Chlorophyll fluorescence imaging of photosynthetic activity and pigment contents of the resurrection plants *Ramonda serbica* and *Ramonda nathaliae* during dehydration and rehydration

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Abstract The desiccation-tolerant plants of the *R. serbica* and *R. nathaliae* are resurrection plants which are able to fully recover their physiological function after anabiosis. A comparison of chlorophyll fluorescence imaging and photosynthetic pigment contents responses of *R. serbica* and, for the first time, *R. nathaliae* to dehydration and rehydration were investigated. For this purpose, plants after collection from their natural habitats were kept fully watered for 14 days at natural condition. The experiment was conducted with mature leaves of both species. *R. serbica* and *R. nathaliae* plants were dehydrated to 5.88 % and 7.87 % relative water content (RWC) by withholding water for 15 days, afterwards the plants were rehydrated for 72 hours to 94.67 % and 97.02 % RWC. During desiccation, *R. serbica* plants preserved the chlorophyll content about 84 %, while *R. nathaliae* about 90 %. During dehydration when RWC were more than 40 %, photochemical efficiency of PSII for photochemistry, the *Fv/Fm* ratio, decreased about 40 % in *R. nathaliae* plants, but a strong reduction with 60 % was recorded for *R. serbica*. Following rehydration, the *Fv/Fm* ratio recovered more rapidly in *R. nathaliae*. The higher photosynthetic rates could also be detected *via* imaging the chlorophyll fluorescence decrease ratio *Rfd*, which possessed higher values after rehydration leaves of *R.*

nathaliae as compared to *R. serbica*. The results showed that the photosynthetic activity and chlorophyll contents after rehydration are recovered more rapidly in *R. nathaliae* in comparison to *R. serbica*.

Keywords Resurrection plants · Dehydration-rehydration · Photosynthetic activity · Chlorophyll fluorescence imaging

Abbreviations

RWC Relative water content
Fv/Fm Maximal photochemical efficiency
Rfd Chlorophyll fluorescence decrease ratio

Introduction

Desiccation tolerance occurs throughout the plant kingdom, this process is widespread and is found in most of the taxonomic groups ranging from pteridophytes to dicotyledons but not observed in gymnosperms (Gaff 1971, 1977; Oliver 1996; Alpert 2000). Desiccation tolerance of the vegetative tissues in vascular plants has been demonstrated in some 350 species, making up less than 0.2 % of the total flora (Porembski and Barthlott 2000), but the list is constantly being extended. These desiccation-tolerant plants are also referred to as resurrection plants, because they can be resurrected by rehydration (Toldi et al. 2009). Desiccation-tolerant plants are capable of withstanding severe water loss to almost complete desiccation while maintaining some metabolic functions, and maintaining the ability to quickly restore normal physiological activity upon rehydration (Schwab et al. 1989).

Resurrection or desiccation-tolerant plants may be subdivided into homoiochlorophyllous and poikilo chlorophyllous types (Tuba et al. 1998). During desiccation homoiochlorophyllous plants retain their chlorophyll

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contents while poikilochlorophyllous plants lose their chlorophyll contents which they are still able to re-synthesize following rehydration (Bewley 1979; Gaff 1989; Navari-Izzo and Rascio 1999). In homoiochlorophyllous plants, during dehydration, amounts of chlorophyll are comparable to those of fresh tissue (Navari-Izzo et al. 1994).

The European flowering homoiochlorophyllous desiccation-tolerant plant species are restricted to two genera of the family Gesneriaceae—*Ramonda* and *Haberlea* (Gaff 1971; Müller et al. 1997; Péli et al. 2012). Examples of homoiochlorophyllous are *Ramonda serbica* and *Ramonda nathaliae*, the endemic and relict plants from Balkan Peninsula. These species are good examples of ecophysiological divergence regarding the plant water relations and the adaptations to the conditions of water regime in the habitat. Under the conditions of water deficit in the habitat, these plants gradually wilt and pass to anabiosis.

