Brassica napus surface wax: understanding and manipulating surface chemistry for crop improvement.

Mark Smith.

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Smith. Lipid lab

- Seed oil: Pathways of biosynthesis and chemical diversity.

- Lipids and seedling performance at sub-optimal temperature.
- Surface lipids: Chemistry, structure, biosynthesis, function.

- *Camelina sativa*: Seed oil and induced genetic diversity (mutagenesis)

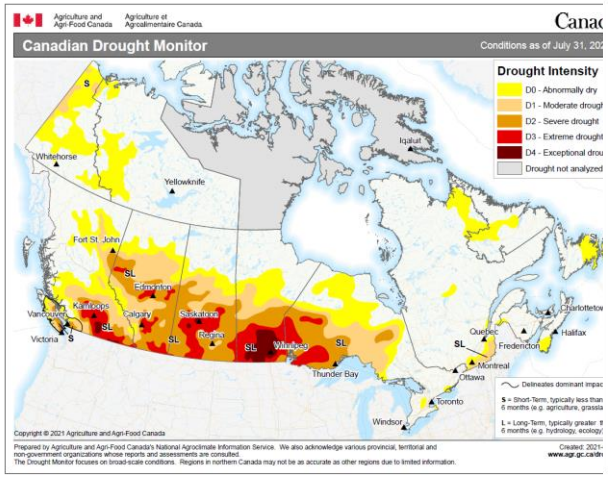
What is it?

How is it made ?

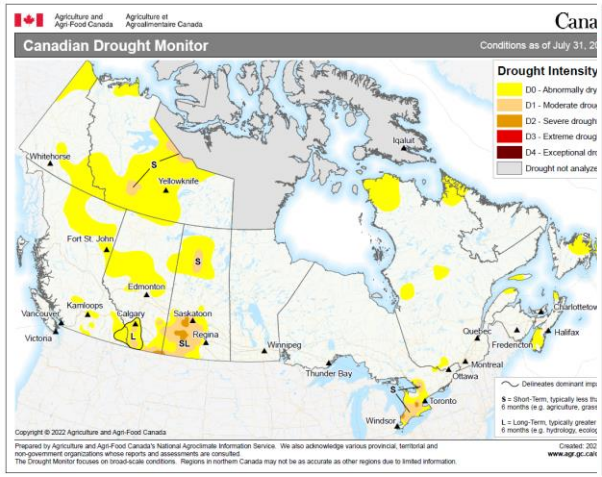
Why is it there?

Could wax manipulation be a target for crop improvement?

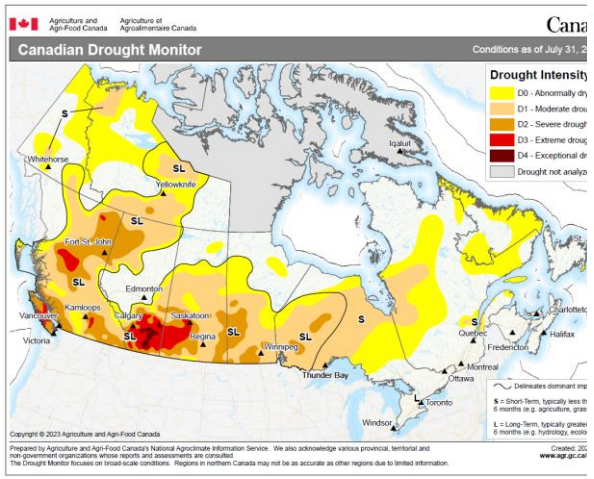
Canadian Drought Monitor. Month of July.



