011679
Bienville	0.0000031	0.0000031	0.0027544	0.0017336	0.0059608	0.0033131



Many of the PIs produced scant yields despite extended time in the field (155 days in 2008 and 163 days in 2009) and yield comparisons between healthy and infected are not reliable for these genotypes. There was a wide variation in yields of SPLCV-infected plants as a percentage of virus-tested plants, but there was no correlation between SPLCV titers of tissue cultures and absolute yields of either healthy or SPLCV-infected plants or more importantly with yield of SPLCV-infected as a percent of healthy (coeff. = -0.149, Pr. = 0.433 for 2008 and coeff. = -0.049, Pr. = 0.797 for 2009). This suggests that resistance/susceptibility to the virus is to some degree independent of tolerance/sensitivity to the virus. For example, Maria Angola (PI-606264) was the only line to consistently produce leaf curl symptoms in the field and had high SPLCV titers but actually produced greater yield in the infected plants than in the controls in both years, while Yanshu (PI-599391) which has SPLCV titers below the limits of detection, had 36% yield from SPLCV-infected plants compared to controls. While leaf curl severity may have been slightly greater in plant beds than in the field, the same clones that showed symptoms in the field also showed symptoms in the plant bed (Table 2).

In field plots, very few lines developed any discernible leaf curl symptoms (Table 2). Of the forty accessions that were inoculated with SPLCV and evaluated in the field in 2008, only one, Maria Angola (PI-606264) showed severe leaf curl symptoms. In 2009, leaf curl severity was systematically rated on 5 vine terminals per plot using a scale of 0-3 where 0 = no symptoms, 1 = barely perceptible curling at edge of leaf, 2 = pronounced curling at leaf edges, and 3 = severe curling, opposing edges of leaves meet in middle. Again, most lines did not show any leaf curl symptoms, the exceptions being UNPRG 64 (PI-531164), Liaoshu 40 (PI-599388), Maria Angola (PI-606264), and Boca de Chisco (PI-531128). Bienville, although it showed leaf curl symptoms in plant beds previously, developed only mild symptoms on a few plants in the field plots.

Left = leaf curl symptoms on Maria Angola 29 days after planting in the field, right = leaf curl symptoms on Bienville in plant beds.

Since the primary goal of this project was to identify SPLCV-resistant germplasm that could be used in U.S. sweetpotato breeding programs, in 2010, efforts focused on further evaluating the status of Covington and Hernandez, both high-yielding orange-fleshed cultivars that have been accepted in the U.S. sweetpotato industry. A major difficulty in working with SPLCV in sweetpotato is that the virus is by itself usually symptomless, making it difficult to confirm whether plants are infected. Unfortunately, we discovered that plants we inoculated by grafting in the Spring to use in field tests were not infected. The postponement of this test meant that it had to be conducted in the greenhouse. Four replicates of virus-tested plants were compared to SPLCV-infected by growing them in soil mix in large plastic pots for four months. We collected SPLCV titer data at six weeks after planting and yield of storage roots greater than 1-cm in diameter which are presented in the table below.

Representative storage root yields per plant from the 2010-2011 greenhouse test. Top row is virus-tested control, bottom row is SPLCV-infected. Left to right = Bienville, Covington, Hernandez, and Xushu-18.

			Yield (g/plant)			
	Rel. SPLCV Titer		VT		SPLCV	
Clone	**mean**	**St. Dev.**	**mean**	**St. Dev.**	**mean**	**St. Dev.**
Bienville	0.00002581	0.00002879	100	78	15	31
Covington	0.00002598	0.00003461	87	45	87	45
Hernandez	0.00000615	0.00001024	213	36	244	49
Xushu-18	0.00000006	0.00000005	293	73	246	110
	Pr > F		Pr > F			
Cultivar	0.363		<0.001			
Treatment			0.236			
Cv x Trt			0.244			
Rep	0.647		0.108			

