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Research Article

SCoT, ISSR, and SDS-PAGE Investigation of Genetic Diversity in Several Egyptian Wheat Genotypes under Normal and Drought Conditions

Abdelghany S. Shaban,[1] **S. A. Arab**,[2] **Mostafa M. Basuoni**,[1] **Mahmoud S. Abozahra**,[1] **Aisha M. Abdelkawy**,[3] **and M. M. Mohamed**[4]

[1]*Botany and Microbiology Department, Faculty of Science (Boys), Al-Azhar University, Cairo 11884, Egypt*
[2]*National Gene Bank and Genetic Resources, Agricultural Research Center, Giza, Egypt*
[3]*Botany and Microbiology Department, Faculty of Science (Girls Branch), Al-Azhar University, Cairo, Egypt*
[4]*Wheat Research Department, Field Crops Research Institute, Agricultural Research Center, Giza, Egypt*

Correspondence should be addressed to Abdelghany S. Shaban; abdelghanysobhy@azhar.edu.eg

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Using agronomic parameters, ISSR (inter simple sequence repeat), and SCoT (start codon targeted) markers, ten potential wheat genotypes were examined for genetic diversity under normal and drought conditions. Significant agronomic features have been identified, as well as a low drought susceptibility index. Using seven SCoT and seven ISSR primers, a total of 112 amplified DNA fragments were synthesized, resulting in 61 and 51 bands, respectively. For SCoT and ISSRs, the percentage of polymorphism was 93.4% and 78.4%, respectively. Two markers, ISSR and SCoT, were found to be effective in detecting polymorphism among the examined genotypes, with mean PIC values of 0.61 and 0.62, respectively. In terms of marker index (MI), resolving power (Rp), and polymorphism percentage, SCoT markers exhibited the most significant values. The examination of seed storage proteins revealed 21 subunits with a mass ranging from 22 to 110 kDa. A cluster analysis of the data and morphological features contributed to identifying different molecular and biochemical bands that could be linked to genotype 4's drought-resistance capabilities.

1. Introduction

Wheat (*Triticum* spp.) is one of the most economically significant cereal crops on a global scale, serving as a critical raw material for most food industries and providing food for billions of people [1]. Wheat is one of the most important crops in Egypt, producing approximately 8.5 million tons per year compared to the annual requirement of approximately 13.5 million tons [2]. As a result, despite being the first crop planted, Egypt remains a significant importer of wheat. High wheat production in Egyptian agriculture is one of the primary goals of increasing food production in order to close the food gap created by the continuous population growth [3]. Therefore, wheat productivity in Egypt must be increased to close the production-to-consumption gap. Consequently, increasing wheat production is critical to meeting the needs of the world's rapidly growing population of 9 billion people by 2050 [4]. This can be accomplished by expanding the cultivable area and increasing productivity by examining and utilizing available wheat germplasm's genetic diversity, as well as by improving cultivar genetics and crop management practices [5].

The assessment of genetic diversity within a gene pool aids in genotype selection, promotes optimal genetic improvement, and shortens breeding time [6]. It is critical in defining breeding lines, cultivars, or species and serves as the basis for selecting appropriate parental forms during crossing development [7]. In general, a variety of techniques are used to assess genetic diversity, including qualitative and quantitative morphological and agronomic evaluation, biochemical protein analysis (SDS-PAGE, isozyme assay), and DNA analysis (molecular markers) [8].

Traditional methods for estimating genetic variation in plants are based on morphological characteristics; nevertheless, these phenotypic characteristics have limitations due to their influence by environmental factors and plant developmental stages [9, 10]. The majority of efforts to improve drought-tolerant wheat cultivars have focused on yield-enhancing morphological characteristics [11, 12], with less emphasis on biochemical, physiological, and molecular characteristics. Agronomic and morphological data have been extensively used to screen wheat varieties for resistance to stress, such as salinity, and drought [13–16]. Nonetheless, morphological measurements alone are inadequate for the genetic identification of wheat cultivars/lines. Using biochemical and molecular markers to select for genetic variability has proven to be more advantageous than using phenotypic markers [17]. Biochemical markers are essential for species identification and the establishment of genetic variability. Due to its simplicity and effectiveness in estimating crop germplasm genetic structure [18], it is also a low-cost, simple, and widely applicable method for displaying protein profiles of plants under various conditions as well as calculating an accurate genetic diversity index [19].

Molecular markers enable a more precise calculation of genetic variation, which is essential for future breeding programs aiming to safeguard better and utilize genetic resources [6]. SCoT markers have been used in several genetic applications, including cultivar identification, QTL mapping, and DNA fingerprinting [20]. In addition, it has a number of advantages over RAPD, ISSR, and AFLP; for instance, it is more stable, provides more repeatable and reliable bands, and may be utilized well for population studies, genetic mapping in various plants, and marker-assisted selection programs [21]. Additionally, ISSR markers are one of the most powerful marker systems available, producing many informative bands [22]. ISSR markers are believed to be capable of amplifying DNA regions between two microsatellites, which explains their widespread use. Due to their use of random markers, ISSRs demonstrate the selectivity of microsatellite markers and can be synthesized without precise sequence information [23]. ISSR primers vary in terms of polymorphism, resolving power (Rp), and informativeness of the bands (Ib), making them robust molecular markers capable of distinguishing between cultivars [23]. If the genome contains enough ISSR motifs, they can be used on any plant species [24]. It is also possible to employ the ISSR marker in wheat genotypes to measure genetic variation and population structure properly [25, 26].

This study aimed to investigate the genetic relationships and differentiation of ten wheat genotypes utilizing biochemical and molecular markers (SCoT and ISSR), in addition to screening and analyzing the degree of variation in morphological features between different wheat genotypes in response to drought stress.

2. Materials and Methods

Two experiments were carried out at Sids Research Station (Latitude 29°04′27″N and 30°50′53″ Longitude E) to study the water stress effect on some wheat genotypes during the two winter seasons of 2019/2020 and 2020/2021. Eight wheat genotypes and two wheat cultivars (Giza 171 and Nubaria2) were regenerated and evaluated for drought tolerance (Table 1). Each plot consisted of 6 rows × 3 m in length and 20 cm apart (plot size = 3.6 m^2). The used design was a randomized complete block design (RCBD) with four replicates. Wheat genotypes were subjected to two water treatments where each water treatment was planted in a separate experiment; the first experiment was typically irrigated (five times), and the second experiment was irrigated only once at 20 days after planting. Table 2 contains a list of the morphological traits that have been investigated, as well as the codes that have been assigned to them.

