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Revealing Genetic Diversity and Drought Tolerance in Durum Wheat through Agro-Physiological and Molecular Approaches

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ABSTRACT

Under changing climate conditions, evaluating both genetic and environmental factors is essential for crop improvement. Limitations of conventional breeding have increased the use of molecular markers to obtain precise genotypic information and improve selection efficiency. This study evaluated drought tolerance in 15 durum wheat genotypes across two cropping seasons under rainfed and irrigated conditions. A randomized complete block design with four replications was used to assess agro-physiological traits, quality parameters, drought tolerance indices, and molecular diversity using iPBS and SCoT markers. Significant genotypic variation was observed for grain yield, 1000-kernel weight, hectoliter weight, protein and starch content, and color traits under both conditions. Ovidio had the highest grain yield and starch content, while Seckin 21 showed the highest 1000-kernel weight. Drought tolerance indices identified NZFM-41 and Maestrale as the most drought-resilient, and Ovidio as highly adaptable with stable yield. 12 indices (SSI, TOL, MP, GMP, STI, YI, YSI, HM, SDI, DI, RDI, SSPI) were calculated from yields under stress (Ys) and non-stress (Yp) conditions. Molecular analysis with 14 iPBS and 10 SCoT primers showed greater polymorphism and allelic diversity for iPBS (PIC = 0.287, Nei's distance = 0.5348) than SCoT (PIC = 0.191, Nei's distance = 0.2700). Clustering and population structure revealed two subpopulations, with iPBS providing higher resolution (FST = 0.3443). MP, GMP, STI, YI, HM, and DI were the most reliable indices for identifying drought-tolerant genotypes. In conclusion, iPBS markers exhibited higher discriminatory power than SCoT for assessing genetic diversity, while specific indices (MP, GMP, and STI) proved most effective for identifying drought-tolerant durum wheat genotypes, with NZFM-41 and Maestrale emerging as promising genetic resources for breeding programs aimed at enhancing climate resilience.

1. INTRODUCTION

Durum wheat (*Triticum turgidum* var. *durum* Desf.) is a minor cereal crop, accounting for less than 5% of global wheat production (Almarri et al., 2023). It is one of the earliest domesticated food crops in the Mediterranean region and remains widely cultivated across this region (Yiğit, 2025). Currently, durum wheat is grown on approximately 13.5 million hectares worldwide, producing about 33.8 million tonses annually (IGC, 2022). It is widely cultivated globally to meet the growing demand of the pasta industry. In Türkiye, durum wheat production is lower than that of bread wheat due to its cultivation in more limited areas. In 2022, Türkiye produced 3.5 million tonnes of durum wheat on 1.2 million hectares (Ceylan, 2022). The Southeastern Anatolia Region of Türkiye, recognized as a gene center of durum wheat (Ozkan & Deniz, 2025), offers favorable environmental conditions for its cultivation (Tekdal & Yildirim, 2015). Nevertheless, the different responses of genotypes to various environmental conditions, namely genotype × environment interactions, make it difficult to select stable and well-adapted genotypes (Göçmen, 2025). However, irregular variations in rainfall and temperature within and between growing seasons pose significant constraints to wheat production in these environments (Martínez-Peña et al., 2023). Drought and high temperatures during the grain-filling period are among the major factors limiting yield and adversely affecting grain quality (Schmidt, 2003; Yildirim, 2013). Drought stress represents a widespread challenge for global wheat production and is projected to intensify with climate change, making wheat cultivation, especially in rainfed areas, increasingly vulnerable due to its dependence on irregular seasonal precipitation (Ayranci et al., 2014; Khatiwada & Curtis, 2021). Therefore, evaluating crop yields under both biotic and abiotic stress conditions is essential for identifying tolerant genotypes, capable of maintaining high yields in both optimal and stress-affected environments (Clarke, 1992). Consequently, wheat breeders are striving to develop high-yielding, high-quality, and highly adaptable varieties to meet the nutritional needs of future generations and to ensure the sustainability of cereal production under increasingly challenging climatic conditions (Krishnappa et al., 2022).

Selecting plants for drought tolerance within conventional breeding programs remains a significant challenge due to the unpredictability of field conditions, variability in weather patterns, and the complex genetic architecture underlying drought tolerance. This trait is controlled by multiple genes and is characterized by substantial genotype × environment and QTL × environment interactions, which markedly influence yield under stress conditions. Consequently, progress in breeding for drought tolerance has been relatively slow (Karkhaneh et al., 2025). To address this challenge, regional yield trials are essential for identifying promising genotypes and classifying them based on yield performance across multiple test sites within target regions. Therefore, many researchers prefer to conduct evaluations under both stress and non-stress conditions. Although certain genotypes may exhibit high yield potential, they do not necessarily possess inherent stress tolerance. Their superior performance under drought conditions may primarily reflect their intrinsic yield potential rather than true resilience to stress (Blum, 1996). To more accurately assess genotype performance under varying environmental conditions, numerous selection indices have been developed, including the geometric mean productivity (GMP), stress susceptibility index (SSI), tolerance index (TOL), mean productivity (MP), harmonic mean (HM), stress tolerance index (STI), yield index (YI), yield stability index (YSI), sensitivity drought index (SDI), drought response index (DSI), drought resistance index (DI), relative drought index (RDI), stress susceptibility percentage index (SSPI), and the modified stress tolerance index (MSTI). These indices are widely used to identify genotypes exhibiting stable performance under both drought stress and optimal growing conditions (Mursalova et al., 2015). Recent studies have demonstrated the effectiveness of indices such as MP and GMP in selecting genotypes with high adaptability to both irrigated and rainfed environments (Mohammadi & Geravandi, 2024; Kizılgeci & Tebrizli, 2025).

The survival and adaptability of plant species largely depend on the availability of genetically diverse gene pools. A high level of genetic diversity increases the likelihood of identifying superior germplasm, which is critical for developing new cultivars molecular marker technologies (Swarup et al., 2021). However, there is growing concern among breeders that successive breeding cycles have progressively narrowed the genetic base of cultivated genotypes. This erosion of genetic diversity poses a significant challenge for future breeding programs, as the remaining variability may not be sufficient to meet emerging demands (Altaf et al., 2025). The integration of molecular techniques into breeding programs has substantially accelerated the identification and selection of traits associated with stress tolerance and quality attributes, providing a semi-quantitative framework for trait evaluation. Molecular markers have long been recognized as essential tools for assessing the extent and structure of genetic diversity (Yildiz et al., 2023). In population genetics, they offer a direct means of measuring genetic variation at the DNA level and serve as a critical link between phenotypic expression and underlying genotypic differences (Peng et al., 2024). With the rapid advancement and increasing affordability of molecular marker technologies, along with the development of cost-effective, high-throughput genotyping platforms, there is growing on employing these tools to dissect complex quantitative traits, including yield potential (Pretini et al., 2025). To be effective, molecular markers must display high levels of polymorphism, be technically straightforward, rapid to apply, and economically feasible (Miah et al., 2013).

To date, numerous studies have investigated the genotyping of wheat using various molecular markers, including genotyping-by-sequencing (GBS) (Ballesta et al., 2019) and simple sequence repeats (SSR) (El-Rawy & Hassan, 2021), under both irrigated and rainfed conditions. These studies consistently demonstrated that the integration of phenotypic data with molecular marker analysis is effective for evaluating genetic diversity in wheat. However, to date, there is no evidence in the literature regarding the application of inter-primer binding site (iPBS) and start codon targeted (SCoT) markers for this purpose. Over the past two decades, both iPBS and SCoT marker systems have been widely utilized in genetic studies. The iPBS method is a retrotransposon-based DNA marker system that amplifies genomic regions flanked by primer binding sites of reverse transcriptase regions in adjacent retrotransposons oriented in opposite directions. Due to their ubiquitous presence, high copy number, and widespread chromosomal distribution within plant genomes, iPBS elements hold considerable potential for developing robust, reliable, and highly informative DNA marker systems (Kalendar et al., 2010). These characteristics make iPBS markers valuable tools for investigating genetic diversity, genome structure, and evolutionary relationships in a wide range of plant species (Demirel et al., 2024; Haliloglu et al., 2023; Kizilgeci et al., 2022). In contrast, the SCoT technique targets short, conserved regions flanking the ATG start codon in plant genes. Similar to iPBS, this approach like iPBS, is valuable for its simplicity, high reproducibility, and effectiveness in assessing genetic diversity, population structure, and gene-associated variation across diverse plant species (Yalinkilic et al., 2024). Molecular breeding employing SCoT markers has proven to be a reliable method for evaluating genetic variability among wheat cultivars and for identifying superior cultivars for further breeding programs (Abouseada et al., 2023). Given the increasing frequency of drought events driven by climate change in Türkiye and the introduction of newly developed breeding lines of durum wheat in farmers' fields, it is timely to test the hypothesis that these new genotypes can deliver stable and reliable yields under rainfed conditions. Assessing the performance of these genotypes under climate-induced stress is essential for informing breeding programs aimed at enhancing yield stability and contributing to global food security (Mohammadi et al., 2023). Furthermore, the adoption of high-yielding and stable cultivars is critical for maintaining agricultural productivity. In this context, it is also important to consider complementary adaptation strategies beyond cultivar improvement to mitigate the adverse impacts of climate change. Therefore, the objective of this study was to identify drought-tolerant, moderately tolerant, and drought-sensitive durum wheat genotypes using specific selection indices, while concurrently evaluating their genetic diversity and population structure through iPBS and SCoT molecular markers. Additionally, the study aimed to classify these genotypes into distinct tolerance groups. By integrating agronomic and molecular analysis, the research seeks to generate accurate and comprehensive insights to support durum wheat breeding efforts and provide practical recommendations for field-level cultivation.

