

The Ethiopian staple food crop enset (*Ensete ventricosum*) assessed for the first time for resistance against the root-lesion nematode *Pratylenchus goodeyi*

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Summary – *Pratylenchus goodeyi* appears to be the most prevalent nematode pest of enset in Ethiopia, where it can occur in extremely high densities. However, the damage to yield or how different enset cultivars react to the nematode has yet to be determined. The current study therefore sought to establish a first assessment of these reactions by enset to *P. goodeyi* infection. Determining pest-resistant cultivars is an important task in developing management strategies. Our study evaluated nine enset cultivars to establish host response and identify potential sources of resistance. In addition, the pathogenicity of *P. goodeyi* was assessed on three enset cultivars. After 9 months' growth, significant differences in final population densities (P_f) and reproduction factor (RF) were observed amongst the nine cultivars, with 'Gefetanuwa' the most susceptible ($P_f = 25\,799$ and $RF = 12.9$), and similarly in a repeat experiment for 4.5 months ($P_f = 126\,534$ and $RF = 63.3$). 'Maziya' and 'Heila' were the most resistant in the first experiment ($P_f < 455$ and $RF < 0.2$) as well as in the repeat, together with 'Kellisa' ($P_f < 5255$ and $RF < 2.6$). In the pathogenicity experiment four inoculum densities significantly affected the P_f and RF but not among the three cultivars 'Maziya', 'Arkiya' and 'Heila'. This is the first known study to assess genotype reaction to *P. goodeyi*, which shows that there are significant differences in the reactions of different cultivars and that resistance appears to be present in enset.

Keywords – cultivar, Ethiopia, food crop, management, pathogenicity, reproduction factor.

Ensete ventricosum is a large herbaceous plant that belongs to the Musaceae family, the same as bananas. The genus *Ensete* comprises seven species (*E. ventricosum*, *E. homblei*, *E. livingstonianum*, *E. superbum*, *E. perrieri*, *E. lecongkietii* and *E. glaucum*) (Cheesman, 1947; Simmonds, 1962; Luu *et al.*, 2012). Wild *E. ventricosum* species are found distributed in sub-Saharan Africa and Asia, but it is domesticated and cultivated as a food crop only in Ethiopia. Unlike banana, enset does not produce edible fruit, but rather the pseudostem and corm are harvested after 3-12 years and processed into food products. Major food products prepared from enset are kocho

(obtained through fermentation of decorticated leaf sheath and corms), bulla (powder from the liquid squeezed out of leaf sheath and pulverised corm) and amicho (boiled corms) (Brandt *et al.*, 1997). In the south and southwestern part of the country, enset serves as a key staple food crop for about 20% of the Ethiopian population (Borrell *et al.*, 2019). It is also important as the key signature crop of the complex enset-based cropping systems in this area, creating stability in relation to food security, as well as the agroecology. As a perennial crop that can be harvested at any time of the year, enset offers food security when other crops are less available, providing a year-round availabil-

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ity of nutritious food. It is also generally perceived to tolerate drought, with a broad agroecological distribution and is easily cultivated around the home with low input and management requirements. Consequently, the crop represents an important position in household food security. In Ethiopia, enset is reported to be more productive per unit area than other starch crops (Tsegaye & Struik, 2001). In addition to food, enset is also used for a multitude of other purposes, such as for feed, medicine, building and fibre. As an orphan crop, with restricted geography, it has received relatively limited attention in terms of crop improvement. This is beginning to change, however, as the importance of this crop becomes better understood, with a few genetic diversity studies being undertaken, as well as research to identify pest and disease resistance (Brandt *et al.*, 1997; Harrison *et al.*, 2014; Borrell *et al.*, 2020).

More than 600 enset landraces collected from major enset-growing areas in Ethiopia have been conserved *ex situ* in the gene bank in the Areka Agricultural Research Center (Yemataw *et al.*, 2017). Molecular characterisation of enset landraces using amplified fragment length polymorphism (AFLP) (Negash *et al.*, 2002), random amplified polymorphic DNA (RAPD) (Birmeta *et al.*, 2002, 2004), simple sequence repeat markers (SSR) (Olango *et al.*, 2015; Gerura *et al.*, 2019) and inter-simple sequence repeat (ISSR) (Tobiaw & Bekele, 2011) techniques have revealed high genetic diversity amongst various landraces. Despite the progress in genetic studies and the potential of the crop, genetic improvement and conservation are based on conventional methods and have remained stagnant (Olango *et al.*, 2015). To date, breeding enset using conventional or biotechnology applications has yet to materialise in improved varieties for any trait (Merga *et al.*, 2019). Its perennial life cycle, with its extended duration to flowering and seed set, its complex vernacular naming, the absence of known traits such as disease resistance and reliance on vegetative propagation make genetic improvement tedious, expensive and time consuming (Olango *et al.*, 2015). Consequently, enset is by far the least studied food security crop (Borrell *et al.*, 2019).