2.1. Statistical Analysis. The data were subjected to analysis of variance, and the observed values were equated with estimating the variance components. The formula proposed by Burton [27] was used to estimate the variance components and coefficients of variation. A combined analysis of the two growing seasons was carried out. Means were compared using the least significant difference (LSD) [28] at the 5% probability level, using the "MSTAT-C" computer software package.

The drought tolerance indices were calculated as follows:

(1) $SSI = 1 - (Ys/Yp)/SI$, where $SI = 1 - (\hat{Y}s/\hat{Y}p)$, whereas SI is the stress intensity and $\hat{Y}s$ and $\hat{Y}p$ are the means of all genotypes under stress and well water conditions, respectively [29].

(2) $STI = (Ys \times Yp)/\bar{Y}p2$ [30].

(3) $MP = (Ys + Yp)/2$ [31].

(4) $GMP = (Ys/Yp)\ 1/2$ [29].

(5) $SDI = (Ys - Yp)/Yp$ [32].

(6) $DI = Ys \times (Ys/Yp)/\bar{Y}S$ [33].

(7) $DTE = (Ys/Yp) \times 100$ [34].

(8) $TOL = Yp - Ys$ [31].

(9) $RDI = (Ys/Yp)/(\hat{Y}s/\hat{Y}p)$ [29].

(10) $SSPI = \{(Yp - Ys)/(2 \times \hat{Y}p)\} \times 100$ [35].

(11) $HM = 2\ (Ys \times Yp)/(Ys + Yp)$ [28].

The phenotypic correlation among all studied traits was calculated according to [36]. Using PAST Paleontological Statistics version 3.08 [37], the statistical analysis and relationship between the germplasm were calculated by calculating their Euclidean distance and paired group as phenogram.

2.2. Extraction of DNA from Plant Materials. The genomic DNAs were recovered from the young leaves of two-week-old seedlings using the CTAB technique [38]. The quality and quantity of the isolated DNAs were determined using spectrophotometry and agarose gel electrophoresis.

2.3. Analysis of ISSR-PCR and SCoT-PCR. A set of seven primers for ISSR and SCoT markers was employed to amplify the genotypes' genomic DNA (Table 3). The PCR

Table 1: Names and pedigree of genotypes used in the study.

Genotype no.	Name	Pedigree
G1	A (B.W) # 14	Sids 6/5/PARENTS47A-4-1/4/SAKHA61/3/MildressMo73/pol//t.aest-BON/CNO-7c/6/OPATA/RAYON//KAUZ/3/MIANYAG 20
G2	A (B.W) # 19	BECARD/KACHU/3/CRDN/PASTOR//GIZA#168
G3	A (B.W) # 20	BECARD/KACHU/3/CRDN/PASTOR//GIZA#168
G4	A (B.W) # 25	BECARD/KACHU/3/FRET2/KUKUNA//FRET2
G5	A (B.W) # 26	ATTJLA/JUCHI/4/SERI.1B//KAUZ/HEVO/3/AMAD/5/KIRITATI/4/2*BAV92//IRENA/KAUZ/3/HUTTES
G6	A (B.W) # 33	Sids 13//KAMB1*2/BRAMBLING
G7	A (B.W) # 34	Kauz//Altar 84/Aos/3/Sids 4/7/LFN/1158.57//PRL/3/HAHN/4/KAUZ/5/KAUZ/6/Sakha 202/8/CT/CDC//PLO/3/SAKER/4/Sids 4
G8	A (B.W) # 36	Attila/3*Bcn/3/DVERD2/AESQUARROSA (214)//2*BCN./7/LFN/1158.57//PRL/3/HAHN/4/KAUZ/5/KAUZ/6/Sakha 202/8/OASIS/5*BORL95/4/CNDO/R143//ENTE/MEXI75/3/CNDO/R143.
Giza 171	Giza 171	Sakha 93/Gemmeiza 9
Nubaria 2	Nubaria 2	FRET2*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ*2/5/BOW/URES//2*WEAVER/3/CROC_1/AESQUARROSA (213)//POG

Table 2: The studied traits and their code used in this study.

Traits	Code	Traits	Code	Traits	Code
Days to heading	DH	Mean productivity	MP	Number of grains per spike	NK/S
Days to maturity	DM	Geometric mean productivity	GMP	Grain yield per plot (kg)	GY/P
Plant height	PH	Yield stability index	YSI	Stress susceptibility index	SSI
Number of spikes per (m^2)	NS m^2	Sensitivity drought index	SDI	Stress tolerance index	STI
1000-grain weight (g)	1000-KW	Drought index	DI	Tolerance	TOL
Relative drought index	RDI	Stress susceptibility percentage index	SSPI	Harmonic mean	HM

Table 3: Primer sequences, P%, PIC, MI, and Rp in SCoT as well as ISSR primers produced in the ten wheat genotypes.

Marker	Primer	Primer sequences 5′-3′	TAF	PF	MF	UF	P (%)	PIC	MI	Rp	FS (bp)	
											Small	Large
	SCoT1	ACGACATGGCGACCACGC3	5.0	4.0	1.0	1.0	80	0.55	1.76	4.0	350	1500
	SCoT2	ACCATGGCTACCACCGGC	9.0	7.0	2.0	1.0	77.8	0.57	3.1	6.4	510	1520
	SCoT3	ACGACATGGCGACCCACA	6.0	6.0	0.0	0.0	100	0.87	5.22	4.0	205	800
SCoT	SCoT4	ACCATGGCTACCACCGCA	6.0	5.0	1.0	0.0	83.3	0.21	0.87	8.6	575	1500
	SCoT5	CAATGGCTACCACTAGCG	10.0	10.0	0.0	1.0	100	0.80	8.0	8.2	200	1350
	SCoT10	ACAATGGCTACCACCAGC	14.0	14.0	0.0	0.0	100	0.75	10.5	13.0	240	1800
	SCoT12	CAACAATGGCTACCACCG	11.0	11.0	0.0	1.0	100	0.54	5.9	13.8	245	1215
Ave.			8.71	8.14	0.57	0.57	91.58	0.61	5.05	8.28		
	ISSR1	AGAGAGAGAGAGAGAGYC	7.0	4.0	3.0	1.0	57.1	0.34	0.77	4.4	120	780
	ISSR2	AGAGAGAGAGAGAGAGYG	7.0	6.0	1.0	3.0	85.7	.074	3.8	3.2	100	1000
	ISSR3	ACACACACACACACACYT	10.0	9.0	1.0	5.0	90	0.7	5.67	6.4	200	1300
ISSR	ISSR4	ACACACACACACACACYG	9.0	8.0	1.0	2.0	88.9	0.72	5.76	5.6	290	930
	ISSR6	CGCGATAGATAGATAGATA	8.0	6.0	2.0	1.0	75	0.60	2.7	5.0	330	1800
	ISSR11	ACACACACACACACACYA	6.0	4.0	2.0	3.0	66.7	0.58	1.5	2.0	320	1000
	ISSR12	ACACACACACACACACYC	4.0	3.0	1.0	1.0	75	0.38	0.85	3.6	310	495
Ave.			7.28	5.71	1.57	2.28	76.91	0.48	3.0	4.31		

