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Inheritance of Resistance to Race 2 Anthracnose in Watermelon[1]

Kamolvan Suvanprakorn and J. D. Norton[2]
Auburn University Agricultural Experiment Station, Auburn, AL 36830

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Abstract. **Inheritance of resistance to *Colletotrichum lagenarium* (Pass.) Ell. & Halst. race 2 in watermelon (*Citrullus lanatus* (Thunb.) Matsum & Nakai) was determined in progeny from crosses between resistant plant introductions (PIs) 189225, 271778, and 326515 and susceptible cultivars 'Charleston Gray', 'Jubilee', 'Crimson Sweet' and AWB-10 advanced line. Parents and progenies were screened for resistance in field and greenhouse plantings. Resistance of F_1 plants indicated resistance was dominant. The F_2 plants segregated 3 resistant: 1 susceptible. The backcross of the F_1 to the susceptible parent segregated 1:1. Resistance in all PIs tested was controlled by a single dominate gene pair.**

Anthracnose caused by *Colletotrichum lagenarium* is a relatively common and widespread disease on the foliage and fruit of watermelon in humid regions. Due to susceptibility of cultivars, damage has been a limiting factor in commercial production of watermelons in the southeastern U.S. Chemical controls are costly and sometimes ineffective during periods of high humidity and rainfall.

According to Winstead et al. (10) the first anthracnose resistant varieties to find wide acceptance were 'Congo', 'Fairfax', and 'Charleston Gray' released by Andrus in 1949. In 1956 Goode (2) observed severe anthracnose on 'Charleston Gray' watermelon at 4 locations in North Carolina. Later (3) he described the race pathogenic on 'Charleston Gray' as race 2. Good and Winstead (4) reported 3 pathogenic groups and in 1958 Goode (3) designated these as 3 physiologic races in *C. lagenarium.* Races of anthracnose were identified on cucurbits by differential host reaction (1). Race 2 of *C. lagenarium* has developed and spread throughout the southeastern production area. Winstead et al. (10) found that an African citron line W-695 segregated for resistance to race 2 but today, 20 years after resistance was reported in W-695, disease still causes severe damage in some fields. In 1959, Winstead et al. (10) recognized that the *Ar* gene confers resistance to race 1 and 3 in watermelon.

In 1964, Jenkins et al. (6) reported that there are 7 races of *C. lagenarium.* Winstead et al. (10) evaluated the resistance to *C. lagenarium* race 1, 2, and 3 on 86 cultivars of watermelon and found that all cultivars resistant to race 1 were also resistant to race 3 and susceptible to race 2. The inheritance studies showed that resistance to race 1 and 3 is controlled by the same dominant gene (5, 10).

In 1980, Sowell et al. (9) reported that PI 189225, 271775 and 271778 were resistant to race 2 of *C. lagenarium* in 3 stages. PI 189225 and 271778 have the additional advantage also of carrying genes for resistance to gummy stem blight (7). In 1976, an additional source of resistance to *C. lagenarium* race 2 was found in PI 326515 which was also resistant to gummy stem blight and *Fusarium* wilt (unpublished.) The objective of the research reported here was to determine the mode of inheritance of resistance to race 2 anthracnose found in these plant introductions.

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[2]Graduate research Assistant and Professor, Department of Horticulture.

[3]Grover Sowell, Research Pathologist, S. E. Regional Plant Introduction Station, Experiment, GA, supplied spore slants of race 2 *C. lagenarium* for the screening studies.

Materials and Methods

Single plants resistant to *C. lagenarium* race 2 were selected from PI 189225, 271778, 326515, and AWB-1-AR 2 (PI 326515 × PI 271778), and used as parents in crosses with susceptible cultivars, 'Charleston Gray', 'Jubilee', 'Crimson Sweet' and AWB-10 advanced line in the inheritance study. Controlled pollinations necessary to obtain F_1, F_2, and backcross generations were made in the greenhouse.

Culture[3] and inoculation techniques used were developed by Sowell (8). Seedlings of all parents, F_1, F_2, and backcross progenies were evaluated under both greenhouse and field conditions. In the greenhouse screening, seedlings were grown in flats arranged in a completely randomized design with four replications for each entry. They were inoculated by spraying to run off with a suspension containing 50,000 spores per ml. Inoculated seedlings were placed in a dark humidity chamber at 25°C with 100% relative humidity for 48 hr before being moved to the greenhouse. Ratings for severity of anthracnose injury were made 21 days after inoculation.

In the field, seedlings were inoculated in the 2-leaf stage. Inoculation procedures were similar to those used in the greenhouse screening except that the plants were sprayed with inoculum in late afternoon to ensure high humidity for spore germination during the night. Plants were rated for severity of anthracnose injury 21 days after inoculation.

