

Molecular Markers Associated with Genetic Diversity, Stress Tolerance, and Breeding Traits in *Theobroma cacao*: A Review

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Abstract: Global chocolate production depends entirely on *Theobroma cacao*, a perennial tree crop severely bottlenecked by an extended juvenile phase, extreme heterozygosity, and accelerating climate and pathogen pressures. Because conventional phenotypic selection cannot keep pace with rapidly compounding ecological threats, molecular breeding has transitioned from an exploratory tool to an absolute necessity for crop survival. This review critically evaluates the evolutionary trajectory, real-world deployment, and functional breakthroughs of diverse molecular marker systems designed to accelerate cacao genetic improvement. We trace the technological paradigm shift from legacy marker configurations (RAPD, RFLP, AFLP)—which established foundational germplasm architecture among Criollo, Forastero, and Trinitario groups—to ultra-precise, co-dominant platforms (SSR, SNP, cpSSR). Beyond mere identification, our synthesis of major findings highlights how these advanced markers have successfully decoupled target agronomic traits from environmental noise. We detail verified loci mapped via quantitative trait loci (QTL) analysis and genome-wide association studies (GWAS) that govern crucial abiotic stress adaptations (drought, waterlogging, oxidative stress) and destructive disease resistance profiles (black pod, frosty pod rot, witches' broom, and viral or insect vectors). Furthermore, the integration of these genetic resources with advanced tissue culture and micropropagation protocols is evaluated as a vehicle to ensure high-fidelity clonal multiplication of elite genotypes. Looking forward, we map critical future prospects where high-density SNP genotyping, comparative transcriptomics, and genomic selection models converge directly with CRISPR/Cas-mediated genome editing systems. This comprehensive review demonstrates that shifting from reactive field evaluation to marker-driven, genomics-assisted precision design provides the definitive molecular framework required to engineer high-yielding, climate-resilient, and disease-proof cacao cultivars, thereby permanently safeguarding the long-term economic sustainability of global cocoa supply chains.

Keywords: *Theobroma Cacao*; Molecular Markers; Genetic Diversity; Marker-Assisted Selection; Abiotic Stress; Disease Resistance; Genomics-Assisted Breeding.

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I. INTRODUCTION

Theobroma cacao L. (Malvaceae), commonly known as the chocolate tree, is one of the most economically important tropical plantation crops and serves as the primary raw material for the global chocolate industry. Cocoa beans are utilized for the production of cocoa powder, cocoa butter, and cocoa liquor, supporting a multi-billion-dollar international market and providing livelihoods to millions of smallholder farmers across tropical regions (Wood and Lass, 1985). Beyond its economic value, cacao cultivation contributes to environmental sustainability through agroforestry systems that aid in soil conservation, biodiversity maintenance, watershed protection, and carbon sequestration (Rice and Greenberg,

2003). Cacao is a perennial diploid species ($2n = 20$) with a relatively small genome size ranging from approximately 390 to 415 Mb (Figueira et al., 1992; Couch et al., 1993). Despite its global significance, genetic improvement of cacao remains challenging due to its long juvenile phase, high heterozygosity, perennial growth habit, and strong genotype $\times$ environment interactions. These biological characteristics considerably slow breeding progress and complicate the selection of superior genotypes. The sustainability of cacao production is further threatened by numerous biotic and abiotic stresses. Diseases such as witches' broom, black pod, and frosty pod rot continue to cause substantial yield losses in major cacao-producing regions, with global production losses estimated to exceed 30% annually under severe disease

pressure (Keane, 1992; Bowers et al., 2001; Evans, 2007). In addition, increasing incidences of drought, waterlogging, and temperature fluctuations associated with climate change pose significant challenges to cacao productivity and adaptation. Conventional breeding approaches have contributed significantly to cacao improvement; however, their effectiveness is often constrained by lengthy breeding cycles and difficulties in accurately identifying desirable traits at early developmental stages. The advent of molecular marker technologies has revolutionized cacao genetic research by enabling precise assessment of genetic diversity, DNA fingerprinting, phylogenetic analysis, quantitative trait loci (QTL) mapping, and marker-assisted selection (MAS). Marker systems such as RAPD, RFLP, AFLP, SSR, ISSR, and SNPs have provided valuable tools for accelerating breeding programs aimed at improving yield, disease resistance, stress tolerance, and quality traits. The present review summarizes the development and application of molecular markers in *Theobroma cacao*, with particular emphasis on their role in genetic diversity assessment, phylogenetic studies, abiotic and biotic stress tolerance, and modern breeding strategies. Furthermore, recent advances in genomics-assisted breeding and their potential contribution to the development of high-yielding, disease-resistant, and climate-resilient cacao cultivars are discussed.

