Journal of Phytology 2010, 2(1): 84-87
Phytopathology
ISSN: 2075-6240
Available Online: www.journal-phytology.com

REGULAR ARTICLE

EVALUATION OF FOUR SOYBEAN VARIETIES RESISTANCE AGAINST SOYBEAN CYST NEMATODE (*HETERODERA GLYCINES*) IN GREENHOUSE CONDITION

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SUMMARY

In this study, during the years 2005-2006, 70 soil and infected root samples were collected from different regions in Golestan province. Nematodes were extracted from soil and root samples followed by mounting in glycerin. Microscopic slides were prepared and morphological as well as morphometric characteristics were studied using light microscope. Four commonly soybean varieties including Sahar, DPX, Gorgan-3 and Wiliames used for observation synchytia as well as comparison their susceptibility. For this purpose, soybean seedlings were artificially inoculated with 4000 eggs and larvae which obtained from collected cysts on soybean seedlings per pot. The total number of cysts produced in infected roots of four soybean varieties was recorded after 4 month. Resistance of varieties against *Heterodera glycines* was also measured by average length and width of synchytia and nematode reproduction rate. There are no cysts on DPX variety while four cysts in Sahar, eight in Gorgan-3 and 15 cysts on Wiliames could be observed. Also, results showed that the most sensitive varieties were Wiliames, Gorgan-3 and Sahar, respectively and DPX is diagnosed as resistant one.

Key words: Soybean, Cyst nematode, *Heterodera glycines*, Resistance

S. Shahabi et al. Evaluation of Four Soybean Varieties Resistance Against Soybean Cyst Nematode (*Heterodera glycines*) in Greenhouse Condition. J Phytol 2/1 (2010) 84-87.
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1. Introduction

Soybean is an annual Dicotyledon plant belongs to Fabaceae family and one of the four most important crops in the world. It is also one of the most strategic plants not only as food consumption but also for industrial uses. Soybean oil is important component of edible oil and could be inverted to various kinds specially margarine and solid oil. Soybean cyst nematode, *Heterodera glycines* is one of the most destructive pathogen in Canada and United states which causes severe damages to host plant roots and have high reproduction rate with a long time survival in soil. One of the most important hosts for this nematode is soybean. Total losses of this pathogen for farmers of soybean during 1997-1998 estimated about 25 million dollar in Ontario. Since pathogen had no specific sign on host plant so farmers have serious problem for disease identification. It's known in Iran as important quarantine nematode for the first time in 1380 from Dasht-e-Naz in Mazandaran province (1). Now the infected regions are Dasht-e-naz in Mazandaran province as well as Kordkooy, Bandar Gaz, Hashem Abad, Jalin, Taghi Abad, Ghorogh and Esfahan Kalateh regions in Golestan province (2). The objective of current study was measuring some commonly soybean varieties including Sahar, DPX, Gorgan-3 and Wiliames for observation synchytia as well as comparison their susceptibility against *Heterodera glycines*.

2. Materials and Methods

During the years 2005-2006, soil and infected root samples were collected from fields which showed any signs related to causing agent from different regions in

Golestan province. During sampling, roots studied for the presence of cyst with stereomicroscope. Collected samples were transferred to laboratory conditions and kept refrigerated at 4°c temperature. Nematodes were extracted from soil by sieving and centrifugation method followed by fixing and transmission them to pure glycerin according to the modified method proposed by De-Greesse (3). Nematode cysts were also extracted by Fenwick method (4). Microscopic slides were prepared and morphological as well as morphometrical characteristics were studied by light microscope. Nematodes were propagated in order obtaining enough population of egg and larvae for soybean inoculation. Soybean seeds surface sterilized by sodium hypochlorite (2%), then planted in 15cm diameter plastic pots containing sterilized soil (a seedling per pot). Following 5-7 days, when soybean reach to 2 leaves stage, nematode cysts separated and broken by homogenizer, then dilution series of larvae as well as eggs prepared by Hosi *et al* (5) method, and about 4000 eggs and larvae were added per pot. One month after inoculation, roots were studied for formation of cysts. 120 days after planting, the probable host plant cells changes were examined. For this purpose, root washed thoroughly and cut to 0.5 cm pieces and cross sections were prepared by microtome. In order to fix samples, roots immediately treated with FAA using O'brain and Mcculley (6) method and kept for 24 hours. After dehydration, impression, incision, staining and clearing, root samples were studied under microscope.

3. Results

In DPX variety, roots had more bacterial nodules. There was no cyst on DPX variety roots after 120 days, but high population of female nematodes were observed after washing and blending infected roots. The numbers of cysts formed after four months on root surface and average long and width of synchytia in each soybean cultivars were considered as another index for determination host plant cultivars resistance. These characters were indicated in Table 1.

Table 1- Numbers of cysts and synchytia average diameter found in soybean cultivars

Cultivars	Numbers of Cysts	Synchytia Average Diameter (Long×Width) (µm)
Williams	15	64.26×42.08
Gorgan-3	8	56.015×34.23
Sahar	4	31.79×13.85
DPX	0	----------

As indicated above, the most sensitive cultivars were Williams, Gorgan-3 and Sahar, respectively. Also, the most resistant cultivar was DPX.