2. MATERIALS AND METHODS

The Plant material, growth condition, stress treatments and agro-morphological traits evaluation

Field trials were conducted during the 2021–2022 and 2022–2023 growing seasons at the experimental site of Teknobiltar Company in Diyarbakır, Türkiye (37°55′29″N, 40°15′16″E). The study included 10 durum wheat varieties and five advanced breeding lines, designated as follows: G1: DZ21-3, G2: Guney Yıldızı, G3: DZ20-7, G4: Seçkin-21, G5: Zenit, G6: Anzele, G7: NZFM-63, G8: Pitagorasvevo, G9: Svevo, G10: Ovidio, G11: NZFM-41, G12: NZFM-38, G13: Mastrella, G14: NZFM-Ziraat and G15: Surmeli. The experimental site is characterized by clay loam soil with low organic matter content and a pH of 7.87. Total annual precipitation was 314.8 mm in the first year and 389.2 mm in the second year (Figure 1), with notably low rainfall during April 2022 and May 2023, critical months for crop development. Trials were conducted under both rainfed and irrigated; irrigation was applied using a sprinkler system at the stem elongation and heading stages, each irrigation event was applied for approximately seven hours. The experimental layout followed a randomized complete block design with four replications. Each plot measured 4 × 1.2 m and consisted of six rows spaced 0.2 m apart. Nitrogen fertilizer was applied at a rate of 70 kg ha^{-1} at sowing and again at stem elongation, while phosphorus fertilizer was applied at 70 kg ha^{-1} at sowing. The traits assessed included thousand grain weight, hectoliter weight, grain yield, protein content, and starch content. Protein and starch contents were determined using a GrainSense device (GrainSense Ltd., Finland). Grain color parameters (L* brightness, a* redness, and b* yellowness) were determined using a HunterLab ColorFlex colorimeter (Model A60-1010-615, HunterLab Inc., Reston, VA, USA). Tolerance indices were calculated based on grain yield data obtained under both irrigated and drought-stress conditions and including the following indices (Table 1).

Table 1. Mathematical formulas of tolerance and susceptibility indices

Index	Formula	Reference
Tolerance (TOL)	TOL = Yp − Ys	(Rosielle & Hamblin, 1981)
Mean productivity (MP)	MP = (Yp + Ys) / 2	(Rosielle & Hamblin, 1981)
Geometric mean productivity (GMP)	GMP = √(Ys × Yp)	(Fernandez, 1993)
Harmonic mean (HM)	HM = 2 × (Ys × Yp) / (Ys + Yp)	(Bidinger et al., 1987)
Stress susceptibility index (SSI)	SSI = [1 − (Ys / Yp)] / [1 − (Ȳs / Ȳp)]	(Fischer & Maurer, 1978)
Stress tolerance index (STI)	STI = (Ys × Yp) / (Ȳp)²	(Fernandez, 1993)
Yield index (YI)	YI = Ys / Ȳs	(Gavuzzi et al., 1997)
Yield stability index (YSI)	YSI = Ys / Yp	(Bouslama & Schapaugh Jr, 1984)
Relative stress index (RSI)	RSI = (Ys / Yp) / (Ȳs / Ȳp)	(Fischer & Wood, 1979)

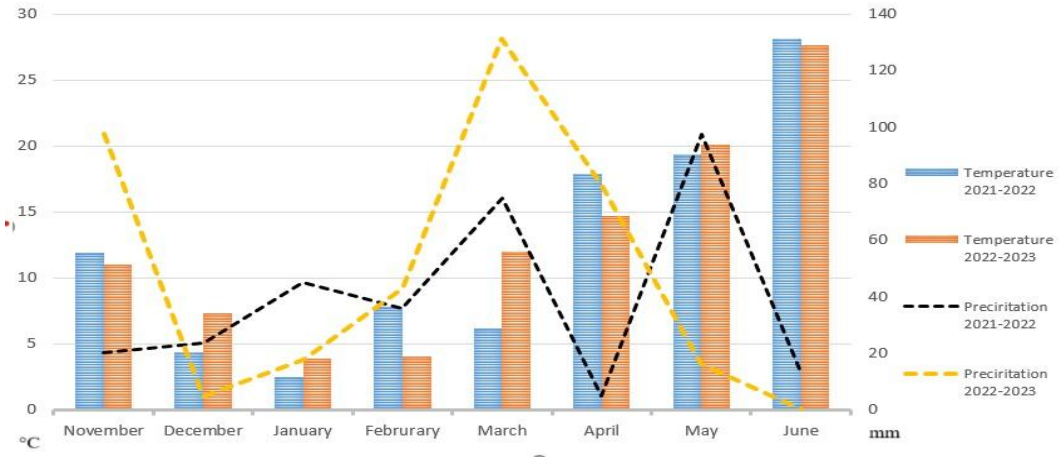

Figure 1. The values of meteorological data for the 2021-2023 season

Data analysis

The experimental data were analyzed using analysis of variance (ANOVA) in JMP Clinical 18 software (SAS Institute Inc., Cary, NC, USA). Mean comparisons among genotypes were performed using the Tukey HSD test at the 5% significance level. Correlation analysis between grain yield and drought tolerance indices was conducted to identify the most informative indices for assessing drought tolerance. Principal component analysis (PCA) and biplot visualization were also performed using JMP Clinical 18. In addition, cluster analysis was carried out using the same software. Pearson's correlation coefficients, biplot analysis, and drought tolerance indices along with their respective ranks were calculated using the iPASTIC toolkit (Pour-Aboughadareh et al., 2019).

iPBS, SCoT-PCR and gel electrophoresis

Genetic variation among the genotypes was evaluated using two molecular marker systems: 14 inter-Primer Binding Site (iPBS) primers (Kalendar et al., 2010) and 10 Start Codon Targeted (SCoT) primers (Collard et al., 2009) to assess the genetic background and diversity of the studied durum wheat genotypes (Table 2). Genomic DNA was extracted from seven-day-old seedlings following the method described by Zeinalzadehtabrizi et al. (2015). To assess DNA quality, 50 ng μl^{-1} of genomic DNA was mixed with 5 ul of bromophenol blue and separated on a 0.8% agarose gel. Electrophoresis was conducted at 90 V for 60 minutes in 1× Tris-Borate-EDTA (TBE) buffer. DNA amplification reactions were performed in a thermal cycler in a total reaction volume of 10 µL, containing 1.2 µL dNTPs (10 mM), 1.2 µL 10× buffer, 0.1 µL $MgCl_2$, 1 µL of each iPBS or SCoT primer, 2 µL genomic DNA, and 0.1 µL DNA polymerase (5 U μL^{-1}, Fermentas). The PCR cycling conditions consisted of an initial denaturation at 94°C for 5 min, followed by 40 cycles of denaturation at 94°C for 60 s, annealing at 50-60°C for 60 s (depending on the primer), and extension at 72°C for 2 min, with a final extension at 72°C for 10 min. Amplified PCR products were mixing with loading dye (7 µl of PCR product with 3 loading dye) and separated on a 1.50% agarose gel in 1× TBE buffer at 90 V for 120 min. DNA fragments were visualized under UV light, and banding patterns were recorded. The presence of polymorphic bands indicated genetic variation among the genotypes (Haliloglu et al., 2022).

Table 2. iPBS and SCoT primers used in the study

No	iPBS Marker	Sequence (5'→3')	No	SCoT Marker	Sequence (5'→3')
1	iPBS-2074	GCT CTG ATA CCA	1	SCoT - 6	CAACAATGGCTACCACGC
2	iPBS-2217	ACTTGGATGTCGATACCA	2	SCoT - 7	CAACAATGGCTACCACGG
3	iPBS-2219	GAACTTATGCCGATACCA	3	SCoT - 14	ACGACATGGCGACCACGC
4	iPBS-2221	ACC TAG CTC ACG ATG CCA	4	SCoT - 15	ACGACATGGCGACCGCGA
5	iPBS-2222	ACTTGGATGCCGATACCA	5	SCoT - 16	ACCATGGCTACCACCGAC
6	iPBS-2238	ACC TAG CTC ATG ATG CCA	6	SCoT - 17	ACCATGGCTACCACCGAG
7	iPBS-2272	GGC TCA GAT GCC A	7	SCoT - 18	ACCATGGCTACCACCGCC
8	iPBS-2353	GCATGGCCTCCA	8	SCoT - 21	ACGACATGGCGACCCACA
9	iPBS-2387	GCG CAA TAC CCA	9	SCoT - 22	AACCATGGCTACCACCAC
10	iPBS-2392	TAGATGGTGCCA	10	SCoT - 23	CACCATGGCTACCACCAG
11	iPBS-2393	TACGGTACGCCA			
12	iPBS-2394	GAG CCT AGG CCA			
13	iPBS 2398	GAACCCTTGCCGATACCA			
14	iPBS-2401	AGTTAAGCTTTGATACCA			

Genotyping assay data analysis

Banding patterns obtained from electrophoresis were converted into a binary data matrix. In this matrix, the presence of an amplified DNA fragment was scored as 1 for presence, and 0 for absence. Following the construction of this dataset, primer efficiency was evaluated using the following parameters; the total number of amplified fragments (NAF), the number of polymorphic fragments (NPF), and the polymorphic information content (PIC), as outlined by Prevost & Wilkinson (1999). To further assess genetic variation within and among populations, additional diversity indices were calculated, including unbiased Nei's gene diversity (uHe), the number of effective alleles (Ne), the percentage of polymorphic loci (PPL), and Shannon's information index (I). Genetic relationships among accessions were inferred through cluster analysis using the neighbor-joining (NJ) method, based on a genetic distance matrix generated using Popgene software (Yeh, 1999). Additionally, principal component analysis (PCA) was conducted in NTSYS to provide a visual representation of the genetic relationships among genotypes (Rohlf, 2002). To explore population structure in greater depth, STRUCTURE software version 2.3.4 (Pritchard et al., 2000) was employed. 10 independent runs were performed for each hypothetical number of genetic clusters (K), ranging from 1 to 10, with each run comprising a burn-in period of 10,000 iterations followed by 100,000 Markov Chain Monte Carlo (MCMC) iterations. The optimal number of genetic clusters (K) was determined using STRUCTURE HARVESTER, a model selection tool developed by Earl & VonHoldt (2012).

