DOI: 10.1007/s11099-015-0116-3 PHOTOSYNTHETICA 53 (3): 471-477, 2015

Differential blockage of photosynthetic electron flow in young and mature leaves of *Arabidopsis thaliana* by exogenous proline

I. SPERDOULI and M. MOUSTAKAS+

Department of Botany, School of Biology, Aristotle University of Thessaloniki, University Campus, 54124 Thessaloniki, Greece

Abstract

Responses of the photosynthetic electron transport system of chloroplasts to exogenous proline application were evaluated in young and mature leaves of *Arabidopsis thaliana* plants under optimal growth conditions. Exogenous proline application (10 mM) during the 4th week of growth increased proline accumulation in young leaves more than in mature leaves, and possibly due to its degradation producing NADPH, decreased significantly the ratio of $NADP^+$/NADPH in both leaf types compared with controls (without proline). However, the ratio of $NADP^+$/NADPH remained significantly higher in the young leaves, suggesting lower proline degradation which resulted in less reduced plastoquinone pool than that in the mature leaves, under both low light [130 μmol(photon) m^{-2} s^{-1}] and high light [1,200 μmol(photon) m^{-2} s^{-1}] treatments. The young leaves seemed to adjust nonphotochemical fluorescence quenching in order to maintain a better PSII quantum yield. We concluded that under optimal growth conditions exogenous proline results in overreduction of the plastoquinone pool and blockage of photosynthetic electron flow due to accumulation of NADPH. We suggest that optimum concentrations of proline are required for optimal PSII photochemistry.

Additional key words: chlorophyll fluorescence; electron transport rate; leaf age; reactive oxygen species.

Introduction

Proline (Pro) accumulation is regarded as a component of stress tolerance mechanism (Szabados and Savouré 2010), and has been reported to occur in response to environmental stresses including heat and water stress, salinity, and metal toxicity (Hare and Cress 1997, Hare *et al.* 1998, De Ronde *et al.* 2004, Ashraf and Foolad 2007, Verbruggen and Hermans 2008, Sperdouli and Moustakas 2012b, 2014b; Khayyat *et al.* 2014). Accumulation of Pro in plant tissue under stressful conditions has been suggested to be the result of a decrease in Pro degradation, increase in Pro biosynthesis, a decrease in protein synthesis or Pro utilization, and increased hydrolysis of proteins (Ashraf and Foolad 2007, Verbruggen and Hermans 2008). Pro is synthesized from glutamate by two successive reductions catalyzed by Δ^1-pyrroline-5-carboxylate (P5C) synthase (P5CS) and P5C reductase (P5CR), respectively (Ashraf and Foolad 2007, Verbruggen and Hermans 2008). An alternative precursor for Pro biosynthesis is ornithine, which can be transaminated to P5C by ornithine-δ-aminotransferase, a mitochondria-located enzyme (Verbruggen and Hermans 2008). Pro degradation is the reverse process of Pro biosynthesis catalyzed by Pro dehydrogenase (PDH) and P5C dehydrogenase (P5CDH) (Ashraf and Foolad 2007, Verbruggen and Hermans 2008).

Pro may serve to protect membrane structures (Chen and Murata 2002), scavenge reactive oxygen species (ROS) (Hong *et al.* 2000), or regulate cellular redox state (Hare and Cress 1997, Poulson *et al.* 2006). Exogenous application of Pro in many, but not all, plant species under stress conditions has led to significant enhancement

Received 8 June 2014, *accepted* 23 October 2014.
+Corresponding author; phone: (+30)2310998335, fax: (+30)2310998389, e-mail: moustak@bio.auth.gr
Abbreviations: AL – actinic light; AOI – area of interest; Chl – chlorophyll; ETR – electron transport rate; F_0, F_m – minimal and maximal chlorophyll *a* fluorescence of the dark-adapted state; F_0', F_m' – minimal and maximal chlorophyll *a* fluorescence of the light-adapted state; F_s – steady-state photosynthesis at a given actinic light; F_v/F_m – potential (maximal) quantum yield of PSII photochemistry; HL – high light; LL – low light; ML – mature leaves; NPQ – nonphotochemical quenching; P5C – Δ^1-pyrroline-5-carboxylate; P5CDH – Δ^1-pyrroline-5-carboxylate dehydrogenase; P5CR – Δ^1-pyrroline-5-carboxylate reductase; P5CS – Δ^1-pyrroline-5-carboxylate synthase; PDH – proline dehydrogenase; PQ – plastoquinone; Pro – proline; q_P – photochemical quenching coefficient; ROS – reactive oxygen species; YL – young leaves; Φ_{PSII} – actual (effective) quantum yield of PSII photochemistry.
Acknowledgements: This work was supported by funds of the Aristotle University of Thessaloniki.

in plant growth (Ashraf and Foolad 2007, Moustakas *et al.* 2011). However, despite its protective role under abiotic stress conditions, external Pro supply was found to be highly toxic for plants (Hellmann *et al.* 2000, Deuschle *et al.* 2001, 2004; Nanjo *et al.* 2003). Nanjo *et al.* (2003) reported that an excess of free Pro results in growth inhibition in *Arabidopsis*, while Deuschle *et al.* (2004) found that external Pro application caused programmed cell death, with callose deposition and ROS production. Thus, how Pro accumulation influences particular regulatory pathways is still unclear (Funck *et al.* 2010, Szabados and Savouré 2010, Sperdouli and Moustakas 2014b).

