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Genetic Dissection of Drought Tolerance in Tomato Using Simple Sequence Repeat Markers: A Comprehensive Review

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ABSTRACT

Tomato (Solanum lycopersicum) is an economically important crop whose productivity is increasingly constrained by drought stress under changing climatic conditions. Conventional breeding for drought tolerance is often slow because this trait is quantitatively inherited and strongly influenced by environmental factors. Molecular markers, particularly Simple Sequence Repeats (SSRs), have become valuable tools for improving selection efficiency and accelerating breeding progress. This review examines the role of SSR markers in the development of drought-tolerant tomato cultivars. The physiological, biochemical, and molecular responses of tomato to drought stress, including growth reduction, photosynthetic impairment, osmotic adjustment, hormonal regulation, and oxidative stress responses, are briefly discussed. The genetic characteristics of SSR markers, including their high polymorphism, co-dominant inheritance, and multi-allelic nature, are highlighted as key advantages for genetic analysis and breeding applications. Furthermore, the review synthesizes recent findings on the use of SSR markers for genetic diversity assessment, Quantitative Trait Loci (QTL) mapping, and identification of genomic regions associated with drought-related traits such as relative water content, chlorophyll stability, root architecture, and proline accumulation. The application of SSRs in Marker-Assisted Selection (MAS), including marker-assisted backcrossing and QTL pyramiding, is also discussed. Finally, the review evaluates the continuing relevance of SSR markers in the genomic era, emphasizing their complementary role alongside Single Nucleotide Polymorphism (SNP) markers, Genome-Wide Association Studies (GWAS), and Genomic Selection (GS). Integrating SSR-based approaches with modern genomic tools offers a promising strategy for developing climate-resilient tomato cultivars and enhancing future food security.

Keywords: *drought tolerance; genomic breeding; QTL mapping; SSR markers; tomato*

Introduction

One of the high-economic-value crops belonging to the *Solanaceae* family is tomato (*Solanum lycopersicum* L.), which originated from the western region of South America and was subsequently domesticated and cultivated widely across the world. Tomato holds significant importance in the agricultural, economic, and health sectors. It is the second most consumed vegetable after potato, with a total global production of 187 million tons (FAO, 2022). Tomato fruits are rich sources of carotenoids, vitamins, antioxidants, particularly lycopene and lutein. Their low-calorie content, high fiber level, and the presence of minerals and phenolic compounds make tomatoes a *functional food* that provides various physiological benefits and contributes to fulfilling basic nutritional needs (Vijayakumar *et al.*, 2021). Tomato consumption can supply approximately 15% of the daily vitamin A

requirement, 8% of potassium, and 10% of iron for men and 7% for women, based on the recommended daily intake (Yadav *et al.*, 2024). The red pigment of tomato, lycopene, acts as a potent antioxidant capable of neutralizing free radicals, thereby protecting cells from oxidative damage and contributing to human health.

Both biotic and abiotic stresses are major limiting factors in tomato productivity. This crop is highly sensitive to environmental fluctuations, particularly drought stress, which can adversely affect seed germination, vegetative growth, and overall plant performance. Such stress conditions may lead to yield losses of up to 79% (Aliche *et al.*, 2018). The impact of drought stress is expected to intensify under future climate change scenarios, with global tomato production projected to decrease by approximately 18–32% during the period 2040–2069 (Dahal *et al.*, 2019). Drought stress during the vegetative phase has been reported to trigger physiological and molecular alterations. Water deficiency at different growth stages also induces morphological and biochemical changes, such as increased root dry weight, root length, and root-to-shoot ratio during early growth stages. These conditions ultimately pose a serious threat to food security.

Enhancing drought tolerance in tomato can be achieved through breeding programs, biotechnological approaches, and improved cultivation techniques (Makhadmeh *et al.*, 2022). According to Korir et al. (2014), more than 7,500 tomato varieties have been developed worldwide for agronomic and commercial purposes. These genetic resources serve as valuable donors of beneficial traits, including disease resistance and adaptation to environmental stresses, while also establishing tomato as a model organism for plant genetic studies. Assessing phenotypic and genetic diversity among tomato varieties is crucial for the conservation and sustainable utilization of genetic resources. Genetic characterization using molecular markers provides opportunities to identify associations between genetic markers and agronomic traits related to abiotic stress tolerance (Brake *et al.*, 2022). Various types of DNA markers have been employed, including RAPD (Random Amplified Polymorphic DNA), RFLP (Restriction Fragment Length Polymorphism), AFLP (Amplified Fragment Length Polymorphism), VNTR (Variable Number of Tandem Repeats), and SSR (Simple Sequence Repeats), each possessing distinct capabilities in detecting genetic polymorphism and elucidating genotype–phenotype relationships in tomato plants (Sheded *et al.*, 2022).

Among the available molecular marker systems, Simple Sequence Repeat (SSR) markers have emerged as reliable tools for assessing genetic diversity, identifying drought-associated loci, and supporting marker-assisted breeding in tomato due to their high polymorphism and co-dominant inheritance (Ditta *et al.*, 2018; Caramante *et al.*, 2021; Scarano *et al.*, 2015). Their application in drought-tolerance research is discussed in detail in the following sections.

Research Methods

A structured narrative literature review was conducted to synthesize and critically evaluate current scientific evidence regarding the role of Simple Sequence Repeat (SSR) markers in improving drought tolerance in tomato (*Solanum lycopersicum* L.). Relevant peer-reviewed publications published between 2000 and 2025 were retrieved from Scopus, Web of Science, ScienceDirect, SpringerLink, and Google Scholar. Literature searches were performed using combinations of keywords related to tomato, drought tolerance, water-deficit stress, SSR markers, quantitative trait loci (QTL), marker-assisted selection (MAS), marker-assisted backcrossing (MABC), genome-wide association studies (GWAS), and genomic selection (GS). Boolean operators (AND, OR) were applied to titles, abstracts, and keyword fields, with search syntax adapted to the indexing requirements of each database.

Publications were included if they were peer-reviewed research articles, review papers, or meta-analyses focusing on drought tolerance, molecular markers, marker-assisted breeding, or genomic approaches in tomato and closely related Solanaceae species. Studies reporting physiological, biochemical, molecular, or genetic traits associated with drought tolerance were also considered. Publications were excluded if they were non-peer-reviewed sources, unrelated to molecular markers or drought-tolerance mechanisms, duplicate records, lacked sufficient methodological information, or were unavailable in full-text form. The literature selection process involved title and abstract screening followed by full-text eligibility assessment according to the predefined criteria. A literature selection workflow adapted from the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) framework is presented in Figure 1.