The severity of anthracnose injury was rated on a 0.5 scale where 0 = no injury, 1 = 0-20%, 2 = 21-40%, 3 = 41-60%, 4 = 61-80%, and 5 = 81-100% of leaf area dead. Plants rated 0, 1, and 2 were considered resistant and the others susceptible.

Results and Discussion

Parental reaction. PI 326515, 189225, 271778, and AWB-1-AR2 showed a high degree of resistance with a disease index rating of 0 both under greenhouse and field conditions (Table 1). The cotyledons of these cultivars exhibited little or no visual symptoms 21 days after inoculation.

Disease indices on commercial cultivars varied from 4-5. 'Charleston Gray' and 'Jubilee' had an index of 5, and developed large lesions causing severe symptoms under both greenhouse and field conditions; they were classified as highly susceptible. Lesions also developed on leaves on 'Crimson Sweet' and AWB-10, but fewer than in 'Charleston Gray' and 'Jubilee'. They were classified as susceptible. The degree of damage to watermelon seedlings was severe for all commercial susceptible cultivars of watermelon in the field.

F_1 generation. All of the F_1 seedlings were rated resistant to anthracnose with a disease index range from 0 to 1 (Table 2). A small number of lesions developed on some leaves after inoculation; however, the plants were classified as resistant. Reaction of the F_1 plants indicated that resistance in PI 189225, 271778, 326515 and AWB-1-AR2 was controlled by a dominant genetic factor.

Table 1. Distribution of anthracnose injury on watermelon parents in the greenhouse and field 21 days after inoculation with *Colletotrichum lagenarium* race 2.

Parents	Greenhouse							Field						
	No. of seedlings tested	Disease index[z]						No. of seedlings tested	Disease index[z]					
		Resistant			Susceptible				Resistant			Susceptible		
		0	1	2	3	4	5		0	1	2	3	4	5
PI 326515	40	40						38	38					
Charleston Gray	37						37	34						34
PI 189225	40	40						40	40					
Jubilee	34						34	31						31
AWB-1-AR2	40	40						40	40					
Crimson Sweet	38				4	34	36						1	35
PI 271778	39	39						37	37					
AWB-10	36				2	34	34						3	31

[z]Disease index: 0 = no injury, 1 = 0 – 20%, 2 = 21 – 40%, 3 = 41 – 60%, 4 = 61 – 80%, 5 = 81 – 100% of leaf area dead.

Table 2. Distribution of anthracnose injury on watermelon F_1 progeny in the greenhouse and field 21 days after inoculation with *Colletotrichum lagenarium* race 2.

Entry	Greenhouse							Field						
	No. of seedlings tested	Disease index[z]						No. of seedlings tested	Disease index[z]					
		Resistant			Susceptible				Resistant			Susceptible		
		0	1	2	3	4	5		0	1	2	3	4	5
PI 326515 × Charleston Gray	39	35	4					35	35					
Charleston Gray × PI 326515														
PI 189225 × Jubilee	38	38						36	34	2				
Jubilee × PI 189225	45	40	5					40	38	2				
AWB-1-AR2 × Crimson Sweet	40	40						38	35	3				
Crimson Sweet × AWB-1-AR2	37	37						34	32	2				
PI 271778 × AWB-10	40	40						38	38					
AWB-10 × PI 271778	37	35	2					36	34	2				

[z]Disease index; 0 = no injury, 1 = 0 – 20%, 2 = 21 – 40%, 3 = 41 – 60%, 4 = 61 – 80%, 5 = 81 – 100% of leaf area dead.

Table 3. Segregation for anthracnose reaction on watermelon F_2 progeny in the greenhouse 21 days after inoculation with *Colletotrichum lagenarium* race 2.

Entry	No. of seedlings tested	F_2 segregation		Expected ratio	x^2 value	P
		Resistant	Susceptible			
PI 326515 × Charleston Gray	78	59	19	3:1	0.02	0.90-0.80
PI 189225 × Jubilee	69	52	17	3:1	0.00	0.95-0.90
Jubilee × PI 189225	53	41	12	3:1	0.16	0.70-0.50
AWB-1-AR2 × Crimson Sweet	196	141	55	3:1	0.98	0.50-0.30
Crimson Sweet × AWB-1-AR2	84	61	23	3:1	0.25	0.70-0.50
PI 271778 × AWB-10	96	74	22	3:1	0.22	0.70-0.50
AWB-10 × PI 271778	81	58	23	3:1	0.50	0.50-0.30
Pooled	657	486	17	3:1	0.36	0.70-0.50

F_2 generation. Segregation of the F_2 population suggested a ratio of 3 resistant:1 susceptible (Tables 3 and 4). The response of the F_2 generation suggested that the resistance to anthracnose in all PIs tested was controlled by a single gene pair with resistance being dominant. Also the reciprocal hybrids under both the greenhouse and the field conditions segregated 3 resistant:1 susceptible. This further confirmed that the resistance in all PI's to anthracnose was controlled by a single dominant gene.

