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Genetic Characterization and Agronomic Evaluation of Drought Tolerance in Ten Egyptian Wheat (*Triticum aestivum* L.) Cultivars

Mohamed A. Emam [1], Amal M. Abd EL-Mageed [2], Gniewko Niedbała [3], Samah A. Sabrey [2], Ahmed S. Fouad [4,*], Tarek Kapiel [4], Magdalena Piekutowska [5] and Soad A. Mahmoud [1]

1 Agronomy Department, Faculty of Agriculture, Suez Canal University, Ismailia 41522, Egypt; m_abdelgawad55@yahoo.com (M.A.E.); soad_atta@yahoo.com (S.A.M.)
2 Department of Agricultural Botany, Faculty of Agriculture, Suez Canal University, Ismailia 41522, Egypt; amal3aaa50@yahoo.com (A.M.A.E.-M.); samahysabry12@gmail.com (S.A.S.)
3 Department of Biosystems Engineering, Faculty of Environmental and Mechanical Engineering, Poznań University of Life Sciences, 60-637 Poznan, Poland; gniewko.niedbala@up.poznan.pl
4 Botany and Microbiology Department, Faculty of Science, Cairo University, Giza 12619, Egypt; tkapiel@sci.cu.edu.eg
5 Department of Geoecology and Geoinformation, Institute of Biology and Earth Sciences, Pomeranian University in Słupsk, Partyzantów 27, 76-200 Słupsk, Poland; magdalena.piekutowska@apsl.edu.pl
* Correspondence: ahmedsfouad@sci.cu.edu.eg

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Abstract: This investigation was carried out for genetic characterization and determination of drought tolerance of ten Egyptian cultivars of wheat (*Triticum aestivum* L.), namely Misr 1, Misr 2, Gemmiza 9, Gemmiza 10, Gemmiza 11, Gemmiza 12, Shandawel 1, Giza 168, Giza 171, and Sids 14. These cultivars were grown in two winter seasons: 2018/2019 and 2019/2020 at the experimental farm Fac. of Agric., Suez Canal Univ., Ismailia, Egypt, under two watering regimes: normal (100%) and stress (50% FC) conditions. Six agronomic traits and five tolerance indices, namely stress tolerance (TOL), mean productivity (MP), geometric mean productivity (GMP), yield stability index (YSI), and drought susceptibility index (DSI), were used to evaluate the impact of drought stress. The results reflected Giza 171, Misr 2, and Giza 168 as precious germplasm for breeding of high-yielding drought-tolerant wheat. A highly significant positive correlation was recorded between yield under normal and stress conditions on the one hand and each of MP and GMP on the other hand. In addition, YSI appeared engaged in a highly significant positive correlation with yield under drought conditions only. TOL and DSI appeared insignificantly correlated with yield. Therefore, MP and GMP were reflected as the first runners among indices suitable to distinguish the high-yielding cultivars under drought conditions. At the molecular level, five primers of Start Codon Targeted (SCoT) markers were able to resolve and characterize the studied cultivars, which reflected SCoT as a potent gene-targeting molecular marker, able to characterize and resolve genetic diversity in wheat at the cultivar level using few primers. Therefore, SCoT is a time-efficient molecular marker, and it can efficiently replace indices in characterization of drought-tolerant genotypes with a high confidence level and reasonable cost.

Keywords: drought; Egyptian wheat cultivars; selection indices; chlorophyll content; yield and yield component; SCoT markers

1. Introduction

Wheat (*Triticum aestivum* L.) is a major cereal crop used in the daily human diet as a main source of carbohydrates and proteins. In addition, the grains provide trace amounts of fats, dietary fibers, minerals, and B-group vitamins [1]. Based on the global annual production of 760 million tons harvested from 219 million hectares (http://faostat.fao.org/, accessed on 27 February 2022), it provides about one-fifth of the food calories and protein for about 4.5 billion people in 94 countries [2].

Water is the amplest constituent of the plant body, comprising about 90% of fresh weight, and performs a crucial role in virtually all vital processes [3,4]. Plants experience drought when their water requirements cannot be satisfied [5]. Thus, plant yield is strongly inhibited by water scarcity, which may finally lead to famines. In addition, global warming has enhanced heat-related drought [6,7]. Between 2005 and 2015, drought was responsible for 30% of crop damage in developing countries of Africa, Asia, Latin America, and the Pacific Islands, which approximated about USD 30 billion in loss [8].

Drought stress negatively influences the morphophysiological traits in wheat crops, including shoot height, relative water content, photosynthetic area, chlorophyll content, stomatal oscillation [9,10], and finally, yield traits, including grain yield, thousand kernel weight, spike length, and the number of grains per spike [11]. Drought severely hinders wheat productivity worldwide; it is estimated that approximately half of the land area cultivated annually with wheat is regularly affected by drought [12]. Under drought conditions, wheat production can be discouraged by 50–90% [13].

