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# Early growth and development of *Ensete ventricosum* (Musaceae) seedlings

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**Abstract:** Enset is drought-tolerant, multi-purpose crop which has, since ancient times, been part of a sustainable cropping system with high agro-biodiversity in Ethiopia. It could improve independent food and livelihood security more than currently and in larger areas. Enset is traditionally propagated vegetatively, and plants are harvested before seed-set. There is request for new cultivars, and traits could be improved by conventional breeding through seeds and selection. However, there is no documented knowledge on seedling growth and development under field conditions. Therefore, we undertook the first study on enset seedling performance. Seeds originating from three cultivated and three wild plants were used. Over 400 newly germinated seeds were planted outdoors in an area with traditional enset cultivation. Seedlings were grown in local soil only or with supplement of cow manure or a locally available inorganic fertilizer (DAP). During the first three weeks after germination, there was no difference due to substrate, while cow manure was efficient and enough for further growth. Within 24 months, seedlings planted in the field and supplied with manure, reached 1.5-2 m pseudostem circumference and four out of ten flowered; much faster and stronger growth than expected. It is concluded that it would be suitable to breed enset through sexual propagation, utilizing the genetic variation for selection and following established procedures for new cultivars. However, seeds must not be distributed for direct use; clones developed from selected seedlings can efficiently be vegetatively propagated and distributed.

**Keywords:** Abyssinian banana, Breeding, Drought tolerant crop, Ethiopia, False banana, Perennial crop, Propagation

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## 1. Introduction

Enset [*Ensete ventricosum* (Welw.) Cheesman] has been cultivated in Ethiopia since ancient times; it is part of a sustainable production system [1]. The genus *Ensete* is close to, but distinctly separated from, the genus *Musa*, the common banana [2]. The vegetative growth habit of enset is similar to banana plants, but *E. ventricosum* does not spontaneously produce suckers and is not grown for the fruits; these contain mostly large and very hard seeds [3], and plants are usually harvested at onset of flowering [4,5].

Enset is a multi-purpose and drought-tolerant crop. There are several hundred landraces (clones), having different characteristics and uses [6]. Enset is used for food [7], fodder [8], fibre [9] and traditional medicine [10]. Enset is

a staple crop for an estimated 15-20 million people in Ethiopia [1] and a reliable food source where failure of annual crops is common [11,12]. The nutritive value of the food is similar to potato, and the fodder composition is suitable for ruminants [13-15]. In terms of edible dry weight and energy, enset gives higher yield than any other crop cultivated in Ethiopia [7]. Enset is most commonly grown in home-gardens, frequently intercropped with peas or beans [16], which is suitable to compensate the low protein level in enset foods [17]. Surface mulching, using a variety of crop residuals, is applied, which will recycle nutrients in a stepwise manner due to differences in composition and decomposition rates, similar to banana cropping systems in Uganda [18].

Being a long-lived monocarp, enset improves local cli-

mate and soil conditions when cultivated [19]; especially the deep roots [20] are important in preventing nitrogen loss and environmental pollution [21]. Ethiopia, being a food insecure country and in protracted crisis [22], would benefit from increased and improved use of enset, and it could be used in additional countries. This would be a straight-forward method to facilitate for people to achieve independent food and livelihood security [23]. However, as noted by the Worldwatch Institute, enset has received little research or extension support [24].

Enset is traditionally propagated vegetatively [4]. Thus, a farmer knows that a new plant will be as well suited to the local situation as the previous one [12]. Traditional vegetative propagation is efficient for enset [25] as for banana; even though there are suitable media and techniques for micropropagation of banana [26], most of the commercial production in Brazil uses traditional methods, partly due to problems with contamination in micropropagation [27].

There is demand for new and improved cultivars of enset, including increased tolerance to diseases and environmental constraints as drought and frost. To make selection possible, there must be genetic variation; this can be achieved by using seeds for propagation, which was suggested already 1987 [28]. A difference in morphology is a good indicator of differences in genotype [29]. Conventional breeding, i.e. utilizing the nearly endless possibility of gene recombination achieved by seed set and thereafter selecting suitable plants for different purposes, can be very successful (compare with the development of apple varieties in Europe). Further, there are currently attempts to use biotechnology to develop enset varieties. To efficiently breed enset, there is need for knowledge about techniques to germinate seeds [25,30,31] and grow seedlings. The purpose of this study was to, for the first time, investigate early growth of enset seedlings under field conditions.

