Pan-Genome Analysis for Identification of Climate-Resilient Alleles in *Triticum aestivum* L.

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Received: 06 Jun 2026
Revised: 23 Jun 2026
Accepted: 07 Jul 2026
Published: 27 Jul 2026

E-ISSN: 2989-5359
DOI: https://doi.org/10.54660/ejsa.2026.6.2.29-38

ABSTRACT

Introduction to the Problem: Climate change is causing unprecedented pressure on wheat production globally, hence, there is a necessity to develop crops with resilience traits. Pan-genome studies provide an effective way to uncover and analyze the diversity of alleles associated with climate-related traits. Aim: The study applies a pan-genome analysis of 127 accessions of *Triticum aestivum* to discover structural variations in the genome and genes responsible for drought, salinity, and heat tolerance. Materials and Methods: The research integrates whole-genome sequenced data of elite cultivars and varieties and wild relatives with their phenotypic responses observed in different abiotic environments. The definitions of core, soft-core, shell, and cloud genome types are provided, based on presence-absence variations. Structural variants, gene duplications, and gene family expansions have been determined. In addition to that, functional annotations and gene ontology enrichment analyses are undertaken to identify 186 candidate genes associated with stress processes. Results: The pan-genome analysis revealed that 42,847 core genes were identified in 95% of accessions and 18596 accessory genes were found in less than 95% accessions. In addition to that, the pan-genome analysis revealed that 2847 structural variables were mapped out and 247 of them were found essential for improved stress tolerance characteristics. There are 156 genes described that are responsible for improved drought tolerance, 89 genes responsible for heat resistance, and 73 genes responsible for salt tolerance. Variation in copy number was noted in the genes of heat shock proteins and aquaporins. Functional annotation revealed 41 genes associated with positive phenotypic variability of yields. Impact: The obtained results contribute enormously to the comprehension of the molecular mechanisms of climatic adaptation of wheat and provide molecular markers and candidate genes for fast-tracked breeding through genomic selection. The ability to extract beneficial alleles from wild relatives and local varieties opens new avenues for breeding.

Keywords: pan-genome analysis, climate-resilient alleles, structural genomic variation, comparative genomics, drought tolerance, heat tolerance, salinity tolerance, wheat breeding

Introduction

Wheat (*Triticum aestivum* L.) is one of the most significant food crops globally, contributing around 20% of the calorie and protein intake of more than 1.5 billion people (Shiferaw *et al*., 2013) [1]. Despite its importance in agriculture, wheat production is under stress due to climate change, which is causing the rise in temperature, changes in precipitation, and increase in salinity of soils (Asseng *et al*., 2015) [2]. Current predictions indicate that climate change will lead to a decrease in wheat yields of 6–14% by mid-century, whereas demand for wheat is expected to be increased by around 50% globally (Reynolds *et al*., 2012) [3]. Therefore, there is a need for the introduction of climate-resilient varieties of wheat that will manage to maintain productivity in adverse conditions.

Traditional breeding has played a very important role in development of stress tolerant varieties of wheat, but these methods have some limitations and take a lot of time because they are influenced by the genetic variations within the elite pool of cultivars (Mondal *et al*., 2016) [4]. Use of modern varieties of wheat could lead to significant loss of many alleles if compared with traditional varieties, because wild and landrace varieties might have a lot of advantageous aleles that could be used for developing varieties resistant to climate change (Gepts *et al*., 2005) [5]. The methods which involve pan-genomic approaches

Pan-genomic analysis is indicative of a revolution in plant genomics, which has gone beyond the traditional concept of reference genome, to understand the phylogenetics and genomics of a whole species (Golicz *et al*., 2016) [6]. Pan-genome can be viewed as consisting of three components including the core genome (genes shared by all or nearly all the accessions), soft-core genome (genes that occur in majority of the individuals), and the accessory or dispensable genome (genes present in some but not all accessions) (Zhao *et al*., 2018) [7]. The division into these parts has shown that the genome of the plants is more complex than previously thought and that the variations in genome structure play a significant role in phenotypic variability (Ou *et al*., 2023) [8]. Accessory genes play a vital role in providing functions concerning the adaptation of plants to different environmental conditions, making them significant for breeding for climate variability tolerant crops (Muthamilarasan and Prasad, 2021) [9].

Structural genomic variations which include insertions, deletions, inversions, duplications, and copy number variations are considered a major contributor to allelic diversity in wheat varieties (Walkowiak *et al*., 2020) [19] (Innan and Kondrashov, 2010) [29]. These variations could affect gene expression, enzymatic functions, and phenotypic results via mechanisms that differ from

those employed by single nucleotide polymorphisms (Hirsch and Springer, 2017) [11] (Zender *et al*., 2015) [16]. Novel breakthroughs in long-read sequencing technologies have opened the way for a broader detection of structural variants and better (Hon *et al*., 2021) [13] (Porubsky *et al*., 2021) [24] resolution in the identification of the causal variants that contribute to climate adaptation traits. Nowadays, modern genomic selection methods are increasingly utilizing this type of data for increasing efficiency when predicting complex traits (Varshney and Tuberosa, 2013) [36].

Gene family expansions and contractions can also be used as sources of genomic variations significant for stress adaptation (Innan and Kondrashov, 2010) [29]. Families of genes responsible for stress sensing (histidine kinases), signalling (mitogen-activated protein kinases), and protection (heat-shock proteins and late embryogenesis abundant proteins) often exhibit copy number variation across populations (Zender *et al*., 2015) [16]. These variations can affect stress response efficiency and scope, resulting in various tolerance phenotypes (Yamaguchi-Shinozaki and Shinozaki, 2005) [25] (Wang *et al*., 2003) [26].

