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Genetic Stability in Potato Germplasm for Resistance to Root Galling Caused by the Pathogen *Spongospora subterranea*

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Abstract *Spongospora subteranea*, the causal agent of potato powdery scab is becoming increasingly important worldwide. Little is known about the genetic basis of resistance to this disease. The present study tested the hypothesis that potato genotypes with stable genetic resistance to "Spongospora root galling" were present in potato germplasm. Root galling index values of 24 genotypes screened for resistance in four field trials (environments) in 2004 and 2005 in Washington State and Idaho were analyzed. Genotypes tested included five resistant, four industry standards and advanced selections from the USDA-ARS, Prosser, WA program. Broad-sense heritability was calculated as 0.76 with a 95% confidence interval of 0.55–0.89, indicating a fairly high genetic component of the trait. Of the 24 genotypes that were tested, eight showed no genotype*environment interactions while six of the remainder had significant variance (i.e., they were unstable) after removal of genotype*environment variance. Among the five resistant genotypes, PA95B2-4 was stable, and PA98N5-2, PA98NM38-1, PO94A009-7 and POR00HG5-1 were stable after the removal of environmental heterogeneity. Among the four industry standards, Shepody was unstable, whereas Ranger Russet, Russet Burbank and Umatilla Russet were stable after the removal of genotype*environment variance. Stable resistance to "Spongospora root galling" was identified. A large portion of the variation was genetic, which will enable breeders to use resistant and stable potato genotypes as parents in future breeding to develop superior commercial potato cultivars with resistance to "Spongospora root galling".

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Resumen *Spongospora subteranea*, el agente causal de la roña polvorienta se está convirtiendo de importancia en aumento en el mundo. Se sabe poco de las bases genéticas de la resistencia a esta enfermedad. El presente estudio probó la hipótesis de que los genotipos de papa con resistencia genética estable al "agallamiento radical por Spongospora" estaban presentes en germoplasma de papa. Se analizaron los valores del índice de agallamiento radical de 24 genotipos probados para resistencia en cuatro ensayos de campo (ambientes) en 2004 y 2005 en los Estados de Washington y Idaho. Los genotipos probados incluyeron cinco resistentes, cuatro estándares para la industria y selecciones avanzadas del programa USDA-ARS, Prosser, WA. Se calculó la heredabilidad en una base amplia como 0.76 con un intervalo de confianza de 95% de 0.55 a 0.89, indicando justamente un componente genético amplio del carácter. De los 24 genotipos probados, ocho no mostraron

interacción genotipo-medio ambiente, mientras que seis del resto tuvieron varianza significativa (por ejemplo, fueron inestables) después de eliminar la varianza genotipo ambiente. Entre los cinco genotipos resistentes, PA95B2-4 fue estable, y PA98N5-2, PA98NM38-1, PO94A009-7 y POR00HG5-1 fueron estables después de eliminar la heterogeneidad del ambiente. Entre los cuatro genotipos estándares para la industria, Shepody fue inestable, mientras que Ranger Russet, Russet Burbank y Umatilla Russet fueron estables después de eliminar la varianza genotipo-ambiente. Se identificó resistencia estable a "agallamiento radical por Spongospora". Una gran proporción de la variación fue genética, lo cual permitirá a los mejoradores usar genotipos resistentes y estables de papa como progenitores en mejoramiento futuro para desarrollar cultivares comerciales de papa superiores con resistencia al "agallamiento radical por Spongospora".

Keywords Powdery scab · *Solanum tuberosum* L. · Resistance · Broad-sense heritability

Introduction

Spongospora subterranea (Wallr.) Lagerh f. sp. *subterranea* (*S. ss*) is a soil and potato tuber-borne pathogen. On potato this pathogen has root infection and tuber infection stages in its life cycle, which result in two diseases: "Spongospora root galling" and potato tuber "powdery scab". The pathogen is classified in the phylum Cercozoa as a member of the Plasmiodiophorida ("plasmodiophorids") in the kingdom Protozoa (Alexopoulos et al. 1996; Merz 2008; Braselton 2001; Cavalier-Smith 2003; Merz and Falloon 2009). The pathogen is biotrophic and is characterized by the formation of a plasmodia in host roots and stolon cells, biflagellated zoospores, infectious activity at temperatures between 10 to 20°C under wet conditions, and the formation of resting spores aggregated in sporosori ("cystosori", "spore balls") (Harrison et al. 1997). The spore balls sporosori aggregate on the roots in galls, and in masses in tuber lesions. Infected potato tubers and infested soils are means of disseminating the spore balls, which can survive in soils for many years (Kole 1954); and also can acquire and transmit the *Potato mop-top virus* (Jones and Harrison 1969; Qu and Christ 2006 b).

