DOI: 10.1007/s11099-015-0164-8 PHOTOSYNTHETICA 54 (1): 93-100, 2016

Growth irradiance affects ureide accumulation and tolerance to photoinhibition in *Eutrema salsugineum* (*Thellungiella salsuginea*)

V.M. MALIK[*,#], J.M. LOBO[*,#], C. STEWART[**], S. IRANI[**], C.D. TODD[**], and G.R. GRAY[*,***,+]

Department of Plant Sciences, University of Saskatchewan, Saskatoon SK S7N 5A8, Canada[*]
Department of Biology, University of Saskatchewan, Saskatoon SK S7N 5E2, Canada[**]
Department of Biochemistry, University of Saskatchewan, Saskatoon SK S7N 5E5, Canada[***]

Abstract

Plants are able to acclimate to their growth light environments by utilizing a number of short- and long-term mechanisms. One strategy is to prevent accumulation of excess reactive oxygen species that can lead to photoinhibition of photosynthesis. Ureides, generated from purine degradation, have been proposed as antioxidants and involved in certain abiotic stress responses. *Eutrema salsugineum* (*Thellungiella salsuginea*) is an extremophilic plant known to exhibit a high degree of tolerance to a variety of abiotic stresses that invariably generate reactive oxygen species. In the present study we have investigated the possible role of the ureide metabolic pathway during acclimation to growth irradiance and its conference of tolerance to photoinhibition in *Eutrema*. Ureide accumulation was greater under high light growth which also conferred tolerance to photoinhibition at low temperature as measured by the maximal quantum yield of PSII photochemistry. This may represent an adaptive plastic response contributing to the extreme tolerance exhibited by this plant. Our results would provide evidence that ureide accumulation may be involved in abiotic stress as another defence mechanism in response to oxidative stress.

Additional key words: acclimation; allantoin; antioxidant; reactive oxygen species.

Introduction

Reactive oxygen species (ROS) are by-products of normal metabolism and include the hydroxyl radical (•OH), superoxide anion (O_2•−), hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$). While ROS have been shown to play an important signaling role, they are also highly reactive and if remained unchecked they can cause wide-spread damage to cellular components and structure. In order to prevent the oxidative damage, the plant controls ROS concentrations through a complex scavenging system consisting of diverse enzymatic and non-enzymatic mechanisms which include low-molecular mass antioxidants such as ascorbic acid (ascorbate) and glutathione (Asada and Takahashi 1987, Foyer and Noctor 2009, Gill and Tuteja 2010, Karuppanapandian *et al*. 2011, Suzuki *et al.* 2012).

Exposure to high light and absorption of energy in excess of what can be effectively consumed or dissipated by chloroplastic metabolism can lead to an inactivation of photosynthesis known as photoinhibition (Powles 1984, Aro *et al.* 1993). Variations in growth environments and the quantity of incident light occur continually, over time-scales ranging from seconds to months. Thus, plants have developed a wide array of both short- and long-term photoprotective and repair mechanisms/strategies at the whole plant, leaf or cellular/organelle levels to deal with excessive light (Demmig-Adams and Adams 1992). Photoinhibition is generally thought of as short-term light stress, growth under high light and the associated excess excitation energy presents more of a long-term challenge.

———

Received 12 March 2015, *accepted* 22 June 2015, *published as online-first* 5 August 2015.
[+]Corresponding author; fax: (+1) 306 9664313, e-mail: gr.gray@usask.ca
Abbreviations: ABA – abscisic acid; *ACT7* – actin7; AAH – allantoate amidohydrolase; ALN – allantoinase; Chl – chlorophyll; FM – fresh mass; F_v/F_m – maximal quantum yield of PSII photochemistry; HL – high light; ML – moderate light; ROS – reactive oxygen species; sqPCR – semiquantitative reverse transcriptase-polymerase chain reaction; UAH – ureidoglycolate amidohydrolase; UGlyAH – ureidoglycine aminohydrolase; UO – urate oxidase; XDH – xanthine dehydrogenase.
Acknowledgments: This project was supported from Discovery funding provided by the Natural Sciences and Engineering Research Council (NSERC) of Canada to G.R. Gray and C.D. Todd, and in part, by an Undergraduate Research Grant, Office of the Vice-President Research, University of Saskatchewan to V.M. Malik. We thank Eva-Maria Aro (University of Turku) for useful discussion.
[#]These authors contributed equally to this work.

Plants are able to acclimate their photosynthetic apparatus by modulating their thylakoid architecture and protein distribution dynamically according to their growth light environments.

This usually involves changes in light-harvesting antenna size, reaction centre stoichiometry, contents of electron transport components and Calvin cycle enzymes. These responses have been well documented for many plant species (Anderson 1986, Anderson *et al.* 1995, Walters 2005).

