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JIP-test in assessing sensitivity to nitrogen deficiency in two cultivars of *Actinidia arguta* (Siebold et Zucc.) Planch. ex Miq.

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Abstract

Optimal nitrogen availability is necessary to keep high photosynthetic efficiency and in consequence, good physiological condition and growth of plants. In order to examine the effect of N nutrition on photosynthetic apparatus performance we used the chlorophyll *a* fluorescence (ChF) technique in two differing *Actinidia* cultivars and we aimed to select the most reliable ChF parameters showing N deficiency. The 4-years-old specimens growing on an experimental plantation were treated with three differing N doses and ChF data were collected during two years. The results showed that PSII performance indices, performance index on the absorption basis and total performance index, are useful to detect the overall decrease in photosynthetic apparatus vitality. Efficiency of electron movement beyond Q_A into the electron transport chain was more susceptible to different N nutritions than maximum quantum efficiency, while a pool of Q_A reducing reaction centres per PSII antenna chlorophyll, RC/ABS, and a number of Q_A reducing reaction centres per cross section of a sample, RC/CS_0, were the best indicators of N deficiency.

Additional key words: fluorescence transient; kiwiberry; mini kiwi; oxygen-evolving complex; photosystem II.

Introduction

Proper nitrogen nutrition of crop plants is one of the crucial factors affecting good physiological conditions of plants, growth, and yield (Yong *et al*. 2010, Wang *et al.* 2012a). Nitrogen, being an important component of amino acids, plays the essential role in protein synthesis and in numerous biochemical processes. It is also a component of chlorophyll (Chl) so that the N-deficiency is clearly reflected by the depletion of Chl content (Chang and Robison 2003, van den Berg and Perkins 2004). Up to 50% of total leaf N may be invested in the photosynthesising structures and CO_2 assimilation, particularly in Rubisco (Makino 2003). Therefore, optimal N availability is necessary to keep a high photosynthetic efficiency (Dordas and Sioulas 2008, Wang *et al*. 2012a) and to enable advantageous sugar production. Moreover, the N availability for plants is highly connected with water availability in soil (Puri and Ashman 1998, Hu and Schmidthalter 2005). Water deficiency in soil may reduce N uptake despite sufficient fertilising (Teixeira *et al*. 2014, Bai *et al*. 2017), as well as N accumulation in leaf tissues (Ashraf *et al*. 2013, da Silva *et al.* 2017).

Chlorophyll *a* fluorescence (ChF) analysis has become recently a popular research method for different purposes: from analysing photosynthetic apparatus structure to assessment of gross primary productivity (Porcar-Castell *et al.* 2014). This method is also used to detect environmental stress in plants, including drought stress (Percival *et al.* 2006, Fini *et al.* 2009, Wang *et al.* 2012b, Guo *et al.* 2016), thermal stress (Brestič *et al.* 2016), high light stress (Živčák *et al.* 2015b), salt stress (Mehta *et al.* 2010), and N deficiency (Kalaji *et al*. 2014a, 2018; Živčák *et al.* 2014a,b; Feng *et al.* 2015). There are two main methods used in examining environmental stress on living leaves. The first one is based on pulse amplitude-modulated (PAM) technique. PAM fluorimeters use a trick to separate the effect of the actinic light, which drives photosynthesis, and the low-intensity measuring light, that is used to probe the state of the photosynthetic system (Kalaji *et al.* 2014b). It has been used for decades to elucidate the organisation, function, and acclimation of the photosynthetic apparatus

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Abbreviations: ABS – average photon absorption; Chl – chlorophyll; ChF – chlorophyll *a* fluorescence; DI – energy dissipation; ET – electron transport; ETC – electron transport chain; F_0 – minimal fluorescence yield of the dark-adapted state; F_m – maximal fluorescence yield of the dark-adapted state; F_v/F_m – maximal quantum yield of PSII photochemistry; OEC – oxygen-evolving complex; PI – performance index; RC – reaction centre; RE – reduction of end electron acceptors at the PSI acceptor side; TR – exciton trapping.

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at the subcellular and leaf levels. The second method is based on application of actinic light that drives photosynthesis also as a measuring light, and is performed using plant efficiency analysers (PEA). Application of light-emitting diodes (LED) of specific wavelength enables quick measurements with a high resolution (Kalaji *et al*. 2014b). Therefore, the latter method facilitates less time-consuming measurements which are necessary to obtain a huge number of samples in a relatively short time (at the same light/temperature conditions) in the field experiment. In most research concerning N nutrition, only a couple of ChF parameters are applied as stress markers, such as F_0, F_m, F_v/F_m, and sometimes additional parameters describing electron transport rate beyond PSII depending on a device used in examination protocol (*e.g*. Janušauskaite and Feiziene 2012, Lin *et al*. 2013, Feng *et al*. 2015). The results confirm advantageous effect of increased N supply on the analysed parameters, which is consistent with the general opinion on significance of N nutrition.

The usage of fluorimeters of high-time resolution measurements (with intervals of 10 μs) provides a data-set for plotting a fluorescence transient which, when shown on a logarithmic time scale, is called a fast (or prompt) fluorescence curve. This curve with visible points marked as O, J, I, P, according to Strasser and Govindjee (1992), enables to get an insight into particular phenomena concerning light absorption and its conversion to biochemical energy. Based on it, so called 'JIP-test' was developed and numerous JIP-test parameters were introduced (Strasser *et al*. 2000, 2004, 2010)), which describe energy fluxes occurring inside and around reaction centres (RCs) of numerous PSII localised in chloroplasts. In this way, the consecutive energy fluxes of average photon absorption (ABS), exciton trapping (TR), energy dissipation (DI), electron transport (ET), and reduction of end electron acceptors at the PSI acceptor side (RE), can be evaluated. Particularly, parameters defining quantum yields and efficiencies (or probabilities) are valuable because of their universal meaning, these are maximum quantum yield of primary photochemistry (at t_0), TR_0/ABS (φ_{Po}); probability that a trapped exciton moves an electron into the ETC beyond Q_A, ET_0/TR_0 (ψ_{Eo}); and the efficiency of ET until PSI acceptors, RE_0/ET_0 (δ_{Ro}) (Strasser *et al*. 2004, 2010). Based on calculated specific absorption flux, ABS/RC, and experimentally accessible phenomenological absorption flux, $ABS/CS_0 = F_0$, the density of active RCs (Q_A reducing RCs) per cross section at point 0 can be defined (Strasser *et al*. 2004). Finally, two integrative parameters, so-called performance indices, PI_{ABS} and PI_{total}, were introduced by Strasser *et al*. (2004, 2010) as multiplications of RC/ABS (a pool of active RCs per PSII antenna Chl) and particular efficiencies.

Selected JIP-test parameters and their description are listed in the following table (*see* the next page).

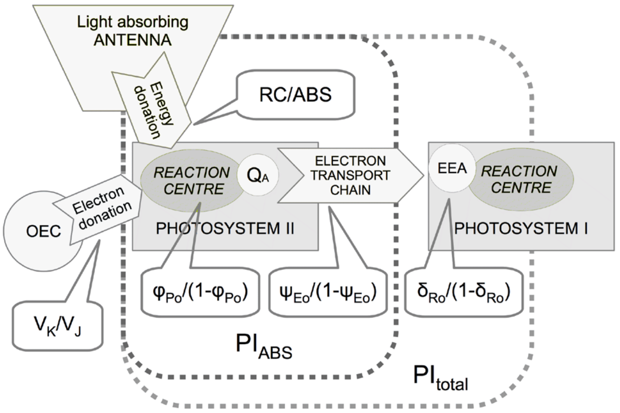

Fig. 1. A simplified scheme of PI_{ABS} and PI_{total} equations. EEA – end electron acceptors at photosystem I, OEC – oxygen-evolving complex (= water-splitting complex), Q_A – plastoquinone A. For details *see* Materials and methods.

