



Enhancing Crop Resilience to Abiotic Stress through Molecular Markers: A Review

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This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Plants are subject to several abiotic stresses, such as extreme heat or cold, drought, and high salinity, which can greatly reduce the yields of agricultural crops. These conditions interfere with nutrient uptake and metabolic functions, causing growth to slow down or stop altogether. Genetically, these stresses challenge plants by impeding their ability to express their genetic potential optimally. A variety of molecular genomic markers are employed to study and improve crops under stress. For example, Random Amplified Polymorphic DNA (RAPD) markers help in identifying and assessing hybrids that are tolerant to drought and salinity, while Simple Sequence Repeat (SSR) markers are crucial for evaluating stress resilience. These markers also play a key role in the Quantitative Trait Loci (QTL) mapping of genes that respond to stress. Genes like

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dehydrins for drought and salinity are essential in the regulation of responses to these stressors. Furthermore, the use of Single Nucleotide Polymorphisms (SNP) markers for single genes assists in the genetic mapping and sequencing of stress-related traits in inbred lines. DNA markers are central to marker-assisted breeding, which enhances tolerance to abiotic stresses through advanced techniques and improvements in markers.

Keywords: DNA; genotype; salinity; drought; temperature stress.

1. INTRODUCTION

Plants encounter a range of challenging environmental conditions throughout their growth cycles, including biotic stresses like herbivory and pathogenic infections, as well as abiotic stresses such as extremes in temperature, drought, nutrient deficiencies, high salinity, and the presence of toxic Metals and metalloids like arsenic, cadmium, and aluminum present in the soil, along with environmental factors such as heat or frost, drought, and salt, are among the most common stressors that substantially decrease agricultural crop yields, posing serious threats to food security and altering the natural geographic distribution of plants. These issues are compounded by climate change, characterized by long-term shifts in weather patterns, which serves as a significant source of abiotic stress [1,2]. In response, plants activate their basal defense systems upon stress detection. Kinase enzymes and phytohormones including jasmonic acid, abscisic acid, ethylene, and salicylic acid regulate pathways that are differentially activated under stress [3,4]. These pathways contribute to the production of reactive oxygen species (ROS), which play a critical role in mitigating biological damage and are essential for plant stress tolerance [5].

Crop yields and production are primarily impacted by abiotic stresses stemming from unfavorable environmental changes. Biologically, any external factor that adversely affects plant growth or health is considered a stress [6,7]. Stressed plants typically undergo three main response phases: the alarm phase, which signals the beginning of stress; the resistance phase, during which defense mechanisms are activated; and the exhaustion phase, which results in damage from prolonged stress. Many plants experience inhibited growth under stress conditions [8]. Salinity specifically restricts growth and productivity by impairing water uptake and disrupting ion balance [9]. Moreover, consistent water shortage, known as drought stress, drastically affects plant survival and growth [10]. Drought is commonly linked to low soil moisture

levels, but it can also be exacerbated by high rates of evapotranspiration caused by elevated air temperatures, creating a discrepancy between water absorption and loss [11].

Genetically speaking, stress is an environmental factor that inhibits plants from realizing their full genetic potential [12,13]. Abiotic stress, which arises independently of interactions with other organisms, adversely affects organisms within an ecosystem and poses a significant challenge in environmental and agricultural contexts. This challenge is now intensified by the effects of industrialization and global warming, further complicating the management and sustainability of ecosystems and agriculture [14,15-18].

DNA markers significantly enhance plant genetics through various applications such as identifying parent genotypes, evaluating genetic variation, and creating detailed genetic linkage maps. Molecular markers, extensively used in crop genetic analysis, are divided into categories such as PCR-based and non-PCR-based markers. RFLP (Restriction Fragment Length Polymorphism) markers, which were widely used in the 20th century, were pivotal in gene mapping and other molecular biology applications. The development of PCR technology by Mullis and Faloona greatly improved DNA marker technologies by making genetic mapping more time-efficient and cost-effective through probe hybridization. PCR, an in vitro method, amplifies DNA sequences of interest using small amounts of a template and primers that match specific gene sequences. Examples of PCR-based markers include Single Nucleotide Polymorphisms (SNP), Sequence-Characterized Amplified Regions (SCAR), and Simple Sequence Repeats (SSR), all requiring gene sequence data from the samples. These markers help delineate the allelic positions (heterozygosity, maternal homozygosity, paternal homozygosity) in progeny or plant lines and are crucial for constructing linkage maps that reflect genetic diversity through recombination analysis in hybrid plant genotypes. This review discusses the effects of abiotic stress on agricultural crops

