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YE.L. KORDYUM, L.YE. KOZEKO, YU.V OVCHARENKO

M.G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine
Tereschenkivska str. 2, 01004, Kyiv, Ukraine

PHENOTYPIC PLASTICITY OF AERIAL-AQUATIC PLANTS *ALISMA PLANTAGO-AQUATICA* L. AND *SIUM LATIFOLIUM* L.: STRUCTURAL AND MOLECULAR ASPECTS

The data of investigations of aerenchyma formation, alcohol dehydrogenase and heat shock protein 70 kDa (Hsp70) levels in *Alisma plantago-aquatica* and *Sium latifolium* plants growing in biotopes with different water regimes are presented. A key role of water regime in development of aerial-aquatic plants was shown. Plant adaptive responses on the changes in water supply are considered in the light of the concept on phenotypic plasticity.

Key words: Alisma plantago-aquatica, Sium latifolium, *adaptation, phenotypic plasticity, anatomy, alcohol dehydrogenase, Hsp70, water regime.*

A genome ability to change its expression and be realized in different phenotypes in a response to various environmental influences is a fundamental property of all living organisms and is known as phenotypic plasticity. Due to phenotypic plasticity, organisms can adapt to environmental variations in space and time. Phenotypic manifestations of changes in gene expression are already defined at the level of transcription efficiency and also RNA processing and translation [18] and include a very broad spectrum of ecologically important traits – physiological, biochemical, anatomical and morphological, peculiarities of developmental biology, time of transferring to the reproductive stage, propagation systems and progeny development. The wide theoretical and experimental investigations of phenotypic plasticity have been carried out in order to elucidate its importance in evolution, specialization, population dynamics and the strategy of survival in heterogenic environment [1, 2, 5, 9, 11, 15, 16, 20, 22, 23].

An idea of plasticity as a general biological phenomenon is emphasized to require special attention to its ecological aspects, as it is supposed that plasticity of organisms significantly affects stability and local diversity of the population and cenosis by action on energy transfer, carbon cycles, a number of trophic levels, turnover of nutrients and primary performance [14, 21]. It is stressed the prospectivity of investigations of plasticity in the ecological aspect to understand both the mechanisms of plant responses on abiotic and biotic factors, and the influence of these responses on interrelations of organisms with their habitat.

Strategy of adaptation is not always easily to understand because particular environmental factors, on which plants react, are not often clear and enough complex. In response to unfavorable fluctuations of ecological factors – drought, flooding, extreme temperature, soil salinity, high light intensity, infection with pathogenic agents – structural and metabolic processes, which counteract stress, occur in plants. As it is well known, water accessibility for plants is a critical external factor of their growth and development [4]. Any limit of water availability leads to dehydration of plants and, thus, affects adversely plant growth and development. For investigations of the influence of water deficit in the soil on plants in nature, aerial-aquatic plants growing in ecotopes with different water regimes are a convenient model to study phenotypic plasticity in plant adaptation to the changes in water supply in natural environment.

Aerial-aquatic *Alisma plantago-aquatica* and *Sium latifolium* plants can grow, develop and bear fruits giving viable seeds on the riverside that is an evidence on the possibilities of aerial-aquatic plants to adapt to limited water consumption. Terrestrial plants perform a “reproductive imperative” – a strategy of adaptation consisting of preservation of a species, i. e. leaving of progeny. Therefore, in the given paper, we consider phenotypic plasticity of some traits of the structural and functional organization (root anatomy, alcohol dehydrogenase (ADH) and heat shock protein 70 kDa (Hsp70) level) in *A. plantago-aquatica* and *S. latifolium* plants growing under different water regimes in the natural conditions.

Material and Methods

Investigations were carried out in the M.G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine during years. The plant material was collected in the vegetative and flowering phases at the river Psjol near Velyka Bagachka in Poltava region during field expeditions. The distance between plants growing in water and on the riverside is only from 1–3 till 20 –25 m that makes possible to sharply determine an action factor – a water quantity in the soil, as light intensity and air humidity are as a rule identical.