NADPH produced by the light reactions of photosynthesis is utilised in the Calvin cycle. The production and utilization of ATP and NADPH may affect the proton gradient and the redox state of PSII (Ögren 1990). While Pro synthesis generates $NADP^+$, its degradation produces NADPH. Thus, a cycle of Pro synthesis and degradation is essential for buffering cellular redox potential in the cytosol, as well as in plastids (Peng *et al.* 1996, Hare and Cress 1997, De Ronde *et al.* 2004). Hare and Cress (1997) suggested that the $NAD(P)^+$/NAD(P)H ratio is important in metabolic regulation and may constitute a form of metabolic signalling within higher plants. Under drought stress, decreased NADPH/$NADP^+$ ratio, resulting in a higher level of oxidized state of the PQ pool and thus in a reduced excitation pressure, contributed to the acclimation of young leaves (YL) of *A. thaliana* to water deficit (Sperdouli and Moustakas 2014b).

Chlorophyll (Chl) fluorescence measurements have become a widely used method to study functioning of the photosynthetic apparatus and are a powerful tool to study the plant's response to environmental stress (Krause and Weis 1991, Morales *et al.* 2001, Gorbe and Calatayud 2012, Sperdouli and Moustakas 2012a, 2014a; Murchie and Lawson 2013, Osório *et al.* 2013, 2014; Guidi and Calatayud 2014, Hanachi *et al.* 2014). Chl fluorescence quenching, predominantly caused by photochemical and energy-dependent mechanisms, is strongly influenced by the utilisation of NADPH and ATP in photosynthesis (Krause and Weis 1991, De Ronde *et al.* 2004). Photosynthetic redox imbalance has recently received a great deal of attention for its potential to act as a retrograde signal (Dietz and Pfannschmidt 2011).

Exogenous application of 10 mM Pro under drought stress conditions has been found to ameliorate drought stress effects on photosynthesis (Moustakas *et al.* 2011), but externally added Pro (10 mM) under optimal growth conditions has been found to be toxic to *Arabidopsis* plants (Mani *et al.* 2002). Recently, a differential photosynthetic response of YL and mature leaves (ML) was shown in *A. thaliana* under drought stress (Sperdouli and Moustakas 2014a). This differential photosynthetic response of *A. thaliana* YL and ML was due to a differential metabolite accumulation under drought stress (Sperdouli and Moustakas 2014b).

The objective of this study was to elucidate (*1*) if external Pro application influences PSII photochemistry under optimal growth conditions and (*2*) if leaf developmental stage modulates the redox potential of photosynthetic electron transport after exogenous Pro application. Thus, we studied the role of exogenous Pro in the $NADP^+$/NADPH redox status maintenance and its impact on photosynthetic electron flow and PSII functioning in YL and ML of the model plant, *A. thaliana*, under both low light [LL, 130 μmol(photon) m^{-2} s^{-1}] and high light [HL, 1,200 μmol(photon) m^{-2} s^{-1}] treatments.

Materials and methods

Plant material and growth conditions: *Arabidopsis thaliana* ecotype Columbia (Col-0) plants were grown in a growth chamber (*EF7*, *Conviron*, Montreal, Canada) with controlled environmental conditions under a long-day photoperiod of 14/10 h, with 50 ± 5/65 ± 5% humidity, temperature of 22 ± 1/18 ± 1°C, and light intensity of 120 ± 20 μmol(photon) m^{-2} s^{-1} (Moustakas *et al.* 2011). Plants were hand-watered every 2 d by spraying. Leaves in the center of the leaf rosette with 1.20 ± 0.22 cm length were assigned as the young leaves (YL), while the average length of mature leaves (ML) in the rosette was 2.4 ± 0.31 cm. During the 4th week of growth, YL and ML were either sprayed (foliar application four times in total, day after day) with water or with 10 mM Pro (Székely *et al.* 2008). This concentration of Pro was found to alleviate drought stress effects in *A. thaliana* (Moustakas *et al.* 2011) but to be toxic under nonstressed conditions (Mani *et al.* 2002). Data shown are means and SE of six replicates per treatment.

Proline determination: YL and ML of four-week-old *A. thaliana* plants sprayed with water or with 10 mM Pro were washed thoroughly with distilled water, cut into small pieces, weighed, placed separately in glass vials containing 10 mL of 80% (v/v) ethanol, and heated at 6°C for 30 min. The extract was then filtered and diluted with 80% (v/v) ethanol up to 20 mL. The concentration of free Pro was determined in this extract following the acid-ninhydrin reagent method (Bates *et al.* 1973), as described by Sperdouli and Moustakas (2014b), using a calibration curve with standard dilutions of L-Pro and expressed as [μmol g^{-1}(FM)].

