EARLY DETECTION OF VERTICILLIUM WILT RESISTANCE IN A POTATO BREEDING PROGRAM

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Abstract

Nine potato clones/cultivars selected for varying degrees of Verticillium wilt (VW) resistance based on degree of vascular colonization, were intercrossed and outcrossed to 13 uncharacterized clones. Based on growth characteristics of 3,535 seedling transplants and an assay quantifying *V. dahliae* in potato vascular tissue, 404 clones were selected for further tests. The average number of *Verticillium dahliae* colony forming units (CFU) expressed as $Log_{10}(CFU + 1)/0.1$ ml of plant sap for the 404 clones was 2.3, and 1.3 and 3.7 for resistant Reddale and susceptible Kennebec checks, respectively. Thirty-five clones (8.66%) had CFU values equal to or less than Reddale suggesting that VW resistance is a readily transmitted trait.

Compendio

Nueve clones/cvs de papa, seleccionados por sus diversos grados de resistencia a la marchitez por *Verticillium* (VW) basados en el grado de colonización vascular, fueron retrocruzados y entrecruzados (menor parentezco) a 13 clones. Basándose en las características del crecimiento de 3 535 plántulas trasplantadas y en un ensayo cuantificativo de *V. dahliae* en el tejido vascular de la papa, se seleccionaron 404 clones para pruebas adicionales. El número promedio de unidades formando colonias de *Verticillium dahliae* (CFU), expresado como $Log_{10}(CFU + 1)/0.1$ cm^3 de savia vegetal, para los 404 clones fue 2.3, y 1.3 y 3.7 para los testigos Reddale (resistente) y Kennebec (susceptible), respectivamente. Treinta y cinco clones (8.66%) tuvieron valores de CFU iguales o menores que Reddale sugiriendo que la resistencia a VW es un atributo transmitido rápidamente.

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Introduction

Precise detection and quantification of resistance to *Verticillium dahliae* in potato is necessary for development of VW resistant cultivars. Several recent papers (4, 6, 9) describe assays to quantify vascular colonization by *V. dahliae* in potato and provide estimates of host resistance and inoculum potential. The tests by Slattery (9) and Davis *et al.* (4) measure number of colony forming units (CFU) of the pathogen per gram of dried tissue. Hoyos *et al.* (6) measured the number of CFU per ml of plant sap in basal stem tissue. Slattery sampled potato tissue at the end of the growing season while Davis and Hoyos sampled approximately 100 days after planting. Significant correlation values were observed between foliar wilt symptoms and CFU/ml of plant sap for 11 clones/cvs grown for 100 days and over a three-year period in a Verticillium wilt plot in North Dakota (6). Similar results were obtained in Idaho where plants were sampled at the same grown stage and the number of CFU/g of *V. dahliae* from tissue that had been dried was highly correlated with foliar wilt symptoms and yield for five clones/cvs in a three-year test (4).

Verticillium wilt resistance in potato has been shown to be heritable (1, 2, 8) and preliminary reports suggest host resistance is stable over space and time (3, 6). A plot for evaluating VW resistance has been established at Grand Forks, ND by inoculating tubers at planting and growing potatoes continuously since 1972 (6). Greenhouse tests using 15 cm pots to screen potato germplasm for resistance to *V. dahliae* were not correlated with field tests (6). When large pots containing 12L or more soil are used, growth of the plants and vascular colonization approximates that of field grown plants (7). An assay procedure quantifying vascular colonization in potato stems has been developed for use in a breeding program where thousands of assays are made annually (6). The strategy presently employed by the Minnesota Potato Breeding Program is to grow seedlings from true seed in a plot where *V. dahliae* is not present and selection is made on these plants for desirable agronomic traits. Tubers from selected clones are then increased and tested for VW resistance in subsequent years. To accelerate our search for resistance to *V. dahliae* we wanted to determine if we could select seedlings from true seed for both VW resistance and desirable agronomic traits in year one of the breeding program. Effective evaluation of VW resistance in seedlings from true seed requires irrigation which is unavailable at the VW plot. To circumvent this problem, we grew seedlings from true seed for eight weeks in the greenhouse and inoculated them just prior to transplanting to a field with irrigation facilities but with a low level of *V. dahliae*. Our objective was to determine if lack of VW symptoms and the vascular colonization assay could be used to 1) select parents and 2) evaluate and select progeny for VW resistance as seedlings.

