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Full Length Article

Impact of osmotic stress on seedling growth observations, membrane characteristics and antioxidant defense system of different wheat genotypes

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ABSTRACT

The objective of the present study was to find out a straightforward technique for screening the tolerance of ten wheat genotypes to two levels of osmotic stress at early seedling stage. Data revealed that polyethylene glycol-induced drought had general negative effect on seedling morphological characters indicated by plumule and radicle length, number of adventitious roots as well as seedling biomass and water content. Water deficit could also suppress membrane integrity by stimulating lipid peroxidation with marked increase in membrane leakage and subsequent decrease in its stability index. For all the addressed germination parameters and seedling membrane features, the impact of severe drought was more pronounced than that of moderate drought. Simultaneously, moderate stress could activate peroxidase, polyphenol oxidase and ascorbic peroxidase of the studied genotypes; but these enzymes were inhibited by severe stress. The activity of catalase, superoxide dismutase and glutathione reductase was conversely retarded by drought whether at moderate or severe level. More interestingly, a novel function "Stress Impact Index; SII" was introduced to rank the estimated morpho-physiological traits (SII_{trait}) as well as the considered genotypes ($SII_{genotype}$) according to their sensitivity to stress. Values of SII_{trait} implied that germination parameters were generally affected by drought more intensively than membrane characteristics and finally came the antioxidant enzymes with the least degree of suppression when applying stress. Based on the magnitudes of $SII_{genotype}$, Sids 13 seemed to be the most drought-tolerant wheat cultivar while Shandawel 1 could be the most sensitive one at their juvenile growth stage.

1. Introduction

Wheat (*Triticum aestivum* L.) is a cereal crop globally cultivated for human consumption as a prime source of carbohydrates, proteins, fats, vitamins, minerals and other nutritional constituents. World production of wheat could be rated in the third level after that of maize and rice [1]. However, great attention is paid to bridge the gap between wheat production and consumption especially with various environmental stresses multiplying readily. Among these stresses, drought is a deleterious factor that can reduce wheat yield by 50–90% [2]. In this context, the final yield of any crop is well known to depend on plant performance during the successive stages of its life cycle; the most critical of which are seed germination and seedling growth [3].

At the same time, seed germination and seedling establishment in the majority of crop species are the most sensitive phases to abiotic stress particularly water deficit [4]. Drought is documented to delay seed germination and suppress its rate. Furthermore, water shortage can induce significant alterations in seedling physiology and biochemistry [5]. Nevertheless, certain plants exhibit a set of physiological adaptations that enable them to withstand water stress. Among these adaptive strategies, enhanced activity of antioxidant enzymes may induce plant tolerance by scavenging reactive oxygen species (ROS) [6]. Overproduction of ROS causes the damage of essential biomolecules present in cell compartments and/ or membranes [7]. Therefore, the status of cellular membranes also indicates the degree of plant acclimation to stress. The impact of water stress on antioxidant defense system and membrane features in wheat and other plants was intensively studied [8,9].

Screening drought-resistant plant genotypes is thus a fundamental goal obviously targeted in arid and semi-arid regions. Nonetheless, drought cannot be easily controlled in the field because of rainfall that can impede water deficit [10]. Therefore, assessing plant response to drought at early seedling stage was commonly achieved using chemical desiccators such as polyethylene glycol (PEG). It was inferred that PEG can be employed to shift the water potential of nutrient media inducing plant-water deficit

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in a relatively programmed manner compatible with experimental protocols [11]. In this regard, Gou et al. [12] and Homayoun et al. [13] evaluated different wheat genotypes under gradual doses of PEG and recorded considerable deterioration in germination indices and seedling traits in susceptible varieties rather than their tolerant synonyms.

Accordingly, the present study aims at exploring differential method to verify the response of different wheat genotypes to two levels of PEG-induced drought during germination and seedling growth stage. In addition, associations between drought-related seedling traits would be investigated *via* in-depth statistical analysis of the addressed traits as well as the checked genotypes.

2. Materials and methods

2.1. Plant material and germination conditions

A collection of different wheat genotypes was kindly supplied by Sakha Agricultural Research Center, Kafr El-Shakh Governorate, Egypt. The obtained wheat cultivars included Masr 1, Masr 2, Gimmaza 9, Gimmaza 11, Sids 12, Sids 13, Sakha 93, Sakha 94, Shandawel 1 and Giza 186. In a germination trial, grains of the studied genotypes were surface sterilized with sodium hypochlorite then soaked for 8 hours in water. The grains of each cultivar were then allowed to germinate in dark at 25 ± 2 °C for 6 days in 3 sets; the first was supplied with water when required to serve as control, while the second and the third ones were treated with PEG 6000 at 15% (−2.95 bar osmotic pressure) and 25% (−7.35 bar); respectively.

2.2. Determination of germination parameters

Plumule and radicle length, the number of adventitious roots as well as the fresh and dry mass of 6-day old wheat seedlings were recorded. Seedling water content, the amount of water per unit seedling fresh mass, was additionally calculated as cited from Mickky [14] where;

$$\text{Water content} = (\text{fresh mass} - \text{dry mass})/\text{fresh mass}$$

2.3. Determination of membrane status

Membrane stability index (MSI) and membrane leakage (ML) were determined following Sairam et al. [15] and Vahala et al. [16], respectively. Furthermore, lipid peroxidation indicated by malondialdehyde (MDA) content was determined according to Doria et al. [17].

2.4. Assay of antioxidant enzymes

Enzyme extracts were prepared as recommended by Agrawal and Shaheen [18] in phosphate buffer at pH 6.8 for peroxidase (POX; EC 1.11.1.7.), polyphenol oxidase (PPO; EC 1.14.18.1.), catalase (CAT; EC 1.11.1.6.) and glutathione reductase (GR; EC 1.8.1.7.); and pH 7.8 for superoxide dismutase (SOD; EC 1.15.1.1.) and ascorbic peroxidase (APX; EC 1.11.1.11.).

The activity of POX and that of PPO were estimated as cited from Devi [19]. CAT and APX were assayed according to Devi [20] and Barka [21], respectively. Meanwhile, the protocol designed by Nishikimi et al. [22] was followed for SOD assay and that of Goldberg and Spooner [23] for GR.

2.5. Data processing

Means of ten determinations for germination parameters and three for the other biochemical investigations were computed along with standard deviation. Obtained data were subjected to one way completely randomized ANOVA (analysis of variance) test at 5% probability level using CoHort/CoStat software. According to the values of LSD (least significance difference), small letters were denoted with different letters referring to significant variation. For each criterion, the mean value was calculated for all genotypes under control as well as moderate and severe drought conditions. Thereafter, the individual % of difference between the values at each drought level and that at control was calculated so that the total % of difference between drought in general and control could be recorded as the average of the two individual percentages.

