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## Physiological Parameters and Drought Tolerance in some F<sub>4</sub> Promising-Glaucousness Families of Bread Wheat

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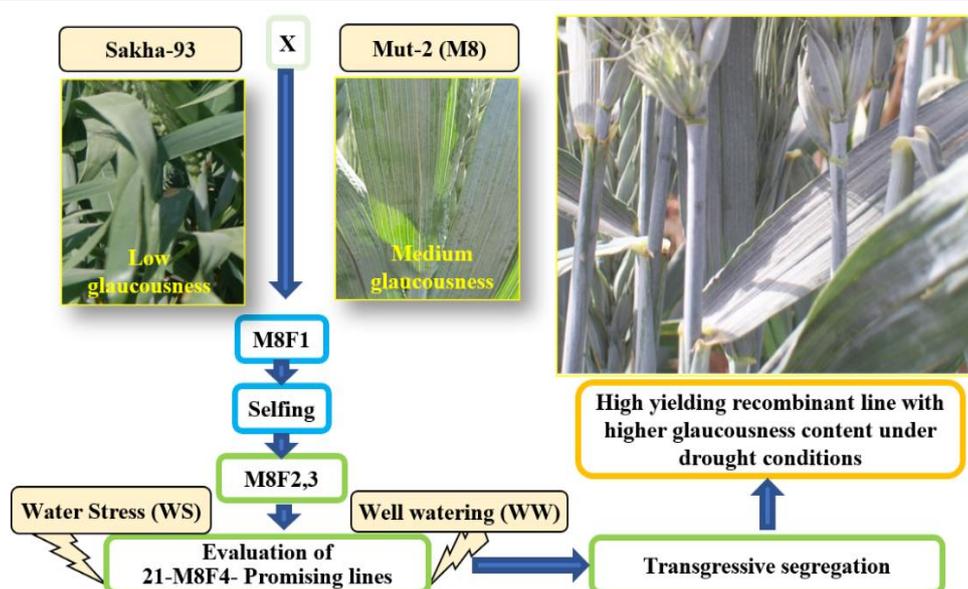
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### HIGHLIGHTS

1. The crosses between wheat mutations and elite genotypes show higher efficiency in pyramiding desirable traits.
2. The glaucous trait is critical to encountering abiotic stresses.
3. Novel F<sub>4</sub> Family has excellent superiority in yield and its attributes.

### GRAPHICAL ABSTRACT



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### ABSTRACT

The selection from segregating generations of hybrid wheat combinations succeeded in developing new genotypes that possess adaptive drought tolerance traits, such as glaucousness. This study was to develop new glaucousness high-yielding recombinants under drought conditions. Twenty-one promising F<sub>4</sub> families selected from segregating generations of a cross between a glaucous mutant and an Egyptian cultivar were evaluated for superiority to their parents and the best check cultivar under water stress (WS) and well watering (WW) conditions. A split-plot design with randomized complete blocks was utilized in three replications of the experiment. A total of 25 genotypes were planted in the main plots and two irrigation regimes were assigned to the subplots (21 F<sub>4</sub> families, two parents and two check cultivars). Transgressive segregation occurred in yield components and physiological attributes, except for spike length. Based on drought tolerance index values, only eleven F<sub>4</sub> families were found tolerant and superior in grain yield/plant (GYPP) to the higher parent and the higher check cultivar under WS and WW. The superiority in GYPP reached in the F<sub>4</sub> family No.4 to 43.27 and 55.65 % and 50.31 and 77.86 % over the higher parent and the check variety under WW and WS, respectively. The top seven grain-producing and drought-resistant families have been discovered. Such genotypes were shown to have a high wax content, a high net photosynthesis rate, and a high stomatal conductance under both WS and WW conditions. These F<sub>4</sub> families should be suggested to wheat breeders seeking to increase yields under drought conditions.

## INTRODUCTION

In a variety of climates and soils, wheat (*Triticum aestivum* L.) is an essential food crop. It is regarded as an integral part of the daily diet in different geographic regions of the world whereas it is a source of energy and supplies 70–75% calories and 10–15% proteins in a daily diet [1]. It is cultivated on 215.9 million hectares area with the estimated production of 765.8 million tons with 3.55 tons /ha grain yield (FAO, 2019). Wheat's demand is likewise rising as the population grows. Thus, careful management of the limited resources, including the efforts of wheat breeders to develop wheat varieties that can thrive in water-stressed environments, is necessary.

Drought is one of the most important abiotic factors that reduce the yield. Whereas, the drought incidence and severity will certainly increase in the coming future as a result of global warming that will directly cause a rigorous decline in the overall production of food [2]. It can cause up to 90% wheat yield loss depending on the growth stage, genotype, intensity and duration of drought [3]. Drought stress results in inhibition of photosynthesis that have been associated with a decrease in chlorophyll content, cell membrane stability, causing loss of membrane permeability and damage to the various physiological and biochemical functions that eventually affect the growth of the plant [4]. Hence, it is important to develop high-yielding wheat cultivars with high drought tolerance in order to improve food security.

Glaucousness, the waxy bloom on the surface of leaves, leaf sheaths and spikes, has been associated with improved grain yields in crops such as wheat [5; 6; 7]. Abiotic and biotic stressors, such as dryness and UV, can be protected by the leaf cuticular wax [8; 9] in previous investigations. Additionally, it can help maintain a reasonably high photosynthetic rate and a comparably high yield under drought stress by reducing the drop in leaf water potential [8].

