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EFFECTS OF WATER DEFICIT ON THE WATER RELATIONS OF *ALISMA PLANTAGO-AQUATICA* L. UNDER NATURAL ENVIRONMENT

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Summary. Water relation properties of two ecological forms of *Alisma plantago-aquatica* L. grown along river beds with partial submergence and on the riverside were examined to determine the possible mechanisms involved in plant response to moderate water deficit. Terrestrial plants in comparison with aerial-aquatic ones are considerably smaller and lower in the entire biomass becoming dwarfs and reaching about 0.5-0.7 m above river. Plant water relations were evaluated by measuring absolute and relative water content, water and osmotic potentials, and the state of water in leaves. The lower water content in the riverside soil led to a higher water deficit and reduction of the absolute and relative water content in the leaves of terrestrial plants in comparison with aerial-aquatic ones. The absolute and relative water content correlated positively with leaf water potentials. It was revealed that osmotic regulation of terrestrial plants was realized to a greater degree by the mineral constituent. The adaptive mechanism of the terrestrial plants was also accompanied with redistribution of water fractional composition, reducing the free and increasing the bound water quantity. The responses of terrestrial plants are discussed in terms of the adaptive mechanisms underlying the maintenance of the water balance under water-stress conditions.

Key words: *Alisma plantago-aquatica*, water deficit, relative

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water content, water potential, osmotic potential of expressed sap, terrestrial ecotype.

Abbreviations**:** RWC - relative water content; FW - leaf fresh weight (g); DW - leaf dry weight (g); WD - leaf water deficit; ψ - leaf water potential (MPa); ES - leaf expressed sap; π - osmotic potential of expressed sap (MPa).

INTRODUCTION

Water is one of the most essential components for plant organisms. It serves as a solvent of different solutes and transporter of solutes between cells and organs. The greater part of water uptake from the soil is consumed by transpiration preventing temperature increases. Water supplies cell turgor pressure, which maintains the cell form and performs the skeletal function in the soft plant tissues. Therefore, plant growth and development severely depends on water availability (Turner, 1991; Yeo, 1998) and it is the primary limiting factor for plant growth in terrestrial ecosystems. Extensive literature has shown that under conditions with low water availability in the soil, plant cells synthesize various kinds of osmotically active solutes, such as minerals, carbohydrates, glycinebetaine, proline etc. (Premachandra et al., 1989; Delauney and Verma, 1993; Good and Zaplachinski, 1994). Osmotic adjustment helps to maintain cell turgor at decreased water potential and is considered to be an important factor in the maintenance of plant in the soil with low water availability.

The main objective of this study was to determine how moderate changes in the soil water availability result a significant reduction in plant growth likewise of two ecological forms of *Alisma plantago-aquatica L.* grown under natural environments. *A. plantago-aquatica* is an aerial-aquatic plant that mostly grows along river beds and is adapted to partial submergence, keeping their foliage above the water surface. Among their adaptive features is the development of internal aerenchyma (Kordyum, 2001; Kordyum et al., 2003) that facilitate aeration of submerged organs. At the same time, *A. plantago-aquatica* can grow on the riverside reaching about 0.5-0.7 meter above river becoming dwarfs. At that, terrestrial plants are significantly lower in the entire biomass. Their average height is 18±5 cm in comparison with 172±13 cm for aerial-aquatic plants (Kordyum et al., 1997; Kordyum et al., 2003); leaf area consists only 3-4 % of the area of aerial-aquatic plants. So, well water supplied aerial-aquatic form of *A. plantago-aquatica* grown at partial submergence and terrestrial form grown at relatively lower water availability form morphologically different ecotypes. It has been shown that plants of two forms vary in their anatomy (Kordyum, 2001; Kordyum et al., 2003), leaf morphology (Belavskaya and Kordyum 1995; Neducha et al., 1998a, 1998b), hormone content (Vedenicheva et al., 1995), and the level of lipid peroxidation (Klymchuk et al., 1998; Baranenko et al., 1999). The plants of two ecotypes also represent the convenient natural "model system" for studying water relation responses related to variability in the soil water availability. We investigated the water content in the soil of aquatic and riverside ecotypes as well as the water relation characteristics of aerial-aquatic and terrestrial forms of *A. plantago-aquatica* plants to evaluate the possible mechanisms by which relative decrease in soil water availability induces the significant reduction in plant growth.

MATERIALS AND METHODS

Plant material

The fully expanded leaves of *Alisma plantago-aquatica* L. were used for determination of water relation parameters. The leaves were taken at midday from not less than twenty plants of each ecotype. The samples of terrestrial form were taken from plants grown about 0.5-0.7 m above river. Measurements were carried out at the budding-flowering (late June) and flowering-yielding (late July) stages of vegetative development. Determinations of leaf FW as well as the level of WD and leaf ψ were carried out in the field conditions.

