Molecular and agro- morphological diversity assessment of some bread wheat genotypes and their crosses for drought tolerance (#103277)

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Molecular and agro- morphological diversity assessment of some bread wheat genotypes and their crosses for drought tolerance

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Wheat is a crucial cereal crop facing climate change and population growth challenges. Maintaining genetic diversity is vital for breeding drought-tolerant cultivars. This study assessed the genetic diversity and drought response of diverse wheat cultivars and their corresponding F1 crosses compared to well-watered conditions. The molecular profiling was conducted utilizing ISSR and SCoT markers. In total of 76 loci were amplified using ISSR and SCoT-PCR primers, out of which 28 were polymorphic and 48 were monomorphic. A statistically significant effect of parental genotypes and their crosses was observed on all investigated agro-morphological traits, including root length, root weight, shoot length, shoot weight, proline content, spikelet number / spike, spike length, grain number / spike, and grain weight/spike. The evaluated genotypes were classified based on their agronomic performance under drought stress into distinct groups ranging from drought-tolerant genotypes (group A) to drought-sensitive ones (group C). The genotypes P5, P2×P5, and P3×P5 were identified as promising genotypes to improve agronomic performance under water deficit conditions. The results demonstrated genotypic variations for drought tolerance and highlighted the potential of ISSR and SCoT markers in wheat breeding programs for developing drought-resistant cultivars.

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Abstract

Wheat is a crucial cereal crop facing climate change and population growth challenges. Maintaining genetic diversity is vital for breeding drought-tolerant cultivars. This study assessed the genetic diversity and drought response of diverse wheat cultivars and their corresponding F1 crosses compared to well-watered conditions. The molecular profiling was conducted utilizing ISSR and SCoT markers. In total of 76 loci were amplified using ISSR and SCoT-PCR primers, out of which 28 were polymorphic and 48 were monomorphic. A statistically significant effect of parental genotypes and their crosses was observed on all investigated agro-morphological traits, including root length, root weight, shoot length, shoot weight, proline content, spikelet number / spike, spike length, grain number / spike, and grain weight/spike. The evaluated genotypes were classified based on their agronomic performance under drought stress into distinct groups ranging from drought-tolerant genotypes (group A) to drought-sensitive ones (group C). The genotypes P5, P2×P5, and P3×P5 were identified as promising genotypes to improve agronomic performance under water deficit conditions. The results demonstrated genotypic variations for drought tolerance and highlighted the potential of ISSR and SCoT markers in wheat breeding programs for developing drought-resistant cultivars.

Keywords: abiotic stress, cereal crops, drought, ISSR markers, molecular diversity, SCoT markers

1. Introduction

Bread wheat (*Triticum aestivum* L.) is an important staple crop, providing a significant portion of the daily caloric intake for a large part of the global population (Alomari et al., 2023). As a hexaploid species, it possesses three sets of related ancestral genomes, each containing 14 chromosomes, resulting in a total of 42 chromosomes (2n = 6x = 42) (Venske et al., 2019).

Climate change is predicted to significantly affect the environment through various factors, including altered rainfall patterns, temperature fluctuations, increased salinity, reduced soil fertility, heightened biological stress, escalating pollution levels, and a concerning decline in biodiversity (Fawzy et al., 2020). These multifaceted environmental changes present a significant threat to crop production, as plant growth and development are intricately influenced by the complex interplay of these factors (Chaudhry and Sidhu, 2022). Wheat production faces a significant challenge in water scarcity, which is increasingly becoming a critical issue in many wheat-growing regions worldwide. Climate change and population growth are predicted to exacerbate water shortage, potentially leading to devastating reductions in wheat productivity (Pequeno et al., 2024; Rezaei et al., 2023). In light of these challenges, assessing wheat genetic resources for future utilization is of paramount importance (Guzzon et al., 2022). Moreover, integrating pre-breeding materials and existing cultivars into genomics-assisted breeding programs offers immense potential for improving the productivity of wheat varieties (Rasheed et al., 2017). Consequently, developing drought-tolerant bread wheat genotypes and maintaining and enhancing wheat production, which relies on harnessing its genetic diversity, is crucial to ensure global food security in these emerging threats.

Recent advancements in molecular biology have resulted in the developing DNA markers, like Inter simple sequence repeat (ISSR), which offer valuable tools for investigating genetic diversity within crop germplasm collections (Abdelghaffar et al., 2023; Al-Khayri et al., 2023; Al-Khayri et al., 2022). ISSRs target regions flanking short microsatellites, tandem repeats of DNA sequences situated nearby and oriented in opposite directions. Amplification of these flanking regions is achieved through PCR (polymerase chain reaction) using either a single primer or a set of primers. The primer design incorporates SSR motifs anchored at the 5' or 3' end, typically consisting of 1-4 pyrimidine or purine residues (Bornet and Branchard, 2001). Moreover, Start Codon Targeted (SCoT) markers offer a reproducible and dominant approach for genetic analysis. SCoT employs a single 18-mer primer targeting the conserved sequence flanking the ATG translation start codon in plant genes. This method necessitates an annealing temperature as low as 50°C (Collard and Mackill, 2009). Both ISSR and SCoT polymorphisms have proven valuable in characterizing cultivars, differentiating genetic resources, and introducing marker-assisted selection in various plant species (Abdelghaffar et al., 2023; Al-Ghamedi et al., 2023; Al-Khayri et al., 2023; Atsbeha et al., 2023; Essa et al., 2023a; Essa et al., 2023b; Golkar and Nourbakhsh, 2019; Gupta et al., 2017).

This study explored the genetic diversity of 15 wheat genotypes, including ten recently developed crosses and their five corresponding parental lines. ISSR and SCoT markers were employed alongside agro-morphological traits to assess genetic variation. We hypothesize that genotypes with high genetic diversity will be prioritized for breeding programs aimed at developing wheat cultivars adaptable to diverse climatic conditions. The combination analysis of both molecular and agro-morphological markers will enhance understanding of the genetic variability within the germplasm under study.

2. Materials and Methods

2.1. Plant materials and experimental treatment

Five wheat genotypes were utilized in this study (Table 1). A half-diallel mating design (5×5) produced 10 F1 hybrids during the winter season of 2020–2021. The genotypes of the parents and their offspring were assessed in field conditions at Experimental Farm of Faculty of Agriculture belongs to Zagazig University, Egypt (30°35′15″ N, 31°30′07″ E, 16 m asl) under ordinary growing conditions during the growing season of 2021 to 2022. The experimental site has an arid climate and receives low precipitation with an average annual rainfall of approximately 55 mm. The experiment was carried out in three replicates using a completely randomized design. The assessed genotypes (parents and F1 crosses) were represented by fifteen seeds planted in pots containing 10 kg of soil. After 15 days, the number of plants per pot was reduced to ten through thinning. Phosphorus and potassium fertilizers were applied as basal doses with a rate of 30 mg P_2O_5 per kg of soil for superphosphate and 50 mg K_2O per kg of soil for potassium sulfate. Nitrogen fertilizer was applied in three installments at a rate of 80 mg N per kg of soil using ammonium sulfate. These installments were done at 20, 35, and 50 days after sowing, along with irrigation water. Intercultural practices such as weeding were performed as needed to maintain optimal growing conditions. To induce drought stress, the irrigation schedule for the pots was adjusted. The stressed pots received water once a week, while the control well-watered pots were irrigated every three days. Soil water tension was measured using a tensiometer to maintain appropriate irrigation levels for both the well-watered and stressed pots were maintained.