## 2. Materials and Methods

Seeds of six plants of *Ensete ventricosum* were collected at maturity (Table 1). The study was performed within a fenced area at Wolaita Sodo University campus (N 06°50'00" E 37°45'08", 1891 m a.s.l.), Ethiopia.

On the 13th of December 2010, seeds were soaked in water for imbibition, and after 24 h the seeds were sown in trays (50 cm diameter, 15 cm high, drainage holes in the bottom) outdoors. The trays were filled with ca. 10 cm local soil, crushed to ca. 0.6-2.0 mm diameter (sand grain size) using local practice to hit a pile of soil again and again with wood sticks. Before sowing, soil was watered with excess water and drained to field capacity. The seeds were placed with minimum the size of one seed distance. Each individual seed was carefully placed with the point for radicle protrusion down and covered with ca. 0.5 cm soil.

The trays were placed in an excavation slightly deeper than the trays, and daily watered to above field capacity (drainage to field capacity occurred within ca. 10 minutes).

**Table 1.** Collection sites for *Ensete ventricosum* seeds. Coordinates are for the exact places, site names are towns in the vicinity

Code <sup>a</sup>	Coordinates (N, E)	Altitude (m a.s.l.)	Site
C1	09°24'55", 49°02'11"	2024	Haramaya
C2	06°50'17", 37°45'43"	1916	Wolaita Sodo
C3	08°18'49", 37°49'05"	1885	Wolkite
W1	07°04'07", 37°11'02"	2195	Tercha
W2 <sup>b</sup>	07°04'02", 37°41'22"	1785	Areka
W3 <sup>b</sup>	07°04'02", 37°41'22"	1785	Areka

<sup>a</sup>C = cultivated, W = wild. <sup>b</sup>Mother plants originated from seeds collected in the wild, around Jimma (N 07°41', E 36°50', 1750 m), and grown at Areka Research Centre until seed ripening.

Protection from direct sun was achieved by a grass cover on 60 cm high sticks. Seeds were checked for emergence every day.

At shoot emergence, seedlings were transplanted to individual polyeten bags (14 cm diameter, 25 cm high), open in bottom. Two studies were conducted: (1) growth of all seed batches in local soil and local soil supplemented with cow manure and (2) growth of one seed batch (W2) in response to a locally available commercial fertilizer (DAP Grade 18-46-0 [N-P-K, Ammophos, Cherepovet, Russia], containing 18% N and 20% P [IPNI, 2013]) compared with the two substrates used in (1). Dry cow manure was collected from a neighbor dairy farm. All substrates were crushed to ca. 0.6-2.0 mm diameter as described above. Soil and manure characteristics (Table 2) were analyzed by Sodo Soil Testing Laboratory. For study (1), we used a mix of ¾ local soil + ¼ sand and a mix of ½ local soil + ¼ sand + ¼ manure. For study (2), either 1, 2, 4 or 8 g fertilizer per pot was added to the soil + sand mix, and local soil + sand and local soil + sand + manure were as above.

The pots with newly transplanted seedlings were placed in a nursery covered with grass roof for 3 weeks and watered twice a day. Thereafter, seedlings were transferred to open sun, watered once a day, if not raining, until harvested. The number of replicates per seed batch was dependent on the number of seeds available and emerging seedlings; we used at least ten replicating seedlings per seed batch and treatment, and 20 per treatment to the fertilizer; totally 412 seedlings were planted.

Seedlings for study (1) were transplanted in January 2011, and seedlings for study (2) were transplanted in February 2011, all were harvested in July, at six (study 1) or five (study 2) months after transplanting. Weather conditions (Table 3) were recorded at a nearby weather station (The National Meteorology Agency of Ethiopia).

After three weeks and either six months (study 1) or five months (study 2) the number of green leaves, plant height (i.e. length of longest leaf), width of longest leaf and pseudostem diameter were measured.