Breeding for drought tolerance is based on genetic resources, including wild relatives and landraces that constitute a natural reservoir of undiscovered beneficial alleles. Having about 800,000 accessions, maintained in gene banks [11], the characterization of tolerant genotypes necessitates the development of time- and cost-efficient markers. Physiological criteria such as relative water content, water use efficiency, turgor pressure, chlorophyll content, photosynthesis, intercellular CO_2 concentration, and stomatal conductance of wheat foliages are monitored under normal and stress conditions [14]. Several tolerance indices based on grain yield under stress and normal conditions have been established and utilized to evaluate drought tolerance. Of these indices, we can mention tolerance index (TOL), mean productivity (MP) [15], geometric mean productivity (GMP) [16], drought susceptibility index (DSI) [17], and yield stability index (YSI) [18]. However, these indices are time-consuming, being calculated at the yielding stage, in addition to suffering from sensitivity to environmental conditions.

Being insensitive to environmental effects and easy to perform at any stage of the life cycle, molecular markers are the recommended tool to characterize drought-tolerant accessions [19]. Several random molecular markers have been applied to distinguish drought-tolerant genotypes in wheat, including RAPD and ISSR [20] and AFLP [21], which do not crave preceding knowledge of the genome sequence and highlight high levels of polymorphism. Depending on high-throughput analyses and mining of the sequenced genomes, DNA markers such as SSR [22] and SNP [23] have been developed and utilized to characterize drought tolerance. However, drought tolerance is a complex quantitative trait controlled by several genes that hinders the establishment of related molecular markers, especially in the large genome of wheat [24,25]. Thus, it is recommended to use functional molecular markers rather than random markers that can be positioned away from the considered gene(s) [26,27].

Based on the opportunity of enormous genomic databases, improvements in recent marker systems placed inside or in the vicinity of the gene or regulatory elements have become more candid [28]. Start codon targeted polymorphism (SCoT) is a powerful gene-targeting marker system in plants that depends on the conserved regions flanking the start codon (ATG) of genes [29]. It is highly reproducible and possesses high genetic resolving potential, being dependent on primers having a relatively high temperature for annealing. SCoT was used successfully to characterize genetic diversity in wheat [30,31]. It was proved to be a potent tool for drought characterization in tomatoes [32].

Therefore, the present investigation aimed to evaluate drought tolerance of ten Egyptian wheat cultivars using agronomic traits and drought tolerance indices. SCoT will be utilized to characterize drought-tolerant cultivars, which facilitates the future breeding of wheat.

2. Materials and Methods

2.1. Plant Material and Experimental Design

Field experiments were conducted at the experimental farm, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt (30°35′ N, 032°16′ E, elevation 13 m), in the 2018–2019 and 2019–2020 winter wheat growing seasons. For both years, the soil at the study site was sandy (96.65% sand, 2.51% silt, and 0.84% clay), with an average pH of 7.45 and electrical conductivity (EC) of 3.91 ds·m^{-2}. The average mineral composition of the soil (%) was 5.2 Ca, 0.72 K, 3.9 Mg,15 Cl, 8.10 SO_4, and 19.6 Na. The research site's weather conditions were semi-arid with winter rains (rainfall of 10 mm, average day/night temperature of 19/10 °C, and relative humidity of 55%). The most common Egyptian wheat cultivars (*Triticum aestivum* L.), namely Maser 1, Maser 2, Gemmiza 9, Gemmiza 10, Gemmiza 11, Gemmiza 12, Shandawell 1, Giza 171 Giza 168, and Sids 14, developed by Field Crops Research Institute (FCRI), Agriculture Research Center (ARC), Giza, Egypt, were used in the present study. The experimental design was a randomized complete block design with three replications. Plots were 3 m × 1.2 m in size, consisting of four rows with 0.3 m spacing. Based on the 100% field capacity of 261 L/m^2, two water regimes were applied using river Nile water: normal (100% Fc) and drought stress (50% Fc). Agronomic practices were conducted when needed, following the regional practices.

2.2. Measurements

At the flowering stage, leaf chlorophyll content was quantified with the aid of a SPAD meter (SPAD-502 chlorophyll meter (Minolta Co., Ltd., Osaka, Japan)) according to Zhu et al. [33]. The heading date for each cultivar was recorded under normal and drought conditions. Plant height (cm), grain yield per plant (gm), and 1000-kernel weight (gm) were recorded at the end of the experiment.

2.3. Tolerance Indices

Tolerance indices were calculated based on grain yield under normal (100% FC) and stress (50% FC) conditions as follows:

Relative Decrease Percent (RD%) = $[(Y_{pi} - Y_{si})/Y_{pi}] \times 100$

Mean Productivity (MP) = $(Y_{pi} + Y_{si})/2$ [34]

Geometric Mean Productivity (GMP) = $(Y_{pi} \times Y_{si})$ [16]

Drought Susceptibility Index (DSI) = $[1 - (Y_{si} - Y_{pi})]/SI$ [17]

Tolerance Index (TOL) = $Y_{pi} - Y_{si}$ [34]

Yield Stability Index (YSI) = Y_{si}/Y_{pi} [18]

where Y_{si}, is the yield of cultivar in stress conditions; Y_{pi} is the yield of cultivar in normal conditions; SI is stress intensity, where: SI = $1 - (Y_s/Y_p)$; Y_s is total yield mean in stress conditions; and Y_p, the total yield mean in normal conditions.

