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# Response of resistant and susceptible soybean cultivars to *Heterodera glycines* races

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**Abstract.** Penetration and reproduction of the soybean cyst nematode (SCN), *Heterodera glycines*, were studied on roots and nodules of soybean cultivars Lee 68, Pickett and PI-90763. Histopathological alterations resulting nematode infection in soybean was also investigated. Penetration rates varied initially but were approximately the same in all cultivars 4 days after inoculation with either SCN race 1 or race 2. Most penetrated juveniles developed into mature females in susceptible Lee 68. Resistant PI-90763 and Pickett exhibited varied degrees of cell necrosis and tissue degeneration following nematode infection. Syncytia associated with SCN in resistant cultivars were characteristically small and filled with material that stained with safranin. Nodular tissues were found unsuitable for normal development of the nematode.

**Key words:** *Glycine max*; Histopathology; Races; Soybean cyst nematode.

## Introduction

Soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, has been studied in relation to reaction against different cultivars of soybean (*Glycine max* (L.) Merrill.). However, most of the work was limited to develop commercial soybean cultivars resistant to SCN with little information on penetration and histopathological changes in susceptible and resistant cultivars. Ross (1962) first reported variation in the ability of different populations of SCN to reproduce on resistant cultivar PI88788. Later findings of differences on soybean led to the designation of four races of SCN based on relative numbers of white females formed on Lee, Pickett, Peking, PI-88788 and PI-90763 (Ichinohe and Ascii, 1956). Triantaphyllou (1975) showed that selection of those few cysts produced on a resistant line (R-line) resulted in increased reproduction on the selecting R-line.

Almost similar rates of penetration was reported in resistant and susceptible cultivars of soybean (Acedo *et al.*, 1984; Endo, 1965). Acedo *et al.* (1984) reported that 14% of those juveniles that entered roots in compatible combinations developed into maturing females, compared with only about 1% in compatible combinations. Selection and reproduction of SCN on resistant cultivars was reported (Ichinohe and Ascii, 1956; Miller, 1970; McCann *et al.*, 1982; Riggs *et al.*, 1973; Starr *et al.*, 1983; Triantaphyllou, 1975; Whitefield *et al.*, 1965). Miller (1970) found lower reproduction in susceptible soybean than with lesser inoculum levels. Riggs *et al.* (1973) observed best reproduction of all races (1, 2, 3 and 4) on Lee and poorest on PI-88788 cultivars. Although histological responses have been reported in resistant and susceptible cultivars of soybeans (Acedo *et al.*, 1984; Endo, 1965; Gipson *et al.*, 1971; Rebois *et al.*, 1970; Ross 1958; Ross and Brim, 1957). No emphasis was given on the study of development of SCN on nodular tissues.

The objective of this investigation were : (i) to determine the rate of penetration and histopathological

changes in three cultivars of soybean infected with *H. glycines* races 1 and 2 with special emphasis on nodules, (ii) to determine the rate of reproduction in infected roots and nodules.

## Materials and Methods

*Penetration and Histology Study*

Seeds of three soybean cultivars, Lee 68. Pickett and PI-90763, were surface sterilized in a mixture of ethanol, commercial bleach (containing 5.25% sodium hypochlorite) and water (1 : 2 : 7, v/v/v) for 3 minutes. After several rinses in sterile water, seeds were germinated in vermiculite for one week at 25°C. Seedlings were transplanted to 15 cm pots containing fine white sand and inoculated with 200 mg of a commercial preparation of *Rhizobium japonicum* (The nitrajin Company, Milwaukee, WI). Plants were inoculated with the soybean cyst nematode (SCN) three weeks after transplantation to ensure proper nodule development. Cysts of races 1 and 2 were collected from established greenhouse stock on Lee 68 by elutriation (Byrd *et al.*, 1976). Cysts were crushed gently in a Ten-Broeck homogenizer and eggs were passed through 65 and 26 $\mu$m screens with the eggs retained in the later. The crushed eggs were cleared of soil by centrifugation at 500 g for 3 minutes into a cushion of 20% sucrose (w/w) in a swinging bucket rotor. The eggs, banded at the interface between aqueous and sucrose layers, were collected in a 26 $\mu$m sieve, rinsed with water and resuspended in water for hatching. Each pot was inoculated with 2,000 juveniles of either race 1 or race 2. Plants were uprooted and washed gently in water to prevent falling of nodules from the roots 1, 2, 4 and 8 days after inoculation. Penetration studies were done by using staining technique of Byrd *et al.* (1983). Total number of juveniles in roots and nodules were counted.

