Bound Water in Durum Wheat under Drought Stress[1]

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ABSTRACT

To study drought stress effects on bound water, adsorption isotherms and pressure-volume curves were constructed for two durum wheat (*Triticum durum* Desf.) cultivars: Capeiti 8 (drought tolerant) and Creso (drought sensitive). Plants were grown under well-watered and water-stressed conditions in a controlled environment. Differential enthalpy (ΔH) was calculated through van't Hoff analysis of adsorption isotherms at 5 and 20°C, which allowed us to determine the strength of water binding. ΔH reached the most negative values at approximately 0.06 gram H_2O/gram dry weight and then increased rapidly for well-watered plants (until 0.10 gram H_2O/gram dry weight) or more slowly for drought-stressed plants (until 0.15–0.20 gram H_2O/gram dry weight). Bound water values from pressure-volume curves were greater for water-stressed (0.17 gram H_2O/gram dry weight) than for well-watered plants (0.09 gram H_2O/gram dry weight). They may be estimates of leaf moisture content where ΔH reaches the less negative values and hence some free water appears. With respect to the well-watered plants, tightly bound water tended to be less bound during drought, and more free water was observed in cv Creso compared to cv Capeiti 8 at moisture contents >0.10 gram H_2O/gram dry weight.

Several studies have shown that in leaves of plants exposed to water stress the increase in osmotic pressure is associated with a decrease in T/D^2 (8, 11, 16, 21). Changes in water content and accumulation of osmotically active and inactive dry matter would lead to a reduction in T/D ratio (16). In durum wheat, the variations in the ratio were accompanied by accumulation of fibrous constituents such as hemicellulose (12). This substance is present as a polymeric gel and has important properties such as ion binding and water-holding capacity. The accumulation of cell wall material may affect the distribution of apoplastic (BW) and symplastic water and may be responsible for some decrease in the osmotic potential values (17).

Among the large number of methods used to determine BW, the PV curve analysis is the most widely accepted. The disadvantages of this technique have been pointed out (7, 15), but a recent comparison of three different techniques of PV curve construction resulted in similar BW estimates (14).

Studies of the thermodynamics of hydration, through the use of water adsorption isotherms on proteins and vegetal tissues, have shown at least three types of BW. According to the strength and nature of water-binding sites, water may be very tightly, weakly, and very weakly bound to the polymer surfaces (3).

Construction of water adsorption isotherms allows one to determine the strength of water binding and the leaf moisture contents corresponding to the different levels of water affinity (18).

In this work, the water adsorption isotherms and the water relations were analyzed in well-watered and water-stressed durum wheat plants, comparing leaf tissues of intolerant (Creso) and tolerant (Capeiti 8) cultivars to water stress (5). Moreover, the BW values obtained from PV curve analysis and the levels of leaf tissue water affinity were compared.

MATERIALS AND METHODS

Plant Materials

Two durum wheat (*Triticum durum* Desf.) cultivars, Creso and Capeiti 8, were grown in a growth chamber under 250-W high-pressure sodium lamps (Philips) and 400-W high-pressure metal halide lamps (Philips); radiation measurements at the pot surface were 1000 μmol photons m^{-2} s^{-1} (400–700 nm) PAR. After vernalization at 4°C for 1 week, six seeds per pot were sown in 2.25-L plastic pots that contained 1000 g of medium-textured soil, 333 g of sand, and 100 g of peat. When the seeds were sown, 20 g of ammonium nitrate fertilizer was applied to each pot, and at tillering a mix of mineral superphosphate (1.2 g/pot), ammonium nitrate (2.0 g/pot), and potassium sulfate (0.1 g/pot) was applied.

The maximum water capacity of the soil was 57% of soil dry weight, and the permanent wilting point was 12%.

A completely randomized design with two replications and two treatments (water stressed and well watered) was adopted. Every 3 d, the pots were brought to about 100% of soil capacity until the booting stage. Then, for half of the pots, irrigation water was reduced to about 35% above the permanent wilting point. The remaining pots were kept well watered as controls. The temperature, light, and RH cycles in the growth chamber are shown in Table I.

