Original Research Article||

In Vivo Induced Sucker Regeneration Efficiency of Enset (*Ensete ventricosum* (Welw.) Cheesman) Landraces Corm Splits Grown under Lath House in Hawassa, Ethiopia

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Abstract

Enset is a vegetatively propagated, drought-tolerant food and income security crop in Ethiopia. However, studies on optimized, economically viable and quick enset propagation methods are limited. This experiment was aimed to explore the in vivo-induced sucker regeneration efficiency of enset landraces corm splits grown under a lath house. Six landraces, namely 'Ado,' 'Astara,' 'Ganticha,' 'Keshicha,' 'Kulle,' and 'Midasho,' were selected. The parent corms were uprooted, and apical buds were removed. The corms were then split into eighths and sun-exposed for 48 hours to heal the cut wounds. The experiment was arranged in a completely randomized design (CRD) with three replications over two years, and the corms were buried in a soil media mixture until sucker harvest. Biometric parameters such as days to 50% emergence, regeneration percentage, and number of suckers per corm, green leaf number, leaf length, leaf width, pseudostem height, pseudostem circumference, and sucker height were recorded. A combined analysis of variance (ANOVA) was conducted using the SAS statistical program, Version 9.4, after normality and homogeneity of variance tests were conducted. All parameters evaluated were significantly ($p < 0.001$) affected by the variation in enset landraces. The landraces 'Midasho' and 'Ado' had the earliest (49.9 days) and longest (82.46 days) days to 50% emergence, respectively. The highest number of suckers (45) per corm split and per whole corm (360) were obtained for the landrace 'Midasho,' while the lowest number of suckers were 9.87 and 78.96 per corm split and whole corm, respectively, for the landrace 'Ado.' The use of an eighth parent corm split in vivo induces sucker regeneration under the lath house technique, providing large quantities of planting material with genotype purity efficiently in a shorter time compared to traditional propagation methods, which typically produce 40 to 200 suckers per mother corm.

Key words: corm split, enset landraces, propagation, sucker number

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INTRODUCTION

Enset (*Ensete ventricosum* (Welw.) Cheesman) is a multipurpose, drought-tolerant, energy-rich crop cultivated for its underground corm and pseudostem processed into starchy and storable food products (Borrell et al., 2019). The crop is a diploid (2n = 18) herbaceous monocot perennial plant in the family Musaceae and the order Zingiberales (Cheesman, 1947). It looks a lot like the banana, which is a close relative (Blomme et al., 2018). The cultivation of enset only occurs in the central, southern, and southwestern highlands of Ethiopia, where it is a staple food of a quarter (20%) of the Ethiopian population, or ~24 million people (Benuzeh and Feleke, 1966; Blomme et al., 2023). It is cultivated as the main crop of a sustainable indigenous African system (Brandt et al., 1997) that is used as food, fiber, feed, construction materials, packaging material, and traditional medicine, as well as a source of income (Azerefegne et al., 2009; Blomme et al., 2023). Generally, the ecological coverage of enset is from 1200 to 3100 meters above sea level (m.a.s.l.), but grows best at altitudes between 2000 and 2750 meters above sea level (m.a.s.l.). However, the wild cultivar grows at a range of 1200–1600 m.a.s.l., which is a relatively narrow range (Brandt et al., 1997; Zippel and Lüdders, 2003). The optimum monthly average temperature for enset growth ranges from 16 ºC up to 20 ºC (Tsegaye and Struik,

2002) and it also requires an optimum of 63–80% relative humidity (Zengele, 2017). Most enset-growing areas receive well-distributed annual rainfall of about 1,100 to 1,500 mm (Brandt et al., 1997). The crop prefers slightly acidic to alkaline soil pH of 5.6–7.3, well-drained, fertile acrisols and nitosols (Tsegaye and Struik, 2002). The genetic structure of enset is mainly shaped by eco-geographic factors, mode of propagation, and cultivation status (Haile et al., 2024). However, its cultivation is characterized by a wide variety of landraces, adapted to varying agro-ecological conditions and with multiple uses by households (Blomme et al., 2023). Farmers make efforts to increase their enset plantation using their indigenous knowledge and methods to grow, harvest, and introduce new landraces (Pijls et al., 1995; Zippel and Lüdders, 2004).

Enset cultivar diversity maintenance significantly contributes to food and livelihood security. Wealthier households tend to have more land to grow more enset landraces compared to households with small landholding. For example, farmers in Sidama maintain more than 72 landraces (Negash, 2001; Haile et al., 2024). Nevertheless, several useful enset genotypes have been lost due to various factors such as biotic factors like genetic degradation, bacterial wilt diseases, root lesion nematodes, and pests (Yemataw et al., 2018; Kidane et al., 2021). Similarly, various abiotic elements, like severe drought and low soil fertility also cause loss of enset genotypes. On the other hand, farmers' selection pressures prioritizing certain clones, human population growth-associated pressures, the introduction of commercial crops and instability in socio-political events, change in land use systems, as well as labor constraints were also reported to contribute (Gebremaryam, 1996; Tsegaye and Struik, 2002; Guzzon and Müller, 2016; Yemataw et al., 2018; Kidane et al., 2021; Feleke and Tekalign, 2022); these factors inhibit the diversity and variability of the crops (Yeshitila et al., 2011).