Measurement of leaf absolute and relative water content

The 8 mm diameter leaf discs were used to determine FW and DW by weighing before and after drying at 105 ºC to a constant weight. The

difference was a measure of the absolute water content in the leaves. RWC in leaves was determined according to equation
$([FW - DW]/[FW_{\text{soaked dist. water}} - DW] \times 100)$ (Turner, 1981).
The difference was a measure of the WD level in the leaves.

Measurement of osmotic and water potentials

The cell sap expressed from the leaves was filtered through Millipore membrane with pore size 0.20 µm. 200 µl of ES was placed into the preliminarily weighted at analytical balances titan crucibles. The sap fresh and dry weights were determined by weighing before and after drying at 105 ºC to a constant weight. Dried sap was then burned in the gas-stove burner and again weighed to determine the mineral constituent of the cell sap (Puyrko et al., 2002). The difference was a measure of the organic constituent in the sap. The π of the mineral and organic constituents of ES were calculated according to the Van't-Hoff equation: $\pi = -CRT$, where R is the universal gas constant, T absolute temperature (293 K), and C molar concentration.

The water potential of leaves was determined using the Shardakov method (Slavik, 1974). 2.0 g of leaf disks were exposed to the range of 5.0 ml sucrose solutions with various water potentials for 2 h. The solution, in which no change occurs, i.e. there has been no loss or gain of water, was identified.

Evaluation of state water in leaves

The state of water in plant leaves was evaluated by curves plotted on basis of a number of experimental points representing the relation between remaining and removed water after the action of water-removing agents of known potentials (Gusev, 1962). The method is based on determination of the gain (or loss) of external solute weight (measured by refractive indices) after exposing the leaf discs. The difference was a measure of the relatively free water content removed from leaf discs. The difference between absolute water content in the leaves and free water content was a measure of the relatively bound water content. It was used 2.0 g of leaf disks exposed to the range of 5.0 ml sucrose solutions (preliminary weighed at analytical balances) for 2 h.

Measurement of total water content in the soil

The samples of ecotype soils were also taken to determine soil water content by weighing before and after drying at 105 °C until a constant weight was obtained. The difference was a measure of the total water content in the soil.

RESULTS

The total water content in the soil of aquatic ecotype was 35.8±0.4 %, whereas the content of water in the soil of the riverside ecotype was 18.8±0.2 and 17.0±0.2 % at the budding-flowering and flowering-yielding stages of vegetative development, respectively.

The absolute and RWC in the leaves of terrestrial plants were reduced at both stages of vegetative development when compared with aerial-aquatic plants (Table 1). The differences in the leaf absolute and RWC between plants of two ecotypes were somewhat higher at the flowering-yielding than budding-flowering stage of vegetative development.

An organic constituent predominated in ES both terrestrial and aerial-aquatic plants (Table 2). However, the contribution of mineral constituent in the π of cell sap was significantly higher than organic constituent in the both ecotype plants. In addition, leaf cell sap of terrestrial plants in comparison with aerial-aquatic ones has shown increased concentration of solutes at both stages of vegetative development. This increase was due to additional accumulation of organic and mineral constituents. The total π of cell sap from leaves of terrestrial plants was lower at both stages of vegetative development when compared with aerial-aquatic ones (Table 3).

Average midday ψ in the samples of terrestrial leaves was –0.79 and –0.89 MPa at the budding-flowering and flowering-yielding stages, respectively in comparison with –0.70 MPa in leaves of aerial-aquatic plants at both stages of vegetative development (Table 3).

As illustrated by the curves of water exchange (Fig. 1), the leaves of terrestrial plants contain lower amount of the relatively free and higher amount of the relatively bound water in comparison with aerial-aquatic plants. In other words, leaves of terrestrial plants lose water in less degree than aerial-aquatic plants at acting of the external water-removing solutions.