Despite its resilience and versatility, several production constraints, including plant-parasitic nematodes, challenge enset. Studies have shown that although a range of nematode species are associated with the crop, the root-lesion nematode *Pratylenchus goodeyi*, root-knot nematodes (*Meloidogyne* spp.) and the foliar nematode *Aphelenchoides ensete* appear the most important nematode

threats (Peregrine & Bridge, 1992; Swart *et al.*, 2000; Bogale *et al.*, 2004; Addis *et al.*, 2006). However, *P. goodeyi* is by far the most common and prevalent species, occurring in all fields sampled, at densities as high as 25 000 (10 g soil)⁻¹ (Bogale *et al.*, 2004; Addis *et al.*, 2006; Kidane *et al.*, 2020). When challenged with densities this high, the crop might undergo considerable stress, with roots straining to maintain water and nutrient supply to the plant. However, the damage potential to enset by these nematodes is yet to be determined, as is the susceptibility to nematodes of the various land races and cultivars used by farmers.

Of the various strategies for the management of nematodes, the use of resistance is most suited for smallholder farmers in Africa, but knowledge of nematode pests and their management is poor and access to, or availability of, quality inputs is limited (Coyne *et al.*, 2009). Commercial banana plantations have mainly relied on chemical nematicides, which are not an option for smallholder enset farmers. Exploiting resistance is an alternative management strategy against nematodes (Speijer & De Waele, 1997). Traditional breeding for genetic traits in members of the Musaceae, however, is fraught with numerous obstacles based on inherent sterility, low genetic base and the long-term nature of the crop (Ortiz, 2011). A first step for the development of a management option is to identify cultivars that are resistant to pests and diseases (Speijer & de Waele, 1997; Pinochet *et al.*, 1998; Coyne & Tenkouano, 2005). To date, there has been no known screening for resistance of enset against plant-parasitic nematodes. Resistance is defined as the ability of a host plant to suppress nematode reproduction and development. Whereas nematodes will reproduce on a susceptible host and cause damage, a tolerant host will support nematode reproduction but suffer limited injury even in the presence of high infection levels, while a sensitive host cannot support even a light infection of nematodes (Bos & Parlevliet, 1995).

The objective of our study was to screen and evaluate the host plant response of nine enset cultivars to inoculation with *P. goodeyi*, in order to identify sources of resistance in the enset germplasm for potential use in nematode management, as well as to assess the pathogenicity of *P. goodeyi* on three selected enset cultivars.

Materials and methods

All experiments were conducted in the screenhouse located at Jimma University College of Agriculture and Veterinary Medicine, Jimma, Ethiopia, 7°42'N, 36°50'E,

at an altitude of 1710 m a.s.l. The area receives an annual rainfall of 1250 mm, average maximum and minimum temperatures of 26°C and 11°C, and an average maximum and minimum relative humidity of 91.4 and 37.9%, respectively.

ENSET CULTIVARS

One-year old enset seedlings, of known cultivars, were obtained from Areka Agricultural Research Centre, Areka, Wolaita. Suckers for each cultivar were regenerated from a single corm to ensure the purity of each cultivar. Prior to planting, roots were removed and the corms peeled before sanitising in boiling water treatment for 20 s (Coyne *et al.*, 2010). The suckers were then trimmed in order to ensure uniformity in size prior to planting. The waste root and corm material was assessed for nematodes before and after boiling water treatment.