To arrange the estimated morpho-physiological traits (germination parameters, membrane features and antioxidant enzymatic defense) according to the degree by which they were affected by water stress, "Stress Impact Coefficient (SIC)" was calculated for each trait as the sum of its individual criteria with signs opposite to those of their total percent of difference. "Stress Impact Index (SII)" was then derived for each trait (SII_{trait}) as the percent of inhibition caused by moderate drought, severe drought and drought in general based on SIC values. To order the addressed genotypes according to their sensitivity to water stress, SII was correspond-

Table 1
Effect of different watering regimes on plumule length, radicle length and number of adventitious roots of ten wheat cultivars. Means for each parameter (±standard deviation) marked with different letters are significantly different at $p \leq 0.05$. The significance degree (degree of significant difference among means) is indicated by asterisks.

Cultivar	Plumule length (cm)			Radicle length (cm)			Number of adventitious roots		
	Control	Moderate drought	Severe drought	Control	Moderate drought	Severe drought	Control	Moderate drought	Severe drought
Masr 1	$9.5^{c} \pm 1.0$	$5.2^{g} \pm 0.7$	$2.8^{ijk} \pm 0.6$	$15.8^{a} \pm 1.0$	$13.7^{cde} \pm 1.3$	$5.8^{mn} \pm 1.4$	$4.2^{ij} \pm 0.9$	$3.7^{kl} \pm 0.8$	$2.9^{m} \pm 0.3$
Masr 2	$8.5^{d} \pm 0.4$	$5.4^{fg} \pm 0.2$	$1.8^{lm} \pm 0.2$	$12.9^{ef} \pm 0.8$	$6.8^{klm} \pm 0.5$	$4.8^{nop} \pm 0.6$	$4.8^{defg} \pm 0.4$	$3.7^{kl} \pm 0.5$	$3.2^{m} \pm 0.4$
Gimmaza 9	$9.4^{c} \pm 0.5$	$3.3^{hi} \pm 0.3$	$1.0^{n} \pm 0.2$	$13.0^{def} \pm 1.2$	$9.6^{hi} \pm 0.8$	$5.8^{lmn} \pm 0.7$	$4.6^{fghi} \pm 0.5$	$4.2^{ij} \pm 0.4$	$3.0^{m} \pm 0$
Gimmaza 11	$10.3^{b} \pm 0.4$	$3.0^{hij} \pm 0.2$	$1.4^{mn} \pm 0.4$	$15.0^{ab} \pm 0.9$	$9.5^{hi} \pm 0.6$	$5.8^{mn} \pm 1.3$	$5.3^{abc} \pm 0.5$	$4.2^{ij} \pm 0.4$	$3.0^{m} \pm 0$
Sids 12	$8.2^{d} \pm 1.0$	$5.0^{g} \pm 0.7$	$2.2^{kl} \pm 0.6$	$13.8^{cde} \pm 0.6$	$10.8^{g} \pm 1.6$	$6.9^{klm} \pm 2.2$	$5.1^{bcde} \pm 0.7$	$5.2^{abcd} \pm 0.4$	$3.3^{lm} \pm 0.5$
Sids 13	$10.6^{b} \pm 1.0$	$6.2^{e} \pm 1.0$	$3.0^{hij} \pm 0.6$	$14.1^{bcd} \pm 1.5$	$10.5^{gh} \pm 1.6$	$7.0^{k} \pm 1.5$	$5.4^{ab} \pm 0.7$	$5.6^{a} \pm 0.5$	$3.2^{m} \pm 0.4$
Sakha 93	$11.3^{a} \pm 0.6$	$5.5^{fg} \pm 0.4$	$3.1^{hij} \pm 0.2$	$12.0^{f} \pm 1.3$	$8.2^{j} \pm 0.8$	$3.9^{p} \pm 0.6$	$4.8^{defg} \pm 0.4$	$4.3^{hij} \pm 0.5$	$3.0^{m} \pm 0.5$
Sakha 94	$10.8^{ab} \pm 1.2$	$5.9^{ef} \pm 1.2$	$3.5^{h} \pm 0.3$	$14.3^{bc} \pm 1.4$	$10.1^{gh} \pm 0.9$	$6.9^{kl} \pm 1.3$	$4.5^{ghij} \pm 0.8$	$5.4^{ab} \pm 0.5$	$4.7^{efgh} \pm 0.7$
Shandawel 1	$10.8^{ab} \pm 0.5$	$5.2^{g} \pm 0.9$	$1.6^{mn} \pm 0.4$	$13.6^{cde} \pm 1.8$	$8.6^{ij} \pm 1.6$	$4.6^{op} \pm 0.6$	$4.9^{cdefg} \pm 0.3$	$4.1^{jk} \pm 0.6$	$3.3^{lm} \pm 0.5$
Giza 186	$10.7^{ab} \pm 0.9$	$6.2^{e} \pm 1.9$	$2.5^{jk} \pm 0.7$	$13.8^{cde} \pm 1.6$	$12.1^{f} \pm 1.4$	$5.2^{no} \pm 1.6$	$5.0^{bcdef} \pm 0$	$5.1^{bcde} \pm 0.6$	$4.3^{hij} \pm 0.8$
Least significant difference	0.65			1.10			0.47		
Significance degree	***			***			***		
Mean	10.0	5.1	2.1	13.8	10.0	5.7	4.9	4.6	3.4
Individual % of difference	0	−49	−79	0	−28	−59	0	−6	−31
Total % of difference	−64			−44			−19		

ingly calculated for each genotype ($SII_{genotype}$) as the average impact index of its traits.

3. Results and discussion

3.1. Seedling growth observations

Water deficit is one of the most serious constraints to agricultural production retarding plant growth especially at its juvenile stage. Data recorded in the current study revealed that PEG-induced drought could negatively affect seedling morphological features of the different assessed wheat genotypes as pointed out by plumule and radicle length, seedling fresh mass and water content, as well as the number of adventitious roots. Only in four genotypes, Sids 12, Sids 13, Sakha 94 and Giza 186, mild stress could increase the number of adventitious roots compared with their unstressed comparatives. At the same time, drought could induce seedling dry mass in all the tested genotypes. For all the scrutinized parameters, severe drought possessed more powerful action than its moderate synonymy (Tables 1 and 2). The inhibitory effect of PEG treatment on seedling growth parameters of different wheat genotypes was similarly documented [24,25].

