Variability for Resistance to *Phytophthora nicotianae* Within a Collection of *Nicotiana rustica* Accessions

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Abstract

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Black shank, caused by *Phytophthora nicotianae*, is one of the most important diseases affecting tobacco (*Nicotiana tabacum*) production worldwide. Many current tobacco cultivars possess immunity to race 0 of this pathogen conferred by introgressed dominant genetic factors. Novel alleles conditioning resistance to alternative races are desired. The objective of this research was to evaluate variability for black shank resistance within a collection of *N. rustica* germplasm using both soilborne disease nurseries and controlled race-specific (race 0 and race 1) inoculations. Nearly all of the 86 accessions studied exhib-

ited very high resistance to race 0, and many displayed levels of race 1 resistance greater than that exhibited by the resistant flue-cured tobacco check, 'K 346'. Materials found to be highly resistant to race 0 and race 1 in growth-chamber experiments also had the best survivability in field disease nurseries. *N. rustica* accessions TR 6, TR 12, TR 16, TR 21, TR 20, TR 48, TR 54, TR 57, and TR 69 could be sources of novel alleles with large effects on black shank resistance, and could have value for burley and flue-cured tobacco breeding.

Black shank, caused by the oomycete *Phytophthora nicotianae* Breda de Haan, is one of the most important diseases affecting commercial tobacco (*Nicotiana tabacum* L.) production worldwide. Genetic resistance is typically the most attractive way to reduce economic losses from disease in crop plants, and both intraspecific and interspecific sources of resistance have been investigated for cultivated tobacco. Immunity to race 0 of *P. nicotianae*, conferred by genes designated as *Php* and *Phl*, has been introgressed into *N. tabacum* from *N. plumbaginifolia* Viv. and *N. longiflora* Cav., respectively (1,4,13,27). These genes provide little or no protection, however, to alternative races of the pathogen that have become increasingly important (3,24). Tobacco breeders have also utilized resistance of the classic quantitative type, where resistance is partial, non-race-specific, and controlled by multiple genes (5,10,21). Based on pedigrees, the cigar tobacco 'Florida 301' (25) is the likely source of the most important polygenes contributing to black shank resistance in current flue-cured and burley tobacco cultivars, although sources with greater levels of resistance have recently been investigated (28). Substantial variability exists among modern tobacco cultivars for level of observed black shank resistance (11,19), presumably resulting from variability in the presence or absence of different components of the Florida 301 polygenic system, or due to their interaction with other genes within the *N. tabacum* genome.

Because complete resistance does not currently exist in tobacco cultivars for black shank races other than race 0, identification of additional genetic factors that extend the range and level of resistance would be of value. Burk and Heggstad (2) reported in their review that, in addition to *N. longiflora* and *N. plumbaginifolia*, some varieties of *N. rustica* L. possess adequate black shank resistance to be potential interspecific donors of resistance to cultivated tobacco. Litton et al. (18) evaluated two accessions of this species and found one accession to be highly resistant to race 1 but not very resistant to an isolate of race 0. The second accession exhib-

ited low levels of resistance to both races. Li et al. (17) tested nine accessions of *N. rustica* for resistance to race 1, and reported varying levels of resistance ranging from low to intermediate. Collectively, the studies conducted to date have evaluated only a small fraction of the genetic variation available in this highly polymorphic, allopolyploid species. Due to the known presence of variable levels of black shank resistance within *N. tabacum* germplasm, it seems reasonable that substantial variability might also exist for resistance within the *N. rustica* gene pool.

A collection of 86 diverse accessions of *N. rustica*, which is maintained by the U.S. *Nicotiana* Germplasm Collection (16), was recently evaluated for a range of morphological characteristics (20). The objective of the research described here was to evaluate this collection of accessions for black shank field resistance and also resistance to specific races (race 0 and race 1) of *P. nicotianae* using controlled growth-chamber experiments. If high levels of resistance were identified, it might then be possible to transfer favorable alleles from this species to cultivated tobacco through interspecific hybridization and backcrossing (6,7,15,29). Results from this study could aid researchers in their choice of the most appropriate source materials for breeding efforts, because alleles affecting resistance to race 1 would be of particular value in applied tobacco cultivar development programs.

