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RESEARCH

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In vivo regeneration efficiency of enset (*Ensete ventricosum* (Welw.) Cheesman) as affected by age of parent plant and corm proportion

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Abstract

In cultivation, *E. ventricosum* is propagated clonally with adventitious buds sprouting from callus formed on the cut surfaces of the corm. The present study was conducted to enhance the efficiency of this indigenous enset propagation technique by identifying the optimum age of parent plants and corm types for maximum shoot production. This study was conducted using corms in the range of 12–18, 20–28, 29–38, and 40–50 cm diameter, corresponding to four age classes (1, 2, 3 & 4 years old) and three corm treatments (whole, half and quarter) were arranged in 3 × 4 factorial combination and layout in randomized complete block design with three replications. The statistical program SAS Version 9.3 was used to examine the data. The result of the study showed that all of the parameters considered were significantly affected by the treatments or their interaction effects. The number of shoots produced per corm was significantly ($p < 0.01$) affected by the interaction of the age of the parent plant and corm treatment. The average number of shoots produced per corm was between 43 and 443 shoots per com. The highest shoot numbers (443 shoots per corm) were recorded on the 3-year plant using quarter corm; whereas the lowest number of shoots (43 shoots per corm) was obtained from whole corms of the 1-year plant. In general, the results of the study showed that the use of quartered corms of 3-year-old parent plants appears to be the efficient technique to regenerate enset in vivo.

Keywords Enset, Corm, In vivo, Regeneration, Shoots, Early growth

Introduction

Enset (*Ensete ventricosum* (Welw.) Cheesman) is a diploid ($2n = 18$) giant monocarpic herbaceous perennial crop belonging to the family Musaceae and in the genus *Ensete* (Cheesman 1947; Simmonds 1953). Until modern times, when it spread widely around the world as an ornamental, the enset was cultivated only in Ethiopia (Simoons 1965); today, Ethiopia is still the only place where it is grown for food (Rossel 1998; Tesfaye 2008). Domesticated enset is restricted to Ethiopia, and *E. ventricosum* is the only *Ensete* species in Ethiopia (Brandt et al. 1997). However, wild *E. ventricosum* also occurs naturally in other countries of Central and East Africa, including Congo, Mozambique, Uganda, Tanzania, and Zambia (Borrell et al. 2019).

Enset is a staple food crop for approximately 20 million people and is part of a successful and sustainable indigenous farming system in south and southwestern Ethiopia (Borrell et al. 2019). *E. ventricosum* occurs naturally in

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large areas of Africa (Brandt et al. 1997; Williams 2017), and its cultivation is practiced over an even wider range of climates, elevations, and soil types (Birmeta et al. 2004; Borrell et al. 2019), therefore, enset agriculture can make an important contribution to improving food security across much larger areas than is currently possible (Bosha et al. 2020). All portions of the plant are used for various purposes, making it a crop with multiple uses (Brandt et al. 1997). The majority of the products produced by enset are utilized for human food, animal feed, fiber, building materials, medicine, and cultural rituals. The three main foods made from enset are *Kocho* (a fermented product made from the corm and pseudostem), *Etino/Bula* (a dehydrated product made from the juice from the decortications of the pseudo stem and grating of the corm), and *Utoo/Amicho*, which is the stripped corm of younger plants of enset that is boiled and eaten (Forsido et al. 2013; Haile et al. 2022).

Enset benefits the neighborhood's ecology by enhancing the soil's nutrient balance (Elias 1998), providing shade, which lowers temperatures, and participating in farming systems with high biodiversity (Tesfaye 2008). Once established, the plant can withstand brief periods of drought. Processed products can be stored for a long time without spoiling, thus ensuring a stable food supply. Fiber, a by-product when enset is processed, is used in local fiber factories and partially substitutes for fiber importation. At the environmental level, enset plantations reduce runoff, improve nutrient recycling, and thus contribute to sustainable agriculture (Forsido et al. 2013).

The enset plant is a giant herbaceous tree that may grow up to 13 m high and have a diameter of 2 m or more (Shigeta 1991; Haile et al. 2023). Like bananas, enset has a pseudo-stem of overlapping leaf sheaths, large paddle-shaped leaves, and a massive pendulous inflorescence with banana-like fruits (Yemataw et al. 2018; Borrell et al. 2019). However, unlike bananas, which are widely farmed for their fruits, it is instead the swollen pseudo-stem base, leaf sheaths, and underground corm that provide a year-round dietary starch source, are harvested before flowering 4–7 years after planting, and may require more than 10 years to complete their life cycle (Cheesman 1947; Tesfaye and Ludders 2003; Borrell et al. 2019).

