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Photon-harvesting efficiency and arbuscular mycorrhiza in amphibious plants

N. ŠRAJ-KRŽIČ*, P. PONGRAC, M. REGVAR, and A. GABERŠČIK

Department of Biology, Biotechnical Faculty, Večna pot 111, SI-1000 Ljubljana, Slovenia

Abstract

The amphibious plant species of intermittent aquatic habitats thrive both submerged and emerged. In order to outline the adaptive characters of these two life forms photochemical efficiency of photosystem 2, leaf contents of chlorophyll (Chl) *a* and *b*, carotenoids (Car), anthocyanins (Ant), and UV-B absorbing compounds (UV-B abs), and root aerenchyma and arbuscular mycorrhizal (AM) colonisation were studied in *Glyceria fluitans, Gratiola officinalis, Ranunculus lingua, Teucrium scordium, Sium latifolium, Sparganium emersum,* and *Veronica anagallis-aquatica*. Water level fluctuations did not exert a severe effect on photon harvesting efficiency. Submerged specimens had higher contents of Car and Ant whereas higher contents of UV-B abs were found in emerged specimens indicating efficient protection against the harmful effects of solar radiation. Roots of all species studied had extensive aerenchyma and were colonised by AM fungi, which were significantly more abundant in emerged specimens. This is the first report on AM symbiosis in *S. latifolium* and *S. emersum*.

Additional key words: aerenchyma; anthocyanins; carotenoids; chlorophylls; intermittent aquatic habitats; photochemical efficiency; photosystem 2; species differences; symbiosis.

Introduction

Alternation of floods and dry periods in intermittent aquatic habitats results in a unique flora, dominated by plants that are able to thrive submerged and emerged, the so called amphibious plants (Braendle and Crawford 1999, Gaberščik *et al*. 2003, Šraj-Kržič and Gaberščik 2005, Šraj-Kržič *et al*. 2006a). Their survival in ecosystem with frequent water level fluctuations depends on several adaptations such as heterophylly that can be induced by several environmental factors (Kuwabara and Nagata 2002). Submerged leaves are thinner, longer, and finely dissected, with higher specific leaf area (SLA), thinner cuticle and mesophyll, with no or few stomata, and lower contents of more efficient photosynthetic pigments than emerged leaves (Rascio *et al*. 1999, Rascio 2002, Kuwabara and Nagata 2002, Enríquez and Sand-Jensen 2003). Submergence affects photosynthetic parameters even in crop plants, *e.g*. rice seedlings (Panda *et al*. 2006).

The majority of amphibious plants depend exclusively on CO_2 as a source of carbon for photosynthesis, only a few utilize HCO_3^- or possess C_4 or CAM strategies (Maberly and Madsen 2002). Lower photosynthesis observed in submerged than in emerged specimens is a result of limited CO_2 availability in water, oxygen saturated aerenchyma, and less efficient nutrient acquisition due to lack of transpiration (Nilsen and Orcutt 1996, Rascio 2002, Boeger and Poulson 2003) even though the so called acropetal water transport takes place in submersed plants (Pedersen and Sand-Jensen 1993). The supply of gases and nutrients can be improved by aerenchyma, supporting respiration of plant organs, oxygenation of rhizosphere, and thus mineralization and providing photosynthetic tissues with CO_2 during submergence (Braendle and Crawford 1999, Rascio 2002, Voesenek *et al*. 2006). Water level fluctuations also affect radiation environment of amphibious plants.

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*Fax: +386 1 25 73 390, e-mail: nina.sray@gmail.com

Abbreviations: A% – arbuscular density; AM – arbuscular mycorrhiza; Ant – anthocyanins; Car – carotenoids; Chl – chlorophyll; DM – dry mass; F% – mycorrhizal frequency; F_v/F_m of PS2 – potential photochemical efficiency; M% – mycorrhizal intensity; PS – photosystem; SLA – specific leaf area; Y – actual photochemical efficiency of PS2.