A simplified scheme presenting graphically PI_{ABS} and PI_{total} calculations is shown in the Fig. 1; however, the structures involved in photochemical reactions are more complicated and differentiated. For instance, based on antenna size, PSII can be classified into three forms; α, β, and γ. Likewise, on the basis of electron transport properties of the acceptor side of reaction centres, two forms of PSII were found, namely, Q_B reducing centres and Q_B nonreducing centres (Mehta *et al.* 2010). The above mentioned performance indices became useful for an assessment of stress influence on plants (Živčák *et al.* 2008, Swoczyna *et al*. 2010, Yusuf *et al*. 2010, Gururani *et al.* 2013, Kalaji *et al*. 2018). They enable to distinguish plant groups affected by stress (when compared to control plants) and to indicate which level of stress is vulnerable for photosynthetic machinery. Additionally, indices on basis of JIP-test can show systemic plant response to local damages, for example changes in cellular pH induced by environmentally affected electrical potential across the plasma membrane, *i.e*. variational potential (Sukhova *et al*. 2018). In practical operation in the field, the more unequivocal identification of stress basis is needed. Therefore, the examination of particular factors of PI multiplications seems to be more useful. RC/ABS reflects a relative number of active RCs per average antenna size (Strasser *et al*. 2004). This parameter is sensitive to thermal stress (Kalaji *et al*. 2011a, Brestič *et al*. 2012), drought (Strasser *et al*. 2010, Guha *et al*. 2013), and salt stress (Kalaji *et al*. 2011b). Elevated temperatures affect the structure of Chl-protein complexes (Allakhverdiev *et al*. 2008) while drought and salt-induced osmotic stress triggers partial closure of RCs (Kalaji *et al*. 2011b, Guha *et al*. 2013). The $\varphi_{Po}/(1 - \varphi_{Po})$ ratio, denoted also as TR_0/DI_0 or F_v/F_0, shows the relation between trapped energy by active RC and dissipated energy (Strasser *et al*. 2004). A decrease in $\varphi_{Po}/(1 - \varphi_{Po})$ is usually connected to drought stress (Fini *et al*. 2009, Swoczyna *et al*. 2015), heat stress (Kalaji *et al*. 2011a, Mathur and Jajoo 2014), or salt stress (Kalaji *et al*. 2011b). It should also be considered when analysing N-deficiency becasuse φ_{Po} (= F_v/F_m) was reported to be sensitive to N nutrition (Janušauskaite and Feiziene 2012, Lin *et al*. 2013, Feng *et al*. 2015). The $\psi_{Eo}/(1 - \psi_{Eo})$ ratio concerns consecutive processes in ETC in which several molecules, such as Rieske FeS protein, cytochromes, plastocyanin, and some more, are involved. Inactivation of those structures affects linear

Fluorescence parameters	Description
$F_0 = ABS/CS_0$	initial fluorescence obtained from measurements, denoted also as ABS/CS_0
$F_K = F_{300}$	fluorescence at 300 µs after illumination of a dark-adapted sample
$F_J = F_{2ms}$	fluorescence at 2 ms after illumination of a dark-adapted sample
$F_I = F_{30ms}$	fluorescence at 30 ms after illumination of a dark-adapted sample
F_m	maximum fluorescence after illumination of a dark-adapted sample
$V_K = (F_{300} - F_0)/(F_m - F_0)$	relative variable fluorescence at 300 µs after illumination of a dark-adapted sample
$V_J = (F_{2ms} - F_0)/(F_m - F_0)$	relative variable fluorescence at 2 ms after illumination of a dark-adapted sample
$V_I = (F_{30ms} - F_0)/(F_m - F_0)$	relative variable fluorescence at 30 ms after illumination of a dark-adapted sample
V_K/V_J	efficiency of electron flow from OEC to PSII reaction centres
$M_0 = 4\ (F_{300} - F_0)/(F_m - F_0)$	approximated initial slope of the fluorescence transient, expressing the rate of RCs' closure
$F_v/F_m = \varphi_{Po} = TR_0/ABS = (F_m - F_0)/F_m$	maximum quantum yield of PSII photochemistry
$\psi_o = ET_0/TR_0 = (F_m - F_{2ms})/(F_m - F_0) = 1 - V_J$	probability that a trapped exciton moves an electron into the electron transport chain beyond Q_A
$\delta_{Ro} = RE_0/ET_0 = (F_m - F_{2ms})/(F_m - F_0)$	probability that an electron from the intersystem electron carriers is transferred to reduce end electron acceptors at the PSI acceptor side
$RC/ABS = \gamma_{RC}/(1 - \gamma_{RC}) = \varphi_{Po}\ (V_J/M_0)$	Q_A reducing RCs per PSII antenna chlorophyll
$RC/CS_0 = \varphi_{Po}\ (V_J/M_0)\ (ABS/CS_0)$	density of active RCs (Q_A reducing RCs) per cross section at point 0
$PI_{ABS} = RC/ABS \times \varphi_{Po}/(1 - \varphi_{Po}) \times \psi_{Eo}/(1 - \psi_{Eo})$	performance index (potential) for energy conservation from photons absorbed by PSII to the reduction of intersystem electron acceptors
$PI_{total} = RC/ABS \times \varphi_{Po}/(1 - \varphi_{Po}) \times \psi_{Eo}/(1 - \psi_{Eo}) \times \delta_{Ro}/(1 - \delta_{Ro})$	performance index (potential) for energy conservation from photons absorbed by PSII to the reduction of PSI end electron acceptors

electron transport towards PSI and, additionally, may contribute to generation of reactive oxygen species. High stress may also disturb redox processes between electron carriers, plastoquinones Q_A and Q_B (Mehta *et al.* 2010). High temperature affects also ATP-generating system (Allakhverdiev *et al.* 2008). Thus, a decrease in $\psi_{Eo}/(1 - \psi_{Eo})$ values, likewise in ψ_{Eo}, reflects thermal stress (Bussotti *et al.* 2010, Brestič *et al.* 2012), salt stress (Baccarin *et al.* 2011), or pathogen infection (Christen *et al.* 2007). Another ChF parameter discussed in previous research is so-called K-band. In case of drought or thermal stress, a new step arises in the fast fluorescence curve at the time of 300 µs, called K-step, or K-band, when only a marked shift of the fluorescence at 300 µs is noted (Strasser *et al.* 2004, Oukarroum *et al.* 2007, Brestič and Živčák 2013). The appearance of K-band is referred to restricted electron flow from OEC to the RC. This might be caused by heat inducing loss of Mn atoms from manganese containing clusters of OEC (Allakhverdiev *et al.* 2008) or by deficit of water molecules which are the source of electrons reoxidising RCs in PSII. The appearance of K-band is detected when analysing the ratio of variable fluorescence at 300 µs and variable fluorescence at 2 ms (V_K/V_J) (Strasser *et al.* 2004, Brestič and Živčák 2013). The V_K/V_J ratio is not included in any PI formula; however, it might be valuable in detecting some kinds of stress.

