

## Analysis of gene action and variance components for cell membrane stability in winter wheat

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**Abstract:** Membrane stability reflects the ability of cell tissues to hold electrolytes under drought condition by retaining the membrane structure of the leaf cell intact. The aim of this research was to analyze the inheritance, gene effects, and the genetic variance component, in order to establish the best parents and crosses for future use in the improvement of wheat tolerance to drought, using leaves membrane stability as a selection criterion. The biological material was composed by seven wheat varieties genetically and ecologically differentiated, and their 21 half-diallel crosses. Data were analyzed using the methods of Hayman and Griffing for diallel crosses. The expression of cell membrane stability for the studied genotypes is conditioned by both dominance and additive gene action, with a higher contribution of dominance one. The direction of dominance for this feature seemed to be variable depending of the parents. Fundulea 4 variety was found to be best general combiner for cell membrane stability, showing a high potential for improving drought resistance of its offspring. The high SCA effects of the cross Fundulea 4 x Alex between parents with high and low GCA, indicates that intermating between crosses and selection allows to obtain genotypes with good cell membrane stability.

### Key words

Wheat, membrane stability, diallel, additive, non additive effects.

Under the backdrop of climate changes from recent decades, the drought tolerance became an important trait in order to ensure economically efficient yields. Drought tolerance refers to the ability of the plant to withstand low tissue water potentials [16]. In terms of yield, the drought tolerance refers to the ability of a genotype to produce with minimal losses in a water-deficient environment in comparisons with a normal water supply environment. Genetic improvement in drought tolerance requires identification of relevant drought resistance mechanism and the development of suitable methodology for their measurement in a large breeding population [4].

Membrane stability reflects the ability of cell tissues to hold electrolytes under drought condition by retaining the membrane structure of the leaf cell intact [19]. The frequency of cell membrane injuries under drought conditions can be estimated by measuring the cell electrolyte leakage [5, 2, 11]. A low frequency of injuries is associated with a high stability of cell membrane, which ensures a better tolerance to water stress [20]. The cell membrane stability has been extensively used as selection criterion for different abiotic stresses including drought and high temperature in wheat [6, 1, 17, 3, 12].

The genetic analysis using the diallel technique developed by Hayman (1954) and Jinks (1954), provides valuable information on the

inheritance type in the early generations and is very useful to make an effective selection. The diallel mating design was extensively used in different self and cross pollinated species to estimate the nature of gene action involved in the inheritance of quantitative traits. The diallel techniques developed by Griffing (1956) relating to the analysis of combining ability, is very useful because it allows to identify the parents with good ability to transfer the desirable traits to offspring.

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### Material and Method

Seven wheat varieties (Fundulea 4, Alex, Apullum, Turda 2000, GKKapos, Apache, Xenos) genetically and ecologically differentiated, and their 21 half-diallel crosses, were studied in a randomized block design on three replications, at Banat's University of Agricultural Sciences and Veterinary Medicine Timisoara..

The determination of cell membrane stability of leaves was made using the method proposed by

Sullivan (1971). Flag leaves were collected at anthesis from each plot of the 28 genotypes. Artificial desiccation was induced by polyethylene glycol (PEG-6000). This treatment affects the cell membrane of leaf and allows the electrolyte leakage. The injury of leaf tissue after desiccation, respectively the cell membrane stability of leaf (CMS) was calculated based on electrical conductivity measurements with the formula:  $CMS (\%) = 100 - (1 - [(1 - T_1/T_2)/(1 - C_1/C_2)]) * 100$ ; where,  $C_1, C_2$ , conductance of control samples before and after autoclaving;  $T_1, T_2$ , conductance of stress samples before and after autoclaving.

Data were analyzed using the method of Hayman (1954) for diallel crosses, regarding: analysis of genetic variance, estimation of variance ( $V_r$ ) and covariance ( $W_r$ ), the establishment of the graph  $W_r - V_r$ .