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During intermittence they are exposed to higher radiation than during flooding (Sand-Jensen in Jacobsen 2002). We presume that adaptations at the biochemical level beside variable plant architecture and life forms optimise photon use efficiency.

Symbiotic relationships, such as arbuscular mycorrhiza (AM), have been reported to enhance tolerance to water excess and improve nutrient acquisition in plants (Wigand *et al*. 1998, Andersen and Andersen 2006, Fougnies *et al*. 2007) in exchange for oxygen and organic carbon in aquatic environments. The presence of AM in wetland ecosystems is spatially (Wolfe *et al*. 2007) and temporarily (Bohrer *et al*. 2004, Escudero and Mendoza 2005) dependent. Functional importance of AM for growth and improved nutrient acquisitions in such ecosystems was demonstrated (Stevens *et al*. 2002, Dunham *et al*. 2003). However, very little information is known on presence, dynamics, and functionality of AM fungi in amphibious plants that are in contrast to wetland plant species at times completely submerged (Miller 2000, Beck-Nielsen and Madsen 2001, Kai and Zhiwei 2006, Šraj-Kržič *et al.* 2006b, Radhika and Rodrigues 2007).

The knowledge on amphibious plants is very important from the aspect of climate changes and more pronounced water level fluctuations in different water bodies, since they are possible candidates for colonisation of disturbed riparian zones. The aim of this study was to delineate (*1*) biochemical and physiological response regarding photon use efficiency, and (*2*) AM colonisation differences between submerged and emerged specimens of selected amphibious plants from an intermittent lake Cerkniško jezero in Slovenia.

Materials and methods

Site description and plant species: This study was undertaken in the summers of 2005 and 2006 at intermittent aquatic habitats of karstic SW Slovenia (Europe). Habitats surveyed are characterised by great water level fluctuations, which are the result of high evapotranspiration rate and water drainage into an extensive underground system in carbonate rocks. Periods of flood lead to complete submergence of plants. During the summer dry period, plants grow on dry land. Due to water level fluctuations amphibious plant species prevail (Gaberščik *et al.* 2003, Šraj-Kržič *et al*. 2006a).

Emerged and submerged specimens of 7 common amphibious plant species were studied: *Glyceria fluitans*, *Gratiola officinalis*, *Ranunculus lingu*a, *Teucrium scordium*, *Sium latifolium*, *Sparganium emersum*, and *Veronica anagallis-aquatica*. Emerged specimens grew on land, while submerged specimens were completely flooded (water depth to max. 1 m) with exception of *G. fluitans* and *S. emersum*.

Photochemical efficiency was estimated by measuring chlorophyll (Chl) a fluorescence of photosystem 2 (PS2) using the modulated fluorometer OS-500 (Opti-Sciences, Tyngsboro, MA, USA). Potential photochemical efficiency (F_v/F_m) and actual photochemical efficiency (Y) were determined (Schreiber et al. 1995). F_v/F_m was measured in dark-adapted samples after 10 minutes of darkness, provided by dark-adaptation clips. Y was determined by providing a saturating pulse of white light to the leaf surface using the standard 60° angle clip. The Y coefficient was defined in as $Fv'/Fm'=(Fm'-Fo')/Fm'$, where Fm′ represents maximal and Fo′ minimal fluorescence of an illuminated sample. Measurements of photochemical efficiency of PS2 were carried out on clear days at noontime. Photosynthetic photon flux density (PPFD) was greater than 1 500 μmol m^{-2} s^{-1}, relative humidity about 45 %, and temperature of 25−30 °C.

Leaf tissue analysis: SLA was calculated as ratio between sample area and dry mass (105 °C, 24 h). Chls and carotenoids (Car) were analysed according to Lichtenthaler and Buschmann (2001a,b) and anthocyanins (Ant) according to Khare and Guruprasad (1993). Total methanol soluble UV-B absorbing compounds (UV-B abs) were determined according to Caldwell (1968). Samples were extracted [methanol : distilled water : HCl (37 %) = 79 : 20 : 1 (v/v)] and absorbances measured by 1 nm step in the spectral range of 280−320 nm and integrated (UV/VIS spectrometer *Lambda 12*, *Perkin-Elmer*, Norwalk, CT, USA). UV-B abs were expressed in relative units per leaf area, as they are synthesised mainly in upper epidermis (Day *et al*. 1996).