Materials and Methods

Field evaluation for black shank resistance. Field experiments were conducted in *P. nicotianae*-infested disease nurseries at the Oxford Tobacco Research Station (Oxford, NC) during the 2005 and 2007 growing seasons. Entries included 86 accessions of *N. rustica* (Table 1; seed obtained from the U.S. *Nicotiana* Germplasm Collection), 'K 346' (TC 569; high level of quantitative resistance) and 'Hicks' (TC 310; susceptible check) tobacco, and one *N. rustica* × *N. tabacum* interspecific hybrid (TR 19) of unknown origin and genetic composition maintained by the Collection. All entries were germinated in float trays and subsequently grown, transplanted, and cultivated according to the standard recommendations for flue-cured tobacco (22,23). The experimental design was a randomized complete block design for each year, with four replications during 2005 and three replications during 2007. Plots consisted of single 12-plant rows with 0.56-m within-row spacing and 1.22-m inter-row spacing. Initial stand counts were recorded approximately 3 weeks after transplanting, and plots

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were evaluated for the number of living plants every other week for a period of 70 days. These data were used to calculate average-days survival (ADS) for each plot using the formula $ADS = (\sum_i x_i y_{i*}) / x_0$, where x_0 is the initial stand count, x_i is the number of living plants at time i , and y_{i*} equals the number of days passed between the stand count at i and the previous stand count ($i - 1$).

Growth-chamber evaluation. An experiment was performed at the North Carolina State University Phytotron to investigate the resistance of this collection of *N. rustica* germplasm to infection with specific races of *P. nicotianae* (race 0 and race 1). The experiment had a split-plot design, with four replicates over time and each replicate in a separate growth chamber. The main-plot factor within each replicate was pathogen race (0 or 1) and the subplot factor was entry (entries the same as for the field experiment described above, with the addition of the *Php*-containing germplasm line 'NC 1071' possessing immunity to race 0 and a very low level of resistance to race 1). Experimental units consisted of six plants contained in a six-compartment segment of a '3601' Com-Pack

plastic tray insert (T.O. Plastics Inc., Clearwater, MN). Seed were germinated on Metro Mix 200 growing medium (Sun Gro Horticulture, Bellevue, WA) in 9-cm plastic pots under plastic domes at 25°C with a photoperiod consisting of 16 h of light and 8 h of darkness. Approximately 2 weeks after emergence, seedlings were transplanted into individual cells of the plastic Com-Pack inserts containing a 50:50 mixture of river-bottom sand and Redi-earth Plug and Seedling Mix (Sun Gro Horticulture). Plants were inoculated 7 to 10 days after transplanting by inserting two *P. nicotianae* race 0- or race 1-infested oat grains into the soil of each cell followed by heavy watering. After inoculation, the temperature during light periods was increased to 30°C, while the temperature in the dark was maintained at 25°C. Data were collected for number of surviving plants beginning 7 days post inoculation (dpi) and continuing twice a week until 26 dpi. ADS values were calculated for each experimental unit according to the formula provided above.

***P. nicotianae* inoculum.** Inoculum for growth-chamber experiments was prepared by growing isolates of either race 0 or race 1 *P.*

Table 1. Mean average-days survival (ADS) for entries evaluated in two North Carolina field environments and race-specific growth-chamber experiments

