

Genetic diversity of coffee germplasm in Sierra Leone: implications for conservation and breeding programs

Paul Musa Lahai^{1#*}, Mohamed Alieu Bah^{2#}, Mohamed Tailu Lahai³, Peter Osobase Aikpokpodion⁴ and Raymonda Adeline Bernadette Johnson⁵

¹ Sierra Leone Agricultural Research Institute (SLARI), IDA, Kenema 42215, Sierra Leone

² Njala University, Mokonde 42215, Sierra Leone

³ Eastern Technical University, Combema Road, Kenema 42215, Sierra Leone

⁴ University of Calabar, Faculty of Biological Sciences, Department of Genetics and Biotechnology, PMB 1115 Calabar, Cross River State, Nigeria

⁵ Ministry of Agriculture and Food Security (MAFS), Freetown 42215, Sierra Leone

These authors contributed equally: Paul Musa Lahai, Mohamed Alieu Bah

* Corresponding author, E-mail: paulmusalahai@yahoo.com

Abstract

Global coffee production is dominated by *Coffea 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. 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. However, more efforts of conservation and genetic improvement on these species, are needed to realize these opportunities. The objective of this paper is to review the coffee genetic resources in Sierra Leone with an emphasis on the wild coffee species including their conservation status, and the phenotypic and molecular characterization. We also present perspectives for future genetic improvement of *C. stenophylla*, and discuss breeding methods, combining ability, and molecular marker-assisted prediction of hybrid vigor. Moreover, with the availability of recently developed single nucleotide polymorphisms (SNP) markers on *C. stenophylla*, we suggest that new technologies of molecular breeding, such as genomic selection can significantly accelerate the breeding progress and deliver improved varieties with high yield, good adaptability, and disease resistance.

Citation: Lahai PM, Bah MA, Lahai MT, Aikpokpodion PO, Johnson RAB. 2023. Genetic diversity of coffee germplasm in Sierra Leone: implications for conservation and breeding programs. *Beverage Plant Research* 3:26 <https://doi.org/10.48130/BPR-2023-0026>

Introduction

As a tropical crop, coffee contributes significantly to the livelihoods of smallholder farmers, especially in rural communities. The coffee species belongs to the genus *Coffea* in the family Rubiaceae and is grown mainly in the tropics^[1]. The genus *Coffea* comprises 124 species^[2], but commercial production depends mainly on two species, *C. arabica* L. and *C. canephora* Pierre and Froehner each accounting for approximately 70% and 30% of the global coffee market, respectively. According to the World Coffee Organization, by geographical distribution and quality group, the top five coffee-producing countries are Brazil, Vietnam, Ethiopia, Colombia and Indonesia^[3,4]. Ethiopia is the 5th largest exporter of *C. arabica* in the world after Brazil, Vietnam, Colombia and Indonesia and the largest coffee producer in Africa. The *C. arabica* is the only allopolyploid species ($2n = 4x = 44$) grown commercially. Studies of its origin have revealed that the primary center of origin of *C. arabica* is the highlands of southwestern Ethiopia and the Boma plateau of South Sudan^[5]. However, other species including *C. canephora* grown commercially are diploid ($2n = 2x = 22$) but are self-sterile except for *C. heterocalyx* and *C. moloundou*, which are diploid and self-compatible^[6,7].

In terms of international trade, coffee has been a valuable commercial commodity since the 1800s and is of great economic importance to developing countries, including least developed countries (LDCs)^[8]. Furthermore, it is of considerable social and economic importance for the consumer countries, where the coffee industry contributes multi-billion dollar to the economy. According to Fairtrade Foundation^[9], more than 125 million smallholder farmers in coffee growing regions around the world earn direct or indirect income along the value chain of growing, processing, transporting, and trading of coffee^[8,10].

Sierra Leone is a globally recognized biodiversity hotspot (of genetic diversity concern) in the forests of Upper Guinea with a rich native flora and fauna including important endemic and rare species at local and international levels^[11]. However, dramatic changes in forest cover have taken place over the past century due to urbanization, population growth and expansion of arable land (agricultural/plantation). In Sierra Leone, the continued depletion of natural resources, especially flora and fauna, has led the government to develop policies aimed at protecting areas such as the Kambui and Kasewe hills, which serve as hot spots for substantial populations of the wild *C.*

stenophylla. The *C. stenophylla* is critically endangered due to the exponential increase in deforestation, mainly logging and charcoal production.

To some extent, the coffee research programs in Sierra Leone have not yet undertaken full-time research to assess the genetic diversity of coffee for further development of new varieties. Despite the foregone, Sierra Leone is one of the places in the world that hosts a diversity of coffee wild relative species and thus potentially allows them to withstand varying environmental factors.

Overview of coffee production in Sierra Leone

The cultivation and production of coffee in Sierra Leone on a commercial scale date back to the 1950s, when the first group of coffee varieties were imported from Uganda, Côte D'Ivoire, and Nigeria, aimed at improving the viability of the economy of the country's agriculture through the formation of the coffee industry. Upon establishing a formidable coffee sector between 1960 and 1980, production volume had already reached 20,000 tons per year^[12]. However, after the 1991 interregnum of the civil conflict, most of the coffee plantations were abandoned and growers had to flee production areas which resulted to a sharp decline in production. Although the conflict ended in the early 2000s, production has never reached the level of previous decades.

Prior to the civil conflict, the world market price of coffee was very attractive which led to massive coffee plantation establishment in Sierra Leone, but the plantations are now too old, and can no longer reach their full production potential. However, the government of Sierra Leone and development partners have developed initiatives to help farmers create new farms and increase production with the available coffee varieties. For instance, in 2018, a European Union project was launched to

restore abandoned coffee plantations in Sierra Leone. Implementation of this initiative involved intensive pruning of aging coffee trees as well as providing smallholder farmers with more agricultural inputs such as fertilizer.

The eastern region of Sierra Leone which consists of Kenema, Kono and Kailahun serves as the hub for coffee cultivation in the country (Fig. 1). Other areas of cultivation include Southern Province in the districts of Pujehun, Moyamba and Bo, while Northern districts include Tonkolili and Koinadugu.

