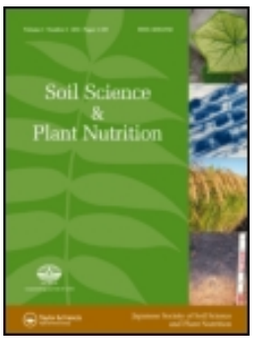

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Calcium Uptake and Resistance to Bacterial Wilt of Mutually Grafted Tomato Seedlings

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Bacterial wilt of tomato (*Lycopersicon esculentum* Mill.) caused by *Ralstonia solanacearum* Smith is a serious disease in Japan. We previously reported that calcium (Ca) nutrition in tomato significantly affected the resistance to the disease, and that highly resistant cultivars were characterized by a high Ca uptake. We examined the relationship between the Ca uptake and resistance using mutually grafted seedlings of tomato cultivars differing in their resistance. A susceptible ('Ponderosa') or moderately resistant ('Zuiei') cultivar (scion) was grafted on the rootstock of a susceptible, moderately resistant, or highly resistant cultivar ('Hawaii 7998'). Roots or petioles of the grafted seedlings were inoculated with the pathogen, and the development of bacterial wilt was observed. Although Ca uptake by shoot increased by grafting on the rootstock of a highly resistant cultivar, the development of the disease was not influenced by the difference in Ca uptake, and depended on the resistance of the cultivar to which the inoculated part of the graft belonged. It was concluded that the differences in Ca uptake of the shoot of the grafted tomato seedlings might not be related to the expression of the resistance to bacterial wilt.

Key Words: **bacterial wilt, calcium, *Lycopersicon esculentum*, *Ralstonia solanacearum*, tomato.**

Bacterial wilt of vegetable crops induced by *Ralstonia solanacearum* is a serious disease in tropical, subtropical, and warm temperate regions of the world (Hayward 1991). In Japan, this soil-borne disease is a constraint on tomato production, and seedlings grafted on the rootstocks of resistant varieties are widely used to control the disease (Lee 1994; Oda 1995). However, the resistance of the rootstocks is unstable (Hayward 1991), and the scion grafted on the rootstock of a highly resistant cultivar has been found to be latently infected with the pathogen (Nakaho et al. 1996). Actually, the disease has recently been found to occur even on grafted plants.

We previously reported that Ca nutrition in tomato significantly affected the resistance to the disease (Yamazaki and Hoshina 1995), and that highly resistant cultivars were characterized by a high Ca uptake capacity (Yamazaki et al. 1996). Similar results have been reported in the tomato-bacterial canker interaction (Berry et al. 1988). These results suggest that there is a close relation between the Ca-dependent resistance and the high Ca uptake in resistant cultivars. However, this relation has not been fully elucidated in detail.

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To reveal the role of the Ca uptake in the resistance of tomato to bacterial wilt, we examined the relationship between the Ca uptake and the resistance of mutually grafted seedlings of tomato cultivars differing in resistance.

Materials and methods

Three tomato cultivars differing in bacterial wilt resistance were selected: the susceptible 'Ponderosa,' the moderately resistant 'Zuiei,' and the highly resistant 'Hawaii 7998.' Seeds were sown and the seedlings were grown in a 1:1 mixture of vermiculite and perlite (v/v) in a greenhouse set at a minimum temperature of 15°C and a maximum temperature of 25°C under natural light. At the four- or five-leaf stage, seedlings of the three cultivars were mutually grafted at the level above the cotyledon by the slant-cut method (Oda 1995). After acclimation, grafted seedlings were transplanted into plastic pots, each filled with 1 kg of commercial soil containing 0.4 g N, 1.9 g P_2O_5, and 0.6 g K_2O (Engei-Baido, Kureha Chemical, Tokyo). After transplanting, the growth of the scion of a highly resistant cultivar ('Hawaii 7998') was very poor. Therefore, the highly resistant cultivar was not used as a scion in this experiment. As a result, six combinations of grafted seedlings, consisting of two scions (susceptible and moderately resistant cultivars) and three rootstocks (susceptible, moderately resistant, and highly resistant cultivars), were used in this study.