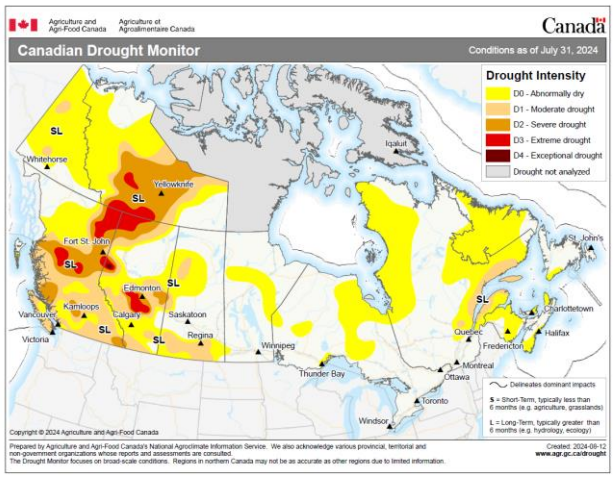
2021



2022



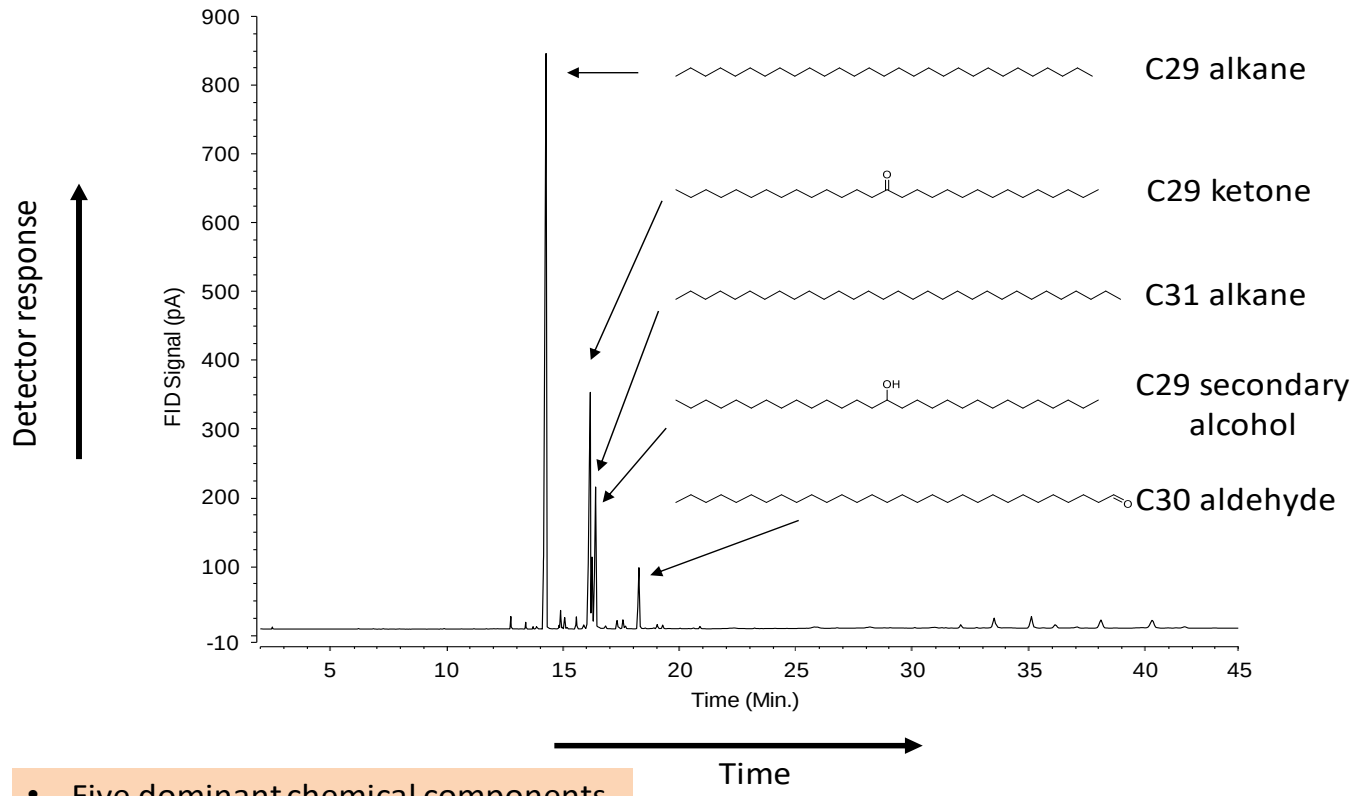
2023



2024

$CHCl_3$

Representative chromatogram of canola leaf wax. (*Brassica napus* DH12075).

