

# GENOME-WIDE ASSOCIATION STUDIES IDENTIFYING ADAPTIVE TRAITS IN CLIMATE-STRESSED CROP VARIETIES

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## ABSTRACT

**Background:** Climate change is expected to be a significant threat to agricultural productivity across the globe by creating more droughts, heat stress, and salinity stress, which will lead to reduced crop growth and yield stability. It is therefore crucial that adaptive genetic traits associated with climate resilience are identified to be used for creating stress tolerant varieties that continue to support food security amidst changes in the environment.

**Objective:** The current study was undertaken to resolve the use of genome wide association studies (GWAS) to uncover the genetic architecture of adaptation to climate stress for crop varieties.

**Methods:** A collection of different populations of crop germplasm were phenotyped under controlled drought and heat stress conditions. To identify significant marker-trait associations related to stress adaptation mechanisms, SNP genotyping was performed at high throughput, the population was structured and genome-wide association mapping was performed.

**Findings:** A number of important adaptive gene-loci associated with drought tolerance, resistance to heat and antioxidant regulation were identified. The Three-way interaction of the two Genus species and 13 biotic factors accounted for 19–28% of the phenotypic variance for adaptive traits explained by the major SNP markers. The stress-tolerant varieties maintained almost a third more chlorophyll than the sensitive varieties, with a relative water content that was 30% higher and increased biomass stability. The relationships among the physiological and yield-related traits were higher in the case of climate stress conditions.

**Conclusion:** GWAS helps generate genomic information that can identify adaptive traits, and it can be used to optimize marker assisted breeding to create crop varieties more tolerant to stress and with improved yield in times of climate change.

**KEYWORDS:** Genome-Wide Association Study, GWAS, Climate Stress, Drought Tolerance, Heat Stress, SNP Markers, Crop Adaptation, Climate-Resilient Crops, Plant Genomics.

## 1 INTRODUCTION

### 1.1 Climate Stress and Crop Productivity

Global climate change has become one of the major threats to agricultural productivity and food security on the global level. The impact of increasing temperatures, irregular rainfall, drought, salinity and extreme weather events on crop growth and stability of crop yield is significant [1]. Abiotic stresses caused by changes in climate have an adverse effect on physiological and biochemical processes in plants leading to decreased leaf photosynthesis, reduced nutrient uptake, oxidative damage and decrease in biomass in plants [2]. Water stress, as well as heat stress, are detrimental stressors especially because they shorten the availability of fresh water, change cellular metabolism and have a negative impact on reproductive development of crops [3]. Salinity stress also stresses crops further by causing ion toxicity and osmotic imbalance. The environmental issues faced are likely to affect sustainable agriculture, the global food production system, particularly in developing nations where crop production is largely reliant on climate sensitive farming methods [4].

### 1.2 Importance of Adaptive Traits

The adaptive traits of crops are physiological, biochemical and molecular features which helps them to survive and to continue production under stress situations. The mechanism of coping to stress includes osmotic adjustment, anti-oxidant defense, ion homeostasis, heat shock protein synthesis, and efficient regulation of water use [5]. Plants and crops with adaptive properties can continue to maintain their chlorophyll content, stability of the cells and productivity when environment conditions are challenging. Crops that display genetic diversity across their populations represent an additional source of alleles and phenotypes that can be identified with respect to a specific stress, and then used to build stress-resilient crops for breeding

programs [6]. Molecular adaptation through stress responsive genes and the signaling pathways are also crucial in drought, salinity and heat resistance in plants. Thus, knowledge of the genetic basis of adaptive traits will be crucial in the creation of new variety while sustaining crop production in the context of changing environmental conditions due to climate change [7].