Conclusions

- This study indicated a wide range of reactions exists within sweetpotato germplasm both in resistance to SPLCV as measured by virus titers, and tolerance to SPLCV as measured by effects on yield. There are however, three caveats that should be considered in future research:
    - Only a single isolate of SPLCV was used in this research. Geminiviruses in general, and the Sweet potato leaf curl virus species complex are hypervariable and there is thus a need to determine if resistance is strain specific or effective against all strains that infect sweetpotato.
    - We measured virus titer at 6 weeks after inoculation or planting, which appears to be the peak in cultivars evaluated to date. However, it would be desirable, though possibly cost prohibitive to assess titer over multiple points in time to determine if some lines such as Maria Angola might have a peak of titer at a different time.
    - The yield data is from relatively small plots, from two years in which adverse weather conditions impacted the results. Thus, yield studies should be repeated before drawing final conclusions on relative tolerance of important cultivars such as Hernandez.
- Since there is **not** a good correlation between SPLCV titer and effects of SPLCV on yield or symptom severity, both titer and yield effects should be measured to reliably determine the status of a line.
    - While a very high proportion of the accessions evaluated had SPLCV titers less than Xushu-18, only a small proportion had yields in the SPLCV-inoculated plots equivalent to or greater than the non-inoculated controls.
    - Symptoms are not reliable indicators of reaction of a genotype.

- Since there appears to be both resistance and tolerance to SPLCV in some high-yielding, moist, orange-fleshed genotypes, such as Hernandez, it may not be necessary to screen slow-yielding lines in order to obtain resistant genotypes suitable for U.S. production.
- Kokkinos (2006) showed that Sweet potato feathery mottle virus (SPFMV) could enhance replication of SPLCV in Beauregard sweetpotato. The accessions used in this study were virus-tested and tissue culture and greenhouse data is not likely affected by SPFMV, other than for one accession, PI-208129 = Camaguey, which tested positive for SPFMV. However, higher titers of SPLCV in field samples could be in part due to presence of SPFMV which spreads in the field very rapidly.
- The micro-grafting inoculation and tissue culture evaluation of SPLCV titers appears to provide relevant data to screening for SPLCV resistance. However, the process was more labor intensive than anticipated and thus, where it is possible to do screening in the greenhouse, it may prove more efficient.
- Data was submitted for entry into the GRIN system with separate ratings for SPLCV susceptibility/resistance based on virus titers and SPLCV sensitivity/tolerance based on yield effects.

References

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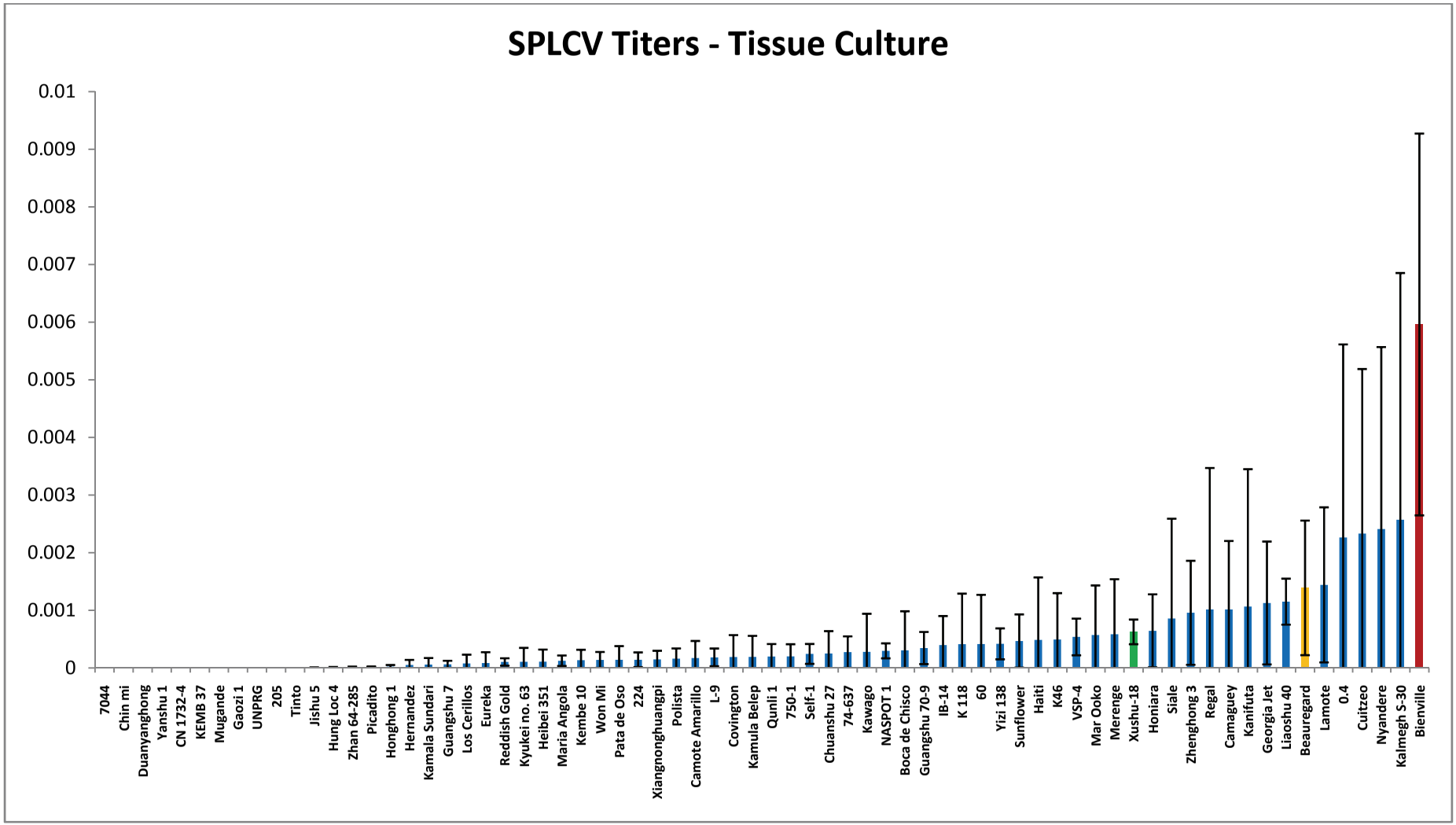

