Evaluation and Characterization of Advanced Potato Breeding Clones for Resistance to Scab by Cluster Analysis

R. W. GOTH, Research Plant Pathologist, K. G. HAYNES, Research Plant Geneticist, and D. R. WILSON, Horticulturist, U.S. Department of Agriculture, Agricultural Research Service, Vegetable Laboratory, Plant Sciences Institute, Beltsville, Maryland 20705

ABSTRACT

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Seventeen potato breeding lines and four cultivars were evaluated in replicated field trials in Presque Isle, Maine, from 1988 to 1991 for their reaction to scab (*Streptomyces scabies*). All tubers were individually scored for type of lesion (0 = none to 5 = pitted scab) and surface area covered (0 = 0% to 12 = 100%). Computations were made for lesion index (LI), surface area index (SAI), and overall scab index (OSI). Analyses of variance of LI, SAI, and OSI revealed significant differences among potato lines in 1989–1991. Cluster analyses on the combination of LI and SAI were superior to clustering on OSI alone. Five clusters were defined in 1989–1991. Cluster analysis provides a means to quantitatively compare the scab reactions of new germ plasm with that of cultivars used as disease standards.

Common scab of potato, *Solanum tuberosum* L., is caused by the actinomycete, *Streptomyces scabies* (Thaxter) Lambert & Loria (9). This disease occurs on all underground parts of the potato (11). On tubers, the disease consists of surface lesions of corky tissue. Symptoms range from superficial lesions to conspicuous, deep-pitted lesions. These lesions can either be discrete and singular or can completely cover the tuber surface (2,3,11,17). Potato cultivars vary in their susceptibility or reaction to infection by *S. scabies,* and no cultivar is completely immune (11). The relative susceptibility of a cultivar is determined by growing it in soil infested with pathogenic strains of the organism. The extent of infection on tubers is compared with infection on standard varieties exposed under the same conditions.

To make a quantitative comparison of cultivars for their reaction to *S. scabies,* a numerical system for scoring the severity of infection is required. Such systems are based either on the proportion of tuber surface covered or on the type of scab lesion. Earlier workers (1,2,10) found a correlation between the lesion type and the tuber surface area covered with lesions, indicating that the relative reaction of cultivars can usually be estimated by either criterion.

In this report, we show that cluster analyses of lesion type and percentage surface area data can be used to compare the scab resistance of advanced breeding clones to standard cultivars.

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MATERIALS AND METHODS

Inoculum production. A modification of the puncture method of Goth (4) was used to isolate *S. scabies* from infected tissue. A heat sterilized needle was inserted into the translucent tissue immediately below the scab lesion, and a tissue section was transferred to water agar (1.5% Difco agar). *Streptomyces* colonies were visually selected from typical bacterial colonies and transferred to Okanishi's R-2 regeneration medium containing 0.1% tyrosine (12). We used this medium because melanin production on tyrosine-containing media has been used to select pathogenic isolates of *S. scabies* (5,8). Isolates that produced melanin were selected for further pathogenicity evaluation on radish (*Raphanus sativus* L). Those isolates that produced scab lesions on radish were used as inoculum for the potato study (5).

In addition to the colonies growing on the R-2 media, scabby potatoes with all types of scab lesions were peeled to provide another source of inoculum. Care was taken to remove only the scab lesion and a minimum of adjacent tissue. The peels were dried at 22–24 C for 72 hr. The inoculum was composed of a mixture of R-2 agar with melanin-

producing isolates of *S. scabies* and the dried scab tissue mixed at a ratio of one part agar culture, one part scabby tissue, and one part distilled water (v/v/v). This mixture was comminuted with a Waring blender for 10 minutes. The resultant mixture was adjusted to contain 3×10^7 cfu/ml of melanin-forming colonies of *S. scabies* on R-2 medium. A total of 50 ml of this mixture was added to each tuber as it was placed in the soil. Inoculated tubers were immediately covered with soil.

