

2種類のレースに分けられるサツマイモネコブセンチュウレースSP6

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[Research Note]**Southern root-knot nematode race SP6 is divided into two races**

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The southern root-knot nematode (SRKN) includes at least nine races, SP1 to SP9, which were identified based on their different responses to five differential sweetpotato cultivars, ‘Norin-1’, ‘Norin-2’, ‘Tanegashimamurasaki-7’, ‘Elegant Summer’, and ‘J-Red’. In this study, we propose a modified method for SRKN race identification in which the threshold of response is 10 egg masses instead of the two-egg-mass threshold used in the original method, when a sweetpotato plant was inoculated with 500 second-stage juveniles. According to this method, two isolates of SP6, Okiishi-12 and Ishigaki-2, showed the same response to each of these five cultivars. We found, however, that three sweetpotato cultivars, ‘Murasakimasari’, ‘Suzukogane’, and ‘Chienoha’, were resistant to the Okiishi-12 isolate and susceptible to the Ishigaki-2 isolate. These results indicated that the Okiishi-12 and Ishigaki-2 isolates were distinct and that SP6 included at least two races. We suggest that the Okiishi-12 and Ishigaki-2 isolates should be distinguished by sub-numbers and designated SP6-1 and SP6-2, respectively. Five isolates of SP6 collected from a sweetpotato field in Chiba prefecture were examined, and all of them were identified as SP6-1. *Nematol. Res.* 47(2), 29–33 (2017)

Key words: *Meloidogyne incognita*, new race, SP6-1, SP6-2, sweetpotato

INTRODUCTION

The southern root-knot nematode (*Meloidogyne*

incognita) (abbreviated as SRKN) causes severe damage to quality and yield in the sweetpotato. SRKN was found in most of the sweetpotato fields in Kyushu, the largest sweetpotato production area in Japan (Iwahori *et al.*, 2000), and was also detected in Kanto, the second largest sweetpotato production area (Yoshida, 1965; Iwahori *et al.*, 2015). Sano and Iwahori (2005) revealed that SRKN included at least nine races, SP1 to SP9, which were identified by their different responses to five differential sweetpotato cultivars, ‘Norin-1’, ‘Norin-2’, ‘Tanegashimamurasaki-7’, ‘Elegant Summer’, and ‘J-Red’ (Table 1). To effectively control SRKN damage by the use of resistant sweetpotato cultivars, it is important to elucidate which SRKN race is primary in a certain region and to which SRKN races the sweetpotato cultivars produced in that region are resistant. As for the former, Sano and Iwahori (2005) indicated that SP1 was predominant in the prefectures of Saga, Nagasaki, and Kumamoto (northern and central Kyushu), SP2 was predominant in Miyazaki and Kagoshima (southern Kyushu), and SP4 and SP6 were predominant in Okinawa. Momota *et al.* (2012) identified SP4 and SP6 in Ibaraki and Chiba (Kanto). As for the latter, Sano *et al.* (2002) and Tabuchi *et al.* (2015) tested the resistance of various sweetpotato cultivars to SRKN races.

In this paper, the following results obtained during resistance testing are reported: (1) the SRKN race identification method should be reconsidered; (2) SP6 included at least two races, designated SP6-1 and SP6-2; (3) some cultivars and lines were resistant to SP6-1 and susceptible to SP6-2; and (4) SP6-1 was identified in Chiba. These results might be useful in controlling SRKN damage.

MATERIALS AND METHODS

Plants and nematodes:

A total of nine sweetpotato cultivars, ‘Norin-1’, ‘Norin-2’, ‘Tanegashimamurasaki-7’, ‘Elegant Summer’, ‘J-Red’, ‘Choshu’, ‘Murasakimasari’, ‘Suzukogane’, and ‘Chienoha’ were used for tests of resistance to SRKN. We also examined the resistance to SRKN of six lines from 52 F₁ progeny plants derived from a cross between ‘J-Red’ and ‘Choshu’. ‘J-Red’ and ‘Choshu’ were resistant and susceptible to SRKN races SP1, SP2, SP3, SP4, and SP6 (Table 2). These cultivars and lines were grown and maintained in a greenhouse and used for the resistance tests.