For histological studies, roots and nodules were fixed in formalin-acetic alcohol (FAA) after 1, 2, 4, 8, 10, 21 and 35 days of inoculation. Material was dehydrated in a tertiary butyl alcohol (TBA) series and embedded in tissuemat. Sections were cut at 12 $\mu$m with a rotary microtome, mounted with Haupt's adhesive and 4% formalin and stained with Johanson's quadruple stain (Johanson, 1940).

*Reproduction of SCN in Roots and Nodules*

Randomized complete block design experiment employed to study the rate of reproduction of SCN races 1 and 2 in three soybean cultivars. One week old seedlings were transplanted to 15 cm pots and inoculated with rhizobia as described above. All plants received one-half strength Hoagland's nutrient solution, minus nitrogen twice a week. Each pot was inoculated with 2000 eggs of SCN race 1 or 2. Samples were collected after 5 weeks of inoculations and number of cysts/plant were counted to determine the reproductive index.

## Results

*Penetration*

There was no significant difference in the number of juveniles in the roots and nodules among the cultivars tested (Table 1). Fewer juveniles were found in roots and nodules of cultivar PI-90763 than in those of cultivars Lee 68 and Pickett.

*Histopathological Changes*

Initial responses of Lee 68, Pickett and PI-90763 to the infection of the SCN were similar one day after inoculation. Juveniles were found in cortex lying in various directions. Intracellular migration of juveniles lead to distortion and killing of cells in the path. After two days. Juveniles were found established in cortex and stelar tissues (Fig. 1). Invaded cells were stained with safranin.

In roots of Lee 68, juveniles underwent molting 4 days after inoculation. More cortical distortion was associated with female than male. Syncytial formation was due to cell wall breakdown. Each syncytia had nuclear enlargement, dense cytoplasm and thick cell wall with transfer cell-like characters. Syncytia with prominent wall ingrowths near xylem elements were noted. Syncytia associated with female remained functional until egg production, whereas those with male showed early degeneration. In Pickett, necrotic response was in cells near to nematode 4 days after inoculation. Syncytia started to degenerate and were heavily stained (Fig. 2). In race 1 infected roots, only few juveniles underwent further molting and most of them developed to males. Few mature females were noted in roots infected with race 2. Resistant reaction was found in the form of disorganized and necrotic cells. Syncytia were small in size, heavily vacuolated

**Table 1.** *Penetration of* ***Heterodera glycines*** *(Races 1 and 2) in three cultivars of soybean*

Each observation is a mean of 10 replicates.

| Soybean cultivar | Days after inoculation | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1 Day | | 2 Day | | 4 Day | | 8 Day | |
| | Root | Nodule | Root | Nodule | Root | Nodule | Root | Nodule |
| Lee 68 (R 1) | 164 | 3.1 | 210 | 15.8 | 337 | 17.3 | 351 | 21.5 |
| Lee 68 (R 2) | 158 | 5.3 | 180 | 9.6 | 335 | 20.0 | 346 | 19.0 |
| Pickett (R 1) | 150 | 4.8 | 207 | 8.3 | 328 | 17.3 | 341 | 22.0 |
| Pickett (R 2) | 167 | 5.6 | 194 | 3.6 | 306 | 22.2 | 318 | 26.0 |
| PI-90763 (R 1) | 129 | 12.0 | 262 | 1.8 | 252 | 8.1 | 320 | 11.0 |
| PI-90763 (R 2) | 88 | 11.3 | 157 | 11.7 | 316 | 8.1 | 321 | 19.7 |

**Table 2.** *Reproduction of* ***Heterodera glycines*** *races 1 and 2 on soybean cultivars Lee 68, Pickett and PI-90763*

