サツマイモネコブセンチュウの感染に影響を与えうるトマトのCLAVATA受容体遺伝子のSNP検出

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SNPs of CLAVATA receptors in tomato, in the context of root-knot nematode infection

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Plant-parasitic nematodes secrete CLAVATA3 (CLV3)/ESR (CLE)-like proteins, which are suggested to function in the process of nematode infection. Here we examined the infection rate of root-knot nematode *Meloidogyne incognita* by using tomato, *Solanum pennellii*, *S. peruvianum*, and seven varieties of *S. lycopersicum* as host plants. *S. lycopersicum* variety Micro-Tom, *S. pervianum*, and *S. pennellii* showed obvious resistance to nematode infection. CLV3 receptors, CLV2 and CORYNE (CRN) / Suppressor of LLP1 2 (SOL2), are known to be responsible for nematode infection in *Arabidopsis*; therefore, we examined SNPs of putative CLV receptor sequences of *CLV2*, *CRN/SOL2*, *CLV1*, and *RECEPTOR LIKE PROTEIN KINASE 2 (RPK2)* in tomato to look for a potential contribution of CLV3 signaling in an *Mi* resistance gene-independent manner. We found many SNPs in the CLV receptors, which might be related to resistance to nematode infection in tomato. Nematol. Res. 41 (2), 35-40 (2011).
Key words: CLV receptors, nematode infection, tomato

INTRODUCTION

Plant-parasitic nematodes are biotrophs that mainly attack the roots of plants and cause over $100 billion in crop damage annually (Sasser and Freckman, 1987). Root-knot nematodes are one of the most economically damaging nematodes; thus it is important to know the molecular mechanisms involved in the nematode infection process. Some of the tomato varieties, for example, *Solanum lycopersicum*, show resistance to nematode infection. The causal gene for this resistance was reported to be *Mi-1*, which encodes a protein with a nucleotide binding site and a leucine-rich repeat region (Milligan *et al.*, 1998). The presence of the *Mi-1* gene has been a classical example of the use of host resistance to reduce the need for pesticide application (Medina-Filho and Stevens, 1980; Roberts and Thomason, 1986). *Mi-1* was introduced into cultivated tomatoes from their wild relative *Lycopersicon peruvianum* in the early 1940s (Smith, 1944).

To gain insight into the establishment of nematode parasitic interactions with host plants, many efforts to identify "parasitic genes" have been carried out with different nematode species (Davis *et al.*, 2000; Davis *et al.*, 2008). In general, parasitic effector proteins produced in the esophageal gland cells of nematodes are secreted from the nematode through its stylet into the plant tissue (Davis *et al.*, 2000; Davis *et al.*, 2008). Attempts to target secretory proteins from the esophageal gland cells of the soybean cyst nematode *Heterodera glycines* at the parasitic stage identified *HgCLE1* (formerly known as *2B10* and identical to *HgSYV46*) and *HgCLE2* (known as *4G12*), which encode a protein that harbors the C-terminal CLE domain (Wang *et al.*, 2001; Gao *et al.*, 2003; Wang *et al.*, 2010). Members of the *CLE* gene family encode small (about 100 amino-acid) proteins that share a conserved structure of a putative N-terminus secretory signal peptide and a conserved 14-amino-acid CLE domain at the C-terminus (Cock and McCormick, 2001; Sharma *et al.*, 2003; Sharma *et al.*, 2005; Strabala *et al.*, 2006; Sawa *et al.*, 2006; Kinoshita *et al.*, 2007; Sawa *et al.*, 2008; Miwa *et al.*, 2009a, b; Sawa and Tabata, 2011; Tabata and Sawa, 2011). *CLE* genes have been found in many plants; however, so far in the animal kingdom, the *CLD* genes have been found only the plant-parasitic nematode. *HgCLE* genes in the nematode encode proteins of about 140 amino-acids with N-terminal signal peptides and their 12-amino-acid CLE domains (Wang *et al.*, 2001; Gao *et al.*, 2003; Wang *et al.*, 2010). Another CLE-like nematode gene, *16D10*, was isolated from a cDNA library derived from the esophageal gland cells of the root-knot nematode *Meloidogyne incognita* at the parasitic stage (Huang *et al.*, 2006a, b). Furthermore, *Arabidopsis* mutants of CLV3 receptors, *clv2* and *crn/sol2*, showed obvious resistance to nematode infection (Replogle *et al.*, 2010). These results indicate that the CLV3 signaling molecules are responsible for successful nematode infection in plants.