Field experiments. Seed tubers of cultivars and breeding lines used in this study were obtained from virus-tested seed stock grown at both Chapman and Aroostook Farms in Presque Isle, Maine. Field studies were conducted at Aroostook State Farm from 1988 to 1991 on a site previously known to produce potato crops affected by scab. The statistical design was a randomized complete block with four replications of five-hill plots. The soil is a Caribou silt loam. In 1988, we relied on the natural inoculum present in this field plot. The pH was 5.7. In 1989 and 1990, all plots received the equivalent of 8.5 t/ha of composted cow manure. In 1989, 1990, and 1991, we adjusted the pH to 6.7 by adding dolomitic lime. After 1988, inoculum was added at the rate of 50 ml of solution containing 3×10^7 cfu/ml to each seed piece. Vines were killed 90 days after planting, and tubers were harvested within 30 days after vine kill.

Scoring. Each tuber was examined and the percentage surface area with scab lesions was estimated with the Horsfall–Barratt rating scale (6). When tubers had multiple lesions, the most severe lesion type was assigned. The rating used to score the type of lesion consisted of six categories based on the assessment key of James (7): 0 = no scab; 1 = superficial lesions less than 10 mm in diameter; 2 = superficial lesions greater than 10 mm in diameter; 3 = raised lesions less than 10 mm in diameter; 4 = raised lesions greater than 10 mm in diameter; and 5 = pitted scabs of all diameters.

Statistical analysis. Four variables for each plot were calculated. An overall scab index (OSI) similar to that suggested by Bjor and Roer (1) was calculated as $OSI = \Sigma_{i=1}^{n}$(lesion type)(surface area infected)/[60(number of tubers)], where n = number of tubers in the plot ($n = 1,2,...$).

In addition, a surface area infected index (SAI) was calculated as $SAI = \Sigma_{i=1}^{n}$(surface area infected)/[12(number of tubers)].

A lesion index (LI) was calculated as $LI = \Sigma_{i=1}^{n}$(lesion severity)/[5(number of tubers)].

Finally, the proportion of scabby tubers (PST) of any kind and amount was calculated as *PST* = (number of scabby tubers)/(total number of tubers).

Analyses of variance of OSI, SAI, LI, and the arcsine transformation of PST were conducted using the general linear models procedure on the Statistical

Table 1. Averages of the overall scab index (OSI), surface area infected index (SAI), lesion type index (LI), and proportion of scabby tubers (PST) over four replications for each potato clone[a]

