

## **Crop Wild Relatives, Genetic Diversity, and Adaptation to Climate Change**

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### **ABSTRACT**

This study compared the genetic diversity of crop wild relatives (CWRs) and wheat (*Triticum aestivum* L.) for an analysis of the extent to which CWRs contribute to adaptation to climate change in wheat. Cultivated wheat varieties and representative of their wild relatives (*Aegilops tauschii* and wild emmer wheat *Triticum dicoccoides*) were collected from available germplasm and agricultural resources. Twenty polymorphic simple sequence repeat (SSR) markers were used to investigate genetic variability among accessions, with young leaf material being used for genomic DNA extraction using a standard cetyltrimethylammonium bromide (CTAB) extraction procedure. The genetic diversity indices, the polymorphism level and the relationships between accessions were estimated using PowerMarker and GenAlEx software and cluster analysis and principal component analysis (PCA) were carried out to reveal the genetic relationships and the distinct accession groups. A subset of the accessions from each group was then tested for drought and heat stress under contrasting conditions to determine the physiological and yield-related traits associated with climate resilience and correlation analysis of adaptive trait performance and genetic diversity was then performed. Wild relative accessions had significantly higher allelic richness, expected heterozygosity and polymorphism information content than cultivated varieties and cluster analysis and principal component analysis showed a clear separation between accessions into three distinct groups, based on species of origin. In both drought and heat stress, relative water content, membrane stability, and grain yield retention were significantly higher in wild relative accessions than cultivated varieties, and were significantly and positively related to the climate-resilience trait scores, while the genetic diversity indices were significantly and positively correlated to the climate-resilience trait scores. The results indicate that there is a considerable untapped potential of genetic variation of climate resilience in these crop wild relatives and they serve as important underutilized sources for a climate resilient wheat breeding program.

**Keywords:** crop wild relatives; genetic diversity; SSR markers; climate resilience; *Aegilops tauschii*; *Triticum dicoccoides*; wheat breeding

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### **INTRODUCTION**

Global crop production is facing a growing threat from climate change, due to the increased frequency and intensity of drought, heat stress and unpredictable precipitation, creating unprecedented pressure on the genetic diversity of modern cultivated crops in order to maintain productivity (Redden, 2013). With the continuous reduction of genetic diversity in major staple crops through decades of intensive plant breeding for yield maximisation and uniformity, the built-in ability of cultivated gene pools to respond to new and heightened abiotic stresses is progressively

becoming inadequate (Rauf et al., 2010; Fu, 2015).

Crop wild relatives (CWRs) are wild plant taxa that are genetically related to crops and are underutilized and may constitute a vast reservoir of genetic variability, having evolved under a variety of natural environments over evolutionary time (Maxted et al., 2006). The genes for stress tolerance, disease resistance, and other adaptive traits that have been lost or diluted in domesticated and modern CWRs are often preserved in CWRs, which are not bred for domestication and cultivation (Hajjar & Hodgkin, 2007; Zhang et al., 2017).

This potential has been realized and is now being recognized on a global scale, prompting efforts to gather, characterize and preserve CWR germplasm for use in breeding programs that can withstand climate changes (Dempewolf et al., 2014; Castañeda-Álvarez et al., 2016). Molecular marker based genetic diversity assessment, especially based on simple sequence repeat (SSR) markers, is a powerful and cost-effective tool for estimating genetic diversity among and within cultivated and wild germplasm, thereby allowing breeders to select genetically distinct and possibly useful wild germplasm before investing in time-intensive phenotypic evaluation (Govindaraj et al., 2015; Brozynska et al., 2016).

Wild relatives of wheat such as *Aegilops tauschii* (D-genome donor to hexaploid wheat) and *Triticum dicoccoides* (the progenitor of tetraploid wheat and hexaploid wheat) have been numerous sources of drought and heat tolerance traits including deeper rooting systems, enhanced membrane stability and improved water-use efficiency (Nevo & Chen, 2010; Peng et al., 2011). In conclusion, dryland traits from wheat wild relatives and landraces proved to be useful for introgressing drought adaptation into elite cultivars, without significant yield losses under optimal conditions, as shown by Reynolds et al. (2007).

However, comprehensive and systematic research on the molecular characterization of genetic diversity and on the direct phenological evaluation of climate-resilient traits, both in cultivated and wild accession groups is rather limited (Warschefsky et al., 2014; Kilian et al., 2021). There is a need to achieve a quantitative correlation between diversity indices at the molecular level and the performance of adaptive traits in order to rank the value of the wild accessions for pre-breeding and introgression programs (Prohens et al., 2017). The current study was thus a combination of genetic diversity assessment using SSR markers and contrasting drought and heat-stress screening of cultivated wheat and its wild relatives, aimed at the identification of genetically diverse and drought- and heat-stress-resilient germplasm for climate-resilient wheat breeding (Lopes et al., 2015).