NEMATODE INOCULUM

Pratylenchus goodeyi was isolated from infected enset roots collected from a high infection ‘hotspot’ highland area in Agena, Guraghe, identified during a recent study (Kidane *et al.*, 2020). A combination of morphometric and molecular data revealed that *P. goodeyi* was the only species of the genus identified from this area (Kidane *et al.*, 2020). Due to there being no monoxenic cultures of *P. goodeyi* available, naturally infected roots were used as inoculum, which has previously been shown to be a successful alternative (Coyne *et al.*, 2010). Monoxenic culturing of some species of *Pratylenchus* is also not always successful using the conventional method on carrot discs (Santos *et al.*, 2012), and *P. goodeyi* has proved difficult to date (Coyne, pers. comm.). Nematodes used for inoculation (P_i) were extracted from a 10 g sub-sample of chopped enset root and corm material using a modified Baermann extraction method over 48 h (Hooper *et al.*, 2005). Nematodes were collected on a 38 μ m sieve, rinsed into beakers, the suspension was reduced to 10 ml, and counted from 1 ml aliquots under a compound microscope.

RESISTANCE SCREENING

Nine cultivars were selected and assessed for resistance to *P. goodeyi*: ‘Gewada’, ‘Zereta’, ‘Maziya’, ‘Heila’, ‘Kellisa’, ‘Gefetanuwa’, ‘Yanbule’, ‘Messana’ and ‘Endale’. These cultivars are among the 623 enset accessions maintained in Areka Agricultural Research Centre, obtained

from single corms of each cultivar. These cultivars have distinct phenotypic variations. They are among the released cultivars for desired characteristics, such as yield and bacterial wilt disease tolerance. The experiments were conducted on raised benches in the screenhouse using 2 l pots containing oven-sterilised sandy soil, arranged in a randomised complete block design (RCBD) with six plants per treatment (cultivar). Suckers were maintained for 2 months to enable enough root development before inoculation with nematodes. At 2 months after planting (MAP) 2000 *P. goodeyi* (mixed juvenile and adult stages) were added to the pots in a 7 ml suspension into three holes made using a pencil around the base of the suckers and then covered. The plants were watered as needed and fertiliser applied as urea, once at 3 months after inoculation (MAI). The experiment was terminated at nine MAI and repeated once; the repeat was terminated at 4.5 MAI (due to the availability of seedlings at a later time and timeline of the study period).

PATHOGENICITY ASSESSMENT

Three enset cultivars (‘Maziya’, ‘Arkiya’ and ‘Heila’) were used to assess *P. goodeyi* pathogenicity. These cultivars are among the cultivars released for their desirable traits and they were also selected, based on results from previous nematode surveys, for supporting either high or low *P. goodeyi* densities. Enset suckers were planted into 2 l pots and inoculated with 500, 1000 and 2000 *P. goodeyi* in a 10 ml suspension and compared with a non-inoculated water control. The pots were prepared and maintained as outlined above in the screening experiment, arranged in a RCBD with four plants per treatment (cultivar \times inoculum density) on raised benches in the screenhouse. The experiment was terminated at 4.5 MAI; unavailability of seedlings prevented a repeat.

GROWTH AND DAMAGE PARAMETERS ASSESSED

At harvest the plant height, shoot fresh weight and root fresh weight were recorded for each plant. Plant height was measured from the soil surface to the tip of the youngest growing leaf. Plants were carefully removed from pots, rinsed free of soil and dabbed dry with paper towels. The roots were removed with a knife and the shoot (including leaves) and roots weighed separately. Roots were chopped into ca 0.5 cm pieces and nematodes extracted from a 10 g sub-sample per plant. The soil from each pot was thoroughly mixed before removing a 100 ml sub-sample. Nematodes from roots and soil were

extracted using a modified Baermann method for 48 h (Hooper *et al.*, 2005). Nematodes were collected on a 38 μm sieve, rinsed into beakers, suspensions reduced to 10 ml and densities assessed from 3×1 ml aliquots under the microscope. The overall nematode root and soil densities per plant were calculated by multiplying the density per g root by the total root weight and per ml soil by soil volume (2000 ml). Final nematode population density (P_f) per plant was calculated as the sum of the root and soil fractions. The reproduction factor (RF) was calculated by dividing P_f by the initial nematode population density (P_i).

STATISTICAL TREATMENT OF DATA

All data were analysed using RStudio[®]. The least significance difference was calculated for separation of means with $P \leq 0.05$. Nematode population densities were $\log(x+1)$ transformed prior to analysis of variance in order that data conformed to a normal distribution. Mean nematode population density data were back-transformed for presentation.