Enset, consisting of an above-ground pseudo-stem made from overlapping leaf sheaths, a short, compact, and fleshy underground stem 'corm', and a conspicuously large leaf (Cheesman 1947; Birmeta et al. 2004). The true stem is a short, compact, and fleshy underground stem, or 'corm'. Ensete plants are monocarpic (i.e., they die off entirely after flowering and fruiting). When a banana plant dies, it is spontaneously replaced by new suckers sprouting from pre-existing buds in the corm (Cheesman 1947). However, in Ensete, shoot production is induced only when the meristem is wounded (Shigeta 1991). The wild populations of ensete are propagated by seeds under natural conditions; seed propagation is the only way for wild ensete to maintain their populations (Shigeta 1991; Rossel 1998; Birmeta et al. 2004).

In cultivation, *E. ventricosum* is propagated vegetatively with adventitious buds sprouting from the corm after the removal of the apical meristem; for the wild enset, reproduction seems to happen mainly through seeds. Cultivated enset also produces seeds, but only after a long juvenile period. Seed germination is very low because of seed dormancy. Moreover, since enset utilizes its stored carbohydrates during fruiting and eventually dies, it is harvested before or shortly after flowering, reducing viable seed production. Therefore, propagation by seed is not common. The plant is usually multiplied vegetatively and grown as clones. Apical buds are removed from the whole or split corms, after uprooting or without uprooting the mother plant to initiate suckering (Alemu and Sandford 1991; Diro and Tabogie 1994; Karlsson et al. 2013).

The apical buds are removed to induce shoot production; if planted without removing them, only one shoot emerges per whole corm and a few suckers per half corm (Diro et al. 1996, 2002; Haile et al. 2021), because the apical buds inhibit the growth of lateral buds. As it has been repeatedly said that Ethiopian farmers have developed this elaborate technique aimed at clonal propagation of the enset plant, the technique could be called in vivo induced shoot regeneration (Haile et al. 2021; Tesfaye 2002). Similar to in vitro regeneration, these authors reported that the induced shoot regeneration method regenerates multiple shoots in vivo from the callus formed on the cut surfaces of the corm and depends on the same principle as shoot formation in in vitro tissue culture by the organogenesis method: as a result of removing apical buds from whole or split corms, adventitious buds differentiate from callus at the cut surface of the corms that develop into shoots (Haile et al. 2021; Tesfaye 2002).

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 (Diro et al. 2002; Kippe 2002; Shumbulo et al. 2012; Karlsson et al. 2015). However, due to the limited research attention given to this indigenous crop, this amazing clonal propagation technique, developed by ancient Ethiopian farmers, has not yet been optimized in a compressive way. There have been no reports showing the effects of parent plant age on in vivo regeneration of enset except for the study by Diro et al. (2001). The existing limited literature explains the existence of considerable differences in shoot production from corm depending on the farmer's practice.

This offered insight into how the regeneration efficiency of enset can be improved by evaluating and optimizing the extensive indigenous knowledge in the country, particularly from southwest Ethiopia. Therefore, the present study was carried out to (i) evaluate the effects of the ages of parent plant and corm type on regeneration and shoot multiplication capacity of enset in vivo, and (ii) determine the optimum age of parent plant and corm type for shoot production in in vivo shoot regeneration.

Hence, shoot regeneration and growth totally depend on the corm's carbohydrate reserves until the regenerated shoot initiates its own roots and reaches the soil surface. Shoot regeneration capability may be impacted by variations in the corm's carbohydrate content, nutritional state, or other growth-promoting elements. Therefore, it is reasonable to determine the optimum age of parent plants and corm types for propagation of this important Ethiopian domesticated crop with due consideration of farmers' indigenous knowledge in the study area. Optimizing both the age of the parent plant and the corm type could improve suckers' production of enset. We hypothesize that the age of the parent plant and corm type have significant effects on shoot regeneration and the multiplication capacity of enset. The key questions to be addressed were: (i) do shoot regeneration and multiplication capacity of enset varies at different age groups? (ii) Does corm splitting enhance the shoot regeneration and multiplication efficiency of enset, and how is it associated with the age of the parent plant? Thus, the outcome of this study would contribute to improving the food security of smallholder farming communities by demonstrating the optimum age of the parent plant for efficient propagation of cultivated enset using a low-cost regeneration method in field conditions.

Material and methods

This experiment was conducted in Chena Woreda at Qocha Wacha primary school in Kefa Zone, in southwestern Ethiopia. The study site is situated at 7.15 N and 36.45 E, and has an elevation of 2012 m.a.s.l. The area receives an average annual rainfall of 1300 mm to 2000 mm and a mean annual average temperature of 14 °C to 28 °C.

