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ORIGINAL ARTICLE

Rooting affects the photosystem II activity: in vitro and ex vitro studies on energy hybrid sorrel

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Abstract *Rumex tianschanicus* × *Rumex patientia* is a high-biomass-yielding plant suitable for fuel and biogas production. The protocol of the hybrid sorrel micropropagation was used to study the changes in the photosystem II (PSII) activity as well as to analyse the ultrastructure of the chloroplasts. The lowest effective PSII quantum yield [Y(II)] and an apparent electron transport rate of PSII [ETR(II)] were observed for adventitious shoots that had been regenerated in vitro, before rooting. These fluorescence parameters were higher and similar for both the leaves of the same adventitious shoots that had been rooted under in vitro conditions and for the shoots that had been acclimated and grown in ex vitro conditions. The analysis indicated that the PSII activity strongly depends on the formation of properly functioning roots and that in vitro or ex vitro culture conditions are, at least to some degree, less important. TEM analysis revealed that chloroplasts from plants rooted in vitro were sufficiently mature and acclimatization processes have less impact on their development. This is the first report concerning the analysis of PSII activity and the ultrastructure of the chloroplasts at all of the stages of micropropagation, i.e. adventitious shoot formation in vitro, rooting in vitro and acclimation to ex vitro conditions. It strongly indicated that rooting under in vitro conditions, rather than the acclimation to ex vitro conditions, plays a key role in the development of a completely functional photosynthetic apparatus in hybrid sorrel.

Keywords *Rumex tianschanicus* × *Rumex patientia* · PSII activity · Chlorophyll *a* fluorescence · Ultrastructure of chloroplasts · In vitro/ex vitro transfer · Photosynthetic apparatus

Introduction

Rumex tianschanicus × *Rumex patientia,* which is a high-biomass-yielding plant, is a cross between English spinach (*R. patientia* L.) as the female line and Tien Shan sorrel (*R. tianschanicus* A. Los.) as the male line (Havlíčková and Suchý 2010). The sorrel, which is also known as the sorrel of Uteush, is one of many newly introduced energy crops that are suitable for manufacturing pellets and briquettes and for producing biogas (Ust'ak and Ust'aková 2004; Myšková et al. 2011; Heděnec et al. 2014). This perennial plant can be harvested dry and can produce high yields continuously for more than 10 years. It also tolerates a broad range of fertilization regimes, climatic conditions and soil types (Heděnec et al. 2015). Moreover, it belongs to the species that are heavy metal tolerant and, therefore, suitable for the phytoremediation of contaminated soils (Zhuang et al. 2005). The sorrel of Uteush is also known as medicinal plant due to the presence of biologically active substances such as carotenoids, ascorbic acid, linolenic acid and flavonoids (Omarova et al. 1998).

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We developed a reproducible and efficient method for the micropropagation of hybrid sorrel through the direct induction of adventitious shoot buds from the hypocotyls (confirmed by histological, SEM and cytometric analyses) (Ślesak et al. 2014). This regeneration protocol could be useful for the rapid clonal propagation of genetically uniform plant material, physiological and biochemical studies or for genetic transformation to improve the properties of this energy plant. The regenerated plantlets were successfully rooted and acclimated to ex vitro conditions with an almost 100% survival rate. It is commonly known that the transfer from in vitro to ex vitro conditions requires adaptation to the new stressful conditions (Pospíšilová et al. 1999; Apóstolo et al. 2005; Joshi et al. 2006; Osório et al. 2010). During this acclimation, plants undergo physiological and morphological changes that enable them to grow in the new environment (Pospíšilová et al. 1999). In the case of many species, the low ex vitro acclimation of regenerated plantlets results from the low photosynthetic capacity that is developed in vitro (Apóstolo et al. 2005; Joshi et al. 2006). The specific environmental conditions inside culture vessels, including high air humidity, the presence of a medium usually supplemented with sugar, low gas exchange, ethylene production and low photosynthetic photon flux density may result in physiological anomalies in the micropropagated plants, such as decreased photosynthetic efficiency, poor water loss control, difficult rooting and a low functionality of the developed roots (Pierik 1990; Pospíšilová et al. 1992, 2000; Kadleček et al. 2001).

Chlorophyll *a* fluorescence is a non-invasive technique that can give detailed information on the photosystem II (PSII) activity and is often used in experimental plant biology. Analysis of the chlorophyll *a* fluorescence induction curves allows to determine physiological conditions that affect photosynthetic electron transport chain in the proximity of PSII (Baker 2008; Murchie and Lawson 2013; Kalaji et al. 2014, 2016, 2017).

The higher biomass production of *R. tianschanicus* × *R. patientia* as an energy plant compared to its parental lines may suggest an enhanced photosynthetic capacity in hybrid form. An analysis of the photosynthetic activity of the hybrid sorrel under in vitro and ex vitro conditions during micropropagation that is based on chlorophyll *a* fluorescence could serve as a source of valuable information related to the development of the photosynthetic apparatus, which is a distinguishing feature of the sorrel of Uteush as an energy plant.