Water Relations

To evaluate the plant water status during the water stress period, the youngest fully expanded leaves were selected at random from one plant of each pot and measurements of leaf Ψ with a pressure chamber (PMS Instruments Co., Corvallis, OR) were obtained. After 15 d of stress (at the flowering

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[1] This paper is dedicated to the memory of Prof. G. Wittmer, director of the Experimental Institute for Cereal Research, Section of Foggia, who died in the summer of 1990.

[2] Abbreviations: T/D, turgid weight to dry weight ratio; BW, bound water; PV, pressure-volume; Ψ, water potential; Ψ_π^{100}, osmotic potential at full turgor; ΔH, enthalpy of water binding; ΔH int, integrated enthalpy.

Table I. *Temperature, Light, and RH Cycles in the Growth Chamber*

Week	Growth Stage	Temperature		Daylight Duration	RH
		Day	Night		
		°C		h	%
I		6	6		70
II	Germination	8	6	10	70
III	Leaflet	12	8	15	55
IV	Third leaf stage	15	10	15	50
V	Tillering	20	10	15	45
VI	Booting[a]	23	18	16	40

[a] The last cycle lasted about 1 month.

stage), three flag leaves from each cultivar and treatment were removed to construct the PV curves following the method previously described (11). For each sample, reciprocals of Ψ ($1/\Psi$) were plotted against water saturation deficit (Fig. 1). BW was estimated by extrapolating a regression fitted to the linear portion of the PV curves to the abscissa, and the BW values were expressed as g H_2O/g dry weight (1). To obtain the values for osmotic potential at full turgor, the method of Wilson *et al.* (21) was used.

Adsorption Isotherms

To construct isotherms, flag leaves were collected, cut in small pieces, and preincubated for 24 h in a drier containing P_2O_5 to standardize the tissue water content. The samples (about 0.5 g) were then put in driers over saturated salt solutions and incubated at 5 and 20°C for 3 d until a constant weight was achieved. To check whether the material size could affect the adsorption characteristics, adsorption isotherms were constructed for whole, ground, and cut leaves in parallel tests, and no significant differences were found among them. Desorption isotherms were also constructed following the same method but avoiding preincubation under P_2O_5, and significant differences between adsorption and desorption curves were not observed. The RH values of the driers were derived from Greenspan (6). The amount of water taken up by the pellets after equilibration was determined gravimetically by heating in an oven at 90°C for 3 d, a time sufficient to achieve a constant dry weight.

The heat of adsorption was calculated according to the Clausius-Clapeyron equation (10, 18) using adsorption isotherm data:

$$\Delta H = R \times \frac{T_1 \times T_2}{T_2 - T_1} \times \ln(aw_1/aw_2)$$

where ΔH is the differential enthalpy (in J/mol water) for a given water content, aw_1 and aw_2 were the relative vapor pressures at the lower (T_1) and higher (T_2) temperatures, respectively. R is the ideal gas constant (8.314 J/mol/°K), and T is temperature expressed in degrees Kelvin.

The integral of ΔH (ΔH int), *i.e.* the total strength of water binding for three ranges of water content (0.04 and 0.05 for Capeiti 8 and Creso, respectively, to 0.10 g H_2O/g dry weight; 0.10 to 0.20 g H_2O/g dry weight; 0.20 to 0.30 g H_2O/g dry weight) was determined indirectly: the points obtained by

plotting ΔH *versus* leaf moisture content were connected by straight segments; the area under the lines was cut out, and its value was determined.

RESULTS

Leaf Water Relations

Rapid and significant decreases in Ψ values of stressed plants were observed 4 d after water reduction, and the minimum leaf Ψ (−3.5 MPa) was observed 10 d later (Fig. 2). Parameters from PV curves showed a great decrease in the osmotic potential values and an increase in BW (Table II) under drought stress. A significant correlation existed between these parameters ($r = 0.69$) and between T/D ratio and osmotic potential values ($r = 0.73$). The measured values of BW were lower than those observed by other authors (16) and by us (unpublished data) in leaves of durum wheat grown in the field.
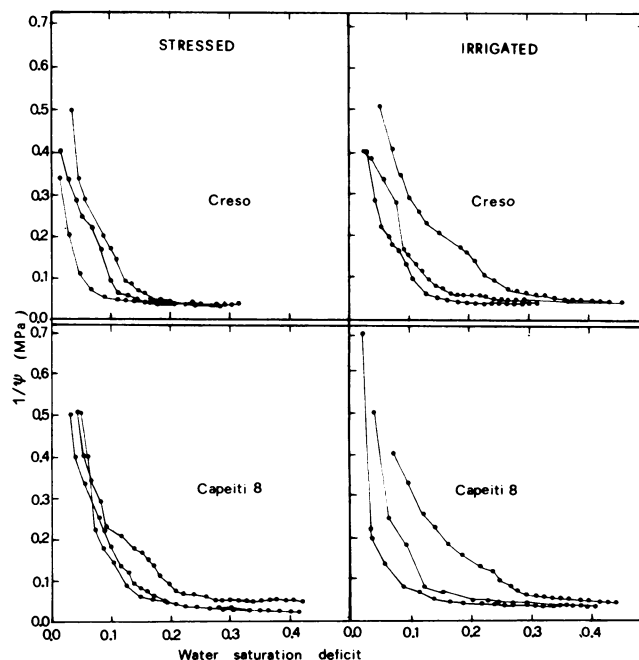

