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SEEDLING RESPONSE TO WATER STRESS INDUCED BY GRADUAL DRYING OF THE SUBSTRATE – A SIMPLE METHOD TO APPROXIMATE DROUGHT RESISTANCE IN WHEAT

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ABSTRACT

Breeding wheat for drought resistance has become a high priority in many parts of the world. Short duration seedling tests under controlled conditions, which can be performed off-season for preliminary selection, could contribute to faster progress in improving wheat response to water stress. Searching for such a simple seedling test, we measured leaf elongation and second leaf drying of seedlings grown in pots filled with 500 cc perlite, and exposed to water stress induced by gradual drying. We found large differences between the studied cultivars in both leaf elongation and drying of the second leaf, and these differences were largely in agreement with the available information about the behaviour of the cultivars under drought in the field. Therefore, we suggest that leaf elongation and leaf drying under water stress induced by gradual drying of the substrate in a seedling test can be used for preliminary characterization and selection of genotypes in breeding for drought resistance.

Key words: drought resistance, seedling test, leaf elongation, wheat.

INTRODUCTION

Drought resistance has become a high priority in many wheat breeding programs, especially in the context of climate changes. Adaptation to water stress is a very complex process, and, as no individual trait is able to describe the plant response to drought, no single test can provide a comprehensive picture of its complexity. However, such tests, and especially seedling tests, can prove very useful for a better understanding of differential responses of genotypes to water stress, and for preliminary selection in breeding.

Morgan (1988) suggested that coleoptile and roots length response to water stress can be used to identify differences for osmoregulation among wheat genotypes. His results opened prospects of seedling tests that could be informative about the behaviour of adult plants under water stress.

Leaf elongation is one of the plant processes most sensitive to water stress conditions (Hsiao, 1973; Hsiao and Acevedo, 1974; Cutler et al., 1980). The role of water in leaf elongation is thought to be mediated by turgour potential, which interacts with cell wall properties to determine the rate of expansion (Lockhart, 1965; Cutler et al., 1980).

Starting from this knowledge, we searched for genetic differences between cultivars in total leaf growth, leaf elongation rate and drying degree of the second leaf, in seedlings under water stress induced by gradual drying of the substrate.

MATERIAL AND METHODS

Following these suggestions, we investigated differences in leaf elongation rate of seedlings exposed to water stress by gradual drying in a set of 11 genotypes of winter wheat, adapted to regions that are contrasting for water availability (Table 1).

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Table 1. The wheat cultivars included in the study

Nr.	Cultivar	Origin	
1	Izvor	Romania	Good performance under drought (Mustățea et al., 2009) High osmotic adjustment ability (Bănică et al., 2008)
2	Retezat	Romania	Derivative of Izvor. Good results under drought
3	Otilia	Romania	Good results under drought
4	Glosa	Romania	Good results under drought (Mustățea et al., 2009)
5	Pitar	Romania	New line not related with Izvor
6	Fundulea 29	Romania	Older cultivar, with modest results under drought (Săulescu et al., 2006)
7	TX86A8072	USA	Good results under drought (Lazar et al., 1997)
8	TX86A5600	USA	Near isogenic with TX86A8072 with poorer results under drought (Lazar et al., 1997)
9	Gerek	Turkey	Known as drought resistant in Anatolia
10	Apache	France	Grown in regions with better water availability

The seeds were left for imbibition in distilled water at 1°C. After 2 days, the seeds were transferred to a substrate of H2 perlite in hydroponic pots (500 cc). This volume of the substrate was established after several attempts (data not shown), as providing the best compromise between the need of a gradual drying that can allow plant adaptation to water stress and the need for a short duration of the test. Three seeds were planted in each pot, in order to have 3 uniform plants per pot, after thinning (Figure 1).

Pots were placed in a growth chamber, with the daily photoperiod set between 8.00 a.m. and 4.00 p.m. The temperature was set at 15°C for the dark phase and 20°C for the light phase. We added an equal volume of distilled water (200 ml) and nutrient solution (1 ml) for each pot.

Gradual stress was induced by the water evaporation and the pots were arranged to an equal distance from each other in order to ensure uniform evaporation conditions. Pots randomization was performed every two days throughout the study. Dynamics measurements started from the fourth day of the emergence and were determined at intervals of 48 hours for the cumulative leaf growth, leaf elongation rate and drying degree of the second leaf.