**Table 2.** Characteristics of substrate components used to enset (*Ensete ventricosum*) seedling early growth study in southern Ethiopia

Component	pH	Total N (%)	Available P (ppm)	Available K (ppm)	Conductivity (ds/m)	Soil texture
Soil	5.7	0.019	2.62	12.6	Not recordable	Loam
Cow dung	8.1	0.810	39.80	99.2	0.01	–

**Table 3.** Monthly average weather in Wolaita Sodo, Ethiopia, during enset (*Ensete ventricosum*) seed germination and seedling early growth (Dec. 2010–Jul. 2011). Daily weather data collected and provided by The National Meteorology Agency of Ethiopia

Month	Precipitation (mm)	Average daily temperature (°C)		Humidity (average RH [%])
		Max	Min	
Dec.	20	27.0	13.8	49
Jan.	5	28.2	14.8	46
Feb.	10	29.3	16.3	39
Mar.	43	28.9	16.9	43
Apr.	68	28.8	16.1	51
May	271	24.9	15.6	74
Jun.	115	22.9	15.3	79
Jul.	203	22.6	14.3	79

At harvest, fresh weight (FW) was recorded directly after harvest and dry weight (DW) was recorded after plants being cut in smaller pieces, dried at 80°C for at least 24 h and checked for constant weight. Aboveground parts (i.e. leaves, including pseudostem, hereafter referred to as 'shoot'), corm and roots were weighted separately. When the seed coats, being very hard and not cracking at germination, detached from plants they were collected and measured in three dimensions, using electronic caliper (precision 0.01 mm).

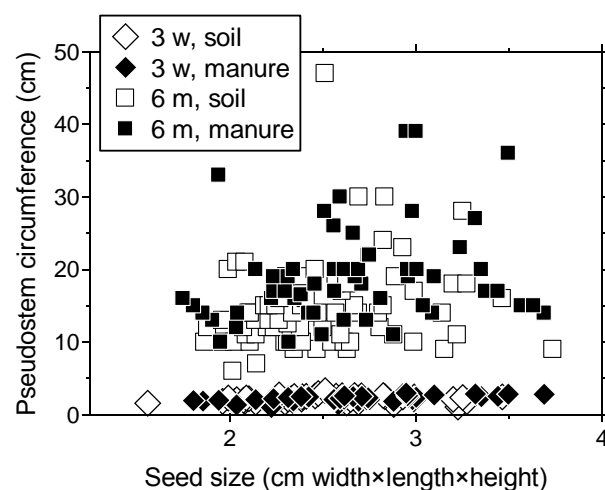
Ten seedlings, grown in pots with soil for six weeks, were planted directly in the field, supplied with manure, watered when needed until established and left to grow freely. These plants were observed until January 2013.

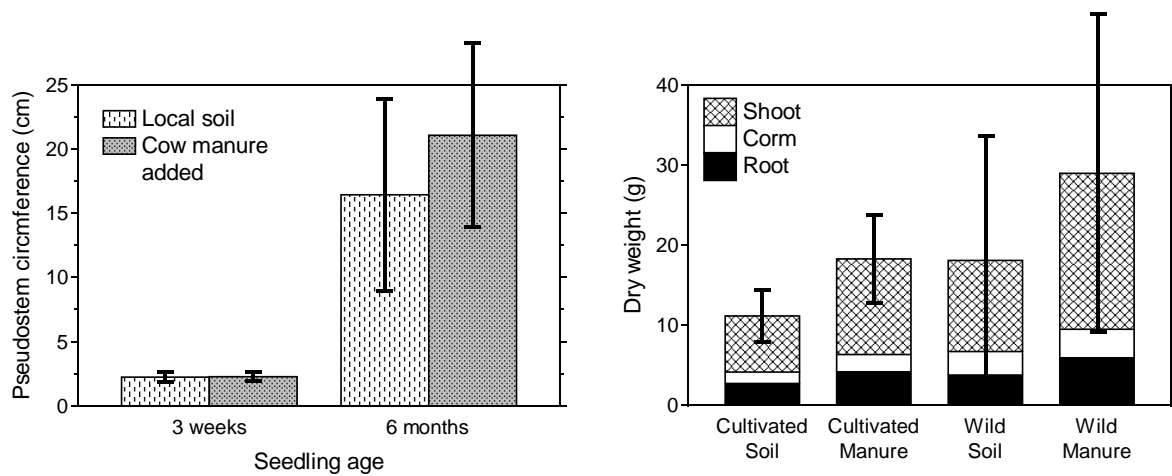
### 3. Results

Total dry weight was strongly correlated to pseudostem circumference (correlation coefficient was 0.92 and 0.93 for plants in local soil and in local soil with manure, respectively). Therefore, pseudostem circumference recorded after 3 weeks and 6 months was used to investigate possible effect of seed size on plant size. Plant size was not generally correlated to seed size (correlation coefficient was 0.08, 0.45, 0.18 and 0.16 for plants after three weeks growth in soil, three weeks in manure, six months in soil and six months in manure, respectively, Fig. 1). Water content in harvested plants was high, on average 91.2% (SD 1.69).