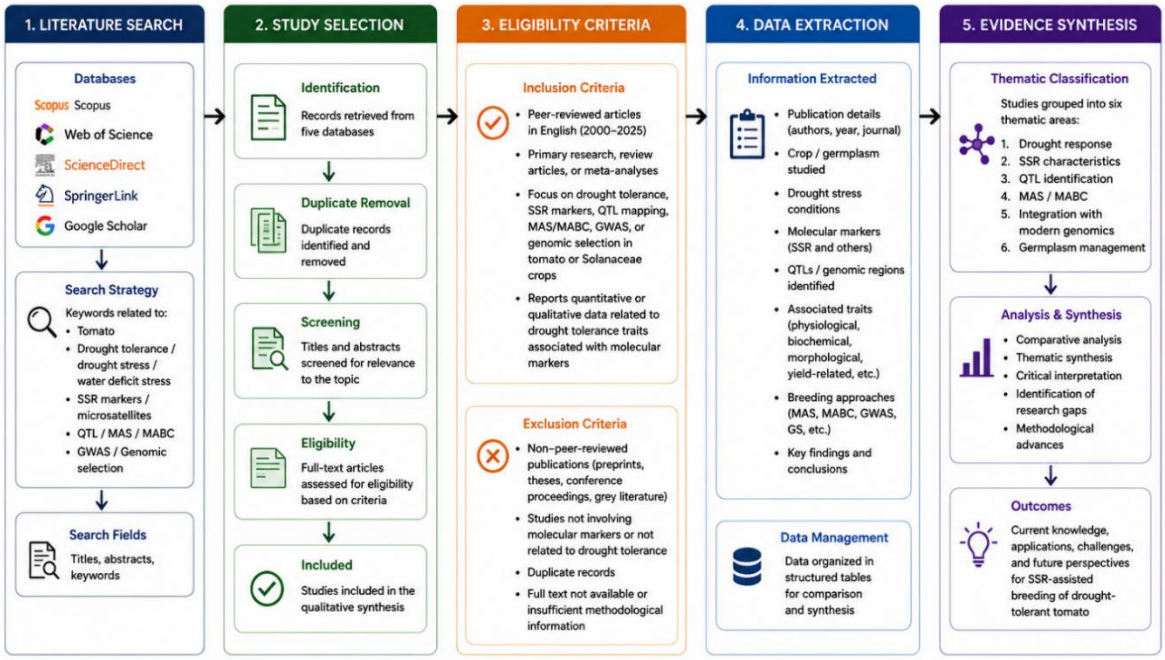

Figure 1. Schematic workflow of the literature review process

The selected literature was categorized into six thematic areas: (1) physiological, biochemical, and molecular responses of tomato to drought stress; (2) characteristics and advantages of SSR markers; (3) applications of SSR markers in genetic diversity analysis and quantitative trait loci (QTL) identification; (4) implementation of marker-assisted selection (MAS) and marker-assisted backcrossing (MABC) for drought-tolerance improvement; (5) integration of SSR markers with modern genomic approaches, including next-generation sequencing (NGS), genome-wide association studies (GWAS), and genomic selection (GS); and (6) the role of SSR markers in germplasm characterization and contemporary plant breeding programs.

The retrieved evidence was synthesized using a descriptive-qualitative approach involving comparative analysis, thematic synthesis, and critical interpretation of previous findings. The synthesis focused on identifying methodological advances, current applications, research gaps, existing limitations, and future perspectives for the integration of SSR markers with contemporary genomic tools in the development of drought-tolerant tomato cultivars.

Results and discussion

1. The Physiological and Molecular Landscape of Drought Stress in Tomato

1.1 Morpho-Physiological Responses to Water Deficit

Drought stress forces crop plants to activate a range of stress-responsive mechanisms to ensure survival while minimizing yield losses (Basu *et al.*, 2016). In tomato, one of the primary adaptive responses involves coordinated morpho-physiological adjustments. These include reductions in leaf area and stomatal

conductance, increases in root depth, accumulation of osmotic solutes, activation of antioxidant systems, and hormone-based signaling networks. For instance, under water deficit, tomato plants rapidly close their stomata to limit transpirational water loss. This response is regulated by a substantial increase in abscisic acid (ABA), as drought conditions can elevate leaf ABA levels several-fold, triggering ABA perception in guard cells and inducing pore closure (Jangid, 2016). In ABA signaling, ABA binds to PYR1/PYL receptors, which then inhibit PP2C phosphatases, releasing their repression of downstream kinases. The activation of OST1/SnRK2 kinases promotes ion efflux from guard cells, leading directly to stomatal closure.
Although stomatal closure restricts CO_2 assimilation, it significantly enhances water-use efficiency during drought stress.

Declining soil moisture also alters biomass allocation patterns in tomato. Water deficit typically stimulates root growth relative to shoot growth, leading to an increased root-to-shoot ratio. ABA plays a key regulatory role here as well under drought conditions, ABA produced not only in leaves but also in root tissues moves upward through the xylem, coordinating whole-plant growth responses. Consequently, drought-stressed tomato plants often develop deeper and more extensive root systems, improving access to water in deeper soil layers. This enhanced root proliferation under limited water availability is strongly associated with improved drought tolerance (Farooq *et al.,* 2012). Water deficit further induces morphological modifications in above-ground organs. Tomato plants commonly develop smaller, more vertically oriented leaves that reduce incident radiation and transpiration load, alongside thicker and waxier cuticles that reduce non-stomatal water loss (Ilyas *et al.,* 2021; Liu *et al.,* 2017). For example, tomato landraces native to arid regions typically exhibit reduced leaf area, more erect leaf angles, and greater investment in root biomass-morphologies that collectively optimize water conservation under prolonged drought. Together, these morpho-physiological adjustments allow tomato plants to maintain survival and limited productivity under fluctuating water availability.

1.2 Biochemical and Photosynthetic Perturbations

Under drought stress, tomato plants accumulate small organic compounds known as compatible solutes (osmolytes) and activate antioxidant defenses to mitigate osmotic and oxidative damage. Plants accumulate these osmolytes as a biochemical adaptation to adverse environmental conditions. Key osmolytes include proline, glycine betaine, polyamines, glycerol, and mannitol, all of which contribute to osmotic regulation, stabilization of cellular structures, and protection of proteins, while also directly scavenging reactive oxygen species (ROS) (Jogawat, 2019; Dikilitaş *et al.,* 2020). Among these, proline plays a central role as an osmoprotectant by stabilizing proteins, membranes, and other subcellular

components, scavenging free radicals, and helping maintain cellular redox balance under stress (Abdelaal *et al.,* 2022). Consistent with previous findings, Turan et al. (2023) reported that drought-stressed tomato seedlings exhibited a strong increase in oxidative stress and osmotic adjustment responses. Hydrogen peroxide (H_2O_2) levels rose from approximately 30–35 mmol kg^{-1} under full irrigation to 55–56 mmol kg^{-1} under drought, indicating substantial ROS accumulation. Concurrently, proline content increased from 0.14–0.16% to about 0.32%, showing that drought stress nearly doubled proline levels. The accumulation of these osmolytes generally increases with drought severity, reflecting the greater osmotic challenge experienced by plants.