Water is a very critical factor during seed germination and early seedling differentiation. Germination begins with water absorption to allow seed coat rupture followed by emergence of radicle and plumule due to consumption of hydrolyzed stored food by the embryo. Therefore, it is logical that water shortage could suppress seed germination with retarded growth rate of the developed seedling. Supporting this assumption, plumule and radicle length in addition to seedling fresh mass and water content of the different wheat genotypes considered herein were all reduced when applying PEG at various levels. On the contrary, the number of adventitious roots was found to upgrade in some genotypes perhaps as a trial to absorb more water from the surrounding medium. In accordance with this belief, the results obtained by Bassirou et al. [26] emphasized that the development of more adventitious roots is an adaptive mechanism exhibited by tolerant plant cultivars to acclimate water deficit circumstances.

Table 2
Effect of different watering regimes on seedling fresh mass, dry mass and water content of ten wheat cultivars. Means for each parameter (±standard deviation) marked with different letters are significantly different at $p \leq 0.05$. The significance degree (degree of significant difference among means) is indicated by asterisks.

Cultivar	Seedling fresh mass (mg)			Seedling dry mass (mg)			Seedling water content (mg H_2O mg^{-1} fresh weight)		
	Control	Moderate drought	Severe drought	Control	Moderate drought	Severe drought	Control	Moderate drought	Severe drought
Masr 1	$120^{g} \pm 25$	$114^{gh} \pm 11$	$95^{jklm} \pm 8$	$35^{efghijkl} \pm 6$	$40^{bcdefg} \pm 6$	$40^{bcdef} \pm 10$	$0.70^{efg} \pm 0.08$	$0.65^{ghij} \pm 0.05$	$0.58^{jkl} \pm 0.10$
Masr 2	$138^{f} \pm 5$	$104^{hijk} \pm 4$	$85^{mnop} \pm 5$	$26^{op} \pm 6$	$31^{jklmno} \pm 4$	$34^{fghijklm} \pm 9$	$0.81^{abc} \pm 0.04$	$0.71^{efg} \pm 0.05$	$0.60^{ijk} \pm 0.09$
Gimmaza 9	$164^{bcd} \pm 6$	$135^{f} \pm 8$	$81^{nop} \pm 6$	$33^{hijklmno} \pm 6$	$42^{bcd} \pm 6$	$39^{cdefgh} \pm 8$	$0.80^{abc} \pm 0.03$	$0.69^{fgh} \pm 0.05$	$0.52^{lmn} \pm 0.11$
Gimmaza 11	$233^{a} \pm 19$	$144^{ef} \pm 12$	$100^{ijkl} \pm 16$	$32^{hijklmno} \pm 7$	$43^{bc} \pm 6$	$46^{ab} \pm 9$	$0.86^{a} \pm 0.03$	$0.70^{efg} \pm 0.05$	$0.52^{lmn} \pm 0.17$
Sids 12	$154^{de} \pm 20$	$118^{gh} \pm 11$	$90^{klmno} \pm 14$	$35^{defghijk} \pm 11$	$41^{bcde} \pm 9$	$50^{a} \pm 8$	$0.77^{bcde} \pm 0.06$	$0.65^{ghij} \pm 0.07$	$0.44^{n} \pm 0.10$
Sids 13	$170^{bc} \pm 33$	$109^{ghij} \pm 9$	$75^{p} \pm 6$	$23^{p} \pm 8$	$28^{mnop} \pm 9$	$37^{cdefghij} \pm 7$	$0.86^{a} \pm 0.04$	$0.74^{cdef} \pm 0.08$	$0.51^{lmn} \pm 0.10$
Sakha 93	$172^{bc} \pm 28$	$112^{ghi} \pm 13$	$87^{lmnop} \pm 7$	$28^{lmnop} \pm 9$	$31^{ijklmno} \pm 6$	$34^{ghijklmn} \pm 8$	$0.83^{ab} \pm 0.07$	$0.72^{defg} \pm 0.07$	$0.61^{ijk} \pm 0.11$
Sakha 94	$158^{cde} \pm 28$	$93^{klmn} \pm 18$	$77^{op} \pm 17$	$27^{nop} \pm 5$	$30^{jklmno} \pm 10$	$32^{hijklmno} \pm 8$	$0.83^{ab} \pm 0.03$	$0.65^{ghij} \pm 0.18$	$0.56^{kl} \pm 0.15$
Shandawel 1	$173^{b} \pm 20$	$92^{klmn} \pm 9$	$85^{mnop} \pm 8$	$30^{klmno} \pm 9$	$35^{defghijk} \pm 8$	$40^{bcdefg} \pm 6$	$0.83^{ab} \pm 0.05$	$0.61^{hijk} \pm 0.09$	$0.52^{lm} \pm 0.10$
Giza 186	$153^{de} \pm 22$	$114^{gh} \pm 18$	$95^{klmn} \pm 13$	$31^{ijklmno} \pm 6$	$38^{cdefghi} \pm 8$	$50^{a} \pm 5$	$0.79^{abcd} \pm 0.04$	$0.66^{ghi} \pm 0.12$	$0.46^{mn} \pm 0.11$
Least significant difference	14			7			0.08		
Significance degree	***			***			***		
Mean	164	114	87	30	36	40	0.81	0.68	0.53
Individual % of difference	0	−30	−47	0	+20	+33	0	−16	−35
Total % of difference	−39			+27			−26		

Table 3
Effect of different watering regimes on membrane stability index, membrane leakage and lipid peroxidation of ten wheat cultivars. Means for each parameter (±standard deviation) marked with different letters are significantly different at $p \leq 0.05$. The significance degree (degree of significant difference among means) is indicated by asterisks.