Assay of $NADP^+$ and NADPH: Extraction of pyridine nucleotides from YL and ML of 4-week-old *A. thaliana* was performed according to Rius *et al.* (2006). $NADP^+$ and NADPH were measured by an enzymatic assay system using glucose-6-phosphate dehydrogenase as described by Zhang *et al.* (2000). The pyridine nucleotides were

assayed spectrophotometrically (*PharmaSpec UV-1700; Shimadzu,* Japan) based on the measurement of the absorbance of the reduced coenzyme at 340 nm (Hald *et al.* 2008). The oxidized nucleotides $NADP^+$ do not show any absorbance at 340 nm.

Chl fluorescence was measured at room temperature in dark-adapted (20 min) *A. thaliana* leaves using an *Imaging-PAM* chlorophyll fluorometer (*Walz*, *Effeltrich*, Germany), as described by Sperdouli and Moustakas (2012b). Five areas of interest (AOI) were selected, one in the centre of the leaf, two in the outer zone of the tip and two in the outer zone of the base of the leaf. First, minimal (F_0) and maximal Chl *a* fluorescence (F_m) were measured with dark-adapted samples, from which F_v/F_m was derived; it represents the potential (maximal) quantum yield. F_m was obtained with a saturating pulse (SP) of white light (2,400 μmol m^{-2} s^{-1} PAR, 800 ms duration) followed by application of actinic light (AL) to assess steady-state photosynthesis (F_s). A low light intensity of AL [130 μmol(photon) m^{-2} s^{-1} (LL, low light)], to match that of the growth light of *A. thaliana* plants, and low enough to avoid photoinhibition, and a high light intensity of AL [1,200 μmol(photon) m^{-2} s^{-1} (HL, high light)] were selected. The illumination time was 2 min with repetitive measurements of F_0' (minimum Chl *a* fluorescence in the light) and F_m' (maximum Chl *a* fluorescence in the light) values every 20 s, from which values of other Chl fluorescence parameters were calculated automatically by *Imaging Win* software (*Walz*, *Effeltrich*, Germany).

The calculated parameters included the effective quantum yield of photochemical energy conversion in PSII [$\Phi_{PSII} = (F_m' - F_s)/F_m'$] that estimates the efficiency at which light absorbed by PSII is used for photochemistry. The photochemical quenching coefficient, q_P, is a measure of the fraction of open PSII reaction centres that represents the redox state of the PQ pool and it was calculated as $(F_m' - F_s)/(F_m' - F_0')$ (Genty *et al.* 1989). NPQ parameter, which was calculated as $(F_m - F_m')/F_m'$, estimates the nonphotochemical quenching that reflects heat dissipation of excitation energy in the antenna system. The relative PSII electron transport rate (ETR) was calculated as $c \times \Phi_{PSII} \times$ PPFD. The coefficient *c* assumes equal distribution of photons between PSII and PSI (0.5), while the homogeneous absorption factor of 0.84 contains assumptions on leaf absorption of PPFD, thus, a value of 0.42 was assumed (Schreiber *et al.* 1994).

Statistical analysis: Each treatment was analyzed with at least six replicates and data are expressed in mean ± SE. Chl fluorescence measurements represented averaged values from two independent experiments with three leaf samples (each with five AOI) from three plants per treatment per experiment. One-way analysis of variance (*ANOVA*) was carried out using the *StatView* computer package (*Abacus Concepts*, *Inc.*, Berkley, CA, USA) and mean was separated at a level of $P<0.05$. For the estimation of the relationships of Pro content with ETR, a linear regression analysis was also performed (Sperdouli and Moustakas 2012b).

Results

Leaf developmental stage influences Pro accumulation and $NADP^+$/NADPH ratio: Exogenously applied Pro increased remarkably (21- and 23-fold) the free Pro content in ML and YL, respectively (Fig. 1*A*), compared with their corresponding controls. Thus, exogenous Pro application resulted in more enhanced Pro accumulation in YL than in ML.

The ratio of $NADP^+$ to NADPH was similar in YL and ML of the control plants. However, exogenous Pro resulted in a decreased $NADP^+$ and an increased NADPH content in both YL and ML. Thus, the ratio of $NADP^+$/NADPH decreased in both YL and ML compared with their controls, but remained higher in YL compared to ML (Fig. 1*B*).

Differential photochemical efficiency of control and Pro-treated young and mature leaves: Different parameters were measured in YL and ML of control and the Pro-treated (10 mM) *A. thaliana* leaves to determine the Pro effects on photosynthesis. The maximal quantum yield of PSII photochemistry (F_v/F_m) was quite high in both YL and ML of the control plants, but YL exhibited lower F_v/F_m values than ML (Fig. 1*C*). However, in YL sprayed with Pro, F_v/F_m decreased less than that in ML. Pro-treated ML had significantly lower F_v/F_m values than YL.

