Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Molecular Marker Based Assessment of Wheat Biodiversity for Conservation and Sustainable Breeding

Mohammed Alqurashi
Taif University
Salman Aloufi
Taif University
Doha A. Albalawi
University of Tabuk
Amenah S. Al-otaibi
University of Tabuk
Basmah M. Alharbi
University of Tabuk
Fahad Mohammed Alzuaibr
University of Tabuk
Hanan khalaf anazi
University of Tabuk
Siham M. AL-Balawi
University of Tabuk
Shafik D. Ibrahim
Agricultural Genetic Engineering Research Institute (AGERI)
Wasima Al-Shammari
University of Hail
Dalal Alenizi
University of Hail
Ahmed F. Abd El-Hakim
Ahmedgyn@azhar.edu.eg

Al-Azhar University

Research Article

Keywords:

Posted Date: July 16th, 2025

DOI: https://doi.org/10.21203/rs.3.rs-6959182/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Biodiversity and Conservation on March 5th, 2026. See the published version at https://doi.org/10.1007/s10531-026-03271-6.

Abstract

Background

Wheat (*Triticum aestivum* L.) is a vital cereal crop, providing a substantial share of dietary calories and protein. However, climate variability, including heat stress, drought, and irregular rainfall, poses serious challenges to wheat production, particularly in regions already experiencing food insecurity. Enhancing genetic diversity and stress tolerance is crucial for sustaining wheat yields under changing environmental conditions. However, intensive breeding for high-yielding cultivars has led to a reduced genetic base, limiting adaptability and resilience.

Objectives

This study aims to assess the genetic diversity of 40 wheat genotypes collected from diverse agro-climatic zones using a combination of molecular marker systems: simple sequence repeats (SSR), inter-simple sequence repeats (ISSR), and amplified fragment length polymorphism coupled with capillary electrophoresis (AFLP-CE). The goal is to characterize genetic relationships, detect polymorphisms, and provide insights to support conservation and sustainable breeding strategies.

Methods

Genotypic differentiation was analyzed using a combination of SSR, ISSR markers, and high-resolution fragment analysis of AFLP-CE to ensure comprehensive genome-wide polymorphism detection. The genetic relationships among wheat genotypes were inferred through similarity matrix analysis and hierarchical clustering, facilitating a robust framework for classification and selection.

Results

The integrated marker approach revealed substantial genetic variability among the studied wheat genotypes, reflecting diverse ecological adaptations. AFLP-CE enhanced the resolution of rare and informative polymorphisms, improving genotype discrimination. These results highlight the richness of genetic resources that can be harnessed to improve stress tolerance and yield stability.

Conclusion

This study underscores the effectiveness of combining SSR, ISSR, and AFLP-CE for the complete evaluation of wheat biodiversity. The findings support conservation and targeted breeding strategies for improved resilience and sustainable yield under climate variability.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is a major worldwide cereal crop, contributing approximately 20% of total caloric and protein intake; thus, ensuring its genetic resilience is crucial for sustaining global food security **(FAO, 2023).** However, climate change presents considerable challenges to wheat production, particularly in areas vulnerable to heat stress, drought, and irregular rainfall patterns **(**Pequeno et al., 2021). Global wheat yields are expected to fall by 1.9% by mid-century, with more severe reductions (15–16%) estimated in North Africa and South Asia, exacerbating food insecurity **(**Pequeno et al., 2021; **USDA, 2025).**

To sustain wheat productivity under increasing pressures of environmental conditions, breeding programs must enhance stress tolerance, yield stability, and genetic diversity **(**Langridge et al., 2021). However, due to decades of intensive selection for high-yielding cultivars, the genetic base of wheat has narrowed, reducing its adaptability to the changing climatic conditions **(**Reif et al., 2005; Voss-Fels et al., 2015). A comprehensive understanding of wheat biodiversity and genetic adaptation is therefore crucial for the development of climate-adaptable cultivars **(**Lopes et al., 2015; Mondal et al., 2016). Egypt, with its diverse agro-climatic zones ranging from hot arid desert to semi-arid environments, serves as an ideal location for studying wheat adaptation to variable climates **(**Tadesse et al., 2019; Hamada et al., 2022; Elkot et al., 2024).

Molecular marker technologies provide efficient techniques for the genetic description of wheat biodiversity, supporting marker-assisted selection (MAS), and finding unique genotypes with adaptive traits. Among these, SSRs and ISSRs have been widely employed because of their specificity and genome coverage **(**Landjeva et al., 2007; Soriano, 2020**).** In parallel, AFLP coupled with capillary electrophoresis (AFLP-CE) provides high-throughput, fine-scale resolution for identifying polymorphisms, particularly those that are undetected on gel-based platforms **(**Papa et al., 2005). The use of AFLP-CE enhances marker sensitivity and reproducibility, making it especially valuable in complex genomes like wheat. Simple sequence repeats (SSR), inter-simple sequence repeats (ISSR), and amplified fragment length polymorphisms (AFLP) have all proven an outstanding ability in genetic differentiation and genome-wide polymorphism identification **(**Landjeva et al., 2007; Noli et al., 2008). However, a comprehensive analysis using integrative and complementary multi**-**marker systems is still required to fill knowledge gaps and gain a precise evaluation of wheat genetic structure, aiding both conservation and breeding efforts.

The present study aims to evaluate the genetic diversity of 40 wheat genotypes across different agro-climatic zones using SSR, ISSR, and AFLP-CE molecular markers. The inferred genetic relationships, based on similarity matrix analysis and hierarchical clustering, will provide a robust framework for genetic classification. Meanwhile, our findings will contribute to wheat conservation efforts and breeding programs aimed at improving stress tolerance and yield potential under climatic variability.

MATERIALS AND METHODS

Plant Materials and Field Trials:

Field experiments were conducted over two consecutive growing seasons (2021/2022–2022/2023) at the Shandaweel Agricultural Research Station in Sohag Governorate, Egypt (26°33'N, 31°42'E). As specified in Table 1 and Figure 1, the present study evaluated forty wheat (*Triticum aestivum* L.) landraces (G1–G40), collected from diverse agro-climatic regions across Egypt, including Upper Egypt, the New Valley, and North Sinai, by the Egyptian National Gene Bank. These genotypes were assigned accession numbers 111320 to 111370, reflecting their official registration in the national germplasm collection **(Egyptian Gene Bank, 2024).**

To investigate genetic diversity and agronomic performance, the wheat genotypes were regenerated and evaluated under controlled field conditions. A randomized complete block design (RCBD) with three replicates was employed for the experiments to ensure reliable statistical analysis. Standard agronomic practices were implemented to ensure optimal growth conditions throughout both seasons. Each genotype was cultivated in six rows, each measuring 2.5 meters in length, with 20 cm spacing between rows. Individual plants within rows were spaced 20 cm apart. The field trials were conducted under conventional irrigation, with five irrigation cycles per season. Data on morphological and yield component traits were collected from ten randomly selected guarded plants per genotype.

Where:

- *Hot Arid*: Extremely high temperatures and low precipitation.
- *Hot Semi-Arid*: High temperatures with slightly higher moisture availability than arid regions.
- *Arid*: Dry conditions with very low annual precipitation.
- *Semi-Arid*: Moderately dry conditions with some seasonal rainfall.

DNA Extraction, Purification, and Quantification:

Genomic DNA was extracted from the young leaves of each accession using the DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany). The quantity and quality of the extracted DNA were determined using spectrophotometric measurements of UV absorbance at 260 nm and 280 nm on a Thermo Scientific NanoDrop 2000™ spectrophotometer.

Simple Sequence Repeat Analysis:

Simple Sequence Repeat (SSR) markers were utilized for genetic diversity assessment and polymorphism detection in the studied wheat genotypes. Ten SSR primers selected for their specificity to key wheat chromosomes and extensive genome coverage, were used for amplification (Table 2). The GrainGenes Database for Triticeae and Avena (http://wheat.pw.usda.gov) included the SSR primer sequences **(Yao et al. 2022).**

PCR reactions were carried out in a 25 µl mixture consisting of 12.5 µl Master Mix (Sigma), 1 µl forward primer, 1 µl reverse primer (10 pmol/µl each), 2.5 µl template DNA (10 ng/µl), and 8 µl dH_2O, following the protocol outlined by **Ibrahim et al. (2016).** Amplification was performed using a Perkin-Elmer/GeneAmp® PCR System 9700 under the following thermal cycling conditions: initial denaturation at 94ºC for 5 minutes, followed by 40 cycles of denaturation at 94ºC for 30 seconds, annealing at primer-specific temperatures for 30 seconds, and extension at 72ºC for 40 seconds. A final extension at 72ºC for 7 minutes was applied to ensure complete fragment synthesis.

Inter Simple Sequence Repeat Analysis

Similarly, Inter Simple Sequence Repeat (ISSR) markers were employed to assess genetic diversity and detect polymorphisms among the examined wheat genotypes. Ten ISSR primers, synthesized by Sigma-Aldrich (USA), were used for amplification (Table 3). PCR reactions were conducted in a 25 µl mixture containing 12.5 µl Master Mix (Sigma), 2.5 µl primer (10 pmol/µl), 3 µl template DNA (10 ng/µl), and 7 µl dH_2O, following the protocol described by **Ibrahim et al. (2019).** Amplification was performed using a Perkin-Elmer/GeneAmp® PCR System 9700 with an initial denaturation at 94ºC for 5 minutes, followed by 40 cycles of denaturation at 94ºC for 1 minute, annealing at 45ºC for 1 minute, and extension at 72ºC for 1.5 minutes. A final extension step at 72ºC for 7 minutes was applied to ensure complete amplification.

Amplified Fragment Length Polymorphism Analysis:

The Amplified Fragment Length Polymorphism (AFLP) assay was performed according to the AFLP Plant Mapping protocol **(Applied Biosystems, 2010).** Genomic DNA was digested with the restriction enzymes *EcoRI* and *MseI* to generate modified restricted fragments for the pre-selective amplification step. Adaptors were ligated to the digested fragments using *T4 DNA ligase* **(New England BioLabs, Ipswich, MA, USA),** according to the manufacturer's instructions, to ensure efficient ligation. Selective amplification was conducted using fluorescence-labeled EcoRI primers and unlabeled MseI primers. Three primer combinations were utilized: Eco-$_{AAG}$/Mse-$_{CTC}$, Eco-$_{ACT}$/Mse-$_{CTT}$, and Eco-$_{AAC}$/Mse-$_{CAG}$ (Table 4).

The amplified fragments were prepared for electrophoresis by mixing 1 µL of the selective product with 12 µL Hi-Di™ formamide and 0.5 µL GeneScan™ 500 ROX™ standard (Applied Biosystems, USA). Prior to analysis, the mixture was denatured and loaded onto the Applied Biosystems 310 Genetic Analyzer, which employs a single-capillary system for high-resolution fragment separation using POP-4™ polymer. Fragment analysis was conducted using GeneMapper® software.

Molecular data analysis:

Throughout the assessment of SSR and ISSR marker results, each gel underwent a duplicate assessment to validate the results, whereas the clearly detectable bands were scored as present (1) or absent (0), while any faint bands were discarded.

Binary data obtained from the molecular marker analysis was used to identify key parameters related to the effectiveness of molecular markers, such as polymorphism percentage (P%), polymorphic information content (PIC), marker index (MI), effective multiplex ratio (EMR), and resolving power. These analyses were executed utilizing the Online Marker Efficiency Calculator (https://irscope.shinyapps.io/iMEC/) as developed by **Amiryousefi et al., (2018).**

Statistical and Multivariate Analysis:

All the results of molecular marker analysis, including SSR, ISSR, and AFLP, were combined to create a comprehensive genetic profile of the studied wheat types. Using Jaccard's coefficient **(Jaccard, 1908),** a similarity matrix was created to evaluate the pairwise genetic relationships. Meanwhile, the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) in Multi-Variate Statistical Package (MVSP) software (Version 3.13d; **Kovach, 2002**) was used to perform cluster analysis, which resulted in a dendrogram that illustrates the genetic relatedness based on polymorphic loci among the studied 40 wheat genotypes.

Principal Component Analysis (PCA):

The principal component analysis (PCA) was performed using PAST software **(version 4.03; Hammer et al., 2001)** to reduce dimensionality and capture main variations in the genetic dataset. The amount of variance explained by each main component was calculated to improve genotype differentiation.

Heat Map Visualization:

A heat map analysis was performed using ClustVis, a web-based tool for clustering and visualizing multivariate data **(Metsalu & Vilo, 2015).** Genetic similarity indices derived from SSR, ISSR, and AFLP markers were used to generate a standardized data matrix.

RESULTS AND DISCUSSION

Genotypic Differentiation Using SSR Marker Analysis:

A comprehensive evaluation of genetic diversity among wheat genotypes was conducted using Simple Sequence Repeat (SSR) markers, revealing genetic diversity and genotype-specific correlations across nine chromosomes: 1A, 1B, 3A, 5A, 5B, 6B, and 7A (Table 5, Figure 2). 823 bands were amplified from 41 SSR amplicons with 10 SSR primers. These 41 alleles comprise 35 polymorphic and 6 monomorphic alleles.,

Fragment sizes varied widely, from 153-105 bp for Xgwm99 (SSR10, 1AL) to 1000-175 bp Xgwm369 (SSR12, 3A), indicating amplification across loci with different size ranges. This diversity in product size improves allele resolution and confirms the extensive genomic coverage of the used primer set. Five primers, WMS273 (SSR2, 1B), Xgwm371 (SSR4, 5B), Xgwm219 (SSR5, 6B), Xgwm99 (SSR10, 1AL), and wmc422 (SSR16, 7A) exhibited full polymorphism among the tested genotypes, confirming their high discriminatory potential. In contrast, Xgwm205a (SSR14, 5A) and Xgwm124 (SSR17, 1B) showed the lowest polymorphism levels (50%), limiting their utility in diversity analysis.

Polymorphic alleles with band frequencies (B.F.) ranging from 0.25 to 0.9 were categorized as rare (BF ≤ 0.2), low (0.2 < BF ≤ 0.5), moderate (0.5 < BF ≤ 0.8), and high (BF > 0.8), in addition to 2 unique bands.