Assessments of AM colonisation were done using washed fresh roots that were cleared with 10 % KOH, and stained in 0.05 % *Trypan* blue (Philips and Hayman 1970). The parameters of AM were determined on fifteen 1-cm long fine root segments. Mycorrhizal frequency (F%) describes the percentage of root segments with fungal colonisation, while mycorrhizal intensity (M%) describes the abundance of colonisation of the root system. Root segments were ranked into six classes of mycorrhizal colonisation [%] (0, <1, 1−10, 11−50, 51−90, >90). $M\% = (95\, n_{>90} + 70\, n_{51\text{-}90} + 30\, n_{11\text{-}50} + 5\, n_{1\text{-}10} + n_{<1})/n$ (n − number of root segments). Arbuscular density (A%) describes the abundance of arbuscules in the root system. The colonised root segments were additionally ranked into four classes of abundance of arbuscules [%]: none (0), low (~10), medium (~50), and high (~100). The calculations of A% also take into account the mycorrhizal intensity (Trouvelot *et al*. 1986).

Root aerenchyma assessment and rhizosphere soil analyses: Root aerenchyma ratios were assessed on cross sections (Ø = 300−1 000 µm) of ethanol-fixed roots (Fig 3). The images of cross sections were obtained with an *Axioscop 2 MOT* microscope (*Carl Zeiss*, Goettingen, Germany), equipped with *Axiocam MRc* colour digital camera using *AxioVision 3.1* software, and analysed by *KS400* image analysis software (*Carl Zeiss Vision*, Halbergmoos, Germany).

The rhizosphere soil (soil in close contact with plant roots) of each parallel sample (~200 g) was dried (48 h, 48 °C), sieved (<1 mm), and pooled into a composite sample. The organic matter content was determined according to Kandeler (1995) and plant available phosphorus following the protocol of *ÖNORM L1087* (1993).

Statistical analyses: Measurements and analyses were carried out on 10 parallel samples. The significance of differences between submerged and emerged specimens of each plant species was tested using the analysis of variance (one-way ANOVA) for parametric data, and Mann-Whitney U-test for non-parametric data. Relationships between two parameters were tested using Spearman's rank-order correlation (r_s). Statistical analyses were performed using *SPSS for Windows 13.0*.

Results

Distinctive differences were observed between submerged and emerged specimens of studied amphibious plants. The SLA was significantly higher in submerged leaves than in emerged ones ($p \le 0.001$) with the exception of *G. fluitans*. Due to the highly dissected submerged leaves of *S. latifolium* specific leaf area was not measured, but it was noticeably higher than in emerged.

The potential photochemical efficiency of PS2 (F_v/F_m) was similar in submerged and emerged specimens, reaching a maximal value of 0.83 (no figure). The actual photochemical efficiency of PS2 (Y) was relatively low but significantly higher in emerged than in submerged specimens, with the exception of *S. latifolium* in which no difference was observed (Fig. 1*A*). The content of Chls *a*+*b* varied greatly and no general difference between submerged and emerged specimens could be outlined (Fig. 1*B*); the ratio Chl *a/b* did not vary significantly (no figure). The contents of Car and Ant were significantly higher in submerged than in emerged specimens (Fig. 1*C,D*). In contrast, the contents of UV-B abs expressed per leaf area were significantly higher in emerged specimens of the majority of species (Fig. 1*E*).

