Screening soybean (*Glycine max* L.) Merrill cultivars for response to root-knot nematodes, *Meloidogyne* (Goeldi) spp. in two agro-ecological zones in Ghana, West Africa

K. Osei[1], H. Adu-Dapaah, S. Ohemeng-Dapaah and J. Sackey-Asante
Crops Research Institute, Council for Scientific and Industrial Research, (CSIR), P. O. Box 3785, Academy Post Office, Kumasi, Ghana.
[1]Corresponding author E-mail: kosei7392@gmail.com

ABSTRACT
Ten soybean cultivars were grown for two seasons in fields heavily infested by *Meloidogyne arenaria*, *M. incognita* and *M. javanica* in two agroecological zones of Ghana in order to assess their reaction to the root-knot nematodes. To evaluate the reaction of the cultivars to the root-knot nematodes, second stage juveniles (J2)/g root, root gall index and number of eggs/ g root, egg mass index, plant growth and dry grain yield were determined. The cultivars TGx 1831-21E and TGx 1802-3F were found to be resistant to all root-knot nematode populations present whilst TGx 1789-7F and TGx 1834-1E were highly susceptible. About 90% of the cultivars recorded an average of 42 days to flowering. Three cultivars; TGx 1830 - 20E, Bengbie and TGx 1833 - 20E were early, while the remaining seven were medium maturing. TGx 1830 - 20E recorded the highest average yield across locations.

Key words: Agroecological zones, *Glycine max*, *Meloidogyne arenaria*, *M. incognita*, *M. javanica*.

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill), is a versatile food legume which contains 40% protein and 21% oil on dry weight basis. The crop is also processed for high protein meal animal supplements, soy flour, soybean milk and curd. It compares favourably with animal protein sources and contains all the essential amino acids required by man (Williams *et al.*, 1987; Buttery *et al.*, 1992).

Soybean is an important grain legume in Ghana. It is very well patronised for its high protein and oil content and low cholesterol level. It is utilised for the preparation of soymilk, pastries, infant feed, porridge and a local dish known as "aprapransa". Soybean cake/meal is a preferred protein source in animal feed in Ghana.

In Ghana, the effect of root-knot nematodes on soybean has not been studied. However, Schmitt and Noel (1984) reported that root-knot nematodes, *Meloidogyne* (Goeldi) spp., are important factors limiting soybean production especially in warm climates (Sikora *et al.*, 2005). They affect kernel quality and quantity (Shane and Barker, 1986) and reduce root nodulation by *Rhizobium leguminosarum* (Frank) thus inhibiting nitrogen fixation by 63% in nodular tissue (Caveness and Ogunfowora, 1985). Soybean yield is negatively affected by root-knot nematodes (Lewis *et al.*, 1993).

The use of resistant cultivars is the cheapest option for pest and disease suppression for the low-resource farmer in Africa (FAO, 1982). Therefore, a host status study was undertaken to assess the reaction of ten soybean cultivars, recommended for their high yield potential, to the most common species of root-knot nematode occurring in Ghana.

Table 1. Characteristics of the soybean cultivars used in the study.

Cultivar	Maturity	Growth type	Seed type	Seed size
TGx 1830-20E	Early	Erect	Cream smooth	Medium oval
Benghie	Early	Erect	Cream with greenish tint	Small oval
TGx 1833-20E	Early	Erect	Cream smooth	Medium oval
TGx 1834-1E	Medium	Erect	Light cream	Oval flat
TGx 1835-10E	Medium	Erect	Cream smooth	Large round
TGx 1789-7E	Medium	Erect	Cream with greenish tint	Oval flat
TGx 1805-33F	Medium	Erect	Cream with greenish tint	Large oval
TGx 1835-12E	Medium	Erect	Cream smooth	Small oval
TGx 1802-3F	Medium	Erect	Cream smooth	Medium oval
TGx 1831-21E	Medium	Erect	Cream smooth	Medium oval

Maturity: Early (< 100 days); Medium (101-114 days). Source : Asafo Adjei *et al.* (2005).

