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Identification of Resistance to *Dickeya dianthicola* Soft Rot in *Solanum microdontum*

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Abstract

Bacteria belonging to the *Pectobacterium* and *Dickeya* genera are globally distributed phytopathogens that are responsible for economically important soft rot and blackleg diseases of potatoes. Since 2014, there have been increased outbreaks of blackleg disease in the Eastern US, with many cases caused by an especially virulent, nearly clonal strain of *Dickeya dianthicola*. This disease is thought to be spread via commercial trade of seed tubers with latent infections of these bacteria. There is an urgent need to develop resistant potato varieties to help reduce the accidental spread and damage caused by these diseases. In this study, we conducted an iterative screen of US Potato Genebank (Sturgeon Bay, WI) collections to find wild potato relatives with resistance to tuber soft rot. We found several *Solanum microdontum* lines with high-level resistance that may be useful as source germplasm for breeding soft rot resistance into commercial potato varieties.

Resumen

Las bacterias pertenecientes a los géneros *Pectobacterium* y *Dickeya* son fitopatógenos distribuidos globalmente que son responsables de las enfermedades de pudrición blanda y pierna negra, económicamente importantes de las papas. Desde 2014, ha habido un aumento de los brotes de la enfermedad de la pierna negra en el este de los Estados Unidos, con muchos casos causados por una cepa especialmente virulenta, casi clonal, de *Dickeya dianthicola*. Se cree que esta enfermedad se propaga a través del comercio de tubérculo-semilla con infecciones latentes de estas bacterias. Existe una necesidad urgente de desarrollar variedades de papa resistentes para ayudar a reducir la propagación accidental y el daño causado por estas enfermedades. En este estudio, realizamos una evaluación repetitiva de las colecciones del Banco de Germoplasma de Papa de los Estados Unidos (Sturgeon Bay, WI) para encontrar parientes de la papa silvestre con resistencia a la pudrición blanda del tubérculo. Encontramos varias líneas de *Solanum microdontum* con resistencia de alto nivel que pueden ser útiles como fuente de germoplasma para mejorar la resistencia a la pudrición blanda en variedades comerciales de papa.

Keywords Wild potato relatives · Potato disease · Blackleg disease · Soft rot disease · Disease resistance · Dickeya · Germplasm · Potato breeding · Resistance screen

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Introduction

Soft rot *Pectobacteriaceae* (SRP), including *Pectobacterium* and *Dickeya* spp., are gram-negative, facultatively anaerobic phytopathogenic bacteria, with the common property of being able to degrade cell wall pectin and cause rotting-type disease symptoms on plants (Ma et al. 2007; Perombelon and Kelman 1980). SRP include at least 18 *Pectobacterium* and 12 *Dickeya* species (Khadka et al. 2020; van der Wolf et al. 2021b), many of which are important pathogens of potato (*Solanum tuberosum*) in many parts of the world (Ma et al. 2007). The potato diseases caused by these pathogens affect different parts

of the plant. For example, when tubers are affected, the disease is known as potato soft rot, which is primarily a postharvest storage problem. Soft rot is characterized by a spreading maceration of the tuber tissue, often with a creamy consistency that darkens when exposed to air. When aerial parts of the plant are affected, the disease is referred to as either potato blackleg disease or aerial stem rot. The symptoms of blackleg typically occur at the base of the stem close to the soil line, beginning as a dark, discoloration that spreads into stems from infected tubers (Czajkowski et al. 2015; Humphris et al. 2015).

The persistence of SRP in soil is usually benefited by the presence of plant materials in soil but reduced by biotic and abiotic challenges (Toth et al. 2021). *Dickeya* species generally have poorer survival ability in soil compared to *Pectobacterium* species (Van der Wolf et al. 2009). Therefore, *Dickeya* species are less likely to be soilborne. In contrast, commercial trade of infected seed tubers is a major route for dispersal and transmission of these diseases (Czajkowski et al. 2011). Unfortunately, inadvertent movement of these pathogens can be difficult to control because the infections are often latent, and the seed tubers can remain asymptomatic until the environment becomes conducive for disease. This can prevent visually identifying contaminated seed lots and underscores the need for improved diagnostic screening methods and for developing host resistant materials. Current management practices are limited to seed hygiene and better cultivation practices (van der Wolf et al. 2021a). An essential addition for disease management is to use host-resistant potato cultivars. There are some tolerant cultivars (i.e., plants less prone to damage by the pathogens), such as cv. Butte, Russet Burbank, and Norgold Russet toward *P. atrocepticum* (Tzeng et al. 1990), but there are no highly resistant cultivated potatoes (i.e., plants that limit pathogen growth) currently available (Bains et al. 1999; Reeves et al. 1999). Other studies found a positive correlation between soft rot resistance and the amount of anthocyanins, soluble phenols and polyphenol oxidase, which explains the higher resistance in color-fleshed potato tubers (Luzzatto et al. 2007; Wegener and Jansen 2007).