3. RESULTS

Characterization of agro-physiological, quality traits grain yield and 1000 kernel weight

Analysis of variance revealed statistically significant differences in grain yield among the tested genotypes (Table 3). Under irrigated conditions, the genotype Ovidio exhibited the highest grain yield (8321.9 kg ha^{-1}), while Güney Yıldızı recorded the lowest yield (5645.1 kg ha^{-1}). Under rainfed conditions, Ovidio again demonstrated superior yield performance (4803.7 kg ha^{-1}), whereas Anzele recorded the lowest yield (3017.5 kg ha^{-1}). The greatest yield reductions in response to rainfed conditions were observed in Anzele (55.9%) and NZFM-63 (54.8%), indicating lower tolerance in these genotypes compared to the others. Significant genotypic variation was also detected for 1000-kernel weight under both irrigated and rainfed conditions. Under rainfed conditions, Seçkin 21 exhibited the highest 1000-kernel weight, while Maestrale recorded the lowest value under irrigated conditions. In the rainfed environment, NZFM Ziraat and Svevo also showed relatively high 1000-kernel weights, whereas Anzele displayed the lowest. When averaged across both conditions, Seçkin 21 demonstrated the highest mean 1000-kernel weight, suggesting superior grain filling capacity and potential resilience under variable environmental conditions.

Table 3. Mean values of grain yield and 1000-kernel weight of durum wheat genotypes grown under irrigated and stress conditions

Genotypes	Grain yield (kg ha^{-1})			1000-kernel weight (g)		
	Irrigated	Rainfed	Mean	Irrigated	Rainfed	Mean
Anzele	6851.5^{b-e1}	3017.5^{de}	4934.5^{e-h}	40.75^{b-e}	39.49^{a-d}	40.12^{bcd}
DZ20-7	7501.2^{ab}	3520.8^{cde}	5511.0^{bcd}	37.20^{ef}	37.57^{b-e}	37.38^{efg}
DZ21-3	7134.9^{bcd}	3510.7^{cde}	5322.8^{cde}	36.35^{f}	36.8^{cde}	36.58^{fg}
GüneyYıldızı	5645.1^{g}	3543.8^{cd}	4594.4^{gh}	38.70^{c-f}	38.71^{a-d}	38.70^{c-f}
Maestrale	6446.9^{d-g}	4452.5^{ab}	5449.7^{b-e}	36.90^{f}	34.58^{e}	35.74^{g}
NZFM-38	6037.0^{efg}	3235^{cde}	4636.0^{gh}	41.24^{bcd}	39.95^{abc}	40.59^{a-d}
NZFM-41	6876.6^{b-e}	4839.4^{a}	5858.0^{b}	43.73^{ab}	37.98^{a-d}	40.86^{abc}
NZFM-63	6303.9^{d-g}	2854.3^{e}	4579.1^{h}	38.41^{def}	36.63^{de}	37.52^{efg}
NZFM Ziraat	6551^{c-f}	3811.1^{bc}	5181.0^{def}	42.07^{abc}	40.85^{a}	41.46^{ab}
Ovidio	8321.9^{a}	4803.7^{a}	6562.8^{a}	39.57^{c-f}	38.80^{a-d}	39.19^{b-e}
Pitagora	6599.2^{c-f}	3827.3^{bc}	5213.3^{def}	39.96^{c-f}	36.87^{cde}	38.42^{def}
Seçkin 21	6945.6^{bcd}	3427.7^{cde}	5186.7^{def}	44.96^{a}	40.62^{ab}	42.79^{a}
Sümerli	7311.5^{bc}	4397.1^{ab}	5854.3^{bc}	38.43^{c-f}	36.79^{cde}	37.61^{efg}
Svevo	6447.9^{d-g}	3781.2^{bc}	5114.5^{d-g}	41.50^{a-d}	40.85^{a}	41.17^{ab}
Zenit	5919.4^{fg}	3482.8^{cde}	4701.1^{fgh}	40.88^{bcd}	36.22^{de}	38.55^{c-f}
F (value)	5.21^{**2}	6.64**	5.43**	3.67**	2.64**	5.07**
LSD (%5)	420	661.5	271.0	1.84	1.65	1.24

[1]Means followed by different letters within the same column are significantly different at the 0.05 probability level according to LSD test. [2]*,** significant at 5% and 1% level respectively.

Hectoliter weight, protein and starch content

Highly significant differences in hectoliter weight were observed among the genotypes under both irrigated and rainfed conditions (Table 4). Under irrigated conditions, the highest hectoliter weight was recorded in the variety Sümerli, followed by Maestrale, Ovidio, DZ20-7, DZ21-3, and NZFM Ziraat. In contrast, NZFM-41 consistently exhibited the lowest hectoliter weight across both environments, with an overall mean of 69.4 kg. Notably, Ovidio and DZ21-3 maintained relatively stable hectoliter weights under both conditions, indicating their potential for stable grain quality under varying moisture regimes.

Significant differences in grain protein content were detected at the 1% level among the genotypes. Under irrigated conditions, Svevo exhibited the highest protein content (17.6%), whereas under rainfed conditions the highest value was recorded for NZFM Ziraat (17.34%). Across both environments, NZFM Ziraat (17.34%) and Svevo (17.32%) showed the highest mean protein contents (Table 4). In contrast, Ovidio exhibited the lowest protein content under both irrigated (14.07%) and rainfed (15.99%) conditions, resulting in an overall mean of 15.03%. Starch content, an important indicator of grain quality and yield potential, also varied significantly among the genotypes. Ovidio exhibited the highest starch content under both irrigated (83.37%) and rainfed (82.87%) conditions, with an overall mean of 83.12%, indicating stable starch accumulation. Conversely, Maestrale recorded the lowest starch content under irrigated conditions (77.23%), although it showed a notable increase under rainfed conditions (82.24%).(Table 4).

Table 4. Mean values of hectoliter weight, protein content and starch content of durum wheat genotypes grown under irrigated and stress conditions

Genotypes	Hectoliter weight (kg)			Protein content (%)			Starch content (%)		
	Irrigated	Rainfed	Mean	Irrigated	Rainfed	Mean	Irrigated	Rainfed	Mean
Anzele	74.59^{ab1}	71.3^{a-e}	72.93^{abc}	15.24^{cde}	15.86^{cde}	15.55^{fgh}	82.71	82.13^{a-d}	82.42^{a-d}
DZ20-7	75.15^{a}	72.2^{a-d}	73.68^{abc}	15.13^{de}	15.13^{ef}	15.13^{gh}	82.82	82.61^{ab}	82.72^{ab}
DZ21-3	75.99^{a}	72.9^{a-d}	74.46^{a}	14.95^{de}	16.01^{cde}	15.48^{fgh}	82.89	81.97^{a-e}	82.43^{a-d}
Güney Yıldızı	73.63^{ab}	74.10^{a}	73.85^{abc}	16.51^{abc}	16.55^{a-d}	16.53^{a-e}	81.42	81.17^{cde}	81.30^{b-e}
Maestrale	76.38^{a}	70.9^{cde}	73.66^{abc}	16.25^{a-d}	16.05^{b-e}	16.15^{c-f}	77.23	82.24^{abc}	79.74^{e}
NZFM-38	74.55^{ab}	73.50^{abc}	74.04^{ab}	16.85^{ab}	16.66^{abc}	16.76^{a-d}	81.04	81.35^{b-e}	81.20^{b-e}
NZFM-41	70.12^{c}	68.70^{e}	69.40^{e}	16.51^{abc}	15.26^{ef}	15.89^{efg}	81.65	82.47^{abc}	82.06^{a-d}
NZFM-63	71,67^{bc}	68.80^{e}	70.24^{de}	15.84^{bcd}	15.94^{cde}	15.89^{efg}	81.85	81.55^{a-e}	81.70^{a-d}
NZFM Ziraat	75.53^{a}	73.20^{abc}	74.36^{a}	17.45^{a}	17.21^{a}	17.34^{a}	80.65	80.78^{e}	80.71^{cde}
Ovidio	76.13^{a}	74.10^{a}	75.10^{a}	14.07^{e}	15.99^{cde}	15.03^{h}	83.37	82.87^{a}	83.12^{a}
Pitagora	73.81^{ab}	73.90^{ab}	73.87^{ab}	16.27^{a-d}	15.68^{def}	15.98^{def}	81.62	82.25^{abc}	81.93^{a-d}
Seçkin 21	74.00^{ab}	70.10^{de}	72.07^{bcd}	16.75^{ab}	16.99^{ab}	16.87^{abc}	81.11	80.84^{de}	80.97^{b-e}
Sümerli	76.51^{a}	71.10^{b-e}	73.80^{abc}	15.54^{bcd}	14.73^{f}	15.13^{gh}	82.35	82.60^{ab}	82.48^{abc}
Svevo	73.34^{abc}	71.00^{cde}	72.15^{bcd}	17.60^{a}	17.03^{a}	17.32^{ab}	80.46	80.86^{de}	80.66^{de}
Zenit	71.66^{bc}	71.70^{a-d}	71.69^{cd}	16.62^{ab}	16.36^{a-d}	16.49^{b-e}	81.32	81.21^{cde}	81.26^{b-e}
F (value)	2.64^{**2}	2.97**	1.98**	4.09**	4.30**	5.59**	1.44^{ns}	2.30*	1.86*
LSD (%5)	1.65	1.46	1.10	0.69	0.49	0.42	1.71	0.68	0.92

[1]Means followed by different letters within the same column are significantly different at the 0.05 probability level according to LSD test. [2*],[**] significant at 5% and 1% level respectively.

L, a and b values

The *L* values represent the yield performance of each variety under both irrigated and rainfed conditions (Table 5). Under irrigated canditions, DZ20-7 exhibited the highest *L* value (55.84), followed closely by NZFM-63 (55.42). In contrast, under rainfed conditions, most varieties exhibited a decline in *L* values, with the greatest reduction observed in Zenit (from 55.69 to 54.75). Svevo consistently exhibited the lowest *L* values under both conditions (53.42 and 53.58, respectively), indicating lower yield stability. The *a* value, indicative of grain weight, was significantly influenced by irrigation. Under irrigation conditions, Maestrale recorded the highest a value (9.64). Under stress, Ovidio and Pitagora maintained relatively high values (9.36 and 9.38, respectively), while DZ20-7 demonstrated consistent performance across conditions, with an *a* value of 8.91 (irrigated) and 8.59 (rainfed), indicating stability in grain weight development under varying moisture conditions. The *b* value varied markedly between environments. Under irrigated canditions, Ovidio achieved the highest b value (27.27), while Maestrale and Sümerli also performing strongly. Under rainfed conditions, a general decline in b values was observed across genotypes, with Svevo and Zenit exhibiting the most pronounced reductions. Notably, Maestrale showed resilience, with only *a* slight decrease in the *b* value (from 27.07 to 24.22), highlighting its potential for maintaining grain quality under rainfed conditions.

