Journal of Science and Development

Volume 12 No. 2, 2024

HAWASSA UNIVERSITY

Journal of Science and Development

Volume 12, No. 2 2024

ISSN (Online): 2789-2123; (Print): 2222-5722

Contents

Journal of Science and Development, JSD

Original Research Article||

Genetic Considerations for Species Choice and Seed Procurement in Ethiopia's Forest Restoration Initiatives

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Abstract

Global commitments to large-scale tree planting and forest restoration are increasing to help restore degraded ecosystems. Ethiopia has pledged to restore 22 million hectares of degraded land, undertaking massive forest restoration campaigns under the umbrella of the "Green Legacy Initiative," with billions of tree seedlings reportedly planted annually. Genetic and ecological research underscores that using native tree species with high genetic diversity is essential for restoration success. This study assessed the consideration of genetic principles in species choice and seed procurement in Ethiopia's restoration initiatives. Data was collected from surveys with seed vendors and nurseries, as well as secondary data from tree seed centers within the national tree seed network. The findings indicated that genetic considerations in species choice and seed procurement are often overlooked in current large-scale restoration practices. Species selection is mainly dominated by a few exotics—Grevillea robusta, *Eucalyptus camaldulensis*, *Acacia decurrens*, and *Cupressus lusitanica*—leaving native species underrepresented. Moreover, seed collection practices frequently disregard guidelines critical for preserving genetic diversity. Notably, 84% of seed collectors source from any available tree, 87% of nurseries receive seeds without passport data, 97% of seed collectors do not consider a minimum number of mother trees for a single collection event, and 88% ignore the required distances between selected mother trees, risking inbred seed collection. These gaps threaten the evolutionary resilience and adaptive capacity of planted seedlings, impacting the long-term success of restoration efforts. To improve outcomes, EFD and other relevant authorities leading the restoration initiatives should devise policies that promote native species use and enforce genetic standards in seed procurement.

Key words: forest restoration, germplasm, genetic diversity, seed procurement, tree nurseries, tree seed

Original submission: November 05, 2024; **Revised submission**: December 08, 2024; **Published online**: December 30, 2024

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INTRODUCTION

Deforestation, land degradation, climate change, desertification, and biodiversity loss continue to pose significant challenges to ecosystems worldwide. One widely adopted strategy to mitigate these environmental crises is forest ecosystem restoration. This imperative action has spurred large-scale tree planting initiatives and ambitious global reforestation commitments (Broadhurst et al., 2008; Thomas et al., 2014; Lamb, 2018; Fagan et al., 2020). Notable examples include the Green Wall of China, spanning 4,500 km and covering 35 million hectares since its inception in 1978, and the Great Green Wall of Africa, which aims to plant a 7,775 km-long tree belt across the Sahel. Initiatives such as the Bonn Challenge and the African Forest Landscape Restoration (AFR100) aim to restore 350 million hectares of degraded land globally, 100 million hectares of which are in Africa, by 2030 (Bozzano et al., 2014; Pistorius et al., 2017; Verdone and Seidl, 2017). To further inspire and accelerate these global restoration initiatives, the United Nations (UN) has designated 2021–2030 as the UN Decade on Ecosystem Restoration

(https://www.decadeonrestoration.org/), followed by the World Economic Forum's 1 Trillion Trees Initiative in support of the UN's goals (Aronson et al., 2020). However, many reforestation projects around the globe have faced limited success, often due to mismatches between planting material and site conditions, poor genetic quality of planting stock, and inadequate management practices (Thomas et al., 2014; Méndez-Toribio et al., 2021).

In Ethiopia, deforestation has been a persistent challenge, dating back to 500 BC (Darbyshire et al., 2003) and re-intensifying since the 16th century (Pohjonen & Pukkala, 1990). Natural forest cover continues to decline (Reusing, 2000; Dessie & Kleman, 2007; Demissie et al., 2017; Etefa et al., 2018), with recent losses confirmed by the FAO (2020). A rapidly growing population exceeding 120 million has intensified pressures on forests, driven by agricultural expansion and wood extraction for household energy (Stebek, 2008; Kindu et al., 2015), resulting in land degradation, erosion, biodiversity loss, and forest fragmentation (Kassa et al., 2017; Mengist et al., 2022). Efforts to combat deforestation began in the 1890s with the introduction of eucalyptus species and expanded in the 1970s with large-scale plantations of fast-growing exotic species of Eucalyptus, Cupressus, and Pinus (Ayana et al., 2013; Lemenih & Kassa, 2014). Recent approaches such as the "climate-resilient green economy strategy" aim to double forest cover by 2025 and cut greenhouse gas emissions by 50% by 2030 (FDRE, 2011; MEFCC, 2018). Ethiopia has also pledged to restore 22 million hectares of degraded land through global programs like the Bonn Challenge and AFR100 (Pistorius et al., 2017; Kassa et al., 2022). Massive tree-planting campaigns, including the "Green Legacy Initiative," are central to these efforts, with billions of tree seedlings reportedly planted annually to restore degraded landscapes (Fikreyesus et al., 2022; Kassa et al., 2022).

While plantations of exotic species in Ethiopia have supported fuelwood and timber supplies, their role in ecosystem restoration remains questionable. Exotics often underperform in maintaining soil quality (Lemenih et al., 2004; Demessie et al., 2012), water-use efficiency (Gindaba et al., 2004, 2005), biodiversity (Abiyu et al., 2011), and pest resistance (Demeke, 2018). Some of them are even becoming invasive (Shiferaw et al., 2004, 2019). Whether exotic species still dominate large-scale planting efforts or if native species are now prioritized remains unclear. Restoration success also depends on the genetic quality of germplasm, which is crucial for resilient and self-sustaining populations capable of adapting to environmental challenges (Broadhurst et al., 2008; Thomas et al., 2014). The extent to which tree seed collections in Ethiopia follow established guidelines, such as those from the World Agroforestry Centre, ICRAF (Kindt et al., 2006), and the Royal Botanical Gardens, Kew (2003), which recommend sampling from at least 30 widely spaced trees to avoid inbreeding, is largely unstudied. A few available studies highlight gaps in the tree seed system and the overlooking of guidelines, with collections often relying on limited mother trees or fragmented populations, leading to reduced genetic diversity (Dedefo et al., 2016; Mehari et al., 2024). As many tree species are naturally outbreeding and carry a genetic load of deleterious recessive alleles (Lowe et al., 2005; Broadhurst & Boshier, 2014), seed collection from isolated trees often results in inbred progeny with slower growth, higher susceptibility to stresses, and reduced resilience (White et al., 2007; Tata et al., 2023). This jeopardizes long-term sustainability as today's plantations often serve as future seed sources. Ensuring genetically diverse germplasm is thus essential for restoration initiatives to yield viable, self-sustaining, and resilient landscapes.

Formal evaluations of the large-scale tree planting campaigns are rare, yet anecdotal reports suggest that seedling survival rates are low, with some estimates indicating that less than 40% of seedlings survive beyond the initial establishment phase (ENA, 2019). A recent study in Tigrai, northern Ethiopia, reported a survival rate of only 53% for planted seedlings, with overall plantation success deemed unsatisfactory (Berhe et al., 2024). However, even these short-term survival rates may not accurately predict long-term restoration outcomes. Effective landscape restoration requires establishment of initial seedling which is accompanied by long-term indicators such as growth, maturation, and reproductive capacity (Le et al., 2011; Bozzano et al., 2014; Thomas et al.,

2014). Ethiopia's 10-year national forest development program document (MEFCC, 2018) acknowledges that "… billions of seedlings are planted each year in the country but hardly grow to become a forest." While poor site conditions and inadequate silvicultural management are often blamed for these failures (MEFCC, 2018; Magaju et al., 2020), the role of genetic quality of the germplasm in planting success remains largely overlooked.

This study aims to assess the extent to which genetic considerations are factored into tree species selection and seed procurement for ongoing forest restoration initiatives in Ethiopia. The results will have substantial practical implications for restoration authorities and practitioners, informing future efforts to enhance the genetic quality of planting material and improve the long-term sustainability of restoration projects.

MATERIALS AND METHODS

Data Sources and Types

Both primary and secondary data were utilized in this study (Table 1). Primary data were collected through a survey of 24 tree nurseries and 23 seed suppliers or vendors (Figure 1). The nurseries and vendors were identified in consultation with the former South Nations, Nationalities, and Peoples Region (SNNPR) Forest and Environment Bureau, the SNNPR Tree Seed Center, and the SNNPR REDD+ Coordination Office. At the time of sampling, the region had not yet been divided; however, since it has been split into four new regions, with the sampled districts now fall within three of them—Sidama, South Ethiopia, and Central Ethiopia. Initially, 14 active tree seed cooperatives, organized by the SNNPR Tree Seed Center and distributed across 11 districts (woredas), were identified and included in the survey.

Private seed vendors, as well as public tree nurseries operating in these districts, were also included in the survey. Since no formal registry of private seed vendors existed, a snowball sampling approach was used to identify them. Additionally, five tree nurseries managed by the regional REDD+ Coordination Office were incorporated into the survey. Once the nurseries and seed vendors were identified, data were gathered through interviews with nursery foremen, cooperative leaders, and individual seed vendors using semi-structured questionnaire. Key informant interviews were also conducted with the heads of the regional Tree Seed Center and the REDD+ Coordination Office.

Secondary data were sourced from the Telegram page of the Ethiopian Tree Seed Network (https://t.me/Ethiopian_TSN). These data comprised monthly seed availability reports from three tree seed centers that regularly posted seed balance updates between January 2020 and August 2022. These centers were the Ethiopian Environment and Forest Research Institute (EEFRI) in Addis Ababa, the Dimma Tree Seed Center in Sebeta (Oromia Region), and the Bahir Dar Tree Seed Center (Amhara Region).

Table 1. Survey entities used as data sources for this study

Survey entities	Data type	Source	No. of entries
National tree seed network			3
EEFRI	secondary	https://t.me/Ethiopian_TSN	
Dimma tree seed center	secondary	https://t.me/Ethiopian_TSN	
Bahirdar tree seed center	secondary	https://t.me/Ethiopian_TSN	
Nurseries			
Government/public	primary	own survey	17
NGO/project	primary	own survey	5
Private	primary	own survey	2
Seed suppliers/ vendors			
Cooperatives	primary	own survey	14
Private	primary	own survey	9

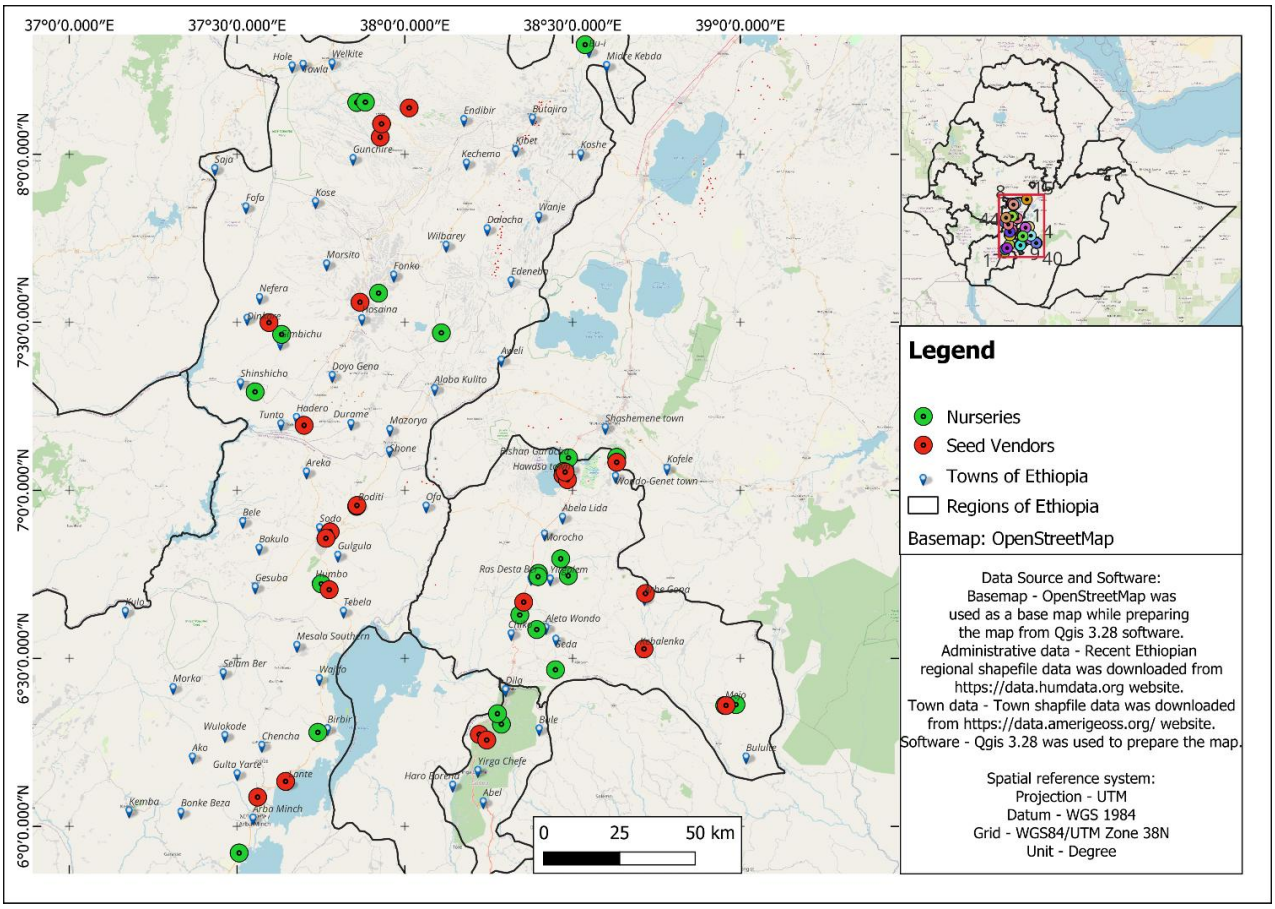

Figure 1. Map showing the locations of surveyed tree nurseries and seed vendors

Data Analysis

The survey data were coded and initially entered into MS Excel. After organizing the data, it was imported into the R statistical software for further analysis and visualization. Most analyses involved summarizing the data using the *dplyr* package (Wickham et al., 2023), followed by graphical visualization with the *ggplot2* package (Wickham, 2016). Additional R packages employed included *xlsx (*Dragulescu & Arendt, 2020) for importing Excel files and *patchwork* (Pedersen, 2024) for combining multiple plots. The results were presented as tables and graphs.

RESULTS AND DISCUSSION

Composition and Ranking of Tree Species Distributed by Vendors and Raised in Nurseries

Figure 2A presents the average seed quantity reported per species in a single monthly entry from the three seed centers. The data reveals that exotic species dominate the largest seed stocks, with the top six species all being exotics. Only two native species, *Cordia africana* and *Olea africana*, make it into the top ten. A larger average seed quantity may reflect either frequent restocking or a large initial acquisition that remains in stock without significant distribution. This dynamic is further explored in Figure 3, where Figure 3A shows the frequency with which species were reported as available, and Figure 3B tracks seed quantity trends over a 32-month period for the 12 most commonly reported species. *Sesbania aculeata*, for example, ranked highest in seed quantity (Figure 2A), but was not frequently reported, ranking 26th

in frequency (Figure 3A). Figure 3B reveals that *S. aculeata* was acquired in bulk in May 2020, remained stable in stock until March 2021, and was fully distributed by May 2021, with no further significant reports thereafter. In contrast, species like *Acacia decurrens*, *Eucalyptus camaldulensis*, and *Cupressus lusitanica* exhibited periodic fluctuations, indicating more frequent acquisitions and distributions over the entire period.

In Figure 2B, seed availability is ranked by the estimated number of seeds per species, further highlighting the dominance of exotic species over native ones. The number of seeds, which develop into potential seedlings in nurseries, is disproportionately higher for exotics. This dominance is even more evident in Figure 2B, where native species are nearly invisible when plotted on the same scale as the exotics. The disparity can be attributed to the finer seeds of exotic species, which yield significantly more seeds per kilogram compared to native species. For instance, exotic species like *Eucalyptus camaldulensis* (1,887,507 seeds/kg), *Eucalyptus saligna* (2,101,282 seeds/kg), and *Cupressus lusitanica* (156,739 seeds/kg) far outnumber the native species *Cordia africana* (6,141 seeds/kg) and *Olea africana* (10,020 seeds/kg), despite these natives being reported in relatively higher quantities in Figure 2A.

Figure 2C breaks down the average amount of seed reported for each species by the three seed centers. Exotic species are clearly dominant in both the Bahirdar and Dimma seed centers, while the Ethiopian Environment and Forest Research Institute (EEFRI) center reported a relatively higher presence of native species. The Bahirdar and Dimma centers also reported higher seed quantities for most of the species compared to EEFRI. Notably, *Acacia decurrens*, *Susbania aculeata*, and *Leucaena leucocephala* have the highest seed quantities at Bahirdar, Dimma, and EEFRI, respectively.

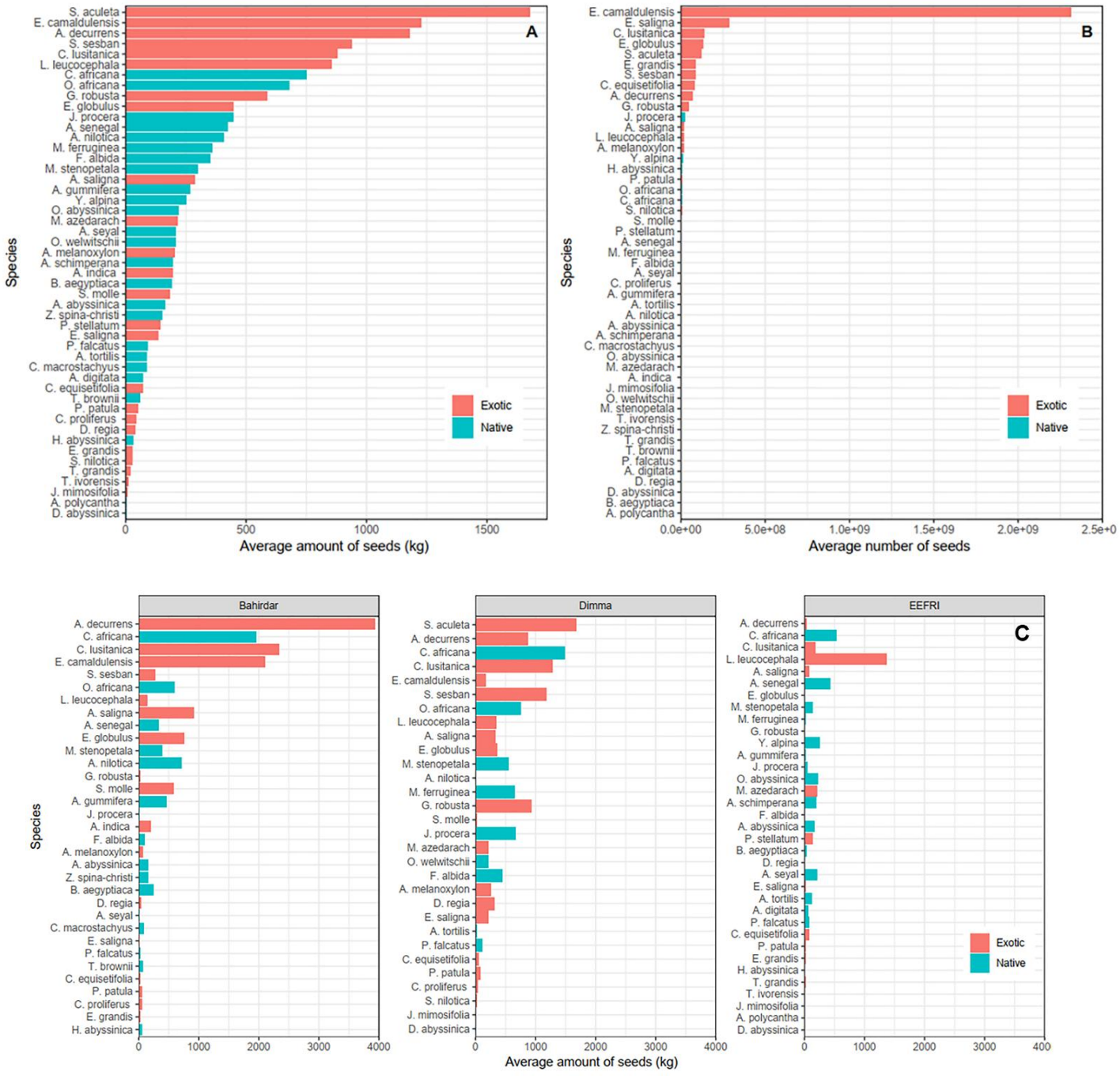

Figure 2. Tree seed availability per species reported by three centers of the national tree seed network of Ethiopia. A) Total amount of seed (in kg) for each species, averaged across all monthly reports.; B) Estimated average number of seeds per species, derived from the average seed amounts reported in A; C) Average seed amounts (in kg) reported by each center (Bahir Dar, Dimma, and EFFRI) for each species. Species are categorized as either exotic (shaded red) or native (shaded blue)

