

(Original Article)



## Selection for Chlorophyll Content and SCoT-Based Molecular Diversity in Wheat Genotypes under Drought Conditions

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### Abstract

Drought stress is a major constraint to wheat productivity, necessitating efficient selection strategies for stress tolerance. In this study, three F<sub>2</sub> wheat populations were evaluated for chlorophyll content, measured as Soil Plant Analysis Development (SPAD) readings, under drought conditions across two cycles of divergent selection. Simultaneously, 1000-grain weight and grain yield per plant were recorded to assess the association between physiological and yield-related traits. Recurrent selection targeting higher SPAD values resulted in significant improvements in chlorophyll content, with population 3 showing the greatest progress. Enhanced SPAD values were positively correlated with yield attributes, indicating the potential of chlorophyll content as a reliable selection criterion under drought stress. Heritability estimates for the studied traits varied from low to high, suggesting differential genetic control among populations and traits. To complement phenotypic evaluation, molecular diversity was assessed using Start Codon Targeted (SCoT) markers. The obtained results from SCoT marker revealed a high level of polymorphism (91.6%) with an average of polymorphic information content (PIC) of 0.36, reflecting substantial genetic variability among the studied genotypes. Overall, the integration of SPAD-based recurrent selection with molecular marker analysis provides a robust and efficient strategy for enhancing drought tolerance and stabilizing yield performance in bread wheat. These findings highlight the value of combining physiological and molecular tools in breeding programs aimed at developing resilient wheat cultivars.

**Keywords:** Drought stress, Heritability, SCoT markers, Selection, SPAD, Triticum.

### Introduction

Wheat (*Triticum aestivum*) is a major global cereal and essential food source. It belongs to the genus *Triticum*, with species differentiated by ploidy level. Common wheat, the most widely cultivated type, has a complex hexaploid genome (AABBDD;  $\sim 1.7 \times 10^{10}$  bp), supporting its broad adaptability and genetic diversity (Maity and Shrivastav, 2024). In Egypt, it represents the main staple crop, providing nearly one-third of daily calories and about 45% of dietary protein, primarily through subsidized *baladi* bread. Globally, wheat is the second most cultivated cereal, with production

estimated at 793.24 million metric tons in 2024/2025, showing a slight increase compared to the previous year (Pingault *et al.*, 2016; Statista, 2024).

Wheat production is increasingly constrained by environmental stresses, especially drought, which is among the most harmful abiotic factors. Since water constitutes 80–95% of plant biomass and is vital for transport, metabolism, and photosynthesis (Seleiman *et al.*, 2021), water deficits cause reduced leaf expansion, shorter stems, and restricted root growth, leading to poor resource uptake, low water-use efficiency, and significant yield losses (Begna, 2020).

Improving drought tolerance in wheat requires integrating genetic, physiological, and molecular approaches. While marker-assisted selection is widely used, its effectiveness is often limited under stress conditions, making phenotypic selection more reliable for identifying tolerant genotypes (Nyaupane *et al.*, 2024; Mohammadi, 2018). Accordingly, breeding programs now focus on enhancing tolerance to drought and heat, as well as resistance to pests and diseases, to sustain yield stability under challenging environments (Reynolds and Braun, 2022). Chlorophyll content is a key physiological indicator of stress tolerance, as its decline under drought and heat is associated with reduced biomass and yield, while breeding for higher retention enhances performance and resilience (Naseer *et al.*, 2024; Sewore and Abe, 2024).

DNA markers are essential for assessing genetic diversity and supporting breeding, with Start Codon Targeted (SCoT) markers offering a PCR-based approach that targets conserved regions near the ATG start codon (Collard and Mackill, 2009). By detecting polymorphisms in functional gene regions, SCoT markers have been effectively used in wheat to evaluate diversity, identify superior genotypes, and analyze population structure (Hamidi *et al.*, 2014; Tiwari *et al.*, 2016; Yalinkiliç *et al.*, 2024), thereby complementing phenotypic selection and strengthening molecular breeding.

The objectives of this study were: (i) to evaluate wheat genotypes selected for drought tolerance, (ii) to assess their response to selection based on physiological and morphological traits, and (iii) to distinguish tolerant and susceptible genotypes using SCoT-based molecular analysis.

## Materials and Methods

### Plant materials and field trials

Three F<sub>2</sub> wheat (*Triticum aestivum* L.) populations, derived from different crosses (Table 1), were used in this study.

**Table 1. Wheat populations used in the study**

Populations	Cross
1	Sakha-08 × Line-6
2	Sakha-08 × Line-1×15
3	PavonF-76 × Line-6

Field trials were conducted at the Experimental Farm, Faculty of Agriculture, Assiut University during three seasons from 2022–2024. Populations were sown at optimal planting dates (late November) under two irrigation regimes: full irrigation (100% field capacity) and limited irrigation (two irrigations, drought-stress). Each F<sub>2</sub>

population included 180 plants, arranged in rows (30 × 30 cm spacing). Chlorophyll content (Soil Plant Analysis Development (SPAD) value) was measured after anthesis, while Spike length and spike weight, 1000-kernel weight, and grain yield per plant were recorded at maturity.

### **Selection for chlorophyll content**

Divergent phenotypic selection was applied across two cycles under drought stress. In cycle 1 ( $F_2 \rightarrow F_3$ ), the three plants with the highest and lowest SPAD values from each population were selected (selection intensity  $\approx 2\%$ ). In cycle 2 ( $F_3 \rightarrow F_4$ ), the top and bottom 10% of plants per family were chosen. Selected families and corresponding unselected bulks were advanced under drought stress using a randomized block design with three replications. In each cycle, chlorophyll content, 1000-kernel weight, and grain yield per plant were evaluated.

