Original article

Early establishment, growth and development response of enset (*Ensete ventricosum*) seedlings to manure in the field

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Summary

Introduction – **In Ethiopia, enset is a food security and livelihood crop, grown merely by vegetative reproduction from selected genotypes (landraces). There are immediate concerns: (*i*) need for variety improvement and (*ii*) preservation of the genetic diversity as the habitat of wild ensets declines. Utilisation of seeds is suggested. Seedling establishment, growth and development responses to manure were studied.** *Methods* – **Experimental field was in southern Ethiopia. Three seed lots were sown, and germinated seeds planted in pots outdoors. At five weeks, 48 seedlings (*N*=4) were planted in the field with 0.0–6.0 kg dry cow manure per plant, watered until onset of the rainy season, and grown for one year. Growth rate and size at harvest were recorded.** *Results* – **Seedlings established well (100%) in the field. Growth and development responses to increased manure supply were strong but generally plateaued from 4.0 to 6.0 kg manure provided per plant. At 12 months, average fresh weights were 15.5, 45.6, 81.8 and 85.5 kg when grown with 0.0, 2.0, 4.0 and 6.0 kg manure, respectively; weights were well correlated to sizes, *e.g.*, analogous pseudostem volumes were 16.6, 53.6, 99.4 and 105.5 dm^3.** *Conclusion* – **Enset seedlings can efficiently be grown in the field with cow manure. Producing quality seedlings with available resources, following a robust method, directly enables the preservation of the existing gene pool and makes breeding programs possible. However, farmers interested in growing enset crop shall be provided with sprouts from vegetative reproduction of known genotypes, to ensure genotype purity for the intended purpose.**

Keywords
Ethiopia, food security, gene pool, indigenous crop, Musaceae, sustainable agriculture

Significance of this study

What is already known on this subject?

- Enset is a traditional food security crop, propagated vegetatively. There is need for variety improvement and gene pool preservation from cultivated and wild plants.

What are the new findings?

- Five-weeks enset seedlings established and grew well in the field: average pseudostem volume was over 100 dm^3 within one year when supplied with 4.0–6.0 kg dry cow manure.

What is the expected impact on horticulture?

- The production of quality seedlings, with a simple and robust method, directly enables the preservation of the existing gene pool and makes breeding programs possible.

Introduction

In Ethiopia, enset (*Ensete ventricosum* [Welw.] Cheesman, Musaceae) is a multipurpose crop, being a main component of a resilient agricultural system (Shack, 1963; Borell *et al.*, 2020), highly valued for efficiently preventing famine and ensuring food security in the densely populated southwestern Ethiopia (Brandt *et al.*, 1997; FAO, 2009; Tsegaye and Struik, 2001; Quinlan *et al.*, 2016). Enset is utilised since ancient times, known for example for the food products providing nutritive values similar to potato (Mohammed *et al.*, 2013; Bosha *et al.*, 2016) achieved at the highest energy yield per area and time unit recorded in Ethiopia (Tsegaye and Struik, 2001). Despite all positive effects of enset agriculture, enset is grown mainly in limited geographical areas of Ethiopia (FAO, 2009) and is receiving little research attention (Stone *et al.*, 2011); even though the number of publications on enset has increased considerably over the later years, the number of papers reporting results from field experiments on growth and management is very low. *Ensete ventricosum* occurs naturally over large parts of Africa (Williams, 2017) and enset agriculture is practised over an even wider range of climates, altitudes, and soil types (Birmeta *et al.*, 2004). Thus, enset agriculture could be an important contributor to improved food security and maintained soil quality over much larger areas than currently.

Enset is traditionally propagated vegetatively and harvested at onset of flowering (Pijls *et al.*, 1995), thus not allowing seed set. Therefore, each landrace is a clone and the genotypes are conserved while utilised. Since ancient times, farmers have selected genotypes suitable for different purposes, thus established hundreds of landraces (Yemataw *et al.*, 2012, 2016; Bekele *et al.*, 2013; Getachew *et al.*, 2014;

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Haile, 2014a), and six of these are released as cultivars with registered official names (Yeshitila *et al*., 2011). There is indication of losses of certain landraces that are not widely used and/or susceptible to certain diseases (Yemataw *et al*., 2016). Such loss would lead to reduced agrobiodiversity and possible loss of unique genes. Currently, there are requests from farmers for new varieties with improved characteristics. To our knowledge, there has not been any attempt to conscious breeding of enset.

