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SURVEY OF PLANT PARASITIC NEMATODES AND DISEASE SEVERITY OF COMMON BEAN LINES EVALUATED FOR REACTION TO ROOT KNOT NEMATODES INFESTATION

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ABSTRACT

Plant parasitic nematodes are important pests in crop production in sub-Saharan Africa. The objective of this study was to identify the occurrence of nematodes associated with common bean (*Phaseolus vulgaris* L.) and evaluate breeding lines for their reaction to *Meloidogyne* spp. in Ghana. Common bean rhizosphere soil was sampled and processed using Modified Baermann Tray method. Five nematode genera, namely *Meloidogyne*, *Pratylenchus*, *Rotylenchulus*, *Helicotylenchus* and *Trichodorus* were extracted. The first four genera listed above were prevalent across locations, with *Trichodorus* present in 30% of the fields sampled. The highest nematode population density of 319 juveniles per 200 cubic centimeter of soil was recorded for *Meloidogyne* spp. compared to 45 juveniles per 200 cubic centimeter, for *Trichodorus*. Twelve breeding lines were evaluated by inoculating roots of two-weeks-old plants with 2000 infective-stage juveniles of *Meloidogyne* sp. Reactions of test lines to *Meloidogyne* sp. infection were assessed by determining the number of egg masses and galling index (GI) on roots. Reproduction index (RI) was used to classify test lines as resistant or susceptible. Significant differences ($P < 0.05$) were observed in the number of eggs, GI and RI among lines tested. No resistant line was identified; however, lines SEF 47, BFS 35 and BFS 60 were moderately resistant, with RI of 13.1, 17.4 and 23.7%, respectively. Line SEF 60, although classified as slightly resistant, recorded a 100 seed weight of 26.0 g, which was 60% higher than line SEF 53 with seed weight of 16.2 g. Moderately resistant common bean lines identified could be used in common bean improvement programmes to develop elite cultivars tolerant to root knot nematodes.

Key Words: Galling index, *Meloidogyne*, *Phaseolus vulgaris*

RÉSUMÉ

Les nématodes phytoparasites sont des ravageurs importants dans la production agricole. L'objectif de cette étude était d'identifier la présence de nématodes associés au haricot commun (*Phaseolus vulgaris* L.) et d'évaluer les lignées généalogiques pour leur réaction à *Meloidogyne* spp. au Ghana.

Le sol de la rhizosphère du haricot commun a été échantillonné et traité à l'aide de la méthode du plateau de Baermann modifié. Cinq genres de nématodes, à savoir *Meloidogyne, Pratylenchus, Rotylenchulus, Helicotylenchus* et *Trichodorus* ont été extraits. Les quatre premiers genres énumérés ci-dessus étaient répandus dans tous les emplacements, avec *Trichodorus* présent dans 30% des champs échantillonnés. La densité de population de nématodes la plus élevée de 319 juvéniles par 200 cm^3 de sol a été enregistrée pour *Meloidogyne* spp. contre 45 juvéniles par 200 cm^3 pour *Trichodorus*. Douze lignées ont été évaluées en inoculant les racines de plantes âgées de deux semaines avec 2000 juvéniles au stade infectieux de *Meloidogyne* sp. Réactions des lignées de test à *Meloidogyne* sp. l'infection ont été évaluées en déterminant le nombre de masse d'œufs et l'indice de galle (IG) sur les racines. L'indice de reproduction (RI) a été utilisé pour classer les lignées de test comme résistantes ou sensibles. Des différences significatives ($P < 0,05$) ont été observées dans le nombre d'œufs, GI et RI parmi les lignées testées. Aucune lignée résistante n'a été identifiée ; cependant, les lignées SEF 47, BFS 35 et BFS 60 étaient modérément résistantes, avec un RI de 13,1, 17,4 et 23,7 %, respectivement. La lignée SEF 60, bien que classée comme légèrement résistante, a enregistré un poids de 100 graines de 26,0 g, soit 60 % de plus que la lignée SEF 53 avec un poids de graines de 16,2 g. Les lignées de haricot commun modérément résistantes identifiées pourraient être utilisées dans les programmes d'amélioration du haricot commun pour développer des cultivars d'élite tolérants aux nématodes à galles.

Mots Clés : Indice de galle, *Meloidogyne, Phaseolus vulgaris*

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is an important crop, comprising of both dry and snap (green) beans grown globally (FAO, 2007). The crop contain a variety of vitamins, protein, fiber, folate, iron, potassium and magnesium, contributing significantly to human nutrition (Thompson *et al*., 2017; Viguiliouk *et al*., 2017).