Previous investigation of *R. serbica* and *R. nathaliae* has brought the evidence to adaptive features necessary to preserve cell membrane integrity (Quartacci et al. 2002), antioxidative capacity (Sgherri et al. 2004; Jovanović et al. 2011), photosynthetic activity (Tuba et al. 1998), CO₂ fixation and chlorophyll *a* fluorescence (Degl'Innocenti et al. 2008), genome size variation and polyploidy (Siljak-Yakovlev et al. 2008), osmotic adjustment (Zivković et al. 2005), seed germination (Gashi et al. 2012a) and *in vitro* cultivation from seeds of *Ramonda* plants (Kongjika et al. 2002; Gashi et al. 2011; Dontcheva et al. 2009; Daskalova et al. 2012).

The present study aims to further investigate the photosynthetic activity responses of *R. serbica* and, for the first time, *R. nathaliae* to dehydration and rehydration. A comparison of chlorophyll fluorescence imaging and photosynthetic pigment contents responses during dehydration and rehydration were made to explore their potential roles in the resurrection phenomenon of these two species.

Materials and methods

Plant material

Resurrection plants of *R. serbica* and *R. nathaliae* of about the same age were collected from their natural habitat together with the layer of soil on which they grew. *R. serbica* plants were collected from Sharri Mountains, near of city Prizren (Kosovo) and *R. nathaliae* plants from Gorge of river Matka, near of city Skopje (F.Y.R. Macedonia). Collected plants were acclimated for 2 weeks keeping them fully watered until the beginning of the experiments. Plants were dehydrated for 15 days by withholding water at room temperature and ambient photoperiod. After 14 days of withholding water, leaves of both species were completely desiccated and plants were in a state of anabiosis. After 5 days in anabiosis, the plants were rehydrated until they recovered their initial

hydrated state. Rehydration was achieved by spraying the plants with water. For analysis, samples of leaves were taken from fully hydrated control plants (C), from plants in different stages of dehydration, after 7 days (D1), 10 days (D2), 14 days (D3) and 15 days (D3), and upon re-watering, after 6 (R1), 12 (R2), 24 (R3) and 48 (R4) hours.

Determination of RWC

Relative water content (RWC) of *Ramonda* leaves was determined at each sampling time during the dehydration and rehydration cycle (D1–R4). Leaves of comparable size (12 replicates) were selected from near the middle of rosettes. Fresh weight (FW) was measured by weighting them before and dry weight (DW) after oven drying for 48 h at 80 °C to a constant mass. Turgid weights (TW) were measured after the leaves had been immersed in distilled water for 24 h at 20 °C in the dark. The above determination was conducted according to Sgherri et al. (1994). Relative water content (RWC) was calculated using the equation:

$$\text{RWC} = [(\text{FW} - \text{DW}) / \text{TW} - \text{FW}] \times 100$$

Photosynthetic pigment determination

Three leaves of similar ages, comparable in size, were taken from five plants from the middle of the rosette to determine photosynthetic pigments content.

The photosynthetic pigments, chlorophylls *a* and *b* as well as carotenoids, were extracted with 80 % acetone. Chlorophyll and carotenoid contents were calculated using absorbance values at 663 nm, 644 nm and 452.5 nm (maximum absorption of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and carotenoids, respectively) measured by a UV–vis spectrophotometer (SECOMAM, Anthelie Advanced 5). The new extinction coefficients and re-evaluated equations of Lichtenthaler were applied (Lichtenthaler 1986; Lichtenthaler and Buschmann 2001a, b). Represented pigment contents were calculated as mg g⁻¹ dry leaf weight (DW).

Chlorophyll fluorescence imaging

Chlorophyll fluorescence imaging was assessed on three leaves of similar ages, comparable in size, taken from five plants from the middle of the rosette.

The chlorophyll (*Chl*) fluorescence induction kinetics of pre-darkened leaves (20 min) was measured using the fluorescence imaging system FlourCam 700MF (Photon Systems Instrument). The fluorescence emission is induced by two sets of 325 super-bright orange light emitting diodes (LED's) (wavelength 605 nm) that provide excitation flashes or a continuous actinic irradiance controlled by defined protocol. Fluorescence images are captured by CCD camera. The

fluorescence images were recorded at 12-bit resolution, in 512×512 pixel. The system was controlled by the FluorCam software. The images of the measured Chl fluorescence intensity were obtained on false colour, whereby black is the lowest (zero) and red the highest fluorescence intensity.

