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Potential for utilization of pepper germplasm with a variable reaction to *Sclerotium rolfsii* Sacc. to develop southern blight-resistant pepper (*Capsicum annuum* L.) cultivars

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Abstract

The bell-type pepper cultivar Golden California Wonder, a source of resistance to southern blight, was crossed with the susceptible cultivar Sweet Banana. The F_1, F_2 and backcross progenies of this cross and the parental lines were evaluated in an inoculated field study for their reactions to *Sclerotium rolfsii* Sacc. A categorical rating system was used to rate plants for severity of wilting and severity of stem-lesion development. The plants within all three non-segregating populations (the resistant parent, the susceptible parent and the F_1) displayed a range of reactions to *S. rolfsii*. Variable reactions to *S. rolfsii* among homogeneous pepper populations were expected because this phenomenon is well documented in the literature and we had observed similar responses in field trials conducted over multiple years. The high level of variation for reactions to *S. rolfsii* exhibited by the parental and F_1 hybrid populations and the categorical system of rating individual plants placed severe limitations on the effective use of any quantitative type of analysis. Accordingly, a weighting procedure was used to correct the F_2 and backcross populations based on frequencies of apparent mis-classifications in the parental and F_1 populations. Examination of the comparative frequency distributions of the parental and progeny populations and examination of the weighted segregation data suggest that the southern blight resistance in 'Golden California Wonder' is conditioned by a single recessive gene. The level of resistance conditioned by this reputed recessive gene appears high enough to recommend it for use in pepper breeding programmes.

Keywords: *Capsicum annuum*; disease resistance; pepper; *Sclerotium rolfsii*; southern blight

Introduction

Southern blight, incited by *Sclerotium rolfsii* Sacc., is a major disease of pepper (*Capsicum* spp.) in most of the tropical and subtropical regions of the world. Although *S. rolfsii* can infect any part of a susceptible pepper plant, it is best known by girdling stem lesions it causes near the soil line. Southern blight often causes extensive premature plant death in pepper plantings (Aycock, 1966). Methyl bromide gives adequate control of *S. rolfsii*, and this fumigant is widely used to treat soil in infested beds (Jenkins and Averre, 1986; Brown *et al.*, 1989). Chellemi (1998) noted that methyl bromide has been the primary pesticide used by Florida pepper and tomato growers in a single tactic approach for over 30 years. He suggests that this success with methyl bromide has limited development of alternative multiple pest

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control tactics for many of the soil-borne pests of these crops. The provisions of the Montreal Protocol will eventually lead to a complete phase out of methyl bromide use by US pepper growers (US Department of Agriculture, 2001). This pending loss of methyl bromide has created a need to develop alternative strategies for controlling southern blight in pepper plantings. Southern blight-resistant cultivars, if available, would provide a cost-effective and environmentally safe method for controlling this disease.

Although there have been several reports of resistance in pepper to southern blight, none of these reports are in the prime scientific literature and little has been published in recent decades (Fulton, 1908; Georgia Agricultural Experiment Station, 1940, 1941; Doolittle, 1953; Amato, 1964; Dempsey, 1976; Dukes and Fery, 1984). Punja (1985) reviewed the literature on the biology, ecology and control of *S. rolfsii*. He stated that an impressive volume of literature has been published on such topics as occurrence, distribution, and methods of control, but noted that 'we have gleaned little information on other very important aspects of the topic, such as the genetic control of pathogenicity or potential sources and mechanisms of resistance'. Aycock (1966) noted that all attempts to incorporate resistance into commercial pepper cultivars have been unsuccessful.

Pepper germplasm was evaluated in field trials at Charleston, South Carolina, for several seasons during the early 1980s to assess reactions to *S. rolfsii* (Dukes and Fery, 1984). This research confirmed that some of the pepper genotypes representing several *Capsicum* spp. identified by earlier researchers do exhibit potentially useful levels of resistance. Additionally, Dukes and Fery (1984) reported that the bell-type cultivar Golden California Wonder (*C. annuum* L.) was resistant. The availability of southern blight resistance in a bell-type pepper is of particular interest to plant breeders wanting to develop southern blight-resistant bell-type cultivars. The breeding value of any southern blight-resistant pepper germplasm would be enhanced greatly if the mode of inheritance was understood. Additionally, most modern bell pepper cultivars are hybrids and the utility of specific genes in development of such cultivars depends on the availability of detailed information about gene action under field conditions. These needs prompted us to assess, under field conditions, the mode of inheritance of the southern blight resistance exhibited by 'Golden California Wonder'.