Cultivar	Membrane stability index (%)			Membrane leakage (%)			Lipid peroxidation (μ mol MDA gm^{-1} fresh weight)		
	Control	Moderate drought	Severe drought	Control	Moderate drought	Severe drought	Control	Moderate drought	Severe drought
Masr 1	$86.02^{d} \pm 1.03$	$81.88^{e} \pm 0.58$	$75.18^{jk} \pm 2.01$	$17.93^{klmn} \pm 0.94$	$22.46^{hij} \pm 0.50$	$27.73^{cd} \pm 0.75$	$1.23^{m} \pm 0.01$	$1.58^{hi} \pm 0.04$	$1.99^{d} \pm 0.08$
Masr 2	$89.74^{a} \pm 0.82$	$82.54^{e} \pm 1.05$	$78.42^{h} \pm 0.88$	$13.27^{st} \pm 0.76$	$18.76^{k} \pm 0.39$	$30.91^{a} \pm 0.76$	$1.05^{n} \pm 0.03$	$1.50^{jk} \pm 0.04$	$1.90^{e} \pm 0.06$
Gimmaza 9	$85.70^{d} \pm 1.17$	$76.80^{i} \pm 1.97$	$67.32^{m} \pm 1.15$	$17.03^{lmno} \pm 0.62$	$22.86^{hi} \pm 0.80$	$31.11^{a} \pm 1.22$	$0.90^{o} \pm 0.06$	$1.83^{e} \pm 0.02$	$1.97^{d} \pm 0.06$
Gimmaza 11	$86.22^{d} \pm 1.12$	$79.96^{fg} \pm 0.62$	$75.90^{ijk} \pm 0.60$	$12.09^{t} \pm 1.10$	$18.51^{klm} \pm 1.42$	$25.51^{ef} \pm 1.29$	$0.91^{o} \pm 0.02$	$1.49^{jk} \pm 0.07$	$2.00^{d} \pm 0.01$
Sids 12	$82.10^{e} \pm 1.34$	$77.08^{i} \pm 0.54$	$71.08^{l} \pm 0.37$	$16.60^{nop} \pm 1.03$	$21.70^{ij} \pm 0.73$	$29.83^{ab} \pm 1.36$	$1.16^{m} \pm 0.02$	$1.66^{g} \pm 0.14$	$2.13^{c} \pm 0.10$
Sids 13	$85.96^{d} \pm 0.18$	$79.16^{gh} \pm 0.44$	$71.94^{l} \pm 0.58$	$16.95^{mno} \pm 1.63$	$22.59^{hij} \pm 1.93$	$29.89^{ab} \pm 1.77$	$1.43^{k} \pm 0.10$	$1.51^{ij} \pm 0.08$	$2.56^{a} \pm 0.07$
Sakha 93	$88.06^{bc} \pm 0.84$	$82.74^{e} \pm 0.75$	$80.46^{f} \pm 1.30$	$13.47^{rst} \pm 0.60$	$18.57^{kl} \pm 1.20$	$25.77^{ef} \pm 1.98$	$1.32^{l} \pm 0.01$	$1.67^{fg} \pm 0.07$	$2.20^{c} \pm 0.03$
Sakha 94	$86.84^{cd} \pm 0.97$	$81.76^{e} \pm 1.12$	$72.26^{l} \pm 1.34$	$14.83^{qrs} \pm 0.87$	$23.74^{gh} \pm 2.55$	$26.92^{de} \pm 1.52$	$0.96^{o} \pm 0.03$	$1.23^{m} \pm 0.04$	$2.45^{b} \pm 0.02$
Shandawel 1	$87.70^{bc} \pm 0.52$	$82.84^{e} \pm 0.61$	$76.22^{ij} \pm 1.29$	$15.02^{pqr} \pm 1.27$	$21.38^{ij} \pm 1.00$	$28.64^{bc} \pm 0.77$	$0.46^{q} \pm 0.01$	$1.19^{m} \pm 0.03$	$1.74^{f} \pm 0.04$
Giza 186	$88.28^{b} \pm 0.44$	$79.32^{fgh} \pm 0.78$	$74.84^{k} \pm 1.18$	$15.80^{opq} \pm 2.14$	$21.18^{j} \pm 0.53$	$25.13^{fg} \pm 0.67$	$0.53^{p} \pm 0.02$	$0.90^{o} \pm 0.04$	$1.61^{gh} \pm 0.04$
Least significant difference	1.27			1.57			0.07		
Significance degree	***			***			***		
Mean	86.66	80.41	74.36	15.30	21.18	28.14	1.00	1.46	2.06
Individual % of difference	0	−7	−14	0	+38	+84	0	+46	+106
Total % of difference	−11			+61			+76		

Table 4
Effect of different watering regimes on peroxidase, polyphenol oxidase and ascorbic peroxidase activity of ten wheat cultivars. Means for each parameter (±standard deviation) marked with different letters are significantly different at $p \leq 0.05$. The significance degree (degree of significant difference among means) is indicated by asterisks.

Cultivar	Peroxidase ($\Delta A * 10^3$)			Polyphenol oxidase ($\Delta A^* 10^3$)			Ascorbic peroxidase ($\Delta A^* 10^3$)		
	Control	Moderate drought	Severe drought	Control	Moderate drought	Severe drought	Control	Moderate drought	Severe drought
Masr 1	$118^{q} \pm 4$	$329^{op} \pm 10$	$152^{q} \pm 10$	$22^{p} \pm 2$	$39^{mn} \pm 3$	$25^{p} \pm 4$	$30^{kl} \pm 2$	$41^{def} \pm 3$	$33^{ijk} \pm 3$
Masr 2	$317^{op} \pm 7$	$345^{o} \pm 12$	$404^{n} \pm 11$	$34^{no} \pm 2$	$43^{lm} \pm 2$	$55^{ij} \pm 3$	$28^{lm} \pm 2$	$38^{efgh} \pm 4$	$38^{fgh} \pm 2$
Gimmaza 9	$415^{mn} \pm 8$	$500^{hi} \pm 116$	$452^{kl} \pm 4$	$35^{n} \pm 1$	$34^{n} \pm 3$	$28^{op} \pm 2$	$38^{efgh} \pm 3$	$59^{a} \pm 4$	$49^{b} \pm 4$
Gimmaza 11	$589^{def} \pm 7$	$587^{ef} \pm 10$	$547^{g} \pm 9$	$53^{jk} \pm 2$	$76^{f} \pm 2$	$66^{gh} \pm 2$	$46^{bc} \pm 5$	$41^{defg} \pm 2$	$33^{ijk} \pm 2$
Sids 12	$624^{d} \pm 6$	$716^{b} \pm 4$	$613^{de} \pm 14$	$93^{d} \pm 2$	$103^{c} \pm 7$	$84^{e} \pm 4$	$39^{efg} \pm 3$	$32^{jkl} \pm 3$	$33^{ijk} \pm 2$
Sids 13	$306^{p} \pm 6$	$449^{lm} \pm 5$	$490^{ij} \pm 1$	$51^{jk} \pm 1$	$65^{gh} \pm 4$	$75^{f} \pm 2$	$25^{mn} \pm 1$	$21^{no} \pm 2$	$19^{o} \pm 3$
Sakha 93	$670^{c} \pm 18$	$461^{jkl} \pm 2$	$499^{hi} \pm 9$	$66^{gh} \pm 4$	$61^{hi} \pm 2$	$61^{hi} \pm 1$	$33^{ijk} \pm 2$	$31^{jkl} \pm 2$	$24^{n} \pm 4$
Sakha 94	$709^{b} \pm 2$	$814^{a} \pm 7$	$407^{n} \pm 5$	$142^{a} \pm 6$	$113^{b} \pm 13$	$66^{gh} \pm 3$	$37^{ghi} \pm 2$	$22^{no} \pm 2$	$34^{hij} \pm 4$
Shandawel 1	$559^{fg} \pm 6$	$487^{ijk} \pm 5$	$402^{n} \pm 8$	$68^{g} \pm 3$	$95^{d} \pm 4$	$91^{d} \pm 9$	$34^{hijk} \pm 2$	$47^{bc} \pm 3$	$43^{cd} \pm 2$
Giza 186	$528^{gh} \pm 5$	$475^{ijkl} \pm 7$	$293^{p} \pm 4$	$78^{f} \pm 2$	$65^{gh} \pm 1$	$48^{kl} \pm 2$	$42^{de} \pm 2$	$43^{cd} \pm 3$	$41^{def} \pm 2$
Least significant difference	37			7			4		
Significance degree	***			***			***		
Mean	484	516	426	64	69	60	35	38	35
Individual % of difference	0	+7	−12	0	+8	−6	0	+9	0
Total % of difference	−3			+1			+5		