Results

RESISTANCE SCREENING

All enset cultivars tested showed different levels of susceptibility to *P. goodeyi* based on the P_f and RF. In the first experiment, the enset cultivars differed significantly ($P < 0.001$) in their host suitability to *P. goodeyi*. ‘Gefetanuwa’ had the highest P_f of 25 799 with a RF = 12.9, followed by ‘Zereta’ ($P_f = 11 196$; RF = 5.6) and ‘Endale’ ($P_f = 3573$; RF = 1.8). Cultivars with the lowest density were ‘Maziya’ ($P_f = 455$; RF = 0.2), ‘Heila’ ($P_f = 350$; RF = 0.2) and ‘Yanbule’ ($P_f = 335$; RF = 0.2). Similarly, in the second experiment, although terminated earlier, there was a significant difference ($P < 0.001$) amongst the enset cultivars. ‘Gefetanuwa’ had the highest P_f of 126 534 with a RF = 63.3, followed by ‘Yanbule’ ($P_f = 22 525$; RF = 11.3) and ‘Zereta’ ($P_f = 20 085$; RF = 10). Cultivars with the lowest density were ‘Heila’ ($P_f = 5255$; RF = 2.6), ‘Kellisa’ ($P_f = 3529$; RF = 1.8) and ‘Maziya’ ($P_f = 2746$; RF = 1.4) (Table 1). Both experiments showed a similar trend except for ‘Yanbule’, which had low P_f in the first experiment, possibly because of low root weight and development, hence resulting in few nematodes. When ‘Yanbule’ was removed from the analysis, there was no

significant difference ($P = 0.02$) between the two sets of experiments (Fig.1; Supplementary Table S1).

PATHOGENICITY ASSESSMENT

Results showed that in the pathogenicity study *P. goodeyi* multiplied on all three cultivars (‘Maziya’, ‘Arkiya’ and ‘Heila’) after 4.5 months but with no differences in P_f or RF among them. Significant differences ($P < 0.001$) on the P_f and RF were observed, based on the four levels of inoculation densities used within each cultivar. We also found that the RF of *P. goodeyi* was low in all three cultivars compared to susceptible cultivars such as ‘Gefetanuwa’ as seen in the cultivar screening experiment (Table 2). No differences in plant growth parameters were observed between the controls and inoculated plants (Table 3).

Discussion

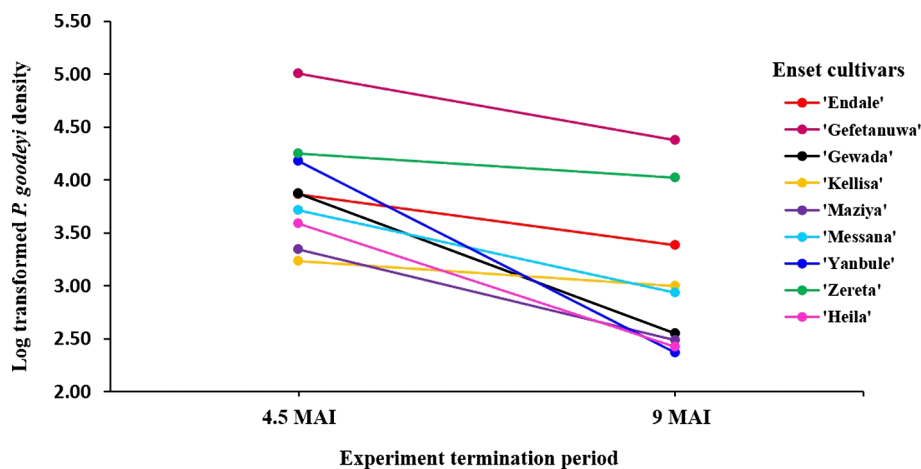
Our study represents the first proper assessment of nematode resistance in enset. Although data from a small number of survey studies indicate possible differences in susceptibility or resistance to plant-parasitic nematodes among enset cultivars (Bogale *et al.*, 2004), there is as yet no information available from any controlled studies. Indeed, there is only limited information on the resistance of enset cultivars against the various pest and diseases. Our results reveal that there does appear to be quite a range in susceptibility to *P. goodeyi* among enset cultivars. The low multiplication of *P. goodeyi* on ‘Maziya’, ‘Heila’ and ‘Arkiya’ also demonstrates a good level of resistance, with a low population build-up, while ‘Gefetanuwa’ was highly susceptible, with a much greater *P. goodeyi* multiplication.

