Assessing Surface Wax chemical diversity as a tool to defend against abiotic and biotic stress in Canola

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Project Code: CARP CCC 2018.18
Final Report: April 2020

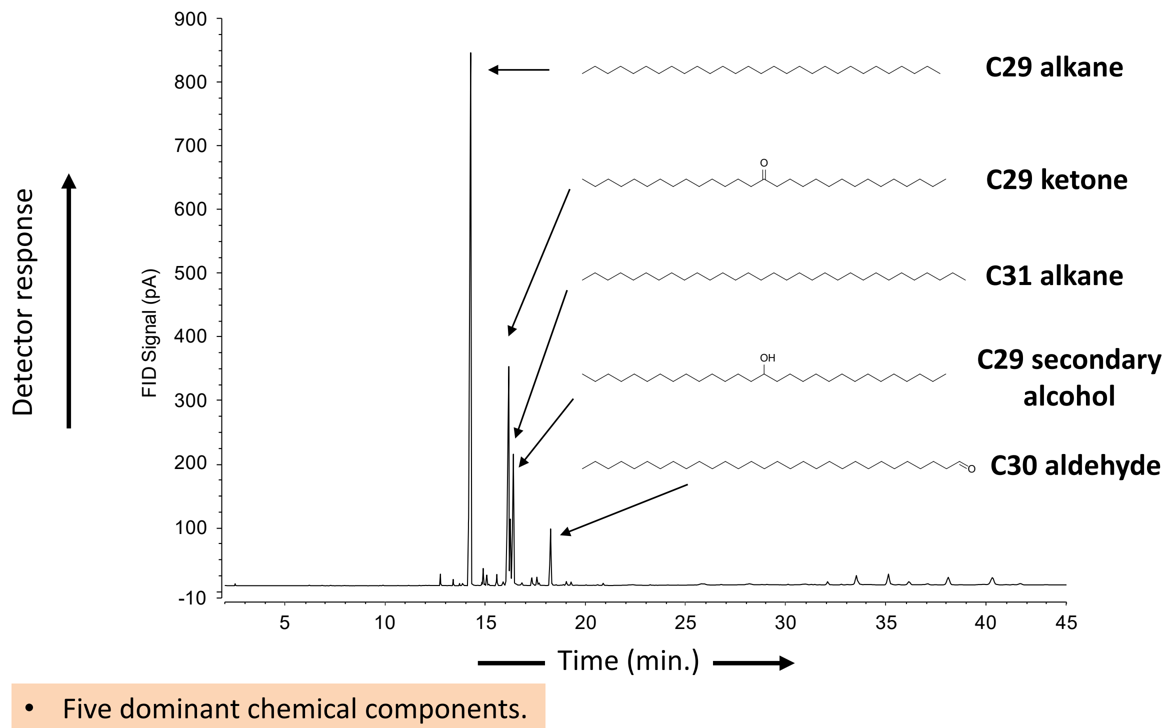
The outer surfaces of land plants are covered by a complex water-repelling material referred to as cuticular wax. This layer plays a fundamental role in prevention of water loss from the plant and in defense against attack by insect pests and fungal pathogens. For canola, little is known about the chemical composition of cuticular wax, its synthesis, biological function, and if there is chemical diversity within Canadian varieties. A 2-year study was conducted by Agriculture and Agri-Food Canada researchers to determine the chemical composition of wax in B. napus and to investigate the distribution of these chemicals on different plant parts and between different canola varieties. To aid in potential breeding efforts for new wax traits, researchers also identified genes encoding enzymes involved in wax biosynthesis and genes that regulate wax production. Results showed that B. napus wax is a complex mixture of aliphatic (chain like) hydrocarbons, with five main components and many minor ones. The chemical composition of wax in B. napus appears relatively uniform over the plant, with significant differences in composition only seen in petals. Low chemical diversity of wax was observed between B. napus varieties. Although this could limit the usefulness of natural diversity in breeding for new wax related traits, searching for induced diversity such as diversity in mutagenized populations may be a way to identify the germplasm needed to manipulate wax profiles if required. This work has considerably enhanced our understanding of wax chemistry and biosynthesis in B. napus and identified gaps where further knowledge is required. For example, knowing the chemistry of wax will help in determining how different components function in plant defense.

Key words: surface wax; chemical composition; tool; defense; stress; canola

The outer surface of a canola plant is covered by a complex mixture of water-repelling material referred to as cuticular wax. This layer plays a fundamental role in prevention of water loss from the plant, helps raindrops roll off the leaf and is the reason why wetting agents must be added to spray formulations. Cuticular wax is also the first material an insect or fungal spore encounters when landing on a leaf or petal. In many plants, natural chemicals within the cuticular wax have a role in defense against biotic stresses, such as attack by insect pests and fungal pathogens. For canola, little is known about the amount and chemical composition of cuticular wax, its diversity, biological function, and pathway of biosynthesis.

