

## Resistance screening of white yam (*Dioscorea rotundata* Poir.) accessions against *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 using yam vines

Joseph ADOMAKO<sup>1,2</sup>, Emmanuel OTOO<sup>3</sup>, Yaw DANSO<sup>1</sup>, David Kwadwo ALHASSAN<sup>3</sup>, Patrick ADEBOLA<sup>4</sup>, Asrat ASFAW<sup>4</sup>

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**Resistance screening of white yam (*Dioscorea rotundata* Poir.) accessions against *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 using yam vines**

**Abstract:** Root-knot nematode (*Meloidogyne incognita*) is an economically important phytoparasitic nematode species. In yam production, therefore, breeding for nematode resistance is an important environmentally friendly tool to manage root-knot nematodes damage. The aim of this study was to determine the reaction of 18 yam accessions to *M. incognita* inoculation under screen house conditions using single node vine cuttings. Vines of each accession were planted in sterilized soil and inoculated with 1000 infective juveniles of *M. incognita*. Resistance level of yam accessions were based on both galling index score and reproductive factor. There were a significant differences in final infective stage nematodes population, galling index, reproduction factor and yield of mini tuber among the accessions tested. Sixteen (89 %) of the accessions showed moderate resistance ( $GI \geq 2$ ,  $Rf \leq 1$ ) to the test pathogen with two accessions classified as susceptible. Accession TDr1515OP16/0030 recorded the highest mini tuber yield mass of 19.4 g, which was 74 % higher than accession 'TDr1515OP16/0108' which recorded the lowest yield of 10.4 g. The moderately resistant accessions identified in the study can be utilized to reduce nematodes reproduction and help manage root-knot nematode in yam production.

**Key words:** host plant resistance; host plant susceptibility; nematode suppression potential; white yams; southern root-knot nematodes

**Preučevanje odpornosti akcesij gvinejskega belega jama (*Dioscorea rotundata* Poir.) na ogorčico *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 z uporabo stebelnih izsečkov**

**Izvelek:** Ogorčica vozlanja korenin (*Meloidogyne incognita*) je ekonomsko pomembna fitoparazitska vrsta. Pri pridelavi jama je v njegovih žlahtniteljskih programih pomembno, okolju prijazno orodje vzgoja na ogorčice odpranih genotipov za uravnavanje škod, ki jo povzročata ta vrsta ogorčice. Namen raziskave je bil določiti odziv 18 akcesij jama na inokulacijo z ogorčico *M. incognita* v rastlinjaku z uporabo enonodijskih izsečkov. Stebelni izsečki jama so bili vsajeni v sterilizirana tla in inokulirani s 1000 kužnimi mladimi primerki *M. incognita*. Stopnja odpornosti akcesij jama je temeljila na indeksu okuženosti korenin z ogorčicami in njihovem reprodukcijskem faktorju. Med preizkuševanimi akcesijami jama je bila značilna razlika v končni stopnji okuženosti, indeksu vozlanja korenin, reprodukcijskem faktorju in v pridelku mini gomoljev jama. Šestnajst (89 %) od preučevanih akcesij je pokazalo zmerno odpornost ( $GI \geq 2$ ,  $Rf \leq 1$ ) na patogena. Dve akcesiji sta se izkazali kot občutljivi. Akcesija TDr1515OP16/0030 je imela največjo maso v pridelku mini gomoljev, 19,4 g, ki je bila za 74 % večja kot pri akcesiji TDr1515OP16/0108, pri kateri je bila masa najmanjša, 10,4 g. Zmerno odporne akcesije jama, identificirane v tej raziskavi, bi lahko uporabili za zmanjševanje razmnoževanja ogorčic in s tem zmanjšali okužbo z njimi pri pridelavi jama.

