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PSII PHOTOCHEMISTRY IN VEGETATIVE BUDS AND NEEDLES OF NORWAY SPRUCE (*PICEA ABIES* L. KARST.) PROBED BY OJIP CHLOROPHYLL *A* FLUORESCENCE MEASUREMENT

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Vegetative buds represent developmental stage of Norway spruce (*Picea abies* L. Karst.) needles where chloroplast biogenesis and photosynthetic activity begin. We used the analyses of polyphasic chlorophyll *a* fluorescence rise (OJIP) to compare photosystem II (PSII) functioning in vegetative buds and fully photosynthetically active mature current-year needles. Considerably decreased performance index (PI_{ABS}) in vegetative buds compared to needles pointed to their low photosynthetic efficiency. Maximum quantum yield of PSII (F_v/F_m) in buds was slightly decreased but above limited value for functionality indicating that primary photochemistry of PSII is not holdback of vegetative buds photosynthetic activity. The most significant difference observed between investigated developmental stages was accumulation of reduced primary quinine acceptor of PSII (Q_A^-) in vegetative buds, as a result of its limited re-oxidation by passing electrons to secondary quinone acceptor, Q_B. We suggest that reduced electron transfer from Q_A^- to Q_B could be the major limiting factor of photosynthesis in vegetative buds.

Keywords: Chlorophyll *a* fluorescence – OJIP transient – photosystem II – *Picea abies* – vegetative buds

INTRODUCTION

In spite of comprehensive investigations the knowledge about development of photosynthetic apparatus is still incomplete. Coordination between chloroplast and nucleocytosolic genetic systems as well as coordination between protein and pigment synthesis has to be studied in detail. Also, investigations concerning the insertion of different components into thylakoid membranes mediated by numerous nucleus- and a few chloroplast- encoded factors are required [24]. Photosystem II (PSII) represents one of the major components of electron transport chain in chloroplasts. This multiprotein complex, composed of more than 20 protein subunits encoded by chloroplast and nuclear genome, is assembled in stepwise manner with several subassemblies

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Abbreviations: Chl *a* – chlorophyll *a*; LHCII – light harvesting complex of photosystem II; NPQ – nonphotochemical quenching; PSII – photosystem II; PSII RC – photosystem II reaction center; PQ – plastoquinone; Q_A – primary electron acceptor of photosystem II; Q_B – secondary electron acceptor of photosystem II.

being identified [25]. Experimental approaches used to investigate building up of PSII include biogenesis of PSII during development of etioplasts to chloroplasts, PSII-specific mutants and PSII turnover in repairing cycle [1]. Also, valuable information can be obtained by investigations of development and growth of photosynthetic organs in field conditions. Jansson et al. [11] suggested that fundamental processes in the assembly of the PSII complex occur in the same way in all plants, although it is differently regulated in gymnosperms and angiosperms. In angiosperms this process is light-dependent while in gymnosperms it is possible in the dark, as well. Gymnosperm plants due to additional light-independent enzymatic conversion of protochlorophyllide *a* to chlorophillide *a* are capable for chlorophyll biogenesis and initiation of assembling the photosynthetic complexes in darkness [2, 7, 26, 31]. Therefore biogenesis of photosynthetically active plastids of Norway spruce (*Picea abies* L. Karst.) begins during formation of vegetative buds.

Vegetative bud of Norway spruce is composed of an embryonic shoot subtended by a cup-like, cataphyll-bearing structure from which it is separated by a crown or nodal diaphragm [4]. The formation of vegetative buds begins in late spring or early summer, after bursting of young shoots. In the autumn spruce vegetative buds obtain characteristic structure and enter a dormant period [18]. Since it is covered with cataphylls, embryonic shoot of vegetative bud is not exposed to light. Despite that, presence of chlorophylls and carotenoids was detected during entire period of dormancy, but in significantly lowered concentration than in fully developed needles [17]. Chloroplasts of vegetative buds with poorly developed thylakoid system and starch grains were found to be distributed in the medulla and needle primordia [18]. During the period of dormancy chloroplast ultrastructure remains unchanged [16]. They were shown to be photosynthetically competent but less efficient regarded to chloroplasts in mature needles. This was contributed to distinct molecular and functional characteristic of their photosynthetic apparatus [16, 19, 20]. So, initiation of photosynthesis occurs long before exposure of young needles to light and it is proven that all components of PSII can already be expressed, translocated and assembled in closed buds [8]. Although lower expression of light-harvesting complexes of PSII, cytochrome b-559 and large subunit of Rubisco (LSU) was observed [20] main reason for limited capability of photosynthesis in vegetative buds it still not clear.

