REACTION OF SOME SELECTED SOYBEAN VARIETIES (Glycine max (L) Merrill) TO ROOT – KNOT NEMATODE INFECTION

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Abstract
Two-year screen-house studies to evaluate the reaction of five soybean varieties (TGX – 1987 – 34F, TGX -1987 – 38F, TGX – 1987 – 95F, TGX – 1986 -3F, TGX – 1985 – 8F) to root – knot nematode, Meloidogyne incognita, were conducted in the Faculty of Agriculture, University of Ilorin. Forty experimental pots containing pasteurized soil were used. Twenty pots were inoculated each with approximately 3,000 eggs of M. incognita while the other twenty which did not receive any treatment served as control. The experimental set up was a completely randomized design having each treatment replicated four times. Results showed that all the varieties were susceptible to M. incognita at varying degrees. While plant height of nematode inoculated varieties were significantly (P =0.05) lower than the un-inoculated plants, among the same varieties, there were significant differences in the number of branches of inoculated and non inoculated plants between different and among the same varieties. There were varietal differences in terms of seed weight which represented actual yield. Generally, the un-inoculated plants gave significantly higher yield than inoculated ones. Root gall infestation rated the same level for all the five varieties. However, variety TGX-1985 – 8F exhibited superior characteristics over the other four varieties because it showed higher level of tolerance to nematode infestation judging from its performance and yield. From the study, it can be deduced that the use of nematode resistant/tolerant Soybean varieties be adopted in the management of root- knot nematode infestation. It is one of the cheapest and safest control methods that pose no form of hazard to man and the environment. In nematode endemic ecological zones, TGX-1985 – 8F is therefore recommended as it proved to contain some specialized genes that conferred a higher level of tolerance against root- knot nematode, Meloidogyne incognita.

Key Words: Glycine max, root – knot nematode, Dominant loci, Mi – 1.2, leucine zipper and R genes.

Introduction
Soybeans and soya beans are common names for an annual leguminous crop belonging to the family Fabacea and genus, Glycine. Soybean has been one of the five main plant foods of China along with rice, barley, wheat and millet (Lance and Garren, 2005). Over the years, it has become one of the most economically versatile crops cultivated worldwide. In Nigeria, soybean cultivation has expanded as a result of its nutritional, economic and diverse domestic usage. Dugje et al. (2009) enumerated some benefits derived from growing soybean to include; source of good food soymilk, soy cheese, tom bran (infant
weaning food). It is a source of excellent vegetable oil. It improves soil fertility and controls parasitic weed *Striga hermontheca*. Soybean cake is an excellent livestock feed especially for poultry. The haulms provide good feed for sheep and goat. It is used in industry.

According to Stallings and Lupo (2009), soybean also has the potential to decrease photo aging of the skin and prevent skin cancers through the oestrogen type and the antioxidant effects of its metabolites. Soybean flour is becoming increasingly important as an ingredient of foodstuffs and bakery products such as bread, biscuits and cakes. Because of its low starch content, the flour forms an ideal ingredient of food for diabetic patients all over the world (Kochlar, 2009). Soybean diet is a low fat diet that can generate positive impulse in the atherosclerosis and formations of artery blocking blood clots are reduced (Shidhaye *et al*., 2009). Being a plant protein, soybean is free from both steroids and antibiotics animal protein content.

As agriculturists try to feed the fast growing world, production of soybeans has been faced with a lot of challenges which result in yield losses or reduction in yield. The reasons attributable to such production constraints range from physiological and growth factors to diseases and pests (Sikora *et al*., 2005). Many pathogenic organisms are responsible for disease manifestations which in turn result in yield loss. They include nematodes, fungi, viruses and bacteria (George, 2004; Singh, 2009; IITA 2009).

Plant parasitic nematodes from several genera including both the economically important cyst and root knot nematodes modify plant cells into feeding sites able to support sedentary females. The most characteristic symptom is the appearance of brownish or dark swellings (galls) all over the root system (Gangawane and Khilare, 2008). The degree of root galling generally depends on three factors: nematode population density, nematode specie and host plant cultivar (Mitkowski and Abawi, 2003).

Root knot nematodes are silent killers that cause high rate of losses in the aggregate and are yield limiting. Hence much attention should be given to nematode control. In view of the hazards associated with the use of chemical nematicides, management strategies which are eco – friendly, effective and sustainable are sought after. The potential of soybean tolerance or resistance was investigated in these trials. The main objective of the study was to evaluate some selected soybean cultivars for nematode tolerance and susceptibility resistance.