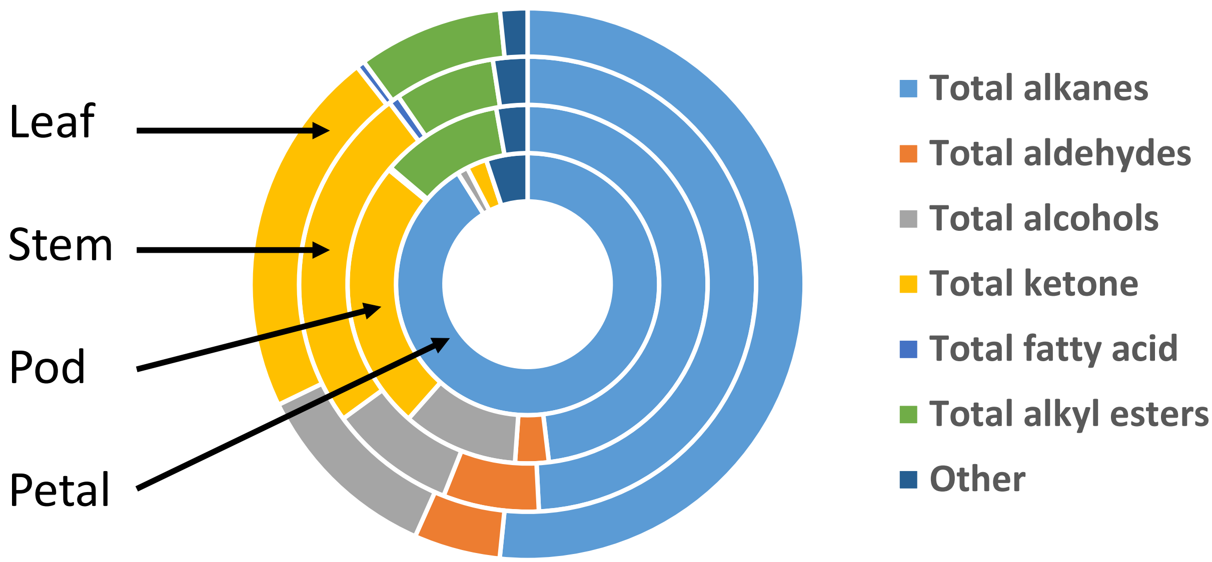
A 2-year study was conducted by Agriculture and Agri-Food Canada researchers at Saskatoon to identify the chemical composition of wax in *B. napus* and to investigate the distribution of these chemicals on different plant parts and between canola varieties. Researchers also identified genes involved in wax biosynthesis and genes that control wax production. The long-term goal of this study is to determine how wax composition can be modified through breeding to enhance the natural ability of the plant to protect itself from abiotic and biotic stresses of drought and attack from insect or fungal pests.

The chemical composition of cuticular wax was characterized by solvent extraction with subsequent separation and identification of components by gas chromatography/mass spectrometry (GC-MS), first using the doubled haploid canola line DH12075 as a model. Research showed that the wax was a complex mixture of aliphatic hydrocarbons, with five main components nonacosane (C29 alkane, 44.9%), nonacosan-15-one (C29 ketone, 21.6%), nonacosan-15-ol (C29 mid-chain alcohol, 10%), hentriacontane (C31 alkane, 4.8%) and triacontanal (C30 aldehyde, 4%). More than 40 additional lower abundance components, including alkanes, primary alcohols, fatty acids and alky esters were also identified.



Representative chromatogram of canola leaf wax (Brassica napus DH12075)

The chemical composition of wax from different parts of the plant: leaf, stem, pod and petiole, appears relatively uniform with some variation in the ratio of components. The wax of petals, on the other hand, was significantly different in composition. It was composed primarily of alkanes (93.6%) with only trace amounts of the ketone, mid-chain alcohol, and aldehyde seen as major components on other parts of the plant. Studying wax chemical diversity with *B. napus* varieties indicates that diversity is low in this species which could limit the usefulness of natural diversity in breeding for new wax related traits. No evidence was found to support major changes in wax composition occurring during the breeding of modern canola varieties. Wax profiles differ primarily in the ratios of components between related *Brassica* species, *B. rapa*, *B. oleracea*, *B. juncea* and *B. carinata*. Searching for induced diversity such as diversity in mutagenized populations may be a way to identify the germplasm needed to manipulate wax profiles if required.



Survey of the leaf wax profiles of different parts of a typical canola plant (Brassica napus DH12075)

Preliminary studies conducted on the effect of the environment on wax production indicates that the amount of wax on the plant increases under drought stress, but with little change in overall composition. Small but significant differences were seen when greenhouse grown plants were compared with the same varieties grown in the field, providing further evidence for the role of the environment in wax biosynthesis. No significant response in wax amount or composition was seen under high or low nitrogen in field grown plants. By looking for genes switched on only in the outer layer of the plant, researchers were able to identify genes involved in wax biosynthesis, and potential regulatory components. As many genes in this pathway are members of multigene families, this approach has enabled the assignment of individual genes to the pathway, and the exclusion of other very similar genes. After validation, identified genes may offer targets for manipulation, or perfect markers for breeding beneficial traits. Now that composition is established, future research will be focused on exploring the role of wax components in insect defense and the early stages of fungal pathogen establishment, and on screening of mutagenized populations for useful diversity.

Overall, this work has considerably enhanced our understanding of wax chemistry and biosynthesis in *B. napus* and identified gaps where further knowledge is required. It has established the groundwork for studies probing the role of wax in abiotic and biotic stress and provided information on the target genes that could be manipulated to modify wax to address specific challenges.

Acknowledgement:

- This research is part of the Canola Agronomic Research Program. Funding was provided by the Saskatchewan Canola Development Commission (SaskCanola).