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Host status of Bambara groundnut accessions to *Meloidogyne incognita* using conventional screenhouse and novel seedling pouch methods

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Summary – Bambara groundnut (*Vigna subterranea*) is an underexploited legume indigenous to sub-Saharan Africa with high protein content. Production constraints of Bambara groundnut include the root-knot nematodes (*Meloidogyne* spp.), which causes substantial losses that present an additional burden that growers need to manage. In this study we: *i*) assessed genetic resistance against *Meloidogyne incognita* in Bambara groundnut; and *ii*) evaluated an improved, more efficient screening method for Bambara. Fifty accessions were evaluated in two screenhouse trials. Following this, the use of seedling pouches was assessed for a high throughput screening method, using two accessions identified from each of three host status categories (resistant, tolerant and susceptible). Variability in host response was observed between the trials but five accessions demonstrated good resistance to *M. incognita*. A general reduction in plant growth parameters was observed on inoculated plants, especially for chlorophyll content at 11 weeks after planting. The pouch screening system confirmed the screenhouse pot results but appears to be more sensitive. From a range of inocula no differences in gall index were observed over the 28 days of assessment in pouches but the number of egg masses proved a useful parameter and differed between inoculum levels, with 500 juveniles/eggs determined as the optimum inoculum level. The use of seedling pouches reduced time, space and cost to screen Bambara groundnuts for resistance against *M. incognita*. Consequently, it is recommended as an efficient, high throughput, rapid screening protocol for Bambara groundnut compared to conventional pot screening methods.

Keywords – food security, high throughput, legume, resistance, root-knot nematodes, screening, sub-Saharan Africa, *Vigna subterranea*.

Bambara groundnut, *Vigna subterranea*, is an interesting but underutilised legume crop, with a high nutritive value (Doku, 1995) and rich in essential amino acids (Bamashiye *et al*., 2011). Although not widely accepted or exploited as a crop, it is the third most important grain legume in semi-arid Africa, after peanut (groundnut; *Arachis hypogaea*) and cowpea (*Vigna unguiculata*). The crop is dispersed across sub-Saharan Africa, where it can be locally important (Ocran, 1998). It tends to be consumed fresh or boiled after drying, as a snack or food supplement rather than as a main staple (Linnemann, 1992). Indigenous to West Africa, from where it is named after the Bambara people (Hepper, 1963), a secondary cultivation centre has developed in South-East Asia, such as Thailand, Indonesia, and parts of Malaysia (BamNetwork, 2020). It is traditionally cultivated in extreme tropical environments under smallholder cropping conditions (Mabhaudi *et al*., 2013). Bambara groundnut is suited

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to marginal soils and is one of Africa's most drought-tolerant native legume food crops. It is a low-growing annual crop and similar to peanut in that it produces stems after fertilisation that grow down into the soil, developing pods, each with one or two smooth round seeds. Due to the low economic status of Bambara groundnut, there has been only limited or no support for research on its cultivation or use, even though it offers numerous benefits (Massawe *et al*., 2005). In order to exploit the crop better and be able to offer more educated recommendations, an assessment of germplasm held in the Genetic Resources Centre of the International Institute of Tropical Agriculture (IITA) is underway to identify beneficial attributes and traits, such as pest and disease resistance.

Although there is only limited information regarding the susceptibility of Bambara groundnuts to plant-parasitic nematodes, there are indications that root-knot nematode (RKN) *Meloidogyne* spp. can be destructive and are likely to be the most important nematode pests of the crop (Fourie *et al*., 2017; Timper *et al*., 2018). As RKN are also viewed as posing one of the greatest biotic threats to crop production across Africa and the tropics (Coyne *et al*., 2018), assessment of germplasm for resistance against these pervasive pests would be useful. The enormous host range of some RKN species, such as *Meloidogyne incognita* and *M. javanica*, makes them extremely persistent and very difficult to manage. The screening of resistance against such pests, therefore, is particularly useful as a basis for use in breeding programmes and towards RKN management. The most commonly recorded nematode pests of Bambara groundnut are *M. incognita* and *M. javanica*, which are both widespread through Africa and tropical cropping systems in general (Hillocks *et al*., 2012; Sikora *et al*., 2018). Studies show that while damage is variable, severe damage to Bambara production can occur (Ogbuji, 1979), especially by *M. javanica* in sandy soils (McDonald & De Waele 1989; Goli, 1995). However, the overall impact of these nematodes on Bambara production is not known, due to the limited available information. Symptoms of infection can include severe root galling damage (Fig. 1), a reduced root system, stunting, chlorosis, reduced plant density and pod yield (McDonald & De Waele, 1989). While most genotypes appear to be susceptible to RKN, some resistance and/or tolerance has been observed (Ogbuji, 1979; McDonald & De Waele, 1989; Kwerepe & Labuschagne, 2004). Establishing which genotypes possess resistance, from those housed in the IITA Genetic Resources Centre repository, would consequently provide important information.

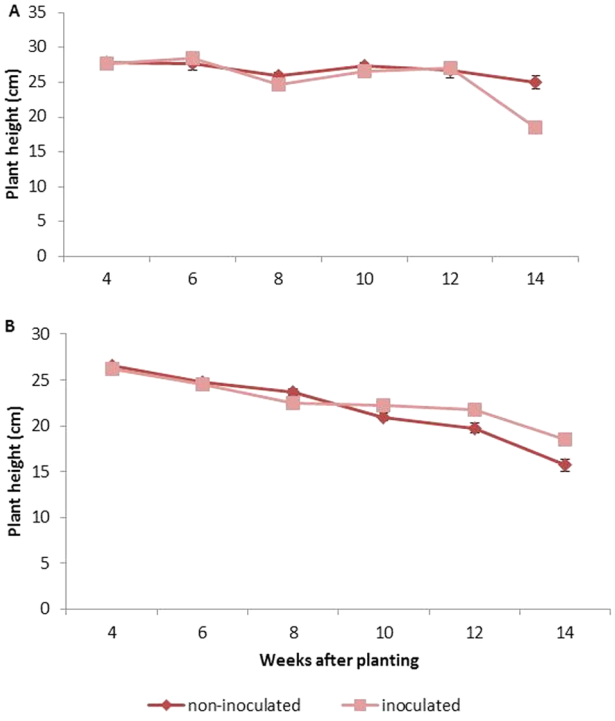

Fig. 1. Mean plant height across 50 accessions of Bambara groundnut in two experiments (A, B) following inoculation with 10 000 second-stage juveniles of *Meloidogyne incognita* in pots in the screenhouse, Ibadan, Nigeria.

The current study aimed at assessing resistance to *M. incognita* from a small sub-set of approximately 2000 Bambara groundnut accessions held in the IITA repository. In addition, we evaluated an alternative RKN resistance screening method, towards determining more efficient screening with higher throughput to enable mass screening of the germplasm collection. To identify resistance against *M. incognita*, diverse Bambara groundnut genotypes, originating from across Africa, were selected from the germplasm collection and screened in pots over 12 weeks in the screenhouse. To explore a more efficient screening mechanism, genotypes identified as resistant, susceptible or tolerant were selected and re-screened using seedling pouches over a 28-day period (Atamian *et al*., 2012). This procedure was also undertaken to adapt the pouch method to Bambara groundnuts and to local conditions, to determine a reliable and more efficient screening protocol (Coyne & Ross, 2014).

Table 1. Bambara groundnut accessions collected from the Genetic Resources Centre, IITA, Ibadan, Nigeria.

