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Breeding for drought stress tolerance in durum wheat using two physiological parameters

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Abstract - *Preliminary experiments using four durum wheat cultivars (Mohamed Ben Bachir, Oued Zenati, Waha, Ofanto) were carried out in order to study two physiological parameters involved during water deficit. The relative water content (RWC) and proline accumulation were used as selection criteria for drought tolerance and determined when plants (coleoptyles) are subjected to 10 and 24 hours water stress. Preliminary results are given in this paper. The evolution in proline accumulation and water loss within each cultivar are considered. Complementary analysis using other morphological parameters should be added in order to give a better understanding to the mechanisms by which plants resist to water deficit.*

durum wheat / drought stress / physiological parameters / R.W.C / proline content

Résumé - *Un essai préliminaire a été réalisé dans le but d'étudier certains paramètres physiologiques liés à la sécheresse. Quatre variétés de blé dur (Mohamed Ben Bachir, Oued Zenati, Waha, Ofanto) ont été utilisées. La teneur relative en eau (TRE) et l'accumulation de proline ont été déterminées quand les plantes (coléoptiles) sont sujettes à 10 et 24 heures de déficit hydriqué (expérience menée au laboratoire dans des conditions contrôlées). Des résultats préliminaires ont été obtenus quant au comportement de chacune des variétés testées face à ce stress.*

blé dur / stress hydrique / paramètres physiologiques / TRE/ teneur en proline

INTRODUCTION

One potentially important mechanism of drought resistance is osmotic adjustment, the accumulation of solutes in plant tissue in response to dehydratation (Turner and Jones, 1980 in Johnson et al., 1984). This mechanism results in the maintenance of turgor pressure to a lower water potentialthan possible in unadjusted plants (Johnson et al, 1984). Tissue turgor pressure has a considerable influence on plant water relations because it controls the diurnal variations of tissue total potential and turgor depending processes such as foliar expansion, stomatal opening, plant transpiration and several enzymatic reactions (Rascio et al.1987).

The plant capability to keep a positive or constant turgor when water potential decrease is an important adaptative feature to water stress (Turner, 1979 in Rascio et al. 1987). R.W.C is frequently proposed as a criterion in selection for drought tolerance. It is influenced by osmotic adjustment and by water absorption and transpiration.

For many authors, tolerant cultivars manifest a higher R.W.C during osmotic stress and consider this parameter as one of the best stress indicator (Al Hakimi et al. 1995). Other physiological parameters can be used to determine the plant water status like: osmotic potential, water potential, leaf tensile strength and membrane integrity.

At biochemical level, the increase in the solute content of cells leading to maintenance of turgor is an adaptative response involved in osmotic adjustment. Sugar, organic-acid and amino-acid are the solutes accumulated during water stress.

Proline is the most common amino-acid thought to play a cardinal role as solute osmoregulator not only in plants but also in eubacteria, protozoa, marine invertebrates and algae (Ashton et al, 1995). The accumulation of this solute seems to be one of the most immediate response to water stress (Aspinall and Paleg, 1981 in Sundaresan and Sudhakaran, 1995) , its level can increase as much as 100 fold (Sundaresan and Sudhakaran, 1995).

As result of general accumulation during several stresses such as low temperature, nutrient deficiency, exposure to heavy metals and high acidity this amino-acid may be considered as a part of general adaptation to adverse environmental conditions (Bohnert et al. 1995).

In our experiment, relative water content (R.W.C) and proline accumulation were used as a preliminary step in the analysis of the effect of 10 and 24 hours water stress in our durum wheat cultivars. The experiments were carried out in the laboratory research of the experimental station I.S.C (Istituto Sperimentale per la Cerealicoltura, Foggia, Italy).

MATERIAL AND METHODS

Plant material and growth conditions

Four durum wheat (*Triticum durum Desf.*) cultivars were used in these present preliminary experiments. Two Algerian cultivars, Mohamed Ben Bachir and Oued Zenati are known to be drought tolerant, the cultivar Waha called also Shaml is from ICARDA (Syria) and considered as high yielding variety in Algeria (where it is cultivated for many years). The fourth cultivar Ofanto is from Italy caracterised by a good quality, a high productivity and drought tolerance.

Seedlings were grown in petri dishes on moist filter paper to the first leaf stage (coleoptyle) and then subjected to water stress by uncovering the dishes letting the air desiccate them for 10h and 24h, unstressed seedlings were kept moist as controls . The samples were harvested and used for relative water content (R.W.C) and proline analysis.

R.W.C method

Stress was applied at coleoptyle stage. The relative water content was determined according to Barrs and Weatherley (1962), method usually used in this laboratory. Cut leaves were weighted (Fresh Weight, F.W), then left in a water saturated for three hours and their turgid weights (T.W.) were calculated. The samples were then dried in an oven at 80°C for 24hours and weighted (D.W.). The relative water content (R.W.C) is determined as follows:
R.W.C = F.W - D.W / T.W - D.W x 100

Proline quantification method

Dried leaves were ground in liquid Nitrogen in a mortar then left to dry at 80°C for 4hours. At 50mg of dried sample were added 10ml of Sulfosalicilic acid (3% W/V). The mixture was vortexed for one hour and shaked every 10mn. The sample solutions were filtrated in funnels with Wathman paper GF/A. 0.5ml of stressed sample and 1ml of controlled sample were used for the proline assay. The method (Bates, 1976 in Rascio et al. 1987) applied in this experiment was as follows:

From proline stock of 10mg/l, six different dilutions were made as standard assay (0.2ml, 0.6ml, 1.2ml, 1.8ml, 2.4ml). In each sample (Standard and sample solution) were added 2ml of ninidrine solution (ninidrine, phosphoric acid 6M, glacial acetic acid) and 2ml of glacial acetic acid. Samples were put in Bain Marie at 100°C for 45mn then transfered in ice. 4ml of toluene were added in each one and mixed. When the two phases are well separated, lectures were made in the spectrophotometer OD520. The quantities of proline were then identified by comparison with standard curve values.