Much of the coffee production in the country is carried out by youths who are in their prime and face challenges such as maintenance of plantations, especially clearing, pruning, and harvesting, most of which is done through local means. According to the International Coffee Organization^[3], the average size of small coffee farms is four hectares, so the contribution of each small farm to the GDP of the country is negligible, unless aggregated. The climate of the main coffee-producing provinces of Sierra Leone could be described as seasonal having six-months of rainy season lasting from May to October and six months of dry season lasting from November to April. The weather is characterised by hot temperature with an average monthly temperature of 26 to 28 °C from June to October and a maximum temperature of 32 °C^[13]. The vegetation in this area is mostly classified as evergreen with six months of continuous rainfall. However, due to the erratic rainfall at the end of March in the production areas, coffee cherries harvested during this period often do not achieve the optimal moisture content (10%–12%) as required for export.

Cleaning of coffee plantations in Sierra Leone is usually done twice during the growing season, i.e., before flowering and around the time of fruit ripening. On the other hand, pruning is immediately done after harvest and consists of pruning old plants and removing young shoots (leaving only four buds or stems per basal tree).

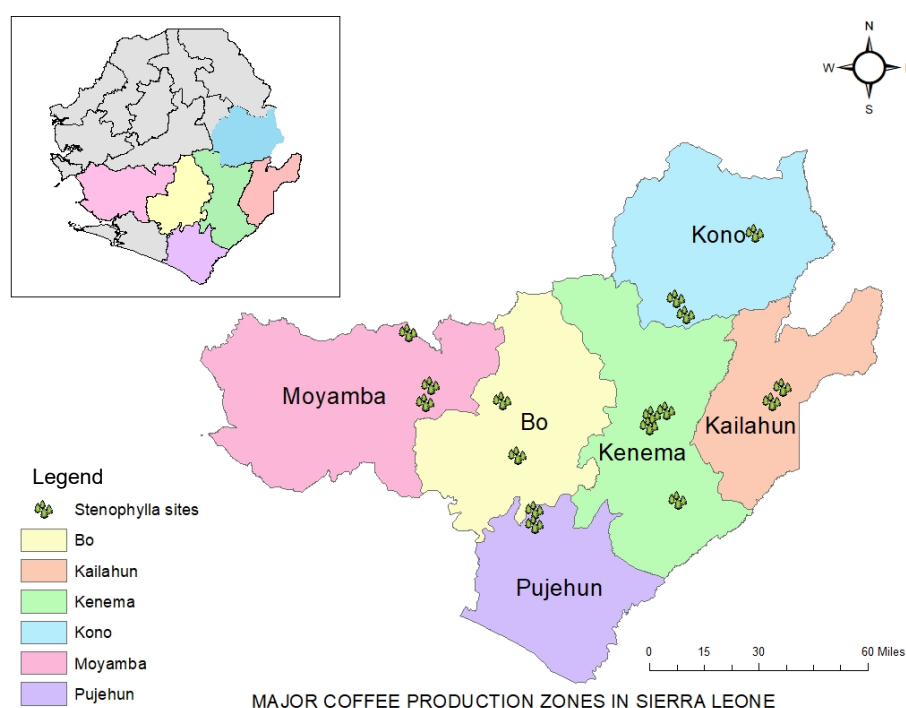


Fig. 1 Map of major coffee production districts in Sierra Leone.

Review of genetic diversity of coffee in Sierra Leone

Harvesting is usually carried out December through March with the labour force consisting of mostly youths and the aged. After harvesting, the cherries are dried on a high or raised platform that had been installed in notable coffee growing communities through the help of the Robusta Coffee Development Project. Other communities usually dry their berries on a drying floor that is protected by mesh, preventing domestic animals from entering the area. In addition to the lack of a domestic cooperative function, most smallholder farmers grow coffee on small areas of land, resulting in low productivity. Labour shortage and lack of advanced tools or implements are the two major constraints to coffee production in Sierra Leone. It is worthy to note that most of the young people who see coffee farming as a lucrative business have migrated to cities and now ride motorbikes as an alternative means of survival.

Coffee germplasm and their conservation status in Sierra Leone

Germplasm of different cultivated *Coffea* species exist in Sierra Leone, including *C. canephora*, *C. liberica*, and *C. arabica*. In addition to the cultivated species, two wild species – *C. stenophylla* (also known as highland coffee) and *C. affinis*, are indigenous to Sierra Leone (Fig. 2).

Since the 11-year interregnum full of civil strife, unrest and political instability, there has been little or no research effort on tree crops in the country. However, the Sierra Leone Agricultural Research Institute (SLARI) is geared up through its donor partners to boost the tree crop sector in terms of research and

infrastructure. In the late 1950s, the Ministry of Agriculture introduced coffee clones from Uganda, Côte D'Ivoire and the Coffee Research Institute of Nigeria as part of its germplasm collection. The introduced germplasm included 'C115', 'A2', 'G98', 'C47', 'C182', 'C107', 'C461', 'C126' and 'C181'. As recent as 2019, the Boosting Agriculture and Food Security (BAFS) project introduced ten varieties of *C. arabica*, including 'Mgscatigua' 3', 'Catuai amarelo' (2SL), 'IAC 125 RN', 'Catuai Amarelo' (IAC 62), 'Paraiso 2', 'Paraiso' (H 419-1), 'Topazio Mg 1190', 'Mundo Novo' (IAC 379-19), 'Catuai Vermelho' (IAC 144) and 'Oeiras Mg 6851' from the Brazilian Agricultural Research Corporation (Embrapa). The morphology traits of the different varieties that are conserved at SLARI research station at Pendembu reveals considerable variability within this pool of germplasm (Fig. 2a, b & c).

The two wild species *C. stenophylla* and *C. affinis*, both found in the hills of Kambui and Kasewe, at an altitude of at least 400 m above sea level, are potential coffee species that the country can utilize in coffee breeding programs through seed or cutting propagation.

