Gall Formation by Root-Knot Nematode, *Meloidogyne incognita*
(KOFOID et WHITE, 1919) CHITWOOD, 1949, in the
Grafted Tomato Plants, and Accumulation
of Phosphates on the Gall Tissues
(Nematoda : Tylenchida)¹

Nobuyoshi ISHIBASHI² and Kei SHIMIZU

Laboratory of Plant Nematology, Central Agricultural Experiment Station, Konosu, Saitama, Japan

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The relations between gall formation and phosphoric metabolism were studied by cross-grafted tomato plants with different susceptibilities. Cross-grafted plants of susceptible tomato (line "Black") and resistant ones (line "Anaf" No. 2 or No. 3) were inoculated with 5,000 larvae of *Meloidogyne incognita* per plant. Fifteen days later, 25 μ Ci of ³²P-orthophosphate was given to the resistant line so as to be absorbed from roots with full nutrients. The data showing the following gall indices, 100 in intact susceptible plants, 0 in intact resistant plants, 85 in resistant scion on susceptible stock, 17.5 in susceptible scion on resistant stock, indicate that the resistant or susceptible factors may be associated with both roots and tops of plants. Autoradiography and wet combustion analysis of plant tissues showed marked accumulation of ³²P-phosphates on the galled roots. Barium insoluble phosphate-esters, which mainly include nucleotides, were more at gall tissues than at healthy roots.

INTRODUCTION

Several reports have been made stating that the unhealthy condition of plants by nematodes is due likely to the abnormal metabolism induced by parasitism rather than the parasite "robbing" the host of its vital elements (DROPKIN and KING, 1956; MYUGE, 1956; OTEIFA and ELGINDI, 1962). In root-knot nematodes, gall formation may give rise to unbalanced distribution and stagnation of nutrients between tops and roots. The authors have investigated the relation between gall formation and phosphorus metabolism in various degrees of gall formation by cross-grafting of resistant and susceptible lines of tomato plants. SHIMIZU (unpublished), one of the authors, found that relative amounts of nucleotides decreased in the leaves, but increased extraordinarily in gall tissues of plants infected with root-knot nematodes. This may indicate greater metabolism in the gall tissues

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2) Present address: Laboratory of Nematology and Entomology, Faculty of Agriculture, Saga University, Saga, Japan.

than in the healthy ones. If the damages to plants by root-knot nematodes result from unbalanced metabolism gall formation itself will become the cause. However, sweet-potato plants, for example, are heavily damaged by the same nematodes, despite less gall formation. In the course of the study to clarify the mechanism of root-knot nematode-resistance in sweet-potato plants., NISHIZAWA (1957) noticed the increase in respiration, abnormal metabolism of phosphates, accumulation of ^{35}S -sulphates, the enhancement of dehydrogenase system, and reduction of catecholase activity in the gall tissues of sweet-potato plants susceptible to *M. incognita* var. *acrita*. The authors have also confirmed the conspicuous accumulation of phosphates in galled roots of tomato plants, and they expected that a certain relation might be obtained between the movement of phosphates from resistant line to gall tissues of susceptible roots and the establishment of nematode-resistance in susceptible roots by cross-grafting.

MATERIALS AND METHODS

Tomato "Anaf" No. 2 and No. 3, which came from Hawaii, U.S.A., were prepared for resistant line to *Meloidogyne incognita*, and Black which also came from Hawaii, for the susceptible line. These tomato varieties were cultured in steam-sterilized sand with full nutrients. When they grew up to a height of about 15 cm, they were cross-grafted in the following 8 ways (C-J). The experimental combination consists of 10 ways including non-grafted intact plants. Each combination had 10 replicates.