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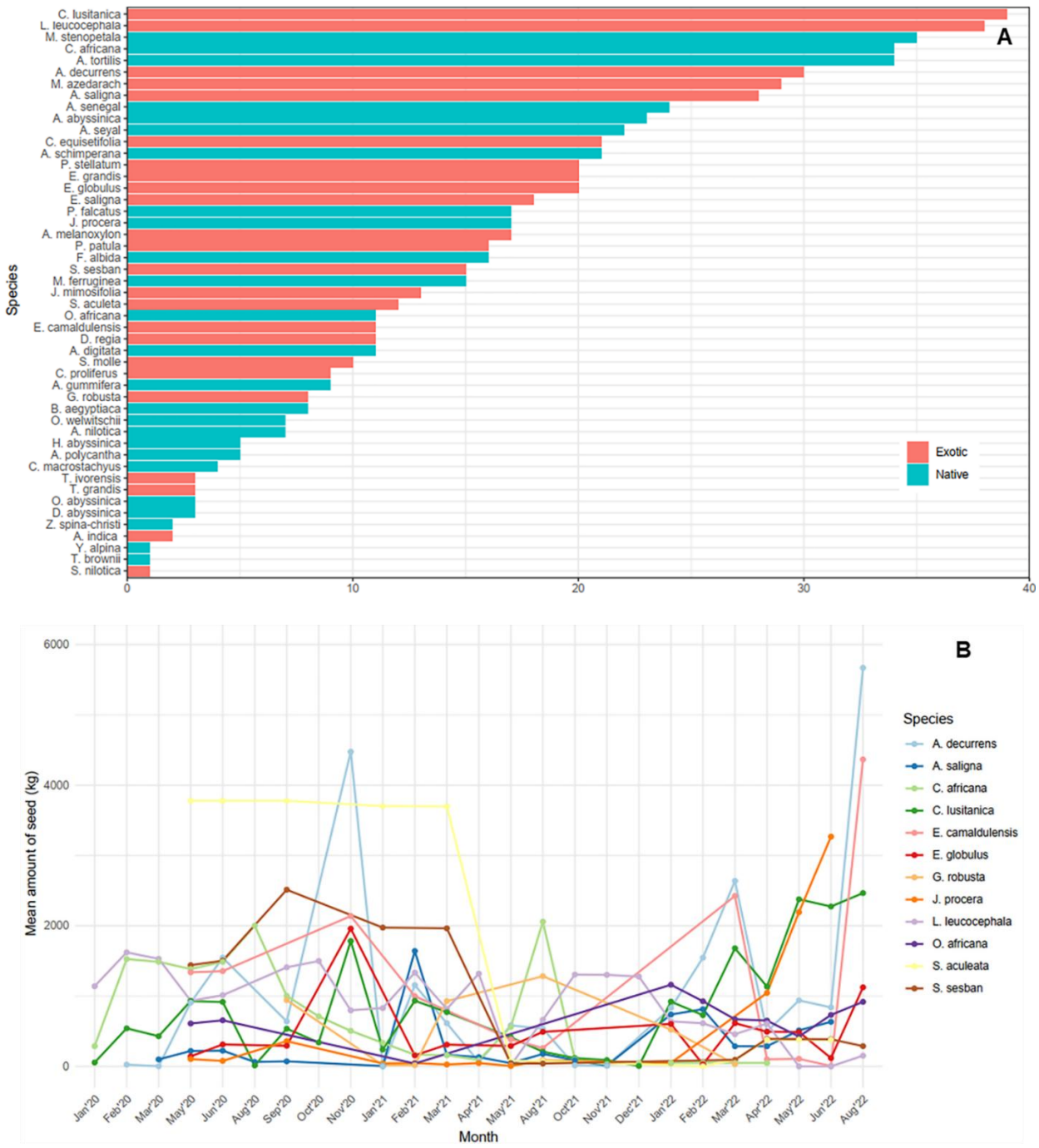

Figure 3. Commonality of Species in the Tree Seed Balance Report. A) The frequency of each species reported in the seed balance by the three seed centers; B) The trend of 12 common species reported over a 32-month period.

Lastly, Figure 4 presents the species preference rankings based on primary survey data from tree nurseries and seed vendors in southern Ethiopia. *Grevillea robusta* ranked first in terms of the volume of seeds and seedlings distributed, followed by *Cupressus lusitanica*, both of which are exotic

species. Among the native species, *Cordia africana* and *Afrocarpus gracilior* were relatively preferred, but the gap between them and the exotics—particularly *G. robusta*—was substantial. Overall, the results from both primary and secondary data sources indicated that exotic species dominated germplasm distribution for tree planting activities in Ethiopia.

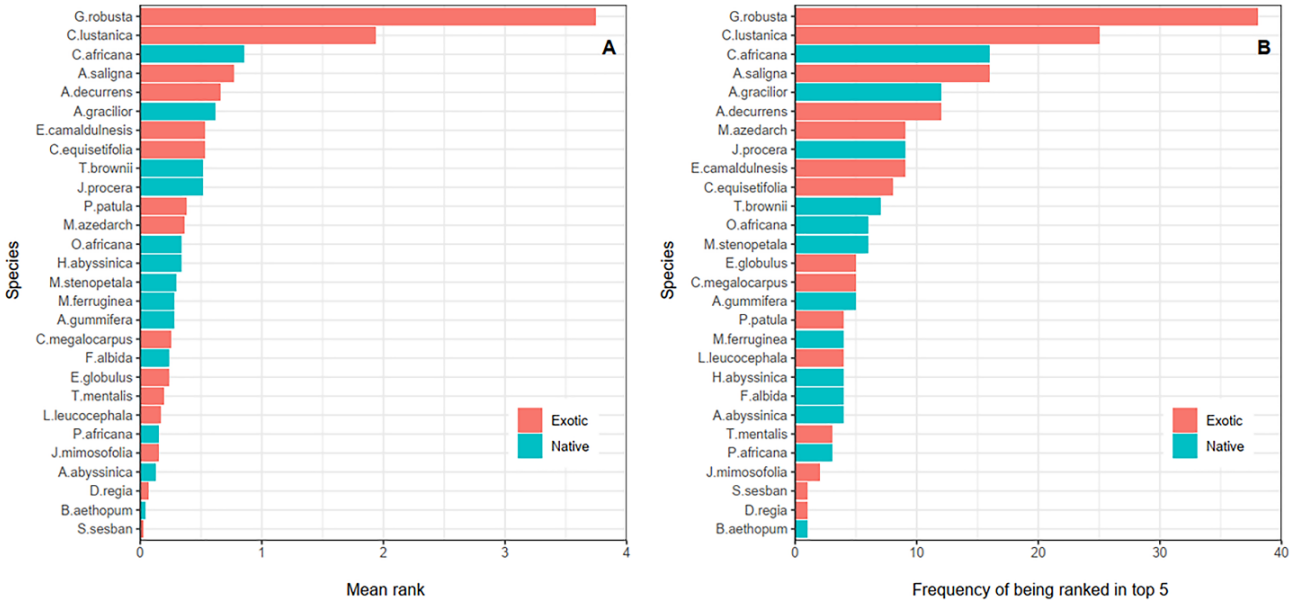

Figure 4. Rank of species preference based on surveyed nurseries and seed vendors. A) Mean rank (on a scale of 1 to 5, with 5 being the highest) for each species, with unranked species scored as 0 in the calculation.; B) The frequency of each species being ranked among the top 5.

Seed Procurement Practices by Nurseries and Seed Vendors

Figures 5, 6 and 7 illustrate the tree seed procurement practices of nurseries and seed vendors. Half of the nurseries surveyed fully outsource their seed requirements, while an additional 46% combine outsourcing with their own seed collection (Figure 5A). This means that 96% of the nurseries rely on some form of outsourced seed to meet their production needs. When outsourcing, 52% of nurseries acquire seed through an open bidding process, 22% purchase directly from local vendors, and another 22% use a combination of both methods (Figure 5B). Among the seed vendors, 65% are cooperatives whose members participate in seed collection, whereas 26% of vendors fully outsource their seed supply, purchasing seeds only after winning bids to provide nurseries (Figure 7B). Regarding seed populations, 84% of seed collectors utilize any available tree or population as a seed source, 10% focus exclusively on natural forests, and only 3% each collect seeds from designated seed production areas and plantations or provenances (Figure 6A). Moreover, only 59% of seed collectors select mother trees based on phenotypic superiority, while the remaining 41% collect from any tree as long as it is seeding (Figure 6B).

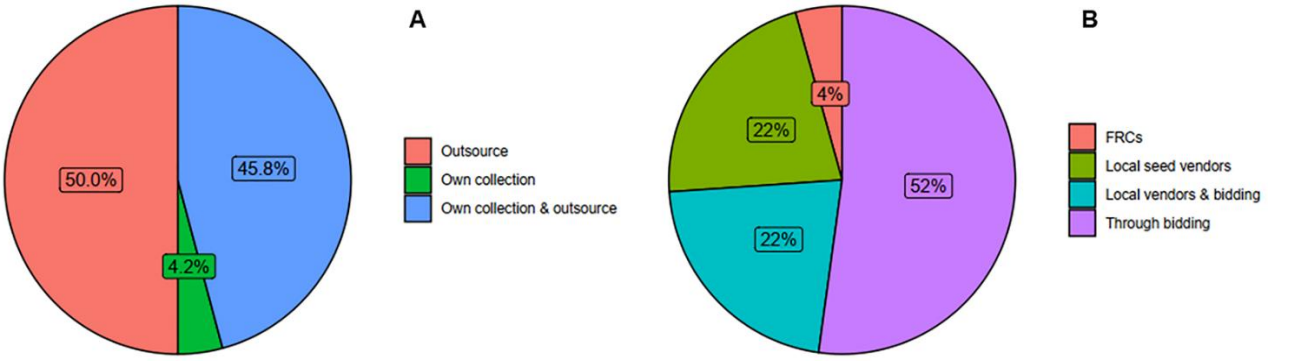

Figure 5. Tree seed procurement approaches by the surveyed nurseries. A) Whether nurseries conduct their own seed collection or outsource their seed supply; B) Methods used by nurseries for outsourcing seed procurement.

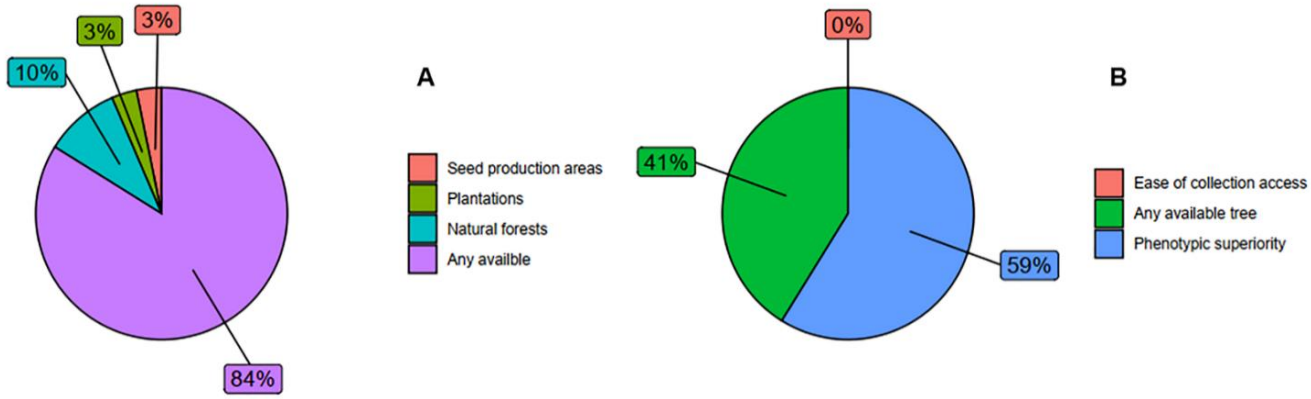

Figure 6. Survey responses on seed source population and mother tree selection. A) Types of populations from which seeds are collected; B) Criteria used for selecting specific mother trees

The majority of tree seed vendors (96%) reported having the required license to operate in this business (Table 2). However, when examining the authorities responsible for issuing these licenses (Figure 7A), the forestry sector is notably absent from the process. Instead, licenses are granted by the Cooperative Commission (65%), the Bureau of Agriculture (26%), and the Trade and Industry Commission (9%), primarily as a legal formality to run the business like any other commodity, without accounting for the peculiarities of the tree seed system.

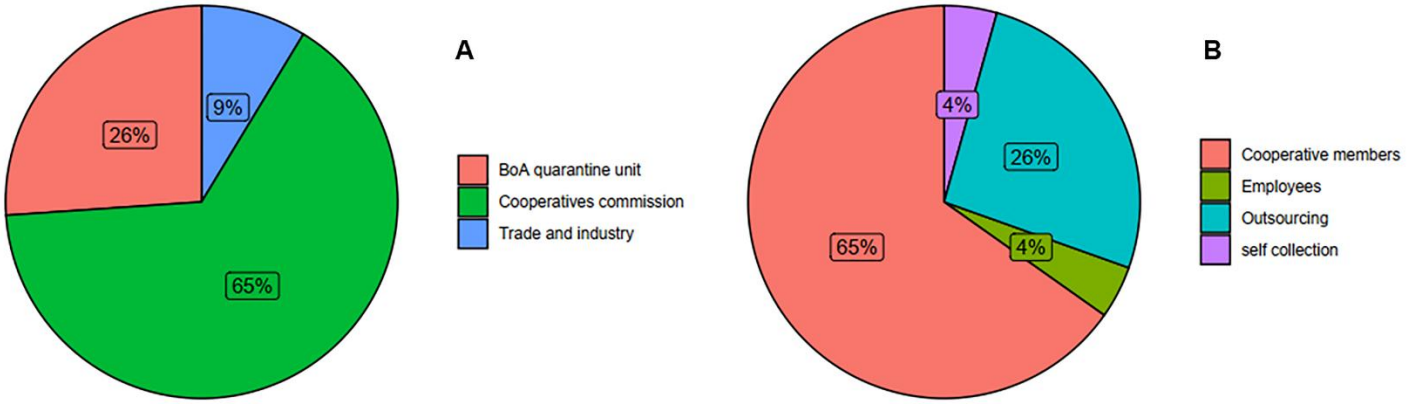

Figure 7. Licensing authorities and seed procurement methods of seed vendors. A) Authorities responsible for issuing licenses to seed vendors; B) Methods used by seed vendors to procure seeds

Table 2. Survey responses on giving genetic consideration during seed sourcing

No.	Questions	Responses		Proportion		Total responses
		Yes	No	Yes (%)	No (%)	
1	Do nurseries receive pertinent information (passport data) on the seed they outsource (e.g., location, climate, altitude, test results)?	3	20	13	87	23
2	Do nurseries face germination issues with outsourced seeds?	20	3	87	13	23
3	Do seed collectors consider the minimum number of mother trees when collecting seeds?	1	33	3	97	34
4	Do seed collectors account for minimum distances between mother trees during collection?	4	30	12	88	34
5	Do seed collectors follow established guidelines for seed collection?	19	10	66	34	29
6	Have seed collectors received training in seed handling?	24	10	71	29	34
7	Do seed vendors have the required license to operate?	22	1	96	4	23

Genetic Considerations in Seed Sourcing

Table 2 summarizes the responses regarding factors affecting the genetic quality of procured tree seeds. A significant proportion of nurseries (87%) reported that the seeds they purchase do not come with labels (or passport data) providing critical information such as the location, seed source population, collection date, or seed test results. The same percentage also noted experiencing germination issues with their outsourced seeds. Furthermore, 97% of seed collectors do not consider a minimum number of mother trees when collecting seeds, and 88% do not consider minimum distances between mother trees, which increases the risk of collecting genetically-related seeds, leading to inbreeding. While 66% of seed collectors claimed to follow established guidelines for tree seed collection, none of the surveyed vendors actually possessed written guidelines. Instead, they relied on training they had received, using their 'knowledge' from these trainings as a substitute for formal guidelines. About 70% of seed collectors indicated they had received some level of training in tree seed handling.

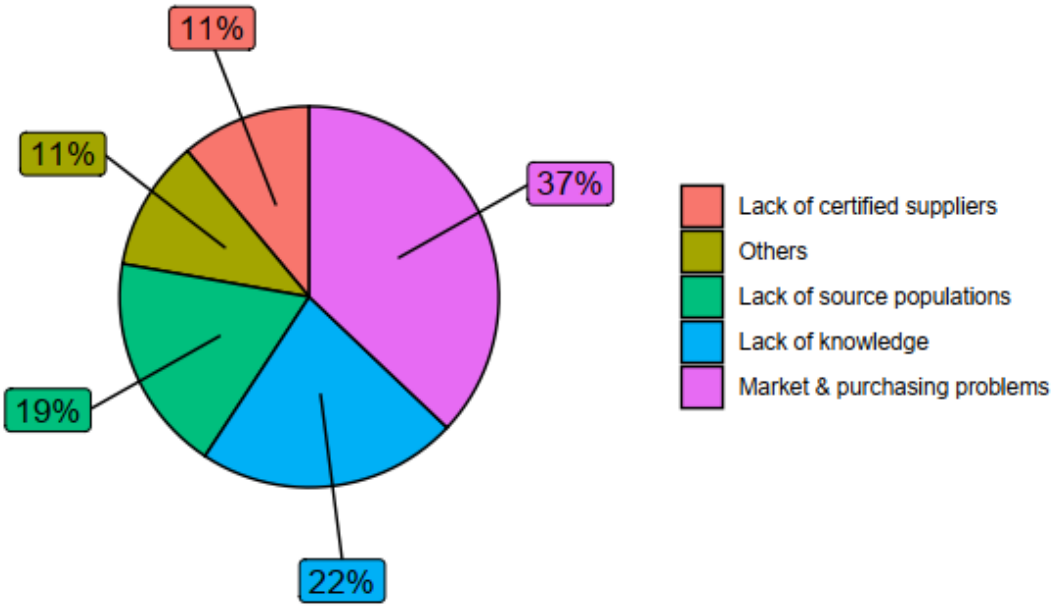

Figure 8. Major seed procurement challenges identified by tree nurseries and seed vendors

Figure 8 highlights the major challenges perceived by survey participants in procuring high-quality tree seeds. The most frequently cited challenge (37%) was the lack of market access, primarily related to the government's bidding-based purchasing system. This was followed by a lack of knowledge and awareness in tree seed collection and handling (22%), and a shortage of good source populations for seed collection (19%). Regarding market issues, respondents expressed concerns that the bidding system allows a few private dealers to dominate the market. These dealers, despite not collecting their own seeds, win bids and then procure any available seed afterward. Respondents attributed this to the government's open bidding system, which doesn't prioritize vendors offering higher-quality seeds but who cannot compete with private dealers on price. They also voiced concerns about corruption, noting that a few regular dealers, leveraging their financial influence, repeatedly manipulate the system to secure winning bids.

DISCUSSION

Native versus Exotic Tree Species

The predominance of exotic species in germplasm distribution for tree planting (Figures 1 – 4) reflects a longstanding preference for fast-growing non-native species over native trees—a trend persisting in current large-scale forest restoration initiatives as well. This preference is largely due to the rapid growth rates typical of exotic species, contrasting with the slower growth and limited silvicultural knowledge available for many native species (Lemenih & Kassa, 2014; Zeleke & Vidal, 2020; Negash, 2021). Although substantial knowledge gaps existed in the domestication, propagation, and management of native trees when exotic species were historically introduced, recent advances in native tree species

propagation techniques now offer alternatives (Negash, 2010; 2021). Yet, nurseries and restoration practitioners still primarily rely only on a limited range of exotic species, hindering the practical application of native species. There is no clear direction or legislative support in the country's 10-year national forest development program (MEFCC, 2018) or the Green Legacy Initiative (Fikreyesus et al., 2022; Beyene & Shumetie, 2023) regarding the extent or specific locations for native tree planting. From a restoration perspective, however, native species should be prioritized for their adaptability to local environmental conditions, support for biodiversity, and enhancing essential ecosystem functions (Thomas et al., 2014; Negash, 2021).