No.	Accession	Country of origin	No.	Accession	Country of origin
1	TVSu-200	Benin	26	TVSu-388	Sudan
2	TVSu-305	Burkina Faso	27	TVSu-378	Tanzania
3	TVSu-315	Burkina Faso	28	TVSu-1628	Togo
4	TVSu-1920	Cameroon	29	TVSu-1626	Togo
5	TVSu-475	Cameroon	30	TVSu-1606	Togo
6	TVSu-527	Cameroon	31	TVSu-1419	Togo
7	TVSu-534	Cameroon	32	TVSu-1420	Togo
8	TVSu-487	Cameroon	33	TVSu-1698	Togo
9	TVSu-506	Cameroon	34	TVSu-2059	Unknown
10	TVSu-465	Cameroon	35	TVSu-1584	Unknown
11	TVSu-401	Cameroon	36	TVSu-2037	Unknown
12	TVSu-424	Cameroon	37	TVSu-787	Zambia
13	TVSu-524	Cameroon	38	TVSu-914	Zambia
14	TVSu-216	Ghana	39	TVSu-1739	Zambia
15	TVSu-1449	Ghana	40	TVSu-689	Zambia
16	TVSu-792	Kenya	41	TVSu-888	Zambia
17	TVSu-1929	Malawi	42	TVSu-779	Zambia
18	TVSu-1794	Malawi	43	TVSu-710	Zambia
19	TVSu-1775	Malawi	44	TVSu-1014	Zimbabwe
20	TVSu-1797	Malawi	45	TVSu-1078	Zimbabwe
21	TVSu-1833	Niger (not confirmed)	46	TVSu-1874	Zimbabwe
22	TVSu-335	Nigeria	47	TVSu-1130	Zimbabwe
23	TVSu-368	Nigeria	48	TVSu-1051	Zimbabwe
24	TVSu-365	Nigeria	49	TVSu-1939	Zimbabwe
25	TVSu-329	Nigeria	50	TVSu-1953	Zimbabwe

TVSu = Tropical *Vigna subterrenea.*

Materials and methods

Pot screening study

The study was conducted in the Nematology Unit at IITA, Ibadan, Nigeria, during 2018 and 2019. Fifty Bambara groundnut accessions originating from various countries in Africa were obtained from the IITA Genetic Research Centre (Table 1). Accessions were randomly selected to represent a range of country origins, although little information is available for many of the selected accessions.

Experimental layout and conditions

Sandy-loamy topsoil was collected, steam-sterilised for 2.5 h, cooled for 24 h then filled into 10 l capacity pots and arranged in the screenhouse. The study included two factors: Bambara accessions (50) and nematode treatments (inoculated and non-inoculated) with three replicate plants (pots) per treatment in the first trial and four replicate pots in the second trial with a total number of 300 and 400 pots, respectively. Each pot was placed on a plastic plate on the floor, arranged in a randomised complete block design for genotypes, with inoculated and non-inoculated (control) treatments separated to further prevent contamination of control pots when watering the inoculated plants. Two seeds of Bambara were sown per pot and thinned to one plant at 3 weeks after planting (WAP). The first trial was conducted in August 2018 with a mean temperature of 25°C and relative humidity (RH) of 85%, and the second trial was in January 2019 with a mean temperature of 30°C and 62% RH. Pots were irrigated at 2-day intervals. From around 5 WAP, flowering was noticed in some plants and soil was manually heaped around the base of plants to cover and protect the pods as they formed. Both trials were terminated at 14 WAP.

Meloidogyne incognita *inoculum*

A population of *M. incognita* originally isolated from tomato in Nigeria and identified using perineal pattern and isozyme techniques (dos Santos *et al*., 2019) was

used in the study. Pure cultures, derived from a single egg mass population, were maintained in the screenhouse on cockscomb (*Celosia argentea*) and tomato (*Solanum lycopersicum*). *Meloidogyne incognita* eggs and second-stage juveniles (J2) were extracted from infected cockscomb and tomato roots using the Hussey & Barker (1973) NaOCl method. Briefly, galled roots were gently removed from pots, washed, chopped and placed in a conical flask with 500 ml 0.5% NaOCl. The flask was manually shaken for 4 min, poured through nested 212 μm, 90 μm and 25 μm sieves, thoroughly rinsed under running water and nematodes collected from the 25 μm sieve into a clean beaker. Nematode density was estimated from 3 × 1 ml aliquots and counted under a Leica 12.5 dissection microscope. Nematodes were surface-sterilised in streptomycin solution (20 g l^{-1} sterile distilled water) for 5 min, rinsed in sterile distilled water and inoculated in the evening at 3 WAP at a rate of 10 000 eggs/J2 plant^{-1}, which were delivered into a furrow made around the plant stem (*ca* 1 cm diam.). Pots were moistened prior to inoculation to aid nematode movement and survival.

Data collection

Data on plant height, canopy width and number of leaves were recorded for each plant at 2-week intervals from 4 WAP, while chlorophyll content was recorded at 8 and 12 WAP using a SPAD-502 leaf chlorophyll meter (Minolta). Plant height was measured by gathering the branches together and measuring from the soil line to the tip of the highest leaf. Data collected per plant at harvest included number of pods per plant, weight of total pods per plant, root fresh weight, root length, nematode population in roots and soil and nematode gall damage index, where 1 = no galling; 2 = $\leqslant$ 25% roots galled; 3 = 26-50% roots galled; 4 = 51-75% roots galled; 5 $\geqslant$ 75% roots galled (Hussey & Janssen, 2002). Roots and pods were gently removed from pots and carefully shaken free of soil before weighing. Eggs and J2 were extracted from the whole root sample after chopping as above (Hussey & Barker, 1973) and J2 were extracted from a 200 ml sub-sample of soil using the modified Baermann pan extraction method after thorough mixing of soil per pot. The final mean nematode population (P_f) for each accession and treatment was calculated from the total nematode counts from the roots and from the total volume of soil calculated from the sub-sample count, and the reproductive factor (RF) obtained by dividing P_f by the initial nematode inoculation (P_i). Accession host status was derived using a combination of gall index, RF and yield, following a modification of Afolami (2000) based on Sasser *et al*. (1984): resistant (GI < 2, RF < 1 and yield of inoculated plant $\geqslant$ control), tolerant (GI $\leqslant$ 2, RF > 1 and yield of inoculated plant $\geqslant$ control), susceptible (GI > 2, RF > 1 and yield of inoculated plant < control).

Pouch experiment

Experimental layout and conditions

Based on the results obtained from the pot experiment, two genotypes each from three host status groups were selected: TVSu-1698 and TVSu-1833 from the resistant group, TVSu-1920 and TVSu-1628 from the susceptible group and TVSu-1953 and TVSu-1797 from the tolerant group. These genotypes were evaluated using a range of nematode inocula in seedling pouches, based on the protocol by Atamian *et al*. (2012) and modified by Coyne & Ross (2014), to assess the suitability of the pouch method for Bambara groundnut and determine an optimum inoculum level.