**Ključne besede:** odpornost gostiteljske rastline; potencial zatiranja ogorčic; beli gvinejski jam; južna ogorčica vozlanja korenin

1 Nematology Unit, Plant Health Division, CSIR-Crops Research Institute, Kumasi, Ghana

2 Corresponding author, e-mail: joeadomako@gmail.com

3 Yam Improvement Programme, Roots and Tubers Division, CSIR-Crops Research Institute, Kumasi, Ghana

4 International Institute for Tropical Agriculture, Ibadan, Nigeria

## 1 INTRODUCTION

White yams (*Dioscorea rotundata* Poir) play an important role in the lives and activities of several people including rural producers, processors and consumers in West Africa (Darkwa et al., 2019). It provides multiple opportunities for poverty reduction and nourishment for poor people in the West African sub-region (Sahore & Kamenan, 2007). Nutritionally, the crop provides substantial amounts of vitamins (thiamine and vitamin C), iron and potassium (Rudrappa, 2013) apart from being an important staple source of starch, sugars and fibers as well as proteins and trace amounts of lipids to consumers in the tropics and sub tropics. *Dioscorea* species also contain important secondary metabolites, steroidal saponins, diterpenoids and alkaloids, which have been exploited in the pharmaceutical industry (Das et al., 2014; Kumar et al., 2017).

Production of the crop is however, constraint by several factors, including low yield potential of local varieties, limited availability of planting materials as well as pests and diseases such as yam anthracnose, virus and nematodes. Plant parasitic nematodes have been implicated as important pest and limiting agent in yam production. Root-knot nematodes pest activities lead to galling and crazy roots syndrome of yam tubers thereby reducing quantity (yield) and quality of tubers. Also, wounds created by the stylets of pest during feeding serves as entry point for other microorganisms which leads to establishment of disease complexes on tubers. This reduces shelf life of infected yam tubers, market value and subsequently increases food insecurity. Phytoparasitic nematodes management in white yams production have depended on the use of synthetic chemicals, application of soil amendment such as neem products prior to planting and crop rotation. Employing most of these management options are limited in use due to high monetary costs, bulkiness, time consumption, feasibility and adverse effects on the environment as well as mammalian toxicity (Plowright & Kwoseh, 2000).

Attempts to develop improved white yam varieties with pests and diseases resistance, wide adaptability and good organoleptic characteristics are being explored by crop protectionist and yam breeders. Identifying resistant white yam cultivars are safe to manage root-knot nematode stress in yam production to reduce the negative impact associated with application of synthetic chemicals on non-targeted soil borne microorganisms and the environment. Plant host resistance management is environmentally friendly, sustainable and at little cost to smallholder farmers. Again, identifying nematodes resistance in white yams would improve breeding activi-

ties by the introgression of resistant genes into adapted varieties with desired traits. In the current study, 18 white yam accessions were evaluated for their reactions to *M. incognita* using single node cuttings.

## 2 MATERIALS AND METHODS

### 2.1 SOURCES OF WHITE YAM ACCESSIONS

Eighteen white yam accessions (Table 1) were obtained from the International Institute of Tropical Agriculture (IITA) and Yam Improvement Programme of the CSIR-Crops Research Institute, Kumasi, Ghana.

### 2.2 SOIL PREPARATION AND STERILIZATION

Soil was prepared by mixing top soil and river sand in a ratio of 3:1 and sterilized in an autoclave at 121 °C for 20 min. The sterilized soil was air dried for a week before use. This was to ensure dissipation of trapped gases. It was also to avoid possible effect of heat on the vine cuttings. The air dried sterilized soil was measured and distributed into one liter plastic screen house pots and placed on concrete benches.

**Table 1:** List of white yam accessions and source of collection

Accessions	Source
TDr 1515 OP16/0108	CSIR-Crops Research Institute
TDr 95/18544	IITA
TDr 1515 OP16/0059	CSIR-Crops Research Institute
TDr 95/19158	IITA
TDr 1515 OP16/0105	CSIR-Crops Research Institute
TDr 1515 OP16/0043	CSIR-Crops Research Institute
TDr 95/19177	IITA
TDr 1515 OP16/0081	CSIR-Crops Research Institute
TDr 00/00362	IITA
TDr 98/01067	IITA
TDr 1515 OP16/0042	CSIR-Crops Research Institute
TDr 98/00604	IITA
TDr 1515 OP16/0092	CSIR-Crops Research Institute
TDr 1515 OP16/0102	CSIR-Crops Research Institute
TDr 1515 OP16/0046	CSIR-Crops Research Institute
TDr 1515 OP16/084	CSIR-Crops Research Institute
TDr 1515 OP16/0176	CSIR-Crops Research Institute
TDr 1515 OP16/0030	CSIR-Crops Research Institute