2.4. DNA Extraction and SCoT-PCR

At the tillering stage, DNA was extracted from fresh young leaves using the DNeasy Plant Mini Kit (QIAGEN, Santa Clarita, CA, USA), following the manufacturer's protocol in the Central Lab, Department of Agricultural Botany, Faculty of Agriculture, Suez Canal University, Egypt. Before PCR amplification, samples of DNA were diluted to a final concentration of 40 ng/µL. Based on our experience with SCoT markers in Egyptian wheat, five primers were selected for our study. Sequences of SCoT primers were presented in Table 1 and designed by Collard and Mackill [29]. PCR amplification was performed in a 20 µL reaction containing 1× PCR buffer, 50 ng sample DNA, 1.5 units of Taq DNA polymerase, 2.5 µM primer, 3 mM $MgCl_2$, and 200 µM of each dNTP. Amplification reactions were carried out as follows: initial denaturation step at 94 °C for 3 min, followed by 35 cycles (denaturation at 93 °C for 1 min, annealing at 48 °C for 1 min, and extension at 72 °C for 2 min), followed by a final extension step at 72 °C for 10 min. PCR products were resolved in 1.2% agarose gels and stained with ethidium bromide before being visualized using the Gel Doc XR + Gel Documentation System (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Table 1. SCoT primer codes and sequences.

Primer Name	Primer Sequence (5′→3′)
SCoT 1	ACG ACA TGG CGA CCA CGC
SCoT 3	ACG ACA TGG CGA CCC ACA
SCoT 4	ACC ATG GCT ACC ACC GCA
SCoT 5	CAA TGG CTA CCA CTA GCG
SCoT 8	CAA TGG CTA CCA CTA CAG

2.5. Data Analysis of DNA

DNA banding patterns generated from the SCoT-PCR reaction were analyzed by the Gel Analyzer 3 program, where bands were scored as (1) for the presence or (0) for absence. Positive or negative unique bands were identified for the specific cultivar. For each primer, the percentage of polymorphism was calculated by dividing the number of polymorphic bands by the total number of scored bands. The similarity between pairs of genotypes was calculated using Jaccard's coefficient [35], and a UPGMA-based dendrogram was constructed to illustrate genetic relationships among the cultivars.

2.6. Statistical Analysis

The studied cultivars were planted in a factorial block design experiment with three replications. According to Steel et al. [36], the least significant difference (LSD) and analysis of variance were used to evaluate the response of each character within treatments in both seasons. According to Hallauer and Miranda [37], the correlation was calculated among grain yield and studied characteristics.

3. Results

3.1. Growth and Yield

Results of the current investigation indicated a significant difference in heading date among the studied cultivars (Table 2). Three categories can be recognized based on mean values under normal and stress conditions. The first included Misr 1 and Misr 2, with the highest mean values for heading date observed under normal and drought stress conditions. The second category contained Gemmiza 9 and Shandaweel 1, with intermediate mean values for heading date originating from intermediate values recorded during stressful and stress-free conditions. Based on the smallest mean values for heading date ranging from 82.17 to 84.33 days, the third category included Gemmiza 10, Gemmiza 11, Gemmiza 12, Giza 168, Giza 171, and Sids 14. In addition, our results reflected a significant decrease in heading date as a common response to drought stress, ranging from 1 day (1.2%) observed in Gemmiza 11 to 8 days (8.7%) recorded in Misr 1, compared with corresponding plants growing under normal conditions.

Based on data recorded for plant height, the cultivars Misr 1, Misr 2, Giza 171, and Sids 14 have the highest mean values for plant height, ranging from 92 to 96 cm, which was more dependent on the values recorded under drought conditions (Table 2). On the other hand, Gimmeza 10 and Gemmiza 12 cultivars have the shortest plants under both conditions, which were summarized in mean values of 82.17 and 84.67 cm measured for plant height in Gemmiza 12 and Gimmeza 10, respectively. Drought stress negatively influenced plant height in all cultivars, which was least pronounced in Seds 14, exhibiting about a 20.6% decrease in response to water deficit. The highest drought-induced reduction in plant height was about 40.6% observed in Gemmiza 9.

Table 2. Mean values of heading date and plant height traits for ten wheat cultivars under normal and drought conditions. Different letters are significantly different, according to the LSD test, at $p < 0.05$.

