OSMOTIC ADJUSTMENT CAPACITY AND CUTICULAR TRANSPIRATION IN SEVERAL WHEAT CULTIVARS CULTIVATED IN ALGERIA

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ABSTRACT

Mostly grown in the semi-arid and even arid areas, Algerian wheat cultivars are potential donors of genes for drought resistance traits. We estimated the osmotic adjustment capacity, based on pollen grain expression, in nine *Triticum durum* and five *Triticum aestivum* cultivars grown in Algeria and found significant differences among them in the reduction of projected cytoplasm area in pollen grains soaked in 50% PEG solution with added KCl, as compared with the pollen grains soaked in 30% PEG solution. In common wheat Mahon Demias and the durum cultivars Mohamed Ben Bachir and Bousselem, the area of the cytoplasm in stressed pollen grains represented more than 90% of the area in non-stressed pollen grains, while in other cultivars the projected cytoplasm area of pollen grains was reduced by more than 25%. This recommends cultivars Mahon Demias, Mohamed Ben Bachir and Bousselem for use as parents in breeding for improved osmotic adjustment. Cuticular transpiration was measured in five Algerian cultivars and showed a relatively low variability for these trait. The average of the five Algerian cultivars was better than the average of Romanian cultivars and the average of all cultivars from the analyzed international collection. However the best Algerian entry was not superior to the best Romanian cultivar or to the best entry from the international collection. This suggests that the analyzed Algerian cultivars may not be useful for use as parents in breeding for reducing cuticular transpiration.

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

INTRODUCTION

Abiotic stress factors, particularly the water deficit limits crop productivity, accounting for more than a 50% of crops in the world (Kramer, 1980; Boyer, 1982). Presently it is estimated that one third of the land, potentially usable for agriculture, is not cultivated because of deficient water availability, and most of the remaining land yields are periodically reduced by drought. This situation will very probably become worse, according to most climate change scenarios (Bănică et al., 2008).

Genetic modification of plants to allow growth and yield under water stress conditions is an important component of the solution to 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). One of them, osmotic adjustment (OA) is receiving increased recognition as a major mechanism of drought resistance in crop plants (Zhang et al., 1999). Osmotic adjustment capacity is one of the important characteristics to adapt to water deficit (Morgan, 1999; Finlayson et al. 1999).

The capacity of osmotic adjustment 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 osmotic adjustment is a cellular mechanism, it is expressed in all plants cell, including pollen grain and this offers a convenient way to characterize germplasm for this trait (Morgan, 1999; Moud and Yamagishi, 2005).

Another potentially useful mechanism for improving plant performance under drought is to reduce non stomatal transpiration. Excised-leaf loss due to cuticular transpiration has been suggested as a technique to identify cereal genotypes adapted to dry environments (Jardat and Konzak, 1983; Clarke et al., 1989). Balotă et al. (1995) found large variation among Romanian wheat cultivars for cuticular transpiration, cultivars with known good performance under drought having low or medium IWC and low WL.

As wheat is mostly grown in the semi-arid and even arid part of Algeria, with rainfall between 200 and 500 mm, Algerian cultivars are potential donors of genes for drought resistance traits.

This paper presents results on osmotic adjustment capacity, estimated using pollen expression of OA, and on cuticular transpiration, in 14 Algerian cultivars.

MATERIAL AND METHODS

Nine *Triticum durum* cultivars and five *Triticum aestivum* cultivars grown in Algeria in different climatic regions, kindly supplied by the Technical Institute for Field Crops

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Algeria (Institut Technique des Grandes Cultures, Alger) were grown at Fundulea, Romania in 2008 and 2009, of which three are local populations and others were introduced from other countries, based on adaptation to the natural conditions of the area and on other technical criteria (Boufenar et Zaghouan, 2006). Table 1 presents the cultivars included in our study.

