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Cultivar Dependent Variation in Pathogen Resistance due to Tissue Culture-propagation of Strawberries

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Abstract. **Tissue culture-propagated (TC) strawberry plants (*Fragaria* × *ananassa* Duch.) had a cultivar-dependent shift in susceptibility to *Phytophthora fragariae* Hickman and *Verticillium albo-atrum* Reinke and Berthold root-rotting fungi. TC-propagated 'Tribute', a resistant cultivar, had a disease reaction similar to that of runner-propagated 'Tribute' plants. TC-propagated and juvenile selfed seedlings of 'Raritan', a susceptible cultivar, were more susceptible to these diseases than 'Raritan' runner plants. The TC plant disease reaction shifted toward the runner plant (or usual clonal) reaction with increasing time out of in vitro culture.**

Micropropagation of strawberry by tissue culture techniques is used widely for producing foundation plants for nurseries (2). Concomitant with the commercial acceptance of strawberry micropropagation have been reports of temporary changes in TC plant vigor, petiole length, runner production, yield, and other characters (6, 9, 12).

The general response of TC-propagated fruit plants to pathogens has not been documented. The present experiment was designed to determine if TC-propagated strawberry plants of a resistant ('Tribute') and susceptible ('Raritan') cultivar react differently than runner-propagated (ST - stolon) plants to 2 major strawberry root rotting fungal diseases: red stele, incited by *Phytophthora fragariae*, and verticillium wilt, incited by *Verticillium albo-atrum*. Selfed seedlings also were screened to determine the juvenile plant reaction; resistance to both pathogens is quantitatively inherited (7, 10).

Twenty-five virus-indexed screenhouse-grown 'Tribute' and 'Raritan' plants were used as source plants thoughout the experiment. ST plants were propagated in early October of 1982 by removing runners and rooting them under intermittent mist. Seedling plants (Sdlg.) were obtained by self-pollinating each cultivar.

The basic medium used for strawberry tissue culture was that of Boxus (1) without growth regulators, substituting 30 g/liter sucrose for glucose, and adding 1.9 g/liter NH_4NO_3. Two methods of TC propagation were compared. TC method "A" was the 3-stage procedure used in commercial and scientific tissue culture facilities. Stage I establishment was on no-hormone medium, and Stage II proliferation was initiated by the addition of 1 mg/liter *N*-(phenylmethyl)-1*H*-purin-6-amine (BA) (1). TC method "B" meristem-tips were placed directly onto basic medium supplemented with either 2 or 5 mg/liter BA. This medium initiates rapid explant proliferation during Stage I (4).

ST, selfed seedlings, TC "B", and most of the TC "A" propagated plantlets were acclimated in a greenhouse 5 to 6 weeks prior to planting in the red stele test. The remaining TC "A" plants were subdivided to include a study investigating the effect of time out of tissue culture on susceptibility. These greenhouse-grown plants were removed from in vitro culture 15 (A_{15}) and 24 (A_{24}) weeks before red stele screening. All plants screened with verticillium wilt were greenhouse grown for 15 to 16 weeks.

Inoculum of red stele, race A-1 (from J.L. Maas, USDA, Beltsville, Md.), was used to test the resistance of the strawberry plants propagated by the various methods. This inoculum is considered less virulent than the combined 5 races used to test breeding seedlings and establish resistance classifications (7, 11). On 16 Nov. 1982, plant roots were dipped in an inoculum slurry and planted immediately in a single greenhouse screening bench (11) in a randomized complete block design with 6 replications. Each 'Tribute' replication contained 48 TC "A", 48 TC "B", 16 Sdlg., and 16 ST plants; each 'Raritan' replication contained 48 TC "A", 12 TC A_{15}, 12 TC A_{24}, 48 TC "B", 16 Sdlg., and 16 ST plants. In addition, 16 seedlings of selfed 'Midland', a susceptible progeny, were used to check test pathogenicity. Two runner plants of 6 clones were included as checks against possible contamination by races other than A-1 (11). All plants were dug after 14 weeks and evaluated for susceptibility to *P. fragariae* using a scale of 1 (most susceptible) to 9 (most resistant) according to root infection percentage (7).

A *Verticillium albo-atrum* isolate (V-09, from strawberry) was used in the verticillium wilt test. Test inoculum consisted of a blend of *Verticillium* grown on 6 liters of vermiculite moistened with 20% aqueous Campbell V-8 juice (Camden, N.J.) and 6 mM $CaCO_3$, mixed with 4 infected potato dextrose agar (PDA) plates (6 cm), and 300 ml of sterile water (3). About 15 ml of this inoculum was placed in each planting hole.

The greenhouse *V. albo-atrum* test was planted on 1 Feb. 1983, in a randomized complete block design with 4 replications, 2 each in 2 metal tubs (1 m × 1 m × 15 cm deep) heated to 25°C with electric cables. Each replication was composed of plots containing, for each cultivar, 6 ST, 6 Sdlg., and 23 TC "B" plants. In addition, 3 plants each of susceptible (MDUS 4234 and 'Cardinal'), and resistant ('Surecrop' and 'Catskill') clones were planted between blocks to test pathogenicity and uniformity (3).

After 12 weeks, plants were dug, washed, and scored on a scale of 1 (most susceptible)

Table 1. Strawberry propagation methods and their associated plant reaction scores to race A-1 of *Phytophthora fragariae*.