The experiment was designed to examine the effects of age of the parent plant and corm types or corm splitting on shoot multiplication efficiency in the traditional propagated induced shoot regeneration method. The experiment was conducted using corms' of Nobo landrace at different size/age classes were sourced from the same farm. Four age groups of parent enset corms from: 1-year-old parent enset (Y1), 2-year-old parent plant (Y2), 3-year-old parent plant (Y3), and 4-year-old parent plant (Y4) with corm sizes of 12–18 cm, 20–28 cm, 29–38 cm, and 40–50 cm diameter ranges, respectively, and three corm types: whole corm (C1), halved corms (C2), and quartered corms (C3) were arranged in factorial combination and laid out in a complete block design with three replicates. For this experimental enset landrace, Nobo was chosen because it is the most chosen landrace in the area due to its high disease, wind, and drought resistance characteristics coupled with high yield and quality products (Kocho, Bula, and fiber) (Haile et al. 2020, 2022).

The experimental land was cleared and ploughed five times by oxen plough according to farmers' practice and applied manure during land preparation. Enset parent plants were obtained on the farmer's field based on their age, i.e., 1 year, 2 years, 3 years and 4 years old. Following the traditional methods described elsewhere (Haile et al. 2021); corms were prepared by carefully removing the meristematic tissue (Fig. 2b) and leaf sheath almost at the joining point of the pseudo-stem and the corm (Fig. 2a). The corms and corm pieces were prepared as whole, half, and quarter and were sun-dried for 48 h. The experimental field was divided into three blocks each comprise 12 plots. Corms of different size/age group plants were planted at 20 cm soil depth in a spacing of 1.0 m × 1.0 m on January 4, 2021. In each plot it was managed to use three corms. However, the number of planted corm pieces varied depending on used corm proportion. When whole corms were used the numbers of corms planted per plot were three. In case of corm pieces, the number of planted corm pieces was the multiple of the corm proportion; i.e. two for halved and four for quartered. Accordingly, six corm pieces and twelve corm pieces per plot were planted for halved and quartered corm pieces, respectively. Each unit plot had dimensions of 12 m^2 (3 × 4 m), with three individual plants' corms or corm pieces being planted in a spacing of 1 × 1 m. Each plot had three rows, each of which comprised four hills. The experimental plants were protected by guard rows; additional extra corms were collected and planted in the outer parts of the plot. Management practices such as weeding and cultivation were done regularly.

To examine the effects of corm type and age, the following data articulating the regeneration and multiplication rate and early growth performance of the regenerated suckers were collected: (1) dates of callus formation (counting number of days when callus formed; one sampled corm and corm piece per plot was pulled out and then poured with water every day for inspection of the changes took place/callus formation on the corm), (2) days to 50% emergence (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), (3) regeneration percentages/frequency (proportion of regenerated corms or corm pieces per treatment), (4) rate of shoot regeneration (speed of shoot regeneration measured by counting the regenerated shoots within seven days interval), (5) shoot multiplication rate (number of suckers per corm, multiplication rate for corm pieces, i.e. for half and quarter was calculated by summing the total number of suckers from each piece), (6) plant height (the length of suckers from the ground to the tip of the longest leaf); (7) length of the leaf (the longest leaf at the point where it starts to diverge from the pseudo-stem and ceases to overlap other leaves to the tip); (8) pseudo-stem height (measured from the ground level up to the point where the bases of the leaves are recorded); (9) leaf width (measured at the point where the leaf is widest on the longest leaf of enset pants); (10) leaf number (counting total numbers of leaf from randomly selected ten plants); and (11) pseudo-stem circumference (the circumference at half the pseudo-stem height). Data on callus formation and emergence were collected starting 30 days after planting corms and corm pieces. Recorded characteristics related to the early growth performance of the regenerated suckers were measured from each shoot according to the method described by Haile et al. (2021). Data were subjected to analysis of variance (ANOVA) using the SAS (9.2 version) statistical system (SAS Institute 2008). Shoot number data were transformed using the log transformation before ANOVA. The differences among means were compared using the least significant difference (LSD) test at a 5% probability level.

Table 1 Effect of parent plant ages and corm proportions on shoot regeneration/multiplication capacity parameters of enset (*Ensete ventricosum* (Welw.) Cheesman)