Table 1. Name and origin of the Algerian wheat cultivars included in the study

Genotype	Species	Origin
Bidi 17	*Triticum durum*	Local
Bousselem	*Triticum durum*	ICARDA/Syria
Chen's	*Triticum durum*	ICARDA/Syiria
Gta Dur	*Triticum durum*	CIMMYT/Mexico
Hedba 3	*Triticum durum*	Local
Mexicali	*Triticum durum*	CIMMYT/Mexico
Mohamed Ben Bachir	*Triticum durum*	Local
Vitron	*Triticum durum*	Spain
Waha	*Triticum durum*	ICARDA/Syria
Ain Abid	*Triticum aestivum*	Spain
Anza	*Triticum aestivum*	USA
Arz	*Triticum aestivum*	CIMMYT/Mexico
Hiddab	*Triticum aestivum*	CIMMYT/Mexico
Mahon Demias	*Triticum aestivum*	Spain

Osmotic adjustment capacity of the 14 cultivars was estimated in plants grown at the National Agricultural Research and Development Institute Fundulea, Romania, in 2007, using the test of pollen developed by Morgan (1999). Pollen grains of matured anthers were soaked in polyethylene glycol (PEG 6000) solutions of several concentrations, over microscope slides, with or without 10 mM KCl added to solutions. PEG concentration of 50% with added KCl proved to be most useful in discriminating the lines according to their OA capacity. After a little agitation to release the pollen grains, the anther sections were removed and the solution covered with a cover slip. Slides were incubated at 20°C for 2 days. Microscopic observations were made using a magnification of 100X and 200X. Pollens grains are usually spherical to ellipsoidal in shape. A stressing concentration of PEG induced shrinkage of pollen grains, which assumed a more conical shape, often with concavities. Modification of pollen grains shape (shrinking) was estimated both visually and by projected area of pollen cytoplasm measurements.

Five of the 14 cultivars were selected for studying residual (cuticular) transpiration in 2009, along wih a large collection of wheat cultivars from Romania and other countries, using a technique similar to that of Clarke and McCaig (1982). Six flag leaves for each replication were detached from field plots and the excised leaves were transported to the laboratory within 30 min. and weighed to obtain the initial water content.

Leaves were then wilted for 5 hours under laboratory conditions (20°C, in the dark), weighed (W_{5h}), wilted for 24 hours at 20°C, re-weighed (W_{24h}), and oven-dried at 70°C to obtain the dry weight (DW). Cuticular transpiration (CT) was estimated as weight of water lost after 24 hours, per gram of dry weight (DW), using the formula:

$$CT = (W_{5h}-W_{24h})/DW$$

where:

- W_{5h} is the weight after 5 hours wilting at 20°C in the dark;
- W_{24h} is the weight after 24 hours wilting at 20°C;
- DW is the dry weight.

RESULTS AND DISCUSSION

There were large differences in the projected area of cytoplasm in pollen grains subjected to various levels of osmotic stress in PEG solutions, among the studied Algerian wheat cultivars (Table 2).

Projected cytoplasm area of pollen grains soaked in 50% PEG solution was smaller by 12.4% than the average area in 30% PEG solution, while adding KCl to the 50% PEG solution, further reduced the area of projected pollen cytoplasm on average by about 20%.

Table 2. Projected area of pollen cytoplasm at various levels of osmotic stress

Genotype	Area measured in pollen grains soaked in solutions of		
	30% PEG	50% PEG	50% PEG+KCl
Triticum durum	*23.56*	*20.87*	*19.13*
Bidi 17	26.40	24.04	22.11
Bousselem	23.75	16.18	22.52
Chen S	23.04	21.92	20.74
Gta Dur	26.22	22.95	15.77
Hedba 3	24.61	21.19	21.26
Mexicali	20.55	19.76	17.82
Mohamed Ben Bachir	21.69	21.60	20.68
Vitron	23.85	18.91	17.56
Waha	21.95	21.29	17.72
Triticum aestivum	*23.21*	*19.92*	*18.25*
Ain Abid	23.67	15.33	15.91
Anza	23.56	24.29	19.52
Arz	22.55	18.46	15.84
Hiddab	26.19	22.87	20.99
Mahon Demias	20.06	18.67	19.00
Average	23.43	20.53	18.81
%	100.0	87.6	80.3

ANOVA shows significant differences among cultivars in size of pollen grains soaked in 30% PEG solution. This concentration is considered non-stressing, so that the size measured in these conditions can be taken as initial size. The average projected cytoplasm area of *Triticum durum* cultivars was not significantly different from the average of *Triticum aestivum* cultivars, but differences between cultivars within each species were significant (Table 3).