Clone	OSI	SAI	LI	PST	Clone	OSI	SAI	LI	PST
B0032-35	...	...	...	...	B0189-45	0.05	0.11	0.29	0.14
	0.19	0.25	0.72	0.92		0.39	0.48	0.80	1.00
	0.03	0.09	0.31	0.28		0.26	0.35	0.70	0.97
	0.19	0.22	0.62	0.74		0.21	0.28	0.72	1.00
B0169-56	0.04	0.10	0.27	0.10	B0221-6	0.03	0.09	0.27	0.10
	0.24	0.34	0.68	0.92		0.19	0.24	0.69	0.86
	0.11	0.21	0.50	0.82		0.08	0.13	0.37	0.30
	0.29	0.39	0.69	0.98		0.12	0.17	0.53	0.76
B0180-31	0.02	0.08	0.20	0.01	B0233-1	0.05	0.11	0.31	0.21
	0.04	0.11	0.26	0.10		0.39	0.49	0.77	1.00
	0.10	0.15	0.51	0.80		0.23	0.27	0.72	1.00
	0.24	0.29	0.78	0.92		0.32	0.35	0.82	1.00
B0180-39	0.03	0.09	0.29	0.12	B0242-31	0.05	0.11	0.36	0.26
	0.24	0.32	0.67	0.78		0.26	0.32	0.80	1.00
	0.42	0.45	0.89	0.92		0.16	0.23	0.56	0.92
	0.39	0.43	0.89	1.00		0.23	0.35	0.72	0.99
B0184-15	0.07	0.12	0.38	0.28	B0243-7	0.05	0.10	0.31	0.20
	0.55	0.65	0.82	0.98		0.33	0.39	0.76	0.92
	0.33	0.40	0.90	0.92		0.23	0.28	0.81	0.99
	0.49	0.54	0.89	1.00		0.46	0.46	0.98	0.98
B0184-16	0.04	0.10	0.29	0.13	B0245-15	0.02	0.09	0.22	0.04
	0.31	0.40	0.76	1.00		0.42	0.51	0.82	0.99
	0.14	0.19	0.69	0.97		0.24	0.30	0.77	0.98
	0.38	0.44	0.91	1.00		0.39	0.44	0.92	0.99
B0184-30	0.02	0.09	0.21	0.01	Green Mountain	0.04	0.11	0.28	0.12
	0.29	0.38	0.72	0.92		0.46	0.58	0.79	1.00
	0.16	0.23	0.61	0.73		0.41	0.46	0.86	0.99
	0.31	0.35	0.83	0.95		0.50	0.50	0.99	1.00
B0186-1	0.05	0.11	0.28	0.12	Ontario	0.04	0.10	0.29	0.16
	0.22	0.28	0.67	0.78		0.22	0.32	0.63	0.90
	0.25	0.34	0.65	0.83		0.13	0.19	0.68	1.00
	0.17	0.20	0.71	0.88		0.13	0.21	0.51	0.80
B0186-3	0.03	0.09	0.24	0.07	Russet Burbank	0.03	0.10	0.25	0.09
	0.24	0.33	0.62	0.83		0.27	0.35	0.76	1.00
	0.17	0.21	0.75	0.94		0.20	0.27	0.69	0.89
	0.34	0.37	0.86	0.95		0.37	0.43	0.88	0.99
B0186-23	0.04	0.11	0.29	0.15	Superior	0.04	0.13	0.31	0.16
	0.40	0.49	0.76	0.92		0.21	0.30	0.71	1.00
	0.35	0.39	0.84	0.96		0.08	0.13	0.57	0.92
	0.22	0.27	0.72	0.90		0.28	0.32	0.85	0.99
B0189-12	0.02	0.09	0.25	0.08	Overall mean	0.04	0.10	0.28	0.13
	0.31	0.39	0.77	0.96		0.29	0.38	0.71	0.89
	0.26	0.35	0.69	0.90		0.21	0.27	0.67	0.86
	0.25	0.35	0.70	1.00		0.30	0.35	0.79	0.94

[a] First line = 1988, second line = 1989, third line = 1990, fourth line = 1991.

Analysis System (15). Pearson product-moment correlations between OSI, SAI, and LI also were determined (16). Where significant differences were detected between clones, the data were subjected to cluster analysis using the unweighted pair-group method by arithmetic averages (UPGMA) (14).

RESULTS AND DISCUSSION

The smallest range of OSI, SAI, LI, and PST values occurred in 1988, indicating that there was not a good separation among clones for these indices (Table 1). There was good separation among clones for OSI, SAI, and LI in 1989–1991 (Table 1).

Analyses of variance revealed significant differences among clones in 1989, 1990, and 1991 for OSI, SAI, and LI, and for PST in 1989 and 1990; but there were no significant differences among clones in 1988 (Table 2). The lack of significant differences in 1988 was caused by a low incidence of *S. scabies* in the uninoculated field plot. Considerable heterogeneity of error variances existed among the four years for all traits, as measured by Bartlett's test of homogeneity of variance (16). Therefore, the data were not combined over years for further analysis.

To decide which variables to include in the cluster analysis, we first looked at the results from the analyses of variance. In 1988, a year that was not conducive to scab development, none of the variables revealed significant differences among the clones. In 1991, a very severe scab year, the analyses of variance revealed that PST was not sufficient to distinguish the scab reaction of these clones. This would be expected because a tuber with just one lesion would fall in the same category as a tuber with several hundred lesions.

The 1991 test was more severe than the 1989 or 1990 tests. These results may be attributed to an increase in the *S. scabies* population in the test plot, coupled with suitable environmental conditions. Therefore, in the two extreme cases of one very poor year and one severe year for scab development, PST was not adequate for discriminating among the reactions of the clones to scab.