The highly informative loci included Xwmc596 (SSR9, 7A) (149 bp) and wmc422 (SSR16, 7A) (150 bp), each amplified in 90% of genotypes (B.F. = 0.9), making them suitable for diversity analysis, population structure assessment, and marker-assisted selection. Moderately frequent loci such as Xgwm234 (SSR13, 5B) (256 bp) (B.F. = 0.775), Xgwm99 (SSR10, 1AL) (113 bp) (B.F. = 0.725), and Xgwm371 (SSR4, 5B) (168 bp) (B.F. = 0.725) were also effective in discriminating among accessions. Conversely, low-frequency loci, including Xgwm371 (SSR4, 5B) (208 bp) (B.F. = 0.25) and Xgwm219 (SSR5, 6B) (176 bp) (B.F. = 0.275), suggest allele restriction to a subset of genotypes, indicating population-specific variations or alleles under selection pressure (**Han et al., 2015).**

Ten rare alleles (B.F. < 0.2) were found; for instance, WMS273 (SSR2, 1B) at 161 bp was found in only three genotypes (G13, G19, and G22), with a B.F. of 0.075, indicating that it is a rare allele with significant discriminating power. Other sorts of bands were found in just one to three genotypes, including WMS273 (SSR2, 1B) at 119 bp (B.F. = 0.05) and 161 bp (B.F. = 0.075); Xgwm369 (SSR12, 3A) at 155 bp (B.F. = 0.05); and Xgwm124 (SSR17, 1B) at 210 bp, demonstrating great discriminatory power. Similar alleles are critical for distinguishing distinct genotypes, such as Xgwm234 (SSR13, 5B) at 263 bp (B.F. = 0.2), Xgwm371 (SSR4, 5B) at 235 bp (B.F. = 0.15), Xgwm99 (SSR10, 1AL) at 105 bp (B.F. = 0.01), and wmc422 (SSR16, 7A) at 160 bp (B.F. = 0.075), and they may represent novel genetic changes important to marker-assisted selection or gene mapping.

Additionally, two markers, Xgwm234 (SSR13, 5B) and wmc422 (SSR16, 7A), produced unique bands, indicating potential for genotype-specific allele identification.

The identified monomorphic markers, each showing a band frequency of 1.0, indicating uniform allele presence across all 40 genotypes, including SSRs targeting Xwmc596 (SSR9) at (279 bp and 237 bp), Xgwm369 (SSR12) at 1000 bp, Xgwm234 (SSR13) at 217 bp, Xgwm205a (SSR14) at 145 bp, and Xgwm124 (SSR17) at 200 bp. While these markers do not contribute to genetic differentiation, they confirm conserved regions in the wheat genome **(Han et al., 2015).** Their presence suggests strong sequence conservation across tested lines, potentially representing core genome regions or housekeeping loci **(Mullan et al., 2005).**

Regarding markers performance, Xgwm371 (SSR4, 5B), Xgwm219 (SSR5, 6B), Xwmc596 (SSR9, 7A), Xgwm99 (SSR10, 1AL), and Xgwm369 (SSR12, 3A), emerged as the most reliable, exhibiting high Polymorphism Information Content (PIC), Effective Multiplex Ratio (EMR), Marker Index (MI), and Discriminating Power (D).

Polymorphic information content (PIC) values ranged from 0.1948 (SSR14) to 0.3750 (SSR13), with an average around 0.35-0.37, categorizing these markers as moderately informative **(Qian et al., 2012).** Higher PIC values observed in SSR4, SSR12, and SSR17 reflect their strong allelic diversity, enhancing their ability to differentiate genotypes. The observed heterozygosity (H.) values were consistent with the polymorphism levels, with SSR17 displaying the highest average heterozygosity (0.0062), suggesting a greater allelic diversity at this locus.

Effective Multiplex Ratio (EMR) values were highest for SSR9 (3.875) and SSR12 (3.325), suggesting efficient polymorphic detection across multiple loci. Corresponding Marker Index (MI) values for these primers (0.0074 and 0.0059, respectively) further emphasize their robustness, combining both informativeness and multiplexing efficiency **(Jahnke et al., 2022).**

Discriminating power (D) and resolving power (Rp) analyses further reinforced the utility of SSR5, SSR12, and SSR16, which showed high D values (≥ 0.77) and Rp values (> 2.0), confirming their strong capacity to distinguish among individuals. Conversely, SSR14 and SSR17 exhibited low D (0.2358 and 0.7275) and very low Rp (0.5 and 0.1), indicating limited effectiveness in resolving genotypic differences.

Overall, SSR4, SSR5, SSR9, SSR10, and SSR12 emerged as the most effective markers, demonstrating high polymorphism, PIC, EMR, MI, and resolving power. These findings recommend their application in future wheat genetic diversity assessments and marker-assisted selection programs.

Xu et al. (2014) employed 34 SSR markers to find polymorphisms in wheat genotypes. Eight markers, including cfa2040, wmc273, and barc32, were discovered to be polymorphic between resistant and susceptible cultivars, allowing the discovery of the stripe rust resistance gene YrC591 for marker-assisted selection in wheat breeding projects. **Wu et al. (2018)** employed SSR and SNP markers to detect wheat genotype differences, identifying a chromosomal area associated with stripe rust resistance on chromosome 1B. The combination of SSR and KASP markers found the resistance gene YrH62, which is critical for wheat breeding selection. **Mohi-Ud-Din et al. (2022)** found 458 alleles in 56 wheat genotypes, with an average of 18.32 per locus, using 30 SSR markers. The polymorphism information content values varied between 0.434 and 0.932, with wmc177 and wms292 having 24 alleles apiece. This genetic diversity is critical for developing drought-tolerant wheat cultivars. Despite their advantages, SSR markers face certain limitations, including time-consuming primer development and relatively high costs, which may hinder broader application **(Nicot et al., 2004).** Nonetheless, their co-dominant inheritance, capacity to distinguish homozygous and heterozygous loci, and overall reliability make them ideal for genetic mapping, cultivar identification, and marker-assisted breeding **(Kalia et al., 2011; Han et al., 2015).** As demonstrated in this study and supported by **Mohamed et al. (2023),** SSR markers offer higher resolution than morphological traits in evaluating genetic diversity among Egyptian wheat genotypes.

Detection of Genome-Wide Variation by ISSR Marker analysis:

The ISSR marker system also demonstrated high utility for assessing genetic variation among the studied wheat genotypes, as illustrated in Table 6 and Figure 3. A total of 3,883 bands (from 124 amplicons) were amplified using ten ISSR primers. Of these, 84 bands were polymorphic, resulting in a mean polymorphism rate of 67.74%, indicating effective allelic diversity detection.

Among the primers, ISSR-7 (92%), ISSR-14 (83%), H24 (83%), and ISSR-9 (80%) recorded the highest polymorphism percentages, highlighting their suitability for distinguishing genotypes. Conversely, H28 and ISSR-8 displayed lower polymorphism levels (27% and 36%, respectively), which limits their effectiveness in diversity studies.

The polymorphism analysis of ISSR markers on wheat genotypes revealed a wide range of allelic diversity, with band frequencies ranging from 0.05 to 0.975. from these polymorphic alleles, 27 high-frequency alleles shared by the majority of wheat genotypes remained polymorphic as a result of continual amplification and varying molecular weight. ISSR5 at 1100 bp, ISSR9 at 1500 bp, ISSR15 at 600 bp, H24 at 970 bp and 1500 bp, H26 at 570 bp, and H28 at 1350 bp (each BF = 0.975) were mainly fixed in the population while staying different from monomorphic loci due to minor but significant genetic exclusions. In addition, 32 moderate-frequency alleles, such as ISSR5 at 830 bp, ISSR14 at 350 bp, and ISSR15 at 1500 bp, have frequencies ranging from 0.55 to 0.6, making them useful for differentiating subpopulations and marker-assisted breeding, as they offer a larger allele distribution with intermediate informativeness. Among the polymorphic alleles, 25 low-frequency alleles, including ISSR5-400 bp and H26-1800 bp, are markers of intermediate variation, useful for analyzing population structure and selection inspections. Alleles like ISSR6 at 530 bp, ISSR7 at 2400 bp, and H26 at 320 bp show variations between accessions. Eight rare alleles were identified, indicating highly discriminative loci in a few genotypes. These include ISSR14 at 460 bp (BF = 0.1), which is found in only two genotypes; ISSR9 at 250 bp (BF = 0.05), 500 bp (BF = 0.2), and 2300 bp (BF = 0.175); ISSR8 at 1000 bp (BF = 0.175); H24 at 650 bp and 350 bp (BF = 0.15 and 0.175, respectively); and H26 at 1650 bp (BF = 0.15), highlighting specific variability, potentially indicating adaptive or unique genotypic traits relevant for breeding.

In contrast, the present investigation identified 40 monomorphic alleles in the 40 wheat genotypes using the ISSR primers (Table 3). including ISSR6, ISSR7, ISSR8, ISSR9, ISSR14, ISSR15, H24, H26, and H28 (Figure 3). Each allele has a band frequency of 1.0, indicating uniformity across all genotypes. These loci, such as ISSR6 at 1500 bp, ISSR8 at 1300 bp, ISSR14 at 940 bp, and H28 at 1900 bp, represent genomic regions with no detectable polymorphism in the population. The identified monomorphic bands, possibly from conserved genomic regions or housekeeping genes, confirm sequence conservation and low mutation rates in regions like ISSR15 at 2000 bp and H24 at 1250 bp, respectively. However, certain primers generate multiple monomorphic bands, suggesting potential primer affinity for tandem repeats or conserved SSR-rich regions **(Kumar et al., 2020).**

PIC values ranged from 0.1364 (ISSR-5) to 0.5739 (ISSR-9). Based on criteria from **Qian et al. (2012)** and **Chen et al. (2021),** four primers, ISSR-9, H24, ISSR-8, and ISSR-14, were classified as highly informative (PIC > 0.5), underscoring their utility in wheat genetic research.

Effective Multiplex Ratio (EMR) was highest in ISSR-6 (12.775), ISSR-H26 (11.775), and ISSR-15 (11.375), reflecting their ability to simultaneously detect multiple polymorphic loci. These values led to a high genotyping resolution, valuable for cultivar differentiation and breeding program support **(Najaphy et al.,**

2011). Marker Index (MI) peaked at H26 (0.0074), followed by ISSR-15 (0.0062), and H24 (0.5606), reinforcing the high efficiency of these primers in capturing genomic variation.

ISSR-9 and H24 also recorded the highest heterozygosity values (0.6467 and 0.5606), indicating rich allelic diversity. Discriminating power (D) was greatest for H26 (0.5206), ISSR-15 (0.3401), and H24 (0.2628), while resolving power (Rp), a key parameter in assessing marker resolution, was highest for H26 (4.75), ISSR-9 (4.65), and ISSR-15 (4.55). These results collectively affirm the utility of these primers for differentiating closely related genotypes **(Xiao et al., 2006).**

Generally, ISSR primers ISSR-9, H24, H26, and ISSR-15 demonstrated superior performance across most of the estimated diversity parameters. These markers are particularly suitable for genetic diversity assessments, cultivar identification, and marker-assisted selection in wheat breeding.

Using ISSR markers, **Vaillancourt et al. (2008)** found 41 polymorphic loci in wheat genotypes and amplified 196 bands from wheat, rye, and triticale DNA samples to demonstrate genetic heterogeneity in the examined accessions. **Najaphy et al. (2011)** revealed high genetic diversity by 86 bands from thirty wheat genotypes, with 69 bands (80.2%) being polymorphic, showing genetic variation among the examined cultivars and breeding lines. **Mohamed et al. (2023)** detected 153 ISSR markers, including 85 polymorphic markers, demonstrating high genetic variation among wheat genotypes, with an average polymorphism band per primer of 7.7. However, a notable limitation of ISSR markers is their dominant inheritance pattern, which prevents differentiation between homozygous and heterozygous loci, reducing their utility in fine-scale genetic mapping **(Grover & Sharma, 2016).** Despite this, their capacity to target both coding and non-coding regions makes them a valuable complement to co-dominant markers like SSRs and AFLPs, contributing to a more comprehensive evaluation of genetic diversity **(Reddy et al., 2002).**

High-Resolution Fingerprinting with AFLP-CE Marker Analysis:

As shown in Table 7, the Amplified Fragment Length Polymorphism) technique, coupled with the capillary electrophoresis system (AFLP-CE), and three selected primer combinations, was highly effective in identifying genetic diversity among 40 wheat genotypes. Each primer pair produced a large number of amplicons (264 to 425), almost all of which were polymorphic. This demonstrates the excellent ability of the AFLP-CE technique to capture the genome-wide variation at high resolution.

The E_{AAC}–M_{CAG} combination produced the highest total number of amplicons (425), all of which were polymorphic, including 65 unique amplicons. It also had the highest resolving power (Rp = 179.65) and discriminating power (D = 0.9273), indicating a greater capacity to detect uncommon alleles and distinguish between genotypes. Although it had the lowest average heterozygosity (2.32×10^{-5}), this might be due to marker saturation or allelic fixation within certain regions. E_{AAG}–M_{CTC} followed closely, producing 391 total amplicons and 33 unique ones, with a high effective multiplex ratio (EMR = 129.90) and Rp = 159.30, indicating its broad genome coverage and informative value. The high polymorphism rate (99.49%) reinforces its use in assessing inter-genotypic variation. E_{ACT}–M_{CTT}, while yielding fewer total amplicons (264), had the highest average heterozygosity (3.99×10^{-5}) and a PIC value of 0.3323, making it valuable for detecting minor allelic differences. Its strong discriminating power (D = 0.9093) suggests its usefulness in genotypic differentiation even with fewer loci.