II. MOLECULAR MARKERS IN CACAO FOR GENETIC DIVERSITY, DNA FINGERPRINTING AND PHYLOGENY

➤ *RAPD Markers:*

The prior sequence information is not needed for RAPD and ISSR markers the survey of plant genomes, but has the problems associated with reproducibility and dominance. RAPD is the simple and cost-effective marker system developed by Williams et al. (1990), and is proven valuable for a number of purposes. RAPD analysis was the first multi-locus protocol based on Polymerase Chain Reaction (PCR) to be applied for the genetic characterization of cacao (Wilde et al. 1992). Genetic relationships among cacao population were determined using RAPD marker by (Russel et al., 1993; Laurent et al., 1993; Lerceteau et al., 1997; Whitkus et al., 1998). N'Goran et al. (1994) analyzed the genetic diversity of 106 genotypes in Cote D'Ivoire belonging to the various morpho-geographic groups within Criollo, using 49 repeatable polymorphic RAPD products. Markers had shown a lucid structure among Forastero and Criollo groups with clear differences between Upper and Lower Amazon Forastero. RAPD analysis was carried out using 10 polymorphic primers to distinguish cocoa accessions (Sane et al., 2002). Accessions were efficiently identified with RAPD markers. Polymorphism was lowest in the Nigerian accessions and highest in the KAU collections. The UPGMA algorithm, based on Jaccard's coefficient, grouped 76 accessions into six groups based on genetic distance, and revealed four highly divergent accessions, viz. BE 10, EQX 78, I-56 and SCA 12. Faleiro et al. (2004) have studied the genetic variability among 19 clonal accessions of *Theobroma cacao* L. from the Brazilian, Ecuadorian, and Peruvian Amazons, using RAPD and microsatellite markers. In assessment, 56 RAPD and 45 microsatellite markers were generated and cluster analysis,

had shown that the different origin groups separately. The RAPD markers are also proven capable for the discrimination of cocoa cultivars in relation to yield components. Molecular analysis showed a close relationship with the genetic distance (GD) and Mahalanobis distance (MD) of yield component data, and between the biplots of GD and MD (Dias et al., 2005). Thirty clonal cocoa accessions showing resistance to witches' broom disease were used for genetic diversity analysis using RAPD marker and pedigree information. The association between genetic similarities was very low (Santos et al., 2005).

➤ *RFLP Markers:*

Laurent et al. (1993) used three types of RFLP probes to assess the genetic diversity of 203 genotypes. Ribosomal nuclear DNA probes distinguished Criollo, American Trinitario and Forastero. Mitochondrial probes revealed considerable variability among Criollo clones and cDNA probes confirmed the original structuring into Forastero and Criollo. Lerceteau et al. (1997) have studied genetic variability in 155 cocoa trees which were belonging to different morphological group's using 18 primers and 43 RFLP probes. RFLP variability observed within the species has reflected the hybridization and introgressions between trees of different origins. However, the Nacional type was different from Forastero, Criollo and Trinitario and genetically specific. The low heterozygosity rate was observed in some genotypes and it represented the original Nacional pool. Risterucci et al., (2000) performed linkage analysis in 181 cacao lines from a cross between Forastero and Trinitario, Linkage map comprised of 424 markers (Five isozymes, six gene loci, 65 genomic RFLPs, 104 cDNA RFLPs, three probes for telomeric region, 30 RAPD markers, 20 microsatellites and 191 AFLPs marker) with an average spacing of 2.1 cM. Markers were dispersed over ten linkage groups and covered 885.4 cM.

➤ *AFLP Markers:*

A linkage map was developed clones based on amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), and simple sequence repeat markers (SSR) markers in F2 population resulted from the cross between the Scavina-6 and ICS-1 cocoa. A total of 273 markers, distributed in 14 linkage groups (LGs) were used and the Linkage group 9 has -one -QTL linked to butter content and for butter stiffness, two QTL were present in linkage groups 9 and 7. QTL associated for butter content and hardness mapped at the LG9 had 51.0 and 28.8 per-cent of the phenotypic variation, respectively (Araújo et al., 2009).

➤ *SSR Markers:*

Microsatellite markers are proven efficient to fingerprint and to resolve identity issues in cacao collections (Figueira, 1998; Risterucci et al., 2001; Saunders et al., 2004; Cryer et al., 2006; Zhang et al., 2006). Brown et al. (2005) have constructed the resistant gene mapping for witches' broom disease in cocoa using the 170 SSR markers and they found two quantitative trait loci for resistance to witches' broom disease, showing important dominance effect. Efombagn et al., (2006) have analyzed the genetic diversity in cocoa using 13 SSR markers. When 282 alleles were analyzed farm