Numerous parameters bring abundant information to specialists, but they are difficult to interpret in common practice. Thus, setting apart few particular valuable parameters detecting N deficiency would be very advantageous for practitioners. *Actinidia arguta* (Siebold & Zucc.) Planch. ex Miq. is a dioecious vine originating from Asia and bearing very sweet aromatic fruit called kiwiberry, mini kiwi, or hardy kiwifruit, which is smaller and hairless in comparison to more popular kiwifruit (*A. chinensis* Planch. var. *deliciosa*). Kiwiberry contains more than 20 essential nutrients and a range of vitamins revealing significant benefits for human health. It is usually referred to as 'healthy fruit' or 'superfood' (Leontowicz *et al.* 2016, Latocha 2017). Among numerous cultivars used for commercial fruit production, 'Weiki' and 'Geneva' show differing growth characteristics. The importance of *A. arguta* as a crop plant has arisen recently but still little information regarding kiwiberry crop management, especially N fertilisation, can be found.

The first aim of the research was to examine the effect of N nutrition on two differing *Actinidia* cultivars, 'Weiki' and 'Geneva'. We hypothesised that, as the both cultivars differ in growth characteristics, they would also differ in light energy management facing nitrogen deficiency. The second objective was to select the most reliable ChF parameters showing N deficiency in *Actinidia*, an example of a perennial crop vine. It was hypothesised plants deficient in nitrogen would have primarily decreased the parameters concerning Chl molecules performance because N deficiency is often reflected by the depletion of Chl content.

Materials and methods

Plant material and experiment design: The experiment was conducted in mature kiwiberry orchard in Bodzew, Mazowieckie State, Poland (51°47'49.9"N, 20°48'44.0"E) in the years 2015–2016. Plants were planted in 2011 with 7:1 female to male ratio. Specimens were set out in rows with 4 × 4 m spacing. *A. arguta* is a climbing plant; the plants were supported by a T-bar construction connected by five carrying wires (2 m high and 2 m wide). Plots for two cultivars ('Geneva' and 'Weiki') with three N treatments; low (LN), standard (SN), and high N supply (HN) [3, 5, and 8 mg(N) 100 g^{-1}(soil DM), respectively], and three repetitions (*i.e.* blocks, each encompassing four specimens) were randomly selected. Fertilisation was conducted with three equal doses (at amounts dependent on N treatment) of ammonium nitrate (34% N) at the beginning of April, end of May, and end of June. The weather data (precipitation and temperature) were collected during whole experiment using *Vantage Pro2 Plus* weather station (Davis, USA).

Chl *a* fluorescence measurements: ChF was measured five times per year with 3-week interval starting from the beginning of June each year. Fast kinetics of ChF was measured using a *HandyPEA* fluorimeter (*Hansatech Instruments Ltd*., King's Lynn, Norfolk, Great Britain). Ten leaves from terminating shoots per each block were collected. It was taken into account to obtain the leaves from the plants of the same height, being of similar size, and representing the typical appearance for particular N treatment. They were immediately put in paper bags and transported to shaded service area at the edge of the plantation. Then the leaves were dark-adapted using light-excluding clips for minimum 20 min. The dark-adapted leaf samples of 4-mm diameter within each clip were illuminated with 660-nm light of 3,444 μmol(photon) m^{-2} s^{-1}.

Based on ChF measurements, the following parameters of the JIP-test were calculated: density of active RCs (Q_A reducing RCs) per cross section at point 0, RC/CS_0; quantum yields and efficiencies, V_K/V_J, φ_{Po} (= F_v/F_m), ψ_{Eo}, δ_{Ro}; and performance indices, PI_{ABS} and PI_{total}. In order to assess which factors of PI_{ABS} and PI_{total} equations were determined by different N supply, the consecutive mathematical factors, RC/ABS, $\varphi_{Po}/(1 - \varphi_{Po})$, $\psi_{Eo}/(1 - \psi_{Eo})$, and $\delta_{Ro}/(1 - \delta_{Ro})$, were analysed.

Chl and leaf N content: Along with Chl *a* fluorescence measurements, total N concentration in leaves of the same plants was determined using the standard Kjeldahl method and expressed as percentage of dry mass. The analyses were carried out in the Regional Laboratory of a Chemical and Agricultural Station in Warsaw, Żółkiewskiego Str. 17, accredited by the Polish Centre for Accreditation. Relative Chl content was measured on the leaves collected for ChF measurements using *CL-01*, a dual-wavelength (620 and 940 nm) optical Chl content meter (*Hansatech Instruments Ltd*, King's Lynn, Norfolk, Great Britain).

Statistical analysis: Two-way analysis of variance (*ANOVA*) was performed on summarised data from each season in order to compare the effect of N treatment and cultivar traits. As in most comparisons, interactions between cultivar traits and N treatment were found, in order to compare the differences between N-supply in each cultivar and at each date, a one-way *ANOVA* was used with the significance of differences being determined by the *Tukey*'s *post-hoc* test at the significant level of $P_{0.05}$. A principal component analysis (PCA) was performed on a correlation matrix of three means from LN, SN, and HN treatment and of all measurement days in each year. All calculations were made using *STATISTICA version 13.0* software (*TIBCO Software Inc. (2017)*, http://statistica.io., USA).

Results

Despite abundant precipitation in April 2015, the next months of the growing season were much drier compared to data from long-term average precipitation records in May, June, and July. The total precipitation in 2015 was 425.4 mm, which was only 80% of the total long-term records. The precipitation in May and June 2016 was lower again, however, in July and August it exceeded the long-term monthly means (Fig. 2*A*). The average temperature in both seasons (2015 and 2016) was higher than long-term average (9.9 and 9.2°C, respectively, *vs*. 7.6°C). The first part of the 2015 season (March–June) was much warmer and the second part (July–September) was colder in comparison to the long-term average. In turn, the year 2016 revealed a similar temperature distribution compared to the long-term average, but at a slightly higher level (Fig 2*B*).

Two *Actinidia arguta* cultivars showed different reactions both to N nutrition and to climatic conditions. In dry June 2015, the maximum quantum efficiency (F_v/F_m) was strongly affected in 'Geneva' showing values not exceeding 0.8. In 'Weiki', low F_v/F_m was noted only in July (Fig. 3*A*). In 2016, both cultivars showed high F_v/F_m in June and July and a depletion of this parameter in August, however, 'Weiki' revealed higher maximum quantum efficiency during the whole season compared to 'Geneva' (Fig. 3*B*), showing no interaction with N treatment (except from most other parameters). Different N treatment did not affect F_v/F_m (φ_{Po}) in 2015 in 'Weiki', except June 24, and in 'Geneva', except August 6, which was also reflected by $\varphi_{Po}/(1 - \varphi_{Po})$ (Table 1).

The efficiency of total electron transport beyond Q_A (ψ_{Eo}) showed significant differences between N treatments in early growing season 2015 ('Weiki') or late growing season 2015 ('Geneva'), and during the whole growing season 2016 (both 'Weiki' and 'Geneva') (Fig. 3*C*,*D*). The same was reflected by mathematically transferred $\psi_{Eo}/(1 - \psi_{Eo})$ (Tables 1, 2). Two-way *ANOVA* indicated the significant effect of N nutrition, as well as of cultivar traits in each season, in every comparison, but no interaction between N nutrition and cultivar traits in 2015 and 2016.

In 2015, the efficiency of total electron transport up to end electron acceptors of PSI (δ_{Ro}) revealed similar pattern in all treatments in both cultivars except June 24, when 'Weiki' showed lower δ_{Ro} in LN, and August 6 and 27, when in LN 'Geneva' the δ_{Ro} value was the highest. The significance of differences in δ_{Ro} followed the differences in $\delta_{Ro}/(1 - \delta_{Ro})$ ratio (Table 1). Likewise, in 2016, 'Weiki' had lower δ_{Ro} in LN on 23 June, and 'Geneva' revealed higher δ_{Ro} in LN at particular measurement days (data not shown, *see* $\delta_{Ro}/(1 - \delta_{Ro})$ ratio in Table 2).