There are over 600 enset cultivars maintained in the Areka gene bank, with a number of studies underway to characterise enset germplasm for genetic and phenotypic variability amongst accessions (Yemataw *et al.*, 2017; Gerura *et al.*, 2019). Screening activities, such as the current study, help contribute to building up the information on the various accessions, towards detecting sources of resistance across a range of constraints and identifying suitable sources for breeding. The current study initiates information gathering for nematode resistance and shows some promising results that provide a basis for further large-scale screening studies. However, the process is time consuming and subject to sensitivity and error, while ambiguity of accession names can be misleading. Conse-

Table 1. *Pratylenchus goodeyi* reproduction on nine enset cultivars.

Cultivar	9 MAI		Cultivar	4.5 MAI	
	Final nematode density (P_f)	Reproduction factor (RF)		Final nematode density (P_f)	Reproduction factor (RF)
'Gefetanuwa'	25 799 a	12.9 a	'Gefetanuwa'	126 534 a	63.3 a
'Zereta'	11 196 ab	5.6 b	'Yanbule'	22 525 ab	11.3 b
'Endale'	3573 bc	1.8 b	'Zereta'	20 085 b	10 b
'Kellisa'	1623 cd	0.8 b	'Endale'	9396 bc	4.7 b
'Messana'	1153 cd	0.6 b	'Gewada'	8455 bc	4.2 b
'Gewada'	591 cd	0.3 b	'Messana'	7691 bc	3.8 b
'Maziya'	455 cd	0.2 b	'Heila'	5255 bc	2.6 b
'Heila'	350 d	0.2 b	'Kellisa'	3529 c	1.8 b
'Yanbule'	335 d	0.2 b	'Maziya'	2746 c	1.4 b

P_f analysis was undertaken using log-transformed data; back-transformed data are presented. MAI = months after inoculation. Mean *Pratylenchus goodeyi* densities and RFs in a column with the same letter are not significantly different ($P \leq 0.05$).

**Fig. 1.** Position of enset cultivars based on log-transformed mean densities of *Pratylenchus goodeyi* at 4.5 and 9 months after inoculation (MAI).**Table 2.** *Pratylenchus goodeyi* reproduction on three cultivars of enset following inoculation at four levels in pots.

Inoculum density ¹	Cultivar ²						Final population density (P_f)	Reproduction factor (RF)
	'Arkiya'		'Maziya'		'Heila'			
	P_f	RF	P_f	RF	P_f	RF		
0	0 a	0 e	0 a	0 e	0 a	0 e	0 a	0 a
500	666 b	1.33 f	582 b	1.16 f	892 b	1.78 f	713 b	1.43 ab
1000	2033 c	2.03 g	2974 c	2.97 g	1297 c	1.29 g	2107 c	2.11 c
2000	5745 d	2.87 h	4143 d	2.07 h	6354 d	3.17 h	5414 d	2.71 d

¹ *P. goodeyi* inoculum (juveniles and adults) per 2 l pot.

² Final nematode density analysis was undertaken using log-transformed data; back-transformed data are presented. Mean *P. goodeyi* densities and RF of each cultivar in a row with the same letter are not significantly different ($P \leq 0.05$). Mean *P. goodeyi* densities and RF across three cultivars in a column with the same letter are not significantly different ($P \leq 0.05$).

Table 3. Plant growth parameters of three enset cultivars following inoculation with *Pratylenchus goodeyi* in pots after 4.5 months.

Inoculum density ¹	'Arkiya'			'Maziya'			'Heila'		
	Root weight (g)	Shoot weight (g)	Plant height (cm)	Root weight (g)	Shoot weight (g)	Plant height (cm)	Root weight (g)	Shoot weight (g)	Plant height (cm)
0	110 a	67 b	25 c	109 d	95 e	27 f	66 g	113 h	20 i
500	114 a	57 b	17 c	108 d	124 e	28 f	119 g	85 h	26 i
1000	135 a	83 b	22 c	110 d	114 e	30 f	48 g	48 h	21 i
2000	146 a	121 b	26 c	95 d	99 e	29 f	89 g	86 h	28 i

Plant growth parameter measurements in a column with the same letter are not significantly different ($P \leq 0.05$).

¹ *Pratylenchus goodeyi* inoculum (juveniles and adults) per 2 l pot.

quently, suitable protocols need to be established, based on the use of accessions that are genetically characterised for conformity of names. Rapid screening procedures targeting single roots and assessing nematode multiplication adopted for banana (De Schutter *et al.*, 2001; Coyne & Tenkouano, 2005) can also be used to screen enset germplasm. The development of tissue culture-based *in vitro* propagation protocols for enset (Negash *et al.*, 2000) could also improve the efficiency and speed of propagating disease-free planting materials for distribution to farmers.