AM colonisation was found in all studied species, except in submerged specimens of *G. fluitans* and *S. emersum*. F% and M% were significantly higher in emerged than in submerged specimens, with the exception of *G. officinalis* and *V. anagallis-aquatica* (Fig. 2*A,B*). Arbuscules were observed in emerged specimens of all species, whereas they were not found in submerged specimens of *G. fluitans*, *S. latifolium*, and *S. emersum*. In general, significantly higher A% was observed in emerged specimens (Fig. 2*C*). Only in *G. fluitans* higher aerenchyma ratio in submerged than in emerged specimens was observed (Fig. 2*D*). Parameters of AM and aerenchyma ratio in roots were significantly negatively correlated in *G. fluitans* ($r_s < -0.579$, $p \le 0.01$) and *S. latifolium* ($r_s < -0.500$, $p \le 0.05$), and positively correlated in *R. lingua* ($r_s > 0.460$, $p \le 0.05$).

The rhizosphere soil of amphibious species had low plant available phosphorous which was in general significantly lower during submergence of plants (Fig. 1*F*). AM parameters correlated positively with plant available phosphorous ($r_s > 0.602$, $p \le 0.001$). Organic matter content was on average lower in submerged (2.25±1.4) than in emerged (5.05±3.5) plant species.

Data analysis revealed only significantly negative correlation between Y and Ant content but only positive between Y and AM parameters in studied amphibious plants (Table 1). There were no significant correlations for *G. officinalis* and *S. latifolium*.

Discussion

Submerged leaves had higher SLA than emerged, which is advantageous for the underwater acquisition of gases, nutrients, and photons (Rascio *et al*. 1999, Enríquez and Sand-Jensen 2003, Mommer *et al.* 2006). The high F_v/F_m of submerged and emerged leaves of amphibious plants is indicative of undisturbed photon harvesting, which is in line with previous reports (Šraj-Kržič and Gaberščik 2005, Panda *et al.* 2008). The Y of submerged specimens was lower than that of emerged specimens, indicating temporarily unfavourable conditions (Schreiber *et al.* 1995). Observed variability in leaf contents of Chls *a* and *b* is attributed to species-specific characteristics (Enríquez and Sand-Jensen 2003, Germ and Gaberščik 2003, Mommer *et al*. 2005). Car, which were found in larger amounts in submerged than in emerged specimens, function as accessory pigments under radiant energy limitation that occur when plants grow under water (Demmig-Adams and Adams 1996). Furthermore, Car prevent photodamage *via* non-photochemical quenching of the Chl *a* fluorescence of PS2 (Adams and Demmig-Adams 2004) although their contents are negatively related to photochemical efficiency of PS2 in some species. Photoinhibition in submerged plants can, besides high irradiances, be a result of nutrient shortage and limited CO_2 availability in water (Steyn *et al*. 2002, Nielsen and Nielsen 2006).

The relatively high contents of UV-B abs in the studied plants are believed to give efficient protection against harmful effects of UV-B radiation (Germ and Gaberščik 2003). The majority of emerged specimens had

