# Differences for bound water content as estimated by pressure–volume and adsorption isotherm curves

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## Abstract

Pressure–volume (*PV*) and adsorption isotherm (AI) curves were constructed on fresh and dry tissues of durum wheat respectively, to assess the relation between the water status of living leaves and the properties of water that is bound (BW) with different strength to ionic, polar or hydrophobic sites of macromolecules. The leaves were collected from six genotypes grown in field, in 2 years. The amounts of the non-osmotic BW fraction, free water and osmotic potential at full turgor were determined by pressure–volume (*PV*) curves. Three parameters that relate to the amounts of the weakly and strongly bound water (quantitative BW properties) and five parameters related to tissues-binding strength for the same water fractions (qualitative BW properties) were calculated by AI-curves.

The non-osmotic volume of *PV*-curves doesn't correspond to the water fraction bound to the charged or polar sites of macromolecules as determined by AI-curves. The qualitative parameters, in contrast to quantitative parameters, may be affected by common physical-chemical factors: the changes in the amount of strongly bound water in tissues were independent from those in the weakly bound fraction; in contrast an increase in tissue affinity for strongly bound water implied a simultaneous increase in the affinity for weakly bound water. The qualitative properties of bound water may be particularly important for drought adaptation in durum wheat, being associated with the solute potential and with the succulence degree of the leaves.
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## 1. Introduction

In all biological systems the ability to retain molecular hydration is a fundamental defensive mechanism against dehydration [1–3]. The structure, mobility and function of biological molecules are affected by the water molecules bound to the ionic, polar and hydrophobic sites of macromolecules [4–6]. During water loss the increase in cell viscosity results in crowding of cellular components, as well as in changes in membrane phase states and in protein hydration shells [7]. The interaction of highly reactive species may be avoided by BW that coats the polymer surfaces [8]. At the molecular level, the same defensive mechanisms to preserve macromolecule hydration have been observed in anhydrobiotic plant systems and in vegetative tissues. Among these are

the production of osmotically active compatible solutes sugars, heat shock proteins and water-replacing proteins such as late embryogenesis abundant (LEA) proteins [9].

The experimental demonstration of the hypothesis that BW plays an important role in the adaptation to moderate dehydration stress, is still lacking and affected by a number of different constrains: (a) the concept of bound (structured, perturbed or nonbulklike) water does not have an univocal meaning to different readers; (b) there are contrasting views about its extension in the cell [10]; (c) results of physiological studies are often non-comparable or contrasting [11,12], being obtained on different subjects grown under dissimilar environments or derived from measurement of unrelated water properties; (d) tolerance to dehydration has been related to the quantity of BW, the strength of binding or the ability to tolerate its removal without damage [13,14,6]; (e) relationships between quantity of BW and water binding strength of plant tissues are unknown.

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In living vegetative tissues, BW is routinely determined [15,16] by pressure–volume (*PV*) curves, i.e. the relationship between tissue water content and tissue water potential. The method gives a quantitative estimate of the water bound to macromolecules of the apoplast cell walls, a water fraction not belonging to the osmotically active solution [17]. Rehydration of dry tissues and construction of AI-curves gives a more analytical picture of BW status. The sigmoidal shape of the isotherms constructed on non-homogeneous plant materials [18,13] provides evidences for the existence of various water fractions: charged or dissociable sites of the macromolecular surfaces, bind water molecules with high affinity (strongly BW); polar groups and non polar residues bind clustered water molecules with decreasing affinity (weakly BW) [19]. The analysis of isotherms constructed at two temperatures and application of the Clausius-Clapeyron equation, allows quantification of thermodynamic properties of the water and gives information on the tissue affinity for water at different hydration degree [18,20].

In this work we tested for the agreement between BW values obtained by *PV*-curves and parameters obtained from AI-curves. We also investigated the relationships among the qualitative and/or qualitative properties of BW and the water status of living leaves.

## 2. Material and methods

Three durum wheat (*Triticum durum* Desf.) cultivars: Capeiti 8, Creso and Trinakria were chosen on the bases of their morpho-physiological traits [21,22] and susceptibility index of Fischer and Maurer [23]. After 5 years long yield trials in Southern (570 mm average rainfall) and Central (840 mm average rainfall) Italy, Capeiti 8 and Trinakria were classified as cultivar adapted to the semi-arid environment; Creso was selected under optimal growth conditions and has great drought susceptibility [24]. Three mutants (108, 364, 290) differing for tissue affinity for water [25] derived from sodium azide (SA) treated seed of Trinakria cv., were included in the experiment. The affinity for water of 108 mutant is affected by the environmental conditions; 364 and 290 mutant lines have greater and lower affinity for BW than Trinakria respectively. To explore the relationship between the BW parameters of living and dry leaves, on samples showing large variation in water status, the experiment was repeated for 2 years at the Experimental Institute for Cereal Crops of Foggia (Italy).

In 1999 and 2000, 400 seeds/m$^2$ of each genotype were sown on medium texture soil, following a randomised complete block design with four replicates. The plots were 1.35 m × 7.5 m. Plants were fertilized at stem elongation with 300 kg ha$^{-1}$ of ammonium nitrate and were grown without irrigation. No disease infections were noted.

Leaf samples were collected early in the morning, at heading stage and immediately placed in water-saturated polyurethane foam, to prevent dehydration. Leaves collected from the same plot, were divided into two groups (sub-samples) to construct adsorption isotherms and pressure–volume curves.

### 2.1. Adsorption Isotherms

Satisfactory reproducibility and repeatability of sorption isotherm measurement may be achieved by standardization of the entire measuring process, because the equilibrium water content values determined depend upon the experimental procedure and preparative treatments. For this reason were used standard equipments and conditions based on an international collaborative study of 32 laboratories within the frame-work: "Cost 90 project" [26]. Leaf samples fulfilled most of the criteria required to allow a reliable judgment of sorption data, among them: absence of hysteresis, relatively rapid rate of equilibration and sigmoidal shape of water sorption. Samples consisted of large 0.5 cm leaf strips that were cut transversely. No significant differences were found between adsorption isotherms constructed in parallel tests for whole, ground and cut leaves.

To determine the samples in water-free condition, leaves were conditioned to the lowest measuring point of the adsorption isotherms by drying at 50 °C for three hours and then in P$_2$O$_5$, for 3 days. The dry weight (DW) of the substrate was determined gravimetrically.

To construct adsorption isotherms (about 0.2 g)

- the dry samples were put into small open weighing bottles (25 mm × 25 mm) standing on trivets above the surface of saturated salt solutions (the sorption device consisted of 1-l glass jars with vapour–tight lids and for temperature control, the jars were submerged in a thermostatically controlled water bath);
- the samples were incubated in parallel at 5 and 20 °C, over 13–15 different saturated salt solutions whose relative humidity values were derived from [27] (sorption values above 80% RH were not determined, because of the possibility of local water condensation and sample deterioration); when a constant weight was achieved after 3 days, the samples were weighted.

Forty-eight adsorption isotherms (plots of tissue moisture content versus the relative vapour pressures of water) were constructed, each of them consisting of 13–15 data points. To calculate the sorption enthalpies from isotherms at 5 °C and 20 °C the Clausius-Clapeyron equation:

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

was used, where $\Delta H$ is the differential enthalpy (in J/mol water) for a given water content, $R$ is the ideal gas constant (8.314 J/mol/K), $aw_1$ and $aw_2$ are the relative vapour pressures of water at the lower ($T_1$) and higher ($T_2$) temperatures, respectively. Very negative $\Delta H$ values are indicative of water that is strongly associated with the polymer surfaces.