### 1.3 Genome-Wide Association Studies (GWAS)

Genome-Wide Association Studies (GWAS) are high dimension genome-based analyses which allow the detection of association of genetic markers with complex diseases within different populations. Genotypes are measured by high-throughput Single Nucleotide polymorphisms (SNPs) and linkage disequilibrium is used to find loci in the genome associated with adaptive traits using GWAS [8]. The GWAS has increased mapping resolution over relation-based mapping and greater genetic diversity for higher resolution mapping of stress responsive loci. With the recent developments of new generation of sequencing techniques like Next Generation Sequencing (NGS), bioinformatics tools have enhanced the efficiency of conducting SNP marker analysis and Marker-trait association studies [9]. GWAS has proved to be a useful tool in the identification of drought, salinity, heat, and stress-tolerant major crop traits such as rice, wheat, and maize [10].

### 1.4 Aim and Objectives

The aim of this study is to identify adaptive genomic traits associated with climate stress tolerance in diverse crop varieties using genome-wide association studies. The objectives include evaluating crop varieties under climate stress conditions, identifying SNP loci associated with adaptive traits, analyzing genotype-phenotype relationships, and supporting climate-resilient crop breeding programs through molecular marker-assisted selection approaches.

## 2 BACKGROUND WORK

### 2.1 Climate Stress Responses in Crops

Significant impacts of climate stress including drought, heat and salinity on crop physiology and metabolic stability. During drought stress when water is limited, stomatal closure causes a lower water availability, which results in less photosynthesis and nutrient transport [11]. Excess heat causes protein instability, compromises of membrane stability and enzymes activity in plant and diminishes suitable growth and reproductive function [12]. These stresses also trigger excessive production of Reactive Oxygen Species (ROS), leading to oxidative damage to lipids, proteins, chloroplasts and nucleic acids [13]. Exposure to stress triggers adaptive responses such as osmotic adjustment, antioxidant defenses, and stress-sensitive signaling pathways in plants.

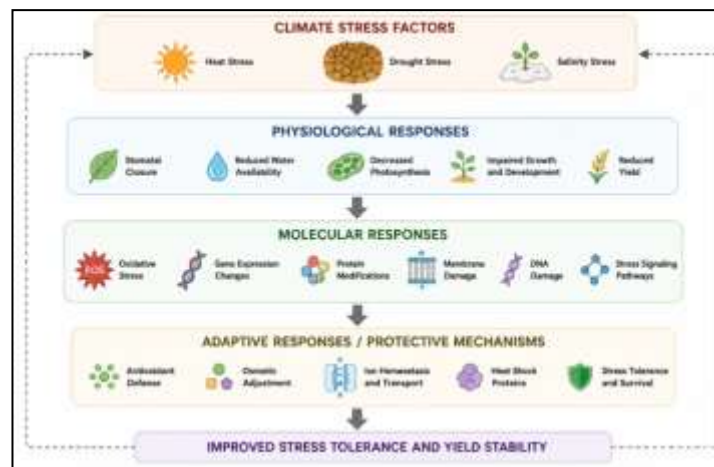


Figure 1. Physiological and Molecular Responses of Crops Under Climate Stress

The major physiological and molecular responses in crops under climate stress conditions e.g. drought, salt and heat stress are shown in Fig. 1. These stresses decrease water availability, photosynthesis, growth, yield, increase oxidative stress and cause cellular damage. Plants thus trigger up adaptive responses such as antioxidant defense, osmotic adjustment, ion homeostasis and signaling pathways that are activated in response to stress. When combined, these stress mitigating measures enhance stress tolerance, cell stability, and yield retention in unfavorable environments.

### 2.2 Genetic Basis of Climate Adaptation

Crop climate adaptation involves several climate stress-responsive genes and molecular processes that include water use efficiency, antioxidant defence, and ion homeostasis, and stress signalling [14]. The function of these genes under environmental stress is to regulate cell's homeostasis. Drought resistance and heat resilience boosts the chances of survival and yield stability for climatically challenged crops.