Eutrema salsugineum (Pall.) Al-Shehbaz and Warwick (also known as *Thellungiella salsuginea* and *Thellungiella halophila*) is a native crucifer that is closely related to the genetic model plant *Arabidopsis thaliana* (Koch and German 2013). While both *Eutrema* and *Arabidopsis* are in the Brassicaceae, they differ greatly in their ability to tolerate adverse environmental conditions. *Eutrema* is often referred to as an extremophile, owed to a high capacity to withstand various abiotic stresses such as freezing, water deficit, and salinity in comparison to *Arabidopsis* (Inan *et al.* 2004, Griffith *et al.* 2007, Amtmann 2009, Guevara *et al.* 2012). Many ecotypes have evolved under diverse stress-prone environmental conditions and their stress tolerance behavior is thought to be appropriate to their natural habitats due, in part, to the high degree of adaptive plasticity shown by *Eutrema* (Amtmann 2009, Guevara *et al.* 2012, Kazachkova *et al.* 2013). The Yukon ecotype, originating from the saline meadows in the Yukon Territories, Canada, a subarctic and semiarid region, also thrives under conditions of very low soil nitrogen (< 1 mM; Guevara *et al.* 2012). While *Eutrema* has been used extensively for salinity studies, there has been relatively little work performed investigating photosynthetic responses and tolerance to high light stress or acclimation to growth irradiance (Wong *et al.* 2006, Stepien and Johnson 2009, Sui and Han 2014).

Purine compounds perform essential metabolic roles and are integral components for many cellular processes (Stasolla *et al.* 2003, Zrenner *et al.* 2006). Ureides are nitrogen containing compounds generated from the purine degradation pathway. The first common intermediate of purine catabolism is xanthine which is oxidized to urate by xanthine dehydrogenase (XDH; EC 1.17.3.2, 1.17.1.4) for which two genes exist in *Arabidopsis* (Hesberg *et al.* 2004). Urate is further metabolized to the ureide allantoin, through the action of urate oxidase (UO; EC 1.7.3.3) and two subsequent enzymatically catalyzed conversions. Plants possess a set of enzymes which further break down allantoin, ultimately releasing CO_2 and ammonia (NH_3). The enzyme allantoinase (ALN; EC 3.5.2.5) converts allantoin to allantoate, followed by allantoate amidohydrolase (AAH; EC 3.5.3.9) which converts to allantoate to ureidoglycine which is enzymatically degraded to ureidoglycolate and NH_3 by ureidoglycine aminohydrolase (UGlyAH; EC 3.5.3.26). In turn, ureidoglycolate is hydrolyzed to glyoxylate, CO_2 and two molecules of NH_3 by ureidoglycolate amidohydrolase (UAH; EC 3.5.1.116) (for review *see* Werner and Witte 2011). In symbiotic legumes, ureides are involved in the storage of fixed nitrogen and its translocation from N_2-fixing nodules to other tissues and organs (Schubert 1986, Todd *et al.* 2006). For a variety of non-N_2-fixing species, purine catabolism and ureide formation is also thought to contribute to the recycling/remobilization of nitrogen from source to sink organs (Reinbothe and Mothes 1962, Werner and Witte 2011). In addition, several studies in *Arabidopsis* using mutants in the purine degradation pathway have indicated that the metabolites of ureide metabolism (allantoin and allantoate) could act as potential antioxidants under abiotic stress conditions (Brychkova *et al*. 2008, Watanabe *et al*. 2010, 2014; Ventura *et al*. 2011). This is supported by recent metabolomic and phytochemical studies examining allantoin accumulation in response to water deficit and salinity that have also implicated a role for ureides in reducing ROS accumulation (Oliver *et al.* 2011, Ventura *et al*. 2011, Yobi *et al*. 2013). In contrast, another study looking at the function of allantoin in rice grains suggested that while allantoin accumulation did not serve directly as an antioxidant, it did show a positive correlation with seedling survival under low temperature and drought stress conditions (Wang *et al.* 2012).

The Yukon ecotype of *Eutrema* is also tolerant to nitrogen stress, a phenotype consistent with the prevailing conditions in its native habitat (Kant *et al*. 2008, Guevara *et al.* 2012). Kant *et al.* (2008) were able to show that *Eutrema* was highly efficient in the mobilization of resources in poor or degraded soils in comparison to *Arabidopsis*.

While not examined by Kant *et al*. (2008), ureides may be involved, consistent with their role in nitrogen recycling and mobilization. Given the potential role of ureides as antioxidants, we hypothesized that ureide accumulation could be playing a protective role in response to oxidative stress, contributing to the overall high degree of abiotic stress tolerance observed in *Eutrema*. Here, we described an investigation into the effects of growth irradiance on ureide accumulation in the Yukon ecotype of *Eutrema* and examined if this accumulation conferred any tolerance to high light (photoinhibitory) treatments at low temperature. Our results would provide evidence that ureides may be involved as an additional class of compound for protection against photo-oxidative stress.

Materials and methods

Plant material and growth conditions: Seeds of *Eutrema salsugineum* (Pall.) Al-Shehbaz and Warwick (Yukon ecotype, stock no. CS22664) were obtained from the Arabidopsis Biological Resource Centre (ABRC,

Columbus, OH, USA) and germinated in 6.5-cm plastic pots containing *Sungrow Sunshine LG3 Mix* germinating soil medium (*Sun Gro Horticulture Inc*., Vancouver, BC, Canada) which were placed in plastic tray flats. Plants were grown in controlled environment chambers (*Conviron*, Winnipeg, MB, Canada) using a day/night temperature (22/10°C) and a light/dark cycle (21/3 h) previously established for the Yukon ecotype of *Eutrema* (Griffith *et al.* 2007). Two different growth irradiances (determined at soil level) were used: moderate light (ML) and high light (HL) which corresponded to PPFDs of 250 and 750 μmol(photon) m^{-2} s^{-1}, respectively. Aerial portions of single plants were harvested and dried for a minimum of 48 h at 90°C or until constant mass was obtained for the determination of dry mass (DM). Total leaf area (LA) measurements were performed using *ImageJ* (Schneider *et al.* 2012; http://imagej.nih.gov/ij/). Specific leaf mass (SLM) was calculated as mg(DM) cm^{-2}. Plants were maintained and fertilized as described by Gray *et al.* (2003).

