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Transmission of Red Stele Resistance by Inbred Strawberry Selections[1,2]

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Abstract. **Seedlings from a diallel set of crosses of inbred and noninbred strawberry (*Fragaria* X *ananassa* Duch.) selections resistant to *Phytophthora fragariae* Hickman were inoculated with the 5 principal Eastern U. S. races of the fungus. The inbred selections, when selfed or intercrossed to other inbreds or to the noninbreds, did not transmit resistance to a greater portion of their progeny than the control cross, a cross of 2 noninbreds. Specific combining ability was found to be important in transmission of resistance to progeny.**

Strawberry cultivars are propagated vegetatively and, hence, do not need to be genetically homozygous. Inbreeding, a breeding approach often used to advantage in certain vegetable and agronomic crops, is not commonly used in strawberry breeding. Though the strawberry is a heterozygous octoploid, it is difficult to obtain or to maintain inbreds because inbreeding depression causes loss of vigor (9). Yet, inbreeding has occassionally been used in strawberry breeding programs with various degrees of success for the past 40 years.

Jones and Singleton (9) and Morrow and Darrow (10) reported on the potential use of strawberry inbreds to concentrate one or more desired characters in an inbred strain and to test its transmission through the inbred. This method was successful in increasing the vitamin C content of strawberry selections (2). 'Albritton' derives from a cross between two S_1 inbreds (6); 'Aliso', an S_1 inbred, was originated by selfing and one parent of 'Sequoia' is an S_1 inbred (4).

The U. S. Department of Agriculture began breeding strawberries for resistance to red stele root rot (incited by *Phytophthora fragariae*) in 1937. Differences among clones were found in the transmission of red stele resistance. Stembridge and Scott (14) showed that inheritance of red stele resistance was partially dominant and quantitatively inherited and that resistant plants were heterozygous in nature. Gooding (7) found general combining ability more important than specific combining ability in the inheritance of field resistance to red stele root rot. Scott and Draper (13) found that 1 generation of inbreeding appeared to increase the homozygosity of genes for resistance to red stele in 1 of 2 selections, as measured by their ability to transmit resistance to progeny. General combining ability was important in the inheritance of resistance to races A-1, A-2, A-3, A-4 and A-6 of the fungus; general and specific combining ability were important when race A-5 was included in the inoculum with the other 5 races.

These results suggested that a breeding strategy based on selfing could produce plants with increased homozygosity for resistance. These plants could then be crossed with other inbred selections to restore progeny vigor and to produce progenies with a larger number of resistant seedlings than those from outcrossed progenies. This study was conducted to compare highly resistant inbred and noninbred strawberry selections for transmission of resistance to red stele root to their progenies.

Materials and Methods

All parental selections (Table 1) in this study rated 8.5 to 9.0 on a scale of 1 to 9, indicating that infection was absent or limited to a few root tips. The backgrounds of the 6 selections used in this study are shown in Table 1.

The crosses were made in all possible combinations, giving a full diallel of 36. The progenies utilized were from 15 crosses, 6 selfs, 4 reciprocal crosses, and a susceptible progeny ('Midland' × self) used as an inoculum check. The 4 progenies from reciprocal crosses were included to detect differences in seed or pollen transmission of resistance. A half diallel was used in the progeny study because Watkins and Spangelo (15) showed that half diallels gave the same results as full diallels in the cultivated strawberry.

The crosses and selfs were made in February, 1978. Seeds from each cross were extracted from the berries by the method of Morrow et al. (11). In August 1978 about 750 seeds per cross were sown on the surface of flats containing damp milled sphagnum moss, were stratified for 2 months at 3°C, and germinated under intermittent mist and supplemental light in October 1978.

Concurrently the 5 principal Eastern U.S. races of the red stele fungus (A-1, A-2, A-3, A-4, and A-6) were cultured on kidney bean meal agar in petri dishes (5). More plates were

Table 1. Derivation of parental selections used in red stele resistance transmission study.