The present study also revealed regional and seed center differences in species preferences and germplasm distributions (Figures 2 and 4). For instance, the Bahir Dar seed center's large distribution of *Acacia decurrens* corresponds to its increasing expansion in northwestern Ethiopia, where it is widely used for charcoal making to meet urban energy needs (Nigussie et al., 2020; Chanie et al., 2021). Similarly, larger distributions of *Sesbania* (at Dimma) and *Leucaena* (at EEFRI) seeds likely result from their roles in agroforestry, particularly as fodder and green manure (Lupwayi et al., 1999; Mengistu et al., 2002; Oosting et al., 2011; Lebrazi & Fikri-Benbrahim, 2021).

In southern Ethiopia, *Grevillea robusta* stands out as the most widely preferred species, both in seed distribution and nursery cultivation (Figure 4). Its popularity arises from its versatile applications as an ornamental and commercial tree, used along roadsides, in parks, homesteads, farm boundaries, and woodlots in restoration efforts. Unlike eucalyptus (described below), *G. robusta* has not been linked to severe ecological impacts, making it a favored agroforestry species despite being a nonlegume. It is commonly intercropped with maize and beans on smallholder farms and serves as a shade tree for coffee and tea in East Africa and Asia (Lott et al., 2009; Kehlenbeck et al., 2011; Nesper et al., 2017). However, assessments results on its performance in intercropping are inconsistent; while some report minimal competition with crops (e.g., Bucagu et al., 2013), others describe significant below-ground competition with herbaceous crops (Smith et al., 1999; Lott et al., 2009). Nevertheless, *G. robusta* remains highly valued for its fast growth and commercial appeal—its wood is prized for fuelwood, construction, and timber (Niyomfura et al., 2022; Bhandari et al., 2023), while its contribution to carbon sequestration makes it attractive for environmental service payment schemes like REDD+ (Kiyingi et al., 2016; Owate et al., 2017). Given its widespread planting in Ethiopia, further research into its agroforestry interactions, ecosystem restoration potential, and timber quality could maximize its economic and ecological benefits.

Another notable finding in this study is the distribution of Eucalyptus germplasm (*E. camaldulensis*, *E. globulus*, *E. saligna*, and *E. grandis*), which was minimal across seed centers except at Bahir Dar (Figure 2). Eucalyptus species were neither highly ranked in species preferences by surveyed nurseries and seed vendors in southern Ethiopia (Figure 4) nor commonly found in public nurseries. The cultivation and distribution of eucalyptus seedlings appear to be discouraged in public nurseries, apparently due to ecological concerns surrounding the species. Nonetheless, farmers' preference for eucalyptus plantations remains strong, especially in northern Ethiopia (Jenbere et al., 2012; Molla et al., 2022; Yimam et al., 2024). Most seedlings for these plantations are either produced by the farmers themselves or supplied by private nurseries (MEFCC, 2018; Tesfaw et al., 2022). Eucalyptus's seeds, which lack hard coats and germinate readily without pretreatment or special nursery facilities, enable easy seedling growth and contribute to the widespread plantings. Additionally, the seeds are very small, allowing large numbers of seedlings to be produced from a small quantity of seed (Figure 2B).

Since its introduction to Ethiopian forestry over a century ago, eucalyptus has been both celebrated and criticized (Jagger & Pender, 2002; Abebe & Tadesse, 2014; Jaleta et al., 2016; Negash, 2021). Critics emphasize ecological concerns, including aggressive water and nutrient consumption that outcompetes other vegetation and allelopathic chemicals that suppress plant growth beneath its canopy, often leaving the ground barren, described as "green on top, but Sahara beneath" (Negash, 2021). Advocates, however, highlight its economic importance in meeting Ethiopia's growing wood demand, alleviating poverty, and protecting remnant natural forests. Its rapid growth, resilience, and capacity to provide consistent cash flow make it a favorite among farmers, with some calling it a "living bank account" (Zerga et al., 2021). Consequently, eucalyptus plantations are expanding rapidly, particularly in the Amhara region, where districts like Mecha and Sinan

have experienced substantial increases (Tesfaw et al., 2022; Molla et al., 2022; Yimam et al., 2024). Despite its benefits, this unregulated expansion poses risks to farmland productivity, food security, and ecological balance, underscoring the need for policies to balance economic gains with environmental sustainability (Bazzana et al., 2021; Alemayehu & Melka, 2022).

Seed Procurement Practices and Genetic Considerations

The survey results revealed that the tree seed procurement system is plagued by noncompliance with quality standards, with most nurseries relying on outsourced seeds with little passport data. Open bidding prioritizes low cost over quality, allowing private dealers to outcompete cooperatives whose members are trained in seed collection. Additionally, licenses issued by non-forestry authorities fail to address the unique needs of tree seed supply, resulting in unregulated participation by uncertified suppliers. This lack of regulation undermines the delivery of high-quality, certified tree seeds essential for successful restoration initiatives.

The results of this survey also indicated that seed dealers frequently overlook essential guidelines for genetic diversity in seed collection, such as those from ICRAF (Kindt et al., 2006) and the Royal Botanical Gardens, Kew (2003). These standards recommend collecting seeds from a minimum of 30 mother trees spaced 50–100 meters apart to reduce inbreeding and ensure genetic diversity. Although it is generally advised to collect small amounts of seeds from many trees rather than large quantities from a few (Rogers and Montalvo, 2004; Bozzano et al., 2014), this practice was not observed among the surveyed vendors. The neglect of these principles may stem from inadequate training or intentional oversight, as regulatory or incentive mechanisms for promoting high-quality seed collection are absent.

Germplasm collected through such practices is often inbred or lacks genetic diversity, which is essential for successful restoration. Seeds of low genetic diversity tend to produce seedlings with limited adaptation, potentially leading to higher mortality, slower growth, and reduced reproductive success over time (Rogers & Montalvo, 2004; Broadhurst et al., 2008; Broadhurst & Boshier, 2014; Thomas et al., 2014). Restoration projects that rely on germplasm from a few parent- trees risk establishing founder populations prone to inbreeding, ultimately reducing fitness in subsequent generations (Broadhurst & Boshier, 2014). Maintaining higher genetic diversity is especially important for restoration sites, which often face challenges like poor soil or low moisture, where inbreeding depression may intensify under such stressful conditions (Fox & Reed, 2010; Thomas et al., 2014; Sandner et al., 2021).

Current large-scale forest landscape restoration initiatives in Ethiopia (Kassa et al., 2022) are likely to face these challenges due to germplasm sourcing practices that capture potentially inbred and low-quality seed. This should be critically evaluated as the use of low genetic quality germplasm affects the success of both current and future restoration attempts. Forests established today are likely to become seed sources for future restoration activities. The Ethiopian Forestry Development (EFD) and other authorities leading restoration initiatives should devise policies, action plans, and regulatory frameworks to ensure the use of high-quality germplasm in restoration projects.

Some efforts are indeed underway to address these challenges, such as those by the EFD and the Provision of Adequate Tree Seed Portfolios (PATSPO) project, which is funded by the Norwegian government and implemented by ICRAF in Ethiopia (https://worldagroforestry.org/project/PATSPO-II). In partnership, the EFD and PATSPO have established a national Tree Seed Network (TSN) to create an effective tree seed system supporting the supply of quality tree seed for restoration efforts in Ethiopia (https://tss.epa.gov.et/ts-devt). However, the implementation of standards promoted by the TSN has largely failed to reach the grassroots level among seed vendors, nurseries, and other participants in the tree seed system. For example, the TSN has developed seed standards for about 30 tree species, specifying requirements for inspecting source populations, seed collection, physical characteristics, seed test results, and labeling. Unfortunately, stakeholders surveyed in this study were often unaware of these standards. Additionally, although TSN intends for seed provision to be done by network members, these practices were not widely followed in seed procurement processes surveyed. For instance, seed cooperatives included in this survey, which were organized by the southern Ethiopia tree seed center (a TSN member), were largely outcompeted by private seed dealers in the market despite providing better quality seed.

To ensure that high-quality germplasm reaches nurseries and that robust seedlings capable of adapting and sustaining on restoration sites are

planted, the EFD and PATSPO should work to implement these standards at the grassroots level. Seeds should be collected and procured following these standards, and provided to the market only by certified seed suppliers who are TSN members. Additionally, introducing niche or specialty markets exclusively for certified suppliers could incentivize quality, offering a better alternative to the current open bidding system.

CONCLUSIONS

This study revealed that genetic principles in species selection and germplasm procurement are largely overlooked in current large-scale forest landscape restoration initiatives in Ethiopia. Survey results showed that few exotic species, such as *Grevillea robusta*, *Acacia decurrens*, *Eucalyptus spp*., and *Cupressus lusitanica* dominate seed distributions and nursery preferences, with native species notably underrepresented. Seed procurement practices also fall short of adhering to standards and guidelines that ensure the capture of intraspecific genetic diversity available in seed source populations. Tree seeds are often collected randomly from any available tree, disregarding standard requirements such as the minimum number of mother trees needed for a single seed collection and the minimum isolation distance between two adjacent mother trees to avoid inbred seed collection. The seeds also lack proper labels (passport data), making it impossible to trace them back to the source populations and match the afforestation sites with seed provenances. These practices risk the loss of evolutionary adaptive potential associated with genetically diverse seeds, which is crucial for survival and long-term sustainability of restoration projects.

These suboptimal practices in sourcing genetic material for forest restoration initiatives call for the EFD and other relevant authorities leading the restoration initiatives to devise policies and regulatory instruments that increase the share of native species in restoration projects and ensure the procurement of high-quality tree seeds. For seed sourcing, this includes strict inspection to ensure seeds are collected and distributed following set standards, and making sure only certified seed dealers participate in the seed market. To facilitate this, the EFD should seek authorization to license and regulate seed dealers, enabling stricter enforcement of compliance and greater control over seed quality. Additionally, the EFD needs to undertake assessments on the performance of restoration projects, including survival, growth, and genetic diversity studies comparing natural forest populations with those in restoration plantations.

CONFLICTS OF INTEREST

Authors declare that they have no conflicts of interest regarding the publication of this paper in the Journal of Science and Development.

ACKNOWLEDGEMENT

The authors acknowledge the Institutional Collaboration Project between Hawassa University and the Norwegian University of Life Sciences – NORAD IV – for providing financial and logistical support for the field survey. Additionally, we would like to extend our heartfelt gratitude to the various nursery foremen, tree seed cooperative leaders, tree seed vendors, heads of tree seed centers, REDD+ officers, and regional and district level officers of the Agriculture and Natural Resource Bureau who participated in and/or facilitated the survey.

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Original Research Article||

Distribution and virulence diversity of wheat stem rust races (*Puccinia graminis* f.sp. *tritici*) in Amhara and Oromia regions, Ethiopia

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Abstract

Wheat stem rust (*Puccinia graminis* f.sp. *tritici*) is the major constraint to wheat production worldwide. Because of the sudden changes in stem rust pathogen race patterns, commercial wheat varieties become vulnerable to the pathogen. The present study aimed to identify the races of stem rust pathogen and examine virulence diversity in the pathogen population in the study areas. To achieve these, surveys were conducted in 464 wheat fields across the Amhara and Oromia regional states of Ethiopia during the 2017 cropping season. Four to six wheat stem samples infected with *Puccinia graminis* f.sp. *tritici* (Pgt) were collected from each farmer and experimental station, where wheat is a key crop and stem rust is known to occur following the international stem rust live sampling collection protocol. Sixty stem rust-infected wheat samples were collected from the study areas, of which only 48 were viable. Three races of the stem rust pathogen, namely, TKTTF, TTKSK, and TTRTF were identified from 48 isolates. Of these, TTRTF which was detected in both regions is a new race for Ethiopia. Most of the genes possessed by the differentials were ineffective against one or more of the tested races except Sr24. The most important resistance gene Sr24, present in the majority of Ethiopian wheat cultivars, was effective against all races identified in this study. Thus, the use of effective Sr genes, either individually or in combination, will be crucial for developing wheat cultivars with a broader base of stem rust resistance. Regular monitoring of further virulence evolution is recommended to identify the emergence of new races for future breeding programs. Due to the limited number of samples analyzed in this study, future study with a large sample sizes is recommended to draw more conclusive findings regarding the Pgt population in the study areas.

Key words: Phenotype, Race analysis, Virulence evolution, Wheat rust

Original submission: October 03, 2024; **Revised submission**: November 23, 2024; **Published online**: December 30, 2024

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INTRODUCTION

Wheat (*Triticum aestivum* L.) is among the leading cereal crops grown worldwide. Annually, it is produced on an area of over 220 million hectares globally (FAOSTAT, 2021). The crop is grown at altitudes up to 3000 meters above sea level and latitude ranges of 30o and 60oN to 27o and 40oS (Deng et al., 2005). Wheat is among the major crops cultivated in the Ethiopian highlands. Ethiopia is the leading wheat producer in Sub-Saharan Africa (FAO, 2021). Wheat is produced on over 1.8 million hectares of land under rainfed conditions and over 3 million ha under irrigation with about 3.1 metric tons/ha annual average production which is by far below the world's average yield (3.5 tons /ha) (CSA, 2022).

Biotic factors such as diseases, insect pests and weeds are among the factors that constraints wheat production in Ethiopia (Abebe et al., 2012). Among these, wheat stem rust (*Puccinia graminis* f. sp. *tritici*) (Pgt) is the most prevalent of all wheat rusts in the country (Admassu et al., 2012; Denbel et al.,

2013). The disease causes complete destruction of the crop over wide areas during epidemic years (Hodson, 2015; Mulu et al., 2022). In Ethiopia, the average yield loss due to stem rust during 2013-2014 was 51% but the disease caused 100% yield loss in some fields and forced the widely cultivated wheat variety Digelu to be out of production (Olivera et al., 2015).

The ability of the stem rust pathogen to quickly form new races makes it difficult to control the disease. According to Singh et al., (2015), genetic recombination and mutation are important mechanisms of new race formation for rust pathogens. Ethiopian highlands are hot spots for the development and dissemination of new stem rust races (Leppick, 1970; Singh and Rajaram, 2006; Periyannan et al., 2013). Most of the previously identified Pgt races were virulent on wheat varieties grown in the country (Admasu et al., 2009; Belayneh and Emebet, 2005). Resistant varieties can be used as an effective method for controlling stem rust (DRRW, 2010). Knowledge of the pathogen virulence diversity, distribution of races and the identification of resistance genes in the host are required for the development of rust-resistant varieties. In Ethiopia, wider virulence diversity of races, the frequent evolution of the pathogen, and the narrow genetic base in wheat lines make the released wheat varieties break up their resistance within short period.

Virulence surveys and pathotype analysis are important to studying the evolution of new races and forecasting the virulence shifts in a pathogen population. These can be used to assesses the origin, occurrence, and dissemination of new pathotypes and to understand how new pathotypes develop. Such studies also help to determine the degree and range of pathogenic variation in particular regions. This study was therefore carried out to identify the physiological race(s) of the wheat stem rust pathogen (*Puccinia graminis* f. sp. *tritici*) in the selected regions of Ethiopia and determine the virulence diversity of the races.

MATERIALS AND METHODS

Description of the Study Area

The wheat stem rust surveys were carried out in the main wheat growing areas of Amhara and Oromia regional states of Ethiopia during the 2017 cropping season (Fig. 1). The two regional states contribute 70% of domestic wheat production. A total of 464 wheat fields were surveyed, of which 224 fields were from Oromia and 240 from Amhara Regional States. The study areas in the Amhara region are located within 10.06172 to 11.96160 N latitude and 36.51448 to 39.64268 E longitudes and had altitude ranges of 1804 to 3450 m.a.s.l. In Oromia regional state, the disease surveys were carried out at altitudes between 1704 and 3334 m.a.s.l, within latitudes of 6.55038 to 8.93800 N and longitudes of 37.16835 to 39.28679 E.

Zones and districts from both regional states were selected based on their wheat production potential. The Bale, East Arsi, and West Arsi areas in Oromia are traditionally referred to as “The Ethiopian Basket of Bread” while East Gojjam, West Gojjam, and North Wello areas in the Amhara regional state are commonly known as “Wheat belts of Amhara”.

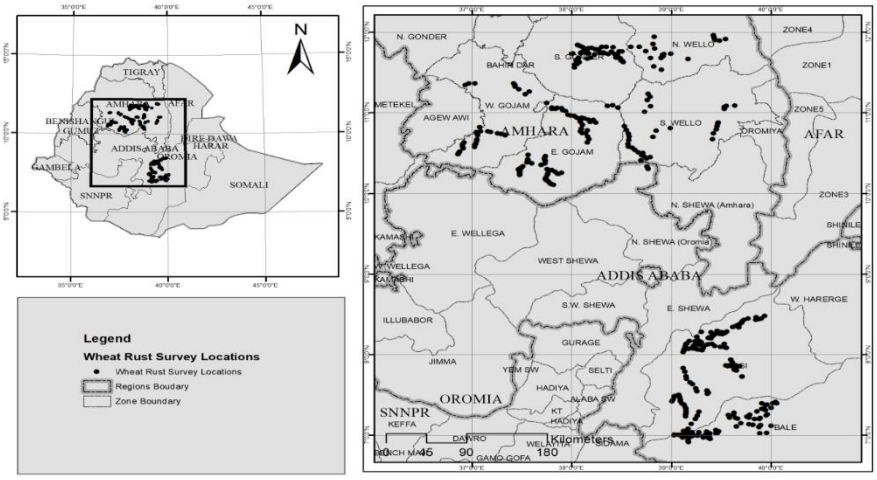

Figure 1. Map showing wheat stem rust survey areas

Rust Samples Collection

Four to six stem samples infected with *Pgt* were collected from farmers' fields and experimental stations, where wheat is important and stem rust is known to occur. The international stem rust live sampling collection protocol was followed during sampling (Park et al., 2013). A total of 464 wheat fields were surveyed of which 224 fields were from Oromia and 240 from Amhara regions. The number of sample was limited to only 464 fields due to budget constraint. During the survey, 60 stem rust-infected wheat samples were collected from the study areas, of which only 48 were viable. The 24 viable samples were collected from Oromia and the remaining 24 viable samples were from Amhara regions. Accordingly, the core tissue and sheaths of the stem samples were removed and cut into small pieces of 5-10 cm. The stem rust infected core sheaths were then put in a glycine paper bag. This method facilitated the easy drying of the samples and prevented prior spore germination before the actual race analysis in the greenhouse. After every sampling and labeling, cutting materials and hands were disinfected using 75% ethanol according to the international rust live sampling protocol (Park et al., 2013). The samples collected were labeled with relevant information, including the name of the zone and district, cultivar name, date of collection, and GPS data (latitude, longitude, and altitude) recorded during the survey. The samples were collected at every 5 km intervals along the main and feeder roads.

Isolation and Multiplication of Single-Pustule Isolates

The urediospores from the collected infected stem samples were harvested into a motorized collector known as "a vacuum and diaphragm pump atomizer" (Vacuubrand Technology, Berlin, Germany). The spores were then multiplied on the universally rust-susceptible variety "Mc Nair701" which does not carry stem rust resistance genes (Roelfs et al., 1992). This variety was obtained from the University of Minnesotra, Cereal Disease Laboratory and CIMMYT wheat rust disease research team. Before using the spore inoculators and collectors, they were decontaminated by 95 % alcohol suspension. They were then immersed in alcohol suspension and stayed for 24 hrs before being used for the green house experiment. Five seedlings grown from seeds of this wheat variety which was confirmed to be stem rust free were raised in 8 cm diameter pots containing sterilized potting media with 2:1:1 ration of soil, sand, and manure, respectively. Greenhouse inoculation was carried out using the methods and procedures developed by Stalkman et al. (1962). Three mg of *Pgt* spores in 1ml of solTrol-130 were inoculated onto seven-day old wheat seedlings using vacuum and diaphragm pump atomizer. Distilled water was used to moisten plants and the moistened plants were then placed in an incubation chamber for 14 hrs in the dark at a temperature of

18-24^{0}C. After incubation, seedlings were supplied with fluorescent light for 4 hrs to provide favorable conditions for infection, and seedlings were allowed to dry/remove their dew/ for about 1-2 hrs. The inoculated seedlings were then placed in glass compartments of the greenhouse, which maintained a 12 hrs photoperiod, a temperature range of 18-25 ^{0}C, and a relative humidity of 60-70%. The remaining rust spores were stored at -80^{0}C and used to replace samples that fail to produce infection on the universally susceptible line Mc Nair701.