Treatments		Shoot regeneration parameters		
Age of parent plant (years)	**Corm proportion**	**DC**	**D50%E**	**SN**
1	Whole	42.66 ± 5^{a}	70 ± 4^{a}	43 ± 3^{h}
	Half	36.66 ± 2^{cd}	64 ± 2^{cde}	65.66 ± 3.6^{fgh}
	Quarter	40.66 ± 3^{ab}	68 ± 2^{ab}	62 ± 5.7^{gh}
2	Whole	40 ± 4^{ab}	68 ± 3^{ab}	95.66 ± 5.4^{fg}
	Half	32.66 ± 1^{e}	62.66 ± 0^{efg}	160 ± 12.6^{de}
	Quarter	36 ± 2^{d}	66.66 ± 1^{bc}	245.33 ± 22.6^{bcd}
3	Whole	39 ± 3^{bc}	66 ± 3^{bcd}	97.66 ± 9.3^{f}
	Half	32.66 ± 1^{e}	60 ± 0^{g}	195.66 ± 23^{cd}
	Quarter	36 ± 2^{d}	63.33 ± 1^{def}	443.33 ± 36^{a}
4	Whole	39.33 ± 3^{bc}	66.66 ± 2^{bc}	102 ± 19^{ef}
	Half	32.66 ± 2^{e}	60.66 ± 0^{fg}	252.33 ± 27^{bc}
	Quarter	36 ± 2^{d}	64.66 ± 1^{cde}	340 ± 42^{ab}
LSD (5%)		2.46	2.23	117
CV (%)		3.92	2.02	5.49

Means followed by the same letter per column are not significantly different at 5% level of significance

DC Date of callus formation, *D50%E* Days to 50% emergence, *SN* Shoot number

The number under shoot in bracket showed that untransformed (normal) data

Results and discussion

Results

We observed differences in the regeneration capacity of enset corms depending on the age of the parent plant and corm proportion (corm types). The interaction effect of age × corm proportion was very highly significant ($p < 0.001$) on days to callus formation, highly significant ($p < 0.01$) on emergence condition (days to 50% emergence), and significant ($p < 0.05$) on multiplication capacity (average number of shoots per corm) of the corms (Table 2). Halved corms from 2-, 3-, and 4-year-old parent plants took significantly earlier days to form callus (33 days) (Table 1), whereas whole corms from tested age groups of parents (1- and 2-year-old plants) showed significantly longer days to form callus (43, 40 days, respectively). Similarly, halved corms have been taken from 3-, 4-, and 2-year-old plants that were earlier to emerge (60, 61, and 63 days, respectively) (Table 1). Days to 50% emergence were significantly delayed (up to 70 days) on the whole corm and 1-year-old treatment combinations, which was statistically similar to that recorded from the whole corm of 2 years and quartered corm of 1 year-old.

The average number of suckers produced per corm varied between 43 and 443 (Table 1). The highest number of shoot (443 per corm) was recorded on the 3-year plants with a quarter corm type, though it is statistically similar to the shoot number (340 per corm) obtained from quartered corms of 4-year-old plants. Significantly ($p < 0.05$) the lowest number of shoots (43 shoots per corm) were obtained from whole corms of a 1-year plant, which is statistically similar to the number of shoots obtained from halved and quartered corms of a 1-year plant and whole corms of a 2- and 3-year plant (65, 62, and 95, 97 suckers per corm, respectively). Shoot number (multiplication rate) was thus influenced by the interaction effect of the age of corms and corm proportion. When quartered corms were used, the mean number of shoots per corm increased with the increasing age of the parent plant and reached its maximum at age three; thereafter, the shoot multiplication rate was no longer increasing (Table 1).

In the present experiment, 83.33% to 100% regeneration frequency was recorded, depending on the age and proportion of corms tested. Quartered corms from year 1 and 4 parent plants showed 83.33% and 91.66% regeneration frequencies, respectively. But there is no variability with regard to their corm regenerability while using any corm proportion from 2- and 3-year-old parent plants; equally, they showed their maximum regeneration potential (100%) in every condition of corm proportion employed. As illustrated in Fig. 1, induction and regeneration of substantial masses of shoots occurred between 5 and 9 weeks in the majority of the studied treatment combinations. But regeneration continued for up to 12 weeks in some cases.

In the present work, we observed statistically significant differences in shoot early growth performance between different age groups of parent enset and corm types used and between their interactions. The interaction effect of age × corm proportion (corm type) was highly significant ($p < 0.01$) on leaf length and leaf number, and very highly significant ($p < 0.001$) on leaf width, pseudo-stem height, and pseudo-stem circumference (Table 2). The result depicted that the mean plant height, leaf length, leaf width, pseudo-stem height, and pseudo-stem circumference of regenerated suckers at their early growth ranged from 34.8 to 57.76, 28.13 to 33.20, 9.93 to 16.53, 6.86 to 21.61, and 6.96 to 11.6 cm, respectively (Table 3). The recorded mean leaf number was between the ranges of 4.66 and 5.73 per sucker.

