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Advances in Coffee Germplasm Enhancement and Utilization in Sierra Leone: A Monograph

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Abstract

Coffee (*Coffea* spp.) is an economically important cash and beverage crop that boosts many livelihoods in developing countries, including Sierra Leone. The country is making significant strides in the enhancement and utilization of the crop, particularly the native species *Coffea stenophylla*. However, dearth of well-articulated knowledge exists on the genetic improvement and utilization of the crop for desired end-user traits. This monograph presents the progress made in the genetic improvement, trait profiling, discovery of quantitative trait loci and candidate genes linked to desired traits of interests. Advances in breeding technologies and methodologies, as well as the availability of high-quality historical trial data, continue to drive improvements in coffee genotypes for desired end-user traits. Information from this review could be useful in designing future marker-based breeding and genetic conservation of coffee. The exploitation of Arbuscular Mycorrhizal Fungi (AMF) for improvement of coffee tolerance to biotic and abiotic stresses, priming strategies, combined utilization of high-throughput genotyping and high-throughput digital phenotyping, and assessment of long-term effects of plant domestication, pleiotropy and selective breeding for desired agronomic traits of coffee forms part of future work on the crop.

Keywords

Trait Profiling, Agronomic and Breeding Constraints, Quantitative Trait Nucleotides, Paternity Determination, White Yam

1. Introduction

Coffee (*Coffea* spp.) is one of the most important beverages and cash crops world-

wide, providing economic livelihood to millions of people in developing countries [1]. It is ranked second in international trade after crude oil [2]. Globally, green coffee production is estimated to be above 134.16 million bags (60 kg capacity per bag) with a retail sales value in excess of $22.7 billion during the 2010-11 sales in the world market [3]. Spanning over 80 countries in Africa, Asia and Latin America, coffee is grown on about 10.2 million hectares of land in the tropical and subtropical regions of the world [2]. Coffee trees belong to the genus *Coffea* in the family Rubiaceae. The genus *Coffea* has a record of more than 130 species, but only three species are commercially cultivated: *C. arabica* L. (2n = 4x = 44) known for its allotetraploidy and self-fertility [4], *C. canephora* P. (2n = 2x = 22), and *C. liberica* L. (2n = 2x = 22). Of recent times, *C. stenophylla* is beginning to gain attention globally due to its superiority taste comparable to *C. arabica* [5]. *C. arabica* is preferred in the consumer market due to its beverage quality, aromatic characteristics, and low-caffeine content, compared to robusta, which is characterized by a stronger bitterness and higher-caffeine content. Globally, *C. arabica* contributes 65% of the total global coffee production [6]. Considering the number of species on record with only a few being commercially viable, this presents limitation for their genetic improvement through conventional breeding programs because of their perennial nature and differences in level of ploidy and incompatibility. Most importantly, characteristics such as resistance to pathogens or pests are seen to be lacking in the available germplasm globally, although conventional and advanced breeding techniques are in progress to address these challenges [1]. Genetic engineering techniques have been utilized to solve this barrier, leading to significant advances during the last decades.

Global coffee production is dominated by *C. arabica* (Arabica coffee) and *C. canephora* (Robusta coffee) due to their relatively high-yielding and quality attributes as opposed to other coffee species. Despite these advantages, production of Arabica and Robusta coffee is facing mounting challenges though not limited to increasing prevalence and severity of biotic and abiotic stresses. These challenges bring forth an indication that the global coffee crop portfolio requires diversification to ensure resilience to the key challenges for sustainable production. Sierra Leone is in the center of genetic diversity of genus *Coffea*, and the country hosts rich coffee genetic resources [5]. The *C. stenophylla*, *C. affinis* and possibly other wild relative species are indigenous to Sierra Leone and these species offer great potential for a new coffee market and income generation [7]. However, more efforts of conservation and genetic improvement on these species are needed to realize these opportunities [5].

C. arabica is mainly native to the highlands of Southwestern Ethiopia with additional populations in South Sudan (Boma Plateau) and North Kenya (Mount Marsabit) [8]. The varieties of *C. arabica* grown all over the world are derivatives from either the "Typica" or "Bourbon" genetic base, resulting in low-genetic diversity among cultivated arabicas. In contrast, however, *C. canephora* has a wide geographic distribution, extending from the western to central tropical and sub-

tropical regions of the African continent, from Guinea, Sierra Leone and Liberia to Sudan and Uganda with high genetic diversity in the Democratic Republic of the Congo [9]. Additionally, *C. canephora* maintains heterozygosity due to its cross-pollinating nature.

More than a decade ago, considerable advances were made in the genetic transformation of coffee. A number of research groups in the world have been able to transform coffee through genetic manipulations for insect resistance, production of decaffeinated coffee, resistance to herbicide and control of fruit maturation. Although the majority of the research is seen to be still limited to laboratory and greenhouse studies, initial field tests with transformed coffee progenies are beginning to appear in the literature.

2. Monograph Methodology

This study collected, collated and synthesized existing evidence on Advances in coffee germplasm enhancement and utilization in Sierra Leone**.** The review followed a structured process comprising literature identification, screening, eligibility assessment, data extraction, and synthesis to ensure transparency and reproducibility.

A search was conducted across Web of Science, Scopus, ScienceDirect, Springer Nature, PubMed/PubMed Central, MDPI, Google Scholar, and the Food and Agriculture Organization (FAO) repository. Additional studies were identified through manual screening of reference lists from relevant publications. Searches were performed using combinations of keywords and Boolean operators related to germplasm collection, characterization, conservation and maintenance; trait profiling, discovery and utilization for coffee germplasm improvement; association mapping of important traits in coffee; breeding for abiotic stress resistance in coffee; and impact of drought stress in the production and productivity of coffee.

Studies were included if they focused on coffee in the highlighted subject areas, and were excluded if they did not focus on the coffee, lacked clear variables of the subject studied, were duplicate publications, or provided insufficient methodological information.

3. Germplasm Collection, Characterization, Conservation and Maintenance

Plant genetic transformation expands the gene pool beyond conventional breeding, enabling efficient gene transfer across species. This technique is vital for perennial crops, allowing rapid introduction of new traits while preserving the original cultivar's genetic base, thus advancing plant science and agriculture. Transgenic crop production is beginning to reach 90 million hectares ten years after release of commercially viable genetically modified plants [10]. Plant transformation aids breeding programs and is essential in research, enabling gene knockout and overexpression. It is valuable across plant physiology, biochemistry, and phytopathology.

Conventional breeding has effectively developed rust-resistant coffee cultivars, but introducing new traits typically takes 20 to 35 years. Genetic engineering can significantly shorten this timeframe, allowing for the quicker development of cultivars with enhanced disease resistance and quality. This technique also facilitates the introduction of traits from different species, enabling characteristics like insect resistance, herbicide resistance, and improved tolerance to abiotic stresses such as drought or frost, which are challenging to achieve through traditional methods.

Genetically Modified (GM) coffee plants have been produced by different research groups in the world [11]-[15]. Despite significant advances over the last 15 years, coffee transformation is still very laborious, with bottlenecks in the methodology that make it far from a routine laboratory technique [16] [17].