Wild and primitive cultivated potato relatives are distributed in a range that extends from the Andes Mountains in the south and into the US Rocky Mountains in the north. These species often have many favorable traits because they are naturally selected to resist abiotic and biotic stresses, whereas cultivated potatoes were selected for traits humans favor (e.g., tuber size, color, starch content, storage qualities, yield etc.). The advancement of genome-wide marker-assisted selection and the artificial breakdown of self-incompatibility of diploid hybrid potato lines (Zhang et al. 2019, 2021) will further facilitate the use of potato germplasm in potato breeding programs. Some soft rot resistant wild potatoes have been found (Bains et al. 1999; De Maine et al. 1998; Huamán et al. 2000; Rousselle-Bourgeois and Priou 1995), and the resistance of *S. chacoense* has been well-characterized (Chung et al. 2017; Joshi et al. 2021). The soft rot resistance of the *S. chacoense* M6 inbred line is associated with rapid wound healing in tubers and the presence of virulence suppressing metabolites. The rapid wound healing contribution to resistance is heritable in hybrid progeny, but quantitative trait loci (QTL) associated with most resistant and reproducible effect are on chromosome I of the diploid hybrid population (Zimnoch-Guzowska et al. 2000). Two strong QTLs that contributed to *D. solani* resistance in tuber were discovered on chromosome II and IV (Lebecka et al. 2021).

Screening exotic potato germplasm for tuber rot has inherent challenges. The US Potato Genebank (USPG) in Sturgeon Bay, WI, has over 5,000 populations representing nearly 100 species (Bamberg et al. 2016), so a scheme for stratified sampling is needed, especially when tubers are to be tested. Wild and primitive cultivated potato species do not tuberize in northern latitude field plantings because they are not adapted to the long-day growing seasons of northern latitudes. So, to obtain tubers, the plants must be grown in pots in short-day length fall-winter greenhouses or three-season screened houses when natural daylengths are conducive, limiting the time of year when tubers can be produced. An additional complicating factor is that stocks are stored as true botanical seed and must be grown for the entire life cycle of the plant to produce tubers. This means that differences in growing environment that occur between replicates (which often span several years) can affect tuber qualities and potentially influence our ability to detect heritable traits, such as disease resistance.

In this study, we conducted a broad screening of USPG collections to identify novel sources of resistance to virulent SRP pathogens that recently emerged in a significant portion of the United States potato production regions. Since 2014, there have been widespread outbreaks of especially virulent strains of *D. dianthicola* in commercial potato growing regions of the Eastern US and Canada (Charkowski 2018). The *D. dianthicola* strains recovered from the State of New York appeared to be clonal (Ma et al. 2021). This pathogen is likely being moved in asymptomatic, latent-infected seed lots, and has caused complete stand loss in some areas. We used a quantitative tuber maceration assay to iteratively screen curated collections of wild potato relatives to identify plants that could be used for future breeding efforts to introduce soft rot resistance and decrease latent transmission of these diseases in cultivated potato. We found *S. microdontum* lines, a self-incompatible diploid species, that exhibited consistent, high-level soft rot resistance.

Table 1 The mini-core potato and other wild species used in the coarse screening for soft rot resistance in potato tubers

Species names	Abbreviation	Number of accessions tested
Solanum acaule Bitt	acl	1
S. berthaultii Hawkes	ber	3
S. bulbocastanum Dun	blb	1
S. boliviense Dun	blv	106
S. brevicaule Bitter	brc	7
S. chacoense Bitt	chc	2
S. commersonii Dun	cmm	2
S. candolleanum P. Berthault	cnd	2
S. demissum Lindl	dms	2
S. hjertingii Hawkes	hjt	2
S. infundibuliforme Phil	ifd	3
S. jamesii Torr	jam	135
S. kurtzianum Bitt. & Wittm	ktz	3
S. kurtzianum Bitt. & Wittm. X *S. microdontum* Bitt	ktz X mcd	30
S. microdontum Bitt	mcd	3
S. okadae Hawkes & Hjerting	oka	3
S. pinnatisectum Dun	pnt	3
S. raphanifolium Card. & Hawkes	rap	3
S. stoloniferum Schlechtd & Bche	sto	22
S. stipuloideum Rusby	stp	2
S. tuberosum L	tbr	48
S. tuberosum L. subsp. *andigenum* (Juz. & Bukasov) Hawkes	tbr adg	7
S. verrucosum Schlechtd	ver	2

Materials and Methods

Ad Hoc Tubers for Survey Screening

An inventory of tubers for systematic resistance testing is not typically maintained at the USPG. Thus, for the initial *Dickeya* resistance screening trials, ad hoc tuber samples available from unrelated research projects were used. All of these were produced with the standard tuberization culture: pot-grown in the greenhouse in the summer and late fall of 2016 in commercial potting medium with standard watering, fertilizing, and insect and disease-control. Tubers were stored in paper bags at room temperature for one week after harvest, then stored at 5 °C until shipped for *Dickeya* resistance testing at Cornell in May of 2017. Ad hoc tubers were as follows:

Mini-core collection: USPG staff have selected a manageable set of 25 of these species for an initial screening when a priori information on the trait of interest is not available. The set aims to maximize representation of ploidies, breeding systems, and geographic origins of potato, but favors species and populations known to have vigorous growth and fertility, as well as species known to have strong expression of useful traits (Bamberg et al. 2016; Hardigan et al. 2015). Each species, including the primitive cultivated species *S. andigenum* is represented by a maximum of three populations, and each population sample tested was from a bulk of tubers from 12 randomly collected individuals. Thus, although these tubers were not produced intentionally for *Dickeya* resistance screening, they were the perfect set for a priori prospecting for high resistance in the USPG.
Criolla: These were "egg yolk" style specialty potato breeding selections crossed to a self-compatibility donor breeding stock, PI 654,351. There were 48 separate F1 hybrid clones with pedigrees primarily involving diploid cultivated potato species *S. andigenum* in the genebank's Germplasm Resources Information Network (GRIN) database, but previously known as *Solanum* species *stenotomum* and *phureja*.
Comprehensive species sets:

S. jamesii. This primitive diploid is the only wild potato species primarily occurring in the US, and has been the subject of extensive collecting and research by genebank staff (Bamberg et al. 2020). Samples were taken from a bulk of tubers from 12 individuals per population.
S. boliviense. These were tuber samples from this wild diploid species from Peru and Bolivia. Tubers from a single randomly selected individual per population were sampled.
S. fendleri. Tubers of multiple individuals from a single population (PI 679,938) of this tetraploid wild species originating in the Santa Catalina Mountains near Tucson, Arizona, USA.

S. kurtzianum (PI 472,923) x *Solanum microdontum* (PI 218,225) hybrids. These were tuber samples of multiple individuals from a single genetic stock experimental population involving research on the trait of high tuber calcium accumulation (Chung et al. 2016).

S. microdontum (SCAN set). One random individual from each of the over 100 populations of *S. microdontum* in the genebank was grown in the greenhouse at USPG, harvested in the first week of November 2017, stored, and shipped for *Dickeya* soft rot disease testing in May 2018.

PI number of each accession tested can be found in supplemental tables (Table S1, S2). Detailed information on all species and populations (PI numbers) used in this study can be obtained through the online GRIN either through the USPG website (https://www.ars.usda.gov/midwest-area/madison-wi/vegetable-crops-research/

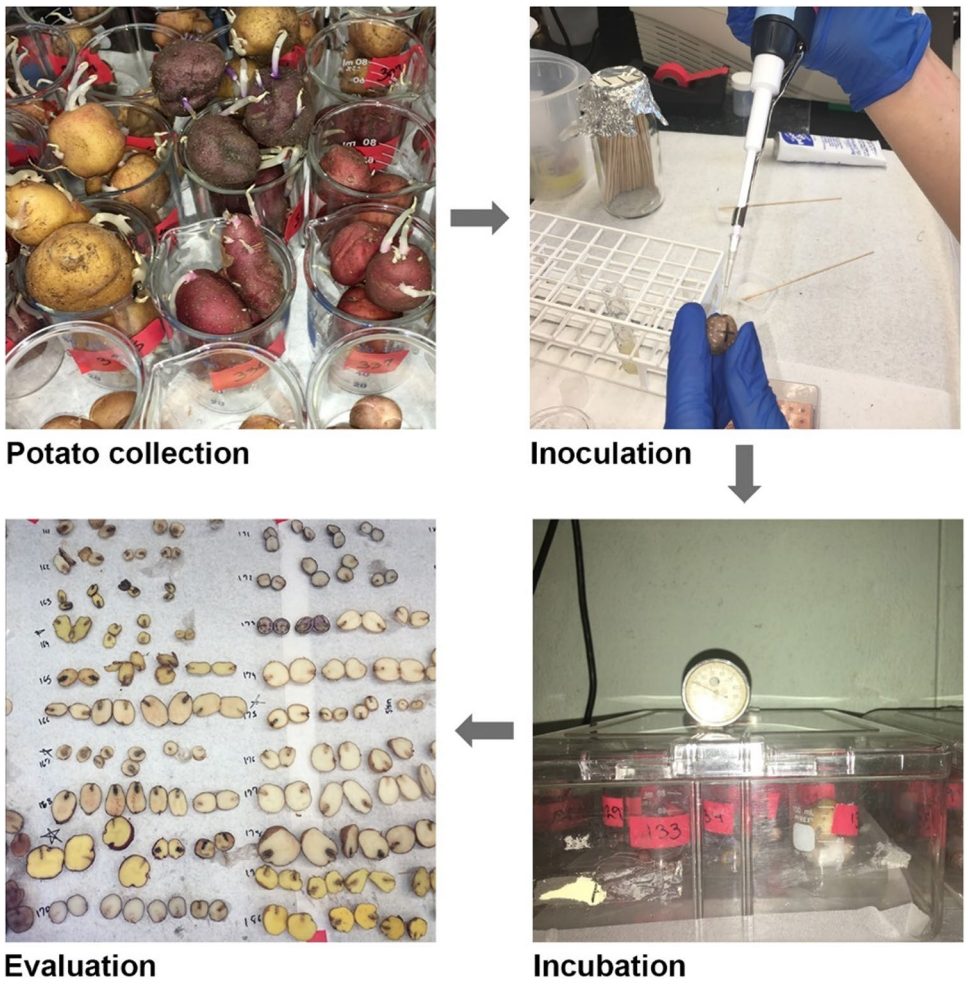

Fig. 1 The procedure for evaluating tuber soft rot resistance

people/john-bamberg/bamberg-lab/) or directly (https://npgsweb.ars-grin.gov/gringlobal/search.aspx).