Varieties	Character					
	Heading Date (Day)		Mean	Plant Height (cm)		Mean
	Normal	Stress		Normal	Stress	
Misr 1	92.33	84.33	88.33 ^{b}	110.67	81.33	96.00 ^{a}
Misr 2	95.33	88.00	91.67 ^{a}	112.33	75.33	93.83 ^{ab}
Gemmiza 9	89.33	83.33	86.33 ^{c}	112.33	66.67	89.50 ^{c}
Gemmiza 10	86.67	81.67	84.17 ^{de}	96.67	72.67	84.67 ^{e}
Gemmiza 11	83.33	82.33	82.83 ^{ef}	104.33	73.00	88.67 ^{cd}
Gemmiza 12	85.00	82.67	83.84 ^{ef}	98.00	66.33	82.17 ^{f}
Giza 168	84.33	82.33	83.33 ^{ef}	101.33	73.00	87.17 ^{d}
Giza 171	85.33	83.33	84.33 ^{de}	107.00	77.00	92.00 ^{b}
Sids 14	83.33	81.00	82.17 ^{f}	103.67	82.33	93.00 ^{b}
Shandweel 1	88.76	83.00	85.88 ^{cd}	104.33	74.00	89.17 ^{cd}
L.S.D.	V = 1.94 and S = 0.25 and V*S = 1.55			V = 2.2 and S = 1.24 and V*S = 1.76		

Results in Table 3 show that plants of Giza 171 have the highest mean value for chlorophyll content, which was insignificantly distinguished from the values measured in Giza 168 and Misr 2. Under normal conditions, Giza 171 plants had the highest chlorophyll content, followed by Giza 168 and Misr 2. However, the highest SPAD readings were observed with Misr 1 plants, followed by Giza 171 and Misr 2 under stress conditions. Compared with measurements recorded during normal conditions, chlorophyll content significantly declined in response to drought stress in all cultivars, which was most obvious for Gemmiza 9 and Gimmeza 10 plants that lost about half of their green pigments following drought stress. On the other hand, the least chlorophyll loss was about 25% calculated in Misr 1 plants.

Table 3. Mean values of SPAD reading for ten wheat cultivars under normal and drought conditions. Different letters are significantly different, according to the LSD test, at $p < 0.05$.

Varieties	SPAD Reading		Mean
	Normal	Stress	
Misr 1	44.50	33.40	38.95 ^{ab}
Misr 2	48.30	28.44	38.37 ^{ab}
Gemmiza 9	45.40	23.50	34.45 ^{e}
Gemmiza 10	47.40	24.30	35.85 ^{de}
Gemmiza 11	44.70	26.60	35.65 ^{de}
Gemmiza 12	45.60	28.00	36.80 ^{cd}
Giza 168	48.80	27.40	38.10 ^{bc}
Giza 171	50.32	31.20	40.76 ^{a}
Sids 14	46.40	25.20	35.80 ^{de}
Shandweel 1	44.50	26.10	35.30 ^{de}
L.S.D.	V = 1.96 and S = 0.745 and V*S = 1.75		

Giza 171, followed by Misr 2, exhibited the highest mean values of grain yield/plant (Table 4). The same results were observed considering the values under normal and drought conditions. On the other hand, Gemmiza 9, Gemmiza 10, Gemmiza 11, and Gemmiza 12 had the smallest grain yield, which was mirrored in values recorded under both growth conditions. The remaining cultivars formed an intermediate class, considering their grain yield/plant, either as mean values or values recorded under different irrigation conditions. In addition to Giza 171 and Misr 2, Gemmiza 11 had the highest mean values for the 1000-kernel weight, which is a reflection of having the highest values under normal and drought conditions. Based on small values under both irrigation conditions, Shandweel 1 had the smallest mean value for the 1000-kernel weight. The remaining cultivars shared a middle class.

Table 4. Mean values of grain yield weight/plant and 1000-kernel weight traits for ten wheat cultivars under normal and stress conditions. Different letters are significantly different, according to the LSD test, at $p < 0.05$.

Varieties	Grain Yield/Plant (g)		Mean	1000-Kernel Weight (g)		Mean
	Normal	Stress		Normal	Stress	
Misr 1	4.33	2.10	3.22 ^{d}	4.60	3.42	4.00 ^{f}
Misr 2	4.77	2.33	3.55 ^{b}	5.37	3.87	4.62 ^{b}
Gemmiza 9	3.87	1.75	2.81 ^{fg}	4.77	3.74	4.25 ^{cd}
Gemmiza 10	3.66	1.80	2.73 ^{g}	4.31	3.74	4.03 ^{ef}
Gemmiza 11	3.96	1.90	2.93 ^{ef}	5.10	3.92	4.51 ^{b}
Gemmiza 12	3.88	2.11	3.00 ^{e}	4.62	3.70	4.16 ^{de}
Giza 168	4.43	2.40	3.42 ^{c}	4.88	3.77	4.33 ^{c}
Giza 171	4.85	2.90	3.88 ^{a}	5.43	4.24	4.83 ^{a}
Sids 14	4.09	2.30	3.20 ^{d}	4.95	3.15	4.05 ^{ef}
Shandweel 1	4.31	2.34	3.33 ^{cd}	4.40	3.28	3.84 ^{g}
L.S.D.	V = 0.132 and S = 0.087 and V*S = 0.10			V = 0.14 and S = 0.044 and V*S = 0.11		

The results of the current study reflected the injurious influence of drought on studied yield-related criteria. However, different sensitivities of grain yield/plant and 1000-kernel weight towards drought stress were demonstrated among cultivars. Compared with the corresponding values recorded under normal conditions, drought was accompanied by a 40.2 to 54.8% decrease in grain yield/plant in Giza 171 and Gemmiza 9, respectively. On the other hand, a 13.2–36.4% decrease was calculated for the 1000-kernel weight in Gemmiza 10 and Sids 14, respectively.