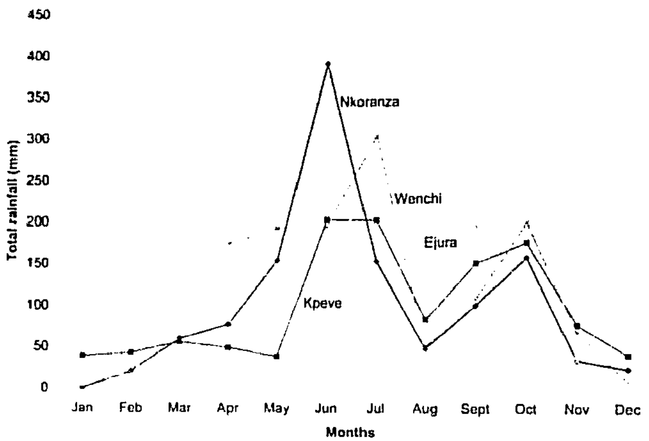

Figure 1. Monthly rainfall totals from Ejura, Kpeve, Nkoranza and Wenchi in 2002.

MATERIALS AND METHODS

The soybean cultivars whose agronomic characteristics are presented in Table 1, were screened during the minor season (July-October) in 2001 and 2002. The cultivars were obtained from the International Institute of Tropical Agriculture, IITA, Ibadan, Nigeria and are recommended for their high yielding potential. No information on their reaction to root-knot nematodes is available, thus the need to evaluate their reaction to the most widespread and pathogenic in the country, the root-knot nematode.

The experiments were conducted in four fields naturally infested with *M. arenaria* (Neal) Chitwood., *M. incognita* (Kofoid and White) Chitwood and *M. javanica* (Treub) Chitwood (Addoh, 1971). The fields were at Ejura (07° 24' N 01° 21' W), Nkoranza (07° 35' N 01° 50' W) and Wenchi (07° 45' N 02° 06' W) all located in the forest-savannah transitional and Kpeve (06° 41' N 00° 20' E) in the forest agroecological zones of Ghana.

The cultivars were planted in a randomized complete block design (RCBD) with three replications. Each plot measured 5 m × 2.4 m and consisted of four rows of 2.4 m long and 75 cm apart. Plant spacing of 15 cm was maintained within rows. Hand weeding was done three times during the trial period. The plots had previously been cultivated with tomato, *Solanum lycopersicum* L.

Before sowing soybean, 450 g of soil was randomly sampled from each plot of the four fields into 6.5 cm diameter plastic pots. The pots were kept on concrete platforms in a plant house. Two-week old tomato seedlings (cv. Asesewa) known to be highly susceptible to root-knot nematodes, were transplanted into the infested soil in a bioassay test to assess the presence and level of root-knot nematode infestation. After 6 weeks, plants were rated for galling on a (0-5) scale (Barker, 1978). The experimental design used was RCBD and the treatments were replicated three times.

Days to maturity and dry grain yield were determined at harvesting time. All other measurements were taken 42 days after planting (DAP) by using five plants/ plot. Growth and yield parameters of the cultivars studied were: plant stand, plant height, days to flowering, maturity period, and dry grain yield. Plant stand was determined by counting germinated hills to assess plant density. Plant height was measured from the first to the last node. Days to flowering and maturity were assessed to determine the maturity periods of cultivars. The reaction of the soybean cultivars to the root-knot nematodes was evaluated by determining second stage juveniles (J2)/g fresh root weight, root gall index after Barker (1978), eggs/ g root and egg mass index after Hussey and Barker (1973). Five plants were randomly selected from the two innermost rows in each plot and uprooted. They were then cut at ca. 5 cm above soil level. Root systems of the cut plants were then lifted up with a shovel, gently shaken in a bucket containing water to remove soil and then placed in polyethylene bags and stored in a cool container. In the laboratory, the roots were washed, cut into 1-cm-long pieces, and thoroughly mixed. Eggs were extracted from 1-g-sub samples by shaking for 3 min in 0.05% NaOCl solution and rinsed for 2 min under running tap water (Stanton and O'Donnell, 1994). Extracted eggs were placed on 20 μm-pore sieve at 20 °C to allow juveniles to hatch. Juveniles were counted under a stereo microscope ×50 after 7 days. Another set of five plants was randomly sampled as described before. Roots were processed as previously described and rated for galling index. Number of eggs /g root and egg mass index was determined.