Overall, the integration of AFLP-CE with capillary electrophoresis enabled precise detection of amplified loci with high polymorphic potential. The combination of high PIC, EMR, and resolving power across primer sets confirms the reliability of this platform in molecular characterization of wheat germplasm. These results support the adoption of AFLP-CE in marker-assisted selection, genetic diversity studies, and pre-breeding programs targeting complex traits **(Papa et al., 2005).**

The AFLP (Amplified Fragment Length Polymorphism) approach, when paired with capillary electrophoresis technology (AFLP-CE), provides an enhanced platform for high-resolution fragment analysis, improved reproducibility, and multiplexing efficiency **(Papa et al., 2005; Blignaut et al., 2013).** Unlike traditional gel-based AFLP technique, which can introduce electrophoretic variability, the use of the Applied Biosystems 310 Genetic Analyzer in this study allowed for precise peak separation using single-capillary electrophoresis and POP-4 polymer technology **(Wenz et al., 1998; Blignaut et al., 2013).** This technique guarantees precise and uniform DNA movement, decreasing signal variability that often affects manual polyacrylamide gel electrophoresis **(Papa et al., 2005; Blignaut et al., 2013).**

The incorporation of GeneMapper software improved the AFLP procedure by automating peak identification, fragment size, and allele binning, reducing subjective bias in AFLP scoring and enhancing data uniformity **(Holland et al., 2008).** Furthermore, fluorescence-based detection increased the sensitivity and multiplexing capability of the AFLP approach, allowing for simultaneous examination of numerous AFLP peaks **(Schwarz et al., 2000).** The absence of post-PCR gel handling significantly reduced contamination hazards, boosting overall data integrity and increasing the dependability of the AFLP technique in high-throughput genetic screening and genome-wide diversity studies **(Papa et al., 2005; Blignaut et al., 2013).**

AFLP markers also provided valuable insights into genotypic differentiation across contrasting agro-climatic zones. Genotypes from Semi-Arid regions (Sohag, Assiut, and Beni Suef) exhibited the highest number of unique AFLP peaks, suggesting the presence of selection-driven genomic variations. Additionally, wheat genotypes from North Sinai (G40) displayed distinct AFLP patterns, potentially reflecting genetic adaptations to salinity stress and fluctuating moisture availability **(Hazen et al., 2002).** The combination of AFLP with capillary electrophoresis enabled the detection of exceptionally high polymorphism levels (99.49%–100%), confirming its effectiveness in wheat molecular breeding and conservation programs **(Papa et al., 2005).** These results underscore the superior capacity of AFLP markers for high-resolution genetic fingerprinting, making them a powerful tool for assessing genetic variability and guiding marker-assisted selection (MAS) strategies.

Integration of SSR, ISSR, and AFLP Markers for Sustainable Wheat Breeding:

The integration of SSR, ISSR, and AFLP markers in this study provided an intensive and complementary approach to analyzing wheat biodiversity. This resulted in higher accuracy for detecting genome-wide variations and locus-specific polymorphisms **(Noli et al., 2008; Malik et al., 2012; Costa et al., 2016).** Meanwhile, SSR markers, due to their co-dominant nature and trait association potential, are widely applied in trait-specific selection, such as drought tolerance, disease resistance, and grain quality **(Han et al., 2015).** ISSR markers facilitate the detection of genome-wide polymorphisms, which is valuable in identifying genotypic responses to abiotic stress **(Reddy et al., 2002; Mohamed et al., 2023).** AFLP markers, despite their complexity, contribute significantly to mapping multigenic traits, like resistance to biotic diseases and yield stability **(Bai et al., 1999; Guo et al., 2003; Liu et al., 2019).** The combined marker analysis is particularly advantageous for marker-assisted selection (MAS) and quantitative trait loci (QTL) mapping,which in turn leads to the identification of climate-resilient genotypes and the preservation of genetic diversity, which are important aspects of sustainable wheat improvement. However, while the integration of these markers enhances genetic resolution, it also presents practical challenges such as data consistency, higher operating costs, and technical demands **(Agarwal et al., 2008; Soriano, 2020).** Along with the integration of molecular marker techniques, future investigations should benefit from the availability of single-nucleotide polymorphism (SNP) arrays and genotyping-by-sequencing (GBS). Combining these high-throughput technologies with marker-assisted selection (MAS) and genome-wide association studies (GWAS) is critical for enhancing breeding efficiency and accelerating genetic gains **(Jiangfeng et al., 2014; Rasheed & Xia, 2019).**

Genetic Similarity Matrix: Assessing Relationships Among Wheat Genotypes

The study of wheat genotypes using molecular marker techniques revealed significant genetic variations, highlighting the importance of multi-locus fingerprinting in understanding population structure and diversity in wheat landraces. The pairwise similarity utilizing Jaccard coefficients ranged from 0.253 to 0.827 (table 8), indicating a broad spectrum of genetic distances and the presence of closely related and genetically divergent members. This variability can be attributed to the rich evolutionary history, local adaptation, and complex seed exchange networks in Egyptian wheat germplasm **(Balfourier et al., 2019).**

According to earlier research, closely related wheat varieties cluster together based on molecular markers **(Peng et al., 2000; Fufa et al., 2005).** The highest similarity (J = 0.827) was found between G28 and G29, which were collected from surrounding villages in Sohag Governorate. This strongly indicates that similarly adapted regional landraces have almost identical genetic histories, most likely due to localized seed circulation, shared environmental pressures, and limited external genetic inflow, as previously reported for closely adapted regional landraces **(Ijaz & Khan, 2009).** Another relatively high similarity (J = 0.698) was found between G38 (Assiut) and G39 (Beni Suef), two landraces from Upper Egypt but different governorates. This inter-regional similarity could reflect a common ancestral lineage or recent seed exchange between the regions, emphasizing the dynamic nature of traditional wheat cultivation systems in Upper Egypt.

By contrast, G19 and G22, both from Upper Egypt, showed only moderate similarity (J = 0.443). This finding contradicts the assumption of regional homogeneity within Upper Egypt and indicates the presence of sub-regional genetic differentiation. Such divergence may arise from distinct evolutionary pathways, segregated seed populations, or variation in farming practices across villages, as is commonly observed in traditional farming systems. The lowest similarity values were recorded between G3 and G35 (J = 0.253) and between G1 and G34 (J = 0.254), indicating substantial genetic divergence. These genotypes may differ in ecogeographic origin, genetic background, or selection pressure, possibly due to genetic drift, historical isolation, or introgression from different breeding pools **(Zhou et al., 2018).** Their genetic distinctiveness enhances their potential as unique sources of alleles for crop improvement, particularly for adaptive traits like drought tolerance or pest resistance **(Mondal et al., 2016).**

As depicted in figure 4, the UPGMA dendrogram constructed from the Jaccard similarity matrix, reveals notable genetic diversity among the 40 wheat genotypes collected from a wide range of agro-climatic regions in Egypt. The similarity ranged approximately from 0.25 to 0.83, reflecting a broad spectrum of genetic divergence and highlighting the rich heritage of wheat germplasm preserved across different environmental and geographic zones.

The dendrogram delineates the genotypes into two main clusters, each consisting of several sub-clusters, with distinguishable correlations to ecogeographic origin. The first cluster, which is genetically distinct from genotypes from the semi-arid and arid regions, includes several genotypes from Sohag Governorate, particularly from Al Monshaah, Sohag city, and Akhmim districts (e.g., G16–G29), along with entries from the New Valley region (e.g., G11–G15). Notably, G22 (Kombedar, Al Monshaah), G19 (Al Salaah, Sohag), and G17 (Shandawil, Sohag) represent some of the most genetically distinct genotypes, clustering separately at low similarity coefficients. Their divergence suggests that these local landraces, collected from isolated rural areas, have preserved unique alleles that may be attuned to specific drought conditions, salinity levels, or selective cultural influences. Genotypes such as G14 and G15 (Farafrah, New Valley) and G26–G29 (Al Monshaah villages) form tight sub-clusters, suggesting shared ancestry and localized adaptation within the oases of the Western Desert and the central semi-arid locations of Upper Egypt. Interestingly, G27 (Awlaad Harron) and G28 (Nagaa Abd El Azeem Salem) are closely grouped, suggesting genetic homogeneity among samples from proximate villages within Al Monshaah .These findings reinforce the importance of semi-arid and arid zones, especially Sohag and New Valley, as central locations for genetic uniqueness and conservation priorities.

The second cluster, which is the larger cluster, has genetically similar genotypes from hot arid and hot semi-arid regions. This second cluster encompasses most of the genotypes from Aswan, Luxor, Qena, Assiut, Beni Suef, and North Sinai, many of which likely underwent modern selection, informal seed exchange, or adaptation to similar agro-ecological niches. Genotypes from Aswan (G1–G3) and Luxor (G4–G6), both categorized under the Hot Arid Region, formed a sub-cluster with moderate similarity, indicating shared traits potentially shaped by high temperature and low precipitation stress conditions. Entries from Qena (G7–G10) and New Valley (G11–G13) grouped relatively closely, underscoring the genetic proximity between genotypes from adjacent semi-arid governorates. G33 (Al Sawamaa Sharq, Akhmim) and G2 (Aswan) clustered tightly with other Upper Egyptian entries like G1 and G3, suggesting gene flow or varietal dissemination across the Nile Valley corridor. A tight sub-cluster including G36 (Bany Aysh, Gerga), G37 (Al Zaraby, Abo Teeg, Assiut), G38 (Assiut city), and G39 (Sayan Abu Ali, Beni Suef) displayed the highest Jaccard similarity values, indicating minimal genetic distance. These may represent improved cultivars or genetically uniform varieties that have diffused widely through national seed systems or farmer networks .Notably, G40 (Rafah, North Sinai)

clustered within this group, suggesting that even distant North Sinai shares a genetic affinity with Upper Egyptian materials, potentially due to varietal introductions or shared breeding stock.

The observed clustering patterns, shaped by both geographic isolation and shared environmental pressures, underscore the importance of preserving region-specific germplasm, particularly from underrepresented areas such as Sohag, New Valley, and North Sinai. Genotypes like G22 (Kombedar) and G19 (Al Salaah), which lie at the borders of the dendrogram, should be prioritized for in situ conservation and GenBank safeguarding, as they harbor rare or divergent alleles valuable for climate resilience .From a breeding perspective, the high divergence among genotypes from different governorates presents an excellent opportunity to exploit heterosis by crossing distant pairs (e.g., G22 × G39 or G17 × G36). Moreover, the tight clustering of certain genotypes highlights redundancy risks, emphasizing the need to avoid overrepresentation of genetically similar lines in breeding pools.

The geographic structuring of genetic diversity revealed in this study holds critical value for conservation planning, germplasm management, and sustainable breeding strategies aimed at enhancing wheat productivity and resilience in arid and semi-arid environments.

PCA Biplot Analysis of Genetic Similarity:

The principal component analysis (PCA) biplot (Figure 5) provides a comprehensive overview of the genetic structure of wheat genotypes. The PCA results complement molecular results by reducing the genetic data into key dimensions that explain the observed variation **(Chou et al., 2022).** The provided biplots illustrate distributions of genotypes and marker contributions to enable the detection of clusters, outliers, and high-contribution markers. The spatial distribution of genotypes in the PCA scatter plot aligns with the dendrogram, reinforcing the robustness of the clustering analysis. The first two components cumulatively explained a substantial portion of the variance, with Component 1 distinguishing genotypes along a gradient likely related to regional adaptation, while Component 2 captured orthogonal variation patterns.

A prominent cluster along Component 1 comprised genotypes from the hot arid and semi-arid regions of Upper Egypt, such as G1, G2 (Aswan), G3 (Al Sayala), G4, G6 (Luxor), G7, and G10 (Qena). Their tight grouping suggests a high degree of genetic similarity, potentially reflecting convergent selection pressure in similarly hot environments. Genotypes G11 and G13 (Dakhla Oasis, New Valley Governorate), from the arid zone, are also shown to be close to this cluster, indicating shared allelic characteristics adapted to harsh desert climates.

In contrast, genotypes from Sohag, especially those from the Al Monshaah district (e.g., G22, G23, G24, G25, G26, G27, G28, and G29), formed a separate sub-cluster oriented more toward Component 2. Their separation implies intra-regional variability, likely due to micro-environmental conditions or farmer selection practices within villages like Al Kawamel Kebly, Al Zook Al Gharbyea, and Kombedar. Genotypes G34 (Al Zook Al Sharkhya, Sohag) and G39 (Sayan Abu Ali, Beni Suef) were positioned in the lower-left quadrant of the biplot, distinctly apart from the central mass. This indicates their unique genetic background, possibly due to geographic isolation or limited germplasm exchange. G40, from Rafah in North Sinai, also displayed considerable divergence, suggesting an adaptation to the cooler and possibly more Mediterranean-influenced coastal climate.

The vectors overlaid on the biplot represent trait or marker loadings, pointing to the traits driving genotype dispersion. Genotypes like G28 (Nagaa Abd El Azeem Salem) and G20 (Al Shoryfat, Sohag) appeared aligned with longer vectors, suggesting strong associations with the underlying variables, potentially representing adaptive or agronomic traits.

Heatmap Analysis of Genetic Similarity:

The heatmap clustering (Figure 6) provides a visual synthesis of pairwise genetic similarity among the 40 wheat genotypes based on the Jaccard coefficient matrix. The hierarchical clustering dendrograms aligned along both axes corroborate the patterns detected in PCA and UPGMA analyses but further emphasize subgroup structures and levels of intragroup homogeneity.

Distinct blocks of high similarity are indicated by warm color gradients (red to orange), while cool tones (light to dark blue) denote dissimilarity. A prominent diagonal line represents perfect self-similarity, as expected, while square clusters along the diagonal highlight groups of closely related genotypes.

One such cluster is observed among genotypes from Sohag (Al Monshaah district), including G22 (Kombedar), G23 (Al Zook Al Gharbyea), G24, G27 (Al Kawamel Kebly to Awlaad Harron), and G28 (Nagaa Abd El Azeem Salem). These genotypes exhibited strong within-group similarity (bright red-orange blocks) and minimal divergence, which likely reflects localized farmer selection and seed exchange within the same ecological and cultural context.

Similarly, G1 and G3 (Aswan and Al Sayala) formed another compact, high-similarity block, reinforcing their close genetic relationship previously seen in PCA space. Their proximity, both geographically and genetically, suggests either a common ancestral landrace or shared selection pressures due to the extreme hot, arid climate of Aswan. The New Valley genotypes, G11 (Bashandy), G12, G13 (El Qasr), G14, and G15 (Farafrah), though slightly more dispersed, still formed a moderately cohesive cluster, consistent with their adaptation to the arid Western Desert conditions.