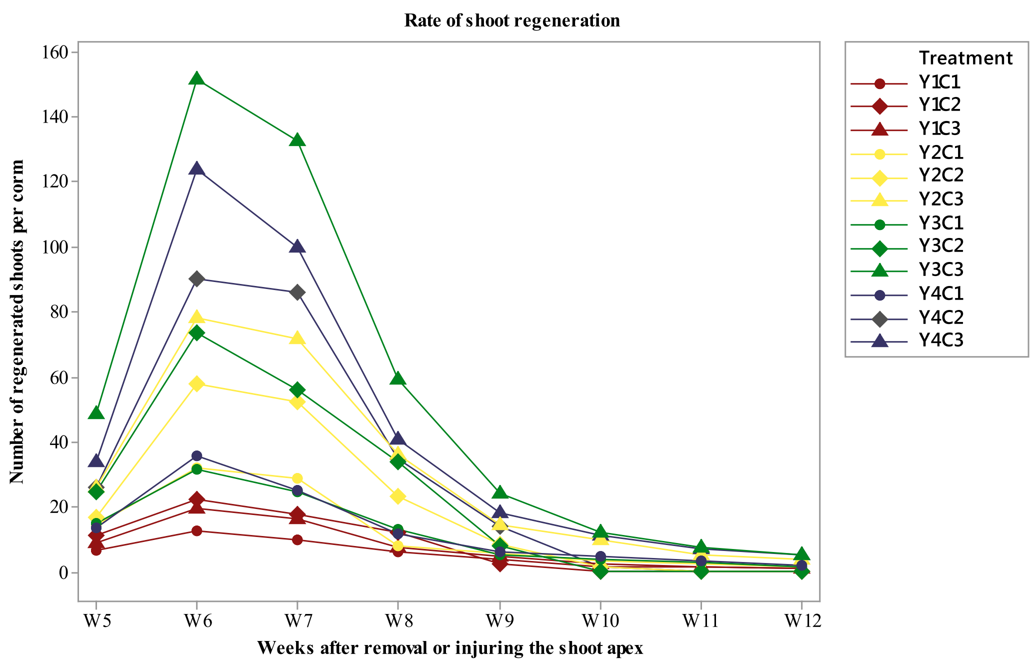

Fig. 1 The speed at which shoot regeneration occurs with different treatments over 12 weeks: W5, W6, W7, W8, W9, W10, W11, W12 indicate 5-, 6-, 7-, 8-, 9-, 10-, 11-, 12-weeks after removal of apical buds, respectively. Y1, Y2, Y3, Y4 represents 1, 2, 3, and 4 year old parent plants; whereas C1, C2, C3 represents whole, halved and quartered corm types

Table 2 Mean square of ages and corm treatment on shoot regeneration and early growth of enset (*Ensete ventricosum* (Welw.) Cheesman)

	Mean square									
	DF	DC	DE	SN	PH	LL	LW	LN	PsH	PsC
Source of variation										
Replication	2	0.77	17.86	0.015	18.90	0.08	11.68	0.02	23.65	5.37
Age	3	17.29***	15.65***	0.63 ***	347.36***	6.67***	22.00***	0.23***	172.78***	15.07***
Corm treatment	2	75.11***	72.86***	0.53***	318.01***	4.15***	17.31***	0.31***	116.33***	8.19***
Age * corm treatment	6	26.74***	6.97**	0.03*	102.36***	1.00**	9.22***	0.11**	68.35***	5.54***
Error	22	2.11	1.73	0.01	15.68	0.20	0.67	0.02	5.61	0.35
CV (%)		3.99	2.04	5.43	8.75	1.48	6.24	2.92	16.91	6.33

*, **, ***F value significant at $p = 0.05$, $p = 0.01$, and $p = 0.001$, respectively

NS non-significant ($P > 0.05$), *DF* degree of freedom, *DC* Date of callus formation, *DE* Date of 50% emergence, *SN* shoot number, *PH* Plant height, *LL* leaf length, *LW* leaf width, *LN* leaf number, *PH* pseudo stem height, *PsC* pseudo stem circumference

Table 3 Early growth performance of enset as affected by interaction effect of parent plant ages and corm proportion