The Φ_{PSII} was downregulated in control YL, compared to that in control ML (Fig. 2*A*) under both LL and HL, indicating that a small fraction of absorbed irradiance was not utilized *via* photochemical reactions in control YL. Compared with control, Φ_{PSII} in Pro-treated ML decreased by 36 and 38% under LL and HL, respectively, while Φ_{PSII} in Pro-treated YL decreased by 16 and 20% under LL and HL, respectively (Fig. 2*A*).

Pro-treated young leaves showed less reduced PQ pool than mature leaves: The plastoquinone pool (PQ) of YL and ML in the control plants was in a more oxidized state compared to that of the Pro-treated plants under both LL and HL (Fig. 2*B*). Pro-treated YL showed the less reduced PQ pool compared to ML, especially under LL (Fig. 2*B*).

Pro-treated young leaves retain higher electron transport rate and nonphotochemical quenching compared to mature leaves: Under HL, NPQ rose in all plants (Fig. 2*C*). Under both LL and HL, control ML exhibited significantly higher NPQ values, whereas Pro-treated YL showed significantly higher NPQ values than that of Pro-treated ML, under both LL and HL.

Electron transport rate (ETR) increased under HL in

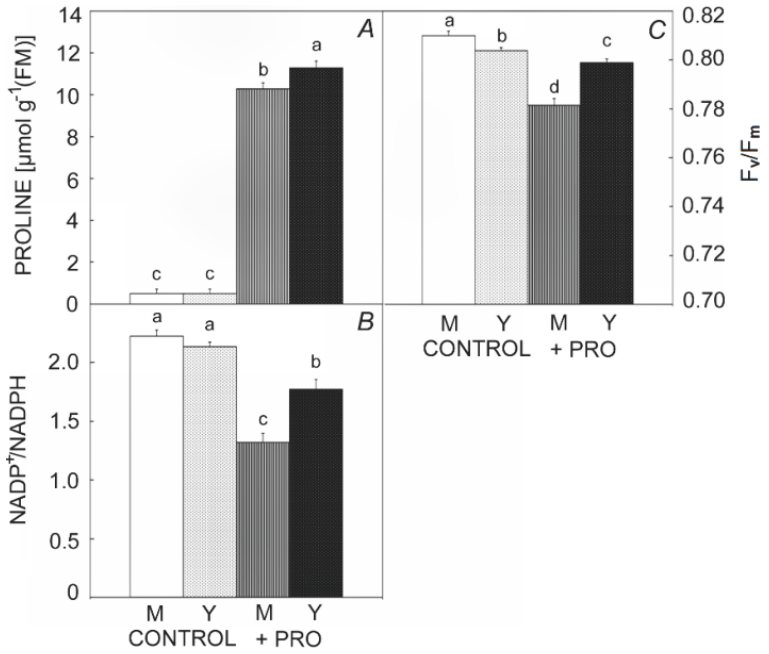

Fig. 1. Free proline (Pro) content (*A*), the ratio of $NADP^+$/NADPH (*B*), and the maximal quantum yield of PSII photochemistry (F_v/F_m) (*C*) of control and Pro (10 mM) treated young (Y) and mature (M) leaves of *Arabidopsis thaliana*. Values are mean ± SE (n = 6). SE is indicated by bars. Bars with different *lowercase letters* are significantly different (P<0.05). FM – fresh mass.

