



Improve Abiotic Stress Tolerance in Crops by Molecular Markers: A Review

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Authors' contributions

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

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ABSTRACT

Abiotic stressors include things like heat, cold, drought, and salt that plants have to withstand. These are the main and ongoing agricultural challenges that lower crop production. In the end, modifications to components, nutrient control, and biosynthetic ability hinder or even stop plant development. Stress is an environmental factor that, in terms of genetics, prevents full genetic expression. These markers are divided into many types based on their intended use: Simple sequence repeat (SSR) markers are a great tool for determining stress tolerance, but RAPD (random amplified polymorphic DNA) markers are useful for identifying and screening hybrids based on salinity and drought stress resistance. These markers are also necessary for the QTL (Quantitative Trait Loci) mapping of genes linked to stress. These ancestor genes control the production of dehydrins and saltol in response to dryness and salinity, respectively. DNA markers enable the use of innovative approaches and marker modification in marker-assisted breeding to increase tolerance to abiotic stress.

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1. INTRODUCTION

A variety of adverse environmental circumstances are faced by plants throughout their development cycles. These circumstances include biotic stressors like pathogen infection and herbivore assault, as well as abiotic stressors like heat, cold, drought, low nutrient availability, elevated salt levels, and dangerous metals and metalloids (including aluminum, cadmium, and arsenic) in the soil. Aridity, salt, and temperature (hot or frost) are the main and most common climatic conditions that lower agricultural crop yields. Such effects affect the geographical distribution of plants in nature and are a concerning indicator for food security [1]. Abiotic stress is mostly caused by climate change, which is defined as long-term changes to weather patterns. Plants' constitutive basal defense mechanisms start working as soon as they detect stress. Different signaling pathways are triggered differently based on the kind of stress. Kinase enzymes and phytohormones control common defense mechanisms. For instance, ethylene, salicylic acid, jasmonic acid, and abscisic acid all activate ion channels, as does the production of reactive oxygen species (ROS). These elements build up and alter the genetic and metabolic machinery. Plant tolerance is built on defense mechanisms that lessen the biological damage brought on by stress [2].

Abiotic stressors primarily affect crop yield and productivity because of unfavorable changes in the surrounding environment [3]. In biological terminology, an external element that negatively affects the development or health of a plant is often referred to as a stress. Stresses are acknowledged as significant deviations from the typical life cycles of plants. Stress-affected plants exhibit three main reaction phases: the alarm phase, which occurs when stress is initially initiated, the resistance phase, which occurs when defensive mechanisms are activated, and the exhaustion phase, which occurs when stress causes loss [4]. Numerous plants exhibit the effects of stress on their systems, which affects the plants' ability to develop. The main climatic element limiting production and development is salinity. It modifies ion homeostasis regulation and encourages water uptake and retention, which affect biological properties [5]. Furthermore, persistent water shortage brought on by drought stress would hinder plant development, growth, and survival. Although low

soil water content is often linked to drought, excessive evapotranspiration may significantly aggravate the condition [6]. A greater temperature in the surrounding environment combined with humid circumstances and high air temperatures might cause this kind of stress. An imbalance between water loss via evapotranspiration flow and soil water absorption is the cause of this stress [7]. In terms of genetics, stress is an environmental factor that prevents a plant from expressing its full genetic potential. Abiotic stress is defined as any stress that affects organisms negatively in their environment and is not brought on by interactions with other species [8]. Abiotic stressors in the agricultural and environmental sectors pose serious risks, which are now being exacerbated by global warming and industrialization [9]. A plant's genetic structure may be enhanced by the use of DNA markers in a variety of ways, including the construction of high-resolution genetic linkage groups, the identification of genetically related parents, and the evaluation of genetic diversity [10]. Using hybridization procedures, DNA markers are classified as restriction fragment length polymorphism, or RFLP, markers. These inheritance hierarchies are recordable and verifiable. In order to create linkage maps (genetic structure), markers are often assembled in a way that represents genetic diversity and linkage assemblies based on recombination that is evaluated using hybrid plant genotypes [11]. This review's goal is to outline the effects of abiotic stress on agricultural crops as well as the DNA markers that are linked to them for gene mapping, genetic control, and the screening of stress-resistant characteristics [12].