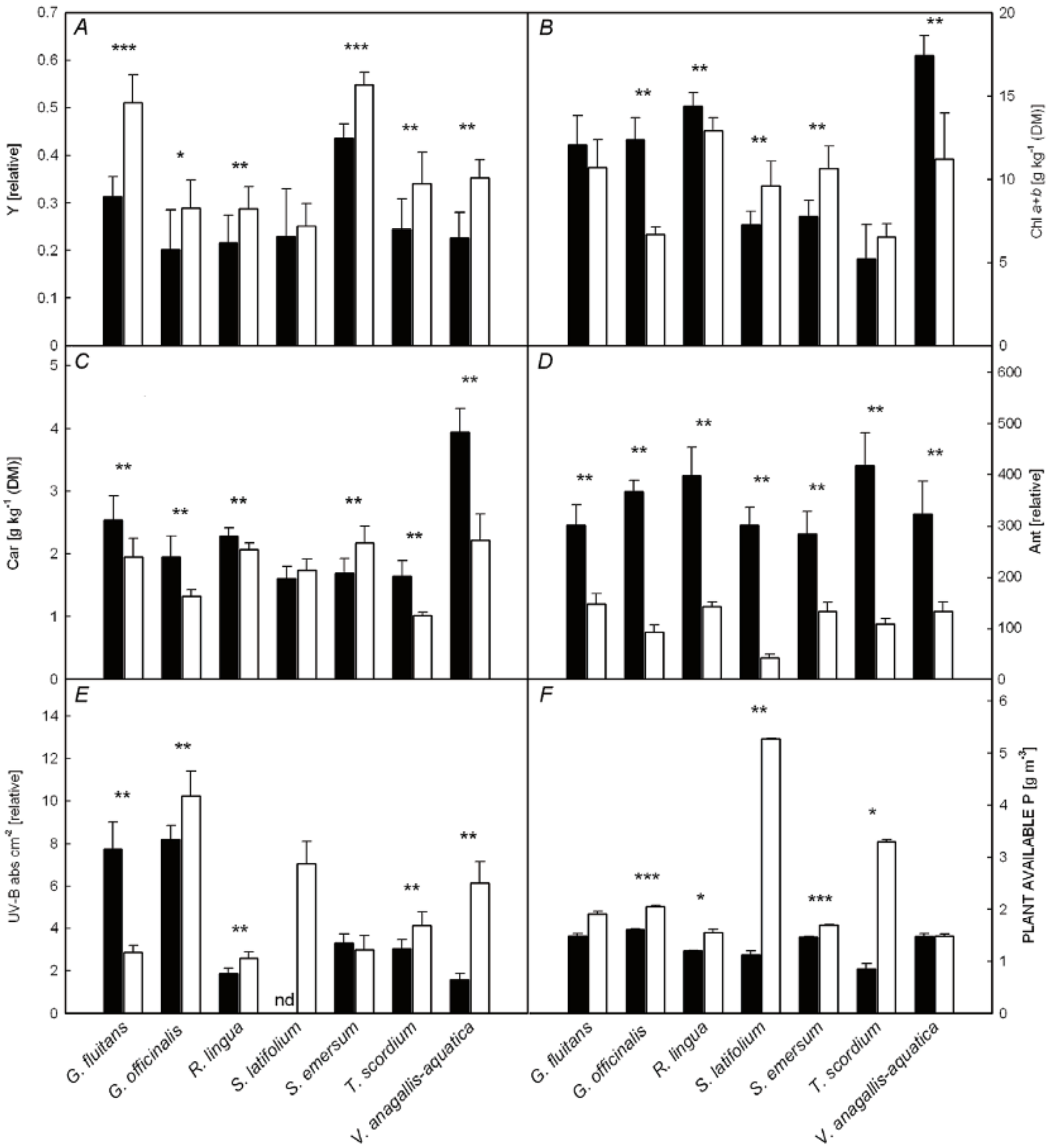

Fig. 1. Physiological and biochemical characteristics of leaf submerged (■) and emerged (□) specimens of amphibious plants: (*A*) actual photochemical efficiency of photosystem 2 (Y), contents of (*B*) chlorophyll (Chl), (*C*) carotenoids (Car), and (*D*) anthocyanins (Ant) calculated per sample dry mass, (*E*) content of total methanol soluble UV-B absorbing compounds (UV-B abs) calculated per sample area, and (*F*) available plant phosphorus. Means ± SD, $n = 10$. One-way ANOVA: $^{*}p\leq0.05$, $^{**}p\leq0.01$, $^{***}p\leq0.001$, nd − no data.

Table 1. Correlations between actual photochemical efficiency of photosystem 2 (Y) and leaf carotenoids (Car), anthocyanins (Ant), and parameters of arbuscular mycorrhiza: mycorrhizal frequency (F%), mycorrhizal intensity (M%), and the density of arbuscules (A%). Two-way Spearman correlation (r_s): ns $p>0.05$, $^{*}p\leq0.05$, $^{**}p\leq0.01$, $^{***}p\leq0.001$.