Plant Genetic Resources (PGR) are crucial for agro-biodiversity, encompassing primitive, modern, obsolete, breeding lines, weedy, and related wild species [18]. Genetic diversity in farmers' fields and wild relatives enhances crop productivity through plant breeding, but PGRs are finite and vulnerable to losses due to new crop varieties, urbanization, and natural hazards [19]. The PGR significantly contributes to achieving the Millennium Development Goals through genebanks, with over six million accessions conserved in over 1400 genebanks worldwide [20]. The Sierra Leone Agricultural Research Institute (SLARI) has responded to this need by establishing a Genetic Resources Unit for assembly, characterization, evaluation, maintenance, conservation, documentation and distribution of germplasm of selected tree crops such as coffee (*Coffea* species), cacao (*Theobroma cacao* L.), cashew (*Anacardium occidentale*) and oil palm (*Elaeis guineensis*).

Genetic variation, once considered unlimited, is rapidly eroding as modern cultivars replace traditional ones, causing the destruction of natural habitats of wild relatives of cultivated species. Several land races of some of the enlisted crops conserved under SLARI's jurisdiction have disappeared, except for *C. stenophylla*, which has resurfaced since 2018 and is beginning to gain momentum due to its superior flavor and ability to withstand changing climatic conditions. Seed conservation is crucial for crop genetic diversity preservation, as it is cost-effective, simple, and maintains genetic stability over long periods. It involves collection, characterization, conservation, distribution, and utilization of plant germplasm for global germplasm conservation.

Germplasm collection is costly and time-consuming, so it's crucial to review past crop collections and secure germplasm from those who have already explored the area. The collection offers protection against genetic erosion, resistance to diseases, pests, and environmental stresses, and improved crop quality and yield traits.

The primary goal of collecting PGR is to capture the maximum amount of genetic variation in the smallest number of samples [21]. Efficient strategies for germplasm collection are influenced by the available information on genetic variation in target taxa populations and their distribution in the target geographical region [22]. However, in cases where there is a lack of information about the target species and the collection area, it may be beneficial to conduct an exploration mission.

The establishment of the Institute of Agricultural Research (IAR), which was later transformed into SLARI in 2009, aimed at the assemblage of germplasm of its mandate crops through donation and exchange with various West African sub-region research institutes. This effort was also complemented by the collection, characterization and management of various land races spanning across tree crops, root and tuber crops, cereals and horticultural crops. The IAR received the first set of introduced *T. cacao* in the 1960s from the West African Cocoa Research Institute (dubbed as WACRI Series I and II). During these periods, Sierra Leone was served alongside her West African Counter parts such as Ghana, Nigeria, Cote d'Ivoire and Togo. This was followed by the introduction of eight varieties of *C. canephora* from Uganda and Nigeria. These materials were initially deposited at the Njala Lower Nursery, Mokonde. They were later propagated and established at SLARI's cocoa and coffee clonal gardens at Kpuwabu and Pendembu. In 2019, SLARI acquired its first-ever set of ten *C. arabica* varieties from Embapra, Brazil through a European Union Funded Project called Boosting Agriculture and Food Security (BAFS). The 10 varieties including MGSCATIGUA' 3, CATUCAI AMARELO (2SL), IAC 125 RN, CATUCAI AMARELO (IAC 62), PAQAISO 2, PAQAISO (H 419-1), TOPAZIO MG 1190, MUNDO NOVO (IAC 379-19), CATUAI VEQMELHO (IAC 144), and OEIRAS MG 6851 were established at Bambawo, Pendembu and Kpuwabu clonal gardens for onward characterization (**Table A1**). The current collections account for 65% - 70% of the available diversity, and there is a continuous need to protect endangered germplasm, especially *C. stenophylla*.

Recently, we obtained wild saplings of *C. stenophylla* from the Kambui and Kasewe Hills for establishment at Bambawo (due to its proximity to the Kambui Forest). Similarly, the Welthungerhilfe (WHH) organization through Greenwich University has been collecting and nursing both seeds and saplings of stenophylla from *ex-situ* and distributing them to smallholder coffee farmers as a form of conservation of wild coffee relatives. Additionally, coffee culture has established 10 ha of wild *C. stenophylla* at Ngegeru (entry point into the Kambui Forest for collection of stenophylla seeds and saplings).

The national agricultural research programs in most countries, universities, regional organizations, and international centers are working on developing crop cultivars/elite breeding lines. Most of these collected lines are conserved in constituent centers of SLARI's genebank for future utilization.

Germplasm utilization by breeders requires accurate characterization of agronomic and morphological traits, continuous screening against stresses, and joint estimation of yield traits with contributions from scientists across different disciplines. For instance, at SLARI, germplasm sets for coffee have been evaluated for agronomic performance by scientists from the Kenema Forestry and Tree Crops Research Centre (KFTCRC) of SLARI. The results of joint evaluation by a team of KFTCRC scientists have resulted in better understanding of the germplasm materials conserved at SLARI's tree crops genebank so that they could be characterized for important morpho-agronomic traits.

Germplasm characterization is the systematic recording of heritable, identifiable traits with precision in fields, contrasting with preliminary evaluation, which records limited agronomic traits for crop improvement. The major objectives of germplasm characterization are as follows:

1) Describe accessions, establish their diagnostic characteristics and identify duplicates;

2) Classify groups of accessions using sound criteria;

3) Identify accessions with desired agronomic traits and select entries for more precise evaluation;

4) Develop interrelationships between, or among traits and between geographic groups of cultivars; and

5) Estimate the extent of variation in the collection.

Achieving the above objectives would lead to the identification of a wide range of sources for desirable traits in the assembled germplasm.

3.1. Phenotypic Diversity of Wild Sierra Leonean Coffee (*Coffea stenophylla*) Collected from Kenema and Moyamba Districts

- The Shannon-Weaver diversity index (H') revealed variations among the samples for the observed 13 morphological traits, which range from 0 for both fruit color and calyx limb persistence to 0.87 for angle of insertion of primary branches on the main stem.
- Among the 13 morphological traits assessed, angle of insertion of primary branches on main stem (0.87), growth habit (0.78), bean size (0.75), young leaf color (0.66), stem habit (0.66) and fruit shape (0.65) exhibited high level of diversity while seed shape (0.58), stipule shape (0.46), leaf shape (0.43), seed uniformity (0.31) and leaf apex shape (0.06) showed low levels of diversity.
- This is the first report of phenotypic diversity of *C. stenophylla* in Sierra Leone and the study thus unraveled existence of diversity among samples.
- It is recommended that these observed variabilities be exploited in order to develop better accessions that are high-yielding yet maintain the same taste.
- Additionally, genetic fingerprinting needs to be applied to provide a complementary assessment of the observed phenotypic diversity.

Phenotypic variations detected in some of the key traits measured among the genotypes are presented in **Figure 1**.

3.2. Unveiling the Genetic Diversity and Demographic History of *Coffea stenophylla* in Sierra Leone Using Genotyping-by-Sequencing

- Using 1037 novel Single Nucleotide Polymorphism (SNP) markers derived from Genotyping-by-Sequencing (GBS), three distinct natural populations (mean Fst = 0.176) of *C. stenophylla* exist in Sierra Leone.
- Evidence of recent bottlenecks and small effective population size (118 - 140) was found across all three populations, reflecting the impact of recent anthropogenic disturbances on this species.