Increased N doses affected positively both performance indices in 2015, however, the two cultivars showed

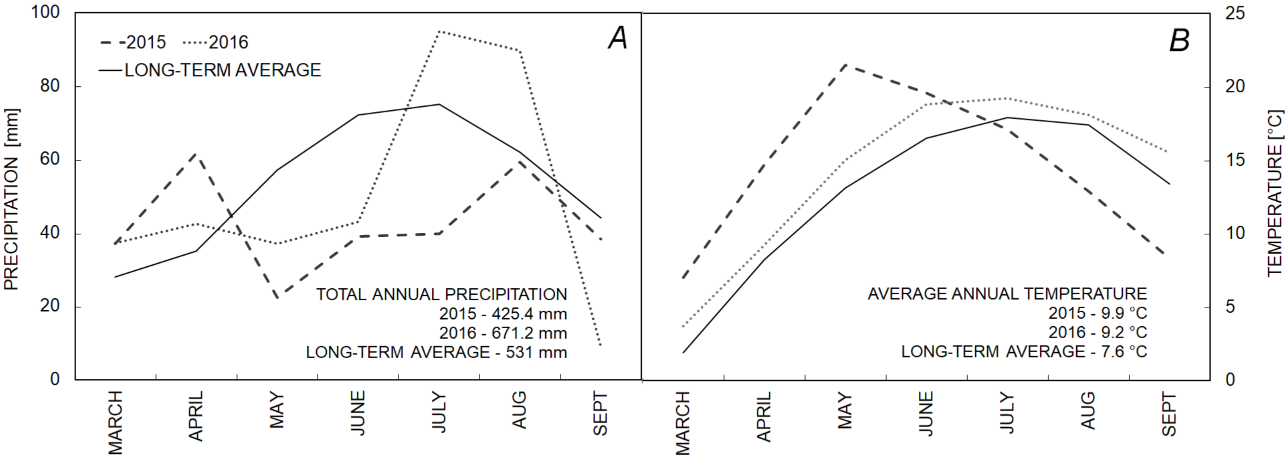

Fig. 2. Mean monthly precipitation (*A*) and temperature (*B*) in 2015 and 2016, and the long-term average monthly precipitation and temperature, respectively.

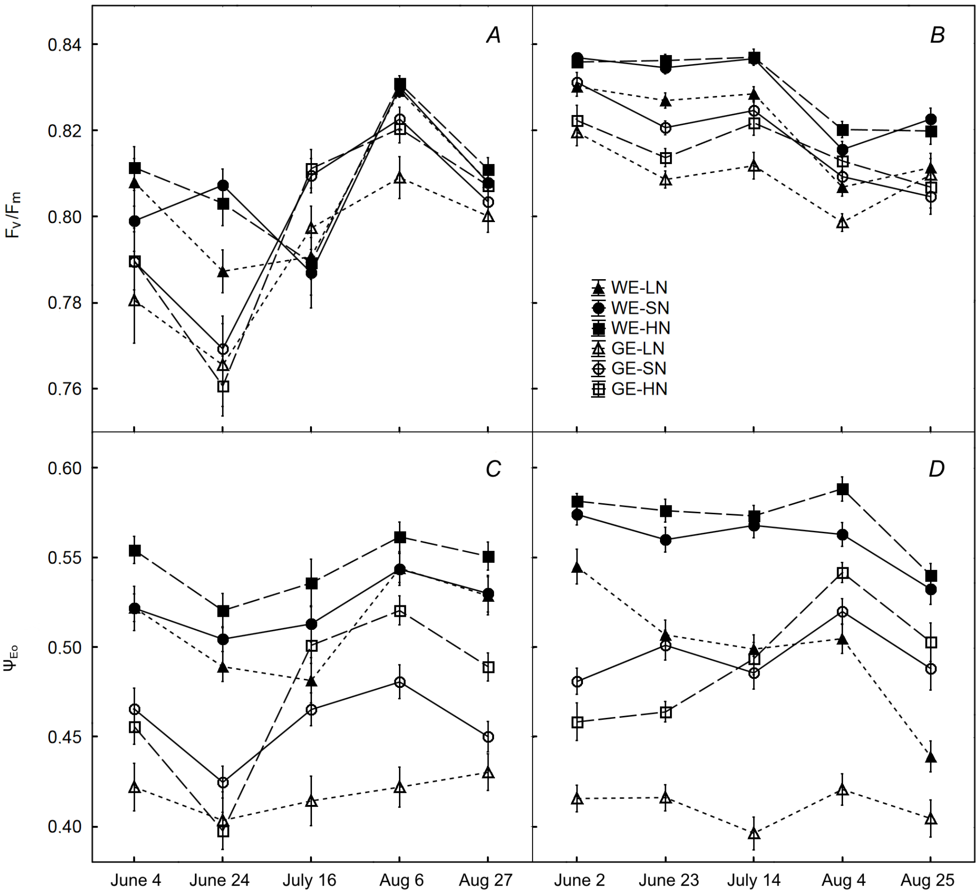

Fig. 3. Maximum quantum efficiency ($F_v/F_m = \varphi_{Po}$) during the growing seasons 2015 (*A*) and 2016 (*B*) and efficiency of total electron transport beyond Q_A (ψ_{Eo}) during the growing seasons 2015 (*C*) and 2016 (*D*) in two *Actinidia arguta* cultivars 'Weiki' (WE) and 'Geneva' (GE). The plants were treated with minimal (LN), standard (SN), and increased (HN) nitrogen supply. Values are means ± SE.

different reactions. 'Weiki' was significantly affected in the early growing season, while 'Geneva' in July and August (Table 1). In 2016, both cultivars revealed significant differences at all measurement days. In 'Weiki', the higher N doses were applied, the higher PI_{ABS} and PI_{total} values were noted. In 'Geneva', the highest PI_{ABS} and PI_{total} were obtained at standard N doses (Table 2). Dry June 2015 contributed to depletion of PI_{ABS} in both cultivars,

Table 1. Chlorophyll *a* fluorescence parameters in ‘Weiki’ and ‘Geneva’ in 2015. For each parameter, *small superscript letters* indicate statistically homogenous groups (*ANOVA*, *Tukey*'s HSD test, $P \leq 0.05$). LN, SN, HN – 3, 5, and 8 mg(N) 100 g^{-1}(soil DM), respectively.

