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Estimation of Gene Action and Combining Ability for Water Loss of Winter Wheat Leaves as an Indicator of Drought Tolerance

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Abstract The rate of leaves water loss is a physiological trait often used as a selection criterion for improving drought tolerance in different species including wheat. The present study was undertaken to evaluate the inheritance type and components of genetic variation for excised-leaf water loss (ELWL), using a half diallel analysis of crosses between seven wheat varieties, in order to obtain information useful for improvement of drought tolerance. The additive gene action is operative for leaves water loss only in the case of ‘Alex’ and ‘Apullum’ varieties. In the case of other parents, the expression of this trait is under the influence of non-allelic gene interactions or environmental conditions. In the case of ‘Xenos’ and ‘Alex’ varieties, the dominance was associated with positive alleles, while in Apache and Fundulea 4 varieties, the dominance was associated with negative alleles. Also, in the case of ‘GKKapos’ variety, the dominant alleles had no definite effect on the leaves water loss. Considering that ‘Turda 2000’ posses a high proportion of recessive alleles for ELWL, F1 crosses of this parents would have more heterozygous loci than the other possible crosses, notably exceeding the parents for this trait. The large dominance effects of leaves water loss make it preferable to select lines in later generations, when the heterozygosity level has decreased to a negligible value. The difference between the negative general combining ability (GCA) effects of Fundulea 4 variety and their specific combining ability (SCA) effect could be an indication of good complementarity among genes involved in the inheritance of this trait. In the case of crosses: ‘Fundulea 4’ x ‘Turda 2000’, ‘GKKapos’ x ‘Alex’ and ‘Xenos’ x ‘Apache’, from parents with high x low GCA, in order to obtain desirable segregants the best breeding strategy is intermating between crosses followed by selection.

Key words

wheat,
excised-leaf water loss,
gene effects, diallel

Climate change during the last decades is predicted to reduce rainfall and water availability of wheat plants, with high negative impact on yield level. Genetic improvement of wheat for drought tolerance requires investigation of the possible elements of drought tolerance and exploration of the available genetic variation [13]. Indirect selection in early generation through traits correlated with drought tolerance is one of the most important strategies in plant breeding [17].

The ability of a plant to survive under severe water deficits depends on its ability to restrict the loss of water through the leaf epidermis after stomata attain minimum aperture [11]. In this regards, differences among genotypes for rate of water loss, which presumably is an estimate of cuticular transpiration rate, could be used for screening wheat genotypes for drought tolerance [9]. The assessment of water loss from excised leaves has shown to be effective for characterising drought tolerance in wheat [9, 23]. This trait is moderately heritable [7], predominantly controlled by additive gene effects [5, 12] and can be easily determined and hence applicable for use in large populations. In wheat genotypic differences in non-stomatal water loss due to variation in cuticular thickness or composition were reported by several studies [3, 4, 19].

Based of several studies the ELWL was usually negatively correlated with other traits: leaf DW, leaf area, leaf length, as well as midrib thickness, implying that a longer path length for water to reach the epidermis slowed the rate of water loss from the leaf surface [10, 25]. Several QTLs for ELWL were identified on chromosomes 1D, 4A, 6B and 6D [28], and on chromosome 3A, 3B, 4B, 5B, 5D, 6B, 7A, 7B, 7D [25].

The diallel analysis method provides breeders the possibility to review the genetic system and nature of gene action involved in the expression of different traits, right in the F_1 generation [18, 22]. The success of an improvement program depends on the selection based on actual performance of the parents as well as their combining ability for traits of main importance [2]. Combining ability indicates the additive or non-additive expression of a trait, and also the appropriate breeding strategy that will be used for an effective improvement [14].

The present study was undertaken to evaluate the inheritance type and components of genetic variation for ELWL, using a half diallel analysis of crosses between seven wheat varieties, in order to obtain information useful for improvement of drought tolerance.

Material and Method

The biological material was composed of seven winter wheat varieties (‘Fundulea 4’, ‘Alex’, ‘Apullum’, ‘Turda 2000’, ‘GKKapos’, ‘Apache’, ‘Xenos’), genetically and ecologically differentiated, used as parents and 21 hybrids of half-diallel crosses among these parents. The experiment was carried out in a randomized block design with three replications.

