Heliyon 7 (2021) e08416

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Methods for vegetative propagation of wild enset (*Ensete ventricosum* (Welw.) Cheesman) that make genotype conservation possible

Bewuketu Haile [a,b,*], Bizuayehu Tesfaye [a], Temesgen Magule Olango [a]

[a] Hawassa University, School of Plant and Horticulture Science, P.O. Box 05, Hawassa, Ethiopia
[b] Mizan-Tepi University, Department of Horticulture, P. O. Box 260, Mizan-Aman, Ethiopia

ARTICLE INFO

Keywords:
Crop wild relative
Wild enset
Genetic resources
Conservation
Regeneration
Vegetative propagation
Ethiopia

ABSTRACT

The declining trends in crop wild relative genetic resources in many crop centers of origins including Ethiopia require short and long-term conservation strategies. Enset (*Ensete ventricosum*) is arguably the most important cultivated food security crop of Ethiopia with dwindling wild stocks. The cultivated enset is propagated clonally through adventitious bud sprouting from the corm after the distraction of the apical meristem. Shoot regeneration in the cultivated enset has been induced by humans and has not been observed to occur naturally. The technique of shoot induction has not been extended to the wild enset. To determine whether the capacity for shoot regeneration existed in wild enset and optimize the technique, a series of experiments were conducted. These involved: (i) sucker production from corms of wild enset with and without apical meristem removal; (ii) sprouting capacity of corms ranging 22–49 cm diameter, with removed apical meristem; and (iii) a factorial experiment involving two populations of wild enset (from *Shebena* and *Getiba* localities in Sheka zone), two ways of preparing or cutting the corms: *tero* and *tubo*, i.e. cutting the pseudostem at the corm junction and cutting it at 25–30 cm height, respectively, and three extents of parting the corm (whole, half, and quarter) using corms with a diameter of 45 ± 2.9 cm. The experiments revealed that wild enset can be successfully propagated vegetatively in the same way as the cultivated enset. It also revealed that the regeneration process involved callus formation and adventurous bud proliferation from corms only after the apical meristem was removed. Corms of different sizes varied in their capacity for regeneration significantly with a linear increase in regeneration frequency with corm size. With a one cm increase in corm diameter, regeneration frequency increased by 3.138 %. The two populations of wild enset showed non-significant differences in regeneration capacity; however, the achieved regeneration was generally analogous to that observed among cultivated enset clones: whole corms resulted in a longer time to emergence and fewer sucker per corm than split corms. Specifically, halved corms emerged significantly ($p < 0.05$) earlier (71 ± 9 and 75 ± 7 days, for *Shebena* and *Getiba* populations, respectively) than whole corms (120 days). Regeneration frequency was higher (75–100%) for split than for whole corms (33–56%). The highest rate of suckering (94 ± 14 per corm) was achieved from quarter corms prepared by cutting the pseudo-stem at the junction. In conclusion, the adventitious bud propagation technique developed by farmers to propagate the cultivated enset can successfully be used for the clonal regeneration of wild enset. We recommend the adoption of this shoot induction to conserve and maintain the rapidly eroding wild enset genetic resources in Ethiopia. In addition, wild enset plants with promising characteristics may be fixed using the method to enrich the gene pool of the cultivated enset.

1. Introduction

Ethiopia has long been recognized as the center of origin and diversity for a large number of crop plants (Vavilov 1951; Harlan 1971). Of these, the enset plant is one of Ethiopian's domesticated crops. It is one of the lesser-known cultivated for food and fiber crops which has nevertheless made a significant impact on the local agriculture of Ethiopia owing to its highly drought-tolerant nature with a broad agro-ecological adaptation (Tesfaye and Ludders 2002; Tsegaye and Struik 2002). About one-fifth of the Ethiopian population (more than 20 million) relies upon this crop (Borrellet al. 2019). Besides its use as a source of large amounts of carbohydrate-rich food, enset (including its wild relative) are also

* Corresponding author.
E-mail address: bewuketu@gmail.com (B. Haile).

https://doi.org/10.1016/j.heliyon.2021.e08416
Received 30 June 2021; Received in revised form 25 September 2021; Accepted 13 November 2021

utilized for animal forage, fiber production, as construction, packing, and wrapping materials, and have ornamental, cultural, and medicinal values (Shigeta 1990; Tsegaye 2002; Garedew et al., 2017). Until modern times, when it spread widely around the world as an ornamental, the enset was cultivated solely in Ethiopia (Simoons 1965); these days Ethiopia continues to be the sole place wherever it's full-grown for food (Rossel 1998; Bizuayehu 2008). *E. ventricosum* is the only Ensete species in Ethiopia (Brandt et al., 1997), and all domesticated enset landraces are believed as it arose from this single species which are extant in Ethiopia.

The geographical range of wild enset (in Ethiopia) is more limited, perhaps due to more specific ecological requirements or loss of habitat (Borrell et al., 2019). Birmeta et al. (2004) report that wild enset occurs mainly around the city of Bonga (Southwest Ethiopia; Kaffa zone) and in an exceedingly smaller space by the Omo River (Southwest Ethiopia; Gamo Gofa zone) whilst Garedew et al. (2017) reported that wild enset is widely distributed in Sheka forest (Southwest; Skeka zone), where most of the remaining forest cover of the country is situated. Ethiopia currently has less than 4 % forest cover (Reusing 2000; Wakjira et al., 2008; Moat et al., 2019), down from a potential climax vegetation maximum of 25–35% (Moat et al., 2019). As a forest species, it is logically assumed that the wild enset distribution is affected by regional rates of forest loss; wild enset may have become extinct in some areas. Moreover, no systematic collection of wild relatives has ever been carried out although these important species are exposed to genetic erosion (Guzzon and Müller 2016). Hence the conservation of endangered wild enset is important, considering the threat represented by the ongoing deforestation that may lead to a decrease of the genetic diversity in the wild populations of enset (Birmeta et al., 2004; Olango et al., 2014).

Nowadays, scientists are on a search for genes in the land where farming began, searching for lost genetic resources that will be crucial for the world to keep feeding itself as climate change and deteriorating agricultural landscapes begin to bite. Crop wild relatives (CWR) are believed to contain higher genetic diversity than crops and harbor traits that can improve crop resilience and yield through plant breeding (Fielder et al., 2015). Crop wild relatives (CWRs) are plant species that are closely related to cultivated crops, their closeness implies that they can contribute beneficial heritable traits for pest or disease resistance or yield improvement to crop varieties. The restricted genetic diversity of crops limits their improvement potential that acts as an inevitable barrier for breeding new (Li et al., 2019). In distinction to their cultivated relatives, CWR have not more experienced the genetic bottleneck of domestication (Tanksley and McCouch 1997). As such, CWR harbor higher levels of genetic diversity and doubtless contain a spread of traits that might be used for crop improvement to extend the resilience and yield of contemporary crop varieties (Heywood et al., 2007; Fielder et al., 2015). Wild relatives have provided traits such as disease resistance, tolerance of extreme temperatures, tolerance of salinity, and resistance to drought (Hajjar and Hodgkin 2007). However, in common with most crop wild relatives, wild *E. ventricosum* is poorly conserved in Ethiopia. There is presently no provision for long-term wild enset genetic resources conservation in situ and comprehensive ex-situ collection. Birmeta et al. (2004) cited that the monocarpic life form and the recalcitrant nature of seeds ought to steadily lead to the extinction of wild enset unless production of suckers is induced by artificial means to ensure its survival by vegetative propagation.