The primary treatment (fixation or freezing of roots and leaves) of the collected material was carried out immediately after collection, the following treatment and analyses – in the laboratory. For light microscopy, segments of the root mature zone were fixed in 16% formaldehyde solution, cut into sections by hand, stained with aniline blue and examined with a Axioscope (Zeiss, Germany). Alcohol dehydrogenase (ADH, EC 1.1.1.1) was resolved by native electrophoresis in 6% polyacrylamide gel (PAG) and isoenzyme patterns were detected by the tetrazolium technique [10]. A cytochemical staining method [19] was used to localize ADH activity in roots. Hsp70 was determined by Western-blot analysis after 10% SDS-PAG electrophoresis of total soluble proteins of the leaves [7]. Monoclonal antibodies against Hsp70 and secondary anti-mouse antibodies coupled to biotin (Sigma) were used.

Results and Discussion

Root anatomy

A filaceous root system mostly consisting of numerous adventive roots is characteristic for adult *A. plantago-aquatica* and *S. latifolium* plants. In these species, as in other aerial-aquatic plants [17], adventive roots are characterized with the presence of aerenchyma – large intercellular spaces, which together with those in stems and leaves provide oxygen uptake by growing root apices and an exchange with exogenous air. Aerenchyma begins to form in the root proximal meristem on a schizo-lysigenous type and fully developed in the root mature part. In monocotyledonous *A. plantago-aquatica*, aerenchyma is surrounded with epidermis and one cortex layer on the root periphery and one cortex layer, which cells closely adjoined, and endoderm on the inner side (Fig. 1, a).

In dicotyledonous *S. latifolium*, a number of cortex layers, which surround aerenchyma on root periphery and on the side of a central cylinder, significantly increased (Fig. 1, b), a secondary growth is typical for roots. Unlike aerial-aquatic plants, in plants of the same species growing on riverside, a root system becomes similar to that of terrestrial plants: adventive roots are characterized with very small intercellular air spaces (Fig. 1, c, d). It needs to note that presence or absence of aerenchyma is not constant and can vary in dependence on changes in water supply. In cases of increasing soil humidity due to long-term rainy weather or the regulation water level in river, the formation of new adventive roots with well developed aerenchyma begins in *A. plantago aquatica* (Fig. 1, e) and *S. latifolium* terrestrial plants. After water abatement in the river, intensive secondary growth begins in the *S. latifolium* roots, owing to this a primary cortex (aerenchyma) appears on the root periphery (Fig. 1, f) and desquamates later, and, as a result, the roots are coated with periderm.