For each genotype, five penultimate leaves /replication were sampled and placed in polythene bags, and transported immediately in the laboratory. In order to calculate ELWL, the leaves were weighed three times using the protocol proposed by Clarke (1987): immediately after sampling to obtain the fresh weight (FW), then dried in an incubator at 28°C at 50% R.H. for 6 h obtaining W6h, and then dried again in an oven for 24 h at 70°C and determine the dry weight (DW). The ELWL was calculated by the formula: ELWL=[(FW - W6h) / (FW-DW)] x 100

Data were analyzed using the method developed by Hayman [18] for diallel crosses, relating to: analysis of genetic variance, estimation of variance (Vr) and covariance (Wr), analysis of the graph Wr-Vr. The relationship between dominance and positive and negative alleles, were analyzed according to the model of Johnson and Aksel [20]. The analysis of combining ability was performed using method 2 (*p* parents and *p(p-1)/2* hybrids), model I (with fixed-effects) as suggested by Griffing [16].

Results and Discussions

Based on the data from Table 1 it can be observed that the dominance variance (*b*) had the greatest contribution to the variability of excised-leaf water loss (ELWL). The additive variance (a) also shows a significant value, having a smaller contribution to the variability of this indicator compared to the dominance variance.

Regarding the dominance and how it acts in the inheritance of ELWL, it was found that the subcomponent b_1 of the dominance variance, which indicates the average deviation of the hybrids from the parental values, or the directional effects of dominance, presents a non-significant value. Because in most cases the average of this trait in F1 hybrids is higher to the parents' mean, the significance of b_1 indicates in most cases the dominance of parents with a higher leaves water loss under drought conditions, but there are also exceptions [6].

Table 1

Analysis of genetic variance for ELWL in wheat

Source of variation	SS	DF	MS	F test
Replications	139.92	2	69.96	1.57
a	1106.53	6	184.42	4.13**
b	4684.18	21	223.06	4.99**
b_1	8.29	1	8.29	0.19
b_2	742.13	6	123.69	2.77*
b_3	3933.75	14	280.98	6.29**
Error	2412.83	54	44.68	
Total	8343.54	83		

The significant value for subcomponent b_2 indicates an asymmetry of the distribution of positive and negative alleles involved in the control of ELWL at the parents. The subcomponent b_3 had also a significant value indicating the existence of differences between the ELWL of hybrids due to dominance and some additive x additive, dominant x dominant or additive x dominant interactions [15]. The differences between the hybrids are not significantly influenced by the variation of growing conditions in the experimental field, considering the F value for replications.

Table 2

The significance of Wr/Vr regression coefficients for ELWL in F_1 hybrids

Parameters	Value	Ho : β=0	Ho : β=1
b	0.059	9.24*	8.23*
s_b	0.057		

Regarding the test of additivity (Table 2), the regression coefficient significantly different from 0 indicates the presence of dominance, while the significance to unity indicates also the presence of non-allelic gene interactions in the inheritance of this trait.

According to the Wr/Vr regression from Figure 1, it is noted that only in the case of ‘Alex’ and ‘Apullum’ parents located near the regression line, additive gene action is operative for this trait. In the case of other parents, the expression of this trait is under the influence of non -allelic gene interactions or environmental conditions. Given the distance between the regression line and the parabola, as well as the position of the parents to the regression line, it follows that both the dominance and additive effects play an important role in the inheritance of ELWL, according with the data from analysis of genetic variance. The negative intercept of the regression line indicated the presence of non-allelic and over dominance in the inheritance of this trait, in accordance with the results of other study on wheat ELWL at tillering stage under glass house conditions [12].

In the case of ‘Turda 2000’ (94.80%) and ‘Apullum’ (71.10%) varieties the highest proportion of recessive alleles was observed, while ‘GKKapos’ (88.20%) and ‘Alex’ (83.80%) varieties have the largest proportion of dominant alleles. The position of ‘Turda 2000’ at the high end of the regression line indicates that this parent is near completely recessive for this trait. So the F1 hybrids derived from crosses with ‘Turda 2000’ would have more heterozygous loci than the other crosses [21].

