RES-FOR HIGHLIGHT #11

November 2020

Chemical Responses in Needles to Drought and Induction Stress in Conifer Seedlings

Overview

We measured the physiological and chemical responses of conifer needles in seedlings subjected to abiotic (drought) and biotic (insect herbivory/induction) stress conditions. In response to repeated drought treatments, lodgepole pine seedlings (2nd growing season) adjusted their levels of circulating osmoprotectants (amino acid-derived molecules) and organic acids compared to well-watered controls. In response to the insect induction treatment, white spruce seedlings altered their concentrations (mg kg $^{-1}$, dry weight) of specific polyphenols and trace elements compared to controls. When the chemical responses arising from the drought treatment were plotted against stomatal conductance (mol. $H_2O.m^{-2}.s^{-1}$), we observed a positive phenotypic correlation after two cycles of drought exposure. Individuals that showed a greater reduction in stomatal conductance also showed a greater increase in the concentrations of metabolites associated with osmotic adjustment (e.g., betaine, tryptophan). This result suggests that some seedlings were able to keep their stomata more open under drought conditions by increasing the amounts of metabolites which protect their needles from dehydration and turgor loss. We will conduct further analyses to test if the ability of these seedlings to increase the production of metabolic compounds is controlled by genetics and heritable. If so, then metabolites could be used as a drought response trait and assist in the selection of drought-tolerant genotypes. An advantage of using metabolites instead of physiological traits for selection is that the former provides a more direct response to stress and is a simpler trait and therefore it may be easier to detect SNP markers associated with them.

Goals & Objectives

1) Our first goal was to test if there was a change in the concentration of primary metabolites in lodgepole pine seedlings in response to drought stress and to examine the correlations between chemical responses and other physiological traits, such as stomatal function, that rely on water availability.
2) Our second goal was to test if the defense chemicals (polyphenols) changed significantly in white spruce seedlings when subjected to drought (abiotic) and insect herbivory (biotic).

Methods

Two greenhouse experiments were conducted to examine the responses of lodgepole pine and white spruce seedlings to 3 dry-down cycles and to a fungus associated with mountain pine beetles (in pine) or spruce budworm defoliation (in spruce). We sampled needles from greenhouse-grown seedlings and subjected them to the liquid chromatography-mass spectrometry (LC-MS) analyses to measure 19 polyphenols and 164 primary metabolites, and 2) an inductively coupled plasma mass spectrometry (ICP-MS) method to measure ~38 inorganic salts and trace metals.

Results

1) Partial least squares discriminant analysis (PLS-DA) showed a distinct separation between drought-treated and well-watered (control) lodgepole pine seedlings for several primary metabolites. The variable importance in projection (VIP) plot showed a number of primary metabolites with high VIP scores. Beta-hydroxybutyric acid and tryptophan were the most significantly different metabolites with a VIP score > 3, while creatine, zeatine, and glyceric acid showed a VIP score > 2 (Fig. 1a). The concentrations of these compounds (except glyceric acid) were higher in drought-treated pine seedlings than in well-watered controls. An accumulation of glycine betaine in drought-treated trees was found to be in agreement with previous literature reports.
2) The concentrations of the significantly different primary metabolites were plotted against stomatal conductance. This showed a positive phenotypic correlation which means that under drought conditions, the

[1]RES-FOR = Resilient Forests: Climate, pests & policy – genomic applications (Genome Canada 2015 LSARP)

lodgepole pine seedlings accumulated beta-hydroxybutyric acid (Fig. 1b), and tryptophan (Fig. 1c), while showing a reduced level of stomatal conductance. However, primary metabolites were not significantly altered between control and insect-treated spruce seedlings.

3) White spruce seedlings showed no changes in polyphenols or monoterpenes under drought or insect-treatments compared with controls (well-watered, no insect) (Fig. 2). Several flavonoid aglycones (myricetin, taxifolin, and apigenin), however, were identified and exhibited higher concentrations in the drought-treated versus well-watered spruce seedlings.