Potato (*Solanum tuberosum* L.) cultivars that are susceptible to the disease demonstrate sponge-like galls on the roots, and/or lesions on the tubers (Harrison et al. 1997). Potatoes vary in susceptibility to these two diseases. Russet skin cultivars do not develop severe or noticeable "powdery scab" tuber lesions as happens with tubers of white or red potatoes with smooth skins. However, roots of most, if not all, commercially successful cultivars demonstrate "Spongospora root galling" to different levels (Merz et al. 2004; Merz 2008). In the Pacific Northwest of the USA the majority of the potato production area is planted to long season (4.5–6 months) russet skinned cultivars used for processing (Schreiber 2006). These cultivars are not severely affected by "powdery scab", but are affected by "Spongospora root galling". Reports from the industry in Washington State indicated that certain processing cultivars with russet skin are no longer grown in areas of the Columbia Basin where soil populations of this pathogen are high due to the damage the pathogen causes to the roots. The observations suggested that losses of 5–12 metric tons/ha may occur due to "Spongospora root galling", causing considerable loss of tuber size and adversely affecting contract incentive payments (Brown et al. 2007).

Nitzan et al. (2008) reported the presence of field resistance to "Spongospora root galling" in potato germplasm. Among 57 genotypes tested for resistance between 2003 and 2007, eight genotypes were identified as more resistant to the root disease than the four industry standards, Russet Burbank, Russet Ranger, Umatilla Russet and Shepody. The resistant genotypes were PA98NM38-1, PO94A009-10, PA95B2-4, PA98N5-2, POR00HG5-1, PO94A009-7, PO94A012-2; and the commercial cultivar Summit Russet. The pedigrees of these genotypes revealed consanguinity in two factors: (i) all were derived from an introgression program to incorporate resistance to the Columbia root-knot nematode *Meloidogyne chitwoodi* from the Mexican wild species *Solanum bulbocastanum*; and (ii) all had the commercial cultivar Summit Russet appearing more than once in their ancestry (Love et al. 2005; Brown et al. 2006; Brown et al. 2007).

The two diseases "Spongospora root galling" and potato "Powdery scab" are of major concern in the Pacific Northwest, and worldwide (Falloon et al. 1995; Falloon et al. 1996; Merz 2000; Merz et al. 2004; Johnson and Cummings 2004; Qu and Christ 2006; Van de Graaf et al. 2007). Therefore, in order to enhance the breeding of resistant genotypes, it was essential to evaluate the genetic stability of the resistance trait. The present study tested the hypothesis that genotypes with stable resistance to "Spongospora root galling" were present among the resistant germplasm previously (Nitzan et al. 2008) identified.

Materials and Methods

The "Spongospora root galling" index values of 24 genotypes (Table 1) previously screened for resistance in four trials (environments) that were carried out in 2004 and in 2005 in Moses Lake, Washington and Rexburg, Idaho were analyzed. Some of these genotypes were identified as resistant, while others were susceptible (Nitzan et al. 2008).

Table 1 List of standardized root gall index values, and genetic stability of 24 potato genotypes that were tested for resistance to "Spongospora root galling" caused by *Spongospora subterranea* in 2004 and 2005 in Washington and Idaho in the field