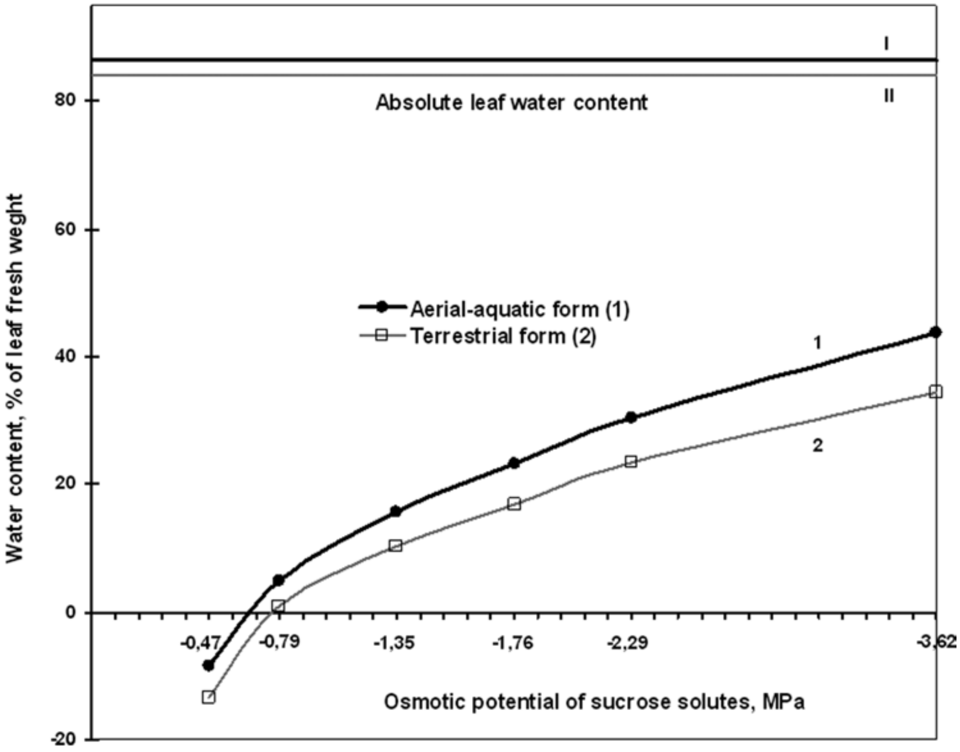
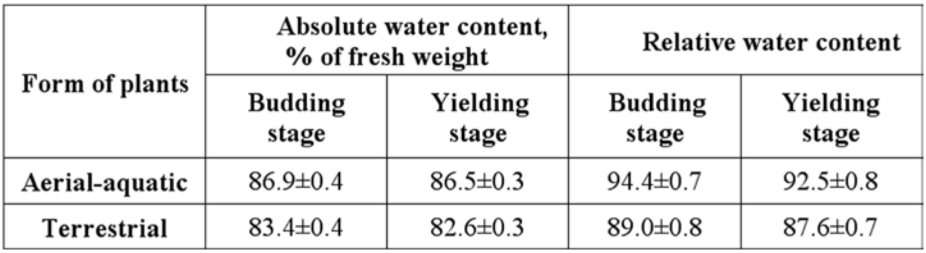


Fig. 1. Water fractional composition in the leaves of aerial-aquatic (1) and terrestrial (2) forms of A. plantago-aquatica at the range of water potential of the external sucrose solutes. The squares between the curves (1, 2) and the straight lines (I, II) of the absolute water contents characterize the amount of residuary (relatively bound) water; the square between the curves (1, 2) and the abscissa line characterize the amount of extracted (relatively free and free-bound) water. Data refer to the budding-flowering stage (2006).

Table 1. Absolute and relative water content in leaves of aerial-aquatic and terrestrial forms of *Alisma plantago aquatica* L. Data are means ± SE (n=3).

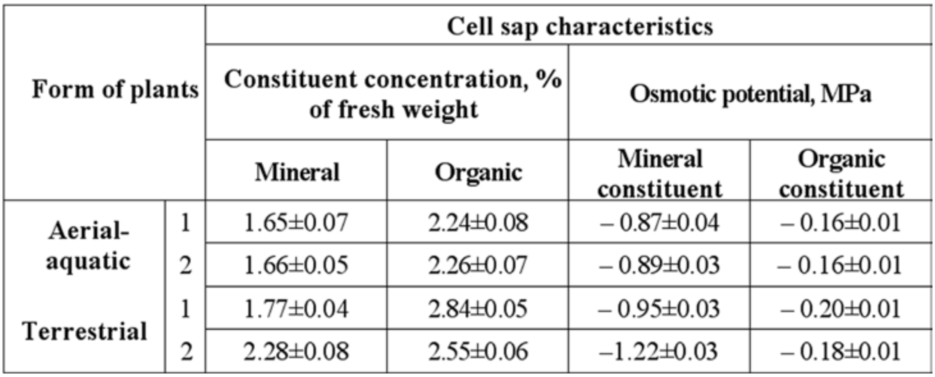


Form of plants	Absolute water content, % of fresh weight		Relative water content	
	Budding stage	Yielding stage	Budding stage	Yielding stage
Aerial-aquatic	86.9±0.4	86.5±0.3	94.4±0.7	92.5±0.8
Terrestrial	83.4±0.4	82.6±0.3	89.0±0.8	87.6±0.7

DISCUSSION

The total water content in the soil of aquatic ecotypes was more or less similar at both growth stages of vegetative development. The water content in the soil of terrestrial ecotypes was lower at both stages of vegetative development. The magnitude of differences in the soil water content between ecotypes was some higher at the flowering-yielding than budding-flowering stage. Data obtained showed that lower water content in the soil of the riverside ecotype was the main cause of the significant reduction in the growth of moisture-lowing plants of *A. plantago-aquatica* during

Table 2. Properties of cell sap from leaves of aerial-aquatic and terrestrial forms of *Alisma plantago aquatica* L. Data are means ± SE (n=3).



