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Greenhouse and Field Nursery Evaluation for Potato Common Scab Tolerance in a Tetraploid Population

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Abstract Potato common scab (*Streptomyces scabies* (Thaxt.) Waksman & Henrici) is a major disease of potato (*Solanum tuberosum* L.), due to the unmarketability of affected tubers. For identification of the most common scab-tolerant material, and for developing molecular markers for common scab tolerance, more information is needed on the genetic basis of common scab tolerance. Phenotyping common scab susceptibility is difficult because of the large variability in disease symptoms among tubers from a single plant, ranging from no common scab to severe pits. Two years of field data were collected for scab reaction on a segregating tetraploid population (MSL603, 160 individuals). Continuous variation in common scab susceptibility phenotype was observed among the progeny, with a normal distribution suggesting common scab disease phenotype is a genetically complex trait. Transgressive segregants were also observed, but they are skewed toward susceptibility. A greenhouse-based screening procedure was evaluated to discern tolerant from susceptible potato lines. A subset of ten individuals from this population were selected (five resistant, five susceptible). For the greenhouse study, soil was inoculated with a pathogenic *S. scabies* strain MSDPZ at a concentration of 3 X 10^8 CFU/ml. This greenhouse assay effectively discerned tolerant and susceptible individuals. There was a moderate correlation between the greenhouse study and the field trial. The greenhouse assay may provide information that would complement field data in identifying resistant clones.

Resumen La sarna común (Streptomyces scabies (Thaxt.) Waksman & Henrici) en una enfermedad muy importante de la papa (Solanum tuberosum L.) debido a que los tubérculos afectados no son comerciables. Para la identificación del material más tolerante a la sarna común y para desarrollar marcadores moleculares para tolerancia a la enfermedad se necesita más información sobre la base genética de la tolerancia. La fenotipificación de la susceptibilidad a la sarna común es difícil por la gran variabilidad en los síntomas de la enfermedad entre los tubérculos de una misma planta, que varían desde la ausencia de sarna a presencia de hoyos profundos en los tubérculos. Se colectaron datos durante dos años sobre la reacción a la sarna en una población tetraploide (MSL603, 160 individuos). La variación continua del fenotipo susceptible a la sarna común fue observada entre la progenie, con una distribución normal que sugiere que el fenotipo de la enfermedad es una característica genéticamente compleja. También se observó variación transgresiva, pero sesgada hacia la susceptibilidad. Un procedimiento de tamizado en invernadero fue evaluado para diferenciar las líneas de papa tolerantes de las susceptibles. Un subgrupo de 10 individuos de la población fue seleccionado (cinco resistentes, cinco susceptibles). Para el estudio de invernadero, el suelo fue inoculado con una cepa patogénica MSDPZ de S. scabies a una concentración de 3 x 10^8 UFC/ml. Este ensayo de invernadero distinguió efectivamente individuos tolerantes y susceptibles. Hubo una moderada correlación entre los estudios de invernadero y la prueba de campo. La prueba de invernadero puede proveer información que complementaría los datos de campo para identificar clones resistentes.

Keywords Breeding · *Streptomyces scabies*

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Introduction

Streptomyces scabies (Thaxt.) Waksman & Henrici, the casual agent of potato common scab, is a soil-borne bacterium that can infect potato (*Solanum tuberosum* L.) tubers. There are over 400 identified species of *Streptomyces* and only a fraction of these are pathogenic on potato tubers (Loria et al. 1997). Those which are pathogenic infect a wide range of hosts, including radish (*Raphanus sativus*), parsnip (*Pastinaca sativa*), beet (*Beta vulgaris*), carrot (*Daucus carota*), and potato (*Solanum tuberosum*) (Goyer and Beaulieu 1997).

Streptomyces scabies is one of the major bacterial pathogens that infect potato. The pathogen produces necrotic, corky-textured lesions on the outer surface of the potato. The lesions may be raised, superficial, or pitted. Chip processors consider pitted lesions a defect because the pit is apparent in the processed chip. Since the marketplace for potatoes is quality driven, the presence of scab lesions, especially those which are pitted, on the outer surface of the potato for both tablestock and chipping varieties significantly lessens their marketability.