2.2. Extraction of genomic DNA

In this experiment, 100 grams of young wheat leaves were employed for the extraction of genomic DNA utilizing a modified CTAB-based protocol (Doyle, 1991; Scobeyeva et al., 2018). The quantity and purity of the extracted DNA were assessed using a NanoDroP2000 spectrophotometer (Thermo Scientific™, Waltham, MA, USA). The DNA concentration was adjusted to 50 ng/µL, and the isolated DNA was stored at -20°C for subsequent amplification procedures.

2.3. Inter-Simple Sequence Repeats (ISSR-PCR)

Genetic polymorphism analysis of wheat cultivars and their F1 hybrids was conducted utilizing Inter simple sequence repeat (ISSR)-PCR. Primers for the analysis were presented in Table 2. The PCR protocol followed the methodology established by Moreno et al. (1998). Each reaction mixture, with a volume of 25 µL, contained the following components: 2 µL of 5x reaction buffer, 20 ng/µL of template DNA, µL of 200 µM dNTPs, 2 µL of 25 mM MgCl2, 22 µL of primer (10 pmol), and 1 unit of Taq DNA polymerase (Promega). The thermocycling protocol commenced with an initial denaturation step at 94°C for 5 minutes, followed by 35 amplification cycles. Each cycle comprised denaturation at 94°C for 1 minute, annealing at a primer-specific temperature for 1 minute and extension at 72°C for 1 minute. The procedure concluded with a final extension at 72°C for 5 minutes..

2.4. Start Codon Targeted (SCoT) amplification

A 25 µL PCR amplification was conducted utilizing a SCoT-PCR based marker system. The reaction mixture consisted of ten µL of GoTaq Green-Master Mix, one µL of template DNA, one µL of primers, and nuclease free water to achieve a final volume of 25 µL. Thermal cycling was carried out using an Applied-Biosystems thermal cycler with the following protocol: initial denaturation at 94°C for 5 mins, followed by 30 cycles of denaturation at 94°C for 1 minute, annealing at 50°C for 1 minute, and extension at 72°C for 1 minute.

2.5. Gel electrophoresis

The amplified products from ISSR and SCoT reactions were separated on 1% agarose gels and visualized using ethidium bromide (MP Biomedicals, Goddard Irvine, CA, USA) staining in TBE buffer (pH 8.5). DNA fragment sizes were estimated using a 1 kbp DNA ladder.

2.6. Agro-morphological characterization

After 60 days from cultivation, measurements were taken for), shoot length (cm), root length (cm), shoot fresh weight (g), shoot dry weight (g), root fresh weight (g), and root dry weight (g). The proline content in the plant samples was assessed as follows: 0.5 g of leaves were ground and mixed with ten mL of 3% aqueous sulfosalicylic-acid to create an extract. After filtration through filter paper, two mL of this extract were combined with two mL of acid ninhydrin-reagent and two mL of glacial-acetic acid. The mixture was heated at 100°C for 1 hour, followed by rapid cooling on ice. To extract the proline, 4 mL of toluene were added to the reaction mixture, and the resulting supernatant was used for proline determination. Absorbance was measured at 520-nm employing a spectrophotometer with toluene used as the blank (Bates et al., 1973). Additionally, the experiment recorded the number of spikelets / spike, spike length (cm), number of grains / spike, and grain weight / spike.

2.7. Data analysis

Using molecular markers, this study explored the genetic diversity and relatedness of wheat genotypes and crosses. Specific PCR loci based on SCot and ISSR techniques were employed. Each locus was classified as either absent (0) or present (1) and all loci were regarded as independent variables. Genetic diversity was assessed by analyzing the banding patterns generated from the PCR amplifications across all genotypes. The polymorphism level, a measure of genetic variation, was determined by dividing the number of loci exhibiting polymorphism (different banding patterns) by the total number of scored loci. Genetic similarities among the wheat cultivars and hybrids were computed using Dice's coefficient (Dice, 1945). This coefficient was determined utilizing SPSS software (Norušis, 1993). A clustering analysis was subsequently performed to generate a dendrogram depicting the phylogenetic relationships among the genotypes (Rokach and Maimon, 2005). The dendrogram, principal component, and heatmap analyses were applied R programming. Statistically significant differences between the evaluated wheat genotypes were identified employing least significant difference (LSD) test at $P < 0.01$.

3. Results

3.1. Molecular analyses

The genetic diversity analysis among the developed crosses and their parental genotypes was assessed via ISSR and SCoT molecular markers using six ISSR primers and two SCoT primers (Figure 1). Seventy-six loci were detected using ISSR and SCoT-PCR primers screened

in 15 genotypes (Table 2). The amplified loci/primer was 9.5. Among 76 ISSR and SCoT-PCR loci, 28 were polymorphic (9.5/primer), and 48 were monomorphic (6/primer). Polymorphism ranged from 58.3% (ISSR3) to 23% (SCoT2), averaging 36.36%. The lowest genetic distance (1.41) was observed between P1×P4 vs. P4×P5, as well as P3×P5 vs P4×P5. This suggests a close genetic similarity between these populations. Conversely, the highest genetic distance (3.61) was detected between P2×P4 vs P2×P5, indicating greater genetic divergence (Table 4). The Dice coefficient was employed to analyze similarity matrices constructed from data obtained with eight primers. According to Table 5, the highest similarity (0.975) was observed between P4×P5 and P1×P4, whereas the lowest similarity (0.818) was found between P2×P5 and P2. These findings may be useful for understanding the genetic relationships between different wheat populations and informing breeding programs.

3.2. Phylogeny Analysis

The clustering analysis based on ISSR and SCoT banding profiles grouped the evaluated wheat genotypes into five groups A-E (Figure 2). Cluster A included only P2×P5, while B contained P1, and C comprised P2. Besides, Group D contained four genotypes P1×P2, P1×P3, P1×P5, and P3×P4. Finally, cluster E comprised eight genotypes P4, P3, P2×P3, P2×P4, P5, P1×P4, P3×P5, and P4×P5.

3.3. Agro-morphological traits

The performance of the studied wheat genotypes and their corresponding F1 crosses for agro-morphological traits under both drought and well-watered conditions is illustrated in Figures 3 to 5. Differences between the assessed genotypes were observed for all studied attributes. P1 and P3 exhibited high shoot fresh and dry weights under well-watered conditions which was reflected in the performance of their F1 crosses P1×P3, P1×P2, P3×P5, and P2×P3 (Figure 3). Moreover, under drought stress, P5, P3×P5, and P4×P5 showed superior performance compared to well-watered conditions, suggesting that the genes controlling these traits were passed from the parents to the offspring. The greatest root fresh and dry weights under water deficit were achieved by P5 (Orabi-1881) and its F1 crosses P2×P5 and P3×P5 (Figure 3), highlighting the significance of these crosses in breeding programs.