For the resistance tests, we used six isolates from single egg mass cultures of SRKN, Nishigoshi, Miyakonojo, Mashiki, Tsukuba, Okiishi-12, and

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Ishigaki-2, Nishigoshi, Miyakonojo, Mashiki, and Ishigaki-2 were referred to as ‘Suya, Nishigoshi’, ‘Mochio, Miyakonojoushi’, ‘Tabaru, Mashiki’, and ‘Isobe, Ishigaki’ and identified as races SP1, SP2, SP3, and SP6, respectively, by Sano and Iwahori (2005). We also used five isolates of SRKN race SP6 designated as KR2, KR5, KR6, KR8, and KR13. These five isolates originated from single egg mass cultures established from soil samples collected in a field naturally infested with SRKN in Katori city in Chiba in 2013 (Kuranouchi *et al.*, 2016).

Test of sweetpotato resistance to SRKN isolates:

Sweetpotato resistance tests were carried out basically according to Sano and Iwahori (2005). Isolates of each SRKN race were propagated in the susceptible tomato cultivar ‘Pritz’ in a greenhouse kept at an average temperature of 25.5°C for 41 to 48 days. Egg masses were picked up from washed root systems and placed on a cotton filter partially submerged in water in a 50 ml beaker at 24°C. The cotton filter was transferred to a new beaker every two or three days, and second-stage juveniles (J2) that emerged in the former beaker were kept at 13°C. J2 individuals from three to five beakers were collected and used for the resistance tests.

During the resistance tests, sweetpotato samples were cultivated at an average temperature of 25.5°C in a greenhouse. Single-node cuttings were prepared from fresh vines of sweetpotato plants and incubated in water for 10 days for rooting. Each cutting was then planted in a pot with a diameter of 9 cm with about 200 g of sterilized soil and shaded for three days. Each pot was then inoculated with 500 J2 and shaded for three days. The root systems were washed after 32 days, and egg masses were stained with 0.2% Phloxine B (Wako Pure Chemical) or 0.02% Erioglaucine (Sigma). The average number and standard deviation of egg masses of each replication were calculated from one to eight plants, and then the final average number and standard deviation

were calculated from two to 14 replications. The number of replications and plants/replication differed for each cultivar and F₁ line.

RESULTS AND DISCUSSION

Reconsideration of the SRKN race identification method:

Initially, tests of the resistance to SRKN isolates Nishigoshi (SP1), Miyakonojo (SP2), Mashiki (SP3), and Ishigaki-2 (SP6) of were carried out for five differential sweetpotato cultivars ‘Norin-1’, ‘Norin-2’, ‘Tanegashimamurasaki-7’, ‘Elegant Summer’, and ‘J-Red’. All of our results were consistent with those of Sano and Iwahori (2005), except in the following two cases; the numbers of egg masses of ‘Elegant Summer’ inoculated with 500 J2 of the Mashiki isolate and ‘J-Red’ inoculated with 500 J2 of the Ishigaki-2 isolate were 2.3 and 4.8, respectively (Table 1, Table 2). Sano and Iwahori (2005) reported that the numbers of egg masses in these two cases were both less than two, and that the Mashiki and Ishigaki-2 isolates were identified as SP3 and SP6, respectively. In our resistance tests, however, the Mashiki isolate was identified as SP6, but the Ishigaki-2 isolate could not be identified as any race according to the identification method of Sano and Iwahori (2005), in which they used a threshold of two egg masses (Table 1). The differences in the numbers of egg masses might have resulted from differences in uncontrollable experimental conditions, such as the activities of SRKN and the sweetpotato, and other factors. The threshold of two seems too severe. Nakayama *et al.* (2012) analyzed F₁ plants derived from a cross between the resistant cultivar ‘Hi-starch’ and the susceptible cultivar ‘Koganesengan’. They indicated that the resistance of ‘Hi-starch’ to SRKN was controlled by a single major dominant gene and that the egg mass number of F₁ progeny harboring this resistance gene was less than 10 when the F₁ progeny was inoculated with 500 J2. Although it is unknown whether or not the resistance genes in the five differential sweetpotato cultivars and ‘Hi-starch’ are identical the

Table 1. SRKN race identification based on the egg mass number of five differential sweetpotato cultivars.

