(Original Article)

Genetic Analysis of Seedling Traits under Drought Stress Conditions in Bread Wheat

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Abstract

In the present study, a laboratory experiment was conducted to study the gene action and estimate general (GCA) and specific (SCA) combining ability of seedling traits in a half diallel cross of bread wheat. Eight bread wheat genotypes and their F_1 hybrids were evaluated under drought stress induced by the treatment with Polyethylene Glycol-6000. The studied traits were shoot length (SHL), root length (ROL), number of lateral roots (NOR), fresh weight of seedling (FRW) and dry weight of seedling (DRW). Highly significant ($P<0.01$) differences were observed among studied genotypes for all seedling traits under drought stress conditions. Highly significant ($P<0.01$) mean squares due to GCA and SCA effects were observed for all seedling traits, indicating the importance of both additive and non-additive gene actions. Obviously, GCA mean squares were larger in magnitude than that of SCA, indicating the importance role of the additive gene action in the inheritance of the studied traits. Compared to their parents, F_1 hybrids produced longer roots under drought stress conditions, indicating the presence of heterosis. The regression analysis indicated a partial adequacy of an additive-dominance model for ROL, NOR, FRW and DRW, while non adequate model was observed for SHL. Based on GCA effects, the parental genotypes P_1, P_2 and P_3 were the best general combiners for most studied traits under drought stress conditions. The highest desirable SCA effects were obtained from the crosses $P_1 \times P_3$, $P_1 \times P_6$, $P_1 \times P_8$, $P_2 \times P_5$ and $P_2 \times P_7$ for most studied traits, indicating their usefulness as best promising combinations under drought stress conditions.

Keywords: Drought, Seedling traits, Bread wheat, Diallel analysis, Combining ability.

Introduction

Drought affects crop plants in different stages of plant development (Hossain *et al.*, 2012). Moreover, Effects of drought stress differ according to several factors including genotype, environmental conditions, and genotype by environment interaction (Hoffman *et al.*, 2009). The tolerance to drought stress is considered as a complex trait controlled by polygenes with a high-level of genotype-by-environment interaction (Cooper *et al.*, 2006).

Development of wheat genotypes tolerant to drought stress is restricted by several factors including the lack of effective selection criteria (El-Rawy and

Hassan, 2014 a,b). Several drought tolerance traits, including various morphological and physiological traits have been long used for the identification of tolerant genotypes evaluated under drought stress conditions (Drikvand *et al.*, 2012; El-Rawy and Hassan, 2021). In this regard, using morphological and physiological traits as indirect selection criteria could be useful to increase yield-based selection procedures in wheat breeding programs (Lonbani and Arzani, 2011).

Polyethylene glycol has long been used to simulate drought stress as an osmotic agent reducing the water potential in vitro (El-Rawy and Hassan, 2014a). Furthermore, the identification of wheat genotypes tolerant to drought or water stress has long been practiced using PEG in vitro (Ahmad *et al.*, 2013). Thus, various growth parameters at seedling stage such as shoot length, root length, number of lateral roots and fresh weight of seedling and dry weight of seedling have been successfully used as indirect selection criteria for drought tolerance (Baloch *et al.*, 2012).

Objectives of the present study were 1) to study the genetic system controlling seedling traits of bread wheat in a half diallel cross evaluated under drought stress induced by PEG, 2) to estimate general and specific combining ability for the studied traits as measures of additive and non-additive gene effects, respectively, 3) to identify the best promising genotypes under drought stress conditions to be used as parents in wheat breeding programs.

Materials and Methods

Plant materials

The initial plant materials used in the study consisted of eight genotypes of bread wheat (*Triticum aestivum* L.) (Table 1). The field experiments were conducted at the experimental Farm of Faculty of Agriculture, Assiut University, Egypt. In 2020/2021 winter season, the eight genotypes were grown in the fields and crossed in a half diallel design to produce 28 F_1 hybrids.