Cultivar	Parameter	N treatment	June 4	June 24	July 16	August 6	August 27
Weiki	V_K/V_J	LN	0.385	0.386^b	0.403	0.360	0.353
		SN	0.390	0.352^a	0.370	0.359	0.354
		HN	0.377	0.353^a	0.374	0.350	0.341
	RC/CS_0	LN	380.5	404.7^a	380.4^a	406.1^a	427.6^a
		SN	375.8	443.3^b	411.1^b	411.2^{ab}	432.4^{ab}
		HN	391.6	442.9^b	414.1^b	429.6^b	451.1^b
	RC/ABS	LN	0.636	0.618^a	0.594	0.698	0.694
		SN	0.619	0.690^b	0.642	0.700	0.693
		HN	0.653	0.686^b	0.647	0.716	0.716
	$\varphi_{Po}/(1 - \varphi_{Po})$	LN	4.278	3.747^a	3.833	4.867	4.268
		SN	4.069	4.223^b	3.793	4.902	4.248
		HN	4.363	4.138^{ab}	3.837	4.935	4.323
	$\psi_{Eo}/(1 - \psi_{Eo})$	LN	1.101^a	0.967^a	0.948^a	1.216	1.152
		SN	1.112^a	1.025^{ab}	1.069^{ab}	1.217	1.158
		HN	1.255^b	1.100^b	1.182^b	1.304	1.246
	PI_{ABS}	LN	3.089	2.297^a	2.248	4.231	3.580
		SN	2.896	3.035^b	2.719	4.303	3.573
		HN	3.648	3.217^b	3.104	4.706	3.959
	$\delta_{Ro}/(1 - \delta_{Ro})$	LN	0.844	0.717^a	0.593	0.561	0.572
		SN	0.887	0.823^b	0.560	0.534	0.548
		HN	0.918	0.832^b	0.579	0.509	0.562
	PI_{total}	LN	2.555^a	1.655^a	1.342	2.369	2.059
		SN	2.590^{ab}	2.519^b	1.588	2.317	1.998
		HN	3.314^b	2.654^b	1.898	2.407	2.226
Geneva	V_K/V_J	LN	0.514^b	0.472	0.516^b	0.520^c	0.468^b
		SN	0.468^a	0.442	0.444^a	0.452^b	0.438^{ab}
		HN	0.471^a	0.475	0.427^a	0.410^a	0.409^a
	RC/CS_0	LN	304.0^a	362.0	321.7^a	307.0^a	330.2^a
		SN	340.5^b	384.9	374.2^b	360.8^b	360.2^b
		HN	337.0^b	366.7	391.1^b	394.3^c	394.7^c
	RC/ABS	LN	0.459^a	0.497	0.471^a	0.479^a	0.524^a
		SN	0.509^b	0.525	0.552^b	0.552^b	0.559^{ab}
		HN	0.507^b	0.485	0.575^b	0.607^c	0.599^b
	$\varphi_{Po}/(1 - \varphi_{Po})$	LN	3.710b	3.374	3.991	4.322^a	4.051
		SN	3.853	3.408	4.288	4.618^{ab}	4.117
		HN	3.845	3.238	4.345	4.678^b	4.229
	$\psi_{Eo}/(1 - \psi_{Eo})$	LN	0.746^a	0.698	0.698	0.750^a	0.772^a
		SN	0.885^b	0.745	0.745	0.945^b	0.831^a
		HN	0.848^{ab}	0.668	0.668	1.103^c	0.970^b
	PI_{ABS}	LN	1.375	1.294	1.451^a	1.685^a	1.739^a
		SN	1.815	1.382	2.156^b	2.530^b	1.984^a
		HN	1.735	1.096	2.628^b	3.202^c	2.535^b
	$\delta_{Ro}/(1 - \delta_{Ro})$	LN	0.847	0.728	0.606	0.663^b	0.655^b
		SN	0.802	0.723	0.548	0.551^{ba}	0.604^{ab}
		HN	0.835	0.736	0.561	0.570^a	0.558^a
	PI_{total}	LN	1.093	0.921	0.848^a	1.041^a	1.074^a
		SN	1.409	0.981	1.156^{ab}	1.357^{ab}	1.149^{ab}
		HN	1.408	0.797	1.394^b	1.774^c	1.369^b

Table 2. Chlorophyll *a* fluorescence parameters in ‘Weiki’ and ‘Geneva’ in 2016. For each parameter, *small superscript letters* indicate statistically homogenous groups (*ANOVA*, *Tukey*'s HSD test, $P \leq 0.05$). LN, SN, HN – 3, 5, and 8 mg(N) 100 g^{-1}(soil DM), respectively.

Cultivar	Parameter	N treatment	June 2	June 23	July 14	August 4	August 25
Weiki	V_K/V_J	LN	0.399^{b}	0.386^{b}	0.382^{b}	0.433^{b}	0.440^{b}
		SN	0.367^{a}	0.350^{a}	0.336^{a}	0.367^{a}	0.346^{a}
		HN	0.370^{a}	0.338^{a}	0.339^{a}	0.355^{a}	0.348^{a}
	RC/CS_0	LN	384.6^{a}	366.2^{a}	373.5^{a}	333.4^{a}	329.3^{a}
		SN	414.7^{b}	406.9^{b}	428.8^{b}	392.3^{b}	433.5^{b}
		HN	414.4^{b}	416.0^{b}	430.1^{b}	410.0^{b}	438.9^{b}
	RC/ABS	LN	0.633^{a}	0.650^{a}	0.650^{a}	0.569^{a}	0.562^{a}
		SN	0.687^{b}	0.719^{b}	0.719^{b}	0.670^{b}	0.717^{b}
		HN	0.680^{b}	0.745^{b}	0.745^{b}	0.696^{b}	0.711^{b}
	$\varphi_{Po}/(1-\varphi_{Po})$	LN	4.903^{a}	4.790^{a}	4.840^{a}	4.190^{a}	4.344^{a}
		SN	5.127^{b}	5.053^{b}	5.122^{b}	4.434^{b}	4.664^{b}
		HN	5.094^{b}	5.110^{b}	5.147^{b}	4.573^{b}	4.589^{ab}
	$\psi_{Eo}/(1-\psi_{Eo})$	LN	1.226^{a}	1.047^{a}	1.012^{a}	1.037^{a}	0.797^{a}
		SN	1.362^{b}	1.289^{b}	1.332^{b}	1.304^{b}	1.161^{b}
		HN	1.397^{b}	1.376^{b}	1.357^{b}	1.449^{c}	1.189^{b}
	PI_{ABS}	LN	3.954^{a}	3.373^{a}	3.319^{a}	2.541^{a}	2.041^{a}
		SN	4.847^{b}	4.776^{b}	5.228^{b}	3.937^{b}	4.018^{b}
		HN	4.870^{b}	5.301^{b}	5.277^{b}	4.705^{c}	3.985^{b}
	$\delta_{Ro}/(1-\delta_{Ro})$	LN	0.800	0.612^{a}	0.606	0.589	0.600
		SN	0.800	0.633^{ab}	0.610	0.617	0.564
		HN	0.806	0.658^{b}	0.622	0.620	0.551
	PI_{total}	LN	3.115^{a}	2.091^{a}	2.006^{a}	1.503^{a}	1.232^{a}
		SN	3.882^{b}	3.055^{b}	3.224^{b}	2.408^{b}	2.307^{b}
		HN	3.944^{b}	3.530^{b}	3.308^{b}	2.907^{c}	2.260^{b}
Geneva	V_K/V_J	LN	0.505^{b}	0.483^{b}	0.491^{b}	0.520^{b}	0.517^{b}
		SN	0.438^{a}	0.409^{a}	0.394^{a}	0.403^{a}	0.404^{a}
		HN	0.459^{a}	0.436^{a}	0.392^{a}	0.398^{a}	0.387^{a}
	RC/CS_0	LN	308.8^{a}	319.6^{a}	322.5^{a}	296.5^{a}	289.7^{a}
		SN	371.9^{b}	377.8^{b}	401.0^{b}	392.4^{b}	408.9^{b}
		HN	371.0^{b}	360.4^{b}	401.9^{b}	387.4^{b}	409.9^{b}
	RC/ABS	LN	0.491^{a}	0.508^{a}	0.504^{a}	0.469^{a}	0.479^{a}
		SN	0.575^{b}	0.608^{c}	0.635^{b}	0.607^{b}	0.604^{b}
		HN	0.543^{b}	0.564^{b}	0.636^{b}	0.614^{b}	0.633^{b}
	$\varphi_{Po}/(1-\varphi_{Po})$	LN	4.575^{a}	4.236^{a}	4.349^{a}	3.977^{a}	4.305
		SN	4.941^{b}	4.581^{b}	4.717^{b}	4.261^{b}	4.172
		HN	4.681^{ab}	4.380^{a}	4.641^{b}	4.363^{b}	4.259
	$\psi_{Eo}/(1-\psi_{Eo})$	LN	0.721^{a}	0.722^{a}	0.669^{a}	0.740^{a}	0.696^{a}
		SN	0.938^{b}	1.022^{c}	0.964^{b}	1.097^{b}	0.984^{b}
		HN	0.867^{b}	0.873^{b}	0.993^{b}	1.193^{c}	1.037^{b}
	PI_{ABS}	LN	1.662^{a}	1.597^{a}	1.547^{a}	1.434^{a}	1.531^{a}
		SN	2.733^{b}	2.919^{c}	3.004^{b}	2.895^{b}	2.603^{b}
		HN	2.317^{b}	2.198^{b}	3.052^{b}	3.235^{b}	2.950^{b}
	$\delta_{Ro}/(1-\delta_{Ro})$	LN	0.780^{b}	0.570^{a}	0.577	0.652^{b}	0.646
		SN	0.655^{a}	0.613^{b}	0.589	0.562^{a}	0.578
		HN	0.589^{a}	0.568^{a}	0.593	0.612^{ab}	0.607
	PI_{total}	LN	1.270^{a}	0.918^{a}	0.875^{a}	0.898^{a}	0.934^{a}
		SN	1.839^{b}	1.791^{c}	1.790^{b}	1.618^{b}	1.522^{b}
		HN	1.436^{a}	1.258^{b}	1.812^{b}	1.945^{c}	1.806^{b}

