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Molecular Description for Some Promising Wheat Accessions Tolerated to water Stress

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ABSTRACT

In light of the risks and challenges facing Egyptian national security, it was found that there are two strategic crises that dominate the Egyptian scene, namely the limited irrigation water and the large deficit in Egyptian bread production. Also, it is noted that the wheat crop is very sensitive to drought stress, especially in the valley and delta regions and areas close to the sea coast. This large deficit in irrigation water causes great damage to the final yield which amounts to 50%. Since, the wheat crop represents the backbone of Egyptian bread production, bread production is severely damaged and affects the vast majority of Egyptians. For all these reasons combined, this investigation was launched with the aim of attempting to genetically improve some wheat entries that are sensitive to drought and raise their tolerance to it besides, reach wheat hybrids that are superior in yield and water stress tolerance so that these entries become new wheat lines in the future. Further, these wheat lines can participate significantly in bridging the large gap in the production of Egyptian bread.

Keywords: Wheat, drought STRESS, GCA & SCA effects, ISSR primers

Introduction

Water stress is considered one of the most serious environmental challenges that threatens agricultural production locally and globally as well. This crisis is at the top of the priorities of countries whose national economy and development depend on agricultural production, such as the Arab Republic of Egypt. On this basis, extensive efforts began by a very large sector of scientists and researchers to try solving this serious environmental problem or reduce its pace in a way that guarantees a reasonable output for agricultural crops (Ahluwalia *et al.,* 2021). Also, it was found that the crisis of water scarcity necessary for irrigation and agriculture has caused an increasing of soil salinity rate, which hinders the growth of field crops and reduces their productivity significantly, and on top of these crops comes the wheat crop because of this strategic crop, it is of nutritional importance locally and globally, (Khatab *et al.,* 2021 a). Further, that the limited irrigation water in the northern Delta regions of Egypt played a major role in the high level of soil salinity and irrigation water, and this represents a major threat to the growth of winter crops such as wheat and barley. Drought stress, especially severe water stress causes a severe decrease in the final wheat output, which may reach from 50 to 60% and this is considered a major destruction of the final product of this strategic crop, (Kizilgeci *et al.,* 2021). Also, the results of a lot of papers conducted in an attempt to produce new plant accessions tolerant to drought are still in their infancy and face many practical obstacles. The reason for this is that the trait of tolerance or resistance to water stress is a complex quantitative trait that depends on the inheritance of a very large number of genes, which are scattered in different genotypes, (Arora 2019). Despite this, many studies have been launched to combine the sciences of plant breeding and molecular genetics or biotechnology as an attempt to produce high-yielding modern wheat lines that are tolerant to salt stress

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through using the technique of genetic transfer of the tolerance trait from wild entries to sensitive local varieties, and the results were very promising. When studying the problem of water stress, we must first learn about the physiological, biochemical and yield components attributes responsible for the tolerance trait to this environmental factor. Physiological traits such as osmotic pressure, osmotic adjustment, chlorophyll content, leaf area index and early maturity besides, some biochemical cattributes such as proline, glycine betaine, trehalose contents and other parameters are considered extremely important in screening and selecting the best resistant accessions, (Al-Kordy *et al.,* 2019). Drought tolerance indices especially for grain yield/plant are considering the most important physiological tests that show the ability of the genotype to environmental stresses tolerance, especially to water stress and give a quick overview of the expected output, (Tawfik & El-Mouhamady 2019). On the other hand, molecular genetic markers are one of the most important aspects of the current study, as it addressed the genetic mechanisms at the molecular level responsible for water stress tolerance in wheat, after identifying it and this will be reviewed in some detail. Results conducted under natural conditions and both salt and water stress treatments demonstrated that some rice entries especially the tolerant ones had given promising data for environmental stresses tolerance especially GZ1368-S-5-4 and Giza 178, (El-Mouhamady 2003 & 2009). The modification of gene expression responsible for salinity stress tolerance in wheat was studied by (El-Seidy *et al.,* 2013) through identification some genes were the genetic basis for this tolerance. Field and physiological tests, especially water stress tolerance indices were strong evidence of the tolerance in some maize varieties to drought during using some modern applications of ISSR markers, (Esmail *et al.,* 2016). Studying the effect of the toxicity of salts and heavy metals was one of the most important studies that was conducted with the aim of determining the safe level of rice cultivation in some areas of the northern Delta by conducting all physiological and biochemical tests. Further, measuring indicators of tolerance to heavy metal toxicity besides, identifying the most important molecular genetic mechanisms responsible for tolerance through using RAPD markers, (Heiba *et al.,* 2016 a). Also, the technology of creating mutations in bread wheat was studied using RAPD and ISSR markers through using the various doses of EMS and the results were very promising, as wheat mutations with high tolerance to environmental stresses, especially water stress were create, (Heiba *et al.,* 2016 b). Many extensive studies have been conducted to study the physiological behavior related to tolerance to environmental stresses, especially water stress, such as the investigation conducted on rice to determine the genetic behavior related to drought tolerance through the use of a selected group of varieties and hybrids through the use of ISSR markers, (Eldessouky *et al.,* 2016) Results were promising in the presence of molecular genetic evidence or special markers or unique fragments linked to tolerance of some rice accessions in the first generation. Also, relying on the chlorophyll pigment composition content as an important physiological indicator of water stress tolerance in wheat was of great importance. Where, the tolerant varieties proved that it can provide a strong chlorophyll content as well, produce the nitrogen element necessary to build sugars and important nutrients for the seedlings during the germination stage, (Kizilgeci *et al.,* 2021). The wheat crop is considered very sensitive to water stress during the flowering and grain filling stages. This confirms beyond doubt that water stress affects all stages of plant growth, starting from germination and seedling formation, through grain filling until the harvest stage and this is the main reason for the significant deterioration in the final grain yield, (Gupta *et al.,* 2024). After all of the above, we can briefly explain the aim of this investigation, which revolves around genetic improvement of the wheat crop to confront the threat of water stress through ancient plant breeding methods and modern molecular genetic techniques, especially molecular genetic markers using ISSR primers.