### 2.3 EXTRACTION AND MAINTENANCE OF *Meloidogyne incognita* EGGS/JUVENILES

A population of *M. incognita* isolated from tomato was maintained and multiplied on susceptible tomato variety 'Pectomech'. Seedlings of the tomato were grown in plastic pots filled with the sterilized soil. Two weeks after planting, the tomato seedlings were inoculated with the eggs of the nematode pest. Eight weeks after inoculation, galled tomato plants were uprooted, washed under running tap water to get rid of all soil and galled roots cut into pieces (ca 2 cm). Nematode eggs were extracted following Hussey and Barker (1973) sodium hypochlorite (NaOCl) method. The extracted eggs were washed into a graduated beaker, and the volume adjusted to 100 ml with sterile distilled water. The nematode egg-water suspension was placed on laboratory benches for 24 hours at  $24 \pm 2$  °C. This was to allow eggs hatching into second stage juveniles. Hatched juveniles were harvested and counted using a counting tray with the aid of a compound microscope.

### 2.4 RESISTANCE SCREENING OF WHITE YAM ACCESSIONS

Single node vines of 2 months old plants of each accession growing on the field was cut and washed under running water to remove debris. The excised vines were planted in sterilized sandy loam soil and placed in the screen house (Fig.1). Two months after planting yam vines which allowed initial rooting to occur, 1000 *M. incognita* infective stage juveniles were introduced approximately 2 cm deep into the soil surrounding roots of each white yam plant. Inoculated plants were arranged



**Fig. 1:** White yam accessions establishment in pots under screen house conditions

in completely randomized design with 3 replications on screen house benches and maintained in the screen house at  $28 \pm 2$  °C. Eighty days after inoculation, white yam mini tubers were harvested, counted and weighed to determine yield. Each mini tuber harvested was examined and the extent of damage due to nematodes were scored on a scale of 1-5 (1 = no symptoms on tuber surface, 2 = slight damage (1-25 % of symptoms on tuber surface), 3 = mild damage (26-50 % symptoms on tuber surface), 4 = heavy damage (51-75 % symptoms on tuber surface), and 5 = severe damage (> 75 % symptoms on tuber surface). Soil samples were collected from each pot and final nematodes population in 200 cubic centimeter (cc) soil extracted and counted. The experiment was carried out in the 2018 and 2019 cropping season, using the same set of cultivars to determine the consistency of differences in nematode resistance.

### 2.5 STATISTICAL ANALYSIS

Data collected for the two years were pooled together for analysis. Data on final nematode numbers were  $\log(x + 1)$  transformed to comply with assumption of normal distribution. Statistical analysis was performed using analysis of variance (ANOVA) with Genstat and differences between significant means separated using Tukey's HSD ( $p < 0.05$ ). The level of resistance or susceptibility of each yam accession was based on both galling and reproduction index (proportion of final nematodes recovered to initial nematodes applied) as described by Afolami et al. (2004) (Table 2). Linear regression analysis was performed to determine the relationship between final nematodes count and galling index using Microsoft Excel.

## 3 RESULTS

### 3.1 PATHOGENICTY AND REPRODUCTION EFFICIENCY OF *M. INCOGNITA* ON YAM ACCESSIONS

White yam plants established successfully from the single node vines cuttings (Fig.1). At harvest, it was observed that mini tubers harvested from uninoculated pots were healthy/clean with no symptoms of *M. incognita* damage (Fig. 2A). It was however not the same for the inoculated pots as they showed varied symptoms of root-knot nematodes infestation. Symptoms of root-knot nematode infestation included appearance of galls on mini tubers and roots as well as crazy rooting syndrome (Fig. 2B).

Results of the study revealed that the various yam

**Table 2:** Resistance rating scale for root-knot nematodes

<sup>a</sup> Plant damage (gall index)	<sup>b</sup> Reproduction Index	Degree of resistance (DR)
≤ 2	≤ 1	Resistant
≤ 2	≥ 1	Tolerant
≥ 2	≤ 1	Moderately resistant
≥ 2	≥ 1	Susceptible