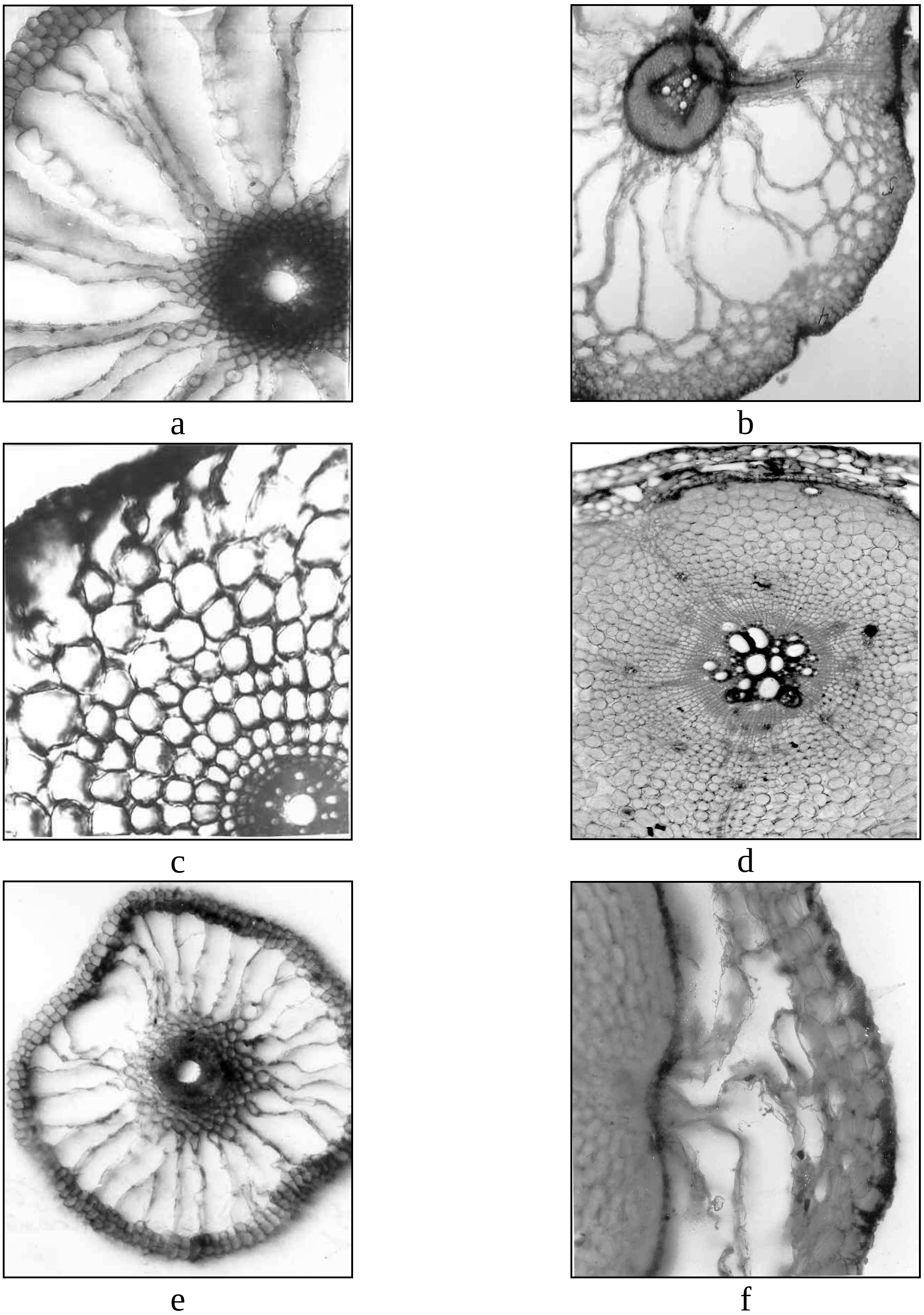

Fig. 1. Transverse sections of the root mature zone of *Alisma plantago-aquatica* (a, c, e) and *Sium latifolium* (b, d, f) grown in different water supply. a, b – an aerial-aquatic plants, c, d – a terrestrial plants, e – a terrestrial plant after long-term raining, f - an aerial-aquatic plant after a certain period of time after water abatement.

ADH expression in roots

Metabolic adaptation of plants to hypoxia includes activation of the ethanol fermentation pathway, in which ADH catalyzes a key reaction – reduction of acetaldehyde to ethanol. It is known that ability of species to ADH induction correlates with their tolerance to hypoxia [13]. Earlier, we showed ADH activity in the roots of *A. plantago-aquatica* and *S. latifolium* growing in water and its absence in the roots of terrestrial plants. The enzyme was induced under flooding of terrestrial plants: ADH activity appeared in the roots after 1 h-flooding and increased after 2 h [8].

In this study, a comparative analysis of aerenchyma and ADH localization in the roots of the both species was performed. Initiation of aerenchyma was observed in the distal elongation zone of root tips of aerial-aquatic plants (Fig. 2, a, b). On the other side, cytochemical analysis of ADH in the roots of plants grown in water and at the riverside revealed the enzymatic activity only in the aerial-aquatic plants (Fig. 2, c-f). It localized mainly in the root apical meristem and the elongation zone. These actively growing apical regions of the root have accelerated oxygen consumption [3] that, apparently, can result in ADH induction as well as initiate of aerenchyma formation.