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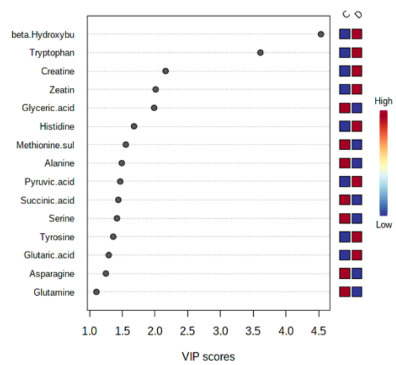

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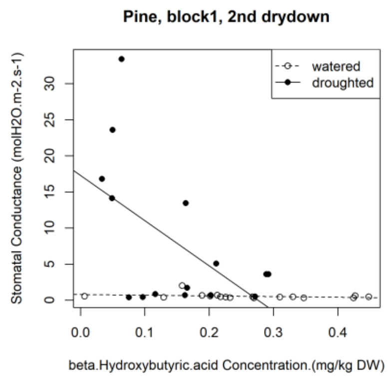

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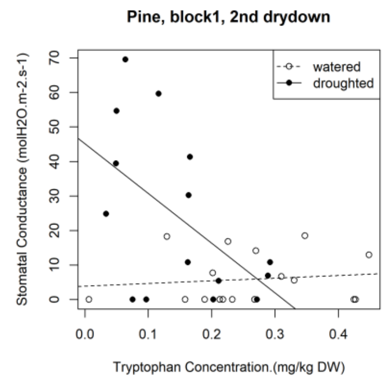


Fig 1. a) VIP scores plot for the well-watered (control) and drought-treated pine trees; Concentrations of beta-hydroxybutyric acid (b), and tryptophan (c) vs stomatal conductance. Solid and dashed lines are the regression lines for drought and well-watered trees, respectively.

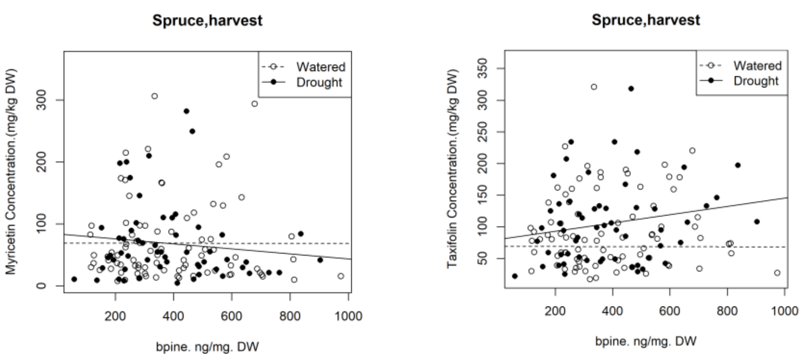
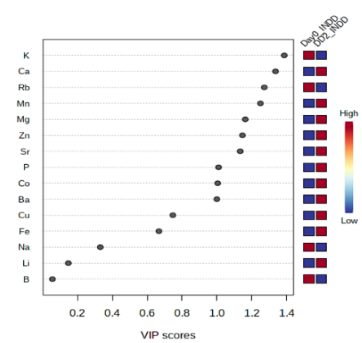


Fig 2. The concentration of myricetin vs beta-pinene (left) and taxifolin vs beta-pinene (right) in well-watered (control) and drought-treated spruce seedlings after three cycles of drought and 23 days of growth; Solid and dashed lines are the regression lines for drought and well-watered trees, respectively.

4) The drought treatment resulted in a decrease of potassium (K) and rubidium (Rb), while there was an increase in the concentrations of calcium (Ca) and manganese (Mn) in the drought-treated spruce seedlings when compared to controls. The VIP plot for the spruce seedlings after the 2nd dry-down cycle is shown in Fig. 3.

Fig 3. VIP plot for the control vs drought treated (after 2nd dry down cycle) white spruce seedlings; trace metal responses.



Conclusions

Based on preliminary data, it is understood that the abilities of the plants to change their metabolites in response to drought have the potential to be used as a drought response trait to assist in the selection of drought-tolerant genotypes.

For more information on the RES-FOR project contact:
Barb Thomas: bthomas@ualberta.ca or
Stacy Bergheim: sberghei@ualberta.ca

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For more information on this highlight sheet please contact:
Sudarshana Reddy: bhumired@ualberta.ca or
David Wishart: dwishart@ualberta.ca