and the role of DNA markers in genetic management, gene mapping, and identifying stress resistance traits. DNA markers significantly enhance plant genetics through various applications such as identifying parent genotypes, evaluating genetic variation, and creating detailed genetic linkage maps. Molecular markers, extensively used in crop genetic analysis, are divided into categories such as PCR-based and non-PCR-based markers. RFLP (Restriction Fragment Length Polymorphism) markers, which were widely used in the 20th century, were pivotal in gene mapping and other molecular biology applications. The development of PCR technology by Mullis and Faloona greatly improved DNA marker technologies by making genetic mapping more time-efficient and cost-effective through probe hybridization. PCR, an in vitro method, amplifies DNA sequences of interest using small amounts of a template and primers that match specific gene sequences. Examples of PCR-based markers include Single Nucleotide Polymorphisms (SNP), Sequence-Characterized Amplified Regions (SCAR), and Simple Sequence Repeats (SSR), all requiring gene sequence data from the samples. These markers help delineate the allelic positions (heterozygosity, maternal homozygosity, paternal homozygosity) in progeny or plant lines and are crucial for constructing linkage maps that reflect genetic diversity through recombination analysis in hybrid plant genotypes. This review discusses the effects of abiotic stress on agricultural crops and the role of DNA markers in genetic management, gene mapping, and identifying stress resistance traits [19,20,21].

2. ABIOTIC STRESS IMPACT ON AGRICULTURAL CROPS

Heat and drought are significant abiotic stresses that dramatically reduce crop yields and productivity, leading to considerable declines in agricultural income and benefits. For example, reducing water availability by 40% can cut maize yields to 40% and wheat yields to 21% of their maximum potential. In Africa, cowpea, a vital crop, sees yield reductions ranging from 34% to 68% due to drought stress. These stresses typically trigger an overproduction of reactive oxygen species (ROS), harmful molecules that can damage carbohydrates, lipids, nucleic acids, and proteins, negatively influencing plant growth. Furthermore, a lack of water and elevated heat stress can inhibit essential functions such as transpiration and stomatal conductance in the leaves of plants [22,23,24,25].

Globally, 91% of the agricultural area is subjected to abiotic stresses, contributing to 50% of the production losses in agriculture. The severity and damaging effects of abiotic stress can intensify with climate changes. Enhancements in agronomic management and the development of stress-resistant genotypes through breeding programs could mitigate these effects [26]. Abiotic stresses disrupt the biochemical and physiological processes in plants. Enhancing the efficiency of light usage and photosynthetic activity could improve tolerance to these stresses. Moreover, the activation of antioxidants and development of stress-related metabolites help protect against cellular damage, though further development of key adaptation strategies to increase stress tolerance in plants is needed [27].

Heat stress arises from rises in air and soil temperatures that surpass the tolerance levels of plants, even if only temporarily, negatively impacting their growth and development. The increasing global temperatures present a significant climatic threat that may sharply heighten the susceptibility of crops. Studying heat stress is essential for understanding how plants react and for developing resilience to these conditions. It is critical to develop new lines of crops that are more heat-tolerant to maintain agricultural productivity. Heat stress detrimentally influences seed germination, photosynthesis, and overall crop yields. It also interferes with the reproductive processes and the operation of tapetal cells in flowers, which can inhibit pollen grain expansion and in the release of less viable pollen [28,29].

