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BRIEF COMMUNICATION

Electrolyte leakage differences between poikilohydrous and homoiohydrous species of *Gesneriaceae*

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Abstract

Comparative analysis of the electrolyte efflux, as a screening test of the membrane tolerance to water stress, was carried out in poikilohydric plants *Ramonda serbica* Panč. and *Ramonda nathaliae* Panč. & Petrov. and homoiohydric plant *Saintpaulia ionantha* Wendl. from the same family *Gesneriaceae*. Water stress was induced by PEG 600. The high degree of solute leakage in the East-African drought-intolerant *Saintpaulia ionantha* points to the loss of membrane integrity. In contrast, Balkan endemites *Ramonda serbica* and *R. nathaliae* show high resistance to water stress due to the specific constitutional drought tolerance mechanisms.

Additional key words: membrane integrity, *Ramonda nathaliae*, *Ramonda serbica*, *Saintpaulia ionantha*.

The species of tropic-subtropic family *Gesneriaceae* are good examples of ecophysiological divergence regarding the plant water relations and the adaptations to the conditions of water regime in the habitat. The majority of these species are homoiohydric, growing in damp thermophilous forests of tropic region (E. Africa, S. America, SE. Asia), while only some of them are poikilohydric or resurrection plants inhabiting sites where dry and wet periods alternate.

A small group of poikilohydric *Gesneriaceae*, including 5 genera and 8 species only (Gaff 1981), survive in dry habitats in tropic and non tropic regions (most frequently in temperate zone). Within this group are also Balkan *Gesneriaceae*,

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Ramonda serbica Panč. and *Ramonda nathaliae* Panč. & Petrov., tertiary relict and rare resurrection plants of northern hemisphere (Košanin 1921, Gaff 1981, 1989). They grow exclusively on northward sides of lime canyons and gorges, although they penetrate slightly into the thermophilous (relict) forests. These plants have wide altitudinal gradient, spanning from lowland to 2000 m above sea. Their habitats on rocky slopes are characterized by harsh environmental conditions: high irradiance and temperature and severe water deficit lasting more than two months.

Both *Ramonda* species are desiccation tolerant plants and capable of undergoing complete protoplasm dehydration without permanent damage. Under the conditions of water deficit in the habitat, these plants gradually wilt and pass to anabiosis. The initial changes, occurring in desiccation tolerant plants during drought are similar to those occurring in water stress intolerant plants (Gaff 1989). However, upon reaching tolerance threshold the intolerant plants sustain irreversible changes (structural and functional) whereas resurrection plants enter anabiosis. *Ramonda* resurrection plants are capable, under favourable water balance in the field, to resume rapidly hydration which means the full biological activity, in the course of 8 to 12 h. Reversible changes during dehydration-rehydration are primarily ascribed to biochemical and physiological properties of the protoplasm, *i.e.* to the preserved cellular integrity. *Ramonda* species lack classical xeromorphic features, through there exist some morphoanatomical adaptations, common to all desiccation tolerant plants (Bewley and Krochko 1982), but they are, in our opinion, of less importance. Namely, the drier the habitats the higher xeromorphic differentiation of mesophyll, which is especially expressed in leaf structure of *R. nathaliae*.

One of the first indications that the protoplasm fine structure in plants is disturbed during water deficit, is the changed permeability of cellular membrane (Senaratna and McKersie 1983) which results in the increased ion leakage (Levitt 1980).

Bearing in mind these results, we wanted to find out to what extent the membrane integrity of resurrection plants differs from that of homoiohydric ones. The measurements of the cellular membrane damage by electrolyte leakage were carried out in order to determine the rank of drought tolerance between poikilohydric *Ramonda serbica* and *R. nathaliae* and homoiohydrous *Saintpaulia ionantha* species from the same family of *Gesneriaceae*.

R. serbica and *R. nathaliae* were collected from their habitats, transferred to the Botanical Garden "Jevremovac" in Belgrade, where they were watered on regular basis and thus maintained in the state of good hydration. *S. ionantha* was cultivated and also well watered in the greenhouse.

The quantity of the electrolytes that permeated through the membranes was measured according to Vasquez-Tello *et al.* (1990). The method was modified regarding the duration (24 instead of 4 h) of the sample treatment with PEG 600 or distilled water. Twenty circular sections (10 mm in diameter) were cut off from the mature leaves and washed in distilled water. Then, 10 of these samples were treated in 10 cm^3 of PEG 600 (water potential ψ = -1.67 MPa) and 10, used as controls, were kept in 10 cm^3 of distilled water only, for 24 h each, and subsequently washed in distilled water again. Then, at each hour, over 10 h, the solution conductivity was measured at 25 °C with conductometer *HI 8733* (*HANNA*, Tokyo). 24 h after the beginning of the measurement the samples were dried at 80 °C for 4 h. Then total conductivity of the extracts was measured at 25 °C.