Materials and Methods

The medium and assay to quantify vascular colonization of potato by *V. dahliae* has been described (6). Nine parents with various levels of vascular colonization were selected after evaluation in the VW plot at Grant Forks, ND (5, 6) (Table 1). These parents were intercrossed and outcrossed to 13 clones unclassified for VW resistance, resulting in 36 crosses (Table 2). In mid-April, seed was sown into pasteurized soil in peat pots in the greenhouse. The plants were inoculated eight weeks after planting by injecting 5 ml of 10^7 conidia and 10^4 microsclerotia per ml of a highly virulent isolate of *V. dahliae* into the soil around the roots of each plant, one day before transplanting. A total of 3,553 seedlings, maximum of 100 per cross, were transplanted on June 24 and 25, at the Sand Plains Experiment Station, Becker, Minnesota, into a plot with low *V. dahliae* infestation but with irrigation ensuring seedling transplant survival. In mid-September, 837 plants that had no wilt symptoms were assayed for presence of *V. dahliae* in the vascular system (Table 2). In mid-October, 399 plants testing negative for *V. dahliae* plus five plants from Cross 607 with low CFU counts all having good vigor and good tuber traits, were harvested.

The following year, these clones were grown in six-hill plots with three replications in a randomized complete block design, in the VW plot at

TABLE 1.—*Characterization of parent resistance by vascular colonization with Verticillium dahliae.*

Parents	Resistance level[1]	Log_{10}(CFU + 1)/0.1 ml[2]	Wilt index[4]
MN11719	R	1.35	1
Reddale	R	1.80	1
MN12761	R	1.93	1
MN82393	R	2.02	1
MN10874	MR	2.34	2
Russette[3]	MR	-	2
Erik	MR	2.61	3
MN82370	MR	2.87	3
Krantz	MS	3.65	4
Kennebec	S	3.95	5

[1]R = resistant, MR = moderately resistant, MS = moderately susceptible, S = susceptible.

[2]CFU for clones/cultivars were obtained from field evaluation 100 days after planting in the Verticillium wilt plot at Grand Forks, ND. LSD 0.01 = 0.77.

[3]MR based on foliar symptoms only.

[4]Wilt Index: 1 = 1-12%, 2 = 13-25%, 3 = 26-50%, 4 = 51-75%, and 5 = 76-100% foliar wilt, determined 100 days after planting.

TABLE 2.—*The progeny of 36 crosses evaluated for Verticillium wilt resistance at the University of Minnesota, Sand Plains Research Station, Becker, MN.*

Cross	Parents	Seedling plants				
		Beginning number	Percent surviving Sept. 15	Number assayed Sept. 15	Number (+) for *V. dahliae*[1]	Number selected Oct. 15
602	Reddale x Krantz	37	35	13	0	3
604	Erik x MN12761	36	33	11	2	5
605	Erik x MN82370	106	18	25	5	3
606	Erik x MN82393	74	11	10	2	8
607	Reddale x AC 84.23	107	6	10	4	11[2]
608	Reddale x Gold. Russ	107	5	9	4	5
609	Reddale x P2	108	25	41	1	26
610	Reddale x P7	108	12	17	4	12
611	Reddale x Russette	105	16	24	7	10
612	Reddale x MN10874	98	19	20	1	3
613	Reddale x MN11719	100	13	13	0	4
614	Reddale x MN12761	106	8	8	0	8
615	Reddale x MN12171	108	42	47	2	25
616	Reddale x MN13181	108	39	43	1	20
617	Reddale x MN13182	106	18	26	7	6
618	Reddale x MN13200	109	21	30	7	7
619	Reddale x MN13205	108	9	12	2	5
620	Reddale x MN82326	95	33	35	3	18
621	Reddale x MN82370	107	14	21	6	15
622	Reddale x MN82393	108	50	59	5	24
623	Reddale x MN83040	107	7	10	2	8
624	MN12761 x MN12761	105	3	3	0	3
625	MN12761 x MN13200	107	7	9	1	8
626	MN12761 x MN13205	105	5	8	3	5
627	MN13227 x AC 84.3	107	34	40	4	20
628	MN82370 x AC 84.3	107	21	24	2	22
629	MN82370 x Gold. Russ	107	26	29	1	15
630	MN82370 x P7	108	19	26	6	19
631	MN82370 x MN13200	107	25	28	1	9
632	MN82370 x MN13205	107	22	26	2	8
633	MN82393 x MN82393	104	5	7	2	5
634	MN82393 x Gold. Russ	104	50	54	2	13
635	MN82393 x P2	108	18	20	1	18
636	MN82393 x MN13181	107	13	15	1	4
637	MN82393 x MN13200	105	43	47	2	14
638	MN82393 x MN13205	107	14	17	2	15
Total		3553	49	837	108	404