Under irrigated conditions, genotypes such as DZ20-7, NZFM-63, and Maestrale exhibited relatively higher values for the color parameters *L*, *a*, and *b*, indicating favorable grain color characteristics. Under water-limited conditions, most genotypes showed reductions in these color parameters; however, NZFM-41, NZFM-63, and Maestrale maintained relatively stable *L, a*, and *b* values, suggesting a greater stability of grain color traits under drought stress. In contrast, Svevo and Zenit exhibited more pronounced declines in especially *b* values under stress conditions, indicating that drought stress negatively affected grain color characteristics in these genotypes.

Table 5. Mean values of L, a and b of durum wheat genotypes grow under irrigated and stress conditions

Genotypes	*L*			*a*			*b*		
	Irrigateed	**Rainfed**	**Mean**	**Irrigated**	**Rainfed**	**Mean**	**Irrigated**	**Rainfed**	**Mean**
Anzele	55.27^{ab1}	54.67^{abc}	54.97	8.79^{cd}	7.84	8.31	26.13^{b-e}	22.55^{ef}	24.34^{def}
DZ20-7	55.84^{a}	53.96^{bc}	54.9	8.91^{cd}	8.59	8.75	26.84^{bbc}	23.28^{c-f}	25.06^{abc}
DZ21-3	55.32^{ab}	54.1^{bc}	54.71	8.86^{cd}	8.63	8.74	26.53^{a-d}	24.48^{a}	25.51^{ab}
Güney Yıldızı	55.14^{ab}	53.59^{c}	54.36	9.2b^{c}	8.71	8.95	26.38^{a-d}	23.54^{bcd}	24.96^{bcd}
Maestrale	54.89^{ab}	54.03^{bc}	54.46	9.64^{a}	8.87	9.26	27.07^{ab}	24.22^{ab}	25.64^{a}
NZFM-38	54.65^{a-d}	53.97^{bc}	54.31	9.18^{bc}	8.6	8.89	26.10^{b-e}	23.69^{abc}	26.10^{b-e}
NZFM-41	55.24^{ab}	55.35^{a}	55.3	8.57^{d}	8.6	8.59	25.57^{de}	23.83^{abc}	25.57^{de}
NZFM-63	55.42^{ab}	55.37^{a}	55.39	8.8^{cd}	8.49	8.65	26.78^{abc}	23.84^{abc}	26.78^{abc}
NZFM Ziraat	53.46^{cd}	53.96^{bc}	53.71	9.42^{ab}	8.58	9.42	25.37^{e}	23.15^{c-f}	24.25^{ef}
Ovidio	54.78^{abc}	55.03^{ab}	54.9	9.36^{ab}	8.67	9.02	27.27^{a}	23.7^{abc}	25.48^{ab}
Pitagora	54.69^{a-d}	54.56^{abc}	54.62	9.38^{ab}	8.79	9.08	26.95^{abc}	23.53^{bcd}	25.24^{abc}
Seçkin 21	54.10^{bcd}	53.56^{c}	53.83	9.12^{bc}	8.58	8.85	26.04^{cde}	23.43^{b-e}	24.74^{cde}
Sümerli	54.27^{bcd}	54.18^{bc}	54.22	9.05^{bc}	8.43	8.74	26.97^{abc}	23.17^{c-f}	25.07^{abc}
Svevo	53.42^{d}	53.58^{c}	53.5	9.17^{bc}	8.51	8.84	25.30^{e}	22.71^{def}	24.00^{f}
Zenit	54.75^{a-d}	55.69^{a}	55.22	9.11^{bc}	7.92	8.51	25.57^{de}	22.37^{f}	23.97^{f}
F (value)	2.10^{*2}	2.67**	1.35^{ns}	3.20**	1.51^{ns}	1.62^{ns}	3.40**	2.86**	2.80**
LSD (%5)	0.67	0.58	0.45	0.22	0.31	0.19	0.50	0.46	0.34

[1]Means followed by different letters within the same column are significantly different at the 0.05 probability level according to LSD test. [2]*,** significant at 5% and 1% level respectively

Characterization of drought tolerance indices

The relative decrease percentage (RD%) quantifies the proportional reduction in yield under rainfed compared to irrigated conditions, serving as an indicator of drought tolerance. Lower RD% values reflect a genotype's ability to maintain yield under rainfed conditions. Among the genotypes evaluated, NZFM-41 (29.63%) and Maestrale (30.94%) exhibited the lowest RD% values, indicating superior drought tolerance. In contrast, Anzele (55.96%) and NZFM-63 (54.72%) showed the highest RD% values, suggesting a high degree of susceptibility to drought-induced yield loss (Table 6).

The tolerance index (TOL), which measures the absolute yield reduction between non-stress and stress environments, further differentiates genotypic responses to drought. Lower TOL values indicate genotypes that maintain stable yields under stress conditions. Maestrale (199.44), NZFM-41 (203.72), and Güney Yıldızı (210.13) demonstrated the lowest TOL values, reflecting strong drought tolerance. Conversely, Anzele (383.40) and DZ20-7 (398.04) exhibited the highest TOL values, indicating greater sensitivity to water stress and reduced suitability for drought-prone environments (Table 6).

The mean productivity (MP) index represents the average yield across both stress and non-stress conditions, providing a comprehensive measure of overall productivity. Genotypes such as Ovidio (656.28), NZFM-41 (585.80), and Sümerli (585.43) recorded the highest MP values, indicating consistently and high yield potential under variable environmental conditions. In contrast, Anzele (493.45) and NZFM-38 (463.60) exhibited substantially lower MP values, suggesting comparatively poorer performance (Table 6).

The geometric mean productivity (GMP) index offers a more conservative estimate of yield stability by incorporating a multiplicative approach that emphasizes the balance between performance under stress and non-stress conditions. Genotypes Ovidio (632.26), NZFM-41 (576.87), and Sümerli (567.01) ranked highest in GMP, reinforcing their adaptability and yield stability across contrasting environments. Conversely, Anzele (454.69) and NZFM-38 (441.92) displayed the lowest GMP values, reflecting heightened sensitivity to environmental stress (Table 6).

The harmonic mean (HM) is a robust index for assessing drought tolerance, as it integrates yield performance under both stress and non-stress conditions, with greater sensitivity to lower yields. In the present study, the highest HM value was recorded for Ovidio (6091.29 kg ha^{-1}), indicating superior yield stability and performance under drought stress. In contrast, NZFM-63 exhibited the lowest HM (3929.42 kg ha^{-1}), suggesting reduced stability and poorer adaptation to water-limited environments (Table 6).

The drought susceptibility index (DSI) measures the relative yield reduction of a genotype under drought stress compared to the mean reduction across all genotypes. DSI values less than 1.0 are indicative of greater drought tolerance. NZFM-41 (0.67) and Maestrale (0.70) recorded the lowest DSI values, reflecting enhanced resilience to water deficit. Conversely, Anzele (1.27) and DZ20-7 (1.21) displayed the highest DSI values, indicating a higher degree of susceptibility to drought-induced yield loss (Table 6).

The Stress Tolerance Index (STI) is a valuable metric for identifying genotypes that maintain high productivity under both stress and non-stress conditions. In this study, genotypes Ovidio (0.88), NZFM-41 (0.74), and Sümerli (0.71) recorded the highest STI values, reflecting strong adaptability and stable yield potential across contrasting environments. In contrast, Anzele (0.46) and NZFM-38 (0.43) exhibited the lowest STI values, indicating limited suitability for cultivation in drought-prone regions (Table 6).

The Yield Index (YI), which compares genotype performance under drought stress relative to the mean performance of all genotypes, revealed considerable variation among entries. YI values ranged from 0.758 in NZFM-63 to 1.285 in NZFM-41. Genotypes such as NZFM-41, Maestrale (1.182), Sümerli (1.167), and Ovidio (1.275) exhibited YI values exceeding 1.0, suggesting superior performance under drought conditions. Conversely, Anzele (0.801) and NZFM-63 (0.758) performed below the population mean, indicating lower drought resilience.

The Yield Stability Index (YSI) represents the proportion of yield achieved under stress relative to yield under optimal conditions, with higher values indicating greater yield stability. Genotypes NZFM-41 (0.70), Maestrale (0.69), and Sümerli (0.60) recorded the highest YSI values, demonstrating stable yield performance under water-limited conditions. In contrast, Anzele (0.44) and NZFM-63 (0.45) presented lower YSI values, suggesting reduced yield stability under drought stress (Table 6).

The Relative Stress Index (RSI), which assesses drought tolerance relative to the mean performance of all genotypes, ranged from 0.786 in Anzele to 1.257 in NZFM-41. Genotypes with RSI values greater than 1, such as NZFM-41, Maestrale (1.233), and Sümerli (1.074), were classified as drought-tolerant. Conversely, genotypes with RSI values below 1, including Anzele (0.786), NZFM-63 (0.808), and DZ20-7 (0.838), were identified as drought-sensitive.

