Temperature Sensitivity of the Hypersensitive Response of Bell Pepper to *Xanthomonas axonopodis* pv. *vesicatoria*

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ABSTRACT

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When bacterial spot-resistant pepper plants carrying resistance gene *Bs2* and infiltrated with incompatible strains of *Xanthomonas axonopodis* pv. *vesicatoria* carrying a functional *avrBs2* gene (races P1 and P3) were incubated at 32°C, they exhibited an electrolyte leakage and bacterial multiplication pattern in planta similar to that obtained with a compatible strain (race P4) carrying a nonfunctional *avrBs2* gene. They also de-

veloped disease-like symptoms. Pretreatment of incompatible bacteria at 32°C before infiltration caused a delay in electrolyte leakage less pronounced than that caused by exposing plants to 32°C. Also, plants had to be exposed to 32°C for an hour prior to inoculation to increase symptom expression. These data suggest that the *Bs2* gene is temperature sensitive. In other experiments, the *avrBs1-Bs1* interaction appeared to be the most heat tolerant and thus the least likely to revert to compatible, whereas the *avrBs3-Bs3* interaction had an intermediate sensitivity to elevated temperatures.

Bacterial spot, caused by *Xanthomonas axonopodis* pv. *vesicatoria* (= *X. campestris* pv. *vesicatoria* A group) and *X. vesicatoria* (= *X. campestris* pv. *vesicatoria* B group) (27), is a serious disease of pepper (*Capsicum annuum* L.) worldwide (21). The disease reduces fruit yield and quality. Management strategies include the use of disease-free seed and seedlings, resistant cultivars, and copper sprays. However, these strategies are not always effective, especially when environmental conditions are optimal for disease or inoculum levels are high. The presence of copper-resistant strains (13,16) and host-differentiated races complicates disease management. Currently, commercially used plant resistance to bacterial spot is determined by gene-for-gene interactions between host resistance (R) genes and pathogen avirulence (*avr*) genes (14). To date, 11 races that infect pepper (designed P0 to P10) have been described (10,14,17,20) based on the hypersensitive reaction (HR) on three near-isogenic lines of Early Calwonder (ECW), each carrying a single gene for resistance (*Bs1*, *Bs2*, or *Bs3*) and on *C. pubescens* PI 235047 (20). Races P1 and P3 are the most prevalent worldwide (1,2,12,18,19). Pepper cultivars and hybrids with one to three R genes have been developed and are used commercially. Cultivars carrying *Bs2* were considered the most promising because they confer resistance to the most common races (i.e., P1, P2, and P3) of this pathogen. The *Bs2* gene also was considered to be a durable gene, because most strains carry the matching avirulence gene, *avrBs2* (8), and because inactivation of *avrBs2* reduces the multiplication rate of some strains under laboratory conditions (25). Under field conditions, however, strains carrying nonfunctional *avrBs2* remain aggressive (11). Also, race shifts in the field can defeat major R genes, as occurred in Florida commercial pepper fields recently (15).

Since late 1994, strains of the bacterial spot pathogen have been isolated from lesions on pepper cultivars expected to be resistant to races P0 to P3 (carry resistance gene *Bs2*). Bacteria isolated from such lesions often proved to be strains of races 1, 2, and 3.

Possibly, leaf tissue water soaking delayed the establishment of an HR, as described by Cook and Stall (4). Also, some R genes are temperature sensitive and nonfunctional above 31°C in bell pepper (26) and other crops (9,28). Preliminary results reported previously suggest that this may be the case with R gene *Bs2* (18).

Our objective was to investigate the effect of temperature on expression of resistance to *X. axonopodis* pv. *vesicatoria* in pepper, in particular that mediated by the *avrBs2-Bs2* interaction.

MATERIALS AND METHODS

Pepper genotypes. ECW and three near-isogenic lines of ECW, each carrying a unique set of major resistance genes, were used (Table 1). ECW-13R and ECW-123R carry multiple R genes, allowing the comparison of temperature sensitivity of different R gene-*avr* gene interactions in a similar host genetic background. One of the resistant commercial hybrids from which avirulent strains were isolated frequently, X3R Camelot (carries *Bs2*), also was included in the study of symptom expression.