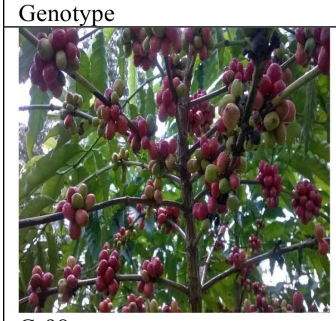
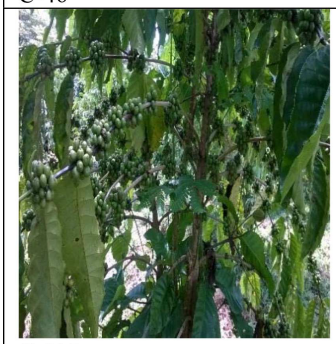
Genotype	**Trait description**
 G-98	**Fruits traits:** Predominantly red when ripe. Occasional yellow-fruited type is rare. Immature fruits are green. Relatively bigger fruits range from 20 to 25 mm. Bean size is smaller than Arabica but uniform. **Plant height**: Typically, 2 to 4 m when cultivated and about 10 m in wild. Taller than Arabica (pruning is essential). **Branching pattern**: Open spreading canopy. Has longer internodes, thus, influences planting density and shading. **Leaf traits**: Broad, large leaves. Glossy, dark green, strong venation. Leaves are larger than Arabica and contributes to canopy. **Adaptation**: Thrives in lowland, hot, humid climates (200-800 m). More tolerant to pest and diseases. Requires high rainfall and temperature.
 A-1	**Fruits traits:** Predominantly red when ripe. No yellow-fruited type. Immature fruits are deeply green. Relatively smaller fruits range from 10 to 12 mm. Bean size is smaller than Arabica and some robusta types but is uniform. **Plant height**: Typically, 2 to 4 m when cultivated and about 10 m in wild. Taller than Arabica (pruning is essential). **Branching pattern**: Open spreading canopy. Has shorter internodes compared to G-98, thus, influences planting density and shading. **Leaf traits**: Broad, large leaves. Glossy, dark green, strong venation. Leaves are larger than Arabica and contributes to canopy. **Adaptation**: Thrives in lowland, hot, humid climates (200-800 m). More tolerant to pest and diseases. Requires high rainfall and temperature.
 C-97	**Fruits traits:** Predominantly pale-red when ripe. Immature fruits are pale-yellow. Relatively smaller fruits range from 12-14 mm. Bean size is smaller than Arabica and some robusta types but size is uniform. **Plant height**: Typically, 2 to 4 m when cultivated and about 10 m in wild. Taller than Arabica (pruning is essential). **Branching pattern**: Open spreading canopy. Has shorter internodes compared to G-98, thus, influences planting density and shading. **Leaf traits**: Broad, large leaves. Glossy, dark green, strong venation. Leaves are larger than Arabica and contributes to canopy. **Adaptation**: Thrives in lowland, hot, humid climates (200-800 m). More tolerant to pest and diseases. Requires high rainfall and temperature.
 C-46	**Fruits traits:** Predominantly red when ripe. Occasional yellow-fruited type is rare. Immature fruits are green. Relatively bigger fruit bean or fruit size ranging from 18 to 22 mm. Bean size is smaller than Arabica but uniform bean size. **Plant height**: Typically, 2 to 4 m when cultivated and about 10 m in wild. Taller than Arabica (pruning is essential). **Branching pattern**: spreading canopy. Has longer internodes, thus, influences planting density and shading. **Leaf traits**: Broad, large leaves. Glossy, dark green, strong venation. Leaves are larger than Arabica and contributes to canopy. **Adaptation**: Thrives in lowland, hot, humid climates (200-800 m). More tolerant to pest and diseases. Requires high rainfall and temperature.
 C-115	**Fruits traits:** Predominantly red when ripe. Occasional yellow-fruited type is rare. Immature fruits are green and become red when ripe. Relatively bigger fruit bean or fruit size ranging from 20 to 24.5 mm. Bean size is smaller than Arabica but uniform bean size. **Plant height**: Typically, 2 to 4 m when cultivated and about 10 m in wild. Taller than Arabica (pruning is essential). **Branching pattern**: Open spreading canopy. Has shorter internodes, thus, influences planting density and shading. **Leaf traits**: Broad, large leaves. Glossy, dark green, strong venation. Leaves are larger than Arabica and contributes to canopy. **Adaptation**: Thrives in lowland, hot, humid climates (200-800 m). More tolerant to pest and diseases. Requires high rainfall and temperature.

Figure 1. Morphological variations in fruit and plant architecture among cocoa genotypes.

- Using a model-flexible inference approach, we unveiled a strong ancient bottleneck approximately 23,000 years ago, coinciding with the Last Glacial Maximum (LGM), followed by post-glacial expansion and divergence into distinct genetic clusters.
- A comparative analysis between *ex-situ* genebanks and natural populations detected a significant gap in genetic diversity, with two out of three natural populations missing from the *ex-situ* genebank collection.
- These findings highlight the urgent need to improve conservation practices for *C. stenophylla* in Sierra Leone.
- The novel SNP markers developed in this study provided valuable tools to support future efforts in conservation and utilization of *C. stenophylla* genetic resources in West Africa.
- The geographic locations of the collection sites, population structure and neighbor-joining tree of *C. stenophylla* are presented in Table 1 and Figure 2 and Figure 3.

Table 1. Populations of *C. stenophylla* and their geographical locations in Sierra Leone.

Population	No. of samples	Latitude	Longitude	Elevation (masl)
Kasewe	25	8˚19'11.694"N	−12˚10'1.620"W	416
Kpumbu	28	7˚59'23.364"N	−11˚11'40.356"W	375
Ngegeru	45	7˚56'50.634"N	−11˚12'16.818"W	466
Bambawo, SLARI	45	8˚0'31.698"N	−11˚8'8.496"W	266
Total	143			

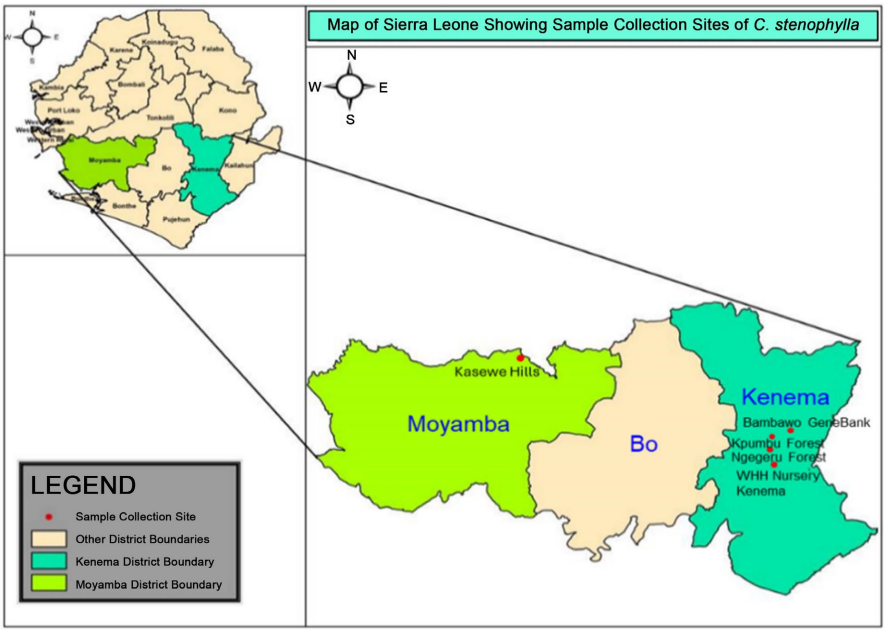


Figure 2. Geographic locations of the four sampled *C. stenophylla* sites.