Figure 1. Relative SPLCV titers of different sweetpotato clones six weeks following micro-graft inoculation and growth in tissue culture.

Figure 2. Variation in SPLCV titer determinations from different sampling dates for standard sweetpotato cultivars Bienville, Beauregard, Hernandez, and Xushu-18.

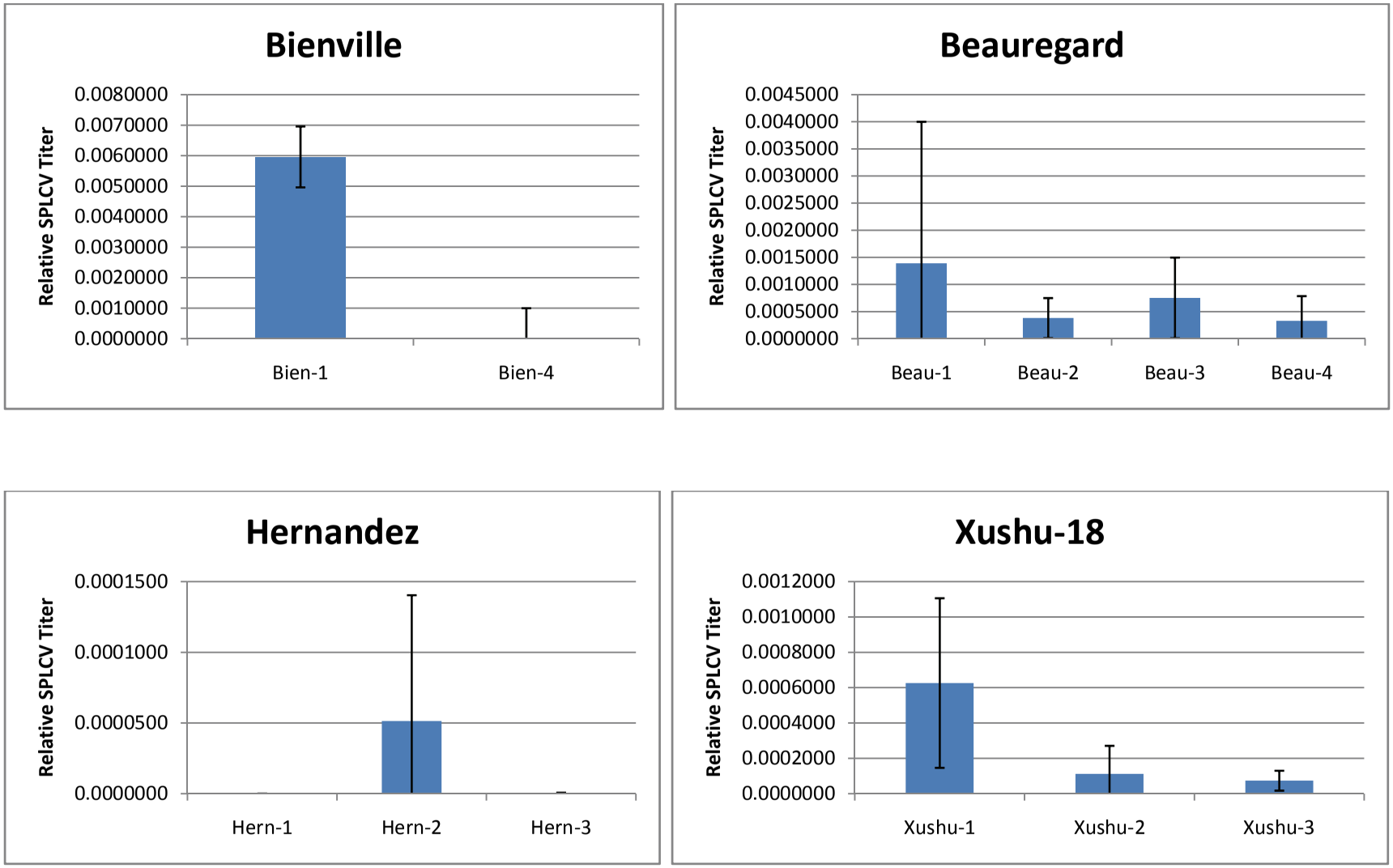

Table 1. Relative SPLCV titers determined by quantitative real-time PCR from micro-graft inoculated tissue culture plantlets and plants grown out in the greenhouse for 6 weeks from those plantlets.

		SPLCV Relative Titer			
PI No.	**Name**	**TC mean**	**TC SEM**	**GH mean**	**GH SEM**
	Bienville	0.0059608	0.0033131		
585061	Kalmegh S-30	0.0025730	0.0042810		
	Nyandere	0.0024100	0.0031590		
556934	Cuitzeo	0.0023310	0.0028570		
286629	0.4	0.0022650	0.0033500		
531134	Lamote	0.0014419	0.0013455		
566613	Beauregard (B-14)	0.0013894	0.0011679	0.0091068	0.0025542
599388	Liaoshu 40	0.0011504	0.0003989		
566627	Georgia Jet	0.0011276	0.0010662		
286621	Kanifuta	0.0010660	0.0023840		
208029	Camaguey	0.0010153	0.0011883		
566650	Regal	0.0010150	0.0024550		
606266	Zhenghong 3	0.0009575	0.0009029		