Cultivar and propagation treatment	Mean disease score[z]	Resistance rating class	Frequency distribution (%)[y]		
			-R-	-T-	-S-
Raritan					
ST	7.6 a	R	71	5	24
A 6 wk (TC)	3.5 b	S	5	39	56
B 6 wk (TC)	3.9 b	S	11	41	48
Sdlg. (Raritan ⊗)	3.2 b	S	17	18	65
Tribute					
ST	9.0 a	R	100	0	0
A 6 wk (TC)	8.9 a	R	99	1	0
B 6 wk (TC)	8.9 a	R	99	1	0
Sdlg. (Tribute ⊗)	7.6 b	R	82	16	2

[z]Means within cultivar, are separated by Duncan's multiple range test, 5% level.
[y]Rating classes; R = resistant (scores 7–9), T = tolerant (scores 4–6), S = susceptible (scores 1–3).

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Table 2. The effect of time interval between in vitro culture and screening on subsequent 'Raritan' *P. fragariae* (race A-1) reaction.

Propagation treatment	Mean disease scores[z]	Resistance rating class	Frequency distribution (%)[y] -R-	-T-	-S-
Sdlg.	3.2 a	S	17	18	65
$A_{6wk(TC)}$	3.5 ab	S	5	39	56
$A_{15wk(TC)}$	4.7 c	T	8	68	24
$A_{24wk(TC)}$	4.2 bc	T	4	65	31
ST	7.6 d	R	71	5	24

[z]Means separated by Duncan's multiple range test, 5% level.
[y]Rating classes; R = resistant (scores 7–9), T = tolerant (scores 4–6), S = susceptible (scores 1–3).

to 9 (most resistant) based on root and top killing, top stunting, and new root production (3). Petiole sections (1 mm) from young leaves were sterilized with sodium hypochorite (0.52%) and cultured on PDA in order to reisolate *Verticillium*. Separate General Linear Model (GLM) procedures of the Statistical Analysis System were used for each cultivar and screen (8). Where these ANOVA tests for significance were met ($P = 0.05$), Duncan's multiple range test was used to separate treatment means (8).

'Tribute' plants from all propagation treatments were, on average, resistant to red stele. The mean score of the selfed seedling population was significantly lower (Table 1). 'Raritan' ST plants were significantly more resistant than TC plants from both methods "A" and "B" or than selfed seedlings. After 15 weeks of greenhouse growth, the disease tolerance of the TC-propagated 'Raritan' plants was greater than that of the juvenile seedlings, but was still less than that of the ST (adult) plants (Table 2). In contrast to the susceptible "A" 6 week TC plants and selfed seedlings, a majority of A_{15} and A_{24} TC plants were tolerant to red stele.

Disease reaction scores of the clones included as checks against possible bench contamination from other *P. fragariae* races indicated that only race A-1 was present.

Table 3. Mean *Verticillium* wilt reaction scores of TC, runner, and selfed seedling populations of 'Raritan' and 'Tribute'

Cultivar and propagation treatment	Mean disease score	Frequency distribution (%)[z] -R-	-T-	-S-
Raritan				
ST	7.0	75	13	12
B (TC)	5.5	49	22	29
Sdlg.	4.1	17	29	54
Tribute				
ST	8.8	100	0	0
B (TC)	7.6	86	10	4
Sdlg.	7.3	71	29	0

[z]Rating classes; R = resistant (scores 7–9), T = tolerant (scores 4–6), S = susceptible (scores 1–3).

'Midland' selfed seedlings (susceptible standard check) gave a mean susceptible disease reaction score of 3.7, indicating the test was pathogenic.

Analysis of the reaction scores of 'Raritan' by ANOVA indicated the verticillium wilt resistance scores of the various propagation treatments were different only at the 10% level (Table 3). TC plants were intermediate between the disease reactions of the more resistant ST plants and the more susceptible Sdlg. population. These results were similar to the *P. fragariae* disease reaction score of TC A_{15} treatment plants grown in the greenhouse for a similar length of time. Mean disease reaction scores of 'Tribute' plants and seedlings were similar, and all fell into the resistant (R) class.

The cultivars and selections included in the *Verticillium* screen as checks indicated that the test inoculum was pathogenic. 'Cardinal' was more resistant than expected, perhaps due to the unexpectedly mild virulence of the *V. albo-atrum* isolate used. *Verticillium* was isolated from plants of cultivars classified as tolerant or susceptible. However, the presence of *Verticillium* in a plant did not necessarily result in detectable disease symptoms.

These results further characterize a temporary change in TC-propagated strawberries. Eventually, TC plants should revert to the ST or normal clonal reaction, since the runners used here were from TC plants grown 3 seasons in a screenhouse. Although disease reaction scores and growth habits (6, 9, 12) are similar to juvenile plants, TC plants must be considered adult, since they can flower under the proper inductive conditions.

The biochemical, anatomical, or related changes in TC strawberry roots which might affect changes in disease resistance were not investigated. Such changes may be nonspecific, since the pathogen-plant interaction is different for each fungus (5).

The altered disease reaction over time (Table 2) indicates that changes in disease susceptibility induced by TC were unlikely to be of permanent genetic origin, i.e., mutations. No readily observable or disease reaction variant plants were detected; however, the pathogens used may not have been virulent enough to detect disease reaction variants, i.e., survivors in a lethal test.

No significant differences in disease reaction were found between TC 'Raritan' plants proliferated by the commercial method (A) and by the immediate exposure to high BA method (B). These results suggest the time of application and amount of BA during in vitro culture were not important to plant disease susceptibility. BA level in vitro has no effect on runnering ability in the field (6).

TC plants of susceptible cultivars should not be grown on soils that are, or possibly can become, infested with these diseases. As commercial nurseries are primarily located on "disease-free" soils, the use of TC for foundation plant production is, in terms of disease resistance, without much added risk.

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