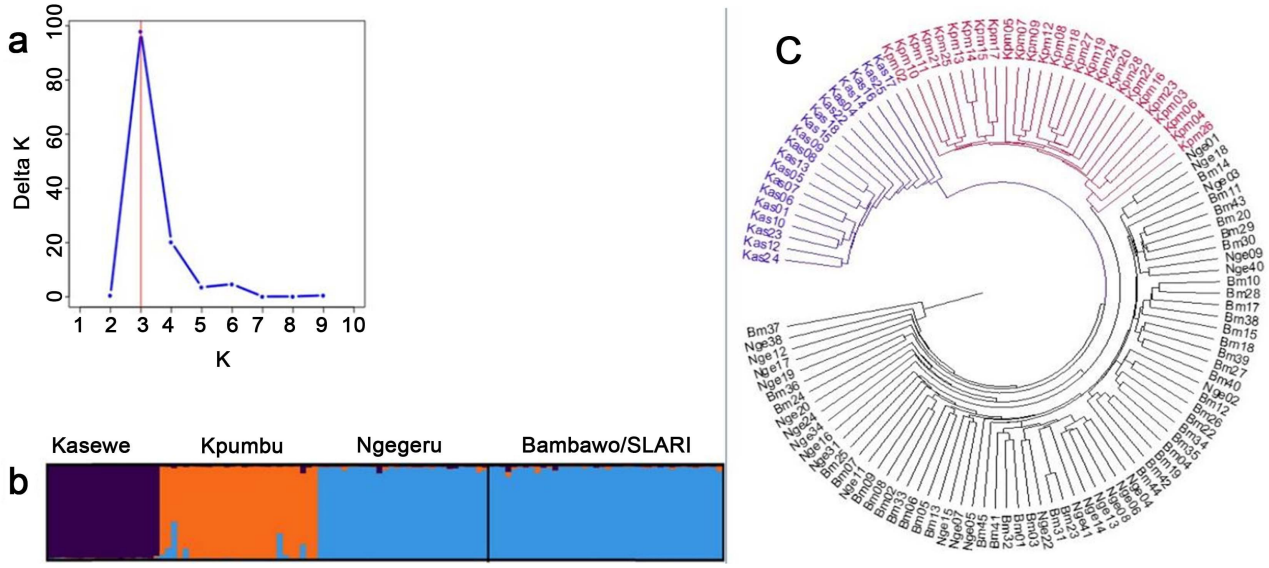


Figure 3. Display of (a) and (b): Bayesian clustering of population structure; (c): Neighbor joining tree of 115 *C. stenophylla* accessions based on 1037 SNP data.

3.3. Germplasm Regeneration

Seeds lose viability even under good storage conditions, necessitating periodic regeneration based on initial viability, rate of loss, and regeneration standard (*i.e.*, the percentage viability at which it is decided to regenerate the accession) [23]. Regeneration is a crucial process in genebank management, aiming to increase seed availability or restore viability in depleted accessions. It's costly, time-consuming, and poses genetic integrity risks, with methods varying by crop species and its reproductive system (inbreeding or out-breeding) [24].

Germplasm regeneration at SLARI is conducted during the post-rainy season, ensuring high-quality seeds due to low disease and pest incidence. The process involves precision field activities, agronomic management, and pollination control measures. It is always ideal for regeneration sites to be carefully selected and ensure that specific cultivar requirements are met, minimizing regeneration frequency and maximizing seed longevity.

It must be noted that seeds are a product of the seed production environment and the genetic constitution of the parent plant [19]. Environmental conditions often influence genetic traits, requiring improved germplasm regeneration programs to focus on effective management and production practices, including pre-harvest, post-harvest, and seed drying techniques [25]. Coffee Wild Relatives (CWR) and critical accessions require proliferation through the use of glasshouse, cleaning procedures for germplasm seeds, and timely processing to avoid losses. A lot of this on germplasm regeneration has been documented by [26].

Efficient regeneration systems are crucial for genetic transformation in coffee, using methods like somatic embryogenesis, meristem and axillary bud culture, and adventitious buds from different tissue types [27] [28].

Somatic embryogenesis: Somatic embryogenesis, the process of forming embryos from somatic tissues without sexual fusion, has been reported in coffee plants, specifically from *C. canephora* internodal explants [29]. Calluses were successfully induced from seeds, leaves, and anthers of Mundo Novo and Bourbon Amarelo cultivars in *C. arabica* [30]. Over the past 35 years, various protocols for somatic embryogenesis have been developed for different coffee genotypes [27]. The protocol for obtaining high embryogenic potential calli from *C. arabica* leaf explants utilized two different culture media compositions: a "conditioning" medium and an "induction" medium [31] [32]. The availability of auxins is crucial for the induction of embryogenic calli [31]. Coffee exhibits High-Frequency Somatic Embryogenesis (HFSE) and Low-Frequency Somatic Embryogenesis (LFSE), with 2,4-D enhancing HFSE and Indole Butyric Acid (IBA) and NAA increasing LFSE. Two cell cluster types are observed during induction [33]. Gene expression differences were observed between embryogenic and nonembryogenic cell clusters, with higher numbers of genes turned off in somatic cells for embryogenic transition [33].

Coffee undergoes two distinct somatic embryogenesis patterns: direct from explants and indirect from callus, both originating from leaf segments and callus, with unicellular origins [34]. Coffee embryogenesis is regulated using triacontanol, $AgNO_3$, salicylic acid, thidiazuron, and purine, with salicylates enhancing somatic embryogenesis in *C. arabica* tissue culture [35]. Triacontanol and silver nitrate, when combined with indole-3-acetic acid and benzyladenine, induced direct somatic embryogenesis in both *C. arabica* and *C. canephora* species [36]. Thidiazuron (TDZ) was found to induce direct somatic embryos from cultured leaf explants of *C. canephora* cv. C × R [36]. Polyamines increase embryogenic response in *C. canephora* explants, but inhibitors like D,L-alpha-difloromethylornithine and D,L-alpha-difloromethylarginine significantly reduce it, highlighting the crucial role of polyamines in coffee somatic embryogenesis [37]. Polyamines, indoleamines, calcium, and calcium ionophores, along with ethylene and dissolved oxygen concentration, are beneficial in inducing somatic embryogenesis in coffee [38].

The industrial-scale use of somatic embryos was achieved by inducing *C. arabica* embryos in liquid medium using bioreactors [39]. The study yielded approximately 46,000 embryos per 3L Erlenmeyer flask after 7 weeks of culture, with other workers also reporting somatic embryo production for industrial use [40].

Micro propagation: The coffee plant has a single apical meristem with 4 - 5 dormant orthotropic buds and two plagiotropic buds. Both types of buds are cultured for plantlets. Microcuttings or nodal culture is a tissue culture method, allowing for 7 - 9 micro-cuttings every eighty days [41]. Numerous studies have been conducted to micropropagate superior coffee genotypes using apical or axillary meristem culture and nodal culture [27]. A maximum of nine shoots was obtained per one shoot explant [42]. The culture of microcuttings in a temporary immersion system led to a 6-fold increase in the multiplication rate compared to

microcuttings multiplied on solid medium [43].