Backcross generation. The backcross of F_1's (resistant × susceptible) to resistant parents (PIs) were uniformly resistant to anthracnose (Tables 5 and 6). The F_1 backcrosses to the susceptible parents segregated in a ratio of 1 resistant:1 susceptible in both field and the greenhouse tests. This supports the theory that resistance in these PIs is controlled by a single dominant gene as indicated by F_2 data.

Conclusion

Results of the greenhouse and field screening tests confirmed the presence of resistance to *C. lagenarium* race 2 in all

Table 4. Segregation for anthracnose reaction on watermelon F_2 progeny in the field 21 days after inoculation with *Colletotrichum lagenarium* race 2.

Entry	No. of seedlings tested	F_2 segregation		Expected ratio	χ^2 value	P
		Resistant	Susceptible			
PI 326515 x Charleston Gray	73	57	16	3:1	0.37	0.70-0.50
PI 189225 x Jubilee	65	49	16	3:1	0.01	0.95-0.90
Jubilee x PI 189225	58	47	11	3:1	1.36	0.30-0.20
AWB-1-AR2 x Crimson Sweet	183	132	51	3:1	0.59	0.50-0.30
Crimson Sweet x AWB-1-AR2	76	55	21	3:1	0.28	0.70-0.50
PI 271778 x AWB-10	84	64	20	3:1	0.06	0.90-0.80
AWB-10 x PI 271778	75	58	17	3:1	0.22	0.70-0.50
Pooled	614	462	152	3:1	0.02	0.90-0.80

Table 5. Segregation of anthracnose reaction on watermelon backcross progeny in the greenhouse 21 days after inoculation with *Colletotrichum lagenarium* race 2.

Entry	No. of seedlings tested	Progeny segregation		Expected ratio	χ^2 value	P
		Resistant	Susceptible			
(PI 326515 x Charleston Gray) x PI 326515	56	54	2	1:0		
(PI 189225 x Jubilee) x PI 189225	64	64	–	1:0		
(Jubilee x PI 189225) x Jubilee	50	22	28	1:1	0.72	0.50-0.30
(AWB-1-AR2 x Crimson Sweet) x AWB-1-AR2	82	77	5	1:0		
(Crimson Sweet x AWB-1-AR2) x Crimson Sweet	52	25	27	1:1	0.08	0.80-0.70
(PI 271778 x AWB-10) x PI 271778	63	59	4	1:0		
(AWB-10 x PI 271778) x AWB-10	54	28	26	1:1	0.07	0.80-0.70
Pooled	156	75	81	1:1	0.23	0.70-0.50

Table 6. Segregation of anthracnose reaction on watermelon backcross progeny in the field 21 days after inoculation with *Colletotrichum lagenarium* race 2.

Entry	No. of seedlings tested	Progeny segregation		Expected ratio	χ^2 value	P
		Resistant	Susceptible			
(PI 326515 x Charleston Gray) x PI 326515	52	52	0	1:0		
(PI 189225 x Jubilee) x PI 189225	55	55	0	1:0		
(Jubilee x PI 189225) x Jubilee	61	31	30	1:1	0.02	0.90-0.80
(AWB-1-AR2 x Crimson Sweet) x AWB-1-AR2	61	58	3	1:0		
(Crimson Sweet x AWB-1-AR2) x Crimson Sweet	48	22	26	1:1	0.33	0.70-0.50
(PI 271778 x AWB-10) x PI 271778	39	37	2	1:0		
(AWB-10 x PI 271778) x AWB-10	46	24	22	1:1	0.09	0.80-0.70
Pooled	155	77	78	1:1	0.01	0.95-0.90

3 PIs tested. Resistance of all F_1 plants indicated that resistance was dominant. Segregation of the F_2 population into an approximate ratio of 3 resistant:1 susceptible indicated that resistance to *C. lagenarium* race 2 in all PIs was governed by a single dominant gene pair. This mode of inheritance of resistance to *C. lagenarium* race 2 was confirmed in the backcross progeny. The segregation of the backcross to the susceptible parents fit a 1:1 ratio which confirmed the results from the F_2 population studies. This conclusion was further supported by resistance of all plants in the backcross to the resistant parents.