### **Statistical analysis**

Trait correlations and normal distribution patterns were analyzed using SPSS. ANOVA was performed to test differences among selected families and bulks. Response to selection was calculated as the relative change (%) of selected families compared with unselected bulks. Heritability was estimated using realized heritability (Ibrahim and Quick, 2001) and parent–offspring regression.

### **SPAD measurement**

Chlorophyll content was estimated using a SPAD-502 Plus meter (Minolta, Tokyo, Japan), which measures relative chlorophyll content non-destructively (Liu *et al.*, 2012; Wakiyama, 2016; Shibaeva *et al.*, 2020). Measurements were taken from the tip, middle, and base of flag leaves, and averaged across three readings.

### **Molecular analysis**

DNA was extracted from  $F_4$  plants of selected high and low SPAD and 1000-kernel weight families, bulked into  $F_5$  seed lots, using the CTAB-based protocol of Youssef *et al.* (2015). DNA concentration and quality were verified by spectrophotometry and gel electrophoresis.

### **SCoT marker analysis**

Genetic diversity was assessed using 15 Start Codon Targeted (SCoT) primers (Collard and Mackill, 2009) (Table 2). PCR amplifications were performed in 10  $\mu$ l reactions containing master mix (Applied Biotechnology), primer, and 15 ng DNA template, with cycling at 94 °C (2 min), followed by 36 cycles of 94 °C (30 s), 58–66 °C optimized for each primer (1 min), 72 °C (2 min), and final extension at 72 °C (2 min). PCR products were separated on 1.3% concentration containing 0.01% ethidium bromide in 0.5× concentration TBE buffer (89 mM Tris-borate; 2.5 mM EDTA). The submarine gel (Ultra-Violet Product, Upland, CA, USA,) was supplied with a direct current of 85 volt for approximately 2–2.5 hours using a Hoefer Scientific Instruments (San Francisco, USA) power supply. The patterns were visualized and photographed using a gel documentation system (Ultra-Violet Product, Upland, CA, USA.).

**Table 2. Primer codes and sequences used in the SCOT analysis**

Serial No.	Codes	Sequence (5' → 3')
1	SCOT 2	5'-ACCATGGCTACCACCGGC-3'
2	SCOT 66	5'-ACCATGGCTACCAGCGAG-3'
3	SCOT 14	5'-ACGACATGGCGACCACGC-3'
4	SCOT 70	5'-ACCATGGCTACCAGCGCG-3'
5	SCOT 34	5'-ACCATGGCTACCACCGCA-3'
6	SCOT 33	5'-CCATGGCTACCACCGCAG-3'
7	SCOT 30	5'-CCATGGCTACCACCGGCG-3'
8	SCOT 15	5'-ACGACATGGCGACCGCGA-3'
9	SCOT 1	5'-ACGACATGGCGACCACGC-3'
10	SCOT 11	5'-AAGCAATGGCTACCACCA-3'
11	SCOT 6	5'-CAATGGCTACCACTACAG-3'
12	SCOT 8	5'-CAACAATGGCTACCACGA-3'
13	SCOT10	5'-CAACAATGGCTACCAGCC-3'
14	SCOT 61	5'-CAACAATGGCTACCACCG-3'
15	SCOT 4	5'-ACCATGGCTACCACCGCA-3'

For molecular data analysis, a binary data matrix was created, recording the presence (1) or absence (0) of bands. Only strong and readable bands were detected and used for analysis. Several polymorphism measures were calculated, including the percentage of polymorphism, the polymorphism information content (PIC), the primer resolution power (Rp), the diversity index (DI), and the marker index (MI). Estimated similarities between wheat genotypes based on the Jaccard coefficient (Jaccard, 1908) were used to perform cluster analysis using the unweighted pair group method with arithmetic mean (UPGMA) by the MVSP software.

## Results

**Correlation among traits** Phenotypic correlations among yield component traits revealed that SPAD value was consistently associated with performance under drought stress (Tables 3–5). In population 1, SPAD value showed significant positive correlations with 1000-kernel weight, grain yield, spike length, and spike weight traits under drought, while most of these correlations weakened under favorable conditions. Similar patterns were observed in Population 3, where the SPAD value was significantly correlated with 1000-kernel weight and grain yield under drought, but not under favorable conditions. In Population 2, the associations were generally weaker, with significant correlations observed mainly under stress conditions.

Overall, the SPAD value tended to be more strongly associated with yield components under drought than under favorable conditions, indicating its potential as a reliable selection criterion for stress tolerance

**Table 3. phenotypic correlation coefficient of 5 traits of bread wheat varieties of the population 1 under favorable, and drought conditions**

Population 1						
Traits	C	1000 Kernel Weight	Chlorophyll content	Grain yield	Spike length	Weight of spike
1000 Kernel Weight	F	1				
	D	1				
Chlorophyll content	F	0.074 <sup>ns</sup>	1			
	D	0.224**	1			
Grain yield	F	0.069 <sup>ns</sup>	-0.102 <sup>ns</sup>	1		
	D	0.226**	0.168*	1		
Spike length	F	0.073 <sup>ns</sup>	0.301**	-0.131 <sup>ns</sup>	1	
	D	0.320**	0.281**	0.325**	1	
Weight of spike	F	0.337**	0.193*	0.129 <sup>ns</sup>	.396**	1
	D	0.356**	0.351**	0.474**	.536**	1

C: Conditions, F: Favorable, D: Drought, ns: non- significance, \* and \*\* denote significance at  $p < 0.05$  and high significance at  $p < 0.01$ .