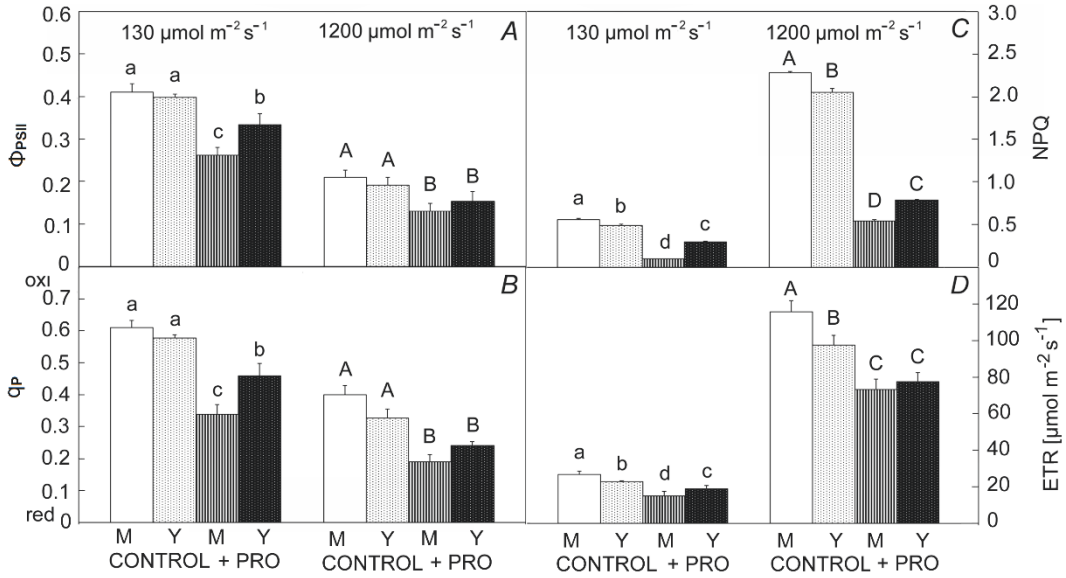

Fig. 2. The quantum efficiency of PSII photochemistry (Φ_{PSII}) (*A*), the redox state of PQ pool (expressed as q_P) (*B*), the nonphotochemical fluorescence quenching (NPQ) (*C*), and the electron transport rate (ETR) (*D*) under low light (130 µmol m^{-2} s^{-1}) and high light (1,200 µmol m^{-2} s^{-1}) of control and Pro (10 mM) treated young (Y) and mature (M) leaves of *Arabidopsis thaliana*. Values are mean ± SE (n = 6). SE is indicated by bars. Bars with different *lowercase letters* are significantly different (P<0.05).

both control and Pro-treated YL and ML (Fig. 2*D*). Control ML had significantly higher ETR values under both LL and HL, whereas Pro-treated YL had significantly higher ETR values than that of Pro-treated ML under LL.

Relationships of electron transport rate and Pro accumulation: ETR was reduced with the increased Pro content and these changes in ETR were negatively correlated with the Pro content under both LL and HL (Fig. 3). ETR, determined at LL, was negatively correlated with the Pro content, but it was not statistically significant (Fig. 3*A*), while it was negatively and significantly correlated (Fig. 3*B*) with the Pro content under HL.

Discussion

Under abiotic stress conditions, Pro accumulation fulfils a number of roles, acting as an osmolyte, protecting enzymes from denaturation, scavenging ROS, and interacting with membranes and other metabolites (Hare and Cress 1997, Verbruggen and Hermans 2008, Sperdouli and Moustakas 2012b). Pro accumulation under stress conditions might serve as a sink for excess reductants, providing the $NADP^+$ necessary for maintenance of the photosynthetic process (Hare and Cress 1997, Ashraf and Foolad 2007). While Pro synthesis generates $NADP^+$, its degradation produces NADPH (Hare and Cress 1997).

The degradation of exogenously applied Pro produced NADPH that decreased the $NADP^+$/NADPH ratio. Accumulation of NADPH in stroma causes over-reduction of the PQ pool by blockage of electron flow, due to depletion of electron acceptors (Marutani *et al.* 2012), and consequently, a less efficient PSII photochemistry. In our experiment, foliar application of Pro led to a greater increase in Pro accumulation in YL compared to ML, suggesting a higher catabolism of Pro in ML that leads to increased accumulation of NADPH. This accumulation of NADPH in ML causes over-reduction of the PQ pool and lowers the electron transport efficiency in PSII by blocking electron flow due to depletion of electron acceptors (Marutani *et al.* 2012). Over-reduction of the PQ pool in ML is considered to be dangerous to PSII (Yamamoto *et al.* 2008), as verified by the observed photoinhibition. In contrast, in YL, a decrease in Pro catabolism resulted in lesser deregulation of the redox status of the PQ pool and decreased blockage of electron flow through PSII. A cycle of Pro synthesis and its degradation has been shown to be essential for buffering a cellular redox potential in cytosol and in plastids (Hare and Cress 1997, De Ronde *et al.* 2004). Pro treatment caused the inhibition of PSII in YL as indicated by the decrease in F_v/F_m. This phenomenon was more pronounced in Pro-treated ML, indicating increased susceptibility of ML to Pro-induced PSII photoinhibition. Reductions in the F_v/F_m ratio were associated with the low ratio of $NADP^+$/NADPH. Φ_{PSII} was lower in YL and ML sprayed with Pro than that in the control plants under both LL and HL, reflecting the overall inhibition of photosynthesis. The degree of inhibition was higher in ML sprayed with Pro than in YL. If the regeneration of $NADP^+$ is limited, redox imbalance is likely to result in photoinhibition and enhanced use of O_2 instead of $NADP^+$ as the electron acceptor in photosynthesis (Hare *et al.* 1998). A decreased $NADP^+$/NADPH ratio would lead to potentially harmful over-reduction and blockage of photosynthetic electron flow (Foyer *et al.* 2012, Marutani *et al.* 2012), as shown in Fig. 2*D*. Blockage of the photosynthetic electron flow indicates an imbalance between energy supply and demand (Dietz and Pfannschmidt 2011). Excess excitation energy has to be safely removed in the antennae complexes of PSII as heat through a process which involves the xanthophyll cycle and a low intrathylakoid pH (Krause and Weis 1991) to prevent formation of ROS (Marutani *et al.* 2012). Both YL and ML from control *A. thaliana* plants dissipated more excitation energy by NPQ than Pro-treated YL and ML, as evidenced by the significantly higher heat dissipation of excitation energy (NPQ). Because NPQ deactivates excited Chl molecules (Havaux *et al.* 2007), thereby avoiding singlet oxygen ($^1O_2$) production, its inhibition in Pro-treated YL and ML leads to photooxidation (Triantaphylidès *et al.* 2008, Sperdouli and Moustakas 2012a).