4. Discussion

Root samples were infected by too many L_2 larvae, so in infected area large amounts of synchytia could be observed in each section (Figures 1, 2, 3, 4). Due to infection of nematode inside cell, infected larvae firstly effect on epidermis followed by intracellular movement and permanent pressure using its stylet to reach in feeding site which could be in internal cortex, endoderm, or vessels parenchyma. Consequently, all of epidermal tissues might be destroyed. During growing of L_2 larvae, its body get swollen and then through molting changed to L_3, L_4 and finally young adult females which fertilized by male nematodes. In this stage, swelling of nematode body pressured cortex tissue cells and caused rupture and breaking in this layer which separated from roots after nematode death but its location on root could be remained. Synchytia in feeding sites play an important role as metabolic sinks or location with several active metabolites

which transmitted after photosynthesis from leaf to roots. These synchytia are composed of 2-120 cells with different size and shape among host plant different varieties.

Penetration pattern was not significantly different among sensitive and resistant varieties. in sensitive cultivars, synchytia were active not only at the end of nematode feeding but also after laying eggs. But in resistant ones, these structures were destroyed followed by nematode stationary phase and formation of feeding site by L_2 due to the host resistance. So, larvae did not have enough energy to move and found a suitable place for synchytium formation. Nematodes differentiated to producing males, exited from roots, so the synchytium filled with parenchyma cells. There was no cyst on DPX variety roots after 120 days, but high population of female nematodes were observed after washing and blending infected roots.

Attachment of Rhizobia to the root surface could be ceased by *Heterodera glycines* due to changing in Lectin production (7) which could be an important factor in assessment of variety resistance. in DPX variety, roots had more bacterial nodules. In sensitive cultivars, synchytium consisted of 2-120 cells so; the size of synchytium could be a suitable index for resistance assays. The numbers of cysts formed after four months on root surface were considered as another index for determination host plant cultivars resistance.

Figure1- Synchytium structure in longitudinal section

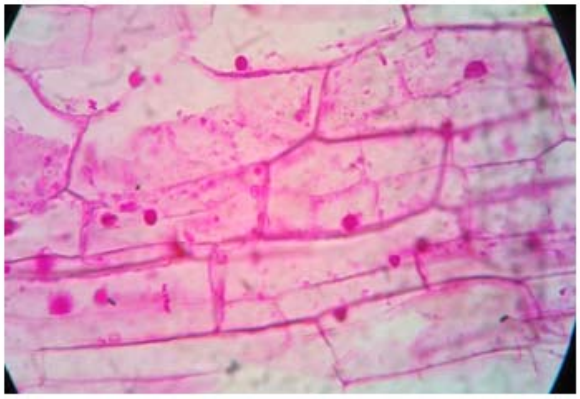


Figure 2- Synchytium structure in cross section

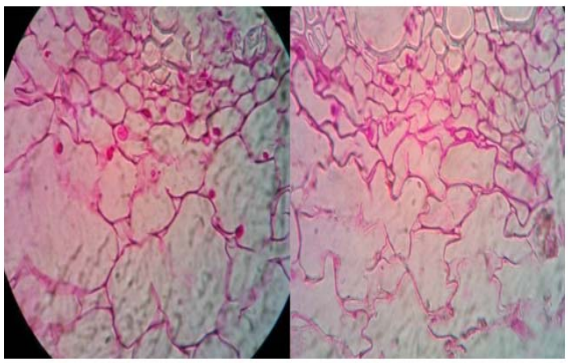


Figure 3- Head and lip region of nematode in cross section of the synchytium

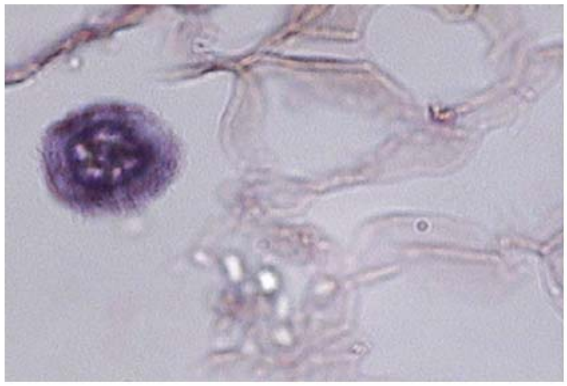
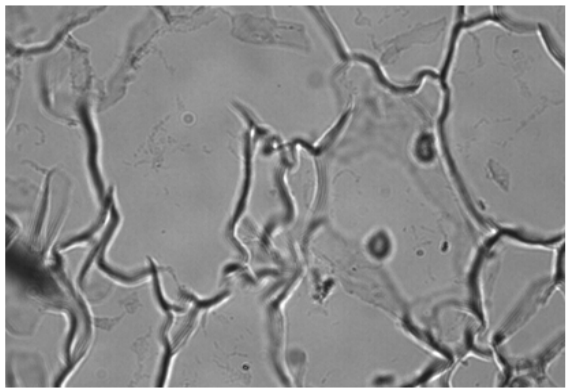


Figure 4-formation of synchytium and thickening of wall



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