**Materials and Methods**

A screen-house experiment was conducted at the Faculty of Agriculture, University of Ilorin for two years. Five soybean varieties were obtained from the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria. The varieties of soybean include TGX – 1987 – 34F, TGX -1987 – 38F, TGX –1987 – 95F, TGX – 1986 -3F, TGX – 1985 – 8F.

Seven and half kg each of pasteurized soil was weighed into 40 perforated plastic buckets. Two seeds sown at 5cm depth per pot and each variety was replicated four times for the infected and for the un-inoculated control. The pots were placed on blocks to avoid reintroduction of microorganisms from the soil. One week after germination, seedlings were thinned down to one vigorous plant per pot. Following the method described by Southey (1986), root – knot nematode eggs were extracted from galled root of *Celosia argentea* (L). The eggs suspension was standardized to contain 300 eggs per ml.
For each variety of the soybean, four buckets were inoculated with 10ml of nematode egg suspension whereas the remaining four pots served as control. The whole set up was a factorial experiment fitted into a randomized complete block design.

Data Collection and Observation

The plant parameters measured are as follows: number of leaves, plant height, number of pods, weight of pods and visual physical conditions of the plants. At harvest, roots were assessed for galling using the rating scale described by Taylor and Sasser (1978).

Table 1: Root Gall Rating

| Rating | Number of galls | Host reaction |
|--------|-----------------|---------------|
| 0      | 0               | Immune        |
| 1      | 1 – 12          | Resistant     |
| 2      | 3 – 10          | Moderately resistant |
| 3      | 11 – 30         | Susceptible   |
| 4      | 31 and above    | Highly susceptible |

Taylor and Sasser (1978)

Result and Discussion

Results of the growth parameters measured on the effect of treatment on plant height and number of leaves followed almost the same trend in the two-year trials. No significant difference was observed between the nematode inoculated and un-inoculated plants of the same variety from week one to week four but significant differences were observed among the different varieties. However, at week five, there were significant differences only in the height of inoculated and un-inoculated plants of the same variety while the number of branches and leaves of inoculated and control plants were not only significantly different among the same variety but also between different varieties (Tables 2, 3 and 4).

Table 5 shows the root – knot nematode effect on the number of pods, pod weight, root galls and seed weight, shoot weight and root weight. No significant differences (p= 0.05) were observed in the number of pods from inoculated and control plants of the same variety but there were significant differences in the number of pods produced by different varieties. Significant differences were recorded in the weight of pods within the same and different varieties. However, variety TGX – 1985 – 8F recorded the highest pod weights of 49.09g and 43.81g in both un-inoculated and inoculated respectively. Variety TGX – 1987 – 95F recorded the lowest pod weight for un-inoculated and inoculated control plants; 32.18g and 27.73g respectively. There were significant differences in the root and shoot weights of treated and control pots within and among varieties.

The root weights of nematode – inoculated plants were significantly lower compared to their un-inoculated counterpart in all the varieties. Shoot weight also followed the same trend. There were significant differences in the weight of seeds produced by all the varieties. Generally, the seed weights of the control plants were significantly higher than those obtained from the nematode inoculated pots. There were no significant differences in the mean number of galls among all the Meloidogyne inoculated varieties while the control plants had no root galls on any of them. Although all the varieties were susceptible to root – knot nematode but the level of response to the pathogen varied from one variety to another. TGX – 1985 – 8F recorded the highest pod and seed weights followed by TGX – 1987 – 3F, TGX -1987 – 38F, TGX – 1987 – 34F and finally TGX – 1986 -95F.