2. AGRICULTURAL CROPS' RESPONSE TO ABIOTIC STRESS

The two main abiotic factors that have a negative impact on a crop's production and output are heat and drought. The advantages of agriculture and farm revenue are decreased by such abiotic pressures. Maize yields are reduced to 40% of the previous yield and wheat yields are reduced to 21% of the previous yield when water is reduced by up to 40% [13]. Drought stress is now affecting cowpea, an important crop in Africa, cutting production by 34% to 68%. Abiotic stressors that encourage the overproduction of reactive oxygen species (ROS) exist. ROS are harmful and reactive substances that harm or

destroy proteins, lipids, carbohydrates, and nucleic acids [14]. Plant development is negatively impacted by this oxidative stress. Moreover, stomatal conductance and transpiration in plant leaves may be harmed by heat stress and water shortage. The output of agriculture is impacted by abiotic stressors. Ninety-one percent of the world's agricultural land is stressed, and this stress accounts for half of the loss in agricultural productivity [15]. Climate change may intensify the negative effects of abiotic stress and hasten their onset. This effect may be lessened by improving agronomic management and encouraging stress-resistant genotypes in breeding programs [16]. Plant biochemical and physiological systems are impacted by abiotic stress. Increased resistance to abiotic stressors may be achieved by improvements in photosynthetic activity and light utilization efficiency. In addition, a number of antioxidants are triggered, and different enzymes may produce stress-based metabolites to aid in preventing cellular damage. To increase stress tolerance in plants, however, important adaptation methods must be developed. Climate and heat stress are intimately related. Crop development and growth may be impacted by air and soil temperature increases that are over tolerance thresholds, even for brief periods of time. Global warming is a significant climatic concern that might negatively impact plant growth and productivity, particularly in the case of crops. Temperature increases will significantly affect the susceptibility of crops [17]. Therefore, studying heat stress is crucial to comprehending how plants react to and tolerate such stressors. The cultivation and propagation of offspring (lines) that are more heat tolerant will ultimately be required to keep agriculture going [18]. Crop yields, photosynthetic activity, and seed germination are all negatively impacted by heat stress. Increased temperatures may also cause the swelling of the pollen grains to halt, resulting in the discharge of weaker pollen. Additionally, anthers may dehisce. For plant crops to adapt to heat stress appropriately, physiological or molecular change may be required. Drought stress causes a variety of physiological changes, including as a decrease in photosynthetic activity, changes in cell wall flexibility, and stomata closure. It is noteworthy that salt and drought have similar effects on plant physiology and eventually overlap in tolerance systems. Drought alters the concentration of ions in plant tissues, which affects the nutritional quality of crops. The amount of accessible soil nutrients that are dissolved onto root surfaces reduces

when moisture levels rise [19]. Variations in biosynthetic capability and alterations in nutrient composition and management are the primary causes of plant growth reduction or cessation. Agricultural sectors need protective mechanisms to ensure plant survival amid abiotic stressors in order to sustain crop growth and productivity levels. Molecular genetics may be used to investigate and comprehend abiotic stress defenses [20].

These elements may affect plants alone or in combination, and they may eventually change the metabolic processes to lower the levels of development, growth, and production. Increased stress levels may become unbearable and cause the death of the plant. There is no such thing as stress freedom [21]. Consequently, in order to live in a stressful environment, plants display metabolic reactions and particular chemicals. Changes in the composition and environmental factors of soil and plant habitats brought on by abiotic stress may result in lower global yields of main agricultural crops. In non-stressed areas, agricultural lands now account for barely 10% of crop output. 90% of the population is still subject to environmental stressors [22]. However, there is always a need for further efforts to increase stress tolerance via the adoption of different strategies, the promotion of resource-saving technologies, and genetically enhancing plant defenses [23].