Breeding for tolerance to potato common scab has been described as the most effective way to combat the disease (McKee 1958), yet relatively few cultivars grown in North America are resistant to potato common scab. Moreover, there is currently no consensus as to the genetic model to explain resistance to potato common scab. Relatively little progress has been made towards developing a marker-based system for the tolerance to potato common scab. Tolerance, however, is believed to be quantitative (Cipar and Lawrence 1972).

To determine the degree of resistance, reliable, reproducible experiments are needed. In field studies the results are variable and many years of testing are required. There have been many attempts to reduce environmental variables to improve the accuracy of screening scab resistance. Keinath and Loria (1991) observed a positive relationship between inoculum density and severity of scab produced on potato. Jellis (1974) was able to reduce variability and distinguish resistant from susceptible varieties by controlling in-field soil moisture by covering field rows with plastic tunnels. Hooker (1950) developed a method for observing scab infection by growing plants in an inverted pot system, allowing scab development to be viewed on a daily basis.

Early studies to test for tolerance commonly used naturally infested field soil. Lapwood et al. (1966, 1970a, 1970b) found that irrigation could significantly suppress disease, and also determined that a potato is physiologically most susceptible in the period immediately following tuber initiation. Wiersema (1970) reported a reliable greenhouse test using field soil in 3 L plastic pots. Watering was done by subirrigation instead of overhead watering, thus promoting disease development. According to Bjor (1974) (Bjor and Roer 1980), field soil produced variable results, and by simply inoculating a sand mixture, results were more consistent. Potato seed pieces were grown in pots with a sand mixture inoculated with *S. scabies* and controlled irrigation. They found that scab severity scores corresponded to field observations and that resistance could be determined based on two to three pots per line.

The objective of this research was to evaluate a segregating population for scab tolerance (MSL603 population) in a field-based common scab test, then compare field results to a selected sub-sample in a greenhouse-based assay.

Materials and Methods

Plant Material

A segregating population (MSL603) for potato common scab tolerance was generated from the cross Jacqueline Lee x MSG227-2. Jacqueline Lee is a commercial release from the Michigan State University potato breeding program (Douches et al. 2001). This variety is oval with cream colored flesh, resistant to late blight and is susceptible to common potato scab. The breeding line MSG227-2 is a round white chip processing line and is classified as tolerant to scab. All 160 MSL603 progeny were evaluated in the field-based scab tests. A subset of the population was selected for evaluation in the greenhouse study.

Field Nursery Study of the MSL603 Mapping Population

Field trials were planted at Michigan State University's Soils Farm in East Lansing, MI. The MSU field scab nursery was developed to promote a high incidence of potato common scab disease by planting susceptible potato varieties, inoculating with the pathogenic MSDPZ strain of *S. scabies*, and incorporating spring and fall manure applications for 3 years prior to the field evaluation. This site has been planted continuously in potatoes with periodic inoculations to maintain potato common scab disease pressure. Seed for the field and greenhouse experiments was grown at field with low scab pressure at the Lake City Experiment Station, Lake City, MI. Tubers with scab lesions were not used for the studies. The 160 MSL603 progeny were planted in 2004 and 2005. In 2004, tuber seed pieces were planted on May 7 and rated during the week of September 6. In 2005, plots were planted on May 9 and rated in the week of September 19. To account for the variability usually found in a scab nursery, the trial was planted as a randomized complete block design with five-hill plots and four replications. Plots were mechanically harvested and the tubers were laid on the soil surface

for immediate evaluation by a three person team. Ratings were based on lesion coverage and lesion type, with higher scores corresponding to an increase in scab severity as described in Table 1.

Greenhouse Assay

Ten individuals (plus the parents) were selected from the MSL603 mapping population for the greenhouse assay. These individuals were chosen based on the 2 years of replicated field trial data. Five individuals were selected from each of the resistant and susceptible categories. The parental clones were used as check varieties.