3.4. Germplasm Conservation and Utilization

The conservation of germplasm in genebanks involves maintaining its integrity over extended periods. Standards for handling and storage of seeds are set based on scientific knowledge and technologies. Seeds are stored for short-term carry-over or long-term germplasm accessions [44]. Pre- and post-harvest factors like crop management, seed production environment, maturity, harvest, and cleaning influence seed quality and longevity. Careful planning and standard protocols are crucial for seed conservation.

The *ex-situ* conservation of coffee genetic resources through seeds is not feasible due to the sensitivity of the seeds to desiccation and low temperatures. Rather, the cryopreservation of zygotic embryos may allow for an efficient and long-term storage for future utilization of coffee germplasm [45].

The SLARI genebank's conserved germplasm has become a crucial source of diversity for researchers globally, benefiting both public and private sectors. Core collections, which represent species diversity, can make large germplasm collections more accessible by reducing handling time by containing about 10% of the entire collection [19]. However, core collections for genetic diversity have limited value unless extensively evaluated for economically important traits. Evaluating these collections will make them more useful to plant breeders and crop improvement scientists.

3.4.1. Methods of Germplasm Conservation

Over the past decade, there has been a significant increase in species disappearing due to environmental pressures such as land clearing, drainage, and soil pollution. This has led to an increase in extinctions, with one in eight species threatened by extinction. The decline is attributed to partial habitat destruction, ecosystem destabilization, climate change, pollution, invasive species, and human activities. Experts are increasingly concerned about the extinction of rare and endangered species, leading to intensified conservation actions and the creation of lists and red books to raise awareness.

Plant genetic resource conservation can be done in natural habitats (*in-situ*) or outside (*ex-situ*), based on the 1992 Convention on Biological Diversity. *In-situ* conservation protects and monitors populations, while *ex-situ* conservation safeguards populations in danger of destruction or replacement [46]. Among these, seed storage is a convenient method for long-term preservation of PGRs, but some species, particularly tropical (e.g., coffee, cocoa etc.) and sub-tropical tree species, are recalcitrant or intermediate, meaning that they cannot stand desiccation below a relatively high critical water content value (10% - 12% or 20% of fresh weight) [47] [48] and cold storage without losing viability [49]. *In-situ* conservation faces limitations due to habitat shrinkage, urbanization, industrialization, and changing policies. *Ex-situ* conservation of crop germplasm is done in seed gene banks by

reducing moisture content and storing at −20˚C. However, economically important plant species produce recalcitrant seeds, making conservation impossible under seed gene banks.

In vitro conservation strategies, including slow growth and cryopreservation, are being proposed as an adjunct to field gene banks for problem species. These strategies help conserve and exchange disease-free germplasm. However, they can only serve short to medium-term conservation strategies and are prone to contamination and genetic instability, making them problematic for large collections. Work is done in the field, often in the open air, thereby increasing the chances of contamination. The factors that affect contamination of *in vitro* collected cultures are as follows:

1) Age: Older plants tissue taken later in the growing season is often more infected than younger plants tissue [50];

2) Position: Underground tissues, such as roots, rhizomes and corms, generally have high levels of endogenous contaminants and can be extremely difficult to clean [51];

3) Complex tissue: Vegetative and floral buds often harbor contaminants in complex tissue, which can protect even external microorganism from surface sterility [52];

4) Environment: Contamination may also be affected by environment. Explants taken from plants in a moist tropical site have higher rate of contamination than those from a temperate site. On the other hand, desert species appear to have less surface contamination by bacteria and fungi and are more easily disinfected than tissue from moister areas [53].

Over the past decade, cryopreservation has been successfully applied to various crops, particularly for the conservation of plant germplasm [54].

3.4.2. Germplasm Utilization

According to Bramel *et al.* [55], coffee field genebanks are highly vulnerable to biotic and abiotic factors. Most field genebanks around the globe suffer from the vagaries of changing climate and weather, inappropriate field conditions, pest and disease outbreaks, fire, and aging plants [55]-[57]. In addition, collections keep growing with time as new accessions are added, and this makes the collection more difficult and expensive to maintain, with the results that the collection is not properly curated, labels are lost from the field, and records are not well kept. These technical, management, and economic constraints severely impact the sustainability of field genebanks, in general, and coffee field genebanks, in particular. However, the accession-by-accession methodology has been useful in effectively monitoring, prioritizing, and rationalizing the field coffee genebanks.

Effective utilization of coffee germplasm necessitates five basic steps are that are often followed in Sierra Leone [57]: 1) gather, collate and analyze information on each coffee genotype; 2) carryout a full inventory of the field collection; 3) determine health status of each plant in the collection; 4) update the field collection database indexed by accession number ensure that all information is contained within

the same database; and 5) develop specific criteria and categories to score and prioritize each accession of the field genebank. The conserved elite accessions reach farmers through participatory selection involving them; establishment of clonal nursery using high-quality planting materials; good management of the clonal nursery for high success rates; on-farm testing; and certification and quality assurance. These steps have fostered clear multiplication pathway, quality evaluation checkpoints, thereby minimizing adoption risks and contamination with wild-derived coffee materials.

4. Trait Profiling, Discovery and Utilization for Coffee Germplasm Improvement

Breeding programs require efficient selection strategies for low heritability traits in univariate or multivariate models for high genetic gains. Multiple trait selection in perennial crops allows for more assertive identification of genetic divergence [58] [59]. High-throughput metabolic phenotyping offers rapid plant characterization, but technical issues and data processing and statistical evaluation strategies must be developed for accurate results [60].

Trait profiling and its subsequent discovery and utilization are crucial for the genetic enhancement of coffee germplasm, particularly in response to challenges like climate change and market dynamics. This process allows for the systematic identification and integration of desirable traits from various coffee accessions into breeding programs to create resilient cultivars. Comprehensive characterization of coffee germplasm evaluates numerous traits such as yield, drought tolerance, and pest resistance, utilizing advanced phenotyping and statistical tools to assess trait variation and stability [61]. Breeding efforts increasingly focus on traits critical for climate adaptation, such as leaf rust resistance and drought tolerance, to bolster agricultural resilience and farmer livelihoods.

Trait discovery in coffee leverages advances in molecular biology, using tools like SNP markers, GBS, and Genome-Wide Association Studies (GWAS) to identify novel genetic markers linked to traits such as heat tolerance and pest resistance. Researchers have therefore taken advantage of these tools to critically examine the genetic architecture of complex traits and uncover rare alleles in wild species such as *C. stenophylla*, *C. liberica*, and *C. racemosa*, offering unique attributes like heat tolerance, pest resistance, and low caffeine content [62]. Techniques such as transcriptomic and metabolomic analyses explain the relationship between gene expression and traits like flavor. To utilize these traits, breeding programs employ marker-assisted and Genomic Selection (GS), alongside hybridization strategies, ensuring alignment with market demands through participatory breeding approaches [62]. Core collections and pre-breeding programs further enhance the use and preservation of genetic diversity.