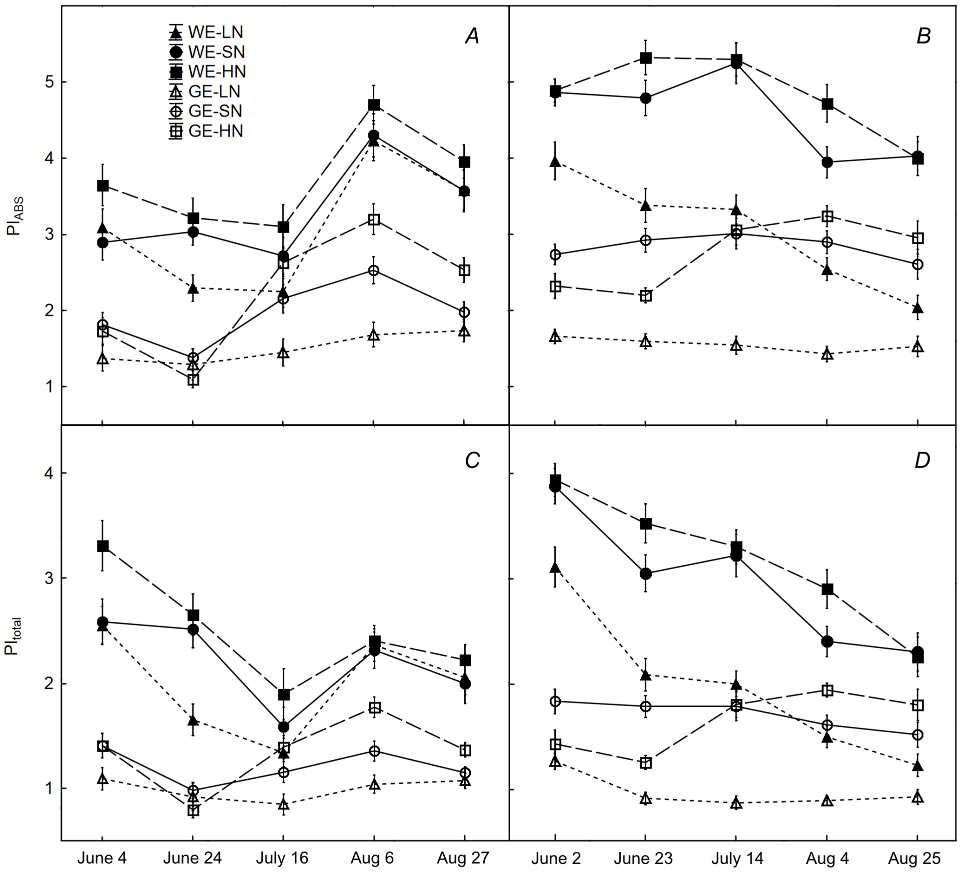

Fig. 4. Performance index (PI_{ABS}) (*A*,*B*) and total performance index (PI_{total}) (*C*,*D*) during the growing seasons 2015 (*A*,*C*) and 2016 (*B*,*D*) in two *Actinidia arguta* cultivars 'Weiki' (WE) and 'Geneva' (GE). The plants were treated with minimal (LN), standard (SN), and increased (HN) nitrogen supply. Values are means ± SE.

and of PI_{total} in 'Geneva' in the beginning of the growing season (Fig. 4*A*,*C*). In 2016, both performance indices showed depletion towards the end of the growing season in 'Weiki' and remained stable to some extent in 'Geneva', however, well N-supported 'Geneva' showed moderate increase (Fig. 4*B*,*D*). Both PI_{ABS} and PI_{total} were dependent also on cultivar traits in each season. Based on the two-way *ANOVA*, we found that in 2015 cultivar traits did not influence the effect of N nutrition on PI_{ABS} and PI_{total}, but in 2016, they did.

Insufficient N nutrition significantly affected the total number of reaction centres per absorption (RC/ABS) and density of RCs per cross section (RC/CS_0) on particular measurement days in 2015 and during the whole growing season in 2016 (Tables 1, 2). The similar pattern was found in V_K/V_J (Tables 1, 2).

PCA showed high effect of 'measurement day × N treatment' combination on the parameters describing PSII performance (PC1, Fig. 5*A*). The parameter V_K/V_J, describing the efficiency of electron flow from OEC to PSII reaction centres, the density of active reaction centres per PSII antenna chlorophyll (RC/ABS), and the efficiency of total electron transport beyond Q_A (ψ_{Eo}) are located directly along the *x* axis and nearly parallelly to total N leaf content. The $\delta_{Ro}/(1-\delta_{Ro})$ ratio allocation on the *y* axis suggests the performance of end electron acceptors at PSI as PC2.

The distribution of 'year × N treatment' combination showed the influence of N treatment on energy fluxes around both photosystems, the coordinates of LN samples were distanced from SN and HN in both directions in most cases. In 'Geneva', LN coordinates indicate higher $\delta_{Ro}/(1-\delta_{Ro})$ values, in 'Weiki', LN coordinates indicate lower $\delta_{Ro}/(1-\delta_{Ro})$ ratio. In both cultivars and both years, the higher N doses were applied, the higher coordinates of PSII performance parameters were shown in the diagram (Fig. 5*B*).