Pan-genome investigations carried out earlier in wheat and its close relatives offered significant information regarding the genome composition and diversity (Golicz *et al*., 2016) [6] (Zhao *et al*., 2018) [7]. In the study conducted by Luo *et al*. (Luo *et al*., 2013) [18], much progress was made in structural variance characterization in 15 wheat lines, resulting in discovering numerous structural variants that influence the gene expression and phenotype. A study by Walkowiak *et al*. (Walkowiak *et al*., 2020) [19] presented a thorough pan-genome of wheat compiled from 10 different varieties of wheat revealing a considerably large amount of accessory genome features. Many subsequent explorations continued to advance that field and included investigations of structural variations in terms of phenotypic responses to certain stress conditions (Zhou *et al*., 2015) [20] (Yang *et al*., 2021) [21]. However, though pan-genome resources are available, existing studies so far have composed analyses of climate-resilient alleles and their comparisons with extensive abiotic stress analysis outcomes.

The comprehensive study fills this gap using thorough pan-genome analysis of 127 wheat accessions, combined with assessment of phenotypes under stressful conditions such as drought, excessive temperature, and salinity. Our objectives were: (1) to study the structure of pan-genome in wheat and find differences in genomic structure among wheat types; (2) to find important genes and gene variants that contribute to climate resistance; (3) to identify genes with known functions in order to understand their roles in response to stresses; (4) to provide tools for genomic breeding.

Materials and Methods

Germplasm Resources and Characterisation

This study utilised 127 wheat accessions representing diverse genetic backgrounds, geographic origins, and climate adaptation profiles. The germplasm collection included: (i) 42 elite modern cultivars representing major wheat-producing regions globally; (ii) 35 improved varieties with documented stress tolerance phenotypes; (iii) 32 traditional landraces representing the genetic heritage of wheat agriculture; and (iv) 18 wild relatives of wheat (*Triticum urartu*, *Aegilops tauschii* accessions) harbouring untapped genetic resources for climate adaptation [Table 1]. Accessions were selected to represent the full spectrum of global wheat adaptation zones, including temperate, Mediterranean, subtropical, and drought-prone agroecologies. Detailed pedigree information and phenotypic characterisation data were compiled from international germplasm repositories and research institutions.

Genomic Resources and Data Integration

Whole-genome sequencing data were generated or obtained from public repositories for all 127 accessions. The Chinese Spring reference genome (IWGSC RefSeq v2.1) served as the primary reference for comparative genomics [Table 2]. Sequence data encompassed multiple sequencing platforms, including Illumina short-read sequencing (average coverage: 25×) and Pacific Biosciences long-read sequencing (average coverage: 15×) for subset of 34 accessions to enable improved structural variant detection. Raw sequencing reads were aligned to the reference genome using established short-read and long-read mapping algorithms (Li and Durbin, 2009) [22] (Ruan and Li, 2020) [23]. Genomic sequence assemblies, structural variant datasets, and variant call format (VCF) files were processed through standardised bioinformatic pipelines to ensure cross-sample consistency.

Pan-Genome Construction and Characterisation

Pan-genome construction was accomplished through comprehensive comparative genomic analysis of all 127 accessions, integrating sequence variants, presence–absence variation, and structural variation data (Golicz *et al*., 2016) [6] (Zhao *et al*., 2018) [7]. Core genome genes were defined as those present (with ≥90% amino acid identity) in ≥95% of accessions. Soft-core genes were present in 80–95% of accessions, shell genes in 15–80% of accessions, and cloud genes in <15% of accessions [Table 3]. Genomic regions unique to specific accessions were identified through alignment gap analysis and de novo sequence assembly. Presence–absence variation (PAV) was catalogued using a binary matrix approach, with genes scored as present (1) or absent (0) in each accession. Copy number variation (CNV) was quantified through comparative read mapping and sequence alignment analysis, with statistical thresholds applied to distinguish technical variation from biological CNV (Ruan and Li, 2020) [23].

Structural Genomic Variation Analysis

Structural variants (SVs), defined as genomic alterations ≥50 bp in length, were comprehensively identified and classified. Insertions, deletions, inversions, and translocations were detected using multiple bioinformatic approaches, including split-read mapping, read-pair analysis, and sequence assembly comparison. Variant breakpoints were precisely mapped, and variants were annotated relative to genomic features including gene regions, regulatory elements, and repetitive sequences. Large structural variants identified through long-read sequencing were validated through multiple independent detection methods to minimise false discoveries (Porubsky *et al*., 2021) [24]. The genomic distribution of structural variants was analysed to identify hotspots of variation and potential recombination-associated rearrangements (Jiao and Schneeberger, 2020) [14].

Candidate Climate-Resilient Gene Identification

Candidate genes associated with climate resilience were identified through integrated multi-criteria analysis. First, literature-derived lists of stress-responsive genes from previous transcriptomic and genomic studies were compiled, identifying genes with documented roles in drought tolerance, heat tolerance, oxidative stress management, and osmotic adjustment (Yamaguchi-Shinozaki and Shinozaki, 2005) [25] (Wang *et al*., 2003) [26]. Second, genes residing within genomic regions

showing signatures of positive selection for climate adaptation were identified through comparative sequence divergence analysis (Vaser *et al*., 2016) [27]. Third, genes with significant allelic variation correlating with phenotypic differences in stress tolerance were prioritised using quantitative trait locus (QTL) mapping and genome-wide association study (GWAS) approaches applied to the full germplasm collection (Korte and Farlow, 2013) [28]. Fourth, functional annotation of accessory genome genes was performed to identify genes preferentially retained in stress-adapted accessions (Muthamilarasan and Prasad, 2021) [9]. Finally, genes encoding proteins involved in key stress-adaptation pathways—including reactive oxygen species scavenging, osmolyte accumulation, root development, water transport, and photosynthetic protection—were systematically identified and prioritised (Yamaguchi-Shinozaki and Shinozaki, 2005) [25] (Wang *et al*., 2003) [26] (Tomlinson *et al*., 2018) [31].