Ensete ventricosum originates from the African continent (Lye and Edwards, 1997). The species has a relatively wide distribution over several nations in the African continent and is currently not regarded being threatened by the Red List (Williams, 2017). However, the populations of wild *E. ventricosum* in the montane and lowland parts of Ethiopia decrease because of large-scale land investments for agriculture; the current African land rush is resulting in social and environmental contradictions (Williams, 2017; Tura, 2018; Schoneveld, 2017). Some wild *E. ventricosum* populations in Ethiopia are growing geographically isolated from each other (Williams, 2017), and therefore it can be expected that unique genetical characteristics would go extinct if a certain population is lost or significantly decreases. There are at least two studies indicating that wild enset populations have relatively high genetic variability: (*i*) Birmeta *et al*. (2004) found larger variation among each of five wild populations than among nine landraces, and (*ii*) Olango *et al*. (2015) analysed five plants of wild origin grown at Areka Research Centre (ARC, the national centre for enset research in Ethiopia): these plants formed one group in relation to landraces studied but had larger difference among each other than what was found among some of the different landraces. The plants analysed by Olango *et al*. (2015) originated from a single seed lot of one mother plant that grew in a wild population outside the town Jimma, collected by Endale Tabogie (personal information, ARC). Thus, results from Birmeta *et al*. (2004) and the genetical differences recorded by Olango *et al*. (2015) support the conclusion that more extensive genetic biodiversity exists among wild plants. Collecting seeds and growing seedlings at protected places is a straight-forward way to maintain the current gene pool.

The genetic variability among wild plants and the large number of existing landraces is a potential for extending the gene pool among cultivated enset and for the development of new and desired cultivars. New genotypes can be achieved by crossing, seed set and selection, and/or by genetic engineering and micropropagation. Due to the difficulty to identify specific genes for complex characteristics (for example different kinds of appreciated food tastes) and the controlled and protected growth conditions needed for sensible plantlets, we suggest the development of conventional breeding program for enset; an action that can be initiated directly without large costs or advanced equipment. For this, seed germination (Karlsson *et al*., 2013a) and seedling growth is crucial. In a pilot study, young seedlings grown in small pots responded well to manure supply (Karlsson *et al*., 2013b). It has been shown that manure supply at corm burial in the field is greatly beneficial for sapling development (Bosha *et al*., 2019) and that sapling size is correlated with plant development several years after being planted individually (Karlsson *et al*., 2020). Thus, providing proper growth conditions at early stage is important for further plant growth, development and maturity. In warm environments, as in Ethiopia, mineralization rate is high and manure supply to plants is beneficial not only as source of nutrients but also by adding organic matter to the soil, thus improving soil texture and water holding capacity (Bayu *et al*., 2006).

Our objective was to advance the knowledge on early establishment of enset seedlings and to set management recommendations for field growth. Therefore, we investigated responses of enset seedlings to different amounts of air-dried cow manure in the field. We hypothesised that seedlings at the three leaves stage, even though much smaller than vegetatively reproduced saplings when normally detached from parent corms, can be planted in the field with appropriate amount of manure and respond with good establishment and strong growth.

Materials and methods

Seeds, experimental field and plant establishment

Mature enset fruits were collected from three plants: from a wild population near Tercha (07°04'N; 37°11'E; 2,200 m a.s.l.), referred to as "Tercha X" and "Tercha Y", and from the landrace, 'Ferezye', grown at Wolkite (08°19'N; 37°49'E; 1,900 m a.s.l.), during 2010–2011. Fruits were peeled, seeds were cleansed from pulp by hand and then stored dry at circa 20 °C until used. On Nov. 30, 2014, circa 150 seeds of each seed lot were soaked and thereafter placed on moist substrate for germination, following the procedure of Karlsson *et al*. (2013a). After three weeks of incubation, circa 50 germinated seeds of each seed lot were transferred to pots (14 cm diameter, 25 cm high) open in the bottom and filled with a mix of ¾ local soil and ¼ sand, following Karlsson *et al*. (2013b).