Drought stress induces a variety of physiological changes in plants, including diminished photosynthetic activity, alterations in cell wall elasticity, and the closure of stomata. Frequently, the effects of drought and salinity on plant physiology overlap and share similar mechanisms for tolerance [30]. Drought impacts the nutritional status of crops by altering the concentration of ions in plant tissues, and the transport of soil nutrients to roots is reduced as moisture levels drop. Variations in nutrient composition and management, coupled with changes in biosynthetic abilities, are key factors that can severely restrict or stop plant growth altogether. Developing robust defense mechanisms to ensure plant survival under such abiotic stresses is essential for sustaining crop growth and productivity in agriculture. These protective strategies against abiotic stress are

currently being investigated through molecular genetics, aiming to better understand and improve stress tolerance [31,32,33].

Abiotic stressors such as salinity, drought, nutrient deficiencies, heavy metals, pollution in water and air, and variations in light photoperiodicity and intensity can individually or collectively trigger stress, potentially disrupting plant metabolic systems and diminishing growth, development, and productivity. High levels of stress may become overwhelming, leading to plant mortality. While completely avoiding stress is unfeasible, plants have developed metabolic adaptations and produce specific molecules to endure stressful conditions. Abiotic stress requires adjustments in soil and plant environmental conditions, which can lead to decreased yields in major agricultural crops globally. Presently, agricultural areas in regions without stress contribute to only 10% of the world's crop production, whereas the remaining 90% endure one or more forms of environmental stress. Plants are continually adapting to abiotic stress through biochemical, physiological, molecular, and phenotypic changes; however, ongoing efforts are crucial to further enhance plant stress tolerance using genetic innovations, resource-saving technologies, and various other strategies [14,34,35].

3. UTILIZING DNA MARKERS FOR ENHANCING ABIOTIC STRESS RESISTANCE IN CROPS

3.1 Employing Rapid Markers to Analyze Salinity and Drought Resistance

Random Amplified Polymorphic DNAs (RAPDs) are PCR-based markers that do not require preliminary sequence data from samples, allowing the analysis of multiple loci from many individuals using limited resources. RAPDs are favored for their straightforward experimental process and are extensively utilized for the genetic screening of intra- and interspecific hybrids [36].

These markers have played a crucial role in identifying genes that confer salinity tolerance in various crops. Plants have genetically controlled mechanisms for managing salinity, which underscores the importance of enhancing salinity tolerance in crops, particularly in areas affected by salinity. Using PCR to amplify specific DNA sequences, RAPD markers aid in the identification of genes resistant to salt. For

instance, research on wheat utilized these markers to evaluate the genetic diversity among salt-tolerant genotypes cultivated in salty soils, successfully differentiating between salt-resistant and salt-sensitive types. This distinction, marked by polymorphic primer pairing, is vital for developing salt-tolerant wheat varieties [37].

Salinity stress can lead to DNA alterations, such as structural disturbances and rearrangements, often triggered by secondary stressors such as oxidative damage linked to the production of reactive oxygen species (ROS) like hydroxyl radicals, singlet oxygen, superoxide, and hydrogen peroxide. In experiments with cotton seedlings affected by salinity and treated with NaCl, RAPD markers have detected genetic instabilities by revealing changes in DNA band patterns on agarose gels. These changes include the disappearance of bands, changes in band intensity, and the emergence of new bands. Such findings demonstrate that RAPD analysis is an effective tool for assessing toxicological stress [38].

Field evaluations, which are labor-intensive and time-consuming, can be complemented by molecular differences identified at the DNA level in plants developed from tissue culturing [39]. RAPD technology, being rapid and requiring only minimal DNA, is sensitive to genetic differences and allows for the efficient processing of large numbers of genomic samples under in vitro conditions. For instance, RAPD analysis in maize identified several primers linked to salt resistance, providing valuable information for breeding programs aimed at developing salt-resistant progeny through marker-assisted selection and direct genetic modifications [40].

The Genomic Template Stability (GTS) assessment, which measures DNA damage and mutations, significantly contributes to molecular marker assemblies for identifying damaged or mutant DNA in plant cells. In a study evaluating the response of various cotton genotypes to saline conditions (200 mM NaCl), RAPD profile variations were quantified using GTS percentages. The highest GTS values were observed in sensitive genotypes, while the lowest were in salt-resistant varieties, indicating a direct relationship between genetic stability and salt tolerance. Such findings underscore the usefulness of RAPD analysis in identifying DNA sequences linked to salt stress and in the early detection of salt-resistant genotypes, particularly in cotton plants [41].