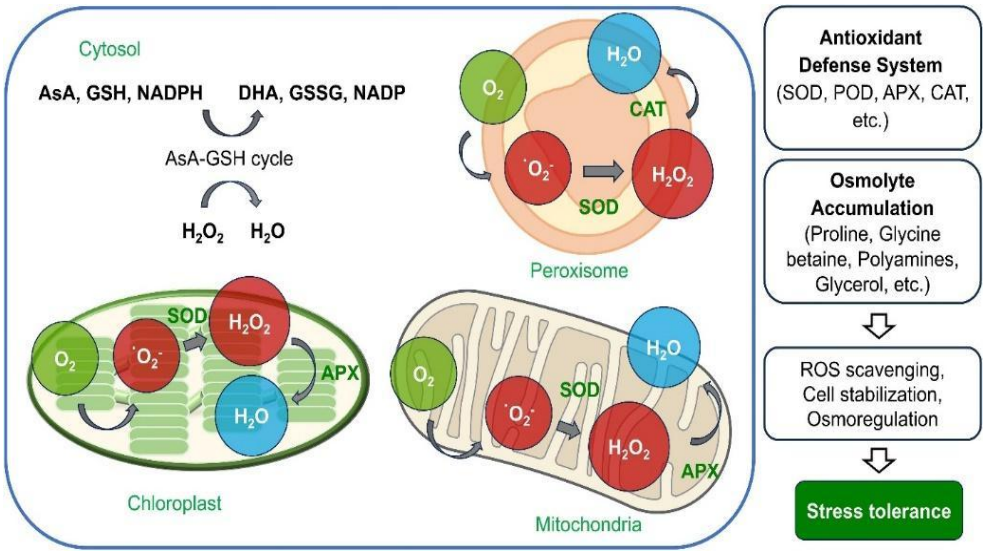

Figure 2. Osmolytes and antioxidants work together to stabilize cells, maintain metabolism, and detoxify ROS during drought stress (Park *et al.,* 2025)

In addition to osmolyte buildup, drought stress triggers overproduction of ROS, prompting plants to enhance their enzymatic antioxidant systems, including superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX) to detoxify superoxide radicals and hydrogen peroxide (Zhou *et al.,* 2019; Laxa *et al.,* 2019; Yang *et al.,* 2022; Fujita and Hasanuzzaman, 2022). Catalase rapidly converts H_2O_2 into water and oxygen, significantly reducing harmful ROS levels. Non-enzymatic antioxidants such as glutathione, ascorbate, tocopherols, and carotenoids also contribute to stress mitigation, mainly by protecting photosynthetic membranes from oxidative damage (Impa *et al.,* 2012). Drought reduces tomato photosynthesis through a combination of stomatal and non-stomatal limitations. Stomatal closure is an essential defense mechanism that minimizes water loss under drought but simultaneously reduces photosynthetic efficiency (Pamungkas and Farid, 2022). The rapid restriction of stomatal aperture limits CO_2 diffusion into the leaf, leading to an immediate decline in carbon assimilation (Liang *et al.,* 2020). Reduced CO_2 availability causes excess excitation

energy in the chloroplast, resulting in the overproduction of reactive oxygen species (ROS) such as H_2O_2 and singlet oxygen. These ROS damage Photosystem II (PSII), decrease the maximum quantum yield (Fv/Fm), and reduce the electron transport rate, contributing to photoinhibition (Sachdev *et al.,* 2021). Under prolonged drought, non-stomatal limitations become more pronounced, while Rubisco and other chloroplast enzymes lose activity, photosynthesis-related genes become down-regulated, and chlorophyll particularly chlorophyll b declines, further diminishing light-harvesting capacity. Carbon metabolism also shifts toward stress tolerance, with plants accumulating osmolytes such as proline and soluble sugars rather than synthesizing starch or exporting sucrose. To manage excess light and oxidative stress, tomato plants enhance photoprotective mechanisms, including increased non-photochemical quenching (NPQ) via the xanthophyll cycle and activation of antioxidant systems and heat-shock proteins. Together, these processes sharply suppress photosynthesis but help maintain minimal physiological function under drought conditions.

1.3 Cellular Stress and Defense Mechanisms

Drought stress is detected through rapid physical and chemical changes at the cellular level, notably reduced turgor and osmotic imbalance, which activate mechanosensitive Ca^{2+} channels. Five major families of mechanosensitive channels have been reported: MscS-like proteins (MSLs), Mid1-complement activity protein channels (MCAs), two-pore potassium channels (TPKs), Piezo channels (PZO), and reduced hyperosmolality-induced $[Ca^{2+}]_i$ increase (OSCA) channels (Gong *et al.,* 2020; Yuan *et al.,* 2014). Among them, OSCA1 serves as a hyper-osmolality–gated Ca^{2+} influx channel that initiates the first cytosolic Ca^{2+} spike under water deficit. Root cap tissues also perceive declining soil water potential and generate hydraulic signals and ROS waves that propagate throughout the plant. Together, Ca^{2+} oscillations, ROS bursts, and hydraulic changes form characteristic stress signatures that activate downstream signaling pathway. These signals feed into ABA-dependent pathways, where rising ABA levels trigger the PYR/PYL–PP2C–SnRK2 cascade leading to activation of AREB/ABF transcription factors, and ABA-independent routes dominated by DREB/CBF transcription factors that bind DRE/CRT elements in dehydration-responsive gene promoters (Maszkowska *et al.,* 2021; Hundertmark and Hincha, 2008).