Seeds were surface-sterilised in 1% NaOCl for 2 min and then rinsed in three changes of sterile distilled water. Filter papers were sprayed with 75% ethanol, allowed to dry, then moistened with sterilised distilled water, placed in 9 cm diam. sterile Petri dishes, covered and sealed. Multiple seeds of the same genotype were placed onto the moistened filter paper to germinate. Once germinated, seeds were transferred singly into a seedling pouch and labelled. The seedling pouches measured 15.5 × 13.5 cm with a thin transparent outer polythene layer lined with absorbent paper internally. The plants were grown in the pouches for 10 days and uniform plants in good condition with three true leaves present were selected for inoculation with 500, 1000, 1500 and 2000 surface-sterilised (2% streptomycin solution) *M. incognita* J2, or distilled water for the non-inoculated control. Seedling pouches were then laid horizontally and maintained in the dark for 2 days. The pouches were watered daily, initially with Hoagland solution (Hoagland & Arnon, 1950) as a nutrient source, at 2 days after inoculation. The pouches were then placed upright in a rack and placed in a controlled environment chamber maintained at 25-28°C and 12 h light/12 h dark cycle. The experiment was arranged in a complete randomised design, replicated four times and repeated once. Data were recorded for plant height, number of leaves, root length and gall index (as above), while the number of egg masses that developed on roots were additionally counted.

Data analysis

The data collected were analysed by two-way Analysis of Variance using the SAS programme and the means separated using least significant difference (LSD) at $P \leqslant 0.05$ for differences between accessions. Differences between nematode inoculation treatments were compared using standard error of means. Nematode count data were transformed prior to analysis using square root transformation (SQRT[$x + 0.5$]) in order that data conformed to a normal distribution. Means of the pouch experiment were partitioned using Duncan Multiple Range Test.

Results

Effect of *Meloidogyne incognita* on the growth of Bambara groundnut accessions

There were no significant differences ($P \leqslant 0.05$) in plant height and canopy width between the non-inoculated plants and inoculated plants across genotypes at 4, 6, 8, 10 and 12 WAP in the first trial. However, at 14 WAP, non-inoculated plants were taller (24.98 cm) and the canopy wider than the inoculated plants (Figs 1A; 2A). In the second trial non-inoculated and inoculated plants had similar plant height at 4, 6 and 8 WAP, while at 10, 12 and 14 WAP the inoculated plants were taller ($P \leqslant 0.05$) than non-inoculated plants (Fig. 1B). No significant differences ($P \leqslant 0.05$) in canopy width were observed for the first 6 weeks between the non-inoculated plants and the inoculated plants after which inoculated plants had a wider canopy (Fig. 2B).

Among the non-inoculated plants in the first trial, TVSu-305 was the tallest followed by TVSu-689, while TVSu-365 and TVSu-1014 were the shortest (Table 2). In the inoculated plants, TVSu-1739, TVSu-689 and TVSu-2059 were taller ($P \leqslant 0.05$) than the other accessions. When comparing the non-inoculated plants and the inoculated plants, 13 accessions of non-inoculated plants were taller ($P \leqslant 0.05$) than inoculated plants, eight accessions were shorter than inoculated plants, and 29 accessions did not differ significantly. At 8 and 11 WAP in the first trial, and at 11 WAP in the second trial, plants inoculated with *M. incognita* had significantly ($P \leqslant 0.05$) lower chlorophyll contents than non-inoculated plants (Fig. 3A). At 8 WAP in the second trial, there was no difference ($P \leqslant 0.05$) between the inoculated and non-inoculated plants but at 11 WAP, non-inoculated plants had significantly higher chlorophyll contents (Fig. 3B). At 4 WAP, there was no difference ($P \leqslant 0.05$) in the number of leaves between the non-inoculated and inoculated plants in the first and second trials. However, at 6 WAP, non-inoculated plants had more leaves ($P \leqslant 0.05$) than inoculated plants (Fig. 4A). In the first and second trial the inoculated plants had more leaves than non-inoculated plants (Fig. 4B). At harvest in the first trial, among the non-inoculated plants (Table 3) TVSu-1078 and TVSu-1797 had the heaviest ($P \leqslant 0.05$) root weights, and TVSu-710 the lowest root weight. In the inoculated pots, TVSu-368 had heavier roots ($P \leqslant 0.05$) compared to all other accessions.

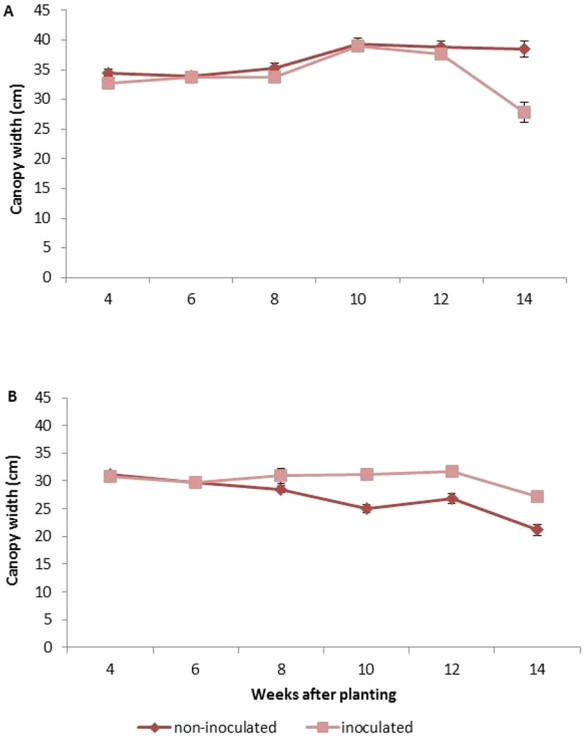

Fig. 2. Mean canopy width across 50 accessions of Bambara groundnut in two experiments (A, B) following inoculation with 10 000 second-stage juveniles of *Meloidogyne incognita* in pots in the screenhouse, Ibadan, Nigeria. Bars indicate standard error.

Yield, nematode damage and host status of Bambara groundnut accessions

Among the non-inoculated plants in the first trial (Table 4), TVSu-335 had the highest yield, and TVSu-787 and TVSu-1794 had no yield. In the inoculated pots, TVSu-487 had the highest yield compared to all other accessions. In the second trial, yield of Bambara was generally low and for many pots there was no podding in the non-inoculated and inoculated plants.

Table 2. Plant height (cm) of Bambara groundnut accessions at 8 weeks after planting in pots in the screenhouse, Ibadan, Nigeria.