<sup>a</sup>Gall index: 0 = no gall formation; 5 = heavy gall formation

<sup>b</sup>Reproductive factor:  $Rf = Pi/Pf$  where  $Pi$  = initial population density, and  $Pf$  = final population density

accessions reacted differently to *M. incognita* infestation. The nematodes incited galling not only on the yam roots but also on the tubers (Fig 2B). Ability of the nematode to reproduce varied significantly ( $p < 0.05$ ) under the different white yam accessions. It was observed that nematode reproduction was highest in accession TDr 98/01067 compared to other accessions. Whilst TDr 98/01067 recorded 1040 juveniles (J2)/200 cc soil, both TDr1515OP16/0105 and TDr 98/00604 recorded 670 J2/200 cc (Table 3). Similarly, galling index significantly ( $p < 0.05$ ) varied between the accessions. The highest galling index of 2.7 was recorded in two accessions namely TDr 00/00362 and TDr 98/01067. Majority (50 %) of the accessions recorded gall indices of 2.0 compared to 16.6, 22.0, and 11.1 % of the accessions recording gall indices of 2.3, 2.5 and 2.7 respectively. The highest reproductive index of 1.4 was recorded in accession TDr 98/01067 which was not significantly different ( $p > 0.05$ ) from TDr 00/00362, which recorded 1.3. However, the lowest reproduction index of 0.7 was recorded in four accessions, namely TDr1515 OP16/0105, TDr 95/19158,

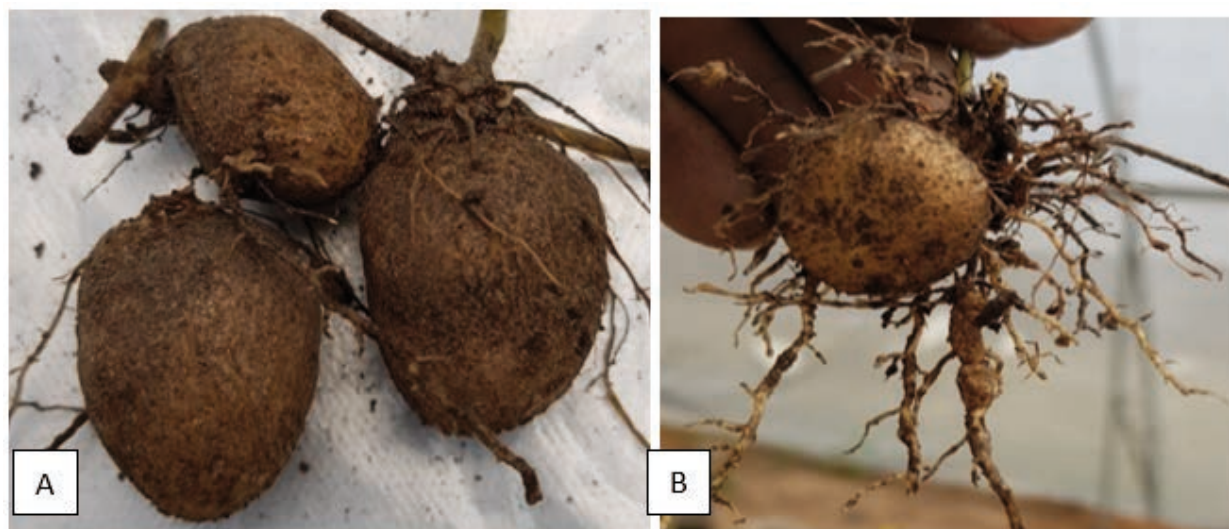
TDr 98/00604 and TDr95/18544 (Table 3). Based on galling reproduction indices, 16 accessions were classified as moderately resistant whilst two namely TDr 00/00362 and TDr 98/01067 were classified as susceptible to the pest.

### 3.2 YIELD OF WHITE YAM MINI TUBERS AND RELATIONSHIP BETWEEN FINAL NEMATODES POPULATION AND MINI TUBER HEALTH

Mini tuber yields were significantly different ( $p < 0.05$ ) with TDr1515OP16/0030 recording the highest mini tuber mass of 40.20 g. This was 74.0 % more than that of TDr1515OP16/0108 which recorded the least (10.4 g) (Table 4). It was also observed that *M. incognita* soil population at harvest had effect on the severity of mini tuber galling. There was a positive relationship between final number of second stage *M. incognita* recovered and tuber damage recorded as galling index (Fig.3). It was observed that increase in the final second stage juvenile population, corresponded significantly with yam mini tuber galling severity.