In Sierra Leone, trait-based coffee improvement has significant potential due to the country's biodiversity and underutilized germplasm, including indigenous *Coffea* species. This approach can lead to the development of climate-resilient and

market-oriented cultivars, supporting national breeding programs and aligning with agricultural innovation, biodiversity conservation, and export competitiveness. Integrating these strategies into policy frameworks and extension systems will enhance their effectiveness, benefiting farmers and stakeholders. Transgenic approaches for coffee focus on utilizing the genetic resources of the native species. *C. stenophylla*, which is rich in genetic diversity [5] [7]. Technologies such as GS and SNP markers have been exploited to enhance breeding progress and produce improved coffee genotypes with better adaptability and disease resistance. These technologies aimed at leveraging the genetic potential of *C. stenophylla* to develop sustainable coffee production in Sierra Leone [5] [7]. To make the recommended approach actionable, the following minimum enabling requirements are essential: establish a robust phenotyping network that includes standardized protocols for capturing and analyzing phenotypic data, ensuring consistency across experiments; 1) ensure assess to high-quality genotyping resources, including tools and databases that support accurate genetic data collection and analysis; 2) implement effective data management practices that facilitate the storage, retrieval, and analysis of phenotypic and genotypic data, ensuring data integrity and accessibility; 3) foster collaborations with researchers, breeders, and industry stakeholders to share knowledge and resources, enhancing the overall effectiveness of the phenotyping and genotyping efforts.

Association Mapping of Important Traits in Coffee

Association mapping, especially GWAS, has become a significant method for analyzing the genetic basis of complex traits in coffee. By utilizing natural genetic diversity from various germplasm collections, scientists can pinpoint marker-trait associations that enhance breeding methods and fast-track the creation of superior cultivars. For instance, in Brazil, research conducted by da Silva *et al.* [63] applied SNP markers to discover candidate genes associated with key agronomic traits in *C. arabica*, such as yield components, bean size, and resistance to leaf rust. This study, carried out by the Federal University of Viçosa and Embrapa, made use of high-density genotyping along with phenotypic data from several environments to reveal loci associated with productivity and stress resilience. In Mexico, José Luis *et al.* [64] utilized GWAS to analyze chemical quality traits in *Coffea* species, with keen interest in metabolites like caffeine, trigonelline, and 5-Caffeoylquinic Acid (5-CQA). Their results indicated strong associations between SNP markers and metabolite levels, providing valuable insights into the genetic factors governing cup quality and facilitating the development of marker-assisted selection focused on flavor characteristics.

Conversely, in Colombia, association mapping has been employed to investigate resistance against coffee leaf rust and coffee berry disease. Research conducted by Moncada *et al.* [65] has pinpointed QTLs and candidate genes associated with pathogen resistance, allowing breeders to cultivate varieties with lasting resistance in the face of high disease pressure. These initiatives are particularly

significant for smallholder farmers in Latin America, where outbreaks can greatly affect their livelihoods. As a novel alternative to conventional linkage disequilibrium, association mapping presents three key benefits: 1) enhanced mapping precision, 2) shorter research duration, and 3) an increased number of alleles [66]. The phenotypic diversity of numerous complex traits that are significant in agriculture or evolution is shaped by various Quantitative Trait Loci (QTLs), their interactions, environmental factors, and the interplay between QTLs and the environment. Linkage analysis and association mapping represent the two primary methods utilized for unraveling complex traits (**Figure 4**). Since it was first applied in plants [67], association mapping has continued to rise in popularity in genetic studies, driven by advancements in high-throughput genomic technologies, a growing interest in discovering new and superior alleles, and improvements in statistical methodologies (**Figure 5**). Both linkage analysis and association studies rely on co-inheritance of functional polymorphisms and neighboring DNA variants. The difference is that in linkage analysis (panel a, using F2 design as an example), there are only a few opportunities for occurrence of recombination within families and pedigrees with known ancestry, resulting in relatively low mapping resolution. In association mapping, on the other hand, (panel b, showing only in haplotype), historical recombination and natural genetic diversity are exploited for both high-resolution mapping. Linkage disequilibrium between a functional locus (yellow diamond for mutated allele) and molecular markers is low except for those within very short distance [68].

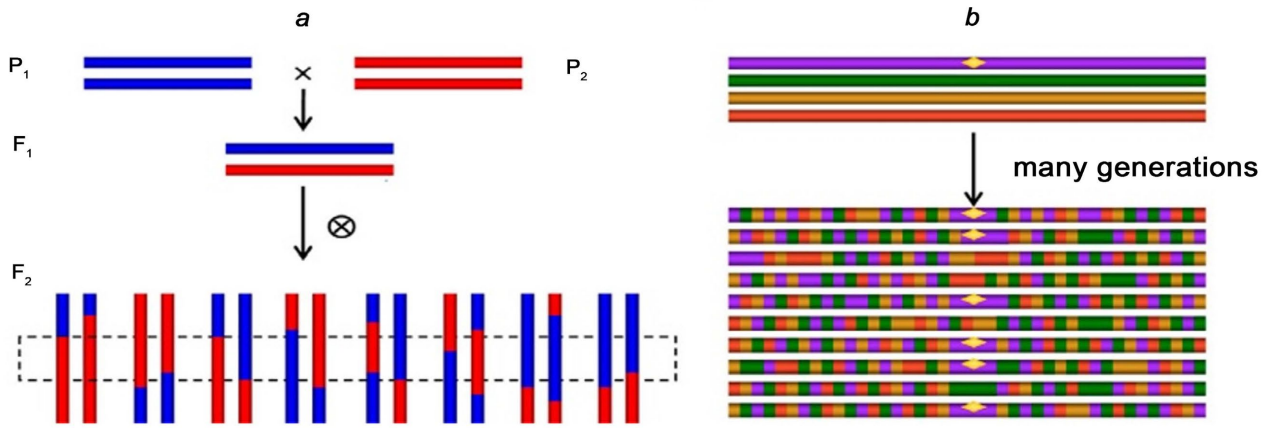


Figure 4. Schematic comparison of linkage analysis with designed mapping populations and association mapping with diverse collections. Adapted from Zhu *et al.* [68].

Based on the scale and emphasis of a particular investigation, association mapping typically divides into two main categories (**Figure 6**): 1) candidate-gene association mapping, which connects polymorphisms in specific candidate genes believed to influence phenotypic variation for certain traits; and 2) genome-wide association mapping, or genome scan, which examines genetic variation across the entire genome to identify association signals for various complex traits [69]. While researchers focusing on a specific trait or a group of traits often utilize can-

didate-gene association mapping, a large consortium of researchers may opt for exhaustive genome-wide analyses of multiple traits by assessing hundreds of thousands of molecular markers spread throughout the genome for association.

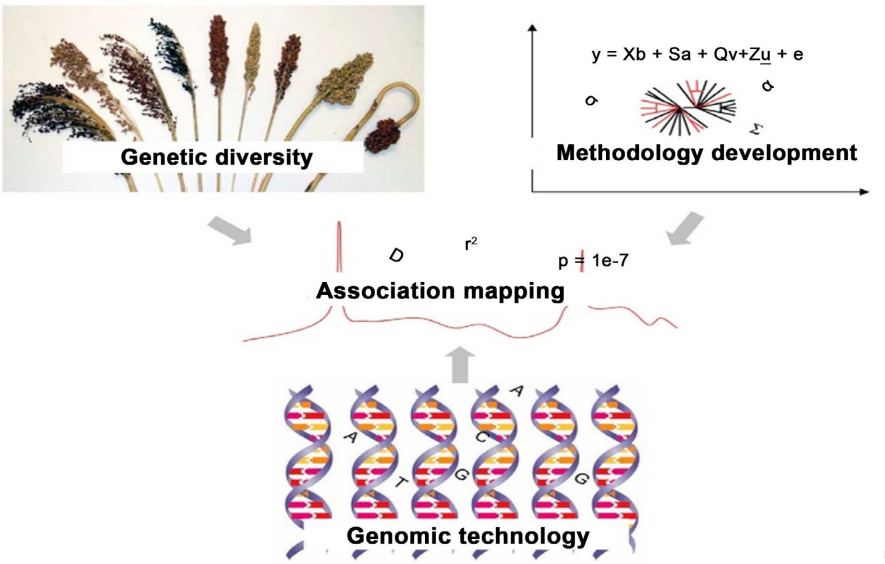


Figure 5. Main driving forces of the current interest in association mapping. Genomic technologies for high-throughput genome sequencing and genotyping made it more affordable to obtain a large amount of marker data across a large diversity panel for complex trait dissection and superior allele mining. Methodology development alleviated the issue of false positives due to population structure. Adapted from Zhu *et al.* [68].