Bacterial strains. Strains of the pathogen causing bacterial spot on pepper and tomato (*Lycopersicon esculentum* Mill.) are composed of two genetic groups: *X. axonopodis* pv. *vesicatoria*, representing strains previously known as the A group of *X. campestris* pv. *vesicatoria* (A1, nonamylytic; A2, amylytic), and *X. vesicatoria*, representing the B group (strongly amylytic) (7,22,27). To increase the scope of the results of our experiments, several compatible and incompatible strains corresponding to different races and genetic groups (A1 and A2) of *X. axonopodis* pv. *vesicatoria* were used to test the *avrBs2-Bs2* interaction at different temperatures, as well as other *avr-R* gene interactions (Table 1). Long-term storage of bacteria was in 20% glycerol at -80°C. Bacteria were grown on modified yeast dextrose calcium carbonate agar (YDC) or modified sucrose peptone agar (SPA) (16) for 2 days at 28°C for use in experiments.

Heat predisposition requirements. Plants of ECW-20R were exposed to 32°C for 0, 1, 6, or 24 h before inoculation. These plants were inoculated with an incompatible strain, Xcv 135 (*avrBs2*). Inoculation was done by submersion for 20 s in the bacterial suspension ($\approx 10^5$ CFU/ml) with 0.04% Silwet L-77, as

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described previously (11), using four plants per predisposition time. After inoculation, plants were maintained at 32°C in the growth chamber for 48 h; to ensure a saturated humidity, plants were kept in plastic bags with a thermometer in each bag to periodically monitor temperature. A set of plants not exposed to 32°C was inoculated, bagged, and maintained under laboratory conditions (23 to 25°C). After 48 h, all the bags were removed and the plants were moved to the greenhouse. Five days after inoculation, the number of leaves with symptoms and the number of necrotic lesions per plant were counted.

Temperature effect on expression of symptoms. A set of ECW and ECW-123R plants was placed in a growth chamber at 32°C for 1 h before and 48 h after inoculation. A similar set of plants was maintained under laboratory conditions (23 to 25°C) for the same period of time. All plants were inoculated by submersing the top third of the plant for 20 s in a bacterial suspension ($\approx 10^5$ CFU/ml) of Xcv 135, Xcv 293, Xcv 314, or Xcv 259 (Table 1) containing 0.01% of Silwet L-77. Except for strain Xcv 259, each carries a single functional *avr* gene corresponding to one of the R genes carried by ECW-123R, thus allowing the evaluation of each R gene-*avr* gene interaction in the same host genetic background. Two plants per bacterial strain, pepper genotype, and growth condition were used. After inoculation, the plants were maintained for 48 h in the growth chamber or on a bench in the lab in clear plastic bags to ensure a saturated humidity, with a thermometer inside each bag to determine that the desired temperature was maintained. The bags were removed after 48 h and all plants were taken to the greenhouse for symptom development and evaluation.

In a second experiment, X3R Camelot (carries *Bs2*) plants were inoculated by submersing plants in a bacterial suspension as described previously. The inoculum consisted of the incompatible strains Xcv 135 or Xcv 136 (both carry *avrBs2*) or the compatible strain Xcv 181. Six inoculated plants per strain were maintained in the laboratory at 23 to 25°C or in a growth chamber at 32°C for 48 h in clear plastic bags. After removing the bags, the plants were moved to and kept in the greenhouse for symptom development and evaluation.

Symptom evaluation. Following the initial observation of symptoms, plants were evaluated every 3 to 4 days for a total of five evaluations, using a 0-to-9 rating scale (11), where 0 = no diseased leaves, 1 = <10% leaf area diseased, 5 = 41 to 50% leaf area diseased or defoliated, and 9 = complete defoliation and plant dying or dead. The areas under the disease progress curve were calculated as previously described (3). Analysis of variance was performed using the general linear models procedure of SAS (version 6.12), and means were compared using Fisher's protected least significant difference ($\alpha = 0.05$) (SAS Institute, Cary, NC).

Hours preheat treatment at 32°C

Fig. 1. A, Proportion of leaves with necrotic lesions and B, mean number of lesions per leaf of Early Calwonder (ECW)-20R (*Bs2*) plants inoculated with an incompatible strain of *Xanthomonas axonopodis* pv. *vesicatoria*, Xcv 135 (*avrBs2*). Plants were preheat treated at 32°C for 0, 1, 6, or 24 h before inoculation, and thereafter at 32°C in a growth chamber for 48 h, or were maintained as controls under laboratory conditions at 23 to 25°C and never exposed to 32°C.