Table 1. Names and pedigree of eight bread wheat genotypes used in the study

Code	Name	Pedigree	Origin
P1	Line-1	Advanced inbred line developed at Assiut University	Egypt
P2	Line-2	Advanced inbred line developed at Assiut University	Egypt
P3	Line-3	Advanced inbred line developed at Assiut University	Egypt
P4	Gemmiza-9	ALD'S'/HUAC'S'//CMH74.630/5X	Egypt
P5	Sids-14	BOW "S"/VEE"S"//BOW"S"/TSI/3/BANI SEWEF 1	Egypt
P6	CHAM-8	Kauz (CM67458)	Syria
P7	L.1x15	Advanced inbred line developed at Assiut University	Egypt
P8	Misr-2	SKAUZ/BAV 92	Egypt

PEG treatment

The eight parental genotypes and their 28 F_1 hybrids (36 genotypes) were subjected to a laboratory experiment to study the gene action as well as to estimate general combining ability (GCA) and specific combining ability (SCA) of different seedling traits under drought stress induced by Polyethylene Glycol-6000 (PEG).

Drought stress was induced by the treatment with 15% PEG (w/v) in the soil. The seedlings were harvested after 10 days. The studied seedling traits were shoot length (SHL), root length (ROL), number of lateral roots (NOR), Fresh weight of seedling (FRW) and Dry weight of seedling (DRW).

Statistical analyses

To test the significance of differences due to genotypes as well as GCA and SCA effects of seedling traits under drought stress induced by 15% PEG, the phenotypic data of the seedling traits studied under drought stress were subjected to an analysis of variance. The diallel analysis was performed following Hayman (1954 a,b), Jones (1965) and Mather and Jinks (1971).

Results

Means of the studied genotypes for different seedling traits evaluated under control and drought stress conditions are presented in Table 2. The result showed that the parental genotypes and their hybrids showed different responses to drought stress simulated by the treatment with PEG. On average, F_1 hybrids produced longer roots compared to their parents under drought stress conditions, indicating the presence of heterosis. Due to drought stress simulated by 15% PEG, overall means of SHL, FRW and DRW were decreased by 12.6, 81.8 and 80.0% for parental genotypes, and by 22.6, 83.9 and 75.0% for F_1 hybrids, respectively. In contrast, ROL was increased by 17.8 and 38.5% for parental genotypes and F_1 hybrids, respectively. Interestingly, drought stress increased NOR by 4.7% overall parental genotypes, while a reduction of 9.7% was observed overall F_1 hybrids (Fig. 1).

Table 2. Mean performance of studied genotypes for different seedling traits studied under control and drought stress simulated by Polyethylene Glycol-6000 (15% PEG-6000)

Traits	Environment	Parental genotypes			F₁ hybrids			LSD (0.05)	LSD (0.01)
		Mean	Min	Max	Mean	Min	Max		
SHL (cm)	Control	15.13	11.24	17.52	10.38	6.47	14.13	2.86	3.79
	Drought	13.23	11.93	15.96	8.03	4.09	12.73	2.60	3.45
ROL (cm)	Control	10.02	8.83	12.28	8.84	5.38	12.77	3.54	4.70
	Drought	11.80	10.27	13.09	12.24	8.47	16.59	2.79	3.70
NOR	Control	3.38	2.95	3.60	2.28	1.97	3.20	0.59	1.25
	Drought	3.54	2.52	4.40	2.06	1.64	2.45	0.44	0.58
FRW (g)	Control	0.55	0.42	0.71	0.31	0.17	0.46	0.15	0.19
	Drought	0.10	0.07	0.12	0.05	0.02	0.07	0.02	0.03
DRW (g)	Control	0.10	0.05	0.12	0.04	0.02	0.05	0.02	0.03
	Drought	0.02	0.02	0.03	0.01	0.004	0.01	0.005	0.01