Chlorophyll fluorescence images of parameters as F_o (minimum fluorescence in the dark-adapted states), F_m (maximum fluorescence in the dark-adapted states), F_p , (initial fluorescence increase caused by the actinic light exposure) and F_s , (steady-state fluorescence in actinic light exposure) were recorded applying quenching analysis protocol. Image fluorescence parameters were calculated by FluoCam software as the mean of the fluorescence signals of all pixels over the leaf area.

Images of various Chl fluorescence ratios obtained by pixel to pixel arithmetic operations performed by FluorCam software were: maximum quantum yields of PS II - F_v/F_m and F_m/F_o ; fluorescence decrease ratio (which assess plant vitality) - $Rfd = (F_p - F_s)/F_s$.

Data analysis

The experiment was performed in a randomized design with five replicates. Differences among parameters and between the stages of dehydration-rehydration were tested using SPSS 17 statistical program. Statistical variance analysis of the all data were performed using one-way ANOVA and mean comparison was performed with Duncan Multiple Range Test's at the 5 % level of significance.

Results and discussions

Relative water content

The decrease in relative water content (RWC) of *R. serbica* and *R. nathaliae* leaves during desiccation under our experimental conditions is shown in Fig. 1. Both *Ramonda* species at the start of experiment (Control) had similar RWC (93–96 %). Following dehydration by withholding water for 15 days (D4), the RWC decreased to the value of 5.88 % in *R. serbica* and 7.87 % in *R. nathaliae*. Initial stage of dehydration (D1) started after 7 days, in *R. serbica* decreasing RWC to ~70% while less resistant in the early stages of dehydration showed *R. nathaliae* plants, where the RWC decreased to ~50%. After 10 days (D2) of dehydration RWC decreased to ~30% for two *Ramonda* species. On rewatering, the RWC was restored rapidly, the leaves started to unfold within the first 6 h following initial rehydration, with relative water content reaching to ~70% (R1) in *R. nathaliae* and ~30% in *R. serbica* respectively. Relative water content (RWC) of *R. nathaliae* and *R. serbica* continued to increase to 95–97% (R4), in leaves harvested at 48 h of rewatering. Similar results reported

from other authors. Degl'Innocenti et al. (2008) showed that in *R. serbica*, the RWC decreased from values of 97 % in fully hydrated control plants to values of only 7 % after 2 weeks dehydration. In addition, the RWC of *R. nathaliae* decreased from 94.5 % to 3.6 % during dehydration (Jovanović et al. 2011). Kinetics of relative water content during dehydration-rehydration cycle was reported from other authors in *R. serbica* (Augusti et al. 2001; Quartacci et al. 2002; Veljovic-Jovanović et al. 2006; Sgherri et al. 2004) and *Haberlea rhodopensis* (Nagy-Deri et al. 2011; Mihailova et al. 2011; Djilianov et al. 2011; Georgieva et al. 2010).

Our results of RWC showed that dehydration of *R. serbica* leaves was very slow, especially in the first stage, while it was more rapid in *R. nathaliae*. On other hand, rehydration was restored rapidly in *R. nathaliae* than *R. serbica*. In addition, for differences during first stages of dehydration (D1–D2) and in R1 of both species, we could hypothesize that *R. serbica* and *R. nathaliae* preserved the natural and molecular mechanisms of adaption on their conditions. In this case, mostly in *R. serbica* habitats present around the rosettes, mosses concur to preserve the soil humidity. Mosses are efficient in absorbing the moisture, and at the same time, they play the role of an isolating buffer, which prevents rapid evaporation from the shallow soil (Rakic et al. 2009). *R. serbica* inhabits in more humid and cooler habitats, primarily sheltered by forest canopy, while *R. nathaliae* is found in more open, drier and warmer habitats. These ecological differences showed that *R. nathaliae* is a more xerophilous species than *R. serbica* (Stevanovic et al. 1991). Therefore, the preservation of the RWC in *R. serbica* more than in *R. nathaliae*, in first stage of dehydration (D1), could be as a result of these conditions. Differences between these species are also in the chromosome number and ploidy level. *R. serbica* is polyploid species, while *R. nathaliae* is diploid (Siljak-Yakovlev et al. 2008; Daskalova et al. 2012).