Ralstonia solanacearum strain MAFF03-01487, confirmed to be highly virulent to tomato, was used. A single colony of the virulent type was grown at 30°C for 48 h on TZC medium (Kelman 1954). The inoculum was cultured on CPG liquid medium (Kelman 1954) by shaking in a water bath at 32°C for 24 h. After incubation, the population in the suspension was adjusted to 10^8 cfu mL^{-1} by measuring the optical density ($OD_{600\ nm}=0.1$ corresponding to 10^8 cfu mL^{-1}).

One week after transplanting, in ten grafted seedlings of each combination the roots of the rootstocks or petioles of the scions were inoculated with the bacterial suspension. For root inoculation, 10 mL of the suspension was poured into the soil gap after the roots were cut by inserting a knife into the soil near the plant. For the petiole inoculation, the petiole of the third leaf from the top was inoculated by cutting with scissors that had been dipped in the bacterial suspension. After the inoculation, the grafted seedlings were grown in a phytotron maintained under the following conditions: 28/22°C day/night (12 h day length) regime, 60% relative humidity, and 320 μmol m^{-2} s^{-1} (photosynthetic photon flux). Disease severity was visually evaluated and recorded on a scale of 0 to 4 (0: healthy and 4: dead) every other day for 20 d after inoculation.

Four days after the inoculation, four non-inoculated seedlings of each combination were cut at the base of their stems, and oven-dried at 65°C. After measurement of the dry weight, samples were ground with a ball mill and subjected to wet-digestion in HNO_3 and $HClO_4$. The concentrations of Ca, K, and Mg were determined with an atomic absorption spectrophotometer (model AA-670, Shimadzu, Tokyo). The data were subjected to an analysis of variance.

Twenty days after the inoculation, latent infection of the scion grafted on the rootstock inoculated with the pathogen was determined. Stem segments (each 5 cm long) were collected from the scions without any symptoms at 20 cm above the ground, and were surface-sterilized with 70% ethanol. A cross-section of each segment was stamped on a TZC medium after cutting with a sterile razor blade, and the plates were incubated at 30°C for 48 h. The pathogen causing latent infection was detected based on the colony appearance on the plate (Kelman 1954).

Results

The growth of the non-inoculated grafted seedlings was not significantly different among the grafting combinations (Table 1). The concentration of Ca of the scion was significantly high when the scion was grafted on the rootstock of a highly resistant cultivar, resulting in a significant increase in the amount of accumulated Ca per shoot. The concentration of K in the shoot (scion) was not significantly affected by the resistance of the cultivar used as the rootstock, while the concentration of Mg in the shoot grafted on the rootstock of a highly resistant cultivar was higher than that in the shoot grafted on the rootstock of a susceptible one. The concentrations and the amounts of Ca, K, and Mg accumulated in the shoot did not vary with the cultivar used as the scion.

The symptoms of bacterial wilt in the grafted seedlings inoculated at either the level of roots or petioles depended on the resistance of the cultivar to which the inoculated part of the graft belonged (Figs. 1 and 2). Among the root-inoculated seedlings, the disease was mild in those grafted on the rootstock of a resistant cultivar and was severe in those grafted on a susceptible cultivar (Fig. 1). Among the seedlings inoculated at the level of the petioles, the disease was severe in the scions of the susceptible cultivars irrespective of the resistance of the cultivar used as the rootstock (Fig. 2). The resistance of the cultivar used as the rootstock did not affect the disease severity.

Twenty days after the inoculation, the scions grafted on the rootstocks inoculated with the pathogen were examined in terms of disease symptoms and latent infection. The percentage of diseased scions decreased with the increase of the resistance of the cultivar used as the rootstock (Table 2). However, all the symptomless scions tested were latently infected

Table 1. Shoot dry weight, and concentration and accumulation of Ca, K, and Mg in shoots of non-inoculated grafted tomato seedlings.