SHL: Shoot length, ROL: Root length, NOR: No. of lateral roots, FRW: Fresh weight of the seedling and DRW: Dry weight of the seedling.
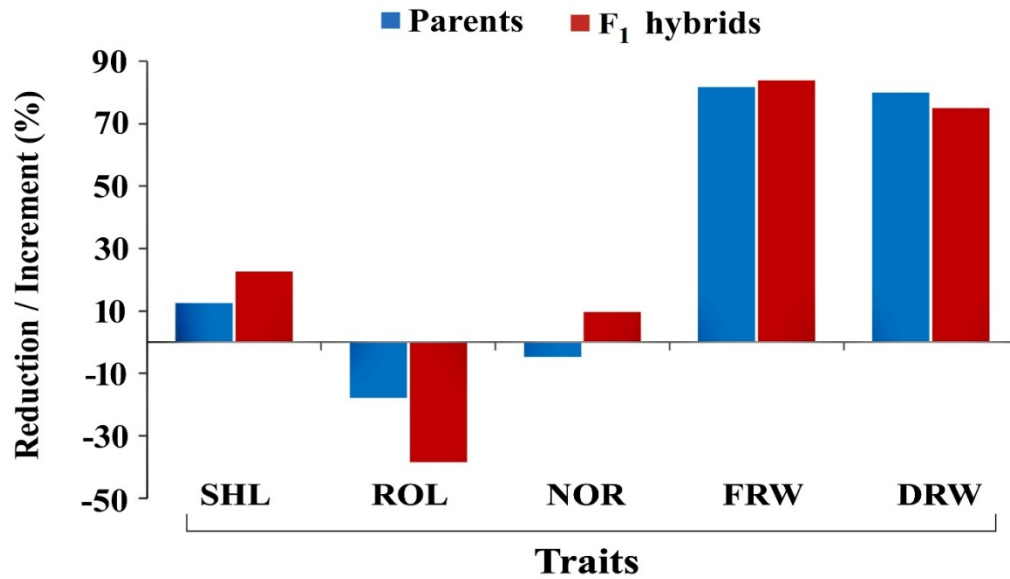
Figure 1. The percentage of reduction (above the x-axis) and increment (below the x-axis) resulted in seedling traits due to drought stress induced by 15% PEG. SHL: Shoot length, ROL: Root length, NOR: No. of lateral roots, FRW: Fresh weight of the seedling and DRW: Dry weight of the seedling.

Highly significant differences (P<0.01) among parental genotypes and their F_1's were observed for all traits evaluated under drought stress conditions. The mean squares due to GCA and SCA effects were also highly significant (P<0.01) for all studied seedling traits, with the mean squares of GCA being much higher compared to mean squares of SCA (Table 3).

Table 3. The diallel analysis of variance under drought stress simulated by 15% PEG

Traits		SHL	ROL	NOR	FRW	DRW
S.O.V.	**d.f**			Mean squares		
Replicates	**2**	1.48	2.80	0.09	0.0006	0.00002
Genotypes	**35**	29.16**	15.04**	1.50**	0.0021**	0.00010**
GCA	**7**	121.69**	52.73**	5.99**	0.0086**	0.00050**
SCA	**28**	6.02**	5.91**	0.38**	0.0005**	0.00004**
Error	**70**	2.54	2.93	0.07	0.0002	0.00001

SHL: Shoot length, ROL: Root length, NOR: No. of lateral roots, FRW: Fresh weight of the seedling and DRW: Dry weight of the seedling. * and **: significant differences at P<0.05 and P<0.01, respectively.

The regression analysis for seedling traits evaluated under drought stress induced by 15% PEG is presented in Table 4. The results showed a partial adequacy of an additive-dominance model for ROL (b= 0.36±0.07), NOR (b= 0.64±0.03), FRW (b= 0.57±0.10) and DRW (b= 0.57±0.15). However, a non-adequate additive-dominance model was observed for SHL (b= 0.14±0.11). Highly significant mean squares (P<0.01) due to the (Wr+Vr) values were observed for SHL and ROL, indicating the existence of a significant dominance variance. Highly significant mean squares (P<0.01) due to the (Wr-Vr) values were also observed for SHL and NOR, indicating epistasis.