We started the screen in 2017 summer with the mini-core wild potato collection and other ad hoc potatoes mentioned above except the SCAN set. The composition of the test collection is summarized in Table 1. The accession PI numbers for each population are included in Table S1. The resistance screening on mini-core and all ad hoc potato tuber accessions were conducted in three replicates.

In the next step, we focused on the systematic assessment of the core collection of 50 accessions *S. microdontum* that possess 98% of the diversity and most of the valuable traits known in the species (Bamberg and Del Rio 2014). *Dickeya* resistance was assessed in tubers of these potatoes grown in two locations, the standard fall-winter greenhouse, and specialized tuberizing growth chambers (referred to as Phytotron thereafter) from CETS Tech LLC (Del Rio et al. 2017). Each population within the two locations was represented by two blocks of five random seedlings. Then, the tuber production in each location was replicated twice in time. The Phytotron tubers were produced almost contemporaneously in two separate chambers in the fall of 2017 with tubers harvested in early 2018. Greenhouse environment tubers were produced in the fall of two separate years, 2017 and 2018, by the same techniques that produced pot-grown ad hoc tubers previously evaluated. The growing environments are detailed in supplemental Appendix A.

In addition to the core collection, an ad hoc collection consisting of 112 accessions of *S. microdontum* from the SCAN set was tested. Due to the extended genetic backgrounds and the different growth environments, these data were analyzed independently to the *S. microdontum* core-collection.

Inoculum and Inoculation

D. dianthicola ME23 (Ma et al. 2019), a representative isolate of the Eastern US *Dickey*a outbreak strain, was used for tuber soft rot resistance screening. To prepare for tuber inoculations, we streak plated LB (Sambrook et al. 1989) agar with *D. dianthicola* ME23 from our culture collection stored at -80 °C in LB broth amended with 16% glycerol. The streak plates were incubated at 28 °C for 24 or 48 h. We then inoculated 5 ml of LB broth with a loopful of *D. dianthicola* ME23 colonies and incubated at 28 °C overnight with shaking at 240 rpm. The

cultures reached approximately 10^9 CFU/ml and were used for tuber inoculations.

We used a quantitative test to determine the soft rot resistance phenotype of tubers produced from plants in each panel. Briefly, 5 µl of *D. dianthicola* ME23 inoculum (final concentration about 10^9 CFU/ml) were placed into a 5 mm deep hole made with a 2 mm diameter sterile wooden applicator. For mock inoculated controls, 5 µl of sterile LB broth were used instead of inoculum. Petroleum gel was applied on the wound inoculations site to prevent drying, and incubated for 24 h at 30 °C. To determine the size of the lesions, we cut a longitudinal section of the tuber, bisecting the inoculation wound, and measured the width (across the inoculation site) of tissue maceration with a digital caliper (Fig. 1). Each trial was replicated using four randomly selected tubers (technical replicates), three of them were inoculated, and one was used as a mock-inoculation control. Tuber inoculation of the initial screen, core microdontum screen, and the SCAN set microdontum screen were conducted in the same manner.

Statistical Analysis

The lesion size of each inoculated tuber was fit to a mixed model using R software v3.4, with the *lmerTest* package v3.0. The model factored in the genotype (potato accession) and genotype by environment interaction as random effects, while environments and blocks as fixed effects. In the course of screening among the mini-core collection and other ad hoc potato tubers, we fit the lesion sizes to the following mixed model to identify the accession with the highest, most consistent resistance phenotypes:

$$y_{ijk} = \mu + g_i + r_k + \varepsilon_{ijk} \quad (1)$$

where y_{ijk} represents the lesion size of ith accession (genotype), μ denotes the overall mean of lesion sizes, g_i denotes the main random effect of *ith* accession, r_k denotes the fixed effect of kth replication. ε_{ijk} represents the random error. In the *S. microdontum* core collection resistance screening, we fit the lesion sizes for the 50 *S. microdontum* accessions in the core collection to the following mixed model:

$$y_{ijk} = \mu + g_i + e_j + (ge)_{ij} + t_k + \varepsilon_{ijk} \quad (2)$$

where y_{ijk} represents the lesion size of ith accession (genotype), jth location, and kth block, μ denotes the overall mean of lesion sizes, g_i denotes the main random effect of ith accession, e_j represents the main effect of jth location, $(ge)_{ij}$ represents the random effect of the interaction of ith accession and jth location, t_k denotes the fixed effect of the kth block, lastly, ε_{ijk} represents the random error. The broad-sense heritability, an indication of the repeatability of a trait among different environments, was calculated based on the following formula (Holland et al. 2003):

$$H^2 = \frac{\sigma_g^2}{\sigma_p^2} = \frac{\sigma_g^2}{\sigma_g^2 + \frac{\sigma_{ge}^2}{m'} + \frac{\sigma_\varepsilon^2}{p'}} \quad (3)$$

where, H^2 is the broad-sense heritability, σ_g^2 represents the genotypic variance, σ_p^2 denotes the phenotypic variance, σ_{ge}^2 is the variance of genotype by the environment, σ_ε^2 represents the variance of replicates per environment, the m′ and p' are divisors of σ_{ge}^2 and σ_ε^2 for unbalanced design (due to the inability to obtain enough tubers from some plants/conditions) calculated in the following method:

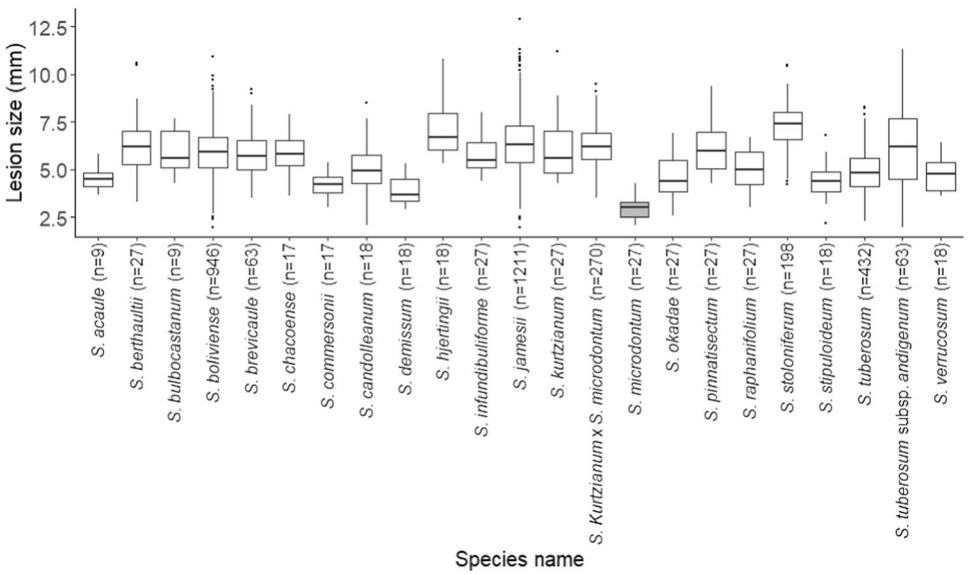

Fig. 2 *Solanum microdontum* exhibited high resistance to soft rot. Lesion size in tubers 24 h after inoculation with *D. dianthicola* ME23. Horizontal lines and boxes show the median and interquartile range, respectively. The dots are outliers beyond 1.5 × the interquartile range above or below each box. Grey-shaded box represented the lesions from *S. microdontum*. The number of samples examined for each species was marked after species name

Table 2 Soft rot lesion size-based ranking for the *S. microdontum* core collection[a]

Accession	Overall[b]			Greenhouse				Phytotron			
PI No.			BLUP	Rep1		Rep2		Rep1		Rep2	
	Rank	n[c]	(mm)	Rank	n	Rank	n	Rank	n	Rank	n
498126	1	101	2.85	2	18	1	27	1	27	5	29
545905	2	100	2.99	1	30	2	25	8	15	8	30
473363	3	110	3.00	14	29	4	28	2	28	3	25
597756	4	120	3.05	3	30	14	30	6	30	4	30
473178	5	112	3.09	5	24	3	30	17	30	9	28
218223	6	111	3.16	11	28	18	26	5	30	7	27
631226	7	83	3.19	4	27	10	27	24	15	2	14
208866	8	111	3.19	18	30	16	27	4	28	6	26
558099	9	95	3.23	6	30	7	30	13	15	20	20
218226	10	115	3.30	19	30	15	27	15	28	10	30
320311	11	112	3.32	9	24	26	30	11	28	14	30
458355	12	112	3.33	12	30	6	27	27	25	17	30
218224	13	117	3.34	7	30	21	30	9	28	24	29
558100	14	92	3.35	21	26	5	27	21	14	11	25
265575	15	119	3.42	23	30	30	30	10	29	13	30
631211	16	97	3.44	27	30	25	30	18	15	1	22
218225	17	113	3.45	15	27	24	27	25	30	19	29
320309	18	117	3.47	16	30	11	30	28	30	26	27
320315	19	115	3.48	17	30	33	29	14	26	22	30
458354	20	119	3.48	13	30	8	30	19	29	41	30
500041	21	99	3.50	25	20	19	30	7	22	28	27
558218	22	96	3.50	22	30	23	30	23	15	16	21
473177	23	115	3.53	28	30	22	30	26	30	12	25
473180	24	98	3.55	8	20	20	27	30	30	29	21
458356	25	118	3.64	32	30	17	29	12	30	37	29
473166	26	111	3.65	10	30	34	27	16	30	45	24
320316	27	115	3.70	24	30	27	30	32	29	31	26
473175	28	111	3.70	41	30	9	27	38	28	21	26
458357	29	111	3.72	26	30	40	21	3	30	46	30
500040	30	100	3.75	29	25	31	24	22	29	38	22
320306	31	116	3.78	20	30	13	29	48	30	35	27
631212	32	88	3.79	36	30	29	30	20	15	32	13
473173	33	116	3.83	45	30	28	27	37	30	18	29
195200	34	119	3.86	37	30	12	30	41	29	33	30
545901	35	117	3.87	31	30	38	27	43	30	23	30
500035	36	107	3.88	30	26	43	27	29	30	30	24
473170	37	111	3.89	39	29	42	24	35	30	15	28
558097	38	103	3.97	33	30	41	30	34	15	42	28
195185	39	115	4.07	43	30	45	30	36	28	25	27
275150	40	115	4.08	40	30	32	26	45	29	34	30
473169	41	114	4.19	44	27	36	27	44	30	39	30
473172	42	117	4.21	42	30	35	30	49	27	43	30
320307	43	120	4.27	46	30	39	30	40	30	44	30
320320	44	105	4.34	38	30	44	18	31	29	49	28
320319	45	111	4.36	34	30	37	28	47	25	50	28
500032	46	117	4.38	49	27	46	30	33	30	40	30
558101	47	90	4.41	47	27	48	27	39	12	27	24
320304	48	117	4.49	35	30	47	27	50	30	47	30
473171	49	110	4.54	48	30	49	30	42	23	36	27
473168	50	119	4.97	50	30	50	30	46	29	48	30