The aim of the present study was to investigate the development of the photosynthetic apparatus of *R. tianschanicus* × *R. patientia* as well as to analyse the ultrastructure of the chloroplasts to answer the following questions:

1. During which culture stage the development of an efficient photosynthetic apparatus takes place—adventitious shoots rooting under in vitro conditions or the adaptation of regenerated plantlets to ex vitro conditions?
2. Is the transfer from in vitro to ex vitro conditions really a critical point in the development of the photosynthetic apparatus during the micropropagation of the hybrid sorrel?

Materials and methods

Plant material and culture conditions

The micropropagation protocol for *R. tianschanicus* × *R. patientia* developed by Ślesak et al. (2014) was used. Hypocotyls that were derived from 7-day-old seedlings were cultured on MS medium (Murashige and Skoog 1962) that had been supplemented with 0.17 mg/l indole-3-acetic acid (IAA), 2.2 mg/l benzylaminopurine (BAP) and 2% sucrose. The regenerated adventitious shoots were rooted on ½ MS medium that had been supplemented with 0.5 mg/l indole-3-butyric acid (IBA) and 2% sucrose after which the plantlets were acclimated to ex vitro conditions. The in vitro cultures and acclimated plantlets were incubated in the same growth room to maintain the same light and temperature conditions: 26 ± 3 °C, a 16-h photoperiod at 100 µmol m^{-2} s^{-1} of white light (cool-white fluorescent tubes).

For the analyses of the chlorophyll *a* fluorescence and the TEM analyses of the ultrastructure of the chloroplasts, plant material (detached leaves) from the following culture stages (Fig. 1a) was used:

1. adventitious shoots that had been regenerated in vitro (12 weeks from the beginning of the culture);
2. the same adventitious shoots that had been rooted under in vitro conditions (17 weeks from the beginning of the culture)—for control the analyses were also done on unrooted adventitious shoots of the same age (Fig. 1b);
3. the same adventitious shoots that had been acclimated and grown under ex vitro conditions for 5 weeks (22 weeks from the beginning of the culture).

Chlorophyll *a* fluorescence

To determine PSII efficiency, a Dual PAM 100 fluorescence system (Heinz Walz GmbH, Effeltrich, Germany) was used. PSII activity was measured by means of the chlorophyll *a* fluorescence on leaves that had been adapted to darkness for 20 min. Dark fluorescence yield (F_o) and maximal fluorescence yield (F_m) were determined by use