The aim of this study was further investigation of PSII photochemistry of spruce vegetative buds as well as to gain more information on functional differences between photosynthetic apparatus in vegetative buds and fully photosynthetically active mature current-year needles. Our previous research [8, 16, 19, 20] revealed that vegetative buds have functional PSII and their photosynthetic performance was not limited by primary photochemistry. Also, compared to needles, vegetative buds had low oxygen production and low electron transport rate. Since photosynthetic quenching in buds was shown to be very low even at low irradiance, we assumed that low rate of electron transport is unlikely to be caused by restricted CO_2 fixation [20]. Based on this, we made the hypothesis that impaired efficiency of electron transport chain is due to disturbed electron flow inside PSII, thus leading to low photosynthetic activity in vegetative buds.

MATERIALS AND METHODS

Two developmental stages (vegetative buds and mature current-year needles), harvested from a single healthy looking Norway spruce (*Picea abies* L. Karst.) tree, were used as plant material for this study. The sampling was done in April 2009 at the beginning of bud proliferation inside the lignificated cataphylls enabling the light impact on bud. It was before bud burst when vegetative buds were approximately 10 months old. Picked branches were immediately transferred to the laboratory with cut ends being held in water to prevent desiccation. After a half of hour period of dark adaptation cataphylls were removed and green embryonic shoots (~0.5 cm in length) were isolated from vegetative buds under dim green light. Mature current-year needles were also dark adapted for half of hour and removed from branches under a dim green light. It was considered that removal of investigated plant material from the branch does not cause changes in chlorophyll *a* fluorescence [9, 22].

Chlorophyll *a* fluorescence measurements were performed at room temperature on dark adapted samples using Handy Plant Efficiency Analyser (Handy-PEA, Hansatech, UK). Saturating red light pulse (3200 $\mu mol_{FOTONS}\ m^{-2}\ s^{-1}$, peak at 650 nm) provided by an array of three light-emitting diodes was used to induce rise from minimal (F_0) to maximal (Fm) fluorescence intensity which was recorded at 12 bit resolution for one secunde and subsequently analyzed using OJIP-test [27, 28].

Increase in fluorescence intensity is caused by a progressive closure of photosystem II reaction centers (PSII RCs). It is accepted that redox state of primary quinine electron acceptor (Q_A) defines state of PSII RC which is called open when Q_A is in oxidized state and closed when it is in reduced state. In dark adapted samples all PSII RCs are assumed to be open so minimal fluorescence intensity (F_0) is measured, while saturating light causes closure of all PSII RCs and increase of fluorescence intensity to maximal value (F_m). In normal physiological condition fluorescence transient is polyphasic with two additional steps, J at ~2 ms and I at ~30 ms, appearing between initial O (F_0) and maximal P (F_m) level. Therefore it is called OJIP transient.

Following recorded data: F_0 (minimal fluorescence intensity), F_m (maximal fluorescence intensity), F_{300} (fluorescence intensity at 300 μs), F_I (fluorescence intensity at 2 ms – I step), F_J (fluorescence intensity at 30 ms – J step), t_{max} (time needed to reach F_m) and AREA (the area above fluorescence transient), were used in OJIP-test for calculation of several biophysical parameters that quantify the stepwise energy flow through PSII [28, 30].