Table 3. ANOVA for the projected cytoplasm area of pollen grain exposed at 30% PEG

Source of variation	Sum of squares	DF	Mean square
Between cultivars	399.0	13	30.7***
- between species	(17.1)	(1)	17.1 n.s.
- within species	(381.9)	(12)	31.8***
Within cultivars	455.5	89	9.7
Total	854.5	102	

After exposure to 50% PEG solutions the average difference between *Triticum durum* and *Triticum aestivum* cultivars, as well as differences among cultivars of both species, were significant (Table 4).

Table 4. ANOVA for the projected cytoplasm area of pollen grain exposed at 50% PEG

Source of variation	Sum of squares	DF	Mean square
Between cultivars	644.48	13	49.57***
- between species	(35.82)	(1)	35.82***
- within species	(606.66)	(12)	50.72***
Within cultivars	348.30	70	4.98
Total	992.78	83	

Adding KCl to the 50% PEG solution also revealed significant differences among cultivars, with the average difference between *Triticum durum* and *Triticum aestivum* cultivars being not significant, but differences among cultivars within both species remaining highly significant (Table 5).

Table 5. ANOVA for the projected cytoplasm area of pollen grain exposed at 50% PEG with addition of KCl

Source of variation	Sum of squares	DF	Mean square
Between cultivars	524.3	13	40.3***
- between species	(2.5)	(1)	2.5 n.s.
- within species	(521.8)	(12)	43.5***
Within cultivars	765.7	79	9.7
Total	1290.0	92	

Correlations between cultivar responses to various levels of osmotic stress applied to pollen grains were not significant (Table 6), suggesting that the projected cytoplasm area under stress was not influenced by the initial size of the pollen. On the other hand, addition of KCl to the PEG solution produced important changes in the cultivar response, with some cultivars (like Anza, Gta Dur and Waha) showing large decreases and others (like Bousselem, Mahon Demias and Hedba3) maintaining or even increasing projected cytoplasm area.

Table 6. Correlation between projected area of pollen cytoplasm at different levels of osmotic stress

Area measured in pollen grains soaked in solutions of	Area measured in pollen grains soaked in solutions of		
	30% PEG	50% PEG	50% PEG+KCl
30% PEG	1		
50% PEG	0.40	1	
50% PEG + KCl	0.32	0.24	1

Morgan (1999) showed that the pollen expression of osmotic adjustment only occurred after addition of potassium chloride to the stressing PEG solution, as a result of the effect of the osmoregulation gene on potassium transport. It means that the most useful characterization of cultivars from the point of view of their osmotic adjustment capacity is given by the behavior of their pollen grains when exposed to PEG solution with addition of KCl.

To avoid a possible influence of the initial size of the pollen grains, the projected cytoplasm area measured after exposure to 50% PEG solution with added KCl was expressed as percentage from the area measured in pollen grains exposed to 30% PEG solution (Table 7). In some cultivars, like the common wheat Mahon Demias and the durum cultivars Mohamed Ben Bachir and Bousselem, the area of the cytoplasm in stressed pollen grains represented more than 90% of the area in non-stressed pollen grains. In contrast, in cultivars Arz and Ain Abid (*Triticum aestivum*) or Vitron and GTA Dur (*Triticum durum*) the projected cytoplasm area after immersion of pollen grains in solution of PEG 50% with 10 mM KCl was reduced by more than 25%.