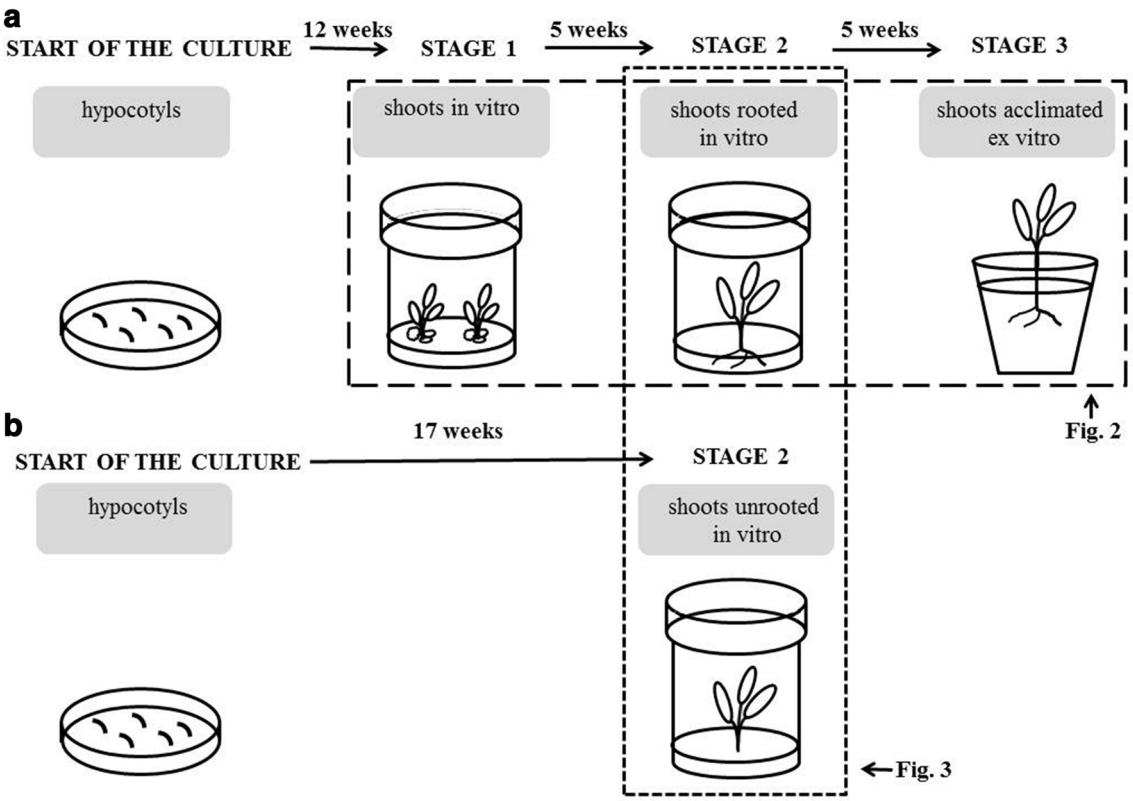

Fig. 1 Scheme of the experiment: **a** hypocotyls that were derived from 7-day-old seedlings were used as the explants, for the chlorophyll *a* fluorescence and TEM analyses of the ultrastructure of the chloroplasts, plant material (detached leaves) from the following culture stages was used—stage 1: adventitious shoots that had been regenerated in vitro (12 weeks from the beginning of the culture), stage 2: the same adventitious shoots rooted under in vitro conditions (17 weeks from the beginning of the culture), stage 3: the same adventitious shoots acclimated and grown in ex vitro conditions for 5 weeks (22 weeks from the beginning of the culture), **b** as the control, the culture was established from 7-day-old seedlings and plant material (detached leaves) that was derived from unrooted adventitious shoots grown under in vitro conditions (17 weeks from the beginning of the culture, the same age as the shoots that had been rooted under in vitro conditions—see stage 2), scheme (**a**) and (**b**) was used for the chlorophyll *a* fluorescence analysis. The scheme of the measurements (results shown in Figs. 2, 3) is indicated by a *dotted line* for better clarity

of weak red light (5 µmol m^{-2} s^{-1}) and strong red light (10^4 µmol m^{-2} s^{-1}) saturating pulses, respectively. All measurements were performed in a light curve with slow kinetic induction curve mode. Therefore, maximal fluorescence yield ($F_{m'}$) and fluorescence yield (F) were measured. The quantum efficiency of PSII in the light-adapted state [$Y(II) = (F_{m'} - F)/F_{m'}$] and apparent electron transport rate of PSII [$ETR(II) = ((F_{m'} - F)/F_{m'}) \times$ intensity of photosynthetically active radiation (PAR) $\times$ 0.5 $\times$ leaf absorptivity] were determined according to Genty et al. (1989) and White and Critchley (1999); the leaf absorptivity was assumed to be 0.84. Nonphotochemical quenching of PSII fluorescence [$NPQ = (F_m - F_{m'})/F_{m'}$] and the quantum yield of regulated energy dissipation [$Y(NPQ) = 1 - Y(II) - 1/(NPQ + 1 + qL(F_m/F_o - 1)$, where qL is coefficient of photochemical quenching] of the PSII fluorescence were quantified according to Kramer et al. (2004). At stages 1 and 2 of the culture (see above; Fig. 1), the leaves were dark adapted under sterile conditions. The Magenta® vessels were opened inside a laminar chamber, the leaves were wrapped in sterile foil for 20 min and then the leaves were detached and immediately placed in the fluorescence system. All of the above-mentioned fluorescence parameters were recorded as rapid light curves at ten steps (the duration of each step was 30 s) of illumination with actinic, i.e. PAR light of an increasing intensity from 11 to 830 µmol m^{-2} s^{-1} (Figs. 2, 3).

TEM analysis

Leaves from the adventitious shoots at each culture stage (Fig. 1a) were detached synchronously with the measurements of PSII activity. Leaf segments (1 mm^2) were cut from the middle part of the leaves for the TEM observation. These segments were then fixed in 2.5% formaldehyde (prepared from paraformaldehyde) and 2.5% glutaraldehyde in a 0.05 M cacodylate buffer (pH 7.0) for