Collection identifier	PI number	Entry name	Origin	Mean ADS (days) ^a		
				Field	Growth chamber	
					Race 0	Race 1
TC 310	PI 552373	<i>Nicotiana tabacum</i> 'Hicks' ^b	United States	29.8 ^k	9.8 ^{kn}	10.7 ^k
TC 364	PI 552715	<i>N. tabacum</i> 'NC 1071' ^c	United States	...	26.0	8.3 ^k
TC 569	PI 549110	<i>N. tabacum</i> 'K 346' ^d	United States	60.9	25.7	19.8
TR 1	PI 499162	4384-HHS	United States	61.7	25.5	19.4
TR 2	PI 499163	4385 L-5-6	United States	58.0	25.2	16.8
TR 3	PI 499164	4386 L-5-6	United States	61.6	24.8	18.5
TR 4	PI 499165	4390 L-5-2-1	United States	62.4	26.0	22.0
TR 5	PI 499166	4398 L-5-2-1	United States	66.9	25.1	22.0
TR 6	PI 499167	4399 L-5-2-1	United States	69.0 ^K	26.0	23.6 ^K
TR 7	PI 499168	43054 L-5-2-1	United States	68.3	25.5	24.2 ^K
TR 8	PI 499169	43101 L-2-1B X L-6-2-1	United States	61.4	25.5	19.5
TR 9	PI 499170	43102-1 L-6-2-1 X L-5-2-1B	United States	60.8	25.5	20.3
TR 10	PI 499171	43103-5 L-5-2-1 X L-6-2-1	United States	62.2	26.0	21.2
TR 11	PI 499172	43104-1 L-5-2-1B X L-6-2-1	United States	63.2	24.9	19.3
TR 12	PI 499173	4401 L-5-2-1B	United States	69.5 ^K	26.0	23.9 ^K
TR 13	PI 499174	Brasilia # 7	Unknown	68.0	26.0	21.7
TR 14	PI 499175	Brasilia # 23	Unknown	68.6 ^K	25.8	23.0
TR 15	PI 34753	Brasilia Selvaggio	Brazil	67.9	26.0	21.2
TR 16	PI 34752	Brasilia Leccese	Brazil	68.6 ^K	26.0	24.8 ^K
TR 17	PI 34754	Erbasanta	Brazil	67.2	26.0	20.5
TR 18	PI 499176	68 Olson	United States	67.7	26.0	23.1
TR 19	PI 499177	C 39-193 (<i>N. tabacum</i> × <i>N. rustica</i>)	United States	70.0	26.0	20.1
TR 20	PI 499178	German # 2	Unknown	69.7 ^K	26.0	25.4 ^K
TR 21	PI 499179	German # 1	Unknown	69.2 ^K	26.0	24.6 ^K
TR 22	PI 499180	Mahorka # 1 AC 18/7	Russia	64.5	26.0	24.0 ^K
TR 23	PI 499181	Mahorka # 2 Armenia	Russia	68.0	25.2	18.6
TR 24	PI 499182	Mahorka # 3 Voronezhskaja	Russia	64.5	26.0	20.0
TR 25	PI 499183	Mahorka # 4 Tall Green	Russia	66.5	25.8	24.9 ^K
TR 26	PI 499184	Mahorka # 5	Russia	67.5	25.2	17.4
TR 27	PI 499185	Mahorka # 6 Yellow # 109	Russia	55.3	24.8	15.4 ^{Hk}
TR 28	PI 499186	Mahorka # 7 Pekhletzenskaia	Russia	65.3	26.0	23.4 ^K
TR 29	PI 499187	Mahorka # 8 Prosechenskaia Local	Russia	66.7	23.9	15.0 ^{Hk}
TR 30	PI 499188	Mahorka # 9 Stapukhenskaia	Russia	65.2	24.4	21.3
TR 31	PI 499189	Mahorka # 10 Sorotooskaia	Russia	68.5 ^K	23.5	22.4
TR 32	PI 499190	Mahorka # 11 Stalingradskaia	Russia	66.1	24.5	16.7
TR 33	PI 499191	Mahorka # 12 Ivisenskaia Local	Russia	65.0	25.1	23.5 ^K
TR 34	PI 499192	Kostoff White Seed # 14	Russia	67.4	25.4	24.6 ^K
TR 35	PI 499193	BAK # 46	Russia	68.8 ^K	24.3	19.0
TR 36	PI 499194	Koriotes Dark-blue	Russia	67.5	25.4	21.2
TR 37	PI 499195	Jainkaya Soldata	Russia	63.0	26.0	23.3 ^K
TR 38	PI 499196	Jainkaya Black-blue # 54	Russia	64.1	25.2	15.2 ^{Hk}
TR 39	PI 499197	Drosgii Black-blue # 45	Russia	67.1	25.2	18.0
TR 40	PI 499198	14 # 23057	Russia	67.1	26.0	22.4