Table 1. Key Adaptive Traits in Climate-Stressed Crops

Adaptive Trait	Biological Function	Stress Response
Drought tolerance	Water conservation	Improved survival
Heat tolerance	Protein stability	Reduced damage
Antioxidant activity	ROS detoxification	Cellular protection

### 2.3 GWAS Approaches in Crop Genetics

To discover genomic regions associated with adaptive traits in crop populations, genome-wide association studies (GWAS) have become key tools. Refining the accuracy of marker-trait association mapping, through the use of high throughput SNP genotyping technology, linkage disequilibrium analysis and population structure assessment, has been achieved [15]. GWAS can identify those loci that respond to stress across a genetically diverse population and can provide markers for use in marker assisted breeding aimed at improving climate resilience [16].

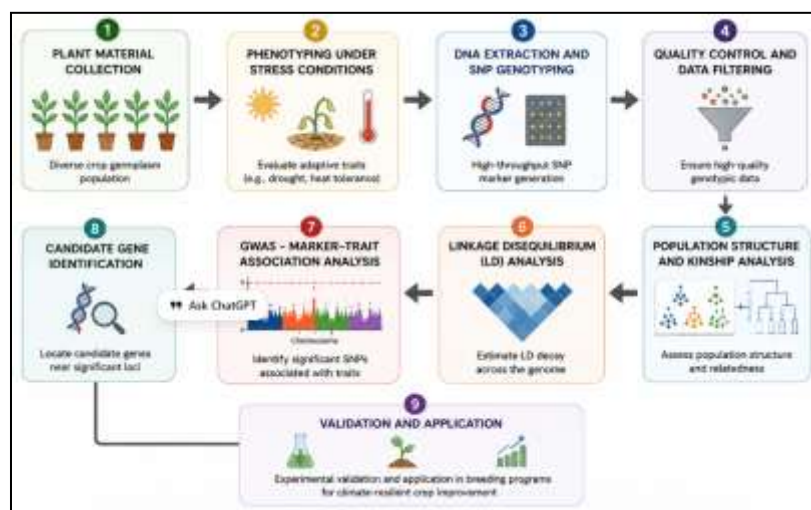


Figure 2. Workflow of Genome-Wide Association Studies for Adaptive Trait Identification

The whole flow of genome-wide association studies (GWAS) for predisposing prestigious plant traits under climate stress is presented in Fig. 2. It starts with collection of broad germplasm along with performance evaluation in stress conditions to evaluate phenotypes; then goes on to DNA isolation and SNP genotyping. Quality control, population structure analysis, linkage disequilibrium mapping then takes place to discover any significant marker-trait associations. Lastly, candidate genes are tested and integrated into breeding programs for the creation of climate-smart crops and stress-tolerant crops, while also creating greater yield stability.

### 2.4 Previous Research Studies

In the major crops, recent GWAS studies have successfully identified drought, heat resistance and salinity tolerant alleles. The results of these studies showed a strong stress resistance and stability in yield using marker-assisted selection (MAS) [17]. Nevertheless, there are still challenges in climate-resilient breeding programs due to interactions with the environment, complex inheritance and reactions caused by genotype.

Table 2. Previous GWAS Studies on Climate Stress Adaptation

Study	Trait Identified	Crop Species	Outcome
Zhao et al.	Drought tolerance loci	Rice	Improved yield stability
Kumar et al.	Heat resistance SNPs	Wheat	Enhanced stress adaptation
Li et al.	Salinity-associated loci	Maize	Improved tolerance

## 3 MATERIALS & METHODS

### 3.1 Experimental Design

The study was done through greenhouse and field-based drought and heat stress experiments to test adaptive traits to drought and heat stress in genetically diverse crops. Comparative analyses for stress-tolerant and stress-sensitive genotypes were conducted, aiming at finding phenotypic and molecular differences in climate resilient genotypes. Experiments were laid out in a Randomized Complete Block Design With 3 biological replications per genotype and each treatment [6]. Environmental stress treatments were applied at vegetative and reproductive growth phases to evaluate the environmental stress response and yield stability.