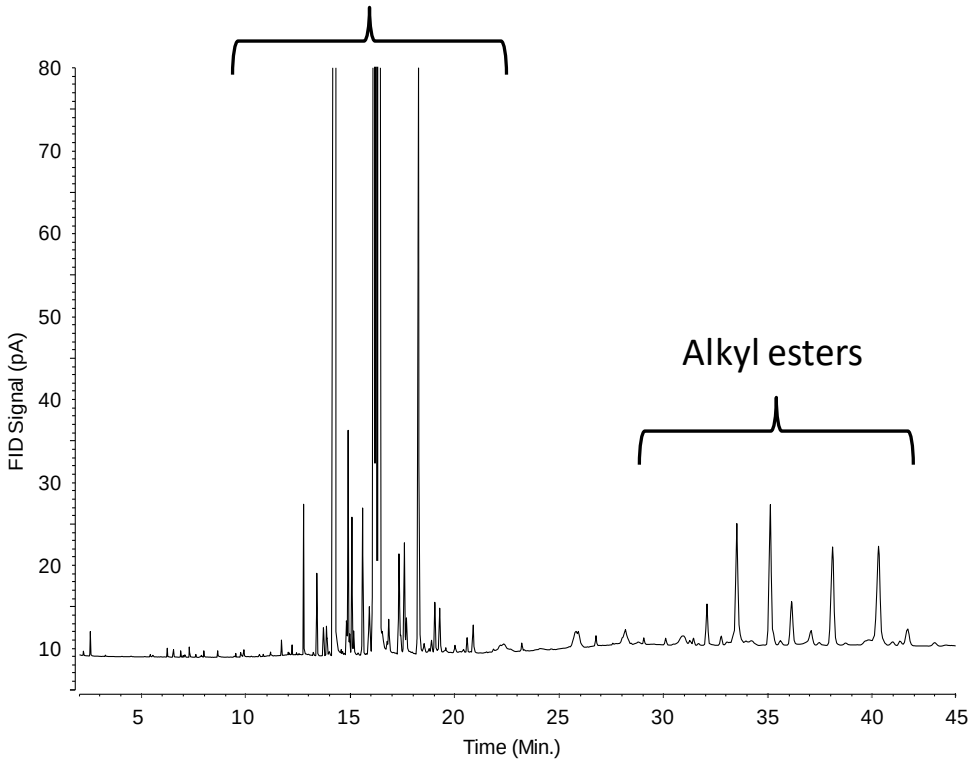


- Five dominant chemical components.

GC FID (Thermo Trace 1310 GC). 30 m TG-SQC capillary column DB5 equivalent.

Representative chromatogram of canola leaf wax. (*Brassica napus* DH12075). **ZOOM IN**

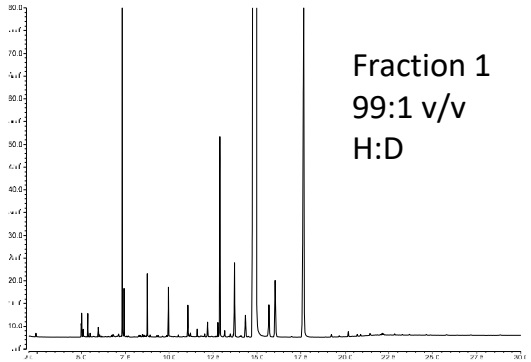
Alkanes, ketones, aldehydes, alcohols, free fatty acids and triterpenes.