There are great differences in their life cycles between the wild and cultivated populations of enset. Wild enset is assumed to reproduce exclusively via seeds, as a result of spontaneous suckering has not been ascertained (Karlsson et al., 2013; Borrell et al., 2019). In cultivation, enset is propagated vegetatively with adventitious buds sprouting from the corm after the removal of the apical meristem (Shigeta 1990; Diro et al., 2001; Bizuayehu 2002). Here, folks have conjointly developed elaborate techniques geared toward clonal propagation of enset plants with desired qualities (Shigeta 1990); as well they maintained significantly genetic variation (Shigeta 1990; Tsegaye and Struik 2002; Bizuayehu 2008; Gerura et al., 2019; Tesfamicael et al., 2020). The method depends on the same principle as shoot formation in vitro tissue culture by organogenesis method (it regenerates mass of adventitious shoots in vivo from the callus formed on the cut surfaces of the corm) (Bizuayehu 2002)). Adventitious regeneration, in general, is the initiation and development of structures from tissues and organs that were not previously organized as meristematic apices (Hartmann and Kester 1983; Hartman et al. 1997, 2010). Adventitious regeneration may involve either organ formation, such as shoots and roots (organogenesis), or the production of somatic embryos (embryogenesis) (Jiménez 2001). In organogenesis cases, shoots and roots are consecutive, and this sort of development is additionally characterized by the presence of vascular connections between the mother tissue and the newly regenerating section (Hartman et al., 2010). According to our current knowledge, this process can be divided into three steps, including activation of regeneration-initial cells, acquisition of competency, and de novo establishment of apical meristems (Sang et al., 2018). In enset, Bizuayehu (2002) described that the in vivo initiation and development of shoot buds involves four distinct phases: dedifferentiation and formation of massive tumor-like callus, differentiation of some of the cells in the callus and formation of meristemoids, proliferation of a large number of buds inside the callus, and the development of these buds into shoots.

Variations in corm preparation are reported between different enset producing areas in Ethiopia, differences are seen in whether or not farmers uproot the rhizome, and whether or not it is split after uprooting, and the age of parent plants (Diro et al., 1996). There are some efforts to assess such farmers' practices and it was reported that the number of suckers produced per corm ranges between 40 and 200 depending on the cultivar, size and age of the mother plant, and corm splitting techniques (Diro et al. 2001, 2002; Karlsson et al., 2015). According to this literature, the time to sucker emergence is shorter for split corms, and a higher number of suckers are generated this way compared with entire corms (Diro et al., 2002; Karlsson et al., 2015). Size/age of the corm have also been reported to affect sucker regeneration capacity of the corm, corms from younger plants (one-year-old) gave significantly less number of suckers, whereas corms from age two to five produced a higher but closer number of suckers (Diro et al., 2001). To the best of our knowledge, however, there is no information available on how the corms of wild enset respond to vegetative propagation. We suggest studying the potential of vegetative reproduction of wild enset to maintain this vital genetic resource through human intervention. Therefore, these studies aimed at exploring the regenerative capacity of wild enset using methods used by farmers to clonally propagate its domesticated form. Also, the studies are expected to analyze the impacts of corm size, cutting methods, corm types, and populations on the regenerative capacity and multiplication rate of wild enset. We hypothesize that wild enset corm has the capacity of regeneration as that of its cultivated form following the same traditional propagation method; the mechanism of regeneration is adventitious, and the regeneration capacity of wild enset is affected by populations, size of corms, cutting techniques and corm types used. Here we explore the regenerative capacity of wild enset by focusing on the following questions: 1) is vegetative reproduction the only feature of domesticated enset or is it already retained capacity in wild enset too? 2) is the mechanism of regeneration in wild enset is similar to that of its domesticated type? 3) would the size of mother plants, populations, corm types, and cutting techniques affect the regeneration and multiplication capacity of wild enset? Hitherto there is no observational or trial report that demonstrates the clonality of wild enset. Thusly, improving our understanding of the vegetative regenerative capacity of wild enset through this traditional propagation method could provide some insights about the early domestication of enset besides its significance to support wild enset conservation efforts.

2. Materials and methods

2.1. Study area

The experiment was conducted on a farmer's field in Anderacha District of Sheka Zone, in Southwestern Ethiopia. The whole Sheka Zone is recognized for its biodiversity-rich Afromontane natural rainy forest and existence of customary biodiversity management practices that UNESCO designated it as 'Sheka Forest Biosphere Reserve'. It was also reported that wild enset is widely distributed throughout this forest (Garedew et al., 2017). The soil of Sheka area is characterized by Acrisol with a sub-surface layer of accumulated Kalonitic clay in the order Oxisol, low Cation exchange capacity, low base saturation, and low pH values (Berhane and Sahelmedhin 2003). The soils are deep, well-drained, and reddish-brown when moist and dark red when dry (Berhane and Sahelmedhin 2003). Anderacha area receives a high amount of rainfall, with an average of 1800–2200 mm annually and mean annual average temperature between 15.1 °C to 27.5 °C (Haile et al., 2015). The study site is situated at 7.056521° latitudes, and 35.045595° longitudes, and at an elevation of 1960 m above sea level.

2.2. Plant materials

Individual plants from two wild enset populations: 1) a wild population at *Shebena* locality, inside Sheka Forest, and 2) a population adjacent to the domesticated population at *Getiba* locality were used. Parent plants within a population that appeared to have good phytosanitary conditions were selected; plants with any disease symptom or damage were excluded from the experiment. In the first experiment, 15 corms were used to observe the mechanism of regeneration in wild enset. In the second experiment: sprouting capacity was studied using corms ranging 22–49 cm diameter from 46 individuals of wild enset. In the third experiment: regenerations methods were studied using 108 corms with a diameter of 45 ± 2.9 cm that was acquired from the two populations of wild enset (54 individuals from each population).

2.3. Treatments, experimental design, and procedures

All the propagation methods and procedures used were adopted from the customary vegetative propagation method used by Shekicho farmers to clonally propagate their domesticated enset. Accordingly, the two ways to detach the corms from the pseudo-stem: *tero* and *tubo*, i.e. cutting the pseudostem at the corm junction and cutting it at 25–30 cm height, respectively, and the existing corm splitting practices (whole, halved, and quartered) were adapted. The cut surfaces of all corms and corm pieces were exposed to sunlight for 48 h before planting. The experimental land was cleared and plowed five times by oxen plow and well manured according to farmers' practice for domesticated enset. The land was used for the cultivation of domesticated enset for several years before it lay fallow for a year. The first and second experiments were conducted between January to June 2019 and the third experiment was conducted from January to June 2020.

First experiment: the possibility of sucker production from corms of wild enset was investigated using corms ranging 40–49 cm diameter. It involved: (1) intact plants, in which cut were made at the junction between the pseudostem and the corm, without damaging meristem; (2) intact plant, a cut was made at the junction whereby meristem tissue has been removed; (3) whole corms, plants were uprooted and their pseudostems were cut off above the crown after which the leaf sheaths and roots were trimmed down without damaging the shoot apex; (4) whole corms, their pseudostems were cut off above the crown and the shoot apex removed; (5) split corms, involving corms sliced into two and four after removing the apical meristem. In this experiment, each corm treatment had three replications. Uprooted corms/corm pieces were laid vertically in the pit with the cut end facing upwards and covered with soil. Corms and corm pieces were pulled out and then poured with water several times for inspection of the changes that took place over each week. At each stage, pictures were taken to document the phenomena.

Second experiment: sprouting capacity was studied on corms ranging 22–49 cm diameter, with removed apical meristem after cutting off the pseudostem at the corm junction. Sprouting capacity was measured by regeneration frequency, the dependence of corm regeneration frequency upon corm size.