After seven to ten days of inoculation (when the flecks/symptoms were clearly visible), leaves containing a single fleck that had produced single pustule were selected from the base of the plant and the remaining seedlings within the pot were removed using scissors. Only 2-3 leaves per pot which contain single pustule were left. Each pot with single pustule was covered with cellophane bags (145 x 235mm) and tied up at the base with a rubber band to avoid cross contamination (Fetch and Dunsmore, 2004). After two weeks (14-15 days) following inoculation (when the mono pustule was developed), each mono pustule was collected using spore collector under vacuum and stored in a separate gelatin capsule. Seven-day old seedlings of a universally susceptible variety "Mc Nair " was inoculated with one gelatin capsule of pgt spore suspension prepared by mixing 3-5 mg urediospores with 1ml of mineral oil (solTrol-130). After inoculation, the seedlings were placed in a humid chamber in dark condition at 18-22^{0}C for 18 hrs and then in the light for 3-4 hrs, after which they were transferred to a greenhouse with temperature of 18 - 25^{0}C and RH of 60 - 70% for 14-15 days. After inoculation, the multiplied spores of each mono pustule/isolate were collected in separate test tubes and stored at -80^{0}C till they were inoculated on the standard differential sets. This procedure was repeated till sufficient number of spores were produced in order to inoculate the set of stem rust differential hosts.

Inoculation of Wheat Stem Rust Differential Lines

Five seeds of each of the 19 stem rust differential hosts with known resistance genes (*Sr5, Sr6, Sr7b, Sr8a, Sr9a, Sr9b, Sr9d, Sr9e, Sr9g, Sr10, Sr11, Sr17, Sr21, Sr24, Sr30 Sr31, Sr36, Sr38,* and *SrTmp*) along with the susceptible line "Mc Nair" (Table 1) were sown separately in 3cm diameter pots and placed in a greenhouse at Ambo Agricultural research center WRRAL (Wheat Rust Race Analysis specialized Laboratory. Twenty pots were used for each of the differential lines and the experiment was arranged in a completely randomized design (CRD) with three replications. Seven-day old seedlings were inoculated with 3-5 mg *Pgt* spores derived from single pustule isolates in 1 ml of distilled water or solTrol-130 using a vacuum pump inoculator, following the procedure described under section 2.3. Once inoculated, plants were supplied with moisture using fine droplets of distilled water produced with an atomizer and placed in an incubation chamber for 14 hrs in the dark at 18-24^{O}C and then the plants in the incubation chamber were supplemented with 4 hrs of additional cool white fluorescent tubes. Once removed from the dew chamber, plants were placed in glass compartments in a greenhouse at temperature of 18-24^{o}C and the relative humidity of 70% until disease develops and evaluated. The experiment was repeated three times to confirm the result.

Data Collection

Scoring for wheat stem rust infection types was carried using the 0-4 scale (Stalkman et al., 1962) 14 days after inoculation. Infection types were grouped into two, where, Low infection type (resistance reaction; 0-2+) are considered as the pathogens are avirulent/low infection. and High infection type (susceptible reaction; 3 to 4 scores) considered as the pathogens are virulent (Figure 2).

Table 1. List of stem rust differential lines used in the study, their corresponding Sr genes and Origin/pedigree.

No.	Differential host	*Sr* genes	Origin/pedigree
1	Lc*Sr*24Ag	24	Little Club/Agent (Cl 13523)
2	W2691*Sr*Tt-1	36	Cl12632 T.timopheevii
3	I*Sr*7b-Ra	7b	Hope/Chinesen Spring
4	I*Sr*8a-Ra	8a	Rieti/Wilhelmina//Akagomughi
5	CnSSrTmp	Tmp	Triumph 64(C/13679)/Chinese Spring
6	*Sr*31 (Benno)/6*LMPG	31	Kavkaz
7	CnS-T-mono-deriv	21	Einkorn Cl 2433
8	Trident	38	Spear *4/VPM(p1519303)
9	I*Sr*9a-Ra	9a	Red Egyptian/Chinese spring
10	I*Sr*9d-Ra	9d	Hope/Chinese spring
11	Combination VII	17	Esp 518/9
12	I*Sr*5-Ra	5	Thatcher/Chinese Spring
13	I*Sr*6-Ra	6	Red Egyptian/Chinese spring
14	W2691*Sr*9b	9b	Kenya 117A
15	Vernsteine	9e	Little club//3*Gabo/2*
16	W2691*Sr*10	10	Marquis*4/Egypt NA95 /2/2*W2691
17	Bt*Sr*30Wst	30	Festival/Uruguay C10837
18	Cns*Sr*9g	9g	Selection from Kubanka (C11516)
19	I*Sr*11-Ra	11	Kenya C6402/pusa4/Dundee
20	McNair701	McN	C115288

Seed source: Ambo Plant Protection Research Center (2017)

Table 2. Naming of *Puccinia graminis* f. sp. *tritici* based on 20 differential wheat

		Infection types produced on near- isogenic Sr lines			
Pgt-code	Set 1	5	21	9e	7b
	Set 2	11	6	8a	9g
	Set 3	36	9b	30	17
	Set 4	9a	9d	10	Tmp
	Set 5	24	31	38	McN
B		Low*	Low	Low	Low
C		Low	Low	Low	High**
D		Low	Low	High	Low
F		Low	Low	High	High
G		Low	High	Low	Low
H		Low	High	Low	High
J		Low	High	High	Low
K		Low	High	High	High
L		High	Low	Low	Low
M		High	Low	Low	High
N		High	Low	High	Low
P		High	Low	High	High
Q		High	High	Low	Low
R		High	High	Low	High
S		High	High	High	Low
T		High	High	High	High

Source: Roelfs and Martens (1988); Jin et al. (2008); *Low: Infection types 0;, 1, and 2 and combinations of these values; **High: Infection types 3 and 4 and a combination of these values.

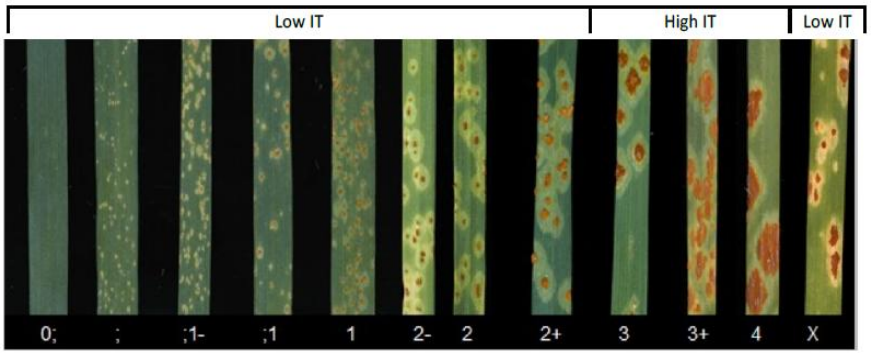

Figure 2. Pictorial description of infection types used in classifying the reactions of stem rust on leaves of wheat seedlings (Stalkman et al., 1962).

Designation of Races

The Northern American Nomenclature System for *Puccinia graminis* f. sp. *tritici* (Roelfs and Martens, 1988) was used to designate races by grouping differential lines, each containing a unique single resistance gene. This approach allows for distinguishing races based on their qualitative reactions to different pathogen isolates. The differentials were put in the order as shown in (Table 2). Set I: Sr5, Sr21, Sr9e, Sr7b, Set II: Sr11, Sr6, Sr8a, Sr9g, Set III: Sr36, Sr9b, Sr30, Sr17, Set IV: Sr9a, Sr9d, Sr10, SrTmp, Set V: Sr24 Sr31, Sr38, SrMcN. The five-letter code was given to each isolate of a race based on the reaction on the differential lines (Fetch and Dunsmore, 2004). For example, letter 'B' and 'T' were assigned for low and high infection types on the four lines, respectively. Hence, when an isolate had a resistant reaction (produce low infection type) on the 20 differential lines, the race was assigned with 'BBBBB' race code. When an isolate produced a high infection type (susceptible reaction) on the 20 wheat differential lines, it was given 'TTTTT' race code. An isolate which produced a low infection type on Sr11, Sr24, and Sr31, but a high infection type on the remaining 17 differential lines was designated as TKTTF. Infection types that were consistent across the differential lines in all three replications were solely used for data analysis. When an infection type of 0, indicating an immune reaction, was observed, the experiment was repeated to rule out the possibility of disease escape.

Data Analysis

SPSS IBM20 and Arc GIS version 10.5.1 computer software programs were used to compute the mean, statistical analysis and to generate maps.

RESULTS

Virulence Structure of Stem Rust Pathogen

Of the total of 60 stem rust isolates collected, 48 were viable and the remaining 12 samples did not yield viable spores after inoculation on the susceptible check McNair 701 in the greenhouse. Therefore, the race analysis was done using 48 viable isolates. The identified races in different wheat varieties and their detection frequencies across the study zones are presented in table 3 and 4, respectively.

Three races namely TKTTF (Digelu race), TTKSK (Ug-99) and TTRTF were identified from the samples collected in the two regions. Among these, TKTTF was the predominant race in the surveyed regions, with a frequency of 75%. The other two races, TTRTF and TTKSK, were detected at frequencies of 22.91% and 4.16%, respectively. Variation in the virulence spectra of these races was observed within the regions. In Oromia, TKTTF was detected in all three surveyed zones (Bale, East Arsi, and West Arsi). The race was most frequent in the East Arsi (45%) followed by Bale (33.33%) zone, while, it was the least abundant in West Arsi zone (8.3%). TKTTF was obtained from the diseased samples from several varieties, including Hidase, Ude, Tesfaye, Ogolcho, Senbete, Kubsa, Dashen, Danda'a, Crossing lines, Ravi-18 and some unknown local varieties in the region.

The race TTKSK (Ug-99) was detected in the East Arsi and West Arsi zones, each with a frequency of 4.2%, from the varieties Ogolcho and Kakaba. However, this race was not observed in Bale zone. In total, the race TIKSK accounted for 8.33% of the *Pgt* population in the surveyed zones. The race TTRTF was detected at a single location in East

Arsi zone with a frequency of 4.2%, specifically from the variety Hidase but it was not detected from samples collected in Bale and West Arsi zones.

In Amhara region, two races, namely TKTTF and TTRTF were detected from the 24-stem rust infected samples analyzed. TKTTF was the predominant race detected in all surveyed zones of the Amhara region. It was most frequently detected in East Gojjam, North Wello, and South Wello zones with frequencies of 20.8%, 16.7%, and 12.5%, respectively. However, it was least abundant (4.2%) in South Gondar.

Race TKTTF was detected from the varieties Digelu, Danda'a, PBW-343, Pavon-76 and some other unknown varieties. In contrast, the race TTKSK was not detected in any of the five zones in the Amhara region. The new race TTRTF was detected in all surveyed zones except West Gojjam, with the highest frequency of 20.8% in South Gondar. TTRTF was obtained from stem rust samples of varieties Digelu, Danda'a, Pavon-76, Israel, Triticale and some Unknown varieties in the region.

Table 3. Wheat varieties infected by the Pgt races identified in selected districts in Oromia and Amhara regions during 2017 main cropping season

Region	Pgt Races identified	Varieties
Oromia	TKTTF	Hidase Ude Tesfaye Ogolcho Senbete Kubsa Dashen Danda'a Crossing line Ravi-18 Unknown
	TTKSK	Ogolcho
	TTRTF	Hidase
Total		
Amhara	TKTTF	Danda'a Unknown Digelu PBW-343 Pavon-76
	TTKSK	-
	TTRTF	Unknown Digelu Danda'a Pavon-76 Israel Triticale
Total		

Table 4. Summary of number of samples collected, analyzed and races obtained from the study areas.

Region	Zone	No of samples	TKTTF	*Freq. (%)	TTKSK	Freq. (%)	TTRTF	Freq(%)
Oromia	Bale	8	8	33.33	-	-	-	-
	East Arsi	13	11	45.83	1	4.17	1	4.17
	West Arsi	3	2	8.33	1	4.17	-	
Sub total		24	21	87.50	2	8.33	1	4.17
Amhara	East Gojjam	6	5	20.83	-	-	1	4.17
	West Gojjam	2	2	8.33	-	-	-	
	North Wello	6	4	16.67	-	-	2	8.33
	South Wello	4	3	12.50	-	-	1	4.17
	South Gondar	6	1	4.17	-	-	5	20.83
Sub total		24	15	62.50	-	-	9	
Total		48	36	75.00	2	4.17	19	

*Freq: Frequency

Virulence Spectra to Sr Resistance Genes

The finding of the present study revealed that 85% of the stem rust resistance genes (Sr) were ineffective against all the races detected, while 30% were effective against one or more of the races identified (Table 5). Fourteen differential hosts with the resistance genes *Sr5, Sr21, Sr9e, Sr7b, Sr6 Sr8a, Sr9g, Sr9b, Sr17, Sr9a, Sr9d, Sr10, Sr38* and *McN* were ineffective to the three races detected, exhibiting a virulence frequency of 100% on these *Sr* genes (Table 6). In contrast, the differential host with the resistance gene *Sr11* was found to be effective against the race TKTTF but ineffective against TTKSK and TTRTF. Similarly, the differential host with *Sr36* was ineffective to race TKTTF and TTKSK, though it was effective against TTRTF. Additionally, the differential host having the *SrTmp* resistance gene was not effective against the races TKTTF and TTRTF but effective against TTKSK. The differential host carrying resistance gene *Sr31* was effective against both TKTTF and TTRTF races, but not against TTKSK. The most important resistant gene Sr24, which the majority of Ethiopian commercial varieties possess (Belayneh, 2010) was found to be effective against all races identified in the study areas. Overall, there was variation in the virulence spectrum among the races identified in the present study.

Table 5. Virulence spectra of the Pgt races identified in Oromia and Amhara regions in 2017

Race	Virulence	Avirulence
TKTTF	*5, 21, 9e, 7b, 6, 8a, 9g, 36, 9b, 30, 17, 9a, 9d, 10, Tmp, 38, McN*	*11, 24,31*
TTKSK	*5, 21, 9e, 7b, 11, 6, 8a, 9g, 9b, 30, 17, 9a, 9d, 10, 31, 38, McN*	*36, Tmp, 24,*
TTRTF	*5, 21, 9e, 7b, 11, 6, 8a, 9g, 36, 9b, 17, 9a, 9d, 10, Tmp, 38, McN*	*30, 24, 31*

Table 6. Virulence frequency of Pgt races collected from the study area to single-gene wheat differentials.

Stem rust resistance gene	Virulence frequency (%)	Stem rust resistance gene	Virulence frequency (%)
*Sr5	100	Sr 30	66.67
Sr 21	100	Sr 17	100
Sr 9e	100	Sr 9a	100
Sr 7b	100	Sr9d	100
Sr 11	66.67	Sr 10	100
Sr 6	100	SrTmp	66.67
Sr 8a	100	Sr 24	0
Sr 9g	100	Sr 31	33.33
Sr 36	66.67	Sr 38	100
Sr 9b	100	McN	100

*Sr: Stem rust

DISCUSSION

From the 48 samples analyzed in this study, three Pgt races (TKTTF, TTKSK, TTRTF) were identified. In contrast, previous studies have reported a broad range of Pgt races in Ethiopia (Belayneh and Emebet 2005; Admasu et al., 2009; Hailu et al., 2015; Abebe et al., 2012; Hei et al., 2018). The smaller number of Pgt races detected in the present study might be due to the limited number of samples analyzed.

The race TTRTF was reported in Georgia in 2014 (Olivera et al., 2019). The race TTRTF identified in this study was the first report of this race in wheat in Ethiopia by Tsegaab et al. (2019). The detection of TTRTF race in Ethiopia concurrent with the massive government ambition of expanding irrigated wheat calls for developing resistant wheat varieties against the race and improving surveillance systems, or implementing policy changes to support farmers.

The race TKTTF is widely distributed in the Middle East region and it has been confirmed to be present in nine countries (Turkey, Iran, Lebanon, Egypt, Ethiopia, Georgia, Azerbaijan, Eritrea, Yemen) (Olivera et al., 2015). This race was the dominant one in Pgt population in Ethiopia and was the major cause for the wheat stem rust epidemics that happened during 2014–15 in Ethiopia (Olivera et al., 2015). The race caused complete yield losses in some fields and led to the widely cultivated wheat variety Digelu being removed from production (Olivera et al., 2015).

The other race identified in this study was race TTKSK (Ug-99). This race pre-dominated the Pgt population of Ethiopia and threatened wheat production in the country (Endale et al., 2016). It had virulence on *Sr31* gene which was resistant for over 40 years. According to Endale et al. (2016) TTKSK was the most dominant and widely distributed Pgt race in Ethiopia after 2003 until the Digelu race (TKTTF) took over the dominance. Similarly, Olivera et al. (2015) reported the dominance of the race TTKSK in Arsi and Bale, before the Digelu (TKTTF) race. The authors also reported that the TTKSK race was responsible for epidemics in 2012. However, in the present study it was detected only from two stem rust samples. This suggests that TTKSK was not predominant in the study areas and this might be due to the release of multiple resistant wheat varieties targeting this race. However, it might be difficult to come up with a conclusive remark due to the limited number of samples collected.

Based on the findings of the present study, Sr 24 is the only stem rust resistance gene that remains resistant against the three races i.e. TKTTF, TTKSK and TTRTF. Virulence on *SrTmp* is considered as the main factor for the complete susceptibility of the variety “Digelu” to the race. Moreover, the detected races had a wider range of virulence spectrum. Broader virulence diversity of Pgt races has also been reported in Ethiopia (Belayneh and Emebet, 2005; Belayneh, 2010). Favorable environmental conditions and continuous wheat production might be the probable reasons for the occurance of virulence diversity in Pgt population in Ethiopia. Similar virulence diversities within Pgt population have been reported in other countries, including

Mexico, USA and Canada and South Africa (Jin, 2005).

The two races TKTTF and TTRTF identified in the present study varied by single gene changes. Both races exhibited avirulence to *Sr24* and *Sr31*. However, a single gene change in virulence occurred on *Sr30 and Sr11*. This entails *Sr30* was broken by the race TKTTF but remained resistant to the race TTRTF and *Sr11* gave up its resistance to the race TTRTF but remains resistant to the race TKTTF. Similarly, Belayneh (2010) and Teklay et al. (2012) noted that single-gene changes are major factors for the variations amongst most Pgt races in Ethiopia. Such single-step changes in virulence have been recognized as a primary mechanism of evolutionary change in *Puccinia graminis* f. sp. *tritici* populations. The findings of the present study are also in agreement with the report of Teklay et al. (2012), which stated that Sr24 is among the effective genes to all stem rust races collected from the northern Ethiopia. However, virulence to Sr24 gene was reported in Kenya in 2006 (Wanyera et al., 2010) and later identified in Ethiopia from samples collected in 2018 (Hei et al., 2020). A variant of Ug99 group (TTKST) that added virulence on stem rust resistance gene Sr24 (Ug99+Sr24 virulence) has further increased the vulnerability of wheat to stem rust worldwide (Jin et al., 2008). Previous reports also indicated the breakdown of the Sr31 resistance gene in Ethiopia (Belayneh, 2010; Teklay et al., 2012; Endale et al., 2016) and this serves as evidence for the existence of the race TTKSK (Ug99) in the country.

CONCLUSIONS

This study revealed that the nature and distribution of diversified soil types along the toposequence of the Qenberenaweti sub-watershed were influenced by the degree of variations in typical topographic positions and key slope features (steepness, aspect, and form). Because these pedogenesis factors directly affect the erosion-deposition and eluviation-illuviation of soil materials via controlling the action of water moving laterally across the surface and percolating vertically into the subsoils, respectively. Generally, the absence of inclusive evidence on the formation, development, and distribution of soils at a site-specific physiographic condition is often a constraint to the improvement of agriculture. Thus, the outputs of such detailed soil characterization, classification, and mapping work give a vital clue for proper planning, management, and utilization of the soil resources at local topographic variability level. However, further research should be done to ensure sustainable agricultural production in the study area.

CONFLICTS OF INTEREST

Authors declare that they have no conflicts of interest.