Figure 1. PV curves for leaves of durum wheat under two different water regimens. Each curve represents one replication.
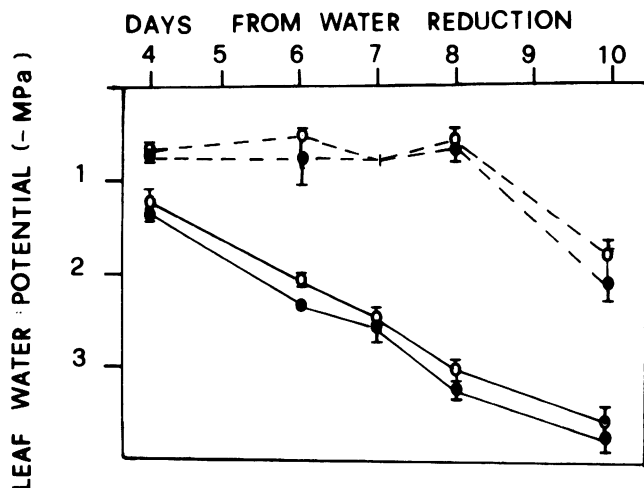
Figure 2. The change with time in leaf Ψ of Creso (O) and Capeiti 8 (●) under well-watered (---) and increasing water stress conditions (——).

Water Adsorption Isotherms

The reverse sigmoidal trend observed in studies of hydration of lysozyme (13), soybean seeds (18), and frond of *Polypodium* species (20) characterized also the adsorption isotherms of durum wheat leaf tissue (Fig. 3). The trend was more evident for curves constructed at 5°C than for those at 20°C and in particular for well-watered Creso leaves. Leaf tissue adsorbed more water at 5°C than at 20°C.

Differential heats of water adsorption (ΔH for a given water content) are shown in Figure 4. ΔH values were always negative: they decreased sharply until 0.06 g H_2O/g dry weight, where the most negative values were observed, and then increased very rapidly (until 0.10 g H_2O/g dry weight) for well-watered and slowly for water-stressed plants (until 0.18 g H_2O/g dry weight). Capeiti 8 showed the most negative values of ΔH (−63 kJ/mol) under drought stress conditions at the lowest moisture content (0.04 g H_2O/g dry weight). For water content lower than about 0.10 g H_2O/g dry weight, the forces of water binding were large and water was presumably chemisorbed.

The water stress effect on the total strength of water binding

was different for the two cultivars. The ratio of the ΔH int for tissue from stressed leaves to that of tissue from well-watered leaves for the same cultivar at the same range of leaf moisture content is shown in Figure 5. At a moisture content <0.10 g H_2O/g dry weight, water-stressed plants lost about 40% of their water affinity; at greater values Creso showed a similar loss of ΔH int, whereas for Capeiti 8 an increase of water affinity up to 100% resulted.

Values of BW inferred from PV curves coincided with the moisture content where ΔH values stopped increasing, i.e. 0.08 to 0.10 g H_2O/g dry weight for well-watered and 0.15 to 0.20 g H_2O/g dry weight for water-stressed plants (Fig. 4).