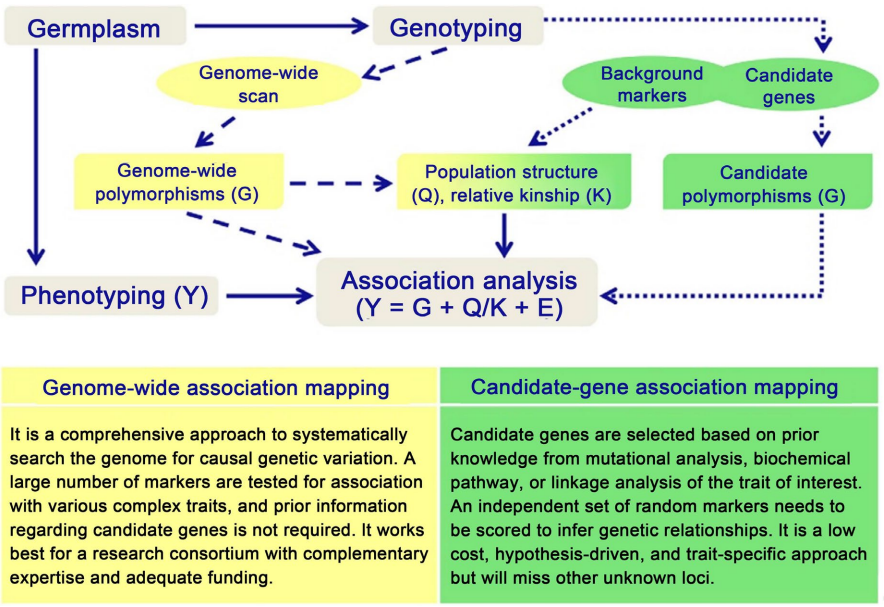


Figure 6. Schematic diagram and contrast of genome-wide association mapping and candidate-gene association mapping. The inclusion of population structure (Q), relative kinship (K), or both in final association analysis depends on the genetic relationship of the association mapping panel and the divergence of the trait examined, and residual variance (E). Adapted from Zhu *et al.* [68].

In East Africa, especially in Ethiopia and Uganda, association mapping is being incorporated into national programs for coffee enhancement in which Scientists are applying GWAS to identify genomic regions linked to drought resilience, bean uniformity, and flowering timing in *C. arabica* landraces and wild relatives [63]. These characteristics are essential for adapting to climate change and ensuring yield stability in rain-fed agricultural systems.

A significant study conducted by Nonato *et al.* [70] in Brazil identified genomic regions linked to bienniality and resistance to biotic stresses, including the coffee leaf miner and leaf rust. Their research emphasized the potential of association mapping to tackle issues related to yield variability and pest pressures, which have been significant challenges in coffee cultivation.

On a global scale, the incorporation of association mapping in coffee breeding is bolstered by initiatives like the World Coffee Research (WCR) breeding hub, which advocates for uniform phenotyping and genotyping methods across various countries. This collaborative strategy improves the transferability of marker-trait associations and aids in the creation of cultivars tailored to specific regions.

In Sierra Leone, Lahai *et al.* [71] suggest that association mapping provides strategic avenues to utilize local and wild *Coffea* germplasm for discovering desirable traits. By connecting phenotypic assessments with molecular data, national research institutes can pinpoint traits that are crucial for climate resilience, quality, and productivity. This knowledge can enhance breeding programs, facilitate the patenting of indigenous varieties such as *C. stenophylla*, and improve the organizational capabilities for cultivar development driven by data.

5. Breeding for Abiotic Stress Resistance in Coffee

Developing coffee varieties that are resilient to abiotic stress has become a critical necessity due to increased variability in climate and environmental challenges. Coffee species, especially *C. arabica* and *C. canephora*, have been observed to be vulnerable to drought, high temperatures, salinity, and excessive sunlight factors that threaten consistency in yield and yield-related parameters, quality of beans, and the livelihoods of farmers in tropical regions. The Sierra Leone scenario seems to be worsened by limited availability of resilient genetic material and insufficient adaptive breeding infrastructure.

Recent developments in coffee breeding have concentrated on the integration of physiological, molecular, and genomic techniques to fast-track the development of cultivars that can withstand prevailing stress. Research by dos Santos *et al.* [72] indicates that genomic-assisted breeding allows for the discovery of Quantitative Trait Loci (QTLs) and genes that respond to stress, which are linked to drought tolerance, heat resilience, and the ability to manage oxidative stress. These findings are being utilized in marker-assisted selection and transcriptomic profiling, enabling breeders to identify and select genotypes with enhanced traits for adaptation. In addition to molecular methods, physiological assessments are crucial. Traits such as stomatal conductance, management of leaf temperature, chlorophyll

fluorescence, and the activity of antioxidant enzymes are increasingly employed to assess the performance of clones under stressful conditions. Borgo *et al.* [73] emphasized on the necessity of integrating plant nutrition with stress physiology in breeding strategies, highlighting that efficiency in nutrient usage and the ability to adjust osmotically are essential indicators of resilience. Additionally, Borgo *et al.* [73] gave a general description of the main effects of drought, heat, excess light, and salinity on plant growth, presenting the mechanisms employed by coffee plants to deal with environmental stresses.

Furthermore, breeding initiatives are embracing a holistic approach that merges traditional selection methods with contemporary stress biology. For instance, Partelli and Vieira [74] presented a scenario that illustrates efforts to create cultivars that are appropriate for various agro-climatic regions through participatory breeding, field experiments, and studies on genotype-by-environment interactions. The goal of these programs is to produce coffee varieties that can withstand abiotic stresses while also fulfilling market and quality expectations.

For the national research institutions and clonal seed garden projects in Sierra Leone, these insights provide a guide for improving local breeding capabilities. By utilizing genomic resources, physiological indicators, and building strategic collaborations, these organizations can enhance the development of climate-resilient coffee clones that align with national goals for restoration and productivity.

6. Impact of Drought Stress in the Production and Productivity of Coffee

Drought stress presents a major challenge to coffee cultivation, especially as climate change exacerbates the intensity and frequency of dry periods in key coffee-producing areas. Researchers globally have taken initiative by investigating the intricate physiological, biochemical, and molecular processes that facilitate drought tolerance in coffee plants.