The graphical analysis was completed with graph of standard deviation for parental ( $y_r$ ) and ( $W_r + V_r$ ) values, according to Johnson and Aksel (1959). This graph indicates the relationship between dominance and positive and negative alleles. Components of genetic variance have been estimated following the model of Jinks and Hayman (1953). The validity of information from diallel analysis was carried out based of the significance of regression coefficient from zero and unity

The analysis of combining ability has been performed using method 2 ( $p$  parents and  $p(p-1)/2$  hybrids), model I (the model with fixed-effects, considering that the biological material is a population to be inferred) as suggested by Griffing (1956), using the following formula:

$$x_{ij} = \mu + g_i + g_j + s_{ij} + \frac{1}{bc} \sum_k \sum_l e_{ijkl}, \text{ where:}$$

$\mu$  - population mean;  $g_i (g_j)$  - effect of general combining ability (GCA) for parent  $i (j)$ ;  $s_{ij}$  - effect of specific combining ability (SCA) for hybrid between parent  $i$  and  $j$ ;  $e_{ijkl}$  - environment effect associated with individual values.

## Results and Discussions

Based on analysis of genetic variance for cell membrane stability from Table 1, it is noted that the dominance effects ( $b$ ) had the highest and significant

contribution to the variability of this trait. Also, the additive effects ( $a$ ) showed a significant value having a lower contribution compared to dominance.

Table 1

**Analysis of genetic variance for cell membrane stability in wheat**

Source of variation	SS	DF	MS	F test
Replications	22.18	2	11.09	0.23
a	812.83	6	135.47	2.85**
b	6209.11	21	295.67	6.22**
b <sub>1</sub>	378.69	1	378.69	7.97**
b <sub>2</sub>	1147.33	6	191.22	4.02**
b <sub>3</sub>	4683.08	14	334.50	7.04**
Error	2566.84	54	47.53	
Total	9610.95	83		

Taking in to account the various components of the dominance variance it is possible to made estimates about dominance and its influence on the cell membrane stability. The item  $b_1$ , which indicates the average deviation of the hybrids to parents, or the directional effects of dominance, had a significant value. Because in most crosses the average frequency of cell membrane injuries is over the parents mean, the significance of  $b_1$  denotes the dominance of parents with lower cell membrane stability under drought conditions. The significant value of component  $b_2$

indicates an asymmetric distribution of alleles with positive and negative effects, involved in the inheritance of cell membrane stability. The item  $b_3$ , had a significant value which shows the existence of differences between the values of hybrids due to dominance and of some interactions types, like: dominant x dominant; additive x additive; additive x dominant. The variation of environmental conditions between replications did not significantly influence the differences between the hybrids concerning cell membrane stability.

Table 2

**The significance of  $W_r/V_r$  regression coefficients for cell membrane stability in  $F_1$  hybrids**

Parameters	Value	$H_0 : \beta=0$	$H_0 : \beta=1$
$b$	0.208	5.97*	22.73***
$s_b$	0.035		

The regression coefficient significantly different from 0 indicates the presence of dominance, while the significant deviation to unity indicates the presence of epistasis (Table 3). Taking in to account the distribution of parents in relation to unity slope

(Fig. 1), it seems that complementary epistasis act in the inheritance of cell membrane stability, considering that most of the parents are below the line of unity slope.

Table 3

**Mean ( $Y_r$ ), variance ( $V_r$ ), covariance ( $W_r$ ) values, and proportion of dominant alleles for parents concerning cell membrane stability**

Parents	Mean $Y_r$	Variance $V_r$	Covariance $W_r$	$V_r + W_r$	Proportion of dominant alleles
Fundulea 4	42.34	88.45	-13.22	75.23	0.420
Xenos	32.57	13.59	19.29	32.88	0.725
GKKapos	33.25	44.44	-8.15	36.29	0.665
Turda 2000	35.08	147.33	40.42	187.75	0.070
Alex	23.38	35.73	12.24	47.97	0.490
Apache	41.77	72.97	25.98	98.95	0.285
Apullum	29.06	100.74	35.97	136.71	0.170

According to Hayman's graphic approach for diallel analysis, the degree of dominance can be estimated based on the point (a) where the regression line intersects the  $W_r$  axis. For this parameter, the regression line cut the covariance axis ( $W_r$ ) above the origin, indicating that the cell membrane stability is conditioned by partial dominance. According to the distribution of parents along the regression line, the following order of dominance was observed: Xenos, GKKapos, Alex, Fundulea 4, Apache, Apullum and Turda 2000. As such, Xenos (72.5%) carries the highest proportion of (partial) dominant alleles, followed by GKKapos (66.5%), while Turda 2000

(93%) and Apullum (83 %) possess the most recessive alleles.