Selection	Parentage	Level of inbreeding
Md-US 4509	Md-US 2856 × self	S_1
Md-US 4515	Surecrop × self	S_1
Md-US 4519	Md-US 4461 × self	S_2
Md-US 4520	Md-US 4461 × self	S_2
Md-US 4355	Raritan × Md-US 3413	N(noninbred)
Md-US 4426	Md-US 3700 × Redchief	N(noninbred)

Md-US 2856 = Md-US 2350 × Surecrop, Md-US 2350 = Fairland × Tennessee Shipper, Md-US 4461 = Md-US 2921 × self, Md-US 2921 = Earlidawn × Md-US 2321, Md-US 2321 = Temple × Md-1972, Md-1972 = Blakemore × Md-683, Md-683 = Fairfax × Scotland BK 46, Md-US 3413 = Md-US 2611 × Earlidawn, Md-US 2611 = US 4152 × Stelemaster, US 4152 = Tennessee Shipper × Maytime, Md-US 3700 = Md-US 2966 × NJ 821-5, Md-US 2966 = Md-US 2386 × Surecrop, Md-US 2386 = Dixieland × Temple.

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[5]Vitamins, hormones, and plant food (VHPF) manufactured by the Miller Fertilizer Company, Baltimore, Maryland.

Table 2. Percentage of resistant (R), intermediate (T), susceptible (S), and very susceptible (YS) seedlings from Md-US crosses and selfs of inbred (S) and noninbred (N) strawberry selections with resistant to red stele, in tests with 5 races of *Phytophthora fragariae.*[z]

Cross[y]	Type of progeny	Progeny classification (%) R (classes 8&9)	I (classes 5-7)	S (classes 2-4)	VS (class 1)
4355 x 4426	N x N	78.3a[x]	3.3	3.8	14.5
4515 x 4426*	S_1 x N	77.1ab	5.4	4.6	12.9
4509 x 4426*	S_1 x N	76.3ab	5.8	4.2	13.0
4426 x 4509*	N x S_1	75.8a-c	3.8	4.6	15.8
4426 x 4515*	N x S_1	74.2a-d	5.0	3.3	17.5
4509 x 4515	S_1 x S_1	73.5a-d	8.3	4.2	15.0
4509 x 4519*	S_1 x S_2	72.5a-d	5.4	2.5	19.5
4515 x 4519*	S_1 x S_2	71.6a-e	8.8	7.5	12.1
4519 x 4515*	S_2 x S_1	71.3a-e	9.2	8.3	11.3
4519 x 4426	S_2 x N	70.8a-e	9.6	5.8	13.8
4355 x 4520	N x S_2	70.8a-e	8.3	3.8	17.1
4520 x 4519	S_2 x S_2	70.7a-e	9.2	4.2	16.7
4520 x 4515	S_2 x S_1	69.2a-e	7.9	8.3	14.6
4355 x 4515	N x S_1	68.3a-e	7.9	2.1	21.6
4509 x 4355	S_1 x N	66.3 a-e	4.2	8.8	20.8
4519 x 4355	S_2 x N	66.6a-e	8.8	8.2	16.3
4520 x 4426	S_2 x N	66.6a-e	5.4	2.5	25.4
4519 x self	S_3	66.3a-e	9.6	5.8	18.3
4509 x 4520	S_1 x S_2	65.4a-e	5.4	8.3	20.8
4355 x self	S_1	65.0a-e	8.3	2.5	24.2
4426 x self	S_1	64.6b-e	9.1	4.2	22.1
4520 x self	S_2	62.5c-e	14.2	4.6	18.8
4509 x self	S_2	61.3de	11.7	3.8	23.3
4519 x 4509*	S_1 xS_1	61.6de	12.1	5.4	20.8
4515 x self	S_2	57.5e	13.3	10.8	18.3

[z]24 plants per plot with 10 replications.

[y]4 crosses and their reciprocals are denoted by an *.

[x]Mean separation by Duncan's multiple range test, 5% level.