Materials and methods

The data reported here are from a field study conducted at the US Vegetable Laboratory, Charleston, South Carolina. Seeds of all parental, F_1, F_2 and backcross populations of the cross 'Golden California Wonder' × 'Sweet Banana' were produced in the greenhouse using standard crossing and selfing procedures. 'Golden California Wonder' was released in 1948 by the Ferry-Morse Seed Company (Minges, 1972). It produces bell-type fruits that turn a chrome yellow colour when ripe. 'Sweet Banana' is a popular sweet pepper cultivar that was released by the Corneli Seed Company in 1940 (Minges, 1972). 'Golden California Wonder' has consistently exhibited a significant level of resistance and 'Sweet Banana' has consistently exhibited susceptibility to southern blight in repeated field tests at Charleston, South Carolina (P. D. Dukes and R. L. Fery, unpublished data). The severity of southern blight symptoms was assessed by assigning each plant subjective scores for severity of wilting and severity of stem-lesion development. The following scale was used to score wilting severity: 1 = no wilting, 2 = slight or partial wilting, 3 = general plant wilting, 4 = permanent wilt, 5 = dead plant. The following scale was used to score stem lesion severity: 1 = no stem lesion, 2 = small stem lesion (≤25% of the stem circumference), 3 = moderate stem lesion (26–50% of the stem circumference), 4 = large stem lesion (>50% of the stem circumference), 5 = dead plant (stem completely girdled).

The isolate of *S. rolfsii* used in this study was recovered from a field-grown pepper plant collected at Charleston, South Carolina, using locally prepared, acidified potato dextrose agar (A-PDA) medium. The isolate was subsequently maintained as dry sclerotia stored in glass vials at room temperature. Inoculum for the inheritance study was prepared using a four-step procedure. First, dry sclerotia were surface-sterilized (dipped in 95% ethanol and flamed), placed in the centre of A-PDA plates and incubated at 30°C until the resulting cultures produced new sclerotia. Second, 9 mm diameter agar plugs of A-PDA media containing both sclerotia and mycelium were used to seed 2.8-litre Fernbach culture flasks (10 plugs per flask) containing an autoclaved mixture of 1000 g of clean sharp sand (air dry), 200 g of yellow corn meal and 300 ml of distilled water. Third, Fernbach flask cultures were incubated at 30°C for 20 days (flasks shaken daily for the first 7 days). Fourth, the medium which contained high concentrations of newly developed, mature sclerotia was removed from the flasks and mixed with sufficient quantities of clean sharp sand to produce inoculum with the desired concentration of sclerotia. Individual plants were prepared for inoculation by removing top soil within ≈ 5 cm radius of the stem to a depth of ≈ 2 cm. A measuring spoon was then used to place one tablespoon (≈ 15 cm^3) of inoculum in direct contact with the entire circumference of the exposed stem.

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Finally, the inoculum placed around the base of each stem was lightly covered with top soil.

Six-week-old, greenhouse-produced, parental, F_1, F_2 and backcross plants were transplanted in the field on 30 May. A completely randomized experimental design was used, and each plot contained a single plant. The test contained 100 plots each of the two parental populations and the F_1 population, 200 plants each of the F_2 population and the $F_1 \times$ 'Golden California Wonder' backcross population, and 201 plants of the $F_1 \times$ 'Sweet Banana' backcross population. The plants (plots) were randomly assigned to sites located in the centre of raised beds. The beds were on 102 cm row centres and the plants on each bed were 91 cm apart. Each plant was inoculated with 24 sclerotia on 24 July, an additional 25 sclerotia on 31 July and a further 100 sclerotia on 21 August. Each plant was scored for degree of wilting on 9 September. On 19 September, each plant was removed from its planting site and the stem scored for degree of lesion development.

Preliminary evaluation of the data on southern blight symptom expression showed variation among plants in the non-segregating parental and F_1 populations. Variation in symptom expression occurred as expected, which created a problem in classification of individual plants accurately enough for genetic analysis. De Jong and Honma (1976) encountered a similar problem in accurately classifying tomato plants for reaction to *Corynebacterium michiganense*, and they used weighting procedures to correct the F_2 and backcross populations for mis-classifications in resistant parent and susceptible F_1 populations. We used the following equation to correct the F_2 population and the $F_1 \times$ 'Golden California Wonder' and $F_1 \times$ 'Sweet Banana' backcross populations for mis-classifications in the resistant parent population, the susceptible parent population and susceptible F_1 population:

$$R_w = R_o + (A/B \times C \times D) - (E/F \times G \times D) - (H/I \times J \times D)$$

where R_w = the weighted number of resistant plants in the segregating population; R_o = the observed number of resistant plants in the segregating population; A = the number of susceptible plants in the resistant parent population; B = the total number of plants in the resistant parent population; C = the expected frequency of plants with the resistant parent genotype in the segregating population; D = the total number of plants in the segregating population; E = the number of resistant plants in the susceptible parent population; F = the total number of plants in the susceptible parent population; G = the expected frequency of plants with the susceptible parent genotype in the segregating population; H = the number of resistant plants in the F_1 population; I = the total number of plants in the F_1 population; and J = the expected frequency of plants with the F_1 genotype in the segregating population. Chi-square tests for goodness-of-fit were used to test genetic hypotheses (weighted observed segregation versus expected segregation).

Results and discussion

The procedures used to evaluate pepper plants in this study for their reactions to *S. rolfsii* were not ideal for an inheritance study because the plants within all three non-segregating populations (the resistant parent, the susceptible parent and the F_1) displayed a range of reactions. However, this was not unexpected because we have seen similar responses in virtually all pepper cultigens evaluated in field trials conducted over multiple years. Aycock (1966) cited a substantial body of published *S. rolfsii* literature about 'the erratic distribution of diseased plants in the field'. He noted that 'it is characteristic that all plants even in a uniformly infested area do not become infected'. Aycock (1966) cited work by Higgins (1927) noting that the distribution of southern blight 'on pepper was quite unlike that of diseases caused by wilt *Fusaria* in which a high percentage of plants in an infested area succumb'. It is our opinion that the variable reactions to *S. rolfsii* observed in our parental and F_1 populations were not due to genetically heterogenous plant material, but to complex interactions of environmental phenomena beyond the scope of the current study.

Our efforts to evaluate the parental and progeny populations using two types of disease-rating criteria were successful. However, average wilt scores for the evaluated populations were lower than average stem lesion scores because not all plants with large stem lesions exhibited plant wilting symptoms that are customarily associated with southern blight. Average wilt and stem lesion scores of the 'Golden California Wonder', 'Sweet Banana', F_1, F_2, $F_1 \times$ 'Golden California Wonder' and $F_1 \times$ 'Sweet Banana' populations were 1.06 versus 1.75, 3.69 versus 4.40, 2.53 versus 3.82, 2.69 versus 3.50, 1.87 versus 2.78, and 3.49 versus 4.07, respectively. Both sets of data were subjected to genetic analyses and the analyses yielded the same results. For the sake of brevity, only the genetic analysis of the stem lesion data will be presented.

Examination of the comparative frequency distributions of observed stem lesion reactions of the 'Golden California Wonder', 'Sweet Banana', F_1, F_2, $F_1 \times$ 'Golden California Wonder' and $F_1 \times$ 'Sweet Banana' populations illustrates several aspects of the genetic system conditioning southern blight resistance in 'Golden California Wonder' (Fig. 1). First, the majority of plants in the 'Golden California Wonder' population exhibit a resistant reaction (stem lesion score ≤3). Second, the majority of the plants in the 'Sweet Banana' population exhibit a susceptible reaction (stem lesion score ≧ 4). Third, the majority of plants in the F_1 population exhibited a susceptible reaction, suggesting that southern blight resistance is inherited as a recessive trait. Fourth, distribution frequencies in the F_2 population and $F_1 \times$ 'Golden California Wonder' backcross population appear to be bimodal, suggesting southern blight resistance is conditioned by a qualitative rather than a quantitative genetic system. Examination of weighted segregation data suggests that southern blight resistance in 'Golden California Wonder' is conditioned by a single recessive gene (Table 1). The F_2 population segregated a ratio of one resistant to three susceptible, the $F_1 \times$ 'Golden California Wonder' backcross population segregated one resistant to three susceptible and all but eight of the 201 plants in the $F_1 \times$ 'Sweet Banana' backcross population were susceptible.