Photosynthetic pigments

The chlorophyll *a* and *b* content slightly decreased upon desiccation of *R. serbica* and *R. nathaliae* leaves and recovered after rehydration (Table 1). Total chlorophyll content on plant control (C) of *R. serbica* was higher than *R. nathaliae*. Similar results were observed by Gashi et al. (2012b), who reported higher chlorophyll amount in *R. serbica* than *R. nathaliae* from different populations. During dehydration, total chlorophyll content of *R. serbica* and *R. nathaliae* did not show significant changes until 14 days after desiccation started (RWC ~30 %). After 15 days of dehydration, total chlorophyll content was decreased and reached the lowest value at RWC ~7 %. At *R. nathaliae* chlorophyll re-synthesis started 6 h (R1) after rehydration and took only 48 h (R4) to regain and exceeded

Fig. 1 Relative water content (RWC) of *R. serbica* and *R. nathaliae* leaves during dehydration and rehydration cycle. Different letters indicate significant differences at the P 0.05 by one-way ANOVA with Duncan multiple range tests. Control plants (C), from plants in different stages of dehydration, after 7 days (D1), 10 days (D2), 14 days (D3) and 15 days (D4), and upon re-watering, after 6 (R1), 12 (R2), 24 (R3) and 48 (R4) hours

the control (fully hydrated plants) values. On other hand, *R. serbica* chlorophyll re-synthesis started 12 h (R2) after rehydration and regained the control values after 48 h (R4). The reduction of total chlorophyll content of *R. serbica* during anabiosis was about 15 % (preserved about 85 %), while *R. nathaliae* preserved more than 89 % of total chlorophyll content during anabiosis. Similar changes during dehydration-rehydration were observed in the carotenoids content of *R. serbica*. In contrast, in *R. nathaliae* leaves during dehydration, carotenoids showed limited change, whereas on rehydration they increased at about 11 % (Table 1).

Similar changes of photosynthetic pigments content during dehydration-rehydration cycle were reported from other authors. In addition, Degl'Innocenti et al. (2008) reported that chlorophyll contents of *R. serbica* did not show significant

changes until RWC=52%, but during anabiosis it reached the lowest value. During dehydration *R. serbica* preserved about 80 % of total chlorophyll, in respect to control plants (Markovska et al. 1994). After rehydration, homoiochlorophyllous resurrection plant increased the chlorophyll content. During rehydration, chlorophyll re-synthesis and their amounts gradually increased and returned to near control levels in fully rehydrated plants of *R. serbica* (Degl'Innocenti et al. 2008; Augusti et al. 2001), *R. nathaliae* (Jovanović et al. 2011) and *H. rhodopesis* (Georgieva et al. 2010; Péli et al. 2012). According to our results and previous investigation from other authors (Dražić et al. 1999; Augusti et al. 2001; Markovska et al. 1994), *R. serbica* and *R. nathaliae* plants may be included among homoiochlorophyllous desiccation-tolerant angiosperms because they retain most of their chlorophyll content during desiccation.

Table 1 Photosynthetic pigments content (mg g⁻¹ dry weight) of *R. serbica* and *R. nathaliae* species during dehydration and rehydration cycle