3.2. Tolerance Indices

The RD% values resolved two classes: the first with RD% less than 50% included Giza 171, Misr 2, Giza 168, Misr 1, Shandweel 1, Sids 14, and Gemmiza 12, whereas Gemmiza 9, Gemmiza 10, and Gemmiza 11 established the second class with RD% higher than 50% (Table 5). The calculated MP and GMP produce the same classes with one exception for Gemmiza 12. The first class hosted Giza 171, Misr 2, Giza 168, Misr 1, Shandweel 1, and Sids 14, with MP and GMP values higher than 3. The second class consisted of Gemmiza 9, Gemmiza 10, Gemmiza 11, and Gemmiza 12, with MP and GMP less than 3.

High TOL values were calculated for Misr 2, Misr 1, Gemmiza 9, Gemmiza 11, and Giza 168, whereas values less than 2 were recorded in Gemmiza 10, Gemmiza 12, Giza 171, Sids 14, and Shandweel 1. The high stability of the genotypes Giza 171, Sids 14, Shandawel 1, Giza 168, and Gemmiza 12, under both conditions, was demonstrated by high values for YSI ranging from 0.54 to 0.6, while the remaining cultivars reflected their low stability through YSI value less than 0.5. On the other hand, high drought tolerance was reflected in the cultivars Gemmiza 12, Giza 171, Sids 14, and Giza 168 by a DSI value higher than 1, while the DSI value less than 1 reflected the sensitivity of Gemmiza 9, Gemmiza 10, Shandawel 1, Misr 2, and Misr 1 to drought stress.

Results of correlation (Table 6) reflected the highly significant positive correlation between yield under normal and stress conditions on the one hand and each of MP and GMP on the other hand. In addition, YSI appeared engaged in a highly significant positive correlation with yield under drought conditions without a similar correlation with yield under normal conditions. Consequently, highly significant correlations were observed among MP, GMP, and YSI. Independent on yield, a positive significant correlation linked YSI and TOL, and a negative one was characterized between the former and DSI. Otherwise, significant correlations were absent between yield and any of the addressed indices or among indices themselves.

Table 5. Relative decrease (RD%), mean productivity (MP), drought susceptibility index (DSI), geometric mean of productivity (GMP), tolerance index (TOL), and yield stability index (YSI) for ten wheat cultivars.

Varieties	RD% of Grain Yield	MP	GMP	TOL	YSI	DSI
Misr 1	51.50	3.22	3.02	2.23	0.48	1.19
Misr 2	51.15	3.55	3.33	2.44	0.49	1.15
Gemmiza 9	54.78	2.81	2.60	2.12	0.45	1.26
Gemmiza 10	50.82	2.73	2.57	1.86	0.49	1.17
Gemmiza 11	52.02	2.93	2.74	2.06	0.48	0.95
Gemmiza 12	45.62	3.00	2.86	1.77	0.54	0.61
Giza 168	45.82	3.42	3.26	2.03	0.54	0.92
Giza 171	40.21	3.88	3.75	1.95	0.60	0.83
Sids 14	43.77	3.20	3.07	1.79	0.56	0.87
Shandweel 1	45.71	3.33	3.18	1.97	0.54	1.05

Table 6. Correlation of yield under 100% FC and 50% FC conditions with each of mean productivity (MP), geometric mean productivity (GMP), tolerance index (TOL), drought susceptibility index (DSI), and yield stability index (YSI). * Correlation is significant at the 0.05 level. ** Correlation is significant at the 0.01 level.

	y_p	y_{is}	MP	GMP	TOL	YSI	DSI
y_p	1	0.853 **	0.967 **	0.940 **	0.503	0.460	−0.056
y_{is}		1	0.958 **	0.980 **	−0.022	0.854 **	−0.446
MP			1	0.996 **	0.265	0.670 *	−0.249
GMP				1	0.179	0.734 *	−0.310
TOL					1	−0.533	0.633 *
YSI						1	−0.728 *
DSI							1

3.3. Start Codon Targeted (SCoT) Markers

Studying the genetic diversity and assessment of the degree of polymorphism among the ten wheat cultivars was conducted using five SCoT primers (Table 1). The primers produced distinct and reproducible band patterns (Supplementary Materials Figure S1). A total of 34 amplicons were obtained, of which 26 were monomorphic (76.48%), while the remaining 8 bands were polymorphic (23.52%).