The fundamental factor of crop biomass during the growing season is photosynthesis [9]. Stomata are critical gateways for gas and water exchange in plants, exerting a considerable influence on photosynthesis and transpiration-related features. Stomata are essential to the plant's survival because they regulate temperature and water use efficiency. Different species and cultivars within a species have a wide range of stomata sizes and densities. Because of this, stomatal traits are heavily influenced by the environment. However, plants often develop leaves with lower maximum stomatal conductance due to changed apical stomatal density and/or size under situations of protracted water deficit in

addition to increasing water use efficiency through stomatal aperture reduction under drought stress [10].

The current study was conducted to evaluate 21 promising F<sub>4</sub> families selected from segregating generations of a hybrid between a glaucous mutant, and high-yielding Egyptian commercial variety for their superiority to its parents and the best check cultivar under water stress and non-stress conditions.

## MATERIALS AND METHODS

### Plant materials and experimental layout

The study was carried out during three growing seasons (2016-2019) at the Experimental Farm of the Plant Research Department, Nuclear Research Center, Inshas, El-Sharkyia Governorate (latitude of 30°24' N; longitude of 31°35' E; altitude 20 m). In season 2017/2018, Twenty-one promising F<sub>4</sub> families (from F<sub>4</sub>-1 to F<sub>4</sub>-21) were selected from crossing between a glaucous mutant (Mut-2) and an Egyptian commercial cultivar (Sakha-93).

In the season 2018/2019, 21 promising F<sub>4</sub> families and its parents (Mut-2 and Sakha-93) and two check cultivars (Shandaweel-1 and Giza-171) were planted under two irrigation levels as well-watered (WW) (every five days) and water-stressed (WS) (every 15 days) regimes in sandy loam soil. The seeds of these families were planted as individual plants in separate rows in a split-plot design with three replications. Days number to 50% heading date (DH), days number to 50% physiological maturity date (DM), plant height (PH), spike length (SL), fertile spikes number per plant (SPP), grains number per spike (GPS), main spike weight (SW), 100-Grain weight (100-GW) and grain yield/plant (GYPP) were recorded. Also, physiological parameters *viz.*, net photosynthesis (PN), and stomatal conductance (gs) were measured on the flag leaf at heading, anthesis, and grain filling stages (110-120 days old) [11]. Gas exchange measurements were done on five samples per plot for 2 minutes per sample in the morning between 8 AM and 12 PM using an Ultra-Light Portable Photosynthesis System (CID, CI- 340 CO<sub>2</sub>, Inc. Vancouver, Washington State, USA).

### Epicuticular wax quantification

The epicuticular wax content was determined according to Ebercon's protocol [12]. Thirty flag leaf blades from each genotype were submerged in 15 ml redistilled chloroform for 15 seconds each. At 35°C, the extracts were filtered and evaporated. The leftovers were weighed after drying for 24 hours at room temperature. The quantity of wax was determined as Mg/dm<sup>2</sup> of each sample against the leaf area (both leaf surfaces).

### Physiological parameters

At grain filling (110-120 days old), different physiological parameters were measured on the flag leaf. Gas exchange measurements were done on five samples per plot for 2 min per sample. Analyses were done from (8 am – 12 pm) using an Ultra-Light Portable Photosynthesis System (CID, CI- 340 CO<sub>2</sub>, Inc. Vancouver, Washington State USA). The instrument was operated within an open system with a leaf chamber. These measurements included net photosynthesis (PN), and stomatal conductance (gs).

### Statistical analysis

The data were analyzed for analysis of variance (ANOVA) by two-factor in split-plot design and mean comparison was carried out by Duncan Multiple Range test using MSTAT-C Statistical software. Pearson's correlation coefficients between studied traits and their significance were calculated using SAS v9.4 software. While the drought tolerance index (DTI) was calculated as the following formula [13]:

$$DTI = (\text{Yield value under WS} / \text{Yield value under WW}) \times 100$$

## RESULTS AND DISCUSSION

### Analysis of variance

Analysis of variance (Table 1) indicated that mean squares due to the watering regime (W), genotype (G)

and W × G interaction were significant ( $P \leq 0.01$  or  $P \leq 0.05$ ) for all studied traits, except W × G interaction for spike length, suggesting that water stress had a significant effect on all studied traits and the existence of significant differences among the 25 studied genotypes (21 F<sub>4</sub> families + 2 parents + 2 check cultivars) for all studied 12 traits. The significance of interaction variance indicates that the rank of genotype differs from well watering to water stress environment for all studied traits, except for spike length. The coefficient of variation (CV) was very small in magnitude (<5%), indicating the accuracy of implementing the field experiment.

### Effect of water stress

Water stress caused a significant ( $P \leq 0.01$  or  $P \leq 0.05$ ) reduction in most studied traits, ranging from 1.31% for days to 50% maturity to 22.55% for net photosynthetic rate and 21.56% for stomatal conductance (Table 2). On the contrary, the wax content trait increased due to water stress by 91.85%. Grain yield/plant (11 %) reduction was associated with decreases in all yield components, including spikes/plant (8.04%), spike length (6.14%), 100-grain weight (1.94%), spike weight (1.37%) and grains/spike (18.22%). Plant height was decreased due to water stress by 3.17%.