573319	Siale	0.0008580	0.0017320		
595869	Honiara	0.0006440	0.0006340		
595873	Xushu-18	0.0006262	0.0002145	0.0003739	0.0002429
642848	Merenge	0.0005860	0.0009540		
	Mar Ooko	0.0005710	0.0008610		
564159	VSP-4	0.0005383	0.0003178		
	K 46	0.0004927	0.0008063		
599369	Haiti	0.0004870	0.0010839		
508521	Yellow sunflower	0.0004672	0.0004619	0.0040923	0.0036329
531164	UNPRG 64	0.0004635	0.0004634		
612676	Yizi 138	0.0004185	0.0002680		
508515	60	0.0004150	0.0008540		
	K 118	0.0004118	0.0008787		
564127	IB-14	0.0003970	0.0005035		
606280	Guangshu 70-9	0.0003458	0.0002788		
531128	Boca de Chisco	0.0003060	0.0006780		
Q44429	NASPOT-1	0.0002970	0.0001300		
612675	Kawago	0.0002790	0.0006610		
508514	74-637	0.0002748	0.0002737		
606263	Chuanshu 27	0.0002510	0.0003888		
508520	Self-fertile No. 1	0.0002440	0.0001727	0.0005716	0.0005716
508519	750-1	0.0002012	0.0002115		
599389	Qunli 1	0.0001981	0.0002169		
	Covington	0.0001900	0.0003800		
573300	L-9	0.0001844	0.0001537	0.0000122	0.0000075
585077	Camote Amarillo	0.0001717	0.0002970		
	Polista	0.0001620	0.0001770		
606273	Xiangnonghuangpi	0.0001491	0.0001491	0.0000007	0.0000007
508518	224	0.0001420	0.0001280		