The total number of amplified bands by each primer ranged from 4 (primer SCoT 8) to 10 (primer SCoT 1) (Table 7). The monomorphic amplicons per primer ranged from three to seven amplified with the primers SCoT 8 and SCoT 1, respectively. On the other hand, the primer SCoT 3 failed to detect polymorphism, while the remaining primers amplified polymorphic bands among the studied cultivars. The overall number of polymorphic bands amplified by each primer ranged from 1 (primers SCoT 4 and SCoT 8) to 3 (primers SCoT 1 and SCoT 5), with polymorphism % ranging from 16.16% (primer SCoT 4) to 37.5% (primer SCoT 5).

The mediocre number of amplicons/primers was 6.8, divided into 5.2 and 1.6 monomorphic and polymorphic bands, respectively, on average. Two unique positive bands appeared: the first of 815 bp amplified with SCoT 4 in Misr 2 and the second of 540 bp amplified with SCoT 5 in Giza 171. In addition, one unique negative band of 900 bp was amplified with SCoT 1 in Gemmeza 12.

Table 7. Primer code, total number of bands, alleles size range, types of bands (monomorphic, polymorphic, and unique), percent of polymorphism (%), and the polymorphism information content (PIC) for each SCoT primer.

Primer Code	Alleles Size Range	Total Number of Bands	Number of Monomorphic Bands	Number of Polymorphic Bands	Unique Bands	Polymorphism %
SCoT 1	215–445	10	7	3	1	30.00
SCoT 3	220–930	6	6	-	-	0.00
SCoT 4	220–1745	6	5	1	1	16.16
SCoT 5	320–1430	8	5	3	1	37.50
SCoT 8	300–865	4	3	1	-	25.00
Total		34	26	8	3	23.52

The phylogenetic tree based on UPGMA analysis of SCoT data was established for the ten wheat cultivars (Figure 1). It encompassed two major clusters. The first included Gemmiza 9 and Gemmiza 11. The second was split into two major subclusters: the first comprised only Misr 2, while the second subcluster was divided into two major branches. The first major branch included Giza 171 and Sids 14, while the second branch contained the last five cultivars arranged in two minor branches. The first carried Shandawell 1, while the second biforked into two groups. The first comprised Misr 1 and Giza 168, while the second comprised Gemmiza 10 and Gemmiza 12.

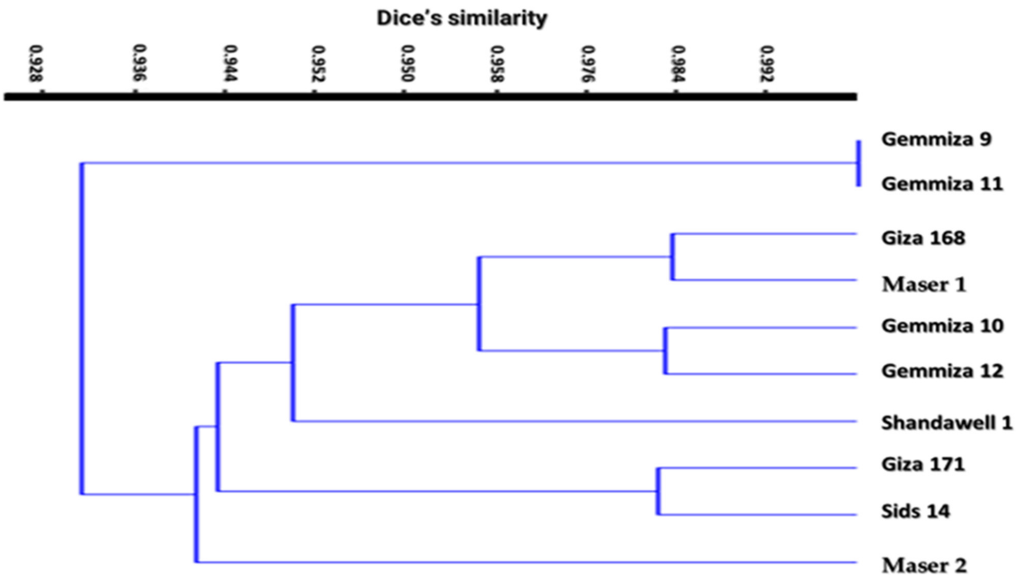

Figure 1. Dendrogram of ten wheat cultivars constructed using SCoT data analysis depending on Dice coefficient and UPGMA method.

On the other hand, the binary scoring achieved by SCoT analysis was used to calculate Jaccard's similarity matrices (Supplementary Materials Table S1). Based on five primers, SCoT resolved nine genotypes from the ten studied cultivars, where Gemmiza 9 and Gemmiza 11 appeared to have a similarity value equal to 1. Among the resolved genotypes, similarity values ranged from 0.897 (calculated between Misr 2 on the one hand and each of Gemmiza 9 and Gemmiza 11 on the other hand) to 0.984 (calculated between Misr 1 on the one hand and each of Giza 186 and Gemmiza 10 on the other hand).