Cultivar		Shoot dry wt. (g)	Nutrient conc. (mg g^{-1})			Nutrient accumulation (mg $shoot^{-1}$)		
Scion	Rootstock		Ca	K	Mg	Ca	K	Mg
Ponderosa	Ponderosa	3.23	15.1	66.0	3.93	48.9	212	12.7
Ponderosa	Zuiei	3.19	15.3	66.1	4.35	49.0	210	14.0
Ponderosa	Hawaii 7998	3.07	20.1	66.6	4.63	60.7	202	14.2
Zuiei	Ponderosa	3.13	15.0	66.2	3.99	46.6	206	12.4
Zuiei	Zuiei	2.96	16.4	70.2	4.21	48.3	209	12.5
Zuiei	Hawaii 7998	3.50	19.1	69.0	4.58	66.4	240	16.0
Variable[a]								
Scion								
Ponderosa (S)		3.16	16.9	66.2	4.30	52.9	208	13.6
Zuiei (MR)		3.19	16.8	68.5	4.26	53.8	218	13.6
Significance		NS	NS	NS	NS	NS	NS	NS
Rootstock								
Ponderosa (S)		3.18	15.1 b[b]	66.1	3.96 b	47.8 b	209	12.6
Zuiei (MR)		3.08	15.9 b	68.2	4.28 ab	48.7 b	210	13.3
Hawaii 7998 (HR)		3.29	19.6 a	67.8	4.61 a	63.6 a	221	15.1
Significance		NS	**	NS	*	**	NS	NS
Interaction								
Significance		NS	NS	NS	NS	NS	NS	NS

[a] S, susceptible; MR, moderately resistant; HR, highly resistant. [b] Mean separation within columns by Tukey's method of multiple comparisons, $\alpha = 0.05$. NS, *, **: nonsignificant, significant at $p < 0.05$, or significant at $p < 0.01$, respectively.

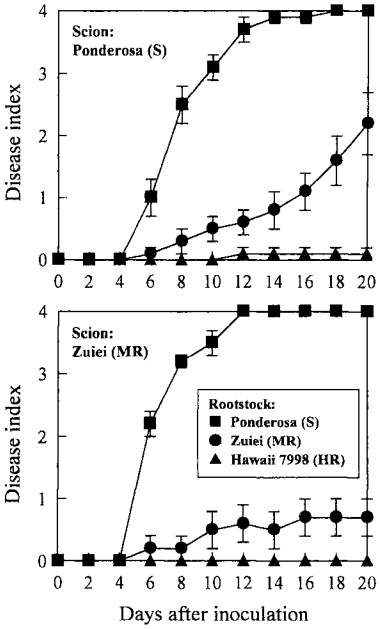

Fig. 1. Development of bacterial wilt on mutually grafted tomato seedlings by root inoculation with *Ralstonia solanacearum*. Scions from susceptible (upper) and moderately resistant (lower) cultivars were grafted on the rootstocks from susceptible (■), moderately resistant (●), and highly resistant (▲) cultivars. Mean disease indices were determined for 10 seedlings, scoring from 0: healthy to 4: dead. Standard errors are represented as vertical bars.

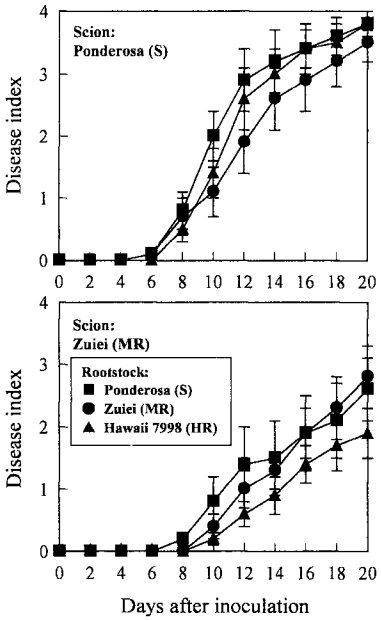

Fig. 2. Development of bacterial wilt on mutually grafted tomato seedlings by inoculation of the petioles of the scions with *Ralstonia solanacearum*. Scions from susceptible (upper) and moderately resistant (lower) cultivars were grafted on the rootstocks from susceptible (■), moderately resistant (●), and highly resistant (▲) cultivars. Mean disease indices were determined for 10 seedlings, scoring from 0: healthy to 4: dead. Standard errors are represented as vertical bars.