Hare *et al.* (2002) suggested that the destructive effects of Pro on chloroplast ultrastructure resulted from feedback inhibition of Pro synthesis that caused over-reduction. Exogenous Pro activates PDH expression (Peng *et al.* 1996) and causes toxicity (Hellmann *et al.* 2000, Deuschle *et al.* 2001, Mani *et al.* 2002). External Pro concentrations (10 mM) have been found to be toxic to *Arabidopsis* plants (Mani *et al.* 2002). Thus, under optimal growth conditions, degradation of Pro leads to toxicity, suggesting that Pro degradation requires an accurate regulation (Hellmann *et al.* 2000).

Measurements of Chl fluorescence allow us to conclude that a more reduced redox state of the PQ pool than that in control ones was observed in both YL and ML sprayed with Pro. At the same time, we observed an over- reduction

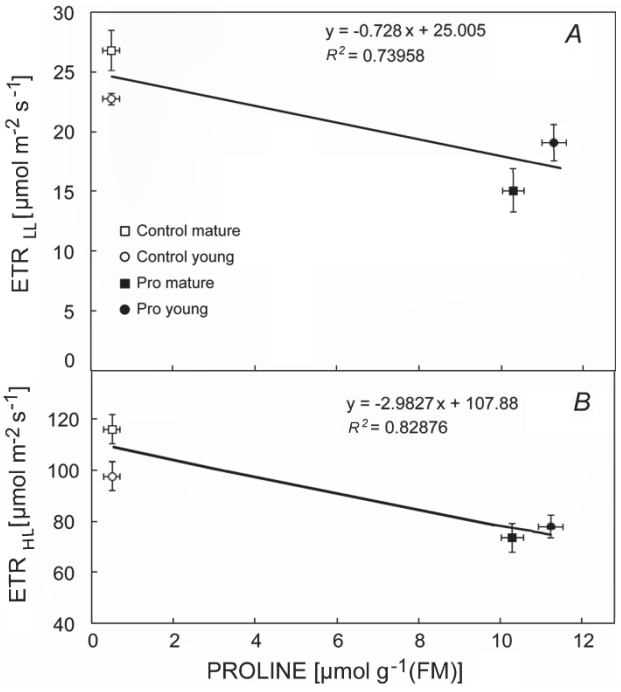

Fig. 3. Relationships between electron transport rate (ETR) and proline (Pro) under low light (LL; 130 µmol m^{-2} s^{-1}; R^2 = 0.7396, P=0.0606) (*A*), and high light (HL; 1,200 µmol m^{-2} s^{-1}; R^2 = 0.8288, P=0.0064) (*B*) of control and Pro (10 mM) treated young and mature leaves of *Arabidopsis thaliana*. Error bars represent ± SE of mean (n = 6). FM – fresh mass.

of the PQ pool in ML sprayed with Pro and consequently, a less efficient PSII photochemistry under both LL and HL conditions. This over-reduced PQ pool in ML is due to the fact that Pro degradation is developmentally regulated in *Arabidopsis* (Nakashima *et al.* 1998, Funck *et al.* 2010). An over-reduction of PQ pool has been shown to initiate a chloroplast retrograde signalling for proper plant responses (Petrillo *et al.* 2014).

It is suggested that external Pro application resulting in high internal Pro accumulation is essential for quenching ROS and contributing to drought tolerance in *Arabidopsis* (Moustakas *et al.* 2011), but under optimal growth conditions external Pro application results in over-reduction of the PQ pool and blockage of photosynthetic electron flow. Under extreme environmental conditions, such as elevated temperatures and high salinity, an enhanced input of reducing equivalents from soluble stromal reductants to the chloroplast electron transport chain was shown to occur (Endo *et al.* 1995, Havaux 1996, Bukhov *et al.* 2002); it resulted in over-reduction of the PQ pool (Marutani *et al.* 2012).

A significant negative correlation of ETR at HL with the Pro content was observed, implying the prevailing influence of Pro metabolism on photosynthetic electron flow. The effect of Pro metabolism on the $NADP^+$/NADPH ratio suggests a developmental link of Pro metabolism to redox buffering in the chloroplast that is critical not only for photosynthetic electron transport but also for ROS generation and signalling (Dietz and Pfannschmidt 2011, Petrillo *et al.* 2014).

References

Ashraf M., Foolad M.R.: Roles of glycine betaine and proline in improving plant abiotic stress resistance. – Environ. Exp. Bot. **59**: 206-216, 2007.

Bates L.S., Waldren R.P., Teare I.D.: Rapid determination of free proline for water stress studies. – Plant Soil **39**: 205-207, 1973.

Bukhov N., Egorova E., Carpentier R.: Electron flow to photosystem I from stromal reductants *in vivo*: the size of the pool of stromal reductants controls the rate of electron donation to both rapidly and slowly reducing photosystem I units. – Planta **215**: 812-820, 2002.

Chen T.H., Murata N.: Enhancement of tolerance of abiotic stress by metabolic engineering of betaines and other compatible solutes. – Curr. Opin. Plant Biol. **5**: 250-257, 2002.