The cultivars reaction to root galling was determined as follows:

Highly resistant	0 – 0.4
Resistant	0.5 – 1.0
Moderately resistant	1.1 – 1.5
Tolerant	1.6 – 2.5
Moderately tolerant	2.6 – 2.8
Susceptible	2.9 – 3.5
Highly susceptible	3.6 – 5.0

The results of 2001 and 2002 were not significant and were therefore combined and the means analysed as one data. Data were subjected to a one-way analysis of variance (ANOVA) using Genstat 8.1 statistical package and treatments were separated using Duncan's Multiple Range Test (DMRT). Data on nematodes were log transformed, {log (x +1)}, before analysis to comply with the assumption of normal distribution.

Table 2. Root-knot nematode gall index* on tomato bioassay on 450 g soil samples taken from the four locations at the start of the trial

Plot No.	Root-knot nematode gall index			
	Ejura	Kpeve	Nkoranza	Wenchi
1	2.3	3.0	2.3	3.3
2	3.3	2.7	3.3	4.7
3	4.0	2.0	3.0	2.5
4	2.3	0.7	2.3	1.0
5	3.3	3.3	1.0	3.3
6	1.3	1.3	0.7	3.0
7	3.3	1.0	1.3	1.7
8	0	4.3	2.7	1.7
9	3.7	2.3	4.3	1.0
10	4.3	3.0	2.7	3.7
CV (%)	48.3	26.5	34.5	28.7
SED	1.3	0.8	0.9	0.9
Significance	**	**	**	**

Data are means of three replications, ** = Significant at 1% level, CV = Coefficient of variation; SED = Standard error difference, * 0-5 scale according to Barker (1978) where 0=no gall formation, 1=1-2, 2=3-10, 3=11-30, 4=31-100, 5=>100 galls/root.

Table 3. Reaction of ten soybean cultivars to root-knot nematode infestation at four locations in Ghana

Cultivar	J2/ g root				Gall index* (GI)				Eggs/ g root				Egg mass index[1] (EI)			
	Eju	Kpe	Nkz	Wen	Eju	Kpe	Nkz	Wen	Eju	Kpe	Nkz	Wen	Eju	Kpe	Nkz	Wen
TGx 1830-20E	162	162	143	117	1.3	1.3	1.0	1.3	90	71	86	15	1.7	2.3	1.3	2.3
Bengbic	397	264	479	410	3.0	1.7	2.3	4.0	280	174	249	391	2.7	3.0	2.7	3.7
TGx 1833-20E	330	190	579	401	2.3	1.0	3.0	3.3	301	131	310	244	2.7	1.3	4.0	3.7
TGx 1834-1E	612	359	495	442	4.3	3.3	2.3	4.3	884	295	327	492	4.3	3.3	2.7	4.7
TGx 1835-10E	433	245	523	388	3.0	1.3	3.0	3.7	414	104	401	384	2.3	2.3	3.0	4.0
TGx 1789-7F	449	501	827	580	3 .7	4.3	5.0	4.7	475	414	1266	894	3.3	5.0	4.7	4.3
TGx 1805-33F	308	316	360	310	2.3	2.7	2.0	2.7	268	251	199	268	2.3	3.0	2.0	2.7
TGx 1835-12E	170	166	145	166	1.3	1.3	1.0	1.7	196	158	86	236	1.7	2.3	1.3	2.3
TGx 1802-3F	132	136	139	137	0.7	1.3	1.0	1.0	128	126	80	97	0.7	0.9	0.7	0.7
TGx 1831-21E	152	155	127	166	0.8	0.8	0.7	0.7	34	0	45	65	0.7	0.9	0.7	0.7
CV (%)	51.6	21.6	38.8	23.7	5 4.7	29.5	43.5	33.5	43	45	46	22	28	26	29	34
SED	62	54	48	74	1.2	0.5	0.9	0.9	132	78	141	72	0.7	0.6	0.8	1.1
Significance	Ns	**	**	**	Ns	**	**	**	**	**	**	**	**			