In order to compare recorded OJIP transients to each other and to reveal differences clearly, in OJIP curve shape double normalization between O and P [28] was used. O-P normalization gives relative variable fluorescence, $V_t = (F_t - F_0)/(F_m - F_0)$ which measures a fraction of closed photosystem II reaction centers (PSII RC), i.e. the fraction of reduced Q_A at any time (t). Fraction of PSII RCs that are closed after a single charge separation is represented by relative variable fluorescence at 2 ms, V_J [28].

The number of Q_A reduction is expressed as turnover number (N). It indicates how many times PSII RCs were closed and re-opened between F_0 and F_m. Normalized area

Table 1
Formulas and definitions of OJIP-test parameters (according to Strasser et al. [28])

Extract and technical parameters	
F_0	minimal fluorescence intensity
F_m	maximal fluorescence intensity
F_{300}	fluorescence intensity at 300 μs
F_I	fluorescence intensity at 2 ms – I step
F_J	fluorescence intensity at 30 ms – J step
t_{max}	time needed to reach F_m
$F_V = F_m - F_0$	maximal variable fluorescence
$V_t = (F_t - F_0)/(F_m - F_0)$	relative variable fluorescence at time t
$V_J = (F_J - F_0)/(F_m - F_0)$	relative variable fluorescence at J step
$V_I = (F_I - F_0)/(F_m - F_0)$	relative variable fluorescence at I step
AREA	area above OJIP transient
$S_m = AREA/(F_m - F_0)$	normalized AREA
$M_0 = (dV/dt)_0$	initial slope of relative variable fluorescence
$N = S_m \cdot M_0 \cdot (1/V_J)$	turnover number
Flux ratios or yields	
$TR_0/ABS = 1 - (F_0/F_m) = F_v/F_m$	maximum quantum yield of PSII
$ET_0/TR_0 = 1 - V_J$	probability that a trapped exciton moves an electron further than Q_A^-
$ET_0/ABS = F_v/F_m \cdot (1 - V_J)$	probability that a absorbed photon moves an electron further than Q_A^-
Specific fluxes or specific activities	
$ABS/RC = M_0 \cdot (1/V_J) \cdot [1/(F_v/F_m)]$ $ABS/RC = (TR_0/RC)/(TR_0/ABS)$	absorption per active reaction centre
$TR_0/RC = M_0 \cdot (1/V_J)$	trapping per active reaction centre
$ET_0/RC = M_0 \cdot (1/V_J) \cdot (1 - V_J)$ $ET_0/RC = (TR_0/RC) \cdot (ET_0/TR_0)$	electron transport per active reaction centre
$DI_0/RC = (ABS/RC) - (TR_0/RC)$	dissipation per active reaction centre
Phenomenological fluxes or phenomenological activity	
$RC/CS_0 = F_v/F_m \cdot (V_J/M_0) \cdot F_0$	density of active reaction centers per excited cross-section
Performance index	
$PI_{ABS} = (RC/ABS) \cdot (TR_0/DI_0) \cdot [ET_0/(TR_0 - ET_0)]$	performance index on absorption basis
$RC/ABS = (RC/TR_0) \cdot (TR_0/ABS)$ $RC/ABS = [(F_J - F_0)/4 \cdot (F_{300} - F_0)] \cdot (F_v/F_m)]$	density of reaction centers on chlorophyll *a* basis
$TR_0/DI_0 = F_v/F_0$	flux ration trapping per dissipation
$ET_0/(TR_0 - ET_0) = (F_m - F_J)/(F_J - F_0)$	electron transport beyond Q_A^-

above fluorescence transient (S_m) gives a measure of the energy needed to close all reaction centres, while the S_m/t_{max} ratio estimates average redox state of Q_A, i.e. average fraction of open RCs during time needed to complete their closure [27, 28].

Three different yields or flux ratios were calculated. The maximum quantum yield of photosystem II ($TR_0/ABS = F_v/F_m$), calculated based only on extreme values of fluorescence intensity, F_0 and F_m, is a measure of efficiency of primary photochemistry of PSII [26]. Probability that the trapped exciton moves an electron into electron transport chain further than Q_A^- (ET_0/TR_0) is calculated as $1–V_J$. Probability that absorbed photon will move an electron into electron transport chain beyond Q_A^- (ET_0/ABS), i.e. quantum yield of electron transport is calculated by multiplying first two flux ratios [27, 28].