Y	Car		Ant		F%		M%		A%	
	p	r_s	*p*	r_s	*p*	r_s	*p*	r_s	*p*	r_s
G. fluitans	**	−0.630	***	−0.713	***	0.745	***	0.717	ns	
G. officinalis	ns		ns		ns		ns		ns	
R. lingua	ns		*	−0.448	**	0.578	*	0.465	ns	
S. latifolium	*	−0.394	ns		ns		ns		ns	
S. emersum	*	0.549	**	−0.675	*	0.564	*	0.589	ns	
T. scordium	ns		ns		*	0.458	*	0.534	**	0.599
V. anagallis-aquatica	**	−0.634	**	−0.649	ns		*	0.474	ns	

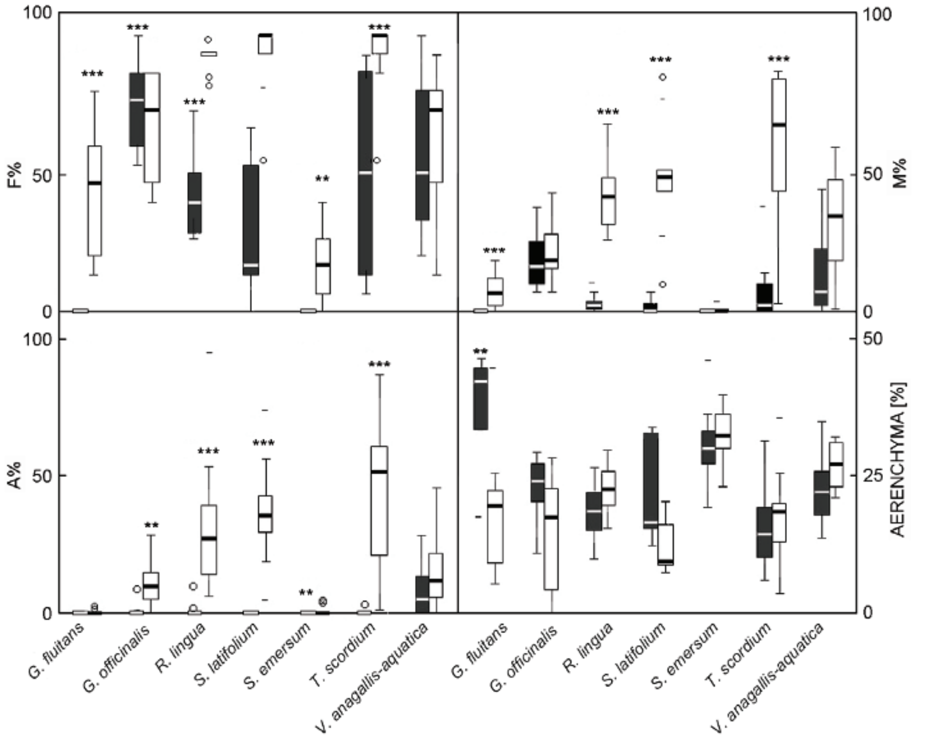

Fig. 2. Parameters of arbuscular mycorrhizal colonisation [mycorrhizal frequency (F%), mycorrhizal intensity (M%), and density of arbuscules (A%)], and aerenchyma ratio [%] in roots of submerged (■) and emerged (□) specimens of amphibious plants. Box-plots represent boxes with 1st quartile, median, and 3rd quartile, whiskers with non-outlier range of data, outliers (○) and extremes (−). $n = 10$. Mann-Whitney U test: $^{*}p \le 0.05$, $^{**}p \le 0.01$, $^{***}p \le 0.001$.