De Ronde J.A., Cress W.A., Krüger G.H.J. *et al.*: Photosynthetic response of transgenic soybean plants, containing an *Arabidopsis* P5CR gene, during heat and drought stress. – J. Plant Physiol. **161**: 1211-1224, 2004.

Deuschle K., Funck D., Hellmann H. *et al.*: A nuclear gene encoding mitochondrial Δ^1-pyrroline-5-carboxylate dehydrogenase and its potential role in protection from proline toxicity. – Plant J. **27**: 345-356, 2001.

Deuschle K., Funck D., Forlani G. *et al.*: The role of Δ^1-pyrroline-5-carboxylate dehydrogenase in proline degradation. – Plant Cell **16**: 3413-3425, 2004.

Dietz K.J., Pfannschmidt T.: Novel regulators in photosynthetic redox control of plant metabolism and gene expression. – Plant Physiol. **155**: 1477-1485, 2011.

Endo T., Schreiber U., Asada K.: Suppression of quantum yield of photosystem II by hyperosmotic stress in *Chlamydomonas reinhardtii*. – Plant Cell Physiol. **36**: 1253-1258, 1995.

Foyer C.H., Neukermans J., Queval G. *et al.*: Photosynthetic control of electron transport and the regulation of gene expression. – J. Exp. Bot. **63**:1637-1661, 2012.

Funck D., Eckard S., Müller G.: Non-redundant functions of two proline dehydrogenase isoforms in *Arabidopsis*. – BMC Plant Biol. **10**: 70, 2010.

Genty B., Briantais J.M., Baker N.R.: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. – Biochim. Biophys. Acta **990**: 87-92, 1989.

Gorbe E., Calatayud A.: Applications of chlorophyll fluorescence imaging technique in horticultural research: a review. – Sci. Hortic.-Amsterdam **138**: 24-35, 2012.

Guidi L., Calatayud A.: Non-invasive tools to estimate stress-induced changes in photosynthetic performance in plants inhabiting Mediterranean areas. – Environ. Exp. Bot. **103**: 42-52, 2014.

Hald S., Nandha B., Gallois P. *et al.*: Feedback regulation of photosynthetic electron transport by NADP(H) redox poise. – Biochim. Biophys. Acta **1777**: 433-440, 2008.

Hanachi S., Van Labeke M.C., Mehouachi T.: Application of chlorophyll fluorescence to screen eggplant (*Solanum melongena* L.) cultivars for salt tolerance. – Photosynthetica **52**: 57-62, 2014.

Hare P.D., Cress W.A.: Metabolic implications of stress-induced proline accumulation in plants. – Plant Growth Regul. **21**: 79-102, 1997.

Hare P.D., Cress W.A., Van Staden J.: Dissecting the roles of osmolyte accumulation during stress. – Plant Cell Environ. **21**: 535-553, 1998.

Hare P.D., Cress W.A., Van Staden J.: Disruptive effects of exogenous proline on chloroplast and mitochondrial ultrastructure in *Arabidopsis* leaves. – S. Afr. J. Bot. **68**: 393-396, 2002.

Havaux M.: Short-term responses of photosystem I to heat stress. – Photosynth. Res. **47**: 85-97, 1996.

Havaux M., Dall'Osto L., Bassi R.: Zeaxanthin has enhanced antioxidant capacity with respect to all other xanthophylls in *Arabidopsis* leaves and functions independent of binding to PSII antennae. – Plant Physiol. **145**: 1506-1520, 2007.

Hellmann H., Funck D., Rentsch D. *et al.*: Hypersensitivity of an *Arabidopsis* sugar signaling mutant toward exogenous proline application. – Plant Physiol. **123**: 779-789, 2000.

Hong Z.L., Lakkineni K., Zhang Z.M. *et al.*: Removal of feedback inhibition of Δ^1-pyrroline-5-carboxylate synthetase results in increased proline accumulation and protection of plants from osmotic stress. – Plant Physiol. **122**: 1129-1136, 2000.

Khayyat M., Tehranifar A., Davarynejad G.H. *et al.*: Vegetative growth, compatible solute accumulation, ion partitioning and chlorophyll fluorescence of 'Malas-e-Saveh' and 'Shishe-Kab' pomegranates in response to salinity stress. – Photosynthetica **52**: 301-312, 2014.

Krause G.H., Weis E.: Chlorophyll fluorescence and photosynthesis: the basics. – Annu. Rev. Plant Phys. **42**: 313-349, 1991.

Mani S., Van de Cotte B., Van Montagu M. *et al.*: Altered levels of proline dehydrogenase cause hypersensitivity to proline and its analogs in *Arabidopsis*. – Plant Physiol. **128**: 73-83, 2002.

Marutani Y., Yamauchi Y., Kimura Y. *et al.*: Damage to photosystem II due to heat stress without light-driven electron flow: involvement of enhanced introduction of reducing power into thylakoid membranes. – Planta **236**: 753-761, 2012.

