The oxidative stress in allelopathy. The participation of prenyllipid antioxidants in the response to juglone.

Beatrycze Nowicka, Bartosz Pluciński, Paulina Kuczyńska, Jerzy Kruk

Department of Plant Physiology and Biochemistry, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Gronostajowa 7, 30-387 Cracow, Poland, corresponding author e-mail: beatrycze.nowicka@uj.edu.pl

Introduction

· **Allelopathy**, according to narrow definition, is the effect of one plant species on another through the release of chemical compounds into the environment [1]. Broader definitions cover also interactions between plant, microbes and fungi [2]. Allelochemicals are secondary metabolites, often produced as inactive precursors and released thorough leaching from aerial parts, root exudation, volatile emissions and decomposition of residues. The modes of action of certain allelochemicals are various, one of them is the induction of oxidative stress [3].

· **Oxidative stress** is a situation, when there is a serious imbalance between the reactive oxygen species (ROS) production and detoxification, resulting in excessive concentration of ROS in cells [4]. ROS are both radical ($O_2^{\cdot-}$, HO_2, OH) and non-radical ($^1O_2$, H_2O_2) forms, usually generated as by-products of aerobic metabolism. They can play a beneficial role in signalling and pathogen defence, but can also damage cellular components. The main site of ROS production in plant and algal cells are chloroplasts; mitochondria and peroxisomes being other important sources of ROS [5].

· **Prenyllipid antioxidants belonging to isoprenoid quinones and chromanols** are amphipathic compounds, occurring ubiquitously in membranes. Prenylquinones act as mobile electron and proton carriers and enzyme cofactors. Members of both groups also participate in signal transduction. These compounds are potent antioxidants, able to quench and scavenge $^1O_2$ and scavenge oxygen and lipid radicals. Important prenyllipid antioxidants present in chloroplasts are tocopherols (α-Toc and γ-Toc) and plastoquinone (PQ)/plastoquinol (PQH_2) [6]

· **Juglone**, 5-hydroxy-1,4-naphthoquinone, belong to the most recognized allelochemicals. Its inactive precursor is produced by trees of genus *Juglans* and released into the environment, where hydrolysis and oxidation results in formation of active compound. Juglone was shown to inhibit germination and growth of sensitive plants, inhibit respiration and photosynthesis and cause wilting [7]. The effect of juglone is pleiotropic and mechanisms of its toxicity have not been fully elucidated yet [8]. Juglone is a strong redox cycler, which means that *in vivo* it can be easily reduced to semiquinol, which can further reduce O_2,to superoxide [3,9]. Reduced juglone can also react with glutathione leading to depletion of this antioxidant [2]. The occurrence of oxidative stress in juglone-exposed plants has been observed [10-13]. Other mechanisms of juglone toxicity are: inhibition of *p*-hydroxyphenylpyruvate dioxygenase, a key enzyme of PQ and Toc biosynthesis [14], inhibition of H^+-ATPase necessary for keeping of water homeostasis [15], inhibition of peptidyl-proly cis/trans isomerases and RNA polymerases [16]

· ***Chlamydomonas reinhardtii*** is a common freshwater green microalga, thought of as a model unicellular photosynthetic organism and often used in environmental studies, as it is easy to grow, metabolically profiled and its genome has been sequenced [17]. Our preliminary experiments have shown that *C. reinhardtii* is sensitive to juglone. Therefore, it can be used as a simplified model for observation of some modes of juglone action.

· **The aim of the study** was to examine the impact of juglone exposure on photosynthetic pigments, prenyllipid content and lipid peroxidation in *C. reinhardtii* (**experiment 1**) and to examine the combined effect of juglone and high light stress (**experiment 2**).

Materials and methods

A *C. reinhardtii* strain 11-32b (SAG collection, Goettingen, Germany) was grown in a modified Sager-Granick medium with 5 mM HEPES pH 6.8 and 0.2 mM sodium acetate as described in [18]. The photosynthetic pigments were extracted with acetone [19] and their concentration was determined spectrophotometrically, as described in [20]. Before the experiments, cultures were diluted with a fresh medium to get a final chlorophyll concentration of 2.5 μg Chl/ml. 10 mM juglone in DMSO was prepared just before application. Juglone was added to the algal culture to obtain final concentration 20 μM.