Wheat quality and production are also severely impacted by drought, with losses comparable to those caused by other climatic factors, a situation exacerbated by global climate change [42,43]. Drought-induced gene expression plays a crucial role in developing drought resistance [44]. In wheat, RAPD analysis using specific primers identified genetic variations associated with drought resistance. For example, a RAPD primer amplified a specific DNA band present in drought-resistant but absent in sensitive varieties, offering insights into the genetic basis of drought resistance in wheat hybrids [45]. Rashed et al. found RAPD markers that validated the method's reliability for identifying drought-resistant wheat genotypes [46].

In horticultural plants like tomatoes, productivity decreases under high temperatures. Traits related to yield are often quantitatively inherited and heavily influenced by environmental changes, complicating the assessment of heat resistance. A study utilizing RAPD markers linked to heat-resistant genes in tomatoes identified specific markers in recombinant inbred lines developed from a cross between heat-sensitive and heat-resistant parents. Several RAPD markers were specifically associated with heat tolerance traits, enhancing the selection of molecular markers for heat tolerance in breeding programs [47]. Further applications of DNA markers in horticultural crops focus on abiotic stress, as detailed in Table 1.

3.2 Enhancing Hybrid Stress Tolerance through Marker-Assisted Selection

Marker-assisted selection (MAS) is an effective method for improving abiotic stress resistance in crops. SSR (Simple Sequence Repeat) markers are frequently used to pinpoint stress-resilient genes. For instance, in wheat, the combination of SSR analysis with bulked segregant analysis has proven crucial for detecting markers related to agronomic characteristics such as grain filling and heat tolerance [48]. Three particular markers, Xgwm132, Xgwm617, and Xgwm577, have been associated with increased grain filling rates under heat stress conditions, contributing to the breeding of heat-tolerant wheat varieties.

Furthermore, SSR markers have been instrumental in identifying drought-resistant hybrids in tetraploid cotton. The use of SSR primers has allowed for the observation of allelic polymorphism, which, when correlated with agricultural characteristics, has yielded notable

findings in hybrids like "Varamin" and "Sayar 314." The "Tabladila" hybrid displayed significant polymorphism with EST-SSR markers, while the drought-resistant "Nazily" hybrid exhibited a 53 percent rate of polymorphism, showcasing the diversity and potential for enhanced drought tolerance through selective breeding [49,50].

3.3 Exploring Genetic Variability in Response to Heat and Frost Stress

Screening for heat-resistant plant varieties in the field is less effective due to unpredictable weather conditions that can compromise the repeatability and accuracy of trials. Additionally, consistently high temperatures in cultivation areas cannot be assured. Therefore, it is crucial to genetically evaluate quantitative traits for adaptive responses. Molecular analysis facilitates the identification of specific genotypes for breeding programs focused on enhancing yield stability and sustainability in harsh stress conditions [48].

Heat resistance is controlled by multiple genetic factors across various tissues and developmental stages. Sequence-Related Amplified Polymorphism (SRAP) markers, which are PCR-based, efficiently extract DNA fragments during a single PCR procedure. These markers are adept at amplifying various polymorphic and reproducible alleles and loci, making them excellent tools for DNA fingerprinting, evaluating genetic diversity, and mapping genes. Nevertheless, their random distribution throughout the plant genome can sometimes restrict their utility. Additionally, another PCR-based marker technique, Target Region Amplified Polymorphism (TRAP), employs two 18-mer DNA primers—one fixed from an expressed sequence (EST) and the other designed with a GC- or AT-rich core to specifically amplify regions within exons or introns [51,52,53,54].

These markers have been applied to wheat genotypes to assess genetic diversity under heat stress. A genetic analysis using SRAP and TRAP markers identified significant genetic diversity in agronomic traits under heat conditions. Although field performance data based on agronomic traits involves complex genetic interactions, marker-assisted data from SRAP and TRAP analyses provided valuable insights into genetic diversity in a more unbiased manner compared to traditional morphological evaluations [55].

