MOLECULAR ANALYSIS OF RED OAK (QUERCUS RUBRA) POPULATIONS FROM A METAL CONTAMINATED REGION IN NORTHERN ONTARIO, CANADA

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Introduction

Metal toxicity is a major cause of abiotic stress in plants. Several taxa have developed different coping mechanisms to soil metal contamination to maintain physiological concentrations of essential plant nutrients and to avoid toxicity. The main objective of the present study is to characterize red oak (*Quercus rubra*) populations from a metal-contaminated region in Northern Ontario by analyzing genetic variation and gene regulation between and within populations located both in metal contaminated soils and reference sites.

Figure 1: Red oak (Quercus rubra)

Materials and Methods

Part 1: Assessing genetic variation

Sampling

Red oak leaves were sampled from seven metal contaminated sites and three reference sites from the Greater Sudbury Region. Secondary leaves were collected and washed. The collected samples were flash frozen and stored at -80°C for the DNA extraction.

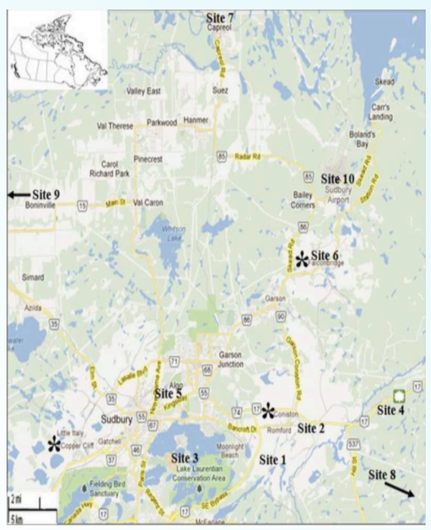

Figure 2: Locations of targeted red oak populations

Materials and Methods

DNA extraction

Extraction of genomic DNA from red oak leaves was performed according to the CTAB protocol (Nkongolo, 1999) and quantified by fluorometry.

PCR

DNA was amplified using six selected RAPD primers (table 1).

Statistical Analysis

Genetic variability parameters were determined using the Popgene software: percentage of polymorphic loci, Nei's gene diversity (h), Shannon's information index (I), observed (Na) and effective (Ne) Number of alleles.

Table 1: The nucleotide sequence of RAPD primers used to amplify DNA from red oak *(Quercus rubra)* population samples.

RAPD Primers	5'→3'	GC content	Number of bands	Size (bp)
UBC 186	GTGCGTCGCT	70%	27	205 - 1950
UBC 402	CCCGCCGTTG	80%	21	330-2000
OPA 16	AGCCAGCGAA	60%	23	230-2000
OPA 19	CAAACGTCGG	60%	25	230-2000
OPB 05	AGGGGTCTTG	60%	28	140-1900
OPT 17	CCAACGTCGT	60%	25	275-1900

Part 2: Determining Gene Expression

Sampling

Red oak roots and leaves were sampled from three metal contaminated sites and three reference sites from the Greater Sudbury Region. Secondary roots were collected and washed. The collected samples were flash frozen and stored at -80°C for the RNA extraction.

RNA extraction

Extraction of total cellular RNA from red oak leaves and roots will be performed according to the Chang *et al.*, 1993 protocol with modifications and quantified via fluorometry and treated with DNAse.

qRT-PCR

Eleven genes involved in nickel and copper resistance in other species were selected for this study (Table 2), and will be used to design primers. The cDNA will be generated using the High-Capacity cDNA Reverse Transcription Kit from Life Technologies. To confirm that the candidate genes are expressed in red oak, regular RT-PCR will be performed on selected samples.

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Table 2**:** Candidate genes for copper (Cu) and nickel (Ni) resistance.