Energy distribution through PSII at the reaction center level was analyzed based on so-called specific fluxes that include absorption (ABS/RC) and trapping of excitation energy (TR_0/RC), electron transport (ET_0/RC) and dissipation of excessive absorbed energy (DI_0/RC) per single active, i.e. Q_A-reducing PSII reaction center [27, 28]. Density of active reaction centers per excited cross-section (RC/CS_0) was also calculated.

The performance index on chlorophyll basis (PI_{ABS}) was used for estimation of overall photosynthetic efficiency. PI_{ABS} is multiparametric expression calculated as a product of three individual parameters related to three main properties that regulate photosynthetic activity: RC/ABS (density of RCs on chlorophyll basis calculated as ratio between fraction of reaction centre chlorophyll and fraction of antenna chlorophyll), TR_0/DI_0 (ratio of trapping flux and dissipation flux which measure contribution of light reaction for primary photochemistry) and $ET_0/(TR_0 – ET_0)$ (component related to electron transfer further than Q_A^-) [28–30]. All above-mentioned parameters are summarized in Table 1.

For each developmental stage, chlorophyll *a* fluorescence rise (OJIP) was measured 15 times and every measurement was done on a different bud or needle. Results of the measurement are presented as mean ± sd. Statistical analysis of calculated OJIP-test parameters was performed using Student *t*-test adjusted for small samples.

RESULTS

The performance index on chlorophyll basis (PI_{ABS}) of vegetative buds was very low, 4 times lower compared to mature current-year needles (Fig. 1) indicating low photosynthetic efficiency of vegetative buds. This was the result of decreased values of all three parameters comprising PI_{ABS} (Fig. 1). RC/ABS and TR_0/DI_0 was 1.3 and 1.39 times lower, respectively, while $ET_0/(TR_0 – ET_0)$ was 2.24 times lower thus mainly contributing to diminished PI_{ABS} for vegetative buds.

Chlorophyll *a* fluorescence transient of vegetative buds and mature current-year needles of Norway spruce without normalization and O – P normalized are shown in Fig. 2A and Fig. 2B, respectively. Both investigated developmental stages exhibited typical fluorescence rise kinetics, but with differences in OJIP curve shape especially

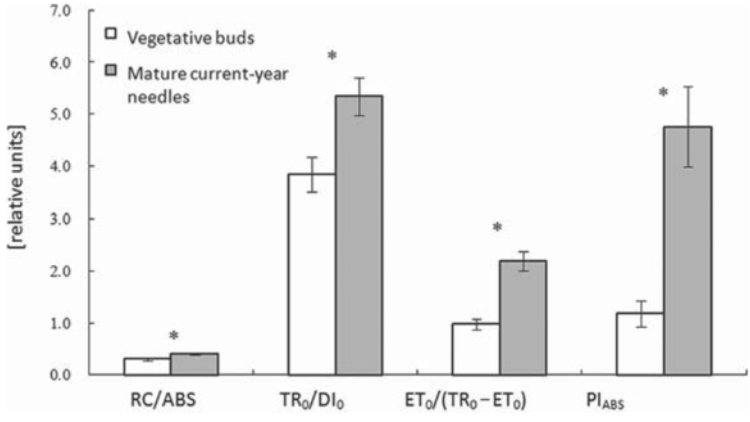

Fig. 1. Performance index (PI_{ABS}) and its components: RC/ABS – density of reaction centers on chlorophyll *a* basis; TR_0/DI_0 – flux ration trapping per dissipation; $ET_0/(TR_0 - ET_0)$ – electron transport beyond Q_A^- in vegetative buds (open bars) and mature current-year needles (full bars); Results are presented as mean of 15 independent measurements ± sd. Statistical analysis was performed using Student *t*-test adjusted for small samples. All tested parameters between buds and needles were significantly different at $P(t) < 0.001$ (*)

in O – J rise. In vegetative buds compared to mature current-year needles initial fluorescence rise from O to J was speeded up (Fig. 2B) with substantial increase in relative variable fluorescence at 2 ms (V_J) (Table 2). This indicated that vegetative buds

Table 2
Chlorophyll *a* fluorescence parameters in vegetative buds and mature current-year needles of Norway spruce (*Picea abies* L. Karst.)