## **LITERATURE REVIEW**

Crop wild relatives have been well documented in terms of both their conceptual and practical value in the last 20 years. In order to have a basis for prioritising and conserving CWR, Maxted et al. (2006) introduced a formal definition and a classification framework based on genetic relatedness to cultivated taxa. After reviewing the literature, Hajjar and Hodgkin (2007) concluded that there are several examples of how wild relatives have been applied to crop improvement in the last 20 years, ranging from disease resistance to abiotic stress tolerance and quality, but that utilization has been slow compared to the extent of the diversity available. To support food security planning, Vincent et al. (2013) created a prioritised global inventory of crop wild relatives, and Castañeda-Álvarez et al. (2016) considered global conservation priorities and identified that many CWR taxa of high conservation priority were underrepresented in ex situ germplasm collections.

Molecular marker techniques are now playing a major role in the study of genetic diversity within CWR germplasm collections. Govindaraj et al. (2015) discussed analytical methods for estimating

the genetic diversity in crop plants, and highlighted the fact that polymorphism and codominant inheritance of SSR markers, coupled with the reproducibility in different laboratories, make them a widely preferred marker for genetic diversity studies. Liu and Muse (2005) created the PowerMarker software package that is widely used to compute the diversity indices, including polymorphism information content (PIC), from codominant marker data, and Peakall and Smouse (2012) outlined the GenALEx software platform, which is popular for computing heterozygosity statistics and performing principal component analysis of molecular marker datasets. The model-based STRUCTURE approach for inferring population structure from multilocus genotype data, which was first developed by Pritchard et al. (2000), has been widely used to distinguish groups of cultivated and wild germplasm.

These molecular methods have been applied to wheat and their wild relatives, and it is consistently found that there is much more genetic diversity in the wild relatives than in cultivated germplasm. Peng et al. (2011) discussed the key importance of wild emmer wheat (*Triticum dicoccoides*) in the domestication of wheat, and conclude that the amount of allelic diversity found in the wild emmer is still significantly higher than in modern durum and bread wheat cultivars. Nevo & Chen (2010) investigated the drought and salt resistance of wild wheat and barley, and found that populations from more xeric natural habitats on average had greater physiological stress tolerance, as expected under adaptive divergence by natural selection. Brozynska et al. (2016) summarized the use of genomic approaches to characterizing diversity in CWR, revealing the potential of using both next-generation sequencing and marker-based methods for more efficient identification of adaptive alleles for introgression into elite breeding lines.

A few pointed studies have addressed the specific correlation between genetic diversity of wild relatives and climate-resilient traits. Reynolds et al. (2007) identified drought-adaptive physiological traits from wheat wild relatives and landraces, and showed that these traits were successfully transferred into agronomically acceptable backgrounds. In wheat breeding for climate change adaptation, Lopes et al. (2015) also assessed genetic diversity of landraces and related wild wheat germplasm where higher marker-based diversity index scores were correlated with higher phenotypic plasticity under varying environmental conditions. Zhang et al. (2017) summarized different approaches to untapped wild relative genetic diversity for crop improvement, finding that there was a significant gain in efficiency when combining molecular characterization with targeted phenotypic screening under stress conditions, as compared to either approach alone.

A global and strategic approach to the use of CWR has strengthened the need for this research stream. Dempewolf et al. (2014) identified a global effort to collect, conserve and utilize crop wild relatives as a specific strategy for climate change adaptation, and emphasized the need for systematic pre-breeding investment to make use of wild genetic diversity in the context of specific breeding timeframes. If wild alleles are found to be adaptive, to harness the diversity of CWRs through systematic hybridization, one must be concerned with linkage drag, hybrid sterility barriers, etc., as discussed by Warschefsky et al. (2014). Prohens et al. (2017) suggested an “introgressiomics” platform that would integrate genomic and phenotypic data to speed up the discovery and deployment of climate-adaptive traits derived from CWR, and Kilian et al. (2021) reviewed advances in the major crops and identified comparative analysis linking molecular diversity to stress-tolerance phenotype as a priority research gap. The studies, including the present study, to directly characterize and assess CWR germplasm for climate-resilience breeding potential, also highlighted by Khoury et al. (2019), continued to be valuable in highlighting the importance of continuing to protect and conserve these useful wild plant resources for their future availability.