Table 6. Estimates of stress tolerance attributes from grain yield for durum wheat genotypes

Genotype	Yp	Ys	RD	TOL	MP	GMP	HM	DSI	STI	YI	YSI	RSI
Anzele	6851.5	3017.5	55.96	3834.0	4934.50	4546.91	4189.77	1.27	0.45	0.801	0.440	0.786
DZ20-7	7501.2	3520.8	53.06	3980.4	5511.00	5139.09	4792.27	1.20	0.58	0.935	0.469	0.838
DZ21-3	7134.9	3510.7	50.80	3624.2	5322.80	5004.85	4705.89	1.15	0.55	0.932	0.492	0.879
Güney Yıldızı	5645.1	3543.8	37.22	2101.3	4594.45	4472.71	4354.19	0.84	0.44	0.941	0.628	1.121
Maestrale	6446.9	4452.5	30.94	1994.4	5449.70	5357.69	5267.23	0.70	0.63	1.182	0.691	1.233
NZFM-38	6037.0	3235.0	46.41	2802.0	4636.00	4419.24	4212.62	1.05	0.43	0.859	0.536	0.957
NZFM-41	6876.6	4839.4	29.63	2037.2	5858.00	5768.76	5680.88	0.67	0.73	1.285	0.704	1.257
NZFM-63	6303.9	2854.3	54.72	3449.6	4579.10	4241.84	3929.42	1.24	0.39	0.758	0.453	0.808
NZFM Ziraat	6551.0	3811.1	41.82	2739.9	5181.05	4996.65	4818.81	0.95	0.55	1.012	0.582	1.039
Ovidio	8321.9	4803.7	42.28	3518.2	6562.80	6322.65	6091.29	0.96	0.88	1.275	0.577	1.031
Pitagora	6599.2	3827.3	42.00	2771.9	5213.25	5025.65	4844.79	0.95	0.55	1.016	0.580	1.036
Seçkin 21	6945.6	3427.7	50.65	3517.9	5186.65	4879.29	4590.14	1.15	0.52	0.910	0.494	0.881
Sümerli	7311.5	4397.1	39.86	2914.4	5854.30	5670.04	5491.59	0.90	0.71	1.167	0.601	1.074
Svevo	6447.9	3781.2	41.36	2666.7	5114.55	4937.69	4766.95	0.94	0.53	1.004	0.586	1.047
Zenit	5919.4	3482.8	41.16	2436.6	4701.10	4540.49	4385.37	0.93	0.45	0.925	0.588	1.051
Mean	6726.24	3766.99	43.86	2959.25	5246.62	5021.57	4808.08	1.00	0.56	1.00	0.56	1.00
Standard deviation	654.13	585.79	-	637.75	532.76	551.08	579.73	0.18	0.13	0.16	0.08	0.14

Ys = Grain yield of genotypes under rain-fed condition, Yp = Grain yield of genotypes under irrigated condition, RD= Relative decrease percentage, TOL = Tolerance index, MP = Mean productivity, GMP = Geometric mean, HM = Harmonic mean, STI = Stress tolerance index, DSI = Drought susceptibility index, YI = Yield index, YSI = Yield stability index and RSI = Relative stress index.

Molecular analysis

Analysis of data for iPBS and SCoT markers

A total of 253 polymorphic bands were generated using 14 iPBS primers across 15 wheat genotypes, all of which exhibited polymorphism, resulting in an average polymorphism rate of 100% (Table 7). The iPBS-2217 primer produced the highest number of bands (35), while the lowest number (2) was generated by iPBS-2401. The average number of alleles per primer was 18.07, and the average number of bands per genotype was 16.86. All primers produced clear and scorable amplification patterns. The polymorphism information content (PIC) values for iPBS primers ranged from 0.198 to 0.427, with an average of 0.287, indicating moderate to high informativeness. In comparison, 10 SCoT primers produced a total of 105 polymorphic bands, also exhibiting a polymorphism rate of 100%. Among these, SCoT-7 and SCoT-14 produced the highest number of alleles (16 bands), while SCoT-17 and SCoT-18 produced the lowest (8 bands). The average number of alleles per primer was 10.50, with an average number of bands per genotype was 7.00. PIC values for the SCoT primers ranged from 0.036 (SCoT-18) to 0.350 (SCoT-6), with a mean value of 0.191, reflecting a lower level of marker informativeness compared to iPBS. Overall, the iPBS marker system exhibited a higher level of polymorphism and greater discriminatory power than the SCoT primers, demonstrating its superior efficiency in evaluating genetic diversity among wheat genotypes.

Table 7. Estimation of informativeness parameters using SCoT and iPBS markers across 15 durum wheat genotypes

Primer	Sequence (5'→3')	NAF[1]	PPB (%)	PIC value
iPBS- 2074	GCTCTGATACCA	23	100	0.307
iPBS- 2217	ACTTGGATGTCGATACCA	35	100	0.280
iPBS- 2219	GAACTTATGCCGATACCA	19	100	0.325
iPBS- 2221	ACCTAGCTCACGATGCCA	20	100	0.296
iPBS- 2222	ACTTGGATGCCGATACCA	25	100	0.383
iPBS- 2238	ACCTAGCTCATGATGCCA	16	100	0.198
iPBS- 2272	GGCTCAGATGCCA	13	100	0.282
iPBS- 2383	GCATGGCCTCCA	16	100	0.259
iPBS- 2387	GCGCAATACCCA	20	100	0.288
iPBS- 2392	TAGATGGTGCCA	13	100	0.427
iPBS- 2393	TACGGTACGCCA	13	100	0.241
iPBS- 2394	GAGCCTAGGCCA	18	100	0.204
iPBS- 2398	GAACCCTTGCCGATACCA	20	100	0.270
iPBS- 2401	AGTTAAGCTTTGATACCA	2	100	0.258
Mean		0.287	100	18.07
SCoT-6	CAACAATGGCTACCACGC	10	100	0.350
SCoT-7	CAACAATGGCTACCACGG	16	100	0.110
SCoT-14	ACGACATGGCGACCACGC	16	100	0.230
SCoT-15	ACGACATGGCGACCGCGA	10	100	0.240
SCoT-16	ACCATGGCTACCACCGAC	8	100	0.090
SCoT-17	ACCATGGCTACCACCGAG	8	100	0.190
SCoT-18	ACCATGGCTACCACCGCC	8	100	0.036
SCoT-21	ACGACATGGCGACCCACA	9	100	0.140
SCoT-22	AACCATGGCTACCACCAC	11	100	0.260
SCoT-23	CACCATGGCTACCACCAG	9	100	0.260
Mean		10.500	100	10.500

[1]NAF: number of amplified fragments; PPB: percentage polymorphic fragments; and PIC: polymorphism information content.

Clustering analysis based on iPBS and SCoT markers

A binary matrix was employed to estimate Nei's unbiased genetic distance coefficients among the 15 durum wheat genotypes. In the iPBS marker analysis, Ne values ranged from 0.1674 (G13) to 1.1514 (G3), with an average of 0.5348. In comparison, Ne coefficients derived from SCoT markers ranged from 0.1214 (G6) to 0.5431 (G11), with a mean value of 0.2700 (Table 8). Principal component analysis (PCA) and cluster analysis based on both iPBS markers and SCoT markers data revealed both congruent and divergent patterns in the genetic relationships among the genotypes. PCA results based on the estimated Ne coefficients indicated that the first two principal components accounted for 31.00%, 42.00%, and 73.00% of the total molecular variation in the iPBS dataset, whereas the corresponding values for the SCoT dataset were 0.35%, 47.00%, and 82.00%, respectively, based on the pooled data (Table 5). In the PCA derived from iPBS data, genotypes G3 and G10 were positioned in the lower left quadrant along Axis 1, whereas G1 was in the upper right quadrant along Axis 2. The remaining genotypes clustered predominantly in the upper left quadrant (Figure 2). For the SCoT-based PCA, G1 was in the lower left quadrant of Axis 1, while G15 was separated in the lower right quadrant along Axis 2, while the remaining genotypes formed a compact cluster in the upper left quadrant. These findings suggest that although both marker systems are effective in detecting genetic diversity, they may capture distinct dimensions of genetic variation, as reflected by the differing placements of key genotypes such as G3, G10, and G15. Cluster analysis based on iPBS markers grouped the genotypes into two main clusters, with G3 forming a distinct cluster and the remaining genotypes comprising a second cluster (Figure 3). In contrast, clustering based on SCoT markers identified G15 as genetically distinct, forming a separate group, with the other genotypes assigned to another cluster. Overall, PCA results largely matched the clustering patterns, supporting the observed genetic differentiation among the genotypes as revealed by each marker system.

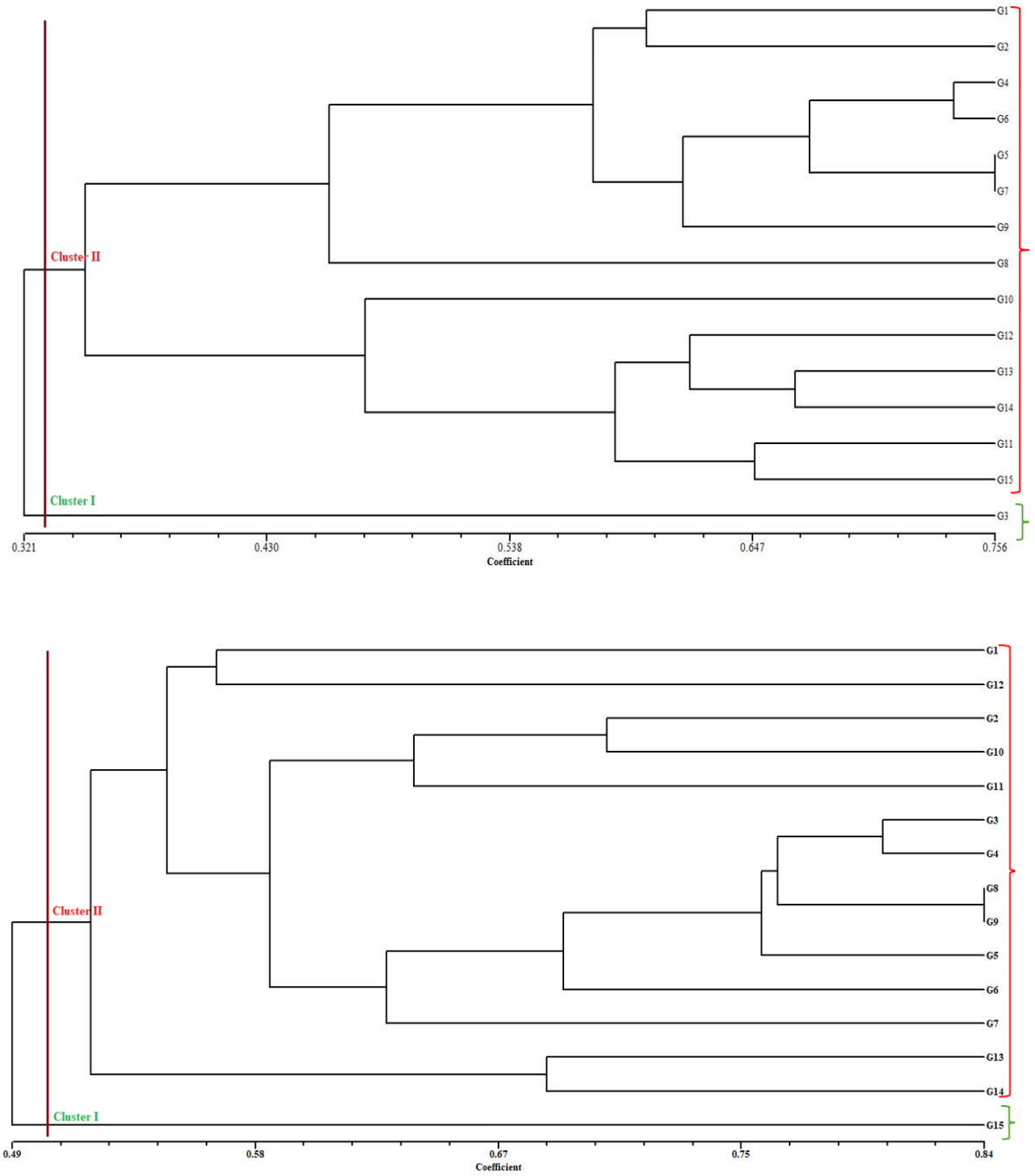

Figure 2. Dendogram generated by the UPGMA method using iPBS and SCoT markers (G1; DZ21-3, G2; Guney Yıldızı, G3; DZ20-7, G4; Seçkın-21, G5; Zenit, G6; Anzele, G7; NZFM-63, G8; Pitagorasvevo, G9; Svevo, G10; Ovidio, G11; NZFM-41, G12; NZFM-38, G13; Mastrella, G14; NZFM-Ziraat and G15; Surmeli)