Differential sweetpotato cultivars	SRKN races ^a								
	SP1	SP2	SP3	SP4	SP5	SP6	SP7	SP8	SP9
Norin-1	+	+	+	+	–	+	–	+	–
Norin-2	–	+	–	+	+	–	+	+	–
Tanegashimamurasaki-7	–	–	+	+	+	+	–	–	–
Elegant Summer	–	–	–	+	–	+	–	–	+
J-Red	–	–	–	–	–	–	–	+	–

^a The races were identified based on the egg mass number per root system of each differential cultivar inoculated with 500 J2. –: egg mass number < 2.0, +: egg mass number ≥ 2.0. From Sano and Iwahori (2005).

Table 2. Egg mass number of nine sweetpotato cultivars and six F₁ lines inoculated with 500 J2 of SRKN races.

Sweetpotato cultivars and lines ^b	SRKN races and isolates ^a																			
	SP1				SP2				SP3				SP4				SP6			
	Nishigoshi				Miyakonojo				Mashiki				Tsukuba				Okiishi-12			
	Ave	SD	N	Rep	Ave	SD	N	Rep	Ave	SD	N	Rep	Ave	SD	N	Rep	Ave	SD	N	Rep
Norin-1	204.9	40.2	14	7	133.6	30.4	13	3	135.5	38.5	12	4	127.0	37.6	11	4	188.8	44.4	12	3
Norin-2	0.2	0.3	25	10	139.2	42.8	20	8	0.6	0.5	12	4	139.8	37.7	15	6	1.1	0.3	12	4
Tanegashimamurasaki-7	1.3	0.4	31	12	0.0	0.0	22	8	128.1	20.2	21	7	237.5	57.4	20	9	163.1	29.4	14	3
Elegant Summer	0.2	0.0	14	6	0.0	0.0	14	5	2.3	2.6	21	8	74.6	13.8	15	6	88.2	23.1	10	5
J-Red	0.5	0.6	31	14	0.5	0.5	21	9	0.2	0.2	16	5	1.8	1.1	24	12	1.2	0.9	12	5
Murasakimasari	1.4	2.4	12	4	71.6	24.3	12	3	0.1	0.2	12	2	1.8	1.2	13	4	0.9	0.9	12	4
Suzukogane	3.9	2.0	12	4	8.1	4.4	14	4	4.6	3.3	11	2	8.6	5.1	13	4	6.3	1.7	12	4
Chienoha	0.4	0.2	12	4	5.0	4.4	12	3	0.1	0.2	12	2	1.6	1.1	13	4	0.8	0.7	12	4
Choshu	171.9	42.6	33	15	203.3	37.9	18	9	215.6	47.2	16	5	254.9	38.8	19	11	202.2	32.1	12	5
JC F ₁ No.15	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8.5	3.5	6	3
JC F ₁ No.18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9.5	5.4	6	3
JC F ₁ No.21	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2	0.2	6	3
JC F ₁ No.24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4.0	0.7	4	2
JC F ₁ No.26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.0	1.4	4	2
JC F ₁ No.28	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.0	0.0	4	2

^a The average number and standard deviation of egg masses of each replication were calculated from one to eight plants, and then the average number and standard deviation indicated in this Table were calculated from two to 14 replications. Ave: average number, SD: standard deviation, N: total number of examined plants, Rep: number of replications, -: not indicated.

^b JC F₁: F₁ lines derived from a cross between 'J-Red' and 'Choshu'.

threshold of 10 egg masses seems proper for identifying SRKN races. Therefore, we propose a modified SRKN race identification method in which a threshold of 10 egg masses is used instead of two when a plant is inoculated with 500 J2 (Table 3). According to this method, the Mashiki and Ishigaki-2 isolates were identified as SP3 and SP6, respectively. The Tsukuba isolates were identified as SP4 (Table 2, Table 3). We applied this method to the following results.