Drought significantly reduces overall wheat growth, which is evident in the substantial reduction in plant height for most genotypes. P1, P3, and their cross P1×P3 showed high shoot length under normal conditions (Figure 4). Under water deficit conditions, P3, P5, P3×P5, and P1×P3 performed best for shoot length. P5 and P2×P5 maintained shoot and root length under both conditions. Root length values of P5, P4×P5, P3×P5, and P3×P4 were higher under drought than under well-watered conditions (Figure 4). All genotypes showed significantly higher proline accumulation under drought stress. P2 had the highest proline content under drought and the lowest under well-watered conditions, while P3 had the opposite (Figure 4).

P2×P3, P3×P5, P3, and P3×P4 had the highest mean spike length under normal conditions, while P1×P5 and P1 had the lowest values (Figure 5). On the other hand, under drought conditions, P1 spike length was less affected compared to P2, P4, and P2×P4. P3×P5 possessed the uppermost number of spikelets per spike under both conditions, while P2 had the lowest number. P3, P1×P4, and P3×P4 showed the highest grain number per spike under well-watered conditions, while P1×P5 and P2 showed the lowest. ~~Otherwise~~, P5, P2×P5, P3×P5, and P3×P4 exhibited the greatest grain number per spik under drought stress. Moreover, P5, P3×P4, P2×P5, and P4×P5 had the highest grain weight per spike. Conversely, P3, P1×P3, P1×P5, and

P4 showed the lowest grain weight per spike under drought, indicating their sensitivity to drought.

3.4. Genotypic classification

The data obtained from agro-morphological characters were employed to illustrate the relatedness among the tested wheat genotypes based on their agronomic performance under drought stress (Figure 6). The analysis grouped wheat genotypes into three distinct clusters (A-C). Group A included three genotypes: P5, P2×P5, and P3×P5, which exhibited the best performance under drought stress, identifying them as highly drought-tolerant. Group B consisted of six genotypes: P4×P5, P1, P1×P3, P3, P3×P4, and P2×P3, which showed intermediate tolerance to drought stress. This indicates that these genotypes possess moderate drought resilience. Group C comprised six genotypes: P1×P5, P2, P1×P4, P4, P1×P2, and P2×P4, which demonstrated the lowest tolerance to drought stress. These genotypes are considered drought-sensitive. This clustering provides valuable insights for selecting genotypes for breeding programs to improve wheat drought tolerance.

3.5. Association among assessed genotypes and evaluated characters

Principal component analysis was performed to illustrate the association among agro-morphological attributes of the wheat crosses and their parental genotypes. The first two PCs displayed the most variance registering around 85.08% (62.36% and 22.72% for PC1 and PC2 in the same order), and were used to construct the PC-biplot (Figure 7). PCA1 effectively categorized the assessed genotypes into groups depending on their position on the positive or negative side. The genotypes on the positive side of PCA1 were associated with high performance, particularly P5, P3×P 5, P2×P5, P3×P4, P 2×P3, P4×P5, and P1. Conversely, the genotypes on the negative side of PCA1 exhibited inferior performance, remarkably P1× P5, P2, P 1×P4, P1×P2, and P2×P4. Yield-contributing traits showed a strong positive correlation with root characteristics. Moreover, heatmap based on the agro-morphological attributes characterized the genotypes into distinct groups (Figure 8). Using a color scale under drought stress, the heatmap analysis illustrated the relationship between the assessed genotypes and the studied traits. High values of measured agronomic characteristics were displayed in blue, while low values were shown in red. The genotypes P5, P3×P5, P2×P5, P2×P3, P3×P4, and P4×P5 exhibited greater values for all agronomic attributes corresponding to blue color in the heatmap. Otherwise, genotypes P1×P5, P2, P1×P4, P1×P2, and P2×P4 had the lowest values, expressed in red under water deficit conditions.

4. Discussion

Genetic diversity analysis employing molecular markers and agro-morphological characterization is fundamental for wheat breeding programs to develop new stress-tolerant genotypes (Bapela et al., 2022)The present study underscored the importance of assessing molecular and agro-morphological diversity in wheat genotypes and their corresponding crosses to improve drought tolerance in breeding programs. Under varying conditions, the observed performance differences among evaluated genotypes provided crucial insights into the genetic factors influencing these traits. This knowledge is instrumental in selecting superior genotypes for future breeding efforts, thereby enhancing the effectiveness of breeding programs.

The genetic diversity analysis utilizing ISSR and SCoT molecular markers (36.36% on average) suggested moderate genetic diversity among the wheat genotypes. The lowermost

genetic distance (1.41) was detected between several cross combinations, indicating close genetic relationships. The uppermost genetic distance (3.61) was detected between P2×P4 and P2×P5, suggesting a more significant difference between these parental lines and their offspring. The Dice coefficient analysis revealed similar trends, with the highest similarity between P4×P5 and P1×P4 (0.975) and the lowest between P2×P5 and P2 (0.818). The genetic distances and similarity coefficients provided further insights into genotype relationships(Herrera et al., 2021; Sheikh et al., 2021).

The clustering based on ISSR and SCoT markers resulted in five clusters (A-E). This suggests that these markers may capture a broader range of genetic variations. ~~This also suggests that ISSR and SCoT markers~~ may be more powerful for discriminating between closely related wheat genotypes(Abouseada et al., 2023; Shaban et al., 2022).Interestingly, P2×P5 formed a distinct cluster (A) in the ISSR/SCoT analysis, suggesting a unique genetic makeup despite its parents belonging to separate clusters (B and C). The ISSR and SCoT molecular markers employed in this study were informative and distinguished in the genetic diversity among the studied genotypes. Numerous studies have explored the molecular diversity of bread wheat using these markers (Abouseada et al., 2023; Atsbeha et al., 2023; Jabari et al., 2023; Shaban et al., 2022). Some studies revealed that SRAP molecular marker has the great potential to determine genetic diversity(Al-Ghamedi et al., 2023; Essa et al., 2023a; Yi et al., 2021; Zhou et al., 2021). Additionally, Several studies have employed SCoT markers alongside ISSR markers ~~in some cases (Etminan et al., 2016),~~ to assess genetic diversity in ~~wheat germplasm. These studies include~~ durum wheat breeding lines and landraces (Etminan et al., 2016), Iranian Triticum species (Pour-Aboughadareh et al., 2017), North African wheat cultivars (Mohamed et al., 2017), and Triticum urartu accessions(Gholamian et al., 2019).

Considerable differences were detected between the parental genotypes and their crosses for all evaluated agro-morphological attributes. Under drought stress, the genotypes P5, P3 × P5, P2×P5, P2 × P3, P3×P4, and P4 × P5 demonstrated superior performance, with enhanced shoot and root growth, underscoring their resilience. These genotypes appear to have inherited drought-tolerant traits, making them vital for breeding programs to improve root and shoot traits under water deficit conditions (Zhang et al., 2017). Moreover, the spike traits of these genotypes were also less affected by water-limited conditions compared to other genotypes. This highlights their potential to enhance drought tolerance in wheat breeding programs (Adel and Carels, 2023). All studied genotypes exhibited significantly higher proline accumulation under drought stress, an indicator of stress tolerance, suggesting varied stress response mechanisms among the genotypes (Guizani et al., 2023). In contrast, under well-watered conditions, P1 and P3 showed excellent agro-morphological performance, which was also reflected in their F1 crosses P1×P3, P1 × P2, P3× P5, and P2×P3. This indicates that these genotypes possess traits that are beneficial for growth in optimal conditions. These findings underscore the importance of specific genotypes and their crosses in breeding programs aimed at both optimal growth and drought tolerance conditions (Lazaridi et al., 2024).