Each observation is a mean of 10 replicates

| *H. glycines* races | Average cyst production per plant on soya cultivars | | | Cyst indices* for races | |
|---|---|---|---|---|---|
| | Lee-68 | Pickett | PI-90763 | Pickett | PI-90763 |
| R 1 | 217 | 6 | 4 | 2.76 | 1.84 |
| R 2 | 192 | 119 | 10 | 61.97 | 5.20 |

$$*\text{Cyst Index} = \frac{\text{No. of cysts on cultivar}}{\text{Average number of cysts on Lee}} \times 100$$

and differed from those of cultivar Lee 68. Degeneration of syncytia took place 8 days after inoculation. Thick cell wall deposition of syncytium nearby nematode head was noted. Cells showed cell wall breakage and cytoplasmic and nuclear degeneration. Large spotted necrotic areas in sections were due to early degeneration of syncytia and limited nematode development.

Cultivar PI-90763 showed brisk resistant reaction with both races of SCN. Syncytia formed near head of juvenile were short lived and found nearby collapsed juvenile. Large necrotic regions with degenerated syncytia and damaged cortical cells were the resistant response of this cultivar. Areas where nematode are present were found to be walled off by secondary wall material (Fig. 3). All the affected cells were dead and filled with dark stained material. Hypersensitive reaction of cultivar is characterized by unusual small syncytia, which degenerate soon after their formation, leading to death of nematode. Except in few instances where Race 1 was found upto 3rd stage, mostly juveniles die before passing through developmental stages. No mature female was observed in this cultivar, however, 4th stage and adult males were observed (Fig. 4).

*Nodules*

Histological changes inside nodules were similar in three cultivars. Usually juvenile penetrate after 1 day of inoculation and finally reside in root cortical zone (Fig. 5). Most of juveniles remained as such without further molting and failed to induce syncytia in these tissues. Cells nearby nematode showed necrotic responses. In cultivar Lee 68, few third stage and young females were observed and in one case, full mature cyst was observed on nodule (Fig. 6). In general, heavy necrotic response lead to death of nematode. No molting beyond second stage was a common feature in cultivars Pickett and PI-90763. Nematodes were not seen inside the nodular cortex and bacteroid zone. Most of juveniles which underwent molting gives rise to male and a few female were observed. Syncytia were similar in size and degenerated very soon showing necrotic response.

*Reproduction of SCN*

Race 1 of the SCN reproduced poorly on cultivars Pickett and PI-90763 as compared to Lee 68 (Table 2). Cyst indices showed higher reproduction rate of race 2 on pickett. There was no significant difference between Pickett and PI-90763 in the number of cysts produced when race 1 was applied. However, there was a significant difference when race 2 was used as inoculum. In both cases, numbers of cysts on Pickett and PI-90763 (average together) were different from Lee 68. These findings confirmed that PI-90763 is incompatible to both races of SCN while Pickett is to race 1. These observations correlate the histological