Table 4. The Joint regression analysis and mean squares due to (Wr+Vr) and (Wr–Vr) values for different seedling traits studied under drought stress simulated by 15% PEG

Traits	Joint regression (b ± se)	Test for b = 0	Test for b = 1	Mean squares of (Wr + Vr)	Mean squares of (Wr – Vr)	Fitness of the model
SHL	0.14±0.11	1.19	-7.54**	40.25**	13.82**	Non adequate
ROL	0.36±0.07	5.36**	-9.55**	29.45**	12.81**	Partial adequacy
NOR	0.64±0.03	21.55**	-12.32**	0.54	0.03	Partial adequacy
FRW	0.57±0.10	5.64**	-4.29**	0.000038	0.0000025	Partial adequacy
DRW	0.57±0.15	3.66**	-2.80**	0.00000081	0.00000029	Partial adequacy

SHL: Shoot length, ROL: Root length, NOR: No. of lateral roots, FRW: Fresh weight of the seedling and DRW: Dry weight of the seedling. * and **: significant differences at P<0.05 and P<0.01, respectively.

The Wr/Vr relationships under 15% PEG (Fig. 2) indicated an overdominance for SHL, NOR and FRW, whereas a partial dominance for ROL. The P1 possessed the most dominant alleles for SHL. In addition, P1 possessed the most dominant alleles for ROL and DRW. Whereas P4, P5 and P3 possessed the most recessive alleles for ROL. The P4 possessed the most dominant alleles for FRW, and P3 possessed the most recessive alleles. The P4, P2 and P3 possessed the most dominant alleles for NOR, whereas P8 possessed the most recessive alleles.

The highest positive GCA effects for SHL was found in the parental genotype P1 (3.67) followed by P3 (1.41) and P2 (1.33). In addition, P2 possessed the highest positive GCA effect for ROL (2.19) followed by P3 (1.40). Furthermore, P1 possessed the highest GCA values for NOR (1.10), FRW (0.04) and DRW (0.01) (Table 5).

Table 5. The GCA effects of parental genotypes for different seedling traits studied under drought stress simulated by 15% PEG

Genotypes	Traits				
	SHL	ROL	NOR	FRW	DRW
P1	3.667**	0.113	1.098**	0.039**	0.010**
P2	1.328**	2.186**	-0.155**	0.006**	0.001**
P3	1.412**	1.404**	-0.224**	0.002**	0.001**
P4	0.218**	0.636**	-0.232**	-0.007**	-0.002**
P5	-1.303**	-0.548**	-0.115**	-0.013**	-0.002**
P6	-1.802**	-1.253**	-0.075**	-0.008**	-0.003**
P7	-1.704**	-1.412**	-0.154**	-0.009**	-0.003**
P8	-1.815**	-1.127**	-0.143**	-0.009**	-0.002**
SD (GI)	0.0741	0.0856	0.0021	0.0003**	0.0001**

GCA: General combining ability, SHL: Shoot length, ROL: Root length, NOR: No. of lateral roots, FRW: Fresh weight of the seedling and DRW: Dry weight of the seedling. * and **: significant differences at P<0.05 and P<0.01, respectively.

As for F_1 hybrids, high and positive SCA effects were observed for SHL in the crosses P2×P7 (2.86), P1×P6 (1.78), P1×P8 (1.76) and P1×P3 (1.70). The cross

P2×P5 showed greater SCA (2.15) for ROL followed by P2×P7 (1.64). Meantime, desirable SCA were found for NOR in the crosses P1×P8 (1.06) followed by P1×P6 (0.71) and P1×P7 (0.34). Moreover, greater positive SCA values were found in the cross P1×P6 for FRW (0.03) and DRW (0.006) (Table 6).

Table 6. The SCA effects of the F$_1$ hybrids for different seedling traits studied under drought stress induced by 15% PEG