Farmers in enset-growing regions of the country implement diverse propagation methods (Zippel and Lüdders, 2004). At the farm level, cultivated enset is most commonly propagated traditionally using vegetative multiplication (macro-propagation) methods with adventitious bud sprouting from the entire corm or corm pieces after apical meristem removal (Tesfaye, 2002; Diro et al., 2002; Yemataw et al., 2018). This method is developed by farmers intending to guarantee enset clonal propagation and could be named in vivo-induced shoot regeneration (Tesfaye 2002; Haile et al. 2021). The technique helps to preserve the characteristics of the landraces, gives rise to offspring that are true to type and genetically identical to their parent (Zippel and Lüdders, 2003), and also provides a high number of plants (Zippel and Lüdders, 2004). Currently, this technique serves to provide the needed suckers at the farm, village, or landscape level (Yemataw et al., 2018). At the altitudinal margins of enset cultivation, high specialization in propagation techniques is found (Zippel and Ludders, 2002). Farmers improve their enset plantation by introducing new landraces; with selections determined by adaptations to climate and palatability (Zippel and Lüdders, 2003). For propagation purposes, immature plants of 2 to 4 years old corms with a 10–35 cm diameter are preferred for the production of suckers (Bezuneh and Feleke, 1966; Yemataw et al., 2018). Mostly, farmers cut down the pseudostem at 10–30 cm above the ground (Diro et al., 1996; Blomme et al., 2018). The corm is then uprooted and the apical meristem is removed, after which the corm may be exposed to sunlight for a few days in order to heal the cut injuries of corm split surfaces (Zippel and Lüdders, 2004; Yemataw et al., 2018). When the apical meristem of a corm is removed and the corm is buried in a loosened soil, numerous shoots emerge from the corm surface (Tsegaye, 2002). This method of vegetative propagation, using buried disease-free corms in the field yields a large amount of healthy and vigorous suckers ideally replanted 9 months after corm burial (Karlsson et al., 2015). Between 4 and 12 weeks, suckers will emerge (Negash, 2001). Farmers usually obtain 20 to 100 suckers per mother corm using conventional methods (Brandt et al., 1997). Some researchers made an effort to assess traditional propagation practices carried out by farmers and reported generally 6-200 suckers produced per mother corm, depending on soil conditions, cultivar type, size and age of the parent plant, amount of rainfall, land preparation, and time of planting (Diro et al., 2002; Negash, 2001; Shumbulo et al., 2012; Karlsson et al., 2015; Yemataw et al., 2018). Tabogie and Diro (1992) also reported an average of 22, 76, and 102 suckers' emerging from whole, half, and quarter corms, respectively. Investigations indicated that 70% of enset landraces produce more than 40 suckers per mother corm (Diro et al., 1996). Farmers produced about 100 suckers from 5 corms at a spacing of 1 × 1 m (Yemataw et al., 2018). Similarly, Diro et al. (2003) indicated that corm splitting gives many small suckers. The highest rate of suckering (94 ± 14 per corm) was obtained from quarter corms prepared by cutting the pseudo-stem at the junction point (collar) (Haile et al., 2021). Suckers obtained from split corms exhibit a lower rate of failure and emerge earlier; which could be linked with more vigorous growth (Diro et al., 1996; Karlsson et al., 2015). In Wolaita area, it was claimed that splitting the corm into four equal parts would produce a large number of suckers (Tsegaye and Struik, 2002). Enset farmers have exceptional knowledge of this crop including farming system, propagation, transplanting, harvesting, and protection from pests and diseases (Garedew et al., 2017).

The planting materials (suckers) produced by farmers, is sold in local markets and can be used as a source of additional income for farmers (Olango et al., 2014). There are very few reports describing enset sucker markets and movement of suckers (Yemataw et al., 2018). Large-scale farmers residing in Hagereselam area of Sidama region propagate enset suckers in their farmlands for commercial purposes in the locality (Egziabher et al., 2020). The production of enset suckers is also the main source of cash income in some areas of Sidama, and hence sucker markets are widely practiced (Woldetensaye, 1997).

Farmers engaged in traditional propagation face several constraints, such as climate change and lack of appropriate planting materials due to accumulation of pests and diseases. Thus, farmers are forced to use raw material for propagation (Yemataw et al., 2018); which requires extended time of 3-5 years to produce corm and low multiplication rate (formation of suckers per corm per year ≤ 10/15 suckers), and less tolerant to drought (Diro and Tabogie, 1992). Besides, different in vitro culture techniques, such as zygotic embryo culture, shoot tip culture, and callus cultures, as well as somatic embryogenesis as methods of propagation have been documented. Research results demonstrated that more than 100 plantlets were generated in 4 months from corm discs isolated from a single in vitro mother plantlet (Tripathi et al., 2017). Birmeta and Welander (2004) reported about 75 shoot buds per explant in 14 weeks from one subculture. Likewise, Negash et al. (2000) obtained 31 plantlets per corm in 16 weeks. Konobo (2014) also reported 2–15 shoots for different enset cultivars using shoot tip explants.

These technologies are useful to provide large numbers of replacement plants rapidly where diseases have reduced plant populations or to locally multiply desired cultivars for distribution (Diro and Van Staden, 2004). The culture enables conservation, rapid propagation, and distribution of clean planting materials (Negash, 2001). However, in vitro propagation of enset is mostly challenged by the presence of extensive blackening, necrosis, and unwanted callus formations (Diro and Van Staden, 2004; Disasa and Diro, 2012). Macro- and micro-propagations of enset are useful technologies to improve sucker production efficiency to provide clean plants and multiply newly introduced cultivars for distribution (Yemataw et al., 2018).

It is very vital to explore propagation methods that help to increase propagation rates of enset landraces but not resource intensive, in terms of space and labor requirements (Yemataw et al., 2018). Consequently, recommendations must be fact-based, reliable, and beneficial for the user (Blackstock et al., 2010). In other words, the conventional system of propagation and production is inefficient to develop acceptable-quality planting materials in short periods of time under different environments.

Hence, selecting the most suitable landraces and corm split types that yield high-quantity and quality suckers for diverse utilization to the user, is essential in order to improve the efficiency of production of enset planting materials. However, research attention given to enset propagation is limited; and hence improvement of propagation techniques have not yet been sufficiently explored. Most previous studies and farmers practices mainly focused on whole, half, and a maximum of quarter corm splits. Conversely, corm splits of more than a quarter split size, or else double quarters have not been evaluated. Even so, there are no reports and experimental evidence that substantiate the response of different enset landraces parent corms to more than a quarter-splitting under controlled environments. We hypothesize that enset sucker production would be enhanced by incorporating variable landraces and increasing corm splits to more than a quarter or doubling a quarter to step up production and improve the traditional propagation method.

