RES-FOR HIGHLIGHT #8

June 2020

Development of Mass Spectrometric Methods for the Absolute Quantification of Trace Metals & Polyphenols from Conifer Needles

Overview

Coniferous trees are known to have a complex defense system involving a range of physiological and chemical responses to both abiotic and biotic stress conditions. In times of drought, plants often adjust their amino acid, sugar, and inorganic salt levels to protect the integrity and stability of plant cells. When attacked by insects, plants often release a range of noxious secondary metabolites such as polyphenols, terpenes, and alkaloids which can drive away or even kill insect attackers. While much is known about the chemical defense systems of crop plants, very little is known about the chemical defense systems of trees, in particular conifers. By monitoring the chemical changes that occur in conifers due to drought, or insect attack (e.g.: spruce budworm or mountain pine beetle), it may be possible to identify chemicals that make certain trees (or seedlings) susceptible or resistant to these biotic or abiotic stressors. The identification of these chemicals could assist tree breeders in selecting trees that are more resilient to stress.

Goals & Objectives

This project had two objectives: 1) develop a targeted, fully quantitative, liquid chromatography-mass spectrometry (LC-MS) method to measure ~ 20 polyphenols in conifer needles; and 2) develop a fast, fully quantitative inductively coupled plasma mass spectrometry (ICP-MS) method to measure ~ 38 inorganic salts or trace metals in conifer needles. *Note: Methods manuscript in preparation for submission to Journal of Analytical and Atomic Spectrometry.*

Method-Absolute quantification of polyphenols by LC-MS

With conifer needle samples collected from seedlings as part of the RES-FOR[1] project, we first froze and then ground the samples to a fine powder using a Geno/Grinder. Next, a quick solvent extraction protocol was used that allowed us to selectively extract polyphenols in high yield using less than 100 mg of needle tissue. A reversed-phase liquid chromatography method was subsequently developed to ensure the effective separation of the polyphenols. By adding (spiking) a known amount of the corresponding ^{13}C/deuterated polyphenol internal standards (ISTDS) to each needle extract, we accurately quantify 19 polyphenols in both white spruce and lodgepole pine needle samples. The accuracy and reproducibility (intraday and interday) of the methods were assessed by analyzing the National Institute of Standards and Technology (NIST)-Certified Standard Reference Material (SRM) for pine needles (1575a, NIST assigned code) on three different days.

Results-Absolute quantification of polyphenols by LC-MS

Matrix-matched calibration curves were determined for all 19 polyphenols and the correlation coefficient (R^2) values were >0.98. The accuracy of the method was evaluated at two different concentrations and calculated as the average of three replicates and expressed as %, while reproducibility was expressed as a relative standard deviation (% RSD) in Table 1. The precision of the method is quite promising with the % RSD less than 9 % for 16 polyphenols. The method we have developed is far superior to any other method developed for the quantitative measurement of polyphenols in conifer needles. It is substantially (2-5X) more sensitive, faster, and more quantitative than any other published method.

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

Table 1. Spiking recovery test: Percent (%) accuracy and reproducibility (% RSD) of the method at two levels of spiking, LS = low spiking, MS = medium spiking for 15 polyphenols. Bold values indicate responses outside the expected range of 100±20%.

Compound	% Accuracy (% RSD); n=3		Compound	% Accuracy (% RSD); n=3		Compound	% Accuracy (% RSD); n=3	
	LS	MS		LS	MS		LS	MS
Apigenin	77 (14)	86 (3)	Taxifolin*	**173** (6)	127 (8)	Quercetin	**66** (4)	75 (5)
Gallic acid	73 (3)	83 (4)	Vanillic acid	115 (2)	82 (2)	Piceol	104 (3)	88 (1)
p-Coumaric acid	79 (1)	78 (6)	Gallocatechin	112 (3)	98 (4)	3-4-DHBA	104 (3)	103 (3)
Caffeic acid	84 (3)	84 (2)	Pungenol	93 (3)	117 (3)	Vanillin	76 (6)	81 (2)
Protocatechuic aldehyde	108 (3)	111 (2)	Ferulic acid	85 (5)	84 (3)	Catechin	101 (4)	89 (2)
LS: Low Spike, MS: Mid Spike			*Quercetin used as an ISTD					

Method-Absolute quantification of inorganic salts by ICP-MS

The method we developed avoids microwave-based sample digestion and instead uses a simple water-bath based method, which minimizes the amount of time required for each sample. In order to minimize the amount of sample needed from each tree, optimization of the amount required was critical. By optimizing the composition of the digestion solvent (concentration of nitric acid and hydrogen peroxide), and digestion time and temperature, sample requirements were reduced. The method was validated with a spiked-recovery test and the accuracy and the precision of the method were calculated.

Results-Absolute quantification of inorganic salts by ICP-MS

Using the method described above, a total of 46 metal ions could be detected, with 38 measured quantitatively, and eight additional metals being measured qualitatively (Ga, Nd, Pd, Nb, Gd, Ho, Hf, and Hg). Calibration curves for the 38 elements/metals yielded R^2 values of 0.99-0.98. The percentage of recovery and precision (% RSD) among the measurements were in an acceptable range (80-120%). Furthermore, the accuracy of the method was substantiated by analyzing NIST-Certified SRM, where the concentrations of the 18 metals/elements were compared to reference concentrations. The accuracy of most of the elements/metals was in the range of 75.1% to 117.7%. Thus, the current method enables the accurate quantification of 38 inorganic salts including several essential micronutrients (cobalt, iron, manganese, molybdenum, nickel, zinc, and copper) which are needed for normal plant growth.

Conclusions

1. The LC-MS method we developed for measuring polyphenols in conifer needles appears to be more sensitive, faster, and more quantitative than any currently published method for polyphenol measurements of needles. The technique is now being applied to the measurement of hundreds of pine/spruce seedling samples and it is expected to yield important new insights into the insect stress response, facilitating the identification of resilient individuals through their chemical response profiles.

2. The ICP-MS method we developed for measuring trace elements and inorganic salts in conifer needles is faster and more sensitive than any currently published method. Our ICP-MS technique is being applied to the measurement of hundreds of pine and spruce seedling needles samples and is expected to yield important new insights into the abiotic (drought) stress response of these trees and help in the identification of informative chemical responses in resilient individuals.

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