Independent Segregation in Potato for Resistance to Verticillium Wilt and Pink-Eye

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ABSTRACT

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In 1991 and 1992, 30 potato families (comprising approximately 1,330 genotypes) were evaluated for resistance to both Verticillium wilt, caused by *V. albo-atrum* and *V. dahliae*, and pink-eye, a disease of unknown etiology. Significant differences were found among families for severity of Verticillium wilt and incidence of pink-eye. Within-family variability accounted for more than 90% of the observed variation for both traits in both years. Homogeneous variances for severity of Verticillium wilt across families were observed in both years; in contrast, heterogeneous variances for incidence of pink-eye across families were observed in both years, with greater variance in those families with a higher incidence of pink-eye. In general, there was no consistent relationship between severity of Verticillium wilt and incidence of pink-eye in segregating families.

Additional keywords: *Solanum tuberosum*

Verticillium wilt of potato (*Solanum tuberosum* L.) is caused by the soilborne fungal pathogens *Verticillium albo-atrum* Reinke & Berthier and *V. dahliae* Kleb. (15). Characteristic symptoms of Verticillium wilt are recoverable true wilting, unilateral permanent wilting, unilateral chlorosis, and necrosis (13). In addition, plants infected with these pathogens have reduced rates of growth of leaves, stems, and tubers and premature maturation or senescence, which is commonly referred to as potato early dying (9). Tubers with pink-eye have pink areas around the eyes, but symptoms can occur on any part of the tuber. The pink condition is ephemeral and is succeeded by brown discoloration (10), referred to as corky patch (14). The specific causes of pink-eye have not been determined.

A major objective of many potato breeding programs worldwide is the development of Verticillium wilt-resistant cultivars that are suitable for processing or fresh table stock markets (8). Corsini et al (2,3) found considerable resistance to infection by *V. dahliae* in four of the 66 tuber-bearing species of *Solanum*, but this resistance was no better than that present in selections of *S. tuberosum* and immunity was not present in the large populations they screened.

Although little is known regarding the inheritance of resistance to Verticillium wilt, Hunter et al (12) found that the frequency of resistant offspring was greater when at least one of the parents was resistant than if neither was resistant. More recently, Treadwell et al (20) suggested that two or more loci may control this trait. There are no published reports on the inheritance of resistance to pink-eye.

Verticillium wilt has been associated with the pink-eye disease complex (4–7). Recent studies have shown that the incidence of pink-eye was enhanced by the severity of Verticillium wilt (6,7) but that Verticillium wilt was not necessary for pink-eye to develop. It was hypothesized that increased resistance to *Verticillium* may reduce the incidence of pink-eye. The objective of this study was to determine if there was a relationship between resistance to Verticillium wilt, caused by combined inoculations with *V. albo-atrum* and *V. dahliae*, and resistance to pink-eye in a segregating potato breeding population.

MATERIALS AND METHODS

Crosses among tetraploid potato parents with varying levels of resistance to Verticillium wilt and pink-eye were used in this study to generate 30 families. These crosses were made among five potato cultivars and 10 advanced breeding selections during the spring of 1988 and 1989. These 30 crosses were composed of a group of five females crossed with each of four males in a design II (1) and 10 other crosses randomly chosen from the remaining successful hybridizations.

The Verticillium wilt and pink-eye disease reactions of the parents used in this study have been reported previously (7). Based on the Horsfall-Barratt scheme (11), where 1 = 0% wilt . . . 12 = 100% wilt, the overall severity of Verticillium wilt in the parents used in this study ranged from 1.4 to 6.8. The overall incidence of pink-eye disease ranged from 1.0 to 45.0% (7). The parentage of the 30 families evaluated for Verticillium and pink-eye disease is given in Table 1.

In August 1989, true seed from these crosses was soaked in 1,500 mg kg^{-1} GA_3 for 24 hr, rinsed with tap water, and dried for 24 hr at room temperature. The seeds were sown in flats of Jiffy Mix and placed on greenhouse benches at Beltsville, Maryland, where the ambient temperature ranged from 20 to 30 C. Natural light was not supplemented. Two to three weeks after planting, approximately 350 seedlings per cross, where available, were transplanted to 8.9-cm clay pots containing Jiffy Mix. In December 1989, the single largest tuber in each pot was harvested and saved for field planting the following spring. Tubers were bulked by family and stored in muslin bags at 95% RH and 4 C until shipped to Presque Isle, Maine, in late April 1990.