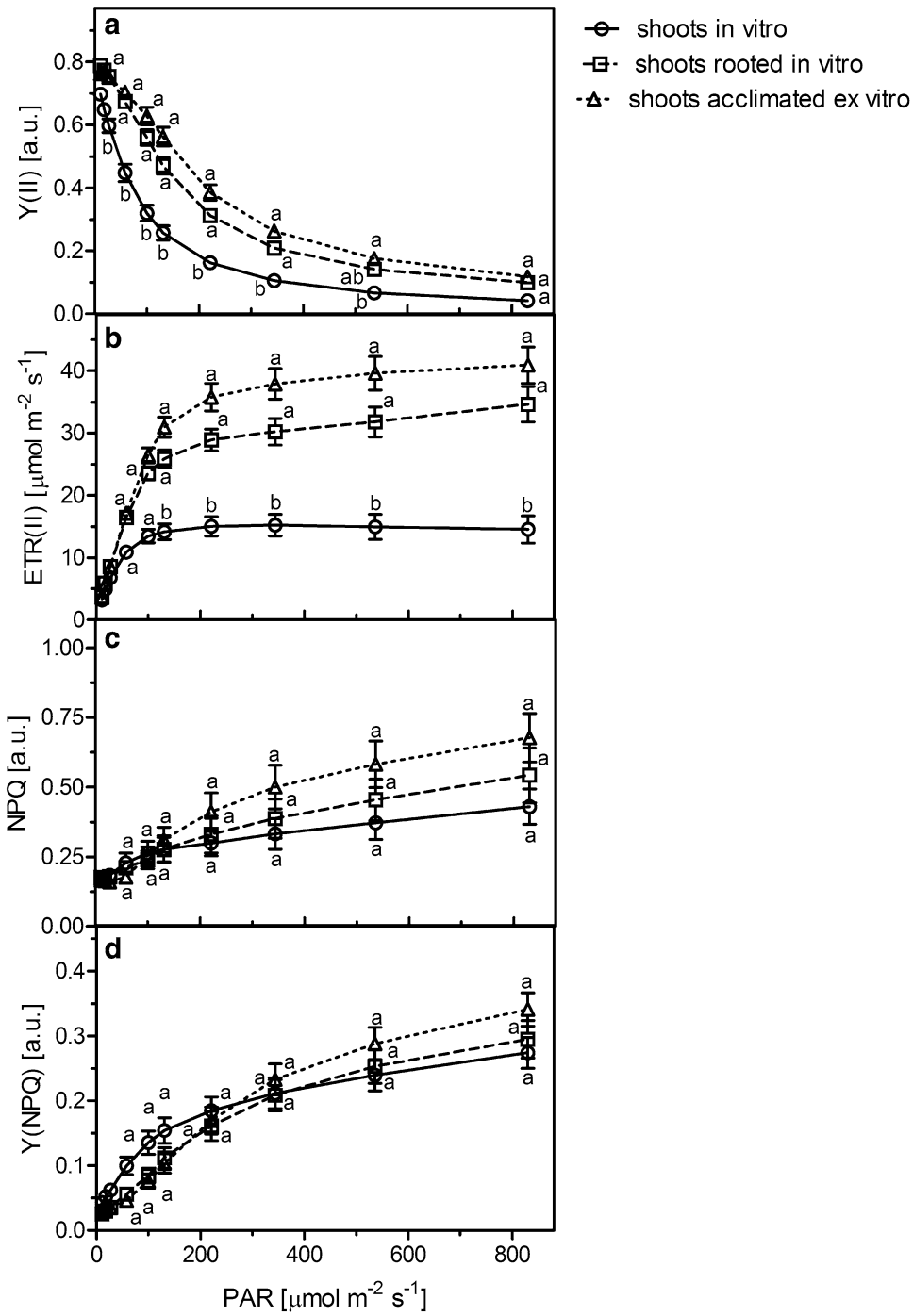

Fig. 2 Light curves for effective PS II quantum yield (Y(II)) (**a**), apparent electron transport rate of PSII [ETR(II)] (**b**), nonphotochemical quenching (NPQ) (**c**), quantum yield of regulated energy dissipation [Y(NPQ)] (**d**) measured at each of the three analysed stages of the *R. tianschanicus* × *R. patientia* culture: in vitro-regenerated adventitious shoots (shoots in vitro), the same adventitious shoots that had been rooted under in vitro conditions (shoots rooted in vitro) and acclimated and grown in ex vitro conditions (shoots acclimated ex vitro) (for details see Fig. 1a); *PAR* photosynthetically active radiation; data are mean ± SE ($n = 8$–15). The *same letters above/near the bars* indicate no significant differences between means, at least at the level of $P < 0.05$. Two-way ANOVA and the Tukey's/Sidak's post tests for the statistical analysis of data were used

4 h at room temperature. The procedure for preparing the samples for TEM was as described earlier (Kowalkowska et al. 2012). The material was dehydrated in a series of graded acetone and embedded in Spurr's low-viscosity resin (Polysciences, Germany). Ultrathin (50–90 nm) sections were cut with a diamond knife on a Leica EM UC7 ultramicrotome. The sections were stained with uranyl acetate and lead citrate and then viewed using a Philips CM 100 transmission electron microscope at 80 kV.

Statistical analysis

Statistics for all of the results are presented as the mean ± SE (standard error). Two-way ANOVA and the Tukey's/Sidak's post tests for the statistical analysis of data were used. Statistical analysis was performed using the GraphPad Prism (v. 5.03 and v.7.0) software.