Table 5
Effect of different watering regimes on catalase, superoxide dismutase and glutathione reductase activity of ten wheat cultivars. Means for each parameter (±standard deviation) marked with different letters are significantly different at $p \leq 0.05$. The significance degree (degree of significant difference among means) is indicated by asterisks.

Cultivar	Catalase (Unit g^{-1} fresh weight)			Superoxide dismutase (Unit ml^{-1})			Glutathione reductase (Unit l^{-1})		
	Control	Moderate drought	Severe drought	Control	Moderate drought	Severe drought	Control	Moderate drought	Severe drought
Masr 1	$1.68^{nop} \pm 0.04$	$2.11^{hijk} \pm 0$	$1.70^{no} \pm 0.09$	$144.9^{fg} \pm 8.5$	$102.3^{ijk} \pm 0$	$180.4^{d} \pm 8.8$	$38.9^{a} \pm 4.0$	$23.3^{defghij} \pm 0.8$	$31.6^{b} \pm 1.7$
Masr 2	$3.31^{c} \pm 0.08$	$3.08^{d} \pm 0.04$	$1.59^{p} \pm 0.04$	$113.6^{hi} \pm 8.9$	$117.9^{h} \pm 2.4$	$117.9^{h} \pm 9.9$	$32.0^{b} \pm 0.9$	$31.4^{b} \pm 2.2$	$30.8^{b} \pm 3.3$
Gimmaza 9	$2.20^{h} \pm 0.04$	$2.06^{jk} \pm 0.04$	$2.18^{hi} \pm 0.07$	$220.2^{b} \pm 8.9$	$254.3^{a} \pm 6.5$	$116.5^{hi} \pm 6.5$	$21.4^{fghijk} \pm 2.6$	$21.7^{efghijk} \pm 4.5$	$19.0^{jk} \pm 0.7$
Gimmaza 11	$3.24^{c} \pm 0.04$	$1.93^{m} \pm 0.04$	$2.04^{kl} \pm 0$	$200.3^{c} \pm 7.4$	$221.6^{b} \pm 18.6$	$198.8^{c} \pm 9.8$	$23.7^{defghi} \pm 0.4$	$24.9^{cdefg} \pm 0$	$23.8^{defghi} \pm 1.7$
Sids 12	$3.26^{c} \pm 0.07$	$2.40^{fg} \pm 0.08$	$1.61^{op} \pm 0.10$	$225.9^{b} \pm 15.4$	$38.4^{n} \pm 4.3$	$108.0^{hij} \pm 8.9$	$28.4^{bc} \pm 0.5$	$25.2^{cdefg} \pm 0.5$	$19.8^{ijk} \pm 1.7$
Sids 13	$2.11^{hijk} \pm 0$	$3.67^{a} \pm 0.07$	$2.88^{e} \pm 0.04$	$82.4^{l} \pm 2.4$	$142.0^{g} \pm 8.9$	$223.0^{b} \pm 16.2$	$18.8^{k} \pm 5.1$	$22.2^{efghijk} \pm 4.4$	$25.7^{cdef} \pm 0.8$
Sakha 93	$3.51^{b} \pm 0.04$	$1.18^{q} \pm 0.04$	$2.47^{f} \pm 0.04$	$159.1^{ef} \pm 10.8$	$90.9^{kl} \pm 13.7$	$157.7^{ef} \pm 7.4$	$24.4^{cdefgh} \pm 5.7$	$24.1^{cdefghi} \pm 3.2$	$20.8^{ghijk} \pm 4.2$
Sakha 94	$3.31^{c} \pm 0.04$	$2.15^{hij} \pm 0.04$	$1.65^{nop} \pm 0.04$	$163.3^{e} \pm 2.5$	$86.7^{l} \pm 6.5$	$44.1^{n} \pm 4.9$	$24.9^{cdefg} \pm 1.6$	$27.6^{bcd} \pm 0.9$	$25.8^{cdef} \pm 1.6$
Shandawel 1	$2.18^{hi} \pm 0.07$	$2.04^{kl} \pm 0.07$	$1.75^{n} \pm 0.04$	$139.2^{g} \pm 8.8$	$45.5^{mn} \pm 2.5$	$93.8^{jkl} \pm 0$	$26.0^{cde} \pm 1.8$	$24.9^{cdefg} \pm 2.8$	$24.9^{cdefg} \pm 4.2$
Giza 186	$2.33^{g} \pm 0.14$	$1.95^{lm} \pm 0.08$	$2.09^{ijk} \pm 0.04$	$186.1^{cd} \pm 6.5$	$115.1^{hi} \pm 4.3$	$59.7^{m} \pm 7.4$	$20.1^{hijk} \pm 4.0$	$23.8^{defghi} \pm 0.5$	$22.0^{efghijk} \pm 1.2$
Least significant difference	0.10			14.3			4.5		
Significance degree	***			***			***		
Mean	2.71	2.26	2.00	163.5	121.5	130.0	25.9	24.9	24.4
Individual % of difference	0	−17	−26	0	−26	−20	0	−4	−6
Total % of difference	−22			−23			−5		

Table 6
Stress impact coefficient (SIC) of different watering regimes on germination parameters, membrane features and antioxidant enzymes of ten wheat cultivars. Means for each parameter (±standard deviation) marked with different letters are significantly different at $p \leq 0.05$. The significance degree (degree of significant difference among means) is indicated by asterisks.