Third experiment: regenerations methods were studied in a factorial experiment using corms with a diameter of 45 ± 2.9 cm from two populations (from *Shebena* and *Getiba* localities in Sheka zone). Two ways to detach the corms from the pseudo-stem: *tero* and *tubo*, i.e. cutting the pseudostem at the corm junction and cutting it at 25–30 cm height, respectively, and three extents of parting the corm: whole, halved, and quartered were arranged in a factorial combination with two populations. The apical meristem was removed from all corms. Corms were buried in randomized complete block design with three replicates. On December 31, 2020 corms from 108 parent plants (54 from each population) were uprooted. Additional extra corms were also collected to use as border plants. The experimental field was divided into three blocks each containing 12 plots. The dimension of every unit plot was 12 m^2 (3 × 4 m), corms/corm pieces were planted in 1 × 1m spacing. A distance of 1m was maintained between the unit plot and blocks. Each plot had three rows which consisted of four hills. In each plot it was managed to use corms from three individual plants, part-records from one original corm were merged to one record for calculations. To avoid the edge effect, experimental plants were protected by guard rows. In plots where few enset corm/corm pieces were planted (in cases of whole and halved corm type), extra corm pieces were planted in the outer parts of the plot. Planting of the corms and corm pieces was done on January 2, 2020. Management practices such as weeding were done regularly.

2.4. Data collection and analysis

Nursery inspection was made every week and data collection on regeneration and emergence had commenced when the first plant emerges. Data articulating the regeneration capacity of wild enset corms: (1) days to emergence (recorded by counting the number of days from the date of planting to the date at which about 50% of the corm or corm piece gave sprout/s), (2) regeneration frequency (the proportion of regenerated corms or corm pieces per treatment), and (3) regeneration/multiplication rate (number of suckers produced per corm) were collected accordingly. Multiplication rate was calculated after harvesting suckers, 90 days after emergence (when the sucker riches to the size for the first transplant, considering farmers' practice in domesticated enset). The multiplication rate for corm pieces (half and quarter) was calculated by summing the total number of suckers from each piece. Recorded characteristics related to the early growth performance of the regenerated suckers, measured from each sucker were: (1) average plant height (measured from the ground to the tip of the longest leaf), (2) average pseudostem circumference (the circumference at half the pseudostem height), (3) average leaf number (number of developed leaves, more than 50% green), (4) average leaf length (length of the longest leaf blade), and (5) average leaf width (blade width of the longest leaf). In the second experiment, linear regression was initially used to quantify the relationship between corm size and regeneration frequency. Data from the third experiment were subjected to ANOVA using SAS statistical software version 9.2 (SAS, 2008) and means compared by using the least significant difference (LSD) test at a 5% probability level. Sucker number data

were transformed using natural logarithms before ANOVA but the untransformed values were used in the discussion. The relationships between parameters were evaluated using correlation analysis.

3. Results

3.1. Experiment I: the mechanism of regeneration in wild enset

Regardless of the cutting techniques and corm type used, adventitious buds were observed from differentiated callus at the cut surface of the corms as the result of removing the shoot apex. The sign of regeneration appeared on the cut surface of the corms (Figure 1b, d) six to eight weeks from the removal of the meristem. The callus tissue formed first on the exposed surface at the site where the meristem was removed (Figure 1b) and enlarged internal to the leaf sheath bases (Figure 1c). Cell proliferation and enlargement of the callus tissue continued along with both horizontal and vertical directions, developed into a compact and massive tumor-like tissue (Figure 1c,d,e) that reached a size of ten centimeters in diameter (this was about 25% diameter of the whole corm used). Callus formation took place only at the upper area of the sub-apical region of the corm while the basal portion remained inactive (Figure 1d, e). After nine weeks, many small swellings appeared on the surface of the callus tissue (Figure 1c, f) these later developed into shoot buds and subsequently to shoots (Figure 1f). Initially, shoots were without roots but after a while started to develop roots at their base. On other hand, simple decapitation of the pseudostem that does not involve injury or damage to the shoot apex is not sufficient to initiate callus proliferation. Corms in which the original growing centers were left undamaged, shoot apices continued their normal development and produced only a single whole shoot (Figure 1a).

3.2. Experiment II: sprouting capacity of wild enset corms

Wild enset of different sizes, ranging from 22 to 49 cm diameter were used to see the sprouting potential of the corms (measured by regeneration frequency). To evaluate the dependence of corm regeneration frequency upon corm size, linear regression analysis was conducted. There was a statistically significant ($p < 0.001$) association between the corm size and regeneration frequency, regeneration frequency increased linearly as corm size increased (Figure 2). With a one cm increase in corm diameter, regeneration frequency increased by 3.138 %. Within the range of values in the data set, the predicted value of regeneration frequency laid in between 0 to 74%. The data indicate that at least 50% regeneration frequency can be guaranteed by using corms of wild enset with 41 cm diameter. The percentage of corm-inducing shoots can be increased between the ranges of 50–74% by using large-sized corms (40–49 cm diameter). Towards this end; we concentrated on corm diameter 40–49 cm to find a specific corm treatment that improves regenerablity of the corms and induces the highest number of shoots per corm.

3.3. Experiment III: Effect of population, corm preparation method, and corm type on regeneration of wild enset

In light of the information obtained from the second experiment, corms with an average diameter of 45 ± 2.9 cm acquired from two wild enset populations in southwest Ethiopia were used to explore the regenerative potential of wild enset (Tables 1 and 2 & Figure 3). This factorial experiment was designed to see the effect of corm preparation methods/cutting techniques and corm types on shoot regeneration and the multiplication capacity of wild enset. We observed differences in the regeneration capacity of wild enset corms depending on populations, corm types, and the cutting techniques employed. The interaction effect of population x mode of corm preparation x corm type was highly significant ($p < 0.01$) on emergence condition of the corm (days to emergence) and significant ($p < 0.05$) on the frequency of regeneration and multiplication capacity (average number of shoot per corm) of the corms. Halved corms prepared by cutting the pseudo-stem at the corm junction were significantly ($p < 0.05$) earlier to emerge (71 ± 9 and 75 ± 7 days) (Table 1). Whole corms prepared using either of the two cutting techniques took longer days to emerge (120 days) but whole corms sourced from *Getiba* locality and prepared by cutting the pseudostem at 25–30 cm height rather took the moderate time (100 days) to emerge.

Regarding regeneration frequency, the lowest regeneration frequency (33.33%) was recorded from whole corms sourced from *Getiba* locality that were prepared by cutting the pseudo-stem at the corm junction. But, slicing the corm into two or four splits had enhanced the regeneration frequency (75–100%) depending on the corm preparation method employed. Similarly, the total number of shoots was higher from split than entire parent corms (Table 1). The higher multiplication rate (94 ± 14 stems/corm) was observed from corms from *Getiba* locality that were prepared by cutting the pseudo-stem at the corm junction and quartering. However, the multiplication capacity of all quartered corms prepared in either of the two methods was statistically similar with this figure except corms acquired from *Getiba* locality, that prepared by cutting at 25–30 cm height. On the other hand, the extremely lowest number of regenerated shoots per corm (2.5 ± 1.5) was recorded from whole corms that were originally obtained from *Shebena* locality and were prepared by cutting the pseudo-stem at corm junction.

