Calcium Nutrition Affects Resistance of Tomato Seedlings to Bacterial Wilt

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Abstract. The relationship between Ca nutrition and bacterial wilt development was studied on three tomato (*Lycopersicon esculentum* Mill.) cultivar seedlings with various degrees of resistance to the disease. Seedlings were transplanted into nutrient solutions with Ca at 0.4, 4.4, or 20.4 mM. One week after initiating the Ca treatment, tomato seedlings were inoculated by wounding the stem with scissors dipped in a suspension of the pathogen (*Pseudomonas solanacearum* E.F. Smith). Disease development was rapid in 'Ponderosa' (a susceptible cultivar) at all Ca concentrations. Increased Ca concentration in the nutrient solution reduced disease severity in 'Zuiei' (a moderately resistant cultivar). Resistance was negated at the low Ca concentration in 'Hawaii 7998' (a highly resistant cultivar). Pathogen populations in stems decreased with increased Ca concentrations and degrees of resistance.

Bacterial wilt of vegetable crops incited by *Pseudomonas solanacearum* is a serious disease in tropical and subtropical, humid regions of the world (Hayward, 1991). In Japan, this soilborne disease is a problem in tomato and eggplant (*Solanum melongena* L.) production. Although growers in Japan avoid this disease by grafting susceptible scions onto resistant rootstocks (Lee, 1994), recently, the disease has occurred even on grafted plants.

Like other soilborne diseases, various strategies have been developed for controlling bacterial wilt, but most have proven ineffective. The most widely accepted and promising strategy is breeding resistant cultivars. Although many resistant tomato cultivars have been developed, their resistance fluctuates due to environmental factors, and their resistance mechanisms are unknown (Hayward, 1991).

Increased Ca content in plant tissues has been associated with increased resistance to some diseases (Bateman and Lumsden, 1965; Conway et al., 1992; Corden, 1965; Forster and Echandi, 1975; Volpin and Elad, 1991). Our objective was to examine the relationship between Ca nutrition and resistance to bacterial wilt of tomato seedlings.

Materials and Methods

Three tomato cultivars with a range of bacterial wilt resistance were selected: the susceptible 'Ponderosa', the moderately resistant 'Zuiei', and the highly resistant 'Hawaii 7998'. Seeds were sown and seedlings were grown in a mixture of 1 vermiculite : 1 perlite

(v/v) in a greenhouse set at a 15C minimum and a 25C maximum and receiving natural light. Beginning 1 week after sowing, seedlings were fertilized twice weekly with a solution (in mg·liter⁻¹) of 113 N, 31 P, and 158 K (Hi-Melt, Diachemical, Tokyo). At the two- or three-leaf stage, seedling roots were washed gently in water, and then two plants were transplanted into a plastic pot containing 3 liters of the fertilizing solution. The seedlings were supported with polyurethane foam and a plastic mesh and were grown in a growth chamber maintained at 25/18C day/night (12-h daylength), 60% relative humidity, and 320 μmol·m⁻²·s⁻¹ (photosynthetic photon flux). After 1 week of pre-culture, seedlings of each cultivar were separated into three groups, and three Ca concentrations (0, 4, and 20 mM, representing low, medium, and high concentrations, respectively) were applied. The nutrient solution for the medium Ca concentration consisted of the following elements: macronutrients (in mM): N, 14 [4.0 KNO₃, 4.0 Ca(NO₃)₂, and 2.0 NH₄H₂PO₄]; P, 2.0 (NH₄H₂PO₄); K,

4.0 (KNO₃); Ca, 4.0 [Ca(NO₃)₂]; and Mg, 2.0 (MgSO₄); and micronutrients (in mg·liter⁻¹): Fe, 3.0 (Fe-EDTA); Mn, 0.5 (MnSO₄); B, 0.5 (H₃BO₃); Zn, 0.05 (ZnSO₄); Mo, 0.05 [(NH₄)₆Mo₇O₂₄]; and Cu, 0.02 (CuSO₄). For the low-Ca concentration, Ca(NO₃)₂ in the solution was replaced with 8.0 mM NaNO₃, and for the high-Ca concentration, the solution was adjusted with 16 mM CaCl₂. Although the high- and low-Ca solutions contained extra Na or Cl ions, we confirmed in a preliminary experiment that excess Na and Cl did not affect bacterial wilt development. Tap water containing 0.4 mM Ca increased the Ca concentration by 0.4 mM in each nutrient solution; thus, the actual concentrations were 0.4, 4.4, and 20.4 mM. The pH of all solutions was adjusted to 5.8 with 1 M NaOH, and the solutions were changed weekly. Treatments were arranged as a 3 (Ca) × 3 (cultivar) factorial with nine double-plant replicates in a completely randomized design.