In **experiment 1** juglone-treated and control cultures were put on the shaker and kept under the weak light (35 μmols photons m^2/s). Samples were taken a t=0, 2.5, 5, 7.5 h.

In **experiment 2** juglone-treated and control cultures (in flat plastic bottles) were preincubated in the weak light on a shaker for 1 h, then either kept in darkness (D) or exposed to white high light (HL, 1000 μmols photons m^2/s) for 40 min (on magnetic stirrers). Samples were taken at t=0, after 1 h of preincubation and after 10, 20, 30, 40 min (for HL series) or 20, 40 min (D series).

Photosynthetic pigments were determined as described above. Prenyllipid antioxidants were extracted with acetone and analyzed using RP-HPLC, as described in [18,19,21]. For lipid hydroperoxides (LOOH) determination samples were extracted with ethanol, then a method with Spy-LHP fluorescent probe was used, as described in [18].

Acknowledgements

This work was supported by grant 2013/11/D/NZ1/00303 from the National Science Center Poland.

Results of experiment 1

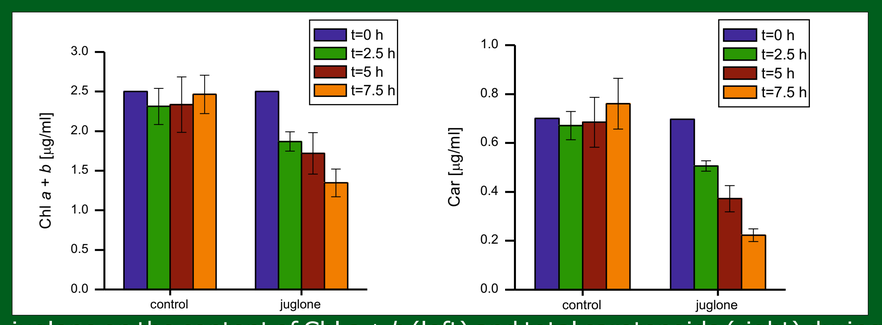
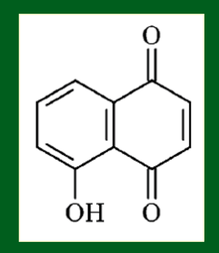


Fig. 1. The effect of exposure to juglone on the content of Chl *a* + *b* (left) and total carotenoids (right) during 7.5 h of incubation in weak light. The data are means ± SD (n=3). Car, total carotenoids; Chl *a* + *b*, sum of chlorophyll *a* and *b*; cont, control series.