Common bean production is threatened by nematodes, infestation (Schwartz *et al*., 2005). Under favourable conditions, nematodes attack leads to the development of common bean diseases, which have been reported to contribute to a yield loss of 80% (de Jesus Junior *et al.,* 2001). Yield losses associated with diseases are further exacerbated by continuous and mono-cropping on the same piece of land caused by limited access to arable lands. Such practice favours the development of pathogens such as plant parasitic nematodes. A wide range of plant parasitic nematodes are associated with common bean; however, root knot nematodes, *Meloidogyne* spp. are of great economic importance. Plant feeding nematodes feed on plant nutrients, reduce root growth, inhibit water and nutrient transportation from roots to shoots and suppresses biological nitrogen fixation by the crop. Root knot nematodes infestation, according to Kimenju *et al.* (1999) can cause a yield loss of up to 60% in common bean production.

Several strategies in Ghana have been developed to control root-knot nematode; but their adoption is still limited in sub-Saharan Africa. Although the use of nematicides is reportedly highly effective in suppressing nematodes, these chemicals are often expensive or not readily available to smallholder farmers, who dominant common bean production in the tropics. On the other hand, use of cultivars with resistance to *Meloidogyne* species offers a cost-effective, easy-to-use, and environmentally friendly management strategy to fight this poikilothermic agent. The objective of the study was to identify nematodes associated with common bean; and evaluate the reaction of breeding lines to *Meloidogyne* spp.

MATERIALS AND METHODS

A survey was carried out in 10 common bean (*Phaseolus vulgaris* L.) growing communities in the semi-deciduous forest and forest-savannah transition agro-ecological zones of Ghana (Table 1). On each field, eighty common bean plants were selected along three W shaped "sample walks" (Wiesel *et al*., 2015). The selected plants were gently uprooted to assess the roots for the presence of symptom of nematode attack. From each field, 10 core soil samples were collected from common bean rhizosphere, bulked together, thoroughly mixed and approximately a kilogramme of the composite soil sample, as well as root samples taken from each field.

Nematodes were extracted from 200 cubic centimeters of soil sampled from each field using the Modified Baermann Tray technique. Identification of nematodes to genus level was done using an identification key and descriptions by Mai and Lyon (1975), using Optika compound microscope. Nematode population levels were determined from a counting slide under a compound microscope and expressed per 200 cubic centimeter soil. Frequency of occurrence, population density and relative abundance of identified nematodes were also determined. To obtain inoculum for the screening experiment, root knot nematodes juveniles extracted were multiplied and maintained on roots of susceptible tomato cultivar Pectomec (Kankam and Adomako, 2014).

Twelve common bean breeding lines (SEF 47, BFS 35, BFS 60, SEF 44, SEF 64, SEF 60, SEF 49, BFS 39, SEF 55, BFS 55, SEF 53, and SEF 17) were obtained from the Legumes and Oilseeds Improvement Programme of the CSIR-Crops Research Institute, Ghana. Two seeds per common bean line were planted in sterilised soil (50:50, sand: soil mixture) in perforated 500 cm^3 plastic pots, arranged in a completely randomised design. The seeds were watered as required. Temperature in the screen house during the period of study was 25 ± 3 ^oC. Two weeks after planting, common bean seedlings were thinned to single plants per pot. The plants were then inoculated with 2000 *Meloidogyne* spp. of infective juveniles, and covered with a moist layer of sand.

Eight weeks after inoculation, common bean plants were harvested by gently uprooting them from the soil. Uprooted roots were gently washed in tap water and the roots assessed for disease (root galling) symptoms. Disease severity for each plant was rated visually, using a 0-5 galling index (GI), as follows: 0 = 0 galls; 1 =1-2 galls; 2=3-10 galls; 3 =11-30 galls; 4 = 31-100 galls; 5 > 100 galls (Karuri *et al.* 2016). Nematode eggs were also identified and counted. Yields of common bean lines were determined as weight of 100 seeds per each test line.

The common bean lines were categorised based on Meloidogyne nematodes reproduction index (RI), which was calculated as final nematodes population divided by the initial number of nematodes juvenile multiplied by 100. The RI rating was as follows: RI < 0 (immune), RI < 1 (highly resistant), 1 < RI < 10 (very resistant), 10 < RI < 25 (moderately resistant), 25 <RI < 50 (slightly resistant) and RI >50 (susceptible) (Taylor, 1967). The experiment was carried out twice, using the same set of lines and resulting data pooled

TABLE 1. Frequency of occurrence and relative abundance of PPN associated with common bean

Nematode genera	Frequency of occurrence	Relative abundance (%)
Meloidogyne	10.0	35.0
Pratylenchus	10.0	22.0
Rotylenchulus	10.0	12.0
Helicotylenchus	10.0	25.0
Trichodorus	3.0	5.0

together before analysis to determine the consistency of differences in nematode resistance.