**Table (1): Analysis of variance of split-plot design for studied traits of 25 genotypes under two irrigation regimes**

SV	df	Mean squares					
		Plant height	Days to 50% Heading	Days to 50% Maturity	Spikes/plant	Spike weight	Spike length
Replication (R)	2	0.25	0.14	1.21	0.19	0.001	0.05
Watering(W)	1	365.04**	94.41**	96.0**	44.83**	0.22**	42.67**
W(R)	2	0.14	1.21	0.62	0.19	0.01	0.13
Genotype (G)	24	304.18**	148.3**	150.2**	42.67**	0.65**	19.82**
W*G	24	4.39**	0.77**	0.82*	0.97*	0.01*	0.28
Error	96	0.67	0.35	0.49	0.53	0.01	0.41
CV%		0.85	0.75	0.58	5.61	1.57	3.77
		Grains/ spike	100-Grain weight	Grain yield/plant	Wax content	Net photo rate	Stomatal conductance
Replication (R)	2	0.09	0.01	18.17	0.14	3.94	11.01
Watering(W)	1	9064**	0.53**	1952**	343.7**	872**	7605**
W(R)	2	0.73	0.01	2.78	0.4	0.04	5.66
Genotype (G)	24	249**	0.85**	466.8**	3.51**	44.83**	298**
W*G	24	41.4**	0.01**	10.97**	1.24**	2.21**	25.4**
Error	96	1.19	0.01	2.22	0.13	0.35	7.52
CV%		1.41	1.51	2.4	7.62	3.11	4.65

\*and\*\* indicate significant at 0.05 and 0.01 probability levels, respectively.

**Table (2): Effect of water stress on studied traits**

Trait	WW	WS	Change%
Plant height (cm)	98.57	95.45	3.17**
50% Heading (day)	79.69	78.11	1.98**
50% Maturity (day)	122.5	120.9	1.31**
Spikes/plant	13.56	12.47	8.04**
Spike weight (g)	5.11	5.04	1.37**
Spike length (cm)	17.43	16.36	6.14**
Grains/spike	85.35	69.8	18.22**
100-Grain weight (g)	5.67	5.56	1.94**
Grain yield/plant (g)	65.62	58.4	11.00**
Wax content (mg/dm <sup>2</sup> )	3.3	6.3	-91.85**
Net photosynthetic rate (CO <sub>2</sub> μmol.m <sup>-2</sup> .s <sup>-1</sup> )	21.37	16.55	22.55**
Stomatal conductance (mmol.m <sup>-2</sup> .s <sup>-1</sup> )	66.1	51.8	21.56**

\*and\*\* indicate significant at 0.05 and 0.01 probability levels, respectively, – indicates decrease.

Shortage of water at any growth stage in the crop life cycle is likely to have consequences for yield and there are several ways in which water stress can affect grain yield, the first by modification of early growth and ear development. The simultaneously occurring processes of tiller production and spikelets initiation are followed immediately before anthesis by a period in which a proportion of tillers and florets die. The second major yielding-determining process affected by stress is the production of fertile gametes and fertilization [14], which determines the proportion of the potential grain number realized. These processes are probably responsible for determining the critical period before anthesis, during which water stress usually has the most detrimental effect on yield [15].

Seed production and kernel weight are two of the most critical aspects of grain yield that can be affected by drought during flowering and grain filling. It is common practice for breeders to use indirect selection and well-correlated features to improve production in dry environments because grain yield is a multi-gene trait [16]. Grain assimilation is reduced during grain filling when wheat plants are subjected to drought or heat stress due to a quick drop in photosynthesis. Kernel dry weight decreased significantly as a result [17]. It is consistent with our findings that drought stress reduces wheat grain production [18; 19; 20]. Several investigators also

reported that water stress had a strong negative effect on the number of spikes per plant [21], grains/spike [18; 22], and 100-grain weight [23].

The observed increase in cuticle wax content due to water stress might be considered as a plant mechanism to tolerate drought *via* reduction of transpiration rate in order to retain its cell's water state and to reduce the rate of water loss through the cuticle [6; 7; 24].

### Effect of genotype

The two parents of the hybrid (Mut-2 and Sakha 93), from which the 21 F<sub>3</sub> families were derived, differed significantly ( $P \leq 0.01$ ) for all studied traits (Table 3). Their divergence was more pronounced for spike length, grain yield/plant, 50% heading and maturity, spike weight, grains/spike and wax content. The parent Mut-2 had a long spike, few number of spikes/plant, heavier grain, higher grain yield, later in heading and maturity, heavier spike weight, a higher number of grains/spike, higher wax content, net photosynthetic rate and stomatal conductance than the parent Sakha-93. This divergence between the two parents of the hybrid is helpful to the plant breeder to produce variability in the segregating generations amenable to effective selection and to get useful recombination and transgressive segregation [6; 24].

They tested 21 F<sub>4</sub>-families varied significantly and differed from their parents and the check cultivars (Shandawel-1 and Giza-171) for all studied traits (Table 3). Plant height ranged from 83.33 cm for F<sub>4</sub>-21 to 105.5 cm for F<sub>4</sub>-6 with an average of 97.01 cm. Days to heading ranged from 73 for F<sub>4</sub>-16 and F<sub>4</sub>-18 to 93 for Mutant-2 with an average of 78.9. Days to maturity ranged from 114.84 for F<sub>4</sub>-16 to 137.67 for Mutant-2 with an average of 121.65 days. Spike length ranged from 11.17 cm for (Sakha) to 20.50 cm for Mutant-2 with an average of 16.89 cm. The number of spikes/plant ranged from 7.67 for Mut-2 to 22.33 for F<sub>4</sub>-3 with an average of 13.01. The 100-grains weight ranged from 4.53 g for (Shandawel-1; Sh.) to 6.07 g for F<sub>4</sub>-18 with an average of 5.62 g. Grain yield/plant ranged from 39.85 g for Sakha-93 to 81.17 g for F<sub>4</sub>-3 with an average of 62.01 g.