**Table 4. phenotypic correlation coefficient of 5 traits of bread wheat varieties of the population 2 under favorable and drought conditions.**

Population 2						
Traits	C	1000 Kernel Weight	Chlorophyll content	Grain yield	Spike length	Weight of spike
1000 Kernel Weight	F	1				
	D	1				
Chlorophyll content	F	0.003 <sup>ns</sup>	1			
	D	0.074 <sup>ns</sup>	1			
Grain yield	F	0.381**	0.234**	1		
	D	0.336**	0.412**	1		
Spike length	F	0.121 <sup>ns</sup>	0.111 <sup>ns</sup>	0.344**	1	
	D	0.226**	0.351**	0.409**	1	
Weight of spike	F	0.538**	0.095 <sup>ns</sup>	0.317**	0.201**	1
	D	0.163*	0.172*	0.267**	0.128 <sup>ns</sup>	1

C (Conditions), F (Favorable), D (Drought), <sup>ns</sup> (non- significance) \* and \*\* denote significance at  $p < 0.05$  and high significance at  $p < 0.01$ .

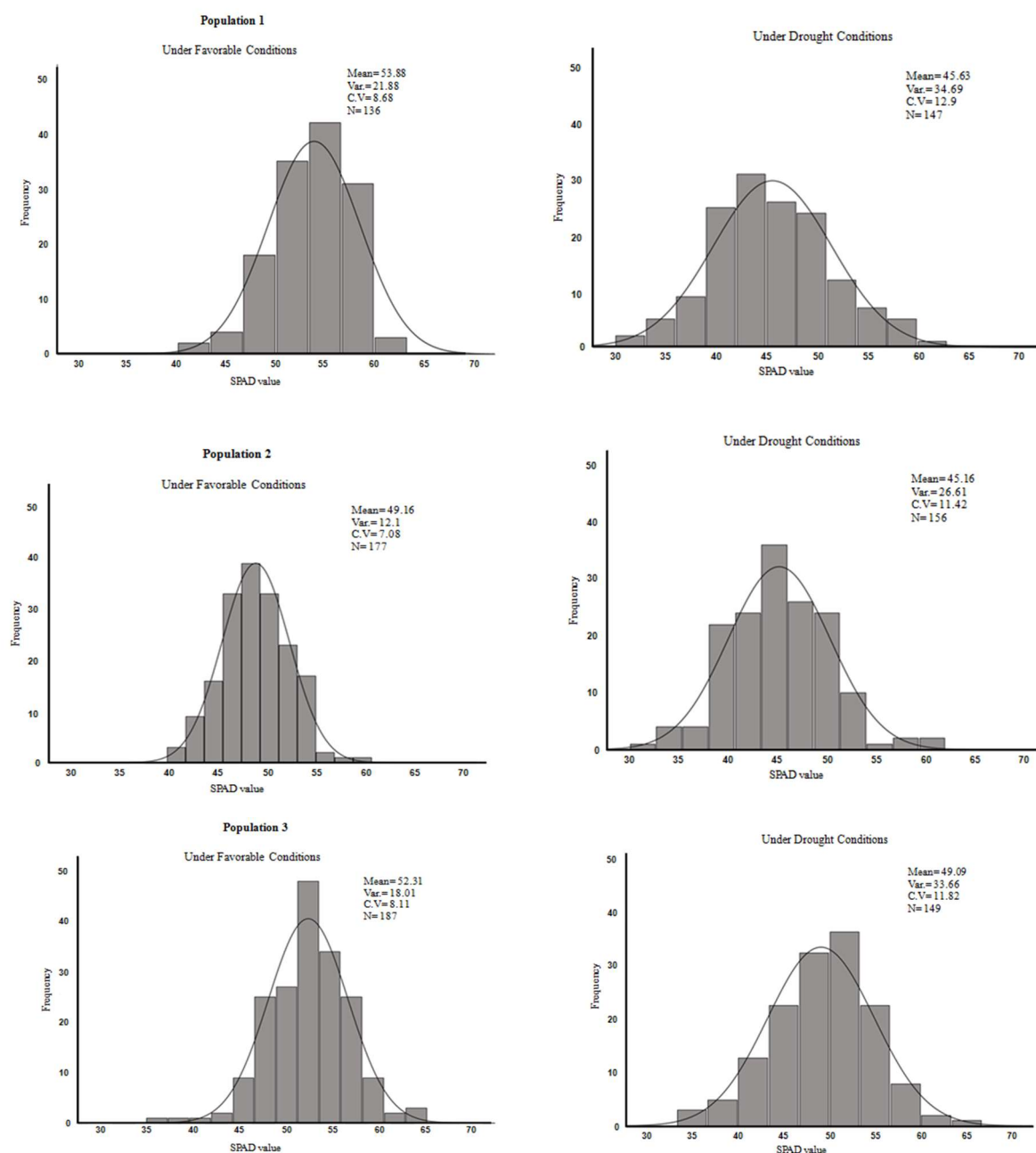
**Table 5. Phenotypic correlation coefficient of 5 traits of bread wheat varieties of the population 3 under favorable and drought conditions**