(continued on next page)

^a Based on the least significant differences (LSDs) in this table, superscript capital letters indicate positive significant differences between the entry and a check variety, while lowercase letters indicate negative significant differences. Check variety codes: H/h = Hicks, K/k = K 346, N/n = NC 1071.

^b Tobacco cultivar chosen as the susceptible check for all experiments.

^c Tobacco breeding line chosen as an additional check for *Phytophthora nicotianae* race-specific experiments due to its differential response (immune to infection by race 0 due to a dominant genomic introgression from *N. plumbaginifolia* known as *Php*; very susceptible to race 1 infection).

^d Tobacco cultivar chosen as the resistant check because it has a high level of quantitative resistance to infection by multiple races of *P. nicotianae*.

nicotianae in sterile petri dishes containing carrot agar (50 ml of organic carrot juice, 950 ml of distilled water, and 20 g of agar) in the dark at room temperature. Single layers of autoclaved oat grains were placed on cultures after 3 to 4 days of growth. Oat grains were considered ready for use when they were visibly covered with hyphae, generally 12 to 16 days after they were added to the cultures.

Data analysis. An analysis of variance for ADS was carried out for the field and growth chamber experiments using PROC GLM of SAS (v. 9.1; SAS Institute, Cary, NC). For the field experiment, replication and year were considered random variables while entry was fixed. For the growth-chamber experiment, pathogen race and entry were considered fixed variables while replication was considered a random factor. Entry means were calculated using the LSMEANS procedure of SAS and compared using the least significant difference test as outlined by Gomez and Gomez (12).

Results

Field study. An analysis of variance of field data from two North Carolina environments with unknown *P. nicotianae* race

structures revealed significant differences among entries for ADS ($P < 0.0001$; Table 2). The susceptible check cultivar, Hicks, exhibited by far the lowest ADS (29.8 days; Table 1), while the greatest ADS was observed for TR 19 (70.0 days), the *N. tabacum* × *N. rustica* synthetic hybrid. The *N. rustica* accessions exhibited a relatively narrow range for ADS (54.0 days for TR 50 to 69.8 days for TR 48). Field resistance of all *N. rustica* accessions was excellent, with all of the entries having a significantly greater value for ADS than Hicks ($P < 0.0001$). Nineteen accessions, including the interspecific hybrid TR 19, survived significantly longer than K 346, the highly resistant flue-cured tobacco check cultivar possessing polygenic resistance, and no *N. rustica* accession exhibited an ADS that was significantly less than that of K 346 ($P < 0.05$).

Race-specific growth chamber experiments. An analysis of variance on data obtained from inoculation with specific *P. nicotianae* races (race 0 and race 1) revealed significant differences among both races ($P < 0.01$) and entries ($P < 0.0001$) for ADS (Table 3). A significant race–entry interaction was also observed ($P < 0.0001$), and race-specific means for ADS in the growth chamber

Table 1. (continued from preceding page)