Ureide extraction and determination: Plant tissue (50–100 mg of fresh mass, FM) from fully expanded leaves was harvested and homogenized using a mortar and pestle in 10 volumes of ice-cold deionized H_2O. The homogenate was centrifuged at 4°C and 14,000 rpm for 5 min to remove cell debris. The supernatant was removed and centrifuged for an additional 5 min at 4°C and 14,000 rpm. The resultant crude extract was kept on ice and used immediately to estimate a total ureide content.

A colorimetric assay of differential glyoxylate derivatives was performed to quantify total ureides as described by Vogels and Van der Drift (1970). In this assay, allantoin and allantoate are independently determined after their chemical hydrolysis to glyoxylate. Total ureides in crude extracts are the sum of allantoin, allantoate, ureidoglycolate and glyoxylate. Results were expressed in nmol mg^{-1}(FM) of glyoxlyate equivalents and were calculated using a glyoxylate standard curve (0–140 μM; $R^2 \geq 0.994$) prepared from a 10 mM stock solution (*Sigma-Aldrich*, St. Louis, MO, USA).

Semiquantitative reverse transcriptase-polymerase chain reaction (sqRT-PCR): Total RNA was isolated from 300 mg of leaf tissue using a urea-LiCl method (Kansal *et al.* 2008) with modifications. The samples were quantified using a *Nanodrop 2000* spectrophotometer (*Thermo Fisher Scientific*, Waltham, MA, USA) followed by gel electrophoresis to examine the quality of the RNA. Agarose gels 1% (w/v) were run in 1× TAE buffer and stained with ethidium bromide according to standard procedures (Sambrook and Russell 2001). Samples were stored at –80°C and used as templates for subsequent sqRT-PCR.

Transcript levels were established by sqRT-PCR using an *iCycler* thermocycler (*Bio-Rad Laboratories*, Hercules, CA, USA) and a *Verso 1-Step RT-PCR ReddyMix*™ Kit (*Thermo Fisher Scientific*) as recommended by the supplier. Total RNA (60 ng) was amplified with gene-specific primer pairs (final concentration of 200 nM each forward and reverse) in 25 μl reactions. PCR products were visualized on a 1% (w/v) agarose gel run in 1× TAE and stained with ethidium bromide using standard procedures (Sambrook and Russell 2001). Primers were designed based on gene orthologs in *Arabidopsis* using the *Primer-BLAST* tool at the National Center for Biotechnology Information (NCBI; Ye *et al.* 2012) and are indicated in Table 1. *ACT7* was used as a reference gene. A *GeneRuler* 1 kb DNA ladder (*Thermo Scientific*) was also used for sizing.

Photoinhibition and chlorophyll (Chl) fluorescence: The maximal quantum yield of PSII photochemistry (F_v/F_m) was determined *in vivo* using a hand-held portable fluorometer (*FluorPen FP100*; *Photon Systems Instruments*, Brno, Czech Republic) and calculated as $(F_m - F_o)/F_m$, where F_o and F_m represent the minimal and maximal fluorescence yields of the dark-adapted state, respectively. Measurements were made on detached leaves at room temperature following a 15 min dark-adaptation as described previously (Chytyk *et al.* 2011).

Photoinhibition of photosynthesis was induced in a cold room set at 2°C under ambient air conditions as described by Gray *et al.* (2003). Detached leaves were floated adaxial side up in a divided Petri dish containing deionized water. Susceptibility to photoinhibition was quantified by monitoring changes in F_v/F_m as a function of a 2 h exposure to PPFD of 1,750 μmol(photon) m^{-2} s^{-1}

Table 1. Oligonucleotide primers used for sqRT-PCR. F – forward; R – reverse; *AAH* – allantoate amidohydrolase; *UGlyAH* – ureidoglycine aminohydrolase; *ACT7* – actin7.

Arabidopsis gene	Abbreviation	Sequence [5'→3']	Size [bp]
		R: TTCAGGTTCCCACACAACAA	
At4g20070	*AAH*	F: AAGGGACGAAGCTGTAGCAA	872
		R: TAGCACTTGGCCATGTTGAG	
At4g17050	*UGlyAH*	F: GCCCTTTACCTGACTGGACA	611
		R: AGTCTTTCCGAGTGCAGCAT	
At5g09810	*ACT7*	F: GATATTCAGCCACTTGTCTGTGAC	187
		R: CATGTTCGATTGGATACTTCAGAG	

at the leaf surface, which was provided by a metal halide lamp (400 W; *Philips Lighting Canada*, Markham, ON, Canada).

Pigment analyses: Total chlorophyll (Chl) (*a* + *b*) and carotenoid (Car) contents were determined photometrically from acetone extracts using a *SmartSpec Plus* spectrophotometer (*Bio-Rad Laboratories*). Leaf material was ground in 80% (v/v) acetone using a mortar and a pestle. Following centrifugation for 10 min at 13,200 rpm at 4°C, contents of the pigments in the supernatant were calculated according to the equations of Lichtenthaler and Wellburn (1983) and expressed on a FM basis.