ACKNOWLEDGEMENT

This research was funded by Ethiopian Institute of Agricultural Research (EIAR) and Delivering Genetic Gain in Wheat (DGGW) Project. The authors thank Ambo Agricultural Research Center for its technical support

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Original Research Article||

Genetic Diversity of Avocado (*Persea americana Mill.*) From Southern Ethiopia Using SSR Markers

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Abstract

Avocado (*Persea americana* M.) Is a vital cash crop in Ethiopia. Understanding its population structure and allelic poly morphism is crucial for genetic improvement. However, the genetic diversity of Ethiopian avocado remains underexplor ed. This study investigated the genetic diversity of avocado in Southern Ethiopia using Simple Sequence Repeat (SSR) markers. A total of 109 avocado trees from 16 districts were sampled, and 12 SSR markers were employed for analysis. The study detected 140 alleles across the 12 loci, averaging 11.7 alleles per locus. The average expected heterozygosity was 0.63 ± 0.12, while the observed heterozygosity was 0.48 ± 0.19, with all loci showing significant deviation from har dyweinberg Equilibrium (HWE). The Analysis of Molecular Variance (AMOVA) indicated that approximately 5% of th e genetic variation was among the 16 populations. Pairwise comparisons of population FST values revealed a lack of gen etic differentiation in seven out of 48 paired comparisons. The Unweighted Pair Group Method with Arithmetic Mean (UPGMA), based on the Nie and Li similarity index, grouped the genotypes into three major clusters with bootstrap value s of 100 and 5. Genetic distance analyses showed mixing of avocado trees from different districts. Discriminant Analysis of Principal Components (DAPC) categorized the samples into three groups, while model-based structure analysis subdivided them into two main genetic clusters. The moderate genetic diversity observed in the avocado germplasm is promising for the future of avocado cultivation in Ethiopia, suggesting the germplasm is a valuable source of alleles for genetic improvement. The mixing of avocado trees across districts indicates strong gene flow among populations. Furthermore, the significant variation among tree populations from different districts offers numerous opportunities for avocado breeding programs, providing hope for potential advancements in avocado cultivation.

Key words: Avocado, Genetic diversity, SSR markers, Population structure, Southern Ethiopia

Original submission: August 12, 2024; **Revised submission**: December 09, 2024; **Published online**: December 30, 2024

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INTRODUCTION

Avocado (*Persea americana* Mill) originated in Mexico, Central or South America, and was first cultivated in Mexico as early as 500 BC (Galindo-Tovar et al., 2008). Avocado is a highly heterozygous diploid species with 24 chromosomes (2n = 24) and a genome size of 841.6 Mbp (Yang et al., 2024). It is a cross-pollinating species with outcrossing rates ranging from 74% to 96% (Borrone et al., 2008). The plant produces edible, nutritious, and commercially used fruits.Avocado is a polymorphic species with numerous taxa adapted to different climates and altitudinal ranges. These include P. americana var. drymifolia, P. Americana var. Guatemalans, and P. Americana var. Americana is commonly called the Mexican, Guatemalan, and West Indian horticultural races, respectively (Galindo-Tovar et al., 2008). The Mexican and Guatemalan races are adapted to cooler

climates, whereas the West Indian race requires warmer weather for optimum development. The three avocado races are cross-compatible, and hybridization can occur between trees of different races when grown near each other (Juma et al., 2020). Most commercial avocado cultivars are interracial hybrids developed from chance seedlings. Thus, agriculturally important cultivars in subtropical climates, such as "Hass," "Bacon," and "Fuerte" are Guatemalan-Mexican hybrids with different degrees of hybridization (Boza et al., 2018). Genetic characterization and diversity analysis in avocado enable researchers to achieve agronomic improvement by developing disease-resistant new varieties and cultivars that are more profitable in terms of fruit production, fruit quality, and fruit maturation precocity (Guzman et al., 2017).

Avocado was first introduced to Ethiopia around 1938 by privet orchardists in Hirna (Eastern Highlands of Ethiopia) and Wando-genet (Southern Highlands of Ethiopia) and has since been distributed to different ago-ecological zones in Ethiopia (Berhanu, 2013). Despite its long history of introduction and the diverse agroecology of Ethiopia, its distribution is limited to a few areas of the country (Jalata, 2021). Currently, the main avocado-producing areas in Ethiopia include the Sidama and Wolaita areas in the south, the Jimma and Mizan areas in the southwest, and the Hararge area in the country's eastern region.

In Ethiopia, avocado trees are mainly grown as an integral component of coffee (Coffea arabica L.) and enset (Ensete ventricosum) production systems (Biazin et al., 2018). Smallholder producers consider avocados to be shade trees and benefit from the sale of avocado fruits (Biazin et al., 2018).

The potential for commercial avocado production in Ethiopia is promising, with a recent surge in interest (Terheggen, 2019). Although several high yielding andbiotic stressresistant varieties are suitable for commercial farming, they are currently scarce in Ethiopia. This underscores the need to gather, charact erize, and select accessions from the existing germpla sm to achieve higher yield and quality. However, ther e is limited information available on the genetic diver sity of avocados in Ethiopia, which is a crucial factor for successful breeding and conservation efforts. This lack of comprehensive data remains a significant cha llenge for advancing avocado breeding programs.

Molecular markers are used for the genetic characterization of germplasm because they exhibit higher heritability and greater polymorphism than morphological markers, making them more effective for distinguishing between genotypes (Bunjkar et al., 2024). Additionally, breeding perennial crops poses challenges due to their long life spans.

Molecular markers, such as Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeats (SSRs), and Single Nucleotide Polymorphisms (SNPs), are employed to identify accessions and variations at the earliest stages of development. These markers have been used to analyze the genetic diversity of avocado accessions globally (Degu et al., 2024). In Ethiopia, SSR markers are particularly preferred for avocado diversity analysis because of their high polymorphism and their ability to detect numerous alleles at specific loci. This makes SSRs highly suitable for genetic diversity assessments.

As co-dominant markers, SSRs allow for the differentiation of both homozygous and heterozygous individuals, providing a comprehensive view of the genetic structure within populations. Moreover, SSR markers are reproducible, reliable, relatively cost-effective, and robust tools for genetic mapping and population structure analysis. Their versatility is invaluable for identifying genomic regions associated with key traits and exploring gene flow and genetic differentiation across avocado populations in Ethiopia.

Although avocado cultivation in Ethiopia has a long history, comprehensive reports have yet to be made on avocado diversity. This research was aimed to fill this gap by using SSR markers to estimate the population structure and diversity of Ethiopian avocados. The findings of this study will enhance the understanding of avocado diversity in Ethiopia and will be helpful for future research and breeding programs.

MATERIALS AND METHODS

One hundred nine avocado accessions, from three regions in southern Ethiopia and from a germplasm bank at the Wondo-Genet Research Center (WGRC), were used for genetic diversity analysis (Fig. 1 and Table S1). Young leaves were collected in a falcon tube (15 ml) filled with the color indicator Silica Gel Desiccant and shipped to the Czech University of Life

Sciences Prague, Faculty of Tropical Agrisciences, Prague, for SSR marker molecular analysis.

Total genomic DNA was isolated using a modified cetyltrimethylammonium bromide (CTAB) extraction protocol developed by (Doyle and Doyle, 1987). The concentration and purity of DNA were measured on a nanodrop spectrophotometer. The DNA samples were diluted to final concentrations of 20 ng/µl for polymerase chain reactions (PCR).

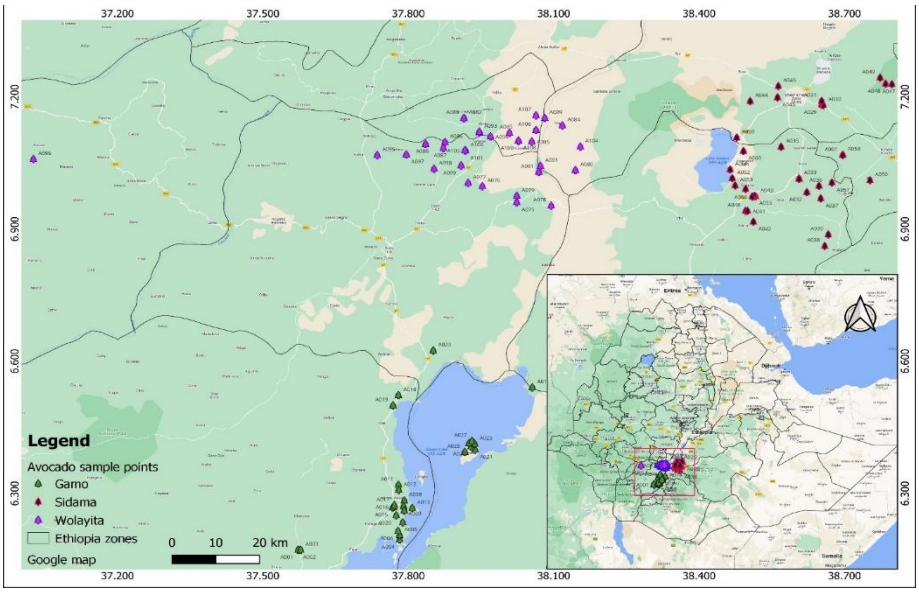

Figure 1. Map of Southern Ethiopia, displaying the geographical location of sampled avocado populations.

Twelve microsatellite primers (Ashworth, 2003; Sharon et al., 1997) (Table 1) were used to analyze the collected avocado samples. The methodology was adapted from Ruiz-chutan et al. 2022. Forward primers were fluorescently labeled for detection in four colors: 6-FAM, PET, NED, and VIC. PCR amplifications were conducted separately for each multiplex in a T100 Thermal Cycler (Bio-Rad, USA) by performing the following steps: Denaturation at 95 °C for 5 minutes, followed by 35 cycles of 30 seconds each, a specific alignment temperature for each multiplex (one minute), elongation at 72 °C for one minute, and a final extension of 10 minutes at 72 °C. Fragment analysis was performed with the Genetic Analyzer 3500 (Applied Biosystems, USA). Microsatellite alleles were scored using GENEMARKER v. 2.4.0 (Soft Genetics, USA).

Table 1. Diversity Statistics of the 12 SSR loci in 109 avocado samples in sixteen districts in southern Ethiopia.

Locus	SSR Motif	Na	Ne	Ho	He	I	HW	PIC
AVAG05	(AG)10	10	2.03	0.3	0.51	1.19	***	0.72
AVAG13	(CT)18	16	5.15	0.72	0.8	1.92	***	0.87
AVT436	(AG)20	6	2.33	0.58	0.57	1.01	***	0.68
AVAG11	(ATC)9	11	2.38	0.28	0.58	1.22	***	0.78
AVAG07	(CT)22	11	2.39	0.62	0.58	1.21	***	0.7
AVMIX04	(GA)15	13	2.8	0.49	0.64	1.34	***	0.82
AVAG21	(AG)12 (CAA)5 (ACAG)10	9	4.48	0.62	0.78	1.68	***	0.85
AUCR418	(TC)14	15	5.11	0.58	0.8	1.88	***	0.89
AVAG22	(TC)15	10	1.76	0.42	0.43	0.94	***	0.65
AVAG25	(GT)12(GA)13	12	1.8	0.07	0.44	0.93	***	0.68
AVD001	(CT)12	15	4.65	0.46	0.78	1.81	***	0.97
AVD022	(TC)13	12	3.41	0.67	0.71	1.46	***	0.89
Mean		11.67	3.19	0.48	0.63	1.38	***	0.79

NA, Number of alleles; Ne, Effective number of alleles; Ho, observed heterozygosity; He, expected Observed heterozygosity; I, Shannon's Information Index; HWE, Hardy Weinberg Equilibrium; PIC, Polymorphic Information content

Data Analysis

The corresponding peaks' peak features and fragment sizes were counted in the data. The GenAlEx (Peakall and Smouse, 2012) program (version 6.5.03) was used to estimate locus-based diversity indices such as the number of alleles (Na), an adequate number of alleles (Ne), observed heterozygosity (Ho), expected heterozygosity (He), or gene diversity (GD). HWE, or inter-population diversity, was calculated with Popgene32 software version 1.32 (Yeh and Boyle, 1997). AMOVA and average gene diversity were estimated across the loci for each population using the GenAlEx version 6.5.03 program (Peakall and Smouse, 2012). The GenAlEx was used to compute fixation indices (FST, FIT, FIS, FCT, and FSC) and pairwise comparisons between populations. Nei's genetic distance was computed in GenAlEx and imported into MEGA6 (Tamura et al., 2013), where the dendrogram in the Newick format was produced using the unweighted pair group method with arithmetic mean UPGMA. The dendrogram was visualized and customized in the Interactive Tree of Life (iTOL) version 4 (Letunic and Bork, 2019). Population structure is constructed using a Bayesian algorithm implemented in Structure software, version 2.3.4 (Pritchard et al., 2000). The analysis was based on an admixture ancestral model with correlated allele frequencies. To determine the number of population clusters (K), a burn-in period of 50,000 and a run length of the Monte Carlo Markov chain (MCMC) of 100,000 were used for K = 1 to K = 10, using ten replications for each K in the STRUCTURE software. The optimum K value was predicted using POPHELPER, an R package (Francis, 2017).

RESULTS AND DISCUSSION

SSR Polymorphism and Genetic Diversity

In this study 12 loci on 109 sampled avocado plants yielded a significant discovery of 140 distinct alleles (Table 1). The average number of alleles per locus was 11.70 ± 2.84 (Table 1). Each marker detected at least six alleles and was polymorphic, a finding that adds to our understanding of avocado plant diversity. AVAG13 was the locus with the most alleles (16), followed by 15 (AUCR418 and AVD001). Locus AVT436 has the lowest alleles (6). Similar analyses detected 11.5 alleles using 10 SSR loci across 71 avocado plants, with alleles ranging from 5 to 22 per locus (Janice and Jemmy, 2014), and 167 alleles using 10 SSR loci across 226 plants, with the number of alleles per locus ranging from 10 to 23 (Juma et al., 2020). The smallest number of alleles per locus, 3.1, was reported by (Liu et al., 2020) for 56 avocado trees examined in Hainan Province, China. In Gross-German and Virue2013) and Janice and Jemmy (2014) reports, it was 11.5. The differences between the present study and the previously reported results might be due to the markers' polymorphism, sample size, population size, the diversity of the germplasm investigated, and the platforms employed for quantifying amplified products. The quality of genomic DNA used in PCR amplification, optimization of PCR protocols, and differences in allele scoring accuracy could also account for these differences. Capillary electrophoresis might have brought about the difference in the number of alleles

discovered in avocado trees from previous studies, except published by Schnell et al., (2003).

The effective allele count varied from 5.15 (AVAG13) to 1.76 (AVAG22). Shannon's information index (I) range was 1.19 (AVAG05) to 0.93 (AVAG25). The average observed heterozygosity was 0.63 ± 0.19, with minimum and maximum values of 0.43 (AVAG22) and 0.83 (AVAG13 and AUCR418), respectively. The average polymorphism information content was 0.79 ± 0.04, indicating a wide range of genetic variation from 0.65 (AVAG22) to 0.97 (AVD001). All loci deviated from the Hardy-Weinberg equilibrium, suggesting the collected avocado samples do not represent random sampling. Potential factors such as non-random mating, genetic drift, or selection may influence the avocado populations' genetic structure. The wide range of polymorphism information content is a significant finding that highlights the genetic diversity among the sixteen avocado populations. In the current analysis, there were 12 alleles on average per locus. According to Juma et al. (2020), Schnell et al., and Guzmán et al. there are more alleles per locus (16.7, 18.8, and 19.5, respectively).

The average observed heterozygosity was 0.48, comparable to the levels of genetic diversity shown for the studied samples (Boza et al., 2018; Janice and Jemmy, 2014). The current observed heterozygosity (0.48), were lower than 0.65 (Schnell et al., 2003), and 0.6 (Guzmán et al., 2017) respectively. This suggests that avocado from Ethiopia has a limited genetic foundation. This finding coincides with the fact that the primary introduction of avocado in Ethiopia was by orchids (Berhanu, 2013). Observed heterozygosity with a lower value (0.39) has been reported by Liu et al. (2020). The finding suggests in the number of populations, and types of accessions used may account for the variation in the allele's number and heterozygosity.

Shannon's information index (I) is another metric for analyzing gene diversity. If markers change in their score and score close to one on the Shannon information index, they indicate the presence of variability and their suitability for genetic diversity investigations in that group (Nassiry et al., 2009). The results of this study's measurements (0.93 to 1.92), which supported the existence of variability and the usefulness of the markers for examining genetic diversity in the Ethiopian avocado population, were thus confirmed. The PIC value of each marker employed in this study was more informative and scored 0.65 and above, indicating that they are all highly informative and enable the diverse investigation of Ethiopian avocado accessions (Smith et al., 1997). Overall, the findings demonstrated the presence of increased genetic diversity, the strength of marker polymorphisms, and their capacity to provide pertinent information that aids in developing effective conservation and avocado improvement programs.

Genetic Diversity among the Collected Population

When avocado samples were analyzed at the population level, the WGRC exhibited the highest number of alleles (Na), 8.1± 0.6, followed by the Haroma (4) and Sake (3.9) with the average allele of 3.5 ± 0.1 (Table 2). On the other hand, Sadeka population had the lowest (2.7 ± 0.2) number of alleles (Table 2). The effective number of alleles also showed similar pattern where WGRC has the highest (5.8) followed by Yirbaduwanch (2.9) and Sake (2.8) with an average value of 2.6±0.1 among the population. The observed heterozygosity (Ho) varied from 0.6 for the WGRC, Sadake, and Sake population to 0.4 for the majority of the population. Additionally, the expected heterozygosity (He) was higher in the WGRC (0.8), followed by Adehora, Haroma, and Sake (0.6), respectively (Table 3). The Shannon's Information Index (I) value for WGRC (1.9) was the highest (Table 3) followed by the Adeyhora population (1.7 ± 0.01), and the lowest for Sadamodicha, Youwo, Sadeka, Xarmesa, Dalboatuawaro, and Warzalasho, at 0.8 ±0.1. Since gene diversity expresses the probability in which two randomly chosen alleles from the population are different, a Nei's unbiased gene diversity was calculated (Table 2). Genetic diversity was high for WGRC (0.8), followed by Haroma (0.7), and Yirbaduwanch (0.7) with an average value of 0.6±0.01 (Table 2).

Table 2. Diversity information among the sixteen geographic populations (districts)

Population	N	Na	Ne	I	Ho	He	Gene Diversity
Shara	10	3.6±0.5	2.3±0.3	0.9±0.13	0.4±0.1	0.5±0.1	0.5±0.1
Mole	10	3.5±0.5	2.6±0.3	1.0±0.1	0.5±0.8	0.6±0.5	0.6±0.5
Delbo	8	3.2±0.5	2.6±0.4	0.9±0.2	0.5±0.1	0.5±0.1	0.5±0.1
Haroma	6	4.0±0.3	2.7±0.2	1.1±0.1	0.6±0.1	0.6±0.02	0.7±0.03
Youwo	6	2.8±0.3	2.1±0.2	0.8±0.1	0.4±0.1	0.5±0.1	0.5±0.1
Sadeka	5	2.7±0.2	2.2±0.2	0.8±0.1	0.6±0.1	0.5±0.05	0.6±0.06
Xarmesa	5	2.8±0.3	2.1±0.2	0.8±0.1	0.4±0.1	0.5±0.05	0.5±0.06
Sadamodicha	5	2.9±0.3	2.1±0.2	0.8±0.1	0.5±0.1	0.5±0.05	0.5±0.06
Yirbaduwancho	6	3.5±0.3	2.9±0.3	1.1±0.1	0.5±0.1	0.6±0.05	0.7±0.05
WGRC	12	8.1±0.6	5.8±0.5	1.9±0.1	0.6±0.07	0.8±0.02	0.8±0.02
Adeyhora	6	3.7±0.4	2.6±0.2	1.0±0.1	0.6±0.1	0.6±0.1	0.6±0.1
Adeykoisha	6	3.4±0.4	2.5±0.3	1.0±0.1	0.5±0.1	0.5±0.05	0.6±0.05
Dalboatuawaro	6	2.8±0.2	2.2±0.2	0.8±0.1	0.4±0.1	0.5±0.05	0.6±0.05
Warzalasho	8	2.8±0.3	2.2±0.2	0.8±0.1	0.4±0.1	0.5±0.1	0.5±0.1
Galacha	5	2.8±0.2	2.2±0.2	0.9±0.1	0.5±0.7	0.5±0.4	0.6±0.5
Sake	5	3.9±0.4	2.8±0.3	1.1±0.1	0.6±0.1	0.6±0.1	0.6±0.1
Mean	7	3.5±0.1	2.6±0.1	1.0±0.03	0.5±0.02	0.5±0.01	0.6±0.01

N, Number of samples; NA, number of allele; Ne, Effective number of alleles; Ho, observed heterozygosity; He, expected Observed heterozygosity; I, Shannon's Information Index.