Coffee beans cannot be stored for a long time in the gene bank because of their recalcitrant nature. Although they are resistant to desiccation when they are at a moisture content of 6%–8% fresh weight, they are still unorthodox due to their sensitivity to cold and desiccation^[14]. The maximum storage time achieved when fully hydrated seeds were stored at 19 °C and 100% relative humidity was 36 months for *C. arabica* and 15 months for *C. canephora* and *C. stenophylla*. Although *ex situ* gene bank in the field is the most common alternative for conserving wild seeds, this approach can still be difficult to preserve seeds of primary interest. However, this method of seed preservation provides easy access to planting materials for characterization, evaluation, and subsequent use for the intended purpose. However, the number of genotypes that a field gene bank can hold is limited with the constraints of available land and financial resources. Therefore, only limited genetic diversity can be conserved in the gene bank^[5]. *Ex situ* conservation of coffee genetic resources in the field can be characterized by the risk of loss of valuable genetic material due to pest invasion, disease infestation and poor adaptation to the environment.

Unlike the *ex situ* method, *in situ* conservation preserves genetic resources in their natural habitat by protecting the area from human interference. This method allows the dynamic evolution of the species to be carried out in its natural habitat, including the creation of new plant varieties thought to be resistant to pests and disease, thus remains an important factor for long-term conservation of coffee genetic resources. However, the *in situ* conservation often faces risk when the natural habitat is subject to destruction due to natural disasters and climate changes, resulting in genetic erosion. For example, the hills of Kambui and Kasewe, known as the source of *C. stenophylla* and the recently discovered variant *C. affinis*, are under serious threat from loggers, charcoal-burners, farmers, and mining activities from the surrounding communities.

The phenotypic diversity of *C. stenophylla* was evaluated by Lahai et al.^[15] in which a total of 203 samples from Kenema and Moyamba districts were evaluated according to the IPGRI morphological descriptors^[16]. Data analysis using the Shannon-Weaver diversity index (*H'*) revealed inter-sample differences



Fig. 2 (a), (b) High yielding G-98 and Unripe A-2 *C. canephora*. (c) Branch of *C. liberica* with young fruit bunches. (d) Relatively low yielding *C. stenophylla* branch. (e) Ripe fruits of *C. stenophylla*. (f) Secondary branch with leaves of *C. stenophylla*.

for the 13 observed morphological traits, including stalk strength, growth habit, seed size, color of young leaves, stem habits, fruit shape, seed shape, leaflets, leafshape, angle of primary branches on the main stem. Significant variation was observed at the species, population, and individual levels^[15]. The presence of *C. arabica*, *C. excelsa*, *C. canephora*, *C. stenophylla*, *C. affinis* and possibly other wild relatives suggest that Sierra Leone is also part of the primary gene pool of *Coffea* species.

Various researchers^[17–19] have reported that the species *C. canephora* and *C. liberica* have higher genetic diversity than *C. arabica* because *C. canephora* and *C. liberica* are native to different geographical regions while cultivated *C. arabica* is thought to have a narrow genetic base and geographical origin. Variations have been shown to exist within and between *C. stenophylla*, *C. congensis*, *C. racemosa* and *C. eugenioides* species^[19]. These reports therefore indicate that these species possess many valuable genetic variations that can be exploited for the development of new commercial varieties, using traditional breeding approaches and advanced breeding methods.

Over the years, assessing genetic identity and diversity of coffee genetic resources in Sierra Leone has been a major challenge. To address the problem of genetic integrity in coffee germplasm, leaf samples of *C. stenophylla*, *C. canephora*, *C. liberica* and *C. arabica* were collected in Sierra Leone and sent to the USDA ARS, Sustainable Perennial Crop laboratory (SPCL) for DNA fingerprinting. The single nucleotide polymorphisms (SNP) panels developed by USDA-ARS^[20,21] were used for the characterization of *C. stenophylla*. Since there is no molecular marker for this species, efforts are being made to develop SNP markers using genotyping by sequencing (GBS) technology. A total of 143 *C. stenophylla* samples were sequenced using dual digestibility restriction site-associated sequencing (ddRAD-seq), which is a cost-effective strategy to generate SNP data. The variants were mapped onto the *C. canephora* genome and pruned based on linkage disequilibrium (LD).

After further filtering with call rate and minor allele frequency, a total of 7,464 high-quality SNP markers were selected for *C. stenophylla*. The information index values of this selected SNP panel ranged from 0.184 to 0.693 with a mean of 0.446. The mean observed heterozygosity was 0.351, ranging from 0.05 to 1.00, while the mean expected heterozygosity was 0.285, ranging from 0.056 to 0.500. Clustering and division analysis of the population structure showed that there is a clear substructure in cluster of *C. stenophylla*, suggesting that genetically distinct populations exist at the origin of this species (Lahai et al., unpublished data).

The results therefore suggest that geographic representation is an important factor for sampling the *C. stenophylla* gene pool, as well as for the design of an *in situ* conservation strategy in Sierra Leone. In addition, a subset of 192 SNPs was selected (out of 7,467 SNPs) for larger-scale genotyping of the *C. stenophylla* germplasm collected from different geographic regions of Sierra Leone. After determining the genetic identity and diversity of these coffee plants, the cuttings of the selected trees will be propagated and planted at the SLARI field trials for evaluation. Promising clones will be selected and used as parent clones for developing new varieties of *C. stenophylla* and *C. canephora*.

Prospects of coffee genetic improvement in Sierra Leone

Despite the economic importance of coffee production in Sierra Leone, to date no systematic breeding effort has been undertaken to develop coffee varieties in the country. The planting materials of Robusta, Liberica and Arabica coffees used by farmers are all based on introduced varieties. The *C. stenophylla* is native but it's not currently grown commercially because the low yield and small fruit make it inferior to other species. However, there are large phenotypic differences in the genetic material of *C. stenophylla*^[15], justifying efforts to enhance *C. stenophylla* genetic resources and develop improved varieties.

