RELATIONSHIP BETWEEN GENETIC DIFFERENCES IN THE CAPACITY OF OSMOTIC ADJUSTMENT AND OTHER PHYSIOLOGICAL MEASURES OF DROUGHT RESISTANCE IN WINTER WHEAT (*TRITICUM AESTIVUM* L.)

Constantina Bănică[1], Elena Petcu[1], Aurel Giura[1], Nicolae N. Săulescu[1]

ABSTRACT

Pollen expression of osmotic adjustment showed large differences among the studied winter wheat cultivars. Three classes of OA capacity could be distinguished: two Romanian cultivars (Izvor and Faur) had high OA, two Romanian cultivars along with the two analyzed French ones had low OA, while most of Romanian cultivars had an intermediate response. Doubled haploid lines from a cross between a high OA cultivar and an intermediate OA cultivar could be classified into the two corresponding classes, the number of lines in each classes being not significantly different. DH lines with high OA had significantly slower water stress induced senescence of leaves, lower drought induced membrane injury and smaller grain yield differences between irrigated and non-irrigated plots. Residual (cuticular) transpiration was not different between high and low OA lines.

Key words: wheat, drought resistance, osmotic adjustment (OA), cuticular transpiration, membrane stability

INTRODUCTION

Presently it is estimated that one third of the land, potentially usable for agriculture, is not cultivated because of deficient water availability, and on most of the remaining land yields are periodically reduced by drought. This situation will very probably become worse, according to most climate change scenarios.

Genetic modification of plants to allow growth and yield under water stress conditions is an important component of the solution to the drought problem (Zhang et al., 1999). Many mechanisms and traits, potentially useful for improving plant performance under drought have been described (Blum, 1996, 1998; Ginkel et al., 1998). Among them, osmotic adjustment (OA) is receiving increased recognition as a major mechanism of drought resistance in crop plants (Zhang et al., 1999). The capacity to adjust osmotically is an inherited trait, which in wheat is controlled by alternative alleles at one locus on chromosome 7A, that controls primarily differences in potassium accumulation (Morgan, 1983, 1991).

As OA is a cellular mechanism it is expressed in all plant cells, including pollen and this offers a convenient way to characterize germplasm for this trait (Morgan, 1999; Moud and Yamagishi, 2005).

This paper presents results about the effect of genetic differences in the osmotic adjustment capacity, estimated using pollen expression of OA, on other traits related to drought resistance.

MATERIAL AND METHODS

Ten Romanian and two French winter wheat cultivars, previously characterized for their field response to drought (Săulescu et al., 1998; 2006; Mustăţea et al., 2003), were characterized for their OA expressed in pollen grains. Based on the results, crosses between cultivars with different OA ability were made.

Fifty six doubled haploid (DH) lines from the cross between cultivars Izvor (drought resistant) and Jiana (medium drought resistance), were obtained using the „*Zea* system” (Giura, 1993), and later characterized for several traits related to drought resistance.

The osmotic adjustment capacity was estimated using the pollen test developed by Morgan (1999). Pollen grains of matured anthers, at or near the point of dehiscing, were soaked in polyethylene glycol (PEG 6000) solutions of several concentrations, over microscope slides, with or without 10 mM KCl added to the solutions. PEG concentrations from 30 to 55% were used for testing cultivars, while for the DH lines a PEG concentration of 55% with and without KCl was used to discriminate the lines according to their OA capacity (Bănică et al., 2008). After a little agitation to release the pollen grains, the anther sections were removed and the solution covered

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[1] National Agricultural Research and Development Institute Fundulea, 915200 Fundulea , Călăraşi County, Romania.
Email: tinabanica@yahoo.com

with a cover slip. Slides were incubated at 20°C for 2 days. Microscopic observations were made using a magnification of 100X and 200X. Pollen grains are usually spherical/ ellipsoidal in shape. A stressing concentration of PEG (40%-55%, depending on genotype) induced a maximum shrinkage of pollen grains that assume a more conical shape, often with concavities. Modification of pollen grains shape (shrinking) was visually estimated.