4. Discussion

Results of the present investigation reflected the negative effect of drought stress on plant height and chlorophyll content regardless of the genotype. Turgidity is a prerequisite for cell expansion; therefore, the low turgor pressure associated with drought stress leads to reduction or even cessation of growth [38]. Drought is a major factor responsible for the overall decrease in growth in wheat [2,39]. Specifically, the reduction in plant height can be attributed to the decrease in GA_3, necessary for stem elongation, associated with drought stress [32,40,41].

Stomatal closure is an acclaimed tactic to diminish water loss during water deficit, but it simultaneously limits the entry of carbon dioxide and subsequently injures photosynthetic activities and enhances ROS generation [42]. The accumulated ROS damages cellular macromolecules [43] and is responsible for the decline in chlorophyll content observed in the current study in response to drought stress. The decrease in shoot height and/or chlorophyll content in wheat plants in response to water shortage was also documented by Pour-Aboughadareh et al. [44] and Ahmad et al. [14]. The injurious effect of drought was also recorded in other crops, including maize [45], rice [46], barley [47], and soybean [6].

The current study reflected the significant impact of drought stress on the timing of anthesis indicated with a decrease in heading date in response to drought stress. Drought escaping is a classical adaptive strategy through which the plant fastens its life cycle prior to a coming water shortage. It is well documented in wild plants but is also recorded in cereals, including wheat. Early flowering time reduces exposure to water stress during the vulnerable stages of flowering and post-anthesis grain filling [48]. It occurs as a consequence of a drought-induced accumulation of abscisic acid (ABA) and its effect on the circadian clock through the synchronous manipulation of many flowering-related genes to accelerate floral development [49]. A decrease in heading date as a consequence of drought stress was documented in wheat by Pour-Aboughadareh et al. [44] and Sharma et al. [50]. It was also documented as a drought response in rice [49] and barley [47].

Drought is associated with a decrease in photosynthetic yield due to oxidative stress-related damage of chlorophyll and prevention of electron flow in PSI [51]. Moreover, there is a decline in CO_2 conductance following stomatal closure [52] and a shortage in several Calvin cycle proteins, including Rubisco [53], which limits the grain filling and appears as a decrease in kernel weight [54]. In addition, the drought-enhanced accumulation of ABA hinders pollination and encourages seed abortion [55], which generally contributes to yield reduction and specifically decreases grain yield per plant. Consequently, our results reflected a decrease in 1000-kernel weight and grain yield per plant under drought conditions. Similar results were recorded in wheat by Pour-Aboughadareh et al. [44], Ahmad et al. [14], and [56]. A decrease in yield is a universal result of drought stress recorded in maize [45], rice [46], barley [47], tomato [32], and soybean [6].

The different drought resistance indices reflected different rankings of the studied cultivars. MP and GMP provided superiority to Giza 171, Misr 2, Giza 168, Misr 1, Shandweel 1, and Sids 14, of which, Misr 1, Misr 2, and Giza 168 were highlighted by TOL, while Giza 171, Giza 168, and Sids 14 were characterized by both YSI and DSI. Lastly, Shandweel 1 tolerance appeared only in the classification based on YSI. Ignoring MP and GMP, bothYSI and DSI reflected tolerance in Gemmiza 12, while TOL reflected tolerance in Gemmiza 9 and Gemmiza 11. Our results also reflected Giza 168 as a tolerant cultivar considering any of the utilized indices. Away from TOL, Giza 171 and Sids 14 appeared tolerant, consulting any of the remaining indices. Tolerance of Misr 1 and Misr 2 was confirmed by MP, GMP, and TOL. A similar result was highlighted for Shandweel 1 following replacing TOL with YSI. Finally, Gemmiza 12 tolerance was confirmed by two indices, i.e., YSI and DSI. The dependence of drought tolerance ranking on the index applied was documented in wheat by Anwaar et al. [57], Dorostkar et al. [58], Aktaş [59], and Farshadfar et al. [60].

Our results put MP and GMP as first runners among indices to select high-yielding cultivars under normal and drought conditions, as mirrored by the positive, highly significant correlations between MP and GMP and yield under normal and drought conditions. In addition, YSI was reflected as a suitable index to characterize the high-yielding cultivar under drought conditions only supported by a positive, highly significant correlation with yield under drought conditions and insignificant correlation with yield under normal irrigation. Otherwise, the addressed indices failed to highlight yield abundance under normal or drought conditions.