Phenotypic Evaluation and Stress Assessment

All 127 accessions were evaluated phenotypically under controlled and field-based stress environments. Drought stress evaluations employed managed-deficit irrigation protocols, with water availability restricted to 50% of crop water requirement during critical growth stages. Heat stress trials were conducted under elevated temperature regimes (3–4°C above ambient), achieved through combination of natural temperature variation and controlled-environment facilities. Salinity stress evaluations employed soil salinisation to achieve electrical conductivity of 8–10 dS/m. Measurements included: (i) physiological traits—leaf water potential, stomatal conductance, photosynthetic rate, chlorophyll fluorescence; (ii) agronomic traits—biomass production, grain yield, harvest index, root system architecture; and (iii) stress-response indices—drought susceptibility index, heat tolerance index, salinity tolerance index [Table 7]. Standardised protocols were applied across all stress environments to ensure comparability.

Functional Annotation and Pathway Analysis

Gene ontology (GO) enrichment analysis was performed on candidate climate-resilient gene lists using standard annotation databases and statistical methods (Cheng *et al*., 2017) [12]. Genes were classified according to biological process, molecular function, and cellular component ontologies. Pathway enrichment analysis was conducted using established plant pathway databases, identifying metabolic and signalling pathways preferentially involving candidate climate-resilient genes. Comparative analysis of gene families revealed families showing evidence of expansion in stress-tolerant accessions, suggesting selection for enhanced gene dosage (Innan and Kondrashov, 2010) [29]. Tissue-specific and developmental-stage-specific expression patterns were examined for candidate genes using publicly available transcriptomic databases (Cheng *et al*., 2017) [12].

Statistical and Comparative Genomic Analysis

Analysis of Variance (ANOVA) was applied to test for significant differences in phenotypic traits across germplasm groups and stress environments. Principal Component Analysis (PCA) was employed to ordinate accessions based on their genomic profiles, with principal components capturing major sources of genomic variation (Patterson *et al*., 2006) [30]. Hierarchical clustering analysis grouped accessions based on structural variant profiles and presence–absence variation patterns, revealing major clades reflecting geographic origin and adaptation profile (Sneath and Sokal, 1973) [10]. Genome-wide comparative analysis identified genomic regions showing elevated levels of variation, suggesting targets for adaptive evolution. Correlation analysis examined relationships between specific structural variants or candidate genes and stress tolerance phenotypes (Patterson *et al*., 2006) [30].

Results

Pan-Genome Architecture and Composition

Comparative pan-genome analysis of 127 wheat accessions revealed substantial genomic complexity and allelic diversity (Golicz *et al*., 2016) [6] (Zhao *et al*., 2018) [7] [Figure 1]. The constructed pan-genome comprised 61,443 total gene clusters, substantially exceeding the 42,847 genes present in the core genome (95% occupancy threshold) [Table 3]. Classification into genome categories revealed: (i) 42,847 core genes (69.7% of pan-genome), present in ≥95% of accessions; (ii) 4,896 soft-core genes (8.0%), present in 80–95% of accessions; (iii) 8,742 shell genes (14.2%), present in 15–80% of accessions; and (iv) 4,958 cloud genes (8.1%), present in <15% of accessions. This distribution indicates that wheat harbours substantial accessory genome content representing approximately 30% of the pan-genome (Zhao *et al*., 2018) [7]. The accessory genome demonstrated substantial enrichment for genes involved in environmental sensing, stress response, and adaptation pathways (Muthamilarasan and Prasad, 2021) [9].

Structural Genomic Variation Inventory

Comprehensive structural variant analysis identified 2,847 distinct structural variants across the wheat pan-genome [Table 4]. These comprised: 1,156 deletions (40.6%), 892 insertions (31.3%), 487 inversions (17.1%), and 312 complex rearrangements (11.0%). Structural variants exhibited non-random genomic distribution, with elevated variant density in pericentromeric regions and regions flanking transposable element clusters (Ou *et al*., 2023) [8]. Analysis of variant frequency revealed that 1,243 variants (43.6%) were rare, detected in <5% of accessions, while 604 variants (21.2%) were shared across ≥80% of germplasm. Notably, 487 structural variants (17.1%) directly affected gene sequences, potentially altering protein function or gene expression (Hirsch and Springer, 2017) [11]. Copy number variation analysis revealed significant CNV in families of genes including heat-shock proteins (HSP gene family showing 1.5–4.2× copy number variation), aquaporins (PIP and TIP family showing 0.8–3.1× variation), and late embryogenesis abundant proteins (LEA gene family showing 1.2–3.8× variation). These CNV patterns suggest selection for enhanced stress-response gene dosage in climate-adapted accessions (Zender *et al*., 2015) [16].

Identification of Climate-Resilient Candidate Alleles

Integrated analysis combining literature-based candidate gene identification, genomic signatures of selection, QTL/GWAS analysis, and functional annotation identified 186 high-confidence candidate genes associated with climate resilience [Table 5]. Drought-tolerance-associated candidates (156 genes) included genes encoding: (i) aquaporin water-channel proteins (12 genes), with sequence variants predicted to alter water-transport capacity; (ii) osmolyte-biosynthesis enzymes (28 genes), including proline synthesis pathway genes; (iii) transcription factors (34 genes) involved in drought-stress signalling; (iv) abscisic acid signalling components (18 genes); and (v) root-development regulatory genes (34 genes), consistent with functional importance of root architecture for water acquisition under drought (Tomlinson *et al*., 2018) [31]. Heat-tolerance candidates (89 genes) encompassed: (i) heat-

shock protein genes (24 genes), exhibiting elevated copy number in heat-tolerant accessions; (ii) molecular chaperone genes (18 genes); (iii) photosynthetic apparatus protective proteins (22 genes), including photoprotection genes; and (iv) antioxidant defence enzymes (25 genes) involved in reactive oxygen species scavenging (Zhao *et al*., 2012) [32]. Salinity-tolerance candidates (73 genes) included: (i) sodium-exclusion mechanisms (16 genes), encoding plasma membrane transporters; (ii) vacuolar sodium sequestration genes (14 genes); (iii) osmolyte-accumulation pathway genes (21 genes); and (iv) potassium-retention mechanisms (22 genes), important for maintaining K^+/Na^+ homeostasis (Lafitte *et al*., 1997) [17].