Treatments		Early growth performance parameters					
Age of parent plant (years)	**Corm proportion**	**PH (cm)**	**LL (cm)**	**LW (cm)**	**LN**	**PsH (cm)**	**PsC (cm)**
1	Whole	38.63 ± 0.6^{ef}	29.43 ± 0.33^{ef}	11.71 ± 0.3^{efg}	5 ± 0.12^{bcd}	9.43 ± 0.4^{ef}	8.23 ± 0.51^{efg}
	Half	36.10 ± 0.5^{f}	29.30 ± 0.4^{f}	11.33 ± 0.4^{fg}	4.9 ± 0.14^{cd}	6.90 ± 0.43^{f}	7.60 ± 0.55^{fg}
	Quarter	34.80 ± 1.1^{f}	28.13 ± 0.24^{g}	9.93 ± 0.36^{g}	4.66 ± 0.15^{d}	6.86 ± 0.3^{f}	6.96 ± 0.45^{g}
2	Whole	50.73 ± 0.9^{bc}	30.96 ± 0.09^{bc}	14.50 ± 0.55^{bc}	5.23 ± 0.1^{bc}	19.26 ± 0.5^{ab}	10.0 ± 0.61^{bcd}
	Half	43.83 ± 0.6^{de}	30.06 ± 0.13^{def}	12.46 ± 0.5^{def}	5 ± 0.11^{bcd}	13.56 ± 0.47^{cde}	9 ± 0.49^{cdef}
	Quarter	40.50 ± 1.5^{ef}	30.40 ± 0.11^{cd}	12.40 ± 0.6^{def}	4.9 ± 0.17^{cd}	10.10 ± 0.7^{ef}	8.80 ± 0.58^{def}
3	Whole	57.76 ± 1.4^{a}	33.20 ± 0.08^{a}	16.53 ± 0.44^{a}	5.73 ± 0.12^{a}	21.60 ± 0.6^{a}	11.60 ± 0.63^{a}
	Half	49.26 ± 0.8^{bcd}	30.70 ± 0.1^{bcd}	14.53 ± 0.3^{bc}	5 ± 0.2^{bcd}	17.36 ± 0.35^{bc}	10.40 ± 0.37^{abc}
	Quarter	43.63 ± 1.5^{de}	30.40 ± 0.17^{cd}	12.20 ± 0.6^{def}	5.10 ± 0.21^{bc}	13.76 ± 0.54^{cde}	9.10 ± 0.43^{bcde}
4	Whole	55.86 ± 1.2^{ab}	31.40 ± 0.9^{b}	15.93 ± 0.6^{ab}	5.33 ± 0.14^{b}	20.93 ± 0.55^{a}	11.60 ± 0.36^{a}
	Half	48.00 ± 1.4^{cd}	30.70 ± 0.62^{bcd}	13.86 ± 0.47^{cd}	5.03 ± 0.23^{bc}	16.13 ± 0.66^{bcd}	10.46 ± 0.53^{ab}
	Quarter	43.36 ± 1.4^{de}	30.23 ± 0.78^{cde}	13.36 ± 0.5^{cde}	5.03 ± 0.22^{bc}	12.76 ± 0.72^{de}	9.26 ± 0.47^{bcde}
LSD (5%)		6.71	0.76	1.39	0.24	4.01	1
CV (%)		8.75	1.48	6.24	2.92	16.91	6.33

Means followed by the same letter per column are not significantly different at 5% level of significance

Y1 Year one, *Y2* Year two, *Y3* Year three, *Y4* Year four, *C1* Whole corm, *C2* Half corm, *C3* Quarter corm proportion, *PH* Plant height, *LL* Leaf length, *LW* Leaf width, *LN* Leaf number, *PsH* pseudo stem height, *PsC* pseudo stem circumference

Fig. 2 Propagation of enset through indigenous induced shoot regeneration method: **a** enset corm from a 3 year old parent plant; **b** removing the meristematic tissue of a 3-year-old enset; **c** quartered corm; **d** formation of adventitious buds from callus; **e** formation of sprouting shoot and developed suckers

The highest mean shoot height (57.76 cm) was observed from a shoot regenerated using a 3-year-old whole corm. However, this value is statistically similar to the recorded plant height from a whole corm taken from 4-year-old parent plants (55.86 cm). The highest mean leaf length (33.20 cm) was scored from 3-year-olds with whole corms. Similarly, the highest mean leaf width (16.53 cm) was recorded in 3-year-olds with whole corm, followed by 4-year-olds with whole corm (15.93 cm). In the same manner, the highest mean leaf number (5.73 cm) was observed by 3-year-olds with a whole corm, followed by 4 years with a whole corm and 2 years with a whole corm (5.33, and 5.23 cm, respectively). The lowest mean leaf width (9.93 cm) and leaf number (4.66) were recorded from quarter corms sourced from a year-old parent plant. The highest mean pseudo-stem height (21.61, 20.93, and 19.26 cm) was scored by 3-year-olds with whole corm, 4 years with whole corm, and 2 years with whole corm, respectively. The lowest mean pseudo-stem height (6.86, 6.9, and 9.43 cm) was recorded by a 1-year-old with a quarter corm, 1 year with a half corm, and 1 year with a whole corm, respectively. In the same way, the highest mean pseudo-stem circumference (11.60, 11.60, 10.40, and 10.46 cm) was recorded from 3-year-olds with whole corm, 4-year-olds with whole corm, 3-year-olds with half corm, and 4-year-olds with half corm, respectively. Whereas, the lowest values (6.96, 7.60, and 8.23 cm) were recorded from 1 year with quarter corm, 1 year with half corm, and 1 year with whole corm, respectively.