Collection identifier	PI number	Entry name	Origin	Mean ADS (days) ^a		
				Field	Growth chamber	
					Race 0	Race 1
TR 41	PI 499199	Edinburg # 25	Russia	67.0	25.8	17.4
TR 42	PI 499200	Ja. Bot. Gar. # 30	France	66.7	25.4	16.6
TR 43	PI 499201	R. Bot. Gar. # 29	Lithuania	69.1 ^K	25.7	22.7
TR 44	PI 499202	Harbin # 6	China	68.2	25.4	21.1
TR 45	PI 269933	...	Pakistan	68.5 ^K	25.2	20.4
TR 46	PI 269934	...	Pakistan	64.7	25.3	19.2
TR 47	PI 269935	...	Pakistan	68.2	24.8	16.4
TR 48	PI 269936	...	Pakistan	69.8 ^K	24.9	23.7 ^K
TR 49	PI 499203	Chinensis	Turkey	68.8 ^K	24.6	18.8
TR 50	PI 499204	Campanulata	New Zealand	54.0	23.0	13.3 ^K
TR 51	PI 499205	Acutiflora	New Zealand	63.2	21.3 ^{kn}	16.3 ^{Hk}
TR 52	PI 499206	Fruticora	New Zealand	68.4 ^K	25.2	22.4
TR 53	PI 499207	Acutifolia	New Zealand	64.3	23.6	18.8
TR 54	PI 240355	Nordugel	New Guinea	69.3 ^K	26.0	24.0 ^K
TR 55	PI 478886	GC-1	India	68.2	25.5	17.8
TR 56	PI 481453	Hasankeyf	Turkey	65.0	24.0	14.9 ^{Hk}
TR 57	PI 475761	PNE 241-5	Pakistan	68.9 ^K	24.6	24.1 ^K
TR 58	PI 475762	PNE 362-4	Pakistan	67.5	23.2	17.8
TR 59	PI 475763	PNE 369-3	Pakistan	66.0	25.7	23.3 ^K
TR 60	PI 475764	PNE 373-13	Pakistan	64.4	25.4	20.8
TR 61	PI 475765	PNE 407-5	Pakistan	66.1	25.3	22.2
TR 62	PI 475766	PNE 412-8	Pakistan	68.1	25.7	22.4 ^K
TR 63	PI 475767	PNE 417-4	Pakistan	66.5	22.9	24.2 ^K
TR 64	PI 475768	PNE 418-6	Pakistan	67.7	25.0	22.0
TR 65	PI 475769	PNE 420-6	Pakistan	64.5	26.0	20.6
TR 66	PI 475770	PNE 427-4	Pakistan	62.0	26.0	21.3
TR 67	PI 494878	ZFA 3544	Zambia	67.8	25.5	24.3 ^K
TR 68	PI 494879	ZFA 3561	Zambia	67.7	26.0	20.3
TR 69	PI 494880	ZFA 3564	Zambia	69.2 ^K	24.8	23.5 ^K
TR 70	PI 500927	...	Zambia	68.3	23.2	14.3 ^{Hk}
TR 71	PI 500931	...	Zambia	69.3 ^K	25.7	19.2
TR 72	PI 500932	...	Zambia	67.6	24.6	21.0
TR 73	PI 500933	...	Zambia	68.1	25.0	20.6
TR 74	PI 500936	...	Zambia	68.4 ^K	25.5	21.1
TR 75	PI 505664	ZM/A5133	Zambia	68.1	25.2	23.2 ^K
TR 76	PI 481861	Maras	Turkey	69.3 ^K	26.0	22.4
TR 77	PI 481867	Karabaglar	Turkey	62.3	21.7 ^{kn}	14.7 ^{Hk}
TR 78	...	Selection from TR36	United States	67.9	24.8	23.3 ^K
TR 79	...	Selection from TR42	United States	67.8	26.0	21.5
TR 80	...	Selection from TR52	United States	66.5	26.0	23.0 ^K
TR 81	...	...	Unknown	67.9	25.2	15.5 ^{Hk}
TR 82	PI 555556	...	Unknown	63.1	24.3	17.3
TW 116	PI 243561	<i>N. rustica</i> var. <i>pavonii</i>	Peru	58.7	18.8 ^{kn}	15.0 ^{Hk}
TW 117	PI 555554	<i>N. rustica</i> var. <i>brasilia</i>	Unknown	68.3	25.1	19.9
TW 118	PI 555555	<i>N. rustica</i> var. <i>pumila</i>	Unknown	63.1	23.0	19.9
TW 119	PI 555692	<i>N. rustica</i>	Unknown	64.3	26.0	18.1
TW 120	PI 555693	<i>N. rustica</i>	Unknown	64.3	24.3	16.5
LSD (0.05)	...	...	...	7.4	3.4	3.4
Coefficient of variation (%)	...	...	...	11.4	11.0	11.0

