Expression of Nickel Resistance Genes in Hardwood Populations from a Metal-Contaminated Region.

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Background

- Heavy metal (HM) contamination in mining regions has become a growing concern worldwide. HM can be toxic to virtually all-living organisms, but plants are among the most affected organisms because they are not mobile and depend on the soil they reside in for nutrients and water. HMs have been known to lower soil nutrient availability (complexion) (Koptsik et al. 2003). However, they are most commonly associated with oxidative stress. Excess levels of heavy metals can lead to membrane instability, protein disruption and nucleic acid damage, either by the metal itself or the production of reactive oxygen species (ROS) (Yadav 2010). This is especially troublesome for mining regions of the Canadian Boreal Forest since the acidic nature of the soil further increases heavy metal availability.
- Key genes associated with nickel tolerance in model and non – model plant species have been identified and characterized. They include some genes coding for transporters of the NRAMP and IREG family, metabolic proteins involved in glutathione synthesis and chelating peptides such as methalothiones (Moreno-Jiménez et al. 2012; Merlot et al. 2014). There is also some evidence that transcription factors from the ZAT family play a role in the regulation of genes involved in nickel resistance (Liu et al. 2014). The expression of these genes in white birch (*Betula papyrifera*) and red oak (*Quercus rubra*) in a metal contaminated environment has yet to be investigated.

Objective

- **To determine the level of regulation of AT2G16800 and glutathione reductase in white birch (*Betula papyrifera*) and red oak (*Quercus rubra*) genotypes growing in soils with different nickel contamination levels.**

Methods

Field experiment

- *B. papyrifera and Q. rubra* leaves and roots were sampled from six locations in the Greater Sudbury Region, three metal contaminated sites and three references.

Nickel treatments

- White birch (*Betula papyrifera*) seeds were collected from a Laurentian University research field site located in the Greater Sudbury Region in Northern Ontario (Canada). The site has been contaminated with metals for > 100 years. Six month seedlings were treated with nickel nitrate as described in Theriault *et al.,* (2016). Gene expression in genotypes resistant and susceptible to a soil nickel concentration of 1,600 mg/kg was analyzed in details.

Gene expression

- The total RNA was extracted from samples from growth chamber and fields trials using the Plant/Fungi Total RNA Purification kit by Norgen Biotek Corporation (Thorold, Canada) or by methods previously described by (Chang et al. 1993).
- Quantitative PCR was carried out by using the Dynamo HS SYBR Green Kit by Life Technologies according to the manufacturer's protocol.

Results

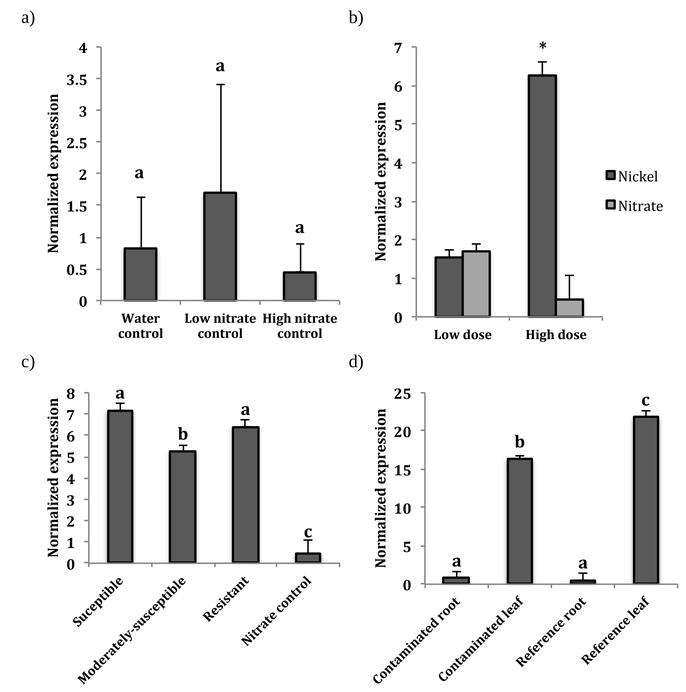

Figure 1. Expression of AT2G16800 (high-affinity nickel-transport family protein) in white birch (*Betula papyrifera*) for **a)** water and nitrate controls; **b)** nickel treatments at low dose of 5.56 mg/kg and high dose of 1,600 mg/kg of nickel; **c)** susceptible, moderately susceptible, resistant plants treated at 1,600 mg /kg; **d)** field samples from metal-contaminated and reference root and leaves. Expression of AT2G16800 was standardized based on the housekeeping gene ef1α. Significant differences were found among groups using the Tukey's multiple comparison or t-test ($p < 0.05$).