Discussion

Considering the difference in climatic conditions between two experimental growing seasons, some obtained ChF parameters reflected an influence of insufficient precipitation despite regular irrigation applied on the experimental plantation. The first part of 2015 growing season was extremely dry and a general trend in F_v/F_m, ψ_{Eo}, and PI_{ABS} in 2015 suggest increased photoinhibition at that time (Figs. 3*A*,*C*; 4*A*). These findings were also shown by Fini *et al*. (2009), Wang *et al*. (2012a), and Swoczyna *et al*. (2015). The 2016 growing season was more abundant

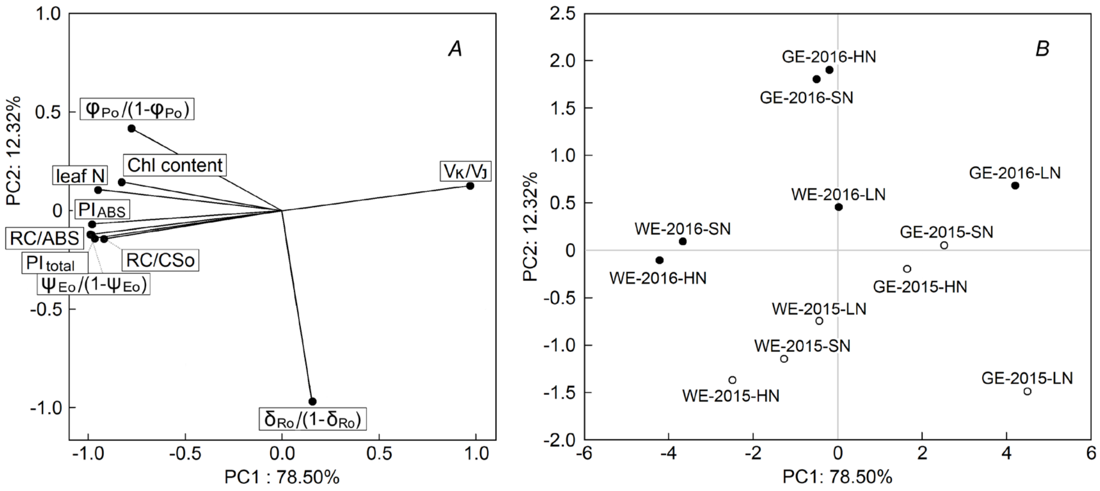

Fig. 5. Principal Component Analysis (PCA) on parameters obtained during 2015 and 2016 for minimum N treatment (LN), standard N treatment (SN), and increased N treatment (HN) in *Actinidia arguta* 'Weiki' (WE) and 'Geneva' (GE). Correlation between variables along two PCA axes (*A*) and ordination of cases along two PCA axes (*B*) was based on data from three averaged samples of LN, SN, and HN from particular measurement days.

in precipitation and the higher F_v/F_m, ψ_{Eo}, and PI_{ABS} values recorded in 2016 compared to 2015 might be an answer to more favourable growing conditions.

Beside the reaction to climatic conditions, recorded ChF parameters showed also some differences between two cultivars. Throughout two years of the experiment, 'Weiki' revealed higher values of the parameters describing efficiencies of particular processes connected with energy transfer around PSII, F_v/F_m, ψ_{Eo}, and additionally, PI_{ABS}, which is the parameter integrating the previous ones. These findings indicate that 'Weiki' has generally a higher potential in solar energy conversion for biochemical processes and confirm better vitality and plasticity of this cultivar.

As diurnal changes may affect PI_{ABS} in field conditions (Živčák *et al.* 2014b), PI_{total} should also be considered. PI_{total} was in average also higher in 'Weiki' than that in 'Geneva', although the fourth factor in PI_{total} calculation, $\delta_{Ro}/(1-\delta_{Ro})$ ratio, was higher in 'Geneva'. Both parameters, δ_{Ro} and $\delta_{Ro}/(1-\delta_{Ro})$ ratio, describe electron flow through end electron acceptors (carriers) at PSI (plastocyanin, ferredoxin) but not exactly the PSI (Živčák *et al.* 2015a). The analysis of δ_{Ro} and $\delta_{Ro}/(1-\delta_{Ro})$ ratio is not very common in environmental research because results are not unambiguous in field experiments (Ugolini *et al.* 2012). In controlled conditions, while gradual leaf desiccation evoked decrease in F_v/F_m (denoted also as φ_{Po} or TR_0/ABS), and often in ψ_{Eo} (denoted also as ET_0/TR_0), δ_{Ro} values were increasing during the first steps of stress and only severe stress caused depletion of the efficiency of electron transport up to end electron acceptors of PSI (Strasser *et al.* 2010, Goltsev *et al.* 2012). Shifted δ_{Ro} values were found in heat-stressed young leaves compared to heat-stressed mature leaves (Brestič *et al.* 2012, Duan *et al.* 2015). Those findings suggest a certain role of end electron acceptors at PSI in recovery of electron transport processes between PSII and PSI. In our experiment, higher δ_{Ro} values, exceeding 0.4, were recorded on 4 and 24 June, 2015 (dry season), and on 2 June 2016 (moderate season), in both cultivars (data not shown, for statistical differences *see* $\delta_{Ro}/(1-\delta_{Ro})$ ratio in Tables 1, 2). A minor δ_{Ro} increase was found in August 2015 and 2016 in N-deficient 'Geneva' specimens. Therefore, we found higher δ_{Ro} values as a symptom of some environmentally evoked imbalance in electron transport between PSII and PSI. Nevertheless, the $\delta_{Ro}/(1-\delta_{Ro})$ ratio was the least sensitive parameter in our experiment, and it did not show evident differences between cultivars, growing seasons, and in most cases, N treatments. Similar results were found in tomato plants by Kalaji *et al.* (2014a). Some researchers combine efficiency of electron transport up to end electron acceptors of PSI, δ_{Ro}, and the efficiency of a movement of electron into the electron transport chain beyond Q_A, ψ_{Eo}. The combined parameter, denoted as ψ_{REo} or ΔV_{IP}, shows total efficiency of electron transport from PSII to PSI (Živčák *et al.* 2015a). It illustrates the increased pool of reduced Q_A^- in plants facing ozone exposure (Desotgiu *et al.* 2012), salinity or drought stress (Salvatori *et al.* 2014), and thermal stress connected with drought (Pšidová *et al.* 2018). In our experiment, ψ_{REo} was significantly lower in N-deficient plants both in 2015 and 2016, and both cultivars revealed the same pattern (data not shown). This was in contrast with δ_{Ro} results and indicated that plastoquinone pool and/or cytochrome b_6f became the bottle-neck for electron transport in case of nitrogen deficiency.

Taking into account other parameters, the different N nutrition affected both cultivars, however, not in the same manner. F_v/F_m revealed poor reaction to different N doses in 'Weiki' showing simultaneously higher values in June, compared to 'Geneva' in 2015. Minor or none F_v/F_m reaction to N deficit was reported by Lu *et al.* (2001). Maximum quantum efficiency ($F_v/F_m = \varphi_{Po}$) and its calculated ratio $\varphi_{Po}/(1-\varphi_{Po})$ were reported as susceptible to N deficit (Kalaji 2011, Živčák *et al.* 2014a, Feng *et al.*

2015). But these parameters are also affected by drought or heat (Kalaji *et al*. 2011a,b; Wang *et al*. 2012b). We suppose that the more drought-resistant 'Weiki' revealed higher and more stable F_v/F_m as a result of acclimation to drought prolonging since May. This was also reflected by PI_{ABS} in 'Weiki'. PI_{ABS} in 'Geneva' was more affected by different N nutrition, especially on 6 August, when photosynthetic performance was significantly different between three N treatments. Here, PI_{ABS} was influenced by significantly different RC/ABS and ψ_{Eo} values according to different N treatment. In 2016, low N doses induced significant decrease in RC/ABS, F_v/F_m, ψ_{Eo}, PI_{ABS}, and PI_{total}. On particular measurement days, high N doses resulted in the presence of an additional statistically homogenous group for HN (Table 2). This suggests that 2016 season was more favourable for N uptake and utilisation by plants. Favourable water conditions were previously reported to be crucial for N uptake (Bai *et al*. 2017), and for an increase of the efficiency of N utilisation in photosynthesising tissues (Teixeira *et al*. 2014).