## 4 DISCUSSION

Nematode-resistant genotypes of crop plants are generally unaffected or little affected by nematodes attack and greatly contribute to reducing nematode infestations. Eighteen white yam accessions evaluated in the present study is critical in the effort of identifying genetic sources to manage root-knot nematode, which is



**Fig. 2:** Healthy (A) and *M. incognita* infested (B) mini tubers from inoculated and uninoculated pots respectively

**Table 3:** Reproduction of *Meloidogyne incognita*, galling index, reproduction index (RI) and resistance levels of white yam accessions

Accession	<sup>a</sup> Nematodes count/200cc soil	<sup>b</sup> GI	<sup>c</sup> RI	<sup>d</sup> Resistance Level
TDr1515 OP16/0105	670.0 (2.83)	2.0	0.7	MR
TDr 95/19158	671.7 (2.83)	2.0	0.7	MR
TDr 98/00604	670.0 (2.83)	2.0	0.7	MR
TDr95/18544	673.3 (2.83)	2.0	0.7	MR
TDr 1515 OP16/0030	680.0 (2.83)	2.0	0.7	MR
TDr 1515 OP16/0042	682.3 (2.83)	2.0	0.7	MR
TDr 1515 OP16/0059	680.0 (2.83)	2.0	0.7	MR
TDr95/19177	682.7 (2.83)	2.0	0.7	MR
TDr 1515 OP16/0043	686.7 (2.84)	2.0	0.7	MR
TDr 1515 OP16/0108	776.7 (2.89)	2.3	0.8	MR
TDr 1515 OP16/084	780.0 (2.89)	2.3	0.8	MR
TDr 1515 OP16/0176	786.7 (2.89)	2.3	0.8	MR
TDr 1515 OP16/0102	846.7 (2.92)	2.5	0.9	MR
TDr 1515 OP16/0046	850.0 (2.92)	2.5	0.9	MR
TDr 1515 OP16/0092	850.0 (2.93)	2.5	0.9	MR
TDr 1515 OP16/0081	863.3 (2.93)	2.5	0.9	MR
TDr 00/00362	1030.0 (3.01)	2.7	1.3	S
TDr 98/01067	1040.0 (3.02)	2.7	1.4	S
HSD ( $p < 5\%$ )	(0.01)	0.08	0.01	
CV	(1.7)	4.5	1.7	

<sup>a</sup>Final *M. incognita* extracted from 200 cm<sup>3</sup> soil, <sup>b</sup>Gall index: 0 = no gall formation; 5 = heavy gall formation, <sup>c</sup>Reproduction index: RI = Pi/Pf where Pi = initial population density, and Pf = final population density, <sup>d</sup>Resistance level based on the RI and GI where MR-Moderately Resistant and S-Susceptible

currently not controlled in yam production. There was a varied response of the white yam accessions to *M. incognita* infestation. Differential responses of plant genotypes to nematodes infection were reported in previous studies (Kagoda et al., 2004; Osei et al., 2015; Kankam et al., 2019). Accessions TDr 00/00362 and TDr 98/01067 found to be susceptible to the test pest allowed higher nematodes reproduction with increased population densities and a higher disease severity compared to other accessions screened. Susceptibility of plants to nematodes according to Cervantes-Flores et al. (2008) may be due to the presence of unfavorable alleles that reduce their level of resistance. Sixteen of the yam accessions screened in this study were identified to be moderately resistant with none being categorized as highly resistant or immune to the test pathogen. Clearly, results obtained showed a reduced root-knot nematode reproduction and galling severity ( $R_f < 1$ ,  $GI < 2$ ) in moderately resistant accessions compared to those rated to be susceptible. Moderately resistant accessions according to Roberts (2002) and Zwart et al. (2019), supports low or intermediate reproduc-

tion compared to susceptible genotypes. Identification of moderately resistant accessions in this study agrees with previous screening studies. Karuri et al. (2017) and Aydinli et al. (2019) identified accessions of *Cucurbita maxima* Duchesne, *Cucurbita moschata* Duchesne ex Poir. and sweet potato that were moderately resistant to root-knot nematode. Moderately resistant plants according to Singh et al. (2012) provides durable resistance against pathogens since it is controlled by multiple resistant genes that reduce multiplication of nematodes within their host (Cervantes-Flores et al., 2008; Lee et al., 2021). High reproduction of nematodes in their host increases extent of damage caused. The positive relationship between nematodes population and galling index scores as observed in the present study agrees with findings of El-Sherif et al. (2007) and Charegani et al. (2012). This may explain why TDr 00/00362 and TDr 98/01067 rated as susceptible in the current study and supported higher *M. incognita* reproduction recorded higher galling index scores.