The correlations between OSI, SAI, and LI were all high (Table 3). However, those correlations involving SAI with LI were the lowest within their respective years. Therefore, from the three variables that remained after eliminating PST from consideration, it seemed appropriate that either OSI or the combination of SAI and LI would be suitable variables for cluster analyses. OSI was derived from surface area covered with scab and lesion type. SAI and LI were derived from surface area covered with scab and lesion type in two separate indices. Analyses of variance on all three variables revealed that they were adequate to distinguish differences among the clones tested in 1989, 1990, and 1991. Therefore, two sets of cluster analyses were conducted for each of the three years where significant differences were found.

The decision on how many clusters to define is a subjective one. We decided to use the squared multiple correlation coefficient (R^2) to determine where to ignore further clustering. Five clusters were chosen because there was a fairly steep decrease in the R^2 value between four and five defined clusters for OSI and the combination of SAI and LI for each year.

The mean values for OSI, SAI, and LI for the five defined clusters in each year and the standard error of the mean are shown in Table 4. The first cluster is composed of the least scab susceptible clones with OSI $\leq$ 0.15 or SAI $\leq$ 0.19 and LI $\leq$ 0.52. The fifth cluster represents the most scab susceptible clones, with OSI $\geq$ 0.41 or SAI $\geq$ 0.43 and LI $\geq$ 0.81. Clusters 2, 3, and 4 are composed of clones in increasing order of scab susceptibility. With these analyses, the number of clones in each cluster varied from year to year and between the OSI cluster analyses and the combination of the SAI and LI cluster analyses. The clones are listed (Table 5) along with their associated clusters.

There was general agreement between the two clustering techniques. However, the cluster analyses conducted on the combination of SAI and LI were better at defining a difference between clusters 2 and 3 in 1990 and between clusters 3 and 4 in 1991 than were the cluster analyses conducted on OSI (Table 5). The difference between clusters 2 and 3 in 1990 and 3 and 4 in 1991 was attributable more to a difference in LI than to a difference in SAI. This difference tends to be minimized in the calculation of OSI. Thus, cluster analysis on the combination of SAI and LI seems to be more sensitive in defining differences in the clones, particularly when differences are observed in only one of the two indices.

Cluster analysis on the combination of SAI and LI also was superior to the cluster analysis of OSI in terms of assigning the check cultivars (Green Mountain, Ontario, Russet Burbank, and Superior) to different clusters in 1989. Again, this was most likely due to the fact that differences in the type of lesion or surface area covered tend to be minimized in the calculation of OSI.

Table 2. Analyses of variance of the overall scab index (OSI), surface area infected index (SAI), and lesion type index (LI), and the arcsine transformation of proportion of scabby potato tubers (PST) for each of 4 yr

Source	df[a]	OSI MS[b]	SAI MS	LI MS	PST MS
1988					
Rep	3	0.000367	0.000044	0.007574	0.021244
Clone	19	0.000556	0.000535	0.008348	0.022186
Error	57	0.000556	0.000576	0.006825	0.014658
Total	79				
1989					
Rep	3	0.006269	0.003808	0.093364**[c]	0.647500**
Clone	20	0.050751**	0.060748**	0.058125**	0.413310**
Error	60	0.005213	0.006398	0.062721	0.101923
Total	83				
1990					
Rep	3	0.020450	0.012199	0.086475**	0.217808
Clone	20	0.047306**	0.044591**	0.101604**	0.487319**
Error	60	0.009720	0.011103	0.018689	0.125566
Total	83				
1991					
Rep	3	0.011437	0.020502	0.055982	0.151653
Clone	20	0.048388**	0.040444**	0.069363**	0.120901
Error	60	0.048387	0.008617	0.022460	0.081620
Total	83				

[a] Degrees of freedom.
[b] Mean-squares from the analyses of variance.
[c] ** = Significant at the 1% level.

