Investigating the contribution of proteotoxicity to juglone's mechanism of action

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Abstract

Juglone is a phytotoxic allelochemical synthesized by black walnut trees (*Juglans nigra*). Within the systems of susceptible plants, juglone causes oxidative stress through redox cycling. It remains unknown whether juglone's propensity to react with nucleophiles, including thiol groups of cysteine residues, also contributes to juglone's allelopathic mechanism of action. Previously, the Widhalm lab demonstrated juglone binds to cysteine thiol groups in glutathione and inhibits the enzyme urease. Furthermore, they found that Arabidopsis plants increase expression of transcription factors (*NAC53* and *NAC78*) that regulate the proteasome, the enzyme responsible for breaking down misfolded proteins. Using *nac53/78* knockout mutants and *in vitro* enzyme assays from Arabidopsis root protein extracts, we tested out hypothesis that juglone broadly inhibits cellular enzyme activities and the proteasome stress regulon is necessary to respond to juglone stress. We also investigated other allelochemicals for their ability to induce proteotoxicity by monitoring the expression of NAC transcription factors using qPCR. We found that each allelochemical tested causes an upregulation in proteasome transcription factors, though only three of the six compounds appeared to have a negative effect on growth. Further tests are necessary to understand the significance of our findings, and to expand our knowledge on the biochemical mechanism of action of juglone and other allelochemicals, which facilitate many plant-biotic interactions.

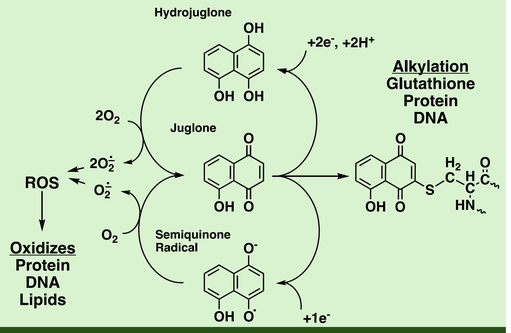


Figure 1. Juglone spontaneously reacts in vitro with nucleophiles. Juglone autoxidizes into hydrojuglone or its semiquinone radical to release reactive oxygen species. Reactive oxygen species oxidizing macromolecules or alkylation of nucleophiles are potential mechanism of action for juglone.

Juglone Inhibits PAL Activity

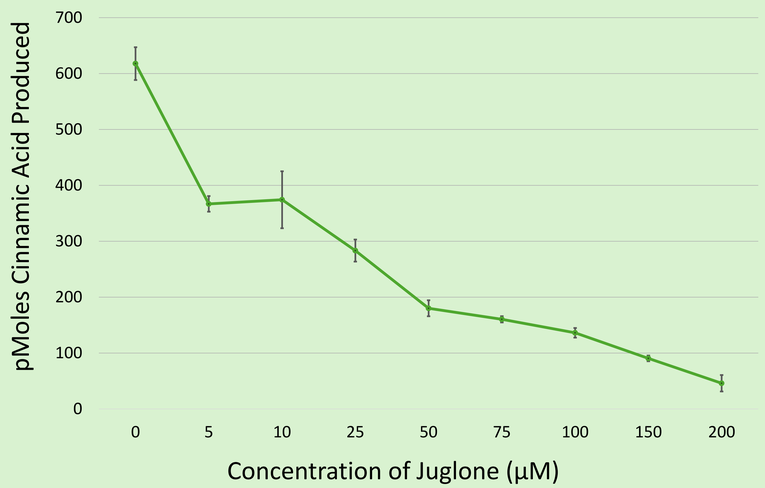


Figure 2. Protein was extracted from ground root tissue and incubated with increasing concentrations of juglone. The reaction was started with the addition of phenylalanine and allowed to go for 45 minutes. The reaction was stopped and the formation of product was measured using HPLC.