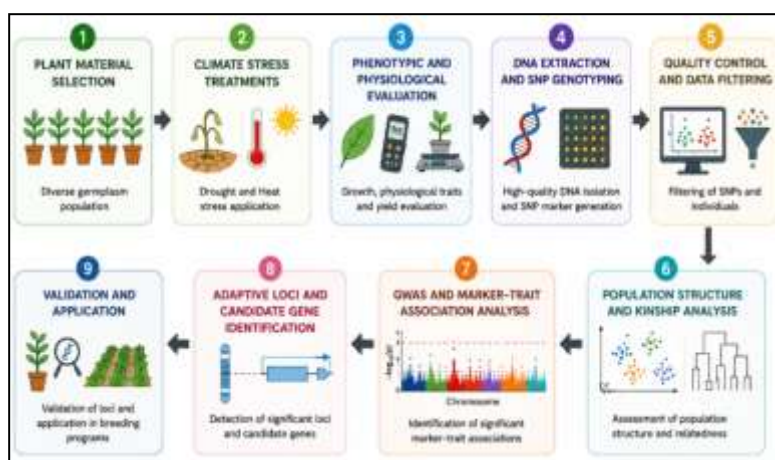


Figure 3. Experimental Workflow for GWAS-Based Adaptive Trait Identification

The GWAS-based Adaptive Trait identification of climate stressed crops is depicted in Figure 3, showing the experimental workflow. It starts by collecting the germplasm and subjecting them to stress in a greenhouse and field environment, then phenotyping for adaptive traits like biomass and chlorophyll and yield stability. For GWAS, genomic DNA is then extracted from the samples and they are genotyped for SNP. Important marker-trait associations and adaptive loci are discovered, confirmed, and implemented in breeding for climate-resilient cereal varieties to boost their resistance to stress and productivity.

### 3.2 Plant Material Selection

200 genetically diverse drought-tolerant (DT), heat-tolerant (HT) and stress-sensitive crop genotypes from international germplasm collections were selected. In order to identify the widest possible spectrum of allelic variation and facilitate genome-wide association mapping, diverse populations were selected. In order to increase genotype-phenotype association analysis, both RILs and natural populations were used.

### 3.3 Climate Stress Treatments

#### 3.3.1 Drought Stress

Soil moisture was kept at about 30% FC for 21 days creating drought stress treatments. Moisture sensors were deployed in the soil to measure and monitor soil water content continuously and ensure a uniform stress application to experimental units.

#### 3.3.2 Heat Stress

A heat stress treatment consisted of subjecting plants to a range of temperature between 35-42°C for 10 days during the day. Recovery analysis was carried out after exposure to stress to assess for resilience and survival capacity in changing environmental conditions.

### 3.4 Phenotypic and Physiological Analysis

Plant height, root length, shoot biomass and grain yield under stress conditions were some of the phenotypic measurements. Physiological studies were carried out through determination of chlorophyll content (SPAD - chlorophyll meter), photosynthetic efficiency and relative water content (RWC). Other symptom parameters such as the stress injury, survival rates and yield stability indices were also observed in order to evaluate the adaptive performance among genotypes [13].

### 3.5 Molecular and Genomic Analysis

#### 3.5.1-Extraction of DNA and determination of its SNP genotype.

To extract genomic DNA, all genotypes young leaf tissues were collected and genomic DNA extracted using the method of cetyltrimethylammonium bromide (CTAB). Quality and concentration of DNA was authenticated by agarose gel analysis and spectrophotometric analysis. Genome-wide SNP arrays and next generation sequencing platforms were used to perform high-throughput SNP genotyping.

#### 3.5.2 GWAS Analysis

Population structure and kinship analyses were carried out to help reduce false positive marker associations. To find out any significant SNP loci associated with adaptive traits, linkage disequilibrium (LD) mapping and genome-wide association analysis (GWAA) were performed with TASSEL and GAPIT software packages [17].