5. Conclusions

Exploring genetic diversity employing molecular markers and agro-morphological characterization is essential for developing stress-tolerant wheat genotypes. Genetic diversity analysis utilizing ISSR and SCoT markers showed a moderate diversity level, with unique genetic makeups in specific crosses such as P2×P5. The genotypes P5, P3 × P5, P2×P5, P2 × P3, P3 × P4, and P4×P5 performed well under drought stress, indicating their resilience and

suitability for drought-tolerance breeding programs. Otherwise, genotypes P1 and P3 and their F1 crosses exhibited better agro-morphological performance under well-watered conditions. These findings highlight the importance of selecting specific genotypes for improving both drought tolerance and growth performance and demonstrate that integrating molecular markers with agro-morphological traits is a broad approach to advancing wheat breeding strategies and enhancing crop resilience and productivity.

Author Contributions

Conceptualization, S.S.S., M.S., A.A.H.; methodology, M.A.E., N.M.A., S.S.S., M.S., R.M.H., M.M.K., D.A., W.F.F., N.M.A., M.A., I.B.A., E.M., A.A.H.; software, M.A.E., N.M.A., S.S.S., M.S., R.M.H., M.M.K., D.A., W.F.F., N.M.A., M.A., I.B.A., E.M., A.A.H.; validation, M.A.E., N.M.A., S.S.S., M.S., R.M.H., M.M.K., D.A., W.F.F., N.M.A., M.A., I.B.A., E.M., A.A.H.; formal analysis, M.A.E., N.M.A., S.S.S., M.S., R.M.H., M.M.K., D.A., W.F.F., N.M.A., M.A., I.B.A., E.M., A.A.H.; investigation, M.A.E., N.M.A., S.S.S., M.S., R.M.H., M.M.K., D.A., W.F.F., N.M.A., M.A., I.B.A., E.M., A.A.H.; resources, M.A.E., N.M.A., S.S.S., M.S., R.M.H., M.M.K., D.A., W.F.F., N.M.A., E.M., A.A.H.; data curation, M.A.E., N.M.A., S.S.S., M.S., R.M.H., M.M.K., D.A., W.F.F., N.M.A., M.A., I.B.A., E.M., A.A.H.; writing—original draft preparation, M.A.E., N.M.A., S.S.S., M.S., R.M.H., M.M.K., D.A., W.F.F., N.M.A., M.A., I.B.A., E.M., A.A.H.; writing—review and editing, M.A.E., N.M.A., S.S.S., M.S., R.M.H., M.M.K., D.A., W.F.F., N.M.A., M.A., I.B.A., E.M., A.A.H.; visualization, M.A.E., N.M.A., S.S.S., M.S., R.M.H., M.M.K., D.A., W.F.F., N.M.A., M.A., I.B.A., E.M., A.A.H.; supervision, S.S.S., M.S., A.A.H.; project administration, S.S.S., M.S., A.A.H.; funding acquisition, S.S.S., M.S., N.M.A., A.A.H.. All authors have read and agreed to the published version of the manuscript.

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446

Figure 1

ISSR and SCoT-PCR amplification patterns of 15 wheat genotypes

ISSR and SCoT-PCR amplification patterns of 15 wheat genotypes using six ISSR primers (A-F)and two SCoT primers (G and H). M=1kbp DNA ladder

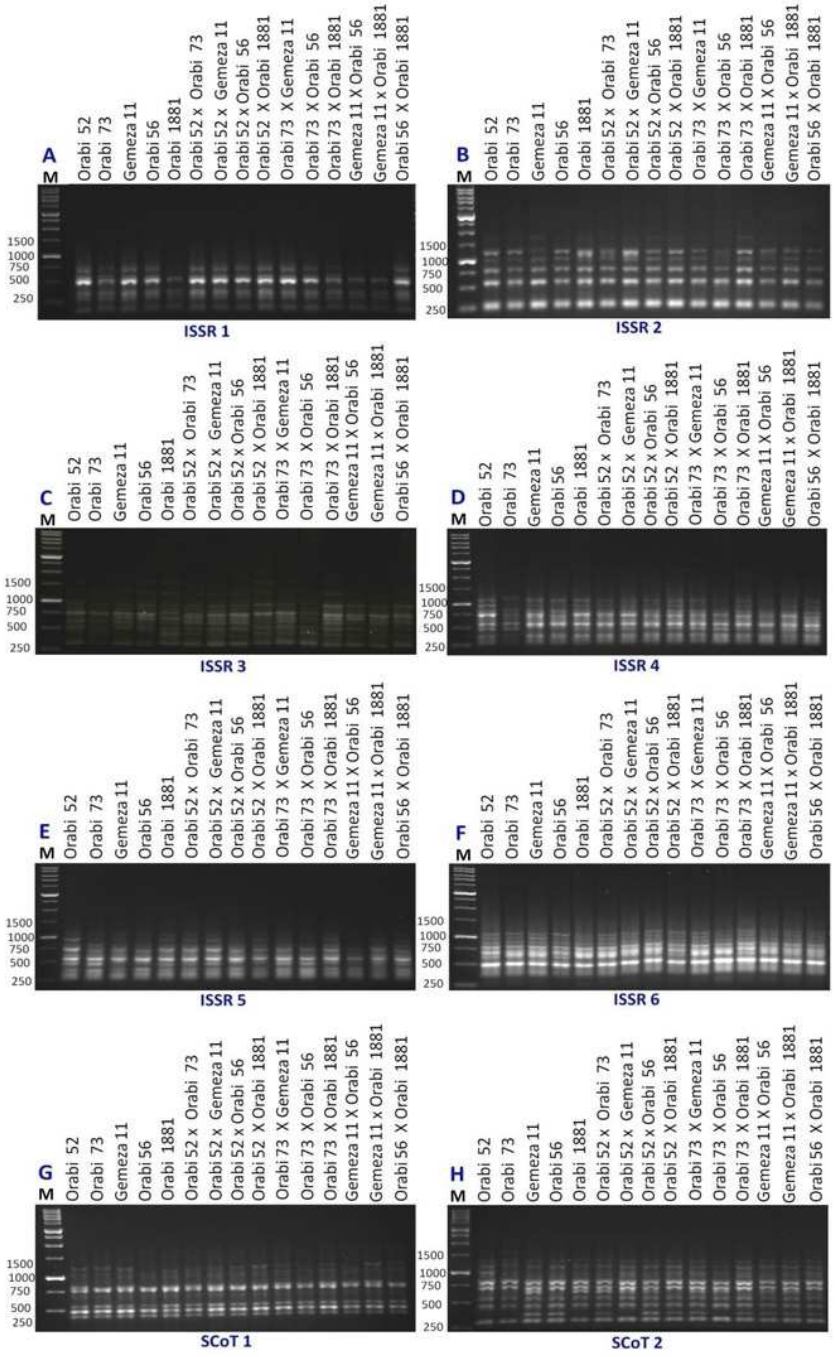
A
M
Orabi 52
Orabi 73
Gemeza 11
Orabi 56
Orabi 1881
Orabi 52 x Orabi 73
Orabi 52 x Gemeza 11
Orabi 52 x Orabi 56
Orabi 52 X Orabi 1881
Orabi 73 X Gemeza 11
Orabi 73 X Orabi 56
Orabi 73 X Orabi 1881
Gemeza 11 X Orabi 56
Gemeza 11 x Orabi 1881
Orabi 56 X Orabi 1881
1500
1000
750
500
250
ISSR 1
B
ISSR 2
C
ISSR 3
D
ISSR 4
E
ISSR 5
F
ISSR 6
G
SCoT 1
H
SCoT 2

Figure 1.

