Resistance to Two Races of *Meloidogyne incognita* and Resistance Mechanism in Diploid *Ipomoea trifida*

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The southern root-knot nematode, *Meloidogyne incognita* is a noxious and widespread plant-parasitic nematode, that causes serious damage to sweet potato, *Ipomoea batatas* storage root both in quality and yields (Clark and Moyer 1988). In this crop, therefore, many resistant cultivars have been developed to deal with this destructive pest. One such cultivar, ‘Minamiyutaka’ was developed using a wild hexaploid accession (K123-11) can be crossed with sweet potato and is highly resistant to nematode (Ono et al. 1977). Tokui et al. (1992) examined the mode of inheritance of the resistance to an *M. incognita* population in diploid *I. trifida*. However, in *M. incognita*, several races with different pathogenicity to sweet potato have recently been identified (Sano et al. 2002). It is thus necessary to evaluate the resistance of *I. trifida* to these pathogenic races and clarify the mechanism of the resistance. In the present study, the resistance of diploid *I. trifida* to two major races of *M. incognita* was evaluated and the histological responses occurring in resistant and susceptible plants were examined.

Two major races of *M. incognita*, SP1 and SP2, both widely prevalent in Kyushu, Japan, were used in this study. The latter race reproduces on a differential sweet potato host, ‘Norin No. 2’, unlike the former (Sano et al. 2002). These two races were raised from an egg-mass and maintained on tomato (cv. Pritz) at a temperature of 25°C. Egg-masses produced on the root systems of the tomato plant were extracted and second stage juvenile (J2) worms, freshly hatched in tap water at 25°C, were used for inoculation. The *F₁* plants were produced from two original (Mexico, Tehuantepec) resistant diploid accessions (4FR15-3 × 4FR18-1), and these populations were crossed again to a sensitive clonal line (G5-6) (unpublished). Seeds from seven cross combinations were provided from Dr. I. Shiotani. The nineteen plants (Fig. 1) from 7 cross combinations of *I. trifida* (diploid) were evaluated for the above two races. Sweet potato cultivars ‘Kokei No.14’ and ‘Minamiyutaka’ were used as susceptible and resistant controls, respectively. Each of the plants was rooted *in vitro* using a culture medium of MS (Murashige and Skoog 1962) for all plants and the cultivars were transplanted into a pot with 200 g of sterilized soil. After five days in a growth chamber at 25°C, each pot was inoculated with 500 J2 worms and plants were grown in the same growth chamber. Forty days after inoculation, all root systems were washed and stained with Phloxine B and the egg-masses produced were counted. The experiments were repeated four times (two plants each time).

Based on the results of the resistance evaluation, a highly susceptible plant ‘C-3’ and a highly resistant plant ‘C-1’, both derived from the same parents, were selected and subjected to histological examination. Plant materials were inoculated with J2 worms of *M. incognita* SP1 in the same manner as described above. Three, five and seven days after inoculation, the five replicated root systems were harvested and stained with aniline blue by a modified cold stain method (De Guiran 1967) to count the number of penetrated J2 worms and to observe the histological reactions.

In addition, the root tips with J2 worms penetrating the

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**Note**

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conducting tissue were collected three days after inoculation. The collected materials were fixed in FAA (a mixture of 50% ethanol, formalin and acetic acid, 90:5:5), cut transversely or longitudinally into 15 µm thick sections by the ordinary paraffin method and stained with Safranin O and Fast Green for anatomical observation.

A similar tendency was observed in the production of egg-masses between the two races except for the case of plant ‘F-2’ where only SP2 produced a considerably large number of egg-masses (Fig. 2). Both races produced large numbers of egg-masses on a susceptible cultivar ‘Kokei No.14’, but few on the resistant cultivar ‘Minamiyutaka’. Thus the resistance factors retained in *I. trifida* used may similarly affect the two major races. In some F₁ families of *I. trifida*, clear segregation was observed in the production of egg-masses. The numbers of egg-masses produced varied greatly in the C, D and F families, suggesting that the parents of these progenies were genetically heterogeneous in resistance to *M. incognita*. Among the family C members, only ‘C-1’ completely suppressed the production of egg-masses by the two races (Fig. 2).

Penetration of the nematode was apparent as early as three days after inoculation in the root systems of both *I. trifida* and sweet potato, whether resistant or susceptible respectively. Even in ‘C-1’, which showed extremely high resistance, and the resistant cultivar ‘Minamiyutaka’, a large number of nematodes were observed in their root systems (Table 1). No significant correlation was observed between the number of days after inoculation and the number of nematodes having penetrated (data not shown). However, in the root systems of resistant plant ‘C-1’, abnormal cells were observed adjacent to the penetrated nematodes. On the other hand, no such cells appeared in the susceptible plant ‘C-3’.