** ** **

Data are means of three replications

** = Significant at 1% level; Ns = Not significant at 5% level

CV = Coefficient of variation; SED = Standard error difference

* 0-5 scale according to Barker (1978); [1]0-5 scale according to Hussey and Barker (1973)

Locations in Ghana: Eju = Ejura, Kpe = Kpeve, Nkz = Nkoranza, Wen = Wenc

RESULTS AND DISCUSSION

The bioassay (Table 2) showed that all the plots at the four locations, except one (plot eight at Ejura) were infested with root-knot nematodes. ***Insert Table 2 here.*** Although the days to flowering differed significantly ($P < 0.01$) from cultivar to cultivar at three locations, most cultivars flowered between 41and 43 DAP. Only TGx 1835-12 E flowered beyond 43 days in all four locations. Days to maturity differed significantly ($P < 0.01$) between cultivars which can be classified as early (< 100 days) and medium (101-114 days) maturing. Flowering and maturation are influenced by agroecological zone and genotype of soybean.

There were differences in plant stand at two locations; Ejura and Kpeve. The cultivar TGx 1805-33 F consistently recorded >100 plant stand across all locations. Plant height differed ($P < 0.05$) at Ejura and Wenchi but not at Kpeve and Nkoranza. Plant height ranged from 30.3 cm at Ejura to 91.3 cm at Wenchi. At Ejura plant height and yield were in most cases relatively lower than at other locations. While this was universally true for plant height, it was not true for yield, since both TGx 1830-20 E and TGx 1802-3 F at Ejura had mean yields higher than those of any other cultivar at any location (Table 4).

The number of J2/g root, number of eggs/g root, gall and egg mass indices differed significantly among cultivars ($P < 0.01$) (Table 3) at three of the locations except at Ejura, where the number of J2/g root and GI of cultivars were not significant. The cultivars TGx 1831-21 E and TGx 1802-3F recorded significantly lower means (152, 155, 127 and 166) and (132, 136, 139 and 137) juveniles/g root respectively across the locations compared to TGx 1789-7 F and TGx 1834-1E which recorded higher means (449, 501, 827 and 580) and (612, 359,495 and 442) juveniles/g root respectively. At all four locations, TGx 1831-21 E and TGx 1802-3 F recorded lowest gall indices (0.8, 0.8, 0.7 and 0.7) and (0.7, 1.3, 1.0 and 1.0) respectively compared to TGx 1789-7 F (3.7, 4.3, 5.0 and 4.7) and TGx 1834-1 E (4.3, 3.3, 2.3 and 4.3).

This study showed TGx 1831-21 E and TGx 1802-3 F were resistant to root-knot nematode, regarding low population density/g root, low gall and egg mass indices. The cultivars TGx 1789-7 F and TGx 1834-1 E were found to be highly susceptible to the nematodes. However, differences in yield were significant only at Ejura where the growing conditions were less favourable.

The low plant height and low yields recorded at Ejura is difficult to explain. Nematode density at Ejura was not significantly higher than the other locations (Table 4), neither was rainfall comparatively low (Fig. 1). Other biotic and abiotic factors might account for the relatively poor vegetative growth and yield. In related studies, Saka (1993) reported varietal differences when cowpea lines were evaluated for resistance to root-knot nematodes. Again, Agu (2006) reported from south-western Ethiopia that eight out of twenty three soybean cultivars were susceptible to *M. incognita*. The cultivars TGx 1830-20 E and TGx 1835-12 E were moderately resistant while TGx 1805-33 F and TGx 1833-20 E were tolerant. Bengbie and TGx 1835-10 E were moderately tolerant.