Parameters	Vegetative buds	Current-year needles	t	P(t) <
F_0	331 ± 40	462 ± 55	7.464	0.001
F_m	1611 ± 267	2925 ± 337	11.835	0.001
V_J	0.508 ± 0.027	0.315 ± 0.020	22.316	0.001
V_I	0.897 ± 0.019	0.801 ± 0.032	9.918	0.001
t_{max}	486.667 ± 118.72	302.667 ± 43.006	5.644	0.001
S_m	15.491 ± 2.156	21.29 ± 3.592	5.361	0.001
S_m/t_{max}	0.034 ± 0.010	0.071 ± 0.009	10.382	0.001
N	39325 ± 5.480	43993 ± 5.686	2.289	0.001
RC/CS_0	104.301 ± 20.160	186.589 ± 16.891	12.117	0.001

F_0 – minimal fluorescence intensity; F_m – maximal fluorescence intensity; V_J – relative variable fluorescence at J step; V_I – relative variable fluorescence at I step; t_{max} – time needed to reach F_m; S_m – normalized AREA above flurescence transient; S_m/t_{max} – average fraction of open PSII RCs; N – turnover number; RC/CS_0 – density of active reaction centre per excited cross-section. Results are presented as mean of 15 independent measurements ± sd. Statistical analysis was performed using Student *t*-test adjusted for small samples. All tested parameters between buds and needles were significantly different at $P(t) < 0.001$.