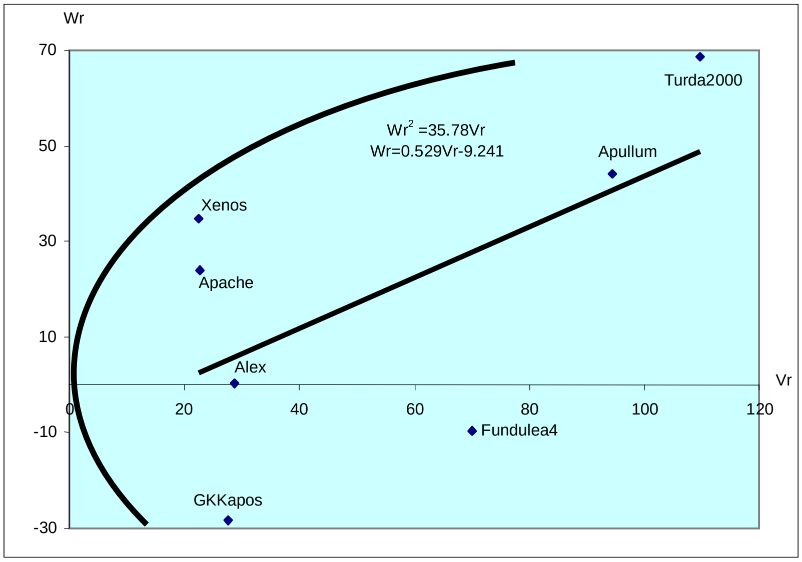

Figure 1. **Wr/Vr regression for RWC in wheat**

Table 3

Mean (Yr), variance (Vr), covariance (Wr) values, and proportion of dominant alleles for parents concerning ELWL

Parents	Mean Yr	Variance Vr	Covariance Wr	Vr + Wr	Proportion of dominant alleles
Fundulea 4	60.02	70.06	-9.54	60.52	0.589
Xenos	76.63	22.40	34.77	57.17	0.605
GKKapos	72.44	27.69	-28.41	-0.72	0.868
Turda 2000	72.79	109.59	68.74	178.33	0.052
Alex	82.10	28.77	0.39	29.16	0.721
Apache	63.34	22.70	24.02	46.72	0.642
Apullum	79.71	94.35	44.17	138.52	0.289

The genetic diversity between the parents for ELWL can be appreciated based on the position of array points along the regression line in association with the Wr+Vr values.

Thus, it was observed that the studied varieties were divided into three groups: ‘Turda 2000’-‘Apulum’; ‘Fundulea 4’ and ‘Xenos’-‘Apache’-‘Alex’-‘GKKapos’, inside which there is a certain genetic similarity. Given that the parent’s mean of this trait was not correlated with the proportion of dominated alleles, it turns out that dominant alleles did not especially control tolerance to leaves water loss in these parents and therefore the selection for this trait will be difficult [1].

The distribution of the parents varieties based on the standardized deviations (Wr+Vr) and the leaves water loss of parents (yr), indicates a large proportion of recessive alleles that increase the values of this trait in ‘Turda 2000’ and ‘Apullum’ varieties. In the case of ‘Xenos’ and ‘Alex’ varieties, the dominance was associated with alleles with a positive effect, while in ‘Apache’ and ‘Fundulea 4’ varieties, the dominance was associated with negative alleles. Also, in the case of ‘GKKapos’ variety, the dominant alleles had no definite effect on the leaves water loss.

The asymmetrical effect of the different alleles on this indicator is also confirmed by the low and non-significant values of the correlation coefficient (r=0.135) between the standardized deviations and the parents' means. In this regard, the order of dominance of parents was ‘GKKapos’, ‘Alex’, ‘Apache’, ‘Xenos’, ‘Fundulea 4’, ‘Apullum’, ‘Turda 2000’, and the order of parental performance was ‘Alex’, ‘Apullum,’ ‘Xenos’, ‘Turda 2000’, ‘GKKapos’, ‘Apache’ and ‘Fundulea 4’ showing week association.

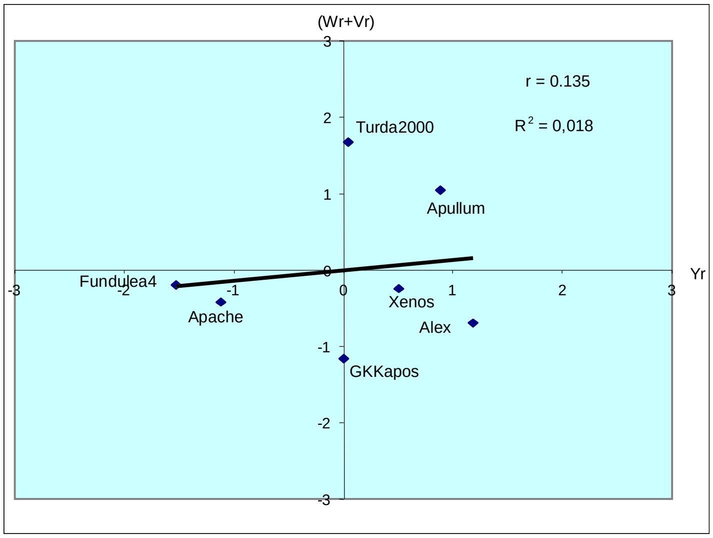

Figure 2. **Standardized deviations (Wr+Vr) and parents mean (yr) for ELWL in wheat**