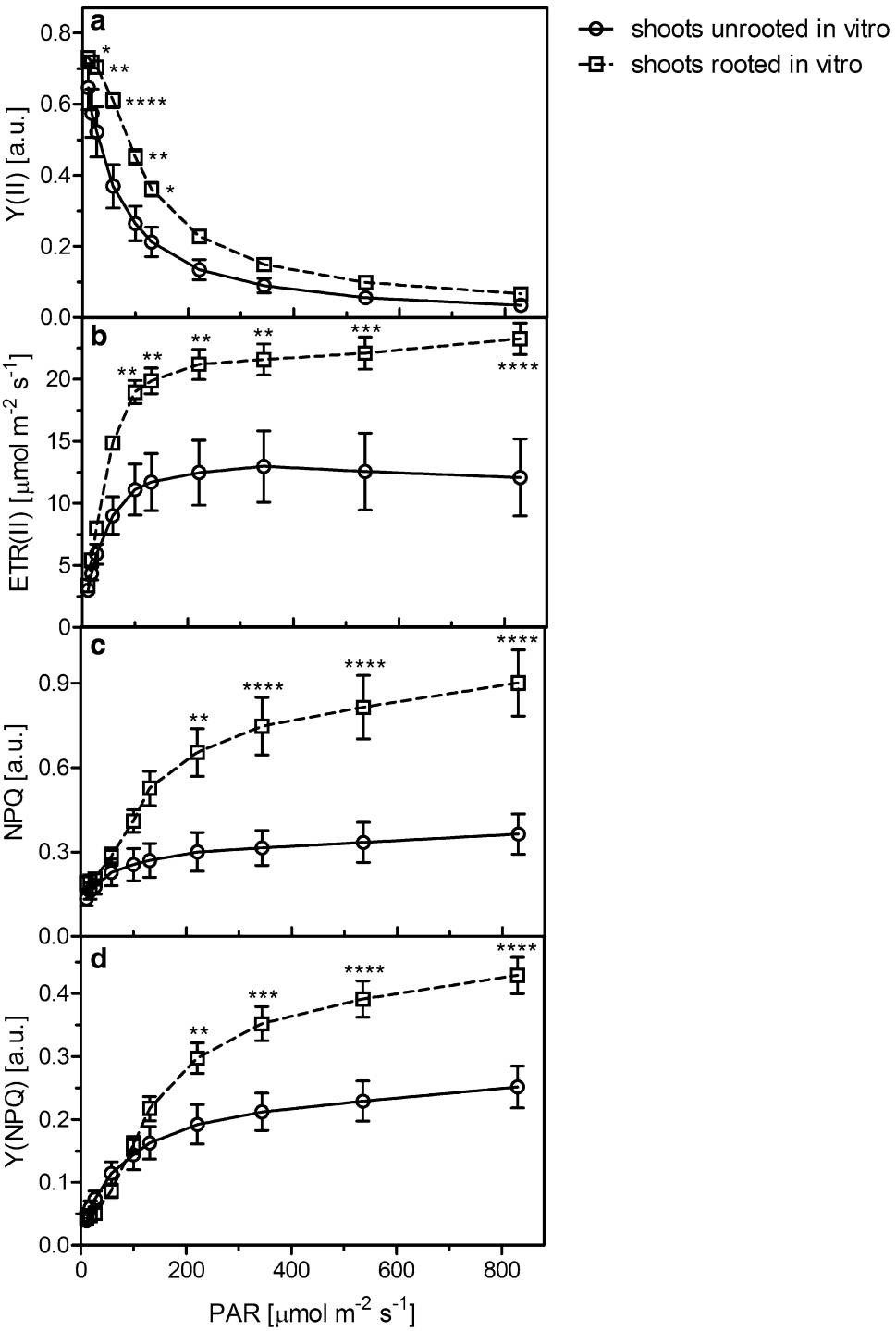

Fig. 3 Light curves for effective PSII quantum yield [Y(II)] (**a**), apparent electron transport rate of PSII [ETR(II)] (**b**), nonphotochemical quenching (NPQ) (**c**), quantum yield of regulated energy dissipation [Y(NPQ)] (**d**) measured in rooted (shoots rooted in vitro) and unrooted (shoots unrooted in vitro) adventitious shoots of the same age, grown under in vitro conditions (17 weeks from the beginning of the culture) (for details see Fig. 1a, b); *PAR* photosynthetically active radiation; data are mean ± SE ($n = 8$–15). *Asterisks* indicate significant differences between means, at the level of $P < 0.05$ (*), $P < 0.1$ (**), $P < 0.01$ (***), $P < 0.001$ (****). Two-way ANOVA and the Tukey's/Sidak's post tests for the statistical analysis of data were used

Results

Chlorophyll *a* fluorescence

Statistical analysis of the fluorescence parameters, which were measured at all of the stages of the *R. tianschanicus* × *R. patientia* micropropagation procedure (i.e. formation of adventitious shoots in vitro, rooting in vitro and acclimation to ex vitro conditions; Fig. 1a), revealed that the lowest values of Y(II) (Fig. 2a) and apparent electron transport rate of PSII [ETR(II)] (Fig. 2b) were found in the adventitious shoots that had been

regenerated in vitro, before rooting (12 weeks from the beginning of the culture). On the other hand, Y(II) and ETR(II) were higher and were similar for both the leaves of the same adventitious shoots, but that had been rooted under in vitro conditions (17 weeks from the beginning of the culture; Figs. 1, 2a, b) and for the same shoots that had been acclimated and grown in ex vitro conditions for 5 weeks (22 weeks from the beginning of the culture). A trend that the shoots acclimated ex vitro had a slightly higher photosynthetic capacity [ETR(II)] compared to rooted shoots growing under in vitro conditions was only observed, but the differences were statistically insignificant (Fig. 2b). Y(II) and ETR(II) showed that linear electron flux through PSII at the given PAR was more intensive after root formation and that PSII has a higher photosynthetic capacity in a wider range of PAR intensities. However, the lack of significant differences between all of the analysed plant samples for NPQ and quantum yield of regulated energy dissipation [Y(NPQ)] (Fig. 2c, d) demonstrates that some of the processes that are associated with nonradiative energy dissipation in PSII were not affected by the growth conditions. The above-analysed samples were at various ages (Fig. 1). Therefore, to eliminate an age effect, we decided to determine the chlorophyll fluorescence for plants of the same age. An analysis of the PSII photochemistry of the leaves that had been detached from the adventitious shoots that had been rooted under in vitro conditions and of the adventitious shoots of the same age (17 weeks from the beginning of the culture), but that were unrooted (Fig. 1b) revealed that most of the differences between the values of the analysed fluorescence parameters were statistically significant (Fig. 3). Y(II) (Fig. 3a), ETR(II) (Fig. 3b), NPQ (Fig. 3c) and Y(NPQ) (Fig. 3d) were significantly higher for the in vitro-rooted adventitious shoots compared to shoots of the same age that had been grown in vitro without roots. Strong differences in parameters linked to nonradiative energy dissipation in PSII, i.e. NPQ, and Y(NPQ) between rooted and unrooted shoots, even more clearly than data presented in Fig. 2, demonstrated a substantial disruption of PSII activity that includes photochemistry and nonphotochemical reactions (Fig. 3c, d).