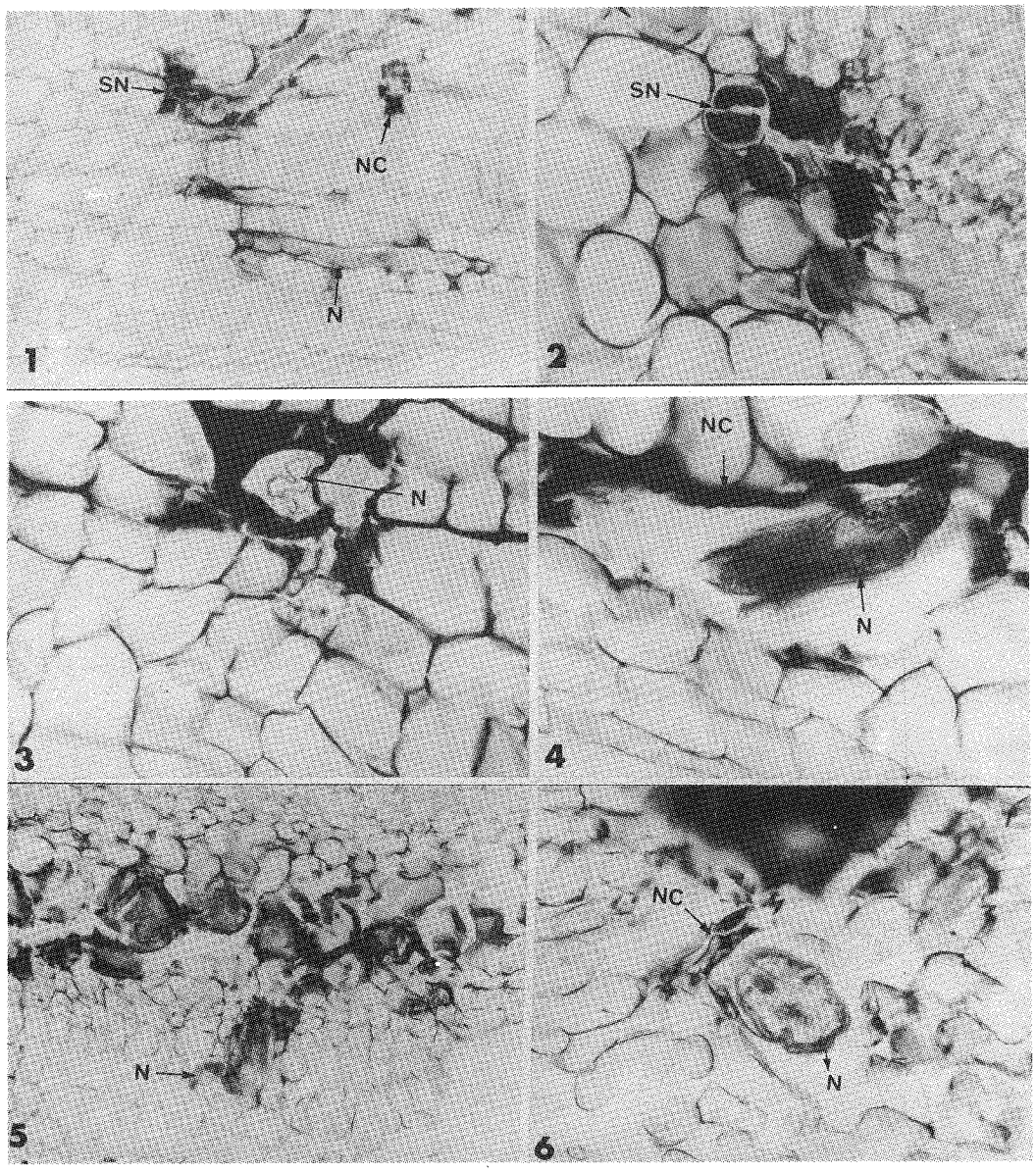


Fig. 1-6 Response of resistant and susceptible soybean cultivars to *Heterodera glycines* races.

Fig. 1 L.S. root cultivar Lee-68, Second stage larvae (R-1) in cortex and stele, Syncytium, Nectosis.

Fig. 2 T.S. root cultivar. Pickett (R-1), Syncytia degenerate, Necrotic response.

Fig. 3 T.S. root cultivar PI-90763 (R-2), Nematode is walled off by secondary wall material.

Fig. 4 T.S. root cultivar PI-90763 (R-1), Adult male, Necrosis.

Fig. 5 T.S. nodule cultivar Pickett (R-1), Nematode in root cortical zones.

Fig. 6 T.S. nodule cultivar Lee -68 (R-2), Mature female in cortical zone.

(N=Nematode; NC=Necrosis; SN=Syncytia; LS=Longitudinal sections; T.S.=Tangential sections).

findings where suppression of nematode development was noted in resistant cultivars.

## Discussion

Juvenile penetration of roots was generally similar in resistant and susceptible cultivars infected with SCN races 1 and 2 after 4 days of inoculation. Similar findings were observed for other soybean cultivars (Acedo *et al.*, 1984; Endo, 1965). No suitable explanation can be given to initial variation in rate of juvenile penetration in susceptible and resistant cultivars. Variation in observed data's may be due to problem in collecting such type of information. Preferential juvenile penetration in root to nodule may possibly be due to easy penetration in tender root as compared to nodular tissues which have thick epidermal tissue.