Population 3						
Traits	C	1000 Kernel Weight	Chlorophyll content	Grain yield	Spike length	Weight of spike
1000 Kernel Weight	F	1				
	D	1				
Chlorophyll content	F	0.156*	1			
	D	0.107 <sup>ns</sup>	1			
Grain yield	F	0.165*	0.013 <sup>ns</sup>	1		
	D	0.231**	0.347**	1		
Spike length	F	0.014 <sup>ns</sup>	0.142 <sup>ns</sup>	0.225**	1	
	D	0.083 <sup>ns</sup>	0.543**	0.386**	1	
Weight of spike	F	0.283**	0.166*	0.291**	0.246**	1
	D	0.071 <sup>n.s</sup>	0.354**	0.246**	0.330**	1

C: Conditions, F: Favorable, D: Drought, ns: non- significance, \* and \*\* denote significance at  $p < 0.05$  and high significance at  $p < 0.01$ .

## Distribution of SPAD values

Frequency distributions of  $F_2$  populations (Fig. 1) showed continuous and approximately normal distributions for SPAD values under both favorable and drought conditions. This indicates polygenic inheritance of chlorophyll content, making it amenable to selection.



**Figure 1: Distributions of the  $F_2$  segregates of population 3 under favorable conditions (above) and drought (below) for SPAD values.**

### First selection cycle

Under drought, the mean of SPAD values of unselected F<sub>2</sub> bulks ranged from 45.16 to 49.09 across the three populations. High-performing individuals reached values of 50.34–59.90, while the lowest values ranged from 34.65 to 38.36 (Table 6). The largest positive selection differential occurred in Population 1 (11.41).

**Table 6. Means of (SPAD value) of three unselected F<sub>2</sub> bulk families of each of three populations grown under drought conditions with the mean of the plants selected for higher and lower Chlorophyll content (SPAD value) and the selection differentials**

Population No.	Population Mean	Mean of the selected F <sub>2</sub> plants		Selection differential	
		High	Low	High	Low
1	45.72	57.12	38.36	11.41	7.36
2	45.16	50.34	34.65	5.18	10.52
3	49.09	59.90	36.21	10.81	12.88

Responses to selection in the F<sub>3</sub> generation confirmed the effectiveness of divergent selection. High-SPAD families recorded percentage of mean improvements of 9.85% in Population 1, 0.43% in Population 2, and 12.54% in Population 3, averaging 7.61% (Table 7). Selection for low SPAD also produced significant responses, ranging from 1.23% to 8.34% with an average of 5.60%. Realized heritability estimates were moderate in Population 1 (0.49) but lower than those in Populations 2 and 3 (0.24–0.28). Parent–offspring regressions confirmed these values, showing close agreement with realized estimates.

**Table 7. Means of SPAD value of the high and low F<sub>3</sub> selections and F<sub>3</sub> bulks of the three populations as measured under drought conditions with the % observed responses, realized heritability and parent-offspring regressions**

Populations		Population 1	Population 1	Population 3
	High	55.45	49.51	55.03
	Bulk	50.48	49.30	48.90
	Low	46.27	45.73	48.30
Response %	High	9.85*	0.43	12.54**
	Low	8.34*	7.24**	1.23
H %	Realized	0.49	0.24	0.28
	b <sub>po</sub>	0.49** ± 0.08	0.23 ± 0.11	0.24 ± 0.17

\*p < 0.05, \*\*p < 0.01 and H %: Heritability %.

Correlated responses indicated that selection for higher SPAD led to significant increases in both grain yield per plant and 1000-kernel weight traits across populations (Tables 8–9). Moreover, yield gains ranged from 16.7 to 23.4%, while kernel weight gains ranged from 4.2 to 6.7%. Selection for lower SPAD also produced positive responses in yield and kernel weight, although the effects were less consistent across populations.

**Table 8. Means of grain yield per plant of the high and low SPAD value F<sub>3</sub> selections as well as F<sub>3</sub> bulks of the three populations grown in drought conditions with the % correlated responses to selections, realized heritability and parent-offspring regressions.**

Populations		Population 1	Population 2	Population 3
High		20.38	29.30	25.33
Bulk		17.46	23.74	21.16
Low		15.31	17.11	16.95
C.R %	High	16.72*	23.42**	19.71*
	Low	12.31*	27.93**	19.90*
H %	Realized	0.83	0.60	0.14
	bpo.	0.11 ± 0.17	0.35* ± 0.09	0.36 ± 0.4

\*p < 0.05, \*\*p < 0.01, C.R %: Correlated Responses % and H %: Heritability %.

**Table 9. Means of 1000-kernel weight of the high and low SPAD value F<sub>3</sub> selections as well as F<sub>3</sub> bulks of the three populations grown in drought conditions with the % correlated responses to selections, realized heritability and parent-offspring regressions.**

Populations		Population 1	Population 2	Population 3
High		47.19	45.63	46.71
Bulk		44.22	43.38	44.81
Low		41.87	40.43	42.55
C.R %	High	6.72*	5.19*	4.24*
	Low	5.31**	6.80**	5.04*
H %	Realized	0.30	0.45	0.15
	bpo.	0.39 ± 0.21	0.17 ± 0.17	0.16 ± 0.09

\*p < 0.05, \*\*p < 0.01, C.R %: Correlated Responses % and H %: Heritability %.

## Second selection cycle

The F<sub>3</sub> bulks used as base populations for the second selection cycle showed mean SPAD values between 48.18 and 50.74, with selected individuals again showing wide contrasts (38.4–63.3, Table 10).

