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Relative water content (RWC) and leaf senescence as screening tools for drought tolerance in wheat

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SUMMARY – An experimental study has been conducted in order to analyse the response to water stress of one durum wheat variety and one bread wheat variety. The drought resistance of the cultivars was measured as the difference in their performances under two water regimes. Indicators such as relative water content (RWC) and leaf senescence were used. The RWC follow up study showed that the durum wheat, in situations of water stress, loses much more water than the bread wheat, whereas in the maximal evapotranspiration (MET) situation there are no differences. In the MET condition, the leaf life duration is the same for the two varieties. However, it is more accelerated in durum wheat than in the bread wheat in a situation of water stress.

Key words: Water stress, drought, wheat, RWC, leaf senescence.

***RÉSUMÉ –** "Teneur relative en eau et sénescence foliaire comme instruments de sélection pour la tolérance à la sécheresse chez le blé". Une étude expérimentale a été conduite dans le but d'analyser la réponse au stress hydrique chez une variété de blé dur et une variété de blé tendre. La résistance des deux cultivars a été mesurée comme étant la différence de leurs performances selon deux régimes hydriques. Des indicateurs comme la teneur relative en eau (RWC) et la sénescence de la dernière feuille ont été utilisés. L'étude de l'évolution de la teneur relative en eau (RWC) a montré que le blé dur en situation de stress hydrique perd beaucoup plus d'eau que le blé tendre. Par contre, il n'y a pas de différence en situation d'évapotranspiration maximale (ETM). En absence de la contrainte hydrique, la durée de vie de la dernière feuille est la même pour les deux variétés. Par contre, elle est beaucoup plus accélérée chez le blé dur en présence de stress hydrique.*

***Mots-clés :** Stress hydrique, sécheresse, blé, RWC, sénescence foliaire.*

Introduction

Water is among the most widespread abiotic stresses limiting wheat distribution and productivity in the Mediterranean environment (Mastrangelo *et al.,* 2000). Although drought tolerance is considered as a valid breeding target in the stabilization of crop performance, by breeders and molecular biologists, at the moment there is a lack of information to be able to measure with precision the plant resistance under drought stress conditions (Blum, 1996). Plant response to drought can be studied by identification of traits that are related to drought tolerance at the physiological, cellular, biochemical and molecular levels. Hence, the study of the diversity of drought tolerance mechanisms can give interesting information on the different possibilities of adaptation. Developing plants that have an advantage under abiotic stress conditions is a major challenge for wheat improvement programmes. Genotypes possessing the ability to maintain green leaf area duration "stay green" and high relative water content traits throughout grain filling are potential candidates to assure yield in semi-arid regions.

In the present work the mechanisms involved in the response to drought were investigated in two wheat cultivars with contrasting drought tolerance capacities, in order to study the relationship between the physiological basis of drought response and plant stress tolerance.

Materials and methods

The experimental study was carried out at the experimental farm Boucheraine Mohamed in Bouira (Algeria) on an silty-clay soil. A criss-cross experimental design was used with four replicates and two studied factors (variety and irrigation). The variety factor was represented by one durum wheat variety (Vitron) from Spain and one bread wheat variety (HD1220) from Mexico. The agronomical practices were the usual ones in the area. The precipitation was low and not well distributed throughout the growing season. February, March, May and June were dry and temperatures were above the long-term averages. The deficit of precipitations is about 94.3 mm accumulated between February and June.

The irrigation factor was represented with two water treatments: (i) T1, plants were exposed to drought from the beginning of the stem elongation stage; and (ii) T2, treated at MET (maximal evapotranspiration) during the whole crop cycle.

To evaluate the water status during the stress period, relative water content RWC was used, determined according to Barrs and Weatherly (1962). Cut leaves were weighed (fresh weight, FW), then left saturated in water for three hours and their turgid weights (TW) were calculated. The samples were then dried in an oven at 80 °C for 24 hours and weighed (OW). The RWC is determined as follows: RWC = (FW – DW)/(TW – DW) x 100. The flag leaf senescence was noted visually. The date when 75% of the flag leaves had senesced and turned yellow was estimate visually and the number of days between heading and 75% senescence was determined.

Results and discussion

Water deficit has exerted a negative effect on RWC, thus in the presence of water stress (T1), the two varieties lose much more water than under non-limited water conditions (T2). This result confirms previous works on durum wheat and bread wheat (Mekliche *et al.*, 1992), showing the effect of water stress on RWC in wheat plants.