- (A) Black (intact susceptible)
- (B) No. 2 (intact resistant)
- (C) No. 2 was grafted on Black.
- (D) Black was grafted on No. 2.
- (E) No. 2 was grafted on Black, while root systems of the two plants were allowed to grow in separate glass pots.
- (F) Contrary to E.
- (G) The top of No. 3 was cut in a way by which No. 3 was grafted on Black, while top of Black which was employed for stock was allowed to grow. The root systems of the two plants were cultured individually in separate pots.
- (H) Contrary to G.
- (I) No. 2 was grafted on Black with allowance of growth of both tops as well as each root systems in separate pots.
- (J) Contrary to I.

Grafting was accomplished by surrounding the stems of the two plants with lead tape (0.2 mm in thickness). After confirming complete grafting about one week later, tops or root stems were cut as shown in Fig. 1 (C-H). About 5,000 larvae of *M. incognita* were inoculated into each pot. On the 15th day after larval inoculation, 25 μCi of ^{32}P -orthophosphate (pH 6.5 adjusted with dilute NaHCO_3) was given to the pot of No. 2 or No. 3 with KASUGAI's solution (referred in ISHIBASHI et al., 1964) so as to be taken by resistant roots. The plants were examined on the 2nd day after the application of ^{32}P . Gall index, gall weight per total weight of roots (hereinafter referred to as gall weight per cent) and specific

Fig. 1. Schematic drawing to show various combinations of grafting ways and infectivity of *Meloidogyne incognita* on resistant line No. 2 or No. 3 and on susceptible line Black.

radioactivity of each part of the treated plants by the method of wet combustion with boiling nitric acid were investigated. Phosphate-esters in galled and healthy roots were fractionated into two parts according to LEPAGE (1951), viz., soluble and insoluble in barium acetate. Their specific radioactivity per mg living tissue weight were estimated by a G-M scaler.

"Anaf" No. 2 and No. 3 tomato varieties are susceptible to *M. hapla*, though resistant to *M. incognita*. Therefore, another experiment was conducted to see whether or not the movement of ^{32}P was stagnated from No. 2 infected with *M. hapla* to nematode-free roots of Black. About 5,000 larvae of *M. hapla* were inoculated on to No. 2 plant alone. Investigations were made as mentioned above.

RESULTS

Intact susceptible tomato variety Black plants (A) were heavily infested with *M. incognita*. Their gall weight per cent was 54.1. There were no galls in the roots of intact resistant variety No. 2 (B). Table 1 shows the infectivity of nematodes on tomato roots of various combinations in cross-grafting. Fig. 1 shows the schema. In Black roots on which resistant No. 2 was grafted (C), the gall weight per cent became 33.4 which indicated reduction by approximately 38 per cent from that of (A). The roots of resistant No. 2 on which susceptible Black was grafted (D) showed a slight galling of 1.3 per cent in weight. In (E), there were no galls in the roots of No. 2 and Black had 32.3 per cent in gall weight. The roots of No. 2 in (F), contrary to (E), had 0.8 per cent galls in weight. This is not significantly different from (D). In (G) and (H), in which No. 3 was used

Table 1. INFECTIVITY OF *M. incognita* ON TOMATO PLANTS WITH VARIOUS COMBINATION OF CROSS-GRAFTING

Combination	(A)	(B)	(C)	(D)	(E)		(F)	
Tops	Black	No. 2	No. 2	Black	No. 2	—	Black	—
Roots	Black	No. 2	Black	No. 2	No. 2	Black	Black	No. 2
Grafting way	—	—	Stock	Stock	Scion	Stock	Scion	Stock
Gall index	100.0	0	86.6	17.5	0	85.5	95.5	11.1
Gall weight %	54.1	0	33.4	1.3	0	32.3	38.9	0.8

Combination	(G)	(H)		(I)		(J)		
Tops	Black	—	—	No. 3	No. 2	Black	Black	No. 2
Roots	Black	No. 3	Black	No. 3	No. 2	Black	Black	No. 2
Grafting way	Stock	Scion	Scion	Stock	Scion	Stock	Scion	Stock
Gall index	100.0	37.0 ^a	58.0	10.1 ^a	0	81.2	100.0	0
Gall weight %	56.5	5.8	—	—	0	35.0	55.9	0

5,000 larvae were inoculated to each plant after the completion of grafting. Investigations were made on the 15th day after inoculation.