DISCUSSION

Water relations of durum wheat were altered by a 10-d exposure to a maximum drought stress rate of about 0.35 MPa d^{-1}. The significant correlation between BW and Ψ_π^{100} suggests that a portion of the total water in leaf cells may be nonosmotic. However, the small increase in BW cannot be completely responsible for the large reduction in Ψ_π^{100} values. In fact, to have a reduction in Ψ_π^{100} from −1.6 to −2.4 MPa, about 30% of osmotically active water (−0.9 g H_2O/dry weight) would be redistributed from symplasm to apoplasm under drought stress, producing a very large increase of BW compared to the result showed in Table II.

Durum wheat leaves hydrated in a manner similar to other vegetable tissue (18, 19, 20) and stressed plants adsorbed more water than the controls with the exception of isotherms determined for cv Creso at 5°C.

At moisture contents <0.10 g H_2O/g dry weight, water stress decreased water affinity, but ΔH values for Capeiti 8 increased to a peak at 0.04 g H_2O/g dry weight, and Capeiti 8 had a smaller loss of water affinity compared with Creso. This result is consistent with the finding of Vertucci and Leopold (20), who, comparing different *Polypodium* species, observed a higher capacity for tightly BW in fronds of *Polypodium polypodioides*, a resurrection fern tolerant to severe desiccation.

The loss of water affinity (less negative ΔH values) at very low water content may be due to the absence of components that bind water or to their physical alteration. As this structural water is removed, lowering of (a) seed viability (4), (b) enzyme activity (13), and (c) structural integrity of biomole-

Table II. *PV Curve Parameters from Well-Watered Leaves and Leaves Water Stressed for 15 d of Two Durum Wheat Cultivars*

Significance of F (Fisher's test) values are shown.

Parameters	Cultivar	Treatment		F Values		
		Well watered	Water stressed	Treatment	Cultivar	Interaction
Ψ_π^{100} (MPa)	Creso	1.7	2.4	***a*	NS	NS
	Capeiti 8	1.5	2.4			
BW (g H_2O/g dry wt)	Creso	0.11	0.15	*b*	NS	NS
	Capeiti 8	0.07	0.19			
T/D	Creso	4.4	3.8	NS	NS	NS
	Capeiti 8	4.1	3.9			

[a] P = 0.01.   [b] P = 0.05.

Figure 4. Differential energies of water adsorption of Creso and Capeiti 8 leaves at different moisture contents. Comparison of plant growth under well-watered and water-stressed conditions. Arrows, BW values from PV curves.
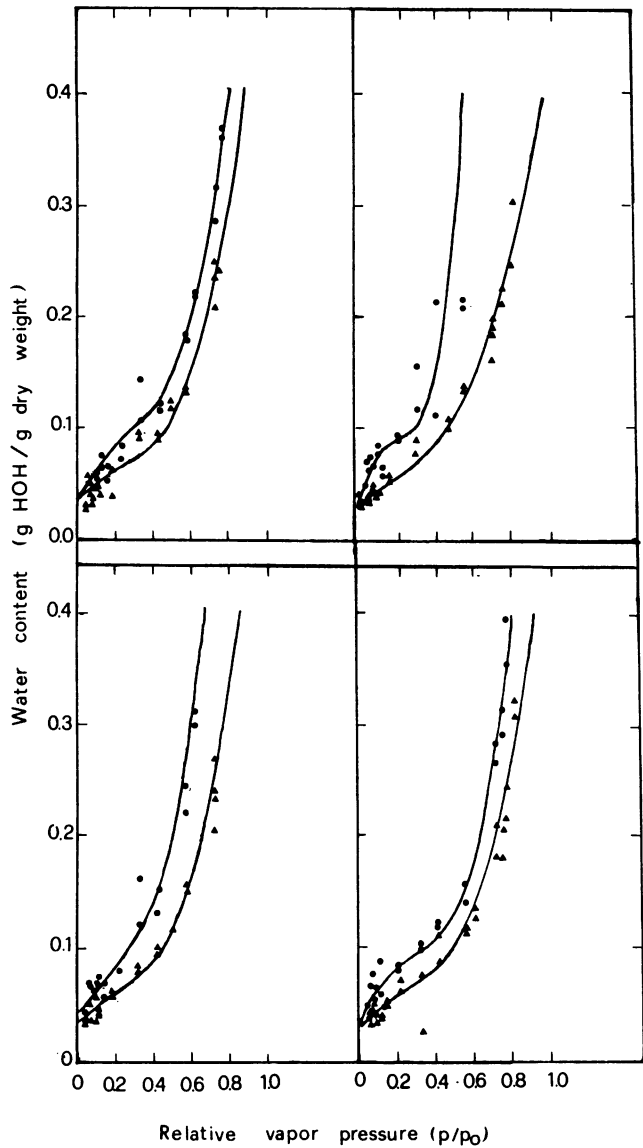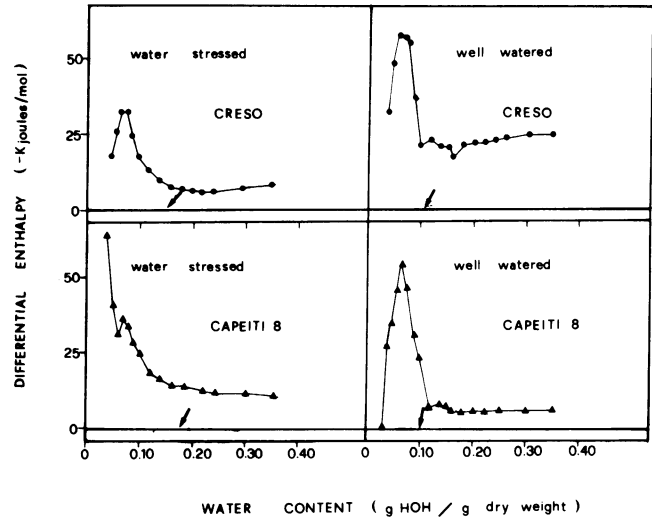