CONCLUSION AND RECOMMENDATION

The cultivars TGx 1831-21E and TGx 1802-3F were found to be resistant to root-knot nematodes under the conditions of this study. The resistance gene/s from these two cultivars could be incorporated into adapted soybean cultivars already commonly available and planted in Ghana which are susceptible to root-knot nematodes. It could be economically helpful to most of the soybean growers in Ghana who are resource-poor with limited access to funding for the purchase of chemicals to control these pathogenic nematodes.

The use of resistant cultivars has been identified as an inexpensive option for pest and disease management for the low-resource farmer in Africa (FAO, 1982). In addition, Castagnone-Sereno (2002) explained that plant resistance is currently the most effective and environmentally safe method to control nematodes. Therefore, incorporating resistant soybean varieties in breeding programmes with the varieties with desirable agronomic characteristics would complement the efforts of a holistic integrated pest management (IPM) strategy. Sustainable soybean production particularly in Africa depends on a pragmatic IPM approach with plant resistance as the pivot.

Table 4. Effect of root-knot nematode on growth and yield parameters in ten soybean cultivars at four locations in Ghana

Cultivar	Plant stand				Plant ht (cm)				Days to flowering				Days to maturity				Yield (t/ha)			
	Eju	Kpe	Nkz	Wen	Eju	Kpe	Nkz	Wen	Eju	Kpe	Nkz	Wen	Eju	Kpe	Nkz	Wen	Eju	Kpe	Nkz	Wen
TGx 1830-20E	121	141	105	211	31.3	63.7	46.3	79.3	39	37	40	42	85	88	99	101	1.74	1.32	1.46	1.40
Benghie	153	146	169	219	38.3	78.7	51.0	69.0	41	42	41	46	91	92	105	102	0.72	1.50	1.64	1.08
TGX 1833-20E	107	122	88	221	30.3	74.3	61.7	79.3	39	37	39	41	86	89	98	102	0.80	1.51	1.13	1.34
TGx 1834-1E	98	141	85	236	31.7	67.0	58.3	85.7	38	37	41	41	85	89	111	101	0.21	1.49	1.53	1.41
TGx 1835-10E	67	101	156	220	36.7	82.7	54.7	70.3	41	40	41	44	89	89	120	99	0.55	1.07	1.36	1.07
TGx 1789-7F	95	120	95	204	38.3	82.3	61.0	90.0	40	41	42	43	88	89	108	97	0.66	1.24	1.57	1.10
TGx 1805-33F	109	116	116	233	39.3	81.3	60.7	78.3	40	38	43	45	92	90	103	102	0.86	1.20	1.52	1.36
TGx 1835-12E	132	155	108	220	47.3	84.3	50.7	79.7	49	44	46	47	94	94	104	103	0.76	1.04	1.53	1.44
TGx 1802-3F	105	154	114	251	42.7	71.7	50.3	91.3	41	37	41	47	97	100	109	104	2.02	1.22	1.37	1.21
TGx 1831-21E	123	140	87	221	39.7	77.7	58.0	88.0	41	37	42	46	93	91	105	102	0.88	1.20	1.19	1.32
CV (%)	14.9	11.0	30.4	11.1	11.8	17.9	15.3	6.2	6.1	7.2	2.8	0.9	3.5	1.6	4.1	0.6	24	17	23	28
SED	16.5	14.7	34.2	24.8	4.4	13.7	8.5	5.0	0.6	2.8	1.2	0.4	3.2	1.5	4.3	0.6	0.23	0.22	0.33	0.35
Significance	**	*	Ns	Ns	*	Ns	Ns	*	**	Ns	**	**	**	**	**	**	**	Ns	Ns	Ns

Data are means of three replications. ** = Significant at 1% level; Ns = Not significant at 5% level, * = Significant at 5% level
CV = Coefficient of variation; SED = Standard error difference, Locations in Ghana: Eju = Ejura, Kpe = Kpeve, Nkz = Nkoranza, Wen = Wenchi.

