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BRIEF COMMUNICATION

Fatty acid content during reconstitution of the photosynthetic apparatus in the air-dried leaves of *Xerophyta scabrida* after rehydration

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Abstract

Desiccation of *Xerophyta scabrida* caused considerable damage of chloroplast ultrastructure together with a complete loss of chlorophyll. Upon rehydration, the relative water content of the pale-green leaves almost reached that of the dark-green ones, however, the Chl content and photosynthetic activity remained lower. The process of reconstitution of the photosynthetic apparatus in the re-greening leaves was accompanied by changes in fatty acid (FA) content. The amount of the FA methyl esters was more than 2-fold higher in the green leaves as compared to the dry ones and slightly increased after rehydration in the pale-green leaves. Among the three main fatty acids in the leaves, oleic, palmitic and linoleic acid, the latter increased more than 3-fold during rehydration. This acid is concentrated mainly in the glycolipids and this was an indirect indication for the restoration of the photosynthetic apparatus. Our results showed that rehydration of *X. scabrida* led to a decrease of the saturated FA in parallel with an increase of the unsaturated FA, thus indicating increased membrane permeability. The observed changes in the lipid content can be considered as a characteristic feature of *X. scabrida* and most probably of other poikilochlorophyllous species.

Additional key words: resurrection plants, chlorophyll fluorescence, chloroplast ultrastructure, oxygen evolution.

Desiccation-tolerant plants, often referred to as resurrection plants, can survive total dehydration of various durations in an abiotic state and will revive on rewetting. Following a recovery phase they show similar metabolic and physiological activities to those prior to desiccation. Two types of desiccation-tolerant angiosperms have been recognized - those that lose chlorophyll (poikilochlorophyllous, PDT) and those that retain chlorophyll (homoiochlorophyllous, HDT) on drying (Gaff 1971). The main function of pokilochlorophylly is probably to limit photo-oxidative damage that can result from the uncoupling of carbon fixation from the electron transport pathway under water deficit (Smirnoff 1993). This advantage, together with the possible benefits from not having to maintain the photosynthetic apparatus intact through long periods of desiccation, presumably outweighs the disadvantage of slow recovery (Tuba *et al.* 1998). The reassembly of the chloroplasts and restoration of the photosynthetic activity is one of the major activities in the rehydration phase. Ultrastructural

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Abbreviations: Chl - chlorophyll; FA - fatty acids; FAME - fatty acids methyl esters; FID - flame ionization detector; F_0 - initial yield of chlorophyll fluorescence in the dark adapted leaves; F_m - maximum chlorophyll fluorescence of dark adapted leaves; F_m' - maximum fluorescence in light-adapted leaves; F_s - steady state fluorescence of light-adapted leaves; F_v - variable fluorescence of dark adapted leaves; F_v/F_m - maximum photochemical quantum efficiency of PS 2; F_v'/F_m' - excitation capture efficiency of PS 2; $1 - F_v'/F_m'$ - proportion of the energy dissipated as heat in the PS 2 antenna; FR - far-red; HDT - homoiochlorophyllous; PDT - poikilochlorophyllous; PPFD - photosynthetic photon flux density; PS 1 - photosystem 1; PS 2 - photosystem 2; P_{700} - reaction center of PS 1; P_{680} - reaction center of PS 2; RWC - relative water content; Rfd - chlorophyll fluorescence decrease ratio; Φ_{PS2} - quantum yield of Photosystem 2 photochemistry in the light-adapted state.

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studies have shown that the outer membranes of organelles remain intact and there is no apparent requirement for macromolecular synthesis for maintenance of their integrity during rehydration of the PDT plant *Xerophyta humilis* (Collet *et al.* 2003). Several components of the photosynthetic apparatus, however, are degraded during drying and they have to be re-synthesised during rehydration. The mRNAs for chlorophyll biosynthesis and components involved in photosystem 2 activity appear to be stored in the dry leaves. These mRNAs together with the protein synthetic apparatus must be stabilized in the dry state and re-activated on rehydration.

It is well known that the composition and physical state of the lipid bilayer influence lipid-protein and protein-protein associations, membrane-bound enzyme activities and the carrier-mediated transport capacity of membranes (Navari-Izzo *et al.* 2000). However, data on the changes in the composition of lipids extracted from thylakoids or intact leaves in relation to desiccation tolerance in resurrection plants remain still insufficient (Stefanov *et al.* 1992, Navari-Izzo *et al.* 1995, Quartacci *et al.* 2002). The aim of this study was to investigate the relationship between the composition of fatty acids and structure and function of the photosynthetic apparatus during re-greening of leaves of *Xerophyta scabrida* upon rewatering.