Whole seed of the seedling families were planted in May 1990 on Chapman Farm, Presque Isle, in rows 0.9 m apart

Table 1. Parentage of the 30 potato families evaluated for Verticillium wilt and pink-eye

Family	Parentage
B1071	Abnaki × B0184-30
B1072	Abnaki × B0233-1
B1073	Abnaki × B0243-10
B1074	Abnaki × Russette
B1075	BelRus × B0177-20
B1076	BelRus × B0233-1
B1077	Cherokee × Russette
B1078	Cherokee × B0184-30
B1079	Cherokee × B0233-1
B1080	Cherokee × B0243-10
B1081	Russette × B0177-20
B1082	Russette × B0243-10
B1083	Superior × B0209-1
B1084	Superior × B0178-35
B1085	B0169-56 × B0184-30
B1086	B0169-56 × Russette
B1087	B0169-56 × B0233-1
B1088	B0169-56 × B0243-10
B1089	B0172-22 × Cherokee
B1090	B0172-22 × B0184-30
B1091	B0177-20 × Russette
B1092	B0177-20 × B0184-30
B1093	B0177-20 × B0233-1
B1094	B0177-20 × B0243-10
B1096	B0179-3 × Russette
B1097	B0179-3 × B0184-30
B1098	B0179-3 × B0233-1
B1099	B0179-3 × B0243-10
B1100	B0209-1 × Cherokee
B1101	B0169-56 × B0183-25

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at 0.3-m spacing within the row in a Caribou silt soil. Standard cultural practices, including virus testing and other disease evaluations, were followed during the growing season. At harvest, the first 50 hills of each family with a least 12 seed pieces per hill were saved for evaluation in 1991. No other selection pressure was applied to this tuberling generation. In 1991, a planting of these segregating offspring was maintained on Chapman Farm to furnish clean seed for evaluation in the Verticillium wilt plot in 1992.

In 1991 and 1992, the severity of Verticillium wilt and the incidence of pink-eye in the 1,332 segregating offspring from these crosses were evaluated in a field plot on Aroostook State Farm, Presque Isle. The soil type in the plot was a Caribou silt loam. This plot had been used for Verticillium wilt and pink-eye evaluations for many years (5–7). Four hills in 1991 and five hills in 1992 of each offspring were planted in rows 0.9 m apart at 0.3-m spacing within the row in a completely randomized design.

The isolates of *V. albo-atrum* and *V. dahliae* used in this study originated from stems of potato with early dying symptoms. Stem cross sections (5–10 mm thick) were placed in 100 × 15 mm plastic petri dishes containing 20 ml of 2% water agar (Difco). Conidia that developed on characteristic verticillate conidiophores in the vascular tissue of the sections were picked up with a dissecting needle and streaked on the surface of the agar. After 24 hr, individual germinating conidia viewed under the microscope were transferred to 100 × 15 mm petri dishes containing 20 ml of a medium consisting of 17.5 g of Czapek-Dox broth (Difco) and 19.5 g of potato-dextrose agar (Difco) and to dishes containing 20 ml of Talboy's prune extract agar (prepared by decanting what remains of simmering 5 g of chopped prunes in 100 ml of distilled water for 30 min, then combining 5 g of lactose, 1 g of yeast extract, 30 g of agar, and distilled water to make 1 L) (19). Colonies of *V. dahliae* and *V. albo-atrum* without bacterial contamination were subcultured onto both media for inoculum production. The colonies of *V. dahliae* were differentiated from those of *V. albo-atrum* by the presence or absence of sclerotia production. A 0.5-ml conidial suspension of the test isolate was streaked onto the agar and, 10–20 days later, the culture was comminuted with water in blenders. The inoculum was adjusted to 10^9 conidia per milliliter of distilled water using a hemacytometer. Each seed tuber was cut into pieces weighing 40–60 g, immersed in the inoculum, and hand-planted in rows. Immediately after seeding, an additional 20 ml of inoculum was poured over the seed piece, and the inoculated seed pieces were covered with approximately 15 cm of soil.