Therefore, this study was initiated to investigate double quarter splits, otherwise known as eighth splits, on regeneration performance and sucker proliferation potential of qualitative phenotypes of different enset landraces under lath house conditions.

MATERIALS AND METHODS

Description of the study area

In general field propagation of enset in Sidama, practical ways in February and March regardless of altitude but most farmers at low altitudes complained about quality of sprouts and they often bought all from highland farmers (Zippel and Lüdders, 2002). However, this lath house two years (June, 2020 - 2022) experiment was conducted in Hawassa University, College of Agriculture campus owned lath house. The lath house experiment was conducted in lath house nursery bed made up of wooden box field with soil media mix. Hawassa, especially the lath house is located between 07º05.5'7.2" N latitude and 38º47'27.2" E longitude in the northern tip of Sidama regional state capital at an altitude of 1688 meters above sea-level (masl), situated 275 km away from Addis Ababa, Ethiopia. The area has moist to humid, warm subtropical climate and receives 1000 to 1800 mm mean annual rainfall. The mean monthly temperature of Hawassa is in the range of 15 to 20ºC. Soil and manure characteristics analysis were conducted at Hawassa University, Soil Testing Laboratory. The climatic data were obtained from the National Meteorology Agency of Ethiopia.

Treatments, Experimental Design, and Media Mix Preparation

The two-year repeated lath house experiment was designed to examine the sucker proliferation potential of enset landraces and eighth corm splits in vivo macro propagation. A completely randomized design with three replications was used. The soil media mixtures contained an equal proportion of sand, sawdust, topsoil, dry cow dung as manure, and forest soil in a 1:1:1:1 ratio to provide balanced nutrients for good sucker growth. The media mix was sterilized using the solarization method by covering it with a transparent plastic sheet for two weeks and tested for its chemical composition (Table 1). Each bed was divided into nine partitions, and the center was pegged and arranged in a randomized manner in the lath house. Two experimental beds (Figure 3d) were prepared from wood with dimensions of 3m by 3m and a height of 30 cm, capable of accommodating all eighteen enset landraces, each cut into eighth corm splits to generate additional competitive information compared to whole, half, and quarter corm splits. Sidama and Ari area farmers produce suckers using either split corm or whole corm preparation (Diro et al., 1996; Tsegaye and Struik, 2002). In this study, a total of 144 corm splits were prepared for the eighth corm splits, enough to replicate each landrace three times. The beds were filled with the prepared soil media mix. Farmers produced ± 100 suckers from a 5 m² nursery with 5 corms at a spacing of 1 × 1 m (Yemataw et al., 2018). Adapting farmers' nursery spacing reported by Yemataw et al. (2018), spacing was demarcated as 1m between plantings with 9 holes in each bed, capable of accommodating all eight splits per hole (Figure 3e), was considered, resulting in a net bed size of 9 m² per bed. The characteristics of the prepared soil media mix were analyzed at Hawassa University, College of Agriculture, School of Plant and Horticultural Sciences' Soil Testing Laboratory (Table 1)

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Table 1. Characteristics of analyzed soil media mix used in lath house for enset landraces corm split in vivo macro propagation.

Chemical composition	pH	Organic C (%)	Available P (mg Kg^{-1})	Available K (mg Kg^{-1})	Total N (%)	Exchangeable Ca (mg Kg^{-1})	Electrical Conductivity (ms m^{-1})
Soil media mix	7.18	2.9	4,8	11.4	0.21	26	9.55
Manure	7.4	4.01	39.0	117.8	0.33	1230	0.18

Experimental Enset Plant Selection

Enset landraces used for this lath house experiment were selected based on farmer-generated classification priorities for cultivar choice, focusing on quality and quantity of food yield, drought tolerance, and disease resistance, which are important criteria for selecting enset clones (Endale et al., 2003). The selection process also considered ecological adaptation, growth rate, maturity, fiber quality and quantity, ease of decortication, corm size, post-cooking taste, fodder quality, and medicinal aspects (Negash, 2001; Tsegaye and Struik, 2002; Yemataw et al., 2014). Morphological characteristics were assessed following the procedures outlined by Yemataw et al. (2018), using qualitative phenotype traits such as upper-side (adaxial) midrib color, under-side (abaxial) midrib color, upper-side petiole color, under-side petiole color, leaf lamina color, and leaf tip edge color (Figure 1) to identify the landraces for this research. The selected landraces (Figure 1) locally known as 'Ado', 'Astara', 'Ganticha', 'Keshicha', 'Kulle', and 'Midasho' were used as parent plants, each being 3 years old based on farmers' experience and previous experimental evidence on the effect of corm age on sucker yield (Yemataw et al., 2014).

Figure 1. Qualitative phenotype of different enset landraces selected for *In-vivo* macro propagation).

Corms from immature enset plants at vegetative stage, aged between 2 and 4 years, are preferred for sucker production (Bezuneh and Feleke, 1966; Negash, 2001; Yemataw et al., 2014). The selected plants for this research were three years old (the average of 2-4 years), popular in the study area, well-known for their use, tolerance to drought, propagation capacity, and marketability of suckers in Hawassa and the entire Sidama area (Figure 2). Sidama and Ari area farmers produce suckers using either split corm or whole corm (Diro et al., 1996; Tsegaye and Struik, 2002). The first suckers started emerging at 50 days after corm burial. Time to sucker emergence was longer for entire corms than for split corms, while a higher number of suckers were obtained per corm when it was split. Less than 60 suckers were recorded for landraces with entire corms, while between 60 and 140 suckers were most often recorded per corm when corms were split into two or four pieces, respectively (Yemataw et al., 2018). Depending on soil conditions, cultivar type, size and age of mother plant, amount of rainfall, land preparation, and time of planting, the number of suckers produced ranges between 40 and 200 per corm (Shambulo et al., 2012).