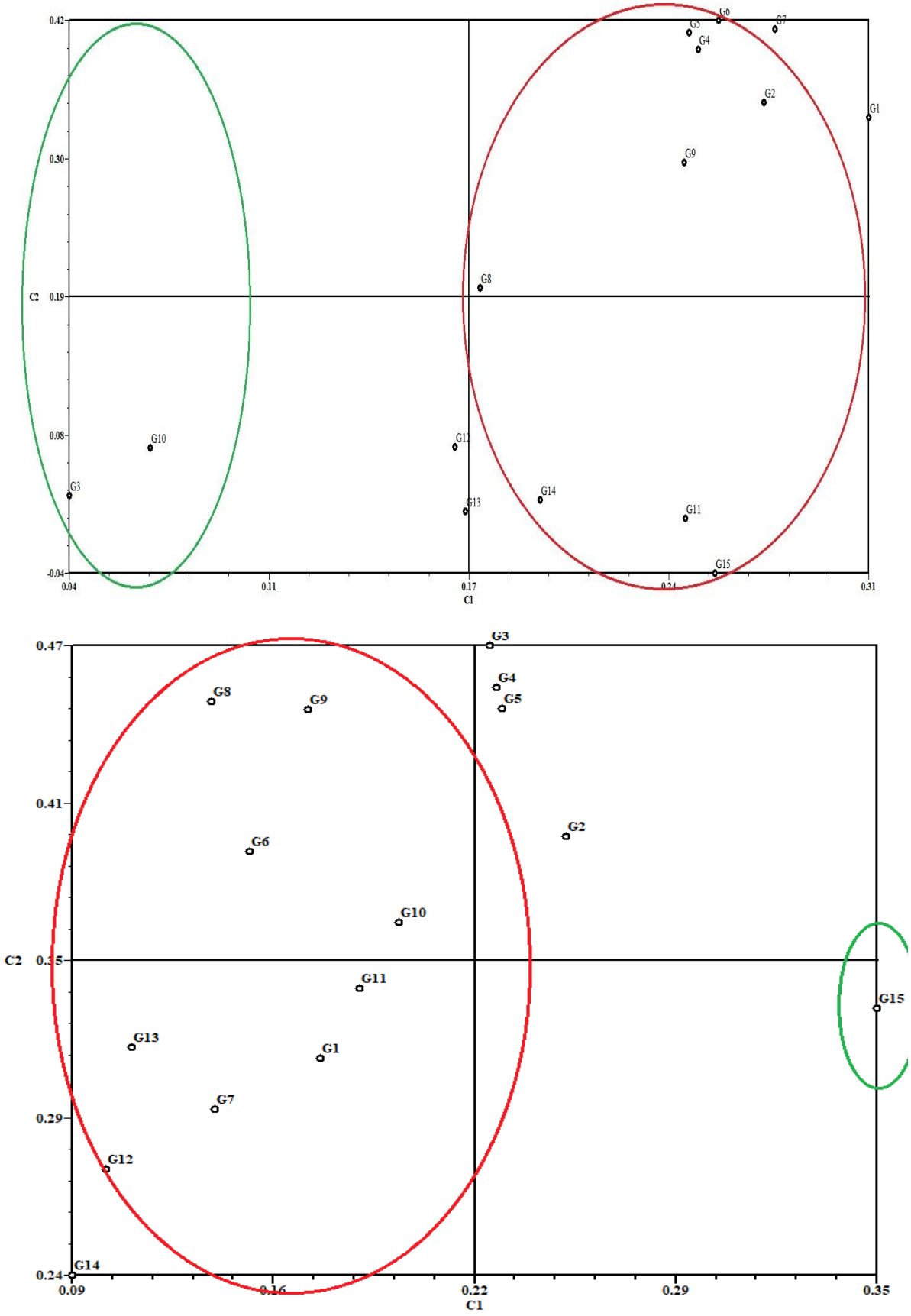

Figure 3. Principal component analysis (PCA) using iPBS and SCoT markers and its separation on two-dimensional diagram (G1; DZ21-3, G2; Guney Yıldızı, G3; DZ20-7, G4; Seçkın-21, G5; Zenit, G6; Anzele, G7; NZFM-63, G8; Pitagorasvevo, G9; Svevo, G10; Ovidio, G11; NZFM-41, G12; NZFM-38, G13; Mastrella, G14; NZFM-Ziraat and G15; Surmeli)

Table 8. Pairwise population matrix of Nei unbiased genetic distance based on iPBS and SCoT markers.

Marker	Population	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12	G13	G14	G15
iPBS	G1	-														
	G2	0.313	-													
	G3	0.842	0.8794	-												
	G4	0.2811	0.3023	1.0225	-											
	G5	0.3404	0.2969	0.9484	0.2351	-										
	G6	0.2916	0.2811	0.842	0.1815	0.1959	-									
	G7	0.3516	0.2969	0.87	0.2655	0.1721	0.2451	-								
	G8	0.4582	0.3743	1.1514	0.3294	0.3239	0.3629	0.3349	-							
	G9	0.543	0.4396	1.0225	0.3686	0.3294	0.3686	0.3185	0.3859	-						
	G10	0.7212	0.7212	0.1815	0.7376	0.7294	0.6582	0.6506	0.8329	0.7885	-					
	G11	0.6659	0.6207	0.969	0.5095	0.543	0.5636	0.543	0.3801	0.4582	0.7459	-				
	G12	0.5776	0.6061	0.8329	0.5228	0.6134	0.5776	0.5705	0.4771	0.5776	0.6207	0.4274	-			
	G13	0.5705	0.5294	1.1145	0.4645	0.4964	0.4771	0.5095	0.3404	0.4771	0.9183	0.2251	0.4335	-		
	G14	0.6061	0.5776	1.0561	0.4964	0.49	0.4582	0.5161	0.346	0.4458	0.8513	0.2401	0.452	0.1674	-	
	G15	0.6134	0.6582	0.9383	0.6281	0.6659	0.7376	0.6061	0.4214	0.6892	0.6892	0.2759	0.3516	0.313	0.3185	-
SCoT	G1	-														
	G2	0.2719	-													
	G3	0.2973	0.2472	-												
	G4	0.3232	0.2973	0.1431	-											
	G5	0.2595	0.3499	0.1653	0.1881	-										
	G6	0.2595	0.2845	0.2231	0.2472	0.2472	-									
	G7	0.2113	0.3913	0.2973	0.2472	0.2472	0.1881	-								
	G8	0.2719	0.2595	0.1322	0.1542	0.1542	0.1214	0.1881	-							
	G9	0.3635	0.3232	0.1653	0.1653	0.1653	0.1542	0.1542	0.0896	-						
	G10	0.3102	0.2113	0.3232	0.3232	0.3232	0.2113	0.3635	0.2351	0.2472	-					
	G11	0.2113	0.2845	0.3232	0.3232	0.2973	0.2113	0.2845	0.1881	0.2231	0.2113	-				
	G12	0.2845	0.2595	0.2973	0.2973	0.3232	0.1653	0.2351	0.1653	0.2472	0.2351	0.2845	-			
	G13	0.2845	0.3102	0.2472	0.2719	0.2231	0.1881	0.2845	0.1431	0.1766	0.1881	0.2595	0.2351	-		
	G14	0.4199	0.3635	0.3232	0.2973	0.3232	0.2113	0.2595	0.1881	0.2231	0.2595	0.2845	0.2113	0.1214	-	
	G15	0.2595	0.4199	0.3499	0.4055	0.4345	0.4796	0.5108	0.3913	0.4055	0.3913	0.5431	0.4199	0.4199	0.4796	-

G1; DZ21-3, G2; Guney Yıldızı, G3; DZ20-7, G4; Seçkın-21, G5; Zenit, G6; Anzele, G7; NZFM-63, G8; Pitagorasvevo, G9; Svevo, G10; Ovidio, G11; NZFM-41, G12; NZFM-38, G13; Mastrella, G14; NZFM-Ziraat and G15; Surmeli.

Monitoring the genetic diversity based on iPBS and SCoT markers

The average number of alleles (Ne), Nei's genetic diversity (h), and Shannon's information index (I) for the 15 wheat genotypes, as calculated from the iPBS and SCoT marker data, are summarized in Table 9. Among the iPBS primers, iPBS-2074 exhibited the highest values across all three parameters (Ne = 2.000, h = 0.364, I = 0.535), while iPBS-2401 had the lowest (Ne = 1.791, h = 0.251, I = 0.392). The overall mean values across all iPBS primers were Ne = 1.900, h = 0.303, and I = 0.459. In comparison, among the SCoT primers, SCoT-18 produced the highest values (Ne = 1.630, h = 0.358, I = 0.532), whereas SCoT-7 showed the lowest values (Ne = 1.159, h = 0.106, I = 0.184). The average values across all SCoT primers were lower than those of the iPBS markers, with Ne = 1.350, h = 0.218, and I = 0.340. These results indicate that the iPBS marker system exhibited greater genetic diversity and provided higher informativeness than the SCoT markers in the evaluated wheat genotypes.

Table 9. Estimation of informativeness parameters using SCoT and iPBS primers across 15 durum wheat genotypes.