2. Materials and Methods

2.1. Materials

The present investigation was carried out under two conditions (The normal and water stress conditions) in 2020 season. Each location was included using 15 wheat entries (the five wheat parents and their ten F1 wheat crosses) through half diallel analysis. Further, the five wheat cultivars with various reaction for drought tolerance, table (1). The first experiment was normal irrigation or control treatment of wheat crop in winter season where the first irrigate was at agriculture time and the other four irrigates were with one month intervals at Al-Noubaria Farm in Beheira Governorate. While, the second treatment was two irrigates. Where, the first one was at sown time and the second one was after

45 days from agriculture and no irrigation was done till harvesting (Low put system). The two experiments were isolated from each other one.

Table 1: Classification of the five Wheat Parents used in a half diallel analysis.

Serial No.	Names of Genotypes	Origin	Reaction to Drought tolerance
1 or (P1)	Sakha 8	Egypt	Tolerance
2 or (P2)	Misr 1	Egypt	Tolerance
3 or (P3)	Sakha 94	Egypt	Moderate
4 or (P4)	Gimeaza 11	Egypt	Moderate
5 or (P5)	Gimeaza 12	Egypt	Moderate

2.1.1. Sowing

The five wheat parents were sown in three planting dates with 7 days interval in order to overcome the differences in flowering time among parents for crossing in seasons 2018 and 2019 to obtain a large quantity of first generation hybrid seeds. All entries (parents and their F1 crosses) were grown under normal and drought conditions in a randomized complete block design with three replicates for each experiment in season 2020.

2.1.2. Studied traits

Fifty plant were taken from each genotype for the two experiments (normal and drought treatments) to evaluate the following traits:

1): Number of filled grains per panicle: Filled grains of the main panicle with separated and counted.
2):1000-grain weight (g): It was recorded as the weight of 1000 random filled grains per plant.
3): Grain yield per plant (g): was recorded as the weight of grain yield of each individual plant, and adjusted to 14% moisture content.
4): The proline content: was determined from a standard curve and calculated on a fresh basis is as follows: [(µg proline / ml C m1 toluence) / 115.5 µg / µ mole] / [(g sample/5)] = µ moles proline / g of fresh weight material. The results related with proline content are average values at least 3-4 samples for each species, according to Chinard (1942) and modified method by Bates *et al.* (1973).

5): Glycine betaine content: - It was carried out according to the method of Grieve & Grattan (1983).
6): Osmotic adjustment: - It was determined as follows:

$$\frac{\text{O.P. x R.W.C.}}{100}(\text{Normal}) - \frac{\text{O.P x R.W.C.}}{100}(\text{drought})\ ^{100};$$

Where: O.P = Osmotic pressure, R.W.C. = Relative water content.

2.2. Methods

2.2.1. Statistical analysis

All calculated data from all studied traits under the two experiments were analyzed using half diallel analysis by Griffing (1956) model I, method II including heterosis over better-parent, general and specific combining ability effects where GCA/SCA ratio: - MSe of GCA-MS error term /Number of parent + 2/ MSe of SCA-MS error term, respectively.

2.2.2. Estimation of tolerance indices

All drought tolerance indices were estimated according to Fischer & Maurer, (1978); Bouslama & Schapaugh, (1984); Fernandez (1992); Gavuzzi *et al.,* (1997) & Golestani & Assad (1998) as follows in table (10):- 1):- GYP: Is meaning the grain yield/plant for the control experiment, 2):- GYS: Is meaning the grain yield/plant for the stress experiment, 3):- YSI: Is meaning yield stability index = YS/YP where: YS the average of yield under stress and YP= The average of yield under the control experiment, 4):- YI: Is meaning yield index (YS for each genotype/mean of YS for all genotypes), 5):- MP: Is means (Average yield for both trials): YS + YP/2 , 6):- STI: Is meaning drought tolerance index

(YP X YS/ (mean of YP) 2 , 7):- GMP: (YP X YS) 0.5 , 8):- YR: Is meaning yield reduction (1-YS/YP). 9):- DSI: Is meaning salinity susceptibility index = (1-YS/YN)/D where YS = mean yield under salt stress, YN = mean yield under control or normal condition, and D = environmental stress intensity= 1-(mean yield of all genotypes under stress/mean yield of all genotypes under irrigated conditions). Note: - Osmotic adjustment was conducted according to Jones & Turner (1978).

2.3. Molecular Characterization

2.3.1. Genomic DNA extraction and PCR condition

Total genomic DNA of all samples was extracted from 12 green wheat leaves (The five parents and the best seven F1 crosses) using Qiagen DNeasy Plant Minikit following the protocol of the manufacturer (Qiagen Inc, Valencia, CA). The quality of the extracted DNA was assessed on agarose gel electrophoresis. PCR was performed using five preselected ISSR primers based on their ability to generate reproducible and informative amplification patterns. Amplification reactions were carried out in Biometra T One Thermal Cycler (Analytik Jena, Jena, Germany). PCR amplification was performed in 25 µl reaction mix which contained 20-30 ng DNA template, 10 pmole of each primer, 2.5 µl of 2mM Thermo dNTPs, 5 µl of 5x Promega Green GoTaq Flexi Reaction Buffer, 2.5 µl of 25 mM Promega $MgCL_2$ and 0.125 µl of 5 U/µl Promega GoTaq Flexi DNA polymerase. The reaction was assembled on ice, amplification was performed at certain conditions as follows: an initial denaturing step at 94 ^{0}C for 5 min followed by 35 cycles at 94 ^{0}C for 30 sec., annealing at 50 ^{0}C for 1 min, an extension at 72 ^{0}C for 1 min and final extension at 72 ^{0}C for 7 mins. The PCR products was assessed on 1.6% agarose gel (Sambrook *et al.,* 1989; Zietkiewicz *et al.,* 1994 & Gezahegn *et al.,* 2010). Banding profile of ISSR were scored using Labimage program and the polymorphism percentage was estimated as follow :- Percent of polymorphism = (Number of polymorphic bands/Total Number of Bands) X 100.