Some growth parameters were also collected to see the early growth of regenerated shoots. As the data depicted, the interaction effect of population x mode of corm preparation x corm type was significant (P <

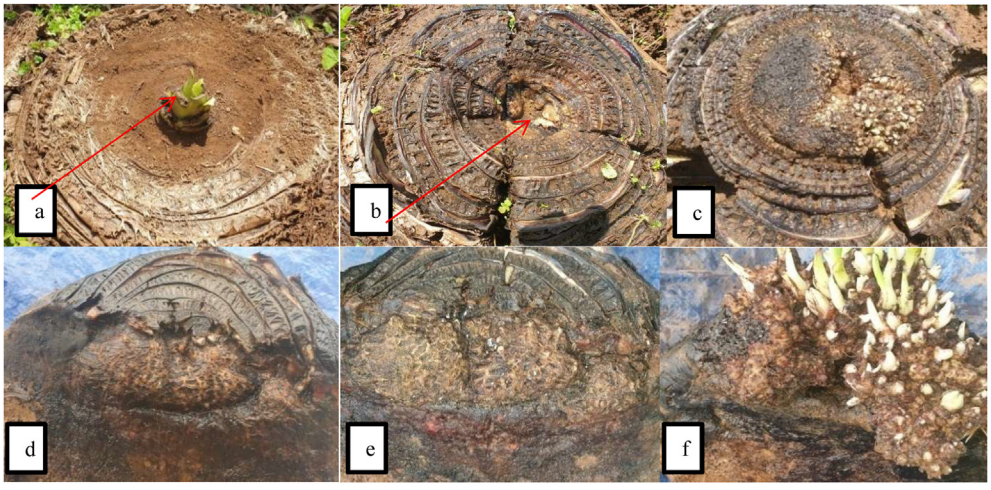

Figure 1. *In vivo* regeneration of wild enset involved dedifferentiation and formation of massive tumor-like callus, proliferation of a large number of buds inside the callus, and the development of buds into shoots. a) Decapitated pseudostem without injuring the shoot apex, 4 weeks after decapitation. b) Sign of repair/callus formation in intact corm at damaged meristem site, 6 weeks after wounding. c) Intact corms of wild enset with massive tumor-like callus and proliferating buds, 9 weeks after wounding. d) Development of compact and massive tumor-like tissue from halved corm, 6 weeks after injury. e) Enlarged tumor-like callus on halved com at the upper area of the sub-apical region, 7 weeks after injury. f) Proliferation and early emergence of shoot buds on the callus, and development of wild enset buds into shoots, 9 weeks after injury.

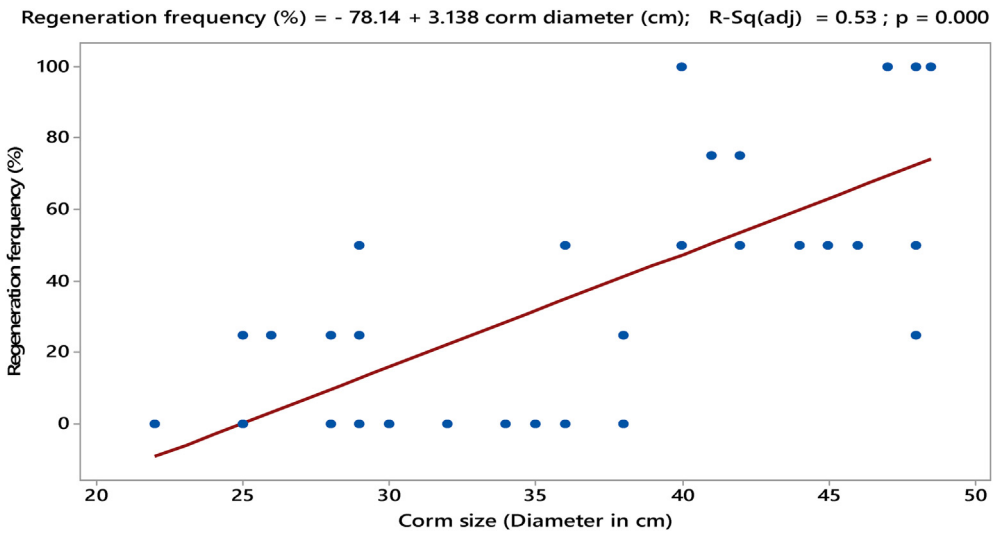

Figure 2. Regeneration frequency of wild *Ensete ventricosum* under different corm size: Linear regression examination of regeneration frequency versus corm size.

Table 1. Regeneration capacity and early growth performance of wild *Ensete ventricosum* as affected by populations, mode of corm preparations/cutting techniques, and corm types.

Treatments			Days to Emergence	Regeneration frequency (%)	Sucker number per corm	Leaf width (cm)	Leaf length (cm)	Pseudo-stem circumference (cm)	Plant height (cm)
P1	MCP1	Whole	120 ± 0^{A}	55.56 ± 19.2^{BC}	2.5 ± 1.5^{G}	$8.25 \pm 0{,}25^{DE}$	13 ± 1^{F}	9 ± 0^{DE}	13.50 ± 0.5^{G}
		Halved	71 ± 9^{F}	50 ± 0^{BC}	21 ± 3^{BC}	11.5 ± 2.5^{BCD}	20.41 ± 4.1^{C_F}	10.41 ± 0.41^{CDE}	38.50 ± 0.5^{CDE}
		Quartered	79 ± 1^{DE}	50 ± 0^{BC}	58 ± 42^{AB}	10.16 ± 1.84^{CDE}	22.21 ± 0.38^{CD}	9.25 ± 1.25^{CDE}	31.50 ± 5.5^{DE}
	MCP2	Whole	120 ± 0^{A}	44.44 ± 19.2^{C}	3.5 ± 2.5^{FG}	7.75 ± 2.25^{E}	13.50 ± 5.5^{EF}	11 ± 4^{BCD}	18 ± 3^{FG}
		Halved	90 ± 0^{C}	75 ± 25^{AB}	10 ± 6^{DE}	$11.25 \pm 0.25^{B-E}$	21.30 ± 1.3^{C_F}	8.62 ± 0.62^{DE}	43 ± 7^{BCD}
		Quartered	90 ± 0^{C}	100 ± 0^{A}	82 ± 6^{A}	$10.99 \pm 0.81^{B-E}$	19.30 ± 4.9^{DEF}	10.05 ± 0.55^{CDE}	43.50 ± 4.5^{BCD}
P2	MCP1	Whole	115 ± 5^{A}	33.33 ± 0^{C}	8 ± 1^{DE}	10.3 ± 0.5^{CDE}	23.40 ± 1.4^{BCD}	12.15 ± 1.15^{BC}	29.50 ± 1.5^{EF}
		Halved	75 ± 7^{EF}	75 ± 25^{AB}	21 ± 7^{BC}	9.5 ± 0.5^{DF}	$20.30 \pm 3.5^{C-F}$	7.60 ± 0.2^{E}	29.75 ± 2.25^{EF}
		Quartered	86.5 ± 3^{CD}	75 ± 0^{AB}	94 ± 14^{A}	13.30 ± 3.8^{ABC}	27.85 ± 10.35^{BC}	9.37 ± 2.63^{CDE}	49.50 ± 19.5^{ABC}
	MCP2	Whole	100 ± 0^{B}	55.56 ± 19.2^{BC}	5.5 ± 1.5^{EF}	16.05 ± 1.55^{A}	40.10 ± 1.1^{A}	15.30 ± 1.3^{A}	60.50 ± 1.5^{A}
		Halved	85 ± 10^{CD}	100 ± 0^{A}	16 ± 2^{CD}	13.92 ± 3.33^{AB}	30.87 ± 6.88^{B}	13.80 ± 2.7^{AB}	52 ± 8^{AB}
		Quartered	90 ± 0^{C}	75 ± 25^{AB}	32 ± 8^{BC}	10.75 ± 2.15^{BCD}	21.50 ± 5.3^{CDE}	8.7 ± 1.3^{DE}	30.50 ± 5.5^{E}
LSD (P = 0.05)			7.611	27.838	23.353	3.544	8.412	3.048	12.279

Mean ± SE for a variable on the same column with the same letter(s) are not significantly different at $P < 0.05$. P1 = corms sourced from a wild population at *Shebena* locality, inside Sheka Forest, P2 = corms sourced from a population adjacent to the domesticated population at *Getiba* locality, Sheka zone. MCP1 = Mode of Corm Preparation 1 (*Tero*), done by carefully removing both the true stem and leaf sheath (pseudostem) almost at the joining point of the pseudostem and the corm. MCP2 = Mode of Corm Preparation 2 (*Tubo*), done by carefully removing (damaging) the short true stem while keeping 25–30 cm pseudo stem with the corm.