Figure 2. Different enset landraces sucker market in kebado town, Dara Woreda, Sidama Region, Ethiopia

Despite the high demand and potential for enset production in various agro-ecological zones, farmers face constraints related to market information, limited government support, and market access (Yemataw et al., 2018). The two-year repeated experiments involved three enset parent plants representing each landrace, totaling thirty-six enset parent plants purchased and used for the study.

Experimental Procedures

The method and procedures used for this propagation experiment were fully adopted from indigenous knowledge of farmers on vegetative propagation practices. Parent corms of all eighteen enset plants from six morphologically different enset landraces were uprooted. Subsequently, their pseudostems were cut off following farmers' practices reported by Diro et al. (1996) at a height of 10-15 cm above the corm junction, just above the collar point to the corm. This helps to ensure the apical meristem's visibility and to avoid removing a large portion of the tissues around the apical meristem that can give rise to numerous suckers. This method was based on the technique reported by Diro et al. (1996) and took into account farmers' experience. Lastly, the corms were washed to remove dirt, and the roots of the plants were trimmed off. The apical bud of all landraces was removed during the day of harvest on June 6, 2020.

Corm splitting was done using a large machete, sharp at the point and along both edges, following traditional practice (Pijls et al., 1995). The clean corms of the parent plants were carefully split into double quarters or eight pieces using a sharp machete (Figure 3c). A total of 144 corm splits were prepared for a one-year experiment, which was then doubled for the second year of study. Subsequently, six groups of corm splits (24 corm pieces from each landrace of three plants as replication corms) were prepared and grouped separately.

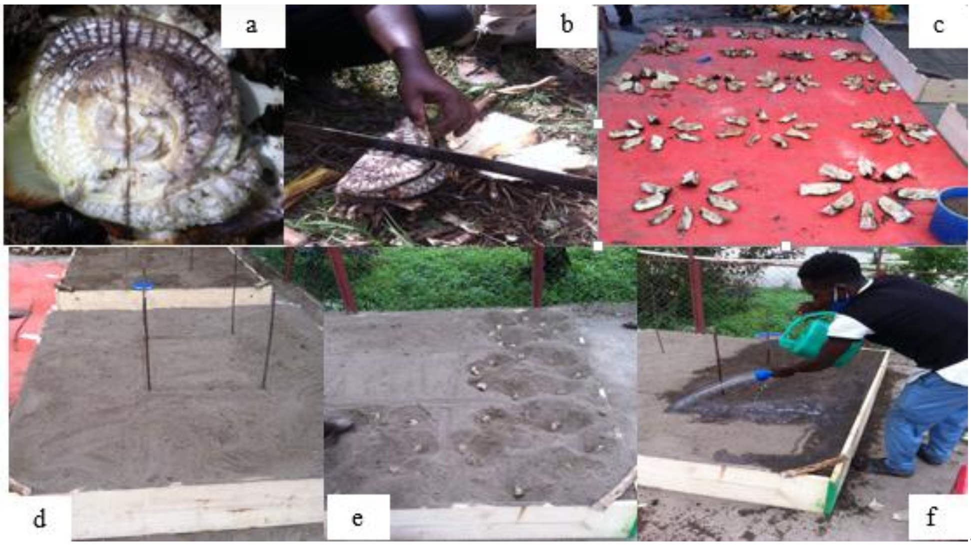

Figure 3. Enset landraces corm splits in-vivo macro propagation methods under a lath house.
(a) Removal of meristematic tissue. (b) Corm split preparation using a sharp machete. (c) Corm splits exposed to diffused light for 48 hours under a lath house. (d) Prepared soil media mix filled in a wooden box and stacked. (e) Corm split burial in a circular technique. (f) Watering of the buried corm splits

The corm splits were handled as a separate treatment (Figure 3c) to maintain the landrace corm split mix-up process. The corm pieces were left in the sun under a lath house shade for 2 days (48 hours) before burying (Figure 3e) to heal the cut wounds.

Corm Split Burying and Management

After the completion of corm piece preparation solar pretreatment, every selected landrace plant corm splits in 18 groups with six corm pieces each were randomly assigned to both two beds in the lath house separately. On June 08, 2020, corm splits were buried in soil media mix on a wood box, with 1m between the centers of two neighboring holes. Subsequently, all experimental plants (each eight corm pieces or double quarter) were buried in the same hole in a circular manner without contact with each other, representing each plant corm (Figure 3e). Following the protocol developed by Diro and Tsegaye (2012), holes were refilled after corm splits were buried with 10 cm of soil media mix, and the beds were watered with enough water (the loosened soil media mix was dry during preparation) (Figure 3f).

Consequently, the presence of newly emerging young suckers was observed carefully, and the days of newly emerging suckers were recorded separately for each sucker that survived by consuming the food stored in the corm at an early stage. The mineralization rate is high in warm environments in Ethiopia; in such cases, manure supply to plants is beneficial as a source of nutrients and also adds organic matter to the soil, improving soil texture and water-holding capacity (Bayu et al., 2006).

Composted 36 kg of air-dried pulverized cow manure per all beds (2kg/m^2) or 2.0 kg per individual buried parent corm were applied. The split applications method was used by dividing the whole share into three, and it was practiced every third month on the surface of the media mixture in liquid form by diluting with 1/3rd water in a container and then applied over the suckers as liquid organic fertilizer using a watering can after the soil media mixture softened. The experimental corm split suckers were visited daily, and weeding and other cultural practices were done when needed. Sprouting was recorded every day after sucker development. Soil softening during the growth period was carried out, and other enset pest management practices were applied for each treatment under the lath house in this experiment.