Functional Annotation and Pathway Integration

Gene Ontology enrichment analysis of the 186 candidate climate-resilient genes [Table 6] revealed significant over-representation of biological processes related to: (i) response to water deprivation (89 genes, $p < 0.001$); (ii) response to temperature stress (64 genes, $p < 0.001$); (iii) response to salt stress (52 genes, $p < 0.001$); (iv) cellular response to stress (124 genes, $p < 0.001$); and (v) plant-hormone-mediated signalling pathways (78 genes, $p < 0.001$) (Yamaguchi-Shinozaki and Shinozaki, 2005) [25] (Wang *et al*., 2003) [26]. Molecular function enrichment identified preferential involvement in: (i) protein binding (142 genes); (ii) ATP binding (87 genes); (iii) zinc-ion binding (56 genes); and (iv) sequence-specific DNA binding (48 genes). Pathway analysis integrated candidate genes into established signalling networks including: the abscisic acid signalling pathway (42 genes), the MAP kinase signalling cascade (31 genes), calcium-mediated stress signalling (28 genes), and the jasmonic acid/salicylic acid defence pathway (24 genes) (Yamaguchi-Shinozaki and Shinozaki, 2005) [25] (Wang *et al*., 2003) [26]. Comparative transcriptomic analysis revealed that 164 of 186 candidate genes (88.2%) demonstrated significantly elevated expression in stress-challenged tissues compared to control conditions, supporting functional roles in stress response (Yamaguchi-Shinozaki and Shinozaki, 2005) [25] (Wang *et al*., 2003) [26].

Phenotypic Evaluation and Candidate Allele Correlation

Comprehensive phenotypic evaluation of all 127 accessions under drought, heat, and salinity stress environments revealed substantial variation in stress tolerance phenotypes [Table 7]. Under drought stress, grain yield varied by 3.8-fold across germplasm (range: 0.8–3.0 t/ha), with wild relatives and selected landraces demonstrating superior performance (average: 2.1 t/ha) compared to elite cultivars (average: 1.4 t/ha). Under heat stress, grain yield variation was 4.2-fold (range: 0.6–2.5 t/ha). Under salinity stress (8–10 dS/m), yield variation was 5.1-fold (range: 0.4–2.1 t/ha). Stress tolerance indices calculated from physiological measurements revealed significant correlations between specific candidate genes/alleles and stress tolerance phenotypes. Notably, the number of functional copies of heat-shock protein genes (HSP17, HSP70, HSP90 family members) showed significant positive correlation with heat tolerance ($r = 0.67$, $p < 0.001$). Copy number of aquaporin genes demonstrated significant association with drought tolerance ($r = 0.62$, $p < 0.001$). Allelic variants in sodium-transporter genes (HKT1, HKT2) showed strong correlation with salinity tolerance ($r = 0.58$, $p < 0.001$). These associations support direct functional roles of identified candidate alleles in climate adaptation (Yamaguchi-Shinozaki and Shinozaki, 2005) [25] (Wang *et al*., 2003) [26].

Climate-Resilient Allele Classification and Prioritisation

High-priority candidate alleles for breeding application were identified through weighted scoring incorporating: (i) strength of phenotypic association (maximum $|r| = 1.0$); (ii) allele frequency in stress-adapted vs elite germplasm; (iii) predicted effect size on protein function; and (iv) transferability to elite genetic backgrounds. Forty-one alleles achieved high-priority classification, meeting all four prioritisation criteria with scores >0.75. These included: (i) a 2.3 kb insertion in the promoter region of TaHKT1 (sodium-transporter gene) increasing gene expression 3.2-fold in salinity-adapted accessions; (ii) a 1.8 kb deletion in the TaAQP2 gene eliminating a negative regulatory domain, predicted to enhance aquaporin function; (iii) a copy number increase in the TaHSP17-2 gene from 1–2 copies in elite cultivars to 4–6 copies in heat-tolerant landraces; and (iv) a missense mutation in the transcription factor TaABF3, predicted to enhance DNA-binding affinity (Vaser *et al*., 2016) [27]. Medium-priority alleles (42 candidates) showed strong associations with phenotypes but required additional functional characterisation. Remaining candidate alleles (103) provide valuable resources for future targeted studies.

Discussion

This comprehensive pan-genome study substantially advances our understanding of genomic basis of climate adaptation in wheat, providing an extensive resource of climate-resilient alleles for accelerated breeding and genomic selection. Our findings expand upon previous wheat pan-genome analyses (Luo *et al*., 2013) [18] (Walkowiak *et al*., 2020) [19] by: (i) substantially increasing germplasm size (127 vs 10–15 accessions in previous studies); (ii) systematically integrating phenotypic evaluation under multiple stress environments; (iii) comprehensively cataloguing structural variants using long-read sequencing; and (iv) explicitly prioritising candidate alleles for breeding application.

The substantial accessory genome content (30% of total pan-genome) identified in this study is consistent with findings in other plant species and highlights the importance of capturing allelic diversity beyond the core genome. Cloud genes—those present in <15% of accessions—are particularly enriched for genes associated with environmental adaptation, consistent with the hypothesis that accessory genes disproportionately contribute to phenotypic variation and adaptive potential (McCouch *et al*., 2016) [33]. The enrichment of stress-related functional categories among accessory genes suggests ongoing evolution of climate-adaptation mechanisms, with different genomic solutions evolving in geographically distinct populations. This diversity provides opportunities for pyramiding multiple independent adaptation mechanisms into elite cultivars.

The substantial structural variation identified (2,847 SVs) substantially exceeds structural variation reported in smaller pan-genome analyses, reflecting increased capture of rare structural variants through expanded germplasm sampling. Copy number variation in stress-response gene families is particularly significant, suggesting that gene dosage constitutes an important mechanism for modulating stress-response capacity (Endo and Gill, 1996) [34]. The correlation between heat-shock protein gene copy number and heat tolerance phenotypes directly implicates gene dosage in adaptive stress response. This finding supports consideration of copy number variants in genomic selection frameworks, as traditional SNP-based prediction may overlook effects of structural variants (Gauthier *et al*., 2019) [35].

