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

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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 plants, microbes and fungi [2]. Allelochemicals are secondary metabolites, often produced as inactive precursors and released throughleaching from aerial parts, root exudation, volatile emissions and decomposition of residues. The mode of action of most allelochemicals remains various, one of them is the induction of oxidative stress [3].

- Oxidative stress is a situation, where 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 (\( \cdot \)O, \( \cdot \)H, OH) and non-radical (\( \cdot \)O₂, H₂O₂) forms, usually generated as by-products of aerobic metabolism. They can play a beneficial role in signaling and pathogen defence, but can also damage cellular components. The main site of ROS production in plant and algal cells are chloroplasts; mitochondrial peroxisomes being other important sources of ROS [5].

- Prenyllipid antioxidants belonging to isoprenoid quinones and chromanols are amphipathic compounds, occurring ubiquitously in membranes. Prenyllipides 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 \( \cdot \)O, and scavenge oxygen and lipid radicals. Important prenyl lipid antioxidants present in chloroplasts are tocopherols (\( \gamma \)-Toc and \( \gamma \)-Toc) and plastoquinone (\( \text{PQ} \)) / plastoquinol (\( \text{PQH}_2 \)) [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 cycle, which means that in vivo it can be easily reduced to \( \text{PQ}\) or \( \text{PQH}_2 \), which can further reduce \( \cdot \)O₂ to \( \cdot \)OH [9,10]. Reduced juglone can also react with glutathione leading to depletion of this antioxidant [2]. The occurrence of oxidative stress in jugulone-exposed plants has been observed [10-15]. Other mechanisms of juglone toxicity are: inhibition of \( \text{p}-\text{hydroxyphenylpyruvate dioxygenase}, \text{a key enzyme of PQ and Toc biosynthesis} [16] \). Inhibition of H⁺/ATPase necessary for keeping of water homeostasis [15], inhibition of pyridol-prolyl cis/trans isomerases and RNA polymerases [16].

- Chlamydomonas reinhardtii is a common freshwater green alga, enough work has been done on the field of isoprenoid quinones and chromanols during oxidative stress responses [17]. The alga has been used for screening powerful antioxidants in higher species [18]. Several allelochemicals are toxic to 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 11-33b (SAG collection, Guntingen, Germany) was grown in a modified Sager–Grancik medium with 5 mM PEPES pH 6.8 and 0.2 mM 

- Photosynthetic pigments were determined as described above. Prenyllipid content and lipid peroxidation in C. reinhardtii (experiment 1) and to examine the combined effect of juglone and high light stress (experiment 2).

Results of experiment 1

- The oxidative stress of juglone treatment of C. reinhardtii and prenyl lipid antioxidants participate in ROS scavenging.

- The decrease of prenyl lipid antioxidants may result not only from increased degradation due to ROS scavenging, but also from inhibition of biosynthesis of these compounds.

Conclusions

- Exposure to juglone causes oxidative stress in C. reinhardtii and prenyl lipid 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].

Acknowledgements

This work was supported by grant 2013/11/D/NZ2/00303 from the National Science Center Poland.