Chlorosis was observed at varying degrees across all the inoculated plants at the termination of the experiment. Galls were present in all the inoculated varieties and ranged between eleven (11) and thirty on their roots.
From the results obtained, all the five varieties of soybean screened were susceptible to root–knot nematode infection. This was informed by the fact that all the nematode inoculated plants performed significantly lower when compared with their uninoculated counterparts in terms of growth, yield parameters and galling of roots. Apparently, the nematode population of the soil was high to cause damage and reduce yield. As a result of nematode feeding, galls of varying sizes and numbers were formed around the root systems of the infected plants. It is believed that upon perception of food signal, parasitic nematodes (including the root–knot nematode, *Meloidogyne spp.*) penetrate the root cell, establish a feeding site, induce cellular modification in root tissues, leading to formation of galls (Bird and Kaloshian, 2003). Plant nutrients are diverted to the galls, invariably leading to reduced translocation of food to other parts of the plant thereby translating to poor growth and low yield. Studies have shown that root–knot nematode infestation on host crops results in root galling, stunted growth and general low productivity (Pandey and Kalra, 2003; Mitkowski and Abawi, 2003). The significantly higher yield produced by TGX – 1985 – 8F shows that the variety is more tolerant to nematode infection. This variety therefore, could be cultivated in nematode infested soils when a selection is to be made from the five varieties evaluated. However, identifying the genes responsible for the resistance would help breeders facilitate their search for the resistant varieties. It had been reported that dominant loci conferring resistance to root–knot nematode have been identified in a number of plants (Bird and Kolashian, 2003). The best studied nematode–resistance gene is *Mi* – 1.2, which has been cloned and found to be a member of the leucine zipper, nucleotide binding, leucine – rich repeat family of plant R genes (Millligan et al., 1998). This constitutively – expressed gene (Martinez de Ilarduya and Kaloshian, 2001) confers resistance to *Meloidogyne incognita*, *M. javanica* and *M. arenaria*, but not to *M. Hapla*, even though these four species are present sympatrically. Recently, Science News line (2012) reported that scientists have identified three neighbouring genes that make soybeans resistant to the most damaging nematode disease (cyst nematode) of soybean. They explained that the genes exist side by side on a stretch of chromosomes, but only give resistance when the stretch is duplicated several times. Since all the tested varieties were susceptible to root- knot nematodes with just one variety showing higher tolerance to *M. incognita*, one could infer that the three neighbouring genes on a stretch of chromosomes may have duplicated minimally but more in TGX – 1985- 8F to allow a level of tolerance developed, making it superior to other varieties in terms of response to nematodes infection and yield. High yield recorded was not dependent on vegetative growth as taller varieties gave lower yield.

The present study has therefore broadened our knowledge on the interactions between some soybean varieties and root–knot nematode, *M. incognita*. However there is need to understand the exact genes responsible for *M. incognita* resistance or tolerance in soybeans. This will help breeders focus on the development of soybean varieties that are not only resistant to root–knot nematode but are also high yielding. According to Wall (2012), understanding this interaction will lead to the development of new novel strategies to enhance the nematode resistance of soybean. Moreover, resistant plant varieties are generally cheap and safe to use as they pose no environmental hazards.
Table 2: Effect of treatment on plant height in cm (mean of 4 replicates)

| Variety   | Treatment    | Week1  | Week2  | Week3  | Week4  | Week5  | Week6  | Week7  |
|-----------|--------------|--------|--------|--------|--------|--------|--------|--------|
| TGX –     | Inoculated   | 18.3a  | 24.0a  | 29.5a  | 37.8bc | 37.8bc | 42.3c  | 45.5c  |
| 1987-34F  | Un-inoculated| 17.8a  | 24.0   | 29.5   | 41.5a  | 52.8a  | 55.0ab | 57.0ab |
| TGX –     | Inoculated   | 13.0c  | 19.0ab | 25.5ab | 38.0ab | 39.3bc | 40.8cd | 41.8cd |
| 1985-8F   | Un-inoculated| 14.5bc | 19.5ab | 25.5ab | 36.5ab | 40.8ab | 42.8c  | 43.88c |
| TGX –     | Inoculated   | 12.3c  | 16.8d  | 21.0bc | 31.3bc | 37.3bc | 38.3cd | 39.5cd |
| 1987- 38F | Un-inoculated| 12.3c  | 17.3b  | 20.3bc | 31.5bc | 40.8bc | 44.5c  | 43.3c  |
| TGX –     | Inoculated   | 12.8c  | 16.0b  | 18.3c  | 27.5c  | 31.8c  | 34.0d  | 35.3d  |
| 1986-3F   | Un-inoculated| 12.5c  | 18.5ab | 24.5abc| 34.5abc| 39.3bc | 38.0cd | 40.5cd |
| TGX –     | Inoculated   | 14.0c  | 19.5ab | 24.0abc| 37.0ab | 47.5ab | 52.0b  | 58.8ab |
| 1987-95F  | Un-inoculated| 12.8c  | 21.3ab | 25.5ab | 41.0a  | 52.5a  | 60.8a  | 65.5a  |
| S.E       |              | 1.2    | 1.8    | 2.1    | 2.3    | 3.2    | 2.3    | 2.4    |

Mean values with different letters (a, b, c, d) in the same column are significantly different at p= 0.05 using Duncan’s Multiple Range Test.