Determining germplasm with good resistance to key pests, diseases and abiotic constraints is important for improving crop productivity, especially in Africa, where losses are particularly large (Coyne *et al.*, 2018). Identifying accessions that have multiple resistance is therefore of even greater value when determining germplasm for use in breeding programmes, or providing recommendations to farmers. For instance, 'Maziya' is regarded as less susceptible to bacterial wilt disease (*Xanthomonas vasicola* pv. *musacearum*), whilst 'Gefetanuwa', which supported the highest reproduction of *P. goodeyi*, also supports rapid *X. vasicola* pv. *musacearum* development, as does 'Arkiya', which has been used as a susceptible control in evaluation studies (Muzemil *et al.*, 2019). Although 'Arkiya' was regarded as one of the cultivars with higher densities of *P. goodeyi* in a previous survey (Bogale *et al.*, 2004), the P_f and RF were similar to the other two cultivars ('Maziya' and 'Heila'). As nematode infection is known to predispose banana to bacterial wilt (Shehabu *et al.*, 2010) and fusarium wilt diseases (Almeida *et al.*, 2018), it further serves a purpose to have nematode resistance traits in banana, as well as enset. Studies such as ours can be very important to identify cultivars to use for studying the relationship of nematodes and bacterial wilt disease.

In our study we found that infection with *P. goodeyi* did not result in any decrease in growth parameters of the enset suckers over the 4.5 months of assessment, as compared to similar studies with banana (Van den Bergh *et al.*, 2002; Dochez *et al.*, 2009; Coyne *et al.*, 2013). This could be explained by the long perennial nature of the enset crop, with about 7 years to maturity, and the period of assessment being too short to detect differences. Alternatively, it may be that the enset cultivars assessed in the current study all exhibit a level of tolerance to the nematodes. This may also explain the high *P. goodeyi* densities experienced on enset roots during recent surveys (Bogale *et al.*, 2004; Addis *et al.*, 2006; Kidane *et al.*, 2020). Similarly, unlike other studies on banana, root necrosis damage was not readily observed or visualised, possibly due to the thin enset roots, combined with the short duration of the experiment, or possibly due to host tolerance. Infection of enset roots with *P. goodeyi* does result in necrosis, however, which can be considerable, as seen during field studies (Bogale *et al.*, 2004; Addis *et al.*, 2006; Kidane *et al.*, 2020) and which is undoubtedly detrimental to growth and production of enset. In any case, it is clear that further investigations are necessary to determine more effectively host damage potential by *P. goodeyi*, possibly over a longer duration, and with a greater range of germplasm assessed using methods such as the single-root inoculation (Coyne & Tenkouano, 2005), which should be repeated to confirm results.

Although the current study screened a few cultivars from the enset germplasm and over a short duration compared to the perennial nature of the crop, this study demonstrates that there are indeed differences in the resistance of cultivars to *P. goodeyi*. Being the first study conducted on enset resistance against nematodes, it can be used as a base for further studies such as screening and interaction of other nematodes and other pathogens.

Most synthetic chemical nematicides have been removed from the market due to environmental and human health concerns and so it is important to select the best performing cultivars in terms of resistance to nematodes and other diseases. Chemical pesticide use on enset is currently very low under the predominantly subsistence manner of production around homesteads. Therefore, the identification of cultivars resistant to the predominant nematode species is a first step towards using those in future breeding efforts.

Despite the importance of enset in Ethiopia, there has been little attention given to the genetic improvement of the crop. Baseline studies on the identification of nematode resistance, such as ours, accompanied by information on the molecular characterisation and genome-wide sequence data of enset (Harrison *et al.*, 2014) will enhance research on this important but neglected crop towards its genetic improvement. Having established tissue culture propagation and *in vitro* conservation protocols for enset will additionally provide a basis for extending such screening work (Negash *et al.*, 2000; Birmeta, 2004).

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Supplementary Table S1. Summary of analysis of variance of log-transformed mean densities of nine enset cultivars in the two sets of experiments (4.5 and 9 months after inoculation).

	df	Sum squares	Mean squares	F value	Pr(> F)
Cultivar	7	25.7	3.7	24.4	3.62e-16 ^{***}
Experiment repeat	1	10.1	10.1	67.1	1.09e-11 ^{***}
Cultivar × Experiment repeat	7	2.8	0.4	2.7	0.018 [*]
Residuals	67	10.1	0.2		