Three weeks after germination, there was no difference between plants grown with or without cow manure, while after six months plants grown with manure were significantly larger (t-test,  $p = 0.006$ , Fig. 2), even though with considerably variations within treatments (Fig. 1). There was no significant difference between seedlings originating from wild and cultivated plants regarding dry weight of entire plants or for root, corm and shoot separately (ANOVA, all  $p > 0.05$ , Fig. 2). There was no significant differences between substrates or between cultivated or wild origin regarding dry weight of the fractions root/shoot, root/(shoot+corm) or corm/shoot (ANOVA, all  $p > 0.05$ ). The seed batches performed differently (Fig. 3) but there was no significant difference between average of wild and cultivated origin regarding any recorded plant characteristic (all  $p > 0.05$ ).

Compared to growth in local soil, plants became larger regarding all investigated characteristics when grown in soil supplemented with manure (t-test:  $p < 0.001$ , Fig. 4), while plants grown with supply from local available commercial fertilizer showed no effect (1, 2 and 8 g of fertilizer, t-test:  $p = 0.26-0.42$ ) or small negative difference (4 g of fertilizer,  $p = 0.044$ ) from local soil (Fig. 4). Seedling mortality was 3-6% in local soil, in soil with manure and for 1 g and 2 g of added fertilizer, 30% for 4 g of fertilizer and 82% for 8 g (Fig. 4).

**Figure 1.** Pseudostem circumference of *Ensete ventricosum* seedlings at three weeks and at six months, plotted against seed size. Plants were grown in southern Ethiopia, with either local soil or soil with cow manure added. CI was 0.08 (3 w soil), 0.45 (3 w manure), 0.18 (6 m soil) and 0.16 (6 m manure) for the relation between seed size and pseudostem circumference.



**Figure 2.** Pseudostem circumference and biomass of *Ensete ventricosum* seedlings grown outdoors at Wolaita Sodo University campus, Ethiopia. Substrate was either local soil or soil with cow manure added. Seeds originated from three cultivated and three wild enset plants. Biomass (dry weight) was recorded after six months of growth. Error bars show SD; for biomass SD of total dry weight. The 'shoot' record is for leaves, including pseudostem; the corm, botanically the stem, was recorded separately.

The ten seedlings planted directly in the field, with manure, had reached a leaf height of circa 2 m after nine months of growth. After 24 months of growth, the base pseudostem circumferences were ca. 1.5-2 m (the largest was 2.67 m), the plants were ca. 6-7 m high (leaf height, including pseudostem) and four were flowering; the first flowered 21 months after germination.