The high level of variation for reactions to *S. rolfsii* exhibited by the parental and F_1 hybrid populations used in this study and the categorical system of rating individual plants placed severe limitations on the effective use of any quantitative type of analysis. Our rationale for using a weighting procedure to correct the segregating F_2 and backcross populations using the mis-classification frequencies observed in the resistant parent population, the susceptible parent population and the susceptible F_1 population is based on the following assumptions: (i) the underlying genetic system conditioning resistance is qualitative rather than quantitative in nature, (ii) the parental populations are not heterogeneous for resistance or susceptibility to *S. rolfsii*, and (iii) the homozygous resistant and susceptible genotypes and heterozygous F_1 genotype respond in the segregating populations in the same manner as in the non-segregating parental and F_1 populations. The weighting procedure used in this study corrected intentional mis-classification of plants among F_2 and backcross populations in a set of theoretical genetic data in which resistance is conditioned by a single recessive gene. This mis-classification correction strategy could be advantageous for evaluation of inheritance in other biological systems where mis-classification is a difficult problem.

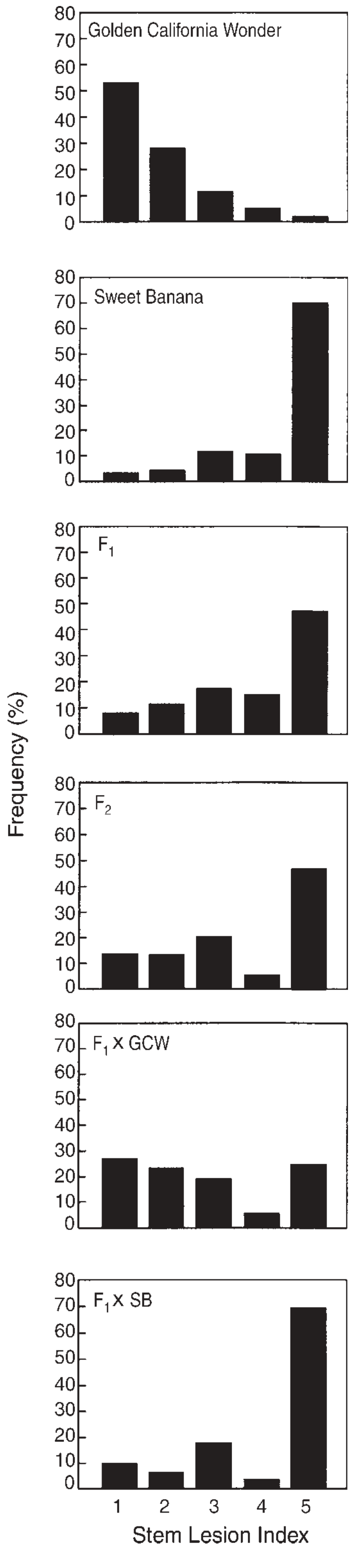

Fig. 1. Comparative frequency distributions of reactions (1 = no stem lesion; 5 = dead plant or stem completely girdled) of field-grown plants from the parental, F_1, F_2 and backcross populations of the cross 'Golden California Wonder' × 'Sweet Banana' inoculated with *Sclerotium rolfsii*.

Conclusions

This study suggests that a single recessive gene conditions southern blight resistance in 'Golden California Wonder'. The level of resistance conditioned by this reputed gene appears high enough to recommend it for use in pepper

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Table 1. Segregation for resistance to southern blight (*Sclerotium rolfsii*) in parental, F_1, F_2 and backcross populations of the cross 'Golden California Wonder' × 'Sweet Banana'

	Observed segregation				Expected segregation				
	Unweighted		Weighted		No. of plants				
Population	R[a]	S[b]	R	S	R	S	Expected ratio (R:S)	χ^2	*P*
Golden California Wonder (P_1)	89	7			96	0	1:0		
Sweet Banana (P_2)	18	76			0	94	0:1		
F_1	32	54			0	86	0:1		
F_2	86	95	47	134	45	136	1:3	0.12	0.80–0.50
$F_1 \times P_1$	134	59	105	88	97	96	1:1	1.33	0.50–0.20
$F_1 \times P_2$	65	136	8	193	0	201	0:1		

[a] R, number of resistant plants (stem lesion index ≤3).
[b] S, number of susceptible plants (stem lesion index ≧4).

breeding programmes. The availability of this reputed gene in a bell-type pepper background should make the development of southern blight-resistant bell pepper cultivars an achievable objective. However, it should be noted that this reputed gene is rather difficult to work with using traditional plant breeding methods and plant pathology evaluation procedures because it is hard to accurately classify the reaction of pepper plants that have been inoculated with *S. rolfsii*. Since most modern bell pepper cultivars are F_1 hybrids, the use of this reputed recessive gene will require its incorporation into both parental lines. The development of a molecular marker for this reputed gene would be of considerable value to bell pepper breeding programmes utilizing marker-assisted selection methodology.

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