Later, each sucker was carefully evaluated and detached from the parent corm split. Parameters such as sucker height, pseudostem height, and circumferences were recorded separately. Data collection began from the first date of the first emergence of sucker sprout after corm pieces burial and continued at 15-day intervals until the 11th month. Lastly, suckers were evaluated for their market maturity stage and then harvested for the preparation of the second experiment repetition. The final data were used for analysis and comparison of corm split sucker proliferation potential. The characteristics of suckers recorded were: days to 50% of the sucker emergence, number of suckers per split, percentage of regeneration (calculated considering the number of corm pieces regenerated divided by the number of corm pieces buried representing each landrace and multiplied by 100), the leaf number of more than 50% green, leaf length, leaf width of the broadest leaf, pseudostem height, sucker height, were measured using a measuring tape, and pseudostem circumferences were measured using a vernier caliper from four randomly selected suckers of each landrace replication.

Data Analysis

In this two-year study, the efficiency of in vivo induced sucker regeneration in Enset (*Ensete ventricosum*) landraces grown in a lath house was evaluated. Data from the study underwent normality and homogeneity of variance tests using the Shapiro-Wilk test and Levene's test, respectively. The data were found to be normally distributed and the variances were homogeneous. An analysis of variance (ANOVA) was conducted on the combined data from the two years to evaluate the significance of variation among treatments using SAS statistical software version 9.4 (SAS, 2022). Since there was no control in the lath house experiment, Tukey's HSD Post-Hoc test was used for mean separation at a 5% significance level. The results were compared with farmers' practices to demonstrate the effectiveness of the method. Graphs and tables were created using MS-Excel.

RESULTS

In vivo macro propagation of qualitative phenotype varying enset landraces corm splits in to 8th had shown a highly significant ($p<0.001$) variation in mean days to 50% emergence, mean number of suckers, mean regeneration percentage, mean green leaf number, mean sucker height, mean leaf width and length, mean psudostem length and circumference (Figure 5).

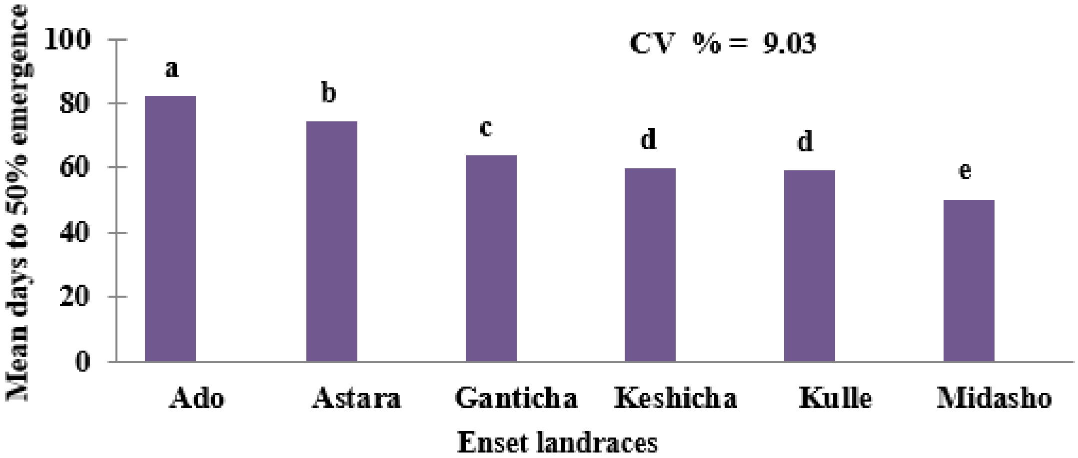

Figure 4. Effect of enset (*Ensete ventricosum* (Welw.) Cheesman) landraces parent corm splits *in vivo* macro propagation on mean days to 50% emergence. Mean differences of bars with the same letter(s) are statistically non-significant and different letters represent significant differences at $p < 0.05$.

Highly significantly ($p < 0.001$) earlier days to emergence (49.9 days) was recorded for landrace 'Midasho' obtained parent plant corm split followed by 'Kulle' (59.29 days). Whereas, significantly delayed (up to 82.46 days) days to 50% emergence was recorded for landrace 'Ado' 3-years-old parent corm split(Figure 4).

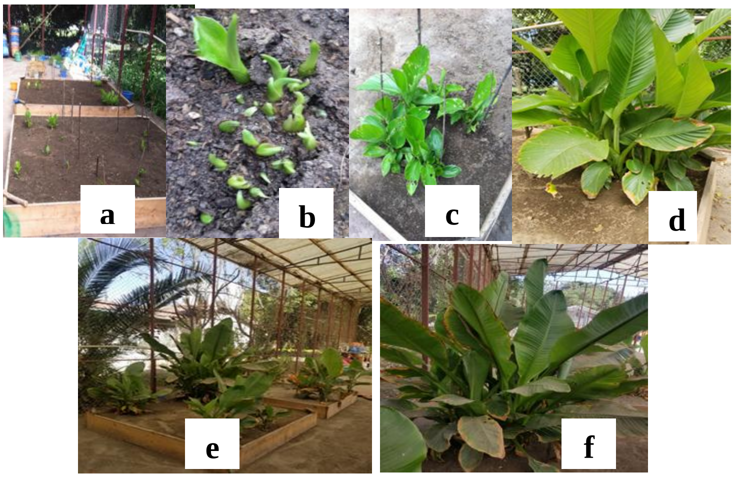

Figure 5. Enset landraces corm splits in-vivo macro propagation sucker growth under lath house. (a) First 50% sucker emergence on bed, (b) Sucker sprouting at 50 days, (c) Sucker growth after 86 days, (d) Sucker development on the Soil media mix, (e) Enset corm splits grown sucker differences at 9th month, and (f) Landrace 'Kulle' sucker growth under lath house.

In our present in vivo corm split into 1/8th macro propagation under lath house experiment, we have observed very highly significant ($p< 0.001$) variation in percentage of enset landraces corm split regenerated sucker between (82.41% to 92. 22%).