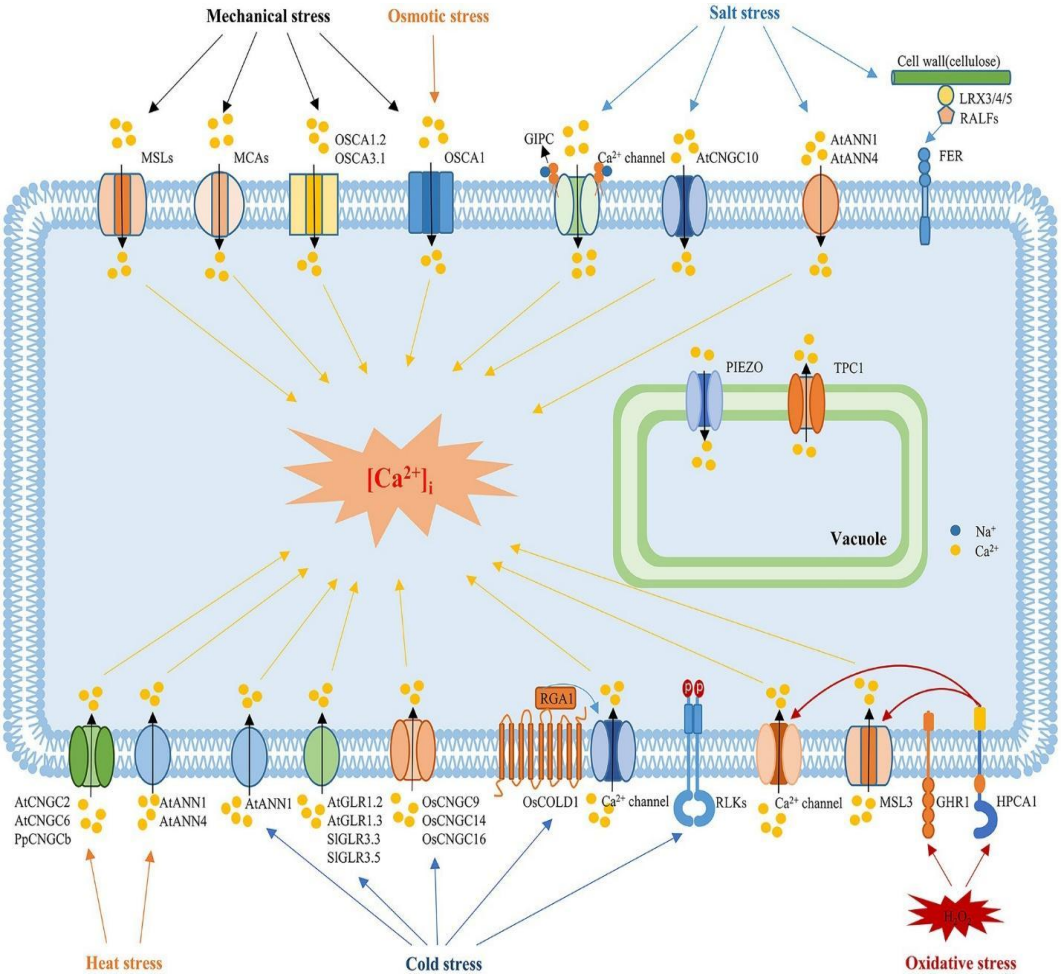

Figure 3. Mechanosensitive channels (MSLs, MCA, Piezos, OSCAs) detect abiotic stress and trigger Ca^{2+} influx as an early cellular signal (Xu *et al.*, 2022).

Once transduced, these signals trigger a coordinated defense response to manage drought-induced oxidative stress. ROS such as H_2O_2 and O_2^- function as essential secondary messengers at moderate concentrations but cause oxidative injury when overproduced, leading to lipid peroxidation, protein oxidation, and elevated MDA levels (Zhou *et al.*, 2019; Hasanuzzaman *et al.*, 2020). Maintaining cellular redox balance is therefore critical. Under drought, tomato plants intensify both enzymatic and non-enzymatic antioxidant systems: SOD, CAT, APX, and glutathione reductase are upregulated to detoxify ROS, while the ascorbate–glutathione cycle reinforces the removal of H_2O_2 through coordinated activity across chloroplasts, cytosol, and peroxisomes. Concurrently, non-enzymatic antioxidants ascorbate, glutathione, tocopherols, flavonoids, and carotenoids accumulate to stabilize membranes and scavenge free radicals (Laxa *et al.*, 2019; Tron *et al.*, 2015). Together, these integrated sensing, signaling, and detoxification mechanisms enable tomato cells to maintain redox homeostasis and limit cellular damage under drought stress.

2. Simple Sequence Repeat (SSR) Markers: A Versatile Tool for Plant Genomics

2.1 The Architecture and Nature of SSRs

Simple Sequence Repeat (SSR), also known as microsatellite markers, are DNA fragments consisting of short tandem repeats ranging from one to six base pairs (Ramzan *et al.,* 2018). Based on the number of nucleotides in each repeat unit, SSRs are classified into mononucleotide, dinucleotide, trinucleotide, tetranucleotide, pentanucleotide, and hexanucleotide repeats. SSRs are found throughout almost the entire eukaryotic genome, in both coding and non-coding regions, and are generally multi-allelic due to variations in the number of repeat units at each locus (Kumar, 2015). SSRs exhibit high levels of polymorphism and stability, making them suitable for detecting genetic variation across plant genomes. Their applications in tomato drought breeding are discussed in the subsequent sections.

2.2 Key Genetic Properties and Advantages

Polymorphism in SSRs results primarily from variation in the number of repeat units caused by DNA strand slippage during replication or unequal recombination (Chen *et al.,* 2025). These length polymorphisms are readily detected through PCR amplification using primers flanking the repeat region. Together with their co-dominant inheritance and high reproducibility, these characteristics make SSRs highly informative for genotype discrimination, linkage mapping, and genetic diversity studies.

2.3 A Comparative Analysis of Molecular Marker Systems

Each type of DNA marker possesses distinct technical characteristics in terms of analytical speed, cost efficiency, level of detectable polymorphism, DNA quantity requirements, and accuracy in estimating genetic distance. Several molecular marker systems are widely used in genetic analyses, including RFLP, RAPD, ISSR, SSR (microsatellites), and SNPs (Mudaningrat *et al.,* 2023). SSR markers are co-dominant, capable of distinguishing heterozygous and homozygous alleles, making them particularly valuable for parentage analysis, kinship studies, and genetic diversity assessment. Single Nucleotide Polymorphisms (SNPs) represent single-based variations occurring within the genome and constitute the most abundant form of genetic polymorphism. While SNPs are widely employed for genome-wide studies, SSRs retain unique advantages in high-resolution discrimination and targeted breeding applications.

3. Deploying SSR Markers to Unravel Drought Tolerance in Tomato

3.1 Characterizing Genetic Diversity in Tomato Germplasm

Genetic diversity represents a fundamental prerequisite for breeding drought-tolerant tomato genotypes because landraces and wild relatives often harbor rare alleles and unique trait combinations that enhance stress resilience. SSR markers have been widely applied to characterize genetic diversity among tomato

germplasm, enabling the identification of genetically divergent parents for drought-tolerance breeding (Sheded *et al.,* 2022; Younis *et al.,* 2020). As reported by Sheded et al. (2022), 4 to 6 alleles per locus (mean 4.8) with polymorphic information content (PIC) values reaching 0.95 in drought-selected tomato lines, indicating highly informative loci (PIC > 0.5). Similarly, SSR screening across diverse tomato panels can generate extensive allelic datasets enabling robust analyses of population structure.

Clustering methods including UPGMA and PCoA delineate genetically distinct groups that often correspond to geographic origin, domestication level, or contrasting drought responses. SSR-derived metrics such as PIC, allele richness, and genetic distance thus provide a robust framework for quantifying diversity, identifying divergent subpopulations, and guiding parent selection. Selecting parents from different SSR-defined clusters maximizes genetic distance and allows breeders to combine complementary drought-tolerance alleles.

3.2 Mapping the Genetic Architecture of Drought Tolerance: QTL Analysis

SSR markers serve as genetic landmarks linking phenotypic variation to specific chromosomal regions. In linkage mapping, an SSR-based genetic map is constructed from biparental populations—for instance F_2 or RILs—which are phenotyped under drought for traits such as proline content, Relative Water Content (RWC), root length, shoot biomass, or yield stability. QTL (Quantitative Trait Loci) analysis then identifies SSR loci whose allelic states co-segregate with variation in these traits. SSR loci frequently lie adjacent to, or within, stress-related genes; for instance, SSRs developed from tomato chromosome 3 were positioned near 96 genes associated with stress physiology, including growth regulators and ROS-scavenging enzymes (Brake *et al.,* 2022). Key outcomes of SSR-based QTL work include the identification of diagnostic flanking markers for drought-related QTL hotspots and the localization of candidate genes near informative SSRs.

3.3 Case Studies of Informative SSR Markers in Tomato

Several SSR loci have emerged as particularly informative for drought-related traits in tomato and closely related species. Notably, the Asr2 SSR is located within an ABA/WDS (water-deficit stress) gene, and genotypes carrying its favorable allele exhibit enhanced osmolyte accumulation and increased proline under drought (Makhadmeh, 2022). The LEta020 SSR lies within a thioredoxin gene, and polymorphisms at this locus correlate with elevated antioxidant enzyme activities, including superoxide dismutase (SOD) and peroxidase. Likewise, LEta014, linked to an auxin-response factor (ARF8), has been associated with variation in root architecture and stomatal regulation, contributing to improved water-use efficiency (WUE).