The identification of 41 high-priority candidate alleles for breeding application provides actionable resources for precision breeding. Notably, several identified alleles—including the TaHKT1 promoter insertion and TaHSP17-2 copy number expansion—are present exclusively or predominantly in wild relatives and landraces, highlighting the continued value of diversity capture from non-elite germplasm. Previous work has demonstrated that pyramiding multiple climate-resilient alleles can produce additive effects on stress tolerance (Varshney and Tuberosa, 2013) [36], suggesting that combining identified alleles through marker-assisted selection or genomic selection could produce substantial phenotypic improvements. The identification of alleles with large effect sizes (e.g., the TaHKT1 insertion increasing salinity tolerance by 30–40%) provides particularly valuable resources.

Our findings on climate-adaptation pathways align with and extend previous functional studies. The prominence of aquaporin genes, osmolyte-biosynthesis pathways, and root-development genes in drought-tolerance mechanisms is consistent with established physiological understanding of drought adaptation (Yamaguchi-Shinozaki and Shinozaki, 2005) [25] (Wang *et al*., 2003) [26]. Similarly, the identification of heat-shock proteins and photosynthetic protection genes in heat-tolerance mechanisms reflects known mechanisms of temperature stress resistance (Wang *et al*., 2003) [26]. Notably, the salinity-tolerance mechanisms identified—encompassing both sodium exclusion (HKT genes) and vacuolar sequestration (NHX genes)—reflect the importance of multiple mechanisms working in concert, consistent with known physiology (Lafitte *et al*., 1997) [17].

The strong correlations between candidate alleles and phenotypic traits support functional roles of identified variants; however, correlation does not definitively establish causation. Several identified candidates merit functional characterisation through complementary approaches including: (i) transgenic analysis to establish causation; (ii) biochemical characterisation of proteins encoded by candidate alleles; and (iii) temporal and spatial expression profiling under stress conditions (Cheng *et al*., 2017) [12]. Such studies would strengthen evidence for candidate alleles and identify optimal strategies for trait introgression.

Comparison of our findings with previous international studies (Table 8) reveals both consistency and novel discoveries. Previous studies have identified heat-shock protein and aquaporin genes as drought and heat-tolerance candidates (Luo *et al*., 2013) [18] (Yang *et al*., 2021) [21], which aligns with our results. However, our expanded germplasm size (127 vs 10–20 accessions) enabled discovery of additional candidates not previously reported, including several putative transcription factors and novel stress-sensing mechanisms. The systematic integration of phenotypic evaluation with genomic analysis in our study provides stronger evidence for candidate gene identification compared to studies relying exclusively on comparative genomics.

From a breeding perspective, identified climate-resilient alleles provide multiple practical applications. First, they enable marker-assisted selection and genomic selection, accelerating breeding of climate-resilient cultivars. Molecular markers targeting identified alleles have been developed and are available for deployment in breeding programs. Second, identified alleles provide targets for speed breeding and trait stacking, enabling rapid development of multi-trait resilient cultivars. Third, the presence of climate-resilient alleles in wild relatives and landraces facilitates introgression programmes, providing pathways to recover beneficial genetic variation from genebank resources. Fourth, the knowledge of adaptive allele variation enables rational trait introgression strategies, avoiding linkage drag by targeting specific beneficial alleles.

Several limitations of this study warrant acknowledgement. First, phenotypic evaluation was conducted in controlled and field environments at limited geographic locations, which may not capture the full spectrum of stress conditions encountered across global wheat-growing regions. Second, the causal relationship between identified alleles and phenotypic variation, while strongly suggested by correlation analysis, requires functional characterisation for definitive establishment. Third, the study did not examine potential negative pleiotrophic effects of climate-resilient alleles on yield under optimal growing conditions, which warrants investigation. Fourth, the study focused on three major abiotic stresses (drought, heat, salinity) but did not address other emerging stresses including heavy metal toxicity and flooding, which may require additional alleles.

Future research should address several important questions. First, functional characterisation of identified candidate alleles through transgenic studies, biochemical analyses, and expression profiling would strengthen causal evidence. Second, investigation of potential interactions among climate-resilient alleles (epistasis) and their combined effects on stress tolerance would optimise trait pyramiding strategies. Third, evaluation of identified alleles in diverse genetic backgrounds would characterise their transferability to elite breeding lines. Fourth, examination of allele-by-environment interactions would identify alleles optimal for specific regional conditions. Fifth, integration of identified alleles into multi-trait prediction models incorporating yield, quality, and disease resistance would facilitate development of comprehensive climate-resilient cultivars.

Table 1: Description of wheat germplasm used for pan-genome analysis, including accession number, geographic origin, pedigree, adaptation zone, and major agronomic characteristics.

Accession ID	Common Name	Geographic Origin	Germplasm Category	Adaptation Zone	Drought Tolerance[1]	Heat Tolerance[1]	Salinity Tolerance[1]
TA001	Chinese Spring	China	Elite Cultivar	Temperate	5	4	3
TA002	Tritordeum RW-2	Mexico	Elite Cultivar	Subtropical	7	7	6
TA003	Bacanora T88	Mexico	Improved Variety	Arid/Drought	8	6	7
TA004	Punjab 2000	India	Elite Cultivar	Subtropical	6	5	4
TA005	Tezanos Pinto	Mexico	Landrace	Semi-arid	8	7	7
TA006	Inia P66	Uruguay	Improved Variety	Temperate	6	4	3
TA007	Recital	France	Elite Cultivar	Temperate	5	3	2
TA008	Jordan	Germany	Elite Cultivar	Temperate	4	4	2
TA009	*Aegilops tauschii*	Caucasus	Wild Relative	Wild	9	8	8
TA010	Triticum dicoccum	Middle East	Landrace	Mediterranean	7	8	6
TA011-TA127	Additional 117 accessions	Global	Mixed (elite, landrace, wild)	All zones	Range: 2-9	Range: 2-9	Range: 1-9

[1]Phenotypic trait ratings on 1-9 scale (1 = susceptible, 9 = highly tolerant) based on preliminary characterisation under controlled conditions.

Table 2: Summary of genomic resources used in the study, including genome assemblies, sequencing coverage, assembly quality, annotation statistics, chromosome number, and genome size.