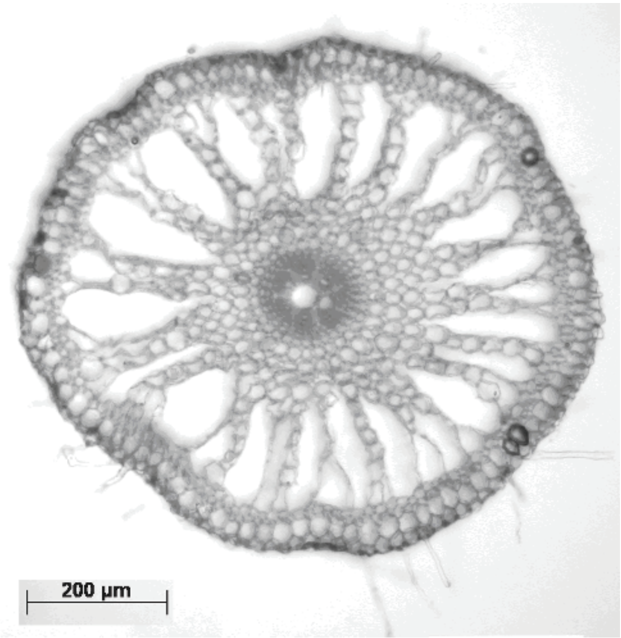

Fig. 3. Cross section of *Spargonium emersum* root.

higher contents of UV-B absorbing compounds than submerged, which is consistent with the higher levels of UV-B radiation on land than in water (Rozema *et al.* 2002). Štroch *et al*. (2008) proposed that photoprotection by xanthophyll cycle reduced the demand for UV-B absorbing compounds' synthesis that might be the case in submerged specimens. Ant absorb excess radiant energy, substituting for the protective role of the xanthophyll cycle in some species (Manetas *et al.* 2002), which may have contributed to the observed higher Ant contents in submerged specimens. Metabolically costly Ant are believed to be indirectly beneficial for plant survival under nutrient shortage (Steyn *et al*. 2002) as nutrient uptake *via* roots of amphibious plants under submergence is diminished (Nilsen and Orcutt 1996, Rascio 2002). This leads to reduced growth, excess of saccharides, and consequently depressed photosynthesis. In such conditions synthesis of Ant can act as a sink of saccharides (Sand-Jensen and Jacobsen 2002, Steyn *et al*. 2002).

AM colonisation of roots was established in all seven species confirming that AM is abundant in plants of aquatic environments (Kai and Zhiwei 2006, Šraj-Kržič *et al.* 2006b, Radhika and Rodrigues 2007). To our knowledge this is the first report on AM in species *S. latifolium* and *S. emersum*. Only submerged specimens of *G. fluitans* and *S. emersum* lacked arbuscules, although Šraj-Kržič *et al.* (2006b) found them in *G. fluitans*. Other factors than water fluctuation may have affected AM colonisation in this species, for instance phenological rhythm which influences AM presence in some wetland species (Bohrer *et al*. 2004).

Lower AM levels in submerged than in emerged specimens were observed in studied plant species which is in line with the reports on AM colonisation decrease with water depth or flood duration due to shortage of oxygen (Stevens and Peterson 1996, Miller 2000). Similarly, arbuscule formation decreases in *Lotus tenius* under water excess compared to control treatment more strongly than in water deficit (García *et al*. 2008). During submergence plants supply rhizosphere soil and thus AM fungi with oxygen (Wigand *et al*. 1998, Karjalainen *et al.* 2001, Voesenek *et al.* 2006) *via* extensive aerenchyma (Karjalainen *et al*. 2001, Mendoza *et al.* 2005), even though the conditions that induce the development of aerenchyma, *i.e*. inundation, and thus oxygen shortage, are otherwise a reason for decreased AM colonisation (Stevens and Peterson 1996, Braendle and Crawford 1999, Miller 2000, Rascio 2002). Therefore significant correlations between AM parameters and aerenchyma were observed in *R. lingua* only.

In general, higher content of plant available P was measured in the rhizosphere soil of emerged specimens and a positive correlation between plant available P and AM colonisation contradict well accepted fact of the AM importance in nutrient poor environment in terms of improved nutrient acquisition. Our results are thus in line with previous suggestions that AM colonisation is not principally related to plant available phosphorous in aquatic habitat (Miller 2000).

In conclusion, submerged and emerged specimens of amphibious plants maintained high photochemical efficiency of PS2 under contrasting environment. The protection of leaves against the harmful effects of solar radiation was attributed to high contents of Car and Ant in most of submerged specimens, and high contents of UV-B abs in emerged specimens. Roots of studied species had extensive aerenchyma and were colonised by AM fungi.

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