Discussion

It has been shown that wounding is the first event that provides signals for triggering the entire regenerative process in plants (Iwase et al. 2011). Corms type/splitting and different sizes or ages of the corm affected the callus formation process (Fig. 2). Large and/or old corms produced greater callus mass and more adventitious buds, and sustained a more vigorous shoot growth than smaller age and younger corms. As suggested by Haile et al. (2021), the size of the parent plants likely played an important role in the in vivo shoot regeneration of enset corm. In a previous experiment on wild enset, it was shown that the regeneration frequency had increased linearly as corm size increased (Haile et al. 2021). Earlier callus formation was recorded on half-corm and larger-size corms from older parent plants with larger corm sizes than whole or quarter corms from younger parent plants. Correlation analysis showed that days to callus formation correlated significantly ($p < 0.05$) and positively with days to 50% emergence ($r = 0.77$) (Table 4). This means that early callus formation resulted in earlier shoot emergence, or delay in callus formation will also delay shoot emergence. Conversely, a highly significant ($p < 0.01$) negative correlation between regenerated shoot number and days to callus formation ($r = -0.63$) and days to 50% emergence ($r = -0.64$) was observed (Table 4). This implies that those corms that took more days to form a callus and that showed delayed emergence exhibited a small number of regenerated shoots, or that faster callus formation resulted in early emergence of a large number of shoots.

Increasing the size of the corms or corm pieces up to a certain age could increase the amount of reserves for cell division, callus proliferation, and the size of the resulting callus which will also influence the number of buds to be initiated. Similarly, this result is in agreement with Tesfaye (2002), who reported earlier callus formation on half corm than other corm proportions. However, quartered corms took a relatively longer time to callus formation than halved corm; this might be due to size effect. Necessary, damaging, or eliminating the shoot apex is required to achieve callus formation (Haile et al. 2021). Similarly,

Table 4 Pearson correlation coefficient for regeneration and early growth parameters

	DC	DE	SN	SH	LL	LW	LN	SPH	SPC
DC		0.77*	−0.63**	−0.246^{ns}	−0.28^{ns}	−0.22^{ns}	−0.42^{ns}	−0.39^{ns}	−0.01^{ns}
DE		1	−0.64**	0.02^{ns}	−0.02521^{ns}	−0.13832ns	−0.11907ns	−0.12896ns	−0.11326^{ns}
SN			1	0.31161^{ns}	0.50092^{ns}	0.27047^{ns}	0.51507^{ns}	0.41474^{ns}	0.14707^{ns}
SH				1	0.85**	0.75**	0.94**	0.84981**	−0.78487**
LL					1	0.76**	0.85270**	0.82505**	−0.59404**
LW						1	0.70194**	0.60841**	−0.77448**
LN							1	0.89160**	−0.61896**
SPH								1	−0.60907**
SPC									1

ns, *, ** indicate non-significant, significant at 5% and 1%probabilitylevel respectively

DC Date to callus formation, *DE* Days to 50%emergence, *SN* Shoot Number, *SH* Sucker Height, *LL* Leaf Length, *LW* Leaf Width, *LN* Leaf Number, *SPH* Sucker pseudo-stem height, *SPC* Sucker pseudo-stem circumference in our further observation, we noticed that simple decapitation of the pseudo-stem that does not involve injury or damage to the shoot apex is not sufficient to initiate callus proliferation.

Splitting or wounding and the size of the corm or age influenced the time of callus formation and shoot emergence. As a result, earlier emergence was observed in the case of half corms than in whole and quarter corms. Delayed shoot emergence was observed on quartered corms from 1 to 4 years old. Quartering initially smaller corms that were sourced from 1-year-old parent plants that failed suckers to emerge could be due to low energy to support the regeneration process. The result of this study is in agreement with the previously reported findings of Tabogie and Diro (1992), and Karlsson et al. (2015), which reported longer shoot emergence for whole corms and shorter for corm splitting. However, the result was also contrary to Buke et al. (2016) report that day to 50% emergence almost at the same time.