Anthocyanins were extracted from leaf discs which had been agitated gently in the dark for 24 h at 4°C in 1 ml of 3 M HCl:H_2O:MeOH (1:3:16, v/v/v) and quantified photometrically as described by Gould *et al.* (2000). Anthocyanin contents were estimated from the methanol extracts as absorbance at 530 nm and expressed on a FM basis.

Statistical methods: All descriptive statistics and analyses were performed using *SigmaPlot 12* (*Systat Software, Inc*., San Jose, CA, USA). Values are derived from three independent biological experiments which were carried out in a minimum of triplicate. A *Shapiro-Wilk* test was performed to examine normality of the data followed by a *Student'*s *t*-test to determine significant differences at P=0.05. Alternatively, a one-way analysis of variance (*ANOVA*) at P=0.05, followed by a *Holm-Sidak* pairwise multiple comparison (P=0.05) was utilized.

Results and discussion

Growth characteristics of *Eutrema* under ML and HL growth regimes: Since the irradiance conditions utilized resulted in different growth rates, plants were harvested at different times which corresponded to approximately 27 and 24 d after germination for ML and HL growth regimes, respectively. Growth kinetic analyses have indicated that despite minor differences in growth phenotype, plants of these chronological ages were at the same point in their growth cycle (Lobo and Gray, unpublished results). Overall, growth under HL resulted in plants with 1.4-fold increase in SLM at these developmental ages compared to the ML conditions (Table 2). These results are consistent with leaf-level traits seen in plants acclimated to high and low irradiance for many species (Boardman 1977, Givnish 1988).

The changes occurring at the chloroplast level during acclimation to light have been examined intensively and are in part, reflected by changes in the ratio of Chl *a/b* (Anderson 1986, Walters 2005). Total Chl, estimated on a FM basis, demonstrated 37% decrease for plants grown at HL when compared to those developed under the ML growth regime which was accompanied by a 1.9-fold increase in the Chl *a*/*b* ratio, similar to what has been observed for acclimation to growth irradiance previously (Table 2; Boardman 1977, Anderson 1986). Anthocyanin accumulation in leaves is thought to protect against photoinhibitory damage caused by high irradiance either by light attenuation or antioxidant effects (Chalker-Scott 1999, Havaux and Kloppstech 2001, Neill and Gould 2003). In *Eutrema*, minimal changes were observed in bulk anthocyanins or total Car contents (xanthophylls and carotenes; Table 2). Car are involved in the prevention of photooxidation and the nonphotochemical dissipation of excess light energy through the xanthophyll cycle pigment interconversions (Demmig-Adams and Adams 1992). These results are not unexpected as we did not determine the epoxidation state of the xanthophylls which is more reflective of photoprotection than are Car pool sizes (Demmig-Adams and Adams 1992).

Ureide accumulation in *Eutrema* is dependent on growth irradiance: The data presented in Fig. 1*A* demonstrate an interesting trend with regard to ureide accumulation in leaf tissue developed under varying irradiance conditions. Growth at HL resulted in a 2.5-fold increase in total ureides when compared to the ML regime (Fig. 1*A*). Besides the Yukon ecotype of *Eutrema*, ureide accumulation was also observed in the ecotype Shandong grown at HL (data not shown). To our knowledge, this is the first report of ureide accumulation

Table 2. Leaf pigment contents for *Eutrema* (Yukon ecotype) developed at different growth irradiance. Plants were grown under either 250 (ML) or 750 µmol(photon) m^{-2} s^{-1} (HL) PPFD. Values represent means ± SD (n = 3). *Different letters* within columns indicate a significant difference at P=0.05 based on a *Student'*s *t*-test. Car – carotenoids; Chl – chlorophyll; DM – dry mass; FM – fresh mass; HL – high light; ML – moderate light; SLM – specific leaf mass.

Growth regime	SLM [mg(DM) cm^{-2}]	Total Chl [µg g^{-1}(FM)]	Chl *a/b*	Total Car [µg g^{-1}(FM)]	Anthocyanin [A_{530} g^{-1}(FM)]
ML	2.94 ± 0.32[a]	1,255.79 ± 66.95[a]	3.56 ± 0.13[a]	204.53 ± 10.88[a]	9.60 ± 0.58[a]
HL	3.97 ± 0.29[b]	794.80 ± 100.010[b]	6.78 ± 1.18[b]	198.42 ± 8.18[a]	12.64 ± 2.67[a]

in response to growth irradiance, although a previous study has implicated photoperiod as a factor in allantoin accumulation (Castro *et al.* 2001).