accessions were differentiated according to the geographical area. Accessions from the East province clustered together with local Trinitario accessions from the GenBank. Nagai et al. (2009) have fingerprinted 100 cocoa trees using eleven SSR markers. The survey group was found to include Criollo, Trinitario, Forastero and their hybrid types, and 77 trees were genetically unique among the samples. The large genetic variation was observed among cacao trees grown in Hawaii. SSR variation and individual identity in cocoa was analyzed using a capillary electrophoresis in 612 accessions of cacao collected from the Nanay Marañon, and Ucayali river systems of the Peruvian Amazon. The definite structure of genetic diversity, stratified by the river systems of the Peruvian Amazon was confirmed by Bayesian clustering method and principal coordinate analyses (Zhang et al., 2009). Lima et al. (2010) have reported EST SSR markers for resistance to witches' broom disease, clear polymorphism was obtained using 12 markers, having 47 alleles in total, with an average of 3.92 alleles per locus. The 11 genomic SSR markers have covered a total of 47 alleles, with an average of 5.22 alleles per locus. The relationship of genomic SSR with EST-SSR has improved the analysis of genetic distance between the genotypes. Yang et al. (2011) have reported the Theobroma cacao cpSSRs markers for genetic variation within the Trinitario cultivars. Eight haplotypes were identified out of 95 accessions sampled. The pentameric repeat (CaCrSSR1) was the most polymorphic and it has proven as a complementary tool for genetic identification of hybrid cultivars between Forastero and Criollo accessions and among all cacao accessions. Polymorphism between 27 cocoa trees in the major cocoa cultivating regions of Tamil Nadu was detected using ten SSR markers. Nearly 54 per-cent genetic similarity was observed and it has resulted in nine clusters (Thondaiman et al., 2013). Suwastika et al. (2015) have studied the genetics and clear pot morphology variation based on SSR marker in numerous clones of cocoa, collected from Central Sulawesi farms. The characterization of clone was done based on pod performance including; shape, size, bean properties. The polymorphism among the samples indicated high evolution rate in cacao trees over Sulawesi Island.

➤ *ISSR Markers:*

ISSR markers are considered useful in gene mapping studies (Godwin et al., 1997). Genome analysis in cacao was performed using ISSR (Charters and Wilkinson, 2000). Charters and Wilkinson, (2000) have characterized the cocoa genotypes using ISSR markers. Three pairs of the 62 accessions were distinguished and dendrogram of UPGMA was used to provide a measure of the genetic variability among genotypes. Chia et al. (2011) have reported ISSR markers to differentiate 46 cacao accessions maintained in Tingo Maria -Perú. ISSR marker, in spite of its dominance nature, established clear grouping of Trinitario accessions into a common cluster. Rivas et al. (2013) have used ISSR markers in three three geographically separate natural populations of *T. subincanum* for genetic diversity. Fifty-nine individuals were characterized by Nei's genetic distance using 13 polymorphic primers.

III. MOLECULAR MARKERS LINKED TO ABIOTIC STRESS

Plants are exposed to several abiotic stresses, including drought, salinity, and temperature extremes. These are the primary and most common stressors that diminish agricultural crop productivity. Changes in nutrient management and components, as well as variability in biosynthetic capacity, often decrease or cease plant growth (Chen et al., 2019). Stress is a climate condition which interacts with full genetic expression. A wide variety of molecular markers are present for studying abiotic stress in *Theobroma cacao*.

➤ *GST and HSP Markers for Oxidative Stress:*

In the early phase, water scarcity induced oxidative stress, as indicated by the increased expression of the oxidative stress markers and genes that encode ROS scavenging enzymes. The impact of oxidative stress on the energy metabolism was assumed based on the up gradation of enzymes related to fermentative-coding genes (Juby et al. 2021). Osorio Zambrano et al. 2021 reported that oxidative stress-related gene activation appeared to be focused to protect leaf cells because the expression of marker genes GST and HSP related to canonical oxidative stress repair showed high induction in Cacao investigated at D23 (DAY 23), which was most likely introduced much earlier. These results could be linked to cacao plants' efforts to mitigate the harmful consequences of reactive oxygen species (ROS) enhance by oxidative stress imposed. As reported by Sharma et al. 2019, over-expression of these genes stimulates initial responses in crops for protecting the structure of protein and lipids from the potential ROS damage and seems to assemble as a result of photorespiration.

➤ *Microsatellite Markers for Drought and Waterlogging Tolerance:*

Microsatellite markers (SSR) have greatly improved the accuracy of molecular analysis of cacao germplasm accessions and have also been helpful for assessing drought stress (DS) tolerant genes in cacao. Apshara et al. (2013) studied that drought stress reduced photosynthetic parameters in 11 genotypes from different geographical areas. There was a huge amount of clonal diversity, and those that responded positively could be recognized as drought tolerant. In due course, high yielding clones with drought tolerance may be selected. There was no clear link between physiological responses and molecular outcomes. Microsatellites were not associated directly with inter-clonal genetic variability in physiological responses because they are genomic in nature and not closely correlated with any specific phenotypic character. Waterlogging precludes air from entering the soil, lowering oxygen concentration and making a diminishing environment.