Table 3. Correlation coefficients of the overall scab index (OSI), surface area infected index (SAI), and lesion type index (LI) in potatoes for each of 4 yr

	OSI	SAI	LI
1988			
OSI	1.00	0.83	0.93
SAI		1.00	0.77
LI			1.00
1989			
OSI	1.00	0.99	0.77
SAI		1.00	0.74
LI			1.00
1990			
OSI	1.00	0.99	0.87
SAI		1.00	0.83
LI			1.00
1991			
OSI	1.00	0.96	0.92
SAI		1.00	0.85
LI			1.00

Table 4. Mean overall potato scab index (OSI), surface area infected index (SAI), and lesion type index (LI), and standard error of the mean for the five clusters defined by cluster analysis on OSI or the combination of SAI and LI

	OSI			SAI			LI	
Cluster	n[a]	Mean	SE	n	Mean	SE	Mean	SE
1	1[b]	0.04	...	1	0.11	...	0.26	...
	5[c]	0.08	0.01	2	0.11	0.02	0.34	0.03
	4[d]	0.15	0.02	2	0.19	0.02	0.52	0.01
2	10	0.23	0.01	8	0.30	0.01	0.67	0.01
	5	0.15	0.01	5	0.19	0.02	0.55	0.02
	5	0.23	0.01	2	0.21	0.01	0.67	0.05
3	4	0.31	0.01	6	0.37	0.01	0.76	0.01
	7	0.24	0.01	3	0.20	0.01	0.71	0.02
	5	0.31	0.01	6	0.32	0.02	0.72	0.01
4	5	0.41	0.01	4	0.49	0.01	0.79	0.01
	2	0.34	0.01	7	0.31	0.01	0.72	0.02
	4	0.38	0.01	4	0.35	0.01	0.84	0.01
5	1	0.55	...	2	0.62	0.04	0.81	0.02
	2	0.41	0.01	4	0.43	0.02	0.87	0.01
	3	0.48	0.01	7	0.46	0.02	0.92	0.02

[a]Number of clones in each cluster.
[b]First line = 1989.
[c]Second line = 1990.
[d]Third line = 1991.

Table 5. Assignment of potato clones to clusters based on cluster analysis of overall scab index (OSI), surface area infected index (SAI), and lesion type index (LI) for 1989–1991

	1989		1990		1991	
Clones	OSI	SAI + LI	OSI	SAI + LI	OSI	SAI + LI
B0032-35	2	2	1	1	1	2
B0169-56	2	2	1	2	3	3
B0180-31	1	1	1	2	2	3
B0180-39	2	2	5	5	4	5
B0184-15	5	5	4	5	5	5
B0184-16	3	3	2	3	4	5
B0184-30	3	3	2	2	3	4
B0186-1	2	2	3	4	1	2
B0186-3	2	2	2	3	3	4
B0186-23	4	4	4	5	2	3
B0189-12	3	3	3	4	2	3
B0189-45	4	4	3	4	2	3
B0221-6	2	2	1	1	1	1
B0233-1	4	4	3	4	3	4
B0242-31	2	3	2	2	2	3
B0243-7	3	3	3	4	5	5
B0245-15	4	4	3	4	4	5
Green Mountain	4	5	5	5	5	5
Ontario	2	2	2	3	1	1
Russet Burbank	2	3	3	4	4	5
Superior	2	2	1	2	3	4

In general, cluster analysis on SAI and LI provided a rapid method of classifying the relative resistance or susceptibility of an unknown clone in relation to known cultivar standards. For example, Green Mountain has been our susceptible check, and it was in the most susceptible cluster based on SAI and LI from 1989 to 1991. Only one clone, B0184-15, was as susceptible to scab. Ontario has been our resistant standard, and it was consistently placed in one of the more resistant clusters. Two clones, B0032-35 and B0221-6, were consistently placed in one of the more resistant clusters, either with or exceeding Ontario. Grouping clones into discrete clusters avoids the problems inherent in generating overlapping classifications by mean separation techniques, which have been well documented (13,18). Our goal was to compare the previously unknown scab reactions of these clones to the reactions of known standards, and not to compare these clones to each other as is normally done using mean separation techniques. Because a breeder would prefer to tell a potential grower that the disease reaction of a new cultivar is similar to the disease reaction of a standard cultivar with which the grower is familiar, it would appear that for evaluating scab reaction, cluster analysis on the combination of SAI and LI is superior to cluster analysis on OSI.

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