Table 3. Weighted mean scores for red stele resistance in seedling (Md-US) progenies from crosses and selfs of inbred (S) and noninbred (N) strawberries selected for red stele resistance and inoculated with 5 races of *P. fragariae.*

Cross	Type of progeny	Weighted mean score[z]
4355 x 4426	N x N	7.37a
4515 x 4426	S_1 x N	7.35a
4509 x 4426	S_1 x N	7.33a
4426 x 4509	N x S_1	7.23ab
4509 x 4515	S_1 x S_1	7.18abc
4519 x 4515	S_2 x S_1	7.16abc
4519 x 4426	S_2 x N	7.16 abc
4426 x 4515	N x S_1	7.15abc
4515 x 4519	S_1 x S_2	7.07abc
4509 x 4519	S_1 x S_2	7.04abc
4355 x 4520	N x S_2	7.02abc
4520 x 4519	S_2 x S_2	7.00abc
4520 x 4515	S_2 x S_1	6.91abc
4519 x 4355	S_2 x N	6.81abc
4355 x 4515	N x S_1	6.78abc
4520 x self	S_3	6.68abc
4519 x self	S_3	6.65abc
4509 x 4520	S_1 x S_2	6.55abc
4426 x self	S_1	6.55abc
4520 x 4426	S_2 x N	6.52abc
4509 x 4355	S_1 x N	6.51abc
4355 x self	S_1	6.40abc
4519 x 4509	S_2 x S_1	6.41bc
4509 x self	S_2	6.40bc
4515 x self	S_2	6.32c
'Midland' x self (susceptible check)	S_1	1.90d

[z]Retransformed weighted mean scores from arcsin transformation used for analysis of variance (1 = dead, 9 = no detectable root damage). Mean separation by Duncan's multiple range test, 5% level.

prepared for the slower-growing races (A-2 and A-6); the plates were incubated at 5°C for 21 days. In November 1978, the roots of each seedling were dipped into a fungal slurry containing a mixture of the 5 principal races. The seedlings were spaced 5 x 5 cm and planted at 24 plants per plot in a randomized complete block design with 10 replications. They were grown in greenhouse concrete benches filled with steam-sterilized sand and received supplemental light at night.

During the screening the seedlings were fertilized 3 times weekly with 1,292 g of VHPF, a complete fertilizer,[5] 190 g of ammonium nitrate, and 30.4 g of iron chelate dissolved in 722 liters of water. The plants were grown at 20-24°C during the day and at 15-16°C during the night until they were about 8 cm in height. Then the temperature was lowered to 10-12°C (day and night) and the benches were flooded twice weekly to promote fungal zoospore production and dissemination.

In late March 1979 the progenies were evaluated for red stele infection using a scale of 1 to 9 according to root infection percentage: Class 9, no infection; Class 8, infection restricted to some root tips; Class 7, 25% of root system infected and no crown infection; Class 6, 37% of root system infected and no crown infection; Class 5, 50% of root system infected and no crown infection; Class 4, 67% of root system infected and no crown infection; Class 3, 75% of root system and no crown infection; Class 2, roots completely infected and crown infected but alive; Class 1, entire plant killed. Data were subjected to diallel analysis according to a method developed by Schaffer and Usanis (12) using Griffings Method 4 Model 1 (8). Percentages and disease scores were transformed using the arcsine transformation before being analyzed. All statistical work was performed in conjunction with the USDA statistical branch of the National Agricultural Library using the statistical analysis system (SAS).

Results and Discussion

Table 2 presents the frequency distributions of resistant, intermediate, susceptible, and very susceptible seedlings produced from cross-and self-pollinations of the parent strawberry selections. The progenies of 'Midland' selfed, included in the test as a susceptible check, had a weighted mean rating of 1.9 for red stele resistance (Table 3), indicating uniform distribution of inoculum. Progenies from the highly resistant parents had 58 to 78% resistant seedlings. The largest number of resistant seedlings was produced by the control cross (N x N); however, it's percentage differed from only 5 progenies, at the bottom of the range. In general, those clustered at the bottom were inbred progenies resulting from 1, 2, and 3 generations of inbreeding. One or 2 generations of inbreeding before crossing did not apparently affect the percentage of resistant seedlings produced. It should be noted, however, that the progeny of the cross of the two resistant noninbreds in this study was about 80% resistant, whereas routine screening of progenies of similar crosses averaged 50% resistant seedlings over the past three years. Had two other noninbreds been used, the number of resistant seedlings produced might have been lower and inbreeding might have been shown to increase heritability of resistance.

