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Resistance to *Candidatus* Liberibacter Solanacearum (Lso) in the Wild Potato *Solanum microdontum*

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Abstract

Zebra chip is an increasingly serious disease in commercial potato production globally. Resistance can be pursued by control of the insect vector, the tomato-potato psyllid, *Bactericera cockerelli* or the bacterial causal agent, *Candidatus* Liberibacter solanacearum (Lso). Some Lso-infected plants of the wild potato species *S. microdontum* (mcd) had been observed to have low symptom expression. Thus, we evaluated a representative core collection of 86 individuals from 50 mcd populations in the US Potato Genebank (USPG). Real-time quantitative PCR on tissue from infected leaves was used as a proxy for bacterial titer of Lso. Russet Burbank control had 56% of the MLT of the most susceptible mcd individual. The average for all mcd was 67%, and the lowest, most resistant six mcd individuals were 0%. Repeated testing of those six individuals identified two as most reliably resistant: mcd15B2 from PI 265575 and mcd62B1 from PI 498126. All of these mcd individuals are available from USPG. They should be useful for research and breeding aimed at better understanding and controlling Zebra chip disease.

Keywords Potato · Zebra chip · Lso · Psyllid · CWR · Germplasm

Resumen

La papa rayada o zebra chip es una enfermedad cada vez más grave en la producción comercial de papa a nivel mundial. La resistencia puede perseguirse mediante el control del insecto vector, el psílido del tomate y la papa, *Bactericera cockerelli* o del agente causal bacteriano, *Candidatus Liberibacter solanacearum* (Lso). Se ha observado que algunas plantas infectadas con Lso de la especie de papa silvestre *S. microdontum* (mcd) tienen una baja expresión de síntomas. Por lo tanto, evaluamos una colección central representativa de 86 individuos de 50 poblaciones de mcd en el Banco de Germoplasma de Papa de los Estados Unidos (USPG). Se utilizó la PCR cuantitativa en tiempo real en tejido de hojas infectadas como indicador del título bacteriano de Lso. El testigo Russet Burbank tenía el 56% de la MLT del individuo de mcd más susceptible. El promedio de todos los mcd fue del 67%, y los seis individuos de mcd más bajos y resistentes fueron del 0%. Las pruebas repetidas de esos seis individuos identificaron a dos como los más confiablemente resistentes: mcd15B2 de PI 265575 y mcd62B1 de PI 498126. Todos estos individuos mcd están disponibles en el USPG. Deberían ser útiles para la investigación y el mejoramiento destinados a comprender y controlar mejor la enfermedad de la papa rayada o zebra chip.

Introduction

Zebra chip disease of potato (ZC) has been the subject of recent comprehensive reviews (Wenninger and Rashed 2024; Prager et al. 2022). The first reports of potato Zebra chip disease in the United States occurred at the turn of the 21st century (Munyaneza et al. 2007) and has since become an increasingly serious economic disease in commercial potato production globally. The phloem-limited bacterium, *Candidatus* Liberibacter solanacearum (Lso), causes ZC

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disease (Munyaneza 2012). Lso is persistently transmitted to plants by adult-stage tomato-potato psyllid *Bactericera cockerelli*. Harmful symptoms manifest in many ways, so control through tolerance is complex and therefore difficult. The logical preference would be to have true resistance—finding potato plants that are unsuitable hosts for both the psyllid and the bacterium. Some populations of the wild potato species *S. verrucosum* (Cooper and Bamberg 2016) and *S. bulbocastanum* (Cooper and Bamberg 2014) have been reported to have strong resistance to the psyllid vector.

For the approach of seeking resistance to the bacterium, Wallis and Rashed (2015) cite wild potato species *S. chacoense*, *tarijense*, *raphanifolium*, *etuberosum*, *berthaultii* as being in the pedigrees of breeding lines showing tolerance. When a spectrum of wild species from USPG was screened by Levy et al. (2018), plants from populations of *S. microdontum* (mcd) appeared to show no symptoms compared to uninfected controls, leading to the current experiment with a focus examining the multiplication of Lso in infected plants of that species. *S. microdontum* is a remarkable species by virtue of its many useful traits (Bamberg and del Rio 2014), and while it has had little documented use in breeding, recent genetic surveys show it has a significant presence in the cultivated potato genome (Hoopes et al. 2022).

With nearly 100 populations in USPG, mcd was a good candidate species from which to select a core subset of about 50 populations that capture nearly all the detected genetic variation, and also captures nearly all of the most favorable state of documented trait variation. The original AFLP-based core subset selected is described in Bamberg and Del Rio (2014), and the SNP-based core of two random clones in vitro from each core population that followed is described in Ma et al. (2022).

Materials and Methods

Detailed information on source populations for the mcd germplasm used in this study can be obtained from the United States Potato Genebank (USPG) using the USDA-ARS Germplasm Resources Information Network (GRIN) (Available at: https://npgsweb.ars-grin.gov/gringlobal/search.aspx).