Pots were placed outdoors under mosquito net in slight shade at Wolaita Sodo University campus (06°50'00"N; 37°45'07"E; 1,882 m a.s.l.), Ethiopia, checked for soil moisture daily and watered when needed. After five weeks, sixteen similar-sized seedlings (having three developed leaves, the largest blade being 12–16 cm long and 5.0–6.2 cm wide and with an average pseudostem circumference of 3.8 cm [sd 0.44]) from each of the three mother plants were randomized to four replicates in four treatments. The experimental field at the University Campus, initially used for grazing, was dug by hand tools several times before onset of experiment. Forty-eight planting holes were dug to 30 cm deep and 30 cm in diameter with 200 cm between centres of two adjacent holes in an experimental field area of 18 × 14 m (8 × 6 holes). Substrates were mixes of local field soil and air-

Table 1. Characteristics of local soil and air-dried cow manure used for early establishment and growth of enset (*Ensete ventricosum*) seedlings for one year (2015) at Wolaita Sodo University Campus, Ethiopia. Substrates were pulverized to approximately sand-grain size.

Substrate characteristics	Dry weight (kg dm^{-3})	pH	Organic C (%)	Available P (mg kg^{-1})	Available K (mg kg^{-1})	Total N (%)	Exchangeable Ca (mg kg^{-1})	Conductivity (dS m^{-1})
Soil	1.020	6.1	0.24	6.4	44.6	0.12	680	0.06
Manure	0.533	7.4	4.20	32.4	96.2	0.37	1,440	0.16

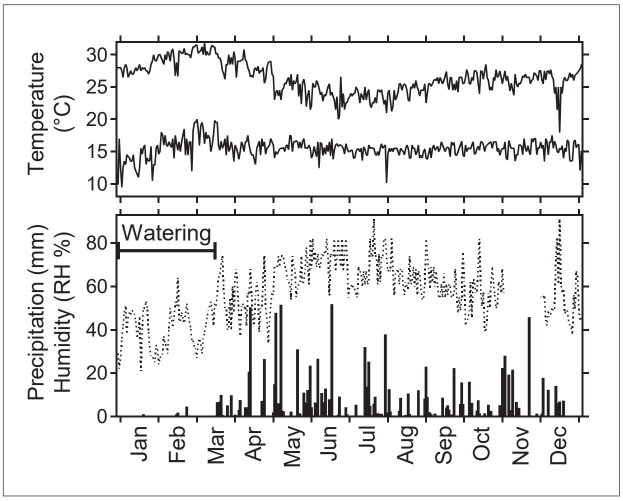

Figure 1. Daily weather (bars: precipitation, dotted line: humidity, solid lines: min and max temperature) at Wolaita Sodo, Ethiopia, during 2015 when *Ensete ventricosum* seedlings were grown. Data provided by the Ethiopian National Meteorology Agency. Watering was done with 5 L per plant, two times per week, during the indicated period.

dried cow manure (Table 1), the substrates were pulverised to approximately sand-grain size by crashing several times with strong sticks and applied with 15 dm^3 per planting hole. Four substrate types were used: 0.0, 2.0, 4.0 or 6.0 kg dry weight (DW) of manure per 15 dm^3 of manure-soil-mix; 2.0, 4.0 and 6.0 kg manure equal 3.75, 7.50 and 11.25 dm^3 air-dried, pulverised, manure, respectively. In each planting hole, 15 dm^3 of well-mixed substrate was placed as a cone. In the centre of these cones, seedlings, with undisturbed roots and soil-sand mix from pots, were planted at Feb. 2, 2015. The distribution of seedlings was random except for not allowing two adjacent plants (neither straight nor diagonally) with the same treatment from the same mother plant. The holes were filled to surrounding soil surface level with local soil and watered. Surrounding the experimental area, two analogous rows with seedlings (72 plants) were planted to avoid edge effects. Thereafter, 5 dm^3 of water per seedling was given two times per week until Mar. 18, 2015, when rain begun (Figure 1). The experimental field was visited daily, and weeding was done when necessary.

Ahead of substrate preparation, soil samples were taken down to 30 cm from 20 spots in regular pattern over the experimental field and well mixed. From the soil sample and from the manure, 2 kg was taken and analysed by Hawassa Regional Soil Laboratory (a governmental standard laboratory, methods reported in Bosha *et al.* [2019]), Hawassa, Ethiopia (Table 1). Daily weather data (Figure 1) were provided by The National Meteorology Agency of Ethiopia.