The universality of MP and GMP to highlight drought tolerance was recorded by many research groups with contradictory results concerning the correlations between other indices and yield. Observing the yield of ten Egyptian wheat cultivars, Shaban et al. [61] recorded a significant correlation between YSI and yield under normal and drought conditions, which was insignificant when replacing YSI with TOL or DSI. Working on 50 genotypes, Anwaar et al. [57] recorded a significant correlation between TOL and yield in drought conditions without similar correlations to link yield with YSI and DSI. Based on monitoring the yield behavior of 36 genotypes under normal and drought conditions, Dorostkar et al. [58] highlighted the significant correlations between TOL and yield in both growth conditions, in addition to a significant correlation between DSI and yield in drought conditions. Studying eight bread wheat genotypes and four landraces, Aktaş [59] observed the significant correlation between TOL and yield in normal conditions and that between YSI and yield in drought conditions without significant correlations between DSI and yield. Farshadfar et al. [60] monitored the indices in a prolonged study that lasted three years. Interestingly, the authors recorded year-to-year variable correlations for TOL, YSI, and DSI with yield under normal and drought conditions.

The current results demonstrated the ability of only five SCoT primers (SCoT 1, 3, 4, 5, and 8) to resolve ten wheat cultivars, which confirmed the high efficiency of SCoT markers to highlight the intraspecific diversity in wheat. This distinguished discriminatory power of SCoT markers can be attributed to the utilization of long primers (18 bp) that produces high polymorphism [62]. Supporting our results, Gowayed and Abd El-Moneim [31] documented the ability of five SCoT primers (SCoT 1, 2, 3, 4, and 8) to resolve 14 Egyptian wheat cultivars. Similarly, Shaban et al. [61] succeeded in resolving ten Egyptian cultivars employing seven SCoT primers (SCoT 1, 2, 3, 4, 5, 10, and 12). In the same context, Etminan et al. [30] resolved 25 breeding lines of Durum wheat (*Triticum turgidum* var. durum) and 18 landraces using six SCoT primers.

The utilized primers amplified 34 bands, of which eight bands were polymorphic, responsible for 23.52% polymorphism. The contradiction between the relatively low percentage of polymorphism and high genotypic resolution can be explained by the accumulation of the sequence heterogeneity in loci amplified by only two primers, viz. SCoT 1 and 5, while the other primers (SCoT 3, 4, and 8) amplified more or less homogenous sequences. A higher percentage of polymorphism (86.7%) was recorded by Gowayed and Abd El-Moneim [31], calculated from profiles of 14 Egyptian wheat cultivars generated with five primers. Compared with our results, the authors calculated a higher percentage of polymorphism for the primers used in our study, reflecting the genetic relatedness of the cultivars manipulated. More or less similar results were calculated for SCoT markers in wheat genotypes by Shaban et al. [61], Nosair [63], and Pavia et al. [64].

Targeting regions in plant genes confers great importance on unique bands amplified with SCoT primers, especially in elite genotypes. The present investigation introduced two unique positive bands, the first of 815 bp in Misr 2 and the second of 540 bp in Giza 171. Both cultivars are high-yielding under normal and drought conditions, which suggests a role for these unique sequences in yield and drought tolerance and provides a start point for a new investigation. The amplification of unique SCoT bands in drought-tolerant wheat genotypes was also recorded by Shaban et al. (Shaban et al., 2022). In harmony with our results, Gowayed and Abd El-Moneim [31] amplified unique SCoT bands in relation to salinity tolerance.

The SCoT-based dendrogram resolved the ten wheat cultivars successfully. In addition, the tree mirrored a grouping of cultivars having a similar response to drought stress, including Giza 186 and Sids 14, as well as Gemmiza 10 and Gemmiza 12. However, ambiguous phylogenetic relationships appeared among the remaining cultivars, characterized by grouping high-yielding cultivars with low-yielding cultivars under normal and drought conditions. The ambiguous grouping can be attributed to the utilization of a small number of primers.

5. Conclusions

Based on yield-related criteria, Giza 171, Misr 2, and Giza 168 appeared as precious germplasm for breeding of high-yielding drought-tolerant wheat. Among TOL, GMP, MP, YS, and DSI, MP and GMP appeared as the most accurate indices to distinguish the high-yielding cultivars under drought conditions. SCoT is a potent gene-targeting molecular marker, able to characterize and resolve genetic diversity in wheat at the cultivar level using few primers. However, establishing a proper phylogenetic relationship requires coverage of a considerable proportion of the genome using many primers. Therefore, molecular markers, especially those targeting genes, e.g., SCoT, can efficiently replace indices in characterization of drought-tolerant genotypes with a high confidence level. In addition, molecular markers can preserve the precious time wasted during cultivation to calculate indices. However, the high cost of molecular markers techniques can be reduced to a reasonable level by comprehensive research to select the minimum efficient combination of primers able to highlight the targeted trait.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12051217/s1, Figure S1: SCoT profiles of the ten wheat cultivars generated by five SCoT primers. Lanes 1 to 11; M: molecular weight of DNA marker,1: Misr 1, 2: Misr 2, 3: Giza 168, 4: Giza 171, 5: Shandawell 1, 6: Sids 14, 7: Gemmiza 9, 8: Gemmiza 10, 9: Gemmiza 11 and 10: Gemmiza 12.; Table S1: Similarity index using SCoT analysis for ten wheat cultivars.

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