Figure 2

The phylogenetic tree of developed crosses and their parental wheat genotypes

The phylogenetic tree of developed crosses and their parental wheat genotypes were revealed according to ISSR and SCoT banding profiles.

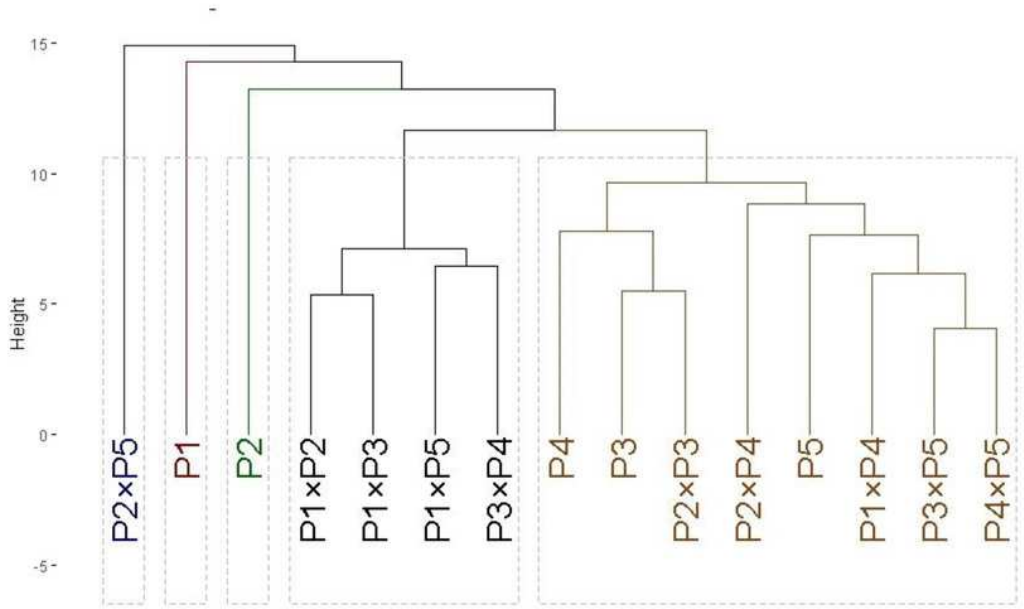
Height
15
10
5
0
-5
P2×P5
P1
P2
P1×P2
P1×P3
P1×P5
P3×P4
P4
P3
P2×P3
P2×P4
P5
P1×P4
P3×P5
P4×P5

Figure 2.

Figure 3

Comparative performance of developed crosses and their parental genotypes

Comparative performance of developed crosses and their parental genotypes: (A) shoot fresh weight, (B) shoot dry weight, (C) root fresh weight, and (D) root fresh weight (D). The bars at the top of the columns indicate the standard error (SE). Different letters on the columns indicate a significant difference using LSD, $p<0.01$. Uppercase letters represent well-watered conditions, while lowercase letters represent water deficit conditions.

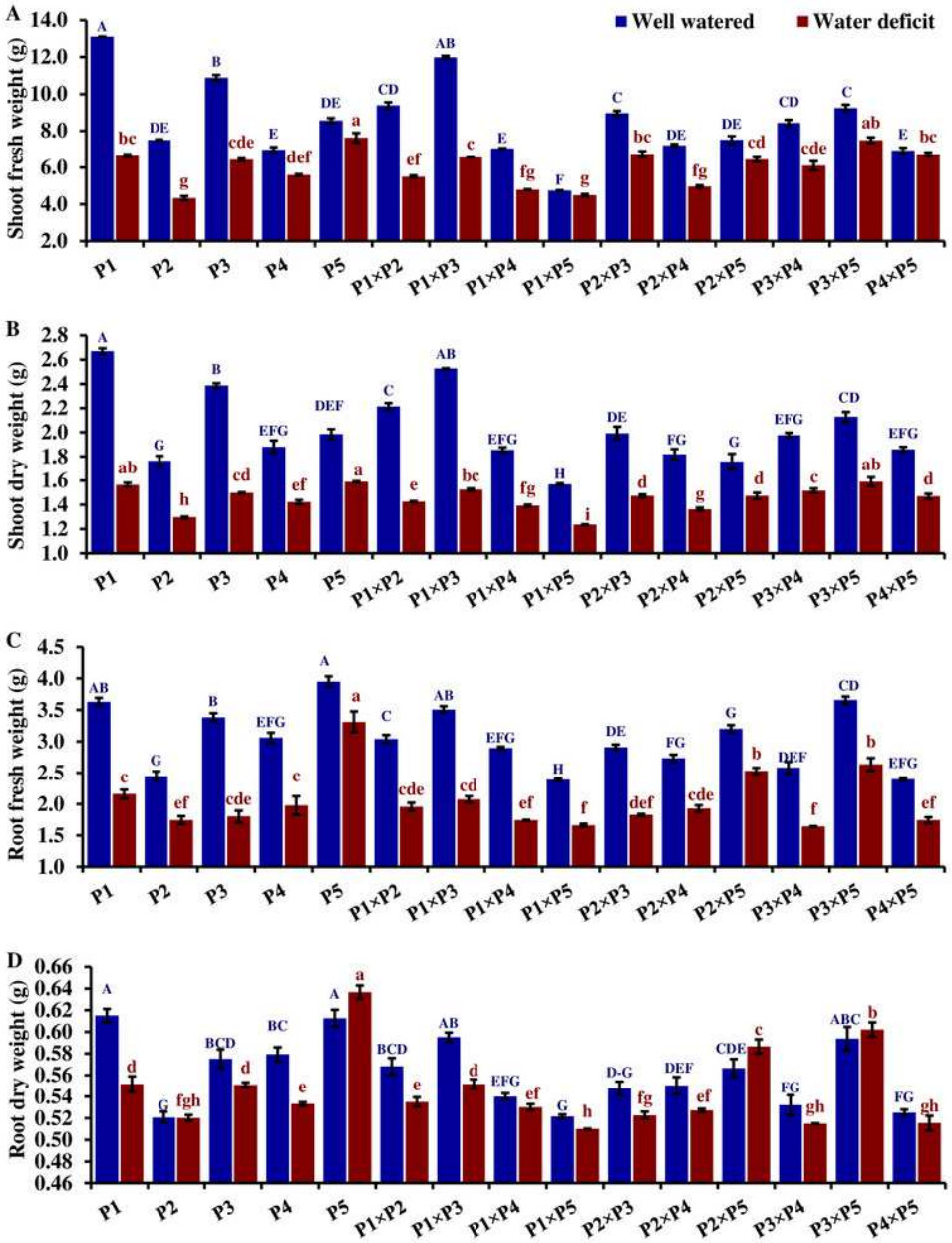
A
Shoot fresh weight (g)
Well watered
Water deficit
B
Shoot dry weight (g)
C
Root fresh weight (g)
D
Root dry weight (g)
P1
P2
P3
P4
P5
P1×P2
P1×P3
P1×P4
P1×P5
P2×P3
P2×P4
P2×P5
P3×P4
P3×P5
P4×P5

Figure 3.