To examine a potential contribution of CLV3 signaling

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to the efficiency of nematode infection, tomatoes, *S. pennellii, S. peruvianum*, and seven varieties of *S. lycopersicum* were used as host plants for *M. incognita* infection. We report here our findings that *S. lycopersicum* variety Micro-Tom, *S. pervianum*, and *S. pennellii* showed obvious resistance to nematode infection. Furthermore, we sequenced putative CLV3 receptor genes in the tomato, and found many SNPs that might be related to the resistance to nematode infection in tomato.

MATERIALS AND METHODS

Root-knot nematode infection assay:

Meloidogyne incognita collected from Koshi (Kumamoto, Japan) was cultivated and used for infection assays. The nematode population was propagated for two to three months on tomato variety, Pritz, in pots under continuous light conditions at 27°C to obtain nematode-containing soil. Tomato seedlings of *S. pennellii*, *S. peruvianum*, and seven varieties of *S. lycopersicum* (Pritz, Micro-Tom, Moneymaker, Aichi-first, Ailsa Craig, M82, and Ponterosa) were grown for four weeks after germination in a soil without nematodes and then transplanted to the well-mixed nematode-containing soil. Transplantation may affect the root growth rate and the balance of such plant hormones as Methyl Jasmonate (MeJa), which affects the nematode infection rate. Our aim here, however, is to compare the nematode infection rate among various tomato species; therefore, we transplanted the tomato plants that had been growing in healthy conditions before nematode infection. After four weeks of growth in the nematode-containing soil, the tomato root systems were recovered from the pots and carefully washed. The root systems were immersed in an aqueous solution of 0.5% Phloxine B for several seconds to stain nematode egg masses. After the roots were washed well with running tap water to rinse off excess dye, the egg masses produced on each root system were counted.

DNA Sequencing:

DNA fragments were amplified with a thermal cycler (DNA Engine Tetrad 1 PTC-240, BioRAD). Cycle conditions for the polymerase chain reaction: 94°C 4 min; [35 cycles: 94°C 30 sec; 55°C 1 min; 72°C 1 min]; 72°C 10 min. KOD DNA polymerase (TOYOBO, Japan) was used. DNA sequencing was conducted by FASMAC Co., Ltd. (Kanagawa, Japan).

Accession numbers:

The ORFs of the putative CLV3 receptors in tomato have been submitted to DDBJ. *Slp, Sml, Spn*, and *Spr* represent the *S. lycopersicum* varieties Pritz and Micro-Tom, *S. pennellii*, and *S. peruvianum*, respectively.

Accession numbers: SlpCLV1 AB645826; SlmCLV1 AB645823; SpnCLV1 AB645824; SprCLV1 AB645825; SlpCLV2 AB645830; SlmCLV2 AB645827; SpnCLV2 AB645828; SprCLV2 AB645829; SlpSOL2 AB645838; SlmSOL2 AB645835; SpnSOL2 AB645836; SprSOL2 AB645837; SlpRPK2 AB645834; SlmRPK2 AB645831; SpnRPK2 AB645832; SprRPK2 AB645833.

RESULTS AND DISCUSSION

Solanum pennellii, *S. peruvianum*, and seven varieties of *S. lycopersicum*, (Pritz, Micro-Tom, Moneymaker, Aichi-first, Ailsa Craig, M82, and Ponterosa) were examined for their susceptibility to the root-knot nematode *M. incognita*. Among them, Pritz was the most susceptible to *M. incognita* (Table 1); whereas, Micro-Tom showed strong resistance to the nematode infection (Table 1). Micro-Tom is one variety of *S. lycopersicum*, which is well known to have an *Mi-1* nematode resistance gene (Smith, 1944). The reduced infection rate in Micro-Tom may be due either to a novel resistance gene or the presence of an *Mi*-like gene. *S. peruvianum* also showed strong resistance (Table 1). The nematode resistance loci *Mi-3* in *S. peruvianum* was mapped on chromosome 12 (Yaghoobi *et al.*, 2005), but has not yet been cloned. We also found that *S. pennellii* showed weak resistance to root-knot nematode infection (Table 1).