On 6 September 1991 and 14 September 1992, every plant in each plot was rated for the degree of wilt by the Horsfall-Barratt scheme, where 1 = 0% . . . 12 = 100% wilt (11). An average of these ratings was computed to give the severity of Verticillium wilt for each plot. Plots were harvested 11–15 October 1991 and 28 and 29 September 1992. Each tuber was inspected for pink-eye symptoms. The incidence of pink-eye was determined from each plot as the percentage of tubers infected with pink-eye. Losses from pink-eye have been estimated to be between 1 and 3% in Wisconsin and North Dakota, although losses on individual farms can be very severe (17). On the basis of previous tests in this field, the incidence of pink-eye could be as high as 79% in a susceptible cultivar (7). Severity of Verticillium wilt and incidence of pink-eye were subjected to analysis of variance using PROC GLM (16). Correlation coefficients between severity of Verticillium wilt and incidence of pink-eye were calculated for each family for each year (18).

RESULTS AND DISCUSSION

Families differed significantly in resistance to Verticillium wilt and pink-eye (Table 2). However, within-family variability accounted for more than 90% of the variation for both traits in both years, suggesting that high segregation for Verticillium wilt resistance or pink-eye re-

Table 2. Analyses of variance on 30 segregating potato families for severity of Verticillium wilt and incidence of pink-eye

	1991			1992		
Source	df	MS[a]	MS[b]	df	MS[a]	MS[b]
Family	29	40.6837** [c]	0.1219**	29	21.0729**	0.0803**
Error	1,301	9.6896	0.0354	1,282	8.0608	0.0421
Total	1,330	...	...	1,311	...	...

[a] Mean squares from analysis of variance on the severity of Verticillium wilt.
[b] Mean squares from the analysis of variance on the incidence of pink-eye.
[c] ** = Significant at the 1% level.

Table 3. Severity of Verticillium wilt, incidence of tubers infected with pink-eye, and within-family variance during 1991 and 1992

	1991				1992			
	Wilt		Pink-eye		Wilt		Pink-eye	
Family	Sev.[a]	Var.[b]	Inc.[c]	Var.[d]	Sev.	Var	Inc.	Var.
B1071	3.5	11.5	0.25	0.06	4.1	10.7	0.26	0.08
B1072	3.5	9.2	0.16	0.03	4.2	9.4	0.15	0.03
B1073	3.3	10.0	0.20	0.05	4.8	8.5	0.22	0.06
B1074	3.4	9.7	0.17	0.03	4.4	8.6	0.22	0.05
B1075	3.8	10.3	0.10	0.02	5.1	8.7	0.14	0.02
B1076	4.2	9.4	0.15	0.03	3.4	3.2	0.15	0.02
B1077	5.8	8.5	0.19	0.05	4.4	7.5	0.14	0.02
B1078	4.3	11.7	0.30	0.06	4.2	9.2	0.26	0.07
B1079	4.2	8.8	0.22	0.06	4.1	5.6	0.14	0.02
B1080	4.5	11.7	0.16	0.03	5.3	6.9	0.21	0.06
B1081	1.1	3.7	0.10	0.01	3.7	6.8	0.21	0.06
B1082	2.8	10.3	0.16	0.03	4.3	6.7	0.18	0.04
B1083	5.0	14.2	0.24	0.04	6.2	8.2	0.25	0.04
B1084	4.9	9.0	0.27	0.04	5.2	11.0	0.27	0.08
B1085	2.8	8.7	0.17	0.04	3.8	9.5	0.21	0.03
B1086	2.5	7.2	0.08	0.01	4.3	9.0	0.14	0.04
B1087	3.2	9.0	0.13	0.02	4.5	6.6	0.14	0.02
B1088	3.0	8.8	0.15	0.03	4.8	7.3	0.18	0.04
B1089	5.1	8.5	0.20	0.03	5.0	6.2	0.16	0.03
B1090	4.4	10.2	0.22	0.07	4.4	7.3	0.23	0.06
B1091	2.9	7.6	0.15	0.04	4.1	7.3	0.22	0.05
B1092	2.4	9.5	0.19	0.04	4.7	7.5	0.25	0.04
B1093	4.1	10.8	0.12	0.02	4.7	6.3	0.19	0.06
B1094	3.9	8.9	0.20	0.04	5.2	8.2	0.19	0.03
B1096	2.3	8.2	0.12	0.03	4.0	8.0	0.13	0.01
B1097	1.8	6.8	0.12	0.02	5.0	10.7	0.14	0.03
B1098	3.5	9.3	0.20	0.03	3.4	7.2	0.21	0.03
B1099	3.4	9.4	0.17	0.02	4.0	8.7	0.16	0.03
B1100	3.9	12.5	0.20	0.03	5.6	8.9	0.20	0.05
B1101	2.8	9.6	0.11	0.01	6.2	8.2	0.18	0.03
Mean	3.7		0.18		4.6		0.19	