Population density (PD) of nematodes, frequency of occurrence (FOC) and relative abundance (RA) were calculated as described by Norton (1978) as follows:

PD = (average number of nematodes in 200 cubic centimeter soil and 10 g roots)

FOC = (number of fields containing a particular nematode genus or species /total number of fields sampled)

RA = (number of individual nematodes genus or species / total number of nematodes identified and counted from 200 cubic centimeter soil and 10 g roots) x 100.

Data on galling index, reproduction index, number of eggs and 100 seed weight from both experiments were pooled together and subjected to Multivariate analysis of variance (MANOVA). Significant means were separated using SED (5%) in GenStat software version 12.0. Before analyses the data on GI, RI, and number of eggs were transformed using log (x + 1).

RESULTS

Survey. Five phyto-parasitic nematodes, namely *Meloidogyne, Pratylenchus, Rotylenchulus, Helicotylenchus* and *Trichodorus*, belonging to different families, were found to be associated with the crop. Four of these nematodes genera, *viz Meloidogyne, Pratylenchus, Rotylenchulus, Helicotylenchus,* were prevalent across all locations, with *Trichodorus* being present in only 30% of fields sampled (Table 1). *Meloidogyne* spp. and *Trichodorus* spp. represented the highest and lowest nematodes population densities, respectively (Fig. 1). Significant differences ($P \leq 0.05$) existed among nematodes population densities (Fig. 1) with *Meloidogyne* being the most prevalent specie.

Resistance screening. Infested plants showed symptoms of leaf chlorosis with galls observed on roots at harvest (Fig. 2).

Common bean lines showed varied reactions to root knot nematodes infestation (Table 2). This was evident ($P \leq 0.05$) in GI

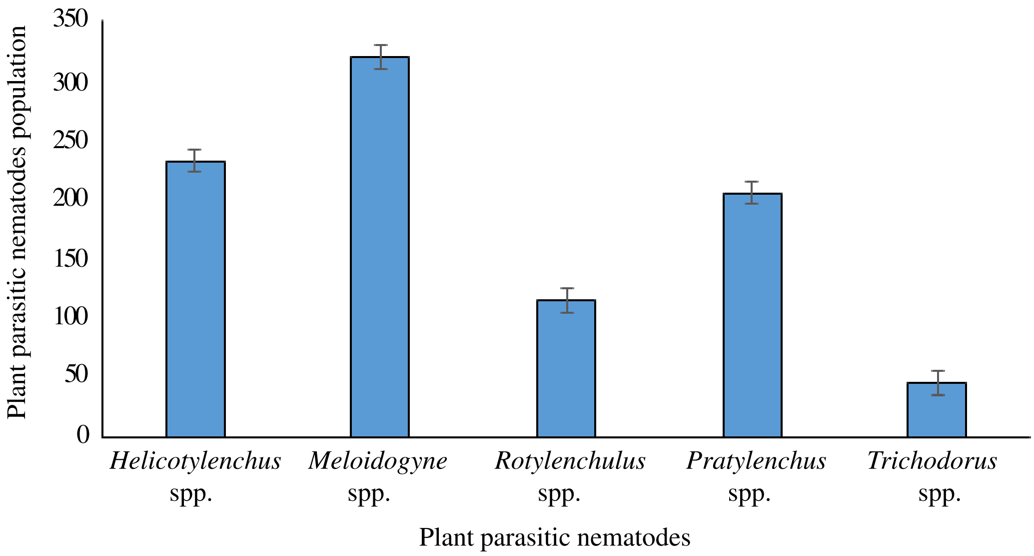

Figure 1. Population of plant parasitic nematodes recovered from common bean fields in Ghana.

A B C

Figure. 2. (A) Uninoculated, (B) inoculated common bean plants showing chlorosis and (C) root galls.

TABLE 2. Galling index, number of eggs, reproduction index and resistance level of common bean lines infected with *Meloidogyne* spp.

Genotype	[a]Galling index	Number of eggs	[b]RI	[c]Resistance level
SEF 47	2.0	59.3 (1.8)	13.1	MR
BFS 35	2.2	32.3 (1.5)	17.4	MR
BFS 60	2.5	59.0 (1.8)	23.7	MR
SEF 44	3.0	49.5 (1.7)	31.0	SR
SEF 64	3.3	63.0 (1.8)	36.2	SR
SEF 60	3.0	64.0 (1.8)	39.2	SR
SEF 49	3.0	43.5 (1.6)	42.6	SR
BFS 39	2.0	33.0 (1.5)	50.0	SR
SEF 55	3.6	28.0 (1.4)	56.7	S
BFS 55	4.0	64.0 (1.8)	85.1	S
SEF 53	4.0	16.7 (1.2)	88.8	S
SEF 17	3.5	57.0 (1.8)	99.4	S
SED (5%)	0.4	0.2	3.5	