Leaves of monocotyledonous plant *Xerophyta scabrida* Dur. *et* Schinz (*Velloziaceae*) stored 5 years in an air-dry state were used. Storage, rehydration and re-greening procedures were carried out according to Tuba *et al.* (1994). Dry (relative water content, RWC 5 %), pale-green (3 d of rehydration, RWC 92.6 %) and fully recovered dark-green leaves (5 d of rehydration, RWC 94.8 %) were used for measurements. The RWC of leaves was measured by gravimetric method as described by Georgieva *et al.* (2005). The pigment content was determined spectrophotometrically according to Lichtenthaler (1987) and the data were calculated on a dry mass basis (80°C for 48 h). Chlorophyll fluorescence emission from the upper leaf surface was measured with a pulse amplitude modulation fluorometer (*PAM 101-103*, *Walz*, Effeltrich, Germany) as described previously (Georgieva *et al.* 2005). The redox state of reaction center of PS 1 P_{700} was monitored *in vivo* as 810/860 nm absorption changes. The *Walz ED 700DW-E* emitter/detector unit was connected to a *PAM 101E* main control unit (Klughammer and Schreiber 1998). Oxygen evolution rate was measured at irradiance of 800 μmol m^{-2} s^{-1} and saturating CO_2 concentration (provided by a 1 M carbonate/bicarbonate buffer) using a leaf disk electrode (*Type LD2/2, Hansatech*, Hoddesdon, UK). The total lipids were extracted and the obtained fatty acids methyl esters (FAME) were analyzed by gas chromatography using *Hewlett Packard 5890* (Palo Alto, CA, USA) as described by Ivanova *et al.* (1999). For transmission electron microscopy (TEM) the samples were fixed, dehydrated, embedded in *Durcupan* and examined with a *Jeol* (Tokyo, Japan) TEM according to Doncheva *et al.* (2009).

Although RWC in desiccated *Xerophyta scabrida* leaves was only 5 %, the leaf water status was restored as usually (Tuba *et al.* 1994) and RWC was almost completely resumed (92.6 %) in the pale green leaves after 3 d of rehydration. The total absence of chlorophyll, the Chl fluorescence signals and the negative value of oxygen evolution rate (-0.266 μmol m^{-2} s^{-1}) in desiccated leaves indicated that the photosynthetic apparatus in *X. scabrida* was fully destroyed during desiccation. This was also confirmed by the dramatic deterioration in chloroplast ultrastructure (Fig. 1*A*). Ultrastructural observations of desiccated leaves showed that the membranes of all organelles were intact, except the chloroplast inner structure. These desiccated chloroplasts of *X. scabrida*, the so-called dessicoplasts (Tuba *et al.* 1993), were rounded and contained groups of osmiophilic globules. In addition, thylakoids and grana were reduced in number and only single visible thylakoid membranes were present in the stroma. The mitochondria cristae were not well-defined. In the cytoplasm there were small vacuoles, shortened profiles of endoplasmic reticulum and nuclei with a nucleolus in which the granular components were distributed around the fibrillar components forming a ring with an irregular border.

In the pale-green leaves some of the cells contained a single central vacuole. Chloroplasts were elongated and the thylakoid membrane had reassembled forming grana stacks. In the stroma near to the envelope translucent vesicles were visible (Fig. 1*B*). As described by Bhatt *et al.* (2009) for *X. viscosa* and *X. retinervis*, tissues reorganized their photosynthetic apparatus upon rehydration. In the green leaves the thylakoid system was organized in several well-defined and regularly distributed grana connected by parallel stroma lamellae (Fig. 1*C*). In the stroma some starch was present. Mitochondria were better structured.