juglone

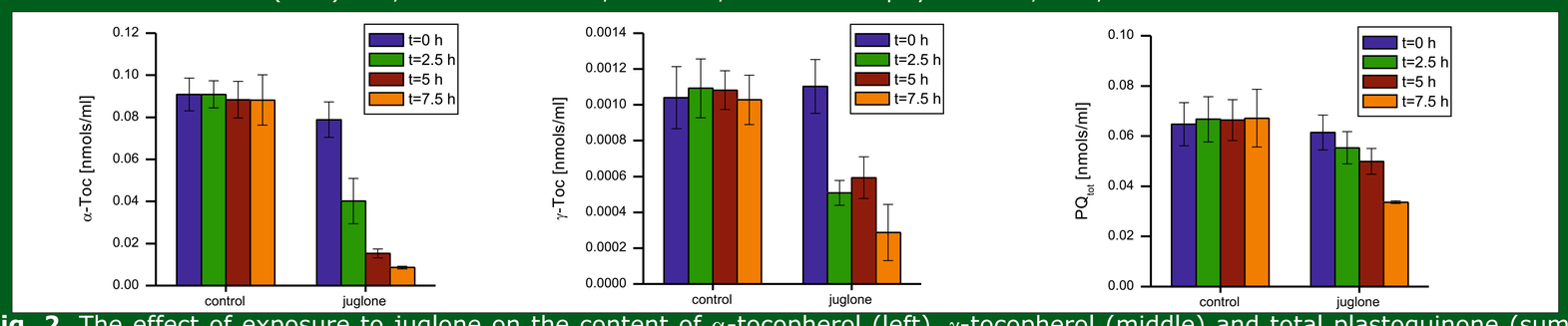


Fig. 2. The effect of exposure to juglone on the content of α-tocopherol (left), γ-tocopherol (middle) and total plastoquinone (sum of plastoquinone and plastoquinol, right) during 7.5 h of incubation in weak light. The data are means ± SD (n=3). cont, control series, PQ_{tot}, total plastoquinone; α-Toc, α-tocopherol; γ-Toc, γ-tocopherol.

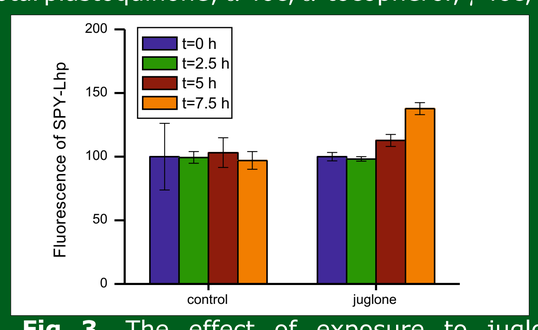


Fig 3. The effect of exposure to juglone on Spy-LHP fluorescence. The fluorescence was normalized on the signal obtained for t=0 samples. The data are means ± SD (n=3).

The exposure to juglone led to significant decrease in photosynthetic pigments and prenyllipid content, which was accompanied with the increase in LOOH content, which points at proceeding lipid peroxidation.

Results of experiment 2

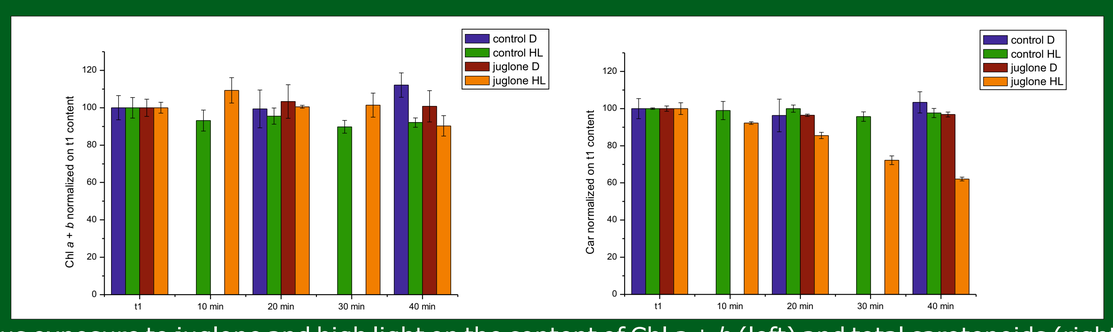


Fig. 4. The effect of simultaneous exposure to juglone and high light on the content of Chl *a* + *b* (left) and total carotenoids (right) during 40 min of incubation in high light or in darkness. The pigment content is expressed as a percentage of content after 1 h of preincubation. The data are means ± SD (n=3). Car, total carotenoids; Chl *a* + *b*, sum of chlorophyll *a* and *b*; cont D, control series kept in darkness, cont HL, control series kept in high light, juglone D, series with juglone kept in darkness, juglone HL, series in high light.