[a]Galling index: 0 = 0 galls; 1 = 1-2 galls; 2= 3-10 galls; 3 = 11-30 galls; 4 = 31-100 galls; 5 > 100 galls
[b]RI = Reproduction index (final nematodes population) /(initial nematodes population)x100
[c]Resistance level based on the RI where MR = Moderately Resistant, SR = Slightly Resistant and S = Susceptible

and number of eggs among various lines. Two lines (SEF 53 and BFS 55) recorded a GI of 4.0 (Table 2). Lines SEF 60, SEF 64 and BFS 55 recorded more than 60 egg masses on their roots compared to line SEF 53, which recorded a GI of 17. The reproductive index ranged from 13 to 99% with the highest and lowest RI observed in lines SEF 17 and SEF 47, respectively (Table 2). Based on the RI, 25.0% of the lines were moderately resistant, with 41.7 and 33.3% being slightly resistant or susceptible to *Meloidogyne,* respctively. Significant differences in seed weight were recorded among the liness (Table 3). The

TABLE 3. Seed weight of common bean lines infested with root knot nematodes

Genotype	Mean seed weight (g)
SEF 47	22.0
BFS 35	20.0
BFS 60	20.7
SEF 44	19.7
SEF 64	21.3
SEF 60	26.0
SEF 49	18.4
BFS 39	18.7
SEF 55	18.1
BFS 55	18.7
SEF 53	16.2
SEF 17	16.9
SED (5%)	2.4

highest weight of 26.0 g recorded in line SEF 60 was 60% higher than the weight of line SEF 53 which recorded 16.2 g.

DISCUSSION

Five nematode types, namely *Meloidogyne, Pratylenchus, Rotylenchulus, Helicotylenchus* and *Trichodorus,* belonging to the order tylenchida and triplonchida on common bean were identified. The high frequency of occurrence and relative abundance of multiple parasitic nematodes on the crop, as observed in this study, agrees with Kimenju *et al.* (1999) and Karanja (2003), who reported the association of several nematode pest on common bean in Kenya. Of the different nematodes extracted, high densities of root knot nematodes from common bean rhizosphere soil were observed. This agrees with earlier studies of Sima˜o *et al.*, 2005 and Waceke (2018) that root knot nematodes are major pests associated with common bean, contributing significantly to a reduction in productivity (Karanja, 1988; Kimenju *et al.*, 1999). Like the root knot nematode, the various nematode species identified could be a cause of concern to common bean production in Ghana as their inhibitory effect on several food crops such as sweet potato and yams have been reported (Lutuf *et al.*, 2018; Adomako *et al.*, 2020).

To mitigate the negative impact of nematode pests in common bean production, there is the need to screen for, identify and promote the use of resistant or tolerant cultivars. Our results collaborated with results of Waceke (2018) and Kgabo *et al.* (2019), common bean lines varied in reaction to root knot nematodes infestations. The present study, however, did not identify resistant line contrary to the findings of Waceke (2018) and Kgabo *et al.* (2019), who identified resistant genotypes. Three of the lines (SEF 47, BFS 35, BFS 60) were identified as moderately resistant to root knot nematodes; which agreed with Gomes da Costa *et al.* (2018) and Waceke (2018) that several lines of common bean are moderately resistant to root knot nematodes. Identification of moderately resistant common bean lines will be helpful in the management of root knot nematodes by farmers in Ghana because according to Ribeiro Do Val *et al.* (2001) and Solano *et al.* (2014), moderately resistant cultivars contain several minor resistant genes which are race non-specific; and as such difficult to be overcome by a pathogen. Significant differences in seed weight recorded among the lines may be due to differences in genetic make-up of the lines evaluated as it was observed that accession SEF 60 with a higher reproduction index, out-yielded lines SEF 17, BFS 35 and BFS 60 with lower reproduction index. This suggests that line SEF 60 may contain genes tolerant to root knot nematodes infection; and that although it supported higher reproduction of the nematodes, it had less impact on its yield potential. Earlier studies identified that some leguminous crops tolerant to root knot nematodes produced higher grain yield, although they supported higher nematode populations (Chakrabortey *et al.*, 2016;

Kankam *et al.,* 2019). All lines categorised as susceptible with higher reproduction index, recorded reduced seed weight. This study can, however, not confirm if it was due to the genetic makeup of the crop or the effect of high nematodes reproduction on those lines; although higher nematode populations have been associated with a reduction in crop yield (Fabia *et al*., 2017).

CONCLUSION

The study has shown that several nematodes pests are associated with common bean in Ghana, with root knot nematodes being the most abundant. There were variations in responses of common bean lines to *Meloidogyne* spp. infestation. Moderately resistant lines identified in this study will be highly useful in common bean breeding, release and mass promotion in Ghana.

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