In the next step, the ultrastructure of the leaf chloroplasts from unrooted, rooted and acclimated (ex vitro) shoots was analysed.

The ultrastructure of the chloroplasts

TEM analysis of the mesophyll chloroplasts revealed strong differences in the ultrastructure of the chloroplasts in the leaves of the adventitious shoots that had been regenerated in vitro compared to the same shoots that had been rooted in vitro and then acclimated ex vitro, but not between the chloroplasts of the last two.

There were visible morphological differences in the shape of the chloroplasts, the organization of the internal membrane system and the presence or absence of starch grains (Fig. 4). The mesophyll chloroplasts derived from the leaves (12 weeks from the beginning of the culture) of the adventitious shoots that had been regenerated in vitro were elongated. The grana, which were connected with long stromal thylakoids, were thin and they mostly consisted of five to eight thylakoids. Starch grains were not usually present in the stroma. Moreover, a few plastoglobules were visible (Fig. 4a). The vesicles and cytoplasmic materials, which had varying degrees of disorganization, were observed in the vacuoles, as well as multivesicular bodies were also noted (Fig. 4a).

A TEM analysis of the chloroplasts derived from the leaves of the same adventitious shoots that had been rooted under in vitro conditions (17 weeks from the beginning of the culture) revealed that their size and ultrastructure was the same compared to the chloroplasts in the leaves that were detached from the same adventitious shoots, but that had been acclimated and grown in ex vitro conditions for 5 weeks (22 weeks from the beginning of the culture) (Fig. 4b, c). In both cases, the chloroplasts were convex towards the vacuole and flattened towards the cell wall. Three to four relatively large starch grains were observed inside the stroma. The stromal thylakoids were short and very dense. The grana were built of densely positioned thylakoids, on average 7–19 per granum. All of the thylakoids had a longitudinal orientation in relation to the main axis of the chloroplasts. Numerous plastoglobules were also observed and mitochondria, which were located next to the chloroplasts, were visible (Fig. 4b, c).

In general, changes in the ultrastructure of the chloroplasts in *R. tianschanicus* × *R. patientia* during the micropropagation procedure showed that root development is associated with the photosynthetic activity of the chloroplasts and seems to have a positive effect on chloroplasts development.

Discussion

The analyses of selected chlorophyll *a* fluorescence parameters and the development of the photosynthetic apparatus in leaves from exactly the same shoots of *R. tianschanicus* × *R. patientia* at all stages of micropropagation were performed. According to Kalaji et al. (2016), the recent development of chlorophyll *a* fluorescence measurement methods represents a valuable new approach that can be used to study the photochemical efficiency of