Breeding methods

Different coffee breeding methods have been used in various coffee producing countries of the world with the ultimate goal of improving the yield, quality and diseases resistance of the resulting varieties. However, the application of these breeding methods may vary depending on the amount of genetic variation that exists in the gene pool, ecological conditions, breeding goals, and production issues^[22–25]. The goal of coffee breeding in Sierra Leone is like many other coffee producing countries in Africa. Methods such as climate adaptability, cross-breeding, developing F₁ hybrid and genotypes × environment (G × E) testing have been used with the aim of developing improved varieties through breeding for resistance, yield, quality, and adaptability to different agro-ecological regions^[26]. Efforts by the Sierra Leone Agricultural Research Institute (SLARI) to develop coffee varieties included the use of clonal materials (cuttings) and seeds introduced from prominent coffee producing countries. As an outcrossing diploidy species, the breeding method for *C. stenophylla* may not be the same as those designed for *C. arabica* in which high yielding inbreeding genotypes with high degree of homozygosity are selected and the "pure line" then is used in production. Instead, *C. stenophylla* would likely adopt the breeding method for Robusta varietal development, in which specific parental combinations were crossed and their F₁ generation was evaluated for the traits of interest. Then, hybrids were selected and distributed either as clones or as seeds (produced by crossing the two parental lines in a seed garden)^[27]. During the development of F₁ hybrids, the appropriate selection of parents with known genetic differences from each other and the identification of promising heterozygous combinations is important^[28] as this has implications important for progeny performance at the end of the selection cycle.

Mating between clones of unrelated parents can produce varieties with minimal inbreeding risk^[29]. In other crops, hybrid breeding usually involves the crossing of inbreeding lines developed through multiple rounds of autogenous reproduction. However, like Robusta coffee, *C. stenophylla* is a species that does not readily self-pollinate. Therefore, the parental clones used in hybrid development in *C. stenophylla* are heterozygous. However, progenies developed by mating between clones of heterozygous parents can be considered as hybrids capable of providing superior performance (hybrid vigor). The combination of yield and hybrid vigor are the two most important factors to consider when selecting a superior F₁ hybrid

Review of genetic diversity of coffee in Sierra Leone

for *C. stenophylla* due to the good combining ability of the parents.

Breeding objectives for *C. stenophylla*

The goal of the *C. stenophylla* coffee breeding program in Sierra Leone is to develop high yield, good quality, resistance to diseases and pests (mainly coffee berry diseases), high planting densities, and adaptability to all coffee growing districts in the country. To achieve these goals, breeding strategies will focus on identifying high-yielding wild populations to develop improved purebreds and crosses between more productive plants to produce hybrids.

Breeding for productivity/yield

The coffee breeding program in Sierra Leone is based on the identification of superior parental lines with high yield potential and desirable characteristics. Several coffee breeding centers are currently focusing on hybrid coffee varieties as the best strategy to rapidly increase crop yield. Hybrid coffee varieties have more consistent yields over location and time (less genotype \times environmental interaction effect). The chances of achieving significant hybrid vigor will be increased by bringing together parents selected from genetically diverse populations. Since the traits of yield components (e.g., bean size, bean weight, and number of fruits per branch) typically have moderately high heritability, selection on these traits may achieve larger genetic gain than direct selecting on yield, which usually have low heritability and larger $G \times E$ interaction.

Breeding for disease resistance

Since *C. stenophylla* was reported to be susceptible to coffee leaf rust (CLR) in Sao Tome and Principe^[30], CLR resistance needs to be included as one of the main breeding objectives. CLR, together with coffee berry wilt disease (CBD), are the two main diseases of Arabica coffee. Unlike the CBD, which is endemic to the African continent, especially in the highlands of East Africa, CLR is a widespread disease affecting most of the world's coffee-growing regions. The variability of pathogens in *Hemileia vastatrix* is great, making the development of resistant coffee varieties complicated. To date, more than 30 physiological strains of *H. vastatrix* have been identified and are associated with CLR disease. According to Carvalho^[31], at least seven major dominant genes are associated with resistance and seven dominant genes have been found for resistance to this pathogen, thereby negating the corresponding genetic predisposition of coffee for this resistance. So far there has been no information on breeding efforts of CLR resistance in *C. stenophylla*. Herein we use the information of CLR, CBD and CWD on Arabica coffee to illustrate the potential breeding needs of disease resistance in *C. stenophylla*.

The economic impact of coffee leaf rust on global Arabica coffee production, estimated at 1–2 billion USD per year due to crop failure (20%–25%) and the need to implement control and cultural measures (10% of production costs), can be deductible. According to Guzzo^[32], more than 75% of the coffee grown in the world is susceptible to different pathogenic strains. Most of the breeding initiatives focus on creating disease-resistant varieties. For example, in Ethiopia, a high degree of horizontal (non-specific) resistance to this disease, partial disease resistance, and genetic diversity of Arabica coffee all contribute to the coffee's ability to be protected against disease as part of the CLR prevention program under the current environmental conditions. The presence of such a wide variety of CLR

resistance in wild forest populations provides an opportunity for the development and use of resistant materials to control coffee leaf rust.

CBD is another fungal disease in Arabica coffee caused by *Colletotrichum kahawae*. The pathogen causes anthracnose disease in green and ripe fruit. It was first discovered in 1922 in newly established imported Arabica coffee farms with a limited genetic base in the Sotik region of western Kenya, south of Mount Elgon. CBD is a major risk for the coffee production of Tanzania, Kenya, Ethiopia, and other African countries. The Ethiopian coffee breeding operations, which began about 30 years ago, have succeeded in creating new varieties with long-term CBD resistance^[33]. It's unknown whether *Colletotrichum kahawae* attacks *C. stenophylla*. However, CBD is considered as a potential threat to the production of *C. stenophylla* in Sierra Leone.