Accession[1]	Trial I			Trial 2		
	Control	Inoculated	SEM[2]	Control	Inoculated	SEM[2]
TVSu-1014	17.70	23.57	1.82*	21.25	13.88	2.82*
TVSu-1051	26.80	23.40	1.46*	22.75	18.78	3.08^{NS}
TVSu-1078	25.53	23.27	2.22^{NS}	22.63	24.08	0.75^{NS}
TVSu-1130	28.93	27.70	2.92^{NS}	28.50	23.55	1.23*
TvSu-1419	21.07	21.43	1.40^{NS}	22.93	18.95	1.08*
TVSu-1420	20.77	20.30	1.09^{NS}	21.93	19.63	1.17^{NS}
TVSu-1449	25.90	23.13	0.99*	23.70	24.25	0.73^{NS}
TVSu-1584	28.77	23.63	1.73*	21.88	21.33	0.84^{NS}
TVSu-1606	22.40	21.07	2.17^{NS}	26.00	16.90	3.23*
TVSu-1626	26.53	22.60	1.18*	23.95	22.35	0.80^{NS}
TVSu-1628	30.43	23.63	3.95^{NS}	22.60	22.15	1.14^{NS}
TVSu-1698	21.27	20.20	2.84^{NS}	25.88	24.33	0.64^{NS}
TVSu-1739	27.37	31.97	1.92*	30.23	30.18	0.65^{NS}
TVSu-1775	27.87	25.40	1.36^{NS}	22.88	21.85	0.54^{NS}
TVSu-1794	24.57	22.47	1.88*	24.18	24.20	0.51^{NS}
TVSu-1797	30.37	28.43	1.36^{NS}	28.40	26.78	1.25^{NS}
TVSu-1833	21.23	20.67	1.42^{NS}	15.95	19.85	1.44*
TVSu-1874	32.20	28.07	1.21*	32.65	28.63	0.91*
TVSu-1920	26.27	27.67	1.01^{NS}	25.23	26.33	0.88^{NS}
TVSu-1929	25.37	28.27	1.12*	23.98	23.78	0.92^{NS}
TVSu-1939	25.93	25.93	0.58^{NS}	22.85	22.28	1.37^{NS}
TVSu-1953	20.30	20.97	1.26^{NS}	21.00	21.90	0.61^{NS}
TVSu-200	25.83	18.13	2.98*	20.75	22.28	3.25^{NS}
TVSu-2037	25.57	27.83	1.42^{NS}	25.23	26.20	1.10^{NS}
TVSu-2059	31.47	30.10	1.52^{NS}	33.63	30.73	1.29*
TVSu-216	26.17	26.57	0.75^{NS}	25.40	23.73	0.87^{NS}
TVSu-305	42.33	23.47	8.87*	22.08	21.60	0.74^{NS}
TVSu-315	22.20	27.27	2.09*	21.33	16.83	2.88*
TVSu-329	27.50	26.37	2.04^{NS}	23.83	25.98	1.15^{NS}
TVSu-335	28.43	25.97	1.60^{NS}	25.65	25.40	0.81*
TVSu-365	16.90	22.53	1.45*	16.13	16.38	1.49*
TVSu-368	29.77	20.13	2.88*	22.05	24.20	1.37*
TVSu-378	25.03	25.47	1.04^{NS}	19.00	24.18	1.50*
TVSu-388	29.43	24.97	2.75^{NS}	25.40	18.68	3.24*
TVSu-401	27.63	27.37	1.98^{NS}	20.40	22.08	1.63^{NS}
TVSu-424	23.23	24.60	0.65*	22.83	22.30	0.51^{NS}
TVSu-465	25.30	22.83	1.44^{NS}	23.45	26.28	1.35*
TVSu-475	28.20	25.23	1.51^{NS}	22.95	19.80	0.97*
TVSu-487	23.97	25.73	1.42^{NS}	25.53	23.23	1.06*
TVSu-506	23.77	25.90	0.53*	19.80	11.08	3.46*
TVSu-524	23.93	21.03	2.85^{NS}	25.43	14.90	3.12*
TVSu-527	23.20	28.43	1.63*	24.20	23.75	0.97^{NS}
TVSu-534	27.63	24.50	1.51*	21.93	24.00	1.38^{NS}
TVSu-689	33.97	30.10	1.59*	26.10	26.83	1.35^{NS}
TVSu-710	18.37	18.67	1.49^{NS}	18.85	18.15	1.40^{NS}
TVSu-779	26.23	22.07	1.19*	23.35	23.43	0.81^{NS}
TVSu-787	28.97	27.07	0.91*	25.03	24.28	0.72^{NS}

Table 2. (Continued.)

Accession[1]	Trial I			Trial 2		
	Control	Inoculated	SEM[2]	Control	Inoculated	SEM[2]
TVSu-792	22.67	24.83	1.15^{NS}	21.15	21.75	0.87^{NS}
TVSu-888	26.70	24.73	2.67^{NS}	23.60	25.23	1.16^{NS}
TVSu-914	28.33	27.13	0.75^{NS}	27.20	27.35	0.72^{NS}
LSD[3]	3.64	2.45		1.92	2.41	

[1] n = 3 replicates in the first trial and 4 replicates in the second trial.
[2] SEM = standard error of mean for comparison between rows of inoculated and control for each trial.
[3] LSD = least significance difference ($P \leqslant 0.05$) for comparison within a column (Bambara accessions).
* Significant difference at $P \leqslant 0.05$; NS = no significant difference.

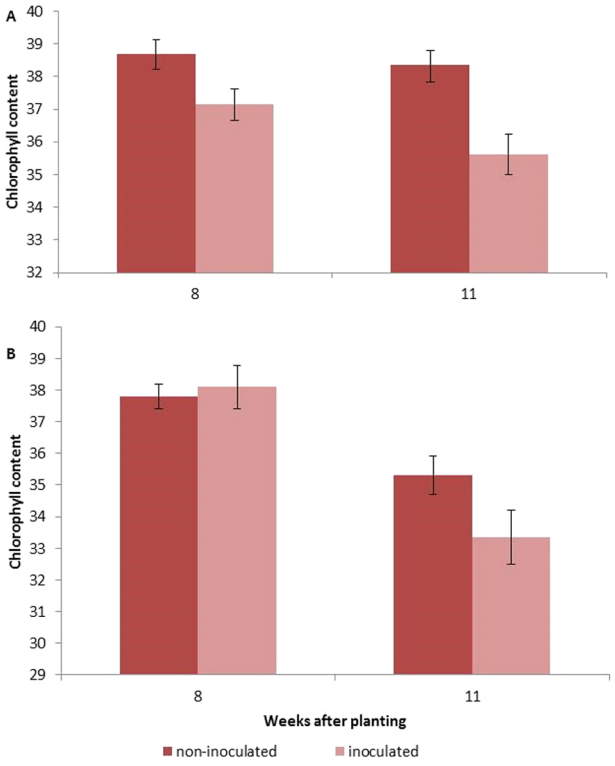

Fig. 3. Mean leaf chlorophyll content (using a SPAD leaf chlorophyll meter) across 50 accessions of Bambara groundnut in two experiments (A, B) following inoculation with 10 000 second-stage juveniles of *Meloidogyne incognita* in pots in the screenhouse, Ibadan, Nigeria. Bars indicate standard error.

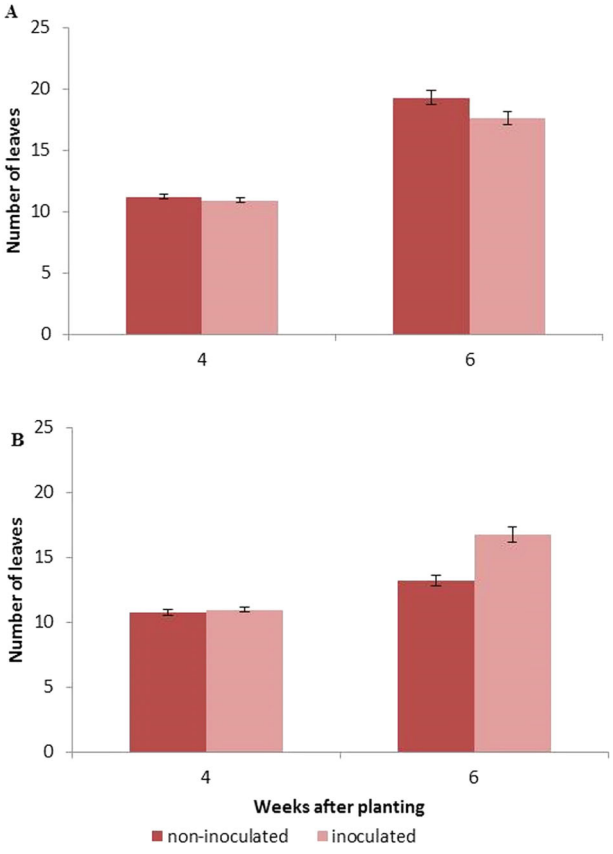

Fig. 4. Mean number of leaves across 50 accessions of Bambara groundnut in two experiments (A, B) following inoculation with 10 000 second-stage juveniles of *Meloidogyne incognita* in pots in the screenhouse, Ibadan, Nigeria. Bars indicate standard error.