3. USE OF DNA MARKERS IN ABIOTIC STRESS TOLERANCE APPLICATIONS

3.1 Using Rapid Markers to Analyze Drought and Salinity Stresses

PCR-based markers are known as random amplified polymorphic DNAs, or RAMDs. For RAPD analysis, preliminary sequencing data for the samples are not required. With little resources, a large number of loci from several people may be evaluated for screening purposes. Because of their simple experimental procedures and superior genetic screening of intra- and interspecific hybrids, RAPDs are extensively used. These markers have been helpful in locating genes in various crops that are resistant to salt stress. Plants have a variety of defense and tolerance mechanisms against salt stress. These reactions are genetically controlled [24]. Enhancing agricultural crops' ability to withstand salt is also essential, particularly in places affected by salinity. Salt-resistant genotypes may be categorized and identified

with the use of DNA markers. A fundamental method for identifying genes resistant to salt is to use PCR for RAPD amplification of certain DNA sequences. Wheat planted in a saline-affected area was utilized in a research to assess the genetic diversity of genotypes resistant to salt. The genotypes that were salt-sensitive and salt-resistant were effectively distinguished by these DNA markers. Genetic variation was demonstrated by polymorphic primer matching between genotypes that were tolerant and sensitive [25]. Stress due to salt may produce structural breaks and rearrangements in DNA, among other alterations. The genetic instability of cotton seedlings treated with NaCl and saline-infected is detectable by RAPD markers. When compared to control plant DNA, RAPD markers revealed the existence of new bands, weak or high band intensity, and missing DNA bands on agarose gels [26]. Prior research verified that using RAPD to study toxicological stress was effective. The OPA08 RAPD primer was useful and had a good chance of detecting DNA changes caused by saline stress, or NaCl. Regrettably, there are still a number of problems with using the RAPD approach. These problems include DNA contamination, identical band appearance, and rivalry in DNA amplification, among other amplification and electrophoretic separation-related concerns. It takes a lot of work and effort to categorize quality criteria, stress resistance, and crop yield via field assessment [27]. DNA-level molecular differences in plants grown using tissue culture were effectively characterized. Using DNA marker technology, plants under harsh circumstances have been genetically screened. RAPD uses very little DNA and is quick and easy to use. The method can handle a large number of genomic samples in vitro fast and is sensitive to genetic variations. For instance, RAPDs identified polymorphic bands in maize that corresponded to genes resistant to salt. In order to produce offspring that are resistant to salt, a number of primers were connected to sequences that showed signs of salt resistance. By directly modifying the genotypes of these progenies, salt resistance may be produced and further tested for using marker-assisted selection. A qualitative indicator of changes in RAPD profiles is called GTS. NIAB 78, abbreviated as N78, Deltapine 50, abbreviated as DP50, Aleppo 118, abbreviated as A118, Deir-Ezzor 22, abbreviated as DE22, and *Gossypium hirsutum* L [28]. (cotton) genotypes were studied to determine how different genotypes farmed in non-saline (control) and salt-affected fields (200 mM NaCl)

responded to different treatment conditions. GTS computed variations in the RAPID profile as a percentage. Two sensitive genotypes, A118 and DP50, showed maximum values, with GTS of 58.2% and 79.1%, respectively. On the other hand, two salt-resistant types, DE22 and N78, which showed 36.7% and 26.4% GTS, respectively, showed minimal values [29]. DNA alterations may potentially be used in plant breeding techniques to increase germplasm tolerance to saline stress. Furthermore, the genotypes DE22 and NB78, which are salt-resistant and have the lowest GTS values, exhibited the largest polymorphic expression of RAPDs. These findings suggest that RAPD analysis may be used to find DNA sequences associated with salt (NaCl) stress [30].

As a result, these DNA markers may lead to advances in the first identification of cotton plant genotypes resistant to salt. The impact of drought on wheat output and quality varies greatly across different locales. Losses resulting from other climatic variables are equivalent to losses caused by drought. The effects of global climate change exacerbate this disease. Stress resistance is directly influenced by the expression of many genes that are triggered by drought. In early DNA fingerprinting for RAPD analysis, utilizing drought-resistant-related DNA primers, the genetic diversity of wheat cultivars was examined. A 717 base pair RAPD band was also present in the B genome that was extracted from the drought-resistant "Barakatli-95" strain. Finding two negative and four positive RAPD markers validated the method's applicability in determining drought-resistant wheat genotypes. Tomato harvests from horticulture plants produce less as temps rise. It is believed that traits associated to yield are quantitatively inherited and heavily impacted by environmental changes. Because of their many different characteristics, crops are challenging to assess, especially in terms of heat resistance [31]. Heat resistance-related RAPD markers showed a positive gene impact as a consequence of the CL5915 gene's contribution [32].