0.05) on leaf width, leaf length, pseudostem circumference, and plant height (Tables 1 and 2). But leaf numbers showed no significant ($p > 0.05$) differences between wild enset populations and between corm types and the method of preparation or between their interactions. The observed leaf width, leaf length, pseudostem circumference, and plant height ranged from 7.75 ± 2.25 to 16.05 ± 1.55, 13 ± 1 to 40.1 ± 1.1, 7.6 ± 0.2 to 15.3 ± 1.3 and 13.5 ± 0.5 to 60.50 ± 1.5 cm, respectively (Table 1). But no significant ($p > 0.05$) correlation was observed between regenerated sucker number and all these measured growth parameters (Table 3).

4. Discussion

4.1. Methods for vegetative propagation of wild Ensete ventricosum

Opposite to the common banana plant, cultivated enset genotypes are known to produce multiple suckers only after cutting off the apical meristem. It was revealed that cell division, callus formation, and adventurous bud proliferation in wild enset took place only after the growing center/apical meristem had been removed. Like that in the cultivated case (Bizuayehu 2002), the current results suggested that damaging or eliminating the shoot apex is required to achieve cell division and regeneration in wild enset. Wounding or destruction of the apical meristem invigorates regeneration in wild enset: both callus formation and bud proliferation took place only in the absence of intact shoot tips. This can be interpreted as the shoot apex does exercise a controlling influence on adventurous bud formation by the cells in the sub-apical region. This phenomenon could be explained from the viewpoint of plant hormone; the formation of advantageous buds in enset following the removal of the shoot apex may be related to a decrease in auxin concentration and an increase cytokine concentration in corms. As the polar transport of auxin from the shoot apex to root determines apical dominance (Kojima et al., 2002), removing the shoot apex must decrease the concentration of auxin. It is clear that morphogenesis is greatly influenced by the ratio of auxin and cytokinin concentrations; a low auxin/cytokinin ratio was found to stimulate regeneration of shoots on cuttings (Schaller et al., 2015; Zinabu et al., 2021). Callus proliferation that involved dedifferentiation and differentiation with the clear

Table 2. Mean squares for shoot regeneration and early shoot growth parameters.

Source of Variation	Mean Squares								
	DF	Days to 50% Emergence	Regeneration frequency	Sucker number/ corm (Logit)	Leaf number	Leaf width (cm)	Leaf length (cm)	Pseudo stem circumference (cm)	Plant height (cm)
Block	2	46.02	13.50	0.003	0.04	0.29	0.199	1.69	14.63
Population (P)	1	85.56*	378.04^{NS}	0.33*	1.32^{NS}	48.44**	736.99***	18.42*	984.39**
Mode of corm preparation (MCP)	1	203.06**	3086.54**	0.15^{NS}	5.52^{NS}	14.82^{NS}	94.04*	23.45*	735.77**
Corm type (CT)	2	2479.56***	3086.54***	4.07***	0.65^{NS}	2.96^{NS}	1.65^{NS}	20.00**	354.42**
P*MCP	1	248.06**	69.39^{NS}	0.13^{NS}	0.06^{NS}	14.25^{NS}	126.06*	14.69*	37.52^{NS}
P*CT	2	651.81***	794.79^{NS}	0.19*	1.64^{NS}	19.26*	201.46**	14.25*	800.73***
MCP*CT	2	1040.81***	378.04^{NS}	0.04^{NS}	0.91^{NS}	10.58^{NS}	145.36**	5.52**	371.73**
P*MCP*CT	2	248.06**	1319.64*	0.23*	0.05^{NS}	19.99*	75.02*	17.91*	710.17***
Error	22	20.203	270.278	0.050	0.716	4.381	24.680	3.241	52.585
CV (%)		4.81	25.01	19.21	14.48	18.78	21.78	17.25	19.83

DF = degree of freedom; CV = coefficient of variance. *, **, ***F value significant at P = 0.05, P = 0.01, and P = 0.001, respectively. NS = non-significant (P > 0.05)

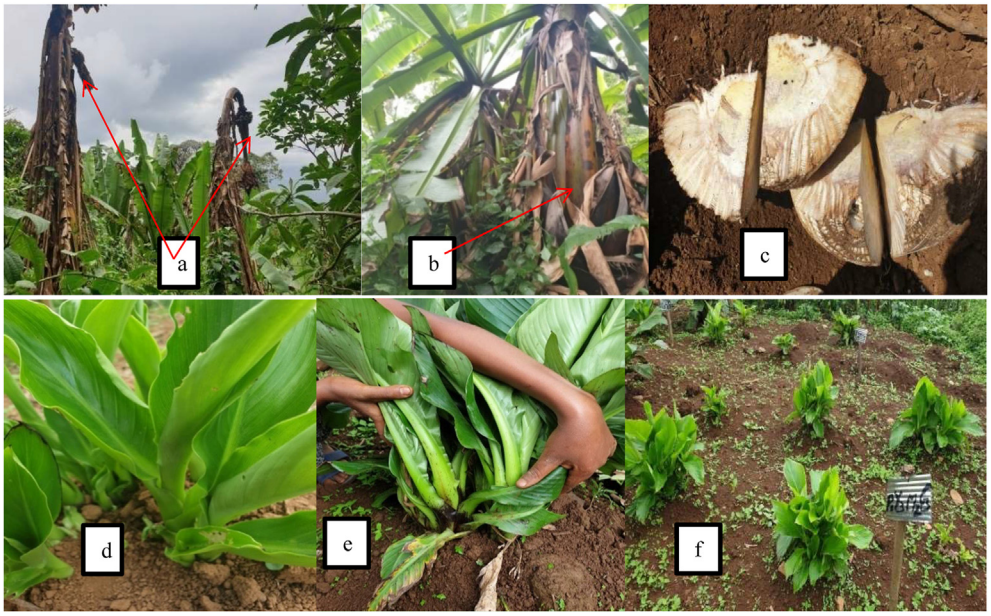

Figure 3. Propagation of the monocarpic wild enset through traditional induced shoot regeneration method. a) Monocarpic wild enset at senescence stage after producing seeds. b) Representative parent plant in its original population, with corm diameter of 45 ± 2.9 cm c) Quartered corm pieces of wild enset. d) Newly regenerated shoots of wild enset. e) Well-grown shoots of wild enset, 90 days after regeneration. f) Partial views of the experimental field, 90 days after shoot regeneration.

Table 3. Pearson correlation coefficient for relationships between regeneration and early growth parameters.

	DE	RF	SN	LN	LW	LL	PsC	PH
DE	1	-0.25^{NS}	-0.4*	0.07^{NS}	-0.33^{NS}	-0.29^{NS}	0.14^{NS}	-0.46**
RF		1	0.35*	-0.44**	0.24^{NS}	0.15^{NS}	-0.07^{NS}	0.39*
SN			1	-0.14^{NS}	0.17^{NS}	0.05^{NS}	-0.23^{NS}	0.27^{NS}
LN				1	0.15^{NS}	0.14^{NS}	0.38*	0.01^{NS}
LW					1	0.93***	0.70***	0.89***
LL						1	0.72***	0.87***
PsC							1	0.54***
PH								1

Ns, *, **, *** indicate non-significant, significant at 5%, 1%, and 0.1% probability level respectively. DE = days to 50% emergence; RF = regeneration frequency; SN = shoot number; LN = leaf number; LW = leaf width; LL = leaf length; PsC = pseudo stem circumference; PH = plant height.

sequences of developmental changes demonstrated that the regenerated mass of shoots in wild enset did not arise from the further development of dormant axillary/lateral buds that were already present as organized shoot apices in the corm. Instead, the corm switched off its normal developmental program from one of integrated and organized growth into a disorganized state, which culminated in the formation of a massive tumor-like callus. Already Bizuayehu (2002) did a histological examination on tissue from the central part of corms at leaf-corm intersections

and previously proven the nonexistence of any organized latent axillary/lateral bud primordial subtending leaf axils in the corms of domesticated enset (Bizuayehu 2002). Henceforth all the buds and subsequently the shoots that were formed by the corm seem to be produced in response to injury and therefore are adventitious in origin. These findings support our hypothesis that wild enset corms have a capacity of regeneration as that of their cultivated form following the same traditional propagation method and the mechanism of regeneration is adventitious. The mode of origin and development of shoots in enset and its wild relative thus contrasts sharply with the mode of sucker development that is typical of banana and other plants, where shoots arise from buds that are located in the axils of leaves and there is a coordinated connection between a bud and suckers (Fisher 1978; Bizuayehu 2002).