599384	Pata de Oso	0.0001413	0.0002376		
585071	Won Mi	0.0001400	0.0001380		
595890	Kembe 10	0.0001347	0.0001813		
606264	Maria Angola	0.0001250	0.0000915		
612677	Hebei 351	0.0001082	0.0002107		
633965	Kyukei no. 63	0.0001080	0.0002410		
573334	Reddish Gold	0.0001042	0.0000643	0.0002968	0.0001732
566624	Eureka	0.0000845	0.0001887		
633442	Los Cerillos	0.0000790	0.0001520	0.0007773	0.0007759
614795	Guangshu 7	0.0000629	0.0000617		
585065	Kamala Sundari	0.0000580	0.0001150		
566632	Hernandez	0.0000514	0.0000890		
612678	Honghong 1	0.0000230	0.0000290		
	Picadito	0.0000155	0.0000097		
585059	Hung Loc 4	0.0000053	0.0000116		
599390	Jishu 5	0.0000049	0.0000067		
585100	Tinto	0.0000004	0.0000003		
318848	Kamula Belep	0.0000004	0.0000006		
508517	205	0.0000002	0.0000003		
599392	Gaozi 1	0.0000001	0.0000001		
614801	Mugande	0.0000000	0.0000000		
595891	Kemb 37	0.0000000	0.0000000		
633964	CN 1732-4	0.0000000	0.0000000		
531135	7044	0.0000000	0.0000000		
599391	Yanshu 1	0.0000000	0.0000000		
573299	Chin mi	0.0000000	0.0000000		
606252	Duanyanghong	0.0000000	0.0000000	0.0000034	0.0000032
508516	78-2				

Table 2. Yield and leaf curl symptom evaluations from field trials of sweetpotato accessions comparing non-inoculated with SPLCV-inoculated plants at the Burden Center in Baton Rouge for 2008 and 2009.

		2008				Leaf Curl in 2009 Plant Beds	2009			
		Leaf Curl in the field	Total Yield (lbs per 5 plants)		LC as % of Control		Leaf Curl in the field	Total Yield (lbs per 5 plants)		LC as % of Control
PI No.	Name		Control	LC				Control	LC	
606280	Guangshu 70-9		6.4	9.5	148	0.4	0.00	0.5	3.8	760
599384	Pata de Oso	Mild	1.2	0.7	58		0.00	0.1	0.5	450
606264	Maria Angola	++	3.9	9.4	241	2.0	0.83	1.4	4.7	344
642848	Merenge	+		1.5			0.00	0.3	0.7	233
508515	60		30.9				0.00	4.8	8.7	181
508521	Yellow sunflower		1.4				0.00	3.4	5.2	151
595873	Xushu-18						0.07	7.2	7.8	108
	Covington						0.00	5.1	5.3	104
633964	CN 1732-4	+	2.6	0.3	12		0.00	7.9	8.1	103
614801	Mugande		1.3	0.1	8		0.00	0.7	0.7	100
634399	Picadito						0.00	0.8	0.8	100
599390	Jishu 5		1.7	12.7	747	0.0	0.00	3.6	3.6	99
	Polista		1.7	0	0		0.00	1.6	1.5	92
	Nyandere		1	0.1	10		0.00	1.7	1.6	91
531135	7044		10	9.2	92	0.4	0.00	9.9	8.9	90
286621	Kanifuta		15.1	9.2	61	0.0	0.00	3.7	3.3	90
566613	Beauregard (B-63)					1.0	0.00	5	4.4	87
566632	Hernandez		23.5	15	64	0.0	0.20	7.7	6.5	84
606266	Zhenghong 3	Mild	22	19.2	87	0.0	0.00	4.6	3.8	83
508518	224		22.7				0.00	12	9.9	83
612676	Yizi 138	Mild	28.7	5	17	0.0	0.00	8	6.5	81
612677	Hebei 351		12.5				0.00	5.1	4	78
585077	Camote Amarillo		18.1				0.00	7.7	5.8	75
508516	78-2	Mild	3	1.3	43	0.0	0.00	5.7	4.2	73
585059	Hung Loc 4		4.8				0.00	9.7	7	73
595891	Kemb 37	Mild	5.1	5.9	116		0.00	2.6	1.8	71
595869	Honiara		3.3	0.4	12		0.00	3.3	2.3	71
573334	Reddish Gold		1.5	6.3	420	0.0	0.00	1.9	1.3	68
599389	Qunli 1		17.1	14.5	85	0.0	0.00	5.1	3.5	68
566624	Eureka		14.1				0.00	8.3	5.4	64
566627	Georgia Jet		10.7	1.4	13	0.0	0.00	4.8	3	63
566613	Beauregard (B-14)		24.7	6.4	26		0.00	7.8	4.8	62
599369	Haiti		8.9	1.7	19	0.0	0.00	5.6	3.4	61
606252	Duanyanghong						0.00	9	5.4	60
	Bienville		9.8	5.7	58					
208029	Camaguey		12.9				0.00	3.5	2	57
585065	Kamala Sundari		2.5				0.00	2.4	1.3	54
633965	Kyukei no. 63		9.7	2.5	26	0.0	0.00	6.6	3.5	53

