



## Inheritance of cell membrane stability under PEG and atmospheric induced osmotic stress in barely genotypes

Maryam Fathollahzadeh Ardabili\*, Hossein Shahbazi

*Department of Agronomy and Plant Breeding, Ardabil Branch, Islamic Azad University, Ardabil, Iran*

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### Abstract

To evaluate the inheritance of cell membrane stability of barely, the F<sub>1</sub> seeds of a 6×6 half diallel, along with their parents were grown in greenhouse in randomized complete block design with three replications. After flowering, leaf blades were used for evaluation of membrane injury Index under atmospheric and PEG Induced osmotic stress. The experiment was carried out at Islamic Azad University, Ardabil, Iran in 2014. Results showed that additive-dominant model was adequate for determining the inheritance of the traits. In spite of high broad sense heritability of the traits, they had relatively low narrow sense heritability. Both of the traits governed with over dominance, while the magnitude of average degree of dominance was greater in Membrane Injury index PEG. Higher frequency of dominant alleles in the parents was also demonstrated in both of the traits. Results showed that there was not a clear relation between dominance and favorability of Traits. The significant GCA mean square suggests that genetic gain is achievable through selection over the segregant populations. However, due to high average degree of dominance, selecting for traits under study must be done in advanced generations of barely breeding programs. Results of combining ability analysis also showed that parent number 1,3 and 4 had good additive genes for membrane injury Index.

\* **Corresponding Author:** Maryam Fathollahzadeh Ardabili ✉ [m.fathollahzadehardabili@gmail.com](mailto:m.fathollahzadehardabili@gmail.com)

## Introduction

Drought is a major abiotic stress, limiting crop production in arid and semi-arid climates. Stress resistance in plants is a complex character that depends on many genes and thus is determined by the interactions of many morphological, physiological and biochemical processes. Oxidative stress is common feature of abiotic stresses in which formation of reactive oxygen species (ROS) can seriously disrupt normal metabolism, resulting in lipid peroxidation and consequently membrane injury (Pan *et al.*, 2006; Quiles and López, 2004). Measurement of solute leakage from tissue can be used to estimate the damage to membranes. Therefore, cell membrane stability (CMS) in plants under stress, usually regarded as indicators of the tolerance of genotypes against stress conditions (Sairam *et al.*, 1998; Grzesiak *et al.*, 2003; Dhanda and Munjal, 2006; Sayar *et al.*, 2008). The continuous distribution of CMS and its broad-sense heritability (34%) indicates that CMS should be polygenic in nature. Composite interval mapping identified nine putative QTLs for CMS located on chromosomes 1, 3, 7, 8, 9, 11 and 12 of rice (Tripathy *et al.*, 2000). Results of Shahbazi *et al.* (2013) showed that cell membrane stability under heat and PEG induced stresses in wheat had high broad sense heritability and low narrow sense heritability. Findings of Yildirim *et al.*, (2009) revealed that membrane stability parameters of wheat genotypes decreased during the later developmental stages, and the membrane thermal stability was mediated mainly by non-additive gene actions. Based on the results of Dhanda and Munjal, (2009) The components of genetic variance indicated considerable influence of dominance variance in determining inheritance of cell membrane stability. Cell membrane stability methods also can be measured easily and are nondestructive to whole plant, so potentially can be considered as selection criteria if they also have high heritability. The objective of this study was comparing the inheritance of cell membrane injury induced by heat and osmotic stresses in barely genotypes.

## Materials and methods

### *Plant material and growth conditions*

Six barely varieties (table 1) were crossed in half diallel fashion. The F<sub>1</sub> seeds, along with their parents were grown in greenhouse in well watered condition using randomized complete block design with three replications at Islamic Azad University, Ardabil, Iran in 2014. Seeds of the genotypes were sown in plastic pots filled with 10 kg of soil composed of a mixture of garden soil, compost and sand (1:1:1, v/v). Two weeks after anthesis, flag leaves were collected for cell membrane injury under atmospheric and PEG induced osmotic stresses.

