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Research article

Effects of nitrogen-deficiency on efficiency of light-harvesting apparatus in radish

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ABSTRACT

Nitrogen starvation has been stated to reduce chlorophyll *a* and accessory pigments, decrease photosynthetic efficiency, as well as modify chloroplast thylakoid membranes. However, the impact of N-deficiency on light-dependent reactions of photosynthesis has not been well understood. In this study, efficiency and structure of light-harvesting complex under N-deficiency conditions were investigated in two radish cultivars (*Raphanus sativus* var. *sativus* 'Fluo HF1' and 'Suntella F1'). Light-dependent reactions of photosynthesis were investigated by measuring in vivo chlorophyll *a* prompt fluorescence signal. Acquired data were utilised in two ways: by plotting fast induction curves and calculating OJIP-test biophysical parameters. Detailed analysis of difference curves as well as OJIP-test results showed that major disturbances were associated with photosystem II and its subunits, including decoupling of light-harvesting complexes, dysfunction of oxygen-evolving complex, and reaction centres inactivation. The maximum quantum yield of photosystem II primary photochemistry was severely restricted, causing an inhibition in electron transport through successive protein complexes in the thylakoid membrane. Structural changes were demonstrated by recording images using Transmission Electron Microscopy (TEM). TEM investigations showed intensive starch accumulation under N-deficiency. Rare thylakoid stacks distributed in tiny layers of stroma around grains and chloroplast periphery were observed in cells of N-deficient plants. The application of principal component analysis (PCA) on OJIP-test results allowed characterizing the dynamics of stress response and separating parameters according to their influence on plants stress response. 'Suntella F1' genotype was found to be more sensitive to nitrogen deficiency as compared to 'Fluo HF1' genotype.

1. Introduction

Photosynthesis is a highly susceptible process to most known stressors (Ahmad and Rasool, 2014). Nitrogen is an essential constituent of several of the most important plant substances, including amino acids and chlorophyll. About 75% of the nitrogen in a leaf is contained in chloroplast proteins (Hörtensteiner and Feller, 2002). More than 20% of total leaf nitrogen in C3 multicellular species may amount to the concentration of RuBisCO (Suzuki et al., 2009). A substantial proportion of cell nitrogen is associated with the light harvesting apparatus: the major integral protein complexes, including photosystem I (PSI), photosystem II (PSII), the oxygen evolving complex (OEC), and the cytochrome b_6f complex

Abbreviations: ADP, adenosine diphosphate; ATP, adenosine triphosphate; Comp1, 2, principal component 1, 2; CS, control sample; DAT, day after treatment; ETC, electron transport chain; FNR, ferredoxin-NADP oxidoreductase; LHCII, PSII light-harvesting complex; $NADP^+$, – nicotinamide adenine dinucleotide phosphate; N-def., nitrogen-deficient; OEC, oxygen evolving complex, Mn_4CaO_5 cluster; PCA, principal component analysis; PF, prompt fluorescence; PQ/PQH_2, a mobile plastoquinone/plastoquinol; PSI, II, photosystem I, II; Q_A, a plastoquinon bounded with PSII subunit D2; Q_B, a plastoquinone that binds and unbinds from PSII subunit D1; RC, reaction centre; RuBisCO, ribulose-1,5-bisphosphate carboxylase/oxygenase; SEM, standard error of mean.

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as well as the smaller, peripheral proteins: plastocyanin, ferrodoxin, ferredoxin-$NADP^+$ reductase, and ATP synthase (Kumar et al., 2002; Makino et al., 2003). However, the impact of N-deficiency on photosynthetic apparatus efficiency and its structure has not been well understood.

A positive correlation between nitrogen supply and photosynthetic efficiency has been reported by many researchers. Nitrogen starvation has been stated to reduce chlorophyll *a* and accessory pigments as well as modify chloroplast thylakoid membranes (Osborne and Geider, 1986; Laza et al., 1993; Nakano et al., 1997). Studies on N-deficient plants showed a decrease in the light-saturated photosynthetic rate (Terashima and Evans, 1988; Amtmann and Armengaud, 2009) and the quantum yield of photosynthesis (Nunes et al., 1993). Modifications in photosynthesis due to variations in nitrogen supply have been associated withthe primary CO_2 fixing enzyme RuBisCO content (Ferrar and Osmond, 1986; Evans and Terashima, 1987; Haup-Herting and Fock, 2000) and ATP synthase (Tezara et al., 1999; Redillas et al., 2011).

Scientific reports on nitrogen deficiency impact on PSII primary photochemistry and electron transport are not unanimous. Some studies have suggested that nitrogen deficiency induces some damage to PSII which is revealed by a decrease in the quantum yield of PSII electron transport and the maximal efficiency of PSII photochemistry (Nunes et al., 1993; Verhoeven et al., 1997; Lu et al., 2001; Jin et al., 2015). von Caemmerer and Farquhar (1981) concluded that nitrogen deficiency in *Phaseolus* reduced *in vitro* both RuBisCO activity and electron transport chain to the same relative extent. Boussadia et al. (2015) stated that modifications in PSII photochemistry observed in olive trees subjected to N-deficiency resulted from a down-regulation mechanism balancing production of adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH) with the decreased demand in the Calvin cycle. Other researchers reported no effect on the quantum yield of PSII electron transport, thus no damage to PSII induced by nitrogen deficiency (Khamis et al., 1990; Henley et al., 1991; Bungard et al., 1997; Lu and Zhang, 2000).