In addition, CWD is a potential biotic constraint to produce *C. stenophylla*. CWD is a vascular disease that can result in complete death of infected coffee trees. This fungal pathogen is known by its teleomorph *Gibberella xylarioides* (*Fusarium xylarioides*). Hosts of CWD include *C. arabica*, *C. canephora*, and *C. liberica*^[34]. Resistance to wilt depends on the genetic potential of the coffee plant for virulence in the pathogen population, inoculum concentration and host genetic potential^[34]. Efforts to control CWD are based on the selection of disease-resistant plants, environmental management, and the use of synthetic fungicides^[35]. Twelve Arabica coffee genotypes were tested in different agro-ecological regions with different resistant responses based on artificial inoculation trials on CWD-infected natural soil to verify previous results to select promising resistant genotypes^[36]. The breeding method of CWD resistance is potentially useful to other coffee species, including *C. stenophylla*.

Breeding for quality attributes

The selection of Arabica coffee has attracted positive attention in terms of bean and cup quality, especially in light (washed) coffee producing countries. Most coffee quality parameters show significant (additive) genetic variation, while environmental factors also have an impact^[37]. Among the genotypes of Arabica coffee, three types of plants can be distinguished: wild genotypes from the Sudano-Ethiopia region; non-invasive cultivation lines (Typica and Bourbon); and invasive variants, mainly consisting of Timor hybrid genotypes. A study on the diversity and correlation between quality and biochemical properties of different potential genetic resources of *C. arabica* in southwestern Ethiopia was performed by Abeyot et al.^[38]. The results showed that, compared to coffee collections from other origins, the collections from Sheko, Dizi and Meanit exhibited significantly different organoleptic and biochemical characteristics. In addition, they found that at the phenotypic level, caffeine, bitterness, and astringency were inversely related to measures of high cup quality.

Combining ability

The concept of combining ability analysis identifies parents who are likely to pass on their desired traits to their offspring by following a recommended breeding strategy. Furthermore, it suggests the best hybrid combinations and provides genetic information highlighting different agronomic traits. Hybridization in coffee usually refers to the behavioural pattern of the parent clones during the crossbreeding process, either directly

or back and forth to obtain hybrids. The general composability (GCA) of a clone refers to the behavior of the clone in a series of crosses based on the mean of the obtained F_1 hybrids. From the results of Allard^[39], the deviation from the mean of the GCA due to the performance of a single clone is called the specific combining ability (SCA). So far, studies on the combining ability of coffee have only been done on *C. arabica*. The results showed that both additive and non-additive types of types of gene actions are important in the inheritance of these traits^[40–43]. However, for most of the interesting characteristics of Arabica coffee, the non-additive genetic impact proved more interesting than the additive ingredients. Using crop yield data obtained over 14 years, Cilas et al.^[44] estimated the genetic parameters of several bean traits and the yield of Robusta coffee using two mating alternatives of 18 parents. The authors found that the general combining ability (GCA) was the main cause of significant variation for the studied traits. This result enabled the identification of superior parents with good GCA in terms of yield and bean trait. Montagnon & Bouharmont^[45] analyzed the between population crosses (Congolese vs Guinean), combining yield and susceptibility to coffee leaf rust (SCLR), using other heterozygous assays. Their findings showed no interaction between tested genotypes and testers, although the correlation between test values obtained from different testers was significant. In addition, test values of tested genotypes can be used to predict the yield and SCLR of cross-population hybrids.

Heterosis

The phenomenon known as heterozygosity refers to F_1 hybrids that are robust or stronger than their parents. Therefore, the terms hybrid dominance and hybrid vigor are often used interchangeably^[46]. This is usually manifested by an increase in vigor, size, growth rate, yield, or some other characteristics. Information on the hybrid superiority of coffee is generally scanty compared to other crops as crossbreeding studies have begun quite recently^[47]. The perennial nature of the crop poses a challenge as it takes several years to achieve significant results^[44]. Since hybrid performance depends on parental selection, the efficiency of hybridization can be improved by predicting hybrid performance from parental genetic distance (GD). Therefore, it is suggested that such predictions of relevance are possible if there is a positive relationship between GD and hybrid performance^[48]. The hybrid vigor of coffee can be useful for imparting establishment like early growth and high early yield. However, prediction of hybrid yield using genetic distance has been under-investigated in coffee than in other economically valuable species.

In *C. arabica*, hybrid vigor was exploited, and its existence was demonstrated by hybridization between different genetic groups^[44,49–53]. Likewise, in *C. canephora*, combinations of different parents exhibit a greater heterozygous effect, and it is suggested that parental selection in this species should consider both genetic differences and superior agricultural value^[54]. Berthaud^[55] and Montagnon et al.^[56] also observed that the crossing of genotypes with high genetic variation produced progeny that were 20% to 50% more productive than the average for the clones. As evident in *C. canephora*, Leroy et al.^[57] and Ferrao et al.^[58] also reported the heterozygous potential of different combinations. In addition, Leroy et al.^[59] and Montagnon et al.^[60] showed that hybrids between

Guinean and Congolese species (the two major genetic groups of *C. canephora*) as identified by Berthaud^[61] exhibit heterozygosity due to the presence of high genetic variability in the species. DNA markers were used to estimate genetic distance and assess its relationship with hybridization performance. The SNP marker is more suitable for high throughput applications at a lower cost than other marker systems^[62,63]. Recently, the SNP was used to estimate the genetic distance between the *C. canephora* genotypes and observed the highest genetic distance between the Conilon (*Coffea arabica*) and Robusta genotypes. These markers have been shown to be effective in assessing the genetic diversity and population structure of *C. canephora*. Selection was performed within and between cultivar groups and hybrids with greater genetic distance were selected as they were considered important for *C. canephora* breeding programs.