Records and statistics

Total plant height (*i.e.*, aboveground plant height: pseudostem height and continued leaf length of longest leaf, cm), number of leaves, length and width of blade of longest leaf (cm) and pseudostem height (measured from soil surface to the point where the lowest green leaf begun to detach from pseudostem, cm) were recorded monthly from end of first month to end of fourth month of field transplanting, and pseudostem circumference (at the middle of pseudostem height, cm) until the tenth month. The above characteristics and corm circumference (cm) were recorded at harvest (Jan. 4, 2016), after 12 months of growth. At harvest, the three fractions corm, pseudostem and remaining aboveground parts (*i.e.*, leaf blades and leaf stalks not being part of pseudostem) were parted in pieces and weighted (fresh weight [FW]) with a 50-kg spring balance having a precision of 0.5 kg. Volume of pseudostem was approximated to a cylinder with radius calculated from circumference recorded at the middle of pseudostem height: $radius^2 \times \pi \times$ pseudostem height (Karlsson *et al.*, 2015), corm volume was calculated as $radius^3 \times \pi \times 4/3$ (globe) and leaf blade area was calculated as (length × width)/2 (rhombus).

Records of the characteristics (*i*) number of leaves, (*ii*) plant height, (*iii*) area of blade of longest leaf, (*iv*) pseudostem height from the first four months of growth, and (*v*) pseudostem circumference during the ten first months of growth were subjected to linear regression with all data of each characteristic over time. After confirming overall significant linear relation between time and plant phenology characteristics (R^2 varied from 0.52 [plant height] to 0.83 [number of leaves], all $p < 0.001$), linear regression was performed for each characteristic and individual plant over time. Thereafter, the individual growth rates (the coefficients of inclination, *i.e.*, the "b" in "a = bx + c") were analysed with two-way ANOVA ($N = 4$), manure amount and mother plant being independent factors. Records at harvest (total weight, corm volume, pseudostem volume, number of leaves, total plant height, area of blade of longest leaf) were analysed as

Table 2. ANOVA results (F-value and p-value notation: *** = $p \leq 0.001$, ** = $p \leq 0.01$, * = $p \leq 0.05$, ns = $p > 0.05$) on records of different characteristics from 48 five-weeks *Ensete ventricosum* seedlings established in the field and grown for 12 months in southern Ethiopia. Seeds originated from three different mother plants. Plants were grown with 0–6.0 kg DW of air-dried cow manure per plant ($N = 4$). Records were taken during growth and at harvest (Figures 2 and 3).

Factor		df	Pseudostem circumference	Pseudostem height	Number of leaves	Total height	Blade area
Growth rate	Mother plant	2	0.16^{ns}	0.54^{ns}	3.85*	0.40^{ns}	2.93^{ns}
	Manure amount	3	28.19***	30.61***	28.50***	7.95***	38.76***
	Mother plant × Manure amount	6	0.75^{ns}	0.48^{ns}	0.93^{ns}	0.71^{ns}	0.48^{ns}

Factor		df	Biomass (FW)	Corm volume	Pseudostem volume	Number of leaves	Total height	Blade area
Harvest	Mother plant	2	1.28^{ns}	2.44^{ns}	0.90^{ns}	0.67^{ns}	2.51^{ns}	0.94^{ns}
	Manure amount	3	24.89***	39.13***	15.83***	9.26***	19.13***	15.24***
	Mother plant × Manure amount	6	1.68^{ns}	0.37^{ns}	1.20^{ns}	1.67^{ns}	1.23^{ns}	1.11^{ns}