Table 2. Percentage of diseased or latently infected scions of mutually grafted tomato seedlings inoculated with *Ralstonia solanacearum* at their roots.

Cultivar[a]		Diseased scions (%)	Latently infected scions (%)	Percentage of total infection of scions (%)
Rootstock	Scion			
Ponderosa (S)	Ponderosa (S)	100	0	100
Ponderosa (S)	Zuiei (MR)	100	0	100
Zuiei (MR)	Ponderosa (S)	80	20	100
Zuiei (MR)	Zuiei (MR)	30	70	100
Hawaii 7998 (HR)	Ponderosa (S)	10	90	100
Hawaii 7998 (HR)	Zuiei (MR)	0	100	100

[a] S, susceptible; MR, moderately resistant; HR, highly resistant.

with the pathogen. All the colonies that appeared on the plates were of the virulent type.

Discussion

The concentration and the accumulation of Ca in the shoots of the grafted tomato

seedlings were comparable to those in the non-grafted seedlings (Yamazaki et al. 1996). The shoots of the seedlings grafted on a highly resistant cultivar contained a large amount of Ca (Table 1). Varietal difference in Ca uptake in tomato has been reported (English and Maynard 1981), and has been attributed to the difference in the efficiency of Ca utilization or ability to absorb Ca (Giordano et al. 1982). In this study, high Ca uptake capacity of highly resistant cultivars (Yamazaki et al. 1996) was reflected in the high Ca content in the shoots grafted on the highly resistant rootstock, i.e., the roots of the highly resistant cultivar supplied more Ca to the scions. The high Ca uptake capacity might be due to differences in the morphological characteristics of roots or efficiency of Ca uptake mechanisms (Gerloff and Gabelman 1983).

In this study, we used two inoculation methods, root or petiole inoculation. The resistance of the rootstock or the scion of the grafted seedlings could be estimated by these methods, respectively. Moreover, in the case of petiole inoculation, the differences in the resistance due to the nutritional status of the shoot could be revealed.

When the roots of the grafted seedlings were inoculated, the development of the disease depended on the resistance of the cultivar used as the rootstock (Fig. 1). However, multiplication of the pathogen in the roots did not vary with the resistance of the cultivar at an early stage of infection, and the resistance to the disease depended on the degree of multiplication of the pathogen in the shoots and/or migration to the shoots (Grimault et al. 1993; Yamazaki and Hoshina 1995; Nakaho 1997). Thus, the resistance of the grafted seedlings, in the case of root infection, might be due to the suppression of multiplication and migration from roots to scions of the pathogen.

In the petiole-inoculated seedlings, disease development depended on the resistance of the cultivar used as the scion, and not on that of the cultivar used as the rootstock (Fig. 2). Although the scion grafted on the rootstock of a highly resistant cultivar contained a large amount of Ca (Table 1), disease development was not related to the high Ca uptake. Although the resistance of tomato seedlings to bacterial wilt was enhanced by a high Ca status (Yamazaki and Hoshina 1995), these results suggest that the high Ca uptake by the shoots grafted on the rootstock of a highly resistant cultivar was not related to the expression of the resistance to bacterial wilt.

All of the scions grafted on the inoculated rootstock either developed the disease or were latently infected with the pathogen (Table 2). A high rate of latent infection in the tomato seedlings of resistant cultivars or those grafted on a rootstock of a resistant cultivar had been observed in other studies (Grimault et al. 1993; Nakaho et al. 1996). These results indicate that the factor(s) related to the suppression of the multiplication and migration of the pathogen are present in shoots of resistant cultivars or in grafted scions. However, the factor(s) related to the resistance of tomato to bacterial wilt remain unknown. Further studies are necessary to elucidate the mechanisms of Ca-dependent resistance to the disease.

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