Akperter et al.^[21] used SNP markers to determine the genetic distance between the parental clones of *C. canephora* and evaluate the relationship between genetic distance and hybrid yield. The experiment included 64 parental clones and 56 crosses that were evaluated over 11 years. Their results showed a significant correlation ($r = 0.351$, $p < 0.01$) between the genetic distance of the parental clones and the yield of their hybrids. Subsequent selection of hybrids with the highest genetic distance showed an increase in yield relative to the overall average as the genetic distance between the parent clones of the hybrids increased. Inter-group hybrids outperformed intra-group hybrids in cumulative yield, according to cross-population and inter-population comparisons determined by Bayesian clustering. The results support the hypothesis that genetic distance is related to the yield of hybrid Robusta coffee varieties and is an effective predictor of hybrid yield. The information from this study will help coffee breeders identify promising parental genotypes and confirm whether predicting hybrid yield based on SNP genetic distance is feasible. The established method can be applied to the development of *C. stenophylla* cultivars. Given the availability of recently developed SNP markers and information on the genetic diversity of *C. stenophylla*, parental clonal selection can be performed based on known relationships to explore hybrid predominance.

In addition, the recently developed SNP markers (Lahai et al., unpublished data) will allow research and application of advanced molecular selection methods on *C. stenophylla*. Among these technologies, genomic selection is increasingly being adopted by plant breeders, and the benefits are even more significant for perennial crops such as coffee, which have a prolonged juvenile phase. Genomic selection aims to predict the breeding value of selected individuals based on information provided by genome-wide molecular markers. The SNP marker is the most used molecular marker for genomic selection. Unlike other molecular breeding methods such as genome-wide association analysis (GWAS), which use only markers that are significantly associated with phenotypic features, genomic selection uses all genotyped SNP markers. Therefore, the selection is based on the complete genotype, not the phenotype. This type of method can improve the accuracy in the selection of young coffee trees and shorten the long coffee growth cycle, which requires a minimum of 15–20 years to complete the development of a new variety^[21]. Ferrao et al.^[58] investigated the effectiveness of gene prediction models in Robusta coffee, using data from two periodic

breeding populations evaluated at two sites. The performance of 13 statistical methods was evaluated based on their ability to predict three traits such as coffee bean yield, leaf rust incidence and green bean yield. Their results showed that the difference in predictive accuracy of competing models is small. In addition, the prediction accuracy of the analysis in the environment was on average higher than those predicted between sites and between populations. Their results support the potential of genomic selection to accelerate the breeding program of *C. stenophylla* and *C. canephora*.

Conclusions

The wild coffee species such as *C. stenophylla*, *C. affinis* and other related species are native to Sierra Leone. These species have great potential as a heat stress tolerant coffee for the West African lowland environments, such as the eastern and southern Sierra Leone. Compared to *C. arabica*, *C. stenophylla* has unique characteristics of flavor and is described as complex and naturally sweet with medium-high acidity, fruity and palatability. The organoleptic properties may complement locally grown Robusta and Arabica coffees. Their ecological adaptability and natural resistance to some of the major pests and diseases of Robusta and Arabica coffee makes them ideal for lowlands and rapidly changing climatic conditions. However, this species is still in its primitive stage, with low productivity. Investment in the research of these species is insignificant in Sierra Leone. Thus, a breeding program for *C. stenophylla* is needed for Sierra Leone. Germplasm is available from original native populations and potentially introduced from neighbouring countries. However, understanding local adaptation and genotypes \times environment ($G \times E$) will be essential for a new crop such as *C. stenophylla*. Robusta coffee breeding methods can be used as examples for breeding hybrid type varieties of *C. stenophylla*, based on the knowledge gained from the combination of fitness and hybrid superiority. A new generation of molecular markers such as SNPs can be applied to predict the hybrid viability of the F_1 generation. Like Robusta coffee, cuttings can be taken from the best plants and then propagated by continuous vegetative propagation as an elite clone. Furthermore, with the availability of recently developed SNP markers on *C. stenophylla*, new molecular breeding technologies, such as genomic selection, could play an important role in increasing breeding accuracy, thereby speeding up breeding and providing improved varieties with high yield, good adaptability, and good disease resistance.

Author contributions

The authors confirm contribution to the paper as follows: conceptualization and writing of manuscript: Lahai PM; proof reading and technical advice: Bah MA; advice on the design of the manuscript and data collection: Bah MT; proof reading and structural arrangement of manuscript: Aikpokpodion PO; aided in providing relevant literature and data collection: Johnson RAB. All the authors read and approved the final manuscript.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

The authors declare that they have no conflict of interest.

Dates

Received 30 May 2023; Revised 7 September 2023; Accepted 17 September 2023; Published online 20 October 2023

References

- Berthaud J, Charrier A. 1988. Genetic resources of *Coffea*. In *Coffee, Agronomy*, eds. Clarke RJ, Macrae R. vol. 4. London and New York: Elsevier Applied Science. pp. 1–42.
- Davis AP, Tosh J, Ruch N, Fay MF. 2011. Growing coffee: *Psilanthus* (Rubiaceae) subsumed based on molecular and morphological data; implications for the size, morphology, distribution, and evolutionary history of *Coffea*. *Botanical Journal of the Linnean Society* 167(4):357–77
- International Coffee Organization. 2012. *Trade Statistics Tables from International Coffee Organization*. www.ico.org/documents/1112-e.asp.
- Davis A, Gole TW, Baena S, Moat J. 2012. The impact of climate change on indigenous Arabica coffee (*Coffea arabica*): Predicting Future Trends and Identifying Priorities. *PLoS One* 7(11):e47981
- Aga E. 2005. Molecular genetic diversity study of forest coffee tree (*Coffea arabica* L.) populations in Ethiopia: implications for conservation and breeding. Doctoral Dissertation. Swedish University of Agricultural Sciences, Alnarp, Sweden. pp. 38–79
- Lashermes P, Combes M, Robert J, Trouslot P, D'Hont A, et al. 1999. Molecular characterisation and origin of the *Coffea arabica* L. genome. *Molecular and General Genetics* 261:259–66
- Coulibaly I, Noirot M, Lorieux M, Charrier A, Hamon SC et al. 2002. Introgression of self-compatibility from *Coffea heterocalyx* to the cultivated species *Coffea canephora*. *Theoretical and Applied Genetics* 105:994–99
- Labouisse J, Bellachew B, Kotecha S, Bertrand B. 2008. Status of coffee (*Coffea arabica* L.) genetic resources in Ethiopia: implications for conservation. *Genetic Resources and Crop Evolution* 55:1079–93
- Fairtrade Foundation. 2023. Coffee Farmers. www.fairtrade.org.uk/Farmers-and-Workers/Coffee/
- Geromel C, Ferreira LP, Guerreiro SMC, Cavalari AA, Pot D. et al. 2006. Biochemical and genomic analysis of sucrose metabolism during coffee (*Coffea arabica*) fruit development. *Journal of Experimental Botany* 57:3243–58
- Bangura W. 2017. *Brief Report on the state of the biodiversity for food and agriculture in Sierra Leone*. Country Report 001. Forestry Division of the Ministry of Agriculture Forestry and Food Security. www.fao.org/3/CA3424EN/ca3424en.pdf
- Grind PD. 2022. Exploring Coffee Production in Sierra Leone. <https://perfectdailygrind.com/2022/10/exploring-coffee-production-in-sierra-leone/>
- Government of Sierra Leone, United Nations Development Programme. 2007. *Integrated Resilient Urban Mobility Project (IRUMP). Environmental and Social Management Framework*. pp 5–20. https://mof.gov.sl/wp-content/uploads/2019/03/ESMF_IRUMP-March-2019.pdf
- McCouch SR, Kochert G, Yu Z, Wang Z, Khush GS. et al. 1988. Molecular mapping of rice chromosomes. *Theoretical and Applied Genetics* 76:815–29
- Lahai PM, Aikpokpodion PO, Lahai MT, Bah MA, Gboku MLS. 2023. Phenotypic diversity of wild Sierra Leonean coffee (*Coffea stenophylla*) collected from Kenema and Moyamba districts. *Beverage Plant Research* 3:12