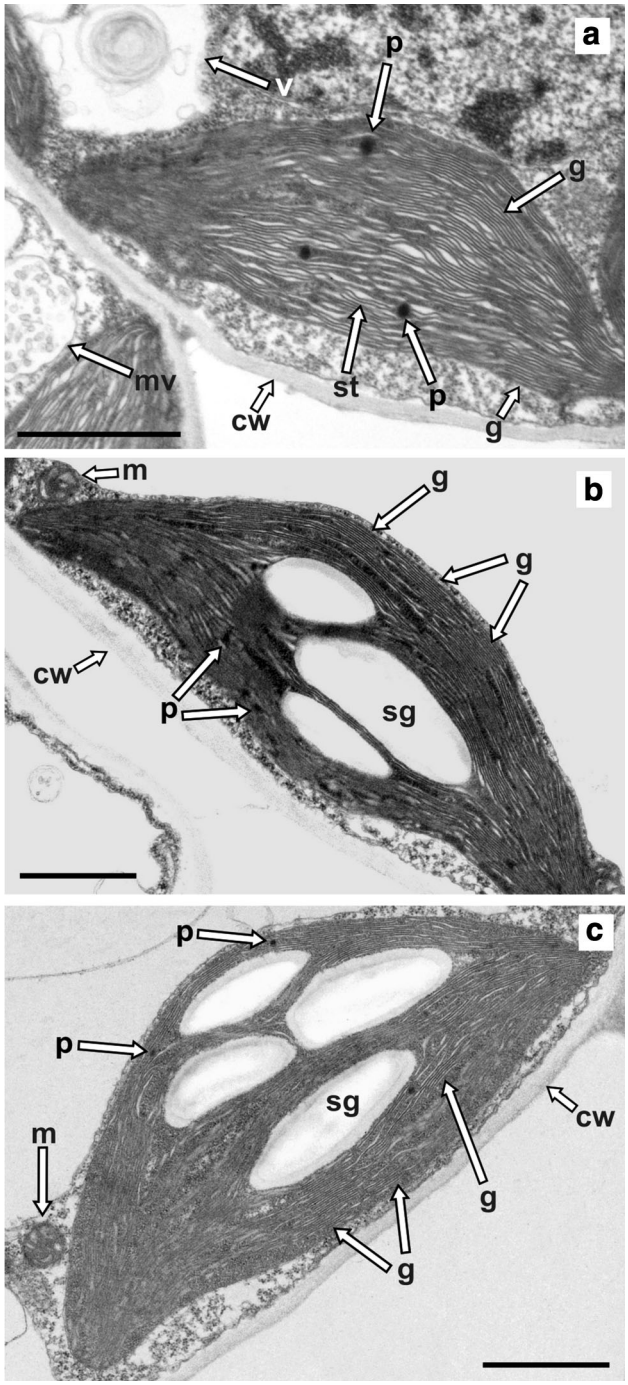

Fig. 4 Electron micrographs of the chloroplasts of the *R. tianschanicus* × *R. patientia* leaves that were detached from the adventitious shoots at each culture stages: adventitious shoots that had been regenerated in vitro (**a**), the same adventitious shoots that had been rooted under in vitro conditions (**b**) and acclimated and grown in ex vitro conditions (**c**); *cw* cell wall, *g* granum, *m* mitochondrion, *mv* multivesicular body, *p* plastoglobules, *sg* starch grains, *st* stromal thylakoids, *v* vacuole. *Bar* 1 µm

leaves and provide details on the status and function of the PSII reaction centres, the donor and acceptor sides of PSII and light-harvesting antenna complexes. Because photosynthesis is sensitive to environmental factors, fluorescence measurements are important components of plant stress studies (Kalaji et al. 2012; Murchie and Lawson 2013).

It was shown during our experiments that rooting plays a crucial role in the PSII activity. Y(II) and ETR(II) were similar for the leaves of the same adventitious shoots that had been rooted under in vitro conditions and for the same shoots that had been acclimated and grown in ex vitro conditions for 5 weeks. Conversely, Sáez et al. (2012) reported that in *Castanea sativa* plants, the net photosynthetic rate of microshoots that had been rooted in vitro was limited by the Rubisco carboxylation capacity and/or RuBP regeneration in the Calvin–Benson–Bassham (CBB) cycle. They also found low ETR(II). Kadleček et al. (2001) observed a decrease in the starch content and fluorescence parameters several days after transferring plantlets from in vitro to ex vitro conditions. These were interpreted as stress reactions to the shock connected with the removal of the roots from the agar medium and transplanting the plantlets into soil. During this procedure, the root hairs may be damaged and, therefore, the absorption of nutrients and water by the roots could be temporarily impaired. In the case of the hybrid sorrel, the micropropagated in vitro plantlets were acclimated to the ex vitro conditions with almost a 100% survival rate. The analysis of the PSII photochemistry made during our experiments on hybrid sorrel leaves that were detached from the adventitious shoots that had been rooted under in vitro conditions and of adventitious shoots of the same age, which were unrooted, revealed that Y(II), ETR(II), NPQ and Y(NPQ) were significantly higher for the in vitro-rooted adventitious shoots compared to the shoots of the same age that had been grown in vitro without roots. The data that were obtained during our experiments indicated that PSII activity in *R. tianschanicus* × *R. patientia* strongly depends on the formation of properly functioning roots and that in vitro or ex vitro culture conditions are of secondary importance.

It is well known that in vitro culture conditions are significantly different compared to field conditions. This can be a source of stress for the plants (Us-Camas et al. 2014). Most in vitro cultures are characterized by low light intensity, increased humidity, poor gas exchange and the presence of sugars in the culture medium. Under in vitro conditions, sugars play specific roles (Sami et al. 2016). Exogenous sugar in the plant system could suppress photosynthetic gene expression and reduce CBB cycle enzymes, leading to low photosynthetic rate (Matysiak and Gabryszewska 2016 and the literature therein). However, in some cases, exogenous sugars improve photosynthesis in vitro and facilitate acclimatization to ex vitro conditions. The effects of sugar can be concentration dependent. In *Arabidopsis thaliana*, exogenous sugars (3%) present in the

medium showed a positive effect on photosynthesis (Eckstein et al. 2012). However, according to Van Dingenen et al. (2016) the transfer of seedlings to sucrose-supplemented medium resulted in significant differences in chloroplast morphology. They were smaller and showed fewer differentiated thylakoid membranes and starch granules compared with control leaves. Sucrose treatment also negatively affects chloroplast division. It was hypothesized that, during early leaf development in *A. thaliana*, exogenously applied sucrose or sucrose produced by source leaves delays chloroplast differentiation in sink leaves, by which cell expansion is postponed and cell proliferation is stimulated (Van Dingenen et al. 2016).