RESULTS AND DISCUSSION

It has been reported, that low levels of proline are found among cereal species in absence of stress (Hanson, 1980). We can notice from the table I that the means of the three replications made for proline content in unstressed samples are not very variable from a variety to the other. Its amounts in absence of stress are situated between approximatively 6 and 11.5 for both 10h and 24h controls.

Proline accumulation is known to increase when plants are subjected to stresses (Hanson, 1980; Irigoyen et al. 1992, Ashton et al. 1993; Sundaresan and Sudhakaran, 1995). This appear on the table when samples are under stress for more than 10h. The value obtained on 24h stress confirm this remark in which a high accumulation of proline is observed.

We notice also that under 10h stress the cultivar Oued Zenati present the higher value but the increase of proline accumulation when the stress is prolonged is not very important (at 10h and 24h stress, the accumulation is respectively 37.93 and 54.32).

The cultivar Ofanto manifest a low value at 10h stress (13.16), the proline accumulation increase for more than 5 times when the stress is prolonged to 24h (72.14). In this experiment, this variety is the one in which the higher accumulation has been obtained.

For RWC, we can notice that approximatively more than 60% of water are maintained for all the cultivars when samples are under 10h stress. The cultivar Mohamed Ben Bachir under 24h stress show a value corresponding to 48.3 , this means that the samples have lost less than 50% of water. This result is noticed for only this cultivar. With the cultivar Ofanto lost of water have been important, the lower value is obtained comparatively to the other (29.32) at 24h stress.

By correlating the two parameters proline and RWC, we confirm that the higher is the decrease of water when plants are under stress (24h stress in our case), higher is the proline accumulation (Al Hakimi et al. 1995) . The cultivar Ofanto is in our experiment a good example in which the higher value of proline and the lower one for RWC are obtained.

Comparing the table II to the precedent one and by analysing the evolution in proline content and relative water content between the samples subjected to water stress and the ones unstressed, we can observe that the cultivar Oued Zenati is isolated from the other by a high significative difference for both proline and RWC quantifications under 10h stress. Parallely, the cultivar Ofanto, under 24h stress show the higher values for the two parameters studied. The cultivar Waha present approximatively similar results comparing to Ofanto, particulary for the RWC

Table I. Proline and RWC means of the four cultivars under stressed (10h and 24h) and unstressed conditions.

Param.	*Proline accumulation (nM/gDW)*				*Relative water content (RWC) (%)*			
Treat.	*Control*		*Stress*		*Control*		*Stress*	
S/treat.	10h	24h	10h	24h	10h	24h	10h	24h
O.Zenati	9.58	9.66	37.93	54.32	91.95	92.90	54.97	44.26
M.B.Ba.	7.50	10.51	10.29	52.30	94.42	97.28	77.50	48.30
Waha	11.64	11.65	13.98	61.77	78.29	94.30	61.89	36.10
Ofanto	5.96	6.75	13.16	72.14	95.00	95.60	69.12	29.32

Table II. Mean values for the variations of proline content and RWC between stressed and unstressed samples

Param.	*Proline accumulation (nM/gDW)*				*RWC (%)*			
Treat.	*10h*		*24h*		*10h*		*24h*	
cv. O.Zenati	28.350	a	44.660	c	36.97	e	48.64	g
cv. M.B.Bachir	2.915	b	41.610	c	15.03	f	48.98	g
cv. Waha	2.340	b	50.120	c	22.02	f	58.20	h
cv. Ofanto	7.20	b	65.460	d	25.86	f	66.34	h

Means with equal letters do not differ significantly at P= 5% (Bonferroni pairwise comparison).

Depending upon these preliminary results and in conclusion to numerous studies (Jones and Turner, 1978; Al Dakheel, 1989; Henson et al, 1982; Johnson et al, 1984; Al Hakimi et al, 1995), we will have a better understanding if we take in consideration other components which contribute to the response of the plants to water stress.

Both proline and RWC are influenced by osmotic adjustment. This mechanism whereby plants maintain positive turgor has been recognised to be an important adaptative response to water stress. Al Dakheel (1989) reported numerous techniques of osmotic adjustment measurements estimated by the determination of water and osmotic potentials of the plants in complementarity to the proline and RWC quantifications.

CONCLUSION

These preliminary experiments gave us many informations about what happen when plants are subjected to water deficit. By determining the R.W.C and proline content used as selection criteria, we can partially have an understanding of many physiological mechanisms that impart drought tolerance and lead to the development of wheat better adapted to our cereal regions. Repeated experiments and elaborated studies including other physiological parameters (osmotic and water potentials, leaf tensile strength, membrane integrity...) may be developed to identify drought adaptation mechanisms of our local cultivars. The mechanisms deduced should be completed by molecular and genetic analysis of stress tolerance.

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