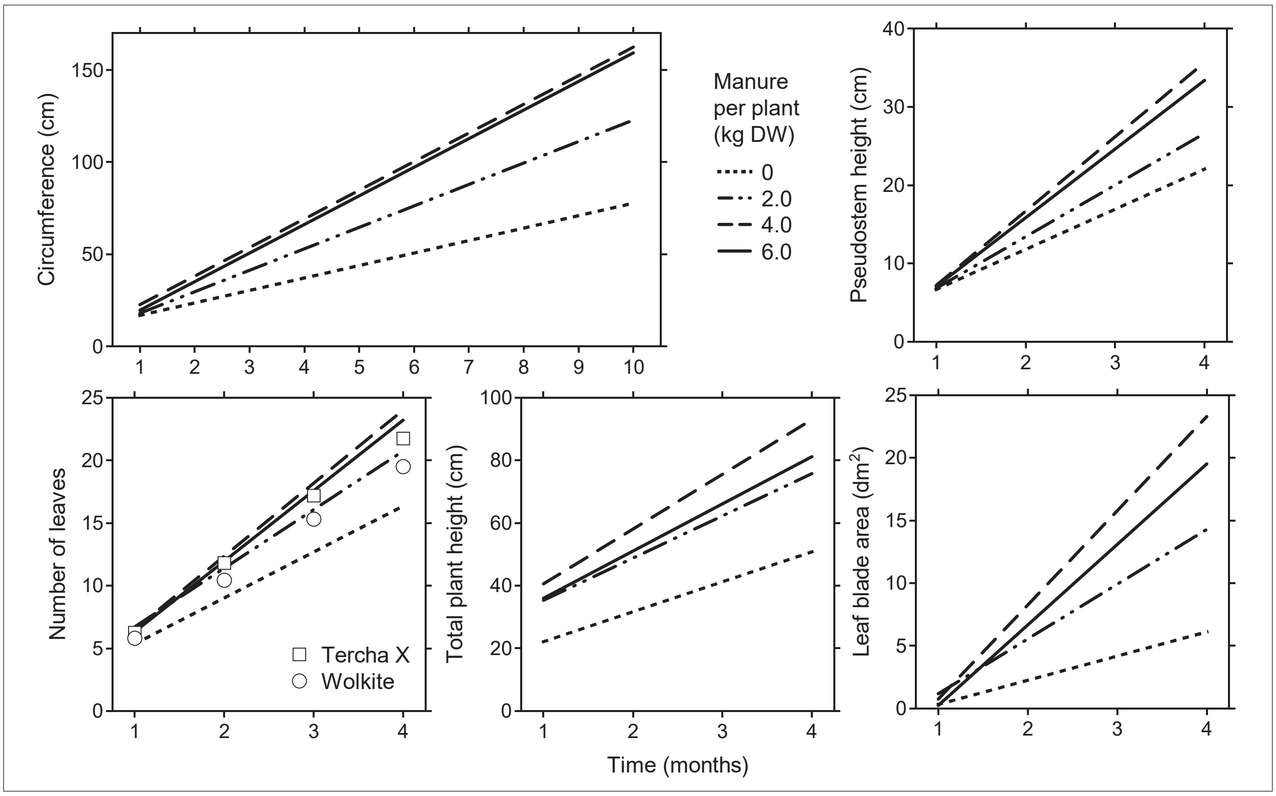

FIGURE 2. Growth rate, linear regression from monthly records, of *Ensete ventricosum* seedlings. Plants ($N = 48$) were grown outdoors at Wolaita Sodo University, Ethiopia, with different amounts of air-dried cow manure. Seed lots were from three mother plants, two from Tercha (wild genotypes) and one from Wolkite (landrace Ferezye) in Ethiopia. ANOVA (Table 2) followed by Tukey HSD test for significant explanatory factors were applied. The one significant difference due to mother plant (growth rate of number of leaves) is shown with symbols, and significant differences in responses to manure amounts are reported in Table 3.