The spike weight ranged from 4.14 g for Sakha-93 to 5.38 g for F<sub>4</sub>-18 with an average of 5.07 g. The number of grains/spike ranged from 58.5 for Sakha-93 to 84.0 for F<sub>4</sub>-17 with an average of 77.57. Wax content ranged from 3.0 mg/dm<sup>2</sup> for Gaiza.171 to 5.75 mg/dm<sup>2</sup> for F<sub>4</sub>-1 with an average of 4.81 mg/dm<sup>2</sup>. The net photosynthetic rate ranged from 14.15 CO<sub>2</sub> μmol.m<sup>-2</sup>s<sup>-1</sup> for Sakha-93 to

23.4 CO<sub>2</sub> μmol.m<sup>-2</sup>s<sup>-1</sup> for F<sub>4</sub>-3 with an average of 58.93 CO<sub>2</sub> μmol.m<sup>-2</sup>s<sup>-1</sup>. Stomatal conductance ranged from 46.72 mmol.m<sup>-2</sup>s<sup>-1</sup> for Sakha-93 to 71.17 for F<sub>4</sub>-3 with an average of 58.93 mmol.m<sup>-2</sup>s<sup>-1</sup>.

Under both water stress and non-water stress circumstances, bread wheat genotypes differ in grain production [18; 25; 26]. Additionally, some researchers identified genotypic variations in wheat grown under drought-stressed and non-stressed conditions for a number of spikes/plant [22; 25; 27; 28], grains per spike [18; 29; 30], 100-grain weight [22; 23] and plant height [18; 25; 26].

### Effect of genotype × watering regime interaction

Analysis of variance (Table 1) revealed that genotypes differed significantly from well watering to the water-stressed environment for all studied traits, except for the spike length trait. Averages, maximum and minimum values of all studied traits for selected 21 F<sub>4</sub> families, their parents (Mut-2 and Sakha-93) and the check cultivars (Shandawel-1; Sh.) and Giza-171 under well watering (WW) and water stress (WS) conditions are presented in Table (4).

**Table (3): Effect of genotype on studied traits**

Trait	Mean	Max	Min	LSD <sub>0.05</sub>	LSD <sub>0.01</sub>
Plant height (cm)	97.01	105.5 (6)	83.33(21)	0.94	1.24
Days to 50% heading	78.90	93.0(Mut-2)	73.0 (16,18)	0.68	0.89
Days to 50% Maturity	121.65	137.7(Mut-2)	114.84(16)	0.80	1.10
Spikes/plant	13.01	22.34(3)	7.67(Mut-2)	0.84	1.11
Spike weight (g)	5.07	5.38(18)	4.14(Sakha)	0.11	0.15
Spike length (cm)	16.89	20.5 (Mut-2)	11.17(Sakha)	0.73	0.97
Grains/spike	77.57	84.0 (17)	58.5 (Sakha)	1.25	1.66
100-Grain weight (g)	5.62	6.07(18)	4.54(Sh.)	0.10	0.13
Grain yield/plant (g)	62.01	81.17(3)	39.9(Sakha)	1.71	2.26
Wax content (mg/dm <sup>2</sup> )	4.81	5.75(1)	3.00(Gz.171)	0.09	0.12
Net photosynthetic rate (CO <sub>2</sub> μmol.m <sup>-2</sup> s <sup>-1</sup> )	18.96	23.40(3)	14.15(Sakha)	0.68	0.90
Stomatal conductance (mmol.m <sup>-2</sup> .s <sup>-1</sup> )	58.93	71.17(3)	46.72(Sakha)	3.14	4.16

Maximum and minimum values are followed by genotype No. or name (between brackets).

**Table (4): Mean performance (Aver.), maximum (Max) and minimum (Min) values of studied traits for 21 F<sub>4</sub> selected bread wheat families, their parents and two check cultivars grown under well watering (WW) and water stress (WS)**