Table 3. Climate Stress Experimental Conditions

Parameter	Condition
Temperature	35–42°C
Soil moisture	30% field capacity

Photoperiod	16 h light / 8 h dark
Relative humidity	65%
Stress duration	10–21 days

### 3.6 Statistical Analysis

Analysis of variance (ANOVA), regression and correlation analysis were carried out to compare stress responses between the various genotypes using both phenotypic and genomic data. To identify associations between markers and traits that are robust, false discovery rate (FDR) and logarithm of odds (LOD) values were used as thresholds in GWAA.

## 4 RESULTS & DISCUSSION

The GWAS was able to successfully identify traits with global association which were important for climate stress in various crop populations. Significant genotype x drought x heat stress (GxDxH) variability was detected. The tolerant materials showed better physiological response such as better ability to retain chlorophyll, stabilize biomass, and maintain yield than sensitive ones. Several key drought tolerance, heat tolerance and antioxidant regulation associated SNP sites were found through GWAS analysis. Molecular validation also showed high marker trait associations and responsiveness of genes to stress in climate adaptation.

### 4.1 Phenotypic Response to Climate Stress

There was considerable differences in growth performance and stress injury symptoms among the crop genotypes subjected to drought and heat stress. Wilting was significantly observed, biomass growth was decreased and grain yield was significantly reduced in stress sensitive varieties compared to the tolerant varieties. Stress-tolerant varieties, however, were able to successfully maintain water status, chlorophyll content and yield stability during stress conditions.

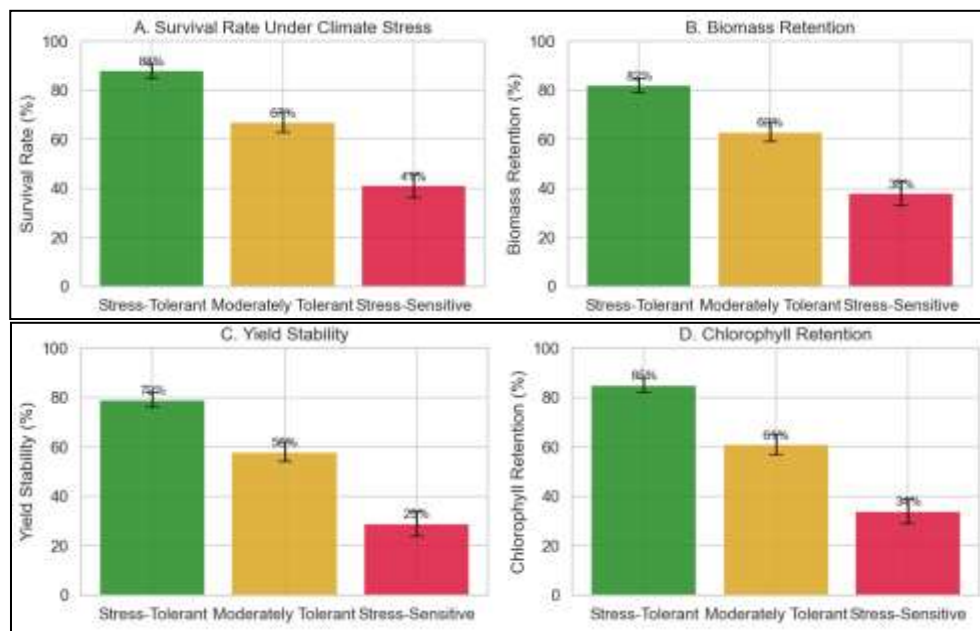


Figure 4. Comparative Growth Performance of Crop Varieties Under Climate Stress

The comparative growth performance of stress-tolerant crop varieties, stress-tolerant crop varieties moderately tolerant to stress, and stress sensitive crop varieties is shown in Figure 4, when exposed to climate stress conditions. The results show that survival rate, biomass retention, yield stability and chlorophyll content of stress tolerant genotypes remained significantly higher than the sensitive varieties. Under drought and heat stress, AOT and other growth parameters showed significant decrease in stress-sensitive crops, suggesting non-good physiological adaptation. The moderately tolerant genotypes performed somewhere between the expressly tolerant and the intolerant ones. The figure highlights the strong relationship between adaptive physiological traits and climate resilience. This increased photosynthetic efficiency, water-use regulation, and continued productivity under stress conditions was an outcome of improved biomass stability and/or chlorophyll retention of the tolerant varieties.