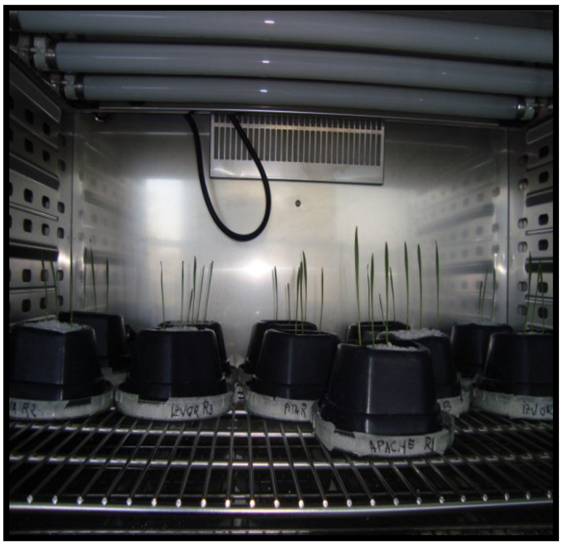


Figure 1. Seedlings in the growth chamber, in the 6th day of the study

Figure 2. Comparative reaction to water stress of two contrasting varieties: Izvor (drought resistant) and Apache (drought susceptible)

RESULTS

Cumulative leaf growth of each cultivar reached a plateau sooner or later, according to the effect of water stress on leaf elongation. Figures 2 and 3 illustrates the differential response of four contrasting cultivars to the gradual drying of the substrate. The French cultivar Apache already stopped growing after 12 days, while cultivar Izvor continued growing even after 20 days. In the first days, the cultivar Fundulea 29 had higher leaf elongation rate than Izvor, but towards the end of the experiment, as the stress was increasingly stronger, leaf elongation rate of Fundulea 29 was noticeably reduced in comparison with Izvor.

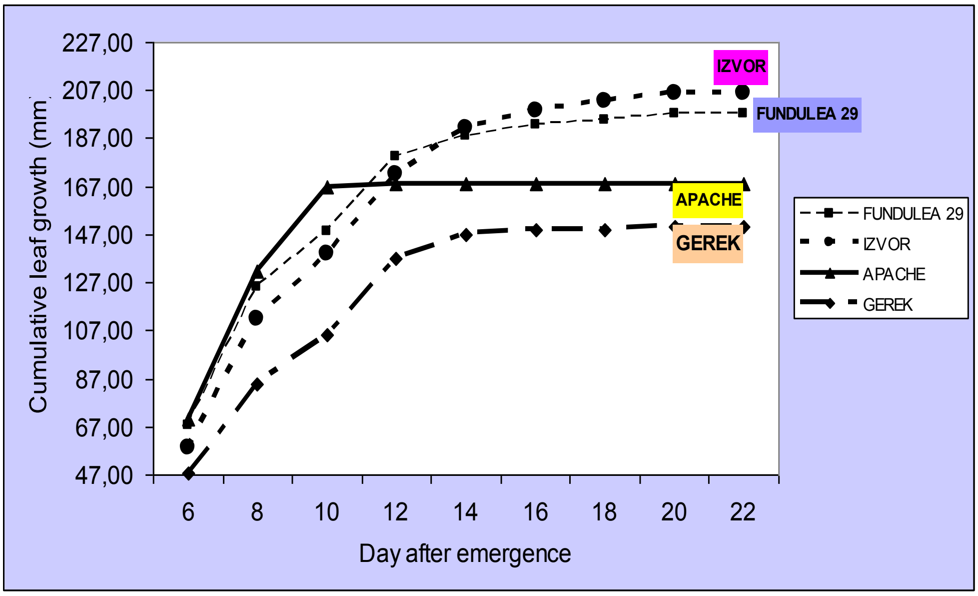


Figure 3. Cumulative leaf growth in 4 cultivars under gradual drying of the substrate

The evolution of leaf elongation with increasing water stress, suggests that best differentiation of cultivar behaviour under stress can be obtained at days 16 or 18, when the most susceptible cultivar stopped elongation and one can still see differences among other cultivars (Figure 4).