ACKNOWLEDGEMENTS

The authors are grateful to the Food Crops Development Project (FCDP) of Ghana for funding the studies. We are equally indebted to the Crops Research Institute (CRI) Technicians of the Legume Improvement Programme at the sub-stations where the studies were conducted for their assistance in data collection.

REFERENCES

Addoh, P. G. (1971). The distribution and economic importance of plant-parasitic nematodes in Ghana. *Ghana Journal of Agricultural Science* 4: 21-32.

Agu, C. M. (2006). Susceptibility of soybean cultivars to root nematodes in south-western Ethiopia. *Tropical Science* 46: 143-146.Asafo Adjei, B., Owusu Ansah, I., Asuboah, R. A., Dapaah, H., Haruna, M. and Oti-Boateng, C. (2005). Soybean Production Guide, 40 pp.

Barker, K. R. (1978). Determining nematode population responses to control agents. *In*: Methods for evaluating plant fungicides, nematicides and bactericide. Zehr, E. (ed.). Pp. 114-125. American Phytopathological Society, St. Paul, Minnesota.

Buttery, B. R., Park, S. J. and Hume, D. J. (1992). Potential for increasing N fixation in grain legumes. *Canadian Journal of Plant Science* 72: 323-349.

Castagnone-Sereno, P. (2002). Genetic variability in parthenogenetic root-knot nematode, *Meloidogyne* spp. and their ability to overcome plant resistance genes. *Nematology* 4: 605-608.

Caveness, F. E. and Ogunfowora, A. O. (1985). Nematological Studies Worldwide. *In*: Cowpea research, production and utilization. Singh, S.R. and Rachie, R. O. (eds.). Pp. 274-285. John Wiley and Sons Ltd., New York, USA.

Food and Agricultural Organisation (FAO). (1982). Breeding for durable disease and pest resistance. Production and Protection Paper 55. FAO, Rome, Italy, 167 pp.

Hussey, R. S. and Barker, K. R. (1973). A comparison of methods of collecting inocula of *Meloidogyne* spp including a new technique. *Plant Disease Reporter* 57: 1025-1028.

Lewis, S. A., Drye, C. E., Saunders, J. A., Shipe, E. R. and Halbrendt, J. M. (1993). Plant parasitic nematodes on soybean in South Carolina. *Journal of Nematology* 25: 890-894.

Saka, V. M. (1993). Evaluation of cowpea (*Vigna unguiculata* L.) Walp cultivars against root-knot nematodes. Trends in cowpea Research. *In*: Proceedings of the Cowpea Research Seminar, Harare, Zimbabwe 25-26 September, 1991, pp.97-99.

Schmitt, D. P. and Noel, G. R. (1984). Nematode parasites of soybeans. *In*: Plant and insect nematodes. Nickel, W. R. (Ed.). Pp.13-59. Marcel Dekker, New York, USA.

Shane, W. W. and Barker, K. R. (1986). Effects of temperature, plant age, soil texture and *Meloidogyne incognita* on early growth of soybean. *Journal of Nematology* 18: 320-327.

Sikora, R. A., Bridge, J. and Starr, J. L. (2005). Management Practices: an Overview of Integrated Nematode Management Technologies. *In*: Plant Parasitic Nematodes in Subtropical and Tropical Agriculture - Second edition. Luc, M. Sikora, R. A. and Bridge, J. (Eds.). Pp. 793-825. CABI Publishing Wallingford, UK.

Stanton, J. M. and O'Donnell, W. E. (1994). Hatching, motility and infectivity of root-knot nematode, *Meloidogyne javanica* following exposure to sodium hypochlorite. *Australian Journal of Experimental Agriculture* 34: 105-108.

Williams, C. N., Chew, W. Y. and Rajaratnam, J. A. (1987). Trees and Field crops of the wetter regions of the tropics, pp. 262. Longman Scientific and Technical, Essex, UK.