The root system's anaerobic respiration reduces the productivity of energy by getting involved with various housekeeping biosynthetic routes like chlorophyll synthesis (Dennis et al. 2000). The use of such molecular markers has been widely applied to analyze genetic variability since it allows for the comfortability of analyzing the huge number of polymorphisms at the DNA level in a short time period even

without the involvement of environmental conditions (Pires et al. 2000). Besides that, Bertolde et al. (2010) assessed genetic

variability among cacao clones affected by waterlogging using fluorochrome-labeled microsatellite (SSR).

Table 1 Characteristics and Applications of Molecular Marker Systems Used in Theobroma Cacao

Marker System	Inheritance Type	Polymorphism Level	Reproducibility	Major Applications in Cacao	Limitations
RAPD	Dominant	Moderate	Low	Genetic diversity assessment, cultivar identification	Poor reproducibility, dominant inheritance
RFLP	Co-dominant	High	High	Genetic mapping, phylogenetic studies	Labor-intensive, requires large DNA quantities
AFLP	Dominant	High	High	Linkage mapping, QTL detection	Complex data interpretation
SSR	Co-dominant	Very High	Very High	DNA fingerprinting, germplasm characterization, MAS	Development cost relatively high
ISSR	Dominant	Moderate-High	Moderate	Diversity analysis, population genetics	Dominant nature limits heterozygosity estimation
SNP	Co-dominant	Very High	Very High	GWAS, genomic selection, high-resolution mapping	Requires advanced sequencing and bioinformatics

MAS = Marker-Assisted Selection; GWAS = Genome-Wide Association Studies; QTL = Quantitative Trait Loci

IV. MOLECULAR MARKERS LINKED TO BIOTIC STRESS

Biotic stress is caused by various living organisms like viruses, bacteria, parasites, fungi, and harmful insects that damage to plants. Because of the enormous economic damages done by biotic stress to cash crops, it is a primary priority of agricultural research. Fungicides and insecticides are used sparingly in plants due to their high cost and concerns about human and environmental safety (Pokou et al., 2019). The production of resistant varieties is one of the most successful tactics for pest and disease control. Molecular markers are an efficient way to facilitate the introduction of resistance genes into one adapted breeding material.

➤ *SNP Markers for Fungal Disease Resistance:*

Single nucleotide polymorphism (SNP) markers are among the most informative and widely used DNA markers in modern plant breeding. Thus, is widely used in marker-assisted selection (MAS) and MAB of agricultural crops. Because of their robustness and effectiveness, SNPs are considered the most suitable DNA markers such as microsatellite markers. Based on SNPs developed by the genotyping-by-sequencing (GBS) method, Osorio-Guarn et al. 2020 reported the first genome-wide association study (GWAS) of disease resistance trait for witches' broom disease (WBD) caused by *Moniliophthora perniciosa* and frosty pod rot disease (FPRD) caused by *Moniliophthora roreri* in cacao. In addition, Royaert et al. (2016) explained genomic regions associated in WBD resistance using a data range of SNPs, and McElroy et al. (2018) noted numerous markers associated with production and disease resistance to *Moniliophthora* spp.

➤ *COI (Cytochrome C Oxidase Subunit I) Markers for Insect Pest Identification:*

The most informative marker for insects and most living organisms is COI. Even so, in some groups, like mealybugs, it has proven to be difficult to amplify, due to the formation of various diverse primer pairs (Malausa et al. 2011). Puig et al. 2021 used different markers such as COI1, 28S, and the ITS2 to identify mealybugs that were connected with cacao plants in North America. All these markers were very interesting for *Maconellicoccus hirsutus* (n=8) and *Pseudococcus comstocki* (n=4) but yet only COI1 delivered unequivocal recognition for *Pseudococcus jackbeardsleyi* (n=11), so this data explains molecular diagnostic protocols for trying to identify cacao-associated mealybugs and also helps to detect the existence of some parasitoids.

➤ *SSR Markers for Disease Resistance:*

Various molecular markers, most commonly Single Sequence Repeats or Microsatellites (SSR), have been used in the identification of QTLs associated with black pod (BP) resistance. The BP disease, caused by *Phytophthora de Bary* species, is one of the world's most severe diseases in the case of cocoa cultivation due to its strong impact on the fall down of productivity (Akaza et al. 2016). Mucherino Muoz et al. 2021 identified the latest report of verification of SSR markers in *Theobroma cacao* L. that were linked with resistance to *Phytophthora palmivora* (Butler) that can be used in local varieties of Brazil, Bahia, markers that had never previously been checked in association to rigidity towards the pathogen within those varieties having high economical value for the area as well as in the international chocolate market.