Marker	ne[1]	h	I	Marker	ne[1]	h	I
iPBS- 2074	2.000	0.364	0.535	SCoT-6	1.571	0.350	0.529
iPBS- 2217	1.983	0.319	0.488	SCoT-7	1.159	0.106	0.184
iPBS- 2219	1.966	0.316	0.482	SCoT-14	1.389	0.234	0.355
iPBS- 2221	1.965	0.325	0.492	SCoT-15	1.371	0.242	0.385
iPBS- 2222	1.948	0.344	0.518	SCoT-16	1.142	0.093	0.148
iPBS- 2238	1.880	0.314	0.467	SCoT-17	1.306	0.191	0.305
iPBS- 2272	1.827	0.301	0.446	SCoT-18	1.630	0.358	0.532
iPBS- 2383	1.856	0.271	0.407	SCoT-21	1.222	0.140	0.218
iPBS- 2387	1.856	0.277	0.422	SCoT-22	1.382	0.255	0.412
iPBS- 2392	1.889	0.312	0.467	SCoT-23	1.436	0.259	0.392
iPBS- 2393	1.907	0.309	0.467	Mean	1.350	0.218	0.340
iPBS- 2394	1.875	0.284	0.433				
iPBS- 2398	1.859	0.261	0.404				
iPBS- 2401	1.791	0.251	0.392				
Mean	1.900	0.303	0.459				

[1] Ne; number of alleles, h; Nei's genetic diversity, and I; Shannon's information index.

Genetic structure analysis of the population based on iPBS and SCoT markers

The population structure analysis based on the iPBS marker data is presented in Figure 4. The optimal number of genetic clusters (K) was determined using the ΔK method, which indicated the highest ΔK value at K = 2 (Figure 4). The results of the population structure analysis were consistent with those obtained from the cluster analysis. The low K value suggests a high level of gene flow among the regions from which the genotypes were collected. Based on the membership coefficient values (Figure 4; Table 10), seven genotypes (G1, G2, G4, G5, G6, and G7) were assigned to Subpopulation I (red), while five genotypes (G6, G11, G12, G14, and G15) were assigned to Subpopulation II (green). The remaining three genotypes (G3, G8, and G10) exhibited an admixed genetic background. The expected heterozygosity values for the two subpopulations were 0.2743 and 0.2588, respectively, with an average of 0.2666 (Tables 10 and 11). The F-statistics (FST) values were calculated as 0.3023 for Subpopulation I and 0.3863 for Subpopulation II, with a mean FST of 0.3443. Similarly, the population structure analysis based on SCoT marker data also indicated K = 2 as the optimal number of clusters according to the ΔK method (Figure 4). The clustering results were congruent with those obtained using the iPBS markers. According to the membership coefficient values (Figure 4; Table 11), three genotypes (G2, G10, and G15) were assigned to Subpopulation I (green), while two genotypes (G8 and G9) were assigned to Subpopulation II (red). The remaining three genotypes displayed an admixed genetic structure. The expected heterozygosity values for the two subpopulations were 0.0067 and 0.5589, respectively, with a mean value of 0.5656. The FST values were 0.1432 for Subpopulation I and 0.3050 for Subpopulation II, with an average of 0.4482. The genetic differentiation (FST) between the two subpopulations identified through the population structure analysis was estimated as 0.1219 for the iPBS markers and 0.0398 for the SCoT markers, indicating moderate and low levels of genetic differentiation, respectively. Overall, these results demonstrate that although both marker systems support the presence of two genetic groups, the iPBS markers provide a more robust resolution of genetic differentiation, whereas the SCoT markers may be more sensitive to capturing within-subpopulation diversity.

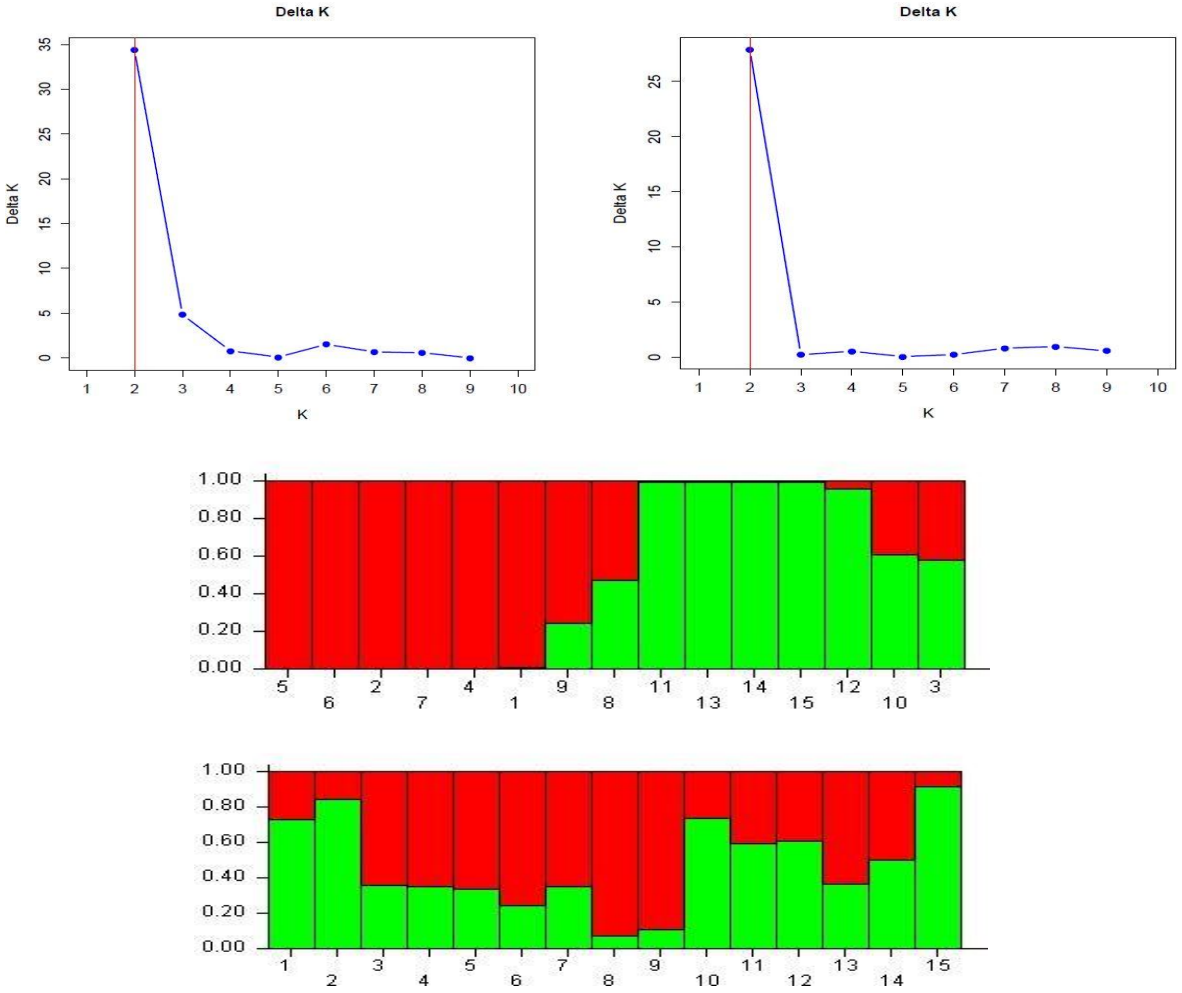

Figure 4. The Bayesian clustering algorithm of 15 investigated durum wheat using the iPBS and SCoT marker. Bars indicate the membership coefficients of accessions based on allele frequencies for each subpopulation. Numbers on the horizontal axes correspond to accession codes in Table 1

Table 10. Expected heterozygosity and Fst values in wheat plant subpopulations based on iPBS and SCoT markers

Markers	Sub-population (K)	Expected heterozygosity	F_{ST}
iPBS	Sub-Pop I	0.2743	0.3023
	Sub-Pop II	0.2588	0.3863
	Mean	0.2666	0.3443
SCoT	Sub-Pop I	0.5589	0.1432
	Sub-Pop II	0.0067	0.3050
	Mean	0.5656	0.4482

Table 11. Genetic differentiation based on Fst values among two subpopulations identified by population structure analysis in wheat based on iPBS and SCoT markers

Markers	Sub-Pop (K)	Sub-Pop A	Sub-Pop (K) B
iPBS	Sub-Pop I	-	0.1219
	Sub-Pop II	0.1219	-
SCoT	Sub-Pop I	-	0.0398
	Sub-Pop II	0.0398	-

4. DISCUSSION

Wheat has a crucial role for human diet especially in developing countries (Tatar et al., 2020). The need to develop drought-tolerant and high-yielding wheat genotypes has become increasingly critical for sustaining wheat production and ensuring global food security in the context of a growing population (El-Aty et al., 2024). Agro-morphological and molecular characterization of durum wheat represents a fundamental approach for uncovering genetic diversity, which is vital for the identification and introgression of desirable traits, particularly under stress conditions (Fatemi et al., 2022). Such comprehensive analyses facilitate the effective utilization of genotypes in breeding programs aimed at enhancing stress resilience and overall agronomic performance (Almarri et al., 2023).

Agro-physiological and quality characteristics

The significant differences observed among genotypes for various traits underscore the presence of substantial genetic variability, which is fundamental for the improvement of both qualitative and quantitative traits. This variation provides a valuable foundation for plant breeders to enhance targeted agronomic and quality characteristics through systematic breeding strategies (Tahir et al., 2022). The existence of both genotypic and phenotypic variation is critical for developing improved lines and establishing effective breeding programs (Bhandari et al., 2017). A key objective of wheat improvement initiatives is the development of genotypes that are not only drought-tolerant but also high-yielding and agronomically stable (Sharma et al., 2021). However, designing experiments that consistently yield reproducible results remains challenging due to the variable nature, intensity, and duration of drought stress (Spitkó et al., 2014). Selection strategies are generally categorized as direct or indirect and are applied under both stress and non-stress conditions. Given that drought stress involves multiple interacting factors, breeding for drought resistance often relies on indirect selection approaches to address the complex physiological and environmental interactions involved (Haussmann et al., 2012). In the present study, we investigated the genetic diversity of 15 durum wheat genotypes, including 10 durum wheat varieties and 5 advanced breeding lines. The observed genotypic differences in grain yield and 1000-kernel weight under both irrigated and drought-stress conditions highlight the impact of water deficit on yield stability. Notably, the genotypes Ovidio and Seçkin 21 maintained superior performance under stress, indicating their potential suitability for cultivation in drought-prone regions. In contrast, Anzele and NZFM-63 exhibited pronounced yield reductions, suggesting lower drought tolerance. Grain yield and its stability under various environmental stress conditions have long served as key criteria for the selection and recommendation of genotypes (Yani & Rashidi, 2012). Our findings align with previous studies demonstrating that drought stress exerts a significant influence on grain yield and 1000-kernel weight, contributing substantially to yield declines in wheat (El-Aty et al., 2024).