The tested plants listed in Table 1. were 84 individual seedling clones from 50 mcd populations maintained in vitro at USPG (Bamberg et al. 2016), and grown and tested for Lso at Wapato. *S. tuberosum* cultivar 'Russet Burbank', obtained as commercial seed tubers was used as a control. Plants were grown in 900 ml plastic pots using standard methods of potting medium, fertilization, and lighting at 16 h days to produce uniform plants.

Psyllids were obtained from a laboratory colony maintained on potato cultivar 'Ranger Russet' and the tomato cultivar 'Moneymaker'. The psyllid colony was established initially from the western haplotype collected from potato fields near Prosser, WA in the spring of 2012, and new psyllids were periodically introduced to maintain genetic diversity. Subsets of colony insects were confirmed by PCR to harbor Lso.

Assays were conducted in two separate trials started from tissue culture plantlets in a completely randomized design with two replicates. Inoculation with Lso was done by confining three infected reproductively mature (> 7 day old) female psyllids to separate terminal leaves of plants in a cage of fine mesh in a greenhouse. The psyllids were removed from plants after one week and leaves were collected after four weeks. DNA was extracted from psyllids and plants using commercial kits (Qiagen). Real-time quantitative PCR (qPCR) was used as an indirect measure of Lso titers in leaves. Each reaction consisted of 10 µL of 2X SYBR Green Master Mix (Roche), 8 µl PCR grade water, 0.5 µl of each primer (final concentration of 125 nM), and 1 µL of template cDNA. The forward primer used was LsoF (CGA GCG CTT ATT TTT AAT AGG AGC) and the reverse primer used was HLBr (GCG TTA TCC CGT AGA AAA AGG TAG) (Li et al. 2009). Samples were run on a Roche Lightcycler 480 with an initial denaturation step of 10 min at 95 °C, 45 cycles of 95 °C for 10 s, 60 °C for 10 s, 72 °C for 30 s, and a final melt curve. The melt curves were checked at the end of each run to ensure there was no off-target amplification. Ct values were compared to those of serial dilutions of plasmid standards to quantify the number of copies of Liberibacter present in each sample. Clone susceptibility was expressed as mean log titer (MLT) with a conservative Ct of 45 set as the standard for zero Lso. Five of most resistant and five of the most susceptible clones were retested in a second trial in by the same method except in triplicate, assessed at both 2 and 4 weeks, and including an average symptom score (1 to 5, where a lower score is more healthy).

Results and Discussion

All mcd are not resistant to Lso. In fact, most (74 of 84) were less resistant to Lso than the susceptible cultivar control (up to 177% MLT of Russet Burbank control). However, 12 were more resistant than control, six having 61–93% MLT of Russet Burbank control, and 6 having a zero MLT value. When 5 clones with zero MLT were retested in a second trial in triplicate, all were confirmed more resistant than the control. Two clones were confirmed as 0% of control MLT, mcd14B1 and mcd62B1. However, considering plant vigor