In 2015, the most sensitive parameter to N nutrition was density of active RCs per cross section (RC/CS_0). In 'Weiki', it was the only parameter which showed the effectiveness of increased N nutrition at most measurement days. In 'Geneva', the increased number of active RCs per cross section was additionally reflected by higher RC/ABS. This suggests that both 'Weiki' and 'Geneva' tend to activate a higher number of RCs while better supported with N nutrition. It is well known that higher N content results in higher Chl concentration in leaves (Loh *et al*. 2002, Chang and Robison 2003), but it is also important to conclude that better N supplementation was proven to increase a number of reaction centres in photosystems and to better utilise the energy harvested by chloroplast antennas. The results from moderate 2016 season show that standard N nutrition accompanied by proper water availability supports the optimal number of Chl molecules needed for the RCs in photosystems. The similar patterns of both RC/CS_0 and RC/ABS statistics (Table 2) suggest that these parameters are good indicators for N deficiency which was also demonstrated by Kalaji (2011) and Redillas *et al*. (2011). Moreover, regardless of climatic conditions, in most cases RC/CS_0 did not exceed values of 360 and 400 in 'Geneva' and 'Weiki', respectively. This indicates that RC/CS_0 is directly dependent on N supplementation and it is possible to define a threshold specific for particular species/cultivars.

In the early season 2015, the effect of N supplementation on the efficiency of total electron transport beyond Q_A (ψ_{Eo}) was not clearly visible (in 'Weiki', only the highest N supply caused the statistically higher ψ_{Eo}) and this might be a result of limited water availability in June (Fig. 3*C*,*D*). In July and August 2015, more sensitive 'Geneva' showed high dependence on N nutrition. Thus, we can suppose that different reactions to N supply in dry year indicate different characteristics of the examined cultivars based probably on their provenance (Pšidová *et al.* 2018). 'Geneva' seems to be more sensitive to drought and poor nitrogen availability, 'Weiki' seems to show more continental-like traits. In 2016, both cultivars revealed higher sensibility of ψ_{Eo} depending on N supply. Moreover, not only the standard doses but also the highest doses of N increased significantly ψ_{Eo} on particular measurement days in 2015 and 2016. This indicates that electron movement in ETC beyond Q_A is visibly dependent on N availability which was also confirmed by Lu *et al*. (2001), Lin *et al*. (2013), and Kalaji *et al*. (2014a), as well as by differences between ψ_{REo} and δ_{Ro} discussed above. N is not only a component of Chl molecules but also of proteins involved in numerous biochemical processes and of electron, proton, and energy carriers (NADPH, ATP). Thus, the different N supply in *Actinidia* may affect also the efficiency of total electron transport beyond Q_A (ψ_{Eo}), *i.e.* the PSII acceptor side, and the based-on-it calculated parameter $\psi_{Eo}/(1-\psi_{Eo})$ which is the component of two performance indices. These results were also confirmed by Kalaji (2011).

Discussing ChF parameters describing consecutive processes of light energy conversion it is worthy to analyse the effect of N supplementation on PSII donor side. In previous research, it was noticed that heat or drought stress conditions may evoke an additional shift in fast fluorescence records, so called K-step (Strasser *et al*. 2004, Oukarroum *et al*. 2007), which is related to an inactivation or disconnection of water-splitting complex. Schmitz *et al*. (2001) showed that the higher N deficit was set to *Vigna uncuiculata* plants, the more visible fluorescence increase appeared at K point. Likewise, Redillas *et al.* (2011) reported the formation of K-band in N-deficient rise seedlings. On the contrary, additional N supplementation may diminish the ratio V_K/V_J in plants growing in field top soil (Li *et al*. 2012). In our research, V_K/V_J revealed a significant shift in N-deficient plants in both cultivars in moderate season 2016. This suggests the important role of N in the processes connected with oxygen evolving and electron donation to RCs of PSII. In water-deficient season 2015, V_K/V_J ratio increased in N-deficient 'Geneva' and incidentally in N-deficient 'Weiki'.

In order to summarise which components of PI_{ABS} and PI_{total} equations are the most dependent on N nutrition, the principal component analysis was performed. PCA showed higher effect of 'climate-conditions × N treatment' combination on the parameters describing PSII performance (PC1, Fig. 5*A*). The density of active RCs per PSII antenna Chl (RC/ABS) and efficiency of electron movement by trapped exciton into the electron transport chain beyond the Q_A [$\psi_{Eo}/(1-\psi_{Eo})$] along with V_K/V_J, describing the efficiency of electron flow from OEC to PSII reaction centres, and RC/CS_0, are located directly along the *x* axis and almost parallel to total N leaf content. These parameters seem to be directly conditioned by N availability for plants. The probability that the absorbed photon will be trapped by RC, expressed as $\varphi_{Po}/(1-\varphi_{Po})$, is also affected by N nutrition to a certain extent, but beside PSII structure other factors may determine the maximum quantum efficiency of PSII. De Castro *et al*. (2014) found that φ_{Po} (F_v/F_m) was affected by Chl content only when Chl was lacking. The $\delta_{Ro}/(1-\delta_{Ro})$ ratio allocation on the *y* axis suggests the lower dependence of the performance of end electron acceptors around PSI (PC2 = 12.32%, Fig. 5*A*) upon N content.

Ordination of cases along two PCA axes showed different characteristics of two examined cultivars. 'Geneva' appeared more susceptible both to climatic conditions and N deficiency. GE-2015 and GE-2016 clusters are more separated from each other than WE-2015 and WE-2016 clusters. In both years low N nutrition affected visibly the performance of PSII in 'Geneva'. High N nutrition amended it to some extent in dry season 2015, but had no effect in 2016. Furthermore, in 'Geneva', the efficiency of end electron acceptors at PSI positively supported PSII performance in plants treated with low N supply in both years. Conversely, in 'Weiki', the efficiency of end electron acceptors at PSI was not much affected by N nutrition. Climatic conditions had no influence here, as well. Considering the performance of PSII, the N nutrition in 'Weiki' was effective only in the growing season with sufficient precipitation, in former drier season, the effect was less visible. These findings show that 'Weiki' is not only able to cope better with scarce water availability but also reveals higher independence from N availability when affected by drought conditions. The differences in nitrogen-use efficiency between different cultivars were found also in winter wheat (Fan and Li 2001).

Conclusions: Two examined cultivars differ in the efficiency of light energy conversion when facing nitrogen deficiency. The highest differences were found in the efficiency of electron transport chain performance indicating ecophysiological differences between both cultivars. Chlorophyll *a* fluorescence measurements in the field experiment allowed us to evaluate usefulness of the ChF parameters in detecting N deficiency. In field conditions, several factors may determinate plant vitality. The integrating ChF parameters, PI_{ABS} and PI_{total}, are useful to detect the overall decrease in plant performance. They are very sensible, but cannot indicate a factor responsible for negative changes. Considering all components of PI_{ABS} and PI_{total} calculations, in our experiment, $\psi_{Eo}/(1-\psi_{Eo})$ was more susceptible to different N nutrition and RC/ABS was the best indicator. Beside the above mentioned parameters, we recommend to analyse the RC/CS_0, which is strictly connected with N availability for chlorophyll synthesis. We found that regardless of the climatic conditions, the RC/CS_0 values beneath experimentally defined thresholds may indicate insufficient N supply.

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