Although SSR loci such as Asr2, LEta020, and LEta014 have been associated with osmotic adjustment, antioxidant capacity, and root architecture, respectively

(Makhadmeh, 2022), these associations remain largely correlative. Functional validation through gene knockout, overexpression, or CRISPR-based editing has rarely been performed for these candidate loci in tomato, leaving their causal contribution to drought tolerance unconfirmed.

Collectively, these case studies demonstrate how individual SSR loci can tag specific mechanisms of drought tolerance, including osmotic adjustment, antioxidant defense, and root or leaf physiological regulation. These informative markers can be combined into low-density panels for marker-assisted selection. Because SSRs are co-dominant, they allow precise tracking of allele dosage, heterozygosity, and pyramid during selection (Brake, 2022; Sheded *et al.*, 2022). Beyond the three SSR markers discussed, gene annotation was performed for all SSR markers (Makhadmeh, 2022). The corresponding BLAST results are summarized in Table 1.

Table 1. Corresponding *Solanum lycopersicum* genes and functional annotations of the SSR markers identified by BLAST analysis

SSR Name	Solyc Gene	Annotation Result
LEat018	Solyc11g005330	ACTIN_RELATED PROTEIN contains InterPro domain IPR004000 (actin family domain).
LEct004	Solyc02g089940	HOMEOBOX PROTEIN TRANSCRIPTION FACTORS contain InterPro domain(s) IPR009057 (Homeodomain-like), IPR006563 (POX domain), IPR008422 (homeobox KN domain), IPR016039 (lipolase-like), and IPR001356 (homeobox domain).
LEta014	Solyc03g031970	AUXIN RESPONSE FACTOR 8 contains InterPro domain(s) IPR003340 (B3 DNA binding domain), IPR010525 (auxin response factor), IPR003311 (AUX/IAA protein), and IPR015300 (DNA-binding pseudobarrel domain).
LEta020	Solyc01g097450	THIOREDOXIN FAMILY TRP26 contains InterPro domain(s) IPR008979 (galactose-binding domain-like) and IPR010400 (PITH domain).
CT114	Solyc10g011690	Protein suppressor of PHYA-105 1 (SPA1) contains InterPro domain(s) IPR000719 (protein kinase domain), IPR017986 (WD40 repeat-containing domain), IPR002290 (serine/threonine/dual-specificity protein kinase, catalytic domain), IPR001680 (WD40 repeat), IPR015943 (WD40/YVTN repeat-like-containing domain), and IPR011009 (protein kinase-like domain).
Asr2	Solyc04g071580	ABA/WDS-induced protein (ABA_WDS) contains InterPro domain IPR003496 (ABA/WDS-induced protein).

4. Marker-Assisted Selection (MAS) for Drought-Tolerant Tomato Varieties

4.1 Principles and Workflow of Marker-Assisted Breeding

Marker-assisted selection (MAS) uses molecular markers linked to target loci to complement conventional phenotypic selection and improve breeding efficiency. By enabling early identification of desirable alleles, MAS reduces the influence of environmental variation and shortens breeding cycles, making it particularly valuable for improving complex traits such as drought tolerance (Cobb *et al.,* 2019; Govindaraj *et al.,* 2015). Databases such as Q-TARO list more than one hundred cloned genes with natural variants affecting key agronomic traits (Ebana *et al.,* 2010), and MAS continues to accelerate the transfer of adaptive traits from wild species like Solanum habrochaites into elite tomato lines.

The MAS workflow integrates molecular tools into routine breeding. It begins with parental selection and population development, where elite lines are crossed with donors carrying validated drought-tolerance alleles to generate segregating populations such as F2 or backcross progenies. This is followed by DNA extraction and genotyping, in which tissue samples are analyzed using PCR-based markers, often SSRs, linked to the target allele. A key advantage of MAS is that selection can occur at the seedling stage, allowing breeders to identify desirable genotypes early and shorten breeding cycles. In the final phase, marker data are combined with agronomic criteria to identify superior individuals for advancement through backcrossing or selfing, ensuring rapid and efficient incorporation of valuable alleles into breeding pipelines (Cobb *et al.,* 2019)

Phenotypic screening for drought tolerance is laborious, environmentally sensitive, and often unreliable. By targeting genotypes rather than phenotypes, MAS enables accurate identification of favorable individuals regardless of stress expression in the field. This reduces uncertainty caused by genotype-by-environment interactions and strengthens selection for complex traits such as drought tolerance (Yadav *et al.,* 2025).

4.2 Accelerating Genetic Gain with MAS

One of the principal advantages of marker-assisted selection (MAS) over phenotypic selection is its ability to accelerate genetic gain by increasing selection accuracy and reducing generation intervals. According to the breeder's equation, genetic gain (ΔG) is determined by selection intensity, selection accuracy, additive genetic variance, and generation interval (Cobb *et al.,* 2019). In tomato drought tolerance breeding, traits such as root architecture, osmotic adjustment, relative water content, and yield stability often require extensive phenotypic evaluation, resulting in lengthy breeding cycles (Foolad, 2007). SSR-based MAS enables

selection at the seedling stage, thereby shortening breeding cycles while maintaining selection accuracy (Collard and Mackill, 2007; Cobb *et al.,* 2019).

Marker-assisted backcrossing (MABC) is among the most effective MAS strategies for introgressing drought tolerance QTLs into elite cultivars. Through the combined application of foreground selection for target loci and background selection for recurrent parent genome recovery, MABC accelerates allele introgression and reduces linkage drag (Hasan *et al.,* 2021). Co-dominant SSR markers enable accurate discrimination of donor and recurrent parent alleles across successive backcross generations, improving selection efficiency and shortening breeding time compared with conventional backcrossing approaches (Hospital, 2005; Hasan *et al.,* 2015). The effectiveness of MABC has been further enhanced by advances in genomic-assisted breeding and high-throughput marker technologies (Herzog and Frisch, 2011; Chang-Brahim *et al.,* 2024).

Controlled-environment breeding technologies can further increase breeding efficiency by accelerating allele fixation and generation turnover. Double-haploid (DH) technology enables the rapid production of completely homozygous lines within a single generation (Dwivedi *et al.,* 2015), whereas speed breeding reduces generation intervals through extended photoperiods and optimized growth conditions (Watson *et al.,* 2018; Naqvi *et al.,* 2022). When integrated with MAS, these approaches facilitate rapid recombination, fixation, and pyramid of favorable drought-tolerance alleles, thereby increasing annual genetic gain and accelerating the development of climate-resilient cultivars (Hickey *et al.,* 2019; Naqvi *et al.,* 2022).