SP6 races:

The Okiishi-12 and Ishigaki-2 isolates showed the same response to five differential sweetpotato cultivars and were identified as SP6 (Table 1, Table 2). In three cultivars, ‘Murasakimasari’, ‘Suzukogane’ and ‘Chienoha’, however, fewer than 10 egg masses of the Okiishi-12 isolate were formed, but more than 10 egg masses of the Ishigaki-2 isolate were formed. We also found that less than 10 and more than 10 egg masses of the Okiishi-12 and Ishigaki-2 isolates, respectively, were formed in six F_1 lines derived from a cross between ‘J-Red’ and ‘Choshu’ (Table 2). These results suggested that the Okiishi-12 and Ishigaki-2 isolates were distinct, that SP6 included at least two races, and that the resistance genes of the sweetpotato to the two isolates were distinct. As more cultivars are investigated by using resistance testing, reports of similar results might increase; nematode isolates that were identified as the same race by an old method may be found to belong to more than two races by a new method. The question then arises of how a new race should be designated. If we designate the Okiishi-12 or Ishigaki-2 isolates as a new race with a new number, such as SP10, it will be necessary to perform resistance testing of the resistance of the new differential cultivar ‘Murasakimasari’ to all nine races. We could not perform resistance tests with respect to SP5, SP7, SP8, and SP9, however, because these races were not kept in our laboratories. Therefore, we propose that the

Okiishi-12 and Ishigaki-2 isolates be identified by sub-numbers of SP6 and, thus, be designated SP6-1 and SP6-2, respectively. ‘Murasakimasari’ was chosen as the sixth differential cultivar, because ‘Murasakimasari’ is more popular than any other cultivars and lines that were resistant to the Okiishi-12 isolate and susceptible to the Ishigaki-2 isolate (Table 3). The SRKN race identification method should be adapted to various SRKN populations from now on.

Isolates of SP6 in a sweetpotato field in Chiba:

Although it was reported that SP6 was predominant in Okinawa (Sano and Iwahori, 2005) and identified in Ibaraki and Chiba (Momota *et al.*, 2012), it is unknown whether these “SP6” isolates belonged to SP6-1 or SP6-2. Tests of the resistance of ‘Murasakimasari’ to five isolates of SP6 (KR2, KR5, KR6, KR8, and KR13) that originated from single egg mass cultures collected from a sweetpotato field in Katori in Chiba were carried out. All of them were identified as belonging to SP6-1, because no egg masses were formed on ‘Murasakimasari’ in two independent experiments using a total of 10 inoculated plants (data not shown). Such re-researching of the SRKN races in other sweetpotato fields where SP6 was detected could improve the control of SRKN by resistant sweetpotato cultivars.

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Table 3. SRKN race identification based on the egg mass number of six differential sweetpotato cultivars.

Differential sweetpotato cultivars	SRKN races ^a					
	SP1	SP2	SP3	SP4	SP6-1	SP6-2
Norin-1	+	+	+	+	+	+
Norin-2	–	+	–	+	–	–
Tanegashimamurasaki-7	–	–	+	+	+	+
Elegant Summer	–	–	–	+	+	+
J-Red	–	–	–	–	–	–
Murasakimasari	–	+	–	–	–	+

^a The races were identified based on the egg mass number per root system of each differential cultivar inoculated with 500 J2. –: egg mass number < 10, +: egg mass number $\geq$ 10. SP6-1: Okiishi-12 isolate, SP6-2: Ishigaki-2 isolate. Resistance tests of ‘Murasakimasari’ to SP5, SP7, SP8, and SP9 were not performed.

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英文報文（原著論文）の和文摘要

2種類のレースに分けられるサツマイモネコブセンチュウ
レース SP6田淵宏朗・藏之内利和・小林 晃・門田有希・岸本和樹・
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サツマイモネコブセンチュウには5種類のサツマイモ判別品種、「農林1号」、「農林2号」、「種子島紫7」、「エレガントサマー」、「ジェイレッド」により同定されるSP1からSP9までの9種類のレースが報告されている。本研究では、1個体のサツマイモに対し500頭の線虫を接種してレース判別を行う場合、従来は着生卵のう数2を閾値として用いていたが、新たに10とする提案を行った。この方法に従うと、SP6の2種類の分離株、石垣2と沖石12はこれら5種類の判別品種には同じ反応性を示す。しかし、3品種のサツマイモ「ムラサキマサリ」、「スズコガネ」、「知恵の葉」は、沖石12には抵抗性だが石垣2には感受性を示した。これらの結果から、沖石12と石垣2は異なっておりSP6には少なくとも2種類のレースが含まれることが明らかとなった。沖石12と石垣2をそれぞれSP6-1とSP6-2と命名した。千葉県1筆のサツマイモ畑から集めたSP6の5種類の分離株について調べたところ、全てSP6-1と判別された。