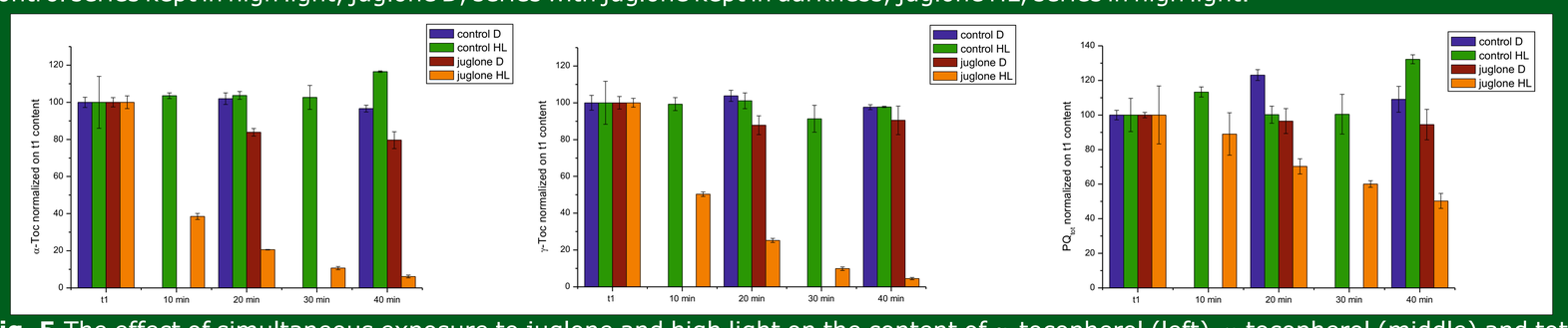


Fig. 5. The effect of simultaneous exposure to juglone and high light on the content of α-tocopherol (left), γ-tocopherol (middle) and total plastoquinone (sum of plastoquinone and plastoquinol, right) during 40 min of incubation in high light or in darkness. The prenyllipid content is expressed as a percentage of content after 1 h of preincubation. The data are means ± SD (n=3). cont D, control series kept in darkness, cont HL, control series kept in high light, juglone D, series with juglone kept in darkness, juglone HL, series in high light. PQ_{tot}, total plastoquinone; α-Toc, α-tocopherol; γ-Toc, γ-tocopherol.

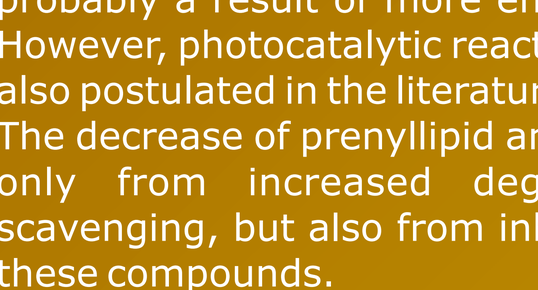


Fig 6. The effect of simultaneous exposure to juglone and high light on the content of α-tocopherolquinone, an oxidation product of α-tocopherol, during 40 min of incubation in high light or darkness. The prenyllipid content is expressed as a percentage of content after 1 h of preincubation. The data are means ± SD (n=3). α-TQ, α -tocopherolquinone.

The simultaneous application of high light and juglone led to a significant decrease in total carotenoid, total PQ and Toc content. High light alone did not cause a decrease in carotenoid and prenyllipids, while in series with juglone alone only a slight decrease (or no decrease) was observed. What is more, there was a significant accumulation of an oxidation product of α-Toc, α-tocopheryloquinone (α-TQ) in series with juglone + high light, while there were no α-TQ increase in darkness (both in juglone and control series) and much smaller increase in high light control.

Conclusions

- Exposure to juglone causes oxidative stress in *C. reinhardtii* and prenyllipid antioxidants participate in ROS scavenging.
- High light significantly increases juglone toxicity. This is probably a result of more effective juglone reduction. However, photocatalytic reactions of juglone itself were also postulated in the literature [7].
- The decrease of prenyllipid antioxidants may result not only from increased degradation due to ROS scavenging, but also from inhibition of biosynthesis of these compounds.