Cultivar	SIC (Unitless)								
	Germination parameters			Membrane features			Antioxidant enzymatic defense		
	Control	Moderate drought	Severe drought	Control	Moderate drought	Severe drought	Control	Moderate drought	Severe drought
Masr 1	$115^{fg} \pm 27$	$97^{hij} \pm 12$	$67^{lm} \pm 10$	$67^{c} \pm 1.9$	$58^{ef} \pm 0.8$	$46^{k} \pm 1.8$	$252^{n} \pm 6$	$377^{k} \pm 11$	$308^{lm} \pm 19$
Masr 2	$139^{e} \pm 7$	$90^{ijk} \pm 7$	$62^{lm} \pm 8$	$75^{a} \pm 1.5$	$62^{d} \pm 1.1$	$46^{k} \pm 1.7$	$404^{k} \pm 12$	$417^{k} \pm 21$	$461^{j} \pm 20$
Gimmaza 9	$159^{cd} \pm 6$	$111^{fgh} \pm 10$	$53^{m} \pm 10$	$68^{c} \pm 1.3$	$52^{i} \pm 2.4$	$34^{n} \pm 1.7$	$586^{g} \pm 9$	$685^{de} \pm 118$	$514^{hi} \pm 2$
Gimmaza 11	$232^{a} \pm 16$	$119^{f} \pm 15$	$64^{lm} \pm 22$	$73^{ab} \pm 2.4$	$60^{e} \pm 1.6$	$49^{j} \pm 1.7$	$717^{cd} \pm 6$	$719^{bcd} \pm 10$	$673^{e} \pm 15$
Sids 12	$146^{de} \pm 19$	$98^{hij} \pm 11$	$53^{m} \pm 15$	$64^{d} \pm 1.8$	$53^{hi} \pm 0.8$	$39^{m} \pm 1.6$	$751^{bc} \pm 11$	$646^{ef} \pm 17$	$625^{fg} \pm 24$
Sids 13	$178^{b} \pm 32$	$104^{fghi} \pm 10$	$52^{m} \pm 9$	$67^{c} \pm 1.7$	$55^{gh} \pm 2.3$	$40^{m} \pm 2.1$	$334^{i} \pm 13$	$531^{h} \pm 14$	$647^{ef} \pm 19$
Sakha 93	$172^{bc} \pm 31$	$99^{hij} \pm 15$	$64^{lm} \pm 12$	$73^{b} \pm 1.0$	$62^{d} \pm 1.5$	$53^{i} \pm 2.3$	$759^{ab} \pm 30$	$486^{ij} \pm 20$	$596^{g} \pm 16$
Sakha 94	$161^{cd} \pm 27$	$85^{jk} \pm 22$	$61^{lm} \pm 19$	$71^{b} \pm 0.8$	$57^{fg} \pm 3.4$	$43^{l} \pm 2.4$	$722^{bcd} \pm 7$	$797^{a} \pm 12$	$379^{k} \pm 10$
Shandawel 1	$173^{bc} \pm 19$	$75^{kl} \pm 12$	$55^{m} \pm 12$	$72^{b} \pm 1.7$	$60^{e} \pm 0.7$	$46^{k} \pm 1.3$	$624^{fg} \pm 11$	$418^{k} \pm 11$	$388^{k} \pm 18$
Giza 186	$152^{de} \pm 21$	$101^{ghi} \pm 24$	$57^{m} \pm 15$	$72^{b} \pm 2.1$	$57^{fg} \pm 0.4$	$48^{l} \pm 1.3$	$617^{fg} \pm 8$	$508^{hi} \pm 8$	$287^{mn} \pm 4$
Least significant difference	15			2			42		
Significance degree	***			***			***		
Mean	163	98	59	70	58	44	577	558	488
Individual % of difference	0	−40	−64	0	−17	−37	0	−3	−15
Total % of difference	−52			−27			−9		

Furthermore, the dry mass of water-unsatisfied wheat seedlings increased compared with their water-unstressed relatives probably because of the larger amount of unutilized stored food in the stressed seedlings. Less utilized nutrients in stressed seedling may accumulate due to little water available for its hydrolysis and/or little food consumption by the weakly-developed radicle and plumule. Matching our finding, a recent study by Mickky [14] recorded an increment in the dry mass of *Vicia faba* seedlings suffering from moderate water stress. Similarly, Guo et al. [27] recorded an increase in the dry mass of *Lycium ruthenicum* seedlings when droughted considering the boost in dry matter allocation as a morphological adaptation to water insufficiency.

3.2. Membrane features

The intensity of membrane damage could indicate plant response to unsuitable conditions. In this regard, membrane stability index (MSI), membrane leakage (ML) and lipid peroxidation are widely-used stress indicators of plant membrane status. In the present study, drought could disrupt membrane integrity of all the checked wheat genotypes by stimulating lipid peroxidation with marked increase in ML and eventual decrease in MSI. Also, severe drought has more intensive effect than moderate drought (Table 3). These results agree with those of Li et al. [28] who observed significant increase in membrane lipid peroxidation of different wheat cultivars grown under water deficit. As a similar response, MDA

