Comprehensive transcriptome analysis of response to nickel stress in white birch (*Betula papyrifera*)

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Background

White birch (*Betula papyrifera*) is a dominant tree species of the boreal forest. It is a pioneer species and rapidly colonizes open areas (Perala et Alm, 1990). Little is known about its adaptation to soil metal contamination even though it plays a key role in forest sustainability. Recent studies have shown that birch is fairly resistant to heavy metal contamination specifically to nickel (Kirkey et al., 2012; Amiro and Courtin, 1981).

Knowledge of regulation of genes associated with metals in higher plants is very unclear. Genome sequence of only one birch species, dwarf birch (*Betula nana*) has been completed (Wang et al., 2013). To date transcriptome analysis in the genus *Betula* has not been investigated. Comparative transcriptome analysis is a novel tool for genetic characterization for stress resistance in plant populations (Barghini et al., 2015; Halimaa et al., 2014; Merlot et al., 2014).

Objectives

1) Develop and characterize the white birch transcriptome
2) Assess gene expression dynamics of white birch in response to nickel stress
3) Describe gene function based on ontology.

Methods

Nickel treatments

To assess the toxicity of nickel, segregating populations were treated with a single dose of $Ni(NO_3)_2$ salt which led to a final concentration of 1600 mg /kg of nickel as previously described (Theriault and Nkongolo, 2015). This concentration corresponds to the total amount of nickel in contaminated sites in the mining region in Northern Ontario. Water treated plants were used as reference. To determine any effect of nitrate on plant damage KNO_3 was also used as an additional control (nitrate control). The experimental design was a completely randomized block with 15 replications.

Damage rating was recorded based on a scale of 1 to 9, 1 being no visible toxicity symptoms and 9 dead plants. Individual plants with a score of 1 to 3 were considered nickel resistant, 4 to 6, moderately resistant and 7 to 9 susceptible.

Transcriptome analysis

The total RNA was extracted using the Plant/Fungi Total RNA Purification kit by Norgen Biotek Corporation (Thorold, Canada).

RNA- seq libraries were generated using the TruSeq RNA-Seq Sample Prep Kit according to the manufacturer's protocol (Illumina Inc. San Diego, CA). The library was quantified using Bioanalyzed 2100 (Agilent Technologies, Santa Clara, CA) and the sequencing was performed on the illumina HISEqSR 1 x50 (illumina Inc.) at Seq Matic (Fremont California, USA). The RNA-Sequence data were used as input to the Trinity program to assemble the transcripts. All transcripts were mapped to protein sequences in the UniProt database and the best match was used to annotate *Betula papyrifera* (white birch) genes and assign gene ontology information. The annotated sequences were run through the go GO-Slim function of the BLAST2GO program to provide a high level summary of functions that include biological process, cellular components, and molecular function.

Results and discussion

A total of 209802 trinity genes were identified and were assembled to 278264 total trinity transcripts. The transcripts were mapped to protein sequences and based on best match; we annotate the white birch genes and assign gene ontology. Overall, 215700 transcripts were annotated and were compared to the published dwarf birch genome. A genomic match for 61% transcripts (169052 out 278264) with the reference genome was found. Expression profiles were generated and 62587 genes were found to be significantly differentially expressed among the nickel resistant, susceptible, and untreated libraries.

Figure 1. White birch (*Betula papyrifera*) from M2 populations treated with 1600 mg/ kg of $Ni(NO_3)_2$. Left plant is resistant and right plant is susceptible 7 days after treatment.

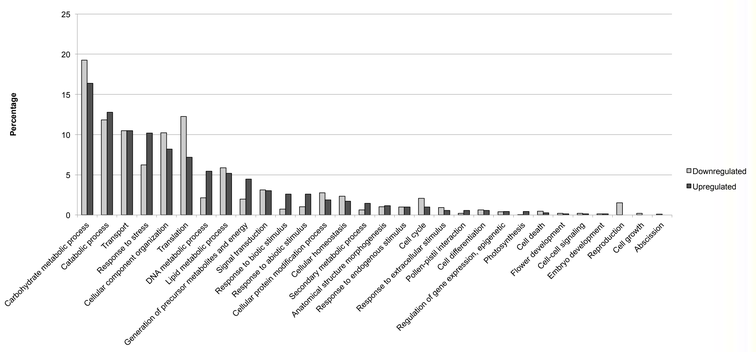

Figure 2: Percentage of upregulated (696) and downregulated (1927) transcripts in the pairwise Resistant vs. Susceptible analysis. Transcripts were assigned gene ontology and grouped by biological function using BLAST2GO.