^a The resistivity of No. 3 to *M. incognita* seemed to be less stronger than No. 2.

Table 2. SPECIFIC RADIOACTIVITY OF PLANT TISSUES BY THE METHOD OF WET COMBUSTION 2 DAYS AFTER THE APPLICATION OF ³²P-ORTHOPHOSPHATE TO ROOTS OF RESISTANT LINE No. 2

cpm/mg dry weight								
	(E)		(F)		(I)		(J)	
Tops	No. 2	—	Black	—	No. 2	Black	Black	No. 2
Roots	No. 2	Black	Black	No. 2	No. 2	Black	Black	No. 2
Grafting way	Scion	Stock	Scion	Stock	Scion	Stock	Scion	Stock
Inoculation of <i>M. incognita</i> to both plants								
Roots gall	—	62.3	41.4	155.5	—	70.4	19.8	—
Roots healthy	424.2	10.1	10.9	125.5	203.2	11.2	7.4	267.4
Ratio to No. 2 ^a	100.0	6.5	18.3	100.0	100.0	15.9	5.8	100.0
Leaves	113.7	—	73.2	—	193.9	14.9	10.6	157.2
Non-inoculation of the nematodes								
Roots					268.8	2.7	3.1	383.3
Ratio to No. 2 ^a					100.0	1.0	0.8	100.0
Leaves					201.3	7.9	56.2	321.8
Inoculation of <i>M. hapla</i> on No. 2 alone								
Roots	41.9	1.8	1.9	22.7	42.6	1.4	1.7	66.7
Ratio to No. 2 ^a	100.0	4.3	8.5	100.0	100.0	3.3	2.5	100.0

^a Ratio of ³²P-activity in whole root system to that of No. 2.

instead of No. 2, the roots the tops of which were cut were also more or less affected by the tops of the partner. In (I) and (J), infectivity of nematodes on susceptible plants depended on whether resistant No. 2 was employed for stock or scion in cross-grafting; the gall weight per cent of Black was 35.0 and 55.9 in (I) and (J)

respectively. The decrease of nematode infection in Black roots in (I) probably resulted from easy translocation of top products of No. 2 to Black root system. As far as tomato plants employed in this experiment are concerned, certain substance or substances which are supposed to exert resistivity to nematodes may be produced more in tops than in the plant roots.

Table 2 shows the specific radioactivity of the treated plants by the method of wet combustion. ^{32}P which was given to No. 2 or No. 3 roots moved remarkably to the gall tissues of Black roots. Accumulation of phosphorous compounds in galled roots was confirmed by autoradiograph, showing high activity in gall tissues. Fig. 2 shows an example in the case of (E). The movement of phosphorus from resistant line to galled roots of susceptible line brought about higher radioactivity in healthy parts of the infected root system than in the roots of Black which were not inoculated with nematodes. By setting the index at 100 for ^{32}P -activity estimated at No. 2 roots 2 days after application of ^{32}P , we found that Black roots with nematodes showed 6.5 in (E), 18.3 in (F), 15.9 in (I), 5.8 in (J), and the average 11.6, while that of Black roots without nematodes showed 1.0 in (I) and 0.8 in (J). Thus, the movement of phosphates from resistant to susceptible roots was increased with nematode infection.

The experiment to see the movement of ^{32}P from infected No. 2 to healthy Black did not yield satisfactory results, because No. 2 plants were too heavily

Fig. 2. Autoradiograph of ^{32}P which was applied to No. 2 roots.

- 1 (left) : Non-inoculated with nematodes.
 - left side : No. 2 which was given ^{32}P -orthophosphate.
 - right side : Black without galled roots.
- 1(right) : Inoculated with nematodes.
 - left side : No. 2.
 - right side : Black with galled roots showing accumulation of ^{32}P -phosphates on the gall tissues.
- 3 : Inoculated with nematodes, photographed with strong contrast.
 - left side : No. 2.
 - right side : Black showing only gall tissues on which ^{32}P -phosphates accumulated.
 - Uppermost : grafting joint.
 - Arrow : gall tissues.