S. scabies cultures (MSDPZ strain) were grown on yeast malt extract (YME) (Schaad et al. 2001) plates for 2 weeks. Spore concentration was quantified using a hemocytometer and was adjusted to 1 X 10^6 spores/ml. One milliliter of spore suspension was used to step culture into 50 ml of liquid YME, and was incubated in a shaker for 3 days at 28°C. Cultures were centrifuged (5,000 g for 5 min) in 10 ml batches. Each culture was resuspended in distilled water and added to its own culture vessel with sterilized vermiculite and 50 ml of SAY solution. Vermiculite was sterilized by autoclaving three times for 1 h every other day at 121°C. The weight of the vermiculite was recorded for later quantification of inoculum (Wanner 2007; Wanner 2004). Five hundred milliliter culture vessels were used to incubate the inoculum for approximately 14 d at 28°C. After incubation, samples of inoculum from all culture vessels were plated on yeast media extract by mixing 1 g of inoculated vermiculite in 9 ml of sterile distilled water. This sample was incubated for 2 days at 28°C to assess population density and ensure that no contamination occurred. Concentration of *S. scabies* in each vessel was determined by plating four 10 µl serial dilutions (10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}) onto yeast media extract and by counting colony forming units (CFU).

The growing medium for the greenhouse assay consisted of a 50:50 mixture of soil and sand that were sterilized by autoclaving three times for 3 h every other day at 121°C. Two liter plastic pots were first filled with a lower layer of a 900 cc mixture of equal parts of sterilized soil and sand. Potato seed pieces were surface sterilized with 5% bleach solution, rinsed, and dried prior to planting. The seed pieces were placed on top of the first layer of growing medium in the pots. The vermiculite inoculum was incorporated into a second 900 cc mixture of growing medium to reach 3 x 10^8 CFU/ml of soil and added to the pots on top of the seed piece. The plants were grown at a 16 h photoperiod with supplemental high-pressure sodium greenhouse lights. Plant watering was reduced to promote scab disease conditions; plants were watered only when they showed early signs of wilting, allowing the soil to dry out between watering. After approximately 14 weeks, tubers were harvested and scored as described above (Table 1). The experiment was conducted as a completely randomized design with three replications each.

Table 1 Rating scale used to classify the severity of common scab infection on potato tubers

	Scab Severity Rating Criteria		
Rating	Scab lesion percent % surface area	Pitted lesion percent % surface area	Depth of pits mm
0	0	0	0
1	1-10	0	0
2	11-25	0	0
3	26-50	1-5	< 1
4	> 50	6-25	> 1
5	> 50	> 25	> 1

Statistical Analysis

Following the F_{Max} test for homogeneity of variances, the data were combined for each of the two field years and also the two greenhouse replications. Statistics for the field scab ratings and greenhouse scab ratings were each conducted using analysis of variance and Fisher's Protected Least Significant Difference for means separation (α=0.05). All analyses of variance were performed using the SAS general linear model procedure at α=0.05 (SAS 2002).

Greenhouse rating data for the ten select MSL603 progeny and parental clones were compared to the field ratings. Pearson's correlation coefficients were determined using the SAS correlation procedure at α=0.05 (SAS 2002).

Results

Field Scab Nursery Results

Data were combined on the MSL603 progeny for years 2004 and 2005. The distribution of the progeny follows a typical bell-shaped curve for a quantitative trait (Fig. 1). The distribution of scab ratings for all 160 progeny in the MSL603 population plus the two parents ranged from 1.1 to 3.6. MSG227-2 ranked 2nd for the scab rating (1.2), while Jacqueline Lee ranked 124th (2.8). Transgressive segregants were observed at both ends of the distribution. The five most tolerant and five most susceptible clones from the MSL603 population were chosen for use in the greenhouse assay (Table 2). Of the 5 most scab tolerant progeny, only one clone (MSL603-272) was more tolerant than MSG227-2. All five of the selected susceptible clones were more susceptible than Jacqueline Lee.

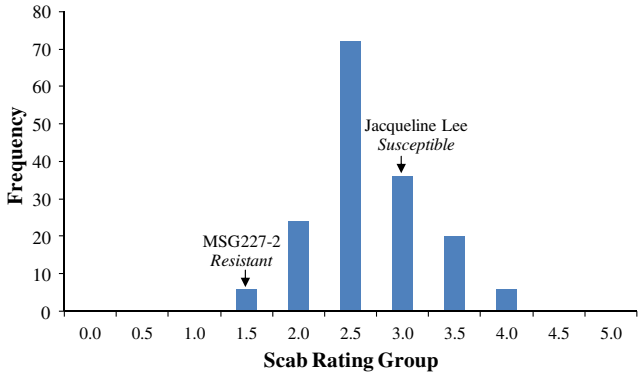

Fig. 1 Frequency distribution of the combined 2004 and 2005 field scab ratings for 160 progeny of MSL603 mapping population. Clones are grouped based on 0.5 ratings on a scale from 0 (no infection)–5 (highly infected with pitted lesions). The parents MSG227-2 (resistant) and Jacqueline Lee (susceptible) are also shown