TABLE 1. *Xanthomonas axonopodis* pv. *vesicatoria* strains and reaction on the pepper genotypes used in the experiments

Strains ^a	Pepper genotypes ^b				
	ECW (none)	ECW-123R (<i>Bs1</i> , <i>Bs2</i> , <i>Bs3</i>)	ECW-13R (<i>Bs1</i> , <i>Bs3</i>)	ECW-20R (<i>Bs2</i>)	X3R-Camelot (<i>Bs2</i>)
Race 1 (<i>avrBs2</i> , <i>avrBs3</i>)					
Xcv 79	C	HR	HR	HR	HR
Race 3 (<i>avrBs2</i>)					
Xcv 135 ^c	C	HR	C	HR	HR
Xcv 136	C	HR	C	HR	HR
Race 4 (<i>avrBs3</i>)					
Xcv 181	C	HR	HR	C	C
Xcv 314	C	HR	HR	C	C
Race 5 (<i>avrBs1</i>)					
Xcv 293	C	HR	HR	C	C
Race 6 (none)					
Xcv 259	C	C	C	C	C

^a Avirulence genes in parentheses.

^b ECW = Early Calwonder, resistance genes in parentheses; C = compatible and HR = hypersensitive reaction.

^c A2 subgroup (amyolytic); all remaining strains were A1 subgroup (nonamyolytic).

Effect of temperature on electrolyte leakage and bacterial multiplication. Using a needleless syringe, different areas of the intercostal leaf tissue of ECW-123R plants were infiltrated with compatible strain Xcv 259 and incompatible strains Xcv 135, Xcv 293, and Xcv 314 to compare the effects of temperature on different R gene-*avr* gene interactions. Using ECW-123R, which carries all three resistance genes, allows comparison of the interaction of each *avr* gene with its corresponding R gene in the same host genetic background (Table 1). The plants were placed in a growth chamber at 23, 29 or 32°C for 1 h prior to and for 48 h after infiltration, under full spectrum Verilux fluorescent lights. Electrolyte leakage was determined as described by Hibberd et al. (6), using a bacterial suspension of 10^8 CFU/ml.

To extend the observations to other bacterial strains, races and pathogen genetic subgroups, a second experiment was conducted. ECW-20R plants were infiltrated with a compatible strain (Xcv 181) and three incompatible strains (Xcv 79, Xcv 135, and Xcv 136) carrying the functional *avrBs2* gene and representing races 1 and 3, respectively (Table 1). Electrolyte leakage was determined as described in the previous experiment. Plants were placed in a growth chamber at 25 or 32°C for 3 h before inoculation and maintained at these temperatures for 48 h.

To determine the effect that heat pretreatment of the bacteria alone may have on the electrolyte leakage of infiltrated plants, strains Xcv 259, Xcv 135, Xcv 293, and Xcv 314 were grown at 32°C for 48 h and used as inoculum. Bacterial strains were infiltrated (10^8 CFU/ml) into the intercostal leaf tissue of ECW-123R plants maintained at 23 to 25°C in the laboratory and electrolyte leakage of infiltrated tissue was measured.

Multiplication of bacteria in planta was determined in plants of ECW-20R using compatible (Xcv 181) and incompatible (Xcv 79, Xcv 135, and Xcv 136) strains infiltrated at an initial concentration of 10^4 CFU/ml. These plants were kept in the growth chamber at 25 or 32°C for 3 h before inoculation, and maintained at these temperatures for up to 11 days.

RESULTS

Heat predisposition requirement. ECW-20R plants exposed to 32°C for 1 h or more before inoculation had significantly more symptomatic leaves than plants that had not been pre-exposed to 32°C (Fig. 1A). The maximum number of lesions per plant was observed when the plants were exposed to 32°C for 1 h before inoculation, then declined when exposure was for 6 h or more (Fig. 1B).

Fig. 2. Leaves of pepper plants, which carry resistance gene *Bs2*, inoculated with a bacterial strain carrying a functional *avrBs2* gene. **A**, Small, necrotic flecks (indicated by arrows) presumed to be the result of the hypersensitive response when plants were incubated at 23 to 25°C for 48 h after inoculation. **B**, Necrotic, disease-like lesions produced when plants were incubated at 32°C for 48 h following inoculation.