Outlier genotypes again stood apart in this heatmap, G34 (Al Zook Al Sharkhya, Sohag) and G39 (Sayan Abu Ali, Beni Suef) clustered weakly with others and displayed mostly cool-toned interactions, emphasizing their unique allelic profiles. G40 (Rafah, North Sinai) formed a distinct branch with limited similarity to other genotypes, which supports its divergent adaptation to the Mediterranean coastal agro-ecology and potential genetic isolation from Upper Egyptian wheat pools.

The heatmap also exposed internal differentiation within broader governorates. For instance, Sohag genotypes such as G16 (Sohag City), G19 (Al Salaah), and G33 (Al Sawamaa Sharq) showed lower similarity with other Sohag landraces, implying that intra-governorate environmental variation and localized seed systems contribute significantly to genetic structuring.

Collectively, the heatmap enriches the understanding of genotype relationships by illustrating fine-scale clustering and suggesting candidate genotypes for breeding crosses. Specifically, crossing highly divergent genotypes such as G40 (Rafah) or G34 (Sohag) with cluster-bound landraces (e.g., G22 and G28) could maximize heterosis and trait recombination potential.

In summary, the integration of similarity matrix, dendrogram, PCA, and heatmap analysis provides a comprehensive assessment of genetic diversity among wheat genotypes. The observed clustering patterns highlight genetic subpopulations essential for wheat breeding and conservation strategies. The identification of genetically diverse and outlier genotypes offers valuable insights into potential parental lines for hybridization, ensuring the sustainable enhancement of wheat biodiversity and resilience in future breeding programs.

Factors Shaping Genetic Diversity in Wheat Genotypes:

The genetic biodiversity observed among the 40 wheat genotypes analyzed in this study reflects a complex interplay of several factors, such as environmental selection pressures, geographical origins, agronomic practices, historical gene flow, genetic drift, and molecular evolution. Over centuries, these components have contributed to wheat adapting to a wide range of environments and cropping strategies **(Sahri et al., 2014).** Understanding these aspects is crucial for enhancing genetic conservation, promoting sustainable breeding practices, and enhancing wheat resistance to changing climate conditions. The integration of SSR, ISSR, and AFLP markers in this study provided a comprehensive framework for assessing genetic diversity, achieving correct genotypic differentiation and genome-wide polymorphism identification, which are required for marker-assisted selection (MAS) and quantitative trait loci (QTL) mapping.

Environmental Selection Pressures and Genetic Adaptation:

Strengthening the link between molecular data and agronomic performance is essential for translating genetic diversity into sustainable yield and food security outcomes. The adaptation of wheat genotypes to diverse agro-climatic zones in Egypt has been a major driver of genetic diversity **(Hamada et al., 2022).** The genotypes analyzed in this study were sourced from Hot Arid, Hot Semi-Arid, Arid, and Semi-Arid regions, each imposing distinct selective pressures that shape genotypic differentiation **(He et al., 2019; Terwisscha van Scheltinga et al., 2021; Hamed et al., 2022).**

Wheat populations in the Hot Arid and Hot Semi-Arid zones (e.g., Aswan, Luxor, and Qena) are subjected to high temperatures and prolonged drought periods, leading to overexpression of the genes involved in water-use efficiency, root system development, and heat tolerance **(Tricker et al., 2018).** The existence of unique SSR alleles and AFLP polymorphism peaks in these genotypes indicates that they include adaptive genomic changes associated with abiotic stress tolerance **(Huang et al., 2016; Du et al., 2023).**

Similarly, wheat genotypes from the Arid region (New Valley: G11–G15) demonstrated distinct ISSR banding patterns, indicating genetic divergence caused by high temperature variations and low soil fertility. The comprehensive genome coverage of ISSR markers allowed for the identification of stress-responsive loci, which are crucial for increasing wheat performance in difficult desert settings **(Kumar et al., 2024).** In contrast, genotypes from Semi-Arid regions (e.g., Sohag, Assiut, and Beni Suef) experience seasonal temperature variations and moderate water availability, leading to the selection of genotypes with increased photosynthetic efficiency, enhanced nutrient uptake, and greater yield stability **(Wasaya et al., 2021; Koua et al., 2022).** The AFLP analysis of these genotypes revealed higher levels of genetic differentiation, confirming that environmental adaptation plays a major role in shaping genetic variation **(Sasanuma et al., 2002).** These findings highlight the necessity of implementing breeding strategies to develop resilient wheat varieties to climate change while maintaining high productivity **(Mondal et al., 2016; Yadav et al., 2022).**

Geographic Isolation, Gene Flow, and Historical Breeding Influences

In addition to the environmental selection pressures and the adaptation of wheat cultivars, geographic isolation and gene flow impact the genetic structure of wheat populations, since they lead to determining the extent of allelic exchange **(Luo et al., 2007; Rufo et al., 2019).** Genotypes from distantly isolated regions (e.g., Dakhla and Farafrah in New Valley) exhibited higher genetic differentiation due to restricted cross-pollination and localized selection pressures **(Bhuri, 2013; Li et al., 2000).** Limited seed exchange and farmer selection in these regions may have contributed to higher genetic uniqueness, as observed in the presence of specific ISSR and AFLP polymorphisms **(Bhuri & Upadhyay, 2013; Balfourier et al., 2019).**

Conversely, some genotypes showed genetic similarities across different regions, indicating possible historical gene flow through seed trade, farmer migration, or cross-regional breeding efforts **(Balfourier et al., 2019; Scott et al., 2019).** For example, wheat genotypes from Sohag (Semi-Arid) and Luxor (Hot Semi-Arid) shared common AFLP markers, suggesting historical introgression of adaptive alleles between these populations. Understanding these patterns of gene flow is critical for the conservation of genetic resources and breeding programs that aim for the optimization of trait introgression for sustainable wheat production **(Royo et al., 2021).**

CONCLUSION

The present study provides a comprehensive assessment of the genetic diversity of 40 wheat genotypes using SSR, ISSR, and AFLP molecular markers and provides insights regarding their genetic structure, phylogenetic relationships, and their potential for breeding applications. The genetic similarity matrix revealed considerable variability, highlighting the impact of environmental selection pressures, geographic origin, and historical breeding practices on wheat populations. The adaptation of genotypes to agro-climatic zones, especially in Hot Arid and Semi-Arid environments, has enabled stress tolerance selection, crucial for developing climate-resilient wheat cultivars. Geographic isolation and gene flow also play a role in detecting genetic diversity, emphasizing the need to conserve both elite and underutilized germplasm for breeding programs. Future research should incorporate next-generation sequencing and genome-wide association studies to improve breeding accuracy and genetic improvement.

Declarations

Acknowledgment:

The authors would like to acknowledge the Deanship of Graduate Studies and Scientific Research, Taif University for funding this work.

Authors' Contributions:

Conceptualization: Shafik D. Ibrahim (SDI), Ahmed F. Abd El-Hakim (AFA),Mohammed Alqurashi (MA), Salman Aloufi (SA), Doha A. Albalawi (DAA), Amenah S. Al-Otaibi (ASA), Basmah M. Alharbi (BMA) Fahad Mohammed Alzuaibr (FMA), Hanan Khalaf Anazi (HKA), Siham M. Al-Balawi (SMA).

Collection and Data Evaluation: SDI.

Methodology – Laboratory and Greenhouse Experiments: SDI, AFA.
Data Analysis: AFA, SDI.
Manuscript Preparation (Drafting): AFA, SDI.
Review and Editing: MA, SA, DAA, ASA, BMA, FMA, HKA, SMA.

Overall guidance: AFA.

All authors have read and approved the final version of the manuscript.

Funding:

The authors would like to acknowledge the Deanship of Graduate Studies and Scientific Research, Taif University for funding this work.

Data availability

All data generated or analyzed during this study are included in this published article.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

College of Science, Taif University- Saudi Arabia, Agricultural Genetic Engineering Research Institute (AGERI), Agriculture Research Center (ARC)-Egypt, Faculty of Science, University of Tabuk-Saudi Arabia, Faculty of Agriculture, Ain Shams University- Egypt, Faculty of Agriculture, Al-Azhar University, Egypt.

References

1. **Agarwal, M., Shrivastava, N., & Padh, H. (2008).** Advances in molecular marker techniques and their applications in plant sciences. *Plant Cell Reports*, *27*(4), 617–631. https://doi.org/10.1007/S00299-008-0507-Z
2. **Amiryousefi, A., Amiryousefi, A., Hyvönen, J., Hyvönen, J., & Poczai, P. (2018).** iMEC: Online Marker Efficiency Calculator. *Applications in Plant Sciences*, *6*(6), 1159. https://doi.org/10.1002/APS3.1159
3. **Applied Biosystems, (2010).** *AFLP® Plant Mapping Protocol*. Foster City, CA.https://assets.thermofisher.com/TFS-Assets/LSG/manuals/cms_040959.pdf
4. **Bai, G., Kolb, F. L., Shaner, G., & Domier, L. L. (1999).** Amplified fragment length polymorphism markers linked to a major quantitative trait locus controlling scab resistance in wheat. *Phytopathology*, *89*(4), 343–348. https://doi.org/10.1094/PHYTO.1999.89.4.343
5. **Balfourier, F., Bouchet, S., Robert, S., De Oliveira, R., Rimbert, H., Kitt, J., Choulet, F., & Paux, E. (2019).** Worldwide phylogeography and history of wheat genetic diversity. *Science Advances*, *5*(5). https://doi.org/10.1126/SCIADV.AAV0536
6. **Bhuri, P. K. (2013).** Genetic Divergence Analysis in Wheat (Triticum aestivum L.). *International Journal of Plant Sciences*, *8*(2), 297–299. http://www.i-scholar.in/index.php/Ijps/article/view/48949
7. **Bhuri, P., & Upadhyay, K. (2013).** Genetic Divergence Analysis in Wheat (Triticum aestivum L.). International Journal of Plant Sciences, 8(2), 297–299. http://www.i-scholar.in/index.php/Ijps/article/view/48949
8. **Blignaut, M., Ellis, A. G., & Le Roux, J. J. (2013).** Towards a transferable and cost-effective plant AFLP protocol. *PLOS ONE*, *8*(4), 1–7. https://doi.org/10.1371/JOURNAL.PONE.0061704