Molecular Variance and Population Divergence

The AMOVA revealed a low but significant ($p < 0.001$) difference among avocado samples collected from different districts, and region which accounted for 5% the total variation (Table 3). However, a lower differentiation (1.0; p value < 0.048) was obtained among groups of samples collected from the two agroecology zones in the study site (Table 3). The variation among individuals within districts and region (20%), and 75% within individuals in the analysis (Table 3) were highly significant ($P < 0.0001$).

Although avocado is usually considered an out-crossing plant, the findings might suggest it does much self-pollination. Another explanation could be that the significant divergence from HWE may be due to linkage disequilibrium with the studied loci under selection in the form of heterozygote disadvantages. The results are more significant than those of Juma et al. (2020), who discovered a difference (1.98%) among avocado population groups in the collection site.

The fixation index, or FIS, is a measure of inbreeding that determines whether a sub population has an excess or deficit of homozygotes (Chakraborty, 1993). Calculation of fixation index indices, based on a prior population hierarchy for the district, region and agroecology of the avocado, using AMOVA, resulted in FST (0.05), FIS (0.02) and FIT (0.205) values (Table 3). The fixation index (FST), which runs from 0 to 1, measures sub-population differentiation. A value near 0 indicates a complete panmixia population, while a number close to 1 indicates fully differentiated populations (Pearse and Crandall, 2004). The current result is indicating the existence of a low district and region based subdivision. This could result from genetic drift, and pressure from indirect selection, often leading to the loss of specific alleles or a change in frequency.The FST values found in the current inquiry were 0.19, 0.22, and 0.25, respectively which were lower than those previously reported by Boza et al. (2018), Guzmán et al. (2017) and Gross-German & Viruel (2013), In contrast to our research, Boza et al. (2018) examined avocado samples from Mexico and the United States that represented Persea americana, P. nubigena, and P. kruguii. Only P. americana that had been gathered in a single country, including regional cultivars, rootstocks, and local selections, was investigated by Guzmán et al. (2017). Samples characterized by Gross-Guzman et al. (2013) comprised P. longipes, P. nubigens, and P. schiedeana. However, the current study's average FST is comparable to Gomez, and Rivera (2017) for 226 avocado samples from Tanzania. The sixteen districts were responsible for 5% of the total genetic diversity (Table 3).

Table 3. AMOVA using 1000 permutations for avocado trees from different districts, region and altitudes

Source	DF	SS	MS	Est. Var.	Perc. Var.	F-Statistics	P-value
Between Districts	15	105.355	7.024	0.192	5	FST = 0.05	0.001
Between samples within District	93	412.063	4.431	0.757	20	FIS = 0.206	0.001
Within Samples	109	318.0	2.917	730.5	75	FIT = 0.245	0.001
Total	217	835.417		3.866	100	Nm =4.8	
Between Region	3	44.183	14.7	0.2	5	FST =0.05	0.001
Between samples within region	105	473.6	4.5	0.8	20	FIS = 0.2	0.001
Within Samples	109	318	2.9	2.9	75	FIT = 0.2	0.001
Total	217	835.4		3.9	100	Nm =4.76	
Between Agroecology	1	6.872	6.872	0.025	1	FST = 0.006	0.048
Between samples within Argo-ecology	107	510.545	4.771	0.927	24	FIS = 0.241	0.001
Within Samples	109	318.0	2.9	2.9	75	FIT = 0.246	0.001
Total	217	835.4		3.9	100	Nm =38.9	

The pairwise FST analysis reveals significant genetic differentiation among the studied populations (Table 4). With 69 out of 120 population pairs exhibiting significant differences ($p<0.001$), it is clear that there is substantial genetic structuring within the sampled groups. The highest FST value of 0.48 observed between the Shara and Warzalasho population indicating a high level of genetic differentiation. Such a high value suggests limited gene flow and genetic isolation between these populations. This could be due to geographical barriers, behavioral differences, or historical separations that have prevented cross pollination. Similarly, the second-highest FST value of 0.47 between Adekoisha and Yaowo ($p<0.05$) further supports the presence of significant genetic differentiation within these groups. The lower p-value threshold ($p<0.05$) indicates that while the differentiation is significant, it may not be as robust as those with $p<0.001$.

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Table 4. Population pairwise FST comparisons between Sixteen avocado populations

Sha	Sha	Mol	Del	Har	You	Sa	Xar	Sad	Yir	WG	Ad	Ade	Dal	War	Gal
Mol	0.02														
Del	0.47*	0.21*													
Har	0.13*	0.42*	0.09												
You	0.41*	0.37*	0.39*	0.08											
Sa	0.31*	0.43*	0.25*	0.38*	0.30*										
Xara	0.34*	0.44*	0.32*	0.08	0.41*	0.33*									
Sad	0.35*	0.08	0.13	0.31*	0.19*	0.11	0.11								
Yir	0	0.15*	0	0.02	0	0.08	0.02	0							
WG	0	0.17*	0.01	0.37*	0.09	0.35*	0.32*	0.26*	0.25*						
Ad	0.39*	0.44*	0.40*	0.14*	0.41*	0.33*	0.41*	0.11	0.01	0.05					
Ade	0.31*	0.15*	0.46*	0.25*	0.47*	0.48*	0.48*	0.35*	0	0.01	0.46*				
Dal	0.41*	0.02	0.36*	0	0.41*	0.02	0.11	0	0	0.01	0.41*	0.46*			
War	0.48*	0.03	0.38*	0.02	0.40*	0.04	0.12	0.14	0	0	0.40*	0.40*	0.16*		
Gal	0.27*	0.44*	0.40*	0.05	0.41*	0.24*	0.27*	0.02	0.01	0.08	0.40*	0.47*	0.11	0.09	
Sak	0.09	0.44*	0.03	0.15	0.05	0.25*	0.26*	0.01	0.39*	0.34*	0.16*	0.23*	0	0	0

Conversely, the lowest FST value of 0.14 between Haroma and Adehora populations suggests a much less pronounced genetic differentiation. This value indicates a higher level of gene flow between these populations, with significant implications. It suggests that these populations are geographically closer, have fewer barriers to gene flow, or share a more recent common ancestry, leading to a more homogenized genetic landscape.

The observed range of FST values underscores the complex population structure and varying levels of genetic connectivity among the studied groups. High FST values suggest that some populations are evolving independently, a process that could lead to local adaptations (De Villemereuil and Gaggiotti, 2015). On the other hand, low FST values show regions of connectivity where gene flow is sufficient to homogenize genetic differences (Izaguirre-Toriz et al., 2024). The influence of geographical barriers and historical events on these values is significant, shaping the genetic structure of these populations. Further research, including detailed ecological studies, is necessary to fully understand the mechanisms driving these genetic patterns and to develop effective conservation and breeding plans.

Hierarchical Cluster Analysis

The genetic distance matrix and the subsequent UPGMA-based dendrogram (Fig. 2) shows the genetic relationships and population structure among the studied avocado samples. These tools help elucidate the underlying genetic differentiation and connectivity within the population by grouping various districts and regions. Populations with lower genetic distances cluster together, indicating closer genetic relationships. Conversely, populations with higher genetic distances form separate clusters, reflecting their genetic isolation. The dendrogram highlights distinct genetic groupings, offering insights into historical and contemporary gene flow patterns (Rheindt and Edwards, 2011). The clustering pattern may reflect ecological niches, or breeding practices that have shaped the current genetic structure.

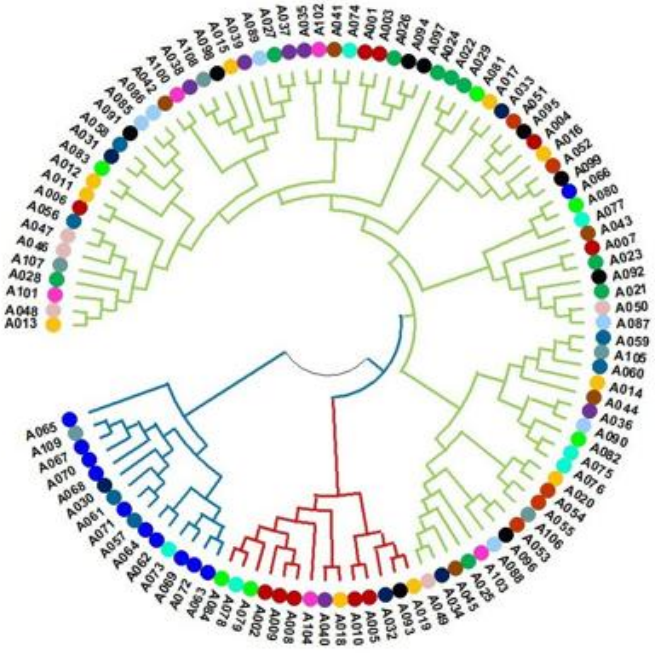

Figure 2. Dendrogram of the 109 avocado trees constructed with UPGMA showing genetic relationships between the analyzed samples. Samples collected from a common district are represented by the same circle color. The different phylogenetic groups are represented by red, blue, and green line colors for Group I, Group II, and Group III phylogenetic groups, respectively

The Discriminant Analysis of Principal Components (DAPC) method, a powerful tool in genetic analysis, has unveiled three distinct clusters among the avocado accessions (Fig. 3). This revelation provides profound insights into the genetic relationships and population structure. The first cluster, an exclusive domain of WGRC accessions, signifies a unique genetic identity for these accessions, possibly owing to their distinct genetic background, selective breeding practices, or specific adaptations to their native environments. The second and third clusters, housing accessions from various districts, hint at a more intricate genetic landscape. These clusters likely represent accessions that have experienced different evolutionary pressures, gene flow, and breeding practices compared to the WGRC accessions. The distinct clustering of WGRC accessions underscores the genetic differentiation between regionally diverse accessions. This differentiation could be attributed to several factors: WGRC accessions may originate from geographically isolated areas (Berhanu, 2013), reducing gene flow with other populations and increasing genetic differentiation. WGRC accessions might have been subject to specific selective breeding practices to preserve certain traits, further distinguishing them from other accessions. Unique environmental conditions and selective pressures in the WGRC regions could have led to the development of distinct genetic traits.

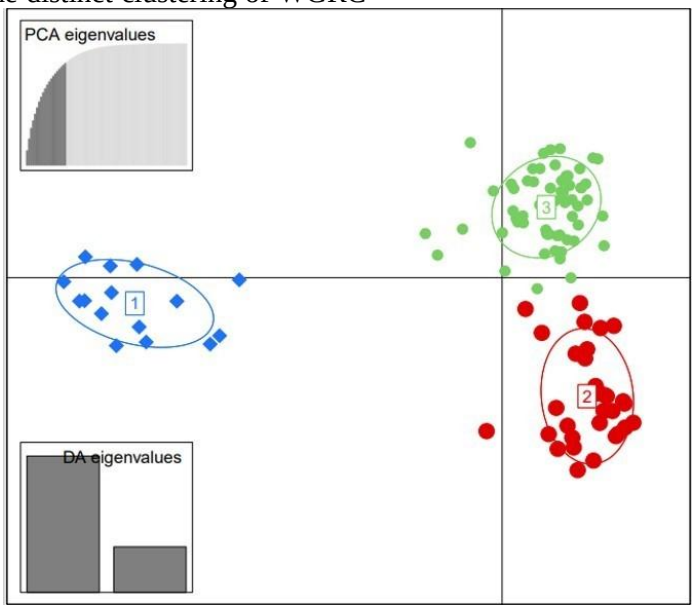

Figure 3. Principal component analysis with discrimination (DAPC) for 109 avocado samples. The first two Linear Discriminants are represented by the axes (LD). Each symbol stands for individual sample, and each circle for a group. The different subpopulations identified by DAPC are represented by numbers.

The clustering of accessions from diverse districts into three separate groups is a testament to the varying levels of genetic differentiation within these regions. This understanding is crucial as it can help us comprehend the different historical events that contributed to the genetic diversity within these clusters. The varied ecological niches across districts could drive adaptation to different environmental conditions, leading to genetic divergence (Sexton et al., 2017). The extent and direction of gene flow between districts can significantly influence genetic structure. These clusters reflect areas with more or less gene exchange. The distinct genetic identity of the WGRC accessions suggests they are valuable genetic resources. Conservation efforts should prioritize preserving their unique genetic traits. Understanding the genetic differentiation within the second and third clusters is not just a scientific endeavor, but a call to action to help identify genetically diverse populations and prioritize them for conservation to maintain overall genetic diversity. The distinct clusters, as revealed by the DAPC method, can serve as a beacon for breeding programs. They identify genetically diverse accessions that can be harnessed to introduce new traits and enhance genetic diversity. The unique genetic traits of the WGRC accessions, a treasure trove for breeding programs, could be instrumental in improving specific traits, such as disease resistance or environmental adaptability. This potential for

enhancement and diversification is a promising prospect for the future of avocado cultivation.

The dendrogram and DAPC could not separate trees based on their districts or regions. This was in line with the findings of AMOVA (Table 3), which showed that 93% of the total genetic variation was between the groups. The dendrogram and DAPC findings were further supported by the population pairwise FST results, which indicated no differences between Shara against Mole, Haroma, WGRC, and Sake. A possible explanation is the trading of avocado seeds, which may have caused genetic mixing in avocado populations similar to the findings from Tanzania (Juma et al., 2020; Juma et al., 2019). The genetic blending of avocado populations is brought by the exchange of seedlings between growers in different districts and the exportation of avocado produce from one area to another, where seeds are later sown or spontaneously sprouted. It might also be due to the introduction of genetic material that is considerably similar to different districts or regions (Juma et al., 2019).

The model-based STRUCTURE showed two ancestral groups (Fig. 4(a)) in the Ethiopian avocado accession. This finding is supported by the population pairwise FST values, which show a lack of difference between the pairs from the same structured population (Table 4). Accordingly, each population (district) has at least two clusters. Numerous indices, including AMOVA, FST, UPGMA, and DAPC, have demonstrated the genetic similarity and low but significant genetic divergence of avocados grown in avocado-producing areas in Ethiopia.

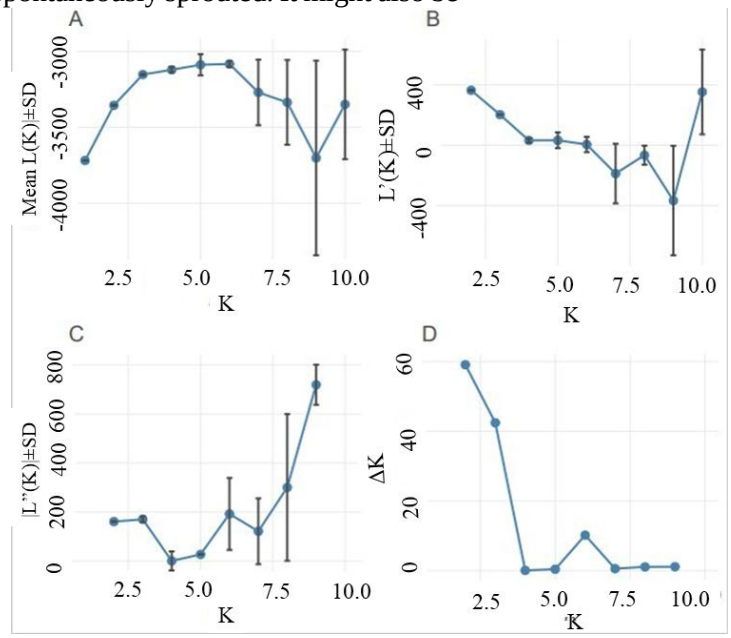

Figure 4a. Estimation of the number of groups in the avocado collection

Figure 4b. Population structure of 109 avocado accessions using STRUCTURE, with each individual represented by a vertical bar, using the expected ideal K = 2, and K =3 populations.

The genetic structure of avocado accessions was obtained using STRUCTURE analysis (Fig.4). The model-based Bayesian algorithm grouped the accessions into two clusters (K=2). This population structure bar plot shows no clear geographic-origin-based structure, indicating a complex genetic structure of avocado populations that may have implications for breeding and conservation strategies.

These findings were supported by DAPC and the UPGMA dendrogram tree, which showed that the 109 examined trees could be separated into three genetic clusters. Due to the genetic material sharing, populations from different growing areas showed less differentiation, indicating population mixing. A shared ancestor, extensive planting material trade, and high levels of gene flow could all contribute to this explanation. Regardless of the geographic distance between populations, the interchange of plant material between locations increases gene flow and the dispersion of alleles. Such a trend maximizes genetic variability among individuals but reduces diversity among the population.

CONCLUSIONS

This study is the first to use micro-satellites to analyze avocado trees introduced to Ethiopia with only three trees. We found a low level of genetic variation in the studied germplasm, as indicated by several diversity indicators. We recommend creating a core collection of the genotypes from the entire range because the genetic diversity of populations is low. The UPGMA tree, DAPC, and STRUCTURE analysis showed mixed trees from different regions and populations, indicating a moderate but significant population structure and a high degree of gene flow between the groups. Ethiopia has diverse avocado germplasm due to gene transfer between populations.

CONFLICTS OF INTEREST

Authors declare that they have no conflicts of interest.

ACKNOWLEDGEMENT

The authors would like to acknowledge the smallholder farmers of the Sidama and the Southern Ethiopia regional governments for allowing us to collect the avocado accessions' leaves for this study.

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Original Research Article||

Prevalence of Retained Fetal Membranes and Associated Risk Factors in Cross Breed Holstein Friesian Dairy Cows Managed in Small and Large Scale Dairy Farms of Selected Districts of Sidama and Oromia Regional States

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Abstract

A retrospective study was carried out to analyze the prevalence and risk factors associated with retained fetal membranes (RFM) in HF crossbred dairy cows managed under various farm scales and farming systems in selected districts of Southern and Oromia regional states. A multi-stage sampling technique was employed to select farms and cows. A total of 120 farm households were selected for household survey. The perceptions of farmers on incidences of RFM, risk factors and possible mitigation practices were collected using the household survey. Prevalence of RFM was estimated by using data from: 1) monthly monitoring of smallholder dairy farms; where a total of 500 calvings were recorded between September 2019 and May 2020; 2) farm records on large scale dairy farms. The result shows that out of the total calving, 69 (13.8%) had RFM. RFM progressively and significantly increased with the advances in age of cows, showing 9.2%, 14.4%, 15.4% and 15.3% respectively, for age groups 2-4, 4-6, 6-8 and >8 years. With increased parities, prevalence of RFM also increased showing 6.9%, 13% and 74.3% respectively, for parity category 1-2, 3-6 and >7. The prevalence of RFM in cows was recorded for poor and medium body conditions as 18.6% and 4.3%, respectively. RFM was also associated with sex of calves born, 10.7% for female and 17.5% for male calves born. The prevalence of RFM was also affected by blood levels of HF crossbred cows where 50%, 75% and 87.5% crossbred had 32.1%, 12.1% and 13.3% RFM, respectively. In conclusion, the prevalence of RFM in the present study area was high, requiring special attention to be given by considering important predisposing factors. In this study, the impacts of other predisposing factors, such as nutritional status of cows, were not considered which might require further studies.

Key words: Ethiopia, reproductive problems, HF crossbred dairy cows, risk factors to retained placenta

Original submission: April 24, 2024; **Revised submission**: December 09, 2024; **Published online**: December 30, 2024

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INTRODUCTION

The livestock sector in Ethiopia, with the largest population in Africa, has a considerable contribution to the economy of the country with 30-35% of GDP and more than 85% of cash income sources for smallholder farmers (Usman et al., 2013).. The total cattle population, which is estimated at 70 million herds, is mainly composed of local breeds (98.2%) (CSA, 2022). Even though there is a large number of cattle in the country their productivity is low due to constraints such as disease, poor nutrition, poor management and poor genetic performance of indigenous cattle breeds.