Trait	Stress	Aver.	Max	Min	Mut-2	Sakha-93	(Shandawel-1; Sh.)	Giza-171	LSD <sub>0.05</sub>
Plant height (cm)	WW	99.50	108.3(6)	84.3(21)	85.30	88.30	95.30	106.70	1.33
	WS	96.60	103.7(4)	82.3(21)	82.30	85.70	92.30	97.30	
Days to 50% heading	WW	79.70	84.7(1)	73.3(18)	94.30	74.30	87.70	89.70	0.96
	WS	78.10	82.7(1)	72.3(16,19,20)	91.70	72.30	85.00	86.70	
Days to 50% Maturity	WW	122.50	127.7(1)	114.7(16)	139.00	118.70	126.30	128.70	1.13
	WS	120.90	125.3(1,3)	115.0(16)	136.30	117.30	124.70	126.70	
Spikes/plant	WW	13.60	24.0(3)	11.7(18)	7.70	10.00	12.70	13.00	1.18
	WS	12.50	20.7(3)	11.0(11)	7.70	10.00	10.30	10.30	
Spike weight (g)	WW	5.10	5.4(18,21)	4.9(4)	5.20	4.10	4.30	4.80	0.16
	WS	5.00	5.3(2,6,8,18)	4.9(4)	5.10	4.20	4.10	4.40	
Spike length (cm)	WW	17.40	19.7(12)	15.7(7,10)	21.70	11.70	16.70	16.00	1.04
	WS	16.40	18.0(12)	14.7(10)	19.30	10.70	15.70	15.00	
Grains/spike	WW	85.30	94.3(17)	81.3(1)	87.30	64.70	68.00	71.30	1.77
	WS	69.80	76.7(1)	67.3(20)	68.00	52.30	56.30	54.30	
100-Grain weight (g)	WW	5.70	6.1(18)	5.4(4)	5.50	4.80	4.70	5.60	0.16
	WS	5.60	6.0(2,18)	5.4(4,5)	5.40	4.50	4.40	5.10	
Grain yield/plant (g)	WW	65.60	84.7(20)	59.0(20)	59.10	42.00	54.30	56.30	2.41
	WS	58.40	77.7(3)	53.0(18)	49.90	37.70	43.70	42.30	
	Change%	<b>10.98</b>			<b>15.57</b>	<b>10.24</b>	<b>19.52</b>	<b>24.87</b>	
Wax content (mg/dm <sup>2</sup> )	WW	3.30	4.3(14)	2.8(11)	1.80	3.00	1.90	1.80	0.58
	WS	6.30	8.1(15)	5.2(6,14)	4.50	5.20	4.60	4.20	
	Change%	<b>-90.91</b>			<b>-150.00</b>	<b>-73.33</b>	<b>-142.11</b>	<b>-133.33</b>	
Net photosynthetic rate (CO <sub>2</sub> μmol.m <sup>-2</sup> .s <sup>-1</sup> )	WW	21.40	26.2(3)	17.3(20)	17.60	15.60	16.50	17.10	0.96
	WS	16.60	20.6(3)	13.3(18)	13.30	12.70	13.20	12.80	
	Change%	<b>22.43</b>			<b>24.43</b>	<b>18.59</b>	<b>20.00</b>	<b>25.15</b>	
Stomatal conductance (mmol.m <sup>-2</sup> .s <sup>-1</sup> )	WW	66.00	81.7(3)	56.0(20)	56.00	53.90	54.30	55.90	4.44
	WS	51.80	60.6(3)	44.8(18)	42.90	39.50	41.60	40.30	
	Change%	<b>21.52</b>			<b>23.39</b>	<b>26.72</b>	<b>23.39</b>	<b>27.91</b>	

Maximum and minimum values are followed by genotype No. or name (between brackets).

The shortest plant height was shown by the F<sub>4</sub> family No. 21 under both of WW and WS environments. The F<sub>4</sub> family No. 3 had the highest, spike length, number of spikes/plant, net photosynthetic rate and stomatal

conductance under WW and WS environments and the highest grain yield under water stress conditions. This family is a promising genotype to produce a promising pure line of wheat that could prove a valuable variety to  
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be grown under water-stressed and non-stressed environments after testing its stability and adaptability. This family produced the highest wax content, net photosynthetic rate and stomatal conductance when was subjected to drought stress. The highest 100-grain weight was shown by family No. 18 under WW and WS conditions and by family No. 2 under WS conditions. The highest spike weight was exhibited by family No. 18 under both water stress, family No.21 under WW conditions and families No.2,6 and 8 under WS conditions. The number of grains/spike was the highest by family No. 17 under WW and No. 1 under WS conditions.

It should be noted that the transgressive segregation occurred in the traits of plant height (No.6 and No. 4), spikes/plant (No.3 and No.3), 100-grain yield (No.2 and No.18), grains/spike (No. 17 and No. 1), wax content (No. 14 and No. 15), net photosynthetic rate (No. 3 and No. 3) and stomatal conductance (No. 3 and No. 3) under WW and WS conditions, respectively. No transgressive segregation was observed for spike length.

Transgression segregation occurs when severe phenotypes or transgressive phenotypes are exhibited in segregated hybrid populations in comparison to the parental lines' phenotypes [6]. Transgressive phenotypes result when progeny plants contain new combinations of multiple genes with more positive effects or negative effects for a quantitative trait than were present in either parent. According to the definition of Vega and Frey [31], a transgressive line has to exceed the parental mean by one "least significant difference" value.

Recombinants and transgressive segregates have already been found in many crops [6; 24; 30; 31; 32]. This phenomenon was studied in hybrid populations, as well as its genetic foundation and the best factors for predicting its prevalence [30]. In just 113 research, extreme phenotypes for at least one character were not recorded, and 336 (58 %) of the 579 characteristics studied throughout the 113 research showed transgression. Transgression appears to be the norm rather than the exception in plant hybrids, according to the researchers. From classical genetics research, there is compelling evidence that transgressive segregation can

be produced by the expression of rare recessive alleles and/or complementing gene activity [6; 24; 31].

The greater genetic divergence between the two parents under study was accompanied by an increase in the number of fixed differences between the parents, resulting in new recombination and transgressive segregations for the number of grains/spike, the number of spikes/plant, 100-grain weight, and grain yield/plant. When it came to grain yield and other associated variables, numerous recombinants showed transgressive segregates. The transgressive segregates for the majority of the qualities examined indicated that the two parents chosen for this study possessed genes associated with high values for these traits. More research will be done to ensure that the transgressive segregates are genetically distinct from their parents at the molecular level. These high-yielding segregates selected in this study will be used as a germplasm sources for enhancing wheat productivity in WW and WS conditions.