The percentage of resistant seedlings produced by each of the crosses Md-US 4509 x Md-US 4519, Md-US 4515 x Md-US 4519, Md-US 4509 x Md-US 4426, and Md-US 4515 x Md-US 4426 did not differ significantly from their corresponding reciprocal crosses (Table 2). Thus, these clones did not differ

Table 4. Transmission of resistance to red stele root rot in strawberry (Md-US) selections used as pollen and seed parents.[z]

Parent	Resistant seedlings (%)	
	Pollen parent	Seed parent
4426 (N)	72a	72a
4519 (S_2)	70ab	67a
4515 (S_1)	69ab	69a
4520 (S_2)	66b	67a
4509 (S_1)	66b	69a
4355 (N)	66b	71a

[z]Mean separation in columns by Duncan's multiple range test, 5% level.

in transmission of red stele resistance when they were used as pollen or seed parents in crosses.

Using the weighted mean scores of progenies from crosses of inbred and noninbred selections (Table 3) resulted in similar rankings and mean separations for the progenies obtained with percentage resistant seedlings (Table 2). The progenies from crosses ranked at the top of the range, and the progenies from selfing tended to be at the bottom.

As seed parents the selections did not differ significantly from each other in ability to transmit resistance to progenies. As pollen parents the selections did differ slightly, but these differences did not appear to be due to level of inbreeding. For example, Md-US 4426, a noninbred, when used as a pollen parent gave the largest percentage of resistant progeny, but inbreds Md-US 4519 (S_2) and Md-US 4515 (S_1) as pollen parents were not significantly different from Md-US 4426.

Table 5 summarizes the mean squares of general combining ability (GCA) and specific combining ability (SCA) for the 4 component classes of reaction to red stele root rot. SCA was significant at the 1 and 5% levels for weighted mean score and resistant classes, respectively. All other classes were not significant for GCA or SCA. In a situation in which all 6 parent selections were highly disease-resistant, the patterns of resistance transmission could not be predicted on the basis of level of parental homozygosity or degree of inbreeding. The results could be best explained by the specific combinations of certain parents. The results are thus attributable to the SCA of individual parents.

The recognition of significant SCA in strawberry inheritance was consistent with findings by Aalders and Craig (1), Bedard and Lauer (3), and Scott and Draper (13). Because transmission of resistance to progeny showed significant SCA, nonadditive variance apparently made up the majority of the total genetic variance for transmission of red stele resistance to progeny of crosses.

Not only was the level of inbreeding not associated with transmission of resistance to progeny in this study, but inbreeding without outcrossing tended to transmit less resistance in general. The other classes (intermediate, susceptible, or very susceptible) did not show a significant transmission pattern either by progeny type or by particular parentage.

It thus appears that neither 1 to 3 generations of selfing nor the subsequent crossing of the inbreds increased the percentage of resistant seedlings in this instance. Even if plants of the S_2 generation were more homozygous for resistance than were the original selfed clones, they did not transmit resistance to a larger number of their progeny. This conclusion supports the findings of Scott and Draper (13), who suggest that current methods of crossing (without inbreeding) and selection adequately increase the number of resistant strawberry varieties with resistance to multiple races of red stele. The greater vigor of seedlings from outcrosses compared with those from inbreds also gives a larger number of seedlings from which to select other desirable characters for breeding improved cultivars.

Table 5. Mean squares of general and specific combining ability for weighted mean score and 4 component classes of reaction to red stele root rot in strawberry lines.

Classes	Source of variation	df	Mean square	F
Weighted mean score	GCA	5	0.7893	.56
	SCA	15	1.3887	2.10**
	Error	220	0.6600	
Resistant	GCA	5	0.0030	.74
	SCA	15	0.0041	2.20*
	Error	220	0.0019	
Intermediate	GCA	5	0.0060	1.41
	SCA	15	0.0039	1.50
	Error	220	0.0026	
Susceptible	GCA	5	0.0041	1.33
	SCA	15	0.0030	1.47
	Error	220	0.0021	
Very susceptible	GCA	5	0.0046	1.30
	SCA	15	0.0035	1.69
	Error	220	0.0021	

*,**Indicates significance at the 5 and 1% levels, respectively.

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