Mutations of *CLV2* and *CRN/SOL2* in *Arabidopsis* conferred resistance against plant parasitic nematode infection; their products CLV2 and CRN/SOL2 are known as CLV3 peptide hormone receptors (Replogle *et al.*, 2010). CLV1 and RPK2 are also known as CLV3 receptors (Miwa *et al.*, 2008; Kinoshita *et al.*, 2010; Betsuyaku *et al.*, 2011). To examine whether the CLV3 receptors are responsible for the regulation of nematode infection rates in tomato, the *CLV1*, *CLV2*, *CRN/SOL2*, and *RPK2* gene homologs of *S. pennellii*,

Table 1. Egg mass number in Tomato plant infected by *Meloidogyne incognita*.

		exp1	exp2
Solanum lycopersicum	Pritz	1174	708
	Micro-Tom	3	9
	Moneymaker	235	417
	Aichi-first	287	112
	Ailsa Craig	382	264
	M82	77	16
	Ponderosa	343	735
Solanum pennellii		12	35
Solanum peruvianum		0	1

Tomato plants were exposed to *Meloidogyne incognita* twice independently, and egg mass number was counted at four weeks after transfer to nematode-infected soil.

S. peruvianum, and two varieties of *S. lycopersicum*, Pritz and Micro-Tom were sequenced.

The full amino-acid sequences of *Arabidopsis* CLV1, CLV2, CRN/SOL2, and RPK2 were used as queries for a TBLASTN search in the tomato genome database (http://solgenomics.net/tools/blast/index.pl). We then constructed phylogenetic trees (Fig. 2). Tomato homologs of CLV2, SOL2, and RPK2 were integrated into the main clade; however, the CLV1 homolog was categorized outside of the main clade. As the amino acid sequence of SlpCLV1 showed the highest similarity with that of *Arabidopsis* CLV1, here we used the SlpCLV1 as a tomato CLV1 homologs. It is possible, however, that other tomato CLV1 ortholog may be encoded in the tomato genome.

As shown in Table 2, we found SNPs in the receptor sequences. These SNPs were compared with the sequence of Pritz (*S. lycopersicum*). Pritz and Micro-Tom showed opposite responses regarding their susceptibility to nematode infection. Nevertheless, the sequences of their putative CLV3 receptors were almost the same. Only one SNP was detected. This indicates that CLV3 signaling might not contribute to the nematode infection resistance in Micro-Tom. In contrast, *S. pennellii* and *S. peruvianum* have many SNPs in comparison with the susceptible lines (Table 2). Forty-six out of the 159 (about 30%) SNPs in *S. pennellii* and *S. peruvianum* shown in Table 2 were nonsynonymous. Interestingly, only *S. pennellii* has a nonsynonymous SNP in the SOL2 homolog in tomato (Table 2, Fig. 1). The nonsynonymous SNPs shown in Table 2 and Fig. 1 may be responsible for the resistance to the nematode infection in both *S. pennellii* and *S. peruvianum*. Further genetic analysis by using this SNPs information would contribute to our understanding about nematode infection/resistance mechanisms in the tomato.

Table 2. SNPs of putative CLV3 peptide receptor genes in tomato.

	CLV2	RPK2	SOL2	CLV1
Micro-Tom (*S. lycopersicum*)	2(1)	0(0)	0(0)	0(0)
S. pennellii	19(7)	33(12)	8(1)	38(10)
S. peruvianum	15(5)	16(4)	6(0)	24(7)

SNPs were found compared with the sequences of the most susceptible cultivar, Pritz (*S. lycopersicum*). Numbers of the SNPs (nonsynonymous SNPs) are shown