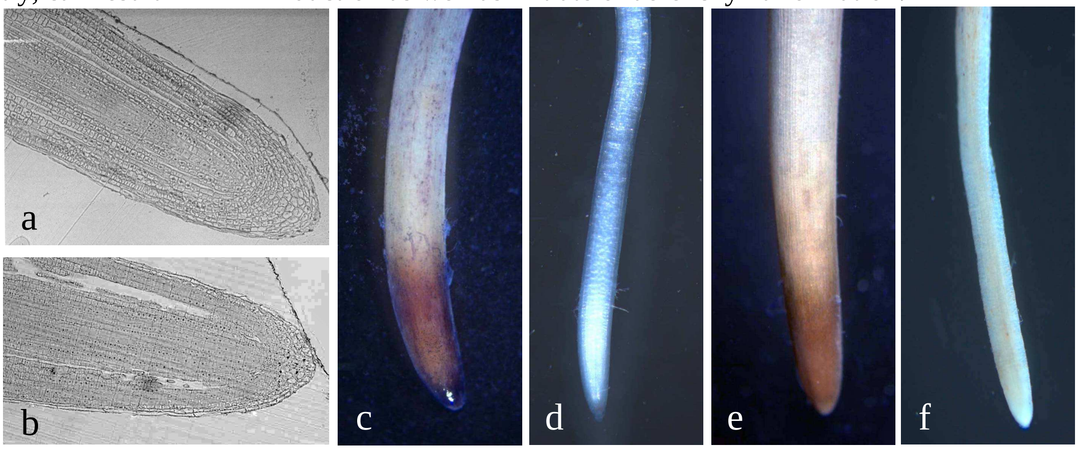

Fig. 2. Root apices of *Alisma plantago-aquatica* (a, c, d) and *Sium latifolium* (b, e, f) grown in different water supply. a, b – Longitudinal sections of the roots of aerial-aquatic plants, c-f – general view of the root apices of aerial-aquatic (c, e) and terrestrial (d, f) plants after cytochemical ADH reaction.

ADH and Hsp70 expression in leaves

Flooded roots undergo hypoxic conditions, but leaves do not suffer from restricted oxygen availability directly. Analysis of leaves of *S. latifolium* also showed ADH expression (Fig. 3) that indicates its systemic induction both in roots and leaves of plants, which undergo root hypoxia. At the same time, the plants growing in different locations showed significant variations in the ADH level not only in the aerial-aquatic plants, but also in the terrestrial ones. The ADH was determined in some plants grown on the riverside but undergone a temporal increase in the soil water content.

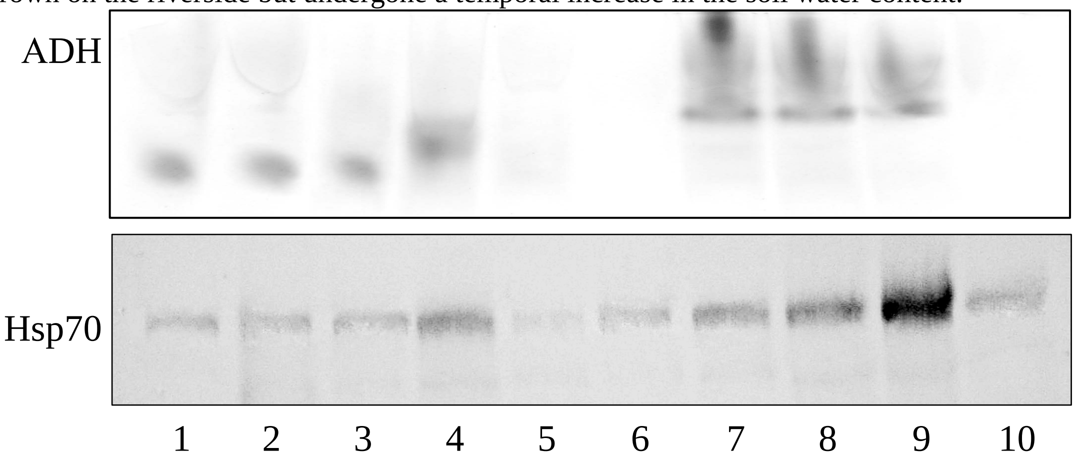

Fig. 3. Zymograms of ADH and Western-blots of Hsp70 of leaves of aerial-aquatic (1-5) and terrestrial (6-10) *Sium latifolium* plants.