Table 1 Ranked relative lso resistance of *S. microdontum* clones

Trial 1				Trial 2			
Clone code	Population Source	MLT 4wks	% of Control	MLT 2wks	MLT 4wks	% of control	Symptoms
14B1	PI218226	0	0	2.18	0.00	0.00	4.00
15B2	PI265575	0	0	2.03	1.54	48.32	1.33
38B1	PI458357	0	0	0.57	2.15	67.50	5.00
45B1	PI473171	0	0	.	.	.	.
45B2	PI473171	0	0	3.52	4.90	154.03	4.00
62B1	PI498126	0	0	0.25	0.00	0.00	2.00
03B1	PI195185	2.37	60.8	.	.	.	.
68B2	PI500035	2.70	69.1	.	.	.	.
01B2	PI195200	2.71	69.4	.	.	.	.
78B1	PI55901	3.19	81.7	.	.	.	.
06B1	PI597756	3.51	89.9	.	.	.	.
74B1	PI500041	3.63	92.9	.	.	.	.
Control	Russet Burbank	3.90	100.0	2.58	3.18	100.00	4.33
02B1	PI275150	3.94	101.0	.	.	.	.
11B1	PI218223	4.09	104.9	.	.	.	.
02B2	PI275150	4.13	105.9	.	.	.	.
40B2	PI473166	4.13	105.9	.	.	.	.
82B1	PI558097	4.22	108.2	.	.	.	.
65B1	PI500032	4.33	111.0	.	.	.	.
36B1	PI458355	4.35	111.4	.	.	.	.
14A1	PI218226	4.35	111.5	.	.	.	.
35B1	PI458354	4.36	111.8	.	.	.	.
86B1	PI558101	4.39	112.6	.	.	.	.
84B2	PI558099	4.40	112.8	.	.	.	.
03B2	PI195185	4.44	113.8	.	.	.	.
07B1	PI473363	4.47	114.6	.	.	.	.
96B2	PI631211	4.48	114.9	.	.	.	.
43B2	PI473169	4.50	115.4	.	.	.	.
52B2	PI473178	4.51	115.5	.	.	.	.
13B1	PI218225	4.54	116.4	.	.	.	.
21B1	PI320307	4.59	117.7	.	.	.	.
11B2	PI218223	4.65	119.2	.	.	.	.
24B1	PI320311	4.68	119.9	.	.	.	.
42B2	PI473168	4.72	121.0	.	.	.	.
85B1	PI558100	4.73	121.2	.	.	.	.
32A2	PI320319	4.76	122.1	.	.	.	.
22B2	PI320309	4.78	122.4	.	.	.	.
49B2	PI473175	4.81	123.3	.	.	.	.
20B1	PI320306	4.82	123.5	.	.	.	.
62B2	PI498126	4.85	124.4	.	.	.	.
43B1	PI473169	4.87	124.7	.	.	.	.
54B2	PI473180	4.88	125.1	.	.	.	.
82A1	PI558097	4.92	126.2	.	.	.	.
46B2	PI473172	4.95	126.9	.	.	.	.
29B1	PI320316	4.98	127.7	.	.	.	.
36B2	PI458355	5.00	128.2	.	.	.	.
24B2	PI320311	5.07	130.0	.	.	.	.
44B2	PI473170	5.09	130.5	.	.	.	.
21B2	PI320307	5.12	131.3	.	.	.	.
18B1	PI320304	5.14	131.8	.	.	.	.
01B1	PI195200	5.16	132.3	.	.	.	.
29B2	PI320316	5.18	132.8	.	.	.	.
35B2	PI458354	5.23	134.1	.	.	.	.
73B2	PI500040	5.25	134.5	.	.	.	.
52B1	PI473178	5.25	134.6	.	.	.	.
18B2	PI320304	5.26	134.9	.	.	.	.
44B1	PI473170	5.27	135.1	.	.	.	.
96B1	PI631211	5.28	135.4	.	.	.	.
86B2	PI558101	5.29	135.6	.	.	.	.
32A1	PI320319	5.31	136.2	.	.	.	.
38B2	PI458357	5.37	137.7	.	.	.	.
33B2	PI320320	5.41	138.7	.	.	.	.
97B2	PI631212	5.41	138.7	.	.	.	.
73B1	PI500040	5.42	138.8	.	.	.	.
65B2	PI500032	5.46	140.0	.	.	.	.
51B1	PI473177	5.47	140.1	.	.	.	.
84B1	PI558099	5.47	140.1	.	.	.	.
15B1	PI265575	5.49	140.6	.	.	.	.
47B2	PI473173	5.54	141.9	.	.	.	.
68B1	PI500035	5.54	141.9	.	.	.	.
07B2	PI473363	5.59	143.3	.	.	.	.
09B1	PI208866	5.60	143.5	.	.	.	.
85B2	PI558100	5.71	146.4	2.44	1.96	61.59	2.00
97A1	PI631212	5.76	147.7	.	.	.	.
12B2	PI218224	5.80	148.6	.	.	.	.
87B2	PI558218	5.85	150.0	.	.	.	.
81B1	PI545905	5.88	150.8	.	.	.	.
28B2	PI320315	5.89	151.0	.	.	.	.
81B2	PI545905	5.93	152.1	.	.	.	.
47B1	PI473173	5.94	152.3	.	.	.	.
98B1	PI631226	6.03	154.5	1.83	0.79	24.87	3.33
49B1	PI473175	6.13	157.2	.	.	.	.
28B1	PI320315	6.43	164.7	2.13	3.38	106.31	4.67
40B1	PI473166	6.59	169.0	0.00	1.49	46.71	4.33
37B1	PI458356	6.91	177.2	3.10	4.06	127.46	2.00

Clones with the first two digits are from the same population. Shaded clones are considered most resistant based on low % of Control Lso MLT in both trials and low disease symptom average in Trial 2.

and symptoms, the two we recommend as having the most robust resistance are clones mcd15B2 and mcd62B1 (as shaded in Table 1.).

The resistance of an individual mcd clone is also not very consistent within its source population. When the average MLT difference of individuals from within the same population was calculated (1.32) it was only slightly less than the difference in random pairs of individuals (1.42). This is not unexpected considering that DNA marker data on this set of materials (unpublished data not shown) fails to most closely pair 8 of 50 possible matches of siblings from the same population as being most genetically similar. Thus, fine screening and population development may need to be done to purify and genetically fix the strongest expression of resistance.

Conclusions

Strong Lso resistance in the standard cultivar breeding pool has been difficult to find, so identifying it here in two *S. microdontum* clones should provide exciting opportunities for mapping the trait, other research, and breeding.

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Declarations

Conflict of Interest The authors affirm that they have no conflict of interest.

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