[a] Severity rated on the Horsfall-Barratt rating system, where 1 = 0% wilt . . . 12 = 100% wilt (11).
[b] Within-family variance for severity of Verticillium wilt.
[c] Incidence rated as proportion of tubers with pink-eye.
[d] Within-family variance for incidence of pink-eye.

Table 4. Correlations between severity of Verticillium wilt and incidence of pink-eye in 30 segregating potato families during 1991 and 1992

Family	1991		1992	
	No. of offspring	Correlation coefficient	No. of offspring	Correlation coefficient
B1071	50	0.07	50	0.09
B1072	47	0.22	47	0.07
B1073	50	0.18	48	0.08
B1074	50	0.11	50	0.14
B1075	42	0.13	41	0.31* [a]
B1076	8	0.07	8	0.32
B1077	50	0.07	47	0.19
B1078	50	0.51**	50	0.19
B1079	50	0.19	49	0.09
B1080	50	−0.05	49	−0.06
B1081	12	0.42	12	−0.59*
B1082	49	0.05	47	0.04
B1083	50	0.10	49	−0.07
B1084	50	0.33*	50	−0.06
B1095	50	−0.08	49	−0.01
B1086	50	−0.06	50	0.12
B1087	15	0.56*	14	−0.47
B1088	50	0.02	49	−0.03
B1089	50	0.09	50	0.22
B1090	49	0.22	50	0.03
B1091	50	−0.03	50	0.00
B1092	50	0.22	49	−0.10
B1093	50	0.11	50	−0.07
B1094	50	−0.20	50	−0.29*
B1096	49	0.25	50	−0.13
B1097	8	0.24	8	−0.58
B1098	50	0.20	50	0.19
B1099	50	0.05	47	−0.02
B1100	50	0.20	50	−0.09
B1101	50	−0.04	50	−0.51**

[a] * = Significant at the 5% level, ** = significant at the 1% level.

sistance was occurring in this population. Verticillium wilt was more severe in 1992 than in 1991 ($t = 8.28$, $P < 0.01$), but the incidence of pink-eye was the same in both years.

Bartlett's test (16) on the homogeneity of family variances for severity of Verticillium wilt and incidence of pink-eye was computed for both years. For 1991 and 1992, the family variances for severity of Verticillium wilt were homogeneous ($\chi^2 = 19.46$ in 1991 and 19.32 in 1992), indicating that the severity rating of the parents had no effect on the variation in severity observed in their progeny (Table 3). In both years, however, the family variances for incidence of pink-eye were heterogeneous ($\chi^2 = 124.90$ in 1991 and 124.63 in 1992), indicating that the greater the family incidence of pink-eye, the greater the variance within that family for incidence of pink-eye (Table 3).

Previous evaluations of the parents used in this study (7) had revealed a significant positive correlation between severity of Verticillium wilt and the incidence of pink-eye in 1988 and 1989. However, no consistent correlation between severity of Verticillium wilt and incidence of pink-eye was observed in the progeny from these parents (Table 4).

On the basis of these results, genes governing resistance to Verticillium wilt and pink-eye appear to segregate independently. Within this large segregating population, resistance to Verticillium wilt appears to follow a normal distribution, whereas resistance to pink-eye does not. However, since plots were not inoculated with the causal organism for pink-eye, which is presently unknown (2,4), this conclusion is tentative. Nevertheless, the mean incidence of pink-eye per family ranged from 8 to 30% in 1991 and from 13 to 27% in 1992 (Table 3), indicating that there was probably adequate disease pressure. In addition, nine plots of the cultivar Cherokee, which was moderately susceptible to pink-eye in this field during 1988–1989 (7), were randomly planted throughout this field in 1992. The incidence of pink-eye did not differ significantly across these plots ($\chi^2 = 13.38$). The field used in this study had been infested with *V. albo-atrum*, *V. dahliae*, and pink-eye for many years; therefore, if an association between Verticillium wilt and the causal organism(s) for pink-eye exists, it should be found across the field.

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