633442	Los Cerillos		18				0.00	3.3	1.7	52
606263	Chuanshu 27			17.4			0.00	19	8.6	45
564127	IB-14	+	11.3	7.3	65	0.0	0.00	4.5	1.8	39
Q44429	NASPOT-1		2.3	0	0		0.00	1.1	0.4	37
573299	Chin mi		16.8				0.00	18.9	7	37
599391	Yanshu 1		18.2	12.1	67	1.4	0.00	12.6	4.6	36
612678	Honghong 1		7.7				0.00	3.5	1.2	35
573319	Siale	Mild	21.3	15.7	74	0.2	0.00	9	2.6	29
599388	Liaoshu 40		34				1.00	6.3	1.5	24
599392	Gaozi 1		17.6	18.4	105	0.0	0.00	5	1	19
566650	Regal						0.00	5.2	1	19
585061	Kalmegh S-30		5.2	7.3	140	0.0	0.00	7	0.8	11
606273	Xiangnonghuangpi		0.6				0.60	2.7	0.2	6
595890	Kembe 10		0	3.4		0.0	0.00	0.1	0	0
614795	Guangshu 7		6.2	11.2	181	0.0	0.00	1.4	0	0
564159	VSP-4		14.3	10.5	73	0.0	0.00	4.6	0	0
318848	Kamula Belep		10.4	0	0	0.0	0.00	0.2	0	0
	Mar Ooko						0.00	0.3	0	0
	K 118	+	2.1	3.2	152	0.2	0.00	0	0	
585100	Tinto		6.5	0.9	14		0.00	0.9		
612675	Kawago	+	4.4	0.1	2		0.20	0	0	
531128	Boca de Chisco	+	1.8	0	0		0.93	0	1.6	
	K 46		0.8	0	0		0.00	0	0	
508517	205		26				0.00	0	0.3	
508514	74-637		19.5				0.00	0	0	
508519	750-1		18.2			0.0	0.00	0	2.2	
573300	L-9		6.1				0.00	0	0.1	
531134	Lamote		5.9				0.00	0	0	
508520	Self-fertile No. 1		2.7				0.00	0	0.9	
531164	UNPRG 64		1				1.87	0	0	
585071	Won Mi		0.8				0.00	0	0	
556934	Cuitzeo		0.3				0.00			
286629	0.4		lost				0.00			

Leaf Curl Symptom Rating Scale

0	no symptoms
1	barely perceptible curling at edge of leaf
2	pronounced curling at leaf edges
3	severe curling, opposing edges of leaves meet in middle