**Table 10. Means of SPAD value of three unselected F<sub>3</sub> bulk families of each of three populations grown under drought conditions with the mean of the plants selected for higher and lower (SPAD value) and the selection differentials.**

Population No.	Population Mean	Mean of the selected F <sub>4</sub> plants		Selection differential	
		High	Low	High	Low
1	50.73	57.82	38.38	7.09	12.35
2	48.18	52.86	39.11	4.67	9.07
3	50.74	63.26	41.46	12.51	9.29

Responses in the F<sub>4</sub> generation were generally stronger than those in the first cycle. High-SPAD selections produced increases of 8.04%, 12.06%, and 34.35% in Populations 1, 2, and 3, respectively, averaging 18.15% (Table 11). Responses for low-SPAD selections were significant with a small mean value of 5.43%. Heritability estimates improved compared to the first cycle, ranging from 0.36 in Population 1 to as



high as 0.89 in Population 3, suggesting that selection became more effective as populations advanced.

**Table 11. Means of SPAD value of the high and low F<sub>4</sub> selections and F<sub>4</sub> bulks of the three populations as measured under drought conditions with the % observed responses, realized heritability and parent-offspring regressions.**

Populations		Population 1	Population 1	Population 3
High		49.18	56.85	71.26
Bulk		45.52	50.73	53.04
Low		42.24	47.30	51.83
Response %	High	8.04**	12.06*	34.35**
	Low	7.21*	6.79*	2.28
H %	Realized	0.36	0.69	0.89
	b <sub>po</sub> .	49.18	56.85	71.26

\*p < 0.05, \*\*p < 0.01 and H %: Heritability %.

Correlated responses in the second cycle again highlighted the yield benefits of selecting for high SPAD. Grain yield per plant was increased by 21.9–34.1% in the three populations (Table 12). In contrast, low-SPAD selections produced no significant yield gains in this cycle. For 1000-kernel weight, selection for high SPAD increased weight by 3.2–8.6%, while selection for low SPAD produced even larger gains in some populations (up to 31.0% in Population 3) (Table 13). Parent-offspring regressions supported these findings, with significance observed in population 2 for kernel weight (0.51).

**Table 12. Means of grain yield per plant of the high and low SPAD value F<sub>4</sub> selections as well as F<sub>4</sub> bulks of the three populations grown in drought conditions with the % correlated responses to selections, realized heritability and parent-offspring regressions.**

Populations		Population 1	Population 2	Population 3
High		26.54	33.25	28.94
Bulk		23.14	24.79	22.65
Low		21.76	23.12	21.25
C.R %	High	21.97*	34.13**	27.77*
	Low	5.96	6.74	6.18
H %	Realized	0.88	0.43	0.64
	b <sub>po</sub> .	0.83 ± 0.43	0.27 ± 0.18	0.96 ± 0.37

\*p < 0.05, \*\*p < 0.01, C.R %: Correlated Responses % and H %: Heritability %.

**Table 13. Means of 1000-kernel weight of the high and low SPAD valueF<sub>4</sub> selections as well as F<sub>4</sub> bulks of the three populations grown in drought conditions with the % correlated responses to selections, realized heritability and parent-offspring regressions.**

Populations		Population 1	Population 2	Population 3
High		59.53	53.64	45.73
Bulk		54.82	52.00	43.36
Low		51.9	46.69	29.92
C.R %	High	8.59**	3.15**	5.47**
	Low	5.33*	10.21**	31.00**
H %	Realized	2.08	0.60	0.71
	b <sub>po</sub> .	0.95 ± 0.56	0.51 ± 0.14*	0.97 ± 1.01

\*p < 0.05, \*\*p < 0.01, C.R %: Correlated Responses % and H %: Heritability %.