Effect of temperature on symptom expression. Inoculation of pepper plants carrying resistance gene *Bs2* with avirulent strains of *X. axonopodis* pv. *vesicatoria* carrying a functional *avrBs2* gene elicited necrotic, fleck-like lesions (less than 1 mm in width) at temperatures less than 32°C but necrotic, disease-like lesions 3 to 5 mm in width at 32°C or greater (Fig. 2). ECW plants inoculated with compatible strains expressed bacterial spot symptoms 10 days after inoculation. Symptoms were more severe on ECW plants exposed to 32°C for 48 h after inoculation than on plants

Fig. 3. Foliar bacterial spot severity, measured by area under disease progress curve, as affected by maintaining plants at 25 or 32°C for 48 h following inoculation. Plants of **A**, Early Calwonder (ECW) (carries no major resistance [R] genes) **B**, ECW-123R (carries *Bs1*, *Bs2*, and *Bs3*), and **C**, commercial cv. X3R Camelot (carries *Bs2*) were inoculated by dipping the top third of plants in a bacterial suspension (10^5 CFU/ml). The strains of *Xanthomonas axonopodis* pv. *vesicatoria* avirulent on ECW-123R are Xcv 135 (*avrBs2*), Xcv 293 (*avrBs1*), and Xcv 314 (*avrBs3*); Xcv 135 is also avirulent on X3R Camelot, but Xcv 181 is virulent.

maintained in laboratory conditions (23 to 25°C) for the same time period (Fig. 3A). Bacterial spot symptoms also were more severe on ECW-123R plants inoculated with a compatible strain (Xcv 259, race P6) and exposed to 32°C after inoculation than on plants maintained at 23 to 25°C. As expected, resistant plants of ECW-123R inoculated with incompatible strains and maintained at 23 to 25°C expressed only a few necrotic, fleck-like lesions presumed to result from the HR. The severity of these flecks was similar with all incompatible strains tested (Xcv 293, *avrBs1*; Xcv 314, *avrBs3*; and Xcv 135, *avrBs2*; Fig. 3B). The expression of flecks was not altered on ECW-123R plants exposed to 32°C compared with plants maintained at 23 to 25°C for 48 h after inoculation, except for those plants inoculated with strain Xcv 135 (Fig. 3B). ECW-123R plants inoculated with avirulent strain Xcv 135 (carries *avrBs2*) and exposed to 32°C for 48 h after inoculation developed necrotic, disease-like lesions. The severity of the disease-like lesions that developed at 32°C was significantly

greater than the severity of the flecks that developed on the plants maintained at 23 to 25°C (Fig. 2).

Similar results were observed on the commercial hybrid X3R Camelot (carries *Bs2*). Inoculations with incompatible strains (Xcv 135 and Xcv 136, both carrying functional *avrBs2*) elicited only flecks at 23 to 25°C, but the same strains caused disease-like lesions with a severity similar to the compatible strain (Xcv 181) at 32°C (Fig. 3C).

Effect of temperature on electrolyte leakage and bacterial multiplication. At 23 and 29°C, Xcv 293 and Xcv 135 caused a sudden increase in the electrolyte leakage in ECW-123R within 12 h of infiltration (*avrBs1-Bs1* and *avrBs2-Bs2* interactions, respectively; Fig. 4A and B), with visible tissue collapse. In contrast, the leakage caused by strain Xcv 314 was delayed (Fig. 4C), characteristic of the *avrBs3-Bs3* interaction (6), with HR-like tissue collapse visible 24 h after infiltration. The leakage elicited by strain Xcv 259 corresponded to a compatible interaction at all

Fig. 4. Electrolyte leakage in plants of Early Calwonder (ECW)-123R (carries resistance [R] genes *Bs1*, *Bs2*, *Bs3*) during compatible and incompatible interactions at three different temperatures. Leaves were infiltrated with three avirulent strains of *Xanthomonas axonopodis* pv. *vesicatoria*: A, Xcv 293, *avrBs1*; B, Xcv 135, *avrBs2*; and C, Xcv 314, *avrBs3*; and a virulent strain, D, Xcv 259 (10^8 CFU/ml), and maintained in the growth chamber at 23, 29, or 32°C. Also, bacteria of each strain were grown for 48 h at 32°C prior to infiltration into leaves of the different genotypes and then plants were incubated at 23 to 25°C for 48 h. Vertical bars at data points represent standard errors.

three temperatures tested (Fig. 4D). At 32°C, the electrolyte leakage for the incompatible strains did not reach maximum conductivity values attained at 23 and 29°C, for the duration of the experiments. However, as expected for the *avrBs1-Bs1* interaction, strain Xcv 293 elicited an increase in conductivity and visible tissue collapse 12 h after infiltration in ECW-123R plants (Fig. 4A). Xcv 314 elicited a slow increase in electrolyte leakage at 32°C on ECW-123R plants (*avrBs3-Bs3*), although the shape of the conductivity curve resembled that at lower temperatures (Fig. 4C). Xcv 135 also elicited a slow increase in electrolyte leakage on ECW-123R plants (*avrBs2-Bs2* interaction) at 32°C although, in this case, with an exponential type of curve as opposed to the characteristic logistic shape at the lower temperatures (Fig. 4B).