The dominance effects (H1) had a main and significant contribution to the inheritance of this trait (Table 4), while the additive effects (D) had a smaller but significant contribution, as revealed by the analysis of genetic variance. The predominant value of the dominance effects was also supported by the high and significant value of the component (h^2), which represents the sum of all heterozygous loci [24].

The dominant alleles (F) that controls the leaves water loss have a higher frequency (53.80%) than the recessive ones, and at the same time an asymmetry of the positive and negative effects of the genes due to dominance was highlighted, considering the significant value of the H2 component. According to the Wr/Vr regression, the average degree of dominance evaluated by the ratios $(H_1/D)^{1/2}$ and kD/(kD+kR) suggests the presence of overdominance in the inheritance of ELWL.

The environmental variance (E), even if it shows a significant value, is lower than the components related to the dominance (H1) and additive (D) effects. Based on the ratios $H_2/4H_1$ ($0.216 \neq 0.250$) it was observed that the dominant genes are in different positive and negative frequencies having positive effects on this indicator in some parents and negative effects in others. The number of genes groups or effective factors ($h^2/H_2 = 0.09$) was underestimated because the dominance effects of all involved genes were not equal in size and direction [20].

The high values of broad sense heritability (0.731) and the low values for narrow sense heritability (0.337) confirm that a considerable part of the ELWL variability is due to the genotype, on the basis of the involvement of some major genes in the determinism of this character [26]. The difference between the two types of heritability suggests a higher environmental influence and therefore difficulty to apply effective selection for drought tolerance using this trait as a criterion.

Table 4

Genetic variance components and genetic parameters for ELWL in wheat

Variance component / ratio	Estimated values
D- additive effects of genes	35.778***
H_1- dominance effects of genes	173.751***
H_2 – corrected dominance effects of genes	150.313***
F- covariance of additive and dominance effects	5.097
h^2- cumulative dominance effects	11.686**
E- environmental variance	25.727***
$(H_1/D)^{1/2}$ – average level of dominance	2.20
$kD/(kD+kR)$ – proportion of dominance genes	0.538
$\overline{F_1} - \overline{P}$ - average direction of dominance	0.07
D-H_1 – average direction of genes effects	-137.97
$H_2 / 4H_1$ – average frequency of positive and negative alleles	0.216
h^2 / H_2 – number of blocks of dominant genes	0.091
D/(D+E) – true sense heritability	0.582
Hb – broad sense heritability	0.731
Hn – narrow sense heritability	0.337

LSD$_{5\%}$ = 7.114 LSD$_{1\%}$ = 9.607 LSD$_{0.1\%}$ = 12.793

Based on the data from Table 5, it can be observed that in the variability of ELWL the additive and non-additive genetic effects had a low influence, in accordance with the results of Farshadfar et al.[15]. As such, very few wheat genotypes differ from the others in terms of concentration of favorable alleles. The GCA/SCA ratio suggested that the SCA effects were small relative to the GCA effects. The importance of GCA effects in the inheritance of ELWL was also reported by Dhanda and Sethii [13].

Table 5

Analysis of variance for combining ability of ELWL in wheat

Variability source	SS	DF	MS	F
Total	10999.65	62		
Replication	4.23	2	2.11	0.01
GCA	1734.17	6	289.03	1.59
SCA	2943.30	14	210.23	1.16
Error	7275.77	40	181.89	0.12
GCA/SCA			1.37	

The effects of GCA present a balanced proportion of negative and positive values, with amplitude of 11.95 from -8.29 in the ‘Fundulea 4’ variety to 3.66 in the ‘Xenos’ variety. As such, ‘Xenos’ (3,66) and ‘Apache’ (3,45) varieties express the highest GCA effects associated with a low potential to improve drought tolerance.