Parameters	Chl a	Chl b	Total Chl	Carotenoids
<i>R. serbica</i>				
C	2.92 ± 0.09 ^{AB}	1.91 ± 0.04 ^{A-C}	4.83 ± 0.13 ^{AB}	1.02 ± 0.02 ^{BC}
D1	3.45 ± 0.08 ^A	2.31 ± 0.05 ^{AB}	5.76 ± 0.12 ^A	1.10 ± 0.04 ^B
D2	3.42 ± 0.32 ^A	2.40 ± 0.22 ^A	5.82 ± 0.54 ^A	1.32 ± 0.12 ^A
D3	2.51 ± 0.27 ^B	1.63 ± 0.28 ^C	4.15 ± 0.55 ^B	0.92 ± 0.09 ^{BC}
D4	2.47 ± 0.24 ^B	1.60 ± 0.26 ^C	4.08 ± 0.50 ^B	0.89 ± 0.07 ^{BC}
R1	2.29 ± 0.01 ^B	1.64 ± 0.01 ^C	3.94 ± 0.01 ^B	0.92 ± 0.01 ^{BC}
R2	2.44 ± 0.26 ^B	1.51 ± 0.14 ^C	3.95 ± 0.41 ^B	0.84 ± 0.01 ^C
R3	2.79 ± 0.03 ^B	1.83 ± 0.09 ^{BC}	4.63 ± 0.12 ^B	0.93 ± 0.01 ^{BC}
R4	2.89 ± 0.01 ^{AB}	2.00 ± 0.01 ^{A-C}	4.90 ± 0.01 ^{AB}	1.00 ± 0.05 ^{BC}
<i>R. nathaliae</i>				
C	2.56 ± 0.07 ^{CD}	1.63 ± 0.06 ^B	4.19 ± 0.12 ^{CD}	0.76 ± 0.01 ^D
D1	2.47 ± 0.06 ^{DE}	1.63 ± 0.04 ^B	4.10 ± 0.10 ^{CD}	0.86 ± 0.02 ^C
D2	2.53 ± 0.04 ^{CD}	1.68 ± 0.01 ^B	4.22 ± 0.04 ^{CD}	0.99 ± 0.02 ^B
D3	2.31 ± 0.02 ^{EF}	1.63 ± 0.02 ^B	3.94 ± 0.04 ^{C-E}	0.81 ± 0.02 ^{CD}
D4	2.22 ± 0.04 ^F	1.56 ± 0.03 ^B	3.78 ± 0.07 ^{DE}	0.81 ± 0.02 ^{CD}
R1	2.33 ± 0.12 ^{EF}	1.59 ± 0.09 ^B	3.92 ± 0.21 ^E	0.86 ± 0.04 ^C
R2	2.67 ± 0.05 ^C	1.57 ± 0.03 ^B	4.24 ± 0.08 ^C	0.82 ± 0.02 ^{CD}
R3	2.96 ± 0.01 ^B	2.05 ± 0.01 ^A	5.02 ± 0.01 ^B	1.08 ± 0.01 ^A
R4	3.16 ± 0.06 ^A	2.15 ± 0.01 ^A	5.31 ± 0.02 ^A	1.14 ± 0.01 ^A

Control plants (C), from plants in different stages of dehydration, after 7 days (D1), 10 days (D2), 14 days (D3) and 15 days (D4), and upon re-watering, after 6 (R1), 12 (R2), 24 (R3) and 48 (R4) hours. The data are present by mean and standard error (±). Mean in each column followed by same letters are not significantly different at the P 0.05 by one-way ANOVA with Duncan's multiple range tests

Fig. 2 Chlorophyll *a/b* ratio of *R. serbica* and *R. nathaliae* leaves during dehydration and rehydration cycle. Different letters indicate significant differences at the *P* 0.05 by one-way ANOVA with Duncan multiple range

tests. Control plants (C), from plants in different stages of dehydration, after 7 days (D1), 10 days (D2), 14 days (D3) and 15 days (D4), and upon rewatering, after 6 (R1), 12 (R2), 24 (R3) and 48 (R4) hours

For the photosynthetic pigment contents changes were reported on other resurrection plants species following dehydration and rehydration cycle, *Boea hygrometrica* (Deng et al. 2000), *Craterostigma plantagineum* (Scott 2000), *Sporobolus stapfianus* (Quartacci et al. 1997), *Boea hygroskopica* (Navari-Izzo et al. 1995), *Xerophyta* sp. (Sherwin and Farrant 1996; Farrant et al. 2003; Perez et al. 2011), *Reaumuria soongorica* (Xu et al. 2008), *H. rhodopensis* (Nagy-Deri et al. 2011).

Chlorophyll *a/b* ratio of *R. serbica* (Fig. 2) was higher during desiccation stage (D3 and D4) than the control plants (C). For this parameter, between days of dehydration-rehydration, statistical significances were observed. On other hand, the highest chlorophyll *a/b* ratio of *R. nathaliae* was 12 h after rehydration (R2) while the lowest 14 and 15 days (D3 and D4) after dehydration (Fig. 2). At this point, we could hypothesize that the rewatering of *R. nathaliae* was more rapid than *R. serbica* during rehydration (R1–R2), and could have an important role to increase the chlorophyll *a* content and its ratio. According to Degl’Innocenti et al. (2008), during dehydration of *R. serbica* plants the chlorophyll *a/b* ratio was reduced and the ratio was restored to the control level after rehydration. Changes in the chlorophyll

a/b ratio during dehydration-rehydration cycle were also reported by other authors in *H. rhodopensis* (Georgieva et al., 2005, 2010).