### *Membrane injury index under PEG induced osmotic stress*

Leaf blades were cut into 1cm-long sections immediately after sampling. Three 0.6g leaf samples were placed into vials. The samples were washed three times with distilled water to remove electrolytes adhering to leaves or released from the cut ends of the tissues. Nine ml of PEG %40 was added to each vial. Vials were covered and held at 10°C for 16 hours. The sample were washed three times and distilled water (10ml) was added to each vial. Vials were held at 10°C for 6 h to allow exosmosis and then warmed to room temperature. Electrolytes were measured directly by an electrical conductivity meter (C<sub>1</sub>). The tubes were heated in boiling water for 10 min and cooled. A second conductivity reading of the aqueous phase (C<sub>2</sub>) was taken at 25°C after the samples were cooled. Membrane stability was expressed as membrane injury Index (MII) proposed by Blum and Ebrecon (1979).

$$MII = (C_1/C_2) \times 100.$$

### *Membrane injury index under atmospheric induced stress*

Fully expanded flag leaf blades were collected, immediately three 0.6 gr weighed and cut into segments (ca. 1 cm), washed for 15 min in sterile deionized water and then were dried by facial tissue exposed to osmotic stress (25°C at %75 humidity in incubator) for 4 h in the light for dehydration. 12 ml distilled water were added on samples and vials were held at 25°C for 16 h. Electrolytes were measured directly by an electrical conductivity meter (C<sub>1</sub>). The

tubes were heated in boiling water for 10 min and cooled. A second conductivity reading of the aqueous phase (C2) was taken at 25°C after the samples were cooled. Membrane stability was expressed as membrane injury Index (MII) proposed by Blum and Ebrecon (1979).

$$MII = (C1/C2) \times 100.$$

#### Statistical analysis

The diallel analysis was done according to the theoretical basis developed by Hayman (1954a), adapted for the half diallel by Walters and Morton (1978). The goodness of fit of the additive-dominant model was performed based on the analysis of variance of  $W_r-V_r$  and linear regression of  $W_r$  on  $V_r$  (Hayman, 1954a). The genetic components: D,  $H_1$ ,  $H_2$ , F and  $h^2$  were estimated according to Singh and Singh (1984). Standard errors of these components were calculated from expected and observed values of  $W_r$ ,  $V_r$ ,  $V_r$ ,  $V_r$  and  $(mL_1 - mL_0)^2$  over replications (Hayman, 1954a). From the estimates of the genetic components, the genetic parameters presented in Table 4 were estimated. Average degree of dominance, broad sense heritability and narrow sense heritability

were calculated according to Mather and Jinks (1971). Combining ability analysis was also carried out following Model I and Method II of Griffing (1956). Following Baker (1978), the variance ratio  $2S^2gca/2S^2gca+S^2sca$  was computed from expected components of mean squares assuming a fixed model, to assess the relative importance of additive and non-additive gene effects. Analysis of variance of Diallel was performed using the DIAL98 software (Ukai, 1989), genetic components were estimated by electronic spreadsheets in the Excel program (Microsoft® Excel 2003). Combining ability analysis was performed using SAS 9.2 Software.

#### Results

##### Goodness of fit of the additive-dominant model

The results of the goodness of fit of the additive dominant model are shown in table 2. Non-significant  $W_r-V_r$  mean squares for treatment (crosses) indicated the adequacy of additive dominant model for both of the traits. The slopes of linear regression were also significantly higher than zero and did not show significant differences with 1 (Table 2).

**Table 1.** List of cultivars used in the study.

Genotype	Pedigree
1	CWB117-77-9-7/4/Rhodes's'/Tb/checkzo/3/Gloria's'
2	U.N.K-80Kelar
3	Probesdwarf / Numar
4	Pamir-065/Sonata
5	Legia/3/LB.IRAN/UN8271//GLORIA
6	Rihane//Toji's'Robur

**Table 2.** Goodness of fit of additive-dominant model for evaluated traits.

Character	Heterogeneity of $W_r-V_r$ (Mean squares)	t-test of b on the null-hypothesis	
		b=0	b=1
MII(PEG)	2070.4 <sup>ns</sup>	0.88*± 0.277	0.88 <sup>ns</sup> ± 0.277
MII(atmospheric)	1537.543 <sup>ns</sup>	0.606*± 0.175	0.606 <sup>ns</sup> ± 0.175

<sup>ns</sup>, \* and \*\* non-significant and significant at the 5% and 1% levels, respectively.

#### Analysis of variance and genetic parameters

The Analysis of variance of the diallel is shown in table 3. Additive variance (a component) was highly significant in both traits indicating the presence of additive effects in their control. The significance of (a) in table 3 was in accordance with the significance of additive effects (D component) in table 4. The

dominant genetic effects (b source of variation) showed highly significant effects in both traits, indicating the importance of dominant genetic effects in these traits. The "b1" component which measures the mean deviations of the F1s from the mid-parental values was not significant for both traits (Table 3).