These constraints results in poor reproductive performance of dairy cattle and hence lower economic benefit from the sector (Bitew and Prased, 2011). Among the major reproductive health problems that have direct impacts on the reproductive performance of dairy cows are abortion, dystocia, retained fetal membrane, repeat

breeding and uterine prolapse. The problems could be classified as postpartum and prepartum (Forar, et al, 1995). Reproductive health problems causes considerable economic losses to the dairy industry due to slower uterine involution, prolonged inter-conception and calving interval, negative effect on fertility, a drop in milk production and early depreciation of potentially useful cows (Mukasa, 1989; Tekelye et al., 1991; Dinka, 2013; Aman, 2023). Diseases have numerous negative impacts on productivity of herds i.e. death of animals, loss of weights, slow down growth, results in poor fertility performance and decrease in physical power (CSA, 2022).

With increases in population size, the demand for milk also increases (Usman et al., 2013). Should the dairy sector improve and play its vital role at both the macro- and micro levels (rural families) those reproduction and production factors needs to be carefully studied and interventions implemented.

Retained fetal membrane (RFM) is one of the most common conditions occurring in farm animals which occur following parturition. It is caused by multiple factors, which might even begin before parturition (Beagley et al., 2010). It is commonly observed in dairy cows, whereas it is less commonly reported in other domestic species. In physiological parturition, the afterbirth of the cow falls away within 3 to 8 hours following calving. The placenta is not said to be retained in cattle until 12 hours after parturition (Raheem et al., 2016)

Retained Fetal Membrane costs farmers in many ways, partly due to the veterinary costs, but mainly because of its effect on milk yield. Most importantly, RFM results in subsequent fertility problems. Studies have shown 5-10% prevalence rates of retained placenta in intensive dairy farms. Veterinary advice should be sought if the rate goes above this figure, especially if it goes above 10% (Laven, 2002). Detachment of the placenta in the cow is initiated by a progressive collogenolysis of the maternal and fetal connective tissues of the placentome during the last month of gestation. The successive weakening of the interface between the maternal and fetal tissue includes re-molding of the connective tissue and influx of leukocytes to the site (Gunnick, 1987).

RFM is a concern in dairy farms, because it can lead to further complications including Septic metritis (infection of the uterus), Septicaemia (infection of the blood), Endotoxemia (toxins in the blood), as well as Laminitis and death (Laven, 2002). In most of the animals affected with RFM, a slight to moderate loss of milk production and a slight to moderate delay in the involution the uterus and subsequent conception rates were reported (Stephen, 2002). With increased smallholder dairy intensifications and use of HF crossbred cows in dairy animals, it is important to asses associated reproductive problems such as RFM, where there is no sufficient data including from the present study area. Therefore, this study was intended to estimate the prevalence of retained fetal membranes and associated risk factors in cross-breed Holstein Friesian dairy cows in selected small and large-scale dairy farms. The specific objectives of the study were to quantify the prevalence of retained fetal membrane in smallholder and large-scale state farms (research and teaching dairy farms) in the study area, and to assess the possible risk factors.

MATERIALS AND METHODS

Description of the Study Area

The study was conducted on dairy farms found in Hawassa (in Sidama regional state), Shashemene (in Oromia regional state) and Wondo Genet area (in both Sidma and Oromia regional states). Hawassa is situated on average altitude of 1750m above sea level and receives average annual rainfall of 955 mm and has mean annual temperature of 20°C. Shashemene is located at 1800m above sea level. The mean annual temperature of Shashemene ranges from 9.02ºC to 19.43ºC. Wondo Genet is situated at 1800- 2400m above sea level, and has mean annual rain fall of 1200 mm.

Household survey, on-farm monitoring as well as farm records were used. The farm monitoring was held between September 2019 to May 2020 on both smallholder and large dairy farms. Farm records from two large dairy farms, both owned by Hawassa University, and found at Hawassa University main campus and Wondo Genet College of Forestry and Natural Resources, were used for the study.

Study Design

Data obtained from farm household cross sectional survey and monthly monitoring were used to estimate the prevalence of retained fetal membranes and also to identify other dairy cows' reproductive problems that occurred in small and large scale dairy farms. The questionnaire was pretested before actual data collection was applied. A regular follow up was also held on purposively selected large scale dairy farms at Wondo Genet (College of Forestry and natural resource dairy farm), Hawassa University main campus dairy farms.

Sample Size, Study Animals and Data collection

Household survey was administered on 120 dairy animal owners to collect information about major reproductive problems they observed. The total number of respondents were computed based on the number of farms in the area and by using Cochran's formula for finite population (n_0 = 384 and N=174 farms in the study areas).

$$n = \frac{n_0}{1 + \frac{(n_0 - 1)}{N}}$$

Where: n_0 = Initial sample size (i.e. already calculated), N = population size, and n = required sample size

From the total households 31 farms were from Hawassa, 41 from Shashemene and 48 Wondo Genet districts. The study animals were HF crossbred dairy cows managed under small- and large-scale production systems. For the farm monitoring, pregnant dairy cows were identified from the study dairy farms. Prior to monitoring of pregnancies and parturitions, the crossbred cows' history were recorded. The breed, blood level, age, body condition scores during parturition, as well as parity were recorded. During farm monitoring, clinical examination was held before confirming RFM. At the time of partition, the sex of claves born as well as the time period elapsed between calving and release of placenta were recorded. If it takes over 12 hrs until the placenta is released, it was recorded as retained placenta (Takagi et al., 2002). Any associated reproductive failures are also recorded. A monthly visit was carried out once a week on 35 dairy farms (7 farms in Hawassa, 12 farms in Shashemene and 16 farms were Wondo Genet area). A total of 253 dairy cows from small scale dairy farms and 247 dairy cows from large-scale dairy farms were monitored. Overall 500 dairy cows were fully monitored from small and large scale dairy farms.

Variables and Working Definitions:

Body Condition Scoring (BCS)

For all of the animals under this study body condition was scored in order to assess the nutritional status of the animal and the prevalence of post parturient reproductive health problems. Therefore, animals were grouped in to 1, 2, 3, 4 and 5 body condition scores according to Richard (1993) and later on classified as poor (score 1 to 2), medium (score 3) and good (score 4 to 5) as referred in Benti and Zewdie (2014). As the proportion of BCS with 4 and 5 were too small the data were not used analysis. The measurement to estimate BCS was done through palpation and visualization of the transverse and spines processes for the lumbar vertebrae (loin) and tail head respectively (Ambaw et al., 2017).

Breed

In this study all study subjects were crosses local breeds (mostly Arsi breed) and HF. All the 500 dairy cows were HF breed, but were categorized into three by their HF blood level as above 87.5% (denoted in this work as 87.5%), between 75% to 87.5% (denoted as 75%), and between 50% and 75% (denoted as 50%).

Parity

The study was undertaken based on number of calvings. Cows were categorized as primiparous (1st calving) or pluriparous (animals with two or more subsequent calvings).

Age

The study also focuses on the age of the cows exhibiting RFM, which were obtained from the records of animals. Age was also used to assess its effect on the prevalence of the RFM. The data was analysed to assess the influence of age on the prevalence of RFM.

Calf Sex

The sex of delivered calves exhibiting RFM were obtained from the records of animals with RFM and the basis of their calf sex, which was used to identify the effect of the calf sex on the incidence or prevalence of RFM.

Data Management and Analysis

The data obtained from the questionnaire and regular farm monitoring were entered into Microsoft Excel spreadsheet, and prepared for descriptive and inferential statistics. The association of RFM and reproductive health problems (abortion, repeat breeding, stillbirth, dystocia and uterine prolapse) retained fetal membrane with various risk factors (age, breed, parity, calf sex and body condition) were analysed by using univariate logistic regression analysis. SPSS version 20 software used for the data analysis.

RESULTS AND DISCUSSION

Prevalence and Risk Factors of RFM

From the total of 500 calved cows, 69 (13.80%) had RFM problems. The current finding is in agreement with Mamo (2004) and Gashaw *et al*. (2011) who reported 14.2% and 19.2% RFM prevalence, respectively. However, it is higher than that of Nigussu *et al*. (2016) and Molalegn and Shiv (2011), who reported 10% and 8.6%, respectively. Higher proportion of RFM was observed in 50% of Holestein cross and poor body condition cows. The Univariable logistic regression analysis revealed that breed, age, parity, body condition score, and sex of calf influenced the prevalence of RFM (Table 1).

Table 1. Prevalence and associated risk factors of RFM in cows

Risk Factors	Categories	No. examined	No with RFM (%)	95% CI
Breed	87.5% HF-Cross	248	33 (13.3%)	9.6-18.2
	75% HF-Cross	224	27 (12.1%)	8.4-17.0
	50% HF-Cross	28	9 (32.1%)	17.4-51.6
Age	2-4	109	10 (9.2%)	5.0-16.3
	4-6	111	16 (14.4%)	9.0-22.3
	6-8	136	21 (15.4%)	10.3-22.6
	>8	144	22 (15.3%)	10.3-22.2
Parity	1-2	288	20 (6.9%)	4.5-10.5
	3-6	177	23 (13.0%)	8.8-18.8
	>7	35	26 (74.3%)	57.2-86.2
BCS	Poor	161	30 (18.6%)	13.3-25.4
	Medium	300	13 (4.3%)	2.5-7.3
Calf sex	Female	272	29 (10.7%)	7.5-14.9
	Male	228	40 (17.5%)	13.1-23.1
Total		500	69 (13.8%)	11.0-17.1

Where, No. of +ve= Number of positive.

The analysis shows that the influences of breed, age, parity, body condition score and calf sex in the occurrence of retained fetal membranes.

Breed

Among the risk factors breed of cows, influenced the occurrence of RFM (Table 2). The prevalence of RFM was significantly higher in 50% HF-cross cows (χ^2=6.77; P< 0.05) than in 87.5% and 75% HF-cross breeds. This might be associated with the variation in the concentration of cholesterol, glucose and total protein; and non-esterified fatty acid, β-hydroxy butyric acid among the crosses (Kumari et al., 2015).

The prevalence of RFM was higher in 50% than in 87.5% and 75% HF blood level. This may be due to the attention differences given by the owners especially in management. Whenever the exotic blood level of cows was increasing, then owners provided more attention to cows in feeding,

watering and health care to get better milk production (Personal observation of researchers).

Table 2. Univariate logistic regression (ULR) on the prevalence of RFM in the study areas.

Risk Factors	Categories (level of risk factors)	No. of cow examined	No affected (%)	Std. Er.	χ^2	P-value	95% CI
Breed	HF-Cross- 87.5%	248	33 (13.3%)	0.02	0.17	0.683	9.6-18.2
	HF-Cross- 75%	224	27 (12.1%)	0.02	Ref		8.4-17.0
	HF-Cross- 50%	28	9 (32.1%)	0.09	8.20	0.004	17.4-51.6
Age	2-4	109	10 (9.2%)	0.03	Ref	-	5.0-16.3
	4-6	111	16 (14.4%)	0.03	1.4	0.229	9.0-22.3
	6-8	136	21 (15.4%)	0.03	2.2	0.143	10.3-22.6
	>8	144	11 (15.3%)	0.03	2.1	0.148	10.3-22.2
Parity	1-2	288	20 (6.9%)	0.02	Ref	-	4.5-10.5
	3-6	177	23 (13%)	0.03	4.8	0.029	8.8-18.8
	>7	35	26 (74.3%)	0.75	7.4	0.000	57.2-86.2
BCS	Poor	161	30 (18.6%)	0.03	25.3	0.000	13.3-25.4
	Medium	300	13 (4.3%)	0.01	Ref		2.5-7.3
Calf sex	Female	272	29 (10.7%)	0.19	Ref	-	7.5-14.9
	Male	228	40 (17.5%)	0.25	4.9	0.029	13.1-23.1

Where, χ^2= Chi-Square, CI=Confidence Interval. BCS= body condition score

Parity

Parity was significantly affecting RFM ($p< 0.05$). As parity increased the occurrence of RFM also increased. Similar results were reported from various areas of the world (Sharma et al., 2017; Khan et al., 2016; Hossain et al., 2015; Gaafar et al., 2010 and Roberts, 1986). When parity increases the cow's milk production also increases and it may cause hypocalcaemia (Sheldon, 2019), and this has impact on the uterine atony (Sheldon, 2019, Qu et al., 2013; Eiler and Fecteau, 2007 and Roberts, 1986) that end up with retention of placenta.

Body Condition Score (BCS)

The occurrence of retained fetal membranes were significantly ($p< 0.05$) influenced by the body condition of the cows. The prevalence of retained fetal membrane was low in medium-body condition cows than in poor conditioned animals. This finding is in general agreement with the report of Hossain et al. (2015) and Benti and Zewdie (2014). Studies have shown that cows with poor body condition were facing a decreasing uterine inertia, and inadequate uterine contraction during the third stage of labour (Sheldon, 2019; Eiler and Fecteau, 2007); and hence, there was poor expulsive force (Robert, 1986). Over-conditioning of cows might also make cows to be more susceptible to metabolic problems and infections making them more likely to have difficulty to give birth, which leads to retained fetal membrane (Ishak et al., 1983). The animals with poor body conditions were more susceptible to RFM, which is due to the weak expulsive force exerted to expel out the fetal membranes leading to secondary complications (Robert, 1986). Induced parturitions, hormonal imbalance, dystocia as well as poor body defence mechanism have been reported to be important predisposing factors for RFM (Ishak et al., 1983; Beagley et al., 2010).

BCS significantly affected ($p<0.05$) the prevalence of RFM. There is evidence of a high incidence of retained fetal membrane when cows' diets are deficient in selenium and/or vitamin E. correction of dietary deficiencies or supplementary feeding of these substances is commonly associated with a reduction of the incidence of retention. The incidence of retained membranes is higher in genetically high-yielding dairy cows and cows on high nutritive planes at parturition which are more prone to RFM, as cows might have disorders with carbohydrate metabolism (fat cow syndrome, ketosis, displaced abomasum) around the time of caving (Noakes et al., 2009).

Calf sex

Retained fetal membrane was significantly ($p< 0.05$) higher in cows calving male calf. In a study by Gaafar et al. (2010), RFM was not associated with calf sex. Hormonal imbalance, nutritional differences among farms, and physiological status like gestation length between individual cows were found as important predisposing factors for RFM (Eiler and Fecteau, 2007; Beagley et al.,. 2010).

District and Farm Scale Level Prevalence of RFM

The observed prevalence of RFP is shown in Table 3 below.

Table 3. Prevalence of RFM disaggregated by farm scales and study districts

Categories	Farm scale			Prevalence (%)
	Total Observed	Negative	Positive	
By farm scale				
Small scale	253	222	31	13.9%
Large scale	247	209	38	15.4%
Overall	500		69	13.8%
By district				
Wondo Genet	221	-	42	19.0%
Shashemene	150	-	15	10%
Hawassa	129	-	12	9.3%
Overall	500			

Prevalence of RFM at District Level

From the three study areas, the effect and prevalence of retained fetal membranes were highly observed around Wondo Genet area which is n=42 (19%), then n=15 (10%) in Shashemene and least in Hawassa n=12 (9.3%).

Perceptions of Farmers on Reproductive Problems

From the total 120 farm households surveyed, 80.8% revealed RFM as the top reproductive problems in the study area (Table 4). This result agree with Abunna et al. (2018) and Tolosa et al. (2021) who reported the highest prevalence of RFM. The proportion of RFM recorded during this study is in agreement with Yohannes and Alemu (2019) and Gashaw et al. (2011). A total of seven different types of reproductive problems were reported from the study areas. According to farmers RFM, abortion and repeated breeding were the top three major reproductive problems. Such reproductive problems were frequently reported from various parts of the country (Tolosa et al., 2021; Yohannes and Alemu, 2019; Abunna et al., 2018; Tigabneh et al., 2017). Such high rates of reproductive problems on dairy cows might heavily contribute to the low productivity of dairy cows in the study areas.

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Table 4. Reproductive disease identified in the study area

Reproductive Problems	Response Categories	District (n=120)			n	Proportion (%)
		Wondo Genet (n=52)	Shashemene (n=37)	Hawassa (n=31)		
Dystocia	Yes	5	5	2	12	10%
	No	47	32	29	108	90%
Stillbirth	Yes	5	3	3	11	9.2%
	No	47	34	28	109	90.8%
Uterine prolapse	Yes	6	4	1	11	9.2%
	No	46	33	30	109	90.8%
Milk fever	Yes	11	8	8	27	22.5%
	No	41	29	23	93	77.5%
Abortion	Yes	19	8	11	38	31.7%
	No	33	31	20	82	68.3%
RFM	Yes	42	32	23	97	80.8%
	No	10	5	8	23	19.2%
Repeat breeding	Yes	15	8	6	29	24.2%
	No	37	29	25	91	75.8%

From a total of 120 respondents about 22.5% of them reported that milk fever was the fourth problem in the study areas. Milk fever was reported from various areas of the country with different prevalence rates (Anteneh et al., 2012; Tolosa et al., 2021; Fasil et al., 2016). It mainly occurs due to deficiency of metabolizable Calcium ions in good milk-yielding cows, and weak management.

Relatively high prevalence of repeated breeding (24.2%) has been reported in this study. This problem was also reported from various scholars studied in different parts of the country (Haile et al., 2014; Hadush et al., 2013; Michael, 2003). The main reason of repeated breeding in healthy cows is related to poor management practices such as faulty heat detection, incorrect insemination time, inappropriate semen handling and technical problems or skill of technician (Arthur et al., 2016). Dystocia that accounted for 10% of the farms is an important predisposing factor for the occurrence of RFM. However, the current finding is higher than the prevalence of 5.9% reported by Fasil et al. (2016), 5.79% reported by Mamo (2004, 7.7% reported by Tesfaye and Shamble (2013), and 3.8% by Gashaw et al. (2011). Age and parity of the dam as well as breed of the sire were found as important factors. Inseminating cows with semen collected from large-sized bulls without taking into account the size and age of cows is an important factor in predisposing cows to dystocia (Noekes 1986).

CONCLUSIONS

From the results, it can be concluded that breed, parity, BCS and sex of calves had pronounced effects on the prevalence of RFM. This study revealed that RFM, abortion, repeated breeding and milk fever were the major reproductive problems in the area. Considering the higher prevalence and distribution of the problems in the area animal health extension work shall be strengthen to aware animal owners the impact of such reproductive problems on cows' productivity. Further epidemiological study to identify the main causes of each problems is very important; and this will play a key role in the designing of control options.

CONFLICTS OF INTEREST

Authors declare that they have no conflicts of interest.

ACKNOWLEDGEMENT

The authors are grateful to dairy farm owners in Wondo Genet, Shashemene and Hawassa for their willingness and cooperation. Also we extend our appreciation to Dr. Shewakena Nigussie for his great help and cooperation during data collection and provision of necessary information.

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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

Original submission: October 07, 2022; **Revised submission**: November 23, 2023; **Published online**: December 30, 2023
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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; Yematow et al., 2014). Morphological characteristics were assessed following the procedures outlined by Yematow 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 (Yematow 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; Yematow 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 (Yematow 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.