Figure 4

Comparative performance of developed crosses and their parental genotypes

Comparative performance of developed crosses and their parental genotypes: (A) shoot length, (B) root length, and (C) proline content . The bars at the top of the columns indicate the standard error (SE). Different letters on the columns indicate a significant difference using LSD, $p<0.01$. Uppercase letters represent well-watered conditions, while lowercase letters represent water deficit conditions.

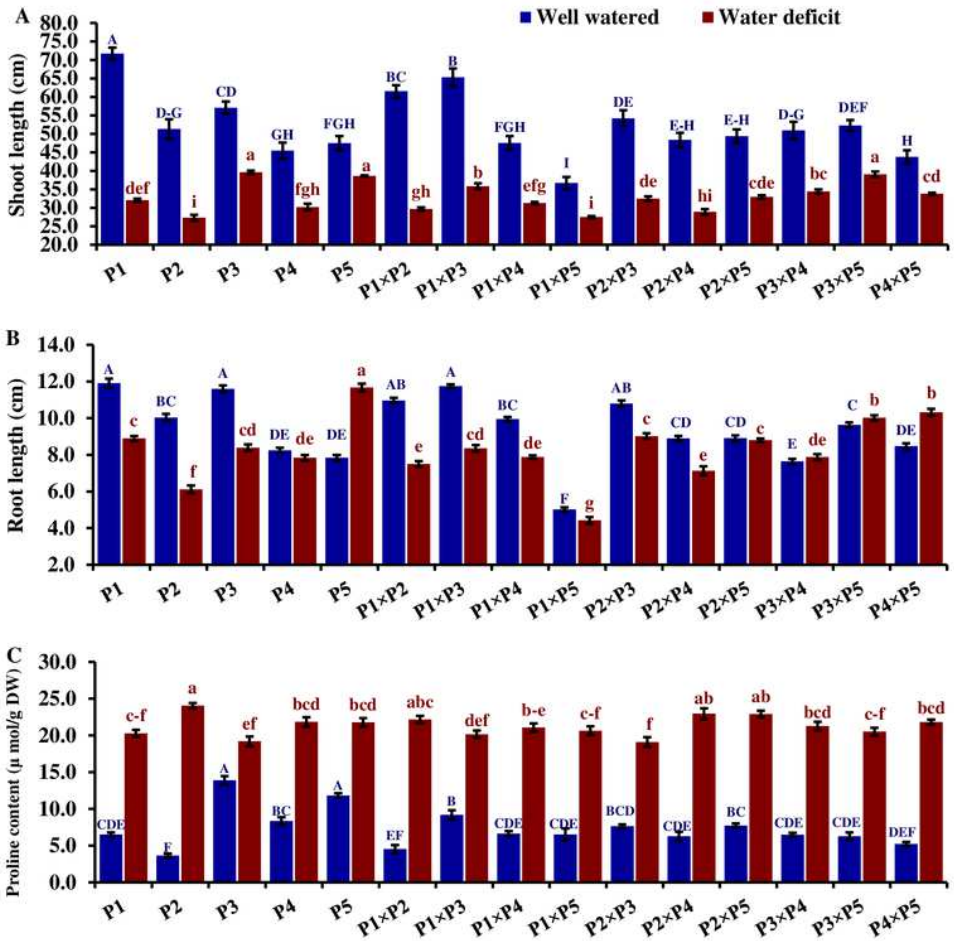
A
Well watered
Water deficit
Shoot length (cm)
B
Root length (cm)
C
Proline content (μ mol/g DW)
P1
P2
P3
P4
P5
P1×P2
P1×P3
P1×P4
P1×P5
P2×P3
P2×P4
P2×P5
P3×P4
P3×P5
P4×P5

Figure 4.

Figure 5

Comparative performance of developed crosses and their parental genotypes

Comparative performance of developed crosses and their parental genotypes: (A) spike length, (B) spikelet number/spike, (C) grain number/spike and (D) grain weight/spike (D). The bars at the top of the columns indicate the standard error (SE). Different letters on the columns indicate a significant difference using LSD, $p<0.01$. Uppercase letters represent well-watered conditions, while lowercase letters represent water deficit conditions.

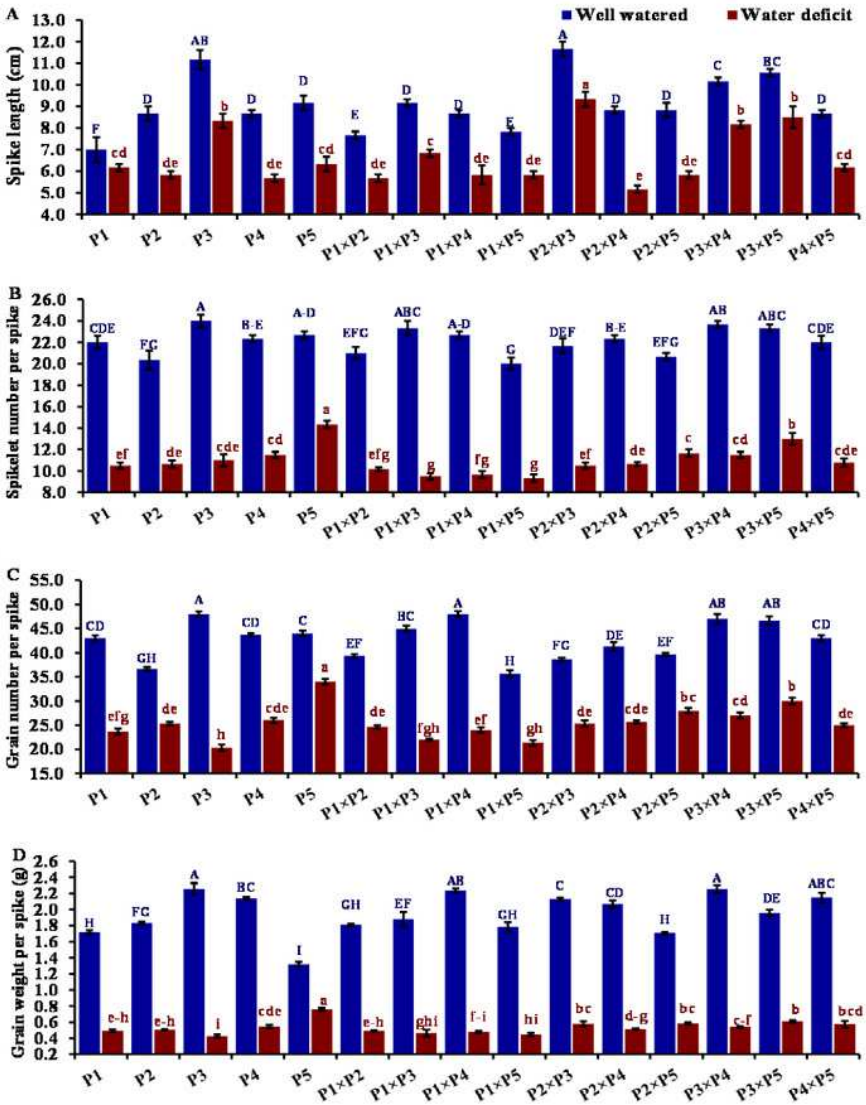
A
Well watered
Water deficit
Spike length (cm)
B
Spikelet number per spike
C
Grain number per spike
D
Grain weight per spike (g)
P1
P2
P3
P4
P5
P1×P2
P1×P3
P1×P4
P1×P5
P2×P3
P2×P4
P2×P5
P3×P4
P3×P5
P4×P5

Figure 5.