16. International Plant Genetic Resource Institute. 1996. Diversity for development. Rome: International Plant Genetic Resources Institute. www.echocommunity.org/resources/f046001b-1de1-4eeef-803c-82bb19e38f76
17. Lashermes P, Trouslot P, Anthony F, Combes MC, Charrier A. 1996. Genetic diversity for RAPD markers between cultivated and wild accessions of *Coffea arabica*. *Euphytica* 87:59–64
18. Moncada P, McCouch S. 2004. Simple sequence repeat diversity in diploid and tetraploid *Coffea* species. *Genome* 47:501–9
19. Silvestrini M, Maluf MP, Silvarolla MB, Guerreiro-Filho O, Medina-Filho HP, et al. 2007. Genetic diversity of a *Coffea* germplasm collection assessed by RAPD markers. *Genetic Resources and Crop Evolution* 55:901–10
20. Gutiérrez OA, Martínez K, Zhang D, Livingstone DS, Turnbull CJ, et al. 2021. Selecting SNP markers reflecting population origin for cacao (*Theobroma cacao* L.) germplasm identification. *Beverage Plant Research* 1:15
21. Akpertey A, Padi FK, Meinhardt L, Zhang D. 2021. Effectiveness of single nucleotide polymorphism markers in genotyping germplasm collections of *Coffea canephora* using KASP assay. *Frontiers in Plant Science* 11:612593
22. Van der Vossen HAM. 2001. Agronomy I: Coffee breeding practices. In *Coffee: Recent Development*, eds. Clark RJ, Vitzthum OG. London, UK: Blackwell Science. pp. 184–201. <https://doi.org/10.1002/9780470690499.ch9>
23. William HU. 2009. All about Coffee. New York: Adams Media. 796 pp.
24. Prado SG, Collazo JA, Stevenson PC, Irwin RE. 2019. A comparison of coffee floral traits under two different agricultural practices. *Scientific Reports* 9:7331
25. World Agroforestry Centre. 2009. *Agroforestry Database. World Agroforestry Centre, agroforestry database 4.0*. <https://apps.worldagroforestry.org/treedb/>
26. EIAR. 2020. Research on coffee and tea. www.eiar.gov.et/jarc/index.php/jarc-research/coffee-and-tea (Accessed on 20th August, 2023).
27. Anim-Kwapong E, Anim-Kwapong G, Adomako B. 2011. Variation and association among characters genetically related to yield and yield stability in *Coffea canephora* genotypes. *Journal of Plant Breeding and Crop Science* 12:311–20
28. Keerthi CM, Ramesh S, Byregowda M, Mohan Rao A. 2018. Frequency of heterotic hybrids in relation to parental genetic divergence and general combining ability in *Dolichos* bean. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences* 88:923–33
29. Kokko H, Ots I. 2006. When not to avoid Inbreeding. *Evolution* 60:467–75
30. Lains E. 1958. Sao Tome and Principe and the cultivation of coffee. In *Memorias Junta Investig. do Ultramar* No. 1. www.aler-renovaveis.org/contents/files/aler-relatorio-stp-nov2020.pdf
31. Carvalho A. 1988. Principles and practice of coffee plant breeding for productivity and quality factors: *Coffea arabica*. In *Coffee: Agronomy*, eds. Clarke RJ, Macrae R. volume 4. London: Elsevier Applied Science. pp. 129–65.
32. Guzzo SD. 2004. Molecular and biochemical aspects of acquired systemic resistance. *International Journal of Research Agriculture* 7:32
33. Melese YY, Kolech SA. 2021. Coffee (*Coffea arabica* L.): Methods, objectives, and future strategies of breeding in Ethiopia—Review. *Sustainability* 13:10814
34. Beckman H. 1987. The nature of wilt diseases of plants. St. Paul, Minnesota, USA: APS Press. 175 pp.
35. Strange RN. 1993. Plant disease control towards environmentally acceptable methods. New York, USA: Chapman and Hall. Springer Dordrecht. 356 pp.
36. Admikew G. 2017. Mode of inheritance of resistance to coffee wilt disease (*G. xylarioides* Heim and Saccas) in Arabica coffee (*Coffea arabica* L.) genotypes. Master's Thesis. Jimma University, Jimma, Ethiopia. pp. 57
37. Clarke R, Vitzthum OG. 2008. Coffee: Recent developments. Hoboken, NJ, USA: John Wiley and Sons. Elsevier Applied Science. pp. 272.
38. Tessema A, Alamerew S, Kufa T, Garedew W. 2011. Variability and association of quality and biochemical attributes in some promising *Coffea arabica* germplasm collections in southwestern Ethiopia. *International Journal of Plant Breeding and Genetics* 5:302–16
39. Allard RW. 1999. Principles of plant breeding. 2nd edition. Canada: John Wiley & Sons. 264 pp.
40. Bayetta B. 2001. Arabica coffee breeding for yield and resistance to coffee berry disease (*Colletotichum kahawae* sp. nor). Ph. D. Thesis. Imperial College of Wye, University of London. pp. 55
41. Wassu M, Bayetta B, Harjit S. 2004. Heterosis and combining ability for yield and yield-related trait in Arabica coffee. M. Sc. Dissertation. Haramaya University, Ethiopia. pp. 1530–36.
42. Ayano A, Sentayehu A, Abush T. 2014. Combining ability for yield and morphological characters in southwestern Ethiopian origin coffee hybrids. *Sky Journal of Agricultural Research* 3:128–36
43. Dula G. 2019. Review on heterosis and combining ability study for yield and morphological characters of coffee (*Coffea arabica* L.) in Ethiopia. *Journal of Environment and Earth Science* 9:24–49
44. Cilas C, Bouharmont P, Boccara M, Eskes AB, Baradat PH. 1998. Prediction of genetic value for coffee production in *Coffea arabica* from a half-diallel with lines and hybrids. *Euphytica* 104:49–59
45. Montagnon C, Bouharmont P. 1996. Multivariate analysis of phenotypic diversity of *Coffea arabica*. *Genetic Resources and Crop Evolution* 43:221–227
46. Bangarwa SK. 2017. Heterosis, its type and theories. <https://biotecharticles.com/Agriculture-Article/Heterosis-Its-Types-and-Theories-4178.html>
47. Geneti D, Bellachew B, Habte E. 2019. Heterosis evaluation for morphological characters of diallel cross in western Ethiopian origin coffee (*Coffea arabica* L.). *International Journal of Scientific Foot Prints* 7:11–20
48. Falconer DS, Mackay FC. 1996. Introduction to quantitative genetics. Longman Scientific and Technical, New York. <https://archive.org/details/IntroductionToQuantitativeGenetics>
49. Charrier A, Berthaud J. 1985. Botanical classification of coffee. In *Coffee: botany, biochemistry and production of beans and beverages*, eds. Clifford MN, Wilson KC. Boston, MA: Springer. pp. 13–47.
50. Ameha M. 1990. Heterosis and Arabica coffee breeding in Ethiopia. *Plant Breeding* 6:593–98
51. Fazuoli LC, Carvalho A, da Costa WM. 1993. Dialectic hybridizations in the Mundo Novo cultivar of *Coffea arabica*. 19th Brazilian Congress of Research. IAC, Campinas Brazil. Brazil: Kluwer Academic Publishers. pp. 14–18.
52. Netto KA, Miguel AE, Queiroz AR, Pereira JBD. 1993. Hybrid studies of *Coffea Arabica* Catimor versus Catuai Catindu versus Catuai and others. 19th Brazilian Congress of IAC, Campinas Brazil. pp. 38–40.
53. Bellachew B. 1997. Arabica coffee breeding in Ethiopia: A review. Proceedings of the 17th ASIC Colloquium, Nairobi, Kenya. ASIC Paris France. pp. 406–14.
54. Resende MDV. 2002. Biometric genetics and statistics in perennial plant breeding. *Embrapa Information Technology Brazil*. pp. 975
55. Berthaud J. 1980. Incompatibility in *Coffea Canephora* - Test method and genetic determinism. *African Journal of Agricultural Research* 24:267–74
56. Montagnon C, Leroy T, Cilas C, Legnaté H, Charrier A. 2008. Heterozygous genotypes are efficient testers for assessing between population combining ability in the reciprocal recurrent selection of *Coffea canephora*. *Euphytica* 160:101–10
57. Leroy T, Montagnon C, Cilas C, Yapo A, Charmetant P, et al. 1997. Reciprocal recurrent selection applied to *Coffea canephora* Pierre.