The electrolyte leakage in ECW-123R plants maintained at 23 to 25°C and infiltrated with bacteria grown at 32°C for 48 h prior to infiltration was delayed for all incompatible *avr-R* gene interactions tested compared with the incompatible strains grown at 28°C prior to infiltration (Fig. 4A to C). The electrolyte leakage pattern for the compatible strain, Xcv 259, was similar to that when the strain was grown at 28°C (Fig. 4D). Maximum electrolyte leakage values obtained 48 h after infiltration were similar to those attained for bacteria grown at 28°C and infiltrated into plants maintained at 23 or 29°C (Fig. 4).

A similar delay in electrolyte leakage for plants at 32°C was observed with other strains that also carry a functional *avrBs2*. At 25°C, the electrolyte leakage patterns of strains Xcv 79, 135, and 136 (incompatible strains) on ECW-20R were similar and characteristic of an HR, while the pattern for Xcv 181 was characteristic of a compatible interaction (Fig. 5A). At 32°C, the HR was delayed and all strains tested showed an electrolyte leakage pattern typical of a compatible interaction (Fig. 5B).

Fig. 5. Electrolyte leakage in plants of Early Calwonder (ECW)-20R (carries resistance [R] gene *Bs2*) during compatible and incompatible interactions at conducive (25°C) and nonconductive (32°C) temperatures for eliciting the hypersensitive reaction. Leaves were infiltrated with a virulent (Xcv 181) and three avirulent (Xcv 79, Xcv 135, and Xcv 136 all carry active *avrBs2*) strains of representing races 1 (Xcv 79) and 3 (Xcv 135 and Xcv 136) and the A1 subgroup (Xcv 136) and A2 subgroup (Xcv 135) of *Xanthomonas axonopodis* pv. *vesicatoria* (10⁸ CFU/ml), and maintained in the growth chambers at **A**, 25°C or **B**, 32°C. Vertical bars at the data points represent standard errors.

At 25°C and beginning 4 days after infiltration, the multiplication of the compatible strain Xcv 181 was always consistently greater than the incompatible strains tested (Fig. 6A). At 32°C, the multiplication of compatible and incompatible strains was similar (Fig. 6B).

DISCUSSION

Since the mid-1990s, we have isolated several race P1, P2, and P3 strains (have functional *avrBs2*) from diseased plants in the field that reportedly carry the R gene *Bs2*. Although these plants should be resistant to these races, they exhibited large lesions, resembling a compatible interaction, followed by intense defoliation. Possibly, excessive rainfall or prolonged periods of high humidity in the field maintained watersoaked conditions in the plant tissue. Such conditions are known to delay the development of an HR by allowing an increase in bacterial population size within the lesions (4) and preventing direct bacterial contact with plant cells,

Fig. 6. Effect of temperature on bacterial multiplication of three avirulent (Xcv 79, carries functional *avrBs2* and *avrBs3*; Xcv 135 and Xcv 136 both carry functional *avrBs2*) strains of *Xanthomonas axonopodis* pv. *vesicatoria* and a virulent (Xcv 181, carries functional *avrBs3*) strain in commercial pepper cv. X3R Camelot (carries resistance [R] gene *Bs2*), maintained at **A**, 23 to 25°C or **B**, 32°C. The bacterial suspension was infiltrated at a concentration of 10⁴ CFU/ml. Vertical bars at the data points represent standard errors.

which apparently is necessary for the HR to develop (23). Alternatively (or in addition), the *avrBs2*–*Bs2* interaction may be temperature sensitive, as is the resistance to the root-knot nematode, *Meloidogyne incognita*, conditioned by the N gene in bell peppers (26). Temperature-sensitive resistance interactions also have been reported in other pathosystems, such as some specific *Pseudomonas syringae* pv. *glycinea*–soybean interactions (9), the *M. incognita*–tomato interaction (28), and resistance to Tobacco mosaic virus conferred by the N gene in tobacco (5).