**Table 3.** Analysis of variance of the diallel tables for the evaluated traits.

S.V.	d.f	MII(PEG)	MII(atmospheric)
REP	2	14.88 <sup>ns</sup>	13.29 <sup>ns</sup>
a	5	141.31 <sup>**</sup>	131.57 <sup>**</sup>
b	15	120.28 <sup>**</sup>	69.46 <sup>*</sup>
b1	1	0.2346 <sup>ns</sup>	14.91 <sup>ns</sup>
b2	5	103.81 <sup>**</sup>	25.21 <sup>ns</sup>
b3	9	142.77 <sup>**</sup>	100.11 <sup>**</sup>
Error	40	14.58	28.16

<sup>ns</sup>, \* and \*\* non-significant and significant at the 5% and 1% levels, respectively.

The significance of the “b1” component indicates that the dominance was predominantly in one direction and measures average heterosis (Singh and Singh, 1984). Non Significance of b1 component was generally in accordance with lower magnitude of dominance ( $\bar{F}_1 - \bar{P}$ ). Since the mean dominance effect of the heterozygote locus ( $h^2$ ) was not significant only for traits, high heterotic effect values would not be expected among crosses (Table4). The “b2” component was significant only for MII under peg induced stress. The significance of the b2 item indicated that the mean dominance deviations of the

Fis from their mid parental values differed significantly over the  $F_1$  arrays; this implies the presence of asymmetry in the distribution of alleles among the parents (Hayman, 1954b). This also means that there was evidence that some parents had a significantly better performance than others (Ramalho *et al.*, 1993). Since b2 is significant for peg induced stress, the 'a' item will not measure additive variance unambiguously, but it will be contaminated with non-additive variance also (Singh and Singh, 1984; Chaudhary *et al.*, 1977). The proportion of positive and negative genes was estimated by calculating (H2/4H1) in table 4.

**Table 4.** Estimates of genetic components and related statistics in half- diallel design.

	MII(PEG)	MII(atmospheric)
D=	50.2 <sup>**</sup> ±4.66	67.04 <sup>**</sup> ±4.32
H1=	208.89 <sup>**</sup> ±11.82	106.54 <sup>**</sup> ±10.97
H2=	180.77 <sup>**</sup> ±10.5573	100.5 <sup>**</sup> ±9.80
F=	61.49 <sup>**</sup> ±11.37	45.329 <sup>*</sup> ±10.57762
$h^2$	4.099 <sup>ns</sup> ±7.105	-0.417 <sup>ns</sup> ±6.596
E	4.85 <sup>ns</sup> ±1.759	9.41 <sup>**</sup> ±1.633
H2/4H1	0.216343	0.235957
KD/KR	1.858199	1.732828
Averag d	2.039892	1.260625
Hn	0.143935	0.286167
Hb	0.917033	0.805587
$F_1$ -P(%)	-0.13509	1.062426
rYr(Wr+Vr)	0.561 <sup>*</sup>	0.0012 <sup>ns</sup>
A(intercept)	37.19 <sup>ns</sup>	-1.36684 <sup>ns</sup>

<sup>ns</sup>, \* and \*\* non-significant and significant at the 5% and 1% levels, respectively.

This ratio was lower than 0.25 in both traits, indicating the presence of asymmetry in the distribution of the positive and negative alleles in the parents. This is also substantiated by H1 being greater than H2 in these traits. The “b3” component which is equivalent to specific combining ability variance was

significant both traits. b3 estimates residual dominance effects combining from additive × additive, additive × dominance and dominance × dominance interaction effects that are not attributed to b1 and b2 (Chaudhary *et al.*, 1977). The estimate of the genetic component F was significant in both cases

which is an indication of asymmetry in the distribution of dominant and recessive alleles in the parents. The ratio of the total number of dominant and recessive alleles in the parents (KD/KR) was higher than one in both traits, demonstrating a higher frequency of dominant alleles in the parents. Positive values for F substantiated by (KD/KR) being greater than one. The degree of average dominance was higher than one, indicating the presence of over dominance in control of both traits. Over dominance also was confirmed by negative intercept of regression line in both cases (Table 4). Yildirim *et al.*, (2009) and Shahbazi *et al.* (2013) also showed that membrane thermal stability was mediated mainly by non-

additive gene actions. Despite the high broad sense heritability (Hb) in both cases, Narrow sense heritability (Hn) of the traits was relatively low (table 4). The differences observed between the Hn and Hb reflected the presence of the dominant effects. Ibrahim and Quick (2001) in parent-offspring regression using F<sub>3</sub> plants and their F<sub>4</sub> progeny means found that, narrow sense heritability was relatively low (0.32–0.38) for RI. Correlation coefficients between the parental means and order of dominance “rYr(Wr+Vr)” was positive in PEG induced stress that recessive alleles are favorable, however such relation was not clearly observed in case of atmospheric induced stress.