Table 2. Applications of SSR Markers in Tomato Drought-Tolerance Breeding

Application	Purpose	Breeding Outcome
Genetic diversity analysis	Characterize germplasm	Parent selection
QTL mapping	Identify genomic regions controlling drought traits	Marker discovery
Marker-assisted selection (MAS)	Early selection of favorable alleles	Increased breeding efficiency
Marker-assisted backcrossing (MABC)	Introgress drought-tolerance QTLs	Elite cultivar development
QTL pyramiding	Combine multiple favorable alleles	Improved and stable drought tolerance
Application	Purpose	Breeding Outcome

4.3 Strategic Implementation for Complex Traits

Drought tolerance in tomatoes is a complex quantitative trait controlled by numerous loci with small to moderate effects that interact with environmental

factors and epistatic networks (Tuberosa, 2012). Therefore, selection based on a single QTL is often insufficient to achieve stable genetic improvement (Collard and Mackill, 2007). Effective marker-assisted selection (MAS) requires the integration of multiple validated markers, phenotypic evaluation across environments, and continuous refinement of marker-trait associations. One of the most widely adopted approaches is QTL pyramiding, which combines favorable alleles governing complementary drought-adaptive mechanisms within a single elite genotype (Servin *et al.*, 2004; Hasan *et al.,* 2021). In tomato, targeted traits include root architecture, osmotic adjustment, photosynthetic maintenance, stomatal regulation, and antioxidant defense, all of which contribute to improved drought resilience and yield stability (Tuberosa, 2012; Park *et al.,* 2025; Hasan *et al.*, 2021).

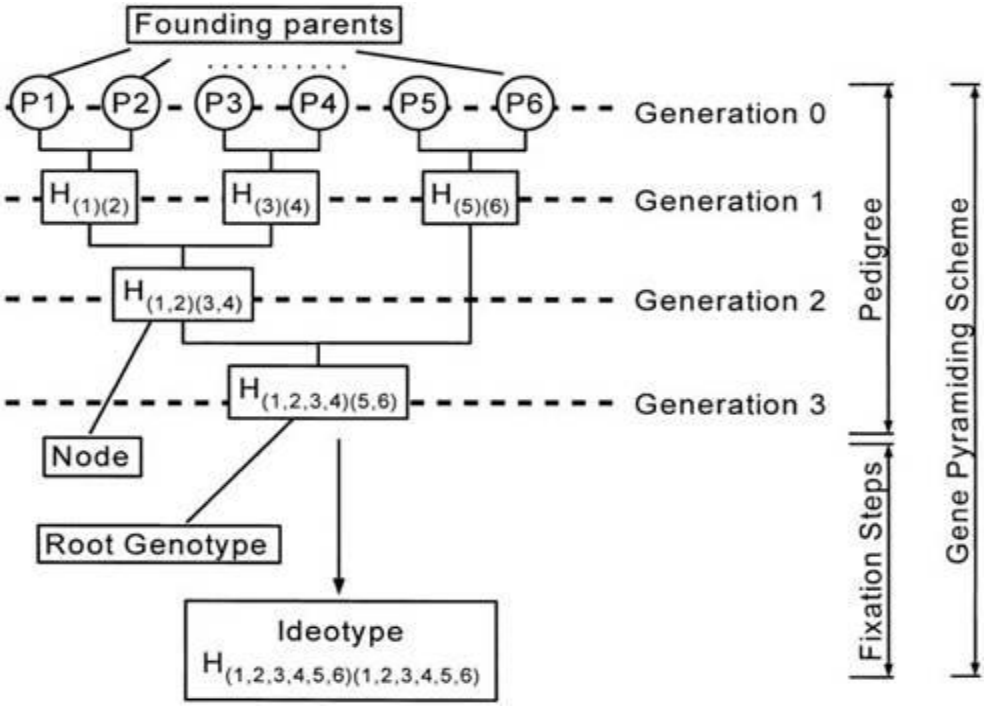

Figure 4. Marker-Assisted Pyramiding of Drought-Tolerance Traits (Servin *et al.*, 2004)

Successful QTL pyramiding depends on appropriate crossing strategies, including sequential backcrossing and convergent crossing, to assemble favorable alleles from multiple donor sources (Servin *et al.,* 2004; Hospital, 2005). SSR markers facilitate simultaneous monitoring of multiple target loci during QTL pyramiding, thereby improving the efficiency of combining favorable drought-tolerance alleles within elite breeding lines (Collard and Mackill, 2007). Furthermore, dense SSR-based linkage maps improve the detection of rare recombination events and enhance the efficiency of combining favorable alleles located on the same chromosome (Collard *et al.,* 2005).

Multi-environment testing (MET) remains essential for validating marker-selected lines because many drought-related QTLs exhibit strong genotype-by-

environment interactions (Tuberosa, 2012; Xu and Crouch, 2008). SSR markers can be employed within MET populations to reassess marker-trait associations, identify stable QTLs, and detect environment-specific loci that contribute to adaptation across diverse production systems (Collard *et al.,* 2005). In practical breeding programs, marker-assisted backcrossing is commonly implemented through foreground selection of target QTLs and background selection to accelerate recovery of the recurrent parent genome (Hospital and Charcosset, 1997). Optimizing marker density and genome coverage can reduce genotyping costs while maintaining selection efficiency and accelerating genome recovery (Frisch *et al.,* 1999; Herzog and Frisch, 2011).

5. Future Perspectives: The Evolving Role of SSRs in the Genomic Era

5.1 Overcoming Traditional Limitations with Next-Generation Sequencing (NGS)

Despite their considerable strengths, conventional SSR systems face inherent limitations in scalability. Traditional marker discovery required microsatellite-enriched library construction, colony hybridization, Sanger sequencing, and individual primer optimization, a process that was laborious, costly, and typically yielded only a few hundred polymorphic loci per study, insufficient for high-resolution QTL mapping or genome-wide association analyses (Guichoux *et al.,* 2011; Davey *et al.,* 2011). Electrophoretic fragment-size genotyping, though accurate and co-dominant, further limits throughput; fluorescent multiplexing is constrained to approximately 3–5 loci per assay due to fragment size overlap, restricting simultaneous analysis of large marker panels (Guichoux *et al.,* 2011; Varshney *et al.,* 2009). These constraints have motivated widespread adoption of next-generation sequencing (NGS) technologies, which enable rapid genome-wide marker discovery and high-throughput genotyping at unprecedented scale (Davey *et al.*, 2011; Kumar *et al.*, 2021).

Next-generation sequencing (NGS) has fundamentally expanded SSR utility by enabling *silico* genome-wide locus discovery. The reference genome of *Solanum lycopersicum* (Tomato Genome Consortium, 2012) enabled computational mining of tens of thousands of microsatellite loci across 12 chromosomes using bioinformatic pipelines such as MISA, Primer3, and BatchPrimer3, yielding dense, genome-wide SSR panels at a scale unachievable through conventional library-based approaches (Thiel *et al.,* 2003; You *et al.,* 2008). Reduced-representation sequencing methods including GBS, RADseq, and ddRAD-seq have further enabled simultaneous variant discovery and population-scale genotyping (Elshire *et al.,* 2011; Peterson *et al.*, 2012). Beyond primary SNP datasets, these NGS platforms recover SSR allele-length variants through read-alignment analyses, facilitating more specific and reliable primer design (Vieira *et al.*, 2016). Large-scale resequencing initiatives, including the *Solanum* diversity panel characterized by Aflitos et al. (2014), provide

targeted resources for identifying drought-associated polymorphic SSR loci, substantially increasing the efficiency and precision of marker-assisted selection for drought-tolerance breeding (Causse *et al.*, 2013; Hasan *et al.*, 2021).