The size of the parent plants is likely to play an important role in the *in vivo* shoot regeneration of wild enset corm. In the second experiment, it was shown that the regeneration frequency had increased linearly as corm size increased. The data indicate that at least 50% regeneration frequency can be guaranteed by using corms of wild enset with 41 cm diameter. Regeneration frequency can be increased between the ranges of 50–74% by using large-sized corms (40–49 cm diameter). These results corroborate with those found by Diro et al. (2001), in which larger corms from cultivated enset of two to five years old plant produced a higher but closer number of suckers than from smaller corms of one year old. Hence shoot regeneration and growth totally depends on the corm's carbohydrate reserves until the regenerated shoot initiates its roots and reaches the soil surface, differences in carbohydrate levels, nutrient status, or other growth-contributing resources within the corm could affect sucker regeneration potential. It seems that the physiological ability of the parent wild enset corm to initiate strong regeneration of suckers reaches the optimum when its corm reaches a diameter of 40 cm or above. In light of this information obtained from the second experiment, corms with an average diameter of 45 $\pm$ 2.9 cm from two populations were used to further explore the regenerative potential of wild enset using different cutting techniques and corm types. In general, the results of the third experiment direct that the split corm has a better emergence condition, higher regeneration frequency, and induces a better shoot number than the entire corms. However, the extent of the response varied among population/and mode of preparation being used, as demonstrated by the significant interaction effect. Thus, these findings support our hypothesis that the regeneration capacity of wild enset is affected by populations, size of corms, cutting techniques, and corm types used. From our first experiment as well from the previous report on domesticated enset by Bizuayehu (2002), we learned that the regeneration process begins at the cut end of the corms. It appears to be slicing the corm into many pieces has provided an increased surface area for callus formation and the main induction stimulus for the regeneration phenomenon could be attributed to wound stimuli. The reason may be that for the mechanically damaged explants, wounding treatment may cause many physical and chemical changes, and those changes can somehow lead to the production of a more appropriate level of growth hormones for in *vitro* or *in vivo* plant morphogenesis (Houmani et al., 2018; Xu 2018). Wounding causes an enhanced cytokinin biosynthesis, which in turn increases cell proliferation and callus formation (Ikeuchi et al., 2017). Similar results have been reported on cultivated enset, where the shorter time to sucker emergence and a higher number of suckers were observed from split corms compared with the entire corm (Diro et al., 2002; Karlsson et al., 2015).

It is worth mentioning that size of the corm where also early regeneration capacity, hence halved corm showed early emergence than quartered corm when prepared by cutting the pseudostem at the corm junction. The noticed early development for this situation may be ascribed because of high corm sugar or carbohydrate reserve to support regeneration in halved corms than in quarter. Several cases reviewed by Simberloff (2009) show that increased propagule size can increase the likelihood of establishment. However, the size of the corm in the whole corm type doesn't subsidize for better regeneration and emergence, hence it induced less number of shoots and showed lower regeneration frequency and late emergence than other corm types. This may strengthen the idea that mentioning slicing is more attributor for early callus initiation/shoot regeneration and emergence in enset when the appropriate size of parent plant corm is used. Any treatment combination that improved sucker regeneration efficiency is expected to negatively affect sucker early growth due to the production of a high number of shoots per corm or corm pieces that consequently created a severely competitive environment for resources. However, amazingly no significant ($p > 0.05$) correlation observed between regenerated sucker number and all measured growth parameters. This implies that corms of enset plants possess enough stored reserve to support the early growth of regenerated sucker regardless of the number of suckers obtained per corm.

4.2. Implication for genetic resources conservation and evolution of enset

To the biotechnician, regeneration refers to the process whereby a hopeful mass of callus differentiates into a plant (Harada et al., 2005; Gitonga et al., 2010). While ecologists often discuss regeneration as being either seed restricted (i.e. seed production, dispersal processes) or establishment limited (i.e. germination to establishment processes) (Nathan and Muller-Landau 2000; Myers and Harms 2009). Nonetheless, the dispersal of propagules from vegetative organs could have substantial and under-reported ecological impacts (Zobel et al., 2010). As a sexually reproducing species, much of the variability that exists in enset is expected to arise through sexual recombination. However, the fact that domesticated ensets are harvested before they reach the age of flowering (Brandt et al., 1997) implies that enset infrequently produces seeds in farmers' fields. Additionally, few studies published about *E. ventricosum* seed germination appeared as it has meager germination behavior (Karlsson et al., 2013). It is in this manner possible that a substantial part of the extraordinary clonal variety that exists in Ethiopia may have emerged through somatic mutation during vegetative proliferation through adventitious bud techniques (Shigeta 1990; Tsegaye and Struik 2002; Bizuayehu 2008). Similarly, the latest reports on enset are likewise showing the presence of high heterozygosity in cultivated enset (that routinely propagated in vegetative method) than in its wild relative that only reproducing through seeds (Gerura et al., 2019; Tesfamicael et al., 2020), they noted that the decrease in effective population size may have added to the noticed lower heterozygosity because of the increment of chances of inbreeding in wild enset populations. In this manner, the demonstrated regeneration method has great implications for the conservation of wild CWRs like enset that have recalcitrant seed nature and monocarpic life form; or when mate limitation/and activity of pollinators are expected to be problems for seed reproduction. In such conditions, this traditional vegetative propagation method could bring an alternative regeneration pathway that can be used to maintain wild enset genetic resources, for the reason that clonality provides reproductive assurance by allowing genotypes to persist and propagate without the involvement of sex. Moreover, clonality is believed to increase the evolutionary costs of self-incompatibility by restricting pollen transfer between genotypes (Vallejo-Marín and O'Brien 2007); and curve natural hybridization between crops and CWRs that may lead to severe genetic erosion of CWRs (Ellstrand et al., 2013).

The current works presented that the adventitious bud propagation technique developed to perfection by farmers can aid in replenishing wild enset stocks and maintaining populations through human intervention. We have demonstrated that wild enset can regenerate a substantial number of suckers from a corm (94 $\pm$ 14 suckers/corm) which is somehow comparable with the previously reported sucker production rate of domesticated enset (40 and 141 suckers per corm) by Diro and his coworkers (2002). These pieces of evidence suggest that the studied propagation method is efficient enough to clonally propagate wild enset as it has been working for its domesticated form since antiquity when appropriate corm size and preparation techniques are used.

Moreover, as this is the first work to show that wild enset possess sucker regeneration capacity when propagated in the same traditional vegetative propagation method that has been used to regenerate the domesticated enset since antiquity, therefore, it would also offer insight into how domesticated enset landraces arose from wild *E. ventricosum*. The current indication appears to reinforce previously postulated hypothesis on domestication process of enset; "domestication in enset involves the selection of individuals from wild populations based on desirable morpho-agronomic characters; once identified and selected, the wild individuals are brought to home gardens, named and added to cultivated landraces and maintained through vegetative propagation" (Shigeta 1996; Olango et al., 2015).