Figure 3. Adsorption isotherms for durum wheat leaves at 5 (●) and (▲) 20°C under two different water regimens. Regression curves are shown. Correlation coefficients were significant at 1% probability level.

cules (10) may be observed. Also, if this water is not removed under physiological conditions, the finding of very strongly BW in a resistant durum wheat cultivar and *Polypodium* species suggests that desiccation tolerance may be related to the strength of water binding in the first region of adsorption isotherms.

Major varietal differences in response to water stress were observed in ΔH int at moisture contents >0.10 and <0.35 g H_2O/g dry weight, where, according to the Brunauer *et al.* model (2), water has little interaction with the polymer surface and has bulk water properties. In this range, water stress effects were opposite for the two cultivars: the integrated ΔH increased 100% for Capeiti 8 and decreased 70% for Creso compared with the controls. In this region, water-tissue inter-

actions are weak, hydrogen bonds dominate, and hydrophobic interactions create strong water-tissue bonds (multimolecular sorption) that form a cage-like structure around the nonpolar moieties (19). The structure of macromolecules, organelles, and membranes depends on hydrophylic and hydrophobic interactions and is regarded as a primary site of dessication damage (9). Structural changes induced by water stress on BW were different for the two cultivars: in general, water was more strongly bound in the resistant cultivar (Capeiti 8), and there was more free water in the sensitive one (Creso). BW values obtained from PV curves may be an estimate of the point at which free water appears. The average apoplastic water from PV curves (0.09 g for well-watered and 0.17 g
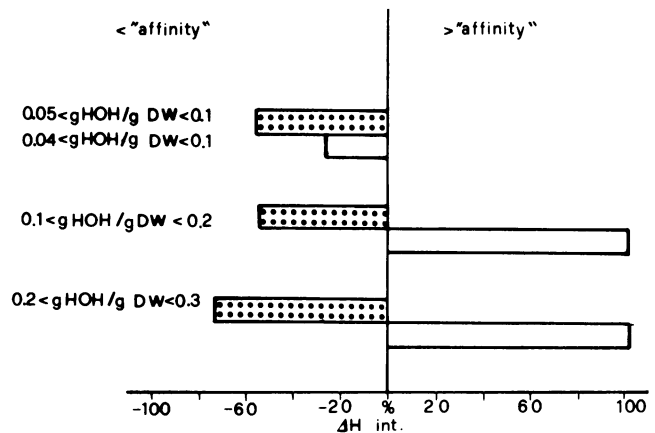

Figure 5. Percentage decrease (left) and increase (right) of ΔH int for unirrigated plants, of Creso (bars with dots) and Capeiti 8 (bars without dots), with respect to values of well-watered plants at three ranges of leaf moisture content (g HOH/g dry weight).

H_2O/g dry weight for water-stressed plants) approximated the water content where ΔH reached the highest values.

ACKNOWLEDGMENTS

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