TAF, total amplified fragments; PF, polymorphic fragments; MF, monomorphic fragments; UF, unique fragments; P%, percentage of polymorphism; PIC, polymorphism information content; MI, marker index; Rp, resolving power; and FS, fragment size.

reactions were performed in a 20 μl volume containing 10 μl master mix 2X PCR (ready-to-use PCR master mix 2X; Ampliqon), 6 μl double distilled water, 2 μl template DNA from each sample, and 1 μl of primers (10 pmol/μl). The amplifications were performed at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 s, primer annealing at 45°C for 30 s, and primer elongation at 72°C for 2 minutes, followed by a final extension at 72°C for 10 minutes using a Bio-Rad (T100) thermal cycler. The DNA was diluted to a concentration of 50 ng/l for the experiment.

2.4. Analyses Using SDS-PAGE. SDS-PAGE was used to determine the variability of total seed storage proteins [39]. The samples were prepared by dissolving the homogenized wheat in an equivalent volume of 2% SDS sampling buffer

containing 100 mM Tris-Cl (pH 6.8), 4% SDS, 0.2 percent bromophenol blue, 20% glycerol, and 14 M mercaptoethanol. After five minutes in a boiling water bath, the samples are centrifuged at 14,000 rpm for one minute and then placed on ice to cool. Then A stacking was prepared, and polyacrylamide gel was separated. Subsequently, SDS gel electrophoresis is performed in 1x Tris-glycine buffer at 100 V for 60 to 90 min. After running, the gel was incubated overnight in a Coomassie staining solution. Finally, the excess stain was removed for 3–4 hours at room temperature using destaining solution buffer, and the gel was prepared for imaging by placing it under white light.

2.5. Analyses and Visualization of Data Derived from Amplified Fragments. On 1.5 percent agarose gels, the PCR products were separated using electrophoresis. They were then stained with Safe View-IITM. Bands were visualized under UV light using gel documentation. The presence (1) or absence (0) of PCR products was determined visually using their gel patterns. DARwin was utilized to analyze the produced data matrices [22]. The preferred power of the primers was determined using three critical metrics: polymorphism information content (PIC), Rp, and MI. Consequently, PIC was determined using the formula $PIC = 1 - Spi^2$, where *pi* denotes the frequency of the locus's *i*-th allele [40]. Kumar et al. [41] developed a formula for calculating MI. The distance coefficient matrix for the three-marker data was computed using the Jaccard distance index, and to visualize the genetic relationships among the analyzed genotypes, a dendrogram based on the unweighted pair group method with arithmetic mean algorithm (UPGMA) was constructed using NTYSYS 2.02 [42].

3. Results and Discussion

The analysis of variance for the ten wheat genotypes under normal and water stress conditions for yield and its components is presented in Table 4. Mean squares of highly significant genotypes were detected for all the traits studied. Furthermore, the presence of significant differences between genotypes would suggest the presence of genotypic variance, which indicates the wide diversity between genotypes and water conditions. These results agree with the study results of Arab et al. [43].

3.1. Mean Performance. The mean performance of the ten wheat genotypes tested under normal irrigation and drought stress are presented in Table 5. Genotype 4 was the earlier genotype under normal irrigation and drought stress because of the early heading date. In contrast, genotype 4 had the latest heading date at normal irrigation and at drought stress. Genotype 1 had the lowest values for the number of days to maturity under normal irrigation and drought stress. However, genotype 2 had the highest value for the number of days to maturity. Genotype 2 demonstrated the shortest plants under normal irrigation and drought stress. In contrast, Nubaria 2 at normal irrigation and genotype 4 at drought stress showed the tallest plants. Genotypes 2 and 4 had the highest value for the number of spikes per m^2 under normal irrigation and drought stress.

On the contrary, genotype 1 had the lowest value for the number of spikes per m^2 under normal irrigation and drought stress. Genotype 2 at normal irrigation and drought stress had the highest 1000-kernel weight value, whereas genotype 1 at normal irrigation and genotype 6 at drought stress had the lowest 1000-kernel weight value. Genotype 2 had the highest value for the number of kernels/spike under normal irrigation and drought stress, whereas genotype 3 had the lowest value for the number of kernels per spike under normal irrigation and drought stress. The grain yield per plot under normal irrigation conditions ranged from 2.02 kg (genotype 1) to the maximum of 3.21 kg (genotype 2), followed by 3.06 kg/plot (genotype 4) and 2.97 kg/plot (Nubaria 2). However, the grain yield under stress treatment ranged from 1.08 kg/plot for genotype 1 to 2.19 kg/plot for genotype 4 with an average of 1.76 kg/plot, while the average grain yield under normal irrigation was 2.71 kg/plot with a total reduction of 0.91 kg/plot.

3.2. Drought Indices. Eleven indexes have been calculated to evaluate genotypes' drought tolerance, in addition to the mean of grain yield under normal conditions and grain yield under water stress, as depicted in Table 6. Based on the stress susceptibility index (SSI), genotypes 1 and 8 were classified as highly drought tolerant. The higher the value of SSI, the more significant the drought tolerance under stress, and the cultivars with greater SSI have higher drought sensitivity [44]. On the contrary, STI, MP, and GMP indexes were higher in genotypes 2 and 4. Genotypes with higher values of stress tolerance index (STI) are generally recognized as drought-tolerant genotypes [45]. The yield stability index (YSI) was more critical in discriminating drought-tolerant from susceptible genotypes. Greater YSI index values were observed in genotype 4. Genotypes with high YSI values were yielding high under stress and yielding low under nonstress conditions. Based on the sensitivity drought index (SDI), the four genotypes 1, 6, 8, and 3 revealed the highest values and were identified as tolerant under stress conditions. Based on the drought index (DI), the three genotypes 4, 2, and 10 displayed higher DI values than the other genotypes and were classified as drought-tolerant genotypes. Tolerance (TOL) of genotypes 2, 3, and 8 were highly sensitive. Genotype 4 was the most drought-tolerant genotype, according to the relative drought index (RDI). Genotype 2 had the highest stress susceptibility percentage index (SSPI), while genotypes 2, 3, and 8 had the lowest. Compared with the other genotypes, genotypes 2 and 3 had the highest harmonic mean (HM), indicating more stress tolerance mechanism. These findings are consistent with [46].