Regardless of the similar RWC of both the pale-green (92.6 %) and the dark-green leaves (94.8 %) the Chl content of the former was lower by 50 % (Table 1). However, the content of total carotenoids did not change during further greening of the pale green leaves. The Chl *a*/*b* ratio and the ratio of Chls to total carotenoids reached the usual values in the dark-green leaves. The relative fluorescence decrease ratio (Rfd) is a good indicator of the photosynthetic activity and vitality of plants (Lichtenthaler and Rinderle 1988). The Rfd value of the pale-green leaves was below 1 (Table 1) indicating not fully developed photosynthetic function, which was in accordance with the low value for oxygen evolution (3.39 μmol m^{-2} s^{-1}) measured in these leaves. Both Rfd and the photosynthetic activity measured in the pale green leaves were decreased by 70 % in comparison with those measured in the dark-green leaves confirming high sensitivity of the photosynthetic apparatus to drought

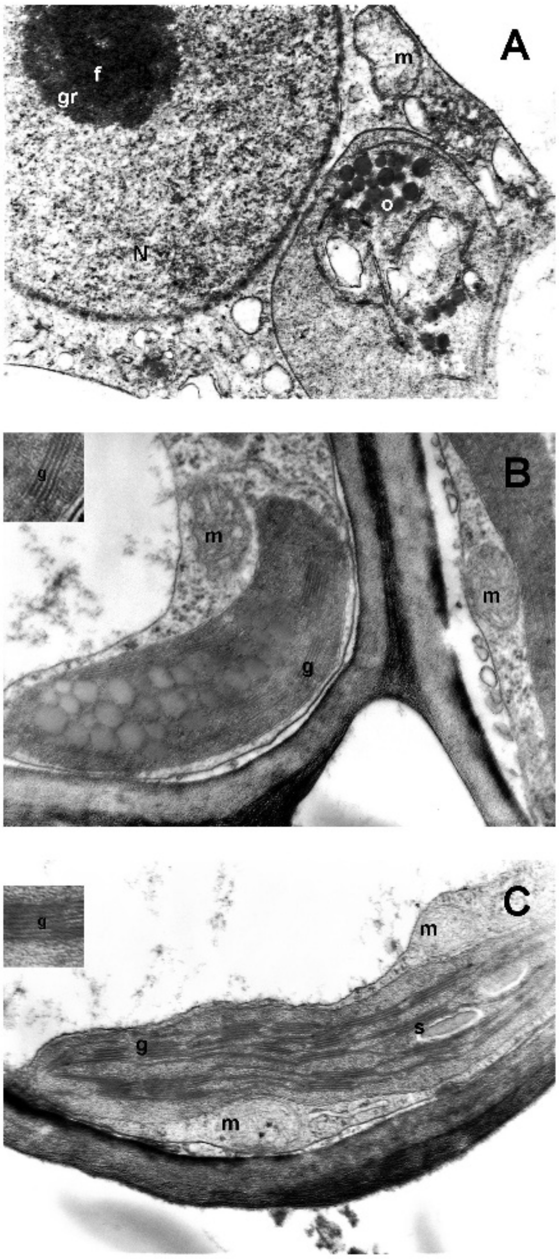

Fig. 1. Micrographs of chloroplasts of *Xerophyta scabrida* leaves. *A* - desiccated; *B* - 3 d of rehydration; *C* - 6 d of rehydration. Chloroplasts (C), starch granules (s), grana (g), plastoglobuli (o), nucleus (N), nucleolus (n) built up by fibrillar (f) and granular (gr) components, mitochondria (m) are indicated.

stress (Santos *et al.* 2009, Cai *et al.* 2010). The low photosynthetic activity of the pale-green leaves correlated with high proportion of the energy dissipated as heat in the PS 2 antenna (determined as 1 - F_v'/F_m', Table 1). The maximum quantum efficiency of PS 2 photochemistry (F_v/F_m) and the actual efficiency of PS 2 electron transport (Φ_{PS2}) were reduced by 30 and 40 %, respectively, in the pale-green leaves. Thus, our results showed that the photochemical activity of PS 2 recovered faster than the oxygen evolution. The increase in leaf absorption after FR irradiance ($\Delta A_{810-860}$) reflected increased PS 1 activity, which was accompanied with increased oxidized form of PS 1 reaction center P_{700}^{+}. Our results showed that rehydration increased the maximum of FR induced P_{700} oxidation (Table 1). The comparison of the extent of recovery of PS 2-related fluorescence parameters ($F_v/F_{m,}$ Φ_{PS2}) and $\Delta A_{810-860}$ revealed that unlike PS 2, PS 1 functional activity recovered more slowly which could be due to limitation of the electron flow between PS 2 and PS 1.