Table 1. Use of dna markers in horticultural crops for abiotic stress management

CROP	DNA Marker	Abiotic Stress	Objective	Reference
<i>Petunia x atkinsiana</i>	Random Amplified Polymorphic DNA	Salinity	- Determining genetic diversity in mutant clones - Screening for mutants associated with salt resistance	[56]
D. Don cv. Prism Red. Tall fescue (<i>Festuca arundinacea</i>)	ISSR (Inter-Simple Sequence Repeat) and SCAR (Sequence Characterized Amplified Region)	Summer stress	Creating sequence-related markers to identify plants resistant to summer stress.	[57]
Perennial grass (<i>Miscanthus sinensis</i>)	SSR	Drought	To develop SSR markers associated with drought resistance through transcriptome sequencing.	[58]
Salvia (<i>Salvia miltiorrhiza</i>)	AFLP	Drought	- To separate drought-related genes in sterile male and fertile near-isogenic lines of S. Miltiorrhiza. - To assess changes in plant fertility during periods of drought stress.	[59]

Conversely, frost has a significant effect on the yield and survival of pea crops. A field study examining 672 varied pea genotypes across three different locations utilized trait-based marker association to assess frost resistance with 267 SSR markers. This analysis identified 16 genotypes as highly tolerant to winter conditions, with survival noted at all test sites. Population analysis showed a structured population divided into two subpopulations, featuring various combinations within the 672 genotypes. The study highlighted seven SSR markers that consistently correlated with frost resistance in at least two distinct environmental settings and under two different statistical models. Notably, one specific marker, EST1109 located on LG VI, is thought to be associated with a gene that influences glycoprotein metabolism responsive to cold stress, indicating a potential pathway for improving frost tolerance in pea crops. These results underscore the value of employing winter-hardy germplasms and cold-tolerance-linked markers in marker-assisted breeding programs to develop frost-resistant pea varieties [60].

4. MAPPING OF QTL GENES ASSOCIATED WITH ABIOTIC STRESS IN CROPS

4.1 Saltol

Saltol, a significant salt-resistance quantitative trait locus (QTL), is located on chromosome 1 of the rice genome. This QTL encompasses a gene vital for decreasing sodium (Na⁺) intake while increasing potassium (K⁺) uptake, resulting in a phenotype with a low sodium ratio under high salinity conditions. Simple Sequence Repeat (SSR) markers, known for their accuracy and reliability, are used to identify genetic variations in various crops, including rice. These markers were instrumental in a study examining genetic diversity among rice genotypes on chromosome 1, where Saltol is found. Utilizing "Pokkali," a key source for Saltol expression, as a benchmark, SSR markers pinpointed distinct salt-resistant haplotypes. The research included eight-generation inbred hybrids (Pokkali x IR29), employing 33 SSR markers, particularly RM8094, which was essential in highlighting genetic distinctions. Cluster analysis sorted the genotypes into three categories based on their salt tolerance, with the most tolerant varieties forming a separate group from those moderately tolerant and sensitive. Through marker-assisted breeding, the Saltol gene was transferred to a popularly grown rice variety, with SSR markers

RM493 and RM3411b used effectively to track the gene's integration. This transfer from the donor parent, FL478, successfully introduced salt tolerance into the BT7 progeny genome [61,62,63].

4.2 Dehydrin

Dehydration stress triggers the production of hydrophilic proteins, including dehydrins, which play a crucial role in enhancing a plant's drought, frost, and salt tolerance. These proteins are synthesized in response to severe dehydration conditions and the presence of abscisic acid. In barley, the expression of YnSKm-type dehydrins is particularly notable, with the species expressing ten out of thirteen dehydrin genes, similar to wheat. These proteins support the protective function during cellular dehydration by maintaining enzyme activity and efficiency under limited water availability [64]. Research in barley has shown that two dehydrin-related genes, regulated by the Hv-WRKY38 gene, significantly enhance drought and salt tolerance. These genes are located near the QTL regions associated with these stress responses [65,66]. Additionally, other genes like aquaporin and CBF also contribute to osmotic stress tolerance, with the CBF gene particularly active in salt and drought stress signaling pathways [67,68]. In *Citrullus colocynthis*, a study utilizing ISSR markers identified drought-tolerant genes in individuals from diverse locations, with specific primers targeting genes like UB, PEPKS, Dehydrin, ACT, and P5CS, confirming their role in drought resistance [69].