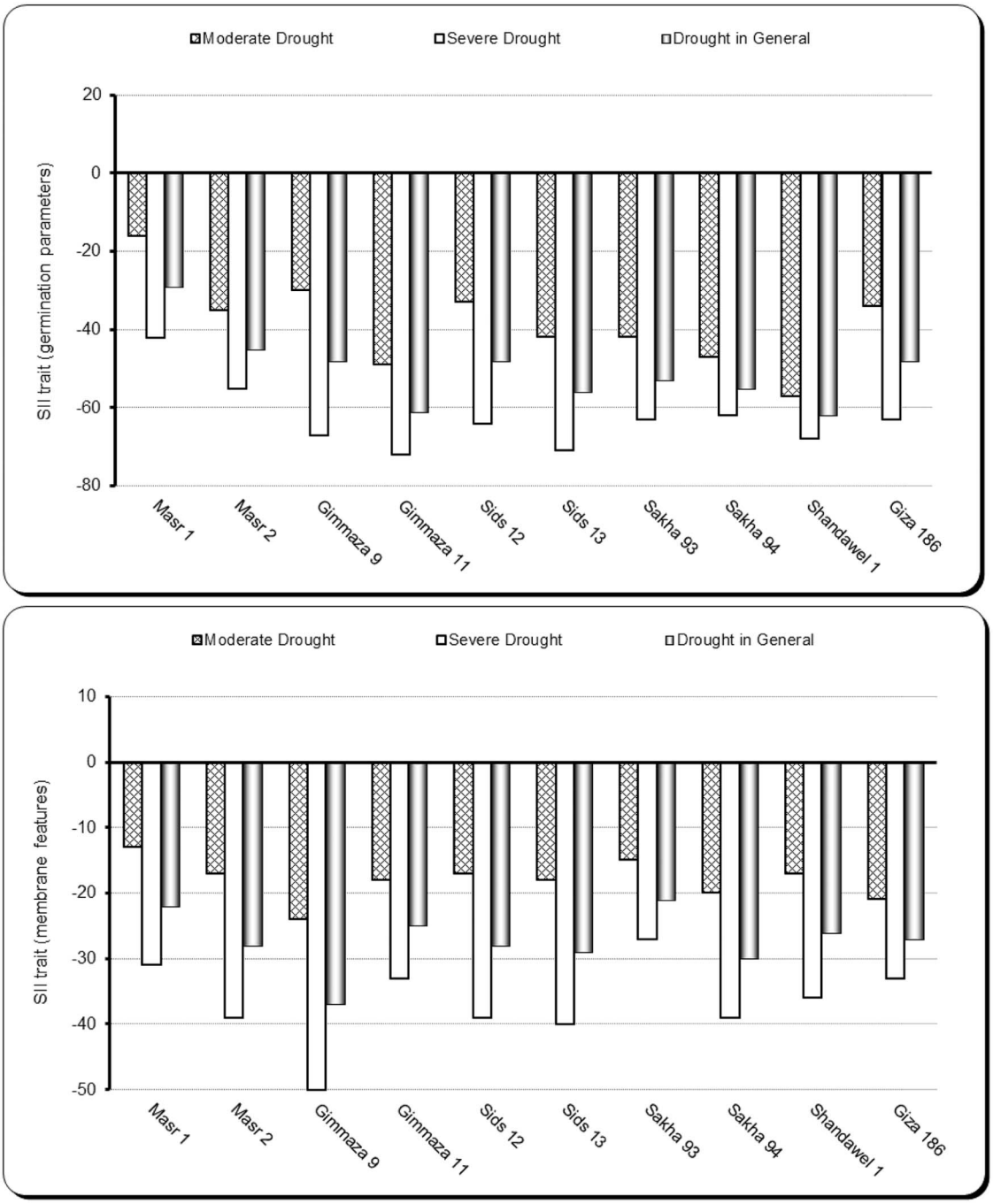

Fig. 1. Stress impact index (%) for different traits (SII_{trait}) of wheat seedlings under moderate and severe levels of drought as well as drought in general.

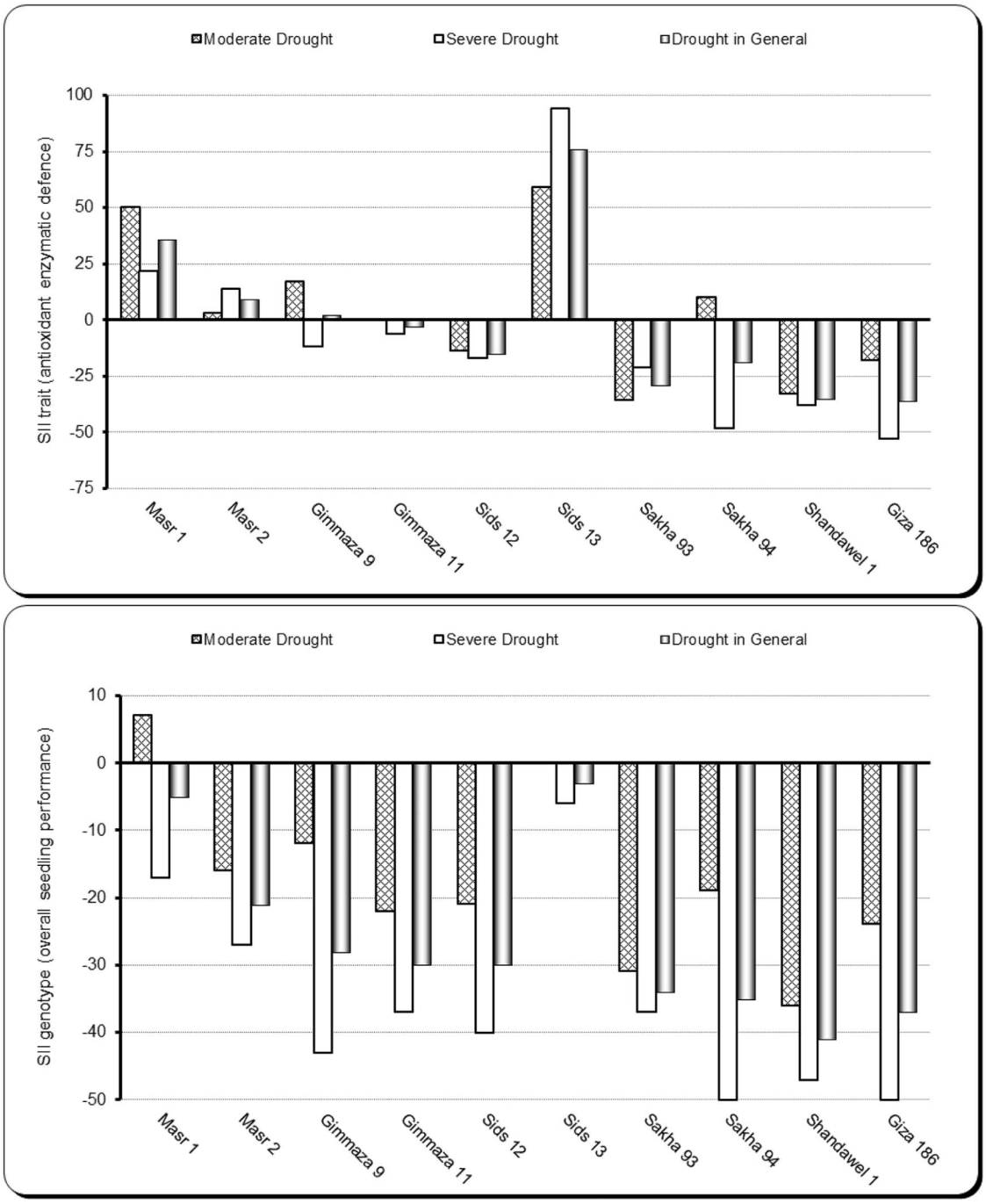

Fig. 2. Stress impact index (%) for different traits (SII_{trait}) and different genotypes ($SII_{genotype}$) of wheat seedlings under moderate and severe levels of drought as well as drought in general.

content and electrolyte leakage from huckleberry seedlings increased under mild and severe water shortage [29].