‘Fundulea 4’ recorded the most valuable additive effects proved to be the most promising parents for breeding of this trait. The mean values of GCA effects are not significantly (r=0.469) correlated with the parents mean, confirming the low influence of the additive effects.

Table 6

Effects of the general and specific combining ability for ELWL in wheat

Parents	GCA	SCA					
		Xenos	GKKapos	Turda 2000	Alex	Apache	Apullum
Fundulea 4	-8.29^0	-	8.48	5.42	-15.97^0	6.44	5.53
Xenos	3.66		-	-1.42	2.71	-1.59	-10.11
GKKapos	-2.11			-	5.45	-10.50	5.48
Turda 2000	2.39				-	2.56	-4.99
Alex	2.78					-	2.50
Apache	3.45						-
Apullum	-1.88						

GCA -LSD$_{5\%}$ =7.21; LSD$_{1\%}$ =9.61; LSD$_{0.1\%}$ =12.56
SCA -LSD$_{5\%}$ = 13.74; LSD$_{1\%}$ =18.33; LSD$_{0.1\%}$ =23.96

The SCA effects presented different values, predominantly positive and with an amplitude of 26.21, ranging from -15.97 for 'Fundulea 4' x 'Turda 2000' to 10.24 for 'Turda 2000' x 'Apullum' hybrid. The highest non additive effects were observed at different hybrids of 'Fundulea 4' variety: 'Fundulea 4' x 'Xenos' (8.48) şi 'Fundulea 4' x 'Apache' (6.44), 'Fundulea 4' x 'Apullum' (5.53). The effects of SCA with different sign for crosses of 'Fundulea 4' variety show that selection among progenies of their crosses may have unpredictable results. The lowest SCA effects were recorded by crosses 'Fundulea 4' x 'Turda 2000' (-15.97), 'GKKapos' x 'Alex' (-10.50) and 'Xenos' x 'Apache' (-10.11), which offers the possibility of obtaining some valuable segregants

Conclusions

The additive gene action is operative for leaves water loss only in the case of 'Alex' and 'Apullum' varieties. In the case of other parents, the expression of this trait is under the influence of non-allelic gene interactions or environmental conditions. In the case of 'Xenos' and 'Alex' varieties, the dominance was associated with positive alleles, while in 'Apache' and 'Fundulea 4' varieties, the dominance was associated with negative alleles. Also, in the case of 'GKKapos' variety, the dominant alleles had no definite effect on the leaves water loss. Considering that 'Turda 2000' posses a high proportion of recessive alleles for ELWL, F_1 crosses of this parents would have more heterozygous loci than the other possible crosses, notably exceeding the parents for this trait. The large dominance effects of leaves water loss make it preferable to select lines in later generations, when the heterozygosity level has decreased to a negligible value.

The difference between the negative GCA effects of 'Fundulea 4' variety and their SCA effect could be an indication of good complementarity among genes involved in the inheritance of this trait. In the case of crosses: 'Fundulea 4' x 'Turda 2000', 'GKKapos' x 'Alex' and 'Xenos' x 'Apache', from parents with high x low GCA, in order to obtain desirable segregants the best breeding strategy is intermating between crosses followed by selection.

References

[1] Abate Z.A., McKendry A.L. 2010. Diallel analysis of Fusarium head blight resistance in genetically diverse winter wheat germplasm. Euphytica, 175, 409–421;

[2] Bertan F., Carvalho D., Oliveira A.D. 2007. Parental selection strategies in plant breeding programs. Journal of Crop Science and Biotechnology, 10 (4), 211–222;

[3] Bi H., Kovalchuk N., Langridge P., Tricker P.J., Lopato S., Borisjuk N. 2017a. The impact of drought on wheat leaf cuticle properties. BMC Plant Biology, 17, 85;

[4] Bi H., Luang S., Li Y., Bazanova N., Borisjuk N., Hrmova M., Sergiy Lopato S. 2017b. Wheat drought-responsive WXPL transcription factors regulate cuticle biosynthesis genes. Plant Molecular Biology, 94, 15-32;

[5] Chandra D., Islam M.A. 2003. Genetic variation and heritability of excised-leaf water loss and its relationship with yield and yield components of F5 bulks in five wheat crosses. Journal of Biological Sciences, 3, 1032-1039;

[6] Ciulca S. 2006. Elemente de genetică cantitativă şi genetica populaţiilor [Elements of population and quantitative genetics]. Ed. Agroprint, Timisoara;