The best seven crosses were:- H1: Sakha 8 X Sakha 94, H2: Sakha 8 X Gimeaza 11, H3: Sakha 8 X Gimeaza 12, H4: Misr 1 X Sakha 94 , H5: Misr 1 X Gimeaza 12, H6: Sakha 94 X Gimeaza 12 and H7: Gimeaza 11 X Gimeaza 12 respectively. **Note:-** Molecular Sizes of marker used in analyses were as follow: 100, 200, 300, 400, 500, 600, 700, 800, 900, 1000, 1500 and 3000 bp. Also: - T.B: Total bands, M.B: Monomorphic bands, P.B: Polymorphic bands, U.B or P.S.M: Unique bands or positive specific marker, P%: Polymorphism percentage and R.S (bp): Range size.

Table 2: Name and sequences of the five selected ISSR primers used in PCR profile analysis.

Primer Code	Sequence(5'→3')	Abbrev.	Mer
SR-14	AGAGAGAGAGAGAGAGYG	(AG)8YG	18
SR-22	CACCACCACCACCAC	(CAC)5	15
SR-29	GAGAGAGAGAGAGAGAT	(GA)8T	17
SR-35	GACAGACAGACAGACAAT	(GACA)4AT	18
SR-37	GATAGATAGATAGATAGC	(GATA)4GC	18

2.3.2. Data Handling and cluster analysis (Phylogenetic Tree)

Data was scored for computer analysis on the basis of the presence or absence of the amplified products for each primer. Pairwise components of the twelve wheat accessions based on the presence or absence of unique and shared polymorphic products, were used to determine similarity coefficients according to Jaccard (1908). The similarity coefficients were then used to construct dendrograms, using the un weighted pair group method with arithmetic averages (UPGMA) employing the SAHN (Sequential, Agglomerative, Hierarchical and Nested clustering) from the NTSYS-PC (Numerical Taxonomy and Multivariate Analysis System), version 1.80 (Applied Biostatistics Program).

3. Results & Discussion

Wheat is considered one of the most important field crops at the strategic level, as it is a first-class food crop because this crop represents the backbone of global food, mainly for the people of the third World. On this basis, the decline in wheat-cultivated areas represents a major food crisis and requires immediate treatment to contain this serious food problem. In fact, it is noted that the main reason for the decline in the areas planned for wheat cultivation is drought stress and the scarcity of water sources, especially designated for wheat cultivation, (El-Mouhamady 2009 & Al-Kordy *et al.,* 2019). Based on this, this investigation was launched as a serious attempt to study the physiological and biochemical responses related to the wheat crop's tolerance to water stress under Egyptian conditions using a selected group of parents and their F1 hybrids resulting from it using half diallel analysis. The evaluation process was carried out under water stress treatment compared to the standard experiment, and this will be presented in detail.

3.1. Variation & Interaction

Results shown in table (3) confirmed that all differences among all wheat entries, GCA and SCA effects were highly significant. There is no doubt that this result is the required statistical data that shows the extent of the difference in all wheat accessions that is included in the statistical half diallel analysis. Also, these highly significant statistical differences are conclusive evidence that all the wheat under genotypes study were ideal materials for tracing the genetic behavior of drought tolerance from the parents to their hybrids. Further, the difference between all wheat materials is the main input to Griffing model 1, method 2 which includes parents and their F1 hybrids. The effects of general and specific combining ability effects are good evidence of the differences between wheat entries from each other. This confirms that the selection process in early segregation generations will be useful and a promising sign for the success of breeding to drought tolerance in wheat under Egyptian conditions, (El-Mouhamady *et al.,* 2012 a & Khatab *et al.,* 2017).

3.2. Mean performance

Data obtained in table (4) revealed that the wheat accessions; (P1, P2, P3, P1 X P3, P1 X P4, P1 X P5, P2 XP3, P2 X P5, P3 X P5 & P4 X P5) were succeed in showing the highly rank of drought tolerance for all attributes under study and this tolerance was under water stress treatment compared to the standard experiment. In fact, it is noted that the process of tolerating of water stress is the result of a group of physiological, biochemical and genetic factors alike, as all of these factors combined are what created and achieved resistance in promising wheat parents besides, their 7 F1 hybrids under drought conditions compared to the normal treatment, (Ramadan *et al.,* 2016; Esmail *et al.,* 2016 & El-Mouhamdy *et al.,* 2016). It is also more likely to say that physiological factors and mechanisms, such as adjusting plant osmosis and achieving adjusted osmotic pressure (osmotic adjustment), are among the most important mechanisms for inducing water stress tolerance in new wheat hybrids. Where, the seven superior wheat entries mentioned above were able to adjust their osmosis so that they could resist and live under water stress conditions. Further, biochemical traits were the most importance indices for water stress tolerance in wheat accessions. Where, the promising crosses were succeeded in producing highly level of proline and glycine betaine contents under drought conditions compared to the normal treatment. This point confirmed that the seven wheat entries were able to change their physiological behavior to avoid drought conditions unlike the other sensitive genotypes. Also, this physiological change is considering a pure reflection on the succeeding of plant breeding for drought tolerance in wheat, (Parida 2005; El-Mouhamady *et al.,* 2014; Nessem & Kasim 2019; El-Mouhamady & El-Metwally 2020; Khatab *et al.,* 2021 b & El-Mouhamady *et al.,* 2021 a). In the same track, physiological and biochemical parameters were complementary together to enhance the ability of water stress tolerance in plants generally and in wheat crop specifically. Therefore, improving crops using traditional plant breeding and modern techniques of biotechnology for resistance and tolerance abiotic and biotic stress would be the essential tools in this regard. Also, it is noticeable in the data presented in table (4) that grain yield/plant, 1000-grain weight and number of filled grains/ spike attributes were showed a clear moral superiority for the first three wheat parents and the seven their F1 hybrids under drought stress compared to the standard experiment. This result proves beyond doubt the extent to which the first-generation hybrid outperformed their parents in all yield traits and this also proves the extreme of segregation generation in the quantitative inheritance of these traits. Therefore, these results may confirm the good selection of parents included in the hybridization program and the success of the process of transferring important quantitative traits, such as high yield and water stress tolerance from tolerant parents to the new hybrids, which will be effective wheat lines to resist environmental stresses in the future. On this basis, the strategic goal of this investigation is to bring about a radical change in the genetic behavior of wheat plants that are sensitive to drought and make it highly tolerant, (El-Mouhamady *et al.,* 2019; El-Mouhamady and Ibrahim 2020; El-Mouhamady *et al.,* 2022 & El-Mouhamady 2023).