9. **Chen, Z., Tang, D., Ni, J., Li, P., Wang, L., Zhou, J., Li, C., Lan, H., Li, L., & Liu, J. (2021).** Development of genic KASP SNP markers from RNA-Seq data for map-based cloning and marker-assisted selection in maize. *BMC Plant Biology*, *21*(1), 157. https://doi.org/10.1186/S12870-021-02932-8
10. **Chou, C. H., Lin, H. S., Wen, C. H., & Tung, C. W. (2022).** Patterns of genetic variation and QTLs controlling grain traits in a collection of global wheat germplasm revealed by high-quality SNP markers. *BMC Plant Biology*, *22*(1), 455.
11. **Costa, R., Pereira, G., Garrido, I., Tavares-de-Sousa, M. M., & Espinosa, F. (2016).** Comparison of RAPD, ISSR, and AFLP Molecular Markers to Reveal and Classify Orchardgrass (Dactylis glomerata L.) Germplasm Variations. *PLOS ONE*, *11*(4). https://doi.org/10.1371/JOURNAL.PONE.0152972
12. **Du, L., Ma, Z., & Mao, H. (2023).** Duplicate Genes Contribute to Variability in Abiotic Stress Resistance in Allopolyploid Wheat. *Plants*, *12*(13), 2465. https://doi.org/10.3390/plants12132465
13. **Egyptian Gene Bank (EGB). (2024).** Wheat germplasm accession data. Agricultural Research Center (ARC), Ministry of Agriculture and Land Reclamation, Egypt. Unpublished database.
14. **Elkot, A. F., Shabana, Y. M., Elsayed, M., Saleh, S. M., Gadallah, M. A. A., Fitt, B. D. L., Richard, B., & Qi, A. (2024).** Yield Responses to Total Water Input from Irrigation and Rainfall in Six Wheat Cultivars Under Different Climatic Zones in Egypt. *Agronomy*, *14*(12), 3057. https://doi.org/10.3390/agronomy14123057
15. **Food and Agriculture Organization of the United Nations (FAO). (2023).** *World food and agriculture – Statistical yearbook 2023*. Rome. https://doi.org/10.4060/cc8166en
16. **Fufa, H., Fufa, H., Baenziger, P. S., Beecher, B. S., Beecher, B. S., Dweikat, I., Graybosch, R. A., & Eskridge, K. M. (2005).** Comparison of phenotypic and molecular marker-based classifications of hard red winter wheat cultivars. *Euphytica*, *145*(1), 133–146. https://doi.org/10.1007/S10681-005-0626-3
17. **Grover, A., & Sharma, P. C. (2016).** Development and use of molecular markers: past and present. *Critical Reviews in Biotechnology*, *36*(2), 290–302. https://doi.org/10.3109/07388551.2014.959891
18. **Guo, P., Bai, G., & Shaner, G. (2003).** AFLP and STS tagging of a major QTL for Fusarium head blight resistance in wheat. *Theoretical and Applied Genetics*, *106*(6), 1011–1017. https://doi.org/10.1007/S00122-002-1129-4
19. **Hamada, A., Said, M., Ibrahim, K. M., Saber, M., & Sayed, M. A. (2022).** A Predictive Study of the Redistribution of Some Bread Wheat Genotypes in Response to Climate Change in Egypt. *Agronomy*, *12*(1), 113. https://doi.org/10.3390/agronomy12010113
20. **Hamed, M. M., Nashwan, M. S., & Shahid, S. (2022).** Climatic zonation of Egypt based on high-resolution dataset using image clustering technique. *Progress in Earth and Planetary Science*, *9*(1), 1–16. https://doi.org/10.1186/s40645-022-00494-3
21. **Hammer, Ø., Harper, D.A.T., and Ryan, P.D. (2001).** PAST: paleontological statistics software package for education and data analysis. *Palaeontologia electronica*, *4*(1), 1-9. Available at: https://past.en.lo4d.com/windows
22. **Han, B., Wang, C., Tang, Z., Ren, Y., Li, Y., Dayong, Z., Dong, Y., & Zhao, X. (2015).** Genome-Wide Analysis of Microsatellite Markers Based on Sequenced Database in Chinese Spring Wheat (Triticum aestivum L.). *PLOS ONE*, *10*(11). https://doi.org/10.1371/JOURNAL.PONE.0141540
23. **Hazen, S. P., Leroy, P., & Ward, R. W. (2002).** AFLP in Triticum aestivum L.: patterns of genetic diversity and genome distribution. *Euphytica*, *125*(1), 89–102. https://doi.org/10.1023/A:1015760802026
24. **He, F., Pasam, R. K., Shi, F., Kant, S., Keeble-Gagnère, G., Kay, P., Forrest, K., Fritz, A. K., Hucl, P., Wiebe, K., Knox, R., Cuthbert, R. D., Pozniak, C. J., Akhunova, A., Morrell, P. L., Davies, J. P., Webb, S. R., Spangenberg, G., Hayes, B. J., … Akhunov, E. (2019).** Exome sequencing highlights the role of wild-relative introgression in shaping the adaptive landscape of the wheat genome. *Nature Genetics*, *51*(5), 896–904. https://doi.org/10.1038/S41588-019-0382-2
25. **Holland, B. R., Clarke, A., Meudt, H. M., & Meudt, H. M. (2008).** Optimizing Automated AFLP Scoring Parameters to Improve Phylogenetic Resolution. *Systematic Biology*, *57*(3), 347–366. https://doi.org/10.1080/10635150802044037
26. **Huang, L., Raats, D., Sela, H., Klymiuk, V., Lidzbarsky, G., Feng, L., Krugman, T., & Fahima, T. (2016).** Evolution and Adaptation of Wild Emmer Wheat Populations to Biotic and Abiotic Stresses. *Annual Review of Phytopathology*, *54*(1), 279–301. https://doi.org/10.1146/ANNUREV-PHYTO-080614-120254
27. **Ibrahim, S. D., Abd El-Hakim, A. F., Ali, H. E., & Abd El-Maksoud, R. M. (2019).** Genetic differentiation using ISSR, SCoT and DNA Barcoding for Quinoa genotypes. *Arab J. Biotech*, *22*(2), 103-118.
28. **Ibrahim, S. D., Adawy, S. S., Atia, M. A. M., Alsamman, A. M., & Mokhtar, M. M. (2016).** Genetic diversity, variety identification and gene detection in some Egyptian grape varieties by SSR and SCoT markers. *Plant Omics*, *9*(5).
29. **Ijaz, S., & Khan, I. A. (2009).** Molecular characterization of wheat germplasm using microsatellite markers. *Genetics and Molecular Research*, *8*(3), 809–815. https://doi.org/10.4238/VOL8-3GMR608
30. **Jaccard, P. (1908).** Nouvelles recherches sur la distribution florale. *Bulletin of the New York Botanical Garden*, *6*(1), 130–178.
31. **Jahnke, G., Smidla, J., & Poczai, P. (2022).** MolMarker: A Simple Tool for DNA Fingerprinting Studies and Polymorphic Information Content Calculation. Diversity, 14(6), 497. https://doi.org/10.3390/d14060497
32. **Jiangfeng, H., Zhao, X., Laroche, A., Lu, Z.-X., Liu, H., & Li, Z. (2014).** Genotyping-by-sequencing (GBS), an ultimate marker-assisted selection (MAS) tool to accelerate plant breeding. *Frontiers in Plant Science*, *5*, 484. https://doi.org/10.3389/FPLS.2014.00484
33. **Kalia, R. K., Rai, M. K., Kalia, S., Singh, R., & Dhawan, A. K. (2011).** Microsatellite markers: an overview of the recent progress in plants. *Euphytica*, *177*(3), 309–334. https://doi.org/10.1007/S10681-010-0286-9
34. **Koua, A. P., Oyiga, B. C., Dadshani, S., Benaouda, S., Sadeqi, M. B., Rascher, U., Léon, J., & Ballvora, A. (2022).** Chromosome 3A harbors several pleiotropic and stable drought-responsive alleles for photosynthetic efficiency selected through wheat breeding. *Plant Direct*, *6*(9). https://doi.org/10.1002/pld3.438
35. **Kovach, W. L. (2002).** *MVSP – A Multivariate Statistical Package for Windows, Version 3.13d*. Kovach Computing Services, Pentraeth, Wales, UK.

36. **Kumar, P., Sharma, V., Sanger, R. S., Kumar, P. R., & Yadav, M. K. (2020).** Analysis of molecular variation among diverse background wheat (Triticum aestivum L) genotypes with the help of ISSR markers. *International Journal of Chemical Studies*, *8*(3), 271–276. https://doi.org/10.22271/CHEMI.2020.V8.I3D.9240
37. **Kumar, M., Nagar, K. K., Pawar, S. V., Pawar, B. S., & Gadekar, D. A. (2024).** Exploring the underlying genetic polymorphism in wheat genotypes employing ISSR markers. *International Journal of Research in Agronomy*, *7*(10S), 410–415. https://doi.org/10.33545/2618060x.2024.v7.i10sf.1803
38. **Landjeva, S., Korzun, V., & Börner, A. (2007).** Molecular markers: actual and potential contributions to wheat genome characterization and breeding. *Euphytica*, *156*(3), 271–296. https://doi.org/10.1007/S10681-007-9371-0
39. **Langridge, P., Braun, H. J., Hulke, B. S., Ober, E. S., & Prasanna, B. M. (2021).** Breeding crops for climate resilience. *Theoretical and Applied Genetics*, *134*(6), 1607–1611. https://doi.org/10.1007/S00122-021-03854-7
40. **Li, Y. C., Li, Y. C., Fahima, T., Krugman, T., Beiles, A., Röder, M. S., Korol, A. B., & Nevo, E. (2000).** Parallel microgeographic patterns of genetic diversity and divergence revealed by allozyme, RAPD, and microsatellites in Triticum dicoccoides at Ammiad, Israel. *Conservation Genetics*, *1*(3), 191–207. https://doi.org/10.1023/A:1011545403198
41. **Liu, C., Khodaee, M., Lopes, M. S., Sansaloni, C., Dreisigacker, S., Sukumaran, S., & Reynolds, M. P. (2019).** Multi-environment QTL analysis using an updated genetic map of a widely distributed Seri × Babax spring wheat population. *Molecular Breeding*, *39*(9), 1–15. https://doi.org/10.1007/S11032-019-1040-1
42. **Lopes, M. S., Elbasyoni, I. S., Baenziger, P. S., Singh, S., Royo, C., Ozbek, K., Aktas, H., Özer, E., Özdemir, F., Manickavelu, A., Ban, T., & Vikram, P. (2015).** Exploiting genetic diversity from landraces in wheat breeding for adaptation to climate change. *Journal of Experimental Botany*, *66*(12), 3477–3486. https://doi.org/10.1093/JXB/ERV122
43. **Luo, M.-C., Yang, Z.-L., You, F. M., Kawahara, T., Waines, J. G., & Dvorak, J. (2007).** The structure of wild and domesticated emmer wheat populations, gene flow between them, and the site of emmer domestication. *Theoretical and Applied Genetics*, *114*(6), 947–959. https://doi.org/10.1007/S00122-006-0474-0
44. **Malik, R., Sareen, S., Kundu, S. S., & Shoran, J. (2012).** The use of SSR and ISSR markers for assessing DNA polymorphism and genetic diversity among Indian bread wheat cultivars. *Progressive Agriculture*, *12*(1), 82–89. https://www.cabdirect.org/cabdirect/abstract/20123151290
45. **Metsalu, T., & Vilo, J. (2015).** ClustVis: A web tool for visualizing clustering of multivariate data using Principal Component Analysis and heatmap. **Nucleic Acids Research**, **43**(W1), W566–W570. https://doi.org/10.1093/nar/gkv468
46. **Mohamed, A., Teleb, S. S., Badr, A., Tantawy, M., Ibrahim, S. D., Ellmouni, F. Y., & Ibrahim, M. F. M. (2023).** Genetic diversity analysis in wheat cultivars using SCoT and ISSR markers, chloroplast DNA barcoding and grain SEM. *BMC Plant Biology*, *23*(1). https://doi.org/10.1186/s12870-023-04196-w
47. **Mondal, S., Rutkoski, J., Velu, G., Singh, P. K., Crespo-Herrera, L., Guzmán, C., Bhavani, S., Lan, C., He, X., & Singh, R. P. (2016).** Harnessing Diversity in Wheat to Enhance Grain Yield, Climate Resilience, Disease and Insect Pest Resistance and Nutrition Through Conventional and Modern Breeding Approaches. *Frontiers in Plant Science*, *7*, 991. https://doi.org/10.3389/FPLS.2016.00991
48. **Mullan, D. J., Platteter, A., Teakle, N., Appels, R., Colmer, T. D., Anderson, J. M., & Francki, M. G. (2005).** EST-derived SSR markers from defined regions of the wheat genome to identify Lophopyrum elongatum specific loci. *Genome*, *48*(5), 811–822. https://doi.org/10.1139/G05-040
49. **Najaphy, A., Ashrafi Parchin, R., & Farshadfar, E. (2011).** Evaluation of genetic diversity in wheat cultivars and breeding lines using inter simple sequence repeat markers. *Biotechnology & Biotechnological Equipment*, *25*(4), 2634–2638. https://doi.org/10.5504/BBEQ.2011.0093
50. **New England BioLabs. (n.d.).** ***T4 DNA Ligase (M0202)*****.** Retrieved from https://www.neb.com/products/m0202-t4-dna-ligase
51. **Nicot, N., Chiquet, V., Gandon, B., Amilhat, L., Legeai, F., Leroy, P., Bernard, M., & Sourdille, P. (2004).** Study of simple sequence repeat (SSR) markers from wheat expressed sequence tags (ESTs). *Theoretical and Applied Genetics*, *109*(4), 800–805. https://doi.org/10.1007/S00122-004-1685-X
52. **Noli, E., Teriaca, M. S., Sanguineti, M. C., & Conti, S. (2008).** Utilization of SSR and AFLP markers for the assessment of distinctness in durum wheat. *Molecular Breeding*, *22*(2), 301–313. https://doi.org/10.1007/S11032-008-9176-4
53. **Papa, R., Troggio, M., Ajmone-Marsan, P., & Nonnis Marzano, F. (2005).** An improved protocol for the production of AFLPTM markers in complex genomes by means of capillary electrophoresis. *Journal of Animal Breeding and Genetics*, *122*(1), 62–68. https://doi.org/10.1111/J.1439-0388.2004.00476.X
54. **Peng, J., Korol, A. B., Fahima, T., Röder, M. S., Ronin, Y., Li, Y. C., & Nevo, E. (2000).** Molecular Genetic Maps in Wild Emmer Wheat, Triticum dicoccoides: Genome-Wide Coverage, Massive Negative Interference, and Putative Quasi-Linkage. *Genome Research*, *10*(10), 1509–1531. https://doi.org/10.1101/GR.150300
55. **Pequeno, D. N. L., Hernandez-Ochoa, I. M., Reynolds, M. P., Sonder, K., Molero-Milan, A., Robertson, R., Lopes, M. S., Xiong, W., Kropff, M. J., Asseng, S., & Asseng, S. (2021).** Climate impact and adaptation to heat and drought stress of regional and global wheat production. *Environmental Research Letters*, *16*(5), 054070. https://doi.org/10.1088/1748-9326/ABD970
56. **Qian, G., Ping, J., Wang, D., Zhang, Z., Luo, S.-Y., & Yang, M.-Z. (2012).** Malt Genotypic Screening of Polymorphism Information Content (PIC) of SSR Markers Based on Physiological Traits in Barley. *Molecular Biology*, *1*(1), 1–6. https://doi.org/10.4172/2168-9547.1000101
57. **Rasheed, A., & Xia, X. (2019).** From markers to genome-based breeding in wheat. *Theoretical and Applied Genetics*, *132*(3), 767–784. https://doi.org/10.1007/S00122-019-03286-4
58. **Reddy, M. P., Sarla, N., & Siddiq, E. (2002).** Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. *Euphytica*, *128*(1), 9–17. https://doi.org/10.1023/A:1020691618797
59. **Reif, J. C., Zhang, P., Dreisigacker, S., Warburton, M. L., Van Ginkel, M., Hoisington, D., Bohn, M. O., & Melchinger, A. E. (2005).** Wheat genetic diversity trends during domestication and breeding. *Theoretical and Applied Genetics*, *110*(5), 859–864. https://doi.org/10.1007/S00122-004-1881-8