[1] *Verticillium* isolated from vascular tissue.

[2] 5 clones were selected with low numbers of CFU/ml of *V. dahliae*.

Grand Forks, ND. The cvs. Reddale and Kennebec were included as resistant and susceptible checks. Each tuber was inoculated at time of planting by spraying with a 5 ml solution containing 10^7 conidia and 10^4 microsclerotia per ml of *V. dahliae* before the furrow was closed. The four center plants of each 6-hill plot were assayed 100 days after planting to determine the number of CFU of *V. dahliae* expressed as $\log_{10}$ (CFU + 1)/0.1 ml of plant sap. At the end of the season, clones having approximately the same or fewer CFU than Reddale were harvested.

Results and Discussion

Seedling progeny survival in mid-September varied from 3 to 50% per cross. The average survival percentage for all crosses was 49 (Table 2). At this time, of 837 vigorous and apparently healthy plants assayed for the presence of *V. dahliae*, 108 were infected. From the remaining 729 plants, 404 vigorous plants were selected for future VW evaluation.

The following year the 404 clones were grown in the VW plot at Grand Forks, ND. At 100 days after planting 4,872 assays (12 per clone/cvs) were made on the 404 replicated clones and the two check cultivars. An ANOVA indicated highly significant differences in *V. dahliae* colonization among the 404 clones. The mean number of CFU/ml for all clones was 2.3 as compared to 1.3 for Reddale and 3.7 for Kennebec (Fig. 1). Four clones had

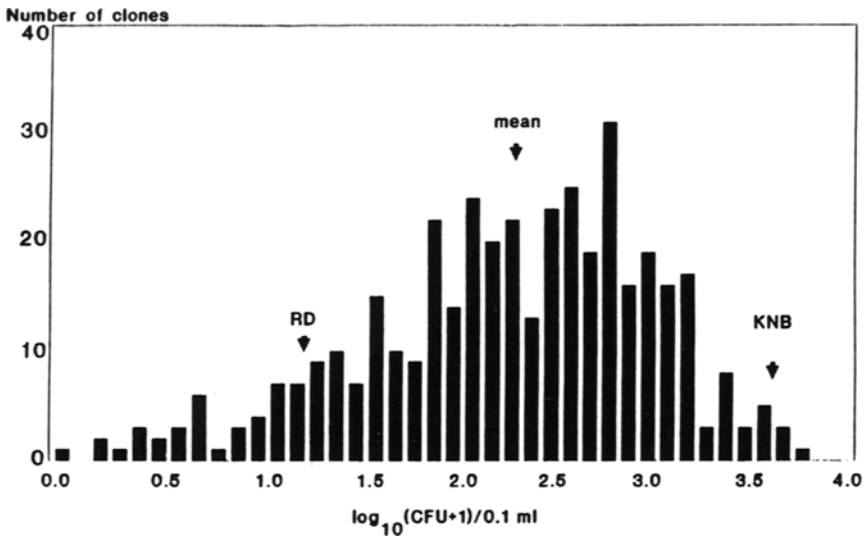

FIG. 1. The vascular colonization of *Verticillium dahliae* in 404 potato clones grown for 100 days in the Verticillium wilt plot Grand Forks, ND. The mean number of colony forming units (CFU) expressed as $\log_{10}$ (CFU + 1)/0.1 ml plant sap for all clones was 2.3; susceptible (KNB) and resistant Reddale (RD) averaged 3.7 and 1.3 respectively. LSD (P.01) = 0.9737.