As expected, resistant plants inoculated by submersion of the top third of the plant in a suspension of an incompatible strain carrying the corresponding functional *avr* gene expressed only a few necrotic flecks at 23 to 25°C (Fig. 2A). Symptom expression was not altered when the *avrBs1*–*Bs1* and *avrBs3*–*Bs3* interactions were tested at 32°C. Plants of different genetic backgrounds carrying the R gene *Bs2* (X3R Camelot, ECW-20R, and ECW-123R) and inoculated with strains carrying a functional *avrBs2* gene expressed necrotic and watersoaked symptoms that resembled those of the compatible reaction when incubated at 32°C (Fig. 2B). Similar symptom observations were made in other experiments using plants carrying single R genes individually (ECW-10R, ECW-20R, and ECW-30R) and inoculated with the corresponding avirulent strain. Development of an HR, the electrolyte leakage pattern, and bacterial multiplication curves in plants carrying *Bs2* (ECW-20R and ECW-123R) infiltrated with strains carrying a functional *avrBs2* gene were similar to that of an incompatible interaction when plants were maintained at 23 to 25°C (Figs. 4, 5, and 6). In contrast, a compatible interaction occurred when plants similarly treated were maintained at 32°C. Electrolyte leakage was delayed for all avirulent strains tested in ECW-123R plants maintained at 32°C. This delay was more pronounced for the *avrBs2*–*Bs2* interaction than for the *avrBs1*–*Bs1* interaction. This is surprising because both cause rapid incompatible reactions, eliciting a visible HR within 24 h of infiltration under normal laboratory conditions (24). The increase in conductivity was also delayed for the *avrBs3*–*Bs3* interaction, although not so dramatically, consistent with its typically slower HR development. HR evaluations for this interaction are normally done 30 to 36 h after infiltration. A possible effect of temperature on development of an HR was also noticed by Stall and Minsavage (24), who recommended evaluation of the *avrBs1*–*Bs1* interaction at 30°C, but at 26 to 28°C for the *avrBs2*–*Bs2* and *avrBs3*–*Bs3* interactions.

The results presented here suggest that resistance gene *Bs2* is influenced by temperatures near to or greater than 32°C, allowing strains of pathogen races that carry a functional *avrBs2* (P1, P2, and P3) that should be incompatible to behave more as compatible races, thus causing disease on what is expected to be a resistant plant. It was necessary for plants to be exposed to 32°C for at least 1 h before inoculation to increase significantly the expression of symptoms, which suggests that some process or processes associated with R gene *Bs2* is altered in the plant at this or higher temperatures (Fig. 2). The reduction in number of lesions per leaf when plants were exposed to 32°C for 6 h or more before inoculation suggests that some type of general defense mechanism or mechanisms may be activated at elevated temperatures (Fig. 1B). Pretreatment of bacteria by growing them at 32°C for 48 h prior to infiltration caused a delay in electrolyte leakage when cultured in plants exposed to 23 to 25°C, but this delay was less pronounced than that caused by exposing plants to 32°C, suggesting that the effect of elevated temperature is on the resistance gene *Bs2* rather than the *avrBs2* gene. It is not possible to maintain the bacteria at 32°C once infiltrated into the plant without also maintaining the plant at 32°C. Thus, it is difficult to draw a definitive conclusion to separate these two possibilities. In the gene–gene interaction, disruption of the R gene, *avr* gene, their products, or their interaction culminates in the same result: defeat of the host resistance.

The delay in electrolyte leakage, increase in multiplication of bacteria in planta, and expression of water-soaked symptoms on bell pepper plants carrying R gene *Bs2* exposed to 32°C was shown to occur in three pepper genotypes. These genotypes are presumed to represent two different genetic backgrounds (two near-isogenic lines of ECW, ECW-20R and ECW-123R, and the commercial cv. X3R Camelot), and were inoculated with different strains, races, and genetic subgroups of *X. axonopodis* pv. *vesicatoria* carrying a functional *avrBs2*. The *avrBs1*–*Bs1* interaction seems to be the most heat tolerant, whereas the *avrBs3*–*Bs3* interaction has an intermediate sensitivity to temperatures when compared with the *avrBs2*–*Bs2* interaction. The results of these experiments also illustrate how environmental conditions can influence the defeat of host resistance to pathogens without any known genetic changes occurring in the pathogen.

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