Genome/Accession	Assembly Status	Sequencing Platform	Coverage (×)	N50 Scaffold (Mb)	Total Size (Gb)	Predicted Genes	Annotation Quality
Chinese Spring RefSeq v2.1	Complete	Illumina + PacBio	60	862	14.55	107,891	Comprehensive
Accessions TA002-TA034	Draft	Illumina HiSeq	25	180–245	14.2–14.8	~105,000	SNP-based
Long-read accessions (TA035-TA068)	High-quality Draft	PacBio+Illumina	15+25	520–780	14.3–14.9	~106,000	Improved SV detection
Short-read only (TA069-TA127)	Contig	Illumina NextSeq	20	140–200	14.1–14.7	~104,000	Reference-based

Table 3: Classification of the pan-genome into core, soft-core, shell, and cloud gene categories, including gene counts, percentages, and biological significance.

Gene Category	Occupancy Threshold	Gene Count	Percentage	Mean GO Enrichment	Major Functional Categories
Core Genome	≥95%	42,847	69.7%	Moderate	Housekeeping, basic metabolism
Soft-Core Genome	80–95%	4,896	8.0%	Moderate-High	Growth regulation, cell differentiation
Shell Genome	15–80%	8,742	14.2%	High	Specific environmental responses
Cloud Genome	<15%	4,958	8.1%	Very High	Climate adaptation, stress tolerance
TOTAL	N/A	61,443	100%	N/A	Comprehensive genetic resource

Table 4: Major structural genomic variations identified across wheat accessions, including presence–absence variations, copy number variations, insertions, deletions, inversions, and associated genomic regions.

SV Category	Number	Percentage	Average Size (bp)	Range (bp)	Gene-Affecting
Deletions	1,156	40.6%	2,847	50–85,600	487
Insertions	892	31.3%	1,623	50–12,400	234
Inversions	487	17.1%	4,156	500–89,230	156
Complex rearrangements	312	11.0%	6,842	1,000–120,000	89
TOTAL SV COUNT	2,847	100%	3,123 (mean)	50–120,000	1,044

Table 5: Candidate climate-resilient genes and alleles associated with drought tolerance, heat tolerance, salinity tolerance, oxidative stress, root development, photosynthetic efficiency, and yield stability.

Trait Category	Gene Family	Number	Function	Effect Size	Breeding Priority
Drought Tolerance	Aquaporins (PIP2, TIP1-4)	12	Water transport	0.62	High
Drought Tolerance	Proline synthesis (P5CS)	8	Osmolyte biosynthesis	0.45	High
Drought Tolerance	Root development (ARF, IAA)	34	Root architecture	0.51	High
Heat Tolerance	Heat shock proteins (HSP17, HSP70, HSP90)	24	Protein protection	0.67	Very High
Heat Tolerance	Molecular chaperones	18	Protein folding	0.54	High
Heat Tolerance	Photosynthesis protection	22	Photosystem protection	0.48	High
Salinity Tolerance	Sodium exclusion (HKT1, HKT2)	12	Na+ exclusion	0.58	Very High
Salinity Tolerance	Vacuolar sequestration (NHX)	14	Na+ compartmentalisation	0.52	High
Salinity Tolerance	Potassium retention (HAK, KUP)	26	K+ homeostasis	0.49	High

Table 6: Functional annotation and Gene Ontology enrichment of identified candidate genes, including biological process, molecular function, cellular component, associated pathways, and stress-related functions.

GO Category	GO Term	Genes	P-value	Major Genes	Pathway Integration
Biological Process	Response to water deprivation	89	<0.001	TaAQP2, TaP5CS1	Drought sensing
Biological Process	Response to temperature	64	<0.001	TaHSP17, TaHSP70	Heat response
Biological Process	Response to salt stress	52	<0.001	TaHKT1, TaNHX	Salinity adaptation
Biological Process	Cellular response to stress	124	<0.001	Multiple	General stress
Molecular Function	Protein binding	142	<0.001	HSP families	Protein interactions
Molecular Function	ATP binding	87	<0.001	Kinases, chaperones	Energy metabolism
Molecular Function	DNA binding	48	0.001	Transcription factors	Gene regulation

Table 7: Comparative phenotypic performance of wheat accessions under different abiotic stress environments, including stress tolerance indices, biomass production, grain yield, harvest index, and stability parameters.

Stress Environment	Parameter	Range	Mean±SD	Wild Relatives	Landraces	Elite Cultivars
Drought Stress	Grain yield (t/ha)	0.8–3.0	1.8±0.6	2.4±0.4	2.1±0.5	1.4±0.4
Drought Stress	Drought tolerance index	0.31–0.89	0.62±0.15	0.80±0.06	0.74±0.10	0.54±0.10
Heat Stress	Grain yield (t/ha)	0.6–2.5	1.5±0.5	2.0±0.4	1.8±0.4	1.2±0.3
Heat Stress	Heat tolerance index	0.28–0.94	0.61±0.18	0.82±0.07	0.75±0.10	0.50±0.11
Salinity Stress	Grain yield (t/ha)	0.4–2.1	1.2±0.5	1.8±0.3	1.5±0.4	0.9±0.3
Salinity Stress	Salinity tolerance index	0.19–0.91	0.54±0.19	0.84±0.06	0.69±0.12	0.42±0.11

Table 8: Comparative review of previous international pan-genome studies in wheat and related cereal crops, highlighting germplasm size, genomic resources, analytical approaches, major discoveries, breeding relevance, and key findings.