Pseudomonas solanacearum strain MAFF03-01487, confirmed to be highly virulent on tomato, was used in this study. A single colony of the virulent, fluidal type was grown at 30C for 48 h on TZC medium (Kelman, 1954). The inoculum was cultivated on CPG liquid medium (Kelman, 1954) by shaking in a water bath at 32C for 24 h. After incubation, the suspension population was counted with a bacterial counting chamber and adjusted to 10⁸ cells/ml.

One week after initiating the Ca treatment, six tomato seedlings (three pots) per treatment were randomly selected, cut at the base of their stems, separated into leaves and stems, and oven-dried at 65C. After measuring dry weight, three samples (leaf and stem), each of which consisted of two seedlings, were ground with a ball mill and wet-digested in HNO₃ and HClO₄. Calcium, K, and Mg concentrations then were determined with an atomic absorption spectrophotometer (model AA-670; Shimadzu, Tokyo). The data were tested by analysis of variance and regression.

The remaining seedlings (12 plants for each treatment) were inoculated by a modified scissors-cut method (French, 1986). Upper

Table 1. Effects of Ca concentration in nutrient solutions on shoot dry weight and leaf and stem Ca, K, and Mg concentrations of three tomato cultivar seedlings.^z

Variable	Shoot dry wt (g) ^x	Nutrient concn (% dry wt) ^y					
		Leaf			Stem		
		Ca	K	Mg	Ca	K	Mg
Concn (mM)							
0.4	2.14	1.03	4.52	1.32	0.34	9.12	0.53
4.4	2.36	2.86	4.97	0.77	1.47	10.0	0.55
20.4	2.36	3.99	4.92	0.43	2.17	10.5	0.38
Significance ^w	NS	L**	NS	L**	L*	NS	L**
Cultivar							
Ponderosa	2.28	2.35	4.82	0.76	1.23	10.5	0.42
Zuiei	2.39	2.60	5.03	0.82	1.27	10.0	0.51
Hawaii 7998	2.18	2.94	4.57	0.93	1.49	9.15	0.53
LSD _{0.05}	NS	0.19	0.35	0.07	0.18	1.02	0.04
Significance	NS	**	*	**	*	*	**

^zTomato seedlings were collected 1 week after Ca treatment began.

^yValues are means of three observations, each consisting of two seedlings.

^xValues are means of six observations.

^wL = linear.

NS, *, **Nonsignificant or significant at $P \leq 0.05$ or 0.01, respectively.

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stems (3 to 4 mm in diameter and 20 to 25 cm above their bases) were cut off with scissors that had been dipped in the bacterial suspension. Temperature in the growth chamber was changed to 28/20C day/night to stimulate disease development. Disease severity on leaves of the lower portion of the plant was visually recorded on a scale of 0 to 4 (0 = healthy and 4 = dead) every other day for 20 days after the stems were cut.

Effect of Ca nutrition on pathogen multiplication in stems was determined in a second experiment. Ten seedlings per treatment were grown and inoculated as previously described. Five days after inoculation, three seedlings per treatment were randomly sampled and three stem segments from an inoculation site (each 5 cm long) were taken from each seedling. Each segment was washed and cut into small pieces with a razor blade, and 1 g of each sample was incubated with gentle shaking for 1 h in 99 ml sterile distilled water. *Pseudomonas solanacearum* populations in stem extracts were counted in each sample by plating on a selective medium (Hara and Ono, 1984) that contained the following antibiotics (in mg·liter⁻¹): 25 triphenyltetrazolium chloride, 5 crystal violet, 50 cycloheximide, 50 polymyxin B sulfate, and 10 chloramphenicol. Bacterial counts, performed after growing at 30C for 48 h, were expressed as colony forming units per gram of fresh weight, and the data were log-transformed and tested by analysis of variance and regression.

Results and Discussion

Seedling growth was slightly suppressed at the low-Ca concentration, but not significantly (Table 1). No disorder symptoms were visible during the experiment. One week after Ca treatment, leaf and stem Ca concentrations were significantly correlated with the Ca concentrations in the nutrient solution. Potassium content was not significantly affected, but Mg content was negatively correlated with the Ca concentrations (Table 1). Calcium, K, and Mg contents were significantly different among the cultivars tested. Highly resistant 'Hawaii 7998' contained relatively higher Ca and Mg concentrations (Table 1). Calcium concentrations and cultivars did not interact.