In the first trial, inoculated plants of TVSu-1920 had a higher gall index ($P \leqslant 0.05$) than other accessions, while TVSu-1419, TVSu-1698, TVSu-1833, TVSu-315 and TVSu-524 had the lowest gall index (Table 4). In the second trial, TVSu-1739 had the highest gall index ($P \leqslant 0.05$) (Table 4). Inoculated plants of TVSu-524, TVSu-315, TVSu-1698 and TVSu-506, however, had no galls. In the first trial, TVSu-1628 and TVSu-1920 had a higher ($P \leqslant 0.05$) RF than other inoculated accessions, while TVSu-1698 and TVSu-1833 had the lowest RF

Table 3. Root weight (g) of Bambara groundnut accessions at harvest of pots in the screenhouse, Ibadan, Nigeria.

Accession[1]	Trial 1			Trial 2		
	Control	Inoculated	SEM[2]	Control	Inoculated	SEM[2]
TVSu-1014	7.59	21.43	4.98*	1.81	2.31	0.62^{NS}
TVSu-1051	8.87	5.94	1.92^{NS}	4.82	6.88	2.24^{NS}
TVSu-1078	19.64	23.64	5.02^{NS}	0.97	10.33	2.06*
TVSu-1130	14.24	9.77	5.04^{NS}	2.85	16.26	2.80*
TVSu-1419	6.24	7.69	3.54^{NS}	1.70	9.64	1.97*
TVSu-1420	9.53	2.54	1.64*	1.54	6.60	1.18*
TVSu-1449	14.85	8.55	1.85*	2.69	5.04	1.13*
TVSu-1584	5.10	16.31	3.71*	1.89	3.79	0.67*
TVSu-1606	8.05	5.3	2.95^{NS}	2.22	4.22	1.31^{NS}
TVSu-1626	9.41	2.29	2.53*	2.37	14.2	4.90*
TVSu-1628	6.78	11.25	2.03*	1.03	7.29	1.85*
TVSu-1698	2.56	1.19	0.49^{NS}	1.47	3.71	1.05^{NS}
TVSu-1739	9.98	11.76	2.73^{NS}	2.20	8.62	1.41*
TVSu-1775	14.89	11.46	2.64^{NS}	2.75	11.64	2.148
TVSu-1794	6.93	7.70	2.23^{NS}	1.99	10.97	2.38*
TVSu-1797	19.19	4.87	3.94*	1.56	6.28	1.03*
TVSu-1833	4.82	2.19	0.93*	0.66	3.41	0.56*
TVSu-1874	16.59	14.49	2.28^{NS}	2.38	9.47	2.08*
TVSu-1920	8.24	16.46	2.49*	1.72	6.10	1.07*
TVSu-1929	5.27	15.83	3.59*	1.84	6.00	0.96*
TVSu-1939	12.6	8.26	2.44^{NS}	1.97	6.58	1.70*
TVSu-1953	4.66	6.85	2.74^{NS}	1.45	5.35	0.97*
TVSu-200	5.77	3.73	1.66^{NS}	1.28	7.32	1.27^{NS}
TVSu-2037	14.08	13.34	1.94^{NS}	1.92	7.00	1.37*
TVSu-2059	6.06	18.32	3.48*	5.67	5.65	1.98^{NS}
TVSu-216	12.24	13.93	5.39^{NS}	4.31	12.65	2.07*
TVSu-305	3.68	5.36	1.28^{NS}	1.66	7.28	1.82*
TVSu-315	3.28	2.14	1.23*	1.14	3.28	0.82*
TVSu-329	10.96	8.40	2.38^{NS}	1.28	13.6	2.88*
TVSu-335	14.48	13.97	3.02^{NS}	2.65	12.98	2.61*
TVSu-365	5.21	5.80	1.58^{NS}	1.86	4.74	1.30*
TVSu-368	6.31	29.21	12.98^{NS}	2.19	7.29	1.46*
TVSu-378	16.34	6.34	3.27*	1.02	7.43	1.67*
TVSu-388	4.48	3.45	1.07^{NS}	1.37	1.62	0.40^{NS}
TVSu-401	6.12	7.19	2.59^{NS}	2.39	3.97	0.41*
TVSu-424	7.07	2.06	2.05^{NS}	1.32	7.32	1.39*
TVSu-465	6.38	2.77	2.42*	1.98	5.04	1.33*
TVSu-475	12.04	10.78	2.76^{NS}	2.20	9.78	1.70*
TVSu-487	6.17	7.42	2.11^{NS}	2.56	8.35	1.33*
TVSu-506	8.10	10.23	1.66^{NS}	0.81	2.35	0.98*
TVSu-524	6.77	2.47	1.92*	2.49	3.06	0.98^{NS}
TVSu-527	3.26	15.52	4.40*	2.09	9.79	2.43*
TVSu-534	14.93	11.63	2.56^{NS}	1.80	3.81	0.908
TVSu-689	8.56	9.67	3.05^{NS}	1.64	14.05	3.06*
TVSu-710	1.90	1.90	0.52^{NS}	1.01	6.00	1.78*
TVSu-779	11.96	5.86	1.56*	2.07	8.73	1.36*
TVSu-787	16.49	14.75	3.30^{NS}	1.44	9.96	2.67*

Table 3. (Continued.)

Accession[1]	Trial 1			Trial 2		
	Control	Inoculated	SEM[2]	Control	Inoculated	SEM[2]
TVSu-792	5.59	8.83	3.08^{NS}	1.99	4.97	0.65*
TVSu-888	7.58	13.52	3.72*	2.22	8.97	2.06*
TVSu-914	15.15	15.00	3.21^{NS}	1.03	9.39	3.02*
LSD[3]	4.84	7.71		2.56	4.34	

[1] n = 3 replicates in the first trial and 4 replicates in the second trial.
[2] SEM = standard error of mean for comparison between rows of inoculated and control for each trial.
[3] LSD = least significance difference ($P \leqslant 0.05$) for comparison within a column (Bambara accessions).
* Significant difference at $P \leqslant 0.05$; NS = no significant difference.

(Table 4). In the second trial, TVSu-1920 had the highest ($P \leqslant 0.05$) RF with TVSu-1698 and TVSu-1833 again having the lowest RF.

From the first trial, five accessions: TVSu-1419, TVSu-1698, TVSu-1833, TVSu-315 and TVSu-524, were rated as resistant to *M. incognita* (Table 4). TVSu-1051, TVSu-1628, TVSu-1739, TVSu-1775, TVSu-1794, TVSu-2037, TVSu-329, TVSu-368, TVSu-527, TVSu-689, TVSu-779, TVSu-914 and TVSu-1920 were rated susceptible, whilst the remaining 32 accessions were rated tolerant to *M. incognita*. In the second trial, TVSu-1833, TVSu-1628, TVSu-1739, TVSu-1775, TVSu-1794, TVSu-368, TVSu-1078, TVSu-1420, TVSu-1797 and TVSu-1929 were tolerant and the remaining 40 accessions were rated resistant.