Table 3: Mean Squares of the half diallel analysis for all studied traits under the control and drought conditions.

S.O.V	D.F	Number of filled grains/Spike		1000-grain weight (gm)		Grain yield/plant (gm)		Proline cont.		Glycine betaine cont.		Osmotic adjustment
		N	S	N	S	N	S	N	S	N	S	
Reps	2	1.53	1.65	1.22	1.08	1.82	1.47	1.03	1.40	1.76	1.94	0.39
Genotypes	14	22.34**	17.54**	26.94**	31.17**	13.08**	20.07**	10.34**	14.75**	10.05**	12.17**	8.42**
GCA	4	78.23**	112.05**	100.05**	83.23**	91.03**	71.05**	123.05**	112.08**	100.05**	93.04**	212.65**
SCA	10	34.32**	67.06**	71.19**	45.03**	69.04**	28.94**	56.14**	81.56**	71.06**	53.01**	117.95**
Error	28	1.88	1.57	1.42	1.15	1.66	1.72	1.54	1.70	1.62	1.54	1.39
Error term		0.62	0.52	0.47	0.38	0.55	0.57	0.51	0.56	0.54	0.51	0.46
GCA/SCA		2.27	1.67	1.40	1.84	1.31	2.45	2.19	1.37	1.40	1.75	1.80

Table 4: Mean performances of all studied traits in all wheat accessions tested under the control and drought conditions.

Entries	Number of filled grains/Spike		1000-grain weight (gm)		Grain yield/plant (gm)		Proline content		Glycine betaine content		Osmotic adjustment
	N	D	N	D	N	D	N	D	N	D	
P1	55.13	47.13	45.12	32.06	44.12	31.08	38.15	33.14	42.16	46.04	0.27
P2	49.54	38.15	39.27	28.05	38.19	30.04	44.05	32.11	48.19	39.07	0.53
P3	50.04	40.22	42.11	34.22	46.17	34.11	55.16	44.01	49.04	39.18	0.32
P4	32.06	21.09	26.19	17.41	28.14	17.13	25.23	13.02	27.05	18.06	1.15
P5	29.18	17.25	23.18	13.06	31.05	24.14	21.84	12.09	24.15	11.06	1.67
P1 X P2	30.11	24.05	33.16	17.03	28.04	17.62	18.32	7.25	23.07	12.37	1.22
P1 X P3	88.13	71.09	48.52	39.77	55.11	46.13	68.24	55.03	63.11	53.01	0.22
P1 X P4	78.12	69.04	55.14	44.03	60.15	51.08	71.26	63.04	59.14	47.16	0.19
P1 X P5	80.51	68.13	60.19	42.05	57.13	48.03	81.04	68.95	79.13	66.03	0.15
P2 X P3	84.32	68.16	63.15	48.13	62.08	47.15	84.25	64.27	77.11	59.02	0.11
P2 X P4	20.13	11.04	20.02	9.28	25.03	13.04	19.26	6.04	21.08	12.31	1.55
P2 X P5	87.33	71.04	65.83	55.11	64.18	55.02	89.07	73.15	81.05	69.24	0.44
P3 X P4	27.15	14.02	18.04	9.36	21.02	10.06	17.45	8.23	19.14	10.18	1.36
P3 X P5	86.22	74.05	56.42	47.07	64.13	51.02	91.03	78.14	80.07	69.76	0.18
P4 X P5	75.62	63.11	51.13	40.08	68.19	55.14	87.11	71.04	79.05	68.04	0.15
LSD at 0.05	1.90	1.74	1.65	1.48	1.78	1.82	1.72	1.81	1.76	1.72	1.63
LSD at 0.01	2.76	2.52	2.40	2.16	2.59	2.64	2.49	2.62	2.56	2.49	2.37

3.3. Heterosis over Better Parent

The wheat entries (P1, P2 & P3) besides the crosses; (P1 X P3, P1 X P4, P1 X P5, P2 X P3, P2 X P5, P3 X P5 & P4 X P5) exhibited highly significant and positively values of heterosis over better parent for all attributes under study for the two treatments in table (5). This investigation was able to highlight the fruitful and effective role of the actual value of hybrid vigor in the seven wheat hybrids mentioned above.

Further, clarifying the success of quantitative genetics in inheriting high yield and water stress tolerance traits through monitoring the role of additive gene action in this context. On this basis, the plant breeder places the maximum in the breeding program for hybridization with the aim of obtaining the desired quantitative traits such as high yielding and water stress tolerance besides, some physiological traits related to the tolerance that can be observed in the early segregation generation. This will not be achieved unless the required genetic mechanisms and genes are available in the tolerant parents in hybridization program, (El-Mouhamady *et al.,* 2014 & El-Mouhamady *et al.,* 2022).

3.4. Combining Ability Effects

3.4.1. GCA Effects

Data shown in table (6) revealed that the first three wheat parents recorded highly significant and positively values of GCA effects parameter for all traits studied under normal and drought conditions. On the other hand, the wheat parents number 4 and 5 were exhibited the same results but in the opposite direction. Results of the half diallel analysis using Griffing model one method 2 showed that the parents included in the crossbreeding program were considered highly rank in the general combining ability effects and this confirms the extent of success achieved in selecting these promising parents for producing wheat hybrids tolerated to water stress and highly yielding. This highlights the importance of additive effects and their reactions in inheriting of yield components, biochemical and physiological traits related to water stress tolerance in wheat. Also, it determines that the appropriate time to select for these excellent traits is during the early segregation generations, (El-Mouhamady *et al.,* 2012 & 2013; Nessem & Kasim 2019; Kizilgeci *et al.,* 2021 & Gupta *et al.,* 2024).