Genotype [a]	Standardized root gall index by environment (year/location) [b]					Genetic stability [c]		
	2004 ID	2004 WA	2005 ID	2005 WA	Mean	σ_i^2	s_i^2	Outcome
PA00N29-3	0.11	0.34	0.02	0.26	0.18	**	**	Unstable
PA00N32-4	0.10	0.11	0.03	0.23	0.12	**	ns	GxE
PA00N35-2	0.24	0.62	0.10	0.61	0.39	**	**	Unstable
PA00N44-2	0.19	0.58	0.04	0.84	0.41	**	ns	GxE
PA95B1-53	0.15	0.19	0.00	0.35	0.17	ns	ns	Stable
PA95B2-4	0.01	0.08	0.02	0.27	0.10	ns	ns	Stable
PA95B4-67	0.18	0.16	0.05	0.42	0.20	ns	ns	Stable
PA98N5-2	0.05	0.14	0.00	0.14	0.08	**	ns	GxE
PA98NM30-11	0.09	0.13	0.00	0.63	0.21	**	**	Unstable
PA98NM36-16	0.13	0.56	0.07	0.72	0.37	**	ns	GxE
PA98NM38-1	0.03	0.04	0.02	0.08	0.04	**	ns	GxE
PA98NM39-1	0.21	0.31	0.10	0.46	0.27	ns	ns	Stable
PA99N82-4	0.12	0.56	0.02	0.40	0.28	**	**	Unstable
PA99N88-2	0.09	0.38	0.02	0.38	0.22	ns	ns	Stable
PO94A009-10	0.03	0.06	0.00	0.36	0.11	ns	ns	Stable
PO94A009-2	0.11	0.15	0.00	0.35	0.15	ns	ns	Stable
PO94A009-7	0.06	0.06	0.02	0.23	0.09	**	ns	GxE
PO94A010-3	0.10	0.17	0.03	0.44	0.19	ns	ns	Stable
PO94A012-2	0.17	0.10	0.10	0.49	0.22	**	**	Unstable
POR00HG5-1	0.05	0.17	0.02	0.09	0.08	**	ns	GxE
Ranger Russet	0.16	0.44	0.07	0.71	0.35	**	ns	GxE
Russet Burbank	0.12	0.33	0.04	0.76	0.31	**	ns	GxE
Shepody	0.23	0.66	0.18	0.82	0.48	**	*	Unstable
Umatilla Russet	0.20	0.33	0.08	0.69	0.32	*	ns	GxE

[a] Genotypes in bolded font were identified as resistant to "Spongospora root galling" (Nitzan et al. 2008). [b] ID=Rexburg, Idaho; WA=Moses Lake, Washington. [c] (*), (**) and (ns), respectively indicate that the genotype makes a significant (P=0.05), highly significant (P=0.01), or no significant contribution to the genotype*environment interaction before (σ_i^2) or after (s_i^2) removal of the environmental heterogeneity. Stable=genotypes that were stable both before and after removal of environmental heterogeneity; Unstable=genotypes that were unstable both before and after removal of environmental heterogeneity; G×E=genotypes were unstable before removal of environmental heterogeneity but stable after removal of environmental heterogeneity.

The original root galling index, which varied among the trials (Nitzan et al. 2008) was standardized by dividing the original root galling index score values (y_i) by the highest value the scale could obtain (y_{max}), or y_i/y_{max} (Haynes et al. 1997). The 0–4 scale used in 2004 in Washington was divided by 4. The 0–6 scale used in 2004 in Idaho was divided by 6. The 0–7 scale used in 2005 in Washington was divided by 7, and the 0–3 scale used in 2005 in Idaho was divided by 3 (Table 1).

Estimates of broad-sense heritability for resistance to "Spongospora root galling" were calculated from the standardized root galling index. The data were analyzed using the mixed models and general linear models procedures in SAS (SAS 9.1, Cary, N.C.). Genotypes, environments and reps were the model's main effects and were considered random. Estimates of the variance components from the mixed models procedure were used to calculate broad-sense heritability (H) as the ratio of the genetic variance over total phenotypic variance: $H = \sigma^2{}_G / \left[\left(\sigma^2{}_{error}/16\right) + \left(\sigma^2{}_{G\times E}/4\right) + \sigma^2{}_G\right]$. Estimates of the genotype and genotype*environment mean squares from the general linear models procedure were used to calculate the 95% confidence interval for H. The upper confidence interval is 1-[(MS1/MS2) $F(1\text{-}\alpha/2\text{:df2,df1})]^{-1}$, while the lower confidence interval is 1-[(MS1/MS2) $F(\alpha/2\text{:df2,df1})]^{-1}$, where MS1=mean squares for genotype and MS2=mean squares for genotype* environment (Knapp et al. 1985). The genotype*environment G×E interaction was partitioned into stability variance components σ_i^2 assignable to each genotype (Shukla 1972) using a SAS program written by Kang (1989). An environmental index for each environment was calculated by subtracting the grand mean over all environments from the mean for each environment. Heterogeneity due to this index

was removed from the genotype*environment interaction and the remainder partitioned into s_i^2 assignable to each potato genotype thus constituting variance not explainable by genotype or environment.