The expression level of two genes (*AAH* and *UGlyAH*), involved in allantoate degradation, were analyzed by sqRT-PCR to determine whether the effect of growth irradiance could influence the concentration of ureides by the transcriptional regulation of these genes (Fig. 1*B*). Both genes, *AAH* and *UGlyAH,* showed a clear up-regulation in response to HL growth (Fig. 1*B*). In addition, *ALN* and *XDH2* were absent under ML conditions but showed a very faint increase under the HL growth regime (data not shown). Genes involved in ureide synthesis and degradation have been shown to be induced upon stress conditions. Higher ureide concentrations, mainly in form of allantoate, were previously reported in shoots and leaves of common bean (*Phaseolus vulgaris*) during drought treatment and this was not a consequence of suppression of allantoate degradation, but rather, an induction of biosynthesis regulated at the transcript level (Alamillo *et al.* 2010, Coleto *et al*. 2014). While we were unable to determine specifically which ureide was contributing to this increase, as only bulk measurements were performed,

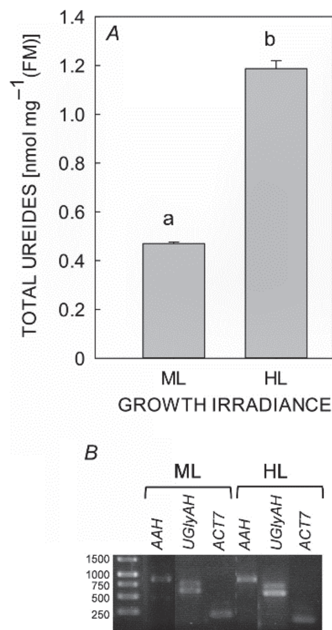

Fig. 1. Effect of growth irradiance on ureide content and transcript level of ureide metabolic genes in leaves of *Eutrema* (Yukon ecotype). Plants were grown under either 250 (ML) or 750 μmol(photon) m^{-2} s^{-1} (HL) PPFD. *A:* Total ureide content. Total ureides are the sum of allantoin, allantoate, ureidoglycolate and glyoxylate and are expressed in glyoxylate equivalents. Values represent means ± SE of three biological replicates. Bars with *different letters* are significantly different at P=0.05 based on a *Student*'s *t*-test. *B:* Transcript level of genes involved in allantoate degradation. A representative image is shown. FM – fresh mass; HL – high light; ML – moderate light; *AAH* – allantoate amidohydrolase; *UGlyAH* – ureidoglycine aminohydrolase; *ACT7* – actin7.

it also appears that in *Eutrema* growth irradiance may influence ureide accumulation at the level of transcription. Our experiment only indicated light responsiveness and did not allow us to speculate as to whether anabolic or catabolic processes are responsible for the observed accumulation in *Eutrema*.

Tolerance to photoinhibition: Exposure to high light and absorption of energy in excess of what can be effectively consumed or dissipated by chloroplast metabolism can lead to an inactivation of photosynthesis known as photoinhibition (Powles 1984, Aro *et al.* 1993).

Excessive ROS production can also lead to oxidative damage of PSII and a reduction in photosynthetic capacity (Aro *et al.* 1993). Furthermore, environmental stresses (salinity, cold, heat and drought) have all been shown to accelerate photoinhibition by limiting the photosynthetic fixation of CO_2, resulting in ROS accumulation and decreased PSII repair (Takahashi and Murata 2008, Murata *et al.* 2012). Detached leaves of *Eutrema* were exposed to a PPFD of 1,750 μmol(photon) m^{-2} s^{-1} at low temperature (2°C) to examine responses to photoinhibition. Similar values of F_v/F_m (ranging from 0.75 to 0.80) were obtained prior to the photoinhibitory treatment for plants grown under either the ML or HL regimes (Fig. 2). Although HL plants generally presented slightly lower values, no significant differences were observed. Following photoinhibition, F_v/F_m had decreased by approximately 50 and 14% for plants grown at ML and HL, respectively (Fig. 2). Thus, in *Eutrema*, acclimation to increased growth irradiance appeared to maintain optimal photochemical efficiency and increase the tolerance to photoinhibition as has been reported earlier for other species (Anderson and Osmond 1987, Anderson *et al.* 1995, Walters 2005).

Ureides and abiotic stress: The accumulation of allantoin and allantoate are frequently in leguminous species as response to water limitation (Serraj *et al*. 1999, King and Purcell 2005). Watanabe *et al.* (2010) reported that in *Arabidopsis*, the silencing of key genes (*XDH1* and *XDH2*) in the ureide pathway negatively influenced seedling biomass as well as cell survival rate when exposed to drought stress conditions. Moreover, mutant plants accumulated higher concentrations of H_2O_2 than wild type, suggesting that the intermediates of ureide metabolism might be involved in protection of the plant in drought by an antioxidative mechanism (Watanabe *et al.* 2010). A recent study by Watanabe *et al.* (2014) using a wide variety of mutants in the ureide degradation pathway studied the role of purine intermediates in response to drought and osmotic stress. Not only did allantoin content increase in wild type plants in response to stress conditions, but an ALN mutant, which constitutively accumulates allantoin, was more tolerant to the stresses than the wild type (Watanabe *et al.* 2014).