#### **Drought tolerance index (DTI) and productivity of F<sub>4</sub> families, their parents and checks**

The values for the drought tolerance index (DTI) for the genotypes studied are listed in Table (5). They were estimated under stressful conditions using the Fageria (1992) equation. When DTI is  $\geq 1.0$ , the genotype is tolerant (T), and when DTI is  $< 1.0$ , the genotype is sensitive (S). The wheat genotypes investigated were classified into two groups based on their DTI values: tolerant (11 F<sub>4</sub> families) and sensitive (10 F<sub>4</sub> families, two parents and two check varieties). The highest DTI under the drought-stressed environment was exhibited by the F<sub>4</sub> family No.3. The 2<sup>nd</sup> highest genotype in DTI was the F<sub>4</sub> family No.7. The 3<sup>rd</sup>, 4<sup>th</sup> and 5<sup>th</sup> highest genotypes in DTI were the F<sub>4</sub> families No.1, No. 2 and No. 4, respectively. In general, the five most tolerant genotypes were F<sub>4</sub> families No. 3, 7, 1, 2 and 4, in descending order; their GYPP was the highest under water-stressed and non-stressed environments (Table 5). These genotypes should be suggested to wheat breeding groups pursuing drought tolerance improvement. On the contrary, the parent (Sakha-93) was the most drought-sensitive genotype; its grain production was the lowest (Table 5).

**Table (5): Drought tolerance index (DTI) and grain yield/plant (GYPP) under well watering (WW) and water stress (WS) of 21 F<sub>4</sub> families and their superiority to higher parent and check**

Genotype	DTI	GYPP (g)		Superiority % under WW		Superiority % under WS	
		WW	WS	Higher Parent	Higher check	Higher parent	Higher check
F <sub>4</sub> -1	1.31	73.37	68.5	24.15	30.25	37.27	56.86
F <sub>4</sub> -2	1.30	73.03	68.03	23.57	29.65	36.33	55.78
F <sub>4</sub> -3	1.72	84.67	77.67	43.27	50.31	55.65	77.86
F <sub>4</sub> -4	1.27	73.4	66.47	24.20	30.30	33.21	52.21
F <sub>4</sub> -5	1.20	72.2	63.73	22.17	28.17	27.72	45.94
F <sub>4</sub> -6	1.12	67.7	63.37	14.55	20.18	26.99	45.11
F <sub>4</sub> -7	1.33	76.23	66.8	28.98	35.33	33.87	52.97
F <sub>4</sub> -8	1.18	70.6	63.93	19.46	25.33	28.12	46.39
F <sub>4</sub> -9	1.24	72.97	65.07	23.47	29.54	30.40	49.00
F <sub>4</sub> -10	1.22	73.87	63.47	24.99	31.14	27.19	45.34
F <sub>4</sub> -11	1.15	66.43	66.43	12.40	17.93	33.13	52.12
F <sub>4</sub> -12	0.94	63.63	56.43	7.66	12.96	13.09	29.22
F <sub>4</sub> -13	0.97	64.93	57.27	9.86	15.27	14.77	31.14
F <sub>4</sub> -14	0.98	65.9	56.97	11.51	16.99	14.17	30.46
F <sub>4</sub> -15	0.92	63.13	55.93	6.82	12.07	12.08	28.07
F <sub>4</sub> -16	0.92	64.37	55.07	8.92	14.27	10.36	26.10
F <sub>4</sub> -17	0.86	61.33	54.03	3.77	8.88	8.28	23.72
F <sub>4</sub> -18	0.83	60.03	53.00	1.57	6.57	6.21	21.36
F <sub>4</sub> -19	0.87	60.27	55.37	1.98	6.99	10.96	26.79
F <sub>4</sub> -20	0.82	58.97	53.27	-0.22	4.69	6.75	21.98
F <sub>4</sub> -21	0.90	61.67	55.70	4.35	9.48	11.62	27.55
Mut-2	0.77	59.1	49.9				
Sakha-93	0.41	42	37.7				
Shandawel-1	0.62	54.33	43.67				
Giza-171	0.62	56.33	42.33				
LSD0.05		2.41					

Grain yield/plant of the F<sub>4</sub> family No. 3 was 84.67 and 77.67 g, while the yield of its parents was 59.1 and 49.9 g for Mut-2 and 42.0 and 37.7 g for Sakha-93 under WW and WS, respectively, indicating the occurrence of transgressive segregates and that its superiority to the higher-yielding parent (Mut-2) is 43.27 % and 55.65 % under WW and WS, respectively (Table 5). Also, GYPP was superior to the best check cultivar under WW (Giza-171) (56.33 g) by 50.31 % and under WS (Shandawel-1) (43.67 g) by 77.86 %. It is clear that the superiority of the F<sub>4</sub> family in GYPP is more pronounced under water-stressed environment, which could be explained by its superiority in the physiological processes, namely extra production of wax content (glaucousness), high photosynthetic rate and high stomatal conductance (Table 4), which keeps the water status of the cells in good condition under drought stress. Under WW, the

2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup> 6<sup>th</sup> and 7<sup>th</sup> places in superiority to the higher parent or higher check came to the F<sub>4</sub> families No. 7, 10, 4, 1, 2 and 9 in descending order. Under WS, the 2<sup>nd</sup>, 3<sup>rd</sup>, 4<sup>th</sup>, 5<sup>th</sup> 6<sup>th</sup> and 7<sup>th</sup> places in superiority to the higher parent or higher check came to the F<sub>4</sub> families No. 1, 2, 7, 4, 11 and 9 in descending order. These F<sub>4</sub> families should be suggested to wheat breeding projects whose objectives include increasing productivity and drought tolerance.