Table 2 Molecular Markers Associated with Important Biotic and Abiotic Stress Traits in Theobroma Cacao

Stress Category	Stress / Disease	Marker / Gene System	Reported Application
Abiotic	Drought stress	SSR markers	Assessment of drought-tolerant genotypes and genetic variability
Abiotic	Oxidative stress	GST, HSP genes	Monitoring ROS-scavenging and stress-response pathways
Abiotic	Waterlogging	SSR markers	Evaluation of genotypic responses to hypoxic conditions
Biotic	Witches' broom disease	SNP markers	Identification of resistance-associated loci and GWAS studies
Biotic	Frosty pod rot disease	SNP markers	Detection of genomic regions linked to resistance
Biotic	Black pod disease	SSR markers, QTLs	Marker-assisted selection for disease resistance
Biotic	Mealybug infestation	COI, ITS2, 28S markers	Molecular identification of insect pests
Biotic	<i>Phytophthora palmivora</i>	SSR markers	Validation of resistance-linked loci for breeding

In associated with early resistance screening methods, the use of these markers in Marker Assisted Selection strategies reflects an efficient and powerful tool for cocoa breeding programs (Schnell et al. 2007). The rapid development of molecular marker technologies has significantly enhanced research on disease resistance and trait inheritance in cacao and the genetic basis of QTL for disease resistance in cacao. Such data would be extremely useful in marker-assisted selection (MAS), which will currently be used in several crop breeding programs to develop better cultivars, including cacao.

V. BREEDING ATTEMPTS IN THEOBROMA CACAO

Breeding efforts in Theobroma cacao have evolved significantly over the past century, transitioning from conventional selection methods to molecular and genomics-assisted strategies. The primary objectives of cacao breeding have consistently focused on improving yield, enhancing resistance to major diseases such as black pod, witches' broom, and frosty pod rot, and maintaining desirable quality traits (Eskes and Efron, 2006; Ploetz, 2016).

➤ Conventional Breeding:

Early cacao improvement programs relied heavily on mass selection and hybridization among genetically distinct groups such as Criollo, Forastero, and Trinitario. Selection of elite trees based on phenotypic performance was widely practiced in major cacao-growing regions, particularly in West Africa and Latin America. Hybridization between high-yielding Forastero types and fine-flavor Criollo types led to the development of Trinitario cultivars, which combined vigor and quality (Motamayor et al., 2008). Recurrent selection schemes were later introduced to accumulate favorable alleles for yield and disease resistance. Notably, the use of Scavina clones (e.g., SCA 6) as resistance donors has been instrumental in breeding for witches' broom resistance in Brazil (Brown et al., 2007). However, conventional breeding in cacao is constrained by its long juvenile phase (3–5 years), high heterozygosity, and strong environmental influence on phenotypic traits.

➤ Disease Resistance Breeding:

Disease resistance breeding has been a cornerstone of cacao improvement due to the severe yield losses caused by pathogens. Resistance to black pod disease (*Phytophthora* spp.) has been shown to be quantitatively inherited, involving multiple genes with moderate effects (Flament et al., 2001). Similarly, resistance to witches' broom disease has been mapped to several genomic regions, with significant contributions from Scavina-derived germplasm (Brown et al., 2007). Field-based selection for resistance, although effective, is often inconsistent due to environmental variability and pathogen diversity. Therefore, integrating genetic information has become essential for improving selection efficiency.

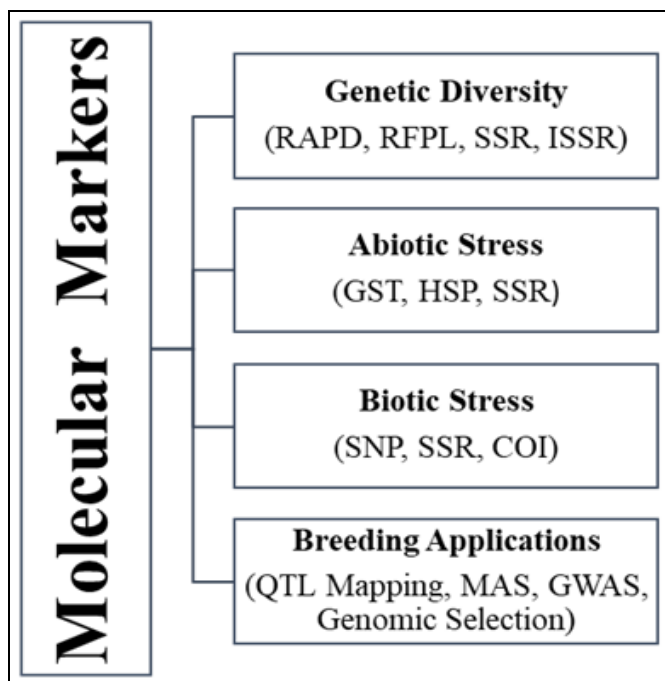


Fig 1 Schematic Representation of the Integration of Molecular Marker Technologies in Cacao Improvement.