The number of induced shoots was dependent on the size or age of the corms used; larger corms and more corms splitting produced more shoots. Splitting or cutting the corm into more pieces increased the number of suckers produced compared with whole corms. Therefore, compared to complete corms, half corms produced more than twice as many suckers, whereas quarter pieces produced three times as many plants. The observed large number of suckers in 3 years and quartered corm might be due to the high amount of stored reserves such as carbohydrates and growth hormone contributing to better regeneration at this age. Splitting into four also improved shoot production, and cutting the corm into many pieces has provided an increased surface area for callus formation. The result of the present finding is also in agreement with the previous results reported by Tesfaye (2002), who observed a higher shoot multiplication rate when using large or older (2 to 3 years old) corms than in smaller and younger corms (1 year old), and Diro et al. (1992), who obtained a higher number of suckers using quarter and half corm pieces than in whole corm proportion. However, in previous studies, it was reported that the number of suckers produced per corm was limited to between 40 and 141 suckers per corm (Diro et al. 1992, 2002; Tesfaye 2002). Nonetheless, in our work, the regeneration capacity of enset was much higher; up to 443 shoots per corm were recorded, depending on the age and proportion of corm used. In the present experiment aiming to optimize the age of parent plants and corm splitting using farmers' knowledge in Southwest Ethiopia, the present authors were able to produce a three-fold higher shoot multiplication rate compared to previous works. However, Tesfaye and Haile's most recent work (2024) reported up to 500 shoots per corm, which is consistent with our current observations. This suggests that the presently optimized practice (3-year-old parent plant and quartered corm type) could be taken as the most efficient method to regenerate enset in vivo.

Hence, shoot regeneration depends on the corm's carbohydrate reserves; differences in carbohydrate levels, nutrient status, or other growth-contributing resources within the corm could affect shoot regeneration potential. It seems that the physiological ability of the parent enset corm to initiate strong regeneration of suckers reaches its optimum between 2 and 3 years old. Another possible explanation for the observed low proportion of regeneration in quartered corms may be due to an increased incidence of rotting on more sliced corms, both in extremely younger and older age groups. This finding is in agreement with previous reports by Tesfaye (2002), who observed the higher regeneration frequency using corms of 2 to 3-year-old parents, and Diro et al. (2002), who recorded higher regeneration percentages from half corms than in whole corm.

In all studied treatments, sprouting of the highest proportion of shoots was obtained between 6 and 7 weeks after removal of the shoot apex. Since the studied combination of different ages and corm proportions showed different multiplication capacities, as discussed somewhere else, the number of observed new shoots in different time intervals also varied depending on age and corm proportion. The highest number of shoots was recorded from quartered corms of year three plants, followed by quartered corms from year four. In general, treatment combinations that exhibited the best shoot multiplication capacity also showed the best rate and speed of shoot induction. The observed occurrences regarding the rate and speed of shoot induction are somehow in agreement with Tesfaye's report about shoot morphogenesis in enset, where the development of enset buds from callus into shoot occurs after 6 weeks of injury (Tesfaye 2002).

In terms of shoot early growth, the regenerated suckers from whole and older (lager) corms showed better early growth for plant height, leaf length, leaf width, number of leaves, pseudo-stem height, and pseudo-stem circumference. This may have to do with the higher stored reserve in the corm to support the growth of suckers. On the other hand, the lowest values were recorded from quarter corms sourced from younger parent plants. This might be due to the lower storage reserve in the younger or smaller corms to support the growth of regenerated suckers. This finding was in agreement with previous reports of Diro et al. (2001) and Buke et al. (2016), which showed the influence of corm pieces, their proportion, and their age on early shoot growth. A corm with a high store reserve appears to produce vigorous shoots due to lesser competition for resources at their earlier growth stages. However, no significant ($p > 0.05$) correlation was observed between the regenerated shoot number and all measured growth parameters (Table 4). Similar phenomena were reported by Haile et al. (2021) based on the experiment conducted on wild enset, and they suggested that corms of enset plants possess enough stored reserve to support the early growth of regenerated shoots regardless of the number of shoots obtained per corm.

Conclusions

In general, the overall result of the current study showed that a quarter corm proportion sourced from a 3-year-old parent plant exhibited superior performance in terms of shoot multiplication capacity. In addition, the achieved regeneration capacity of enset in this experiment (up to 443 shoots per corm) was about three-fold higher than the previous report of enset multiplication capacity, suggesting presently optimized practice as the most efficient method to regenerate enset in vivo. Therefore, the current research indicates that quartered corm proportion and enset corm with a diameter of approximately 29–38 cm, which were taken from a 3-year-old plant, can be utilized to successfully and efficiently regenerate enset plants in vivo. As a result, by showcasing an effective, low-cost approach of field regeneration, this study will help smallholder farming communities improve their food security.

Author contributions

FB conceived and designed the experiments, performed field experiments, analyzed and interpreted the data, wrote the draft version of the manuscript, BH proposed the idea, involved in designing and conducting the experiment. Both authors contributed to revising and editing the manuscript. Both authors read and approved the final manuscript.

Availability of data and materials

The data used to support the findings of this study are available from corresponding author upon request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflicting interests.

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