It is well established that water stress could paramountly disturb the stability of plant cellular membranes [30]. This action could be ascribed to ROS overproduced under stressful circumstances. At higher concentration, ROS cause oxidative damage more dominantly by hydrogen peroxide that can bring about decomposition of lipid constituents of biomembranes. Also, stress-induced burst of superoxide anion was found to be accompanied with increased membrane fluidity; an indirect cause of decreased membrane stability [7]. Confirming this assumption, Fan et al. [31] found that PEG-induced drought in cucumber seedlings caused excessive generation of ROS including hydrogen peroxide and superoxide radicals with subsequent increase in MDA content. The formed MDA can react with the free amino groups of proteins as well as phospholipid constituents of cell membranes initiating ethylene production with consequent alterations in membranes characteristics [32]. In this context, Mickky [33] has lately demonstrated that water stress could lower phospholipid content of broad bean seedlings causing marked increase in their ML and lipid peroxidation with significant decline in MSI.

3.3. Antioxidant enzymes

Stress-enhanced leakage of electrons to molecular oxygen is well documented to stimulate the production of ROS that appear to have dual effect according to their overall amounts. Although ROS can behave as secondary signals in stress transduction, excess doses of ROS can cause oxidative cell damage [34]. However, drought-tolerant plant genotypes are equipped to efficiently detoxify ROS by the coordinated action of enzymatic antioxidants; the most important components of which are superoxide dismutase (SOD), catalase (CAT), peroxidase (PRX), ascorbate peroxidase (APX), polyphenol oxidase (PPO) and glutathione reductase (GR) [35].

According to the results represented herein in Tables 4 and 5, moderate water shortfall could induce the activity of POX, PPO and APX in the various genotypes of wheat but these three enzymes were suppressed under severe drought. On the other hand, CAT, SOD and GR were generally inhibited with water shortage whether to moderate or severe intensity. SOD represents the first line of defense against oxidative stress as it detoxifies superoxide radicals by converting it into molecular oxygen and hydrogen peroxide. Plant SOD exists in three forms according to the metal ion of their active site; Cu/Zn, Mn and Fe forms [32]. Detailed perusal of data obtained in the current study manifested that in wheat cultivars Gimmaza 9 and Gimmaza 11, moderate drought enhanced SOD, while severe drought enhanced SOD in Masr 1, and the activity of this enzyme was enhanced by both drought levels in Masr 2 and Sids 13. Otherwise, different levels of stress suppressed SOD activity (Table 5). The enhanced activity of SOD in some wheat varieties under stress reflects a considerable experience to water deficit causing a reasonable degree of tolerance. Conversely, the recorded inhibition of SOD in the other cases may be ascribed to the adverse impact of drought on protein synthesis or the defect in Cu, Zn, Mn and/or Fe – metals activating the enzyme. Coinciding with our results, increased SOD in drought tolerant cultivars and suppressed activity in susceptible ones were formerly highlighted [36,37].

Among the ROS accumulated under water stress conditions, H_2O_2 can be considered as the most relatively stable non-radical without a net charge [38]. High levels of H_2O_2 cause severe injury to essential cell biomolecules. However, some plants respond to stressful factors by enhancing H_2O_2-metabolizing enzymes such as CAT, POX, APX and GR. Without the need for a reductant to catalyze the dismutation reaction, CAT can cleave H_2O_2 into H_2O and O_2 while POX decomposes H_2O_2 by oxidation of co-substrate such as phenolic compounds. Instead, H_2O_2 can be scavenged through ascorbate-glutathione cycle that encompasses oxidation-reduction of ascorbate and glutathione by APX and GR, respectively [39].

In the current investigation, application of water stress inhibited CAT activity in all the studied genotypes except Masr 1 and Sids 13. GR was also inhibited in all the surveyed genotypes except in moderately-droughted Gimmaza 9 and 11 as well as stressed Sids 13, Sakha 94 and Giza 186. Reduction in CAT and GR activity in water-unsatisfied wheat was previously noted [28]. Suppressed activity of CAT and GR under stress may result from the reverse effect of stress on protein biosynthesis. Moreover, POX activity was induced by stress in Masr 1 and 2, Gimmaza 9 and Sids 13, while APX was activated in Masr1 and 2, Gimmaza 9, Shandawel 1 and Giza 186; otherwise these two enzymes were inhibited. Enhanced activity of POX and APX was reported in wheat seedlings as a stress acclimation strategy [40]. Similarly, the activity of PPO generally increased in stressed wheat genotypes except in Gimmaza 9, Sids 12, Sakha 93, Sakha 94 and Giza 186. PPO utilizes O_2 to oxidize phenolics to their corresponding quinones, so higher PPO activity may indicate more degradation of various toxic substances accumulated because of stress.

3.4. Correlations among traits and genotypes

It would be of great prominence to numerically summarize the overall behavior of the surveyed wheat genotypes on the basis of the pooled data. For that, stress impact coefficient (SIF) was formulated as unitless virtual values that link the different criteria of each estimated trait. Moreover, stress impact index (SII) was introduced as a percent to indicate the effect of stress on the estimated parameters whether as positive effect causing an enhancement in the investigated parameter (positive SII values) or negative effect causing a general impairment in the estimated parameter (negative SII values).

Irrespective of the screened genotype, the values of SIC listed in Table 6 indicated that seedling performance was generally deteriorated when suffering little water supply as indicated by germination parameters which were suppressed more intensively than membrane characteristics and finally came the enzymatic antioxidant defense system which was inhibited but to a lower degree compared with germination parameters and membrane features.

With respect to the various genotypes involved in the present investigation, values SII depicted in Figs. 1 and 2 showed that drought in general caused the lowest impact on germination parameters in cultivar Masr 1 and on membrane features in Sakha 93. The maximum titer of the negative impact index was recorded in Shandawel 1 for germination parameters and Gimmaza 9 for membrane features. Regarding the antioxidant enzymes, the highest positive stress impact was recorded in cultivar Sids 13 while the highest negative value was calculated for Giza 186.

4. Conclusion

Chemical desiccation induced by PEG could suppress germination and seedling growth of different wheat genotypes. Regardless of the genotype, stress impact on the estimated morphological traits of wheat seedlings was more vigorous than that on their membrane features and antioxidant enzymes. However, the ability to cope with drought showed significant variation among the considered varieties. Generally, Sids 13 seemed to be the most tolerant variety followed by Masr 1, Masr 2, Gimmaza 9, Gimmaza 11, Sids 12, Sakha 93, Sakha 94, and Giza 186 and finally came Shandawel 1 with the maximum sensitivity.

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