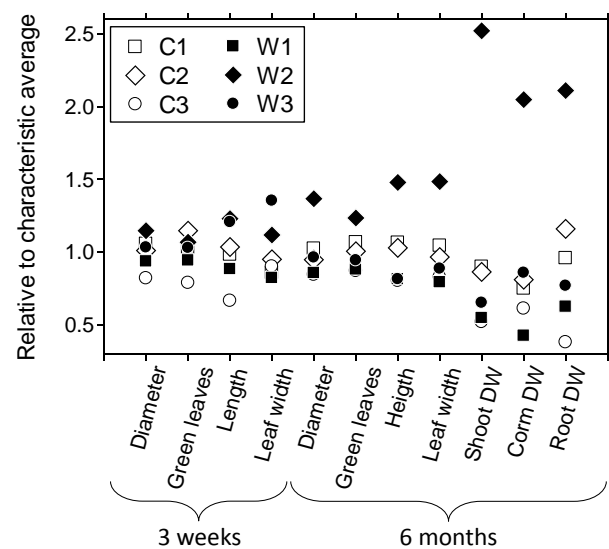
## 4. Discussion

### 4.1. Seedling performance

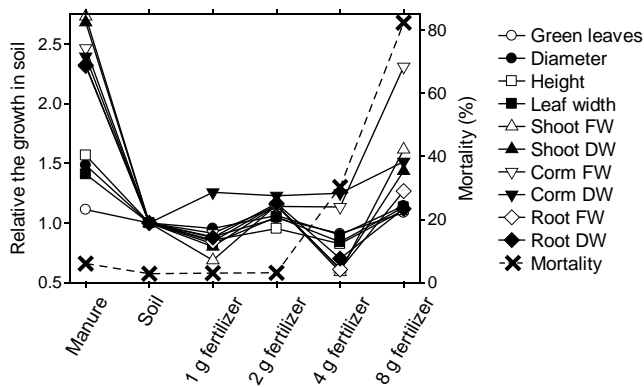
Germinated enset seeds gave a robust impression and developed well. There was no mortality due to microorganisms in the trays used for germination or in pots with seedlings. Further, seedlings from intact seeds readily developed secondary roots: already when the shoot appeared and young seedlings were transplanted to pots there were tens of roots. Thus, when using intact seeds instead of excised embryos, problems with microorganisms, as development of callus and poor development of secondary roots [32], are entirely avoided. Growth of enset seedlings from intact seeds requires no advanced equipment, costly chemicals or laboratory resources but can be performed outdoors with local soil in areas of cultivation. However, germination varies considerably between seed batches: 0-50% [25,31], while [30] reported ca. 90% for three different seed batches. Thus, there is uncertainty of the number of seedlings achieved from any certain seed collection, and there is risk for seeds with suitable genome for requested traits staying ungerminated and therefore not being utilized. Enset seed germination must be further studied.

Enset seedlings are supplied with energy and nutrients from the endosperm after germination, explaining why seedling size was not dependent on fertilization during the

first three weeks after germination (Fig. 2). Enset seeds vary a lot in shape and size but there were enough resources for establishment also in the smaller seeds (Fig. 1). Seedlings from different seed batches differed in size and shape after six months of growth (Fig. 3), but there was no consistent difference between wild and cultivated origin. The difference, which is not significant, between weight averages of seeds from cultivated and wild plants (Fig. 2) was mainly a result of one seed batch of wild origin (W2) giving larger seedlings than the other five seed batches (Fig. 3).



**Figure 3.** Relative performance of seedlings from six seed batches; three originating from cultivated (C) and three from wild (W) plants of *Ensete ventricosum*. Seedlings were grown in pots outdoors at Wolaita Sodo University campus, Ethiopia. The 'shoot' record is for leaves, including pseudostem; the corm, botanically the stem, was recorded separately.



**Figure 4.** Performance, relative plants grown in local soil, of *Ensete ventricosum* seedlings grown with cow manure or different extent of an inorganic fertilizer (DAP). Records are of viable plants after five months of growth, mortality is calculated on the number of newly germinated seeds planted. Plants were grown in pots outdoors at Wolaita Sodo University campus, Ethiopia. The 'shoot' record is for leaves, including pseudostem; the corm, botanically the stem, was recorded separately.

Because of the resource in endosperm, good plant performance during about a month's growth after germination should not be taken as a sign of the used substrate being ideal. This can explain what a farmer, who unconsciously had seed set and germination in his enset plantation, said to us 'they grew well first, but then very slow' (personal communication, Ato Eyoel Fuge, Doyogena, Ethiopia); the early growth was due to resources in the seed, later on the soil was not suitable for continued rapid growth.

As shown in Fig. 2, the growth during the first three weeks after germination is not a reliable predictor for the continued performance. By hearsay, we have got information that plants from seeds require longer time than plants from corm sprouts to reach flowering stage. Plants from corms are expected to reach harvesting stage, the time of flowering, after about six years of growth [4,7]. However, the rapid and strong growth of seedlings planted in the field in our study; some plants flowering within two years, clearly showed that there is no general delay of growth of seedlings compared to vegetative sprouts. The growth rate of enset is probably correlated to the environmental circumstances as temperature, soil quality and moisture, rather than to the propagation method.

Enset seedlings benefited from cow manure (Figs. 2, 4). This is not surprising; soil in warm climates has usually low content of organic matter because of the high rate of mineralization, so the manure contributed not only with nutrients but also with improved soil texture and water-holding capacity [33]. For long-term cultivation, the traditional mulching with residuals is not enough, analogous to banana production in Uganda [18], since resources are withdrawn from the fields at harvest.

The effect of the locally available inorganic fertilizer (DAP) which, in relation to plant requirements, has low N:P ratio (18:20), was not promising. There was no recordable effect of fertilization when supplying 1 or 2 g (Fig. 4). The

plants grown with 4 g of fertilizer were slightly but significantly smaller than those in local soil, while those with 8 g of fertilizer did not differ significantly from seedlings in only local soil (Fig. 4).