Our results demonstrated that the significant genotypic variation in hectolitre weight, protein content, and starch content under both moisture regimes underscores the diverse quality responses among the evaluated durum wheat genotypes. The genotypes Ovidio and DZ21-3 maintained stable hectolitre weights across conditions, while NZFM Ziraat and Svevo exhibited consistently high protein content regardless of environment. Ovidio's consistently elevated starch content further highlights its potential as a dual-purpose genotype, combining yield stability with superior grain quality under drought stress conditions. This variation may be attributed to a combination of environmental influences and inherent genetic differences among genotypes. Numerous studies have emphasized that both the protein content and overall quality of wheat are significantly affected by genotype and environmental factors (Albayrak et al., 2020; Gungor & Dumlupınar, 2019; Mut et al., 2017). These results reinforce the notion that protein content is highly responsive to genotype × environment interactions.

The results indicate that several genotypes, particularly Maestrale and Ovidio, exhibited relatively higher *b* values, indicating superior grain color quality potential associated with higher carotenoid pigment accumulation. However, drought stress generally reduced *b* values in most genotypes, suggesting that water limitation may negatively influence pigment accumulation and consequently grain color quality. Similar findings have been reported in studies conducted in Türkiye, particularly in the Southeastern Anatolia region, where environmental stress during the grain-filling period significantly affects durum wheat quality traits. For instance, Kizılgeci et al. (2016) and Tekdal and Yıldırım (2015) reported that drought and high temperatures during grain filling can reduce carotenoid accumulation and alter grain color parameters in durum wheat grown under rainfed conditions. Likewise, Kendal et al. (2019) observed considerable genotypic variation in color traits and emphasized the strong influence of environmental conditions in semi-arid regions on these characteristics. Collectively, these findings support the results of the present study and highlight the importance of evaluating grain color parameters alongside agronomic performance when selecting durum wheat genotypes adapted to water-limited environments.

Drought Stress index characteristics

The selection of germplasm exhibiting substantial genotypic variability for drought tolerance is a fundamental prerequisite for breeding programs aimed at enhancing stress resilience (Mondal et al., 2023). For the effective development of drought-tolerant durum wheat through conventional breeding approaches, comprehensive knowledge of

the genetic diversity and trait heritability within the breeding material is essential. This includes identifying significant variation in genotypic responses to water deficit conditions and elucidating the genetic basis of key adaptive traits. A thorough understanding of plant responses to drought stress not only facilitates the identification of promising genotypes but also improves the efficiency of selection strategies for developing resilient cultivars (Farshadfar et al., 2013). The assessment of relative yield performance under both stress and non-stress conditions is frequently employed as an initial approach for identifying drought-related traits and selecting promising genotypes for cultivation in water-limited environments. However, when applied to small populations, the reliability of this method may be limited due to reduced genotypic variation. The narrow genetic base in such populations can hinder the accurate differentiation of drought-tolerant genotypes, thereby diminishing the effectiveness of yield-based selection criteria. Consequently, while yield assessments remain valuable, their utility in small populations warrants cautious interpretation and should ideally be complemented by additional physiological or molecular markers. Such indices have been successfully employed to identify stable genotypes based on their performance under both stress and favorable conditions (Mursalova et al., 2015). In the present study, the evaluation of drought tolerance indices revealed clear genotypic differences in yield stability and adaptability across moisture regimes. NZFM-41 and Maestrale consistently ranked among the top performers across RD%, DSI, TOL, YSI, and STI, highlighting their superior drought tolerance and yield resilience. In contrast, Anzele and NZFM-38 exhibited poor performance across most indices, indicating high sensitivity to drought stress and limited suitability for cultivation in arid environments. These findings provide valuable insights for the selection of genotypes with stable performance under water-limited conditions.

Genetic diversity using iPBS and SCoT markers

In this study, iPBS and SCoT markers were employed to assess the genetic diversity among durum wheat genotypes. A total of 253 and 105 polymorphic bands were generated by the iPBS and SCoT primers, respectively, with both marker systems achieving 100% polymorphism. The iPBS primers produced a higher average number of alleles per primer (18.07) and exhibited greater polymorphic information content (PIC) values (mean = 0.287) compared to the SCoT primers (mean alleles = 10.50; PIC = 0.191). These results indicate that the iPBS marker system possesses greater discriminatory power and efficiency for detecting genetic variation among the wheat genotypes analyzed. This difference can be attributed to the higher number of polymorphic bands generated by the iPBS primers (Apana et al., 2021). Overall, the iPBS markers demonstrated higher effectiveness than the SCoT markers and have shown considerable utility in genetic diversity studies across a wide range of plant species. Their superior performance can be ascribed to their capacity to produce a larger number of polymorphic fragments and to deliver greater informativeness, both of which are critical for robust genetic analyses.

The assessment of genetic variation parameters such as the number of effective alleles (Ne), Shannon's information index (I), and unbiased expected heterozygosity (uHe) provides critical insights into the extent of genetic diversity within plant populations. Among the iPBS primers evaluated, iPBS-2074 exhibited the highest values for all three parameters (Ne = 2.000, h = 0.364, I = 0.535), whereas iPBS-2401 showed the lowest (Ne = 1.791, h = 0.251, I = 0.392). The mean values across all iPBS primers were Ne = 1.900, h = 0.303, and I = 0.459. In comparison, among the SCoT markers, SCoT-18 yielded the highest estimates (Ne = 1.630, h = 0.358, I = 0.532), while SCoT-7 exhibited the lowest (Ne = 1.159, h = 0.106, I = 0.184). These findings indicate that the iPBS marker system provided greater genetic diversity and informativeness than the SCoT markers in the durum wheat genotypes examined. Collectively, these genetic diversity metrics contribute to a more comprehensive understanding of population structure, supporting both conservation initiatives and breeding programs by facilitating the identification of genetically diverse individuals (Bokaei et al., 2025). Similar studies employing molecular markers have likewise reported high levels of polymorphism and genetic variability within plant populations (Sobirov et al., 2024).

Estimates of genetic similarity or distance among genotypes are fundamental for the selection of parental lines in breeding programs. Cluster analysis and principal component analysis (PCA) are robust multivariate techniques commonly employed to classify genotypes based on genetic similarity, offering valuable insights into the genetic structure of plant populations (Mohammadi & Prasanna, 2003). Cluster analysis has been successfully utilized to evaluate genetic diversity and to categorize wheat genotypes according to shared stress-related traits (Jamali et al., 2020). The outcomes of such analyses can guide the development and optimization of future wheat breeding strategies (Abdelghany et al., 2023). Observed variations in genetic similarity coefficients may result from differences in the number of genotypes analyzed or the specific microsatellite primers employed to detect DNA polymorphisms (Kara et al., 2017). In the present study, cluster analysis based on inter-primer binding site (iPBS) markers grouped the genotypes into two primary clusters, with genotype G3 forming a distinct group, while the remaining genotypes clustered together. Similarly, cluster analysis using start codon targeted (SCoT) markers identified G15 as genetically distinct from the other genotypes. These clustering patterns were largely corroborated by PCA results, reinforcing the genetic differentiation revealed by both marker systems.

Population structure analysis based on iPBS and SCoT marker data revealed the presence of two major genetic clusters (K = 2) among the studied genotypes. Notably, iPBS markers exhibited a higher level of genetic differentiation between the two subpopulations (Fst= 0.1219), while SCoT markers indicated a lower degree of differentiation (Fst=

0.0398). These results suggest that iPBS markers provide a clearer resolution of genetic separation, while SCoT markers may be more sensitive to genetic variation within subpopulations. Both marker systems detected evidence of admixture in certain genotypes and gene flow between clusters, indicating their effectiveness in capturing population structure. Overall, iPBS markers appear to offer more robust intergroup differentiation, whereas SCoT markers contribute a finer-scale view of intragroup genetic diversity. Integrating agro-physiological performance with molecular characterization provides a robust framework for assessing drought resilience in durum wheat. In this study, a strong concordance was observed between phenotypic stability and the genetic structure of the evaluated genotypes. NZFM-41 and Maestrale, identified as the most drought-resilient genotypes based on high STI, GMP, and HM values, were consistently grouped within the same genetic clusters in the iPBS-based STRUCTURE analysis. This pattern suggests that the relatively high resolution of the iPBS marker system (FST = 0.3443) effectively captures genomic variation associated with adaptation to water-limited environments. Similarly, the identification of Ovidio as an elite, high-yielding genotype corresponded with its distinct position in the PCA, further supporting the reliability of the integrative evaluation framework. The concordance between drought tolerance indices and molecular clustering highlights the value of iPBS markers as complementary tools to phenotypic screening for identifying strategic parental lines in climate-resilient durum wheat breeding programs.

5. CONCLUSION

This study successfully identified genotypic variation in drought tolerance and genetic diversity among durum wheat genotypes, offering valuable insights for breeding programs aimed at enhancing drought resilience. Both morphological and molecular analyses revealed key traits and genetic markers critical for the selection of stable, high-yielding genotypes, with iPBS markers demonstrating greater resolution in detecting genetic differentiation compared to SCoT markers. Drought tolerance indices further supported the identification of genotypes better adapted to water-limited environments. Among the evaluated genotypes, Ovidio consistently exhibited superior grain yield under both irrigated and drought stress conditions, highlighting its adaptability and resilience. These findings make a significant contribution to the development of durum wheat varieties capable of withstanding drought stress, thereby supporting climate-resilient crop improvement strategies. Moreover, cluster analysis revealed clear genetic distinctions among genotypes, facilitating targeted breeding and selection strategies.

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