Effect of *Meloidogyne incognita* inoculum level on Bambara groundnut growth in pouches 10 days post inoculation

There was no significant difference in plant height and the number of leaves of TVSu-1628, TVSu-1833 and TVSu-1953 in either the first or second trials among all inoculum levels (P_i) (Table 5). However, some differences were observed in TVSu-1698, TVSu-1797 and TVSu-1920 between the different inoculum levels. In general, the galling index and number of egg masses were relatively low on all accessions in both trials, and relatively fewer in the first trial (Tables 6, 7). Differences were observed between inoculation levels for some accessions while for some, such as TVSu-1698, differences were observed only in the second trial. There was no significant difference in galling index among the inoculum treatments, which was observed only on inoculated plants (Table 7). Inoculation at 500 and 1000 J2 resulted in the highest gall index in the first trial and 500 in the second trial, followed by 1000 J2. More egg masses were recorded when inoculated with 1000 (2.17) and 500 (1.96) than non-inoculated plants in the first trial, while inoculation with 500 (8.92) and 1000 J2 (5.92) resulted in more egg masses than all other treatments in the second trial.

Discussion

As RKN are viewed as among the most serious threats to crop production in Africa (Onkendi *et al*., 2014; Coyne *et al*., 2018), it is vitally important to identify as many sources of resistance against these pervasive pests towards developing sustainable management strategies (Viaene *et al*., 2013). The current study shows that good levels of resistance are available in Bambara groundnut germplasm, at least against *M. incognita*. Four (8%) accessions were consistently rated as resistant to this nematode across the pot trials. The results of the second trial differed substantially, however, probably due to differences in the environmental conditions, resulting in variability of the data. The first trial was conducted from August to December corresponding to the normal Bambara season, while the second trial was conducted from January to April, under hotter conditions, which may have influenced the performance of the crop. Growth of all accessions was generally lower, with only limited yield, while nematode damage and multiplication was lower, likely due to reduced root growth to support nematode feeding. Such unevenness in results between trials is not necessarily uncommon, however, and has also been reported when assessing Bambara groundnut landraces (Kwerepe & Labuschagne, 2004). It also illustrates the need for more efficient, uniform and less cumbersome screening techniques. However, the current study demonstrates good

Table 4. Reaction of Bambara groundnut accessions to *Meloidogyne incognita* inoculation in pots in the screenhouse, Ibadan, Nigeria.

Accession[1]	Trial I						Trial 2					
	Yield (g)[2]			Gall index[4]	RF[5]	Host status[6]	Yield (g)			Gall index	RF	Host status
	Inoculated	Control	SEM[3]				Inoculated	Control	SEM			
TVSu-1419	19.11	19.44	9.26^{NS}	2.00	0.48	Resistant	0.55	0.57	0.36^{NS}	1.75	0.85	Resistant
TVSu-1698	8.32	24.99	5.71*	2.00	0.15	Resistant	0.89	0.31	0.45^{NS}	1.00	0.72	Resistant
TVSu-1833	15.24	5.89	4.01^{NS}	2.00	0.22	Resistant	1.60	0.43	0.41*	1.50	0.73	Tolerant
TVSu-315	4.03	0.89	1.57^{NS}	2.00	0.45	Resistant	0.00	0.00	0.00^{NS}	1.25	0.77	Resistant
TVSu-524	4.90	16.34	5.79^{NS}	2.00	0.56	Resistant	0.00	0.00	0.00^{NS}	1.00	0.74	Resistant
TVSu-1051	12.98	18.97	3.43^{NS}	3.67	2.52	Susceptible	3.09	2.50	0.94^{NS}	1.63	0.86	Resistant
TVSu-1628	18.07	17.88	7.15^{NS}	3.33	5.23	Susceptible	0.00	0.15	0.08^{NS}	2.00	1.11	Tolerant
TVSu-1739	16.46	11.90	3.40^{NS}	4.00	4.31	Susceptible	0.00	0.00	0.00^{NS}	2.50	1.02	Tolerant
TVSu-1775	5.60	7.15	2.47^{NS}	3.00	2.36	Susceptible	0.00	0.00	0.00^{NS}	2.13	1.03	Tolerant
TVSu-1794	0.00	0.00	0.00^{NS}	3.00	0.71	Susceptible	0.00	0.00	0.00^{NS}	2.13	1.04	Tolerant
TVSu-2037	0.91	8.71	2.02*	4.00	1.15	Susceptible	0.00	0.00	0.00^{NS}	1.63	1.05	Resistant
TVSu-329	7.94	25.12	5.21*	2.33	2.94	Susceptible	0.00	0.00	0.00^{NS}	2.13	1.00	Resistant
TVSu-368	0.56	19.68	6.24*	3.33	2.39	Susceptible	1.40	0.00	0.46*	2.38	0.93	Tolerant
TVSu-527	23.50	18.98	5.50^{NS}	4.00	4.81	Susceptible	0.00	0.00	0.00^{NS}	1.63	0.98	Resistant
TVSu-689	10.41	24.59	4.47*	4.00	4.05	Susceptible	0.00	0.00	0.00^{NS}	2.00	0.96	Resistant
TVSu-779	1.04	1.13	0.54^{NS}	3.67	3.86	Susceptible	0.00	0.00	0.00^{NS}	2.13	0.97	Resistant
TVSu-914	14.24	24.95	4.05*	3.00	4.12	Susceptible	0.86	0.90	0.57^{NS}	2.00	0.89	Resistant
TVSu-1920	5.27	2.45	1.07*	4.67	5.12	Susceptible	0.00	0.00	0.00^{NS}	1.88	1.17	Resistant
TVSu-1014	10.34	0.98	2.80*	3.67	4.72	Tolerant	0.00	0.12	0.06^{NS}	1.25	0.83	Resistant
TVSu-1078	10.44	2.87	2.65*	2.33	2.21	Tolerant	0.00	0.00	0.00^{NS}	2.00	1.02	Tolerant
TVSu-1130	5.54	16.72	6.59^{NS}	2.67	1.30	Tolerant	0.00	0.00	0.00^{NS}	1.75	0.96	Resistant
TVSu-1420	5.01	27.63	7.48*	2.67	1.47	Tolerant	1.02	3.86	1.53^{NS}	2.38	0.98	Tolerant
TVSu-1449	6.40	12.97	2.77*	3.33	2.37	Tolerant	0.00	1.89	0.95^{NS}	1.50	0.84	Resistant
TVSu-1584	5.71	9.11	3.93^{NS}	4.33	0.91	Tolerant	0.00	0.00	0.00^{NS}	1.88	0.80	Resistant
TVSu-1606	4.51	13.87	3.33*	2.33	1.11	Tolerant	0.00	0.00	0.00^{NS}	1.38	0.87	Resistant
TVSu-1626	2.92	22.37	8.67*	3.00	0.45	Tolerant	0.00	0.00	0.00^{NS}	1.50	0.80	Resistant
TVSu-1797	11.59	28.02	5.56*	2.33	1.70	Tolerant	2.70	0.00	0.85*	1.75	0.90	Tolerant
TVSu-1874	2.61	7.49	2.96^{NS}	3.67	4.27	Tolerant	0.00	0.00	0.00^{NS}	1.75	0.86	Resistant
TVSu-1929	18.87	11.15	4.37^{NS}	3.67	4.11	Tolerant	3.57	0.15	1.59*	2.13	0.81	Tolerant
TVSu-1939	6.27	27.12	5.39*	3.67	4.77	Tolerant	0.41	2.24	0.97^{NS}	2.13	0.85	Resistant
TVSu-1953	11.59	11.37	4.99^{NS}	3.00	1.62	Tolerant	0.00	1.20	0.46*	1.63	0.88	Resistant
TVSu-200	7.57	23.75	5.43*	2.67	0.81	Tolerant	0.00	0.00	0.00^{NS}	2.00	0.94	Resistant
TVSu-2059	19.54	2.79	5.96*	3.33	4.54	Tolerant	0.00	0.00	0.00^{NS}	1.88	1.01	Resistant
TVSu-216	6.74	12.63	4.76^{NS}	2.33	2.09	Tolerant	0.00	0.00	0.00^{NS}	2.25	0.98	Resistant
TVSu-305	22.89	10.38	4.29*	3.33	0.32	Tolerant	0.31	0.48	0.27^{NS}	2.00	0.96	Resistant

Table 4. (Continued.)