**Table 5.** Analysis of variance of the diallel tables for the evaluated traits.

Source of variation	Df	Mean Squares	
		MII(PEG)	MII(atmospheric)
General Combining Ability	5	60.9804**	154.0733**
Specific Combining Ability	15	130.0772**	61.9643*
Error	40	14.6275	28.1660
$\frac{2S^2_{GCA}}{2S^2_{GCA} + S^2_{SCA}}$	--	0.48%	0.815%

\* and \*\* significant at the 5% and 1% levels, respectively.

Combining ability analysis by Griffing's method indicated the significance of both GCA and SCA mean squares, which is in accordance with the results of Dhanda and Munjal (2006) and Shahbazi *et al.* (2013). This shows the importance of both additive and dominance effects (Table 5). Higher Baker's ratio for MII under atmospheric stress suggests that additive effects are more important than non-additive effects in this case (Table 5). Dhanda and Munjal (2006) found

that the magnitude of GCA effects was considerably higher than SCA effects for relative injury under heat stress. Dhanda and Munjal (2009) showed that the mean square for GCA was higher in magnitude than that of SCA, but the components of genetic variance indicated considerable influence of dominance variance in determining inheritance of cell membrane stability.

**Table 6.** General combining ability estimates of parents used in the experiment.

Parent	MII(PEG)	MII(atmospheric)
1	0.992 <sup>ns</sup>	-2.274*
2	-1.4733**	0.045 <sup>ns</sup>
3	-1.046 <sup>ns</sup>	4.400**
4	2.752**	1.266 <sup>ns</sup>
5	-0.355 <sup>ns</sup>	-1.876 <sup>ns</sup>
6	0.870 <sup>ns</sup>	-1.562 <sup>ns</sup>
Standard error of GCA	0.542	0.978

The importance of GCA effects was also evident from the higher correlation between the parental means and the GCA effects in the case of MII under peg ( $r = 0.561^*$ ) comparing with mii under atmospheric stress (0.0012). Thus, the combining ability analysis was in good agreement with the conclusions from Hayman's method in showing the gene action in the inheritance of MII (PEG) and MII(atmospheric) .

Results of GCA effects indicated that genotypes number 2, 3 and 5 had lower GCA for ,MII (PEG) (smaller values are favorable) while genotypes number 1, 5 and 6 had favorable gens for MII under atmospheric stress (Table 6).

### Discussion

The fulfillment of assumption for Hayman's analysis indicated that a relatively simple genetic model was involved in the inheritance of traits under study. This result is in agreement with the findings of Shahbazi *et al* (2013). Since the degree of average dominance was higher than 1, the presence of over dominance and greater importance of dominance effects in control of traits was suggested. Similar results were obtained by other scientists (Shahbazi *et al*, 2013; Yildirim *et al.*, (2009; Dhanda and Munjal, 2009 ). The results also showed that, in the case of MII under PEG stress dominance was predominantly in one direction, indicating the presence of heterosis in the control of this trait (Singh and Singh, 1984). Regarding to the high broad sense heritability of traits under study, it can be concluded that they can be considered as candidates for selecting drought tolerance in barley. However relatively lower narrow sense heritability of traits suggests the use of multiple replications during selection to limit environmental effects. Regarding to the low narrow sense heritability of the traits, it can be concluded also that these traits can be considered as selecting criteria for drought tolerance in advanced generations of barley breeding programs. The significant GCA mean squares for both traits indicated variability of GCA among the parents and this suggests that genetic gain is achievable through selection over the segregant population. As additive gene action and additive  $\times$  additive types of epistatic

gene action are exploitable in homozygous genotypes, the estimates of GCA effects of individual lines are a useful predictor for progeny performance in self-fertilizing species like barley (Baker1978). In this regard, genotypes number 2, 3 and 5 had favorable genes for ,MII under PEG stress while genotypes number 1, 5 and 6 had favorable gens for MII under atmospheric stress.

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