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Effect of Chilling on Respiration and Volatiles of California Lemon Fruit

Irving L. Eaks
Department of Biochemistry, University of California, Riverside, CA 92521

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Abstract. **The respiration, ethylene production and ethylene, ethyl alcohol, and acetaldehyde content of the internal atmosphere of citrus fruit increased at 20°C following exposures to chilling temperatures (0° and 5°) compared with fruit placed directly at 20°C. The increases were greater the longer the exposure and greater following exposure to 0° than following exposure to 5°. Exposure to 12.8°, a nonchilling temperature, did not elicit a stimulation of these attributes when transferred to 20°. Ethylene, ethyl alcohol, and acetaldehyde in the internal atmosphere of fruit remained at the same levels during the chilling exposures. During storage at 12.8° the acetaldehyde content in the internal atmosphere increased, but the ethylene and ethyl alcohol content did not. The chilling injury sustained by citrus fruit during storage could be evaluated by transferring samples to 20° and determining the respiratory rate, ethylene production or the volatile content in the internal atmosphere 24 hours after transfer to 20°.**

Physiological disorders, referred to as chilling injury, occur in most fruits of tropical and subtropical origin when held at temperatures below 10°C. The literature on chilling injury is summarized in the excellent review by Lyons (6). Empirical observations on temperature tolerance, time-temperature responses and symptomology have been dilineated. Although the membrane lipid physical-phase change appears to be central to the physiological and biochemical disruption of metabolic activity (7, 11) the mechanism of chilling injury is not completely understood. As a result of this disruption, metabolites such as acetaldehyde and ethanol would be expected and have been reported to accumulate in fruit during chilling (2, 3, 6, 10). Stress such as holding in nitrogen also caused increases in the acetaldehyde and ethanol content of citrus fruit (8, 9).

The anomalous respiratory patterns of chilling-sensitive fruits at chilling temperature and the accelerated respiratory rates when transferred from chilling to a nonchilling temperatures have been reviewed (6). In addition, field chilling (1) and freezing field temperatures have increased the ethylene content of the internal atmosphere of citrus fruits (12).

Reported here are the respiratory rates and ethylene production at 20°C following various chilling exposures and the volatile content of the internal atmosphere during holding at chilling temperatures and after transferred to 20° for lemon fruits.

Materials and Methods

Light green lemon fruits (*Citrus limon* [L.] Burm. f. cv. Eureka) were harvested from Experiment Station trees and held at 10°C until the next day when the experiment was established. Fruits of uniform size and color were selected and randomly placed in the different treatments. Treatments consisted of samples placed directly at 20° and storage for 4, 8 and 12 weeks at 0°, 5° and 12.8° and then transferred to 20°. The recommended storage temperature for California lemons is 12.8°. Each treatment was replicated 6 times using individual fruit (6 fruit for respiration and ethylene production and 6 fruit for volatile determinations of the internal atmosphere). Each fruit was marked and weighed initially and at the time of transfer to 20°. Also, similar experiments were conducted with navel and 'Valencia' oranges.

Fruits for respiratory rate and ethylene production determinations were placed in respiratory chambers at 20°C. Humidified air, with the ethylene removed by passing through a column of Purafil and the CO_2 removed by bubbling through 2 N NaOH was metered through the respiratory chambers at 5 liters/hr by calibrated capillaries. CO_2 production of each fruit was determined by a calibrated Beckman infrared CO_2 analyzer equipped with a switching system to sequence the outlet gas from each fruit chamber through the analyzer. Data were taken from the chart every 6 hours for calculation of respiration as ml CO_2/kg-hr. Ethylene production was determined twice daily (8 AM and 4 PM) on 1 ml samples taken from the outlet of each respiratory chamber by a Varian Aerograph flame ionization gas chromatograph equipped with a 2 m × 3.2 mm column packed with 60-80 mesh activated alumina. At each sampling, the gas chromatograph was calibrated with 1 ml samples of a standard ethylene-nitrogen mixture.

The internal atmospheres were sampled by removing the button, inserting the needle of a 1 ml gas-tight syringe into the pithy central core of the fruit and withdrawing the plunger past the 1 ml mark. A rubber septum was placed on the needle to provide a seal between the fruit and the syringe. After the sample was taken the puncture was sealed with silicone stopcock grease. The effect of repeated sampling the same fruit vs. sampling different fruit after various storage treatments was evaluated in a preliminary experiment. The internal carbon dioxide, oxygen, and volatile concentrations were similar for both conditions. The area penetrated by the needle has a low metabolic activity thus, has essentially no effect on the physiology of the fruit. Therefore, the internal atmospheres of the 6 individual fruit designated for this purpose for each treatment were sampled just prior to transfer to 20°C, 7 hr after transfer and daily for 7 days.

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