An important component of a general adaptation syndrome of different stresses is known to be Hsp70 – a key member of the cellular molecular chaperone system [12, 24]. It can be used as a biomarker of a stress state of plants [6]. So we compared ADH level in the leaves of the *S. latifolium* plants with the Hsp70 level. It was determined that the ADH and the Hsp70 showed similar changes in their expression that indicates, respectively, specific and non-specific adaptive reactions in response to flooding. So, it may suppose that Hsp70 (as molecular chaperones in whole) is an important component of the tolerance of aerial-aquatic plants to changes in water supply that ensures the high adaptive plasticity of the species.

Conclusions

The obtained data on phenotypic plasticity of a number of structural and molecular patterns in aerial-aquatic plants growing in the ecotopes with different water regimes (in water and at the riverside) clearly demonstrate a key role of water supply in development of aerial-aquatic plants, that makes possible for plants to adopt to the unfavorable environmental fluctuations.

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Є.Л. Кордюм, Л.Є. Козеко, Ю.В. Овчаренко
Інститут ботаніки ім. М.Г. Холодного НАН України, Київ

ФЕНОТИПІЧНА ПЛАСТИЧНІСТЬ ПОВІТРЯНО-ВОДНИХ РОСЛИН *ALISMA PLANTAGO-AQUATICA* L. І *SIUM LATIFOLIUM* L.: СТРУКТУРНІ ТА МОЛЕКУЛЯРНІ АСПЕКТИ

Представлені дані дослідження формування аеренхіми, експресії алкогольдегідрогенази та білка теплового шоку 70 кДа (Hsp70) у рослин *Alisma plantago-aquatica* і *Sium latifolium*, що ростуть в біотопах з різним водним режимом. Показана ключова роль водного режиму в розвитку повітряно-водних рослин. Адаптивні відповіді рослин цих видів на зміни у водозабезпеченні розглядаються у світлі концепції фенотипічної пластичності.

Ключові слова: Alisma plantago-aquatica, Sium latifolium*, адаптація, фенотипічна пластичність, анатомія, алкогольдегідрогеназа, Hsp70, водний режим*

Є.Л. Кордюм, Л.Е. Козеко, Ю.В. Овчаренко
Институт ботаники им. Н.Г. Холодного НАН Украины, Киев

ФЕНОТИПИЧЕСКАЯ ПЛАСТИЧНОСТЬ ВОЗДУШНО-ВОДНЫХ РАСТЕНИЙ ALISMA PLANTAGO - AQUATICA L. И SIUM LATIFOLIUM L.: СТРУКТУРНЫЕ И МОЛЕКУЛЯРНЫЕ АСПЕКТЫ

Представленны данные исследования формирования аэренхимы, экспрессии алкогольдегидрогеназы и белка теплового шока 70 кДа (Hsp70) у растений Alisma plantago - aquatica и Sium latifolium, произрастающих в биотопах с разным водным режимом. Показана ключевая роль водного режима в развитии воздушно-водных растений. Адаптивные ответы растений этих видов на изменения в водообеспечении рассматриваются в контексте концепции фенотипической пластичности.

Ключевые слова: Alisma plantago-aquatica, Sium latifolium*, адаптация, фенотипическая пластичность, анатомия, алкогольдегдрогеназа, Hsp70, водный режим*

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Н.О. СТЕЦУЛА
Дрогобицький державний педагогічний університет ім. І. Франка
вул. В. Івасюка 11, Трускавець, 46027, Україна

ІСТОРІЯ ДОСЛІДЖЕНЬ ФЛОРИСТИЧНОГО СКЛАДУ ПЕРЕДКАРПАТТЯ

Проведено історичний аналіз ботанічних досліджень і накопичення наукових знань про рослинність Передкарпаття та лікарських рослин зокрема. Весь період досліджень видового складу угруповань передгірських ландшафтів доцільно розділити на три різних за тривалістю та рівнем досліджень етапи. До першого загально-флористичного етапу можна віднести – дослідження 1809 р.–1940 р., до другого популяційного – дослідження 45–80 років XX ст., до третього етапу – сучасні дослідження, з кінця XX – початку XXI століття.

Ключові слова: флора, історія досліджень, угруповання рослин, ландшафт, лікарські рослини