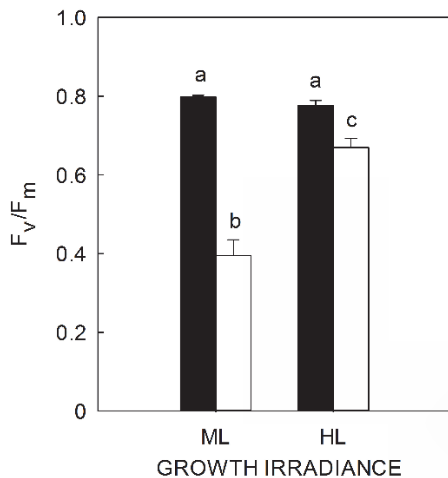

Fig. 2. Photoinhibition of photosynthesis in leaves of *Eutrema* (Yukon ecotype) developed at different growth irradiance. Plants were grown under either 250 (ML) or 750 μmol(photon) $m^{-2} s^{-1}$ (HL) PPFD. Responses were determined by monitoring changes in the F_v/F_m ratio prior photoinhibition (*black bars*) and post photoinhibition (*white bars*). Values represent means ± SD (n = 8). Bars with *different letters* are significantly different at P=0.05 based on a one-way *ANOVA*. F_v/F_m – maximal quantum yield of PSII photochemistry; HL – high light; ML – moderate light.

The phytohormone abscisic acid (ABA) is an important signaling hormone as plays a well-established role in mediating plant responses to abiotic stress (Nakashima *et al.* 2014). Watanabe *et al*. (2014) suggested that increased allantoin content elevated ABA concentrations, which in turn elicited ABA-mediated stress responses, thus linking allantoin accumulation and ABA-dependent signaling, also shown by Alamillo *et al*. (2010). Although we did not examine ABA in this study, it seems likely that contribution of allantoin in abiotic stress responses is both regulatory (ABA accumulation) and perhaps a more direct role in the detoxification of ROS.

Metabolic changes during abiotic and biotic stresses are associated with the remobilization of plant metabolites which is accelerated by catabolic activities (Sagi *et al.* 1998, Werner and Witte 2011). The use of a metabolic intermediate, such as a ureide, in stress responses has been suggested to facilitate rapid responses to environmental fluctuations whereby stress-induced inhibition or down-regulation allows the temporary accumulation of an intermediate with protective or regulatory properties and facilitates environmental acclimation and adaptation (Sagi *et al.* 1998, Watanabe *et al.* 2014). Ureides acting as ROS scavengers under stress would be consistent with their role in nitrogen recycling and mobilization, thereby supplying a nitrogen source under stress conditions (Werner and Witte 2011).

Conclusion: Our results with *Eutrema* demonstrated that ureides accumulated in leaf tissue during acclimation to high irradiance and this was correlated with increased tolerance to photoinhibition in these HL-grown plants. Our preliminary data indicated genes in the pathway are light responsive, suggesting regulation at the level of transcription. We suggest that ureides may be involved as another protective mechanism for ROS scavenging and/or ABA-dependent signaling in response to oxidative and possibly other abiotic stress responses.

References

Alamillo J.M., Díaz-Leal J.L., Sánchez-Moran M.A.V., Pineda M.: Molecular analysis of ureide accumulation under drought stress in *Phaseolus vulgaris* L. – Plant Cell Environ. **33**: 1828-1837, 2010.

Aro E.-M., Virgin I., Andersson B.: Photoinhibition of photosystem II. Inactivation, protein damage and turnover. – Biochim. Biophys. Acta **1143**: 113-134, 1993.

Amtmann A.: Learning from evolution: *Thellungiella* generates new knowledge on essential and critical components of abiotic stress tolerance in plants. – Mol. Plant **2**: 3-12, 2009.

Anderson J.M.: Photoregulation of the composition, function, and structure of thylakoid membranes. – Annu. Rev. Plant Physio. **37**: 93-136, 1986.

Anderson J.M., Chow W.S., Park Y-I.: The grand design of photosynthesis: Acclimation of the photosynthetic apparatus to environmental cues. – Photosynth. Res. **46**: 129-139, 1995.

Anderson J.M., Osmond C.B.: Shade-sun responses: compromises between acclimation and photoinhibition. – In: Kyle D.J., Osmond C.B., Arntzen C.J. (ed.): Topics in Photosynthesis, Vol. 9. Photoinhibition. Pp. 1-38. Elsevier, Amsterdam 1987.

Asada K., Takahashi M.: Production and scavenging of active oxygen in photosynthesis. – In: Kyle D.J., Osmond C.B., Arntzen C.J. (ed.): Topics in Photosynthesis, Vol. 9, Photoinhibition. Pp. 227-287. Elsevier, Amsterdam 1987.

Boardman N.K.: Comparative photosynthesis of sun and shade plants. – Annu. Rev. Plant Physio. **28**: 355-377, 1977.

Brychkova G., Alikulov Z., Fluhr R., Sagi M.: A critical role for ureides in dark and senescence-induced purine remobilization is unmasked in the *Atxdh1* Arabidopsis mutant. – Plant J. **54**: 496-509, 2008.

Castro A.H.F., Young M.C.M., de Alvarenga A.A., Alves J.D.: Influence of photoperiod on the accumulation of allantoin in comfrey plants. – R. Bras. Fisiol. Veg. **13**: 49-54, 2001.

Chalker-Scott L.: Environmental significance of anthocyanins in plant stress responses. – Photochem. Photobiol. **70**: 1-9,1999.

Chytyk C.J., Hucl P.J., Gray G.R.: Leaf photosynthetic properties and biomass accumulation of selected western Canadian spring wheat cultivars. – Can. J. Plant Sci. **91**: 305-314, 2011.

Coleto I., Pineda M., Rodiño A.P. *et al.*: Comparison of inhibition of N_2 fixation and ureide accumulation under water deficit in four common bean genotypes of contrasting drought tolerance. – Ann. Bot.-London **113**: 1071-1082, 2014.