Other 1,4-NQs Inhibit Arabidopsis Growth

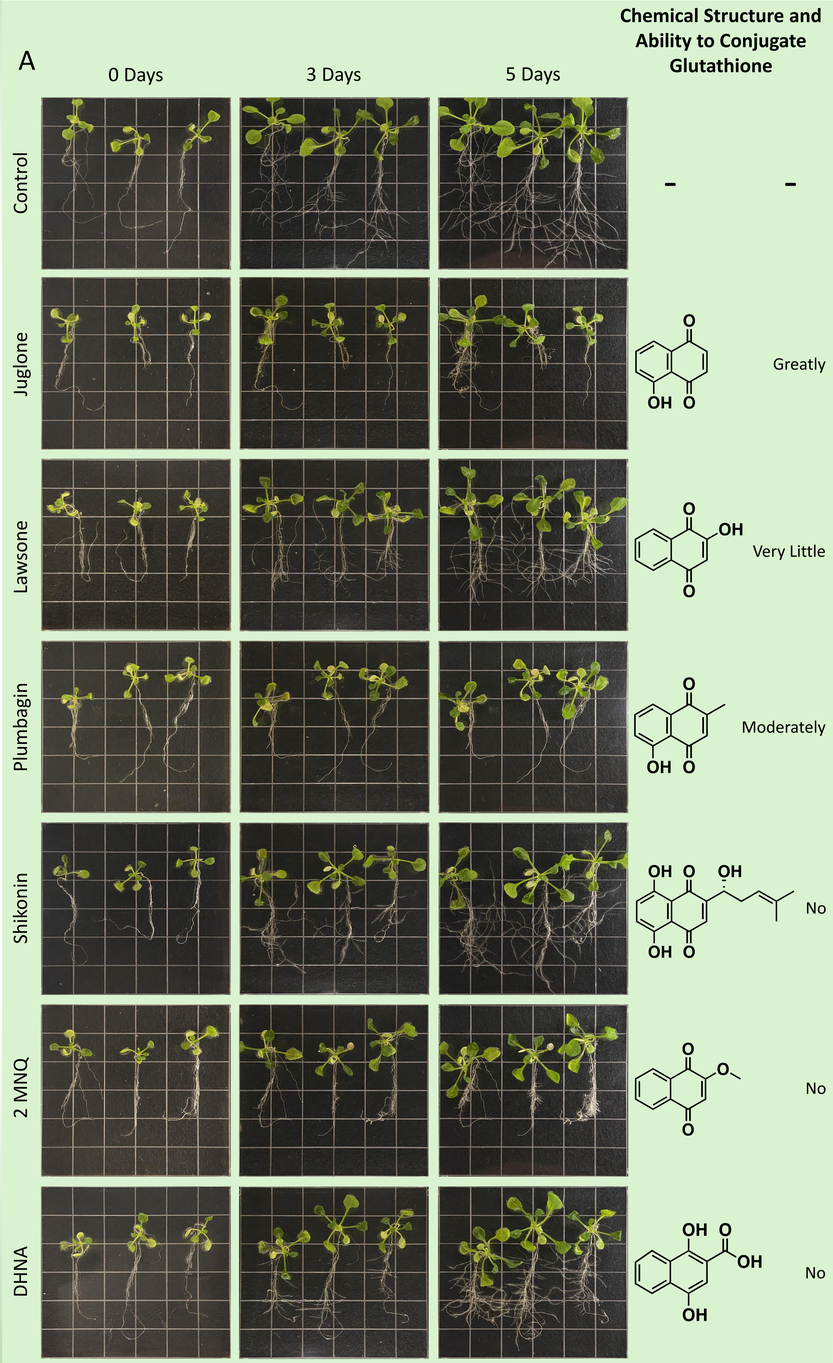
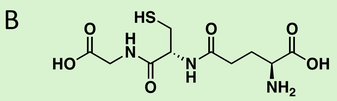


Figure 3. (A) Col-0 plants were germinated for 12 days before being transferred to plates supplemented with 20µM of indicated 1,4-naphthoquinone (1,4-NQ) and photographed over 5 days. The chemical structure of the 1,4-NQ is also shown, as well as a brief description of the quinones ability to alkylate glutathione. Glutathione alkylation was detected by HPLC-MS (data not shown). (B) Glutathione molecule.



1,4-NQ Stress Causes Proteasome Upregulation

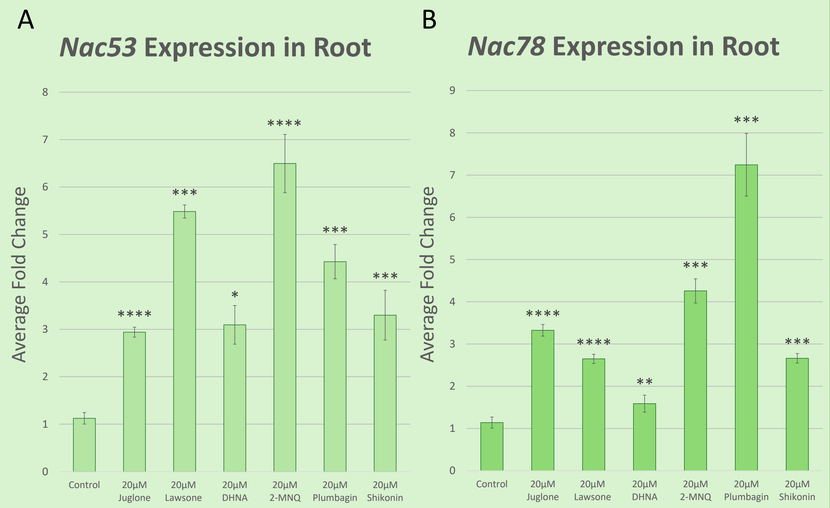


Figure 4. Expression of transcription factors *Nac53* (A) and *Nac78* (B) in 12-day old Arabidopsis roots after 12-hour treatment with indicated quinone. The expression values were calculated using *ACT2* transcripts as a reference and normalized to those from control root expression. Error bars represent standard error from three biological replicates. Statistical differences were calculated using t-test (α=0.05).

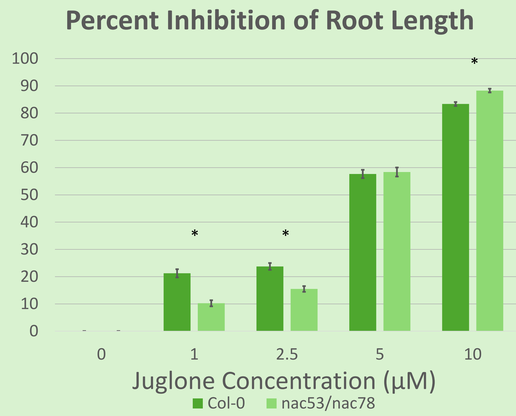


Figure 5. Two-day old Arabidopsis plants, wild type (Col-0) or mutant (*nac53/nac78*), were transferred to plates with increasing juglone concentrations. Root lengths were measured and compared to 0µM juglone control after 6 days. Statistical differences were determined using t-test between both genotypes in each condition (α=0.05). Error bars indicate standard error.

Future Directions

- Perform enzyme inhibition assays with other 1,4-naphthoquinone treatments.
- Phenotype *nac53/78* mutants after other 1,4-naphthoquinone treatments.
- Look for juglone-protein conjugates using Purdue proteomics facilities.

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