#### Grouping based on grain yield and drought tolerance

The mean grain yield per plant under water stress (WS) was plotted against the drought tolerance index of the same genotypes (Fig. 1), allowing for the distinction of four groups: tolerant high yielding, tolerant low yielding, sensitive high yielding, and sensitive low yielding [33].

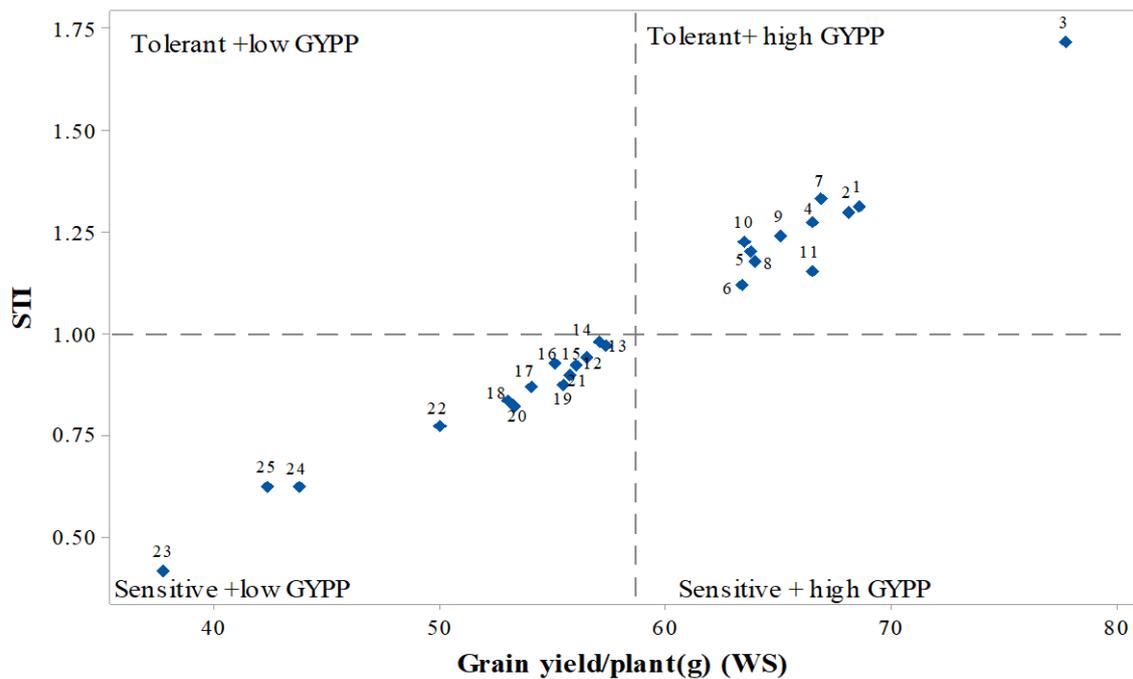


Fig. (1): Relationships between stress tolerance index (STI) of 25 wheat genotypes and GYPP under water-stressed environment. Broken lines represent the mean of STIs and GYPP (numbers from 1 to 25 refer to genotype numbers mentioned in Table 5)

The genotypes No. 3 followed by 1, 2, 7, 4, 11, 9, 10, 8, 5 and 6 were classified as most drought tolerant and high yielding in this study. On the contrary, the rest of the genotypes were classified as water stress-sensitive and low-yielding (Fig. 1).

### Trait interrelationships

One approach to increasing the efficiency of selection in a stressed environment relies on the use of correlated secondary traits. Correlations between grain yield, yield components, phenological, and physiological traits may be of value in determining useful criteria for drought tolerance. Estimates of Pearson's correlation coefficients ( $r$ ) between pairs of studied traits and stress tolerance index across all genotypes, and across stressed and non-stressed environments (Fig. 2).

As previously shown [34; 35], high grain yield is an optimal trait for selecting for high drought tolerance as indicated by the stress tolerance index's (STI's) perfect positive and significant ( $P \leq 0.01$ ) correlation coefficient ( $r=0.99$ ) with grain yield/plant. Net photosynthetic rate (0.94) and stomatal conductance (0.94) were strongly and statistically significant correlated ( $P \leq 0.01$ ) with STI, while grains/spike (0.53) and wax content (0.53) were less strongly correlated ( $P \leq 0.01$ ) (0.42).

A positive and statistically significant association coefficient ( $P \leq 0.01$ ) was found between the following variables and grain yield/plant: spikes/plant, 100-grain weight, grains/spike, net photosynthetic rate, stomatal conductance, and wax content. Between GYPP and DTI, net photosynthetic rate, and stomatal conductance, as well as between net photosynthetic rate and stomatal

conductance, there was a significant correlation. Each day to heading had a significant positive correlation with the number of days to physiological maturity (DTM). There was no correlation between plant height and any of the other characteristics examined. Numerous investigations have discovered a significant correlation between GYPP and wax concentration [36].

We found that drought-resistant genotypes were shown to have high net photosynthetic rates and the ability to store water in their cells in both WS and WW environments, according to this study. Several additional researchers have reached the same result [37; 38; 39; 40; 41]. These characteristics might be used as selection criteria for drought tolerance in wheat.