Demmig-Adams B., Adams W.W. III: Photoprotection and other responses of plants to high light stress. – Annu. Rev. Plant Phys. **43**: 599-626, 1992.

Foyer C.H., Noctor G.: Redox regulation in photosynthetic organisms: signaling, acclimation, and practical implications. –

Antioxid. Redox Sign. **11**: 861-905, 2009.
Gill S.S., Tuteja N.: Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. – Plant Physiol. Bioch. **48**: 909-930, 2010.
Givnish T.J.: Adaptation to sun and shade: A whole-plant perspective. – Aust. J. Plant Physiol. **15**: 63-92, 1988.
Gould K.S., Markham K.R., Smith R.H., Goris J.J.: Functional role of anthocyanins in the leaves of *Quintinia serrata* A. Cunn. – J. Exp. Bot. **51**: 1107-1115, 2000.
Gray G.R., Hope B.J., Qin X.Q. *et al*.: The characterization of photoinhibition and recovery during cold acclimation in *Arabidopsis thaliana* using chlorophyll fluorescence imaging. – Physiol. Plantarum **119**: 365-375, 2003.
Griffith M., Timonin M., Wong A.C. *et al*.: *Thellungiella*: An *Arabidopsis*-related model plant adapted to cold temperatures. – Plant Cell Environ. **30**: 529-538, 2007.
Guevara D.R., Champigny M.J., Tattersall A. *et al.*: Transcriptomic and metabolomic analysis of Yukon *Thellungiella* plants grown in cabinets and their natural habitat show phenotypic plasticity. – BMC Plant Biol. **12**: 175, 2012.
Havaux M., Kloppstech K.: The protective functions of carotenoid and flavonoid pigments against excess visible radiation at chilling temperature investigated in *Arabidopsis npq* and *tt* mutants. – Planta **213**: 953-966, 2001.
Hesberg C., Hänsch R., Mendel R.R., Bittner F.: Tandem orientation of duplicated xanthine dehydrogenase genes from *Arabidopsis thaliana*: differential gene expression and enzyme activities. – J. Biol. Chem. **279**: 13547-13554, 2004.
Inan G., Zhang Q., Li P.H. *et al.*: Salt cress. A halophyte and cryophyte Arabidopsis relative model system and its applicability to molecular genetic analyses of growth and development of extremophiles. – Plant Physiol. **135**: 1718-1737, 2004.
Järvi S., Suorsa M., Aro E.-M.: Photosystem II repair in plant chloroplasts – regulation, assisting proteins and shared components with photosystem II biogenesis. – Biochim. Biophys. Acta **1847**: 900-909, 2015.
Kansal R., Kuhar K., Verma I. *et al*.: Improved and convenient method of RNA isolation from polyphenols and polysaccharide rich plant tissues. – Indian J. Exp. Biol. **47**: 842-845, 2008.
Kant S., Bi Y.M., Weretilnyk E. *et al*.: The *Arabidopsis* halophytic relative *Thellungiella halophila* tolerates nitrogen-limiting conditions by maintaining growth, nitrogen uptake, and assimilation. – Plant Physiol. **147**: 1168-1180, 2008.
Karuppanapandian T., Moon J.C., Kim C. *et al*.: Reactive oxygen species in plants: their generation, signal transduction, and scavenging mechanisms. – Aust. J. Crop Sci. **5**: 709-725, 2011.
Kazachkova Y., Batushansky A., Cisneros A. *et al*.: Growth platform-dependent and -independent phenotypic and metabolic responses of Arabidopsis thaliana and its halophytic relative, *Eutrema salsugineum*, to salt stress. – Plant Physiol. **162**: 1583-1598, 2013.
King C.A., Purcell L.C.: Inhibition of N_2 fixation in soybean is associated with elevated ureides and amino acids. – Plant Physiol. **137**: 1389-1396, 2005.
Koch M.A., German D.A.: Taxonomy and systematics are key to biological information: *Arabidopsis*, *Eutrema* (*Thellungiella*), *Noccaea* and *Schrenkiella* (Brassicaceae) as examples. – Front. Plant Sci. **4**: 267, 2013.
Kono M., Terashima I.: Long-term and short-term responses of the photosynthetic electron transport to fluctuating light. – J. Photoch. Photobio. B **137**: 89-99, 2014.
Lichtenthaler H.K., Wellburn A.R.: Determination of total carotenoids and chlorophylls *a* and *b* of leaf extracts in different solvents. – Biochem. Soc. Trans. **11**: 591-592,1983.
Murata N., Allakhverdiev S.I., Nishiyama Y.: The mechanism of photoinhibition *in vivo*: Re-evaluation of the roles of catalase, α-tocopherol, non-photochemical quenching, and electron transport. – BBA-Bioenergetics **1817**: 1127-1133, 2012.
Nakashima K., Yamaguchi-Shinozaki K., Shinozaki K.: The transcriptional regulatory network in the drought response and its crosstalk in abiotic stress responses including drought, cold and heat. – Front. Plant Sci. **5**: 170, 2014.
Neill S.O., Gould K.S.: Anthocyanins in leaves: light attenuators or antioxidants? – Funct. Plant Biol. **30**: 865-873, 2003.
Oliver M.J., Guo L., Alexander D.C. *et al*: A sister group contrast using untargeted global metabolomic analysis delineates the biochemical regulation underlying desiccation tolerance in *Sporobolus stapfianus*. – Plant Cell **23**: 1231-1248, 2011.
Powles S.B.: Photoinhibition of photosynthesis induced by visible light. – Annu. Rev. Plant Physio. **35**: 15-44, 1984.
Reinbothe H., Mothes K.: Urea, ureides, and guanidines in plants. – Annu. Rev. Plant Phys. **13**: 129-150, 1962.
Sagi M., Omarov R.T., Lips S.H.: The Mo-hydroxylases xanthine dehydrogenase and aldehyde oxidase in ryegrass as affected by nitrogen and salinity. – Plant Sci. **135**: 125-135, 1998.
Sambrook J., Russell D.W.: Molecular Cloning: A Laboratory Manual. 3rd Edition. Pp. 2100. Cold Spring Harbor Laboratory Press, New York 2001.
Schneider C.A., Rasband W.S., Eliceiri K.W.: NIH Image to ImageJ: 25 years of image analysis. – Nat. Methods **9**: 671-675, 2012.
Schubert K.R.: Products of biological nitrogen fixation in higher plants: synthesis, transport, and metabolism. – Annu. Rev. Plant Physio. **37**: 539-574, 1986.
Serraj R., Sinclair T.R., Purcell L.C.: Symbiotic N_2 fixation response to drought. – J. Exp. Bot. **50**: 143-155, 1999.
Stasolla C., Katahira R., Thorpe T.A., Ashihara H.: Purine and pyrimidine nucleotide metabolism in higher plants. – J. Plant Physiol. **160**: 1271-1295, 2003.
Stepien P., Johnson G.N.: Contrasting responses of photosynthesis to salt stress in the glycophyte Arabidopsis and halophyte Thellungiella: Role of the plastid terminal oxidase as an alternative electron sink. – Plant Physiol. **149**: 1154-1165, 2009.
Sui N., Han G.: Salt-induced photoinhibition of PSII is alleviated in halophyte *Thellungiella halophila* by increases of unsaturated fatty acids in membrane lipids. – Acta Physiol. Plant. **36**: 983-992, 2014.
Suzuki N., Koussevitzky S., Mittler R., Miller G.: ROS and redox signalling in the response of plants to abiotic stress. – Plant Cell Environ. **35**: 259-270, 2012.
Takahashi S., Badger M.R.: Photoprotection in plants: a new light on photosystem II damage. – Trends Plant Sci. **16**: 53-60, 2011.
Takahashi S., Murata N.: How do environmental stresses accelerate photoinhibition? – Trends Plant Sci. **13**: 178-182, 2008.
Todd C.D., Tipton P.A., Blevins D.G. *et al*.: Update on ureide degradation in legumes. – J. Exp. Bot. **57**: 5-12, 2006.
Ventura Y., Wuddineh W.A., Myrzabayeva M. *et al.*: Effect of seawater concentration on the productivity and nutritional value of annual *Salicornia* and perennial *Sarcocornia* halophytes as leafy vegetable crops. – Sci. Hortic.-Amsterdam **128**: 189-196, 2011.
Vogels G.D., Van Der Drift C.: Differential analyses of