are presented in Table 1. Hicks was the most race 0-susceptible entry in the experiment (9.8 days ADS), whereas almost all *N. rustica* accessions exhibited little to no infection. Only three accessions (TR 51, TR 77, and TW 116) exhibited a level of race 0 survival significantly lower than that for NC 1071, a tobacco breeding line immune to race 0. Excluding these three entries, the range of *N. rustica* race 0 ADS values was 22.9 to 26.0 days, with a mean of 25.2 days.

A much greater range for ADS values (13.3 to 25.4 days; Table 1) was observed among *N. rustica* accessions after inoculation with race 1 of *P. nicotianae*. In total, 32 accessions had ADS values that were significantly different from those of the resistant check cultivar. Of the 10 entries that had significantly lower ADS values than K 346 (19.8 days; $P < 0.05$), only TR 50 was not significantly different from the susceptible control, Hicks (10.7 days; $P < 0.05$), although TR 50 still survived longer than NC 1071 (8.3 days; $P < 0.05$) in the presence of race 1. Twenty-two *N. rustica* accessions exhibited high levels of resistance to race 1, with ADS values significantly greater ($P < 0.05$) than that observed for K 346. Pearson correlation coefficients describing the relationships between ADS in the field and following race 0 and race 1 inoculations were 0.75 ($P < 0.0001$) and 0.54 ($P < 0.0001$), respectively.

Discussion

N. rustica was previously reported to exhibit moderate to high levels of resistance to one or more races of *P. nicotianae* (17,18), although results have been variable. In order to better understand the scope of black shank resistance in this species, we investigated the largest and most diverse group of *N. rustica* accessions studied to date. Nearly all of the accessions evaluated exhibited very high resistance to race 0 of *P. nicotianae*, with a large proportion that could be rated as immune. By testing such a large collection, our study was also able to reveal more than 20 *N. rustica* accessions that had significantly higher levels of resistance to race 1 than the resistant flue-cured tobacco check, K 346. This information is valuable to tobacco breeders, because resistance to race 1 of the black shank pathogen is scarce not only in *N. tabacum* but also within the genus as a whole (17,18). Specifically, the *N. rustica* accessions designated TR 6, TR 12, TR 16, TR 21, TR 20, TR 48, TR 54, TR 57, and TR 69 would be the best sources of novel alleles with large effects on black shank resistance, and could have value for burley and flue-cured tobacco breeding.

When grown in *P. nicotianae*-infested fields and cultivated using standard practices for commercial tobacco, the majority of *N. rustica* accessions evaluated had excellent field resistance, a quality that was also noted by Tisdale and Kelley (26). In race-specific tests, *N. rustica* accessions exhibited very high levels of resistance to the most common race of *P. nicotianae*, race 0, with only a sin-

gle accession (TW 116) having an intermediate level of resistance. These results are at odds with the observations of Litton et al. (18), who, using an index from 0 (no symptoms; immune) to 5 (all plants dead), reported a race 0 disease index for one *N. rustica* accession as 4.5 and rated PI 555554 (TW 117; 48-G) as 4. In the current study, the latter accession had a race 0 ADS value of 25.0 days, which translates into 87% healthy plants after 26 days of exposure to the pathogen. When this same accession was challenged with race 1, Litton et al. (18) reported a disease index of 3, which is more in line with our results (19.9-day ADS; 64% healthy plants).

When challenged specifically with race 1 of *P. nicotianae*, the *N. rustica* accessions responded with intermediate to high levels of resistance. These results are generally congruent with those of Litton et al. (18), who reported one highly resistant accession (disease index of 1), as well as intermediate resistance for TW 117 (see above). However, after evaluating a group of nine accessions of *N. rustica*, Li et al. (17) documented a range in percentage of healthy plants after inoculation with race 1 of 0 to 32% (very low resistance). For the three accessions that were also included in our study (PI 499162, PI 499163, and PI 499174), Li et al. (17) reported 0, 18, and 24% healthy plants, respectively, while observations from our study were 38, 25, and 52%. The discrepancies between our results and those of Litton et al. (18) and Li et al. (17) are most likely due to differences in experimental procedures, because both of these groups employed a small-plant technique. Plants in the current study were older, with more established root systems, and were not subjected to intraspecific competition for resources.