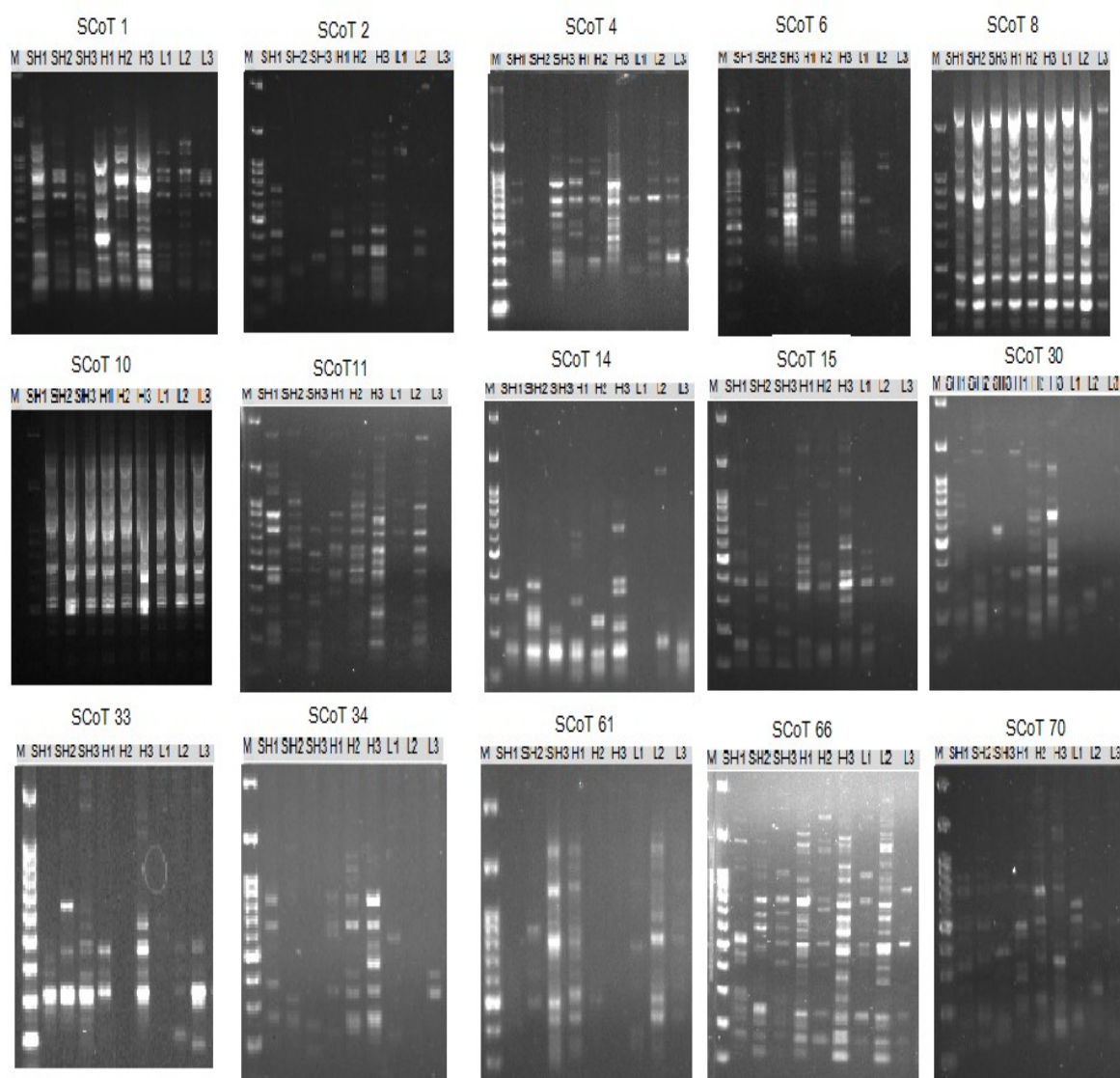
### Molecular analysis (SCoT assay)

Start Codon Targeted (SCoT) markers were used to assess molecular diversity among the selected populations (Fig 2). Fifteen primers produced 190 bands, of which 174 (91.6%) were polymorphic, confirming high genetic variation among populations under drought conditions. Polymorphism levels varied widely across primers, from 28.6% (SCoT 6) to 100% (several primers). PIC values ranged from 0.09 to 0.46, averaging 0.36, while the resolving power averaged 7.22, indicating strong discriminatory capacity (Table 14).

**Table 14. Survey of SCOT analysis showing total number of bands along with genetic diversity measures calculated for wheat populations under drought conditions for SPAD value.**

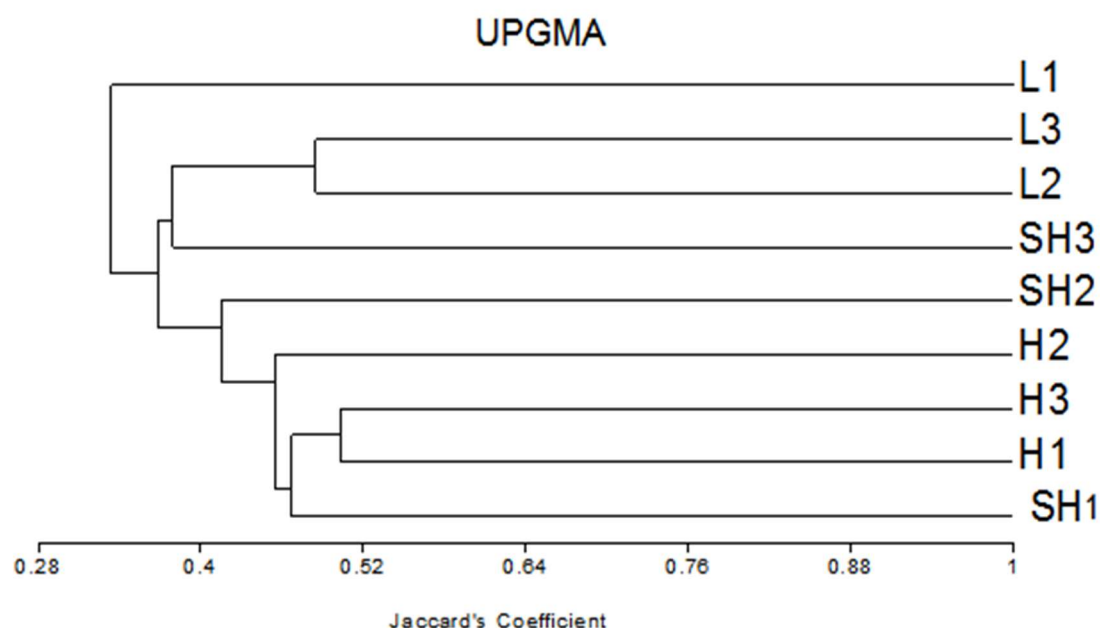
Primers	TNB	NPB	P %	Genetic diversity			
				PIC	Rp	DI	MI
SCOT 1	9	7	77.8	0.30	4.22	1.77	2.11
SCOT 2	12	12	100.0	0.39	7.11	3.62	5.03
SCOT 4	10	10	100.0	0.40	5.11	2.15	3.16
SCOT 6	7	2	28.6	0.43	6.89	3.26	4.30
SCOT 8	16	14	87.5	0.09	1.11	0.43	0.17
SCOT10	17	16	94.1	0.34	8.89	4.39	4.75
SCOT 11	10	10	100.0	0.36	9.11	4.63	5.81
SCOT 14	18	16	88.9	0.38	5.56	3.14	3.75
SCOT 15	16	16	100.0	0.38	10.89	5.41	6.01
SCOT 30	11	11	100.0	0.35	8.00	4.97	5.58
SCOT 33	10	10	100.0	0.38	6.00	3.38	4.20
SCOT 34	9	9	100.0	0.40	6.22	3.26	4.05
SCOT 61	26	24	92.3	0.42	5.78	2.77	4.59
SCOT 66	10	10	100.0	0.38	15.78	7.56	9.59
SCOT 70	9	7	77.8	0.46	7.56	3.24	4.59
Total	190	174	91.6	0.36	7.22	3.60	4.51