3.3. Correlation Coefficients. Correlation coefficients between YP, YS, and other quantitative drought tolerance markers were calculated (Table 7). Grain production has been found to be positively related to YS, STI, MP, GMP,

Table 4: Mean squares of the studied wheat genotypes traits combined over the two seasons of normal and water stress conditions.

	d.f.	Normal irrigation						
		DH	DM	PH	NS m^2	1000-KW	NK/S	GY/P
Year	1.00	14.45**	84.05**	31.25**	5956.55**	435.57**	1597.22**	0.13
Error (a)	6	2.16	2.82	1.88	60.92	0.50	4.00	0.04
Genotype	9.00	110.11**	152.89**	99.44**	110735.68**	20.88**	327.97**	0.99**
Genotype X year	9	16.01	17.69**	27.78	957.97	25.64	93.45	0.07*
Error (b)	54	3.49	8.47	13.19	722.48	3.33	27.94	0.11
Total	79	16.94	26.29	23.91	13405.08	13.09	87.33	0.20
		Water stress						
Year	1.00	211.25**	46.51**	11.25	189.11*	312.68**	1386.11**	0.06
Error (a)	6	8.14	1.11	4.38	16.24	1.94	0.62	0.02
Genotype	9.00	39.99**	148.43**	114.58**	2765.68**	18.94**	249.96**	0.93**
Genotype X year	9	18.31	16.82	27.92	454.45	9.68	56.22	0.06**
Error (b)	54	5.63	8.53	15.28	69.96	4.60	22.65	0.07
Total	79	13.16	25.25	26.82	417.06	10.36	67.91	0.16

*significant at $P < 0.05$, and **significant at $P < 0.01$.

YSI, DI, RDI, and HM, whereas SSI and SDI were negatively correlated with YP. YS had a significant positive association with the STI, MP, GMP, YSI, DI, RDI, and HM. However, the SSI and SDI had a significant negative correlation with YS. The SSI revealed a substantial negative connection with STI, MP, GMP, YSI, DI, RDI, and HM. The STI demonstrated a significant positive association with MP, GMP, YSI, DI, RDI, and HM, but the SDI had a significant negative correlation with the STI. A significant positive association was detected between MP and GMP, YSI, DI, RDI, and HM; nonetheless, there was a negative relationship between MP and SDI. A significant positive association was established between GMP and YSI, DI, RDI, and HM. The YSI was positively correlated with DI and RDI; and HM and SDI were positively correlated with TOL and SSPI. The DI was positively correlated with RDI and HM. TOL was positively correlated with SSPI, and the RDI was positively correlated with HM. As measured by MP, GMP, and STI, a positive and significant association between grain production under normal and stress conditions is adequate for determining genotypes' drought resistance [47]. Grain yield under stress (YS) was positively and strongly linked with STI and DI. Yield in the absence of stress (YP) was found to be significantly and favorably connected with YS, SSI, STI, SDI, and DI but negatively correlated with YSI [49]. Grain yield in stress conditions (YS) had a significant and strong positive correlation with the indices STI, GMP, and YSI and a significant negative correlation with the indices SSI, SDI, SSPI, and TOL [48].

3.4. Cluster Analysis. Cluster analysis has been used extensively to describe genetic diversity and clustering based on the similar characteristic. The studied genotypes were grouped into 4 clusters based on cluster analysis (Figure 1). The first cluster aggregated G1 with a low grain yield (1.55 kg/plot) and is sensitive to drought. The second cluster consisted of four genotypes G3, G6, G7, and G8 that recorded moderate grain yield (2.06 kg/plot) and were sensitive to drought. The third cluster consisting of G5, Giza 171, and Nubaria 2 had a moderate grain yield (2.42 kg/plot) and were moderate sensitive to drought. The fourth cluster consisted of G2 and G4 that recorded a high grain yield (2.06 kg/plot) and tolerance degree to drought. Table 8 presents a summary of ten genotypes based on grain yield under normal and drought conditions.

3.5. SCoT Results. In inbreeding projects that entail interspecific crossings or targeted gene transfer, studying the genetic diversity of wild wheat species is critical [49]. For example, various landraces or wild crops can have distinct characteristics and large geographic distributions [50]. Genetic diversity analyses among and within bread wheat's wild relatives can be practical measures before examining their resilience to biotic and abiotic stress. Two marker systems were employed in this work to investigate the genetic diversity of Egyptian wheat. The results of the molecular genetic variations in genomic DNA among ten wheat cultivars are presented in Figure 2 and Table 3; the analysis of seven SCoT primers, 61 SCoT bands, was amplified. The generated bands ranged in size from 200 to 1800 bp. SCoT10 and SCoT1 primers produced the most (14) and fewest (5) amplified bands, respectively, with an 8.7 bands/primer average. The polymorphic bands ranged from 4 for SCoT1 to 14 for SCoT10, with a median of 7 for each primer.

Meanwhile, the primers SCoT3, SCoT5, SCoT10, and SCoT12 revealed the highest percentage of polymorphism (100 percent) (Table 3). There were four monomorphic bands in total, with an average of 5 monomorphic fragments per primer. The present investigation detected a significant level of polymorphism among the genotypes, with polymorphism percentages of 93.4 for SCoT markers. SCoT and ISSR markers were found to help examine wheat genetic diversity [51]. Additionally, the polymorphism percentage of the studied SCoT marker in this study was higher than that of detected by Abdein et al. [52], who reported that the highest polymorphic rate was 80% for primer SCoT9 and the lowest was 11.11% for primer SCoT11. Moreover, Shahlaei et al. [53] reported that 10 SCoT primers generated 83 bands, of which 30 (36.14%) were polymorphic.

Table 5: Mean values of studied traits for ten wheat genotypes evaluated under normal irrigation and drought stress combined over the two wheat-growing seasons of 2019/2020 and 2020/2021.