The amount of FAME slightly increased in the pale-green leaves whereas it was higher by more than 2-fold in the green leaves (Table 1) as compared to the dry ones

Table 1. Photosynthetic parameters and FAME content in *Xerophyta scabrida* leaves. Means ± SE, *n* = 6. *, **,*** - significant differences between green and pale-green leaves at *P* < 0.05, 0.01 and 0.001, respectively.

Parameters	Pale-green	Green leaves
Chl (*a*+*b*) [mg g^{-1}(d.m.)]	1.055±0.12	2.280±0.43*
Chl *a/b*	1.721±0.44	2.293±0.16*
Car (*x*+*c*) [mg g^{-1}(d.m.)]	0.558±0.02	0.536±0.02
Chl (*a*+*b*)/Car	1.901±0.26	4.202±0.61**
F_v/F_m	0.589±0.007	0.884±0.033***
Φ_{PS2}	0.420±0.009	0.690±0.008***
Rfd	0.725±0.010	2.232±0.088***
1- F_v'/F_m'	0.499±0.009	0.225±0.005***
$\Delta A_{810-860}$	1.102±0.020	1.363±0.019**
O_2 evolution [µmol m^{-2} s^{-1}]	3.39 ±0.30	11.26 ±0.90***
FAME [mg g^{-1}(d.m.)]	1.80 ±0.01	3.80 ±0.03***

Table 2. Changes in fatty acids composition [% of total FA] in *Xerophyta scabrida* leaves. Triplicate values for methyl esters in standard mixtures by gas chromatographic analyses varied within 12 % for minor components (< 5 %) and within 5 % for the others.

Fatty acid	Dry leaves	Pale-green leaves	Green leaves
12:0	0.3	0.3	0.3
14:0	1.2	1.0	1.2
15:0	0.1	0.3	0.3
16:0	27.7	26.0	26.1
16:1	1.2	0.3	0.8
16:2	0.8	0.7	1.0
18:0	15.0	11.7	9.7
18:1	20.6	15.0	14.0
18:2	15.8	20.6	20.7
18:3	4.9	14.7	16.7
20:0	5.2	3.7	3.4
22:0	2.6	1.9	1.6
23:0	1.3	1.1	1.4
24:0	3.2	2.6	2.8

ones (1.7 ± 0.01). Undoubtedly, rehydration led to a significant enhancement of FAME (resp. lipids) content. The same effect has been observed for other desiccation-tolerant plants (Stefanov *et al.* 1992, Navari-Izzo *et al.* 1995, Quartacci *et al.* 2002). During dehydration, however, a decrease in total lipids as well as in membrane fluidity has been observed (Le and McQueen-Mason 2006).

The main fatty acids found in the dry, pale-green and green leaves were palmitic (16:0), stearic (18:0), oleic (18:1) and linoleic (18:2) (Table 2). The content of palmitic acid remained constant during rehydration. While the content of oleic acid decreased, that of linoleic acid increased. The same trend has been observed for other plants (Stefanov *et al.* 1992, Quartacci *et al.* 2002). However, an opposite effect was observed earlier for the total lipids of *Boea hydroscopica* subjected to rehydration (Navari-Izzo *et al.* 1995). A recent study on FA changes during rehydration of the same plant has shown that the amounts of both oleic and linoleic acid increased (Navari-Izzo *et al.* 2000). It should be noted that in both cases the lipids were isolated from the thylakoid membranes. Evidently, desiccation (resp. rehydration) can cause different changes in the FA profiles depending on the plant part studied. Our results showed also that the amount of linolenic acid (18:3) increased more than 3-fold during rehydration. This acid is concentrated mainly in the glycolipids and this is an indirect indication for the restoration of the photosynthetic apparatus. In addition, all samples contained small amounts of short-chain fatty acids - dodecanoic (12:0), tetradecanoic (14:0) and pentadecanoic (15:0) and long-chain FA – eicosanoic (20:0), docosanoic (22:0), tricosanoic (23:0) and tetracosanoic (24:0), which remained unchanged during the rehydration process.

In conclusion, we have demonstrated that the reconstitution of the photosynthetic apparatus in the re-greening leaves of poikilochlorophyllous air-dried *Xerophyta scabrida* upon rehydration is accompanied by changes in the fatty acid composition. While the amount of the saturated FA decreased, the unsaturated FA were found to increase, thus indicating increased membrane permeability. The changes in the lipid content can be considered as a characteristic feature of PDT *Xerophyta scabrida* and most probably of other PDT species.

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