60. **Royo, C., Ammar, K., Villegas, D., & Soriano, J. M. (2021).** Agronomic, Physiological and Genetic Changes Associated with Evolution, Migration and Modern Breeding in Durum Wheat. *Frontiers in Plant Science*, *12*, 674470. https://doi.org/10.3389/FPLS.2021.674470
61. **Rufo, R., Álvaro, F., Royo, C., & Soriano, J. M. (2019).** From landraces to improved cultivars: Assessment of genetic diversity and population structure of Mediterranean wheat using SNP markers. *PLOS ONE*, *14*(7). https://doi.org/10.1371/JOURNAL.PONE.0219867
62. **Sahri, A., Chentoufi, L., Arbaoui, M., Ardisson, M., Belqadi, L., Birouk, A., Roumet, P., & Muller, M.-H. (2014).** Towards a comprehensive characterization of durum wheat landraces in Moroccan traditional agrosystems: analysing genetic diversity in the light of geography, farmers' taxonomy and tetraploid wheat domestication history. *BMC Evolutionary Biology*, *14*(1), 264. https://doi.org/10.1186/S12862-014-0264-2
63. **Sasanuma, T., Chabane, K., Endo, T. R., & Valkoun, J. (2002).** Genetic diversity of wheat wild relatives in the Near East detected by AFLP. *Euphytica*, *127*(1), 81–93. https://doi.org/10.1023/A:1019941817057
64. **Schwarz, G., Herz, M., Huang, X. Q., Michalek, W., Jahoor, A., Wenzel, G., & Mohler, V. (2000).** Application of fluorescence-based semi-automated AFLP analysis in barley and wheat. *Theoretical and Applied Genetics*, *100*(3), 545–551. https://doi.org/10.1007/S001220050071
65. **Scott, M. F., Botigué, L. R., Brace, S., Stevens, C. J., Mullin, V. E., Stevenson, A., Thomas, M. G., Fuller, D. Q., & Mott, R. (2019).** A 3,000-year-old Egyptian emmer wheat genome reveals dispersal and domestication history. *Nature Plants*, *5*(11), 1120–1128. https://doi.org/10.1038/S41477-019-0534-5
66. **Soriano, J. M. (2020).** Molecular Marker Technology for Crop Improvement. *Agronomy*, *10*(10), 1462. https://doi.org/10.3390/AGRONOMY10101462
67. **Tadesse, W., Suleiman, S., Tahir, I. S. A., Sanchez-Garcia, M., Jighly, A., Jighly, A., Hagras, A., Thabet, Sh., & Baum, M. (2019).** Heat-Tolerant QTLs Associated with Grain Yield and Its Components in Spring Bread Wheat under Heat-Stressed Environments of Sudan and Egypt. *Crop Science*, *59*(1), 199–211. https://doi.org/10.2135/CROPSCI2018.06.0389
68. **Terwisscha van Scheltinga, C., de Miguel Garcia, A., Wilbers, G. J., Heesmans, H. I. M., Dankers, R., & Smaling, E. (2021).** Unravelling the interplay between water and food systems in arid and semi-arid environments: the case of Egypt. *Food Security*, *13*(5), 1145–1161. https://doi.org/10.1007/S12571-021-01208-1
69. **Tricker, P. J., ElHabti, A., Schmidt, J., & Fleury, D. (2018).** The physiological and genetic basis of combined drought and heat tolerance in wheat. *Journal of Experimental Botany*, *69*(13), 3195–3210. https://doi.org/10.1093/JXB/ERY081
70. **United States Department of Agriculture Foreign Agricultural Service (USDA). (2025, January).** *Grain: World markets and trade* (p. 18). https://www.fas.usda.gov/sites/default/files/2025-01/grain.pdf
71. **Vaillancourt, A., Nkongolo, K., Michael, P., & Mehes, M. (2008).** Identification, characterisation, and chromosome locations of rye and wheat specific ISSR and SCAR markers useful for breeding purposes. *Euphytica*, *159*(3), 297–306. https://doi.org/10.1007/S10681-007-9492-5
72. **Voss-Fels, K. P., Frisch, M., Qian, L., Kontowski, S., Friedt, W., Gottwald, S., & Snowdon, R. J. (2015).** Subgenomic diversity patterns caused by directional selection in bread wheat gene pools. *The Plant Genome*, *8*(2). https://doi.org/10.3835/PLANTGENOME2015.03.0013
73. **Wasaya, A., Manzoor, S., Yasir, T. A., Sarwar, N., Mubeen, K., Ismail, I. A., Raza, A., Rehman, A., Hossain, A., & Sabagh, A. (2021).** Evaluation of Fourteen Bread Wheat (Triticum aestivum L.) Genotypes by Observing Gas Exchange Parameters, Relative Water and Chlorophyll Content, and Yield Attributes under Drought Stress. *Sustainability*, *13*(9), 4799. https://doi.org/10.3390/SU13094799
74. **Wenz, H. M., Robertson, J. M., Menchen, S., Oaks, F., Demorest, D. M., Scheibler, D., Rosenblum, B. B., Wike, C., Gilbert, D. A., & Efcavitch, J. W. (1998).** High-Precision Genotyping by Denaturing Capillary Electrophoresis. *Genome Research*, *8*(1), 69–80. https://doi.org/10.1101/GR.8.1.69
75. **Wu, J., Wang, Q., Xu, L., Chen, X., Li, B., Mu, J., Zeng, Q., Huang, L., Han, D., & Kang, Z. (2018).** Combining Single Nucleotide Polymorphism Genotyping Array with Bulked Segregant Analysis to Map a Gene Controlling Adult Plant Resistance to Stripe Rust in Wheat Line 03031-1-5 H62. *Phytopathology*, *108*(1), 103–113. https://doi.org/10.1094/PHYTO-04-17-0153-R
76. **Xiao, M., Li, Q., Wang, L., Guo, L., Li, J., Tang, L., & Chen, F. (2006).** ISSR Analysis of the Genetic Diversity of the Endangered Species Sinopodophyllum hexandrum (Royle) Ying from Western Sichuan Province, China. *Journal of Integrative Plant Biology*, *48*(10), 1140–1146. https://doi.org/10.1111/J.1744-7909.2006.00304.
77. **Xu, H., Zhang, J., Zhang, P., Qie, Y., Niu, Y., Li, H., Ma, P., Xu, Y., & An, D. (2014).** Development and validation of molecular markers closely linked to the wheat stripe rust resistance gene YrC591 for marker-assisted selection. *Euphytica*, *198*(3), 317–323. https://doi.org/10.1007/S10681-014-1108-2
78. **Yadav, M. R., Choudhary, M., Singh, J., Lal, M. K., Jha, P. K., Udawat, P., Gupta, N. K., Rajput, V. D., Garg, N., Maheshwari, C., Hasan, M., Gupta, S., Jatwa, T. K., Kumar, R., Yadav, A. K., & Prasad, P. V. V. (2022).** Impacts, Tolerance, Adaptation, and Mitigation of Heat Stress on Wheat under Changing Climates. *International Journal of Molecular Sciences*, *23*(5), 2838. https://doi.org/10.3390/ijms23052838
79. **Yao E., Blake V.C, Cooper L., Wight C.P., Michel S., Cagirici H.B., Lazo G.R., Birkett C.L., Waring D.J., Jannink J.-L., Holmes, I., Waters A.J., Eickholt D.P., Sen, T.Z. (2022).** GrainGenes: a data-rich repository for small grains genetics and genomics. *Database*, *2022*, baac034.
80. **Zhou, Y., Chen, Z., Cheng, M., Chen, J., Zhu, T., Wang, R., Liu, Y., Qi, P., Chen, G., Jiang, Q., Wei, Y., Luo, M.-C., Nevo, E., Allaby, R. G., Liu, D., Wang, J., Dvorak, J., & Zheng, Y.-L. (2018).** Uncovering the dispersion history, adaptive evolution and selection of wheat in China. *Plant Biotechnology Journal*, *16*(1), 280–291. https://doi.org/10.1111/PBI.12770

Tables

Table 1: Classification of the studied wheat genotypes based on climatic regions in Egypt.

Genotype No.	Barcoding No.	Source in Egypt		
		Governorate	District	Village
1. **Hot Arid Region:**				
G1	111320	Aswan	Aswan	Aswan
G2	111321	Aswan	Aswan	Aswan
G3	111322	Aswan	Al Noba	Al Sayala
G4	111323	Luxor	Esna	Tuffnice
G5	111324	Luxor	Esna	Danffic
G6	111325	Luxor	Esna	Danffic
2. **Hot Semi-Arid Region:**				
G7	111326	Qena	Qeft	Qeft
G8	111327	Qena	Nag HamadI	Al HalfayaKebly
G9	111328	Qena	Nag HamadI	Al Rahmanya
G10	111329	Qena	Qena	Dandrah
3. **Arid Region:**				
G11	111330	New Valley	Dakhla	Bashandy
G12	111331	New Valley	Dakhla	El Qasr
G13	111332	New Valley	Dakhla	El Qasr
G14	111333	New valley	Farafrah	Farafrah
G15	111334	New valley	Farafrah	Farafrah
4. **Semi-Arid Region:**				
G16	111335	Sohag	Sohag	Sohag
G17	111336	Sohag	Sohag	Shandawil
G18	111337	Sohag	Sohag	Jazirat-Shandawil
G19	111338	Sohag	Sohag	Al Salaah
G20	111340	Sohag	Sohag	Al Shoryfat
G21	111341	Sohag	Sohag	AwlaadNaseer
G22	111342	Sohag	Al Monshaah	Kombedar
G23	111343	Sohag	Al Monshaah	Al Zook Al Gharbyea
G24	111344	Sohag	Al Monshaah	Al Kawamel Kebly
G25	111345	Sohag	Al Monshaah	Al Kawamel Kebly
G26	111346	Sohag	Al Monshaah	Al Horyzat Al Gharbeya
G27	111347	Sohag	Al Monshaah	AwlaadHarron
G28	111348	Sohag	Al Monshaah	NagaaAbd El Azeem Salem
G29	111349	Sohag	Al Monshaah	Kombedar
G30	111351	Sohag	Idfa	Idfa
G31	111353	Sohag	Akhmim	Al deyabat
G32	111354	Sohag	Akhmim	Arab Al Atawla
G33	111356	Sohag	Akhmim	Al SawamaaSharq
G34	111359	Sohag	Al Monshaah	Al Zook Al sharkhya
G35	111362	Sohag	Gerga	Gerga
G36	111363	Sohag	Gerga	BanyAysh

G37	111364	Assiut	Abo teeg	Al Zaraby
G38	111365	Assiut	Assiut	Assiut
G39	111369	BeniSwif	BeniSwif	Sayan Abu Ali
G40	111370	North Sinai	Rafah	Rafah

Table 2: SSR primer details, including marker name, chromosomal location, annealing temperature, and primer sequences.

Primer Code	Marker Name	Chr.*	Ta (°C)*	Fwd (5'–3')*	Rev (5'–3')*
SSR2	**WMS273**	1B	55	ATTGGACGGACAGATGCTTT	AGCAGTGAGGAAGGGGATC
SSR4	**Xgwm371**	5B	58	GACCAAGATATTCAAACTGGCC	AGCTCAGCTTGCTTGGTACC
SSR5	**Xgwm219**	6B	60	GATGAGCGACACCTAGCCTC	GGGGTCCGAGTCCACAAC
SSR9	**Xwmc596**	7A	61	TCAGCAACAAACATGCTCGG	CCCGTGTAGGCGGTAGCTCTT
SSR10	**Xgwm99**	1AL	57	AAGATGGACGTATGCATCACA	GCCATATTTGATGACGCATA
SSR12	**Xgwm369**	3A	55	CTGCAGGCCATGATGATG	ACCGTGGGTGTTGTGAGC
SSR13	**Xgwm234**	5B	55	GAGTCCTGATGTGAAGCTGTTG	CTCATTGGGGTGTGTACGTG
SSR14	**Xgwm205a**	5A	60	CGACCCGGTTCACTTCAG	AGTCGCCGTTGTATAGTGCC
SSR16	**wmc422**	7A	51	GACTACTGAACTGGAGAGTGTG	GCATTAGAATTTGGAGTTTGGAG
SSR17	**Xgwm124**	1B	60	GCCATGGCTATCACCCAG	ACTGTTCGGTGCAATTTGAG

Abbreviations:

Chr.*: **Chromosome;** ***Ta (°C)**;* **Annealing Temperature (°C);** ***Fwd (5'–3')*;*** **Forward Sequence (5' - 3');** ***Rev (5'–3')**;* **Reverse Sequence (5' - 3').**

Table 3: ISSR primer sequences and details.

Primer Code	Sequence
ISSR-05	5'-GTGTGTGTGTGTGTGTG-3'
ISSR-06	5'-CGCGATAGATAGATAGATA-3'
ISSR-07	5'-GACGATAGATAGATAGATA-3'
ISSR-08	5'-AGACAGACAGACAGACGC-3'
ISSR-09	5'-GATAGATAGATAGATAGC-3'
ISSR-14	5'-CTCCTCCTCCTCCTCTT-3'
ISSR-15	5'-CTCTCTCTCTCTCTCTRG-3'
H24	5'-AGAGAGAGAGAGAGAGYC-3'
H26	5'-AGAGAGAGAGAGAGAGYG-3'
H28	5'-HVHTGTGTGTGTGTGTGT-3'

Table 4: AFLP Adapter and Primer Sequences.

Name	Sequence (5′-3′)
Adapters	
EcoRI Adapter	5′-CTCGTAGACTGCGTACC-3′
	3′-CATCTGACGCATGGTTAA-5′
MseI Adapter	5′-GACGATGAGTCCTGAG-3′
	3′-TACTCAGGACTCAT-5′
Primers	
MseI Primer	GATGAGTCCTGAGTAA
MseI + CTC	GATGAGTCCTGAGTAACTC
MseI + CTT	GATGAGTCCTGAGTAACTT
MseI + CAG	GATGAGTCCTGAGTAACAG
EcoRI Primer	GACTGCGTACCAATTC
EcoRI + AAG (Ned)	GACTGCGTACCAATTCAAG
EcoRI + ACT (Fam)	GACTGCGTACCAATTCACT
EcoRI + AAC (Fam)	GACTGCGTACCAATTCAAC

Table (5): performance parameters of SSR primers used for genetic diversity analysis of the studied wheat genotypes.

Primer code	Fragment size	Total Amplicons	T. AB	Monomorphic Amplicons (MB)	Polymorphic Amplicons (PB)	Unique Bands (UB)	P %	PIC	EMR	MI	H	H. Avg	D
SSR2	161-106	5	82	0	5	0	100.00	0.3668	2.05	0.0050	0.4838	0.0024	0.83
SSR4	235-119	5	103	0	5	0	100.00	0.3748	2.575	0.0064	0.4996	0.0025	0.73
SSR5	201-176	3	43	0	3	0	100.00	0.3541	1.075	0.0041	0.4599	0.0038	0.87
SSR9	279-149	6	155	2	4	0	66.67	0.3528	3.875	0.0074	0.4575	0.0019	0.58
SSR10	153-105	4	75	0	4	0	100.00	0.3740	1.875	0.0058	0.4980	0.0031	0.78
SSR12	1000-175	7	133	1	6	0	85.71	0.3744	3.325	0.0059	0.4988	0.0018	0.77
SSR13	263-217	4	80	1	3	1	75.00	0.3750	2	0.0063	0.5000	0.0031	0.75
SSR14	160-145	2	70	1	1	0	50.00	0.1948	1.75	0.0048	0.2188	0.0027	0.23
SSR16	160-140	3	40	0	3	1	100.00	0.3457	1	0.0037	0.4444	0.0037	0.89
SSR17	210-200	2	42	1	1	0	50.00	0.3744	1.05	0.0065	0.4988	0.0062	0.72
Total		**41**	**823**	**6**	**35**	**2**							

*** Amplicon:** A DNA fragment amplified and detected across the 40 genotypes via SSR marker technique.

****Abbreviations:**

***PIC**:** Polymorphic Information Content, ***EMR**:** Effective Multiplex Ratio, ***H**:** Heterozygosity ***H. Avg**:** Average Heterozygosity, ***MI**:** Marker Index, ***D**:** Discriminatory Power, ***Rp**:** Resolving Power.