[a] The lesion sizes were calculated from the best linear unbiased prediction (BLUP) from a mixed model factoring the genotype, location, genotype by location interaction, and blocks. The rank order of each accession was highlighted with a transitional color from green (Rank 1) to yellow (Rank 50)

[b] The overall ranks were calculated based on the lesion sizes from the two replications conducted in the two locations, respectively

[c] n denotes the number of potato tubers tested

$$m' = \frac{n}{\sum_{i=1}^{n} \frac{1}{m_i}} \quad (4)$$

$$p' = \frac{n}{\sum_{i=1}^{n} \frac{1}{p_i}} \quad (5)$$

where, m_i is the number of environments for the ith genotype, p_i stands for the number of tubers tested for the ith genotype, n denotes the number of genotypes (50 genotypes in this study) (Holland et al. 2003). In the complete *S. microdontum* collection screening, the lesions sizes were measured and fit to the following mixed model:

$$y_{ik} = \mu + g_i + r_k + \varepsilon_{ik} \quad (6)$$

where, y_{ik} is the lesion size of ith accession (genotype), and kth replicate, μ denotes the overall mean of lesion sizes, g_i denotes the main random effect of ith accession, r_k is the fixed effect of the kth replication, and ε_{ik} represents the random error.

The best linear unbiased prediction (BLUP) of lesion sizes for each accession was calculated as the sum of random effects contributed by its genotype and the computed model intercept of the mixed model. This BLUP score was used to compare the resistance phenotype of the potato tubers from different genetic backgrounds. In the core collection screening, we computed the overall BLUP from all four environments (two locations by two replicates) and the BLUP for each environment to test how consistent the soft rot rankings were between different growing environments. The variances of lesions sizes from Phytotron and greenhouse were compared with a one-sided F-test with the R function var.test(), in which the "alternative" argument was set to "less". We also divided the core collection lesion size data into 50 accession groups. Within each accession group, the linear regression model and Tukey's HSD test with the *agricolae* package v1.3–5 were employed to compare lesion sizes from each environment.

Results

Mini-core, *S. boliviense*, and *S. jamesii* Collection Screening

We initiated our search for soft rot resistant plants by conducting a coarse screen of USPG germplasm for *Dickeya* resistant materials. We evaluated the mini-core, *S. boliviense*, and *S. jamesii* and other ad hoc potato tubers, which together entail 392 plant accessions or hybrids that belong to 23 different potato species or subspecies (Table 1). Soft rot lesion sizes were plotted in boxplots to show the median and variance of lesion sizes for each species/subspecies (Fig. 2). We fit the lesion sizes to formula (1). By calculating the random effects and BLUP of lesion sizes, we were able to rank the relative resistance of each accession (Table S1). In the initial screening of mini-core collection and other ad hoc tubers of many species and hybrids, three populations of *S. microdontum* tubers from the Mini-core set had the best *Dickeya* resistance scores (Fig. 2 and Table S1).

S. microdontum Core Collection Screening

Finding that the soft rot lesions in *S. microdontum* tubers were consistently smaller than all other accessions tested motivated us to explore the *S. microdontum* species more thoroughly for resistance to *Dickeya* soft rot. To accomplish this, we tested the soft rot resistance phenotype of the *S. microdontum* core collection, which is a curated set of 50 accessions that were chosen as the minimum number of accessions needed to represent 90% of the variation of the *S. microdontum* species (Bamberg et al. 2016). In this experiment, we fit the lesion sizes to the model described in Formula (2). The BLUP of each genotype was computed based on this mixed model. We ranked the resistance of the 50 *S. microdontum* core collection accessions by their overall BLUP of lesion sizes and ranked the lesion sizes from four different environments (two locations by two replicates) (Table 2). The lesion sizes ranged from 2.85 cm for the most resistant accession to 4.97 cm for the least resistant accession. The overall ranks for each accession were not always consistent with the ranks in each of the four environments. But we observed that in each environment, the high resistant and the low resistant accessions tend to cluster at the two ends of the table (Table 2), suggesting that some accessions are close in resistance and hard to separate in ranks, while others have distinguishable differences in their resistance to *Dickeya*.