3.2 Hybrids' Ability to Tolerate Stress

An effective method for enhancing plant abiotic tolerance is marker-assisted selection. The methods for selecting stress-tolerant hybrids using criteria aided by DNA markers. Typically, SSR markers are used to identify genes that are resistant to stress [33]. Bulk segregant analysis and SSR analysis are helpful in

identifying molecular markers linked to agronomic characteristics in wheat crops, such as heat tolerance and grain fullness. SSR analysis revealed three markers (Xgwm132, Xgwm617, and Xgwm577). In heated conditions, the rate at which grain fills is correlated with these indicators. The creation of a cultivar with enhanced resilience to heat stress was aided by these methods. Additionally, SSR markers for rice representing chromosomes 3 and 4, RM3586 and RM3735, respectively, demonstrated an effective association with heat resistance with 3 and 17 percent total genetic variation. The detection of tetraploid cotton hybrids that are resistant to drought was aided using SSR molecular markers. Significant findings were found in the "Varamin" and "Sayar 314" hybrids based on allele polymorphism utilizing SSR primers and agricultural traits; however, the "Tabladila" hybrid displayed highly polymorphic data using EST-SSR markers [34]. 53 percent polymorphism was found in the drought-tolerant hybrid "Nazily".

3.3 Recognizing Genetic Variability Despite Heat and Frost Stress

It is not advised to do morphological screening, which searches for genotypes or varieties resistant to heat in field conditions, since erratic weather patterns may compromise the precision and repeatability of research findings. Moreover, growth zones cannot be guaranteed to maintain a constant high temperature (heat stress). For adaptive responses, a genetic assessment of quantitative attributes is required. Molecular analysis enables the use of certain genotypes in breeding strategies to increase crop sustainability and production stability under severe stress [35]. Drought-resistant tetraploid cotton hybrids were simpler to detect thanks to SSR genetic markers. Based on allelic polymorphism using SSR primers and agricultural attributes, significant results were reported in the "Varamin" and "Sayar 314" hybrids; in contrast, the "Tabladila" hybrid showed highly polymorphic data using EST-SSR markers. Thirty-three percent of the hybrid "Nazily" was drought-tolerant and polymorphic. Various features of heat resistance are regulated by various gene sets in different tissues or at different phases of development, making heat resistance a complicated genetic characteristic. For DNA fingerprinting, genetic diversity assessment, and gene mapping, these markers are commonly utilized because of their multiallelic and multilocus features. SRAP

markers should not be employed in a random way throughout the plant genome [36]. These markers were used to genotypes of wheat that were resistant to heat. Through the use of SRAP and TRAP markers in a genomic investigation, the genetic diversity of genotypes of durum wheat was evaluated. Under heat stress, genetic variation was discovered in agronomic characteristics. Field performance data based on agronomic traits took the form of complex, multi-genetic structures. Still, with the help of the marker, the procedure of testing offspring for characteristics linked to stress tolerance utilizing DNA markers. Heat resistance is a multifaceted characteristic that is governed by several genes, each of which regulates a distinct aspect of resistance in various tissues or developmental stages. Sequence-related amplified polymorphism markers (SRAP), PCR-type molecular markers, may be used to recover DNA fragments in a single PCR run. These DNA markers amplify a number of polymorphic and repetitive alleles and sites [37]. It is not advisable to use SRAP markers randomly throughout the plant genome. Another useful and active PCR type marker is target region amplified polymorphism (TRAP), which needs two 18-mer DNA primers to work. While the first primer is "fixed" from the EST (expressed sequence tag), the second primer is linked with either a GC- or AT-abundant core to match with an exon or an intron. These markers were used to genotypes of wheat that were resistant to heat [38]. Through the use of SRAP and TRAP markers in a genomic investigation, the genetic diversity of genotypes of durum wheat was evaluated. Under heat stress, genetic variation was discovered in agronomic characteristics. Field performance data based on agronomic characteristics took the form of complex, multi-genetic structures. Marker-assisted data from SRAP and TRAP studies showed useful for the objective detection of genetic diversity, in contrast to traditional morphological evaluation. Frost, on the other hand, has a significant role on the mortality and reduced yield of pea crops. In order to boost the frost resistance of peas, a field research with 672 distinct pea genotypes was conducted in three distinct locations. Frost resistance was assessed using trait-based marker association and 267 SSR molecular markers. Because 16 genotypes were able to thrive in every experimental field utilized in the study, it was determined that these genotypes were the most winter-tolerant. Different combinations of genotypes from the 672 genotypes were seen, as well as two subpopulations within the organized population.