Table (6): performance parameters of ISSR primers used for genetic diversity analysis of the studied wheat genotypes.

Primer Code	Fragment Size	Total Amplicons	T. AB	Monomorphic Amplicons (MB)	Polymorphic Amplicons (PB)	Unique Bands (UB)	P %	PIC	EMR	H	H. Avg.	MI	D
ISSR-5	423-1903	10	368	2	8	0	80%	0.1364	9.2	0.1472	0.0004	0.0034	0.153
ISSR-6	371-1541	14	511	6	8	0	57%	0.1469	12.775	0.1597	0.0003	0.0036	0.167
ISSR-7	266-2684	13	338	1	12	0	92%	0.4432	1	0.5250	0.5250	0.5250	0.253
ISSR-8	364-2526	11	362	7	4	0	36%	0.4604	1	0.5168	0.5168	0.5168	0.109
ISSR-9	282-1581	10	251	2	8	0	80%	0.5739	1	0.6467	0.6467	0.6467	0.192
ISSR-14	310-1961	12	368	2	10	0	83%	0.4539	1	0.5078	0.5078	0.5078	0.194
ISSR-15	218-1319	14	455	4	10	0	71%	0.2583	11.375	0.3047	0.0005	0.0062	0.340
H24	219-1493	12	330	2	10	0	83%	0.4937	1	0.5606	0.5606	0.5606	0.262
H26	226-2184	17	472	6	11	0	65%	0.3351	11.775	0.4258	0.0006	0.0074	0.520
H28	302-1806	11	428	8	3	0	27%	0.3799	1	0.4790	0.4790	0.4790	0.032
Total		**124**	**3883**	**40**	**84**	**0**							

*** Amplicon:** A DNA fragment amplified and detected across the 40 genotypes via ISSR marker technique.

****Abbreviations:**

***PIC**:** Polymorphic Information Content, ***EMR**:** Effective Multiplex Ratio, ***H**:** Heterozygosity ***H. Avg**:** Average Heterozygosity, ***MI**:** Marker Index, ***D**:** Discriminatory Power, ***Rp**:** Resolving Power.

Table (7): Efficiency Parameters of CE-AFLP Primer Combinations for Assessing Genetic Diversity Among 40 Wheat Genotypes.

Primer Combination	Total Amplicons	Monomorphic Amplicons (MF)	Polymorphic Amplicons (PF)	Unique Amplicons (UF)	Polymorphism (%)	PIC	Heterozygosity (H)	Avg. Heterozygosity (H Avg.)	EMR
E_{AAG}-M_{CTC}	391	2	389	33	99.49%	0.3453	0.4437	2.83698E-05	129.9
E_{ACT}-M_{CTT}	264	0	264	26	100.00%	0.3323	0.4209	3.98586E-05	79.5
E_{AAC}-M_{CAG}	425	0	425	65	100.00%	0.3163	0.3939	2.31723E-05	114.625

*** Amplicon:** A DNA fragment amplified and detected across the 40 genotypes via CE-AFLP.

****Abbreviations:**

***PIC*:** Polymorphic Information Content, ***EMR*:** Effective Multiplex Ratio, ***H*:** Heterozygosity ***H. Avg*:** Average Heterozygosity, ***MI*:** Marker Index, ***D*:** Discriminatory Power, ***Rp*:** Resolving Power.

Table 8: Similarity matrix of the examined 40 wheat genotypes based on combined ISSR, SSR, and AFLP marker data.

	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15	G16	G1
G1	**1.000**																
G2	0.485	**1.000**															
G3	0.352	0.439	**1.000**														
G4	0.456	0.595	0.475	**1.000**													
G5	0.454	0.545	0.481	0.620	**1.000**												
G6	0.435	0.543	0.433	0.602	0.658	**1.000**											
G7	0.437	0.525	0.462	0.619	0.690	0.607	**1.000**										
G8	0.526	0.532	0.427	0.550	0.602	0.624	0.612	**1.000**									
G9	0.450	0.609	0.459	0.607	0.625	0.627	0.647	0.629	**1.000**								
G10	0.471	0.543	0.490	0.542	0.551	0.546	0.591	0.649	0.585	**1.000**							
G11	0.421	0.515	0.419	0.633	0.594	0.613	0.612	0.554	0.652	0.554	**1.000**						
G12	0.446	0.532	0.431	0.576	0.658	0.609	0.667	0.684	0.583	0.582	0.597	**1.000**					
G13	0.465	0.559	0.415	0.555	0.588	0.695	0.603	0.639	0.568	0.587	0.594	0.691	**1.000**				
G14	0.379	0.445	0.323	0.460	0.445	0.492	0.500	0.502	0.484	0.489	0.488	0.494	0.543	**1.000**			
G15	0.390	0.467	0.353	0.470	0.464	0.502	0.490	0.502	0.486	0.523	0.517	0.519	0.558	0.684	**1.000**		
G16	0.351	0.439	0.343	0.439	0.412	0.453	0.429	0.444	0.454	0.478	0.469	0.485	0.481	0.544	0.593	**1.000**	
G17	0.304	0.380	0.325	0.386	0.360	0.387	0.385	0.377	0.391	0.400	0.407	0.391	0.413	0.445	0.460	0.583	**1.0**
G18	0.343	0.427	0.314	0.435	0.419	0.468	0.446	0.478	0.456	0.455	0.474	0.493	0.479	0.514	0.562	0.630	0.6
G19	0.386	0.388	0.289	0.379	0.387	0.401	0.379	0.407	0.388	0.392	0.403	0.425	0.420	0.430	0.430	0.414	0.4
G20	0.375	0.420	0.359	0.445	0.476	0.443	0.462	0.457	0.457	0.440	0.458	0.487	0.476	0.484	0.549	0.463	0.4
G21	0.313	0.381	0.373	0.423	0.406	0.386	0.418	0.383	0.413	0.384	0.397	0.396	0.383	0.398	0.436	0.455	0.4
G22	0.312	0.328	0.270	0.346	0.341	0.338	0.353	0.352	0.337	0.358	0.377	0.353	0.376	0.425	0.437	0.373	0.3
G23	0.361	0.451	0.363	0.447	0.497	0.491	0.470	0.539	0.462	0.469	0.449	0.531	0.527	0.513	0.525	0.464	0.4
G24	0.384	0.444	0.389	0.494	0.488	0.470	0.474	0.455	0.473	0.460	0.463	0.485	0.456	0.477	0.479	0.443	0.3
G25	0.340	0.422	0.373	0.473	0.465	0.454	0.447	0.437	0.435	0.425	0.418	0.433	0.419	0.437	0.459	0.433	0.3
G26	0.298	0.384	0.335	0.404	0.402	0.388	0.412	0.375	0.392	0.360	0.384	0.392	0.398	0.434	0.388	0.405	0.3
G27	0.323	0.400	0.334	0.432	0.412	0.398	0.422	0.410	0.422	0.400	0.441	0.433	0.404	0.424	0.434	0.457	0.3
G28	0.334	0.395	0.351	0.420	0.418	0.389	0.402	0.392	0.414	0.395	0.422	0.412	0.403	0.410	0.463	0.415	0.3
G29	0.350	0.394	0.350	0.427	0.409	0.395	0.392	0.389	0.414	0.396	0.432	0.414	0.409	0.423	0.466	0.414	0.3
G30	0.285	0.366	0.346	0.390	0.367	0.367	0.378	0.358	0.384	0.351	0.380	0.380	0.379	0.388	0.369	0.404	0.3
G31	0.280	0.355	0.351	0.357	0.351	0.367	0.360	0.325	0.356	0.338	0.343	0.345	0.360	0.373	0.356	0.373	0.3
G32	0.284	0.366	0.360	0.370	0.370	0.368	0.397	0.378	0.377	0.399	0.350	0.395	0.412	0.410	0.402	0.401	0.4
G33	0.397	0.467	0.380	0.481	0.469	0.472	0.505	0.553	0.516	0.498	0.469	0.539	0.498	0.425	0.414	0.407	0.3
G34	0.254	0.303	0.280	0.306	0.301	0.322	0.313	0.326	0.306	0.313	0.315	0.323	0.329	0.371	0.359	0.376	0.4
G35	0.275	0.292	0.253	0.320	0.302	0.303	0.314	0.299	0.305	0.292	0.317	0.296	0.310	0.358	0.340	0.343	0.3
G36	0.295	0.370	0.294	0.360	0.366	0.380	0.363	0.347	0.384	0.361	0.415	0.340	0.373	0.403	0.436	0.412	0.3
G37	0.300	0.350	0.293	0.373	0.350	0.350	0.376	0.337	0.363	0.359	0.370	0.336	0.354	0.426	0.404	0.404	0.3
G38	0.291	0.329	0.286	0.338	0.327	0.338	0.342	0.335	0.349	0.351	0.375	0.338	0.358	0.406	0.398	0.422	0.3
G39	0.300	0.330	0.286	0.339	0.333	0.339	0.343	0.326	0.318	0.355	0.362	0.335	0.351	0.393	0.397	0.400	0.3
G40	0.268	0.347	0.294	0.347	0.333	0.343	0.352	0.315	0.344	0.367	0.367	0.331	0.333	0.391	0.388	0.391	0.3

Figures

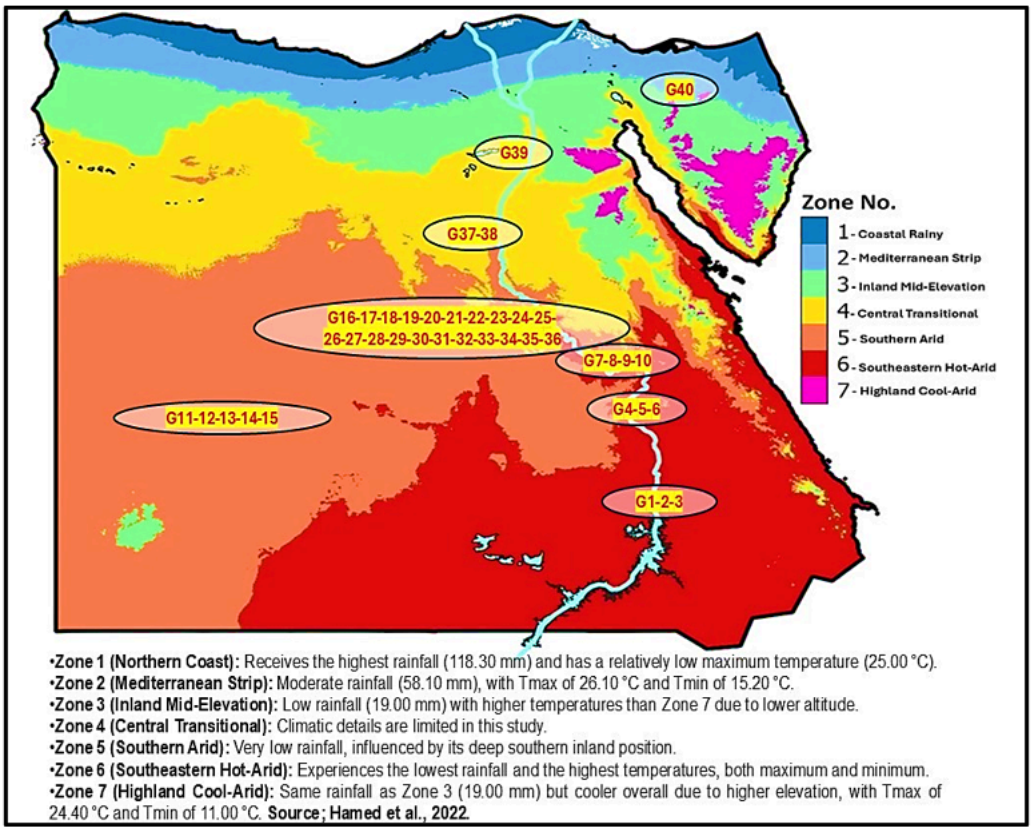


Figure 1

Distribution of 40 wheat genotype collection sites across various climatic zones in Egypt, (Hamed et al., 2022).