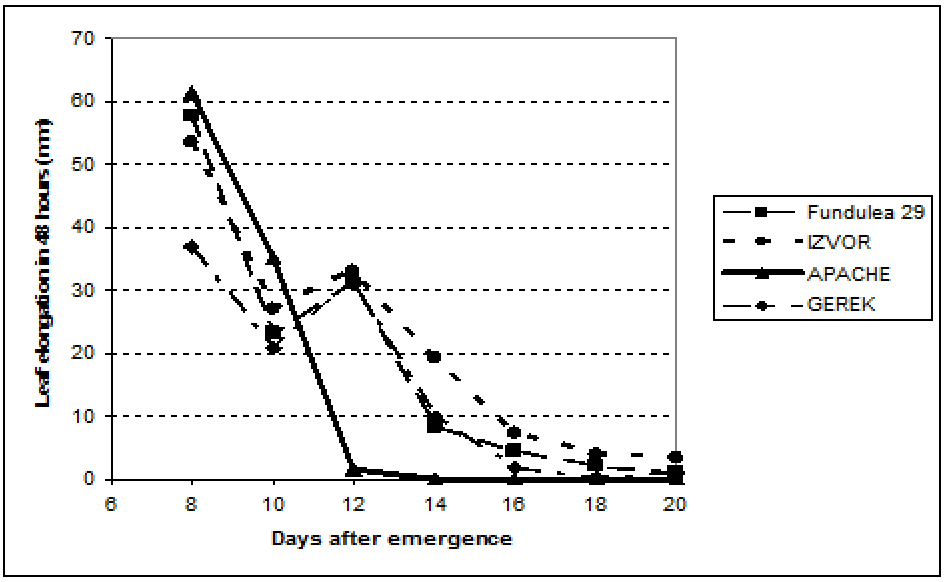


Figure 4. Leaf elongation during 48 hours in 4 cultivars under gradual drying of the substrate

Table 2 presents a classification of the studied cultivars according to leaf elongation estimated at day 18. Highest elongation was found in cultivar Izvor, characterized as having high osmotic adjustment ability (Bănică et al., 2008), and in one of its progenies (Retezat), as well as in the cultivar Otilia, with good performance under drought, in which leaf elongation after 18 days of gradually increasing water stress represented about 92% from the initial leaf elongation at day 8, when water stress effects were not manifested. In contrast, the smallest leaf elongation was found in the French cultivar Apache (with 100% reduction), but also in the Turkish cultivar Gerek, described as drought resistant (with 99% reduction). Obviously, the cultivar Gerek adopts a different strategy for saving water, having a reduced growth even in the absence of water stress and under mild stress. Except under very severe stress, this strategy can be considered less favourable for obtaining better yields.

Table 2. Leaf elongation during 48 hours in several wheat cultivars after 18 days of water stress induced by gradual drying of the substrate

Classi-fication	Cultivar	Leaf elongation at day 18	
		mm	% reduction from initial elongation (at day 8)
1	Retezat	7.95	92.0
2	Otilia	7.72	92.3
3	Izvor	7.14	92.9
4	TX86A80	6.06	93.9
5	TX86A56	5.14	94.9
6	Glosa	4.83	95.2
7	Pitar	4.02	96.0
8	Fundulea 29	3.76	96.2
9	Gerek	0.90	99.1
10	Apache	0	100.0

After 14 days of water stress, we observed drying of the second leaf, which progressed rapidly and significantly differentiated the studied cultivars. Figure 5 illustrates the evolution of the estimated dried area of the second leaf, expressed as percentage of the total leaf area. As seen in the graph, Apache and Fundulea 29 had a larger percentage of dried leaf area than Izvor or Gerek. Differences between cultivars were best expressed in the 20th day of the experiment.

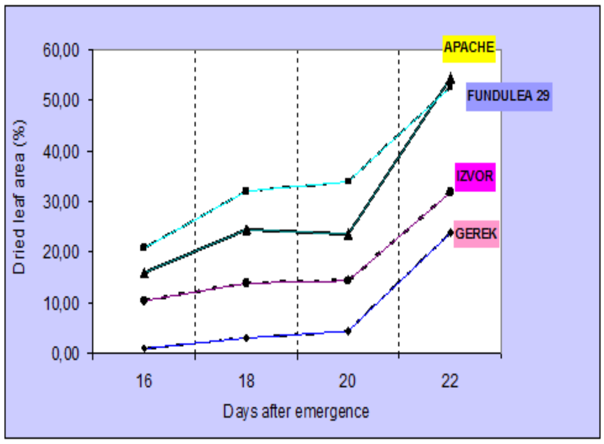


Figure 5. Evolution of second leaf drying in 4 cultivars under gradual drying of the substrate

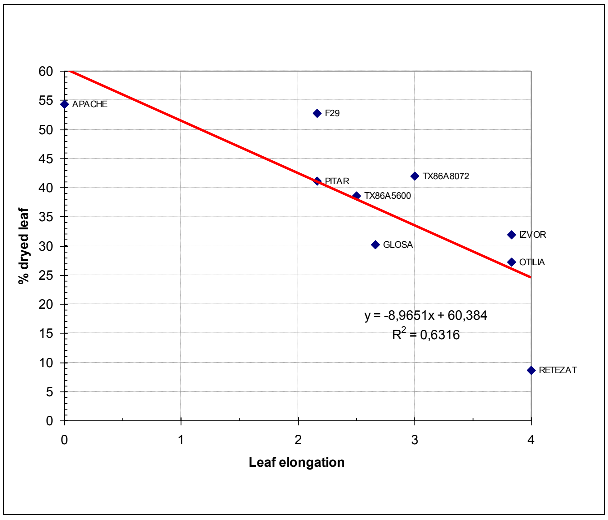
The behaviour of Gerek is interesting, as it shows that the slower growth of this cultivar was efficient in saving water, which was available for slowing down leaf drying.