Seven molecular SSRs were shown to consistently indicate a connection with frost resistance in at least two different climatic circumstances using two statistical models and an association technique [39]. It was proposed that EST1109, one marker on LG VI, is linked to a gene that is involved in the metabolism of glycoproteins in response to germplasms. When it comes to marker-assisted breeding of cold-resistant pea cultivars, linked markers that are resistant to cold are crucial [40].

4. GENOME-BASED QTL MAPPING

Drought is one of the primary abiotic factors that might negatively impact wheat production in different parts of the world. It's possible that hybrids' ability to tolerate drought will be greatly enhanced by the presence of DNA markers linked to drought resistance QTLs [41]. The proximal region of chromosome 4AL is home to the grain yield QTL [42]. Grain filling rate, biomass production, spike density, grain yield, and drought sensitivity index are all connected with this location. Many pressures reduce production and productivity [43]. Components of resistance to salt stress that are complicated include both physiological and genetic variables. Drought restricts *Pisum sativum*, or pea, production output and stability in most areas. Research on the genetic resources associated with drought resistance in peas is lacking. Genetic areas linked to drought tolerance were found in a study. Considerations such as freezing resistance, seed yield, and seed quality must all be made when crossing cold season cultivars. One study looked at the genetics of yield and developmental features, as well as the genetics of cold/frost resistance. Using a recently discovered source of cold tolerance, six different climate scenarios were used to assess recombinant inbred line populations. A 947.1 cM genetic map was created, which includes 679 molecular markers spread over seven linkage groups [44].

5. USING SNP MARKERS TO AID WITH MARKER SELECTION

More alleles in big populations are evaluated using association mapping than by linkage analysis. The advantage of mapping is that it may be used to find many lines with mutational traits and evolutionary recombination [45]. This approach specifically finds the genes linked to phenotypic diversity. As of right now, identifying the genes causing quantitative variation in

complex traits like drought resistance requires the use of gene mapping analysis [46]. In contrast, association mapping is not very effective in locating uncommon alleles in populations of plants. Furthermore, the need for plentiful line sequencing and genotyping drives up expenses [47]. It is more economical and time-efficient to use fixed multiplex SNP chips for genome-wide linking and association mapping. On the other hand, multi-allelic markers and capillary electrophoresis are needed for allele separation in linkage analysis [48]. Research establish that SNP chips provide high-quality data, accurate genotyping information, and comprehensive genetic data. Furthermore, SNPs could be a better option for linkage analysis than more conventional DNA markers like SSR [49]. SNPs are sophisticated molecular markers that are prevalent in relation to variation. SNPs are used to identify functional genes and genetic variations because of features of genome-wide assembly. Using functional genetic differences. 27 SNPs associated with genetic changes in maize lines linked to drought tolerance [50].

6. CONCLUSIONS

Managing abiotic stress is essential to improving agricultural quality and productivity. Numerous DNA markers that investigate genetic alteration, genotypic resistance, stress-tolerant lines, and genetic data linked to abiotic pressures are now widely available thanks to molecular genetics. The emergence of early molecular marker technologies made it possible for DNA markers to provide essential details on stress tolerance. However, the most sophisticated marker programmes on the market today are able to pinpoint the precise gene—or genes—that confer abiotic stress tolerance. QTL mapping in conjunction with DNA markers has the potential to identify a pattern of genes linked to stress tolerance at certain chromosomal regions. Stress tolerance may, however, be assessed even more extensively if environmental circumstances alter, which will need further advancements in DNA marker technology.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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