3.4.2. SCA Effect

Results viewed in table (6) detected that the seven wheat accessions; (P1 X P3, P1 X P4, P1 X P5, P2 X P3, P2 X P5, P3 X P5 & P4 X P5) were recorded highly significant and positively values of SCA effects for all attributes under testing for the two conditions. While, the other three crosses exhibited the data but in negative direction. These results confirm that the seven promising wheat hybrids had achieved great progress in the field of SCA effects parameter and were closely linked to the measure of hybrid vigor, which confirmed their tolerance to water stress. In contrast to the remaining three hybrids, which did not achieve the same results and confirms their sensitivity and intolerance to this dangerous environmental factor and the weakness of their final output. Also, these positive indicators reflect the fruitful role of dominance and dominance X dominance gene interaction in inheriting the aforementioned desirable traits related to drought tolerance and highly yielding, (Khatab *et al.,* 2019 &Tawfik & El-Mouhamady 2024).

3.5. Drought Tolerance Indices

After a comprehensive review of all results of prospective genetic measurements which demonstrated the existence of new genotypes tolerant to water stress in the wheat crop, it was necessary in order to cover all aspects of the study, including testing of drought tolerance indices in grain yield/individual plant in table (7). The most wheat accessions recorded highly rank of water stress tolerance indices were (P1 X P3, P1 X P4, P1 X P5, P2 X P3, P2 X P5, P3 X P5 & P4 X P5) for the two parameters; (YSI & YI) where the values were less than the unity. Further, all entries under study were exhibited values less than one for (YR) parameter which confirms that all wheat entries under testing were able to reduce the losses % in their final yield and this is a strong indication of the extent of their tolerance to water stress. Also, the entries; (P1, P2, P1 X P4, P1 X P5, P2 X P5, P3 X P5 & P4 X P5) were exhibited mean values lower than the unity for DSI parameter which confirmed that these wheat accessions were highly tolerance to water stress, (El-Demardash *et al.,* 2017; Khatab *et al.,* 2019 & El-Mouhamady & Habouh 2019; El-Mouhamady *et al.,* 2021 &Tawfik & El-Mouhamady 2024). Traditional plant breeding programs, particularly using simple hybridization between drought-tolerant

Table 5: Heterosis over better-parent for the 10 Wheat crosses obtained from half diallel analysis in all studied traits under the two conditions.

Entries	Number of filled grains/Spike		1000-grain weight (gm)		Grain yield/plant (gm)		Proline content		Glycine betaine content		Osmotic adjustment
	N	S	N	S	N	S	N	S	N	S	
P1 X P2	-45.38**	-48.97**	-26.50**	-46.88**	-36.44**	-43.30**	-58.41**	-78.12**	-52.12**	-73.13**	351.85**
P1 X P3	59.85**	50.83**	7.53**	16.21**	19.36**	49.75**	23.71**	25.03**	28.69**	15.13**	-18.51**
P1 X P4	41.70**	46.48**	22.20**	37.33**	36.33**	64.35**	86.78**	90.22**	40.34**	2.43**	-33.33**
P1 X P5	46.03**	44.62**	99.66**	31.16**	29.48**	54.53**	112.42**	108.05**	87.68**	43.41**	-44.44**
P2 X P3	68.50**	69.46**	49.96**	40.64**	34.45**	38.22**	52.73**	46.03**	57.23**	50.63**	-65.62**
P2 X P4	-59.36**	-71.06**	-49.01**	-66.91**	-34.45**	-55.92**	-56.27**	-81.18**	-56.25**	-68.49**	192.45**
P2 X P5	76.28**	94.10**	67.63**	96.47**	68.08**	83.15**	102.20**	127.81**	68.18**	77.22**	-16.98**
P3 X P4	-45.74**	-65.14**	-57.15**	-72.64**	-54.47**	-70.50**	-68.36**	-81.29**	-60.97**	-74.01**	325.0**
P3 X P5	72.30**	84.11**	33.98**	37.55**	38.89**	49.57**	65.02**	77.55**	63.27**	78.05**	-43.75**
P4 X P5	135.87**	199.24**	95.22**	130.21**	119.61**	128.41**	245.26**	445.62**	192.23**	276.74**	-86.95**
LSD at 0.05	1.90	1.74	1.65	1.48	1.78	1.82	1.72	1.81	1.76	1.72	1.63
LSD at 0.01	2.76	2.52	2.40	2.16	2.59	2.64	2.49	2.62	2.56	2.49	2.37

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Table 6: GCA and SCA effects in wheat accessions under normal and drought conditions.

Entries	Number of filled grains/Spike		1000-grain weight (gm)		Grain yield/plant (gm)		Proline content		Glycine betaine content		Osmotic adjustment
	N	S	N	S	N	S	N	S	N	S	
P1	7.87**	15.32**	10.56**	8.74**	11.67**	30.01**	13.24**	17.80**	12.77**	14.42**	13.45**
P2	14.01**	11.95**	11.32**	14.33**	25.31**	41.23**	20.06**	25.14**	27.93**	22.06**	22.71**
P3	22.05**	10.63**	18.94**	20.06**	18.91**	18.03**	24.12**	42.03**	32.08**	28.04**	18.40**
P4	-22.69**	-28.11**	-17.88**	-16.98**	-40.89**	-46.38**	-31.28**	-51.69**	-55.23**	-31.69**	-41.28**
P5	-21.24**	-9.79**	-22.94**	-26.15**	-15.0**	-42.89**	-26.14**	-33.28**	-17.55**	-32.83**	-13.28**
LSD at 0.05 (gi)	1.76	1.22	2.55	1.78	1.03	1.08	1.55	1.38	1.93	1.88	1.78
LSD at 0.01 (gi)	2.04	1.84	3.87	2.44	1.47	1.78	2.14	2.73	2.71	2.59	2.85
P1 X P2	-88.15**	-27.70**	-115.80**	-78.44**	-245.11**	-125.61**	-148.13**	-180.05**	-125.13**	-142.06**	-112.14**
P1 X P3	15.22**	7.95**	76.22**	80.05**	23.88**	17.33**	100.03**	78.04**	27.08**	18.52**	25.78**
P1 X P4	34.01**	24.72**	18.45**	22.12**	18.05**	22.08**	67.57**	92.13**	48.05**	63.23**	31.66**
P1 X P5	14.03**	20.03**	30.71**	26.93**	55.61**	47.81**	81.15**	78.23**	37.93**	42.13**	45.93**
P2 X P3	17.08**	12.05**	27.93**	25.41**	60.03**	52.01**	70.32**	62.14**	55.62**	49.52**	55.60**
P2 X P4	-47.66**	-44.16**	-48.13**	-66.80**	-41.55**	-78.15**	-150.23**	-113.80**	-78.11**	-39.40**	-51.33**
P2 X P5	27.04**	16.01**	40.03**	36.07**	100.04**	83.92**	26.92**	30.03**	70.02**	68.23**	71.45**
P3 X P4	-52.67**	-55.84**	-68.34**	-83.93**	-121.85**	-94.09**	-75.89**	-90.33**	-63.61**	-85.96**	-46.35**
P3 X P5	51.06**	27.88**	13.21**	17.51**	70.14**	63.02**	18.22**	27.26**	18.83**	10.04**	20.05**
P4 X P5	30.04**	19.06**	25.72**	21.08**	80.76**	77.68**	10.04**	16.35**	19.32**	16.29**	30.08**
LSD at 0.05 (Sij)	1.31	1.04	1.25	1.88	1.53	1.71	1.21	1.36	1.44	1.59	2.23
LSD at 0.01 (Sij)	1.98	2.11	1.75	2.53	2.45	2.58	1.95	2.29	2.55	2.46	3.01