The divisors used in estimating the broad sense heritability were calculated based on Formulae (4) and (5).

$$m' = \frac{50}{12.5} = 4$$

$$p' = \frac{50}{0.4605} = 108.57$$

The broad sense heritability was calculated based on Formula (3).

$$H^2 = \frac{0.2563}{0.2563 + \frac{0.1342}{4} + \frac{0.9026}{108.57}} = 0.8596$$

The variance of genotype and variance of genotype by environment computed from the mixed model, Formula (2), were used to calculate the broad-sense heritability. The broad-sense heritability is 0.8596, which indicates that

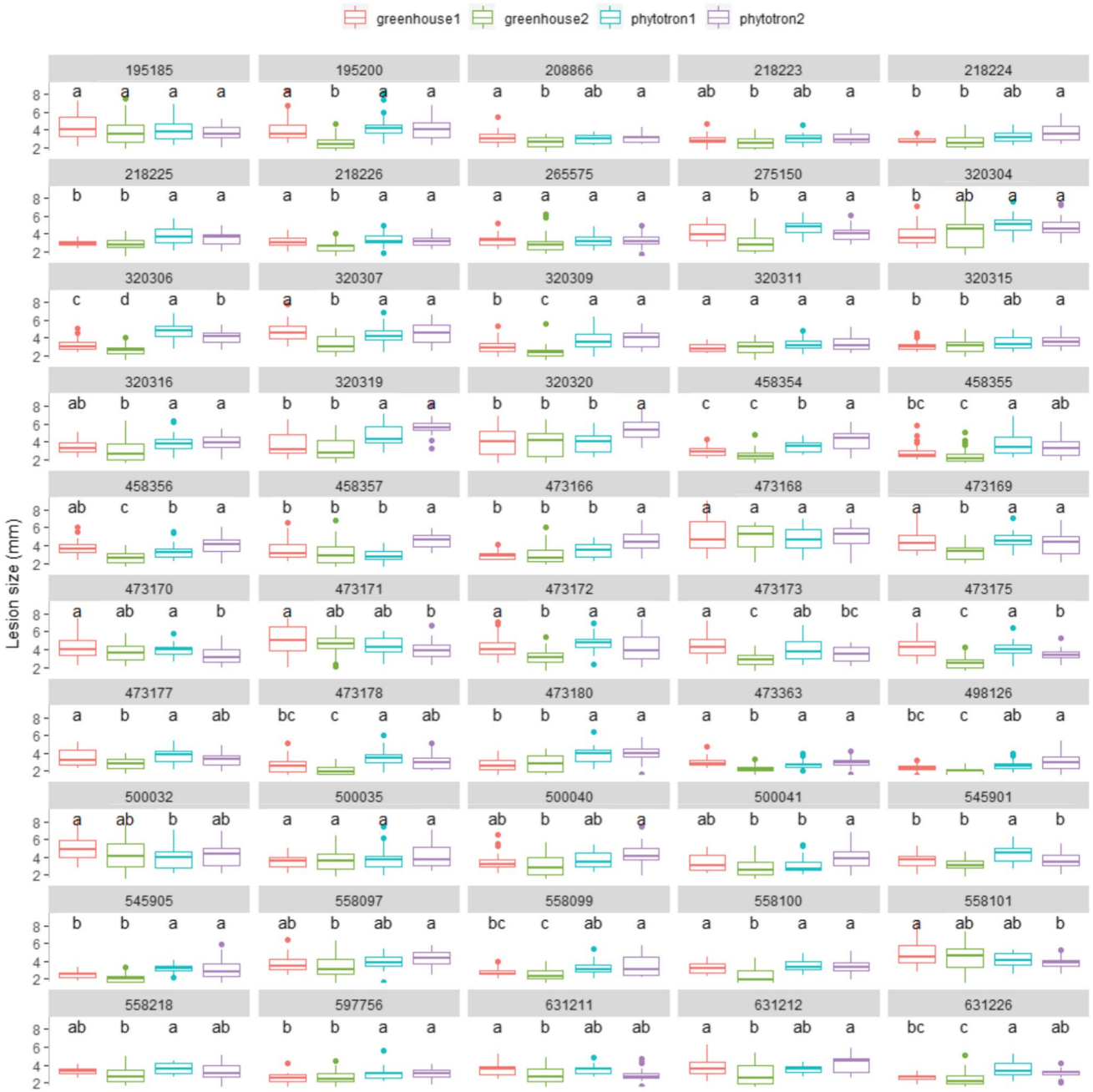

Fig. 3 Comparison of the effects of four environments (greenhouse and Phytotron with two replications, respectively) on the lesion sizes for each individual accession of the 50 *S. microdontum* potato accessions. The numbers on top of each chart panel are the accession PI number. Horizontal lines and boxes show the median and interquartile range, respectively. The dots are outliers beyond 1.5 × the interquartile range above or below each box

85.96% of the total variance in lesion sizes was conferred by plant genotype (i.e., accessions); the rest 14.04% of the variance was contributed by the phenotype-by-environment interaction and random error.