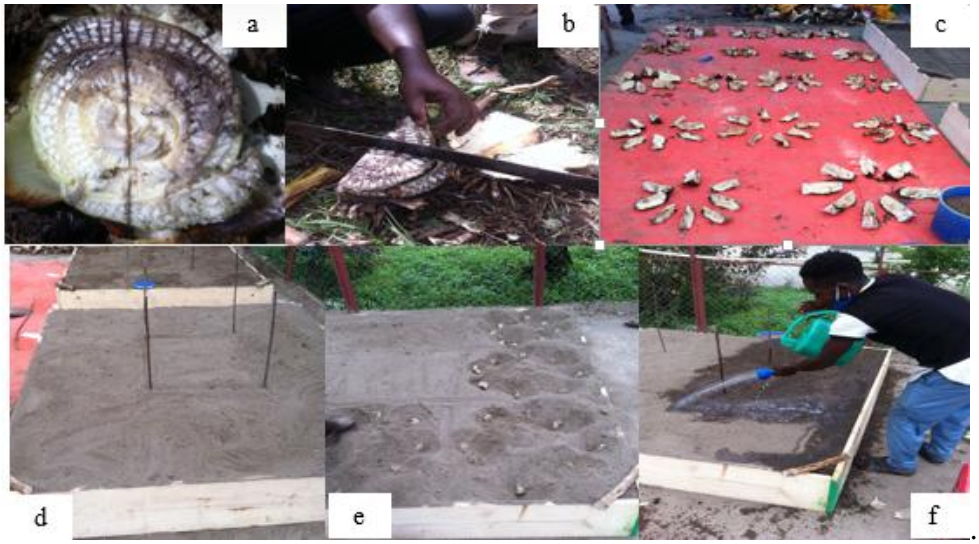

Figure 3. Enset landraces corm splits in-vivo macro propagation methods under a lath house. (a) Removal of meristematic tissue. (b) Corm split preparation using a sharp machete. (c) Corm splits exposed to diffused light for 48 hours under a lath house. (d) Prepared soil media mix filled in a wooden box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

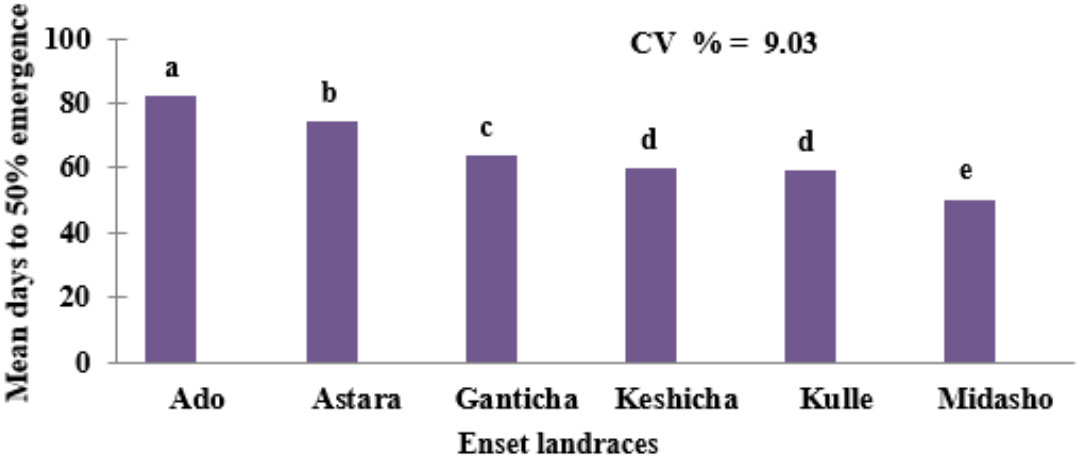

Figure 4. Effect of enset (*Ensete ventricosum* (Welw.) Cheesman) landraces parent corm splits *in vivo* macro propagation on mean days to 50% emergence. Mean differences of bars with the same letter(s) are statistically non-significant and different letters represent significant differences at $p <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

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).

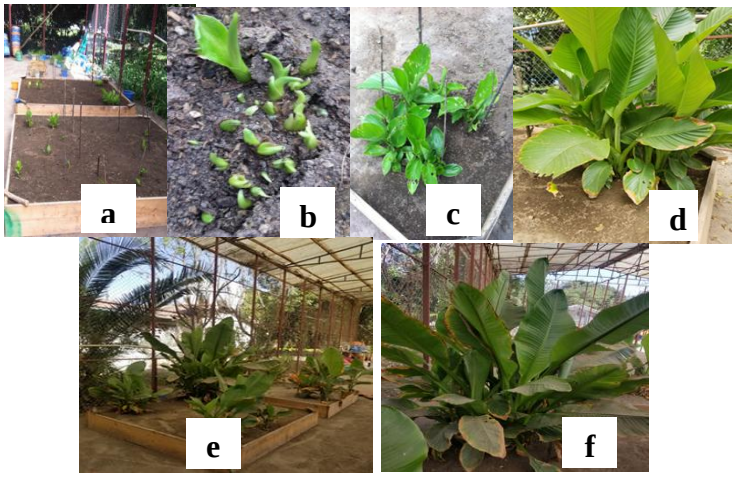

Figure 5. Enset landraces corm splits in-vivo macro propagation sucker growth under lath house. (a) First 50% sucker emergence on bed, (b) Sucker sprouting at 50 days, (c) Sucker growth after 86 days, (d) Sucker development on the Soil media mix, (e) Enset corm splits grown sucker differences at 9th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

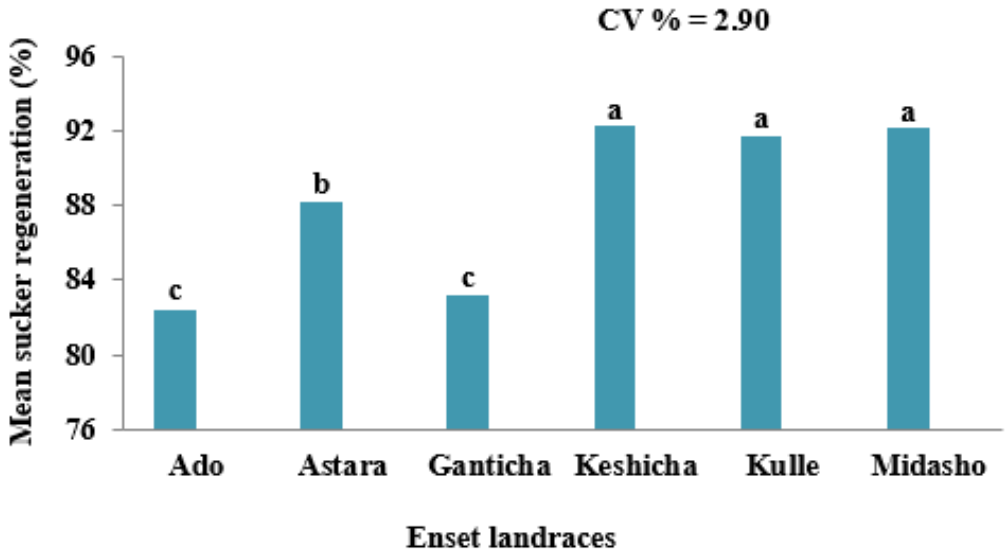

Figure 6. Effect of enset (*Ensete ventricosum* (Welw.) Cheesman) landraces parent corm splits *in vivo* macro propagation on mean sucker regeneration percentage. Mean differences of each bar with similar letter(s) are statistically non-significant and bars with different letters represent significant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

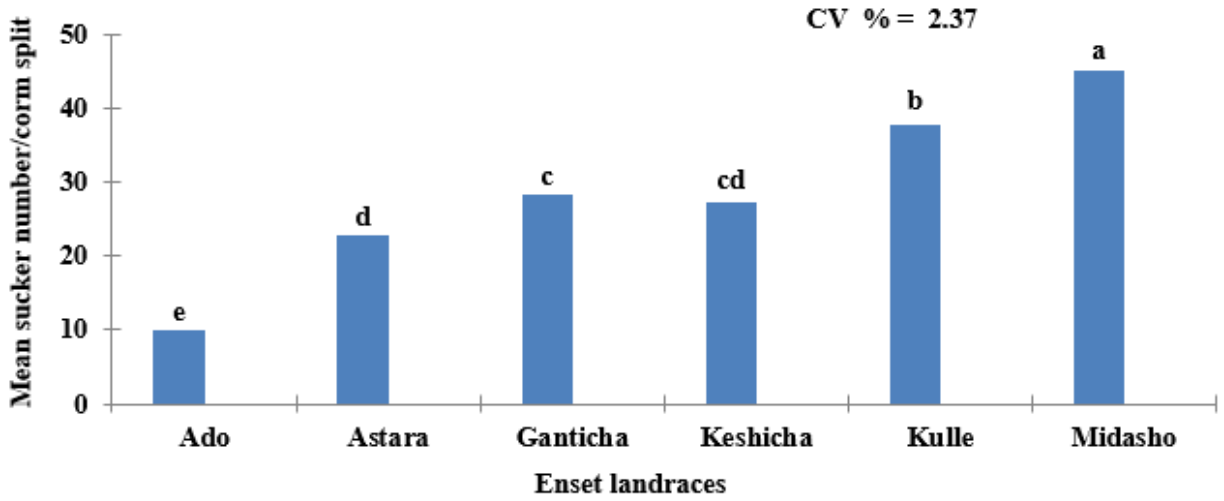

Figure 7. Effect of enset (*Ensete ventricosum* (welw.) Cheesman) landraces parent corm splits *in vivo* macro propagation on mean sucker number/corm split. Mean values of each bar with the same letter(s) are statistically non-significantly different and bars with different letter(s) are statistically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

ACKNOWLEDGEMENTS

We would like to genuinely thank and appreciate Hawassa, University, College of Agriculture, School of Plant and Horticultural Sciences for the delivery of laboratory and transport facility. We would also like to acknowledge the financial contribution and transport facility of NORAD and EWA-Belt Projects for their support to this research work.

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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The ***Journal of Science and Development (JSD)*** is a multi-disciplinary, peer-reviewed **bi-annual journal** published by the Research and Development Directorate of Hawassa University. JSD publishes articles on a wide range of disciplines, articles on a range of disciplines of agriculture and veterinary sciences including, Agricultural Biotechnology, Agribusiness, Agricultural Economics, Agricultural Engineering, Agricultural Microbiology, Agricultural Extension, Agronomy, Animal Healthcare, Animal Genetics, and Breeding, Animal Nutrition, Conservation Agriculture, Forestry and Agroforestry, Horticulture, Livestock Parasitology, Livestock Production, Plant Genetics, and Breeding, Plant Protection, Post-harvest Biology and Management, Community Nutrition, Sustainable Agriculture, Poultry, Soil Science, Veterinary Anatomy and Physiology, Veterinary Clinical and Preventive Medicines, Veterinary Diagnostics, Veterinary Epidemiology, Veterinary Pathology, Veterinary Toxicology.

General requirements

Upon submission of a manuscript, the authors are required to state that the paper has not been submitted for publication to any other journal or will not be submitted elsewhere in the future. Manuscript submission implies that the author and all co-authors agree to assign copyright to *JSD*. Manuscripts should be written in English, with spelling according to recent editions of the Advanced Learner's Dictionary of Current English (OUP). The font size for the text is 11- point Times New Roman, at exactly 1.5-point line spacing throughout (TNR 11/1.5).

Types of articles

Research articles

Research articles should report original research findings. They should not exceed 6000 words in length, including title, abstract and references; 3-4 tables and 5-6 figures are permitted.

Review articles

Review articles cover recent advances in an area in which an author has been actively engaged. Maximum permissible length is 6000 words, including title, abstract and bibliography, or proportionately shorter if the review includes illustrations.

Short communications

Short communications contain news of interest to researchers, including progress reports on ongoing research, records of observations, short comments, correction and reinterpretation of articles previously published in JSD, etc. Maximum permissible length is 1500 words, including title, abstract and references; they may contain no more than two figures and/or two tables.

Book reviews

A critical evaluation of a recently published book in all areas of science and development will be

published under this column. The maximum permissible length of a book review is 1500 words, including any references.

Format of manuscripts

Research articles intended for submission to the Journal of Science and Development (*JSD*) should have the following basic structure.

Research articles

Title: The title of the paper, the name (s) and affiliated institutions. Full postal, telephone and email address of the
corresponding author should be clearly indicated.

Abstract: The abstract must contain (a) the author's or authors' name(s), (b) the full title of the manuscript, (c) an abstract of not more than 300 words indicating the major aims and findings of the paper.

Keywords: 3-6 keywords should be set below the abstract, arranged in alphabetical order and separated by commas.

Introduction: A brief background of the subject, statement of the problem and the aims of the paper.

Materials and methods: Describe the materials and sites used in the study, the procedures, methods or tools used in data collection and analysis.

Results: Describe the results obtained, cross-referencing between text, tables and figures. When applicable, describe the statistical significance of the results.

Discussion: Give interpretations and implications of the results obtained. Compare your findings with related previous studies. The results and discussion sections may be presented together or separately.

Conclusions: Describe the contribution of the study to knowledge, and indicate future research needs (if any). The conclusion may also be included in the discussion.

References: All literature referred to in the text should be cited as exemplified below.

Acknowledgements: (if required). These should be brief, *e.g.* five lines of text.

Short communications

Short communications should essentially follow the structure given for research articles.

Review articles, book reviews

The structure of these articles will largely be determined by their subject-matter. However, they should be clearly
divided into sections by an appropriate choice of headings.

Methods of submission

1. Electronic submission

Manuscripts should be prepared by means of Microsoft Word or an equivalent word-processing program. They should preferably be submitted electronically, by means of the style sheet ***JSD-stylesheet.doc***, which can be downloaded from the Journal webpage. This style sheet consists of two sections:

(1) an *Input section*, into which your final manuscript is pasted from another Word

document, and
(2) a *Help section*.

The Help section contains detailed instructions for preparing a manuscript for *JSD*. Please read it before you begin to prepare your manuscript.

Electronic files containing manuscripts should be named according to the following convention:
Authorname_Brief_title.doc, *e.g.* Bloggs_Podocarps_in_southern_Ethiopia.doc, Where Brief_title is the first 4-5 words of the manuscript's title.

Diagrams should be lettered in a sans-serif font (Arial or Helvetica-at least 12-point), for final reduction to single- column (6.9 cm) or double-column (14.3 cm) width. Single column figures are preferred. Black-and-white diagrams should be submitted as uncompressed TIFF (.tif) files or as .jpg files, at a resolution of 300 dpi. Diagrams created in the default mode of Microsoft Excel (frame, colored background, *etc*.) are not acceptable for publication in *JSD.*

Files containing diagrams should be named according to the following convention: Author name _Figure No xxx.tif,
e.g. Bloggs_Figure 006.tif

Photographs should be submitted as high-resolution (at least 600 dpi) greyscale (8-bit).jpg or uncompressed .tif files. The desired final size ('1-col', '2-col' or 'landscape') should be indicated. Always send photographs as separate files, using the same filename convention as above.
Photographs as described above are preferred, but clear, glossy black and white photographs (100×70 mm) on photographic paper may also be submitted. They should be clearly numbered on the back in **soft** pencil.

Tables should be prepared in MS Word's Table Editor, using (as far as possible) 'Simple1' as the model: (Table … Insert … Table … Auto format …Simple 1),
(see JSD_stylesheet.doc for illustration). Tables taken directly from Microsoft Excel are not generally acceptable for publication in *JSD.*

Use Arabic (1, 2, 3 …), not Roman (I, II, III …), numerals for tables. Footnotes in tables should be indicated by superscript letters beginning with 'a' in each table. Descriptive material not designated as a footnote maybe placed under a table as a Note.

Footnotes should be avoided. Wherever possible, incorporate such material in the text, within parentheses.

2. Submission in paper form

Manuscripts may also be submitted on A4paper, subject to the same limits regarding number of words, tables and
figures as above. Separate the manuscript into three sections: (1) **text section**, with figure and table texts at the end;
(2) **figure section** (one figure per page, for reduction to 6.9-cm and 14.3-cmcolumn width); and
(3) **table section** (one table per page). Type the text itself at double line-spacing on one side of the paper only, with top, left and bottom margins set at 2.5 cm. The right margin should, however, be set at 7.5cm, to leave space for reviewers' and editors' comments. Number all pages in sequence, including figures and tables.

The order of headings and sub-headings should be indicated as shown in the style sheet JSD_stylesheet.doc. Keep all levels of heading as short as possible.

Tables, figures and illustrations should be submitted each on a separate page. When a manuscript is submitted in paper form, a CD containing all sections of the paper, including diagrams, is also required. Diskettes ('floppy disks') are not admissible.

Conventions

Scientific names must be italicized. At first mention, the author (*e.g.* (L.)) should be given, but must not be italicized.

Use single quotation marks '', unless you are giving a quotation within a quotation, in which case use "'"'.

Insert … Symbol … Special characters

All data should be given in the metric system, using SI units of measurement.

Use '.' (point) as the decimal symbol. Thousands are shown spaced, thus: 1 000 000.Use a leading zero with all numbers <1, including probability values (*e.g.*, $p < 0.001$).

Numbers from one to nine should be written out in the text, except when used with units or in percentages (*e.g.*, two occasions, 10 samples, 5 seconds, 3.5%). At the beginning of a sentence, always spell out numbers (*e.g.*, 'Twenty-one trees were sampled...').

Use the 24-hour time format, with a colon '**:**' as separator (*e.g.*, 12:15 h). Use day/month/year as the full date format (*e.g.*, 12 August2001, or 12/08/01 for brevity in tables or figures). Give years in full (*e.g.* '1994–2001', never '94–01'). Use the form '1990s', not '1990's' or '1990ies'.

Use the en-dash – for ranges, as in '1994–2001'

(Insert … Symbol … Special characters En dash).

In stating temperatures, use the degree symbol 'º', thus 'ºC', **not** a superscript zero '0'. (Insert … Symbol … Normal text),

Define all symbols, abbreviations and acronyms the first time they are used, *e.g.*, diameter at breast height (DBH), meters above sea-level (m asl). In the text, use negative exponents, *e.g.*, g m-2, g m-2 sec-1, m3 ha-1 as appropriate.

Use 'h' for hours; do not abbreviate 'day'.

If possible, format mathematical expressions in their final version (*e.g.*, by means of Equation Editor in MS Word or its equivalent in Word Perfect or Open Office); otherwise, make them understandable enough to be formatted during typesetting (*e.g.*, use underlining for fractions and type the numerator and denominator on different lines).

References

Please inspect the examples below carefully, and adhere to the styles and punctuation shown. Capitalize only proper names ('Miocene', 'Afar', 'The Netherlands') and the initial letter of the title of papers and books, e.g., write 'Principles and procedures of statistics', not **'**Principles and Procedures of Statistics'. Do not italicize Latin abbreviations: write 'et al.', **not** '*et al.*'

References in the text should use the 'author-year' (Harvard) format:

(Darwin and Morgan, 1993) or, if more than two authors, (Anderson et al., 1993)

(Hartman and Kester, 1975; Anderson et al., 1993; Darwin and Morgan, 1994) chronologically.

It is highly recommended that Citations/References Management Software programs such as

Mendeley are used for organizing Citations and Bibliographic lists following the style of Crop Science Journal (alphabetical order) as shown in the following examples:

Journal article

Kalb J.E. 1978. Miocene to Pleistocene deposits in the Afar depression, Ethiopia. *SINET: Ethiop. J. Sci*. 1: 87-98.

Books

Whitmore T.C. 1996. *An introduction to tropical rain forests*. Clarendon Press, Oxford, 226pp.

Steel R.G.D. and Torrie J.H. 1980. *Principles and procedures of statistics*. 2nd ed. McGraw-Hill Book Co., New
York. 633 pp.

Contribution as a chapter in books (Book chapter)

Dubin H.J. and Grinkel M. 1991.The status of wheat disease and disease research in warmer areas. In: Lange L.O., Nose1 P.S. and Zeigler H. (eds.) *Encyclopedia of plant physiology. Vol. 2A Physiological plant ecology*. Springer- Verlag, Berlin. pp. 57-107.

Conference/workshop/seminar proceedings

Demel Teketay 2001. Ecological effects of eucalyptus: ground for making wise and informed decision. Proceedings of a national workshop on the eucalyptus dilemma, 15 November 2000, Part II: 1-45, Addis Ababa.

Daniel L.E. and Stubbs R.W. 1992.Virulenceof yellow rust races and types of resistance in wheat cultivars in Kenya.
In: Tanner D.G. and Mwangi W. (eds.). Seventh regional wheat workshop for eastern, central and southern Africa. September 16-19, 1991. Nakuru, Kenya: CIMMYT. pp. 165-175.

Publications of organizations

WHO (World Health Organization) 2005. Make every mother and child count: The 2005 World Health Report. WHO,
Geneva, Switzerland.

CSA (Central Statistical Authority) 1991. Agricultural Statistics. 1991. Addis Ababa, CTA Publications. 250 pp.

Thesis

Roumen E.C.1991. *Partial resistance to blast and how to select for it*. PhD Thesis. Agricultural University,
Wageningen, The Netherlands. 108 pp.

Gatluak Gatkuoth 2008. *Agroforestry potentials of under-exploited multipurpose trees and shrubs (MPTS) in Lare district of Gambella region.* MSc. Thesis, College of Agriculture, Hawassa University, Hawassa. 92 pp.

Publications from websites (URLs)

FAO 2000. Crop and Food Supply Assessment Mission to Ethiopia. FAOIWFP. Rome. (http://www. fao.or~/GIE
WS). (Accessed on 21 July 2000).

Proof correction

Page proofs will be sent to the author, shortly before publication, as an Adobe Acrobat portable document format (PDF) file attachment to an e-mail message. This is essentially the final form in

which the paper will appear. Minor alterations may be made, to conform to scientific, technical, stylistic or grammatical standards.

Although proofs are checked before they are sent to the author(s), it is the responsibility of the author(s) to review page proofs carefully, and to check for correctness of citations, formulae, omissions from the text, *etc*. Author(s) should return their corrections within seven (7) working days from the date on which the proofs were sent to them. Failure to do so will cause the paper to be printed as in the page proofs.

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ISSN (Online): 2789-2123; (Print): 2222-5722