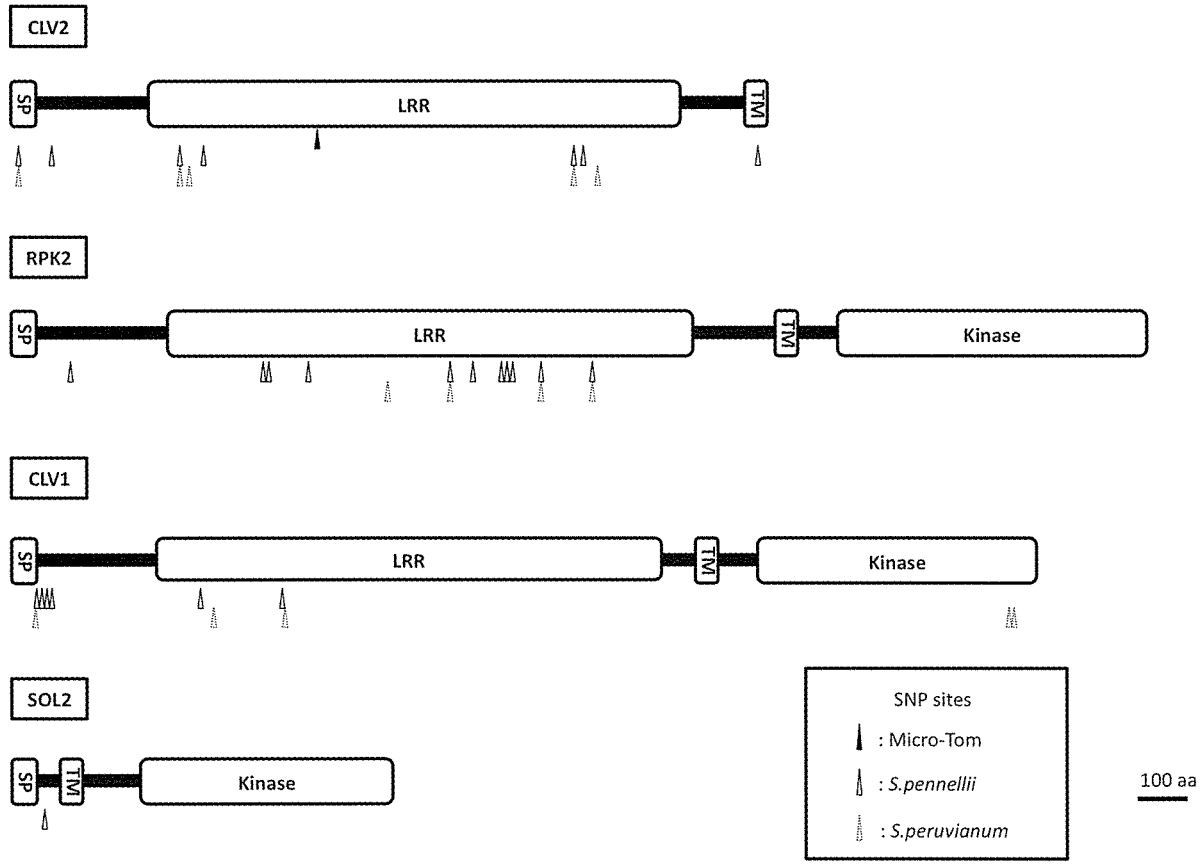

Fig. 1. Nonsynonymous SNPs in putative CLV3 receptors in tomato.
SNPs were detected by comparison with the amino acid sequences of the Pritz tomato (*Solanum lycopersicum*). SNP points are shown as arrow heads. There are few or no nonsynonymous SNPs in the kinase or transmembrane domain, respectively. TM: transmembrane domain; LRR: leucine rich repeat domain; SP: signal peptide.