Figure 6

Dendrogram of developed crosses and their parental wheat genotypes

Dendrogram of developed crosses and their parental wheat genotypes according to the evaluated traits under water deficit conditions.

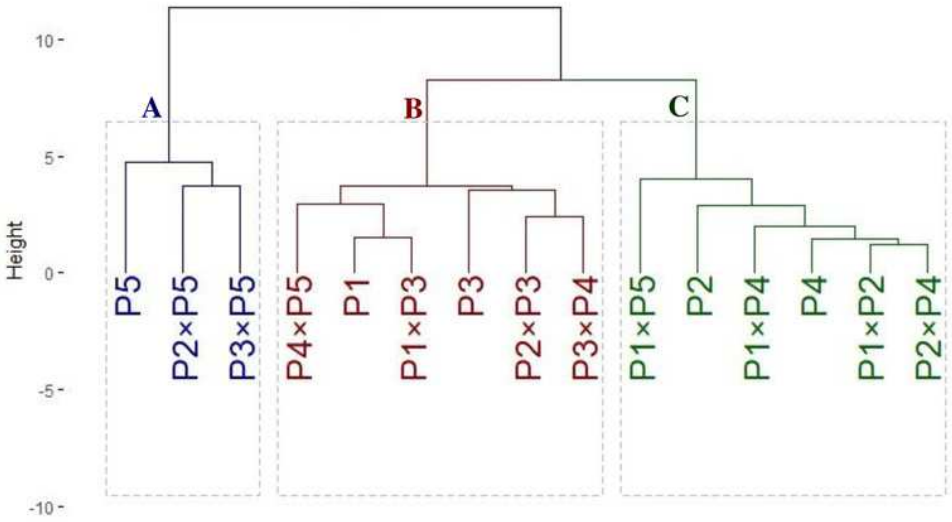
10
5
0
-5
-10
Height
A
B
C
P5
P2×P5
P3×P5
P4×P5
P1
P1×P3
P3
P2×P3
P3×P4
P1×P5
P2
P1×P4
P4
P1×P2
P2×P4

Figure 6.

Figure 7

The principal component biplot for the developed crosses and their parental wheat

The principal component biplot for the developed crosses and their parental wheat according to the traits studied under water deficit conditions.

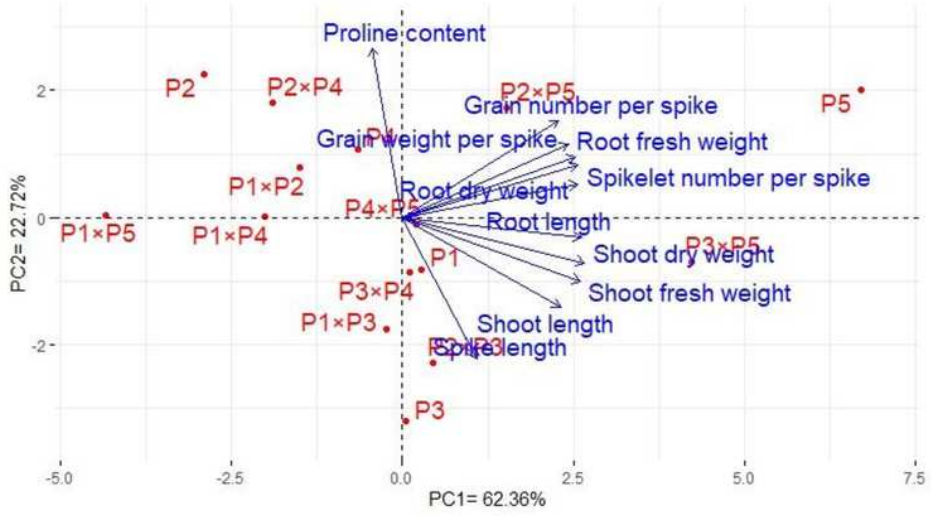
Proline content
P2
P2×P4
P2×P5
Grain number per spike
P5
Grain weight per spike
Root fresh weight
P1×P2
Spikelet number per spike
Root dry weight
P4×P5
Root length
P1×P5
P1×P4
P3×P5
P1
Shoot dry weight
P3×P4
Shoot fresh weight
P1×P3
Shoot length
Spike length
P3
PC2= 22.72%
PC1= 62.36%
2
0
-2
-5.0
-2.5
0.0
2.5
5.0
7.5

Figure 7.

Figure 8

Heatmap categorizing the developed crosses and their parental wheat

Heatmap categorizing the developed crosses and their parental wheat genotypes under water deficit conditions into distinct clusters based on studied traits.

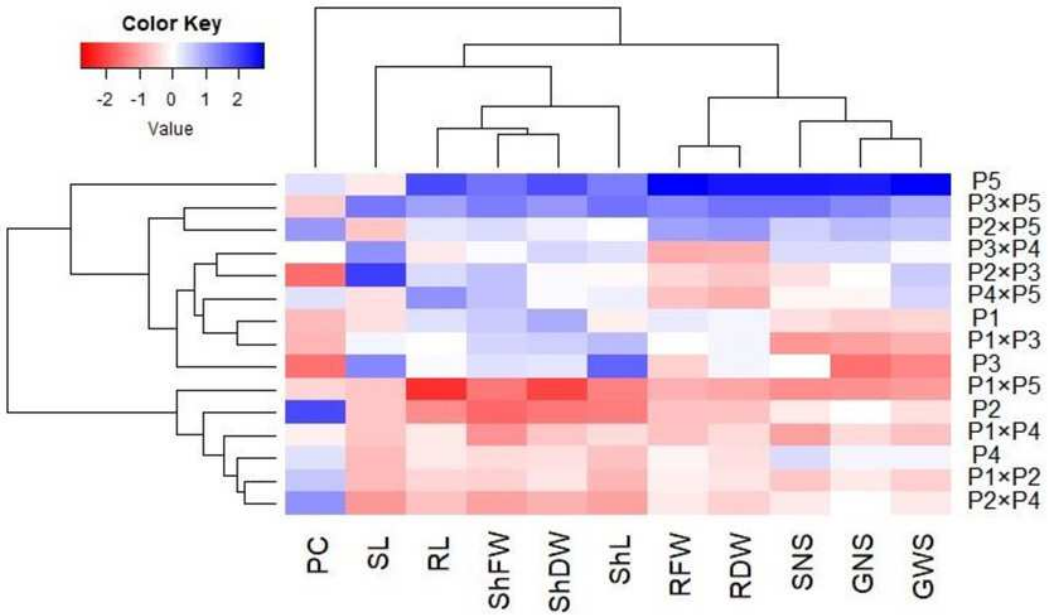
Color Key
-2 -1 0 1 2
Value
P5
P3×P5
P2×P5
P3×P4
P2×P3
P4×P5
P1
P1×P3
P3
P1×P5
P2
P1×P4
P4
P1×P2
P2×P4
PC
SL
RL
ShFW
ShDW
ShL
RFW
RDW
SNS
GNS
GWS

Figure 8.