From Figure 1, according on the distance between the parabola and the regression line, as well as the position of parents to the regression line, it is observed that mainly the dominance effects are involved in the inheritance of membrane stability, while the additive effects have a considerably lower influence. As such, the additive effects are involved in the inheritance of this parameter only in case of Alex variety, located near the regression line. The expression of cell membrane stability for other parents is controlled by non-allelic gene interactions or by the environmental conditions.

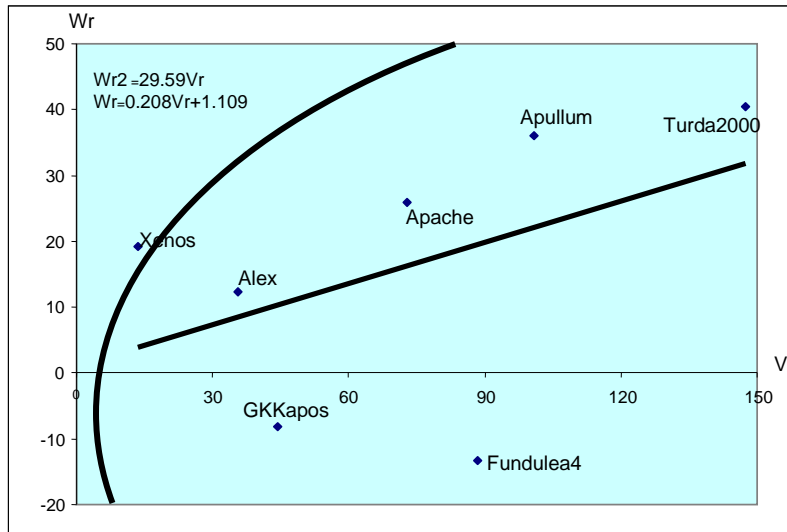


Fig. 1.  $W_r/V_r$  regression for cell membrane stability in wheat

The genetic diversity among parents, estimated according to the scattering of them along the regression line, indicates that they are divided in to three groups: Xenos, GKKapos and Alex; Fundulea 4 and Apache; Apullum and Turda 2000, inside which

there is a certain genetic similarity. The departure of determination coefficient ( $r^2$ ) from unity indicates that it's meaningless to predict the possible limits of selection among dominant genes [23].

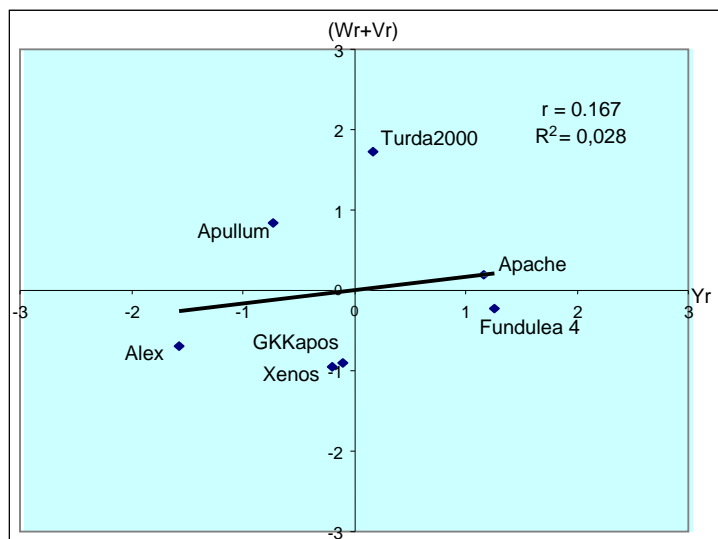


Fig.2. Standardized deviations ( $W_r+V_r$ ) and parents mean ( $y_r$ ) for cell membrane stability in wheat