Wheat grain production and number of spike<sup>-1</sup> grains were shown to be significantly correlated under drought stress [42]. Grain weight and the number of grains per spike had significant, positive, and direct effects on grain yield under both water stress and well-watered circumstances, according to [41]. In the event that these qualities show a high degree of heritability and high predicted genetic advancement as a result of selection, they might be employed as selection criteria for wheat drought toleration. Breeders have employed a variety of yield measures to assess drought stress on wheat plants, including days to heading, days to maturity, spike length, number of grains per spike, hundred kernel weight, and grain yield per plant. Many different traits may be used to measure drought tolerance, or drought indices that accurately measure genotypic yield response to drought stress [16; 43]. The findings of Al-Naggar et al. [35] support this result.

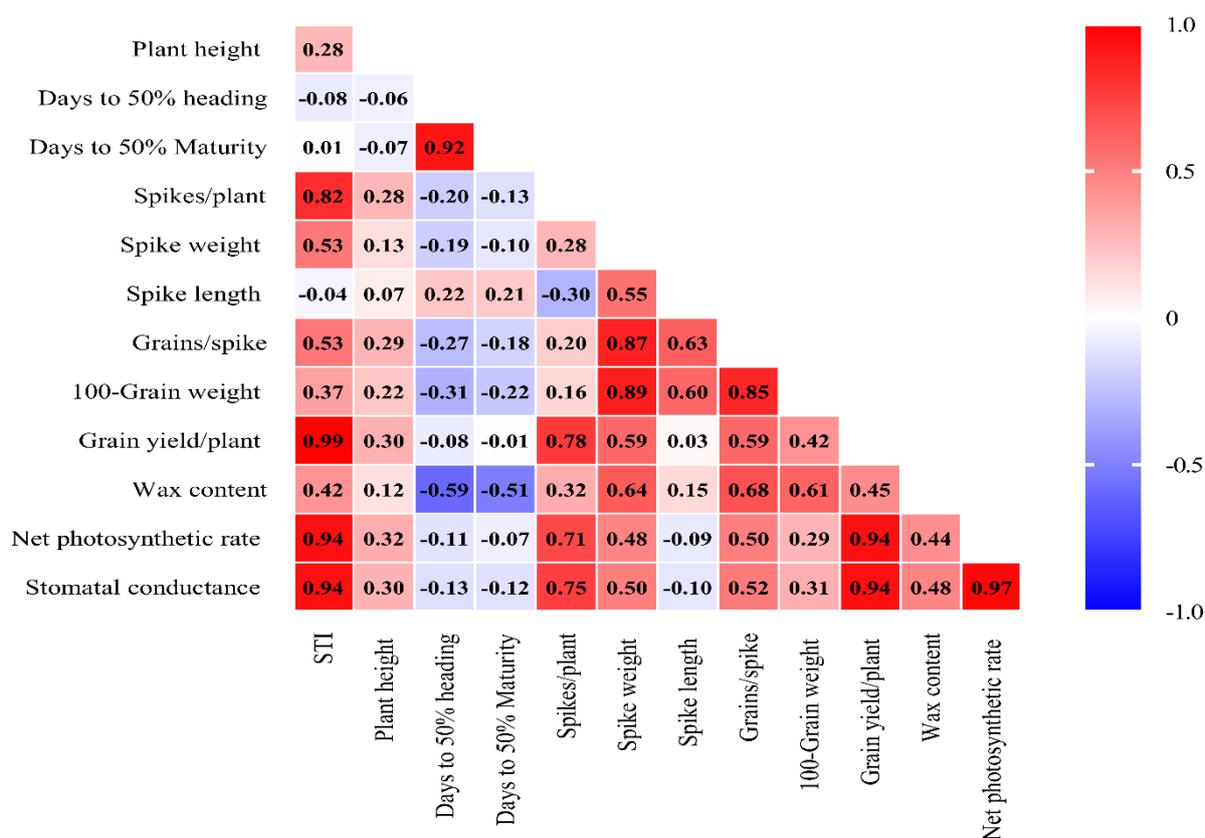


Fig. (2): Pearson's correlation coefficients between the measured traits and stress tolerance index (STI) for all studied genotypes

## CONCLUSION

The observed increase in cuticle wax content due to water stress might be considered as a plant mechanism to tolerate drought *via* reduction of transpiration rate in order to retain its cell's water state and to reduce water loss rate through the cuticle. The transgressive segregation occurred in the traits grain yield/plant, plant height, spikes/plant, 100-grain yield, grains/spike, wax content, net photosynthetic rate and stomatal conductance under WW and WS, respectively. This study was able to identify the most promising F<sub>4</sub> families for high grain yield, glaucous and drought tolerance. The results of this study reveal that drought-tolerant high-yielding genotypes have a high net photosynthetic rate and a high stomatal conductance, i.e. a high capacity to retain water in their cells, under both WS and WW circumstances. Further studies should be conducted to assure the genetic divergence of the transgressive segregates from their parents on the molecular level. Moreover, these segregates selected in this study would be used as a germplasm source for improving wheat productivity under drought stress.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Author contribution

Al-Azab carried out all experimental work, Basyouny has done the physiological work, Younis and Osamy analyzed the data. Al-Naggar wrote the paper, Ayaad and Basyouny designed and guided this study and revised the paper. The authors declare no competing financial interests.

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