Table 3: Effect of treatment on the number of Branches (mean of 4 replicates)

| Variety   | Treatment    | Week1  | Week2  | Week3  | Week4  | Week5  | Week6  | Week7  |
|-----------|--------------|--------|--------|--------|--------|--------|--------|--------|
| TGX –     | Inoculated   | 2.0a   | 3.0a   | 3.3ab  | 4.0b   | 4.0b   | 4.0d   | 4.8c   |
| 1987-34F  | Un-inoculated| 2.0a   | 3.0a   | 3.0b   | 4.5ab  | 5.5a   | 5.8ab  | 6.5a   |
| TGX –     | Inoculated   | 2.0a   | 3.0a   | 3.3a   | 4.0b   | 4.5ab  | 5.0bcd | 5c     |
| 1985-8F   | Un-inoculated| 2.0a   | 3.0a   | 3.3a   | 4.5ab  | 4.8ab  | 5.8ab  | 6.3ab  |
| TGX –     | Inoculated   | 2.0a   | 3.3a   | 3.3a   | 4.3ab  | 4.3b   | 4.8bcd | 5.0c   |
| 1987- 38F | Un-inoculated| 2.0a   | 3.0a   | 3.0a   | 31.5bc | 40.8bc | 44.5c  | 43.3c  |
| TGX –     | Inoculated   | 2.0a   | 3.0a   | 3.3ab  | 4.8a   | 4.5ab  | 5.3bc  | 5.3bc  |
| 1986-3F   | Un-inoculated| 2.3a   | 3.3a   | 3.8a   | 5.0a   | 5.5a   | 6.5a   | 6.5a   |
| TGX –     | Inoculated   | 2.0a   | 3.0a   | 3.0b   | 4.3ab  | 4.3ab  | 4.5cd  | 5.0c   |
| 1987-95F  | Un-inoculated| 2.0a   | 3.0a   | 3.3ab  | 4.5ab  | 4.8ab  | 5.8ab  | 5.8abc |
| S.E       |              | 0.2    | 0.1    | 0.2    | 0.3    | 0.3    | 0.4    | 0.3    |

Mean values with different letters (a, b, c) in the same column are significantly different at p= 0.05 using Duncan’s Multiple Range Test.
Table 4: Effect of treatment on the number of leaves (mean of 4 replicates)

| Variety      | Treatment  | Week1 | Week2 | Week3 | Week4 | Week5 | Week6 | Week7 | Week8 |
|--------------|------------|-------|-------|-------|-------|-------|-------|-------|-------|
| TGX –        | Inoculated | 16.ab | 30.8bc| 46.8cde| 56.8ef| 89.0c | 104.0bc| 115.3c| 122.8cd|
| 1987-34F     | Un-inoculated | 16.5ab| 30.0bc| 48.3cd| 60.3ef| 111.8a| 114.5a| 175.5a| 183.5a|
| TGX –        | Inoculated | 14.8b | 34.5ab| 66.8ab| 80.3bc| 90.3bc| 96.3c | 102.8c| 106.0d|
| 1985-8F      | Un-inoculated | 15.3b | 35.3ab| 66.5ab| 87.5ab| 97.8abc| 110.8bc| 117.8bc| 122.0cd|
| TGX –        | Inoculated | 17.5ab| 36.5a | 53.0a | 65.0de| 92.8bc| 101.8c| 107.0c| 114.5c|
| 1987-38F     | Un-inoculated | 19.4a | 37.3a | 56.3bc| 73.8cde| 107.5ab| 126.5ab| 144.3b| 156.5ab|
| TGX –        | Inoculated | 14.3b | 33.3ab| 65.0ab| 93.8a | 102.5a| 105.0bc| 106.0c| 107.5cd|
| 1986-3F      | Un-inoculated | 15.3b | 33.5ab| 73.0a | 99.5a | 106.0abc| 117.0bc| 122.5bc| 131.5bcd|
| TGX –        | Inoculated | 16.3ab| 25.5c | 32.0e | 50.3f | 97.5ab| 112.5ab| 122.5bc| 129.5bcd|
| 1987-95F     | Un-inoculated | 16.0b | 25.0c | 37.3de| 59.5ef| 112.8a| 118.8bc| 130.0bc| 138.8bc|
| S.E          | 1.1        | 1.8   | 5.0   | 4.2   | 5.4   | 7.4   | 8.7   | 9.5   |

Mean values with different letters (a, b, c, d, e, f) in the same column are not significantly different according to Duncan’s Multiple Range Test.