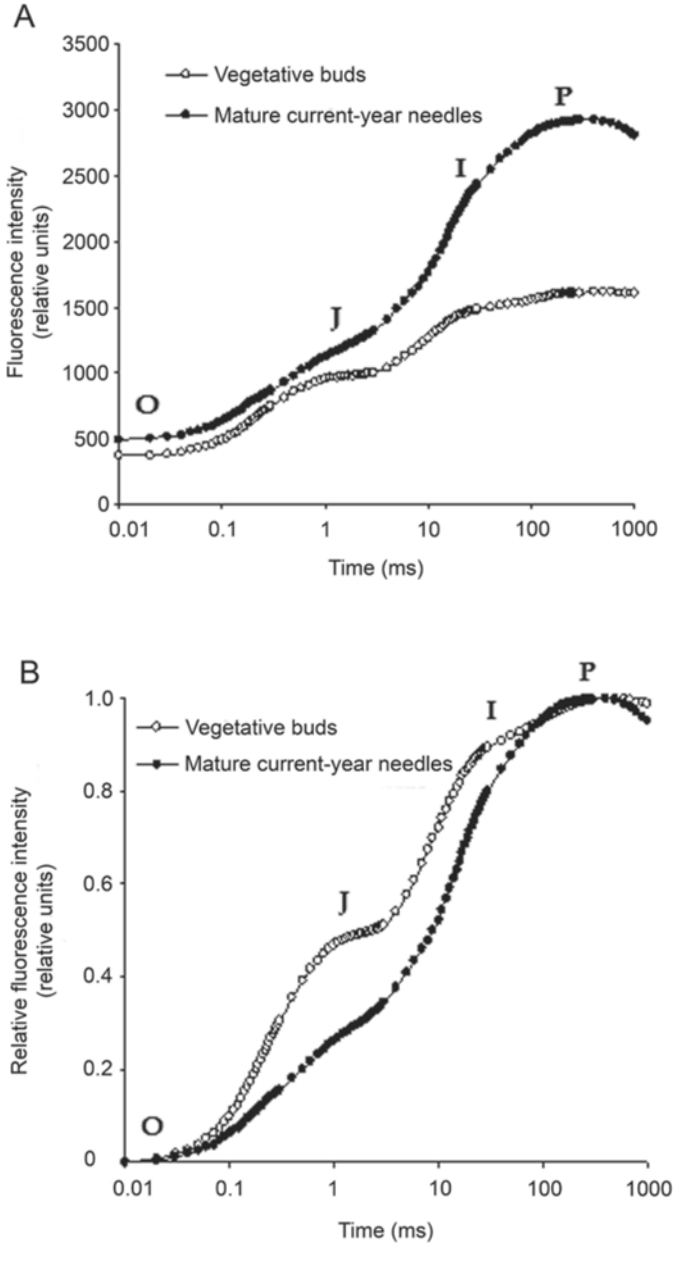

Fig. 2. OJIP chlorophyll *a* fluorescence transients without normalization (A) and O–P normalized (B) in vegetative buds (open symbols) and mature current-year needles (black symbols) plotted on a logarithmic time scale. Each transient represent average of 15 independent measurements per developmental stage

had higher fraction of reduced Q_A after a single charge separation. Also, vegetative buds had slightly increased relative variable fluorescence at 30 ms (V_I) regarded to current-year needles (Table 2) what was assumed to be consequence of accumulation of reduced Q_A and PQ pools.

In correlation with observed differences in OJIP curve shape, turnover number (N) was 1.37 times lower and normalized area above fluorescence transient (S_m) was 1.12 times lower in vegetative buds compared to needles, as well as twice smaller S_m/t_{max} ratio (Table 2). Further more, probability that trapped exciton (ET_0/TR_0) or absorbed photon (ET_0/ABS) will move an electron into electron transport chain beyond Q_A^- was considerable decreased in vegetative buds (28.11% and 32.31%, respectively) (Fig. 3). This pointed to difficulties in electron transfer from Q_A^- to secondary quinine electron acceptor of PSII (Q_B) and further downstream in vegetative buds. It indicates both, reason for observed accumulation of Q_A and low performance index PI_{ABS} in vegetative buds.

In spite of decreased F_0 and F_m values in vegetative buds in comparison with current-year needles (Table 2), the maximum quantum yield of photosystem II ($TR_0/ABS = F_v/F_m$) was only slightly lowered (5.84%) and beyond 0.75 what is considered to be limited value for PSII functionality [3].

Specific energy fluxes are presented in Fig. 4. Surprisingly, vegetative buds had 1.3 times higher absorption per active reaction centre (ABS/RC). Trapping of exciton per active reaction centre (TR_0/RC) was also increased for 1.22 times, but elevation of absorption and trapping was not accompanied with proportional elevation of electron transport beyond Q_A^- (ET_0/RC). In contrary, ET_0/RC was 1.14 times decreased. Values of non-photochemical dissipation per active reaction center (DI_0/RC) in veg-

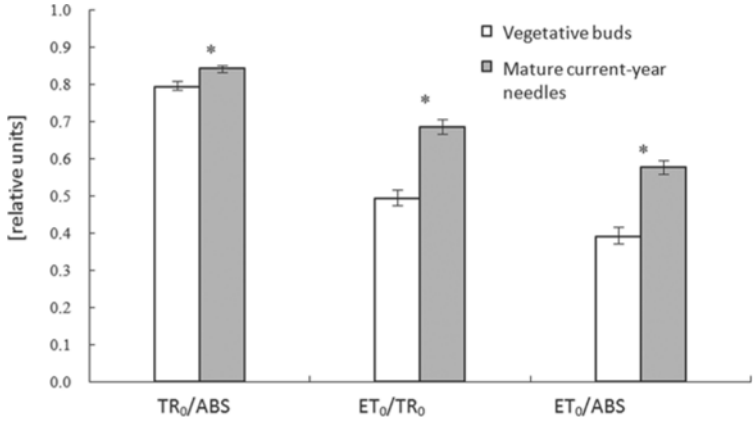

Fig. 3. Flux ratios or yields in vegetative buds (open bars) and mature current-year needles (full bars): TR_0/ABS – maximum quantum yield of PSII; ET_0/TR_0 – probability that a trapped exciton moves an electron further than Q_A^-; ET_0/ABS – probability that a absorbed photon moves an electron further than Q_A^-. Results are presented as mean of 15 independent measurements ± sd. Statistical analysis was performed using Student *t*-test adjusted for small samples. All tested parameters between buds and needles were significantly different at $P(t) < 0.001$ (*)