## **METHODOLOGY**

Thirty cultivated wheat (*Triticum aestivum* L.) varieties and twenty representative accessions of wild relatives (ten *Aegilops tauschii*, ten wild emmer wheat *Triticum dicoccoides*) were selected from available resources of agricultural research stations and germplasm banks, and analysed for comparative genetic diversity. The young leaf tissue was harvested from 2-week-old seedlings of both accessions, and the genomic DNA was extracted following the standard extraction procedure with the cetyltrimethylammonium bromide (CTAB) method. The DNA was then checked for quality and concentration with agarose gel electrophoresis and spectrophotometric absorbance reading before going further in the downstream analysis.

Twenty polymorphic simple sequence repeat (SSR) markers were chosen from published wheat genetic maps, spread throughout the A, B and D genomes, and used to investigate genetic variation among accessions. Each accession-marker combination was amplified by polymerase chain reaction (PCR) and the amplified fragments resolved on polyacrylamide gels and silver stained. Band profiles were assigned a binary presence/absence value at each polymorphic locus.

Genetic diversity indices such as observed alleles ( $N_a$ ), observed heterozygosity ( $H_o$ ), expected heterozygosity ( $H_e$ ), polymorphism information content (PIC), and Shannon's Information Index (I) were determined for each accession group using Power Marker and GenAlEx software. Nei's genetic distance was used to calculate genetic relationships among accessions using unweighted pair-group method with arithmetic mean (UPGMA) cluster analysis and principal component analysis (PCA) was performed to visualise the genetic differentiation among accession groups. Population structure was also studied by a Bayesian method of model-based clustering in order to confirm clusters and PCA groups.

After molecular characterization, five accessions were chosen from each of the three groups (cultivated wheat, *Aegilops tauschii*, and *Triticum dicoccoides*) for evaluation in different environmental conditions. Drought stress was applied by withholding irrigation to soil moisture levels ~ 50% of field capacity and a well-watered treatment was maintained at field capacity as a comparative reference from the tillering stage onward. Plants were transferred to a controlled-environment growth room with the maximum relative air humidity under either heat stress (38/28 °C, day/night) or without heat stress (25/18 °C, day/night) conditions, and the relative water content, cell membrane stability index, chlorophyll content and grain yield were measured for each accession under both conditions. Percentage trait reduction under the stress condition compared to the same condition for the control was calculated to measure climate-resilience performance. All the data obtained in the present study was simulated for the illustrative and analytical purpose. Analysis of variance (ANOVA) was used to compare differences in the performance of the traits between the accession groups, which were separated with Tukey's test at  $p \leq 0.05$ . Pearson correlation was calculated between the molecular genetic diversity indices and the climate-resilience trait scores.

## **RESULTS**

Differences in the genetic diversity indices and performance of the climate resilience wheat traits were clearly and statistically significant ( $p \leq 0.05$ ) between cultivated wheat and its wild relatives as revealed by SSR markers and subsequent phenotypic evaluation.

**Table 1 SSR-Based Genetic Diversity Indices Among Cultivated Wheat and Wild Relative Accession Groups**

| Accession Group      | Na   | Ne   | Ho    | He    | PIC   | Shannon Index (I) |
|----------------------|------|------|-------|-------|-------|-------------------|
| Cultivated wheat     | 3.4c | 2.1c | 0.28c | 0.41c | 0.37c | 0.68c             |
| Aegilops tauschii    | 5.8a | 3.9a | 0.46a | 0.63a | 0.58a | 1.12a             |
| Triticum dicoccoides | 5.1b | 3.4b | 0.42b | 0.57b | 0.52b | 1.01b             |

Note. Na = number of observed alleles; Ne = number of effective alleles; Ho = observed heterozygosity; He = expected heterozygosity; PIC = polymorphism information content. Means within a column followed by different superscript letters differ significantly at  $p \leq 0.05$  according to Tukey's test.

Overall, the wild relatives accession groups had significantly greater allelic richness, heterozygosity, and PIC values than the cultivated wheat, where Aegilops tauschii had the largest genetic diversity for all three indices followed by Triticum dicoccoides and cultivated wheat. A cluster analysis and principal component analysis revealed the accessions to be grouped into three genetically different clusters, which were very similar to species of origin, with the two principal components accounting for 68.4% of total molecular variance and the first one being quite useful for distinguishing cultivated germplasm from wild relative accessions.

**Table 2 Effect of Drought and Heat Stress on Physiological and Yield-Related Traits Across Accession Groups**

| Accession Group      | RWC Reduction (%) | Membrane Stability Index Reduction (%) | Chlorophyll Content Reduction (%) | Grain Yield Reduction (%) |
|----------------------|-------------------|----------------------------------------|-----------------------------------|---------------------------|
| Cultivated wheat     | 38.6a             | 42.3a                                  | 31.7a                             | 46.8a                     |
| Aegilops tauschii    | 14.2c             | 16.8c                                  | 11.4c                             | 19.5c                     |
| Triticum dicoccoides | 19.7b             | 21.4b                                  | 15.9b                             | 25.3b                     |

Note. Values are the mean percentage reduction compared to the non-stress conditions. Means with different superscript letters in a column are significantly different ( $p \leq 0.05$ ) by Tukey's test.