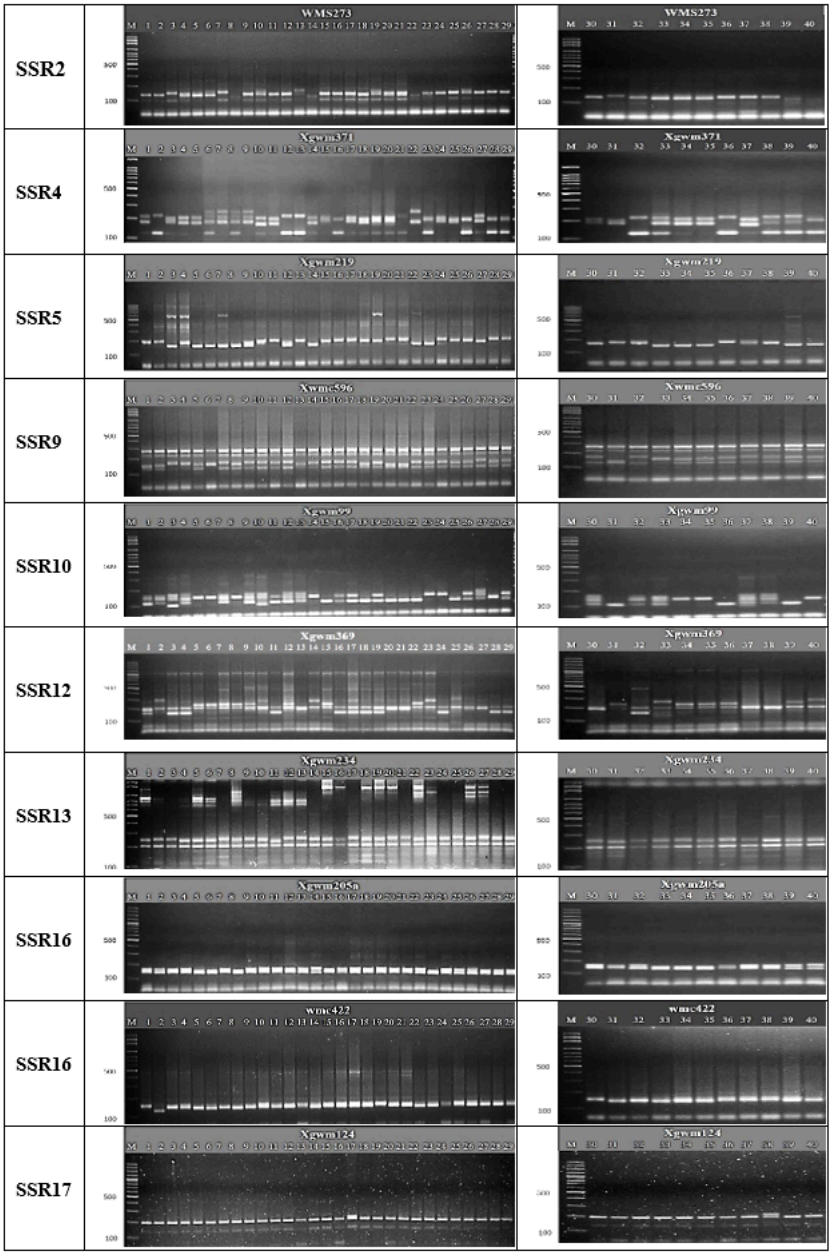


Figure 2

SSR marker profiles of forty wheat landraces amplified using ten primer pairs.

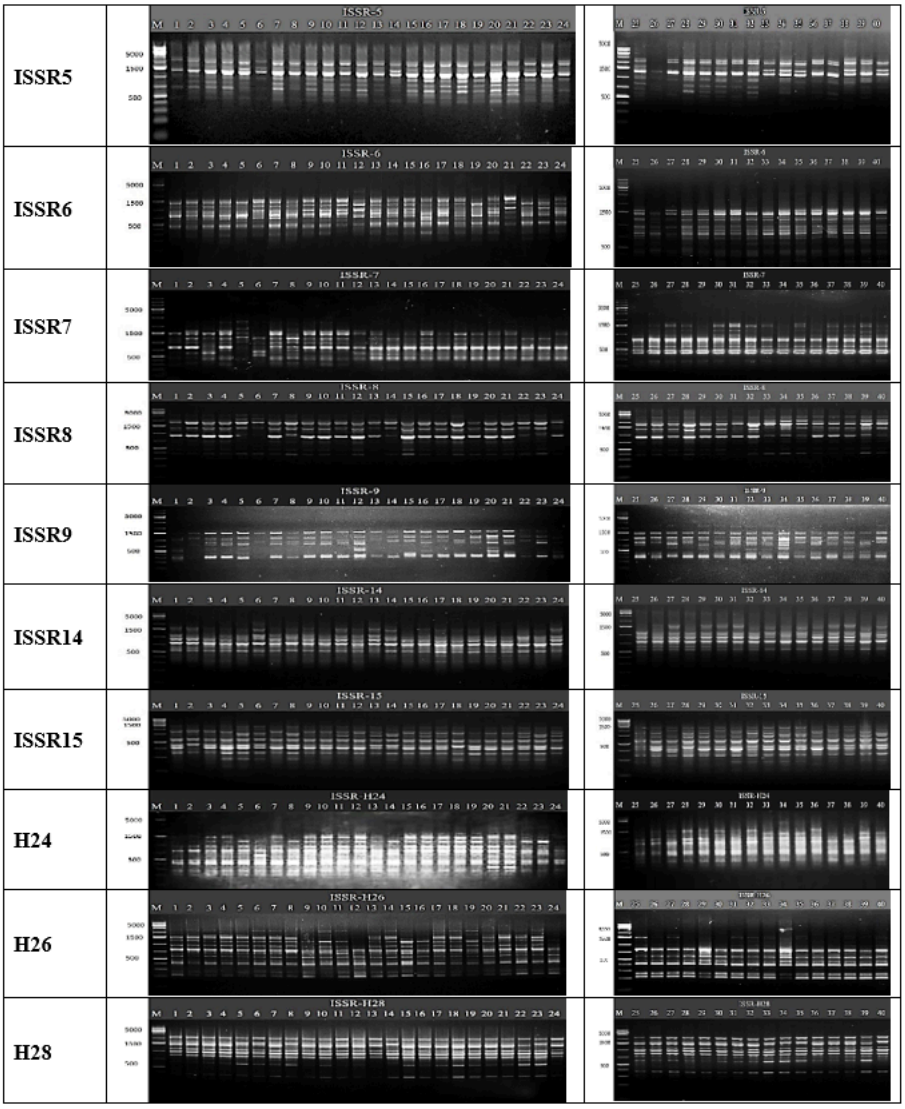


Figure 3

ISSR marker profiles of forty wheat landraces amplified using ten primer pairs.

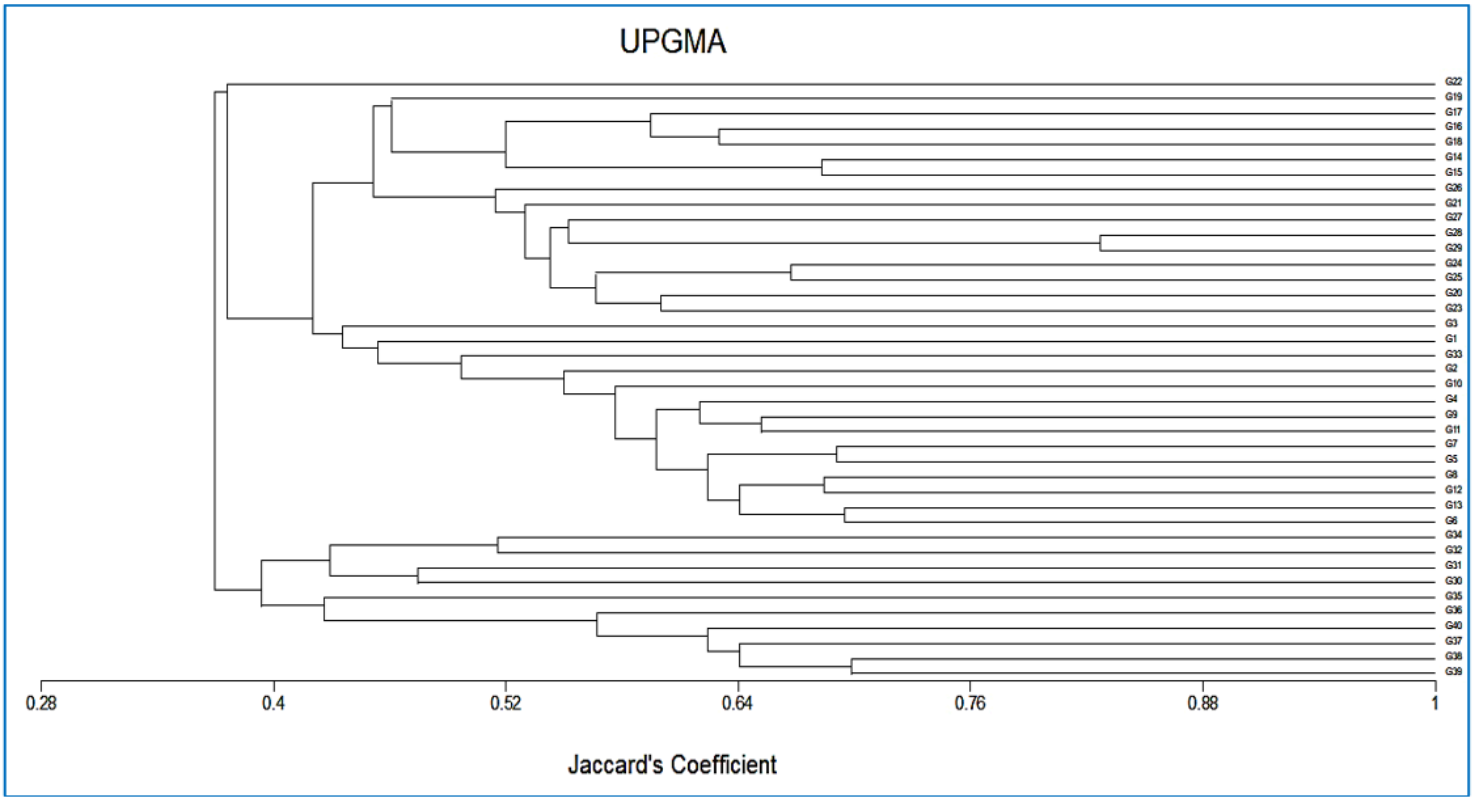


Figure 4

Dendrogram illustrating the relationships among 40 wheat genotypes based on combined ISSR, SSR, and AFLP marker data.

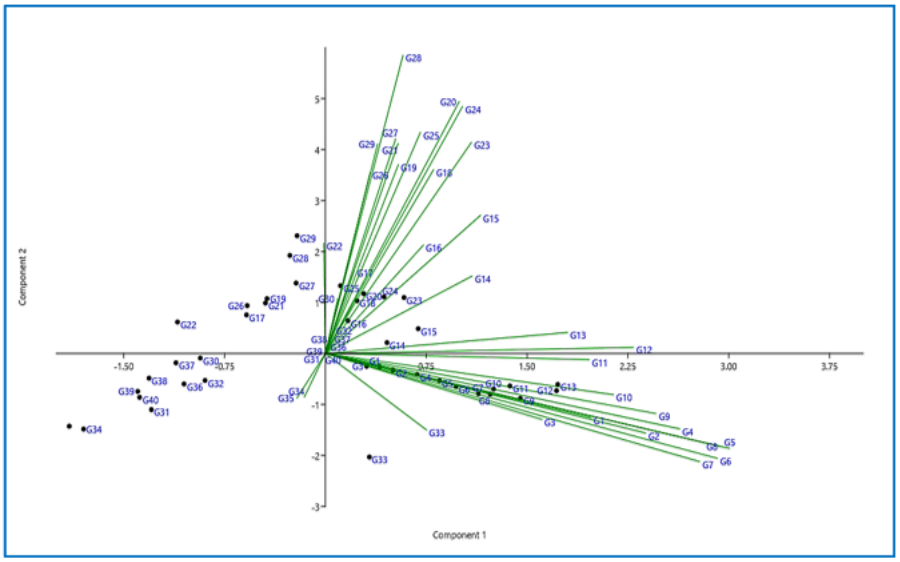


Figure 5

Principal Component Analysis (PCA) biplot of 40 wheat (*Triticum aestivum* L.) genotypes.

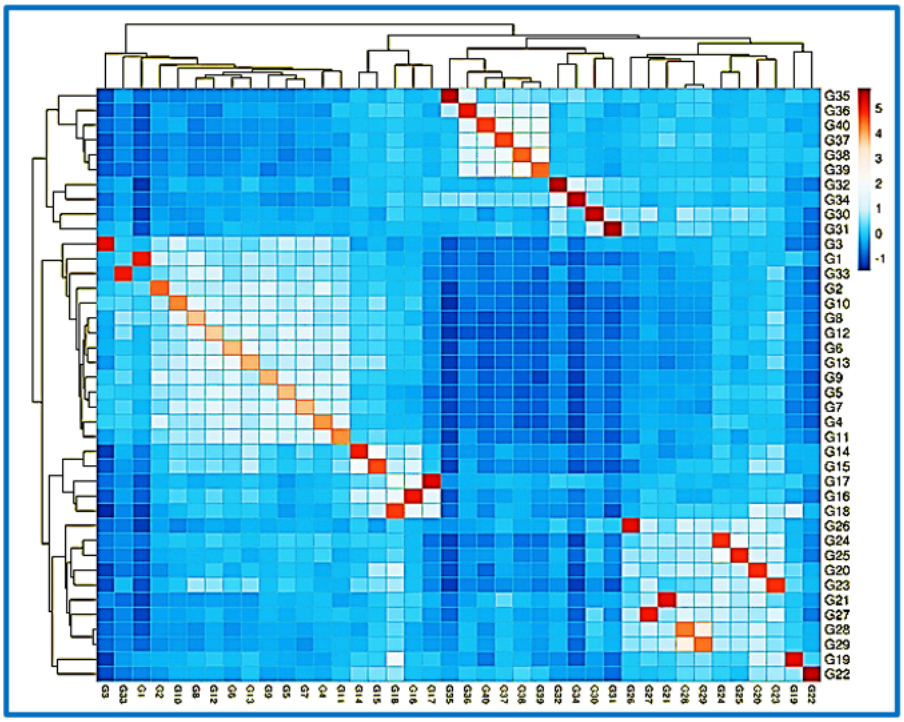


Figure 6

Heatmap of Genetic Similarity Among 40 Wheat (Triticum aestivum L.) Genotypes Based on Molecular Marker Data.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SSR21.tif
- SSR22.tif
- SSR52.tif
- SSR102.tif
- SSR122.tif
- SSR122.tif
- SSR142.tif
- SSR172.tif
- SSR132.tif
- SSR41.tif
- SSR42.tif
- SSR51.tif
- SSR91.tif
- SSR92.tif
- SSR101.tif
- SSR121.tif
- SSR161.tif
- SSR161.tif
- SSR141.tif
- SSR131.tif
- SSR162.tif
- SSR171.tif
- ISSR51.tif
- ISSR52.tif
- ISSR61.tif

- ISSR62.tif
- ISSR71.tif
- ISSR72.tif
- ISSR81.tif
- ISSR82.tif
- ISSR91.tif
- ISSR92.tif
- ISSR141.tif
- ISSR142.tif
- ISSRH241.tif
- ISSRH242.tif
- ISSRH262.tif
- ISSRH282.tif
- ISSR151.tif
- ISSRH261.tif
- ISSR152.tif
- ISSRH281.tif
- AFLPReportanalysis.xlsx
- ISSRReportanalysis.xlsx
- SSRReportanalysis.xlsx