The field response of wheat plants to the severe drought experienced in the very dry spring of 2007 was estimated by counting the number of leaves that remained green on June 10, when drought induced leaf senescence was already severe.

The DH lines were also tested in yield trials on 5 square meter plots in three replications, both with and without irrigation in the dry year 2007. Response to drought was estimated as the difference between grain yields in irrigated and non-irrigated plots.

Membrane stability after water stress was estimated using a conductance test, as described by Petcu et al. (1995). The medium part of flag leaves collected from field plots on May 21, 2007, when wilting was noticeable on medium drought resistant parent Jiana, were cut in 2 cm segments, washed and than placed in the tubs with 25 ml distilled water. The tubes were shaken to mix the content and an initial conductance reading was made after 24 hours. The tubes were then autoclaved at a pressure of 1.5 atm for 15 min. Membrane stability was expressed as relative (RI) injury as follows:

$$RI = (1 - C1/1 - C2) \times 100$$

where C1 and C2 are the first and the second reading of conductance.

A technique similar to that of Clarke and McCaig (1982) to determine residual (cuticular) transpiration was used. Five flag leaves for each replication were detached from field plots on May 17, 2007, about 10 days after heading, and the excised leaves were transported to the laboratory within 30 min., weighed, wilted for 5 h under ambient laboratory condition (22-24°C , in the dark), re-weighed , oven-dried for 4 h at 70°C and weighed again. Cuticular transpiration (CT) was estimated as weight of water lost after 5 h per gram of dry weight (DW), using the formula:

$$CT = (Wi – W5h)/DW$$

where: Wi is the initial weight and W5h is the weight after 5 hours wilting at 22-24°C in the dark.

ANOVA was used to estimate significance between groups of DH lines with different OA pollen expression, by F-testing the variance between groups against the variance within groups.

RESULTS AND DISCUSSION

Using PEG solutions of different concentrations (30%, 40%, 45%, 50% and 55%) we could differentiate three classes of OA capacity for the twelve winter wheat cultivars tested (Table 1).

Table 1. Osmotic adjustment capacity expressed in pollen grains and drought resistance estimated in the field, in twelve winter wheat cultivars.

Cultivar	Pollen expression of osmotic adjustment	Drought resistance estimated by multi-annual observations
Izvor	High	Very resistant
Faur	High	Resistant
Ardeal	Intermediate	Moderately resistant
Boema	Intermediate	Moderately resistant
Dropia	Intermediate	Moderately resistant
Glosa	Intermediate	Resistant
Delabrad	Intermediate	Moderately resistant
Jiana	Intermediate	Moderately resistant
Doina	Low	Susceptible
Fundulea 4	Low	Medium susceptible
Bersée	Low	Very susceptible
Renan	Low	Very susceptible

The first class included cultivars Izvor and Faur, which showed no shrinking of pollen grains even in a high concentration of 55% PEG solution. In the two French cultivars, Bersée and Renan, as well as in two Romanian cultivars, Doina and Fundulea 4, pollen grains severely changed their shape and volume, even when emerged in 40% PEG solutions.

The rest of Romanian cultivars were intermediate in their response to PEG, showing only a slight modification of pollen grain shape in a 50% PEG solution (Figure 1).

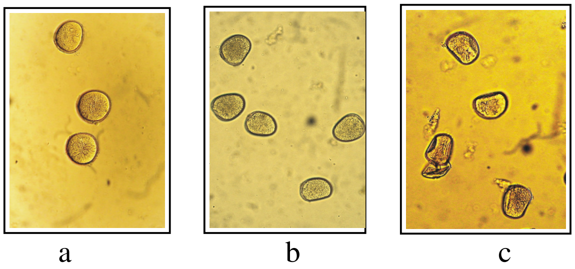

Figure 1. Modifications of pollen grains shape and volume after immersion in PEG solution: a) pollen grains in a non-stressing control solution (30% PEG); b) genotype with low osmoregulation capacity (40% PEG); c) genotype with high osmoregulative capacity (55% PEG).