Form of plants		Cell sap characteristics			
		Constituent concentration, % of fresh weight		Osmotic potential, MPa	
		Mineral	Organic	Mineral constituent	Organic constituent
Aerial-aquatic	1	1.65±0.07	2.24±0.08	– 0.87±0.04	– 0.16±0.01
	2	1.66±0.05	2.26±0.07	– 0.89±0.03	– 0.16±0.01
Terrestrial	1	1.77±0.04	2.84±0.05	– 0.95±0.03	– 0.20±0.01
	2	2.28±0.08	2.55±0.06	–1.22±0.03	– 0.18±0.01

Note: 1 – budding-flowering stage; 2 – flowering-yielding stage.

Table 3. Osmotic potential of cell sap and water potential of leaf cells of aerial-aquatic and terrestrial forms of *Alisma plantago aquatica* L. Data are means ± SE (n=3).

Form of plants	Osmotic potential, MPa		Water potential, MPa	
	Budding stage	Yielding stage	Budding stage	Yielding stage
Aerial-aquatic	–1.03±0.05	–1.05±0.04	– 0.70	– 0.70
Terrestrial	–1.15±0.04	–1.40±0.04	– 0.79	– 0.89

adaptation to the riverside ecotype. Lower water availability in the riverside soil induced the decrease in the absolute and RWC in leaves of the terrestrial plants. Dehydration of terrestrial plant leaves correlated with a decrease in their ψ resulted reduction in the cell size, leaf plate area (Belavskaya, Kordyum, 1995), and an increase in the cuticle width (Neducha et al., 1998a, 1988b). These data agree with those observed by Utrillas and Alegre (1997) in *Cynodon dactylon* grown also under natural conditions. Water stress decreased both leaf RWC and ψ and was accompanied by decreased mesophyll and bundle sheath cell areas and increased cell wall thickness.

At the same time, midday leaf RWC of the terrestrial plants was not lower than 88 % and ψ was not more negative than –0.90 MPa. This means relatively low differences in dehydration between plants of two ecotypes. Thus, terrestrial plants show their relative stability, reflecting the other type of water status according to the conditions of the ecological niche.

The adaptation of *A. plantago-aquatica* plants to riverside ecotype was accompanied by additional accumulation of osmoprotectants resulted in a reduction in the osmotic potential of the cell sap (Table 3). In our early studies, it was revealed that in comparison with aerial-aquatic form terrestrial plants accumulated slightly higher concentrations of proline (by 14 %) and low-molecular water- and ethanol-soluble carbohydrates (by 41 %), (Klymchuk, 2003). Despite of the organic constituent predominated in the cell sap of both plant forms, the contribution of mineral constituent in the cell sap to osmotic potential was more significant. In addition, the osmotic adjustment in leaves of terrestrial plants was first of all due to additional accumulation of mineral constituents (Table 3). In this connection, it is worth noting the data of Puyrko et al. (2002), which demonstrate that mineral component in the cell sap of halophyte plants forms the osmotic potential five times higher than organic one.

As shown in Fig. 1, the adaptation of aerial-aquatic plants to the riverside conditions is accompanied by redistribution of water fractional composition, reducing the quantity of free and increasing the quantity of bound water. The leaves of terrestrial plants lost water to a lesser extent in comparison with aerial-aquatic plants that diminished water dissipation in conditions of its deficit, lowering the osmotic and water potential of leaf cells. This plays an important role in plant adaptation to water deficit (Morgan 1984; Raggi 1994; Rascio et al., 1994). At the same time, our data support the idea that strengthening of intercellular water to binding can lead to a decrease in its motility, intensity of exchanged processes (Grigoryuk et a.l., 1990) and inhibition of plant growth.

The results presented here suggest that accumulation of additional solutes by osmotic adjustment in *A. plantago-aquatica L.* in response to lowered soil water availability is associated with changes in water state and a decreased amount of free water, which leads to a decrease in water mobility between cells and organs (Grigoryuk et a.l., 1990). It may be one of the reasons why aerial-aquatic plants genetically adapted to optimal water supply reduce significantly growth at spreading to terrestrial ecotype with a relatively insignificant water deficit.

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