Accession[1]	Trial I						Trial 2					
	Yield (g)[2]			Gall index[4]	RF[5]	Host status[6]	Yield (g)			Gall index	RF	Host status
	Inoculated	Control	SEM[3]				Inoculated	Control	SEM			
TVSu-335	2.27	39.28	10.36*	3.33	0.55	Tolerant	0.23	0.00	0.12^{NS}	1.88	0.81	Resistant
TVSu-365	20.64	11.08	5.14^{NS}	3.00	1.39	Tolerant	0.00	0.00	0.00^{NS}	1.63	0.88	Resistant
TVSu-378	7.20	10.74	3.93^{NS}	3.00	3.11	Tolerant	0.00	0.00	0.00^{NS}	2.13	0.87	Resistant
TVSu-388	5.45	23.08	4.61*	3.00	0.39	Tolerant	2.41	2.51	1.31^{NS}	1.50	0.86	Resistant
TVSu-401	15.65	14.24	5.60^{NS}	2.33	0.52	Tolerant	2.85	4.90	1.91^{NS}	2.00	0.89	Resistant
TVSu-424	13.06	14.77	2.94^{NS}	2.33	0.73	Tolerant	4.07	1.54	1.79^{NS}	1.63	0.82	Resistant
TVSu-465	16.32	18.04	5.77^{NS}	2.33	0.77	Tolerant	0.61	2.28	1.08^{NS}	1.50	0.80	Resistant
TVSu-475	23.91	25.02	5.73^{NS}	3.00	1.16	Tolerant	0.00	2.29	0.81*	1.75	0.77	Resistant
TVSu-487	33.30	13.46	6.86*	2.67	1.06	Tolerant	2.42	0.10	1.21^{NS}	1.75	0.92	Resistant
TVSu-506	20.97	16.82	2.72^{NS}	2.33	2.37	Tolerant	0.00	0.55	0.28^{NS}	0.88	0.74	Resistant
TVSu-534	18.94	23.77	3.92^{NS}	2.67	3.19	Tolerant	0.00	0.00	0.00^{NS}	1.63	0.81	Resistant
TVSu-710	6.33	7.76	3.05^{NS}	2.67	0.73	Tolerant	3.00	6.40	1.84^{NS}	1.63	0.87	Resistant
TVSu-787	5.83	0.00	2.92*	3.67	2.10	Tolerant	0.00	0.00	0.00^{NS}	2.13	0.87	Resistant
TVSu-792	15.34	16.47	6.73^{NS}	2.67	1.11	Tolerant	0.28	0.16	0.15^{NS}	2.13	0.82	Resistant
TVSu-888	12.82	7.18	4.43^{NS}	3.00	1.85	Tolerant	0.82	0.00	0.41^{NS}	2.13	0.99	Resistant
LSD[6]	13.21	25.03		0.55	3.27		0.02	0.02		1.48	2.04	

[1] n = 3 replicates in the first trial and 4 replicates in the second trial; inoculation with 10 000 eggs/second-stage juveniles pot^{-1}.

[2] Yield = Total weight of pods per plant.

[3] SEM = standard error of means between inoculated and control; * significant difference at $P \leqslant 0.05$; NS = no significant difference.

[4] Gall index where: 1 = no galling, 2 = ⩽ 25% roots galled, 3 = 26-50% roots galled, 4 = 51-75% roots galled, 5 ⩾ 75% roots galled after Hussey & Janssen (2002).

[5] RF = Reproductive Factor: final nematode density/initial nematode density.

[6] LSD = least significance difference ($P \leqslant 0.05$) for means within a column. Host status is derived using gall index, reproductive factor and yield as the factors, according to Afolami (2000) and Sasser *et al*. (1984), where Resistant (GI < 2, RF < 1 and yield of inoculated plant ⩾ control), tolerant (GI ⩽ 2, RF > 1 and yield of inoculated plant ⩾ control), and susceptible (GI > 2, RF > 1 and yield of inoculated plant < control).

Table 5. Growth parameters of selected Bambara groundnut accessions in growth pouches at 10 days after inoculation with *Meloidogyne incognita*, Ibadan, Nigeria.

Accession[1]	Inoculum[2]	Trial 1		Trial 2	
		Plant height	No. of leaves	Plant height	No. of leaves
TVSu-1628	0	18.28	5.00	17.60	5.00
TVSu-1628	500	20.55	3.50	18.13	4.50
TVSu-1628	1000	20.38	3.25	12.65	4.50
TVSu-1628	1500	21.80	3.25	17.85	5.00
TVSu-1628	2000	23.00	4.75	17.85	5.25
LSD		10.00	3.09	7.14	2.71
TVSu-1698	0	24.75	6.00	18.58	5.25
TVSu-1698	500	22.73	5.25	18.03	6.25
TVSu-1698	1000	22.55	4.25	19.93	4.75
TVSu-1698	1500	20.68	5.50	20.05	7.50
TVSu-1698	2000	24.88	6.25	15.40	7.00
LSD		2.72	1.96	8.96	5.79
TVSu-1797	0	15.88	2.50	22.05	4.75
TVSu-1797	500	23.15	3.25	24.93	4.50
TVSu-1797	1000	24.15	4.25	22.83	3.25
TVSu-1797	1500	25.48	3.25	24.18	3.75
TVSu-1797	2000	27.55	3.75	26.90	4.25
LSD		9.02	1.65	7.40	1.23
TVSu-1833	0	22.35	3.75	20.33	7.00
TVSu-1833	500	16.15	3.25	19.23	6.75
TVSu-1833	1000	16.10	2.75	20.35	8.25
TVSu-1833	1500	18.88	3.75	19.15	8.50
TVSu-1833	2000	23.60	5.25	20.30	7.25
LSD		13.07	3.25	5.00	3.05
TVSu-1920	0	11.88	1.50	11.68	3.75
TVSu-1920	500	11.98	3.25	12.08	4.50
TVSu-1920	1000	15.43	4.25	14.95	4.00
TVSu-1920	1500	24.73	6.75	12.33	4.25
TVSu-1920	2000	18.40	5.75	13.13	4.75
LSD		16.69	4.47	12.34	5.12
TVSu-1953	0	14.83	3.00	19.35	5.00
TVSu-1953	500	10.88	2.00	19.80	4.50
TVSu-1953	1000	15.65	3.00	20.60	3.50
TVSu-1953	1500	23.13	3.25	19.43	4.75
TVSu-1953	2000	17.05	2.75	19.63	4.75
LSD		15.62	3.29	3.55	1.63

[1] Means (n = 4) were separated using least significant difference (LSD) at $P \leqslant 0.05$.
[2] Level of *Meloidogyne incognita* inoculum.

resistance and consequently the potential for exploiting this in Bambara breeding programmes. Although there has been only limited assessment of Bambara groundnut, from the few studies available most accessions tend to be susceptible to RKN (Ogbuji, 1979; McDonald & De Waele, 1989), with resistance identified in just a small percentage (<5%) (Kwerepe & Labuschagne, 2004; Asiwe, 2009). This was reflected in the first pot trial, but not the second, indicating that a more sensitive, replicable and cost-efficient method would be useful, particularly given the environmental influences on Bambara groundnut performance. Furthermore, to screen large numbers of accessions, a high throughput method would undoubtedly be of benefit to process them effectively and efficiently.