5. Conclusion

As it has been repeatedly said Ethiopian farmers have developed elaborate techniques aimed at clonal propagation of domesticated enset, in present studies we showed how wild enset behave when propagated in this traditional method. As of in domesticated enset, the mechanism of regeneration in wild enset was shown to be adventitious. Wounding or destruction of the apical meristem stimulates regeneration: both callus formation and bud proliferation took places only in the absence of intact shoot tips. The bigger corms showed better regeneration rate, this can be related to the reserves of assimilates available for regeneration inside corms. Besides the regeneration capacity is dependent on the population, mode of corm preparation and corm types. Results of the experiment direct that as the quartered corm induces better shoot regeneration and good emergence condition than halved and whole corm types. However, the extent of the response varied among population/and mode of preparation being used. We have demonstrated that wild enset can regenerate substantial number of suckers from the corms using the adventitious bud propagation technique developed to perfection by farmers. These suggest that the studied propagation method is efficient enough to clonally propagate wild enset as it has been working for its domesticated form since antiquity and can aid in replenishing wild enset stocks and maintain populations through human intervention. However, further technical and physiological studies are necessary to establish this cost effective traditional mass propagation method for better efficiency. Lastly, we advocate the adoption of this traditional way of shoot induction as an alternative regeneration strategy in both ex-situ and in-situ conservation of wild enset; this could save the highly threatened wild enset from extinction. Further, wild enset plants with promising characteristics may be fixed using the method to enrich the gene pool of the cultivated enset.

Ethical statement

Prior to the research, participants and local authorities were informed about objective of the work along with an institutional letter. Upon their agreement, we proceeded to conduct the experiments. Collection and utilization of the plant material follow the Ethiopian biodiversity guidelines.

Consent to publication

Not applicable.

Declarations

Author contribution statement

Bewuketu Haile: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Bizuayehu Tesfaye: Conceived and designed the experiments; Performed the experiments; Wrote the paper.

Temesgen Magule Olango: Performed the experiments; Wrote the paper.

Funding statement

This study was supported by the Ethiopian Ministry of Science and Higher Education and the Norwegian Programme for Capacity Development in Higher Education and Research for Development (NORHED) administered at Hawassa University. The paper reflects the authors' own research and analysis in a truthful and complete manner. The funding body has no role in the design of the study, analysis, and interpretation of the data and in writing the manuscript.

Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

We would like to thank Hawassa University, School of Graduate Studies, and Mizan-Tepi University, Research Directorate for its facilitation and support. The authors wish to acknowledge with thanks Anderacha District Administration office and the farmers in the study sites whose contribution was vital for the successful completion of the studies.

References

Berhane, F., Sahelmemedhin, S., 2003. Assessment of different phosphorus forms in some agricultural soils of Ethiopia. Ethiop. J. Agric. 5 (2), 193–213.

Birmeta, G., Nybom, M., Bekele, E., 2004. Distinction between wild and cultivated enset (*Ensete ventricosum*) gene pools in Ethiopia using RAPD markers. Hereditas 140, 139–148.

Bizuayehu, T., 2008. On Sidama folk identification, naming and classification of cultivated enset varieties. Genet. Resour. Crop Evol. 55, 1359–1370.

Bizuayehu, T.A., 2002. Studies on landrace diversity. In: Vivo and in Vitro Regeneration of Enset (*Ensete ventricosum* Welw.), PhD Thesis. Humboldt University, Berlin, p. 127.

Borrell, J.S., Biswas, M.K., Goodwin, M., Blomme, G., Schwarzacher, T., Heslop-Harrison, J.S., WendawekAM, Berhanu, A., Kallow, S., Janssens, S., Molla, E.L., Davis, A.P., Woldeyes, F., Willis, K., Demissew, S., Wilkin, P., 2019. Enset in Ethiopia: a poorly characterized but resilient starch staple. Ann. Bot. XX, 1–20.

Brandt, S.A., Spring, A., Hiebsch, C., McCabe, J.T., Tabogie, E., Diro, M., Wolde-Michael, G., Yntiso, G., Shigeta, M., Tesfaye, S., 1997. The "Tree against Hunger". Enset-Based Agricultural Systems in Ethiopia. American Association for the Advancement of Science), Washington DC, USA, p. 66.

Diro, M., Gebremariam, S., Zelleke, A., van Staden, J., 2002. Growth of enset (*Ensete ventricosum*) suckers under different horticultural practices. South Afr. J. Bot. 68, 430–433.

Diro, M., Tabogie, E., Bekele, A., 2001. Effect of age of parent enset (*Ensete ventricosum*) plants on production of suckers. P. 99-103. In: Girma, Bedada, Desalegne, Lemma, Negussie, Mandefro, Weyessa, Bulcha, Lakew, Berhane, Kibebew, Fasil, Bejiga, Geletu (Eds.), Crop Science Society of Ethiopia (CSSE).Sebil, Vol. 9. Proceedings Ofthe Ninth Biannual Conference, 22-23 June 1999, Addis Ababa, Ethiopia.

Diro, M., Haile, B., Tabogie, E., 1996. Enset propagation research review. In: Abate, T., Hiebsch, C., Brandt, S.A., Gebremariam, S. (Eds.), Enset-based Sustainable Agriculture in Ethiopia. Proceedings from the International Workshop on Enset, Addis Ababa, Ethiopia, 13–20 December 1993. Institute of Agricultural Research), pp. 242–250.

Ellstrand, N.C., Meirmans, P., Rong, J., Bartsch, D., Ghosh, A., de Jong, T.J., Haccou, P., Lu, B., Snow, A.A., Stewart, C.N., 2013. Introgression of crop alleles into wild or weedy populations. Annu. Rev. Ecol. Evol. Syst44, 325–345.

Fielder, H., Brotherton, P., Hosking, J., Hopkins, J.J., Ford-Lloyd, B., Maxted, N., 2015. Enhancing the conservation of crop wild relatives in England. PLoS One 10 (6), e0130804.

Fisher, J.B., 1978. Leaf-opposed buds in *Musa*: their development and a comparison with allied monocotyledons. Am. J. Bot. 65, 784–791.

Garedew, B., Ayiza, A., Haile, B., Kasaye, H., 2017. Indigenous knowledge of enset *(Ensete ventricosum* (welw.) cheesman) cultivation and management practice by Shekicho people, southwest Ethiopia. J. Plant Sci. 5 (1), 6–18.