Genetic diversity analysis using molecular markers such as RAPD, RFLP, SSR, and ISSR facilitates marker discovery and identification of candidate genes, which are subsequently utilized in QTL mapping, genome-wide association studies (GWAS), marker-assisted selection (MAS), and genomic selection (GS) for the development of high-yielding, disease-resistant, and climate-resilient cultivars of Theobroma cacao.

➤ *Marker-Assisted Breeding:*

The introduction of molecular markers has greatly enhanced cacao breeding programs. Early linkage maps integrating RFLP, RAPD, AFLP, and SSR markers enabled the identification of quantitative trait loci (QTLs) associated with economically important traits (Risterucci et al., 2000). For instance, QTLs for resistance to black pod disease and butter content have been identified on specific linkage groups, explaining a significant proportion of phenotypic variation (Flament et al., 2001; Araújo et al., 2009). SSR markers, due to their co-dominant nature and high polymorphism, have been extensively used for marker-assisted selection (MAS) and germplasm characterization (Lanaud et al., 1999; Saunders et al., 2004). These markers have facilitated the identification of resistant genotypes at early developmental stages, thereby reducing the reliance on long-term field trials.

➤ *Genetic Diversity and Utilization in Breeding:*

Understanding genetic diversity has been critical for broadening the genetic base of breeding populations. Molecular studies have revealed that domesticated cacao varieties represent only a fraction of the diversity present in wild Amazonian populations (Motamayor et al., 2008). This has prompted increased efforts to incorporate wild germplasm into breeding programs to enhance resilience and adaptability. The identification of distinct genetic groups and population structure has also enabled breeders to design strategic crosses that maximize heterosis and combine desirable traits. However, the introgression of traits from wild genotypes is often associated with linkage drag, requiring careful selection.

➤ *Genomics-Assisted Breeding:*

The availability of the cacao reference genome (Argout et al., 2011) has marked a major milestone in breeding research. High-density SNP markers have enabled genome-wide association studies (GWAS) and genomic selection (GS), allowing the prediction of breeding values based on genome-wide marker effects (Livingstone et al., 2015; Cornejo et al., 2018). Genomic selection is particularly promising for cacao due to its ability to handle complex traits such as yield and disease resistance, which are controlled by many loci with small effects. By reducing the breeding cycle time and increasing selection accuracy, GS represents a significant advancement over traditional MAS. In addition, transcriptomic studies have provided insights into the molecular mechanisms underlying stress responses, including the activation of defense-related pathways during pathogen infection. Emerging technologies such as CRISPR/Cas-mediated genome editing are also being explored for functional validation of candidate genes, although their application in cacao is still in early stages.

➤ *Tissue Culture and Clonal Propagation:*

Biotechnological approaches such as somatic embryogenesis have been developed to facilitate large-scale clonal propagation of elite genotypes. This is particularly important in cacao, where seed propagation leads to high genetic variability. Tissue culture techniques also provide a platform for genetic transformation and conservation of valuable germplasm. However, challenges such as soma-

clonal variation and genotype-dependent responses limit the widespread application of these techniques.

➤ *Constraints in Cacao Breeding:*

Despite considerable advances in cacao improvement, breeding programs continue to face several challenges. The long generation time and perennial nature of cacao result in slow breeding cycles, while the complex polygenic inheritance of economically important traits complicates the selection process. Furthermore, strong genotype × environment interactions often influence trait expression, reducing the reliability of phenotypic evaluations. Breeding efforts are also constrained by the limited availability of high-quality phenotypic data and the high cost and technical expertise required for implementing advanced genomic tools. Collectively, these challenges highlight the need for integrating modern breeding approaches with improved phenotyping platforms, high-throughput data collection, and efficient data management systems to enhance breeding accuracy and accelerate genetic gain.

➤ *Genome Editing and Future Molecular Breeding:*

Recent advances in genome editing technologies have opened new opportunities for precision breeding in *Theobroma cacao*. Among these, the CRISPR/Cas9 system has emerged as a powerful tool for targeted modification of genes associated with disease resistance, abiotic stress tolerance, yield, and quality traits. Unlike conventional breeding approaches, genome editing enables precise alterations at specific genomic loci without extensive backcrossing, thereby significantly reducing the time required for cultivar development. Furthermore, CRISPR-based approaches facilitate functional validation of candidate genes identified through quantitative trait loci (QTL) mapping, genome-wide association studies (GWAS), and transcriptomic analyses, improving our understanding of the genetic mechanisms underlying important agronomic traits. Beyond conventional CRISPR/Cas9-mediated gene knockouts, advanced technologies such as base editing and prime editing have further expanded the scope of genetic improvement. Base editing allows the direct conversion of one nucleotide into another without generating double-stranded DNA breaks, while prime editing enables precise insertions, deletions, and targeted sequence modifications. These technologies offer considerable potential for correcting unfavourable alleles and introducing beneficial genetic variations in cacao breeding programs. Although the application of genome editing in cacao remains at an early stage due to challenges associated with transformation efficiency, tissue culture responsiveness, and regulatory considerations, continued progress in functional genomics and biotechnology is expected to accelerate its adoption. Future molecular breeding strategies will likely integrate molecular markers, genomic selection, transcriptomics, and genome editing technologies to develop high-yielding, disease-resistant, and climate-resilient cacao cultivars capable of meeting the increasing global demand for cocoa.