Genotypes	Traits				
	SHL	ROL	NOR	FRW	DRW
P1 X P2	0.472	-2.637**	-0.662**	-0.003	0.001
P1 X P3	1.698*	-1.387*	-0.043*	0.017**	0.003**
P1 X P4	-0.820	0.121	-0.735**	-0.011*	0.001
P1 X P5	0.376	-1.458*	0.165**	-0.016**	-0.002*
P1 X P6	1.780**	-0.326	0.708**	0.026**	0.006**
P1 X P7	0.797	0.888	0.337**	0.010*	0.005**
P1 X P8	1.755*	0.343	1.059**	0.015**	0.006**
P2 X P3	-0.469	0.151	-0.006	-0.017**	-0.003**
P2 X P4	-1.498*	1.613*	-0.065**	0.001	0.002*
P2 X P5	1.116	2.15**	0.335**	0.016**	0.002*
P2 X P6	-0.412	0.129	0.112**	0.001	0.001
P2 X P7	2.863**	1.641*	0.190**	0.010*	0.002*
P2 X P8	0.634	1.496	-0.088**	0.010*	0.001
P3 X P4	-0.079	0.395	0.137**	0.012*	0.001
P3 X P5	-0.619	0.765	0.121**	-0.007	0.003**
P3 X P6	-0.891	-0.199	0.047*	-0.007	0.001
P3 X P7	-0.314	-0.284	-0.008	0.001	-0.001
P3 X P8	-0.773	-0.095	-0.374**	-0.005	-0.003**
P4 X P5	0.866	-0.110	0.128**	-0.019**	-0.004**
P4 X P6	1.332	0.426	0.122**	0.014**	0.001
P4 X P7	-1.807**	-2.609**	0.001	-0.005	-0.001
P4 X P8	-1.734*	-1.819*	-0.011	-0.011**	-0.001
P5 X P6	-0.716	-0.975	-0.195**	0.001	-0.001
P5 X P7	0.607	0.522	-0.117**	0.012	0.001
P5 X P8	-0.095	-0.378	-0.128**	0.008	-0.001
P6 X P7	-1.595	-1.020	-0.157**	-0.010*	-0.002*
P6 X P8	-1.260*	-0.798	-0.168**	-0.009	-0.002*
P7 X P8	-0.261	-0.153	-0.089**	-0.003	0.001
SD (SIJ)	0.6959	0.8042	0.0197	0.005	0.001

SCA: Specific combining ability, SHL: Shoot length, ROL: Root length, NOR: No. of lateral roots, FRW: Fresh weight of the seedling and DRW: Dry weight of the seedling. * and **: significant differences at P<0.05 and P<0.01, respectively.
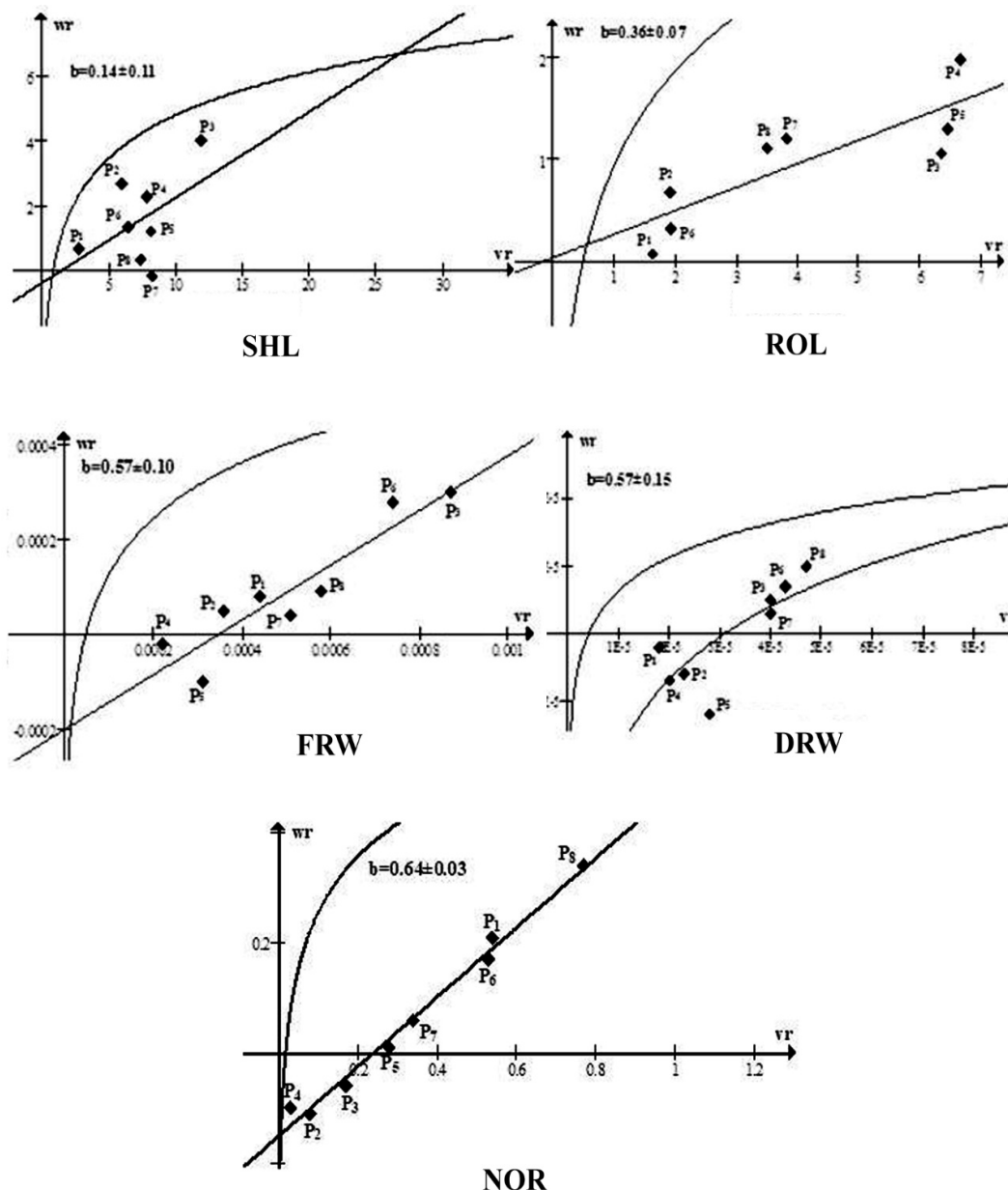
Figure 2. The Wr/Vr graphs of SHL: Shoot length, ROL: Root length, NOR: No. of lateral roots, FRW: Fresh weight of the seedling and DRW: Dry weight of the seedling.