The grouping based on pollen expression of osmotic adjustment is in good agreement with the performance observed in the field in the dry years 2003 and 2007 (Table 1).

Our classification into three groups of osmotic adjustment capacity differs from the results of Morgan (1999), who only distinguished two classes, corresponding to two alleles at one locus „*or*”. We suggest that even if only one gene is involved, this gene might have multiple alleles, causing a more diverse response.

Thirty one out of the 56 DH lines from the cross between a cultivar with high OA (Izvor), and one with intermediate OA (Jiana) had high OA and were considered carriers of the recessive allele „*or*” and the rest of 25 had intermediate pollen expression of osmotic adjustment, and were considered carriers of the dominant allele „*Or*”. The slight excess of high OA lines was not significant according to χ^2 test ($\chi^2 = 0.46$ n.s.).

The number of leaves that were still green after a severe drought period in 2007 was characterized for 55 of the DH lines and varied from 0 to 2 (Figure 2).

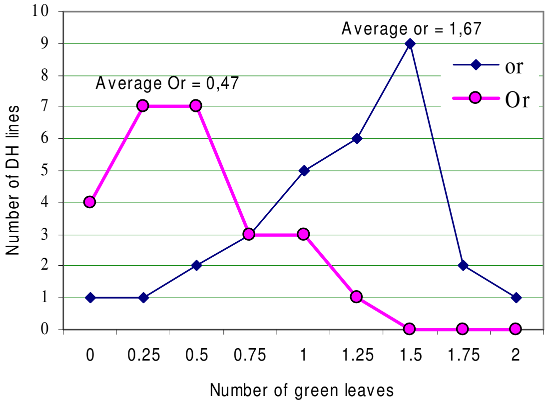

Figure 2. Frequency distribution for the number of green leaves in Izvor x Jiana DH lines with different osmotic adjustme

Although distributions of „*or*” and „*Or*” lines partially overlap, the average value for the „*or*” lines was 1.2 higher and ANOVA shows a very significant effect of the „*or*” alleles on this trait (Table 2).

Table 2. ANOVA for the number of leaves still green following water stress induced leaf senescence in the field

Sources of variation	DF	MS
Between groups with different pollen expression of osmotic adjustment capacity	1	6.62***
Within groups	54	0.17

*** = significant at P<0.1% level

The grain yield difference between irrigated and non-irrigated plots in 2007 ranged between 500 and 1900 kg/ha (Figure 3).

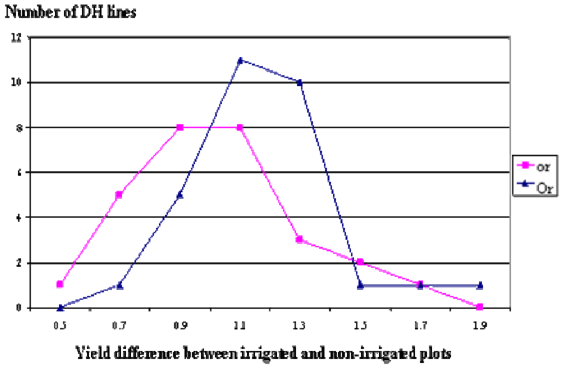

Figure 3. Frequency distribution for the yield differences between irrigated and non-irrigated plots in Izvor x Jiana DH lines with different osmotic adjustment

Distributions of „*or*” and „*Or*” lines largely overlap, a result that was expected, given the large number of factors influencing the grain yield.

Nevertheless, ANOVA shows that the effect of the „*or*” alleles was significant (Table 3).

Table 3. ANOVA for grain yield difference between irrigated and non-irrigated plots in 2003

Sources of variation	DF	MS
Between groups with different pollen expression of osmotic adjustment capacity	1	0.439*
Within groups	54	0.063

***) = significant at P<5% level.

Membrane stability, expressed as % solute leakage after water stress, was characterized in 49 of the DH lines, and ranged from 15 to 65%, with an average of 22.72% for „*or*” lines and 36.85% for „*Or”* lines (Figure 4).