The seemingly contradiction resulting from increasing fertilizer from 2 to 4 and 8 gram can be explained by a toxic effect of fertilizer that appeared at 4 g. DAP is known to increase pH in soil and therefore it is risk that volatile ammonium gets harmful to seedlings [34]. In the soil used, pH was below 6 (Table 2) but could possibly have increased too much above pH 7 due to DAP, and therefore increased release of ammonium gas could occurred. It may be more suitable to let newly germinated enset seedlings grow at least three weeks, using seed resources and no additional nutrients (which is not needed, Fig. 2), before supplying DAP. The few plants that survived 8 g DAP could probably make use of the supplied nutrient, especially the nitrogen that was the limiting factor in the soil (Table 2) and slowly is released from DAP when soil microorganisms convert ammonium [34]. In a six-year study on sorghum fields in north-eastern Ethiopia, manure was much more efficient than DAP to generally improve the soil quality and provide nitrogen and potassium [33].

#### 4.2. Growing Enset Seedlings

The growth rate of seedlings planted in soil with cow manure and left to develop after the six months experimental period was astonishing. After 24 months from germination, plants were very large (some reaching over 2 m in base pseudostem circumference) and four of ten flowered. The usual expectation is that sprouts from corms take about six years from emergence to harvest, i.e. flowering [4,5]. Our results show clearly that breeding through seeds and selection would be a relatively quick way to find new enset cultivars.

Based on current knowledge, we can provide a functional, even probably not an optimal, recommendation for enset seedling production: *i* collect ripe seeds (from orange or brown fruits) and place for germination on moist, not wet, soil. *ii* Dig up germinated seeds with a spoon when shoots are about one centimeter. *iii* Plant each seedling in a poly-eten bag, ca. 7 cm diameter and 20 cm high, filled with local field soil; if necessary mix with sand for drainage. *iv* Place pots with seedlings in a sun-protected place and water daily if not raining. *v* Let grow for three weeks. *vi* Move pots with seedlings to sunny place, similar to final place for growth, let grow for three weeks, water daily if not raining. *vii* Prepare for transplantation to field by digging holes approximately 30 cm deep and 30 cm in diameter. *viii* Mix the dug up soil with approximately ¼ volume of dry, crushed, cow manure and put back the softened soil mix in the hole. *ix* Remove the seedling and soil cylinder from the pot by carefully cutting along the side, plant in the prepared holes. *x* Water once and then as needed until proper establishment.

A practical advice is to have the area carefully protected by fence and ditch: porcupines find corms delicious and green parts are eaten by grazing and browsing animals.

Further, many people regard the enset as a resource from which they can cut leaf blades (for baking, decoration or animal fodder), not caring about that the removal affects the growth; we used border plants that were not included in experiment.

When, within breeding program, trying an enset plant for cooking or other use, the corm (or a piece of it) should be kept and buried for vegetative sprouting, to keep the genotype and allow further selection from its seeds.

Locally, when corms are buried for vegetative propagation and when the sprouts are transferred, manure is usually placed on soil surface; there is a belief the corm will rotten otherwise. However, we have very good experience of mixing soil with manure and therefore provide the roots with close access to nutrients, when growing vegetative sprouts [25] as well as seedlings (Figs. 2, 4).

### 4.3. Conclusions

Given the knowledge from this study, enset should be a suitable species for conventional breeding. Seedlings can be used in addition to the genetic engineering suggested, as it is not always possible to find one or a few genes that allow development of the desired clone, and conventional breeding does not require any expensive or advanced equipment. With the random recombination of the entire genome, which is the result of natural sexual reproduction with seeds, the number of combinations of genes, and thus expressed characteristics, is nearly endless; this is shown also in taro, *Colocasia esculenta*, [35]; a corm crop grown in Ethiopia.

Enset seedlings can be used in research for selection of new cultivars with desired traits, while seed germination must be further studied. Additional characteristics to investigate are pollination requirements, fertilization mechanisms and environmental conditions affecting seed set. Enset seedling growth rate should be further studied to establish optimal conditions and reliable recommendations for plant production.

Finally, we like to point out that seeds or seedlings must not be distributed to farmers to encourage enset cultivation. This way to increase the use of enset is currently suggested by different groups. The recombination of genes in seeds, the reason for breeding and selection, may result in unsuitable plants for the intended purpose. Breeding should be performed in controlled ways, following the variety release procedure. We agree that the cultivation and use of enset should be encouraged; distribution of plant material can efficiently be done with corms or sprouts from corms, thus distributing plants from clones with known performance and qualities.

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