The injury index (I_d), expressed in percentage, was calculated according to the formula given by Flint *et al.* (1966):

$$I_d = [(Rt - Ro)/(1 - Ro)] \times 100$$

where Rt = EC/EC_{total} for stressed samples; Ro = EC/EC_{total} for control; EC = conductivity at the specific hour; EC_{total} = total conductivity.

Experiment was carried out towards the end of April and May. Measurements were repeated three times and the results are the means. Water content [%] was the percentage of water on a fresh mass basis. Water potential [MPa] was measured by a pressure chamber according to Scholander (1964). Osmotic potential [MPa] was measured with a semimicroosmometer (*KNAUER*, Berlin, Germany) according to Walter (1928, 1931).

The results obtained show that water deficit, elicited by osmotically active substance PEG 600 (ψ = -1.67 MPa), caused changes in plants cell membranes. These changes brought about leakage of organic compounds, salts and electrolytes. The index of injury (I_d) of poikilohydric plants extremely differed from that of homoiohydric ones.

Before the beginning of the experiment, leaves of *R. serbica* had water potential -0.9 MPa, osmotic potential -0.6 MPa and water content 76 %. Similar values had also the leaves of *R. nathaliae*: water potential -0.9 MPa, osmotic potential -0.7 MPa, and water content 78 %. After the 24 h treatment with PEG 600, similar values of I_d were found. During the first hour of rehydration I_d was 2.9 % in *R. nathaliae*, and

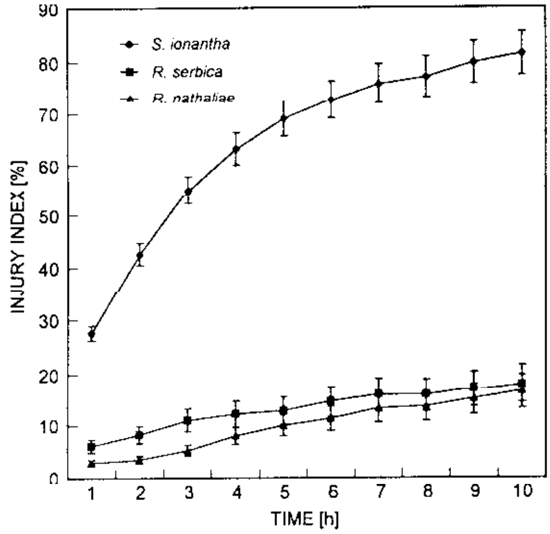

Fig. 1. Electrolyte leakages during rehydration of stressed leaf discs of *Ramonda serbica*, *Ramonda nathaliae* and *Saintpaulia ionantha*.

6 % in *R. serbica*. It gradually increases in the course of rehydration, reaching maximum value at the end of experiment (16.5 % in *R. nathaliae*, and 18 % in *R. serbica*).

Leaves of *S. ionantha* had water potential -0.5 MPa, osmotic potential -0.3 MPa, and water content 97 % before the beginning of the experiment. However, after the first hour I_d was 27.5 %. By the sixth hour of rehydration I_d significantly and continuously increased. From the sixth to the tenth hour of rehydration there was a slight I_d increase reaching its maximum during the tenth hour, 81.5 % (Fig. 1).

Extremely low values of injury index in poikilohydric *R. nathaliae* and *R. serbica* are the indications of the high degree of protoplasmatic tolerance.

By comparing I_d values it is evident that *R. serbica* is somewhat more sensitive in its drought tolerance capacity to water deficit than *R. nathaliae* due to which, probably, their environmental demands are also different (Stevanović *et al.* 1986, 1991). This correlates well with the water deficit tolerance of the species in natural habitats (Stevanović 1986, 1989) and with the total lipids and fatty acid contents during dehydration and rehydration of these plants (Stevanović *et al.* 1992).

Homoiohydric mesophyte *S. ionantha* does not possess structural and functional desiccation tolerant mechanisms, which is reflected on extremely high I_d values at rehydration after induced water deficit. The severe electrolyte leakage may result from the changed metabolic activity, which finally leads to irreversibly disturbed cellular membrane integrity. It persists in the regions with highly wet climate and favourable water relations of the habitat, in mountain regions of E. Africa (Skog 1981).

Generally, such a response of the plants examined (poikilohydric *R. serbica* and *R. nathaliae* and homoiohydric *S. ionantha*) to the induced water stress reminds of the response of more or less resistant species of the genera *Phaseolus* and *Vigna* (Vasquez-Tello *et al.* 1990). However, the injury index of desiccation tolerant plants *R. serbica* and *R. nathaliae* is unlike to that of drought resistant homoiohydric species of the genera *Phaseolus* and *Vigna* being significantly lower. In contrast, the homoiohydric *S. ionantha* is much more susceptible to water deficit than the drought sensitive species of the mentioned genera, *Phaseolus* and *Vigna*. The extent of electrolyte leakage is incomparably lower in desiccation tolerant plants than in either sensitive or resistent homoiohydric ones.

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