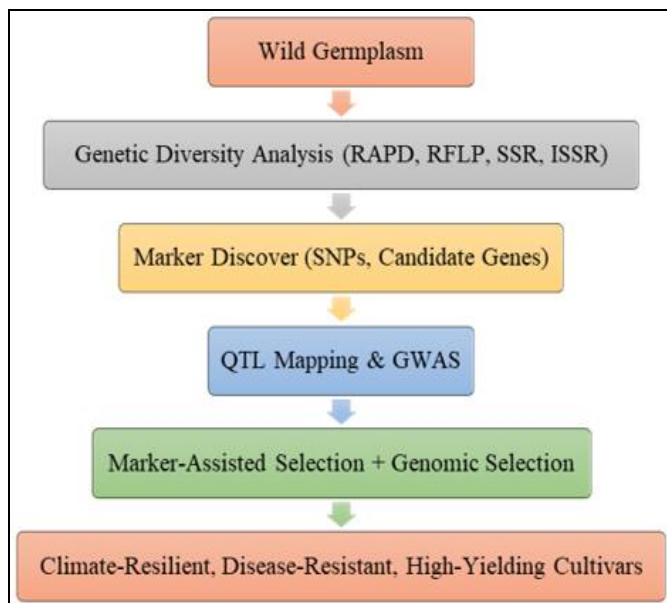


Fig 2 Modern Breeding Pipeline for Genetic Improvement of *Theobroma cacao*.

The breeding process integrates germplasm collection, genetic diversity analysis, molecular marker discovery, QTL mapping, genome-wide association studies (GWAS), marker-assisted selection (MAS), and genomic selection (GS) to accelerate the development of elite cacao cultivars with improved yield, quality, and resistance to biotic and abiotic stresses.

VI. FUTURE PROSPECTIVES AND EMERGING GENOMIC TOOLS

The strategic roadmap for *Theobroma cacao* genetic improvement is undergoing a profound paradigm shift, transitioning from a reactive, phenotype-driven practice to a predictive, data-intensive science. Overcoming cacao's historical biological bottlenecks—namely its protracted juvenile phase, high heterozygosity, and geographically restricted tropical footprint—demands that modern breeding programs move beyond legacy single-locus marker-assisted selection (MAS) and fully embrace high-throughput, multi-dimensional genomic architectures to counter accelerating climate volatility and shifting pathogen pressures.

➤ Re-Architecting Complex Traits: From Single Markers to Genomic Selection:

While traditional MAS struggles to capture polygenic traits controlled by minor-effect loci, Genomic Selection (GS) circumvents this limitation by utilizing genome-wide marker data to calculate genomic estimated breeding values (GEBVs) across entire breeding populations. When coupled with high-density Genome-Wide Association Studies (GWAS), this approach enables breeders to evaluate complex, environmentally sensitive phenotypes such as raw bean quality, long-term yield stability, drought adaptation, and multi-pathogen resistance profiles—years before a tree reaches reproductive maturity. By bypassing decades of field evaluation, genomic selection drastically compresses breeding cycles in this perennial crop.

➤ Precision Genome Architecture Via CRISPR/Cas Systems:

The maturation of high-quality cacao reference genomes alongside high-density SNP arrays has paved the way for direct, surgically precise sequence modifications. CRISPR/Cas-mediated genome editing stands at the vanguard of this transition, offering a mechanism to knock out susceptibility genes or introduce beneficial alleles for biotic and abiotic resilience without the linkage drag inherent to conventional backcrossing. Although tissue culture recalcitrance and transformation efficiencies remain active technical bottlenecks in cacao, advances in functional genomics are rapidly translating candidate gene discoveries into targeted, field-ready genetic variations.

➤ Deciphering the Cacao Interactome Through Multi-Omics Integration:

True precision breeding requires moving past static DNA blueprints to capture the dynamic, real-time physiological responses of the plant. Integrating multi-omics platforms provides a comprehensive understanding of such mechanisms. Genomics & Transcriptomics pinpoint structural variants and map the activation of defense-related pathways during early pathogen infection. Proteomics & Metabolomics characterize the functional downstream proteins and secondary metabolites (such as polyphenols and alkaloids) that actively mediate stress tolerance and determine flavour profiles. Cross-referencing these layers allows researchers to isolate exact functional biomarkers and regulatory hubs rather than relying on tentative genetic correlations.