Imaging of chlorophyll fluorescence and photosynthetic activity

Image fluorescence parameters during induction kinetics at the maximum fluorescence *F_m*, ground fluorescence *F_o* and variable fluorescence *F_v* in the dark-adapted state were decreased during dehydration stage (D2) of *R. serbica* and *R. nathaliae* leaves and quickly recovered after 24 h (R3) (Table 2). After rehydration, these parameters increased above the control level in *R. serbica*, while in *R. nathaliae* exceeded values of control plants (Table 2).

Fluorescence images at *F_m* displayed at the same pseudoscale clearly showed changes of the values of this parameter and their distributions related to the heterogeneity over leaf area between leaves in different status of RWC of *R. serbica* and *R. nathaliae* (Fig. 5-*F_m*). Chlorophyll fluorescence images measured by this method are very important for resurrection plants because it shows the photosynthetic

Table 2 Induced fluorescence image parameters and their ratios of *R. serbica* and *R. nathaliae* leaves grown in conditions of control-full hydrated (C), dehydration after 10 days (D2) and rehydration after 24 hours (R3)

Parameters	<i>R. serbica</i>			<i>R. nathaliae</i>		
	C	D2	R3	C	D2	R3
F _o	120.84 ^A ±2.71	88.20 ^B ±9.14	126.60 ^A ±8.09	146.09 ^A ±1.72	79.12 ^B ±6.79	151.98 ^A ±2.38
F _m	566.83 ^A ±6.43	132.07 ^B ±8.64	523.01 ^A ±47.91	577.87 ^A ±6.08	146.33 ^B ±20.85	632.75 ^A ±11.66
F _v	445.93 ^A ±6.57	43.87 ^B ±6.84	396.40 ^A ±39.89	431.77 ^A ±7.43	67.21 ^B ±14.18	480.76 ^A ±9.59
F _m /F _o	4.69 ^A ±0.11	1.52 ^C ±0.12	4.11 ^B ±0.11	3.96 ^A ±0.08	1.82 ^B ±0.11	4.16 ^A ±0.03
F _v /F _m	0.79 ^A ±0.01	0.33 ^B ±0.05	0.76 ^A ±0.01	0.75 ^A ±0.01	0.45 ^B ±0.03	0.76 ^A ±0.01

The data are present by mean and standard error (±). Mean in each rows followed by same letters are not significantly different at the *P* 0.05 by one-way ANOVA with Duncan’s multiple range tests

activity for all leaf area and differences within each leaves (Fig. 5).

Induction kinetics of chlorophyll *a* fluorescence is often used to monitor in photochemical activities of photosystems (Babani and Lichtenthaler 1996; Lu et al. 1994). Resurrection angiosperms show decrease in chlorophyll *a* fluorescence during dehydration and rapid recovery upon rehydration (Wen-Long et al. 2003).

Two Chl fluorescence ratios F_v/F_m and F_m/F_o were applied to characterize the maximum quantum yield of PSII photochemistry of the photosynthetic apparatus, i.e. quantum efficiency if all PSII centers are opened (Babani and Lichtenthaler 1996; Kitajima and Butler 1975). Many studies have indicated that the photochemical reactions are sensitive indicators to study the physiological state of resurrection plants during desiccation and rehydration (Sherwin and Farrant 1998; Peeva and Cornic 2009). The loss of PSII function correlates with the loss of power to generate potentially damaging reactants and therefore

Fig. 3 Image fluorescence ratios (F_m/F_o and F_v/F_m) of *R. serbica* and *R. nathaliae* leaves grown in conditions of control-full hydrated (C), dehydration after 10 days (D2) and rehydration after 24 hours (R3)

Fig. 4 Fluorescence decline ratio image Rfd of leaves of *R. serbica* and *R. nathaliae* grown in conditions of control-full hydrated (C), dehydration after 10 days (D2) and rehydration after 24 hours (R3) (pseudoscale 0–4)

it acts as a sensor for stress (Van Rensen and Curwiel 2000). The significant decrease in PSII activity in *R. serbica* leaves upon desiccation was suggested to be a protective mechanism to maintain membrane integrity (Degl'Innocenti et al. 2008).