Table 5: Effect of treatment on the number of pods, number of galls, pod weight (g), seed weight (g), root weight (g) (mean of four replicates)

| Variety      | Treatment  | Number of pod | Pod weight | Number of galls (g) | Seed weight (g) | Root weight (g) |
|--------------|------------|---------------|------------|--------------------|-----------------|-----------------|
| TGX –        | Inoculated | 79.3c         | 29.8ef     | 17.5c              | 22.9e           | 41.8c           |
| 1987-34F     | Un-inoculated | 82.5c         | 36.3d      | 0a                 | 30.5c           | 49.4ab          |
| TGX –        | Inoculated | 88.5c         | 43.8b      | 15.5bc             | 35.9b           | 43.3c           |
| 1985-8F      | Un-inoculated | 94a           | 49.1a      | 0a                 | 41.5a           | 54.9a           |
| TGX –        | Inoculated | 80c           | 32.6e      | 16.75bc            | 26.5d           | 40.6c           |
| 1987-38F     | Un-inoculated | 82c           | 40.1c      | 0a                 | 34.7b           | 43.0c           |
| TGX –        | Inoculated | 81.8c         | 41.8bc     | 16.25bc            | 29.6c           | 44.7bc          |
| 1986-3F      | Un-inoculated | 81.5c         | 41.8bc     | 0a                 | 34.2b           | 54.4a           |
| TGX –        | Inoculated | 60d           | 27.7f      | 13.25d             | 20.8e           | 51.2ab          |
| 1987-95F     | Un-inoculated | 62d           | 32.2e      | 0c                 | 25.9d           | 54.3a           |
| S.E          | 1.6        | 1.1           | 1.2        | 1.0                | 2.2             |

Mean values with different letters (a, b, c, d, e, f) in the same column are significantly different at p= 0.05 using Duncan’s Multiple Range Test.

Table 6: Root gall ratings

| Variety      | Degree of infestation | Host reaction |
|--------------|------------------------|---------------|
| TGX – 1987 – 34F | 3                      | Susceptible   |
| TGX – 1985 – 8F  | 3                      | Susceptible   |
| TGX – 1987 – 38F | 3                      | Susceptible   |
| TGX – 1986 – 3F  | 3                      | Susceptible   |
| TGX – 1987 – 95F | 3                      | Susceptible   |
References

Bird, D. and Kaloshian, I. (2003). Are roots special? Nematodes have their say. *Physiological and molecular Plant Pathology*. 62: 115 – 123.

Dugje, I.Y., Omoigui, L.O., Ekeleme, F., Bandyopadhyay, R., Lava Kumar, P. and Kamara, Y. (2009). Cultural practices. *Farmers’ guide to soybean production in Northern Nigeria*; International Institute of Tropical Agriculture 21: 4 – 14

Gangawane, L.V. and Khilare, V.C. (2008). Root - knot nematode. *Crop disease identification and management, a colour handbook*.

George, A. (2004). Biological enemies of horticultural plants. *Horticulture production and Principles*. Pp 256

Kochlar, S.I. (2009). Plant diseases. *Economic Botany in the Tropics* 3rd edition, Macmillian Publishers, India.

Lance, G. and Garren, B. (2005). *Origin, History and uses of soybean (Glycine max)*, Iowa State University, Department of Agronomy.

Martinez de Ilarduya, O. and Kolashian, I. (2001). *Mi – 1.2 transcripts accumulate ubiquitously in root – knot nematode resistant Lycopersicon esculentum*. *Journal of Nematology*, 33: 116 -20

Mitkowski, N.A and Abawi, G.S. (2003). Root – knot nematodes. *The plant health instructor*. Revised 2011

Pandey, R. and Kalra, A. (2003). Root – knot disease of Ashwagandha. *Withania somnifera* and its eco-friendly cost effective management. *J. Mycol. Pl. Pathol.* 33 (2): 240 – 245.

Science news line (2012) *Unusual Genetic Structure Confers Major Disease resistance trait in Soybean*. University of Wisconsin, Madison.

Shidhaye, S., Malke, S., Mandal, S., Sakhare, N. and Kadam, V. (2009). Soy-A Hidden Treasure for Therapeutic, Cosmetic and Pharmaceutical Use. *Internet Journal of Alternative Medicine*, 7(2): 11

Sikora, R.A., Greco, N. and Joao, F.V.S (2005). Nematode parasites of food legumes. *Plant Parasitic nematode in subtropical and tropical Agriculture*. Ed: Luc, M., Sikora R.A. and Bridge J. pp.259 – 318

Southey, J.F. (1986). *Laboratory methods for work with plant and soil nematodes*. 6th edition. Her majesty stationary office, London.

Stallings, A. and Lupo, M. (2009). Practical uses of botanicals in skin care. *The Journal of Clinical and Aesthetic, Dermatology*, 2(1): 36 -40.

Taylor, A.L. and Sasser J.N. (1978). Biology, identification and control of root – knot nematodes, *Meloidogyne* species. North Caroline University Graphics press pg. 111.

Wall, T. (2012). Mystery of nematode pest resistant soybeans cracked by MU Scientists. (*News Bureau*).