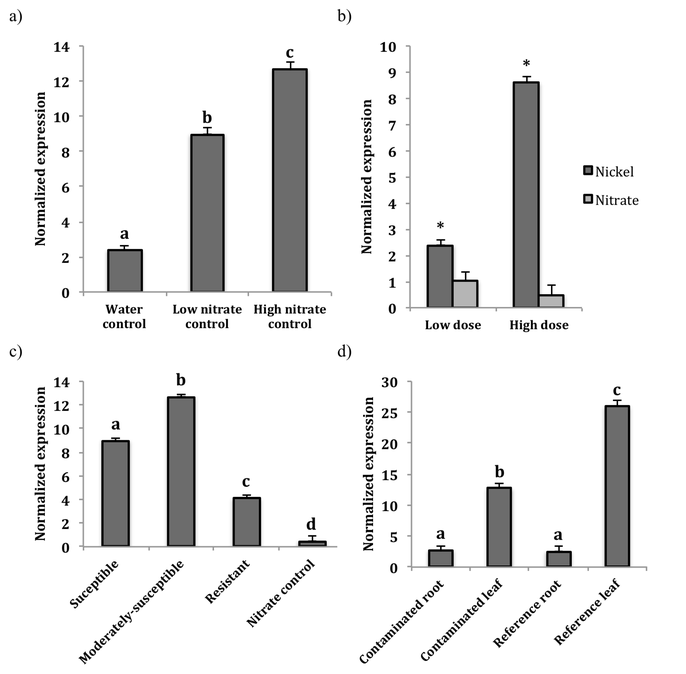

Figure 2. Expression of Glutathione reductase (GR) in white birch (*Betula papyrifera*) for **a)** water and nitrate controls; **b)** nickel treatments at low dose of 5.56 mg/kg and high dose of 1,600 mg/kg of nickel; **c)** susceptible, moderately susceptible, resistant plants treated at 1,600 mg /kg; **d)** field samples from metal-contaminated and reference root and leaves. Expression of GR was standardized based on the housekeeping gene ef1α. Significant differences were found among groups using the Tukey's multiple comparison or t-test ($p < 0.05$).

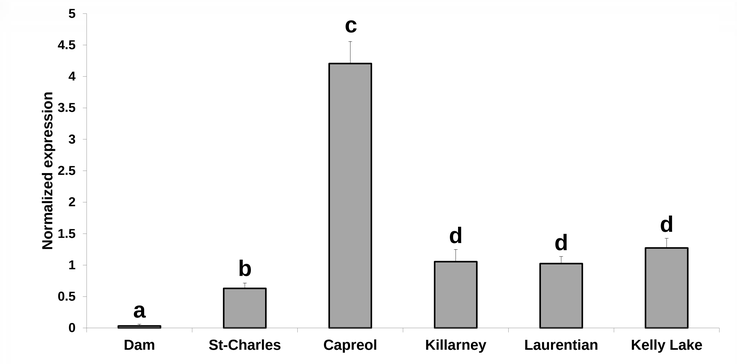

Figure 3. Expression of AT2G16800 (high-affinity nickel-transport family protein) in red oak roots (*Quercus rubra*) **in** field samples from metal-contaminated (Dam, Laurentian and Kelly Lake) and reference (St-Charles, Capreol and Killarney) root and leaves. Expression of AT2G16800 was standardized based on the housekeeping gene ef1α. Significant differences were found among groups using the Tukey's multiple comparison ($p < 0.05$).

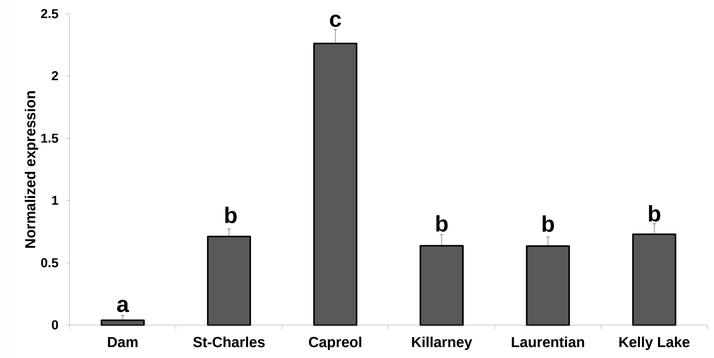

Figure 4. Expression of Glutathione reductase (GR) in red oak roots (*Quercus rubra*) **in** field samples from metal-contaminated (Dam, Laurentian and Kelly Lake) and reference (St-Charles, Capreol and Killarney) root and leaves. Expression of AT2G16800 was standardized based on the housekeeping gene ef1α. Significant differences were found among groups using the Tukey's multiple comparison ($p < 0.05$).

Conclusions

- Low and high nickel doses induced GR expression but only the high dose induced AT2G16800 expression in *B. papyrifera*.
- For *B. papyrifera*, AT2G16800 expression was highest in the susceptible genotypes and lowest in the resistant ones. GR expression was highest in moderately susceptible plants and lowest in water controls.
- No link between Ni-resistance and AT2G16800 was found however, lower GR expression in resistant genotypes could indicate lower Ni toxicity levels.
- For field experiment, expression of the two genes in white birch was significantly higher in leaves compared to roots from all sites. No link was found between metal contamination and expression of AT2G16800 and GR for white birch or red oak.
- Gene expression studies of these genes in red maple (*Acer rubrum*) and trembling aspen (*Populus tremuloides*) are in progress

Acknowledgements

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