### Table 1. Number of penetrated nematodes and percentage occurrence of necrotic reaction in root systems of resistant and susceptible *Ipomoea trifida* plants and *I. batatas* cultivar

<table>
<thead>
<tr>
<th>Plant/Cultivar¹</th>
<th>Number of nematodes/g fresh root</th>
<th>% of necrosis²</th>
</tr>
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<tbody>
<tr>
<td>C-1 (R)</td>
<td>216.4</td>
<td>93.1</td>
</tr>
<tr>
<td>C-4 (R)</td>
<td>175.9</td>
<td>59.1</td>
</tr>
<tr>
<td>C-2 (S)</td>
<td>287.0</td>
<td>0.0</td>
</tr>
<tr>
<td>C-3 (S)</td>
<td>204.5</td>
<td>0.0</td>
</tr>
<tr>
<td>Minamiyutaka (R)</td>
<td>401.2</td>
<td>60.7</td>
</tr>
<tr>
<td>Kokei No.14 (S)</td>
<td>700.9</td>
<td>0.0</td>
</tr>
</tbody>
</table>

¹) R: Resistant, S: Susceptible.

²) (Number of necrotic symptoms/Number of penetrated nematodes) × 100.

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![Fig. 2](image2.png)

**Fig. 2.** Number of egg-masses/g fresh root in 19 F₁ plants of *Ipomoea trifida* and 2 cultivars of *I. batatas* inoculated with races SP1 and SP2 of *Meloidogyne incognita*. Eight root systems per plant were investigated. K: Kokei No.14, susceptible; M: Minamiyutaka, resistant; A-G: Progenies of *I. trifida*, Bar: standard error.

![Fig. 3](image3.png)

**Fig. 3.** Histological reactions observed in the roots of resistant and susceptible *Ipomoea trifida* progenies inoculated with SP1 of *Meloidogyne incognita*. The roots were stained with aniline blue in lactophenol and observed 3 days after inoculation. A: Resistant plant (C-1), necrotic cells were observed adjacent to the penetrated nematode; B: Susceptible plant (C-3), no necrotic cells were visible.

![Fig. 4](image4.png)

**Fig. 4.** Histological reactions observed in the root tips of resistant and susceptible *Ipomoea trifida* progenies inoculated with SP1 of *Meloidogyne incognita*. The tissue sections were stained with Safranin O and Fast Green and observed 3 days after inoculation. A: Resistant plant (C-1), necrotic cells were observed adjacent to the penetrated nematode; B: Susceptible plant (C-3), no necrotic cells were visible.
In tomato, microscopic studies have provided some information on the resistance mechanism (Dropkin 1969, Paulson and Webster 1972, Ho et al. 1992). Nematodes are attracted to and penetrate roots, and they then migrate to the feeding site in a similar manner to resistant and susceptible plants. However, in resistant plants, there is no development of the feeding site. Instead, a localized tissue necrosis or hypersensitive reaction occurs at or near the site where feeding would normally be initiated. Nematodes that fail to establish feeding sites either die or leave the roots. In the sweet potato, the resistance to *M. incognita* also generally occurs via a hypersensitive reaction (Shibuya 1952, Dean and Struble 1953, Sasser 1954, Gentile et al. 1962, Giamalva et al. 1963, Martin and Birchfield 1973, Jones and Dukes 1980). Furthermore, the hypersensitive reaction has been reported in other crops (Okamoto and Mitsui 1974, Sano and Nakasone 1986).

The molecular genetic study of root-knot nematode resistance is far advanced in tomato, and tomato resistances are controlled by the single dominant genes *Mi* (Gilbert 1958). However, Tokui et al. (1992, 1993) examined the mode of inheritance of the resistance to an *M. incognita* in a diploid of *I. trifida* using resistance *F₁* plants and sister test, and concluded that its resistance was controlled by at least two dominant genes. In this study, our result suggests a hypersensitive reaction is also one of the main mechanisms of nematode resistance in diploid *I. trifida*. The same reaction was observed in resistant cultivar ‘Minamiyutaka’ (Table 1), however, SP2 produced a few egg-masses on ‘Minamiyutaka’. Thus, ‘Minamiyutaka’ was not completely resistant to *M. incognita*. These results suggest that ‘Minamiyutaka’ and diploid *I. trifida* may have the same resistance mechanisms by two dominant genes. However, during the breeding process for root-knot nematode resistance, resistance genes were not equally introgressed into ‘Minamiyutaka’. Therefore, isolation of the genes relevant to this resistance (hypersensitive reaction) and production of useful DNA markers is now being conducted using proteome analysis.

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**Literature Cited**


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