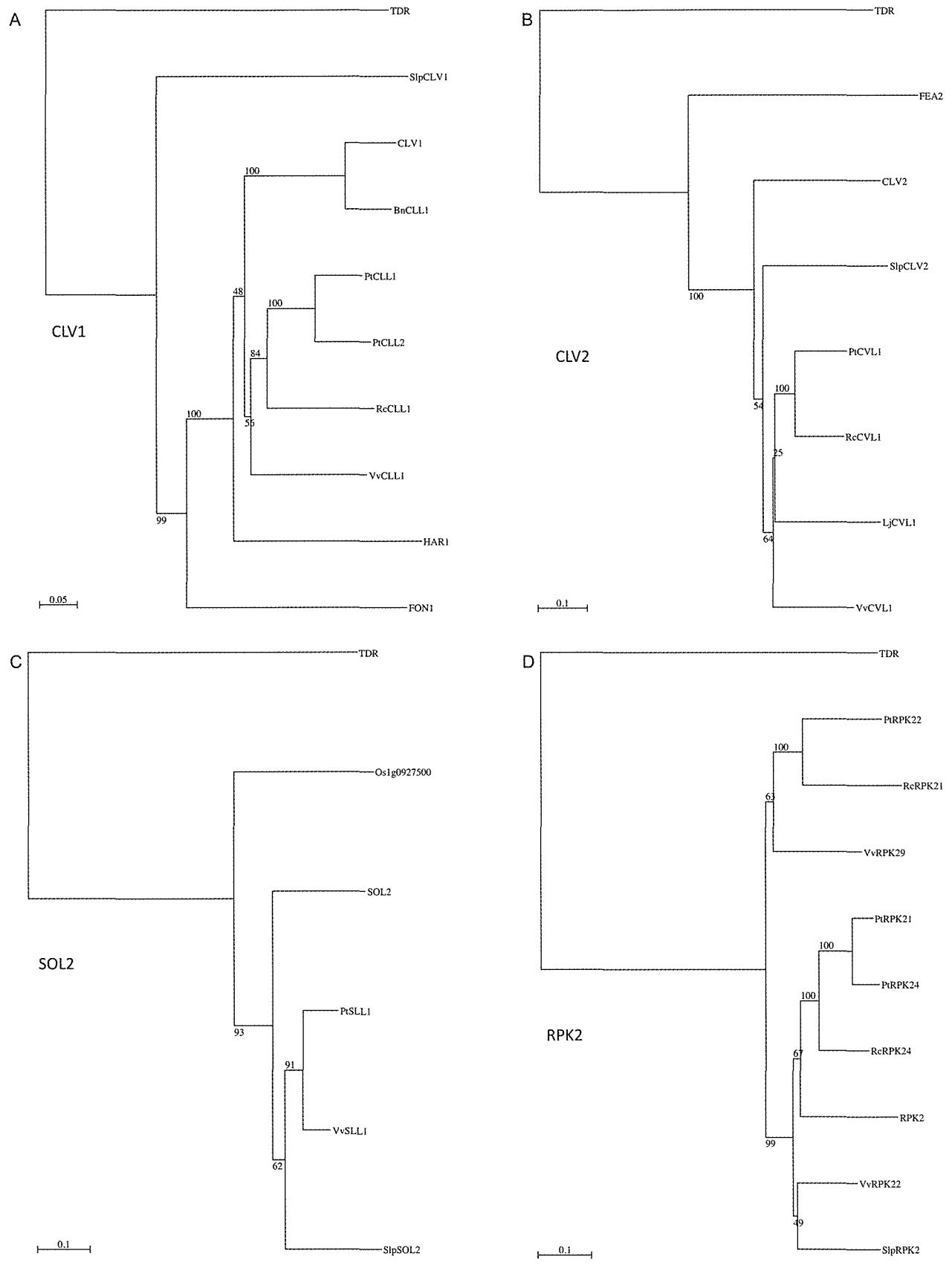

Fig. 2. Comparison of CLV1, CLV2, SOL2, RPK2, and their homologs Phylogenetic relationships of (A) CLV1, (B) CLV2, (C) SOL2, and (D) RPK2 to their counterparts from other plant species, *Solanum lycopersicum* Pritz, *Arabidopsis thaliana*, rice, *Populus trichocarpa*, *Vitis vinifera*, *Ricinus communis*, *Lotus japonicus*, and *Brassica napus*. The phylogenetic tree was calculated and drawn with the MEGA3.1 program from an alignment of complete protein sequences, with gap deletion. Bootstrap values from the neighbor-joining method with Kimura's correction are shown. The amino-acid sequences of *Arabidopsis* TDR were used as an outgroup. The scale bar indicates the number of amino-acid substitutions per site.

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英文論文（本報・短報）の和文摘要

サツマイモネコブセンチュウの感染に影響を与えうるトマトのCLAVATA受容体遺伝子のSNP検出

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シロイヌナズナを用いた研究から、植物のペプチドホルモン、CLAVATA受容体の突然変異体は植物寄生性線虫の感染効率が下がることが知られている。一方、様々なトマト系統において、それぞれ線虫感染効率が異なることも知られている。我々は、3種9系統のトマトを用いて感染効率の検定試験を行い、*Solanum lycopersicum*のMicro-Tom, *S. pennellii*, そして*S. perubianum*が有意にサツマイモネコブセンチュウの感染に対して抵抗性を示すことを明らかにした。さらに、これらのトマト系統からCLAVATA受容体のホモログを単離、シーケンスし、それぞれのトマト系統の線虫感染効率を調べることで、その感染効率を調節しうる遺伝子のSNPを多数検出した。今後、これらのSNPをさらに解析することで、線虫感染に対する抵抗性品種の育種に応用できる可能性があると考えられる。