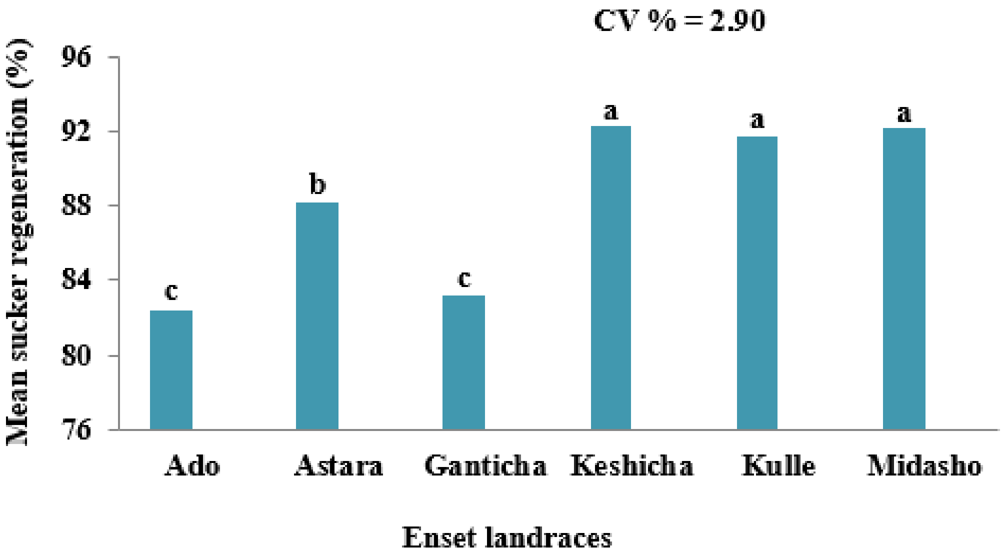

Figure 6. Effect of enset (*Ensete ventricosum* (Welw.) Cheesman) landraces parent corm splits *in vivo* macro propagation on mean sucker regeneration percentage. Mean differences of each bar with similar letter(s) are statistically non-significant and bars with different letters represent significant differences at *p* < 0.05.

All the landraces in this in vivo macro propagation experiments achieved less than 100% regeneration percentage. The results revealed that enset landraces' Keshicha',' Midasho', and' Kulle' gave significantly highest (92.22, 92.10 and 91.72) regeneration percentages respectively. As demonstrated in (Figure

6), the lowest (82.41) regeneration percentage being recorded for landrace 'Ado' sucker regeneration than other landraces assessed in this experiment.

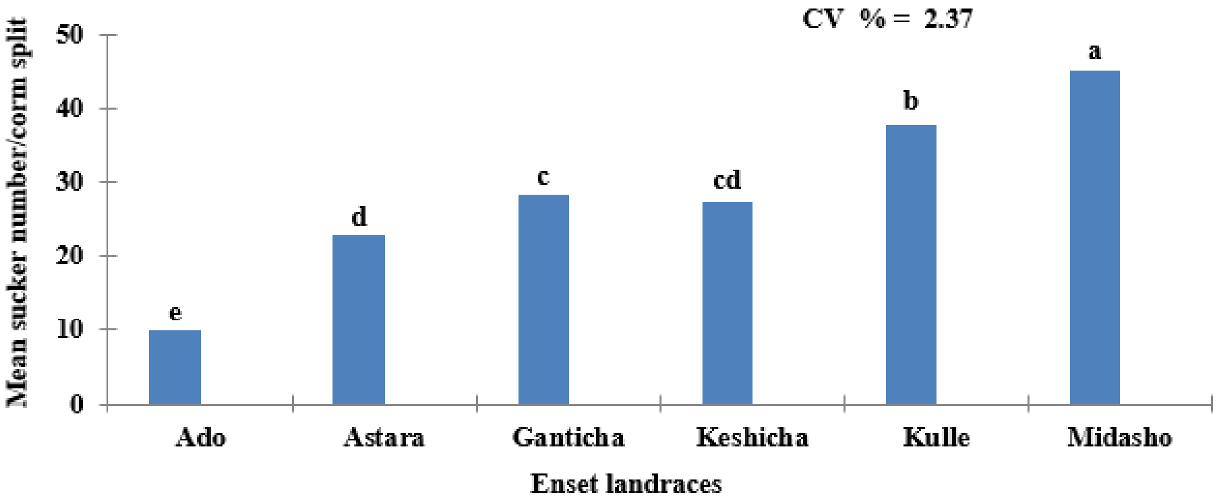

Figure 7. Effect of enset (*Ensete ventricosum* (welw.) Cheesman) landraces parent corm splits *in vivo* macro propagation on mean sucker number/corm split. Mean values of each bar with the same letter(s) are statistically non-significantly different and bars with different letter(s) are statistically significantly different at $p < 0.05$

On the other hand, very highly significant ($p < 0.0001$) variation among enset landraces in mean number of suckers per corm split was observed (Figure 7). The highest number of sucker (45) was recorded on landrace 'Midasho' parent corm1/8th split, followed by landrace 'Kulle' which gave (37.75) which is equivalent to (360 and 300 suckers) per corm for landrace 'Midasho' and 'Kulle' respectively. The lowest mean sucker number per corm split (9.87) was recorded on landrace 'Ado' which is also comparable to (78.96) suckers obtained per corm.

Furthermore, in this double quarter (an eighth) corm split in vivo macro propagation, very highly significant ($p<0.0001$) variation was observed between enset landraces corm split developed suckers leaf width (Table 2). The highest mean leaf width (23.69 cm) was recorded for landrace 'Ganticha'. Statistically significantly narrowest leaf widths (19.18, 19.40, and 19.65 cm) were recorded for landrace 'Kulle', 'Midasho', and 'Keshicha') respectively.