During our experiments, both the regeneration medium and the rooting medium were supplemented with 2% sucrose. Despite the same concentration of sugar in the medium, TEM analysis of the mesophyll chloroplasts of the adventitious *R. tianschanicus* × *R. patientia* shoots revealed strong differences in the ultrastructure of the chloroplasts in the leaves of the adventitious shoots that had been regenerated in vitro, before rooting. Similar to Scheuring et al. (2011), we observed in this case vesicles and cytoplasmic materials in the vacuoles as well as multivesicular bodies.

The structural organization of the chloroplasts in in vitro-cultivated plants is a very important indicator for evaluating the regenerative potential of plant species (Stefanova et al. 2013, 2015). Many studies have indicated that in vitro conditions can alter the shape of the chloroplasts, an accumulation of plastoglobuli, starch or an irregular orientation of the thylakoid system (Majada et al. 2002; Stefanova et al. 2015; Ladygin et al. 2008). Such abnormalities are considered to be responsible for the decreased photosynthetic capacity of regenerated plantlets. Moreover, it has been stated that, e.g. *Stevia* plants had only a slight ability to accumulate starch because of their lower photosynthetic rate as a result of having been grown in closed vessels in which there is a slow diffusion of gases (Ladygin et al. 2008). Sáez et al. (2012) reported that in *C. sativa*, the chloroplasts of plantlets that had been grown in vitro were devoid of starch, probably because of a low photosynthetic activity or because exogenous sucrose created negative feedback on the enzymes in the plastids that are responsible for starch biosynthesis. However, it should be emphasized that starch accumulation also depends on its degradation rate in sink heterotrophic organs (Patrick et al. 2013). In addition, low plastoglobulus content under in vitro conditions may result in a low capacity of in vitro plantlets to prevent oxidative damage in the membrane systems of chloroplasts. During our experiment, there were no differences in chloroplasts ultrastructure when we compared chloroplasts in leaves of the same adventitious shoots rooted growing in in vitro conditions and chloroplast in shoots that had been acclimated to ex vitro conditions (growing in pots, without exogenous sugar supply). We have not observed expected photoinhibition in the case of adventitious shoots rooted and growing under in vitro condition (inside a vessel). Similarly, in the case of regenerants acclimated to ex vitro conditions, in spite of lower air humidity and no additional external carbon source after ex vitro transfer, the chloroplast ultrastructure was not changed. The obtained results of TEM analysis suggest that proper chloroplast maturation is linked to root formation. Therefore, only after rooting, which enabled efficiently uptake of water and micronutrients from the medium, the leaf chloroplasts developed properly functioning thylakoid membrane system that was reflected in PSII activity.

Improving the photosynthetic functioning of in vitro plantlets that are transferred to ex vitro is essential to assure high survival rates after transfer to in vivo conditions (Carvalho et al. 2001). Reduced leaf photosynthetic activity as a result of the poor development of the photosynthetic apparatus, inadequate gas exchange, stomata deformation and suppressed Rubisco activity has been revealed to limit successful in vitro to ex vitro transfer (Zenkteler and Borkowska 2002; Apóstolo et al. 2005; Joshi et al. 2006; de Oliveira et al. 2008 and the literature therein). High mortality during acclimation to ex vitro conditions is mainly associated with the low ability of plantlets to regulate water loss as the result of anatomical abnormalities that had developed in vitro such as increased stomatal density (Joshi et al. 2006) or root system damage during transfer from in vitro to ex vitro conditions. Because the photosynthetic apparatus of plantlets that are cultured in vitro is adapted to relatively low light intensities, they can be considered to be shade plants (Borkowska 2005). These anomalies are evident during the transfer of in vitro plants to a greenhouse or a field with high light intensity and cause their slow establishment and low survival percentage compared to plants that are cultured by traditional propagation (Pospíšilová et al. 2000). In our experiments, light and also temperature conditions were the same for in vitro and ex vitro plants; therefore, an effect of light intensity and the culture temperature was the most likely insignificant.

Conclusions

Here, we show for the first time the analysis of the PSII activity and chloroplast development in leaves from the same shoots of *R. tianschanicus* × *R. patientia* during micropropagation. Chloroplasts from plants rooted in vitro were sufficiently mature and photochemically active. Moreover, acclimation processes, i.e. plants being transferred from in vitro to ex vitro conditions, have relatively little impact on their development.

Our data indicate that in *R. tianschanicus* × *R. patientia*, rooting under in vitro conditions predominantly affects the development of a completely functional photosynthetic apparatus, but not the acclimation to ex vitro conditions per se.

Author contribution statement HŚ—design of the study, conducting in vitro culture experiments, measurements of the chlorophyll *a* fluorescence, data interpretation and writing a manuscript; ML—conducting in vitro culture experiments and measurements of the chlorophyll *a* fluorescence; IŚ—measurements of the chlorophyll *a* fluorescence, statistical analysis, data interpretation and participation in preparing manuscript; MKK—TEM analysis of the ultrastructure of the chloroplasts; MPK—data interpretation and participation in preparing manuscript; AJJ—proofreading the manuscript and critical revision. All authors read and approved the manuscript.

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