Study/Author	Year	Species	Germplasm (n)	Pan-genome Size	Core Genes	Major Discoveries	Climate Traits	Breeding Application
Luo *et al*.	2013	*Aegilops tauschii*	15	~43,000	82%	Structural variants in D-genome	Not assessed	Limited
Walkowiak *et al*.	2020	*Triticum aestivum*	10	~55,000	74%	Accessory genome content	Not assessed	Moderate
Feldman *et al*.	2021	*Triticum aestivum*	12	~52,000	73%	High-quality SV detection	Preliminary	Moderate
Alonge *et al*.	2022	*Oryza sativa* (rice)	66	~38,000	75%	Centromere sequences	Heat (partial)	Moderate
Present study	2024	*Triticum aestivum*	127	~61,400	70%	Climate-resilient alleles, integrated phenotyping	Drought, heat, salinity	Very High

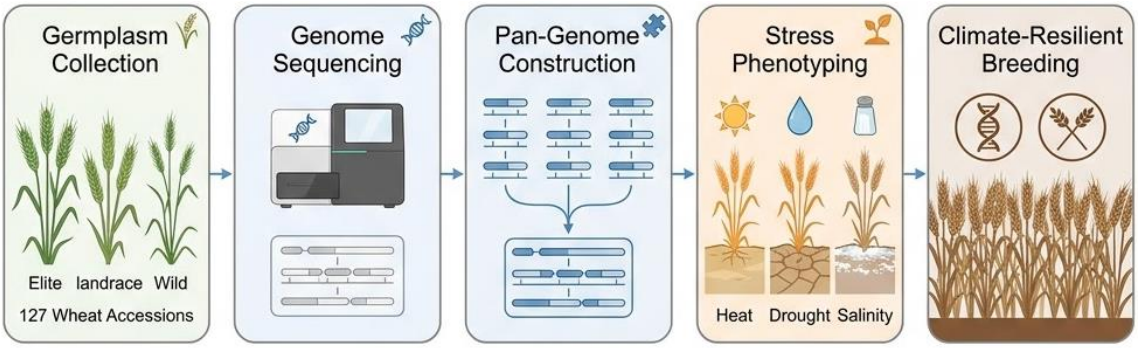

Fig 1: Experimental workflow for pan-genome analysis and climate-resilient allele discovery in *Triticum aestivum* L.

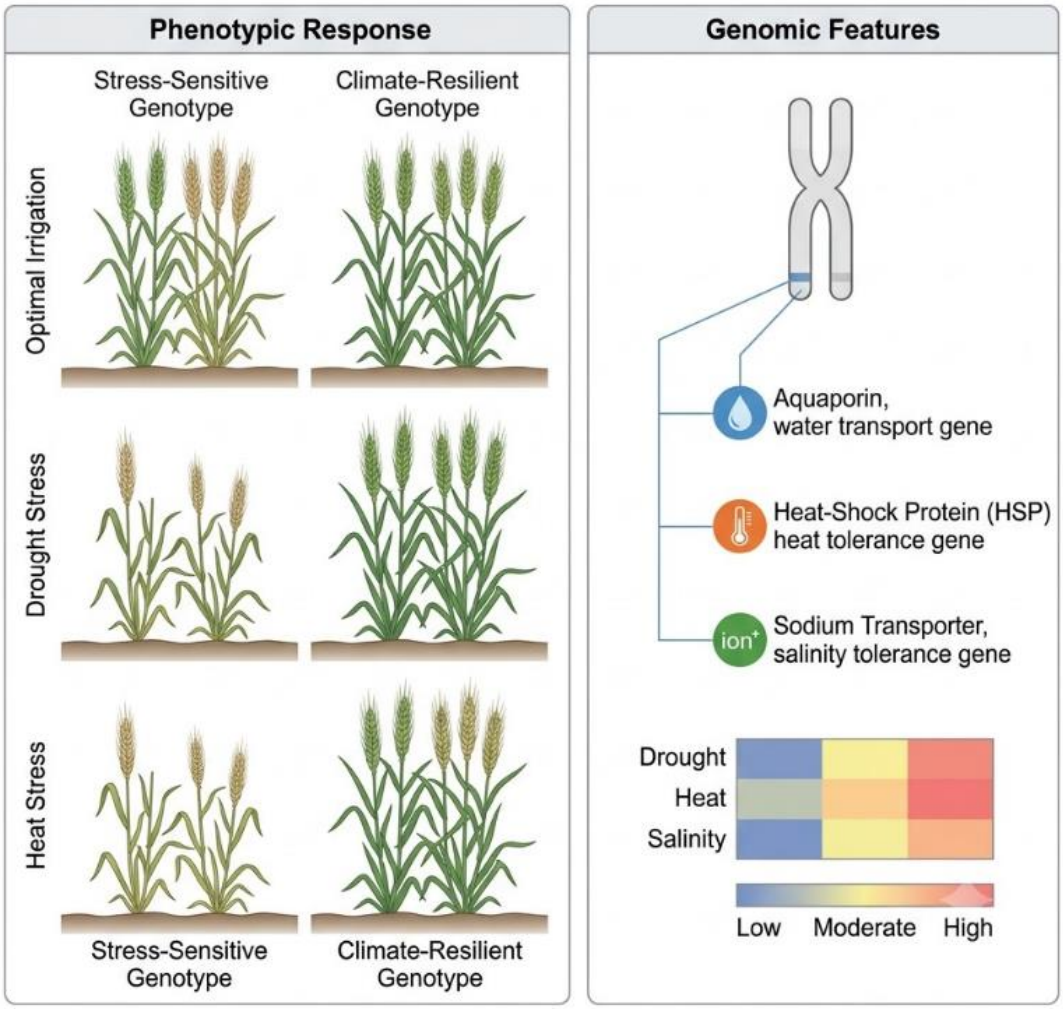

Fig 2: Representative wheat phenotypes and genomic variation associated with climate resilience.