Enset landraces parent an eighth corm split had very highly significant ($p<.0001$) effect on mean number of leaf per parent corm split of regenerated suckers (Table 2). Considering this, there are variations among different landraces in mean leaf number and significantly highest mean leaf number (5.24) was observed for landrace 'Midasho' suckers. The lowest leaf number (3.86) was recorded from landrace 'Astara' (Table 2). There were very highly significant ($p<.0001$) versions in mean leaf length on suckers developed from double quarter split corms. The longest mean leaf lengths values (96.77cm) were scored by landrace 'Ganticha' parent corm split developed sucker (Table 2). The shortest mean leaf length (57.07 cm) was recorded for landrace 'Ado'. In this enset landraces in vivo macro propagation using parent corm split in to 1/8th showed significant ($p<0.007$) effect on mean pseudostem circumference for regenerated suckers (Table 2). The highest mean pseudostem circumference (7.83 cm) was recorded for landrace 'Ado'. The lowest pseudostem circumference (5.86 cm) was recorded on sucker developed from the landrace 'Ganticha' (Table 2).

Table 2. Effect of enset (*Ensete ventricosum* (Welw.) Cheesman) landraces parent corm split in *in-vivo* macro propagation on growth performance of sucker parameters

Landrace Name	Growth performance parameters					
	LeL	LeW	LeN	PsC	PlH	PsH
Ado	57.07^{e}	21.87^{b}	4.52b^{c}	7.83^{a}	69.63^{c}	23.73^{c}
Astara	63.36^{d}	13.82^{d}	3.86^{c}	6.59^{bc}	66.25^{c}	25.66^{bc}
Ganticha	96.77^{a}	23.69^{a}	5.09^{ab}	5.86^{c}	83.70^{b}	26.24^{bc}
Keshicha	80.77^{c}	19.65^{c}	4.72^{bc}	6.88^{ab}	69.67^{c}	29.09^{b}
Kulle	83.88^{b}	19.18^{c}	4.86^{ab}	6.27^{bc}	102.07^{a}	34.09^{a}
Midasho	82.47^{bc}	19.40^{c}	5.24^{a}	6.64^{bc}	99.94^{a}	34.49^{a}
Over all Mean	77.39	19.60	4.71	6.68	81.88	28.88
CV %	2.04764	4.95518	8.24008	12.3461	7.77677	10.19
P value	<.0001	<.0001	<.0001	0.007	<.0001	<.0001

Means within a column followed by the same superscript letter(s) are statistically non-significant and different letters represent significant differences at $p < 0.05$. LeL leaf length, LeW Leaf width, LeN Leaf length, PsC pseudo stem circumference, PlH Plant height, PsH pseudo stem height

Enset landraces parent corm split piece had significant (p<.0.001) effect on mean height of regenerated sucker (Table 2). Significantly longest mean sucker height (102.07 cm) was recorded from landrace ‘Kulle‘parent corm split. The shortest sucker height (66.25 cm) was recorded from ‘Astara’ landrace. Similarly, enset landraces parent corm split also had significant (p<.0.001) effect on pseudostem height (Table 2). The highest pseudostem heights (34.49 and 34.09 cm) were obtained for enset landrace ‘Ado ‘parent corm developed sucker.

DISCUSSION

In general, enset is a vegetatively propagated plant, and it achieves a high rate of propagation by cutting the pseudostem and removing the meristem (Afza et al., 1996). New suckers, which are not previously organized, regenerate adventitiously from tissues and organs as meristematic apices (Hartmann et al., 2010). In some plant species, mechanically disrupting the tissue can separate intact cells of certain organs (Kohlenbach, 1977). In our study, the central growing points of the enset corms were removed, and multiple suckers were formed after callus formation from tested landraces using an eighth (double quarter) corm splits (Figure 7). The findings of our study coincide with Buke et al. (2016), who concluded that when the corm's growing center was removed, callus development was observed first, and then suckers started to grow on the callus. The result of this study reveals that wounding is necessary to induce sucker regeneration (Figure 6).The result is in line with the findings of Diro and Tabogie (1992), who reported that complete damage or physical elimination of the bud apex overrides the influence of the controlling shoot apex. The result of this study is also supported by Tesfaye (2002), who stated that wounding is a potential initiator of mitotic activity in plants linked to the physical elimination of the shoot apex, which releases the cells in the sub-apical region of the corm that the apical meristem imposed inhibition on. Induction of adventitious buds is a normal occurrence in vivo but is regularly limited by time and space (Afza et al., 1996). These methods of propagation utilize relatively large pieces of plants and are hence called 'macro-methods' of propagation (George et al., 2008). Consequently, a highly significant ($p < 0.001$) earlier sucker emergence (49.9 days) was observed from the parent corm of an eighth split of the landrace 'Midasho' (Figure 5). Our study results are in line with Karlsson et al. (2015), who found that the time required for sucker emergence is shorter for split corms. Conversely, our work was found to be contrary to Buke et al. (2016), who reported that the days to emergence was almost the same. The first sucker from landrace 'Midasho' emerged 49.9 days after the parent corm split burial, which is the earliest date for the split corm. It is frequently confirmed that half- and quarter-split-corm suckers emerge earlier than those from the whole corm (Tabogie and Diro, 1992). Our results show earlier emergence than the result (60 days) for 3-year-old plant half corm splits reported by Bora and

Haile (2024). Our result also conflicts with the time to sucker emergence reported by Blomme et al. (2008), ranging from 60 to 65 days for watered corm of the 'Zerita' enset cultivar, whereas for non-watered corm, it ranged from 60 to 85 days, and Tsegaye and Struik (2002) also reported 2–3 months for emergence of suckers after the burial of corm or corm pieces.In other words, the significantly highest regeneration percentages were recorded for landraces 'Keshicha' and 'Midasho'. This might be due to the absence of dormant buds of the true stem apical end that were physically removed during corm splits preparation, initiating dividing cells. As demonstrated in Figure 6, the lowest regeneration percentage was recorded for the landrace 'Ado'. This decrease in regeneration percentage on this landrace might be due to damage to the tissue or possibly the reduced synthesis of growth hormones that influence regeneration in the enset parent corm (double quarter).