In this experiment we tested 2649 tubers produced in Phytotron and 2823 in the greenhouse. Tubers from Phytotron have a mean lesion size of 3.79 cm and a standard error of 0.0215, while that from greenhouse have a mean of 3.27 cm and a standard error of 0.0229. To determine whether the variance of lesion sizes differed between these two growing locations, we compared the variances of the lesions sizes from Phytotron grown tubers to those grown in the greenhouse using a one-sided F-test with the null hypothesis that they have the same variance. The F-test result ($F = 0.83$, $p < 0.001$) rejected the null hypothesis and indicated that the variance observed from tubers produced in Phytotron is

significantly smaller than that in greenhouse. In addition to the examination of the overall variances of lesion sizes from the two locations, we also studied the consistency of lesion sizes from different environments for individual accessions. The lesion sizes from the four environments were separated by Tukey's HSD test for each accession (Fig. 3). The results showed that there were variations in lesion sizes within the same genotype that were caused by different growing environments. Only five out of 50 accessions had no significant difference among the four environments (i.e., PI195185, PI265575, PI320311, PI473168, PI500035). Regarding the variances between the two replicates from the same facility (greenhouse or Phytotron): 17 out of 50 accessions grown in the greenhouse had significantly different lesion sizes, while 10 out of 50 accessions grown in the Phytotron had significantly different lesion sizes (Fig. 3).

Complete *S. microdontum* Collection

We screened the complete *S. microdontum* collection, which consists of 112 accessions. The lesion sizes were fit to Formula (6). All 112 accessions were ranked based on their BLUP of lesion sizes (Table S2), ranging from 1.95 to 5.2 mm. When we compared the results of the *S. microdontum* core collection and the complete *S. microdontum* collection screening, we found that some accessions showed consistent high resistance in both experiments. Tubers from accessions PI458355, PI558099, PI208866, PI545905, PI498126, PI473178 are among the 15 most resistant in both experiments. Meanwhile accession PI320320 had mixed results from the two experiments, which ranked 44th in the initial screen of the *S. microdontum* core collection but ranked eleventh in the complete collection survey. PI473168 and PI500032 are among the least resistant accessions in both experiments (Table S2).

Discussion

We began this study with a coarse screen of 392 accessions or hybrid lines and identified that tubers of three *S. microdontum* populations were among the most resistant to soft rot caused by *D. dianthicola*. This prompted us to systematically evaluate 50 core-collection accessions and 112 complete collection accessions of *S. microdontum* to determine their BLUP of lesion sizes under four environments. In the coarse screen, we also identified that some other species (e.g., *S. andigenum*), amenable to breeding due to their close phylogenetic relationship to cultivated potatoes, had a large variation in lesion sizes between accessions. This included some highly resistant accessions, which should be further examined in other focused studies.

This study is the first to quantify the tuber soft rot resistance of *S. microdontum* populations against *D. dianthicola*, an important pathogen in the Eastern US. *S. microdontum* is a diploid wild potato species with large simple leaves that exists in a range extending from the Andes in central Bolivia to northern Argentina. The *S. microdontum* genome sequence suggests its close relation to cultivated potato and shows the significant historical contribution to the *S. tuberosum* group Tuberosum (Hardigan et al. 2017). The ability of *S. microdontum* to cross with *S. tuberosum* makes it a good subject for research and crop-relevant breeding (Bamberg and Del Rio 2014; Peloquin et al. 1989).

We examined the effect of environment on *S. microdontum* soft rot resistance and found that environment has a minor role in this trait. The broad-sense heritability we observed for tuber soft rot resistance showed that 87% of the variation in lesion sizes among the *S. microdontum* tubers was conferred by plant genotype, which strongly suggests that the observed resistance has a genetic basis and is not primarily a function of the environment by genetic interaction. This broad-sense heritability for soft rot resistance is close to that found in clones of complex diploid potato hybrids (Lebecka and Zimnoch-Guzowska 2004). But it was also much higher than some other cases, such as in *S. chacoense* and dihaploid *S. tuberosum* potato hybrids and other complex diploid potato hybrids (Lebecka et al. 2021; Lee et al. 2019). The high broad-sense heritability for resistance in *S. microdontum* clones makes them strong candidates for use as breeding materials in future projects.

Growing the tubers at multiple locations also allowed us to analyze the effect of growth conditions on the variances of our assay results. We found that Phytotron-grown tubers developed more consistent lesion sizes than in the greenhouse. Phytotrons are state-of-the-art growth chambers that automatically control environmental conditions, which enables rapid tuber production (between 60–70 days) at any time of year (Del Rio et al. 2017). The consistency in our assay results and the flexibility in timing suggest that Phytotrons provide a useful method for producing tubers for potato breeding research.

The mechanistic basis of the *Dickeya* resistance that we observed among *S. microdontum* populations is not known. Previous studies showed that some *S. microdontum* tubers are capable of elevated calcium accumulation (Bamberg et al. 1998), suggesting the possibility that the *S. microdontum* lines found here have elevated calcium ion accumulation in their tubers. There is a positive correlation between the amount of calcium in potato plant tissues and resistance to SRP infection and disease (McGuire and Kelman 1984). Calcium ions help reduce the effects of SRP pectinolytic

enzymes by strengthening plant cell walls (van der Wolf et al. 2021a; White and Broadley 2003). Calcium amendments can also delay the development of blackleg in the field (Bain et al. 1996; Ngadze 2018), but some field trials found that the calcium content in tubers is negatively associated with SRP soft rot symptoms, suggesting that additional research in this area is warranted (Mantsebo et al. 2014; McGuire and Kelman 1984).

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Declarations

Conflicts of Interest The authors have no potential conflicts of interest to disclose.

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