	Days to heading		Days to maturity		Plant height		Number of spikes per m^2		1000-Kernels weight (g)		Number of kernels per spike		Grain yield/plot (kg)		Reduction (%)
	Normal	Drought	Normal	Drought	Normal	Drought	Normal	drought	Normal	Drought	Normal	Drought	Normal	Drought	
G1	97.13	91.63	139.50	135.38	108.13	100.00	352.00	337.25	49.09	42.95	74.25	57.50	2.02	1.08	46.53
G2	104.25	92.88	153.25	148.75	102.50	97.50	419.88	402.88	51.94	46.53	93.13	65.75	3.21	2.13	33.64
G3	94.25	88.38	142.88	138.63	110.00	103.13	370.25	362.13	47.10	42.16	76.61	54.88	2.63	1.64	37.64
G4	99.88	93.25	152.88	148.63	114.38	108.75	412.75	393.25	50.11	46.30	91.75	67.50	3.06	2.19	28.43
G5	102.75	90.88	149.25	145.00	108.13	102.50	389.63	374.00	49.91	42.90	85.75	64.50	2.83	1.91	32.51
G6	94.75	89.63	142.75	138.50	112.50	107.50	366.13	359.88	50.60	45.15	81.58	51.88	2.42	1.47	39.26
G7	94.50	87.88	145.75	141.50	109.38	104.38	370.63	365.88	47.95	42.62	81.75	54.38	2.54	1.71	32.68
G8	93.38	86.88	145.75	141.50	108.75	101.88	365.38	374.50	47.81	44.06	80.00	52.88	2.54	1.55	38.98
Giza171	96.63	91.25	146.75	142.50	108.13	98.75	393.75	380.88	47.00	44.02	78.88	56.25	2.88	1.91	33.68
Nubaria 2	97.25	92.63	147.50	143.25	114.38	106.88	397.88	384.25	49.43	44.98	89.00	58.38	2.97	2.01	32.32
Mean	97.48	90.53	146.63	142.36	109.63	103.13	348.93	373.49	49.09	44.17	83.27	58.39	2.71	1.76	35.06
LSD 0.05%	2.65	3.36	4.13	4.14	5.15	5.54	38.02	11.86	2.59	3.04	7.49	6.75	0.47	0.37	
LSD 0.01%	3.53	4.48	5.50	5.51	6.86	7.38	50.56	15.79	3.44	4.05	9.98	8.99	0.63	0.49	
Minimum	93.38	86.88	139.50	135.38	102.50	97.50	352.00	337.25	47.00	42.16	74.25	51.88	2.02	1.08	
Maximum	104.25	93.25	153.25	148.75	114.38	108.75	419.88	402.88	51.94	46.53	93.13	67.50	3.21	2.19	

Table 6: Estimated sensitivity rate of the ten wheat genotypes by different drought tolerance indices under normal and stress conditions.

	YP	YS	SSI	STI	MP	GMP	YSI	SDI	DI	TOL	RDI	SSPI	HM
G1	2.02	1.08	1.40	0.30	1.55	1.48	0.54	1.33	0.61	0.94	0.82	17.30	1.41
G2	3.21	2.13	1.01	0.93	2.67	2.62	0.66	0.96	1.21	1.08	1.02	19.96	2.56
G3	2.63	1.64	1.13	0.59	2.13	2.07	0.62	1.07	0.93	0.99	0.96	18.23	2.02
G4	3.06	2.19	0.85	0.92	2.63	2.59	0.72	0.81	1.25	0.87	1.10	16.03	2.56
G5	2.83	1.91	0.98	0.74	2.37	2.33	0.67	0.93	1.09	0.92	1.04	17.00	2.28
G6	2.42	1.47	1.18	0.48	1.94	1.88	0.61	1.12	0.83	0.95	0.93	17.53	1.83
G7	2.54	1.71	0.99	0.59	2.12	2.08	0.67	0.94	0.97	0.83	1.03	15.34	2.04
G8	2.54	1.55	1.17	0.54	2.04	1.98	0.61	1.11	0.88	0.99	0.94	18.23	1.92
Giza171	2.88	1.91	1.01	0.75	2.39	2.34	0.66	0.96	1.08	0.97	1.02	17.88	2.29
Nubaria 2	2.97	2.01	0.97	0.81	2.49	2.44	0.68	0.92	1.14	0.96	1.04	17.65	2.40

Grain yield under normal condition (YP), grain yield under water stress (YS), stress susceptibility index (SSI), stress tolerance index (STI), mean productivity (MP), geometric mean productivity (GMP), yield stability index (YSI), sensitivity drought index (SDI), drought index (DI), tolerance (TOL), relative drought index (RDI), stress susceptibility percentage (SSPI), and harmonic mean (HM).

Table 7: Correlation between drought tolerance indices with grain yield under normal irrigation and drought stress conditions.

	YP	YS	SSI	STI	MP	GMP	YSI	SDI	DI	TOL	RDI	SSPI
YS	0.98**											
SSI	−0.86**	−0.94**										
STI	0.99**	0.99**	−0.90**									
MP	1.00**	1.00**	−0.91**	1.00**								
GMP	0.99**	1.00**	−0.91**	1.00**	1.00**							
YSI	0.85**	0.93**	−1.00**	0.89**	0.90**	0.90**						
SDI	−0.86**	−0.94**	1.00**	−0.89**	−0.91**	−0.91**	−1.00**					
DI	0.98**	1.00**	−0.94**	0.99**	0.99**	1.00**	0.94**	−0.94**				
TOL	0.26	0.07	0.24	0.16	0.17	0.15	−0.27	0.24	0.06			
RDI	0.87**	0.94**	−1.00**	0.90**	0.91**	0.91**	1.00**	−1.00**	0.94**	−0.24		
SSPI	0.27	0.08	0.23	0.16	0.17	0.16	−0.27	0.23	0.07	1.00**	−0.23	
HM	0.99**	1.00**	−0.92**	1.00**	1.00**	1.00**	0.91**	−0.92**	1.00**	0.13	0.92**	0.13

*, **P <0.05> *significant at $P < 0.05$, and **significant at $P < 0.01$. Grain yield under normal condition (YP), grain yield under water stress (YS), stress susceptibility index (SSI), stress tolerance index (STI), mean productivity (MP), geometric mean productivity (GMP), yield stability index (YSI), sensitivity drought index (SDI), drought index (DI), tolerance (TOL), relative drought index (RDI), stress susceptibility percentage (SSPI), and harmonic mean (HM).