The distribution of parents based on standardized deviations  $W_r+V_r$  and  $Y_r$  (parents mean) according to the of Johnson and Aksel (1959), indicates at Turda 2000 and Apache varieties the presence of a high proportion of recessive alleles which decrease the frequency of cell membrane injuries, thus increasing the membrane stability, while at Apullum variety the recessive alleles cause a reduction of cell membrane stability (Figure 2). In the case of GKKapos, Xenos and Alex varieties, the dominance is associated with negative alleles which thus cause a

decrease of membrane stability. Also, in Fundulea 4 variety the dominant alleles cause a reduction of the frequency of cell membrane injuries, and implicitly increase its stability. The asymmetric effect of different alleles on cell membrane stability is also confirmed by the low and non-significant value of correlation ( $r = 0.167$ ) between ( $W_r + V_r$ ) and  $y_r$ .

The method developed by Hayman (1954), allows besides the graphical analysis another different approach based on the estimation of genetic variance components and some ratios between them, whose

content is also interpretable on the basis of the theory of diallel crosses. According with the data from Table 4, the dominance ( $H_1$ ,  $H_2$ ) and additive effects ( $D$ ) are significant, but the contribution of dominance is more predominant in the inheritance of this parameter, in accordance with the analysis of genetic variance and the results of Dhanda and Munjal (2009). The

important role of dominance effects is also argued by the high and significant value of  $h^2$ , representing the sum of all heterozygous loci. As such, a simultaneous capitalization of additive and dominant effects can be achieved by applying a reciprocal recurrent selection [7].

Table 4

**Components of variance and genetic parameters for cell membrane stability in wheat**

Variance component / ratio	Estimated values
$D$ - additive effects of genes	29.598***
$H_1$ - dominance effects of genes	252.870***
$H_2$ – corrected dominance effects of genes	206.081***
$F$ - covariance of additive and dominance effects	-5.114
$h^2$ - cumulative dominance effects	63.584***
$E$ - environmental variance	15.559***
$(H_1/D)^{1/2}$ – average level of dominance	2.923
$kD/(kD+kR)$ – proportion of dominance genes	0.404
$\overline{F_1} - \overline{P}$ - average direction of dominance	-4.91
$D-H_1$ – average direction of genes effects	-223.272
$H_2/4H_1$ – average frequency of positive and negative alleles	0.204
$h^2/H_2$ – number of blocks of dominant genes	0.360
$D/(D+E)$ – true sense heritability	0.655
$Hb$ – broad sense heritability	0.856
$Hn$ – narrow sense heritability	0.378

LSD<sub>5%</sub> = 7.094      LSD<sub>1%</sub> = 9.579      LSD<sub>0.1%</sub> = 12.756

Even if the environment variance ( $E$ ) has a significant value, it is lower than the components of the dominance ( $H_1$ ,  $H_2$ ) and additive effects ( $D$ ). Based of the ratio  $H_2 / 4H_1$  (0,204≠0,250) it is noted that the frequencies of positive and negative dominant genes are different, having a positive effect on membrane stability for some parents and negative effects on the others. Considering the negative value of covariance for additive and dominance effects ( $F$ ), it can be seen that the dominance alleles which control the membrane stability have a lower frequency (40.4%) compared to the recessive ones (59.6%). The genes involved in the heredity of this parameter were more positive, given that the value of  $H_1$  is higher than for  $H_2$ . The number of blocks of dominant genes according to the value for  $h/H_2$  was underestimated because the dominance effects of all involved genes were not equal in size and direction, or their distribution was correlated [12].

The high value of broad sense heritability (0.856) and also the low value of narrow sense heritability (0.378), indicates that an important part of the variability for membrane stability is due to dominance effects, on the background of the involvement of some major genes [18]. The true sense heritability based on dominance and environment variance has a value over the limit of 0.5, above which the selection is considered to be effective.