Table 7. Pollen cytoplasm measurements of analyzed pollen grains

Genotype	Species	Projected pollen cytoplasm area in pollen stressed at concentration of 50% PEG with added KCl, expressed as % of area in non-stressed pollen
Mohamed Ben Bachir	*Triticum durum*	95.3
Mahon Demias	*Triticum aestivum*	94.7
Bousselem	*Triticum durum*	94.8
Chen's	*Triticum durum*	90.0
Mexicali	*Triticum durum*	86.7
Hedba 3	*Triticum durum*	86.4
Bidi 17	*Triticum durum*	83.8
Anza	*Triticum aestivum*	82.8
Waha	*Triticum durum*	80.7
Hiddab	*Triticum aestivum*	80.1
Vitron	*Triticum durum*	73.7
Arz	*Triticum aestivum*	70.2
Ain Abid	*Triticum aestivum*	67.2
Gta Dur	*Triticum durum*	60.1

The modifications of pollen grains shape and of the cytoplasm area were easily noticeable under microscope (Figure 1)

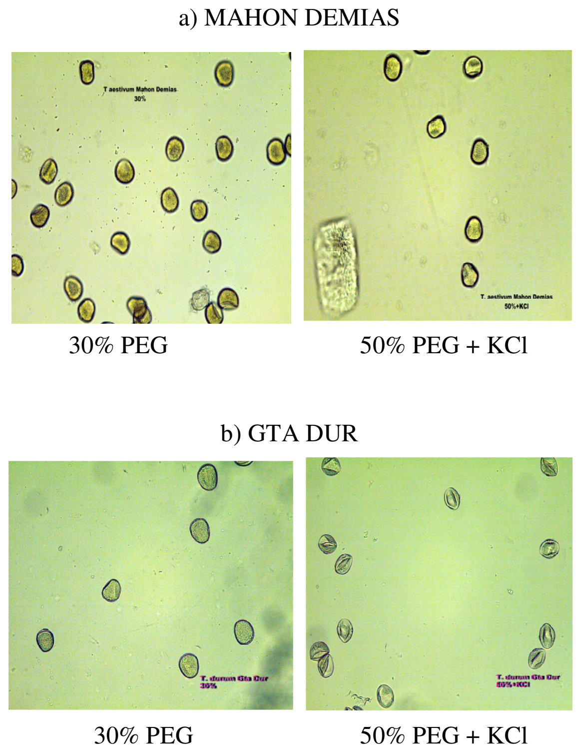

Figure 1. Modification of pollen grain shape and projected area in two Algerian cultivars, contrasting in osmotic adjustment capacity

Our data suggest that the best Algerian cultivars might be superior to the Romanian winter wheat cultivar Izvor, which showed the best osmotic adjustment capacity in previous studies (Bănică et al., 2008). This indicates that several Algerian wheat cultivars are potentially useful for use as parents in the Romanian breeding program to improve the osmotic adjustment potential.

The five Algerian cultivars which were analyzed for initial water content and cuticular transpiration showed a relatively low variability for these traits (Table 8). The average of the five Algerian cultivars was better than the average of Romanian cultivars and the average of all cultivars from the analyzed international collection. However the best Algerian entry was not superior to the best Romanian cultivar (Flamura 85) or to the best entry from the international collection (TX86A5606).

Table 8. Initial water content and cuticular transpiration in wheat cultivars grown in Algeria, as compared with Romanian cultivars and an international collection

Genotype	Initial water content	Cuticular transpiration
Mohamed Ben Bachir	2.343	1.308
Bidi17	2.783	1.328
Ain Abid	2.322	1.395
Vitron	2.451	1.407
Hiddab	2.492	1.517
Algerian cultivars:		
Average	2.478	1.391
Best cultivar	2.322	1.308
Romanian cultivars:		
Average	2.381	1.450
Best cultivar	2.226	1.183
All cultivars:		
Average	2.359	1.400
Best cultivar	1.936	1.170
LSD 5%	0.190	0.239

This suggests that the analyzed Algerian cultivars may not be useful for use as parents in breeding for reducing cuticular transpiration in Romanian germplasm.

CONCLUSIONS

Estimation of the osmotic adjustment capacity, based on pollen grain expression, showed that some Algerian cultivars are outstanding for this trait and could be used as parents in breeding for improved drought resistance.

Cuticular transpiration of tested Algerian cultivars was on average superior to Romanian cultivars, but the best Algerian cultivar was not better than the best Romanian cultivar and was clearly below the best cultivar identified in an international collection. This does not recommend Algerian cultivars as donors of genes for reduced cuticular transpiration.

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