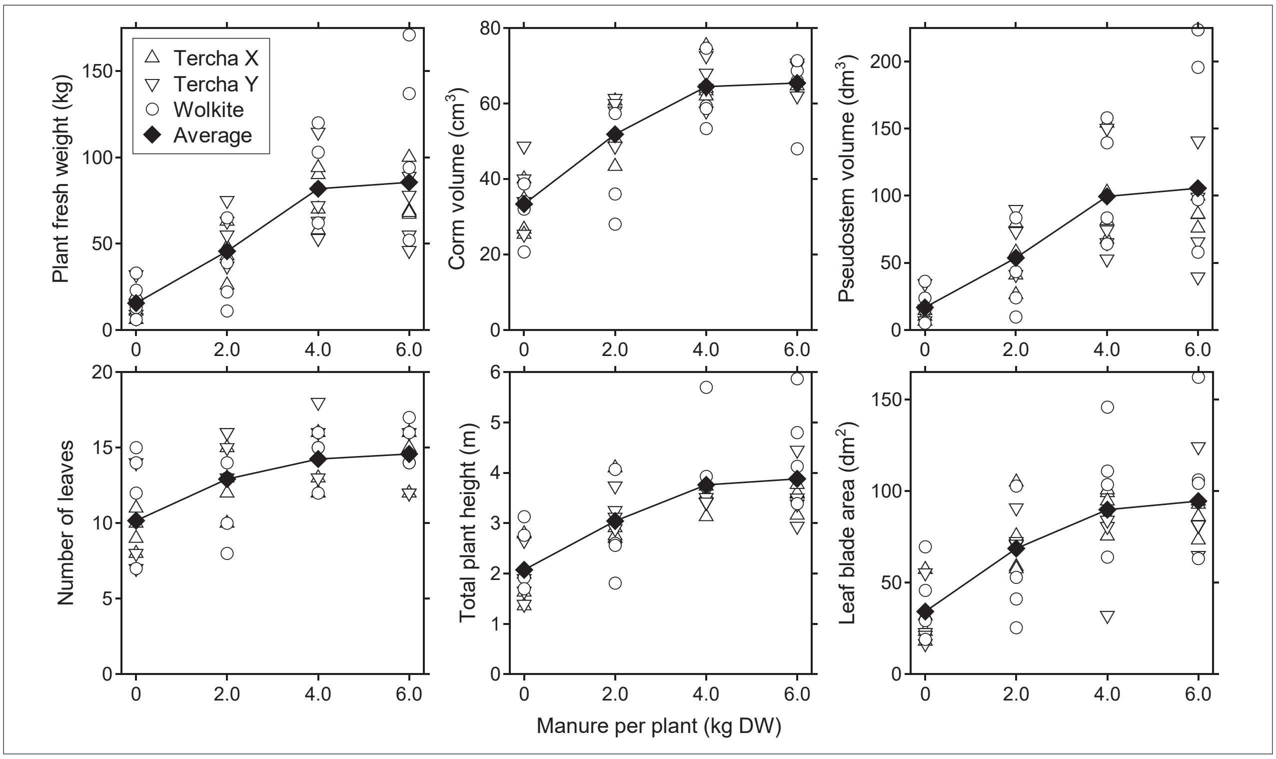

FIGURE 3. Records of *Ensete ventricosum* plants when harvested 12 months after germinated and established in the field (in southern Ethiopia) at the three leaves stage. Seeds originated from three mother plants: two from nature near Tercha and one landrace (Ferezye) grown in Wolkite. Substrate was pulverized mix (15 dm^3) of local field soil and different amounts of air-dried cow manure. Effect of mother plant and manure amount were analysed with ANOVA (Table 2) and evaluation of differences (Tukey HSD test) due to manure amount are reported in Table 3.

Table 3. Tukey HSD test (p-values, significant differences [$p < 0.05$] indicated in bold), following two-way ANOVAs (explanatory factors being "mother plant" and "manure amount", Table 2) on growth rate and size at harvest for 48 *Ensete ventricosum* seedlings grown for 12 months in southern Ethiopia. The explanatory factor "mother plant" had significant effect on growth rate of number of leaves only (Figure 2). For the explanatory factor "manure amount", the larger amount of manure gave the higher result for all significant differences (records subjected to the statistical analyses are shown in Figures 2 and 3).

Response variable		Manure amount (kg DW plant^{-1})	Vis-a-vis manure amount (kg DW plant^{-1})		
			2.0	4.0	6.0
Growth rate	Pseudostem circumference	0	**<0.001**	**<0.001**	**<0.001**
		2.0	–	**0.007**	**0.007**
		4.0	–	–	1.000
	Pseudostem height	0	**0.033**	**<0.001**	**<0.001**
		2.0	–	**<0.001**	**0.001**
		4.0	–	–	0.499
	Total plant height	0	0.108	**<0.001**	**0.011**
		2.0	–	0.094	0.774
		4.0	–	–	0.479
	Number of leaves	0	**0.003**	**<0.001**	**<0.001**
		2.0	–	**0.001**	**0.005**
		4.0	–	–	0.870
	Leaf blade area	0	**0.001**	**<0.001**	**<0.001**
		2.0	–	**<0.001**	**0.004**
		4.0	–	–	0.225
Harvest	Fresh weight	0	**0.014**	**<0.001**	**<0.001**
		2.0	–	**0.003**	**0.001**
		4.0	–	–	0.979
	Pseudostem volume	0	0.079	**<0.001**	**<0.001**
		2.0	–	**0.020**	**0.007**
		4.0	–	–	0.976
	Corm volume	0	**<0.001**	**<0.001**	**<0.001**
		2.0	–	**0.004**	**0.002**
		4.0	–	–	0.992
	Total plant height	0	**0.005**	**<0.001**	**<0.001**
		2.0	–	0.052	**0.018**
		4.0	–	–	0.970
	Number of leaves	0	**0.028**	**0.001**	**<0.001**
		2.0	–	0.491	0.297
		4.0	–	–	0.984
	Leaf blade area	0	**0.008**	**<0.001**	**<0.001**
		2.0	–	0.162	0.062
		4.0	–	–	0.966

Table 4. Linear correlation between fresh weight and sizes calculated from linear measurements at harvest of one-year-old *Ensete ventricosum* plants originating from seeds and grown in the field in southern Ethiopia, $N = 48$.