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lines among the other sensitive entries have made significant scientific progress in the field of wheat improving to drought stress. This was clearly demonstrated by the genetically tests and measurements used in the study and resulting from the hald diallel-analysis for all yield components, biochemical and physiological attributes in all wheat accessions under drought stress compared to the standard experiment. Further, this investigation succeeded in explaining the true meaning of physiological acclimatization by causing osmotic changes in water stress tolerant hybrids, unlike sensitive ones. It is also proved their ability to produce organic compounds and acids, which were a fundamental reason for tolerance. All this ultimately was led to the production of water stress tolerant and high yield wheat hybrids under Egyptian conditions. Also, drought tolerance indices demonstrated the high capacity of these new wheat accessions to endure and simulate harsh environmental conditions.

Table 7: Drought tolerance indices in wheat entries for the two experiments.

Genotypes	1	2	3	4	5	6	7	8	9
P1	**55.13**	**47.13**	0.85	1.01	51.13	44.62	50.97	**0.15**	**0.75**
P2	**49.54**	**38.15**	0.77	0.82	43.84	32.45	43.47	**0.23**	**1.15**
P3	**50.04**	**40.22**	0.80	0.86	45.13	34.56	44.86	**0.20**	1.0
P4	32.06	21.09	0.65	0.45	26.57	11.61	26.0	0.35	1.75
P5	29.18	17.25	0.59	0.37	23,21	8.64	22.43	0.41	2.05
P1 X P2	30.11	24.05	0.79	0.51	27.08	12.43	26.90	0.31	1.55
P1 X P3	**88.13**	**71.09**	0.80	1.52	79.61	107.59	79.15	**0.20**	**1.0**
P1 X P4	**78.12**	**69.04**	0.88	1.48	73.58	92.62	73.43	**0.12**	0.60
P1 X P5	**80.51**	**68.13**	0.84	1.46	74.32	94.19	74.06	**0.16**	**0.80**
P2 X P3	**84.32**	**68.16**	0.80	1.46	76.24	98.69	75.81	**0.20**	**1.0**
P2 X P4	20.13	11.04	0.54	0.23	15.58	3.81	14.90	0.46	2.30
P2 X P5	**87.33**	**71.04**	0.81	1.52	79.18	10.65	78.76	**0.19**	**0.95**
P3 X P4	27.15	14.02	0.51	0.30	20.58	6.53	19.51	0.49	2.45
P3 X P5	**86.22**	**74.05**	0.85	1.59	80.13	109.64	79.90	0.15	0.75
P4 X P5	**75.62**	**63.11**	0.83	1.35	69.36	81.95	69.08	0.17	0.45

3.6. Molecular Description

Molecular markers are considering the biggest importance technique for generic improvement in crops. Where, this method was succeed in change a big jump in the field of increasing yield and its components in several crops such as wheat, barley, rice, maize, soybean and so on. Also, Biotechnology techniques, especially genetic engineering, genetic transport, and tissue culture were among the most successful scientific cultivation of modern or pirate molecular genetics for enhancing and improving crops for abiotic and biotic resistance. Regarding the endurance of water stress, the biotechnology had a major role, especially in the most sensitive and needy for water in strategic crops like wheat. On this basis, molecular markers using ISSR primers will play a major role in identifying genetic mechanisms at the molecular level responsible for water stress tolerance in promising wheat hybrids, with the aim of distinguishing these wheat accessions between it and sensitive crosses in terms of defining the division basis for these entries and their use in other studies for abiotic stress tolerance. Data are viewed in (table 8 and Fig.1) showed that the five ISSR primers exhibited 41 amplified fragments, 31 of them were monomorphic besides, 28 polymorphic included 9 unique bands with 68.29% polymorphism and the molecular weights ranged from226 bp to 1633bp. Also, the highest polymorphic number were showed in ISSR-29, 35 and 37 primers were the values were (5, 6 & 11). While, the highest number of unique amplicons were generated by SR -35 and 37 primers (6 & 3), respectively. Further, the biggest rank of polymorphism % (85.71% & 84.61%) was detected by ISSR-35 & 37 primers. These results confirmed that the five ISSR markers were able to unpack and identify all genetic differences among the twelve wheat genotypes. Also, they were used effectively as a classification and identification basis for wheat hybrids that are highly tolerant to water stress and even succeeded in identifying parents and hybrids that are sensitive to it. These results were agreement and similarity with (Zian *et al.,* 2013; Khatab & El-Mouhamady 2022).