Discussion

Seedling traits of the studied genotypes were affected differently due to drought stress conditions induced by using PEG. These findings agree with other investigations obtained by Singh *et al.* (2008) and Ahmad *et al.* (2013) in wheat. Evidently, drought stress induced by 15% PEG decreased SHL, FRW and DRW. In accordance, it has been reported that the treatment with PEG significantly reduced different growth parameters in wheat at seedling stage (Ahmad *et al.*, 2013). The reduction resulted in shoot length may be due to the impediment of the

cell division and elongation (Khakwani *et al.*, 2011). In contrast, ROL was increased due to drought stress induced by PEG. Similar results were found in wheat by Decuseara and Nedelea (2012) and El-Rawy and Hassan (2014a). Moreover, Khakwani *et al.* (2011) demonstrated that some genotypes increased ROL with 15% PEG to reach deeper water in the soil.

Furthermore, abundant genetic variation was detected in the present study among the studied genotypes for all seedling traits. Thus, wheat genotypes that showed different responses to drought could serve as important sources to study the adaptive responses of plants to drought stress conditions (Bhargava and Sawant, 2013). Furthermore, the genotypic variation detected among different genotypes in response to the PEG treatment could be useful for the selection of desirable wheat genotypes at the seedling stage (El-Rawy and Hassan, 2014a).

The highly significant GCA and SCA estimates observed for all seedling traits evaluated under drought stress induced by 15% PEG indicated that both additive as well as non-additive gene actions are involved in the inheritance of the studied traits. Obviously, GCA mean squares were larger in magnitude than that of SCA, suggesting that the additive gene effects play a major role in the observed variation of these traits, and thus selection in early segregating generations could be effective to develop desirable wheat genotypes. These results are in accordance with the results obtained by Awan *et al.* (2011) and Ashadusjaman *et al.* (2012).