[7] Clarke J.M., Townley-Smith T.F. 1986. Heritability and relationship of excised-leaf water retention in durum wheat. Crop Sci 26, 289–292;

[8] Clarke J.M. 1987. Use of physiological and morphological traits in breeding programmes to improve drought resistance of cereals. In: J.P. Srivastava, E. Porcedo, E. Acevedo & S. Varma (Eds.), Drought Tolerance in Winter Cereals, pp 171–190. John Wiley & Sons, New York;

[9] Clarke, J.M., I. Romagosa, S. Jana, J.P. Srivastava & T.N. McCaig, 1989. Relationship of excised-leaf water loss rate and yield of durum wheat in diverse environments. Can J Plant Sci, 69, 1075–1081;

[10] Czyczyo-Mysza M.I., Skrzypek E., Bocianowski J., Dziurka K., Rancic D., Radosevic R., et al. 2018. Genetic analysis of water loss of excised leaves associated with drought tolerance in wheat. Peer J, 6:e5063;

[11] David M. 2010. Water loss from excised leaves in a collection of *Triticum aestivum* and *Triticum durum* cultivars. Romanian Agricultural Research, 27, 27-34;

[12] Dhanda S.S., Sethi G.S. 1998. Inheritance of excised-leaf water loss and relative water content in bread wheat (*Triticum aestivum* L.). Euphytica, 104, 39-47;

[13] Dhanda S.S., Sethi G.S., Behl R.K. 2004. Indices of drought tolerance in wheat genotypes at early stages of plant growth. J. Agron. Crop Sci, 190, 6-12;

[14] Falconer D.S., Mackay T.F., Frankham R. 1996. Introduction to quantitative genetics (4th edn),"Trends in Genetics, vol. 12, no. 7;

[15] Farshadfar E., Rasoli V., Jaime A. Teixeira da Silva, Farshadfar M. 2011, Inheritance of drought tolerance indicators in bread wheat (Triticum aestivum L.) using a diallel technique. Australian Journal of Crop Sciences, 5(7), 870-878;

[16] Griffing B. 1956. Concept of general and specific combining ability in relation to diallel crossing systems. Australian J. Biol. Sci., 9, 463-493;

[17] Golparvar A.R., Ghasemi-Pirbalouti A., Madani H. 2006. Genetic control of some physiological

attributes in wheat under Drought Stress Conditions. Pakistan Journal of Biological Sciences, 9, 1442-1446*;*

[18] Hayman, B.I. 1954. The theory and analysis of diallel crosses. Genetics, 39: 789-809;

[19] Jäger K, Fábián A, Eitel G, Szabó L, Deák C, Barnabás B, Papp I. 2014. A morpho-physiological approach differentiates bread wheat cultivars of contrasting tolerance under cyclic water stress. Journal of Plant Physiology, 171, 1256-1266;

[20] Johnson L.P.V., Aksel R. 1959. Inheritance of yielding capacity in a fifteen parent diallel cross of barley. Can. J. Genet. Cytol. Mol., 208-265;

[21] Kobayashi K., Horisaki A., Niikura S., Ohsawa R. 2007. Diallel analysis of floral morphology in radish (*Raphanus sativus* L.). Euphytica, 158, 153–165;

[22] Mather K.V., Jinks J.L. 1982. Introduction to biometrical genetics. Chapman and Hall Ltd., London;

[23] McCaig T.N., Romagosa I. 1991. Water status measurements of excised leaves: Position and age effects. Crop Sci 31, 1583–1588;

[24] Sharma J.R. 2006. Statistical and biometrical techniques in plant breeding. New Age International;

[25] Sayed MAA, Bedawy IMA. 2016. Heterosis and inheritance of some physiological criteria imparting drought tolerance of grain sorghum in the irrigated and water-limited environments. Egyptian Journal of Agronomy, 38, 293-318;

[26] Solomon K.F., Labuschagne M,T. 2004. Inheritance of evapotranspiration and transpiration efficiencies in diallel 1 F hybrids of durum wheat (Triticum turgidum L. var. durum). Euphytica, 136, 69-79;

[27] Viana J.M.S., Cardoso A.A. 1999. Theory and analysis of partial diallel crosses. Genetic and Molecular Biology, 22, 591-599;

[28] Yang R.C., Jana S., Clarke J.M. 1991. Phenotypic diversity and associations of some potentially drought-responsive characters in durum wheat. Canadian Journal of Plant Science, 31, 1484-1491.