Weighed plant part (FW)	Size calculated from linear measurements	R^2	p
Corm	Corm (globe)	0.81	<0.001
Pseudostem	Pseudostem (cylinder)	0.92	<0.001
Aboveground parts except pseudostem	Blade area of longest leaf (rhombus)	0.75	<0.001
Entire plant	Corm (globe)	0.83	<0.001
	Pseudostem (cylinder)	0.97	<0.001
	Blade area of longest leaf (rhombus)	0.85	<0.001

dependent factors with two-way ANOVAs ($N=4$), the independent factors were manure amount and mother plant. For significant factors, ANOVA analyses were followed by Tukey HSD test. Relation between FW and calculated volume of corm and pseudostem were analysed with linear regression, as was relation between leaf blade area of the longest green leaf and FW of aboveground parts not being pseudostem. Finally, the relation between entire plant FW and (*i*) volume of corm, (*ii*) volume of pseudostem, and (*iii*) blade area of longest leaf, were evaluated with linear regression. Statistics were performed with the software Dell Statistica (Dell, Inc., 2015).

Results

There was no mortality, all 48 planted seedlings were harvested after 12 months of growth, recorded, and included in statistical analyses. Growth rate was significantly dependent on amount of manure for all recorded characteristics (Table 2). The effect of mother plant was significant for growth rate in number of leaves (Table 2), for which Tercha X had significantly ($p=0.033$) faster growth than Wolkite during the first four months of growth: on average Tercha X had 0.4 more leaves than Wolkite after one month of growth and 2.2 more leaves after four months (Figure 2). There was no significant interaction between mother plant and amount of manure (Table 2). After 12 months of growth, size of plant was dependent on manure amount at planting (Table 2); generally increasing with increasing manure amount while plateauing between 4.0 and 6.0 kg DW of manure (Figure 3). There was no significant effect of mother plant or of the interaction mother plant × manure on any recorded characteristic after 12 months of growth (Table 2).

For all characteristics recorded, for growth rate as well as for size at harvest, 4.0 or 6.0 kg air-dried manure gave faster growth and larger plants than no manure, and there was no significant difference between the results from 4.0 and 6.0 kg air-dried manure (Table 3). For most characteristics, 2.0 kg air-dried manure resulted in significantly faster growth and larger plants than no manure, exceptions being growth rate of plant height and pseudostem volume at harvest (Table 3). Generally, 4.0 or 6.0 kg air-dried manure gave significantly faster growth and larger plants than 2.0 kg manure, exceptions being growth rate of total plant height and number of leaves and blade area of longest leaf at harvest, where there were no statistical significant differences between 2.0, 4.0 or 6.0 kg air-dried manure, and total plant height at harvest where there was no statistical significant differences ($p=0.052$) between 2.0 and 4.0 kg air-dried manure (Table 3).

Fresh weight of corm, pseudostem and aboveground parts excluding pseudostem were significantly linearly correlated to calculated sizes of corm, pseudostem and blade area of longest leaf, respectively, and total plant weight was significantly linearly correlated to each of the above sizes (Table 4).

Discussion

The hypotheses that seedlings of *Ensete ventricosum*, at the three leaves stage, would establish and perform well when grown directly in the field with air-dried cow manure was supported. Within one year from germination, plants grown in the field in southern Ethiopia reached on average 15.5, 45.6, 81.8 and 85.5 kg FW in response to 0.0, 2.0, 4.0 and 6.0 kg air-dried cow manure, respectively (Figure 3). The responses of recorded characteristics to increased manure amount (Figure 3) resembled the well-known dose-response effect, *e.g.*, nitrogen amount in potato cultivation (Vos, 2009), even though responses from 2.0 kg manure were not always statistically significant different from responses to other amounts (Table 3). There was no significant difference between plants grown with 4.0 or 6.0 kg air-dried manure for any recorded characteristics, showing that the fertiliser response plateaued at that level (Figures 2 and 3). Thus, higher amount of manure is not beneficial; on the contrary, surplus of fertilisers is negative for environment and should be avoided (Smith *et al.*, 2002). Thus, 4.0–6.0 kg DW (7.50–11.25 dm^3) of air-dried pulverised cow manure mixed to totally 15 dm^3 with local soil can be recommended per seedling at planting in the field. In farmers' established enset fields, soil fertility is usually higher than what was recorded from the experimental field at onset, while the experimental field was more similar to grazing land or farmers' fields used for annual crops (Table 1; Tensaye *et al.*, 1998; Amede and Tabogie, 2007). The higher soil fertility in established enset fields can be explained by the fact that farmers commonly grow enset close to the homestead and regularly provide organic waste to the field with particular emphasis on young, newly planted, enset (Amede and Tabogie, 2007).