➤ Cognitive Breeding: AI, Machine Learning, and Big-Data Analytics

The next major milestone in cacao breeding is not merely data generation, but automated data synthesis. Modern crop improvement is leveraging artificial intelligence (AI) and machine learning (ML) to parse and fuse massive, heterogeneous datasets. By training predictive algorithms on integrated matrices of genomic profiles, high-throughput automated phenotyping, microclimate variables, and macro-climatic projections, breeders can simulate cross outcomes *in silico*. This AI-driven orchestration optimizes parental selection, maximizes heterosis, and slashes both the field footprint and experimental costs required to identify elite cultivars.

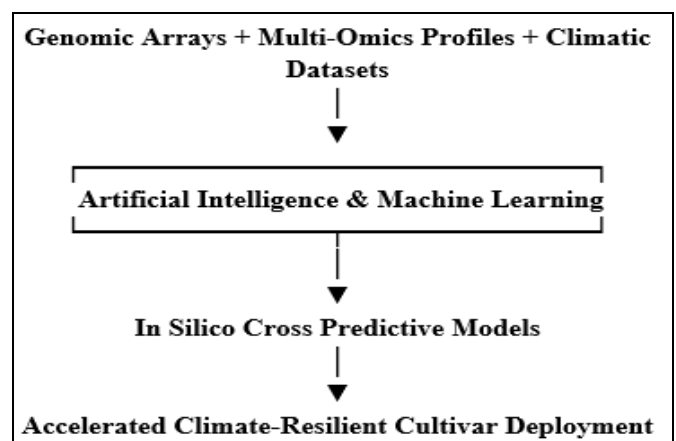


Fig 3 Cognitive Breeding: AI, Machine Learning, and Big-Data Analytics

Ultimately, the evolution of cacao improvement rests on the systemic convergence of these technologies: molecular markers define the baseline, multi-omics decode the functional pathways, CRISPR alters the sequences, and AI maps the predictive trajectory. This unified computational and

biotechnological front is no longer a futuristic luxury; it is the definitive strategy required to stabilize smallholder economies, secure global chocolate supply chains, and engineer a climate-proof future for *Theobroma cacao*.

Table 3 Evolution of Breeding Approaches in *Theobroma cacao*

Breeding Approach	Major Objective	Advantages	Limitations
Conventional Selection	Yield improvement	Simple, low cost	Long breeding cycle
Hybridization	Combine favorable traits	Increased variability	Requires extensive field evaluation
Marker-Assisted Selection	Disease resistance	Early selection possible	Limited by marker availability
Genomic Selection	Complex trait improvement	Higher prediction accuracy	High computational cost
Genome Editing	Targeted trait modification	Precision breeding	Regulatory and technical constraints

VII. CONCLUSION

Molecular markers have helped Cacao breeding by enabling precise diversity profiling, germplasm identification, and efficient trait selection. Marker systems such as RAPD, RFLP, AFLP, SSR, ISSR, and SNPs have collectively contributed to the understanding of cacao phylogeny, population structure, and quantitative trait inheritance, while also facilitating DNA fingerprinting and the establishment of linkage maps. Among these, SSR and SNP markers have emerged as particularly valuable tools due to their high reproducibility, co-dominant inheritance, and suitability for marker-assisted breeding and genome-wide analyses. The review demonstrates that molecular markers play a pivotal role in addressing major challenges in cacao cultivation, especially those associated with biotic stresses such as witches' broom disease, black pod disease, frosty pod rot, and insect infestations, as well as abiotic stresses including drought, oxidative stress, and waterlogging. Identification of stress-responsive genes, QTLs, and resistance-associated genomic regions has enhanced the efficiency of breeding programs and reduced dependence on lengthy field evaluations. Furthermore, the integration of molecular markers with advanced genomic approaches, including GWAS, genomic selection, transcriptomics, and emerging genome editing technologies, has opened new avenues for precision breeding in cacao. Despite substantial progress, several limitations continue to hinder rapid genetic improvement in cacao, including long generation time, complex polygenic inheritance, environmental influences on phenotype expression, and the high cost of advanced genomic tools. Therefore, future breeding programs should prioritize the integration of multi-omics technologies, high-throughput phenotyping, and bioinformatics-driven selection models to improve breeding accuracy and efficiency. Greater utilization of wild Amazonian germplasm and climate-resilient genetic resources will also be essential for broadening the genetic base of cultivated cacao. In conclusion, the continued advancement and integration of molecular marker technologies with conventional and genomics-assisted breeding approaches will be indispensable for the sustainable development of high-yielding, disease-resistant, and climate-adaptive cacao cultivars capable of meeting future global demands.

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