### 4.2 The identification of Adaptive SNP Loci

Several SNPs revealed as significant, were associated with adaptive traits related to climatic stress tolerance, identified in genome-wide association mapping. The major loci were evenly distributed among several chromosomes, and were highly associated with the traits by logarithm of odds (LOD) analysis.

Table 4. Significant Adaptive SNP Loci Identified by GWAS

SNP Marker	Chromosome	Associated Trait	Phenotypic Variance (%)
SNP-DR12	3	Drought tolerance	28%
SNP-HT7	7	Heat tolerance	24%
SNP-ROS5	5	Antioxidant activity	19%

Founded SNPs were responsible for a substantial part of the phenotypic differences in relation to stress adaptation. The SNP-DR12 marker exhibited the highest association with drought tolerance traits and SNP-HT7 marker was associated with heat stress resilience and yield stability under heat stress.

### 4.3 Physiological & Molecular Adaptation

The stress-tolerant genotypes showed higher osmotic adjustment, photosynthetic capacity, and chlorophyll retention than the susceptible ones. Under stress exposure, the activity of antioxidants was significantly enhanced, which decreased the accumulation of reactive oxygen species, and also the level of oxidative damage. Molecular analysis also showed upregulation of stress responsive genes related to osmotic balance, heat shock proteins, and ROS detoxification pathways.