Morales F., Moise N., Quílez R. *et al.*: Iron deficiency interrupts energy transfer from a disconnected part of the antenna to the rest of Photosystem II. – Photosynth. Res. **70**: 207-220, 2001.

Moustakas M., Sperdouli I., Kouna T. *et al.*: Exogenous proline induces soluble sugar accumulation and alleviates drought stress effects on photosystem II functioning of *Arabidopsis thaliana* leaves. – Plant Growth Regul. **65**: 315-325, 2011.

Murchie E.H., Lawson T.: Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. – J. Exp. Bot. **64**: 3983-3998, 2013.

Nakashima K., Satoh R., Kiyosue T. *et al.*: A gene encoding proline dehydrogenase is not only induced by proline and hypoosmolarity, but is also developmentally regulated in the reproductive organs of *Arabidopsis*. – Plant Physiol. **118**: 1233-1241, 1998.

Nanjo T., Fujita M., Seki M. *et al.*: Toxicity of free proline revealed in an *Arabidopsis* T-DNA-tagged mutant deficient in proline dehydrogenase. – Plant Cell Physiol. **44**: 541-548, 2003.

Ögren E.: Evaluation of chlorophyll fluorescence as a probe for drought stress in willow leaves. – Plant Physiol. **93**: 1280-1285, 1990.

Osório M.L., Osório J., Romano A.: Photosynthesis, energy partitioning, and metabolic adjustments of the endangered Cistaceae species *Tuberaria major* under high temperature and drought – Photosynthetica **51**: 75-84, 2013.

Osório J., Osório M.L., Correia P.J. *et al.*: Chlorophyll fluorescence imaging as a tool to understand the impact of iron deficiency and resupply on photosynthetic performance of strawberry plants. – Sci. Hortic.-Amsterdam **165**: 148-155, 2014.

Peng Z., Lu Q., Verma D.P.S.: Reciprocal regulation of Δ^1-pyrroline-5-carboxylate synthetase and proline dehydrogenase genes controls proline levels during and after osmotic stress in plants. – Mol. Gen. Genet. **253**: 334-341, 1996.

Petrillo E., Godoy Herz M.A., Fuchs A. *et al.*: A chloroplast retrograde signal regulates nuclear alternative splicing. – Science **344**: 427-430, 2014.

Poulson M.E., Boeger M.R.T., Donahue R.A.: Response of photosynthesis to high light and drought for *Arabidopsis thaliana* grown under a UV-B enhanced light regime. – Photosynth. Res. **90**: 79-90, 2006.

Rius S.P., Casati P., Iglesias A.A. *et al.*: Characterization of an *Arabidopsis thaliana* mutant lacking a cytosolic non-phosphorylating glyceraldehyde-3-phosphate dehydrogenase. – Plant Mol. Biol. **61**: 945-957, 2006.

Schreiber U., Bilger W., Neubauer C.: Chlorophyll fluorescence as a non-intrusive indicator for rapid assessment of *in vivo* photosynthesis. – In: Schulze E.D., Caldwell M.M. (ed.): Ecophysiology of Photosynthesis. Ecological Studies, vol. 100. Pp. 49-70. Springer, Berlin 1994.

Sperdouli I., Moustakas M.: Spatio-temporal heterogeneity in *Arabidopsis thaliana* leaves under drought stress. – Plant Biol. **14**: 118-128, 2012a.

Sperdouli I., Moustakas M.: Interaction of proline, sugars, and anthocyanins during photosynthetic acclimation of *Arabidopsis thaliana* to drought stress. – J. Plant Physiol. **169**: 577-585, 2012b.

Sperdouli I., Moustakas M.: A better energy allocation of absorbed light in photosystem II and less photooxidative damage contribute to acclimation of *Arabidopsis thaliana* young leaves to water deficit. – J. Plant Physiol. **171**: 587-593, 2014a.

Sperdouli I., Moustakas M.: Leaf developmental stage modulates metabolite accumulation and photosynthesis contributing to acclimation of *Arabidopsis thaliana* to water deficit. – J. Plant Res. **127**: 481-489, 2014b.

Szabados L., Savouré A.: Proline: a multifunctional amino acid. – Trends Plant Sci. **15**: 89-97, 2010.

Székely G., Ábrahám E., Cséplő Á. *et al.*: Duplicated P5CS genes of *Arabidopsis* play distinct roles in stress regulation and developmental control of proline biosynthesis. – Plant J. **53**: 11-28, 2008.

Triantaphylidès C., Krischke M., Hoeberichts F.A. *et al.*: Singlet oxygen is the major reactive oxygen species involved in photooxidative damage to plants. – Plant Physiol. **148**: 960-968, 2008.

Verbruggen N., Hermans C.: Proline accumulation in plants: a review. – Amino Acids **35**: 753-759, 2008.

Yamamoto Y., Aminaka R., Yoshioka M. *et al.*: Quality control of photosystem II: impact of light and heat stresses. – Photosynth. Res. **98**: 589-608, 2008.

Zhang Z., Yu J., Stanton R.: A method for determination of pyridine nucleotides using a single extract. – Anal. Biochem. **285**: 163-167, 2000.