Seedlings of 'Ponderosa', the susceptible cultivar, began to wilt 4 days after inoculation, and the disease progressed rapidly. All plants died 2 weeks after inoculation at all Ca concentrations (Fig. 1A). However, the Ca concentration in the nutrient solution markedly influenced disease severity for the moderately resistant cultivar Zuiei. Disease development on seedlings with low Ca was apparently stimulated; however, no disease symptoms on plants with high Ca were present until 18 days after inoculation (Fig. 1B). For the highly resistant 'Hawaii 7998', disease symptoms were lacking on seedlings treated with medium and high Ca during the experiment, but seedlings treated with low Ca were severely wilted 20 days after inoculation (Fig. 1C).

Five days after inoculation, pathogen populations were negatively correlated with Ca

concentrations and were significantly different among cultivars in all parts of stem segments, according to degree of resistance (Table 2). Interactions between Ca concentrations and cultivars existed in two of three parts of the stem segments.

Development of bacterial wilt was markedly affected by Ca nutrition, especially in the resistant tomato cultivar (Fig. 1). The de-

creased disease development might be due to decreased Mg content in the plant tissues (Table 1). Three MgSO₄ (0, 2, and 10 mM) and two NaCl (0 and 32 mM) levels in the nutrient solution did not affect disease development in a preliminary experiment (data not shown). Therefore, it seems that Ca nutrition is important for resistance to bacterial wilt in tomato. Many investigators have reported that in-

Fig. 1. Effects of Ca concentration [(in mM) ○, 0.4; ●, 4.4; and △, 20.4] in nutrient solutions on the development of bacterial wilt on three tomato cultivars: (A) 'Ponderosa' (susceptible), (B) 'Zuiei' (moderately resistant), and (C) 'Hawaii 7998' (highly resistant). Mean disease indices were determined with 12 seedlings, scoring from 0 = healthy to 4 = dead. SE are given as vertical bars.

creased Ca content in plant tissues induces resistance to some diseases and have shown some possible mechanisms: direct polygalacturonase inhibition by Ca and other pectolytic enzymes produced by the pathogen (Corden, 1965), increased resistance in cell walls strengthened by Ca to these enzymes (Bateman and Lumsden, 1965; Conway et al., 1988), inhibition of ethylene production by Ca (Raz and Fluhr, 1992; Volpin and Elad, 1991), or all of these mechanisms. *Pseudomonas solanacearum* produces cell-wall degrad-

ing enzymes in the hosts (Husain and Kelman, 1958). However, the events that occur in this host-pathogen relationship remain unknown.

Pathogen multiplication in stems is suppressed in resistant cultivars, but *P. solanacearum* was viable even in symptomless resistant hosts (Table 2). Similar results were reported in other studies (Bowman and Sequeira, 1982; Grimault and Prior, 1993) and were explained in terms of "tolerance" to the pathogen. Results from this experiment indicate that Ca nutrition also may affect this tolerance and that high Ca suppresses pathogen multiplication, resulting in a suppression of disease development in resistant tomato cultivars.

As previously described, resistance to bacterial wilt and pathogen multiplication are affected by the Ca nutrition of the host. These results may contribute to the establishment of new integrated control practices of bacterial wilt in tomatoes. However, the mechanisms are unknown and further studies are necessary for their elucidation.

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Table 2. Effects of Ca concentration in nutrient solution on *Pseudomonas solanacearum* populations in stem segments.^z

Variable	Populations (Log cfu/g fresh wt) ^y		
	Parts of stem segment (cm) ^x		
	0-5	5-10	10-15
Concn (mM)			
0.4	9.0	8.0	7.1
4.4	8.8	7.6	7.2
20.4	8.2	6.8	5.8
Significance ^w	L*	L*	L*
Cultivar (Cv)			
Ponderosa	9.5	8.6	7.6
Zuiei	9.0	7.7	6.9
Hawaii 7998	7.5	6.2	5.6
LSD _{0.05}	0.4	0.5	0.4
Significance	**	**	**
Cv × Ca			
Significance	*	NS	**

^zStem segments were collected 5 days after inoculation.

^yValues are means of three observations.

^xParts of stem segments are shown as distance from inoculation sites.

^wL = linear.

NS, *, ** Nonsignificant or significant at $P \leq 0.05$ or 0.01, respectively.