Table 3. SPECIFIC RADIOACTIVITY OF ACID SOLUBLE PHOSPHATE
FRACTION IN GALLED AND HEALTHY ROOTS

	cpm/mg living tissue weight					
	Black				No. 2 or No. 3 into which ³² P was applied	
	Galled roots		Healthy roots			
	Ba-insoluble fraction	Ba-soluble fraction	Ba-insoluble fraction	Ba-soluble fraction	Ba-insoluble fraction	Ba-soluble fraction
E	2.9	0.7	1.2	0.2	18.9	2.7
F	3.2	1.6	2.6	0.5	19.7	2.3
G	4.5	1.8	—	—	16.8 ^a	3.5 ^a
H	7.3	3.1	2.9	0.5	16.2 ^a	2.4 ^a
I	2.5	0.8	1.5	0.3	14.2	3.7
J	2.2	0.6	2.3	1.4	17.3	2.7
Average	3.75	1.45	2.10	0.60	17.21	2.88
	(1.00) ^b	(0.39)	(1.00)	(0.29)	(1.00)	(0.17)

^a: No. 3.^b: Ratio to Barium-insoluble fraction.

damaged by *M. hapla* to absorb the nutrients containing ³²P enough for the purpose of this experiment. Nevertheless, the movement of ³²P to the Black root system was more than expected. This experiment should be conducted again with less nematode infection than the present experiment.

Table 3 shows the specific activity of ³²P in barium acetate soluble and insoluble fractions of acid soluble phosphates in galled and healthy roots. The former fraction (insoluble in ethanol) mainly include glucose-1-phosphate, glucose-6-phosphate, fructose-6-phosphate etc., the latter mainly nucleotides such as ATP or ADP. In general, barium-insoluble phosphate-esters were more in gall tissues than in healthy ones. Ratios of barium-soluble phosphates to insoluble ones were also increased with infection of nematodes; 0.39 at gall tissues, 0.29 at healthy tissues of Black roots, and 0.17 at roots of resistant line.

DISCUSSION

In preliminary tests ³⁵S-compounds did not move so much as ³²P from No. 2 roots to galled roots of Black. Therefore, the present investigations were focused on the movement of phosphorous compounds. One of the abnormality of galled roots may be characterized by the enhancement of phosphate metabolism, though investigations on potassium, calcium, amino-acids etc. were not made. NISHIZAWA (1957) demonstrated active translocation and accumulation of ³²P-phosphate and ³⁵S-sulphate to gall tissues from healthy roots, using the root-separation method, in tomato, sweet-potato and garden balsam, and suggested higher metabolic activities in the gall tissues. OTEIFA and ELGINDI (1962) also demonstrated the accumulation of ³²P-phosphates in gall tissues. The increment of nucleotides in gall tissues suggests active protein synthesis. BIRD (1961) reported that cytoplasm in giant cells induced by nematodes had 10 times as much protein as normal cells. OWENS

and NOVOTNY (1960) also reported that protein, nucleic acids, phosphorus and nitrogen were increased by 2-fold in galls. KOSTOFF and KENDALL (1930) have already postulated the movement of organic materials from healthy to gall tissues. DROPKIN and KING (1956) concluded that the unhealthy condition of the plant is not caused by the parasite "robbing" the host of nutrients. Thus, the rise of metabolic activity in gall tissues may bring about the concentration of vital elements on those sites and monopolize so as to restrict movement to the proper growing points. This will be one of the cause of the nematode damages on host plants. The movement of phosphorus from resistant roots to gall tissues of susceptible roots, depending on various grafting ways, seemed to be inversely related to nematode infection in susceptible roots (see (I) and (J) in table 2). Though there is no evidence that translocation of phosphorus is associated with a revelation of resistivity, the present investigations may offer a route through which the mechanism of resistance can be analyzed from the viewpoint of substance.

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