glyoxylate derivatives. – Anal. Biochem. **33**: 143-157, 1970.
Walters R.G.: Towards an understanding of photosynthetic acclimation. – J. Exp. Bot. **56**: 435-447, 2005.
Wang P., Kong C.H, Sun B., Xu X.H.: Distribution and function of allantoin (5-ureidohydantoin) in rice grains. – J. Agric. Food Chem. **60**: 2793-2798, 2012.
Watanabe S., Matsumoto M., Hakomori Y. *et al*.: The purine metabolite allantoin enhances abiotic stress tolerance through synergistic activation of abscisic acid metabolism. – Plant Cell Environ. **37**: 1022-1036, 2014.
Watanabe S., Nakagawa A., Izumi S. *et al*.: RNA interference-mediated suppression of xanthine dehydrogenase reveals the role of purine metabolism in drought tolerance in Arabidopsis. – FEBS Lett. **584**: 1181-1186, 2010.
Werner A.K., Witte C.P.: The biochemistry of nitrogen mobilization: purine ring catabolism. – Trends Plant Sci. **16**: 381-387, 2011.
Wong C.E., Li Y., Labbe A. *et al.*: Transcriptional profiling implicates novel interactions between abiotic stress and hormonal responses in *Thellungiella*, a close relative of *Arabidopsis*. – Plant Physiol. **140**: 1437-1450, 2006.
Ye J., Coulouris G., Zaretskaya I. *et al*.: Primer-BLAST: A tool to design target-specific primers for polymerase chain reaction. – BMC Bioinformatics **13**: 134, 2012.
Yobi A., Wone B.W., Xu W. *et al.*: Metabolomic profiling in *Selaginella lepidophylla* at various hydration states provides new insights into the mechanistic basis of desiccation tolerance. – Mol. Plant **6**: 369-385, 2013.
Zrenner R., Stitt M., Sonnewald U., Boldt R.: Pyrimidine and purine biosynthesis and degradation in plants. – Annu. Rev. Plant Biol. **57**: 805-836, 2006