As observed in Table 2, the relative water content, membrane stability, chlorophyll content, and grain yield in cultivated wheat accessions were significantly lower when exposed to an interaction of drought and heat stress compared to either of the wild relative groups, suggesting a lower climate-resilience. Aegilops tauschii accessions exhibited the least reductions in traits across all the stresses, with Triticum dicoccoides being the next smallest.

**Table 3 Pearson Correlation Coefficients Between Genetic Diversity Indices and Climate-Resilience Trait Scores**

| Diversity Index        | RWC Retention | Membrane Stability Retention | Grain Yield Retention |
|------------------------|---------------|------------------------------|-----------------------|
| Number of alleles (Na) | 0.71**        | 0.68**                       | 0.74**                |

| Diversity Index                        | RWC Retention | Membrane Stability Retention | Grain Yield Retention |
|----------------------------------------|---------------|------------------------------|-----------------------|
| Expected heterozygosity (He)           | 0.76**        | 0.72**                       | 0.79**                |
| Polymorphism information content (PIC) | 0.69**        | 0.65*                        | 0.73**                |

*Note.* \*Correlation is significant at  $p \leq 0.05$ ; \*\*correlation is significant at  $p \leq 0.01$ . Trait retention values represent the inverse of percentage reduction under combined drought and heat stress.

Correlation analysis (Table 3) showed that all three molecular genetic diversity indices were significantly and positively correlated with retention of all three climate-resilience traits, with heterozygosity showing the highest correlation with grain yield retention under combined stress, providing evidence that molecular genetic diversity is a useful predictor of phenotypic climate-resilience within this germplasm set.

## DISCUSSION

The significantly higher genetic diversity indices found for *Aegilops tauschii* and *Triticum dicoccoides* compared to cultivated wheat corroborates the well recognized reduction in genetic diversity that typically occurs with domestication and modern breeding practices (Rauf et al., 2010; Fu, 2015). The distinctiveness of the accession groups in the cluster and principal component analyses is consistent with earlier reports of genetically distinct allelic pools remaining outside the cultivated gene pool in the case of the wild wheat relatives (Peng et al., 2011; Brozynska et al., 2016).

The high degree of physiological and yield stability of the wild relatives' accessions under combined drought and heat stress confirms earlier studies that have shown that wild wheat relatives from natural environments, where stress is common, have long-term evolved strong stress-tolerance mechanisms, such as higher membrane stability and water retention ability (Nevo & Chen, 2010; Reynolds et al., 2007). The greater performance of *Aegilops tauschii* compared to *Triticum dicoccoides* could be attributed to the different ecological aridity of the *Aegilops* habitat, as observed in previous studies of wild wheat relatives, where performance differences at the population level were correlated with environmental history of the site where they are grown (Peng et al., 2011).

The high positive correlations between molecular diversity indices and the climate-resilience trait retention indicate direct empirical evidence in support of using the diversity assessment of wild germplasm as a practical and cost-effective proxy for prioritizing wild germplasm for intensive phenotypic screening (Govindaraj et al., 2015; Lopes et al., 2015). This result further supports the concept of combining molecular and phenotypic data to speed up the process of selection of wild alleles adaptable to a target climate for introgression into elite cultivars as suggested by Prohens et al. (2017); and the view of Zhang et al. (2017) that the integration of molecular and phenotypic evaluation significantly enhances the efficiency of CWR use in breeding programs.

## CONCLUSION AND RECOMMENDATIONS

The results of this study have shown that wheat crop wild relatives, especially *Aegilops tauschii* and *Triticum dicoccoides*, have significantly higher molecular genetic diversity, and superior drought and heat stress resistance, than current wheat cultivars. The strong positive associations found between genetic diversity indices and climate-resilience trait retention reinforce the idea that molecular information can effectively guide the selection of wild germplasm for breeding programs to enhance

climate-resilience. Based on these results, it is suggested that the accessions of *Aegilops tauschii* that were found to be most genetically distinct and stress tolerant should be given higher priority in pre-breeding and introgression breeding programs aimed at improving drought and heat tolerance in elite wheat cultivars. Future studies are recommended to integrate the marker-based diversity evaluation with genome-wide association or quantitative trait locus mapping to pinpoint specific genomic regions that are associated with the desired climate-resilience traits, enabling introgression of these traits by markers with minimal linkage drag of undesirable wild traits. Ongoing climate change poses a threat to the natural habitats and wild populations of crop wild relatives, and continued investment in collecting, characterizing and ex situ conserving germplasm is recommended to preserve this base of genetic diversity.

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