Compared to their parents, F_1 hybrids produced longer roots under drought stress induced by 15% PEG, indicating the presence of heterosis. A partial adequacy of an additive-dominance model was found for ROL, NOR, FRW and DRW, while non adequate model was observed for SHL. Based on GCA effects, the parental genotypes P1, P2 and P3 were the best general combiners for most seedling traits under drought stress conditions. The highest desirable SCA effects were obtained from the crosses P1×P3, P1×P6, P1×P8, P2×P5 and P2×P7 for most studied traits, indicating their usefulness as best crosses under drought stress conditions. These findings suggested that these genotypes possessed drought tolerance genes. Therefore, the use of these genotypes could be efficient for improving the drought tolerance in wheat. Furthermore, several investigations reported that growth parameters at seedling traits could be successfully used as selection criteria under drought stress conditions in wheat (Rebetzke *et al.*, 2007; Bayoumi *et al.*, 2008; Awan *et al.*, 2011; Datta *et al.*, 2011; Baloch *et al.*, 2012).

In conclusion, abundant genetic diversity was found in the present study among the studied genotypes. The additive gene action played the major role in the observed variation of all studied seedling traits, and thus phenotypic selection at early segregating generations may lead to effective identification of desirable wheat genotypes. Three parental genotypes and five crosses were identified as the most tolerant genotypes at seedling stage, suggesting their advantages as beneficial genetic resources for improving the drought tolerance in wheat.

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التحليل الوراثي لصفات البادرة تحت ظروف إجهاد الجفاف في قمح الخبز

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قسم الوراثة، كلية الزراعة، جامعة أسيوط، مصر.

الملخص

في الدراســــة الحاليــة، أجريت تجربة معملية لدراســــة الفعل الجيني وتقدير القدرة العامة والخاصــــة على الائتلاف لصــفات البادرة في تهجين نصــف دائري من قمح الخبز. تم تقييم ثمانية طرز وراثية وثمانية وعشــــرون هجين جيل أول تحت ظروف الجفاف الناجم عن المعاملة بمادة البولي إيثيلين جلايكول-6000. كانت الصــفات المدروســة هي طول الســويقة وطول الجذر وعدد الجذور الثانوية والوزن الرطب والجاف للبادرة. لوحظت فروق معنوية جداً بين الطرز الوراثية لجميع الصــــفات تحت ظروف إجهاد الجفاف. كانت قيم متوســـط المربعات الراجعة للقدرة العامة والخاصــــة على الائتلاف معنوية جداً لجميع الصــــفات، مما يشــــير إلى أن كل من الفعل الجيني المضيف وغير المضيف كان له دور في وراثة الصفات المدروسة. كانت قيم متوسط المربعات الراجعة للقدرة العامة على الائتلاف أكبر من تلك الراجعة للقدرة الخاصــــة على الائتلاف، مما يشير إلى أهمية دور الفعل الجيني المضيف في وراثة تلك الصفات. بالمقارنة مع آبائهم، أنتجت هجن الجيل الأول جذوراً أطول تحت ظروف الجفاف، مما يشــير إلى وجود قوة هجين. أشــــار تحليل الانحدار إلى ملائمة نموذج جزئية لنموذج إضافة-سيادة بالنسبة لصفات طول الجذر وعدد الجذور الثانوية والوزن الرطب والجاف للبادرة، في حين لوحظ عدم ملائمة النموذج لصفة طول السويقة. اعتمادا على القدرة العامة على الائتلاف، كانت الطرز الوراثية P_1 و P_2 و P_3 أفضــل الآباء ائتلافاً لمعظم الصــفات تحت ظروف الجفاف. تم الحصــول على أفضــل تأثيرات مرغوبة للقدرة الخاصــــة على الائتلاف من الهجن $P_1 \times P_3$ و $P_1 \times P_6$ و $P_1 \times P_8$ و $P_2 \times P_5$ و $P_2 \times P_7$ لمعظم الصــفات، مما يشــير إلى فائدتها كأفضــل التوليفات الواعدة تحت ظروف الجفاف. وبالتالي، فإن إدراج تلك الطرز الوراثية المتفوقة في برامج التربية يمكن أن يكون مفيدا لتحســين تحمل الجفاف في القمح.

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