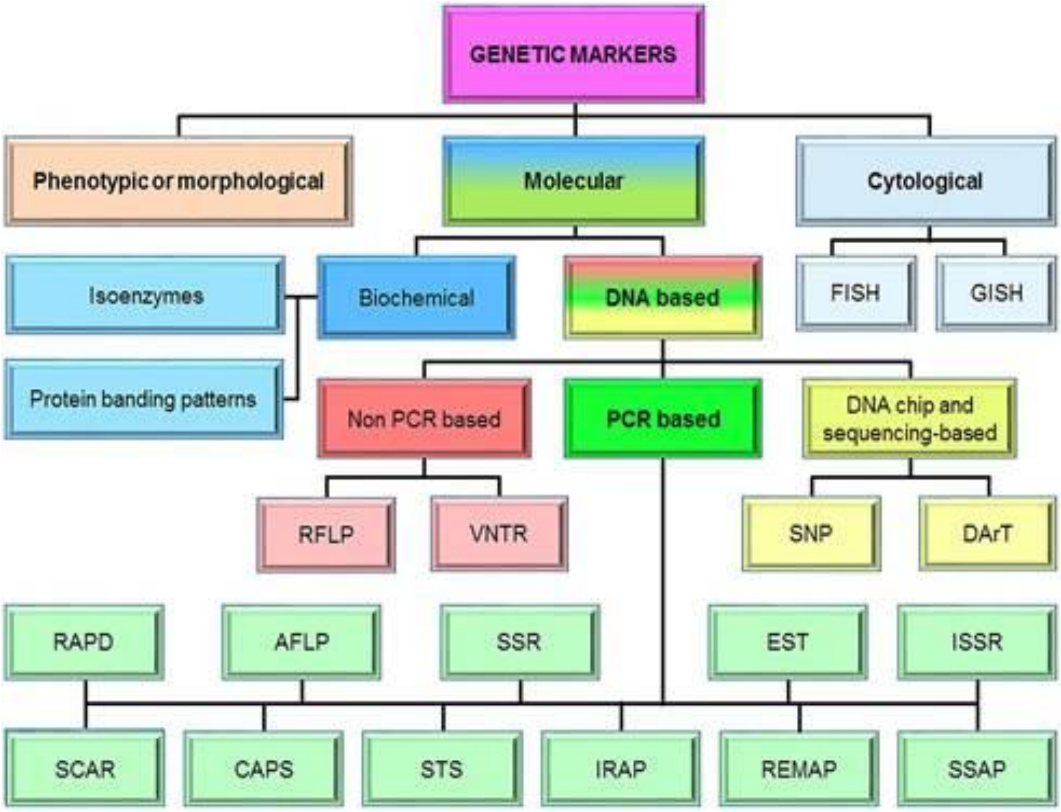

Figure 5. Classification of Genetic Markers Used in Plant Genomics (Kushanov *et al.*, 2021)

Expressed Sequence Tag (EST)-derived SSRs represent a particularly strategic class of NGS-enabled markers because they originate from transcribed genomic regions and therefore directly tag functional gene loci. Mining transcriptomic datasets, including drought-stress RNA-seq experiments, allows discovery of markers associated with stress-adaptive gene expression, protein function, and regulatory pathways (Varshney *et al.,* 2005; Taheri *et al.,* 2018). Polymorphisms at EST-SSR loci are more likely to reflect functional variation than those at anonymous genomic SSRs, making them particularly informative for dissecting mechanisms of drought tolerance (Korir *et al.,* 2014; Varshney *et al.,* 2005). EST-SSRs additionally exhibit high cross-species transferability within the *Solanaceae* family, including pepper (*Capsicum annuum*), potato (*Solanum tuberosum*), and eggplant (*Solanum melongena*), owing to higher coding-region sequence conservation, facilitating comparative mapping and cross-species QTL validation (Ellis and Burke, 2021; Caramante *et al.*, 2021). Collectively, the NGS era has not superseded SSR markers

but dramatically expanded their quality, quantity, and functional relevance for tomato drought-tolerance research (Vieira *et al.*, 2016).

Most SSR-based QTL studies reviewed here were conducted in single-environment trials, despite evidence that drought-related QTLs frequently exhibit strong genotype-by-environment interactions (Tuberosa, 2012). The scarcity of multi-environment testing (MET) data limits confidence in the stability and transferability of SSR-linked QTLs across production environments relevant to tomato breeding programs

5.2 Synergy with Modern Genomics: GWAS and Genomic Selection (GS)

Genome-Wide Association Studies (GWAS) have transformed the genetic dissection of complex traits by exploiting historical recombination events within diverse germplasm collections, thereby circumventing the requirement for structured biparental populations. Through linkage disequilibrium (LD) analysis between molecular markers and causal variants, GWAS identifies trait-associated genomic regions at resolution superior to conventional linkage mapping. Because genome-wide analyses require dense marker coverage, high-throughput SNP arrays and genotyping-by-sequencing (GBS) platforms constitute the principal genotyping tools for GWAS in tomato (Causse *et al.,* 2013; Aflitos *et al.*, 2014). SSR markers, while insufficient in density for genome-wide scans, retain a critical complementary role in post-GWAS phases; EST-SSRs located within or near candidate regions can be deployed to validate favorable alleles and develop practical selection markers, thereby translating GWAS findings into applicable breeding tools (Tomato Genome Consortium, 2012; Varshney *et al.,* 2005).

Genomic Selection (GS) extends beyond conventional MAS by simultaneously incorporating thousands of genome-wide markers to estimate the genomic estimated breeding value (GEBV) of selection candidates, thereby capturing both major and minor genetic effects on complex traits. Statistical models including ridge regression best linear unbiased prediction (rrBLUP), Bayesian approaches, and machine-learning algorithms are trained on populations that have been both phenotyped and genotyped, enabling prediction of breeding values for unphenotyped individuals (Meuwissen *et al.,* 2001; Crossa *et al.*, 2017). Recent studies have demonstrated the potential of GS to accelerate genetic gain for drought tolerance, yield stability, and fruit quality traits, particularly when high-density SNP datasets are employed (Crossa *et al.,* 2017; Budhlakoti *et al.,* 2022). Within GS frameworks, SSR markers retain complementary utility through germplasm characterization, genetic diversity assessment, sample identity verification, and

population quality control, supporting the reliability of training datasets and genomic prediction pipelines.