References:

[1] Rice EL (1979) *Allelopathy: an update,* Bot Rev 45:15–109.
[2] Macias FA, Molinillo JM, Varela RM, Galindo JC (2007) *Allelopathy - a natural alternative for weed control*, Pest Manag Sci 63:327-348
[3] Weir TL, Park SW, Vivanco JM (2004) *Biochemical and physiological mechanisms mediated by allelochemicals*, Curr Op Plant Biol 7:472-479.
[4] Ahmad P, Sarwat M, Sharma S (2008) *Reactive oxygen species, antioxidants and signaling in plants*, J Plant Biol 51:167-173.
[5] Gechev TS, van Breusegem F, Stone JM, Denev I, Laloi C (2006) *Reactive oxygen species as signals that modulate plant stress responses and programmed cell death*, BioEssays 28:1091–1101.
[6] Kruk J, Szymańska R, Nowicka B, Dłużewska J (2016) *Function of isoprenoid quinones and chromanols during oxidative stress in plants*, New Biotechn http://dx.doi.org/10.1016/j.nbt.2016.02.010.
[7] Willis RJ (2000) *Juglans spp., Juglone and allelopathy*, Allelopathy J, 7:1-55.
[8] Dayan FE, Duke SO (2009) *Biological activity of allelochemicals. In Plant-derived Natural Products*, Springer US. pp. 361-384.
[9] Koster AS (1991) *Bioreductive activation of quinones: a mixed blessing*. Pharmaceutisch Weekblad 13:123-126.
[10] Babula P, Adam V, Kizek R, Sladky Z, Havel L (2009) *Naphthoquinones as allelochemical triggers of programmed cell death*, Environ Exp Bot 65:330-337.
[11] Chen SY, Chi WC, Trinh NN, Cheng KT, Chen YA, Lin TC, et al. (2015) *Alleviation of allelochemical juglone-induced phytotoxicity in tobacco plants by proline*, J Plant Inter 10:167-172.
[12] El Hadrami A, Kone D, Lepoivre P (2005) *Effect of juglone on active oxygen species and antioxidant enzymes in susceptible and partially resistant banana cultivars to black leaf streak disease*, Eur J Plant Pathol 113:241-254.
[13] Mylona PV, Polidoros AN, Scandalios JG (2007) *Antioxidant gene responses to ROS-generating xenobiotics in developing and germinated scutella of maize*, J Exp Bot 58:1301-1312.
[14] Meazza G, Scheffler BE, Tellez MR, Rimando AM, Romagni JG, Duke SO, et al. (2002) *The inhibitory activity of natural products on plant p-hydroxyphenylpyruvate dioxygenase*. Phytochemistry 60:281-288.
[15] Hejl AM, Koster KL (2004) *Juglone disrupts root plasma membrane H+-ATPase activity and impairs water uptake, root respiration, and growth in soybean (Glycine max) and corn (Zea mays)*, J Chem Ecol, 30:453-471.
[16] Chao SH, Greenleaf AL, Price DH (2001) *Juglone, an inhibitor of the peptidyl-prolyl isomerase Pin1, also directly blocks transcription*, Nucl Acid Res 29:767-773.
[17] Hanikenne M (2003) *Chlamydomonas reinhardtii as a eukaryotic photosynthetic model for studies of heavy metal homeostasis and tolerance*, New Phytol 159:331–340.
[18] Nowicka B, Pluciński B, Kuczyńska P, Kruk J (2016) *Prenyllipid antioxidants participate in response to acute stress induced by heavy metals in green microalga Chlamydomonas reinhardtii*, Environ Exp Bot 123:98–107.
[19] Nowicka B, Kruk J (2012) *Plastoquinol is more active than α-tocopherol in singlet oxygen scavenging during high light stress of Chlamydomonas reinhardtii*, Biochim Biophys Acta: Bioenerg 1817:389–394.
[20] Lichtenthaler HK (1987) *Chlorophylls and carotenoids: pigments of photosynthetic biomembranes*, Meth Enzymol 148C:350–382.
[21] Nowicka B, Pluciński B, Kuczyńska P, Kruk J (2016) *Physiological characterization of Chlamydomonas reinhardtii acclimated to chronic stress induced by Ag, Cd, Cr, Cu and Hg ions*, Ecotoxicol Environ Saf 130:133-145.