Greenhouse Assay

Both MSG227-2 and Jacqueline Lee were rated as more susceptible in the greenhouse assay compared to their field ratings (Table 2). The breeding line MSG227-2 was rated 0.6 points higher in the greenhouse and Jacqueline Lee was rated 0.3 points higher compared to field ratings, whereas all ten select MSL603 progeny had a lower greenhouse rating compared to their field scores. The greenhouse assay ratings were moderately correlated (r^2=0.347) to field-based scab disease nursery ratings (P<0.0443, Fig. 2).

Table 2 Scab severity ratings of ten select clones from the MSL603 population for field (combined 2004 and 2005) and greenhouse evaluations. MSL603 parents MSG227-2 (R = resistant) and Jacqueline Lee (S = susceptible) are also included

Scab	MSL603	Scab Severity Rating[1]			
Category	Clone	Field		Greenhouse	
R	272	1.1	b	0.4	fg
R	MSG227-2	1.2	b	1.8	bcd
R	219	1.4	b	0.9	defg
R	274	1.5	b	0.1	g
R	281	1.6	b	0.9	defg
R	167	1.9	b	0.7	efg
S	Jacqueline Lee	2.8	a	3.1	a
S	298	3.3	a	1.3	cde
S	234	3.4	a	1.4	cde
S	153	3.5	a	2.2	bc
S	182	3.6	a	2.3	ab
S	291	3.6	a	1.3	def
	$LSD_{0.05}$[2]	0.9		0.9	

[1] Ratings were based on a 0 to 5 scale with 0 = no disease and 5 = most severe symptoms (Table 1)

[2] Means with the same letter designation are not significantly different based on Fisher's Least Significant Difference (LSD, α=0.05) within each trial

Discussion

For identification of the most common scab-tolerant material, and for developing molecular markers for common scab tolerance, more information is needed on the genetic basis of common scab tolerance. The genetics of tolerance/resistance remain unclear, even though several genetic studies to date have been done on haploid or diploid populations (Cipar et al. 1972; Dionne and Lawrence 1961). Phenotyping common scab susceptibility is difficult because of the large variability in disease symptoms among tubers from a single plant, ranging from no common scab to severe pitting. In this study, a comprehensive evaluation of all 160 progeny from the MSL603 population was completed for severity of potato common scab over two field seasons (2004 and 2005). Continuous variation in common scab susceptibility phenotype was observed among the progeny, with a normal distribution (Fig. 1). This suggests common scab disease phenotype is a genetically complex trait and has a strong environmental component to the phenotype. Transgressive segregants were also observed. They are biased toward susceptibility. The genetic inheritance for tolerance to potato common scab is not clearly understood. Tolerance is believed to be quantitative (Cipar and Lawrence 1972). Alam (1972) hypothesized that two loci are responsible for scab tolerance. At one locus (Sc_1), one or more dominant alleles confer resistance while at the other locus (sc_1) a homozygous recessive condition confers resistance. Due to environmental influences, severity of this disease fluctuates from year to year, thereby making the resistance difficult to map.

Our intention was to use this population for quantitative trait locus (QTL) analysis, but the range of scab ratings and the skewing of the population towards susceptible progeny did not allow us to use this population for further genetic studies. For QTL analysis to be conducted in the future, a

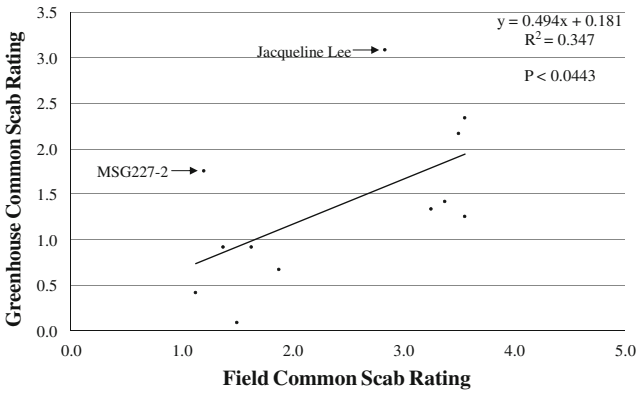

Fig. 2 A moderate correlation between the field (combined 2004 and 2005) and greenhouse ratings for the select MSL603 mapping population clones. Graph displays the ten select progeny from the MSL603 population and the parents

population derived from parents with a greater difference in scab ratings should be used.