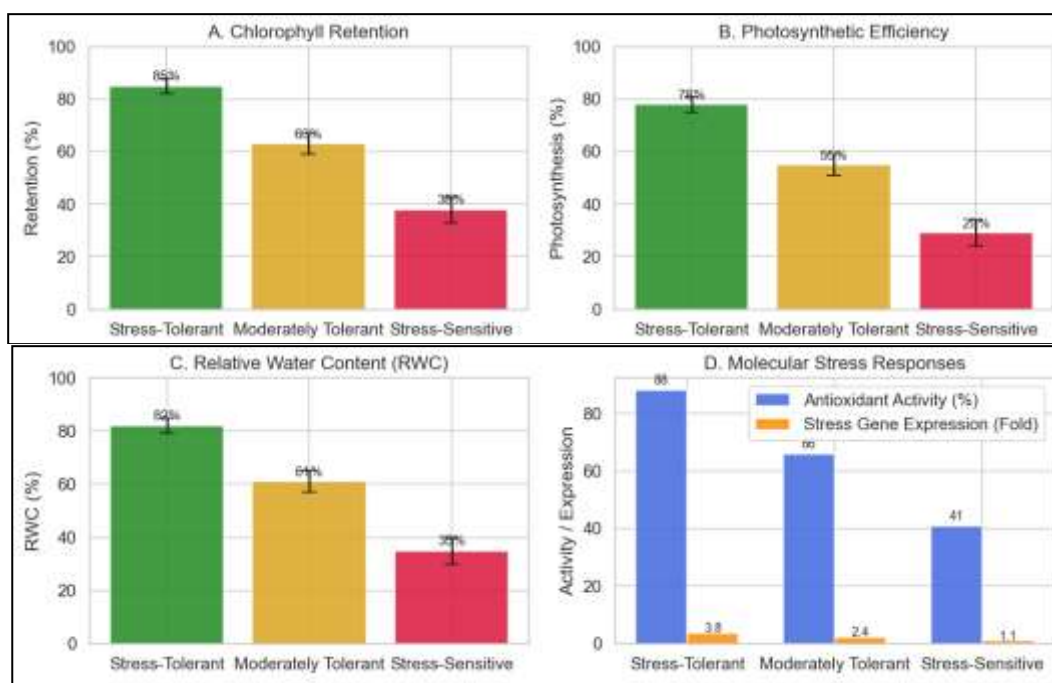


Figure 5. Physiological and Molecular Adaptation Responses Under Climate Stress

Physiological and molecular adaptation responses of the crop varieties in climate stress conditions are shown in Figure 5. Relative Water Content (RWC) of stress-tolerant genotypes was significantly more in comparison to stress-sensitive varieties, while (chlorophyll retention) and (photosynthetic efficiency) was at par. Additionally, the antioxidant response in tolerant plants was found to be stronger and expression of stress-responsive genes was also elevated, suggesting better control of oxidative stress and cellular protection in the tolerant plants. On the other hand, sensitive genotypes exhibited decreased physiological stability and adaptive response to drought and heat stress. This illustrates that, under adverse environmental conditions, the stress-resistant plant varieties show superior stress resilience, biomass maintenance, and yield stability, both as a result of the coordinative physiological and molecular adaptation responses.

### 4.4 Molecular Validation

Validation analyses showed that key marker-trait associations found via GWAS were consistent. No candidate SNP loci were associated with non-stable replicates in various experimental conditions and environmental treatments. Significant upregulation in the expression of stress responsive genes located in the vicinity of adaptive loci, further corroborating drought and heat stress tolerance, was obtained.

#### 4.5 discussion

The adaptive loci identified offer valuable resources for genetic engineering and breeding of climate-smart germplasm via marker assisted breeding programs. All the SNP marker-trait associations corroborate the previous studies of GWAS conducted in rice, wheat and maize populations. Environmental interactions and polygenic inheritance are still important challenges on trait stability and considerations on breeding efficiency. Combination of GWAS with genomics selection and functions could further enhance climate adaptation studies and speed up cultivar genetics for stress resistance.

Table 5. Comparative Evaluation of Adaptive Traits Identified by GWAS

Adaptive Trait	Benefit	Limitation
Drought tolerance	Water-use efficiency	Complex inheritance
Heat tolerance	Yield stability	Environmental sensitivity
Antioxidant regulation	Reduced oxidative damage	Moderate effect size

#### DISCUSSION

Legacy traits associated with drought tolerance and water-use efficiency led to better survival of EWs in a water-stressed environment. Most of the Heat tolerance loci helped in maintaining reproductive stability and yield during hot temperature conditions. An understanding of antioxidant regulation was shown to dampen the damage from oxidative stress, however, exhibited moderate genotypic effects when compared with major adaptive loci. Overall, these findings highlight the critical role of GWAS in the identification of genomic regions related to climate adaptation and in future programs of crop adaptation for climate resilience.

#### 5 CONCLUSION

In the present study, genome-wide association studies (GWAS) was performed on the multiple quantitative traits of the genetically diverse crop population for detection of the significant adaptive SNP loci associated with climate stress tolerance. Climate resilience-related major loci over several chromosomes were identified, showing a high marker-trait association which indicates strong climate resilience genes. Crop's different physiological parameters such as stability of yield, osmotic status, chlorophyll retention, oxidative damage were found to be higher in stressed plants than non-stressed ones generated by stress-tolerant crop varieties than the susceptible ones under drought and heat stress.

The results also further showed that adaptive genetic variability has a significant role in controlling crop responses to environmental stress. Candidates, identified by molecular markers, were confirmed by molecular validation of stress-responsive genes, lending confidence to the validated markers for use in breeding. The results underscore the value of incorporating genomic technologies into crop breeding efforts to speed the creation of climate-smart farming systems.

Furthermore, GWAS was shown to be a useful strategy to discover genomic regions associated with complex adaptive traits in crops. These SNP markers can be used as effective markers for MAS and GAB used to enhance their stress tolerance and productivity in changing climatic conditions. These developments have not, however, solved other problems of stable expression and breeding efficiency, such as interactions with the environment, genotype by environment variability, and polygene inheritance.

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