Table 1(on next page)

Pedigree and origin of the wheat genotypes

Pedigree and origin of the wheat parental genotypes.

1 **Table 1**. Pedigree and origin of the wheat parental genotypes.

Code	Genotype	Pedigree
P1	Orabi-52	New promising mutant line G-168-3-1 of M7 generation by using EMS 0.5 % (Giza168- EMS), DUS no 269, 2018 year
P2	Orabi-73	promising mutant-line of M7 generation by using Gamma rays 300-Gy dose (Seds12), DUS no 270, 2018 year
P3	Gemmiza 11	BOW- s/KVZ/7C-SERI 82/3-GIZA 168-SAKHA 61
P4	Orabi-56	New promising mutant line G- 168-5-1atM7 generation by using EMS 0.5 % (Giza 168-EMS), DUS no, 2023
P5	Orabi-1881	New promising mutant line of M7 generation by using EMS 0.25 % (Sakha93-EMS)), DUS no 284, 2018

2

Table 2(on next page)

Characterization of ISSR and SCoT primers

Characterization of ISSR and SCoT primers.

1 **Table 2.** Characterization of ISSR and SCoT primers.

Primer	Nucleotide sequences (5′-3′)	Tm (°C)	Molecular weight (g mol^{-1})	Primer Length (bp)	GC content (%)
ISSR1	AGAGAGAGAGAGAGAGYC	56.3	5366.6	18	52.94%
ISSR2	CTCTCTCTCTCTCTCAT	53.5	4998.3	17	52.94%
ISSR3	GAGAGAGAGAGAGAGATT	54.3	5685.8	18	44.44%
ISSR4	AGAGAGAGAGAGAGAGC	56.3	5366.6	17	52.94%
ISSR5	GAGAGAGAGAGAGAGC	54.1	5053.4	16	56.25%
ISSR6	ACACACACACACACACG	60.6	5086.4	17	52.94%
SCoT1	ACGACATGGCGACCACGC	68.2	5478.6	18	66.67%
SCoT2	CCATGGCTACCACCGCAG	65.8	5429.6	18	66.67%

2 Y = C or T

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Table 3(on next page)

Number of bands (NB), monomorphic bands (MB) and polymorphic bands (PB) generated by eight primers (six ISSR and two SCoT)

Number of bands (NB), monomorphic bands (MB) and polymorphic bands (PB) generated by eight primers (six ISSR and two SCoT) in 15 wheat genotypes and the related polymorphism (%).

Table 3. Number of bands (NB), monomorphic bands (MB) and polymorphic bands (PB) generated by eight primers (six ISSR and two SCoT) in 15 wheat genotypes and the related polymorphism (%).

Primers	NB	MB	PB	**Polymorphism (%)**
ISSR1	6	4	2	33.3%
ISSR2	8	5	3	37.5%
ISSR3	12	5	7	58.3%
ISSR4	9	5	4	44.4%
ISSR5	9	5	4	44.4%
ISSR6	10	8	2	20%
SCoT1	10	7	3	30%
SCoT2	12	9	3	23%
Total	76	48	28	
Average	9.5	6	3.5	36.36%

Table 4(on next page)

Genetic distance among the five wheat cultivars and their F1 hybrids

Genetic distance among the five wheat cultivars and their F1 hybrids based on SCoT and ISSR banding profiles.

Table 4. Genetic distance among the five wheat cultivars and their F1 hybrids based on SCoT and ISSR banding profiles.

Genotype	P1	P2	P3	P4	P5	P1×P2	P1×P3	P1×P4	P1×P5	P2×P3	P2×P4	P2×P5	P3×P4	P3×P5	P4×P5
P1	0.00														
P2	3.32	0.00													
P3	3.00	2.00	0.00												
P4	2.45	2.65	1.73	0.00											
P5	3.32	3.16	2.83	2.65	0.00										
P1×P2	3.16	3.32	2.65	2.45	2.24	0.00									
P1×P3	2.65	3.16	2.45	2.24	2.45	1.73	0.00								
P1×P4	2.45	3.00	2.24	2.00	2.24	2.00	1.73	0.00							
P1×P5	2.83	3.32	2.65	2.45	3.00	2.00	1.73	2.45	0.00						
P2×P3	3.16	2.65	1.73	2.45	2.24	2.00	2.24	2.00	2.83	0.00					
P2×P4	2.83	2.65	2.65	2.45	2.24	2.45	2.65	2.00	2.83	2.45	0.00				
P2×P5	3.32	3.46	3.16	3.32	3.16	3.00	2.83	3.00	2.65	3.00	3.61	0.00			
P3×P4	2.65	3.16	2.45	2.24	2.45	2.24	2.00	2.24	1.73	2.65	2.65	2.45	0.00		
P3×P5	2.83	2.65	1.73	2.00	2.24	2.45	2.24	2.00	2.45	2.00	2.45	3.00	2.24	0.00	
P4×P5	2.83	3.00	2.24	2.00	1.73	2.00	1.73	1.41	2.45	2.00	2.45	3.00	2.24	1.41	0.00

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Table 5(on next page)

Dice measurement for similarity coefficient of the five wheat cultivars and their F1 hybrids

Dice measurement for similarity coefficient of the five wheat cultivars and their F1 hybrids based on SCoT and ISSR banding profiles.

Table 5. Dice measurement for similarity coefficient of the five wheat cultivars and their F1 hybrids based on SCoT and ISSR banding profiles

Genotype	P1	P2	P3	P4	P5	P1×P2	P1×P3	P1×P4	P1×P5	P2×P3	P2×P4	P2×P5	P3×P4	P3×P5	P4×P5
P1	1.00														
P2	0.84	1.00													
P3	0.87	0.94	1.00												
P4	0.91	0.90	0.96	1.00											
P5	0.85	0.87	0.90	0.91	1.00										
P1×P2	0.86	0.85	0.90	0.92	0.94	1.00									
P1×P3	0.90	0.86	0.92	0.93	0.92	0.96	1.00								
P1×P4	0.92	0.88	0.93	0.95	0.94	0.95	0.96	1.00							
P1×P5	0.88	0.84	0.90	0.91	0.88	0.94	0.96	0.92	1.00						
P2×P3	0.86	0.90	0.96	0.92	0.94	0.95	0.93	0.95	0.89	1.00					
P2×P4	0.90	0.91	0.91	0.92	0.94	0.93	0.91	0.95	0.90	0.93	1.00				
P2×P5	0.83	0.82	0.85	0.84	0.86	0.87	0.88	0.87	0.89	0.87	0.82	1.00			
P3×P4	0.90	0.86	0.91	0.93	0.92	0.93	0.94	0.93	0.96	0.90	0.91	0.91	1.00		
P3×P5	0.89	0.90	0.96	0.95	0.94	0.92	0.93	0.95	0.92	0.95	0.93	0.87	0.93	1.00	
P4×P5	0.89	0.88	0.93	0.95	0.96	0.95	0.96	0.98	0.92	0.95	0.93	0.87	0.93	0.97	1.00