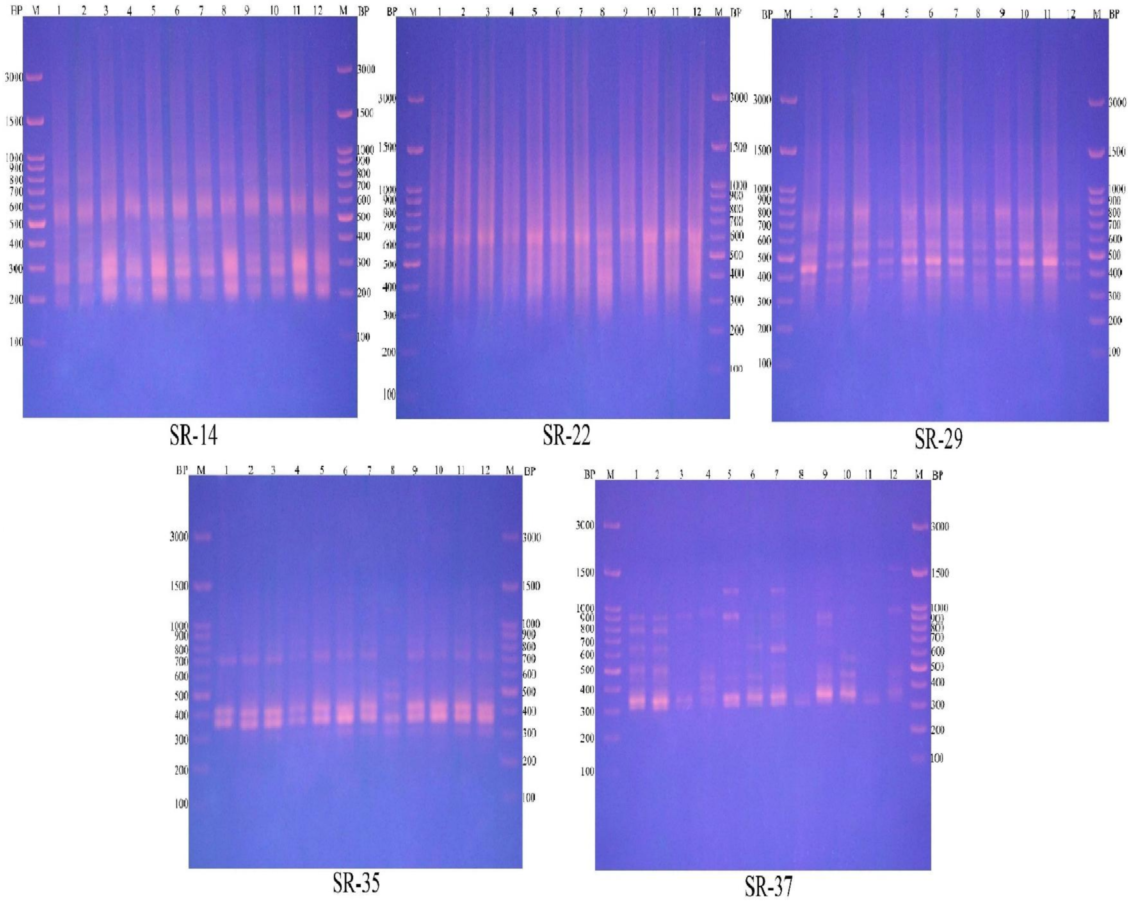

Fig. 1: ISSR primers used in twelve wheat accessions namely; SR-14, 22, 29, 35 and SR-37.

Table 8: Band variation and polymorphism % in the twelve wheat entries using 5 ISSR markers.

No.	ISSR primers	T.B	M.B	P.B	U.B	P %	R.S (bp)	Sequence
1	**SR-14**	8	4	4	**0**	**50.0%**	872-226	AGAGAGAGAGAGAGAGYG
2	**SR-22**	4	2	**2**	0	**50.0%**	662-442	CACCACCACCACCAC
3	**SR-29**	9	4	**5**	0	**55.55%**	806-333	GAGAGAGAGAGAGAGAT
4	**SR-35**	7	1	6	6	**85.71%**	856-333	GACAGACAGACAGACAAT
5	**SR-37**	13	2	11	3	**84.61%**	1633-331	GATAGATAGATAGATAGC
Total		41	13	28	9	68.29%	1633-226	

Data presented in table (9) recorded nine specific markers divided to 4 positive and 5 negative amplified fragments among the twelve wheat accessions (The five parents and the best seven crosses) through using the five ISSR primers. In fact, these specific markers are among the most important outcomes of this investigation, as they were able to be a practical guide to identify and distinguish hybrids that excel in yield and drought tolerance from those that are sensitive to it. Also, these markers will be used in the future to track highly tolerant and yielding wheat lines until they are produced and distributed commercially as new drought-tolerant wheat varieties. The primer SR-35 exhibited six markers as follows; four negative (two for the parent number 1 at sizes of 333bp and 856 bp and two for hybrid 3 at sizes of 441bp and 751bp). While, the other two positive markers were showed in hybrid 3 at sizes of 501 bp and 561bp, respectively. On the same track, the primers SR-37 recorded three specific markers for hybrid number 7 as follows; two positive at molecular sizes of 985bp and 1633bp besides, one negative at size of 331bp. (Khatab *et al.,* 2017 & 2022). Also, data viewed in table (10)

Table 9: Mapping of positive and negative unique bands in wheat genotypes.

ISSR Primers	MS(bp)	Parent 1	Parent 2	Parent 3	Parent 4	Parent 5	Hybrid 1	Hybrid 2	Hybrid 3	Hybrid 4	Hybrid 5	Hybrid (6)	Hybrid (7)	(P or N) Marker
SR-35	856	-	+	+	+	+	+	+	+	+	+	+	+	Negative (P1)
	751	+	+	+	+	+	+	+	-	+	+	+	+	Negative (H3)
	561	-	-	-	-	-	-	-	+	-	-	-	-	Positive (H3)
	501	-	-	-	-	-	-	-	+	-	-	-	-	Positive (H3)
	441	+	+	+	+	+	+	+	-	+	+	+	+	Negative (H3)
	333	-	+	+	+	+	+	+	+	+	+	+	+	Negative (P1)
SR-37	1633	-	-	-	-	-	-	-	-	-	-	-	+	Positive (H7)
	985	-	-	-	-	-	-	-	-	-	-	-	+	Positive (H7)
	331	+	+	+	+	+	+	+	+	+	+	+	-	Negative (H7)
Total														4 (Positive) + 5 (Negative)