Gerura, F.N., et al., 2019. Genetic diversity and population structure of enset (*Ensete ventricosum* Welw Cheesman) landraces of Gurage zone, Ethiopia. Genet. Resour. Crop Evol. 66, 1813–1824.
Gitonga, L.N., Gichuki, S.T., Ngamau, K., Muigai, A.W.T., Kahangi, E.M., Wasilwa, L.A., Wepukhulu, S., Njogu, N., 2010. Effect of explant type, source and genotype on in vitro shoot regeneration in Macadamia (*Macadamia* spp.). J. Agric. Biotechnol. Sustain. Dev. 2, 129–135.
Guzzon, F., Müller, J.V., 2016. Current availability of seed material of enset (*Ensete ventricosum*, Musaceae) and its Sub-Saharan wild relatives. Genet. Resour. Crop Evol. 63, 185–191.
Haile, B., Mohammed, A., Woldegiorgis, G., 2015. Effect of planting date on growth and tuber yield of potato (*Solanum tuberosium* L.) varieties at Anderacha District, southwestern Ethiopia. Int. J. Res. Agric. Sci. 2 (6), 2348–3997.
Hajjar, R., Hodgkin, T., 2007. The use of wild relatives in crop improvement: a survey of developments over the last 20 years. Euphytica 156, 1–13.
Harada, M., Oda, G., Mori, M., Ikeda, H., 2005. Mass regeneration of shoots from cut surface of stems in tomato stock plants. J. Jpn. Soc. Hortic. Sci. 74, 479–481.
Harlan, J.R., 1971. Agricultural origins: centers and non-centers. Science 174, 468–474.
Hartman, H.T., Kester, D.E., Davies, F.T., Geneve, R.L., 1997. Plant Propagation: Principles and Practices. 6thed. Prentice-Hall.
Hartman, H.T., KesterDE, Davies, F.T., Geneve, R., 2010. Hartmann & Kester's Plant Propagation: Principle and Practices, eighth ed. Prentice-Hall, Englewood Cliffs, NJ, USA.
Hartmann, H.T., Kester, D.E., 1983. Plant Propagation; Principles and Practices, sixth ed. Prentice-Hall, Englewood Cliffs, New Jersey, p. 727.
Heywood, V., Casas, A., Ford-Lloyd, B., Kell, S., Maxted, N., 2007. Conservation and sustainable use of crop wild relatives. Agric. Ecosyst. Environ. 121, 245–255.
Houmani, H., Rodriguez-Ruiz, M., Palma, J.M., Corpas, F.J., 2018. Mechanical wounding promotes local and long distance response in the halophyte Cakilemaritima through the involvement of the ROS and RNS metabolism. Nitric Oxide Biol. Chem. 74, 93–101.
Ikeuchi, M., Iwase, A., Rymen, B., Lambolez, A., Kojima, M., Takebayashi, Y., Heyman, J., Watanabe, S., Seo, M., De Veylder, L., et al., 2017. Wounding triggers callus formation via dynamic hormonal and transcriptional changes. Plant Physiol. 175, 1158–1174.
Jiménez, V.M., 2001. Regulation of in vitro somatic embryogenesis with emphasis on the role of endogenous hormones: Review. Rev. Bras. Fisiol. Vegetal 13 (2), 196–223.
Karlsson, L.M., Dalbato, A.L., Tamado, T., Mikias, Y., 2015. Effect of cultivar, traditional corm pretreatment and watering on sprouting and early growth of *Ensete ventricosum* suckers. Exp. Agric. 51, 232–243.
Karlsson, L.M., Tamado, T., Dalbato, A.L., Mikias, Y., 2013. Seed morphology and germination of *Ensete ventricosum* (Musaceae). Seed Sci. Technol. 41, 357–370.
Kojima, K., Ohtake, E., Yu, Z., 2002. Distribution and transport of IAA in tomato plants. Plant Growth Regul. 37, 249–254.
Li, Q., Zhao, Y., Xiang, X., Chen, J., Rong, J., 2019. Genetic diversity of crop wild relatives under threat in yangtze river basin: call for enhanced in situ conservation and utilization. Mol. Plant 12, 1535–1538.
Moat, J.F., Tadesse, W., Davis, A.P., Gole, 2019. Least concern to endangered: applying climate change projections profoundly influences the extinction risk assessment for wild Arabica coffee. Global Change Biol. 25, 390–403.
Myers, J.A., Harms, K.E., 2009. Seed arrival, ecological filters, and plant species richness: a meta-analysis. Ecol. Lett. 12, 1250–1260.
Nathan, R., Muller-Landau, H.C., 2000. Spatial patterns of seed dispersal, their determinants and consequences for recruitment. Trends Ecol. Evol. 15, 278–285.
Olango, T.M., Tesfaye, B., Catellani, M., Enrico Pè, M., 2015. Development of SSR markers and genetic diversity analysis in enset (*Ensete ventricosum* (Welw.) Cheesman), an orphan food security crop from Southern Ethiopia. BMC Genet. 16 (98), 1–16.
Olango, T.M., Tesfaye, B., Catellani, M., Pe`, M.E., 2014. Indigenous knowledge, use and on-farm management of enset (*Ensete ventricosum* (Welw.) Cheesman) diversity in Wolaita, Southern Ethiopia. J. Ethnobiol. Ethnomed. 10, 41.
Reusing, M., 2000. Change detection of natural high forests in Ethiopia using remote sensing and GIS techniques. Int. Arch. Photogram. Rem. Sens. 1253–1258. XXXIII.
Rossel, G., 1998. Taxonomic-linguistic study of plantain in Africa. Res. School CNWS 65, 277.
Sang, Y.L., Cheng, Z.J., Zhang, X.S., 2018. Plant stem cells and de novo organogenesis. New Phytol. 218, 1334–1339.
SAS Institute Inc, 2008. SAS/STAT. 9.2 User's Guide. Cary, NC: SAS Institute Inc., USA.
Schaller, G.E., Bishopp, A., Kieber, J.J., 2015. The yin-yang of hormones: cytokinin and auxin interactions in plant development. Plant Cell 27, 44–63.
Shigeta, M., 1990. Folk in-situ conservation of Ensete. Towards the interpretation of indigenous agricultural science of the Ari, south-western Ethiopia. Afr. Stud. Monogr. 10 (3), 93–107.
Shigeta, M., 1996. Creating landrace diversity: the case of the Ari people and Ensete (*Ensete ventricosum*) in Ethiopia. In: Ellen, R.K.F. (Ed.), Redefining Nature, 1996. Berg: Berg, Oxford, pp. 233–268.
Simberloff, D., 2009. The role of propagule pressure in biological invasions. Annu. Rev. Ecol. Evol. Syst. 40, 81–102.
Simoons, J.F., 1965. Some questions on the Economic prehistory of Ethiopia. J. Afr. Hist. 6 (1), 1–13.
Tanksley, S.D., McCouch, S.R., 1997. Seed banks and molecular maps: unlocking genetic potential from the wild. Science 277, 1063–1066. PMID: 9262467.
Tesfamicael, K.G., Gebre, K., March, T.J., Sznajder, B., Mather, D.E., López, C.M.R., 2020. Accumulation of mutations in genes associated with sexual reproduction contributed to the domestication of a vegetatively propagated staple crop, enset. Hortic. Res. 7 (185), 10, 10.
Tesfaye, B., Ludders, P., 2002. Diversity and distribution patterns of enset landraces in Sidama, Southern Ethiopia. Genet. Resour. Crop Evol. 50, 359–371.
Tsegaye, A., Struik, P.C., 2002. Analysis of enset (*Ensete ventricosum*) indigenous production methods and farm based biodiversity in major enset growing regions of Southern Ethiopia. Exp. Agric. 38, 292–315.
Tsegaye, A., 2002. On Indigenous Production, Genetic Diversity and Crop Ecology of Enset (*Ensete ventricosum*). PhD Thesis. Wageningen University, The Netherlands, p. 198.
Vallejo-Marín, M., O'Brien, H.E., 2007. The paradox of clonality and the evolution of self-incompatibility. New Phytol. 173, 415–421.
Vavilov, N.I., 1951. The origin, variation, immunity, and breeding of cultivated plants. Chron. Bot. 13, 1–366.
Wakjira, D.T., Gole, T.W., Senbeta, F., 2008. Deforestation of afromontane rainforests in Ethiopia. Deforest. Res. Prog. 19–39.
Xu, L., 2018. *De novo* root regeneration from leaf explants: wounding, auxin, and cell fate transition. Curr. Opin. Plant Biol. 41, 39–45.
Zinabu, D., Gebre, E., Asfere, Y., 2021. Genotype and plant growth hormone interaction effect on in vitro propagation of early maturing enset (*Ensete ventricosum* (Welw.) Cheesman) cultivars. Plant Cell Biotechnol. Mol. Biol. 22 (19&20), 89–102.
Zobel, M., Moora, M., Herben, T., 2010. Clonal mobility and its implications for spatio-temporal patterns of plant communities: what do we need to know next? Oikos 119, 802–806.