Given the analysis of variance for combining ability from Table 5, and the significance of GCA and SCA effects, indicates that both additive and non additive variances plays and important role in controlling the expression of cell membrane stability. The value of GCA/SCA ratio indicates a predominant and significantly higher influence of additive effects (71.99%) compared with the non-additive ones (28.01%), in accordance with the findings of Dhanda and Munjal, (2009).

Table 5

**Analysis of variance for combining ability of cell membrane stability in wheat**

Variability source	SS	DF	MS	F
Total	7772.85	62		
Replication	29.36	2	14.68	0.31
GCA	3058.45	6	509.74	10.66**
SCA	2771.93	14	197.99	4.14**
Error	1913.10	40	47.82	

GCA/SCA

2.57\*\*

The GCA effects of showed a high frequency of negative values, with an amplitude of 16.95%, ranging from -5.56 % in Apache variety to 11.39 % for Fundulea 4 variety. The highest gca effects were recorded in Fundulea 4 variety, showing a high potential for improving drought resistance, and able to transmit to its progenies a good stability of cell

membrane. Apache and Alex varieties registered the lowest additive effects for the improvement of this parameter. Taking in to account that the GCA effects were not significant correlated with the parents means ( $r = 0.423$ ), we consider that it will be difficult to predict the additive effects of the parents basis of their performance.

Table 6

**Effects of the general and specific combining ability for cell membrane stability in wheat**

Parents	GCA	SCA					
		Xenos	GKKapos	Turda 2000	Alex	Apache	Apullum
Fundulea 4	11.39*	-	-12.66 <sup>0</sup>	-1.65	13.89*	1.67	-8.54
Xenos	-0.62		-	-1.03	5.90	-1.81	12.81*
GKKapos	1.50			-	-5.36	5.26	-3.65
Turda 2000	-2.76				-	-2.22	-4.13
Alex	-5.51					-	1.53
Apache	-5.56						-
Apullum	1.57						

GCA -LSD<sub>5%</sub> =9.57; LSD<sub>1%</sub> =12.77; LSD<sub>0.1%</sub> =16.68;SCA -LSD<sub>5%</sub> = 12.14; LSD<sub>1%</sub> =16.19; LSD<sub>0.1%</sub> =21.16.

The SCA effects had different values, mostly negative and non significant, with an amplitude of 26.55%, ranging from -12.66% in Fundulea 4 x Xenos cross to 13.89 % at Fundulea 4 x Turda 2000 cross. The highest non- additive effects were observed in the crosses: Fundulea 4 x Turda 2000 , Xenos x Apache and Fundulea 4 x Apullum, which offers the possibility of obtaining valuable genotypes for cell membrane stability and drought tolerance in the segregating generations. The lowest non-additive effects for this parameter were recorded by following crosses: Fundulea 4 x Xenos, Fundulea 4 x Alex and Turda 2000 x Apullum, showing a high frequency of membrane injuries and a low breeding potential for improvement of this feature.

**Conclusions**

The expression of cell membrane stability for the studied genotypes is conditioned by both dominance and additive gene action, with a higher contribution of dominance one. The direction of dominance for this feature seemed to be variable depending of the parents. As such, for GKKapos, Xenos and Alex varieties, the dominance is associated

with negative alleles, but in Fundulea 4 variety the dominant alleles cause an increase of cell membrane stability. In case of Turda 2000 and Apache varieties the high proportion of recessive alleles induces a decrease of the cell membrane injuries.

Additive variance was higher than non-additive variance and accounted around 72% of total, suggesting that the additive gene action were mainly responsible for the inheritance of cell membrane stability in the studied crosses. Fundulea 4 variety was found to be best general combiner for cell membrane stability, showing a high potential for improving drought resistance of its offspring. The high SCA effects of the cross Fundulea 4 x Alex between parents with high and low GCA, indicates that intermating between crosses and selection allows to obtain genotypes with good cell membrane stability.

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