5. QTL MAPPING VIA DNA MARKERS

Drought represents a critical challenge to wheat production in various global regions. Utilizing DNA markers linked to specific Quantitative Trait Loci (QTLs) for drought resistance can greatly improve the drought tolerance of wheat hybrids. Research aimed at identifying QTLs associated with genes affecting grain yield under drought conditions has provided essential genetic information. STS markers have been used to map QTLs in wheat lines that show resistance to drought. A significant QTL affecting grain yield was identified on chromosome 4AL, close to its proximal region. This QTL is associated with important characteristics such as grain filling rate, spike density, grain yield, drought sensitivity index, and overall biomass production. Such stress-related QTLs have been extensively reported in wheat and other agricultural crops, as detailed in various studies, as documented in Tables 2 and 3.

Table 2. Applications of dna markers for qtl mapping in agronomic crops

Crop	Botanical name	DNA Marker	No. of QTLS	NO. of Chromosomes with QTLs LOCI	Objective	Reference
Cotton	<i>Gossypim hirsutum</i>	Simple sequence repeats (SSR);	14	11	-Identification of salt resistance traits - Evaluation of mapping robustness for QTL detection _ - Identification of traits resistant to salt - Assessment of the robustness of mapping for detecting QTLs	[70]
Maize	<i>Zea mays</i>	Single strand conformation polymorphic (SSCP) Single nucleotide polymorphism (SNP)	29	1,3,5	- Development of inbred lines with drought resistance - Detection of QTLs linked to drought resistance Assessing salt resistance in seedlings through the use of unconditional and conditional QTLs.	[71]
Sorghum	<i>Sorghum bicolor</i>	Restriction fragment length polymorphism (RFLP)	7	1 and 2	Detection of drought resistance and lodging resistance prior to flowering.	[72]
Barley	<i>Hordeum vulgare</i>	Single nucleotide polymorphism (SNP)	2 (salt tolerance indices)	1,2	- To estimate the genetic differences in Asian barley for salt resistance. - To identify and screen salt resistance traits in accessions.	[73]

Table 3. Utilization of dna markers for qtl mapping in wheat (*Triticum aestivum*) crops

Dna Markers	No. of QTLs Related to tolerance	Chromosome No. with QTL LOCI	Research objective	References
Simple sequence repeats (SSR) and amplified fragment length polymorphism (AFLP)	3	1,5,7	- To categorize and map QTLs associated with heat resistance. - To identify DNA markers that are linked to QTLs.	[74]
Simple sequence repeats (SSR), diversity array technology (DarT), gene-based marker for Vrn-A1	1	5A	- Understanding the genetic structure of drought tolerance during the reproductive phase. - Developing a morphological approach to enhance drought tolerance by targeting the premature microspore stage of pollen development, thereby reducing stress during flowering.	[75]
Simple sequence repeats (SSR)	8	2A	- To identify the associations between SSR markers and drought resistance traits on chromosome 2A	[76]

Table 4. Utilization of dna markers for qtl mapping in rice (*Oryza sativa*)

DNA Markers	No. OF QTLs related TO tolerance	No. OF chromosomes with QTLs LOCI	Research Objectives	References
Simple sequence repeats (SSR)	47	1–4 and 6–12	- Identifying reliable QTLs for drought tolerance and yield performance under stress conditions. - Implementing marker-assisted selection in rainfed regions	[77]
Single feature polymorphism, simple sequence repeats (SSR)	2	4 and 10	- To identify potential QTLs linked to high-temperature resistance. - Mapping genes associated with heat tolerance.	[78]
Simple sequence repeats (SSR)	1	3	- Identification of QTLs associated with heat tolerance - Detection of markers for use in marker-assisted breeding	[79]

Rice exhibits considerable genetic diversity attributed to its progenitor species and widespread distribution across vast hectares. However, numerous stress factors significantly diminish yield and overall production. Abiotic stresses alone can lead to up to 50% losses in yield. Among these stressors, salinity poses a substantial biophysical challenge to rice production in diverse regions [77]. Resistance to salinity stress is intricately governed by genetic and physiological factors. Multiple stress-related Quantitative Trait Loci (QTLs) have been pinpointed in rice, as summarized in Table 4.