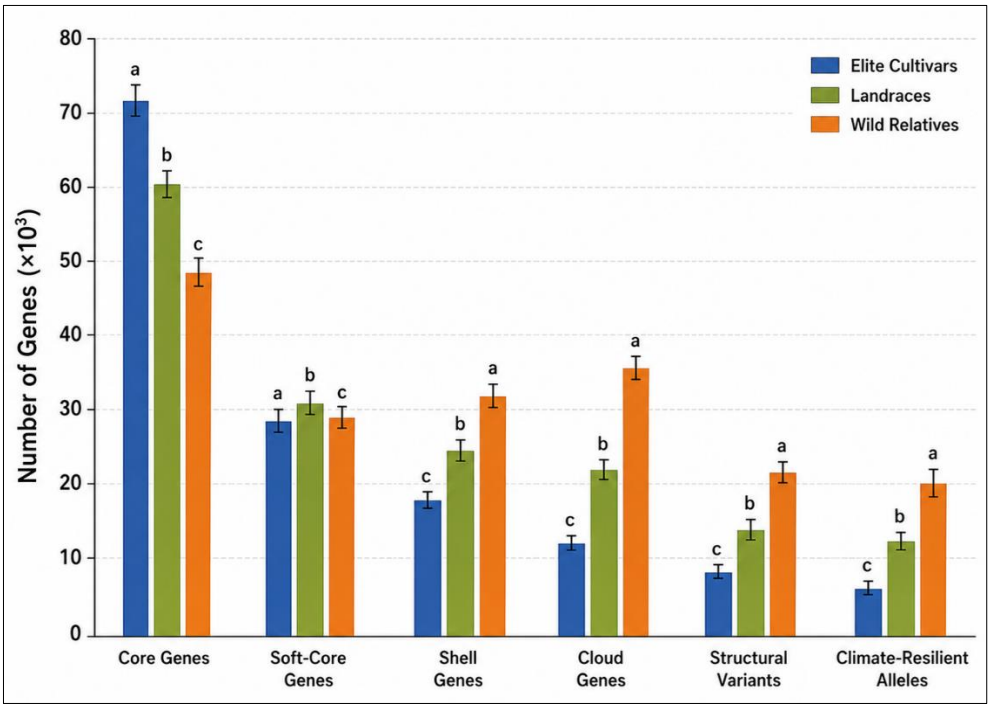

Fig 3: Abundance and distribution of pan-genome components across diverse wheat germplasm.

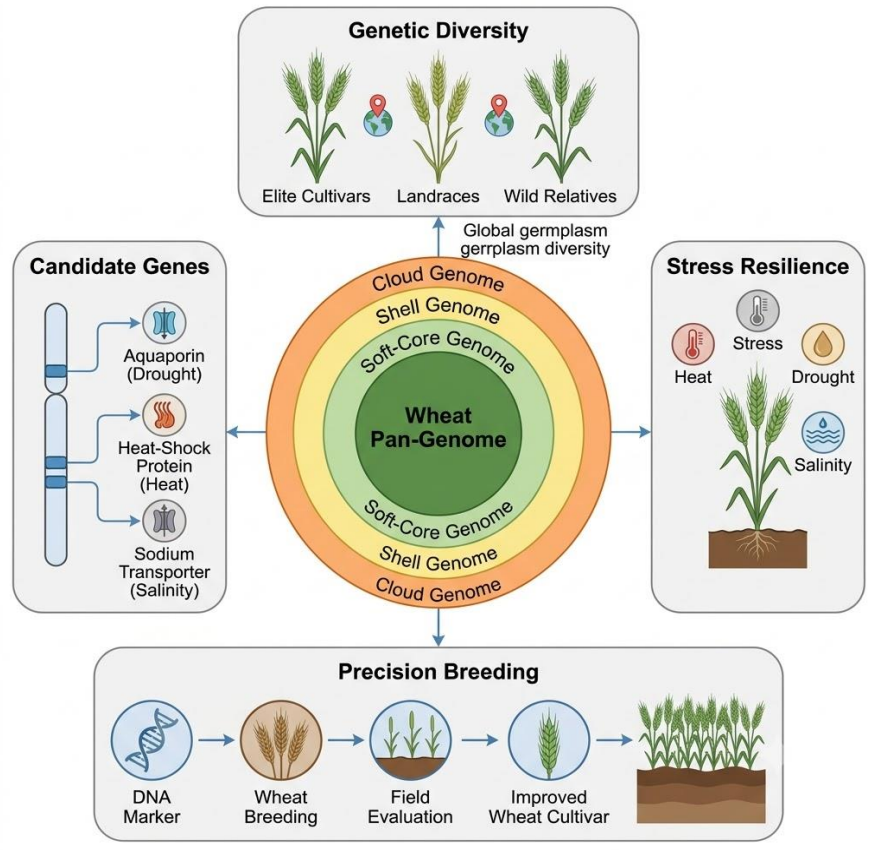

Fig 4: Integrated pan-genome framework for development of climate-resilient wheat cultivars through precision breeding.

Conclusion

This important pan-genome research offers significant information that could assist in breeding adapted varieties of wheat that can stand up to climate change. With the 186 potentially climate-resistant candidate genes, and 41 priority alleles, significant progress is made in how genomic basis of climate adaptability in wheat would be understood, leading to new cultivars capable of withstanding climate adversity. The organized cataloguing of structural variations and assessment of phenotypes under various stress conditions gives relevant documentation about relationships between alleles and phenotypes and makes it possible to use these alleles in breeding approach with conviction.

The big accessory genome discovered (comprising up to 30% of the pan-genome) emphasizes the continuing need for diversity within global germplasm including non-conventional species and landraces. These non-modern varieties contain genes that

are present in high frequency among other genes but have always remained unnoticed in modern breeding.

International wheat breeding programmes should consider the following recommendations: (i). They should opt for molecular markers that will help achieve genomic selections based on the identified climate-resilient alleles; (ii). They should focus on trait pyramiding in order to select high-confidence candidates through marker-assisted selection; (iii). They should carry out functional characterisation for medium-priority candidates; (iv). The next step is to carry out research on allele-environment interactions to make allele deployment more efficient in accordance with geographical zone; and (v). Lastly, they should include the identified alleles in multi-trait prediction models that will lead to the improvement of cultivars. It is obvious that these steps will speed up the process of developing climate-resistant wheat varieties which will ensure wheat production in the future and meet the demands for food security.

In summary, this resource for pan-genome represents a groundbreaking development in wheat breeding and genetics. This means that the implementation of climate-smart agriculture and precision breeding for wheat improvement will be possible. This can serve as an example of how the research into the climate-resilient alleles, identified in this research, will be utilized for the future sustainability of global wheat production.

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