The aim of this research was to study efficiency and structure of light-harvesting apparatus in leaves of radish (*Raphanus sativus* var. *sativus*) grown under condition of nitrogen deficiency. One of approaches to investigate light-dependent reactions of photosynthesis is an analysis of chlorophyll *a* fluorescence. Although it is corresponding to a very small fraction of the energy dissipated from the photosynthetic apparatus, it is widely accepted to provide an access to the understanding of its structure and function (Maxwell and Johnson, 2000; Strasser et al., 2004; Kalaji, 2011; Stirbet et al., 2014). Chlorophyll *a* fluorescence measurements have been used to study plant's reaction to various kinds of environmental stresses (Strasser et al., 2004; Wituszyńska et al., 2013; Kalaji et al., 2014a), including nutrient deficiencies (Goltsev et al., 2012; Aleksandrov et al., 2014; Kalaji et al., 2014b; Živčák et al., 2014; Wang et al., 2015). To monitor structural changes in chloroplasts, transmission electron microscopy (TEM) was used.

2. Material and methods

2.1. Plant material and growing conditions

Two radish hybrid cultivars were selected for the experiment: *Raphanus sativus* var. *sativus* 'Suntella F1' and 'Fluo HF1'. Plants were grown in a plant growth chamber under a set of controlled conditions, close to optimal for both cultivars. The photoperiod was 14 h and day/night air temperature was 18/13 °C respectively. Photosynthetic photon flux (area) density (PPFD) was about 250 μmol m^{-2} s^{-1}. Twenty-four hour average relative air humidity was about 50 per cent. Plant density was 525 plants per m^2. Experiment was performed in 3 replications.

Plants were grown in hydroponics, which allows precise controlling of the mineral composition of a solution. An innovative material: polyethylene pellets, made from a synthetic resin was applied as a growing medium. It is characterized by a low ion absorption capacity as compared to mineral substrates commonly used as growing media for hydroponics.

Both experimental and control solutions were developed based on the Hoagland solution (Hoagland and Arnon, 1950). In solutions with low nitrate concentration the nitrate was replaced mostly by chloride, as in an experiment of Evans and Terashima (1987). Control solution contained following macro nutrients (concentration expressed in mmol/L): N (NO_3) – 11.63; P (PO_4)– 1.00; K – 6.50; Ca – 4.00; Mg – 2.00; Na – 1.13; S (SO_4) – 3.13; Cl – 1.00; while the experimental solution: N (NO_3) – 0.13; P (PO_4)– 1.00; K – 5.00; Ca – 4.00; Mg – 2.00; Na – 1.13; S (SO_4) – 4.13; Cl – 8.99. Both solutions contained the same concentration of micro nutrients (in μmol/L): Fe – 118.48; B – 48.57; Cu – 1.60; Zn – 0.97; Mn – 6.51; and Mo – 0.52. For solution recipes see Tables A.1 and A.2 in Appendix. Deionized water was used to prepare the stock solutions and growing nutrient solutions.

After 7 days of germination, plants were grown in the control solution until the first pair of leaves fully expanded (in 15^{th} day of vegetation). In 16^{th} day of vegetation plants were subjected to the stress conditions: N-deficient solution. 17^{th} day of vegetation will be referred further in this paper as 1 DAT (day after treatment). Stress conditions were sustained for 14 days. On 14 DAT (30^{th} day of vegetation) the control solution was restored. The final, recovery phase lasted 13 days, until 43^{rd} day of vegetation.

2.2. Measuring methods and devices

Leaf weight (excluding cotyledons) was evaluated five times during vegetative period: on 1, 4, 7, 21 and 24 DAT. There were 6–21 randomly selected plants harvested for evaluation in each term per each cultivar and treatment (n = 6–21). Dry matter content was determined by drying foliage, then reweighting and expressed as percentage of the fresh weight. Yield of individual plant organs (leaves, storage bulbs, and roots) was evaluated on 26 DAT (12^{th} day of recovery, 42^{nd} day of vegetation).

Since leaves are considered to be the most satisfactory plant part to use for diagnosis (Barker and Bryson, 2006), collected foliage was further analysed in order to determine its quantitative content of selected elements: N by high-temperature combustion detection TCD, while P, K, Mg, S, and Fe by inductively coupled plasma atomic emission spectroscopy (ICP-AES) (THERMO SCIENTIFIC iCAP 6000, Thermo Fisher Scientific Inc, Waltham, MA, USA), after mineralization in 65% HNO_3 and 70% $HClO_4$ (HNO_3:$HClO_4$ = 4:1). Analyses were performed on pool samples of 6–21 randomly selected plants per each term, cultivar and treatment.

Chlorophyll content was evaluated in vivo using Dualex Scientific+™ Polyphenol & Chlorophyll-Meter (Force-A, France), four times during stress conditions and four times after restoring control solution in 2–4 day intervals. Measurements were conducted on 2–3 fully expanded leaves of different age from 9 plants per treatment, selected and marked with a water-based pen prior to introducing stress conditions (n = 18–27).

Net photosynthesis rate (Pn), transpiration rate (Tr), stomatal conductance (Cond.) and intercellular CO_2 concentration (Ci) were evaluated in vivo using an infra-red gas analyser (IRGA) LI-6400 (LI-COR Inc., USA). Measurements were conducted six times during vegetative period: four times during stress period (on 4, 6, 10 and 13 DAT), and two times during recovery period (on 17 and 21 DAT). Measurements were conducted on four, fully expanded leaves from