P: Positive, **N:** Negative, **MS:** Molecular Size exhibited 55 relationships among 12 wheat genotypes revealed about similarity. The values were ranged from 0.500 to 0.945 with an average of 0.722. Where, the biggest level of similarity was (0.945) among (P5 & H2) and the lowest limit was (0.500) between (P4 & H3). The other values were showed in various rank of genetic similarity. These results demonstrate the utmost importance of similarity and difference relationships in terms of the degree of genetic kinship between parents and their F1 hybrids that are compatible with each other for cultivation and growth under the same conditions. Further, this reflects the basic strategy of this study, which is to identify which wheat genotypes have high genetic compatibility for growth together in order to establish a strong breeding program to obtain high-yielding and water-stress-tolerant wheat lines. Results of phylogenetic tree (Fig, 2) revealed the result of 12 wheat entries were divided to two main cluster. where the first one was (H3) only. While, the second cluster had two sub-cluster. The first one was (H7) only and the second sub-cluster was concluded the other wheat entries, (Morran *et al.,* 2011; Budak *et al.,* 2013; Rana *et al.,* 2016; Ahmad *et al.,* 2018; Khan *et al.,* 2019; Yadav *et al.,* 2019 & Gupta *et al.,* 2024).

Table 10: Similarity % of the twelve Wheat Entries within using 5 ISSR markers.

Similarity %	P1	P2	P3	P4	P5	H1	H2	H3	H4	H5	H6	H7
P1	1.0											
P2	0.909	1.0										
P3	0.696	0.787	1.0									
P4	0.750	0.787	0.625	1.0								
P5	0.805	0.888	0.742	0.742	1.0							
H1	0.771	0.805	0.657	0.705	0.810	1.0						
H2	0.810	0.891	0.702	0.702	**0.945**	0.864	1.0					
H3	0.527	0.567	0.700	**0.500**	0.621	0.676	0.589	1.0				
H4	0.848	0.939	0.781	0.727	0.833	0.800	0.837	0.555	1.0			
H5	0.742	0.828	0.676	0.727	0.885	0.852	0.837	0.647	0.823	1.0		
H6	0.657	0.742	0.741	0.636	0.800	0.818	0.756	0.709	0.787	0.903	1.0	
H7	0.540	0.621	0.514	0.709	0.675	0.685	0.641	0.485	0.657	0.757	0.718	1.0

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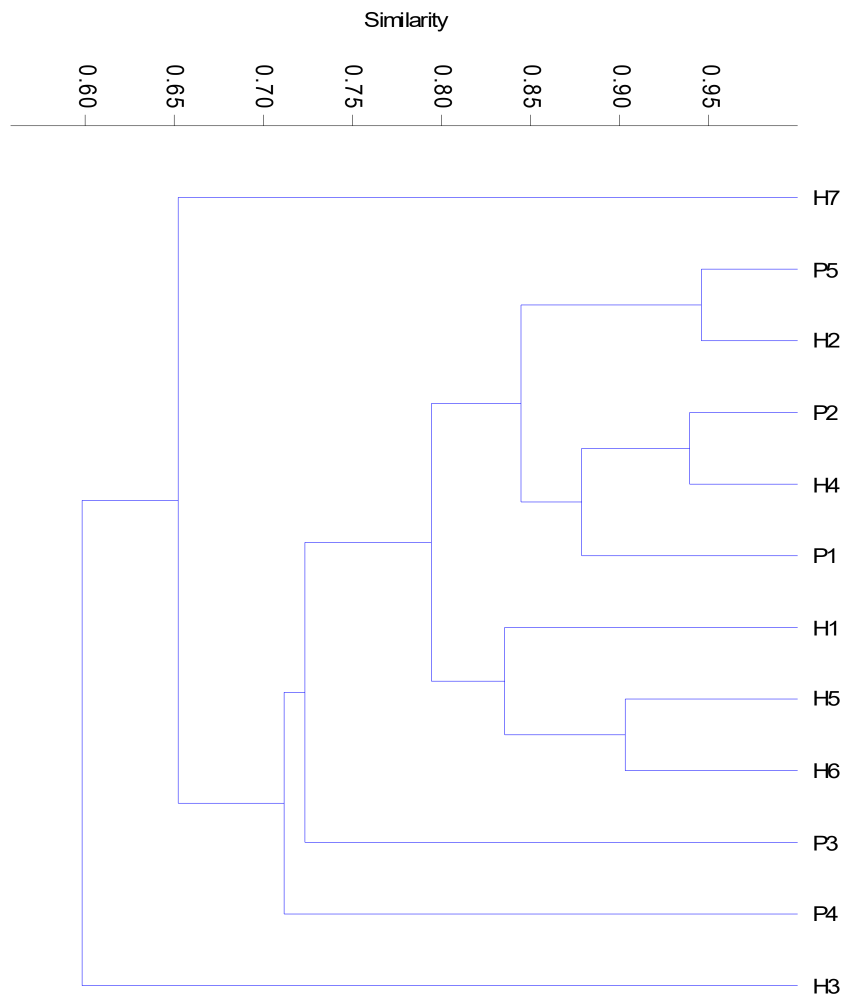

Fig. 2: Dendrogram analysis between 12 Wheat accessions across ISSR markers

4. Conclusion

The present investigation was conducted to study the genetic behavior responsible for water stress tolerance in some promising wheat entries under Egyptian conditions. Also, some yield components, physiological and biochemical attributes were tested for the fifteen wheat accessions (The five parents and their 10 F1 hybrids) under normal and drought conditions. Further, genetic parameters were evaluated in the previous wheat accession under the two conditions such as; heterosis over better-parent, GCA and SCA effects besides, drought tolerance indices test to determine the tolerated wheat genotypes for water stress. Molecular description using five ISSR primers was done for the twelve wheat genotypes (The five parents and the best seven F1 hybrids) which exhibited the highest rank of all attributes calculated under water stress treatment compared to the standard experiment. The final results confirmed that the first wheat parents (P1, P2 & P3) and the seven F1 crosses; (P1 X P3, P1 X P4, P1 X P5, P2 X P3, P2 X P5, P3 X P5 & P4 X P5) were recorded highly level of drought tolerance under the stress treatment compared to the normal conditions. Also, the five ISSR markers exhibited 28 polymorphic fragments with 68.29% polymorphism % among the twelve wheat accessions.

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