A recent investigation conducted by Ramalho *et al.* [75] delivers a comprehensive examination of stress resilience in *C. arabica* and *C. canephora* amid severe drought and heat circumstances. The investigators underscored crucial genes, proteins, and lipid reactions that aid in drought adaptation. Their results emphasize the significance of antioxidant enzymes, osmoprotectants, and membrane-stabilizing lipids in reducing oxidative damage and preserving cellular integrity during periods of water scarcity. Notably, *C. canephora* showed a stronger response, indicating its higher potential for developing drought-resistant cultivars. In addition to this molecular viewpoint, Borgo *et al.* [73] assessed the combined impacts of drought, heat, excessive light, and salinity on coffee physiology and productivity. They highlighted the necessity of integrated mitigation approaches, including plant breeding, soil management, and nutritional strategies. Their research stresses the combined advantages of merging genetic enhancements with agronomic practices to bolster drought resilience. For example, selecting genotypes with deeper root structures and improved water-use efficiency has demonstrated potential in

field experiments across Brazil and East Africa.

Ramirez-Builes *et al.* [76] illustrated that boron supplementation significantly enhances resistance to drought stress in coffee. When paired with calcium, boron not only improves physiological resilience but also boosts long-term yield and seed quality. These findings indicate that managing micronutrients could serve as a cost-effective solution for smallholder farmers dealing with unpredictable rainfall.

Beyond physiological and nutritional methods, researchers are also investigating remote sensing and modeling technologies to forecast drought effects and inform adaptive management practices. Satellite-based indicators and crop simulation models are being customized for coffee growing systems in Latin America and Africa, allowing for early warnings and tailored recommendations.

Sustaining growth and increasing crop yield in highly stressful environmental situations like water scarcity is one of the primary challenges faced by contemporary agriculture [77].

7. Concluding Remarks and Future Outlook

High-Throughput Plant Phenotyping (HTPP) is considered as a very relevant technique used to speed up discoveries in many areas of plant science, including plant physiology [78] [79], genetics [80], breeding [81], and phytopathology [82]. The technique was found to be efficient in detecting plant stress response under open field and greenhouse conditions. A wide gap exists between the discovery and practical use of QTL for crop improvement, especially for several important agronomic traits of interest. This limitation is attributable to the low accuracy in QTL detection resulting from low marker density and the manual collection of phenotypes of complex agronomic traits. Combined utilization of High-Throughput Genotyping (HTG) and high-throughput digital phenotyping has revealed the possibility of increasing marker density and improving the precision and resolution of QTL detection [83]. The quantitative measurement of Drought Resistance (DR) phenotyping markers, including transpiration or leaf moisture content in addition to automated HTPP platforms, provides an opportunity to correlate traditional and novel DR traits as well as perform DR-related genes mining. Using LemnaTec's Scanalyzer3D, Honsdorf *et al.* [84] and Parent *et al.* [85] identified 44 and 21 DR QTLs in a set of wild barley introgression lines and a wheat Recombinant Inbred Line (RIL) population under water stress, respectively. Similarly, 51 DR traits were detected in an association panel and a RIL population. Consequently, 93% of the loci found by GWAS co-localized with previously reported DR-related QTLs and different loci containing known DR-related genes were identified. Through GWAS and linkage analysis, 69 trait-locus associations were identified, and the role of a DR gene, OsPP15, was confirmed by genetic transformation experiments, demonstrating that the combination of HTPP and genetic mapping is a promising technique for the discovery of novel DR genes [86] and other important traits.

Arbuscular Mycorrhizal Fungi (AMF) are among the beneficial soil microbes with a role in improving coffee tolerance to abiotic stresses. Exploitation of AMF has been suggested for consideration in the context of agriculture, since the AM symbiosis is the dominant mycorrhizal symbiosis formed by most crops (except for the Brassicaceae species) and its potentially positive, multifunctional role in plant nutrition, pathogen protection, stress tolerance, and soil structure provision [87]. Applied research focused on these symbioses should consider improving the production and application of the mycorrhizal fungal inoculum, which directly addresses the decline in mycorrhizal abundance in agricultural fields. Mycorrhizal technology involving the set of measures utilized for optimization of local mycorrhizal abundance and diversity in terms of functioning for attaining sustainability of agroecosystems [88], should be supported by research including: 1) a better understanding of the relative contribution of AM symbiosis to any aspect of sustainability; 2) defining which parameters influence symbiosis effectiveness, helping to prevent other agricultural management approaches from interfering with the mycorrhizal-mediated benefit; and 3) expanding the response variables for documenting mycorrhizal effects. Translation of the information derived from fundamental research to innovation in crop, soil, and water management, with the development of beneficial microbial-based strategies and practices designed for environmentally sustainable coffee production and tailored to a specific environment, should be exploited. Such a holistic approach necessitates a tight linkage of highly controlled phenotyping trials, a network of field experiments in contrasting environments, field phenotyping approaches, long-term experiments under field conditions and the development of accessible data repositories. Development and application of bioinformatics tools useful to study the complex interactions among different functional groups of microorganisms should be exploited in future studies. Various bioinformatic approaches have been developed to study the microbial diversity as well as the interaction networks discovered by high-throughput DNA sequencing, although they are often applied separately for bacteria and fungi [89]. Microbiome-based innovations may contribute to the policies for the development of sustainable practices, in line with the Sustainable Development Goals (SDGs, https://sdgs.un.org/goals), and international cooperation in microbiome research projects has been suggested to be an essential point for a sustainable future [90]. Notably, diversity and efficiency of microorganisms, as well as the microbial networks, are correlated to crop plant species/varieties and soil/environmental conditions [89].

Domestication of coffee has focused more on yield-related traits compared to stress tolerance or disease resistance traits, which consequently need to be recovered from wild relatives by introgression that takes long periods, and fails when concurring stress conditions appear. Priming strategies, such as rhizosphere and root-associated microbiota, exhibit a huge potential in the development of a next-generation coffee. These approaches will contribute to boosting the existing endogenous plant potential to endure abiotic and biotic constrictors. Priming strat-

egies also exhibit potential long-lasting effects and non-negative modification of other interesting traits, including quality of edible organs and yield. Moreover, advancement in technology, such as the use of high-throughput platforms to quantify crop performance and tolerance traits of different coffee genotypes upon different treatments (e.g., inoculation with beneficial microbes, plant response to diverse environmental stresses, etc.), provides an opportunity to characterize the plant responses of selected breeding lines under controlled growth conditions or in the field, where real-life agricultural conditions are found.

Advances in breeding technologies and methodologies, as well as the availability of high-quality historical trial data, continue to drive improvements in coffee genotypes for desired end-user traits.

Information from this monograph could be useful in designing future marker-based breeding as well as genetic conservation of coffee. The exploitation of AMF for improvement of coffee tolerance to biotic and abiotic stresses, priming strategies, combined utilization of high-throughput genotyping and high-throughput digital phenotyping, and assessment of long-term effects of plant domestication, pleiotropy and selective breeding for desired agronomic traits of coffee forms part of future work on the crop.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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Appendix

Table A1. Arabica coffee introductions in Sierra Leone.

S/N	Variety	Area Planted (Ha)			
		Bambawo	Pendembu	Kpuwabu	Boajibu
1	MGSCATIGUA' 3	1	1	1	1
2	CATUCAI AMARELO (2SL)	1	1	1	1
3	IAC 125 RN	1	1	1	1
4	CATUCAI AMARELO (IAC 62)	1	1	1	1
5	PAQAISO 2	1	1	1	1
6	PAQAISO (H 419-1)	1	1	1	1
7	TOPAZIO MG 1190	1	1	1	1
8	MUNDO NOVO (IAC 379-19)	1	1	1	1
9	CATUAI VEQMELHO (IAC 144)	1	1	1	1
10	OEIRAS MG 6851	1	1	1	1