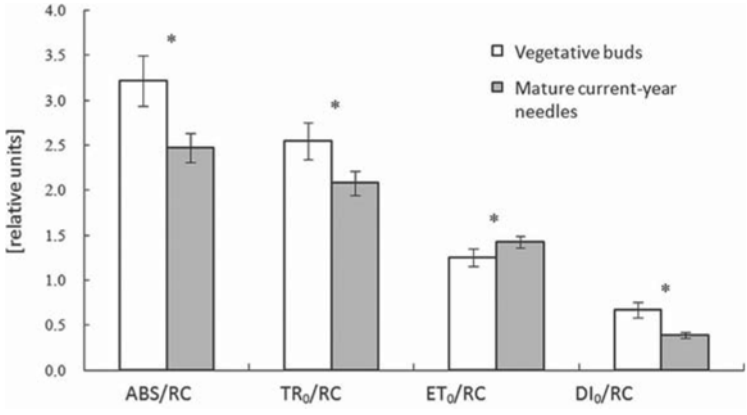

Fig. 4. Specific energy fluxes (b) in vegetative buds (open bars) and mature current-year needles (full bars): ABS/RC – absorption; TR_0/RC – trapping; ET_0/RC – electron transport DI_0/RC – dissipation per active reaction centre. Results are presented as mean of 15 independent measurements ± sd. Statistical analysis was performed using Student t-test adjusted for small samples. All tested parameters between buds and needles were significantly different at $P(t)<0.001$ (*)

etative buds were 1.71 times increased. Density of active reaction centers per excited cross-section (RC/CS_0) was 44.10% lower in vegetative buds (Table 2) in comparison to mature needles.

DISCUSSION

Assembly of photosystem II as well as of the whole photosynthetic apparatus is very complex and highly regulated stepwise process that must be synchronized with physiological and developmental state of plant photosynthetic organs. In Norway spruce this process starts in vegetative buds, but it goes only up to certain point. This was evidenced by very low value of performance index (PI_{ABS}) in vegetative buds regarded to mature current-year needles (Fig. 1) which indicated that vegetative buds have very low photosynthetic efficiency. First step in PSII assembling is formation of reaction center capable for charge separation, followed by association of CP47 and CP43 along with several small subunits and association of water splitting complex. Next, dimerization of PSII occurs and finally attachment of Lhc*b* polypeptides to form PSII-LHCII supracomplexes takes place [24]. Functional PSII RCs capable for primary charge separation seem to exist in vegetative buds, since their TR_0/ABS value was only slightly decreased. Q_A in vegetative buds could be reduced, but accumulated in reduced form after a single charge separation. Also, PQ pool was more reduced in vegetative buds as suggested by increase in V_J and V_I (Table 2) [27, 30]. Beside that, decrease in N, S_m and S_m/t_{max} ratio (Table 2) indicated that Q_A in vegetative buds could be reduced, but not re-oxidized as efficient as in needles. Therefore, vegetative buds may not be fully competent to perform multiple turnovers [14].

Decrease of several OJIP-test parameters in vegetative buds compared to mature needles such as ET_0/TR_0, ET_0/ABS (Fig. 3) and ET_0/RC (Fig. 4) pointed to diminished electron flow form Q_A^- to Q_B and further. Also, main reason for decreased PI_{ABS} in vegetative buds came from decrease in the component related to electron transport, $ET_0/(TR_0 - ET_0)$ (Fig. 1).