TNB: total number of bands, NPB: number of polymorphic bands, %P: percentage of polymorphic, PIC: polymorphism information content, Rp: primer resolving power, DI: diversity index, MI: marker index. Primers codes are related to table (2).



**Figure 2.** SCoT profiles generated by SCoT (1, 2, 4, 6, 8, 10, 11, 14, 15, 30, 33, 34, 61, 66 and 70) primers among the three wheat populations, evaluated under drought and heat stress conditions for 1000-kernel weight and SPAD value.

Cluster analysis using the Jaccard coefficient grouped genotypes into two main clusters (Jaccard, 1908) (Fig. 3). One cluster contained low-SPAD selections (L1, L2, SH3), with L1 being the most genetically distinct. The second cluster contained high-SPAD plants (H1, H2, H3, SH1, SH2), which grouped closely together. This clear separation reflects consistent molecular differences between high- and low-SPAD selections across populations.



**Figure 3. UPGMA-dendrograms show relationship among the three populations under drought conditions for SPAD value based on SCoT molecular markers.**

## Discussion

Drought stress is one of the most critical abiotic constraints on wheat productivity worldwide, as it impairs key physiological processes by reducing relative water content, chlorophyll concentration, and leaf water potential, while increasing diffusion resistance and lowering photosynthetic efficiency (Ahmad *et al.*, 2022; Nyaupane *et al.*, 2024). Since SPAD measurements provide a reliable estimate of leaf chlorophyll content, they are widely used as an indicator of photosynthetic capacity and plant performance under stress (Uddling *et al.*, 2007; Akter *et al.*, 2016; Bunphan *et al.*, 2019). In the present study, SPAD was positively and significantly correlated with grain yield, spick length, and spick weight traits under drought, highlighting its potential as a secondary trait for selecting drought-tolerant genotypes. These results agree with previous reports in wheat, where SPAD was significantly associated with yield under water stress (Javed *et al.*, 2022; El-Saadony *et al.*, 2021), and with studies in maize showing similar associations with yield-promoting traits (TSpitkó *et al.*, 2016; Kandel *et al.*, 2018). Collectively, these findings reinforce SPAD as a robust physiological trait for improving yield stability in environments with limited water availability.

Two successive cycles of divergent selection for SPAD were conducted in this study. The mean responses of 18.15% (high SPAD) and 5.4% (low SPAD) in the  $F_4$  selections, compared with 7.6% and 5.6% in the  $F_3$ , indicate additional genetic gains averaging 10.55% in the high-SPAD direction. When analyzed by population, gains of 8.04%, 12.06%, and 34.35% were achieved in  $F_4$  high-SPAD selections, while two populations also showed modest progress (6.79–7.21%) in the low-SPAD direction. These results suggest that additive genetic variance for SPAD persisted after two cycles, enabling continued progress. The heritability estimates (0.24–0.49 in  $F_3$ , 0.36–0.89 in  $F_4$ ) are consistent with previous findings, where significant genetic variance in SPAD

was reported in durum wheat, barley, and triticale (Giunta *et al.*, 2002), and large genetic variability was observed in chickpea under drought (Kashiwagi *et al.*, 2010). Similarly, Hefena *et al.* (2016) reported moderate heritability (~36%) for chlorophyll content in rice, with substantial expected genetic progress. Together, these results confirm that SPAD is a heritable trait responsive to selection across crops and environments.

In both F<sub>3</sub> and F<sub>4</sub> generations, selection for higher SPAD was consistently associated with significant improvements in grain yield per plant. Similar outcomes have been reported in chickpea, where higher SPAD values were linked with greater seed yield under water stress (Kashiwagi *et al.*, 2010). More recently, Wang *et al.* (2024) observed strong SPAD–yield correlations in spring wheat, with SPAD contributing to high drought tolerance indices, while Mutanda *et al.* (2024) demonstrated that integrating SPAD into canopy reflectance-based models improved yield prediction under stress. Besides yield, this study also showed that both high- and low-SPAD selections enhanced 1000-grain weight under drought. Comparable findings have been reported in wheat, where genotypes with higher SPAD values exhibited superior yield and kernel weight (Javed *et al.*, 2022), and in rice, where SPAD was significantly associated with 1000-kernel weight and yield variation under drought (Xu *et al.*, 2023). Thus, SPAD consistently emerges as a heritable, reliable trait with direct implications for yield improvement in stressed environments.