Results and Discussion

The general linear models procedure explained 88% of the model's variation, though the coefficient of variation was relatively large (43.59). Significant differences among the four environments or among the replicate within environment [rep(environment)] effects were not indicated by the mixed procedure (Pr>Z=0.12 and 0.09, respectively) (Table 2). Broad-sense heritability was calculated as 0.76 with a 95% confidence interval of 0.55–0.89. This indicates a fairly high genetic component of the trait that could be transferred with relative ease to newly developed genotypes. Of the 24 genotypes tested for genetic stability, 8 were genetically stable both before and after removal of environmental heterogeneity, 10 were genetically stable after the removal of environmental heterogeneity, and 6 were genetically unstable both before and after removal of environmental heterogeneity (Table 1). Therefore, the results of the present study indicate the presence of potato genotypes with genetically stable resistance to "Spongospora root galling".

Five of the 24 potato genotypes in this study were previously identified as resistant to "Spongospora root galling" (Nitzan et al. 2008) and four others were industry standard cultivars. All five resistant genotypes were genetically stable. PA95B2-4 was genetically stable both before and after removal of environmental heterogeneity, while PA98N5-2, PA98NM38-1, PO94A009-7 and POR00HG5-1 were stable after the removal of environmental heterogeneity (Table 1). Among the four industry standards, Shepody was unstable, while Ranger Russet, Russet Burbank and Umatilla Russet were stable after the removal of environmental heterogeneity. An important factor in the development and selection of resistant genotypes, during evaluations that extend over many years and locations, is the ability to compare germplasm to a dependable and stable set of standards. The results described here, establish Ranger Russet, Russet Burbank and Umatilla Russet and the five resistant genotypes as a reliable set of industry and resistant standards, respectively. This strengthens the selection process of germplasm with resistance to "Spongospora root galling", making it more reliable, consistent and efficient.

In a previous study, Nitzan et al. (2009) reported the presence of resistance to "black dot" and the genetic stability of this trait among 17 potato genotypes. The broad-sense heritability for resistance to "black dot" was calculated to be 0.13, which was low; and the 95% confidence interval for this estimate was 0.00–0.68. Such a broad confidence interval is indicative of extreme variability, pointing out the difficulty of breeding for resistance to "black dot". In the present study, the broad-sense heritability for resistance to "Spongospora root galling" was calculated as 0.76 with a 95% confidence interval of 0.55–0.89. This indicates that it will be easier to breed for resistance to this disease than to "black dot". Among the 17 genotypes tested for resistance to "black dot" and the genetic stability of the trait (Nitzan et al. 2009) were PA95B2-4, PA98NM38-1, PO94A009-7 and POR00HG5-1, which had stable genetic resistance to "black dot". These also are reported here as having stable resistance to "Spongospora root galling". To our best knowledge, to date, these are the only known potato genotypes possessing genetically stable resistance to both diseases.

In conclusion, potato genotypes with stable resistance to "Spongospora root galling" have been identified and the genetic component of resistance is fairly high. Genotypes with stable resistance to both "Spongospora root galling" and "black dot" have also been identified. The results reported here, together with those previously reported (Nitzan et al. 2008; Nitzan et al. 2009) will enable breeders to use resistant and genetically stable potato genotypes as parents in future breeding, and to efficiently develop superior commercial potato cultivars.

Table 2 Source of variation, mean squares from the general linear models procedure, and estimate of the variance component and Z values from the mixed models procedure

Source	DF [x]	Mean squares [x]	Estimate of the variance component [y]	Pr≥Z [y]
Environment	3	2.88698	0.03153	0.1154
Rep (environment)	12	0.02063	0.00050	0.0914
Genotype	23	0.21557	0.01100	0.0052
Genotype* environment	69	0.05163	0.01119	<.0001
Error	257	0.00948		
Corrected Total	364			
R-square [x]	Coeff var [x]	Root galls mean [x]		
0.881602	43.58592	0.223412		

[x] Values from the general linear models procedures. [y] Values from the mixed models procedure.

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