Review of genetic diversity of coffee in Sierra Leone

- III. Genetic gains and results of first cycle intergroup crosses. *Euphytica* 95:347–54
58. Ferrão RG, Cruz CD, Ferreira A, Cecon PR, Ferrão MAG, et al. 2008. Genetic parameters in coffee conilon. *Brazilian Journal of Agricultural Research* 43:61–69
59. LeRoy CJ, Wymore AS, Davis R, Marks JC. 2014. Indirect influences of a major drought on leaf litter quality and decomposition in a southwestern stream. *Fundamental and Applied Limnology* 184:1–10
60. Montagnon C, Leroy T, Cilas C, Charrier A. 2003. Heritability of *Coffea canephora* yield estimated from several mating designs. *Euphytica* 133:209–18
61. Berthaud J. 1986. Genetic resources for the improvement of diploid African coffee trees. Evaluation of the genetic richness of forest populations and its organizing mechanisms. Implications for the application. In *Coffee*. Vol 4. Paris (FRA), ORSTOM. 379 pp. England: Elsevier Applied Science Publishers.
62. Gupta PK, Rustgi S, Mir RR. 2008. Array-based high-throughput DNA markers for crop improvement. *Heredity* 101:5–18
63. Sousa TV, Caixeta ET, Alkimim ER, de Oliveira ACB, Pereira AA, et al. 2017. Population structure and genetic diversity of coffee progenies derived from Catuaí and Híbrido de Timor revealed by genome-wide SNP marker. *Tree Genetics & Genomes* 13:124



Copyright: © 2023 by the author(s). Published by Maximum Academic Press, Fayetteville, GA. This article is an open access article distributed under Creative Commons Attribution License (CC BY 4.0), visit <https://creativecommons.org/licenses/by/4.0/>.