Root-knot nematode infestation in accessions TDr

**Table 4:** Yield (g) of white yam accessions at 4 months after planting

Yam accessions	Mini tuber mass (g)
TDr 1515 OP16/0108	10.4
TDr95/18544	11.1
TDr 1515 OP16/0059	11.7
TDr 95/19158	12.2
TDr 1515 OP16/0105	13.0
TDr 1515 OP16/0043	13.2
TDr95/19177	14.0
TDr 1515 OP16/0081	14.1
TDr 00/00362	15.0
TDr 98/01067	15.5
TDr 1515 OP16/0042	15.6
TDr 98/00604	15.6
TDr 1515 OP16/0092	19.4
TDr 1515 OP16/0102	19.90
TDr 1515 OP16/0046	31.20
TDr 1515 OP16/084	31.20
TDr 1515 OP16/0176	32.40
TDr 1515 OP16/0030	40.20
HSD (P<5 %)	1.1
CV	1.7

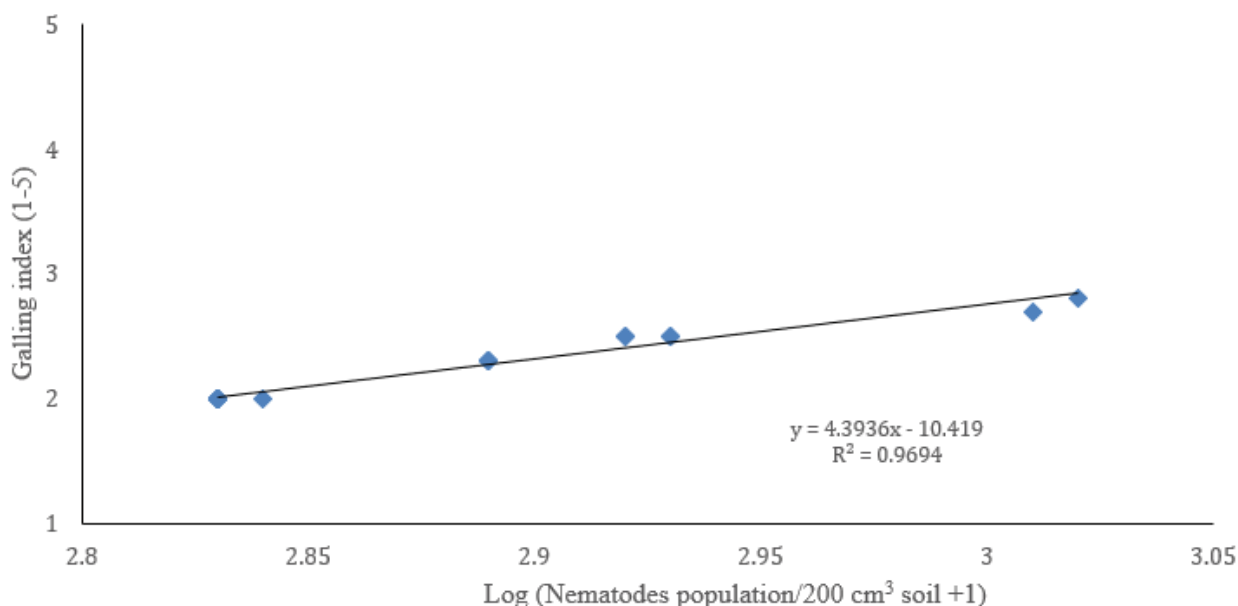
00/00362 and TDr 98/01067 affected their appearance due to galling and crazy rooting on symptoms tubers. However, mass of these two were in some instances higher than moderately resistant accessions. This observation confirms the assertion of Bridge et al. (2005) that *Meloidogyne* spp., do not necessary decrease tuber mass but marketability. Earlier studies reporting on variations in yield of crops have attributed differences in yield performance to genotypic characteristics (Ene et al., 2016; Usman et al., 2017). The moderately resistant white yam accessions identified in this study will help reduce *Meloidogyne incognita* population build up and contribute to the management of root-knot nematode menace in yam production.

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**Fig. 3:** Relationship between *M. incognita* population and galling index

Breeding sections of the CSIR-Crops Research Institute, Kumasi, Ghana.

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