All primers demonstrated an average PIC, MI, and Rp values of 0.61, 5.05, and 8.28, respectively (Table 3). SCoT3 revealed the maximum PIC value (0.87), while SCoT4 demonstrated a minimum PIC value (0.21). Furthermore, the highest MI value (10.5) was revealed by SCoT10, whereas SCoT4 had the lowest MI value (0.87). In contrast, the Rp of the primers ranged from 4 (SCoT1 and SCoT3) to 13.8 (SCoT1, SCoT3, and SCoT12). SCoT primers had a greater average PIC value than the other marker systems (Table 3). According to Botstein et al. [54], primers with a PIC value of 0.25 to 0.50 provide important information for genetic diversity research. In contrast to our findings, Heikrujam et al. [55] revealed that CBDP markers are more successful than SCoT markers in terms of PIC value when investigating genetic diversity among male and female jojoba genotypes. Furthermore, other informative indices such as Rp and MI demonstrated a strong endorsement of the discriminating potential of these markers. The more significant MI and Rp values in SCoT12, SCoT10, and SCoT5 primers indicated that these primers had a higher resolution and potency than other SCoT primers, which may be relevant in future investigations on wheat species.

Three distinct positive bands were detected in genotype 4 at 1500 bp using the SCoT1 primer, 1200 bp using the SCoT10 primer, and 310 bp using the SCoT 12 primer, while primer SCoT5 detected one unique positive marker at 1090 bp in genotype 6.

3.6. ISSR Results. ISSR primers can be used to target microsatellites found throughout the plant genome. Therefore, the markers have been found to be more repeatable than others, such as RAPD [56]. The results of the genetic relationship study between ten wheat cultivars using seven ISSR primers are depicted (Figure 2 and Table 3). The bands formed ranged in size from 100 to 1800 bp. There were 51 bands in total, with the number of bands per primer ranging from 4 in ISSR12 to 10 in ISSR3 and an average of 7 bands per primer. There were also 40 polymorphic bands with a median of 6 polymorphic amplicons/primer and 11 monomorphic bands with a median of 2 monomorphic fragments/primer, and 40 monomorphic bands with a median of 2 monomorphic fragments/primer. The ISSR3 primer generated the most polymorphic bands, while the ISSR4 and ISSR2 primers revealed the highest polymorphism percentage (88 and 85 percent, respectively). Similarly, Emel [57] observed a similar *P%* to that reported here (76.07%). Also, Carvalho et al. [58] found a *P%* of 98.5 in 99 wheat accessions when using 18 ISSR primers. According to Tok et al. [73], the

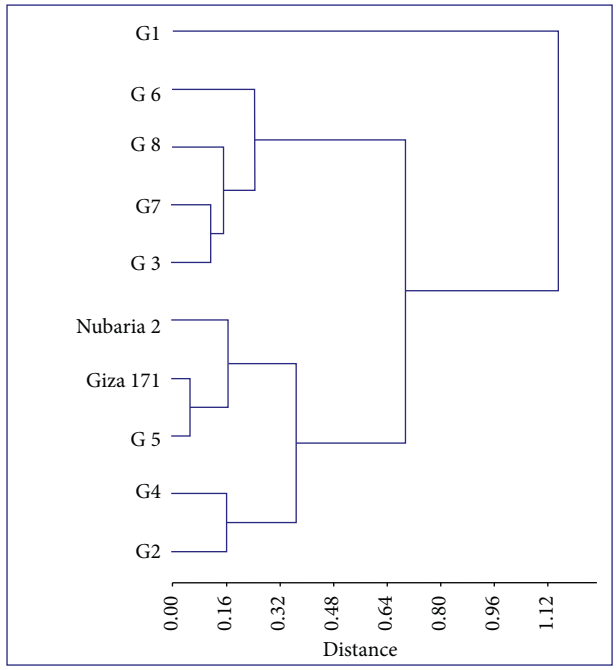

Figure 1: Correlation between drought tolerance indices with grain yield under normal irrigation and drought stress conditions.

Table 8: Summary of hierarchical cluster analysis represents the classification of tested ten wheat genotypes based on grain yield under normal and stress tolerance indices.

Cluster no.	Genotypes	Grain yield		Average grain yield	Grain yield category	Stress tolerance degree
		Normal	Drought			
1	G1	**2.02**	**1.08**	**1.55**	Low	Sensitive
	G3	**2.63**	**1.64**			
	G6	**2.42**	**1.47**			
2	G7	**2.54**	**1.71**	**2.06**	Moderate	Sensitive
	G8	**2.54**	**1.55**			
	Mean	**2.53**	**1.59**			
3	G5	**2.83**	**1.91**			
	Giza 171	**2.88**	**1.91**	**2.42**	Moderate	Moderate
	Nubaria 2	**2.97**	**2.01**			
	Mean	**2.89**	**1.94**			
4	G2	**3.21**	**2.13**			
	G4	**3.06**	**2.19**	**2.65**	High	Tolerant
	Mean	**3.14**	**2.16**			

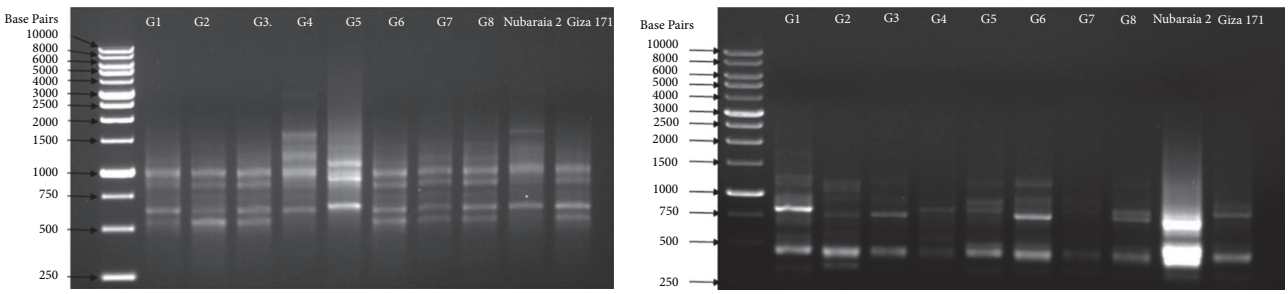

Figure 2: Electrophoretic profile of PCR products using (a) SCoT2 primer and (b) ISSR6 primer for the ten wheat genotypes.

Table 9: Molecular weight analysis of the 10 wheat varieties obtained by SDS-PAGE.