Generally, the responses to the different manure amounts were not dependent on mother plant (Table 2, Figures 2 and 3). From five and six plant characteristics recorded during growth and at harvest, respectively, there was only one characteristic where mother plant was a significant explanatory factor for variation (Table 2): Tercha X increased the number of leaves faster than Wolkite (Figure 2), while the number of leaves did not differ between these two groups at planting (all planted seedlings had three developed leaves) or at harvest (Table 2, Figure 3). The difference shown during the first months of growth can be due to variations in early development rate dependent on mother plant (genome) even though the difference in number of leaves is small: the growth rate in numbers of leaves for Tercha Y did not differ significantly from either Tercha X or Wolkite and the amount of manure was a much stronger predictor for this characteristic (Table 2, Figure 2). For banana, the number of leaves per plant show similar response as enset to increased amount of fertiliser, and there is positive correlation between numbers of leaves and yield (Kuttimani *et al.*, 2013).

Volumes (calculated from recorded linear measurements) of corm and pseudostem were well correlated to fresh weight at harvest; the corms have more unequal shape which can explain less precision in the measurement than for pseudostem (Table 4). The size of leaf blade of the longest leaf alone gave a good estimation of the weight of all green parts that were not pseudostem, and there was close correlation between total plant weight and sizes of corm, pseudostem and largest leaf separately (Table 4). It shall be noted that the pseudostem records of these plants, during the first year of growth (Figure 2), shall not be directly compared with pseudostem circumferences of mature plants, ready for harvest. Until ten months of growth, the growth habit of seedling pseudostems were rather cone-like, while pronounced elongation of pseudostem begun thereafter. Despite this, the increase in circumference (Figure 2) during the first ten months is a measurement of plant growth. For mature plants, pseudostem circumference is positively correlated to yield (Yemataw *et al.*, 2012; Haile, 2014b). For studies on plant growth, pseudostem volume is a robust measurement and well correlated to total plant FW (Table 4; Karlsson *et al.*, 2020), thus suitable for comparisons when applying non-destructive sampling.

As all plants, enset responds positively to suitable amount of nutrients (Figures 2 and 3). Vegetatively produced sprouts of enset increase production in response to increased manure (Bosha *et al*., 2019) and to N and P as inorganic fertiliser (Ayalew and Yeshitila, 2011). In the relatively warm and dry climate where enset is commonly grown, application of manure or other organic matter give long-term benefit to soil quality, as improved water holding capacity and less leakage of nitrogen (Bayu *et al*., 2006; Amede and Tabogie, 2007), while inorganic fertilisers do not provide such benefits. Thus, manure is the preferred fertiliser to grow enset in a sustainable way. In this study, manure was applied directly around the planted seedlings' roots and corms, while farmers often place manure on soil surface when burying enset corms for vegetative reproduction (Diro, 1997), assuming the buried corms would rotten if manure is placed in the planting hole. However, it has been shown that air-dried manure applied with the corm is not harmful but on the contrary is more beneficial than manure applied on the surface (Bosha *et al*., 2019).

Conscious breeding of enset can contribute to farmers' requests of new and improved characteristics and at the same time preserve the existing gene-bank, especially if including rare landraces and wild plants in the program. However, even though enset seedlings grew efficiently (Figures 2 and 3), seeds or seedlings should not be used as plant material for farmers who want to grow enset for a certain purpose. The reason is that the seedlings have new and unknown combinations of genes from the two parent plants, and thus the suitability to an expected purpose cannot be guaranteed before being tested and released through established procedure of a new cultivar. Currently, there is knowledge on efficient sucker production from corms (Karlsson *et al*., 2015; Bosha *et al*., 2019) and suckers of suitable cultivars or landraces can be distributed to farmers interested in growing enset for food and/or other purposes.

In conclusion, for the preservation of the gene pool from wild and cultivated ensets or for a planned breeding of enset, an efficient seedling production can be achieved without use of synthetic or expensive fertilizer input by applying 4.0–6.0 kg DW (7.5 to 11.25 dm^3) of air-dried cow manure, pulverized to sand-grain size and mixed with local soil to a total of 15 dm^3 per planting hole and seedling at the three leaves stage. The genetic variation among cultivated as well as wild enset could be utilised for achieving genotypes with combinations of desired characteristics by crossing and selection.

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