The ability of the plant to survive severe water deficits depends on its ability to restrict water loss through the leaf epidermis after the stomata have attained minimum aperture (El Jaafari, 2000). In fact, several workers have reported the existence of a significant positive correlation between yield and flag water retention in durum wheat. The RWC follow up (Table 1) shows that the durum wheat represented by the variety Vitron maintained under water deficit regime (T1) loses much more water than the bread wheat represented by the variety HD1220.

Table 1. Relative water content (RWC) (%) of the two cultivars under stressed conditions (T1) and unstressed conditions (T2). Greeen surface in (%) of the whole leaf surface

Varieties	Days after anthesis							
	4		8		12		18	
	T1	T2	T1	T2	T1	T2	T1	T2
Vitron	78.52	86.30	65.63	83.90	60.83	82.27	51.31	76.93
HD1220	83.09	86.67	71.17	74.24	66.31	71.43	56.66	67.01

However, in the unstressed conditions (T2), there are no appreciable differences between the two species. Our results confirm those obtained by Gate *et al.* (1992) and Mekliche *et al.* (1992).

Leaf senescence comprises a series of biochemical and physiological events which include the final stage of development, from the fully expanded state until death. During leaf senescence, the photosynthetic apparatus is dismantled and nutrients are exported to young tissues or storage organs. Genetic variation exists for foliar senescence and genotypes and plants with leaves which remain green for longer than normal are defined stay-green (Di Fonzo *et al*., 2000). Over fifty years ago it was realised that the diversity in yield for most crops is mainly a consequence of variation in the duration, rather than the rate of photosynthetic activity (Watson, 1952), and so, delayed leaf senescence (i.e., stay-green) has long been considered to be a desirable trait in cereal breeding. So, the ability to maintain green leaf area duration during the grain filling is one of the important physiological traits that have an implication on yield potential related to increasing assimilate (i.e. Source) availability. The flag leaf senescence (Table 2), provides complementary information. In fact, the leaf duration after anthesis depends strongly on the water conditions. In presence of water deficit (T1), the two varieties have a much shorter flag leaf duration than under non-limited water conditions (T2) and begin to show from day 18 after anthesis.

Table 2. Flag leaf senescence of the two cultivars under stressed conditions (T1) and unstressed conditions (T2). Green surface in (%) of the whole leaf surface

Varieties	Days after anthesis									
	4		8		12		18		20	
	T1	T2	T1	T2	T1	T2	T1	T2	T1	T2
Vitron	92	96	74	81	45	74	14	60	00	43
HD1220	94	95	77	79	50	71	20	43	04	57

We also remark that the leaf senescence also depends on the wheat species. Thus, under water stress, the flag leaf senescence in the durum wheat variety Vitron occurs much sooner than in bread wheat variety HD1220. Similar results are reported by others (Mekliche *et al.*, 1992; Gate *et al.*, 1992).

This behaviour explains in part the decrease in the 1000 grain weight of the studied varieties (much more pronounced in the durum wheat variety). This is by a decrease in grain filling duration due to the acceleration of the flag leaf senescence (Larbi *et al.*, 2000). Similar observations have been reported by Day and Intalap (1970), Gate *et al.* (1992) and Debaeke *et al.* (1996).

Conclusion

These preliminary experiments gave us a great deal of information on what happens when plants are subjected to water stress during the post-anthesis period, and clearly show the difference in behaviour towards water deficit between the two species represented by the varieties Vitron and HD1220. The variety HD 1220 appears more tolerant to water stress during the grain filling period by the ability to maintain green leaf area duration and a high relative water content in limited water conditions. This confirms the results of the numerous previous studies conducted in rainfed and irrigated situations between the durum wheat and the bread wheat varieties (Deumier, 1987; Gate *et al.*, 1992; Mekliche *et al.*, 1992). This is due probably to the osmotic adjustment by accumulation of solutes such as sugars, or by a good regulation of the stomatal conductance.

Studying the RWC and the leaf senescence used as an indirect selection criteria for grain yield (known as "analytical" breeding) is an interesting alternative approach because those traits are easily and rapidly screened, and relatively inexpensive. So we can gain a partial understanding of many physiological mechanisms that confer drought tolerance and lead to the development of wheat better adapted to such environments.

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