In both resistant and susceptible cultivars, most juveniles were found at feeding sites within 2 days of inoculation. Early development was observed to be similar in all the three cultivars but later on pronounced resistant reaction was noted in resistant cultivars. Most of the juveniles degenerated at the feeding site within 5 to 7 days and did not develop beyond second stage in cultivar PI-90763, due to necrotic responses. However, some third stages were noted in PI-90763 infected with race 2. A greater number of males vs female in PI-90763 and Pickett is due to resistant response, where syncytia die soon after their formation, resulting in poor food supply for female development. Thick cell wall deposition on syncytium nearby nematode head prevent feeding of nematode.

Necrotic resistant reactions are similar as reported for other cultivars (Acedo *et al.*, 1984; Endo, 1964, 1965; Golden *et al.*, 1970; Riggs *et al.*, 1977; Ross, 1958). Ross (1958) reported no syncytia in resistant Peking while small syncytia were observed in resistant PI-90763 and Pickett, which degenerate very soon. This is in conformity with observation of Endo (1965) in resistant Peking. Syncytia deterioration followed by collapse of juvenile in resistant plants was due to less available food for further development. Necrosis of syncytia was noted in resistant cultivars as observed by Endo (1965) in Peking, in cereals (*H. avenae*) by cock (1974) and in sugarbeet (H. schachtii) by Yu and Steele (1981). Riggs (1966) reported difference in free amino acids and phenolic compounds in roots of susceptible Lee and resistant Peking while Chambers and Epps (1969) has concluded that resistant is genetically inherent in the entire plant, but is expressed itself as localized tissue reaction at sites of root infection. Most of the histopathological observations on Lee 68 are similar as reported earlier (Acedo *et al.*, 1984; Endo, 1964, 1965; Jones and Dropkin, 1975). Syncytia with characteristic of transfer cells are observed (Jones and Dropkin, 1975). Resistant cultivars have small characteristic syncytia as reported by Acedo *et al.* (1984) in association with P-89 in PI-209352 plants.

Juvenile penetration of nodules varies in different cultivars after 1 and 2 days of inoculation but later on almost similar number of juveniles were counted in all cultivars. Less juveniles in nodules be due to less favourable site for penetration whose epidermal cells are thick and rough as compared to tender root. The failure of penetrated juvenile to reproduce normally in most of the cases clarified that nodular tissues are unsuitable for their development. The observations confirms with earlier report (Barker *et al.*, 1972). However, successful development was noted on clover to *Meloidogyne javanica* and *H. trifolii* (Taha and Raski, 1969) on soybean to *Rotylenchulus reniformis* (Rebois *et al.*, 1970) and *M. incognita* (Barker and Hussey, 1976). No nematode presence was observed in nodular cortex and bacteroid zone, which such findings were reported for *R. reniformis* (Rebois *et al.*, 1970) and *M. incognita* (Barker and Hussey, 1976) on soybean. Thick xylem elements in nodules may be one of the barrier for juvenile to enter deep inside nodule.

Results of reproduction of SCN on three cultivars support the observation made by Riggs *et al.* (1973) and Triantaphyllou (1975) that PI-90763 was resistant to races 1 and 2 and Pickett was resistant to race 1. Normal reproduction was observed on Lee 68.

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# 抗病性與感病性大豆栽培品種對線蟲*Heterodera glycines*之反應

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本文在探討包囊線蟲 *Heterodera glycines* 對大豆根部與根瘤之侵入、生殖，以及對寄主組織病理之變化情形。*H. glycines* 小種 1 與小種 2 對不同大豆品系之侵入速率雖不同，但第 4 天後即趨於一致。多數侵入之幼蟲在感病性之大豆 Lee 60 品系內發育爲成熟之雌蟲，但在抗病性之 PI-90763 和 Pickett 品系內則造成不同程度之細胞壞死與組織退化。因線蟲感染所引起之多核體細胞，在抗病性品系中其細胞小，並充滿可被沙黃染色之物質。此研究亦發現根瘤組織不適於線蟲之正常發育。