Detecting quantitative trait loci (QTLs) for salinity resistance through closely linked DNA markers offers a promising alternative to traditional breeding methods, which heavily rely on morphological analysis. In rice, researchers have identified twenty QTLs distributed across chromosomes 1, 2, 4, 6, 8, 9, and 12 [80]. Notably, distinct QTLs like qSESF12.1 and qSESI12.1 stand out, holding potential for precise mapping of loci and identifying closely linked markers crucial for enhancing salt resistance.

The cultivation of *Pisum sativum*, commonly known as pea, faces challenges posed by drought stress in various climatic conditions, yet research on drought tolerance and associated genetic resources in pea remains limited. Nevertheless, a study has identified genomic regions linked to drought tolerance, leveraging indicators of drought stress and relative soil and leaf water content to assess recombinant inbred pea lines [81]. Ten quantitative trait loci, independently explaining between 9 to 33 percent of the observed morphological differences, were identified. These findings, alongside the discovery of reproducible molecular markers associated with these QTLs, hold promise for selecting individuals with desired QTLs in pea breeding programs focused on drought tolerance.

Furthermore, enhancing cold tolerance in cold-season pea varieties presents a significant agricultural challenge. Breeding efforts must consider not only freezing resistance but also seed yield and quality. Recent research targeted the genetic determinants of cold and frost resistance, utilizing a newly identified cold-tolerant source [82]. Populations of recombinant inbred lines were evaluated across various climatic conditions, resulting in the construction of a comprehensive genetic map comprising 679

molecular markers spread across seven linkage groups. Through this analysis, researchers identified 161 QTLs, explaining between 9 to 71 percent of the observed morphological differences across measured traits. These findings suggest the potential for targeted breeding to improve frost resistance and enhance seed quality and productivity in cold-season pea varieties.

6. MARKER-ASSISTED SELECTION USING SNP MARKERS

Association mapping offers the advantage of assessing a greater number of alleles in large populations compared to linkage analysis, thereby revealing evolutionary recombination and mutational characteristics across various lines. This method specifically targets genes associated with phenotypic diversity, making it instrumental in identifying genes responsible for quantitative variation, such as drought resistance [83,84]. However, association mapping is less effective in identifying rare alleles within plant populations and typically involves higher costs due to the need for genotyping and sequencing numerous lines [84]. Research affirms the reliability of SNP chips in delivering high-quality data, precise genotyping information, and comprehensive genetic insights. Moreover, SNPs may offer advantages over traditional DNA markers like SSRs for linkage analysis, as they are abundant and exhibit genome-wide distribution characteristics, facilitating the identification of functional genes and genetic variations [85].

For instance, Hao et al. [86] identified 27 SNPs associated with genetic variations in drought tolerance among maize lines through the detection of functional genetic differences.

7. CONCLUSIONS

Effective management of abiotic stress in agricultural crops is crucial for improving both quality and yield. The field of molecular genetics has transformed this area by introducing numerous DNA markers, which aid in exploring genetic modifications, genotypic resistance, stress-tolerant lines, and genetic information related to abiotic stresses. Initially, molecular marker technologies provided basic insights into stress resistance, but recent advancements now allow for the identification of specific genes or gene clusters that confer abiotic stress tolerance. The integration of DNA markers with QTL

mapping highlights a clear pattern of stress tolerance genes at specific chromosomal locations. Nevertheless, the changing climate demands ongoing enhancements in DNA marker technology to facilitate even more precise analysis of stress tolerance mechanisms. These continuous advancements are essential for maintaining agricultural resilience amid evolving environmental challenges.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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