Table 6. Reaction of selected Bambara groundnut accessions to *Meloidogyne incognita* inoculation in growth pouches at 28 days after inoculation, Ibadan, Nigeria.

Accession[1]	Inoculum[2]	Trial 1		Trial 2	
		Gall index[3]	No. of egg masses	Gall index	No. of egg masses
TVSu-1628	0	1.00	0.00	1.00	0.00
TVSu-1628	500	1.75	3.50	2.00	11.00
TVSu-1628	1000	2.00	7.00	1.75	7.50
TVSu-1628	1500	2.00	4.50	1.75	8.25
TVSu-1628	2000	1.75	3.75	1.50	4.75
LSD		0.73	5.86	0.62	9.71
TVSu-1698	0	1.00	0.00	1.00	0.00
TVSu-1698	500	1.00	0.00	1.50	6.00
TVSu-1698	1000	1.00	0.00	1.50	4.50
TVSu-1698	1500	1.00	0.00	1.75	5.75
TVSu-1698	2000	1.00	0.00	1.50	5.25
LSD		0.00	0.00	0.75	10.18
TVSu-1797	0	1.00	0.00	1.00	0.00
TVSu-1797	500	1.25	1.25	1.75	7.00
TVSu-1797	1000	1.00	0.00	1.75	6.25
TVSu-1797	1500	1.25	0.25	1.50	0.50
TVSu-1797	2000	1.00	0.25	1.50	1.25
LSD		0.48	1.75	0.73	8.11
TVSu-1833	0	1.00	0.00	1.00	0.00
TVSu-1833	500	1.00	0.00	1.75	10.50
TVSu-1833	1000	1.00	0.00	1.75	2.75
TVSu-1833	1500	1.00	0.00	1.75	6.00
TVSu-1833	2000	1.25	0.75	2.00	7.00
LSD		0.34	1.01	0.58	9.59
TVSu-1920	0	1.00	0.00	1.00	0.00
TVSu-1920	500	2.00	7.00	2.00	17.25
TVSu-1920	1000	2.00	6.00	2.00	13.25
TVSu-1920	1500	1.50	5.25	1.50	5.50
TVSu-1920	2000	1.50	2.00	1.25	0.50
LSD		1.23	9.55	0.51	10.65
TVSu-1953	0	1.00	0.00	1.00	0.00
TVSu-1953	500	1.00	0.00	1.50	1.75
TVSu-1953	1000	1.00	0.00	1.50	1.25
TVSu-1953	1500	1.00	0.00	1.50	1.75
TVSu-1953	2000	1.00	0.00	2.00	2.50
LSD		0.00	0.00	0.67	2.62

[1] Means (n = 4) were separated using least significance difference (LSD) at $P \leqslant 0.05$.
[2] Level of *Meloidogyne incognita* inoculum.
[3] Gall index where: 1 = no galling, 2 = $\leqslant$ 25% roots galled, 3 = 26-50% roots galled, 4 = 51-75% roots galled, 5 = $\geqslant$ 75% roots galled (Hussey & Jansen, 2002).

The current study has helped in identifying a faster, less labour-intensive method. Although there was variability between trials, the pouches appear to provide greater sensitivity than the use of pots, which would enable more robust assessment and identification of the most resistant material. Pouches have been shown to work well for similar crops, and are routinely used in places, such as for cowpea (Atamian *et al*., 2012). To use the method routinely, the pouch screening protocol would likely require refinement and adaptation to local conditions, as well as

Table 7. Reaction of Bambara groundnut to a range of *Meloidogyne incognita* inoculum in growth pouches at 28 days after inoculation, Ibadan, Nigeria.

Level of inoculum	Gall index[1]		No. of egg masses[1]	
	Trial 1	Trial 2	Trial 1	Trial 2
0	1.00b	1.0b	0.00b	0.00c
500	1.33a	1.75a	1.96a	8.92a
1000	1.33a	1.71a	2.17a	5.92ab
1500	1.29a	1.63a	1.67ab	4.63b
2000	1.25a	1.63a	1.13ab	3.54bc

[1] Gall index where: 1 = no galling, 2 = 25% roots galled, 3 = 26-50% roots galled, 4 = 51-75% roots galled, 5 = 75% roots galled (Hussey & Jansen, 2002). Values with the same letters in the same column are not significantly different ($P \leqslant 0.05$) from each other using Duncan's multiple range test.

for different crops. However, the Bambara germplasm in the repository is substantial, as it is for most crops, and ultimately the identification of molecular markers for resistance should ideally be a key objective to further increase the speed and efficiency of screening large germplasm collections (Shi *et al*., 2021). The current study provides progress in this direction, through the identification of genotypes with resistance to *M. incognita*.

Bambara groundnut is affected by at least two species of *Meloidogyne* (Timper *et al*., 2018). It is therefore important to establish genotypes with resistance against the species that most commonly affect Bambara, *M. incognita* and *M. javanica*, which are also the most commonly occurring species of *Meloidogyne* in Africa (Coyne *et al*., 2018). This would enable the deployment of more durable material that can withstand multiple RKN species attack. However, although other species have not been recorded parasitising Bambara groundnut, likely due to the scarcity of such studies, other *Meloidogyne* species may be pests. *Meloidogyne enterolobii*, for example, is extremely polyphagous (Janssen *et al*., 2016), highly prevalent in West Africa (Pagan *et al*., 2015; Dos Santos *et al*., 2019), highly aggressive and able to overcome resistance that is effective against *M. incognita* and *M. javanica* in solanaceous vegetables (Khallouk *et al*., 2013). Identification of germplasm that has resistance against this pest would therefore be advantageous, especially if Bambara groundnuts are susceptible, but also for potential transfer for use in other crops.

Even with the variability experienced in the current study, results demonstrate clearly how highly damaging RKN can be to Bambara groundnut. Crop growth parameters were reduced in inoculated plants, along with chlorophyl content, which was associated with severe galling damage to the roots. Such damage and consequent production losses emphasise the threat that RKN poses to food security, especially in areas of the world where food productivity needs to be drastically improved. Bambara groundnut is indigenous to Africa and described as well adapted to semi-arid conditions and resilient to drought (Mabhaudi *et al*., 2013). With some attention in terms of breeding and developing cultivars with these attributes as well as nematode (and other pest and diseases) resistance, a nutritious, versatile legume crop could potentially be promoted for use in sustainable cropping systems (Mayes *et al*., 2019; Chimonyo *et al*., 2020; Tan *et al*., 2020). In the current study, a single population of *M. incognita* originating from tomato was used. To identify germplasm with durable resistance against additional populations and species of RKN, broader screening is necessary. It is important to establish germplasm with resistance against the species most commonly affecting Bambara groundnut in Africa and potentially others (Coyne *et al*., 2018).

Conclusion

Bambara groundnut accessions TVSu-1698, TVSu-315, TVSu-1419 and TVSu-524 demonstrated good resistance against *M. incognita* and yielded well. There is little information available for these accessions except that TVSu-1698 and TVSu-1419 originated from Togo, TVSu-315 originated from Burkina Faso, whilst TVSu-524 originated from Cameroon. These can be recommended for promotion to farmers, at least in the meantime, as well as for use in breeding programmes. Further screening of germplasm against additional RKN species will help to identify a broader range of accessions for

recommendation. This can be conducted using 500 J2 in pouches, which was found to be more efficient, with potential for higher throughput than the conventional pot method.

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