Development of molecular markers to aid in the identification of western redcedar (Thuja plicata) populations that are resistant to deer browsing and heartwood rot fungi

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Submitted by Jim Mattsson, Project leader

Contact information:
Jim Mattsson, PhD
Associate professor
Department of Biological Sciences
Simon Fraser University
8888 University Drive
Burnaby BC V5A 1S6
Canada
phone: 778-782-4291
jmattsso@sfu.ca

Project purpose and management implications

Based on total logs harvested, western redcedar (Thuja plicata; hereafter abbreviated as WRC) ranks as the fifth most important species in BC. WRC wood is highly valued for its high dimension stability and its natural durability and is used in many exterior applications. The utilization of WRC is not without problems however. In BC, reforestation with WRC is expensive and inefficient due to extensive herbivory by ungulates, i.e. deer and elk. Currently, the industry spends up to $6 per tree in deer/elk areas, which corresponds to most of the BC coast and part of the interior, in order to establish WRC. Although regarded as highly durable, second growth WRC heartwood succumbs to early rot, especially in the interior cedar/hemlock biogeoclimatic zone, where complete trees are culled. However, substantial tree-to-tree variation among second-growth trees with respect to both ungulate herbivory and heartwood rot resistance has been noted and the BC Ministry of Forests (MoF) is currently breeding for browsing resistance and intends to breed for heartwood rot resistance. Here we are proposing research to develop markers that correlate with browsing resistance and heartwood rot resistance. The intended use of these markers is to substantially shorten the time and cost for each breeding cycle with respect to browsing resistance and, for the first time, enable efficient breeding for rot resistance in WRC. The deployment of such markers could have considerable short-term benefits for reforestation with WRC and invaluable long-term benefit with regard to the quality and reputation of WRC heartwood from BC.
Methodology overview, Year 2

There is a strong positive correlation between monoterpenoid content in WRC and resistance to ungulate browsing (Vourc’h et al., 2002 a,b). It is known that induced synthesis of monoterpenoids following attack by stem-boring insects is accompanied by a significant up-regulation of monoterpene synthase (mono-TPS) gene expression (Byun-McKay et al., 2003; Miller et al., 2005). Thus, it is possible to obtain an indirect measure of monoterpenoid content and thereby resistance by quantifying the expression of mono-TPS genes. One of the classes of monoterpenoids implicated in plant defense are the tropolones (Reviewed in Zhao, 2007). Tropolones are natural fungicides found in the heartwood of mature trees where they are involved in WRC resistance to fungal degradation (DeBell et al., 1999; Haluk et al., 2000, 2003). An early screening tool would be invaluable in rapidly identifying durable, rotresistant trees for reforestation. Previous research has shown that there is good correlation between the production of transcripts (mRNAs) from genes encoding monoterpenic synthases (mono-TPS) and production of the corresponding monoterpenes (Reviewed by Keeling and Bohlmann, 2006). In year 1 we cloned complementary DNAs corresponding to the most abundant mono-TPS mRNAs expressed in WRC foliage and cambium. In year 2 we have mapped the expression of the most promising candidate gene in some detail using real time quantitative PCR.

We carried out a series of experiments generating first calli then cell suspension cultures of WRC lines with known high and low tropolone content. We optimize conditions for induction of genes potentially involved in tropolone biosynthesis by scoring the expression of TpSS1 at a series of time points after addition of jasmonic acid or fungal extract, both known elicitors of tropolone production. We identified time points for peak of expression for both treatments. High quality RNA was extracted from control and jasmonic acid (JA) treated suspension cultures and sent for ultra deep Illumina tag sequencing to identify genes those expression is higher JA treated cells. The Genome Science Centre at the BC Cancer Agency performed this service.

Project scope and regional applicability, Year 2

The scope of the study in year two was to: (1) identify among candidate mono-terpene synthases, a gene or genes whose expression correlate with to develop robust markers that can be used for fast and efficient typing of western redcedar (WRC) for predicting herbivory resistance in segregating breeding populations, (2) to identify WRC individuals in segregating breeding populations likely to exhibit resistance to ungulate herbivory, (3) to identify genes those expression correlate with, and based on gene annotation, are likely to be involved in tropolone production. The project applies to all regions in BC where WRC is grown.
Interim conclusions

Identification of a monoterpene synthase-encoding gene whose expression correlates with foliar thujone levels

We have accumulated evidences that one of our candidate genes, which we have named \textit{Thuja plicata} Sabinene Synthase1 (TpSS1) is involved in the production of the major (\sim 85\%) monoterpenoid in WRC foliage – thujone. The evidences are as follows (in point form):
\begin{itemize}
  \item[a.] Among our candidate genes, TpSS1 is the most abundantly expressed monoterpene synthase-encoding gene.
  \item[b.] TpSS1 is expressed primarily in adult foliage (subject to browsing), with minor expression in stem and juvenile foliage.
  \item[c.] TpSS1 expression is elevated more than 30 fold in response to jasmonic acid, an intermediate in response to biotic stress such as browsing.
  \item[d.] We have found that monoterpenes, primarily thujone, are stored in foliar resin glands, with very low levels in non-gland tissues.
  \item[e.] TpSS1 is expressed in the epithelial cells lining the resin glands in foliage, suggesting a function in production of monoterpenes secreted into the gland lumen.
  \item[f.] In collaboration with Joerg Bohlmann’s lab (UBC) we have established that TpSS1 has strong sabinene synthase activity. Sabinene has been described as the precursor monoterpane in the synthesis of the derived monoterpane thujone (Dehal and Croteau, 1987), which in turn is the major monoterpane in WRC foliage.
  \item[g.] We have identified a correlation between the expression of TpSS1 and monoterpene content in MFR material. The strongest correlation is with thujone content, with a weaker correlation with sabinene content.
\end{itemize}

These findings are now described in a manuscript submitted to FORREX in conjunction with this report. It will be submitted for publication after further revisions.

Identification of genes that are involved in tropolone biosynthesis with the prospect of developing markers for heartwood rot resistance.

We have identified a large number of candidate genes for heartwood rot resistance and more genes will be identified in the very extensive data set generated by Illumina tag profiling of fungal jasmonic acid-induced genes. We are currently validating candidate genes by RT-Q-PCR. We are now also collaborating with specialists at FPIInnovations, Forintek division, regarding tropolone qualification.
Cited references


Contact information
Jim Mattsson, see above contact information