higher CFU counts than Kennebec and 35 clones had counts equal to or less than Reddale. There were however, another 140 clones with CFU counts not significantly greater than Reddale and these clones could be important sources of resistance in a breeding program.

The number of CFU in progeny of 17 crosses with the best VW resistance is presented in Fig. 2. The CFU of *V. dahliae* in the Reddale and Kennebec checks are added as reference points. The progeny of cross 622 (Reddale × MN82393) had the most offspring with high resistance (14 clones). Both Reddale and MN82393 have high levels of VW resistance. Another cross, 609 (Reddale × P2), had 7 clones with low CFU counts indicating resistance. The wilt resistance of P2 is unknown. Two other crosses, 616 and 620, had five clones each with CFU counts close to that of Reddale.

These results suggests that low vascular colonization of potato stems by *V. dahliae*, an estimate of VW resistance, can be used to select parents and that the low vascular colonization trait is transmitted to progeny with a fairly high frequency. This study also suggests that selection of VW resistance can be done on potato seedlings inoculated with *V. dahliae* just prior to transplanting to a plot with low VW infestation. To accelerate the search for resistance to *V. dahliae*, however, a more rigorous first year test is needed to eliminate susceptible true seed seedlings. Only 8.6% of the putatively

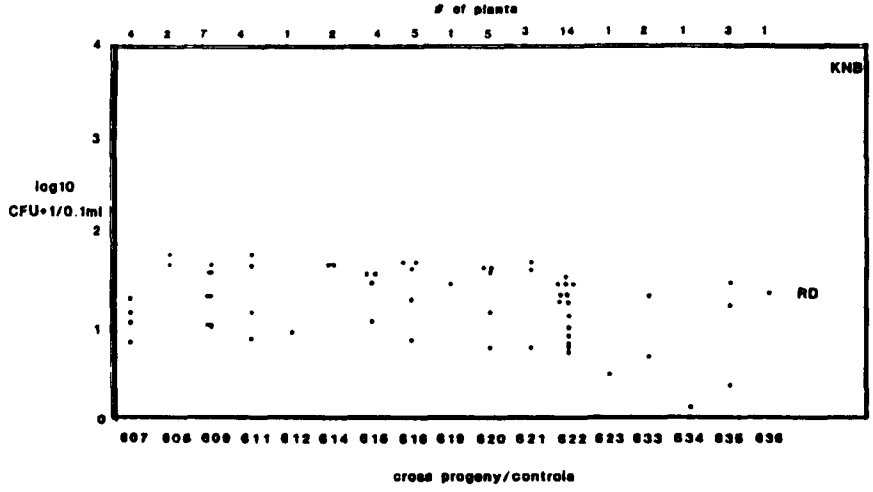

FIG. 2. Number of colony forming units (CFU) *Verticillium dahliae* expressed as $\log_{10}$ (CFU+1)/0.1 ml plant sap of 62 progeny clones of 10 crosses with wilt resistance approximately equal or exceeding that of Reddale. Each dot represents the average vascular colonization of 12 plants per clone from a particular cross. The resistant Reddale (RD) and susceptible Kennebec (KNB) are included as reference cultivars.

resistant 404 clones selected in year one of this test had a resistance level equal to or better than Reddale. For the Minnesota Potato Breeding Program, the ideal situation would probably be to evaluate true seed seedlings immediately in the VW plot at Grand Forks, ND. Our assumption is that the susceptible seedlings could be detected visually and seedlings with moderate to high resistance differentiated by low levels of vascular colonization. Irrigation capability, however, would be needed to insure seedling survival after transplanting.

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