Although our study was not intended to address the inheritance or mechanism of this source of resistance to black shank, it seems possible that *N. rustica* may carry a dominant resistance factor conferring immunity to race 0 of *P. nicotianae*, similar to *Php* or *Phl*, due to the overwhelming lack of symptom development in most accessions of *N. rustica* during both field and race-specific trials. From a practical breeding point of view, race 0-specific resistance genes of *N. rustica* origin probably have limited commercial value because immunity to race 0 has already been introgressed into *N. tabacum* from the species mentioned previously. Alternatively, considering that some accessions also have high levels of resistance to race 1, a multigenic system conferring very high levels of quantitative resistance may be present. More studies need to be conducted to resolve this issue. Interestingly, accessions of *N. longiflora* immune to race 0 have also been shown to possess excellent resistance to race 1 (18), but either the source of this resistance is controlled by a different genetic locus or the *Phl* introgression cannot overcome susceptibility factors present within the *N. tabacum* genome.

Table 2. Analysis of variance for black shank resistance, as measured by average-days survival, for 86 *Nicotiana rustica* accessions and associated checks grown in two North Carolina field environments

Source	df	Sum of squares	Mean squares	Computed <i>F</i>	<i>P</i> value
Year	1	1,134.2	1,134.2	20.28	0.0064
Rep (year)	5	279.7	55.9	3.63	0.0031
Entry	88	17,435.3	198.1	3.59	<0.0001
Entry × year	88	4,853.2	55.2	3.58	<0.0001
Pooled error	440	6,771.2	15.4	...	...

Table 3. Analysis of variance for average-days survival for 86 *Nicotiana rustica* accessions and associated checks after inoculation in a growth chamber with specific races (0 or 1) of *Phytophthora nicotianae*

Source	df	Sum of squares	Mean squares	Computed <i>F</i>	<i>P</i> value
Replication	3	920.0	306.7	5.48	0.0980
Race	1	4,011.5	4,011.5	71.68	0.0035
Error (a)	3	167.9	56.0	...	...
Entry	89	3,952.5	44.4	7.28	<0.0001
Race × entry	89	1,586.0	17.8	2.92	<0.0001
Error (b)	534	3,256.7	6.1	...	...

Although neither the amphidiploid species *N. rustica* nor its likely diploid progenitors (*N. undulata*, and *N. paniculata* or *N. knightiana*; 8,9), are considered to be close relatives of *N. tabacum* or its diploid precursors (14), it is possible to transfer genetic material from *N. rustica* to cultivated tobacco. For example, Woodend and Mudzengerere (29) introgressed a gene controlling resistance to the angular leaf spot pathogen (*Pseudomonas syringae* pv. *tabaci*) from *N. rustica* into cultivated tobacco. Additionally, Chaplin (6) conducted breeding work to transfer an increased genetic capacity to accumulate alkaloids from this species to commercial tobacco, and Legg and Mann (15) demonstrated the effect of introgression of *N. rustica* chromatin on agronomic characteristics of cultivated tobacco after three rounds of backcrossing to *N. tabacum*. Because of the considerable degree of taxonomic divergence between *N. rustica* and *N. tabacum*, opportunities for genetic exchanges between *N. rustica* and recipient *N. tabacum* chromosomes are reduced. Therefore, the likelihood of transferring all components of a multigenic resistance system using interspecific hybridization followed by backcrossing is probably low, especially without adversely affecting yield and quality. It may be possible, however, to transfer a few alleles controlling a large proportion of the quantitative resistance to *Phytophthora nicotianae* race 1 infection from *N. rustica* into *N. tabacum*.

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