Metal	Gene	Species	Reference
Copper	Copper-transporting ATPase (RAN1)	*Arabidopsis thaliana*	Kobayashi *et al.*, 2008
	Multi-Drug resistance associated protein (MRP4)	*Betula pendula*	Keinänen *et al.*, 2007
	Copper transporter protein (COPT1)	*Arabidopsis thaliana*	Sancernón *et al.*, 2004
	Metallothionein (MT2b)	*Arabidopsis thaliana*	Guo *et al.*, 2008
	Serine acetyltransferase (SAT)	*Thlaspi goesingense*	Freeman *et al.*, 2004
	Glutathione reductase (GR)	*Thlaspi goesngense*	Freeman *et al.*, 2004
	Nicotianamine synthase (NAS3)	*Noccaea, Caerulescens, Thlaspi caerulescences*	Visioli *et al.*, 2014 Mari *et al.*, 2006
Nickel	Metal transporter Nramp3	*Noccaea coaerulescens*	Visioli *et al.*, 2014
	Metal transporter Nramp4		
	1-aminocyclopropane-1-carboxylic acid deaminase (ACC)	*Brassica napus*	Stearns *et al.*, 2005
	High affinity nickel transporter family protein (AT2G16800)	*Arabidopsis thaliana*	

Results

All genetic markers were scored based on presence or absence of amplification products and observed as bands on the agarose gel. The data was used to calculate the genetic distances from the 10 populations as illustrated in table 3.

Table 3: Distance matrix generated from RAPD data using Jaccard's similarity coefficient analysis. Values based on a 0 (identical) - 1 (different) scale.

	Daisy Lake	Dam	Laurentian	Kukagami	Kingsway	Falconbridge	Capreol	St.Charles	Onaping Falls	Airport
Daisy Lake		0.20	0.26	0.27	0.27	0.25	0.28	0.23	0.31	0.27
Dam			0.24	0.26	0.32	0.25	0.27	0.25	0.30	0.31
Laurentian				0.29	0.35	0.27	0.34	0.29	0.35	0.30
Kukagami					0.31	0.28	0.32	0.26	0.29	0.35
Kingsway						0.24	0.35	0.26	0.34	0.33
Falconbridge							0.27	0.18	0.31	0.34
Capreol								0.26	0.34	0.35
St.Charles									0.27	0.30
Onaping Falls										0.31
Airport										

The level of polymorphic loci ranged from 61 % to 72 %. The mean values of observed number of alleles (Na), Expected number of alleles (Ne), Nei's gene diversity (h) and Shannon's information index were 1.69, 1.30, 0.19 and 0.29, respectively. The level of population differentiation (G_{ST}) was low (0.17) and the estimated gene flow (N_m) was high (2.39). The total gene diversity (H_T) and the mean gene diversity between populations (H_S) were 0.22 and 0.19, respectively (table 4).

Results

Table 4: Genetic Diversity parameters of red oak (*Quercus rubra)* populations based on RAPD data.

Population	P (%)	Na	Ne	H	I
Daisy Lake	71.81	1.7181	1.3024	0.1859	0.2923
Wahnapitae Hydro Dam	67.79	1.6779	1.2889	0.1787	0.2808
Laurentian	71.14	1.7114	1.3088	0.1898	0.2970
Kukagami	70.47	1.7047	1.2817	0.1754	0.2781
Kingsway	69.80	1.6980	1.3215	0.1963	0.3046
Falconbridge	71.14	1.7114	1.3635	0.2202	0.3373
Capreol	61.07	1.6107	1.2817	0.1701	0.2636
St.Charles	68.46	1.6846	1.3093	0.1875	0.2912
Onaping Falls	71.14	1.7114	1.3034	0.1869	0.2929
Airport	62.42	1.6242	1.2652	0.1616	0.2535
Mean		1.69	1.30	0.19	0.29
		Ht	Hs	Gst	Nm
Overall		0.2239	0.1852	0.1728	2.3941

Conclusion

- There were significant differences in soil metal content between metal contaminated and uncontaminated sites for the main elements (nickel, copper, zinc…).
- No significant difference in polymorphism was observed between metal contaminated and reference populations of red oak.
- Genetic variability within populations was high.
- Genetic distance among populations was low and the gene flow was high.

Future Directions

Transcriptome analysis of red oak samples from metal - contaminated and control areas

Acknowledgements

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