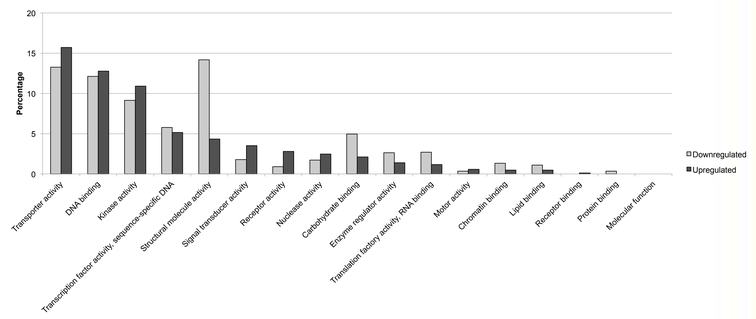

Figure 3: Percentage of upregulated (853) and downregulated (1751) transcripts in the pairwise Resistant vs. Susceptible analysis. Transcripts were assigned gene ontology and grouped by molecular function using BLAST2GO.

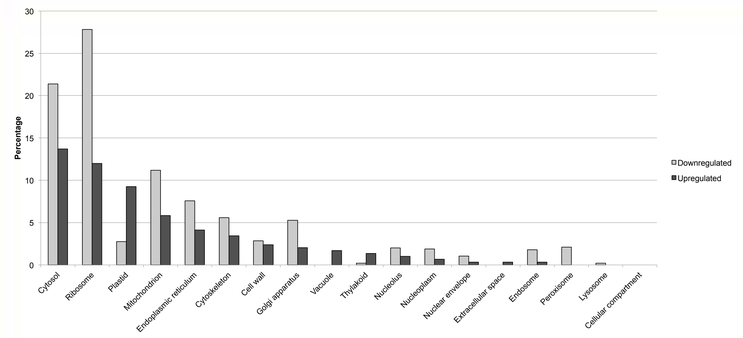

Figure 4: Percentage of upregulated (292) and downregulated (949) transcripts in the pairwise resistant vs. Susceptible analysis. Transcripts were assigned gene ontology and grouped by cellular compartment using BLAST2GO.

Table 1. Candidate genes involved in nickel resistance of white birch

Transcript ID	Protein	Log2FC	Function	Reference
TR73973|c3_g5	Glutathione S-transferase	9.15	Coverts glutathione (GSH) into xenobiotic substrates for the detoxification of free radicals	(Jozefczak et al., 2012; Freeman et al., 2001)
TR101884|c0_g3	Thioredoxin family protein	9.15	Defense against heavy metal stress, metal binding	(Sevilla et al., 2015; Lemaire 1999)
TR109068|c1_g1	Putative transmembrane protein	9.10	Unknown	None
TR56135|c0_g6	Nramp transporter	8.62	Metal transport, found to play a role in Ni resistance and homeostasis in plants.	(Wei et al., 2009; Mizuno et al., 2005)
TR56135|c0_g1	Nramp transporter	8.32	Metal transport, found to play a role in Ni resistance and homeostasis in plants.	(Wei et al., 2009; Mizuno et al., 2005)

note: Log2FC is calculated based on susceptible white birch

Conclusion

- The main nickel resistance mechanism in *B. papyrifera* is a down regulation of genes associated with translation (in ribosome), binding, and transporter activity.
- Five candidate genes associated to nickel resistance were identified. They include Glutathione S – transferase (GST), thioredoxin family protein, putative transmembrane protein and two Nramp transporters.

Acknowledgements

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