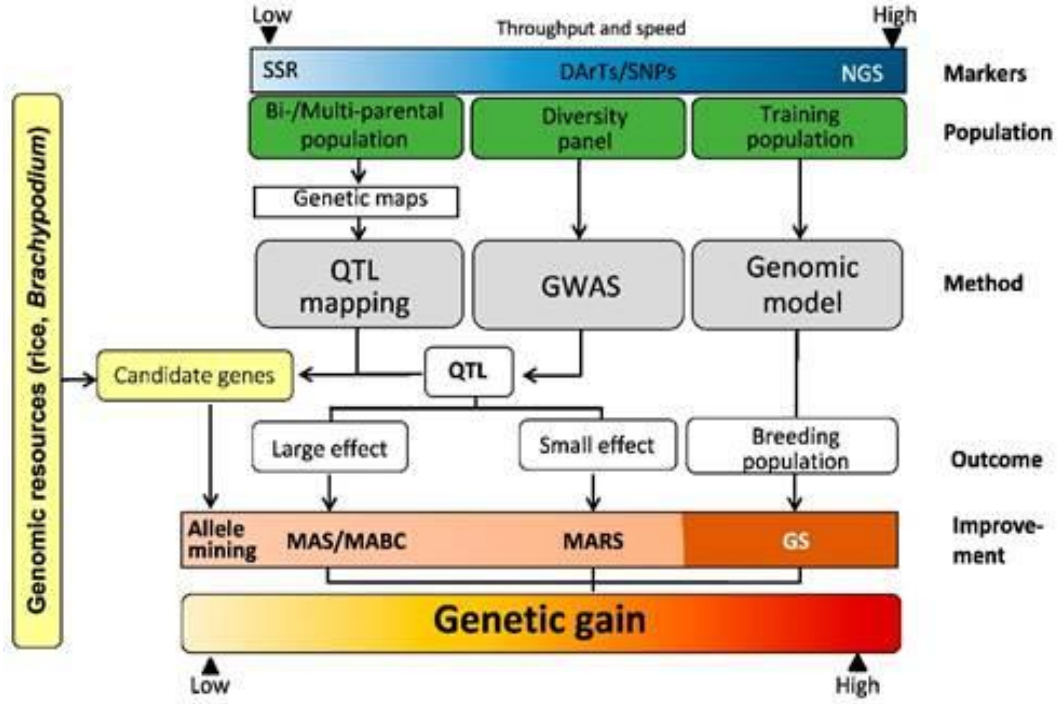

Figure 6. Integration of SSR Markers, GWAS, and Genomic Selection in Modern Breeding Programs (Miedaner *et al.*, 2020)

The integration of GWAS, GS, and SSR-based MAS constitutes a coherent and mutually reinforcing framework for drought-tolerance breeding in tomatoes. GWAS identifies candidate genomic regions and associated genes, GS enables genome-wide prediction of complex trait breeding values, and SSR-based MAS facilitates targeted validation and introgression of favorable alleles into elite materials. Declining genotyping costs and advances in next-generation sequencing have further expanded opportunities to deploy these approaches in combination, improving both the efficiency and precision of modern tomato breeding programs (Elshire *et al.*, 2011; Crossa *et al.*, 2017; Hasan *et al.*, 2021). Although SSR markers are unsuitable for genome-wide analyses because of their relatively low marker density, they remain valuable for validating candidate loci and implementing routine marker-assisted breeding.

5.3 Critical Appraisal of SSR Markers Relative to Modern Genomic Approaches

Despite these complementary roles, SSR markers present several important limitations compared with SNP-based platforms. First, marker density is a fundamental constraint: conventional SSR panels rarely exceed a few hundred loci per study, whereas SNP arrays and genotyping-by-sequencing platforms routinely provide tens of thousands of genome-wide markers, giving SNPs a decisive advantage for high-resolution QTL mapping and GWAS. Second, throughput and

cost-efficiency favor SNPs at large population scale; electrophoretic SSR genotyping is constrained to roughly 3–5 multiplexed loci per assay (Guichoux *et al.,* 2011), making it comparatively labor-intensive for germplasm panels involving hundreds of accessions. Third, most SSR-based QTL studies in tomato drought tolerance rely on biparental mapping populations (F2 or RIL), which sample only the allelic diversity segregating between two parents, in contrast to GWAS panels that exploit historical recombination across diverse germplasm collections and thus detect a broader allelic spectrum. These limitations mean that SSR markers alone are increasingly insufficient for dissecting the fine-scale, polygenic architecture of drought tolerance, a role for which SNP-based GWAS and genomic selection are now better suited.

5.4 The Enduring Relevance of SSR Markers

Despite the widespread adoption of SNP-based platforms and genomic selection approaches. Their co-dominant inheritance, multi-allelic nature, high polymorphism information content (PIC), locus specificity, and relatively simple PCR-based genotyping procedures provide practical advantages for routine genetic analyses (Vieira *et al.,* 2016). Owing to their high discriminatory power, SSRs remain widely used for cultivar identification, genetic purity assessment, germplasm characterization, and seed certification activities. Compared with biallelic SNP markers, a relatively small number of SSR loci can often provide sufficient resolution to distinguish closely related genotypes, making them valuable tools for varietal authentication and genetic resource management (Scarano *et al.*, 2015; Kalia *et al.*, 2011).

Despite the increasing adoption of SNP-based platforms, SSR markers remain valuable for germplasm characterization, cultivar identification, parentage analysis, seed purity testing, and genetic diversity assessment. Their high polymorphism, co-dominant inheritance, and relatively simple PCR-based genotyping make them practical tools for routine breeding programs, particularly in resource-limited laboratories where high-throughput sequencing is not readily available (Vieira et al., 2016; Kalia et al., 2011; Scarano et al., 2015).

While genomic selection has been extensively validated for drought and yield traits in cereal crops (Crossa *et al.,* 2017; Budhlakoti *et al.,* 2022), its combined application with SSR-based marker-assisted selection remains comparatively underexplored in tomato. Few published studies report a unified pipeline in which SSR markers support germplasm quality control while SNP-based genomic prediction drives selection decisions, representing a clear opportunity for methodological development.

Conclusion

This review demonstrates that SSR markers remain a scientifically valuable and practically accessible tool for dissecting the genetic basis of drought tolerance in tomato, particularly for genetic diversity assessment, QTL mapping, marker-assisted backcrossing, and germplasm characterization. However, the evidence synthesized here also reveals that SSR markers alone are increasingly insufficient to resolve the fine-scale, polygenic architecture of drought tolerance. Their comparatively low genome-wide density and throughput place clear limits on their standalone use for genome-wide association studies (GWAS) and genomic selection (GS), both of which require the marker density that only SNP-based platforms currently provide. The most compelling synthesis emerging from the reviewed literature is therefore not a choice between SSR and SNP-based approaches, but their complementary integration: GWAS to identify candidate genomic regions, GS to predict breeding values for complex traits, and SSR-based MAS to validate, track, and introgress favorable alleles into elite breeding materials at comparatively low operational cost. For tomato breeding programs, particularly those in resource-limited settings, this integrated framework offers a pragmatic pathway toward developing climate-resilient cultivars without requiring immediate access to high-throughput sequencing infrastructure.

Suggestion

Future research should focus on the development of more precise QTL mapping populations using advanced genomic tools combined with SSR markers, particularly targeting wild tomato relatives as sources of drought-adaptive alleles. Integration of SSR-based MAS with genomic selection frameworks and high-throughput phenotyping platforms is recommended to accelerate the identification and pyramid of drought-tolerance loci. Collaborative efforts among plant breeders, molecular biologists, and bioinformaticians are encouraged to develop open-access databases of validated SSR markers linked to drought-tolerance traits, which would facilitate the adoption of marker-assisted breeding in both advanced and resource-limited programs.

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