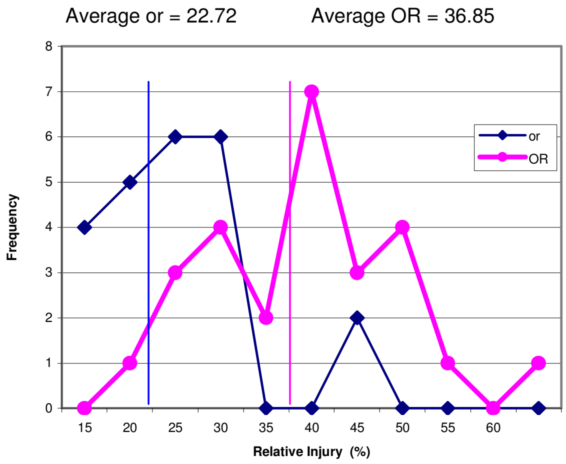

Figure 4. Frequency distribution for injury index in Izvor x Jiana DH lines with different osmotic adjustment

ANOVA shows a very significant effect of the grouping according to pollen expression of OA (Table 4). This is in agreement with the fact that osmotic adjustment is the result of active solutes in the cell (Blum, 1988), and the known effect of solutes accumulated as a result of water stress on stabilization of proteins, including membrane proteins (Bohnert and Jensen, 1996).

Table 4. ANOVA for membrane stability after water stress

Sources of variation	DF	MS
Between groups with different pollen expression of osmotic adjustment capacity	1	2257.9***
Within groups	47	4545.3

*** = significant at P<0.1% level.

Cuticular transpiration in the 49 DH lines which were characterized for this trait ranged from 1 to 1.7, with an average of 1.26 for „*or*” lines and 1.37% for „*Or”* lines (figure 5).

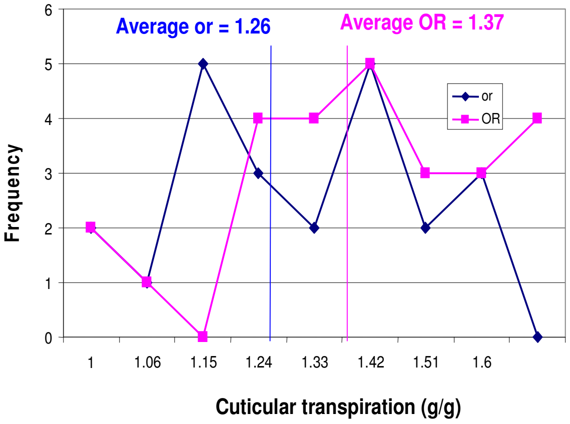

Figure 5. Frequency distribution for cuticular transpiration in Izvor x Jiana DH lines with different osmotic adjustment

Distributions of „*or*” and „*Or*” lines completely overlap, and ANOVA shows that the effect of the OA differences is not significant (Table 5). Obviously, the two traits are independent and this suggests that one approach for improving drought resistance could be to combine high OA with low CT.

Table 5. ANOVA for cuticular transpiration

Sources of variation	DF	MS
Between groups with different pollen expression of osmotic adjustment capacity	1	0.143 n.s.
Within groups	47	2.081

n.s. = not significant at P<5%

CONCLUSIONS

Pollen expression of osmotic adjustment showed large differences among the studied winter wheat cultivars. Three classes of OA capacity could be distinguished: two Romanian cultivars (Izvor and Faur) had high OA, two Romanian cultivars along with the two analyzed French ones had low OA, while most of Romanian cultivars had an intermediate response.

Doubled haploid lines from a cross between a high OA cultivar and an intermediate OA one could be classified into the two corresponding classes, the number of lines in each classes being not significantly different.

DH lines with high OA had significantly slower water stress induced senescence of leaves, lower drought induced membrane injury and smaller grain yield differences between irrigated and non-irrigated plots. Residual (cuticular) transpiration was not different between high and low OA lines.

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