The five most tolerant and the five most susceptible clones were selected for testing in the greenhouse assay. The scab severity ratings for the parents were consistent with previous years' ratings (data not shown). The combined greenhouse tests of the ten select MSL603 progeny produced lesions that were comparable in appearance to those found on field grown tubers. High concentrations of scab inoculum (3 x 10^8 CFU/ml) were used to evaluate the ten select MSL603 progeny. Despite using high concentrations of inoculum, scab incidence was not great enough to strongly discriminate between resistant and intermediate classes of progeny. Future greenhouse tests should employ higher inoculum concentrations than 3 x 10^8 CFU/ml and monitor inoculum concentration levels during the onset of tuberization.

The greenhouse scab ratings from the MSL603 progeny moderately correlated (r^2=0.347, P<0.0443) with the field scab ratings. Keinath and Loria (1991) found that scab severity is positively correlated to inoculum concentrations ranging from 5.5 x 10^2 to 6.8 x 10^5 CFU/g soil-sand under greenhouse conditions. Kobayashi et al. (2005) planted seed pieces in paper pots inoculated with *S. turgidiscabies*, then transplanted to the field after emergence. They observed scab infection with inoculum concentration in the paper pots. In another study, Loria and Kempter (1986) obtained results with a lower concentration (3 x 10^6 CFU/ml) of inoculum. They used stem cuttings and seed pieces to assess disease reaction on cultivars Chippewa, Katahdin, and Superior under greenhouse conditions. Although lesions were similar to those found on field grown tubers, they were not able to generate moderate or deeply pitted lesions. It appears that the infection process of common potato scab is confounded by environmental factors in the field and greenhouse as well as cultivar resistance.

Our results are similar to Bjor and Roer (1980) who found that their inoculated coarse sand greenhouse assay performed more reliably than their field test. They also found that the most resistant varieties retained their resistance across thirty-six different *S. scabies* isolates. The most tolerant progeny (MSL603-272 and MSL603-219) from the MSL603 population demonstrated similar resistance levels in both field and greenhouse ratings (Table 2). Transgressive segregants were observed in both field and greenhouse ratings. Of the 2 years of field data, 36 of MSL603 progeny ranked more susceptible to common potato scab than Jacqueline Lee, the susceptible parent (data not shown). When comparing field data for tolerant progeny with the tolerant parent (MSG227-2), only one clone was more tolerant (MSL603-272). It may be worth pursuing other populations segregating for scab tolerance to see if tolerant transgressive segregants can be identified.

Effective and efficient identification of scab resistant clones is important in a breeding program. The greenhouse assay is time and labor intensive; therefore, depending on resources available, it may be used to test only a limited number of clones. The field evaluation is less resource intensive, but does have environmentally influenced variability, limiting our ability to identify resistant clones in a single environment. Fewer resources are needed to run a field test compared to a greenhouse scab assay. To take advantage of both screens, the breeder could first evaluate a large number of clones in the field eliminate the highly susceptible clones and then select only the clones with less infection for further evaluation in the greenhouse. The greenhouse test may provide further information in identifying clones that were misclassified as resistant in field tests.

Conclusions

Phenotyping common scab susceptibility is difficult because of the large variability in disease symptoms among tubers from a single plant, ranging from no common scab to severe pits. The greenhouse study produced lesions comparable to field grown tubers. A moderate correlation was found between field data and the greenhouse assay using selected progeny from the MSL603 population. The greenhouse results indicate that the average field ratings from both years were always higher than the greenhouse ratings for all progeny. At this time, a combination of field and greenhouse trials could be combined to identify scab resistant progeny in segregating populations. Further investigation is needed to refine a suitable resistance screen to common potato scab. Limiting the amount of variation in disease ratings from the scab nursery field trials should be of primary importance. This will likely be the most challenging aspect of the resistance screen since water, temperature, and host plant resistance will remain as key variables during disease progression. More spread between parental phenotypes and better methods of scoring and visualizing the disease phenotype may be helpful in developing and characterizing potential mapping populations to be used in developing markers for the common scab tolerance.

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