In such condition, when electron transport is inhibited, increased ABS/RC value observed in buds compared to needles (Fig. 4) should not be interpreted as structural increase of antenna complexes, but as a result of decrease in density of PSII RC per absorption (RC/ABS) (Fig. 1) [6]. Such conclusion is corroborated with observation that vegetative buds had lower amount of LHCII complexes in comparison to mature needles [20]. Increase in dissipation per reaction centre (DI_0/RC) in vegetative buds regarded to mature current-year needles (Fig. 4) is correlated with both, increased absorption and low electron transport activity. Plants possess several mechanisms for harmless dissipation of excess absorbed light [22]. Based only on increased DI_0/RC we cannot conclude what is the nature of dissipation process. As the presence of carotenoids was detected in completely covered vegetative buds and efficient non-photochemical quenching was reported in both vegetative buds and mature current-year needles [20], it would be logical to assume that at least some mechanisms for avoiding damage caused by excessive irradiation are already developed in buds insuring protection after a bud break occurs.

The increase in dissipation (DI_0/RC) along with increase in absorption (ABS/RC) and low density of active reaction centers (RC/CS_0) could be explained as a result of presence of PSII Q_A-non-reducing reaction centers [23]. Even if there is a certain fraction of inactive reaction centers, by our opinion, this had not the crucial influence on photosynthetic activity in vegetative buds since TR_0/ABS was enough high to consider PSII as functional.

Our results concerning low photosynthetic efficiency, but high PSII functionality are consistent with previous investigations on development of spruce needles [8, 16, 20]. Functionality of PSII primary photochemistry in vegetative buds is also in accordance with results of PSII photoactivation in dark-grown seedlings of Scots pine (*Pinus sylvestris* L.) [11]. They reported that photoactivation of PSII involved Mn-ligation into oxygen evolving complex (OEC) without changes in primary reaction of PSII. Further more, it seems that significant similarity exists between spruce vegetative buds and young leaves of some angiosperm plants. Photosynthetic capacity in developing leaves of grapevine (*Vitis vinifera* L.) [13] and elm (*Ulmus pumila* L.) seedlings [12] grown under natural irradiance was not limited by primary photochemistry, but by impaired electron flow beyond Q_A^-. Investigation of light-induced development of PSII during conversion of etioplast to chloroplast in etiolated sunflower (*Helianthus annuus* L.) cotyledons done by Lebkuecher et al. [15] showed that concentration of PSII was increasing, while concentration of Q_B-non-reducing PSII reaction centers and slow PSII Q_B-reducing centers relatively to total PSII centers were decreasing. They also concluded, that development of PSII involves assembly of complexes which initially cannot reduce Q_B and that heterogeneity of PSII is related to different developmental states of PSII during chloroplast maturation.

Furthermore, it was indicated that oxygen evolving complex (OEC) is not fully functional at the beginning of development [11, 13] what is consistent with observations made by Lepeduš et al. [18] showing that spruce vegetative buds have very low oxygen production which increased with needles maturation. This could imply that development of PSII acceptor side is coordinated with the development of its donor side. In cyanobacterium *Synechocystis* sp. PCC 6803 partially assembled PSII present in plasma membrane are capable to catalyze single turnover, but not multiple turnovers as it was observed for PSII units in vegetative buds. This contributed to a limitation on the donor side and was supposed to be a protection mechanism to avoid harmful consequences from performing water oxidation before PSII is fully assembled [14].

Based on the presented results we have concluded that low photosynthetic efficiency of vegetative buds was due to an incompletely developed photosynthetic apparatus. It is evident that primary photochemistry of PSII was established in vegetative buds and thus it was not a holdback of photosynthetic activity. Vegetative buds were capable for absorption of light and trapping of exciton resulting in reduction of Q_A. Limitation of electron flow further than Q_A^- was the most significant difference observed between vegetative buds and mature current-year needles. Low rate of Q_A^- re-oxidation may contribute to high portion of Q_B-non-reducing reaction centers in which capability of electron transport from Q_A^- to Q_B was not established yet. This indicates that impaired electron transport between Q_A^- and Q_B is a limiting step of photosynthesis in vegetative buds.

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