At the molecular level, Start Codon Targeted (SCoT) markers provided an efficient tool for assessing genetic diversity. These markers target conserved regions adjacent to the ATG start codon, amplifying protein-coding sequences and revealing functional variation associated with traits of interest (Collard and Mackill, 2009; Xiong *et al.*, 2011). Compared with RAPD and ISSR, SCoT markers are more reproducible and cost-effective, requiring no prior sequence data (Gorji *et al.*, 2011; Rai, 2023). In this study, the high polymorphism (91.6%) observed across populations confirms the utility of SCoT markers for detecting variation under drought. Similar high levels of polymorphism and resolution were reported in wheat and cereals by Amirmoradi *et al.* (2012). Although CBDP and SSR markers sometimes yield higher PIC values, SCoT remains a valuable and economical option, and its integration with complementary systems could improve the precision of diversity assessments (Etminan *et al.*, 2016; El-Esawi *et al.*, 2022).

The effectiveness of SCoT markers in this study was reflected by the high resolving power ( $R_p = 7.22$ ) and diversity index ( $DI = 3.60$ ). In particular, SCoT 66 exhibited the highest resolving power (15.78), suggesting strong potential for marker-assisted selection. The broad range of marker index values (0.17–9.59) also demonstrated their capacity to capture variation, consistent with previous studies in wheat and barley (Etminan *et al.*, 2016). Cluster analysis grouped the genotypes into two main clusters: low-SPAD lines (L1, L2, SH3) and high-SPAD lines (H1, H2, H3, SH1, SH2). The distinct position of L1 suggests a unique genetic background. Comparable clustering patterns were reported in wheat under salt stress, where SCoT and ISSR markers separated tolerant from susceptible genotypes (El-Esawi *et al.*, 2022), and in Tritipyrum and barley populations (Abdel-Lateif and Hewedy, 2018; Etminan *et al.*, 2016). These findings highlight the capacity of SCoT markers to distinguish

drought-tolerant and SPAD-related genotypes, offering practical value for wheat improvement programs.

## Conclusion

This study demonstrates that SPAD is a dependable, heritable, and responsive physiological trait that can serve as an effective secondary selection criterion for enhancing drought tolerance in wheat. Consistent selection for higher SPAD values across generations resulted in significant improvements in both grain yield and 1000-grain weight, confirming its value in maintaining yield stability under water-limited conditions. On the molecular level, the high polymorphism and discriminatory capacity of SCoT markers proved efficient in detecting genetic variation and differentiating drought-tolerant genotypes from susceptible ones. The clustering patterns further suggest that SPAD-related traits are linked to distinct genetic backgrounds, which can be strategically exploited in breeding programs. Taken together, these findings highlight the complementary potential of combining SPAD-based phenotypic selection with SCoT marker-assisted diversity analysis. Such integration offers a promising pathway to accelerate the development of drought-resilient wheat cultivars, thereby contributing to food security and sustainable agriculture under increasing climatic and water constraints.

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## الانتخاب لمحتوى الكلوروفيل والتنوع الجزيئي المعتمد على الواسم الجزيئي الـ SCoT في التراكيب الوراثية للقمح تحت ظروف الجفاف

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### الملخص

إجهاد الجفاف يُعد من أبرز القيود التي تحد من إنتاجية القمح، مما يستلزم تطوير استراتيجيات فعّالة للانتخاب من أجل تحسين القدرة على تحمل الإجهاد. في هذه الدراسة، تم تقييم ثلاث عشائر من الجيل الثاني من القمح لصفة محتوى الكلوروفيل، والمقدّر من خلال قراءات جهاز SPAD، تحت ظروف الجفاف عبر دورتين من الانتخاب التبايني. وفي الوقت ذاته، تم تسجيل وزن الألف حبة وغلة النبات الفردي لتقييم الارتباط بين الصفات الفسيولوجية والصفات المرتبطة بالإنتاج.

أدى الانتخاب التكراري المستهدف لارتفاع قيم SPAD إلى تحسينات معنوية في محتوى الكلوروفيل، حيث أظهرت العشيرة الثالثة أكبر تقدّم. كما ارتبطت القيم المرتفعة لـ SPAD ارتباطاً موجباً بصفات الغلة، مما يشير إلى إمكانية اعتماد محتوى الكلوروفيل كمعيار انتخابي موثوق تحت ظروف الجفاف. تراوحت تقديرات المكافئ الوراثي للصفات المدروسة بين المنخفض والعالي، مما يعكس وجود تحكم وراثي متباين بين العشائر والصفات.

ولإثراء التقييم المظهري، تم تحليل التنوع الجزيئي باستخدام مؤشرات SCoT، حيث أظهرت النتائج مستوى مرتفعاً من التعدد الشكلي بلغ 91.6% بمتوسط قيمة لمعامل المعلومات المتعددة الأشكال (PIC) قدره 0.36، مما يعكس تنوعاً وراثياً كبيراً بين التراكيب المدروسة.

بوجه عام، فإن دمج الانتخاب التكراري المعتمد على SPAD مع تحليل العلامات الجزيئية يوفر إستراتيجية قوية وفعّالة لتعزيز تحمل الجفاف وتحقيق استقرار في إنتاجية قمح الخبز. وتؤكد هذه النتائج على أهمية الجمع بين الأدوات الفسيولوجية والجزيئية في برامج التربية الهادفة إلى تطوير أصناف قمح أكثر تحملاً للإجهاد.

**الكلمات المفتاحية:** إجهاد الجفاف، الانتخاب، المكافئ الوراثي، الواسم الجزيئي الـ SCoT، قمح الخبز، محتوى الكلوروفيل