Bands no.	Protein wheat varieties type	Marker	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	Polymorphism
1		110	1	1	1	0	1	1	1	1	1	1	P M
2	HMW-GS	105	0	0	1	0	1	1	0	0	1	0	P M
3		98	0	0	0	0	0	1	1	0	0	1	P M
4		82	1	1	1	1	1	1	1	1	1	1	M M
5		75	1	0	0	0	1	0	1	0	0	1	P M
6		68	1	0	0	1	1	1	0	0	0	0	P M
7		60	1	1	1	1	1	1	1	1	1	1	M M
8		50	0	1	1	0	1	1	1	1	1	1	P M
9		45	1	1	1	1	1	1	1	1	1	1	M M
10		40	0	0	0	0	0	0	0	1	0	1	P M
11		35	1	1	1	1	1	1	1	1	1	1	M M
12	LMW-GS	33	0	1	1	1	1	1	1	1	1	1	P M
13		31	0	0	0	0	1	0	0	0	0	0	Uni
14		30	1	1	1	1	1	1	1	1	1	1	M M
15		28	1	1	1	0	1	1	1	1	1	1	P M
16		27	0	0	0	0	0	0	0	0	0	1	Uni
17		26	0	0	1	0	0	1	1	0	1	0	P M
18		25	0	0	0	0	0	0	0	0	0	1	Uni
19		24	0	0	1	0	1	1	0	0	0	0	P M
20		23	1	1	1	0	1	1	1	1	1	1	P M
21		22	1	1	1	0	1	1	1	1	1	0	P M
Total		21	11	11	14	7	16	16	14	12	13	15	

maximum percentage of polymorphic loci among wheat genotypes was just 17.59%.

Many genetic diversity studies have used the polymorphism information content (PIC) index [59, 60]. DNA markers can be used for gene mapping, molecular breeding, and germplasm evaluation based on their PIC value [61].

In this study, the PIC for each primer pair ranged from 0.38 ISSR12 to 0.74 ISSR2, with a mean of 0.62. The lowest and greatest MI values of 0.77 and 5.76, respectively, were found in ISSR1 and ISSR4 primers. The Rp of the primers ranged from 2 ISSR11 primers to 6.4 ISSR3 primers, with a mean of 4.31 (Table 3). Etminan et al. [25] detected genetic diversity in durum wheat genotypes using ISSR and SCoT marker systems. According to Etminan et al. and Khodaee et al. [25, 62], Rp and MI were the most relevant indices for measuring marker efficiency, although the ISSR had a higher resolution than SCoT markers, contradicting our findings. Additionally, the current study's findings verified the utility of these markers for detecting wheat and its wild cousin genotypes' genetic diversity [63, 64].

In addition, ISSR primers yielded many distinct positive fragments in different cultivars; for instance, primer ISSR3 yielded five distinct positive bands in genotype 4 at 1100, 850,730, 600, and 410 bp. ISSR displayed the highest number of significant specific markers, followed by SCoT markers. In the same context, 22 RAPD-specific markers were detected by Hassan et al. [65], whereas Abdein et al. [52] reported 24 unique markers after SCoT and ISSR analysis.

3.7. Phylogenetic Relationship Based on Amplified SCoT Fragments. A similarity coefficient was calculated by comparing the SCoT profiles pairwise using the shared amplification products. The genetic similarity of the ten wheat cultivars ranged from 0.30 to 0.90. The cultivars (G1 and Nubaria 2) and (G2 and G7) were found to have the greatest genetic similarity (0.87), indicating a low genetic diversity in the species. However, the lowest similarity value (0.30) was observed among (G2 and Giza 171), reflecting a wider genetic diversity between them.

The dendrogram was constructed based on the similarity matrices using UPGMA. The ten wheat cultivars were categorized into two major groups (Figure 3(b)). The first group was also divided into two subgroups; the first group included (G6 and G5); while the second group contained 4 cultivars (G2, G7, G3, and G8). The second group was divided into two subgroups; subgroup I consisted of 2 closely related cultivars (Giza171 and G4), while subgroup II involved Nubaria 2 and G1.

In ISSR, a similarity coefficient was generated by pairwise comparisons of ISSR profiles based on shared amplification products. The genetic closeness of ten wheat cultivars ranged from 0.57 to 0.90. The maximum genetic closeness (0.90) was found between wheat cultivars (2 and 3), indicating a limited genetic diversity. However, the lowest similarity value (0.57) was observed between 4 and 9, reflecting a more significant genetic diversity.

The phylogenetic tree (Figure 3(a)) delineated the ten wheat cultivars into two main clusters in agreement with two main subgroups. The first central cluster included only one cultivar G4. The second main cluster is divided into two subgroups; the first one contains the Giza171 cultivar, while the other contains the rest eight cultivars.

The two markers yielded promising results and grouping in the current investigation, owing to each marker's ability to recreate distinct sections of the genome [66]. Consequently, these markers provide more detailed and diversified information regarding the genetic diversity of Egyptian wheat accessions and within them [67]. There have been instances

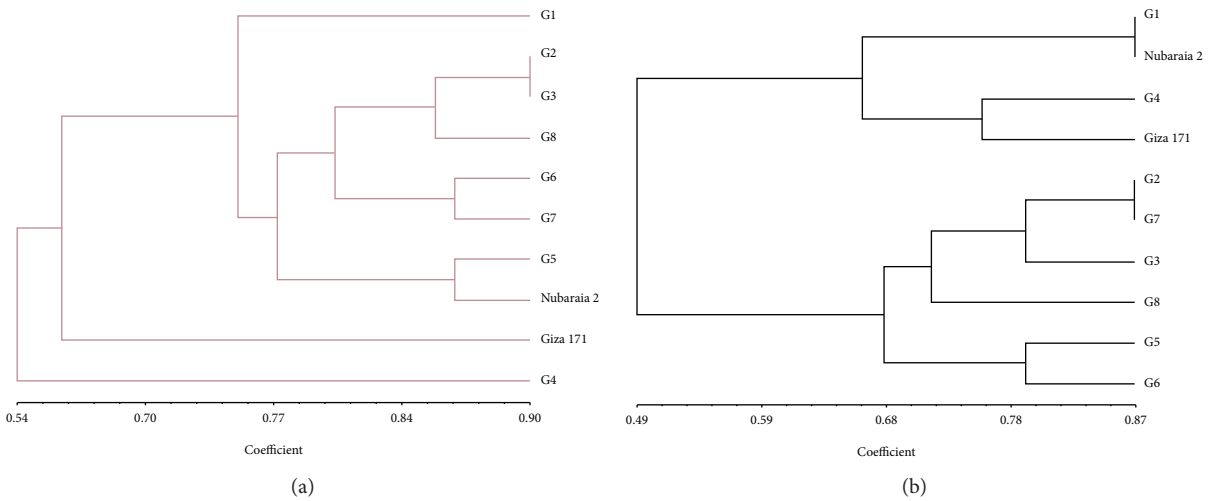

Figure 3: Dendrogram of the 10 wheat genotypes using the UPGMA method based on ISSR (a) and SCoT (b).