On the other hand, the sucker proliferation capacity of enset landraces was variable, and a higher sucker number was recorded on landrace 'Midasho' per corm split (1/8), with a total of maximum (360) and minimum (78.96) suckers per corm recorded for landrace 'Midasho' and 'Ado', respectively. This study was able to produce a higher shoot multiplication rate in a specified area under a lath house within a short period of time compared to previously reported field plot works (Tabogie and Diro, 1992; Diro et al., 2002; Karlsson et al., 2015; Buke et al., 2016; Bora and Haile, 2024). However, Bora and Haile (2024) recently reported up to 443 shoots per corm, which is not consistent with our current observations. The highest number of suckers was recorded in our study on landrace 'Midasho' parent corm cut into double quarter or 1/8th split, followed by landrace 'Kulle' (Figure 7). Our result also revealed that different landraces had different sucker proliferation potential. The sucker number in our study is higher than the report of Buke et al. (2016), who found that enset propagation using corm pieces yields 3.7–38.1 suckers. Nonetheless, our study result is contrary to the previous studies reported by Diro et al. (1994, 2002) and Tesfaye (2002), which confirmed that the number of suckers produced was between 40 and 141 suckers per corm.

Our results are in line with Diro et al. (2002) and Karlsson et al. (2015), who reported a higher number of suckers generated for corm split compared to entire corms. Conversely, Tsegaye (2002) stated that when the apical meristem of a corm is removed (for plants in the vegetative stage) and the corm is buried in loosened soil, numerous shoots will emerge from the corm surface. Also, our study of an eighth (double quarter) corm split in vivo macro-propagation revealed that the sucker proliferation potential is increasing with the increase of parent corm splitting for different landraces. On the other hand, our results are conflicting with Diro and Tabogie (1992), who recorded an average of 22, 76, and 102 suckers from field-grown whole corm, half corm, and quarter corm, respectively. Similarly, Diro et al. (2002) reported 40 to 141 suckers from 'Halla' landrace using 1.5m spacing from halved corms in a three months field experiment. Our result is also supported by Gowen (1995), who concluded the possibility to produce more suckers of plantains from altered traditional propagation techniques.

The results of our study revealed that landrace 'Midasho' gave the highest number of suckers compared with other landraces tested, and we also recommend this landrace for better sucker yield. Our study result is highly conflicting with the study report by Diro and Tabogie (1992) who reported a higher number of suckers from a field plot grown with large spacing (1.5m), grown with half and quarter corm pieces of 'Halla' landrace. But in our study, we managed smaller spacing (1m) on a lath house constructed bed; using different landraces, a smaller area, and spacing between holes compared with the field plot executed study. Resembling results were reported on commonly cultivated enset, for which quicker sucker emergence time and a comparatively greater number of suckers were obtained from corm split than the entire corm (Diro et al., 2002; Karlsson et al., 2015).

Our result is also in line with the result of Bora and Haile (2024), who concluded that splitting or cutting the corm into more pieces increased the number of suckers produced compared with whole corms. Similar results were reported by Zippel (2005), who states that the number and size of suckers are factors during the selection of cultivar, while the specific performance of a plant depends on growth conditions. In our study, the commonly used by farmers (3-years-old parent corms) from different landraces corm splits were used, comparatively to (half and quarter) which is divided into a double quarter or an eighth, which is very small split size compared to half and quarter corm splits. Only 60 suckers were obtained from the entire corm, whereas 60 and 140 suckers were most often documented per corm for the corm split in half and quarter corm splits, respectively (Karlsson et al., 2015). Thus, this eighth corm split practice can increase the efficiency of propagation under a lath house by hastening the time of propagators wait for and providing more suckers than the farmers' practice. Mostly, yield for most clones is more than 40 suckers per whole corm (Diro and Tabogie, 1994). The highest number of suckers (35) per half corms was acquired and reported from a three-year-old clone of 'Halla' left undisturbed mother plant for one year after apical bud removal (Diro et al., 2002).

Generally, our results showed that enset landrace 1/8th (double quarter) parent corm splits in vivo macro-propagation under the lath house technique with good management practices (manure application, watering, disease control, and weeding) was more efficient than the conventional practice. It regenerates large quantities of suckers rapidly in an economical way with genotype purity efficiently in a short time than in the field study in a specified small area. The techniques used in our study might be evidently disseminated to sucker growers and researchers, ensuring successful propagation under a lath house in a short period of time.

CONCLUSIONS

In vivo macro propagation of corm splits was significantly effective for all tested qualitative phenotype-varying landraces. This study, as the first report, revealed that the landrace 'Midasho' sourced from a 3-year-old parent corm showed promising performance compared to the assessed landraces in this lath house experiment. Overall, the 'Midasho' landrace exhibited superior sucker proliferation potential for in vivo macro propagation. Additionally, the sucker regeneration capacity of the landrace 'Midasho' (45 and 360 suckers per corm split and per whole corm, respectively) was found to be effective compared to previously reported enset corm propagation research and traditional sucker production. This research report presents double quarter corm split, which improved the practice to the most efficient end to regenerate suckers of varying enset landraces using corm splits for in vivo macro propagation under lath house conditions. Therefore, all qualitative phenotypes of different enset landraces tested with an eighth corm split performed significantly compared with traditional farmers' methods and can be utilized to regenerate more vigorous enset suckers effectively in a specified area within a short period of time.

Similarly, the method can be practiced anywhere without the demand for excess land to support the enset culture and contribute to achieving food security of the enset growing population compared to farmers' conventional practices. The result of the present study is promising for sucker multiplication of newly developed cultivars in conventional breeding. This eighth corm split propagation under the lath house study will help smallholder farmers and sucker producer groups to maintain landrace diversity and improve income security. However, a study on an eighth corm split in vivo macro propagation incorporating a wide range of enset landraces would need to be carried out for more concrete recommendations. Furthermore, the biochemical reactions that take place within the corm splits, contributing to the sucker growth and yield performance during propagation, need to be investigated and clarified in future studies.

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DECLARATIONS

The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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