Table 3 presents the percentage of dried area in the second leaf for all 10 studied cultivars, after 20 days since the beginning of the experiment. Apache and Fundulea 29 had more than half of the second leaf dried, while Retezat had less than 10%, and Gerek and Otilia less than 30%.

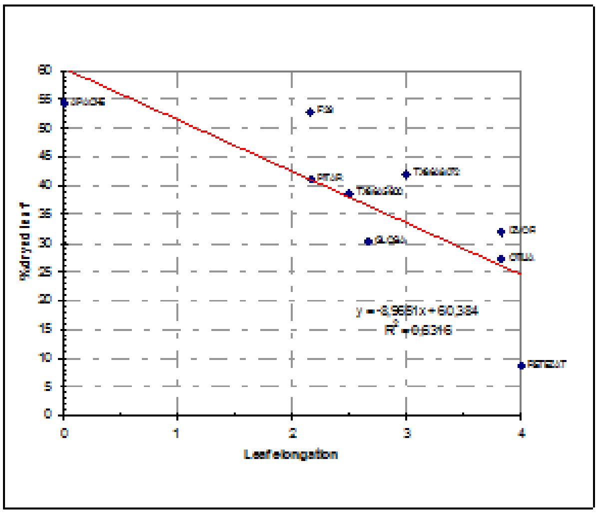
Table 3. Percentage of the second leaf area dried after 20 days since the beginning of the experiment

Classification	Cultivar	% dryed area of the second leaf
1	Retezat	8.66
2	Gerek	23.97
3	Otilia	27.21
4	Glosa	30.23
5	Izvor	31.92
6	TX86A5606	38.62
7	Pitar	41.09
8	TX86A8072	42.01
9	Fundulea 29	52.70
10	Apache	54.36

We can consider that the two parameters that can be observed by growing seedlings under gradual drying of the substrate are both useful for characterization of genotype responses to water stress. Figure 6A shows that, when all studied cultivars were taken into account, the two parameters were not significantly correlated, but when the cultivar Gerek (Figure 6B) was excluded the correlation was highly significant.



A)



B)

Figure 6. Relationship between leaf elongation after 18 days and leaf drying after 20 days of water stress (A - for all 10 studied cultivars; B - excluding the cultivar Gerek)

DISCUSSIONS

Drought resistance is a very complex phenomenon, involving many various mechanisms, acting at different phenophases. Therefore, one cannot expect that a single test, particularly a seedling test, could completely characterise crop behaviour under drought.

On the other hand, seedling tests, of relatively short duration, which can be performed during winter under controlled conditions, therefore, providing early information for preliminary selection, might have an important contribution to increasing genetic progress in breeding for superior performance under drought.

Morgan (1988) suggested that coleoptile responses to water stress might be used to differentiate wheat genotypes for osmoregulation, and it is known that osmoregulation is a property of all cells, therefore manifested all during the vegetation period.

Our results suggest that leaf elongation and drying of first leaves under water stress induced by gradual drying of substrate can also provide useful information about genotypic differences in response to drought. Only a small difference in leaf elongation was found between the near-isogenic lines Tx86A8072 and Tx86A5600. Apparently, the difference between the performance of these lines was due to traits not expressed at seedling stage. Balotă et al. (2007) found that these lines were different in Canopy Temperature Depression.

The behaviour of the Turkish cultivar Gerek suggests that leaf elongation under stress alone can be misleading, if cultivars with different strategies of coping with water stress are compared. Leaf elongation after a long exposure to stress should be considered together with the leaf elongation before stress and with leaf drying. Obviously, this method cannot describe all aspects of drought resistance in wheat, but it can help distinguish ways of adaptation to water stress. Very diverse genetic materials, which differ considerably in plant and leaf size, root traits, or other plant traits that determine the rate of cell elongation, should not be directly compared using leaf elongation alone. However, such comparison can prove very useful when dealing with materials from a breeding program, which are more or less related and use similar strategies for water stress resistance, such as osmotic adjustment.

CONCLUSIONS

We found large differences between the studied cultivars in both leaf elongation and drying of the second leaf, under water stress induced by gradual drying. These differences were largely in agreement with the available information about the behaviour of the cultivars under drought in the field.

Therefore, we suggest that leaf elongation and leaf drying under after water stress induced by gradual drying of the substrate in a seedling test can be used for preliminary characterization and selection of genotypes in breeding for drought resistance.

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