Our results showed that water stress had significant effect on the Fv/Fm and Fm/Fo ratios of *R. nathaliae* and *R. serbica* (Fig. 3 and Table 2). During dehydration (D2), Fv/Fm and Fm/Fo ratios decreased about 40 % in *R. nathaliae*, and more strongly, about 60 % in *R. serbica*. Following 24 h (R3) rehydration of *R. serbica* the Fv/Fm and Fm/Fo ratio increased considerably but it was still lower than that of control. On the other hand, in *R. nathaliae* the Fv/Fm and Fm/Fo ratio increased after rehydration more than the control (C1) plants (Fig. 3 and Table 2). Similar results were reported by Augusti et al. (2001), Fv/Fm ratio of *R. serbica* was significantly decreased after RWC was 30–40 % and increased 24 h after rehydration. In addition, Péli et al. (2005) showed that Fv/Fm ratio of *H. rhodopensis* decreased after 7–10 days of dehydration. Restoration of Fv/Fm ratio after 24 h of rewatering was reported at other resurrection plant *X. scabrada* (Perez et al. 2011). In leaves of *B. hygrometrica*, the Fv/Fm declined from about 0.8 to 0.4 with change in relative water content from 100 % to 1 % (Deng et al. 2000), after that the values of Fv/Fm ratio almost restored to that of normal control leaves upon rehydration. Differences in values of Fv/Fm ratio and other photosynthetic parameters during dehydration and rehydration cycle between *R. serbica* and *R. nathaliae* are in accordance with

other species that are within genera of *Xerophyta*. In addition, Bhatt et al. (2009) reported some differences of Fv/Fm ratio and RWC between *X. viscosa* and *X. retinervis* during dehydration and rehydration cycle.

The values of Rfd ratio as plant vitality indicator demonstrated that full green leaves can be characterized by the high photosynthetic activity (Lichtenthaler and Rinderle 1988). In this case, the Rfd ratio was higher in control plants of *R. nathaliae* than *R. serbica*. During dehydration the Rfd ratio decreased strongly in *R. serbica* and *R. nathaliae*, and 24 h (R3) after rewatering this parameter increased more than the initial values of control plants of *R. serbica* and especially in *R. nathaliae* (Fig. 4 and 5 - Rfd). In addition, significant changes of Rfd ratio were reported from other authors in *R. serbica* (Markovska et al. 1994) and *H. rhodopensis* (Georgieva et al. 2005, 2008).

Conclusions

Based on the obtained results, conclusions are as follows:

Decrease of relative water content (RWC) of leaves from values of 93–96 % in fully hydrated (control plants) to values of only 4–7 % after desiccation were observed at both of *Ramonda* species. After rehydration, plants showed faster recovery of their water content, regaining 95–97% RWC after 48 h. For this parameter, *R. serbica* at

Fig. 5 The fluorescence decline ratio image Rfd leaves and maximum Chl fluorescence in the dark-adapted state Fm of *R. serbica* and *R. nathaliae* grown in conditions of control-full hydrated (C), dehydration after 10 days (D2) and rehydration after 24 hours (R3) (pseudoscale 0–4)

the first stage of dehydration was more resistant, while in *R. nathaliae*, the RWC recovered more rapidly after rehydration.

According to our results, *R. serbica* and *R. nathaliae* plants may be included among homoiochlorophyllous desiccation-tolerant angiosperms because they retain most of their chlorophyll content during desiccation (85 % and 89 %, respectively).

During dehydration (D2), the *Fv/Fm* and *Fm/Fo* ratios decreased about 40 % in *R. nathaliae*, and more strongly, about 60 % in *R. serbica*. Full recovery of photosynthetic activity a few hours after rehydration indicates that *R. serbica* and *R. nathaliae* leaves completely restore their thylakoid membrane integrity and PSII activity, depending on the severity of the stress.

Moreover, the present results suggest that *R. nathaliae* was more resistant during desiccation than *R. serbica*, and recovered more rapidly to normal physiological activity after rewatering.

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