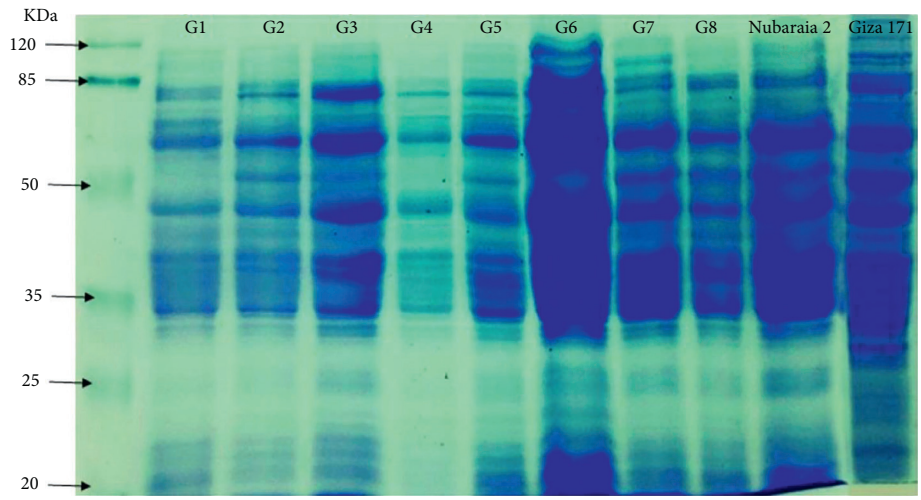

Figure 4: SDS-PAGE profile of the ten wheat genotypes.

of mismatching results between dendrograms generated by various markers in different plants, such as in snake melon [68], sponge gourd [69], and bamboos [70].

3.8. Electrophoretic Patterns of Total Soluble Proteins. Assessment of the application of protein profiling by SDS-PAGE for cultivar identification in the wheat collection showed different banding patterns among the different cultivars. Based on the relative mobility of proteins on the gel, many alterations in protein patterns were observed in wheat leaves; the electrophoretically resolved proteins into multiple 21 detected bands varied from 7 to 16 between different cultivars with different molecular weights ranging from 22 KD to 110 KDa, which were not necessarily being present in all cultivars (Figure 4 and Table 9). Sixteen bands were polymorphic with 76.2%, and five were common bands (monomorphic) with 23.8% monomorphism. The comparison with standard markers reveals that wheat genotype 4 contains three subunits in the range of 60–110 KDa (HMW-GS), which it shares with genotype 2 and genotype 8, while another genotype represents 5 and 6 subunits. Furthermore, genotype 4 has the least number of subunits (4 bands) compared with other genotypes, owing to its low molecular weight of 10–50 kDa (Table 9). Gene silencing occurs in some types that code for these proteins, resulting in diverse subunits of high-molecular-weight proteins [71]. It is challenging to distinguish low-molecular-weight glutenin subunits (LMW-GS) from monomeric gliadin storage proteins, utilizing total protein extracts by SDS-PAGE [72].

At a 0.95 coefficient level, genetic similarity coefficients classified the ten genotypes into two groupings (Figure 5). In contrast to the SCoT study, the clustering of genotypes was comparable to that found by ISSR, although it was quite distinct. Generic genotype 4 (cluster I) was found to have the lowest similarity index among the other nine genotypes (cluster II) based on a UPGMA dendrogram (Figure 5). The

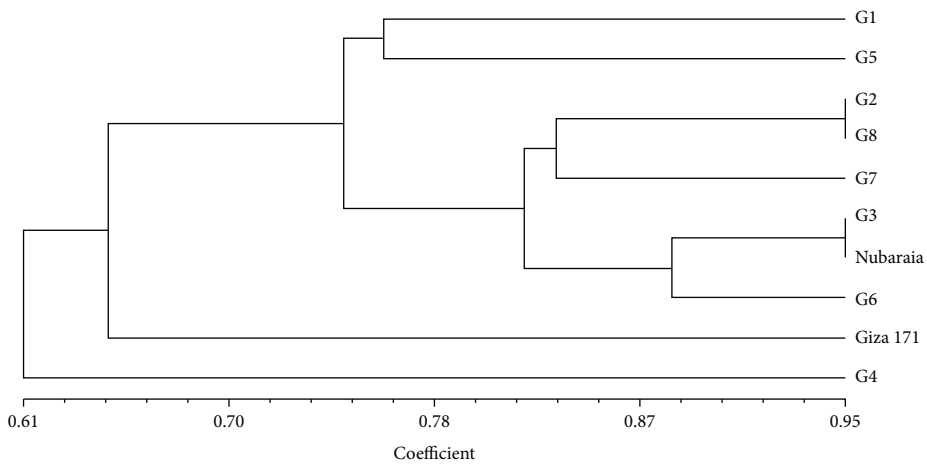

Figure 5: Cluster analyses of the 10 wheat genotypes based on SDS-PAGE analyses.

coefficients of similarity ranged from 0.52 to 0.95 (Figure 5). Using SDS-PAGE data, we could identify the cultivars tested by comparing the amount and quality of protein bands to each other.

Genotype 4 was revealed to cope with water scarcity in terms of morphological findings. Following further investigation, we were able to identify distinct molecular and biochemical bands linked to its drought-resistant abilities.

4. Conclusions

Using agronomic data, ISSR, SCoT markers, and SDS-PAGE, ten wheat genotypes were evaluated for genetic diversity and the identification of specific molecular markers under normal and drought conditions. Drought-resistant genotypes 2 and 4 demonstrated significant agronomic characteristics and a low drought susceptibility index. After extensive research, we were able to construct separate molecular and biochemical bands that might be related to Genotype 4's drought-resistance qualities using the distinct bands of these markers and cluster analysis of the data, as well as morphological features. Two markers, ISSR and SCoT, were demonstrated to detect variation among the genotypes tested effectively. More research on genotype 4 is needed, particularly in the area of DNA sequencing, in order to identify drought-resistant genes.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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