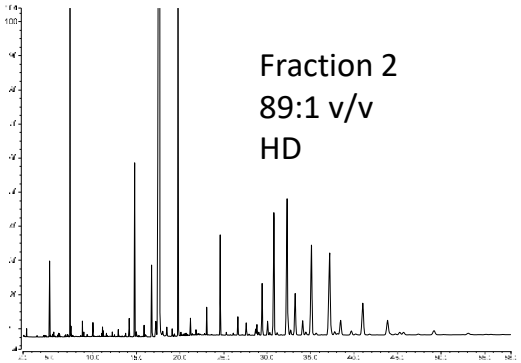
- Five dominant chemical components.
- **Many, diverse minor chemical components**

ID of minor peaks is challenging!

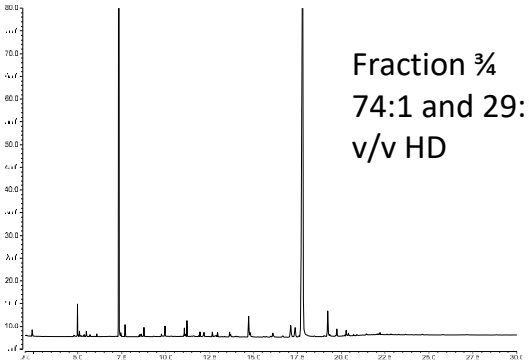
Fractionation of wax extract on a simple Silica gel SPE column.



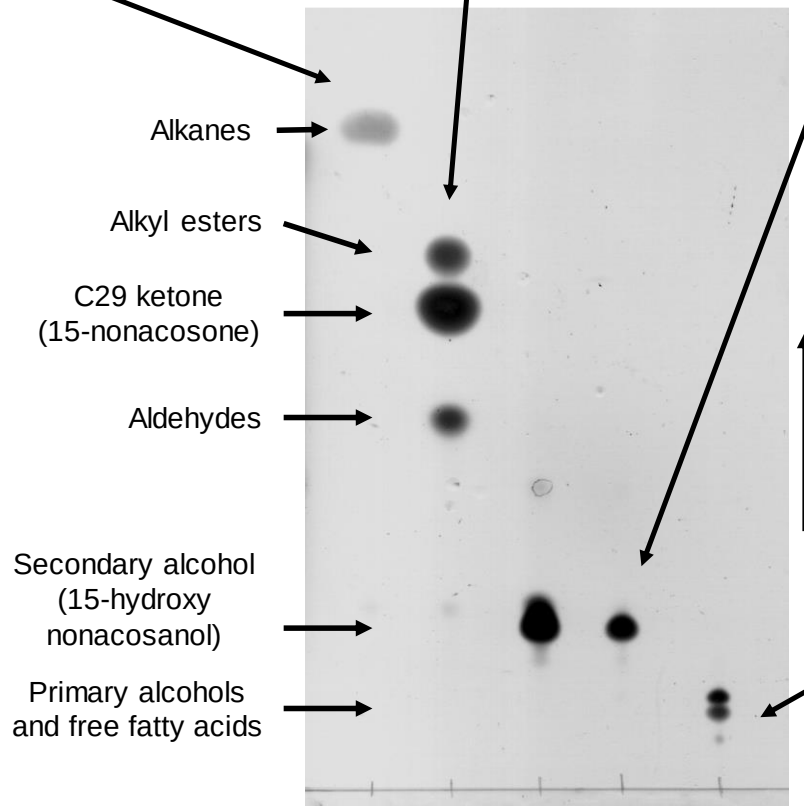
Fraction 1
99:1 v/v
H:D

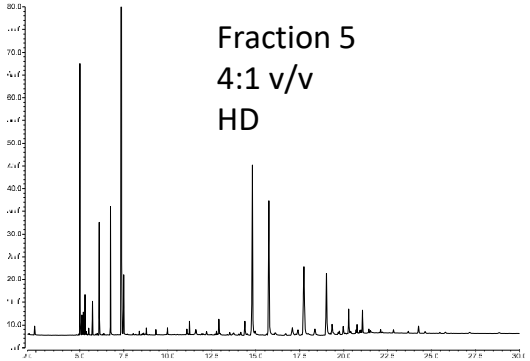


Fraction 2
89:1 v/v
HD



Fraction ¾
74:1 and 29:1
v/v HD



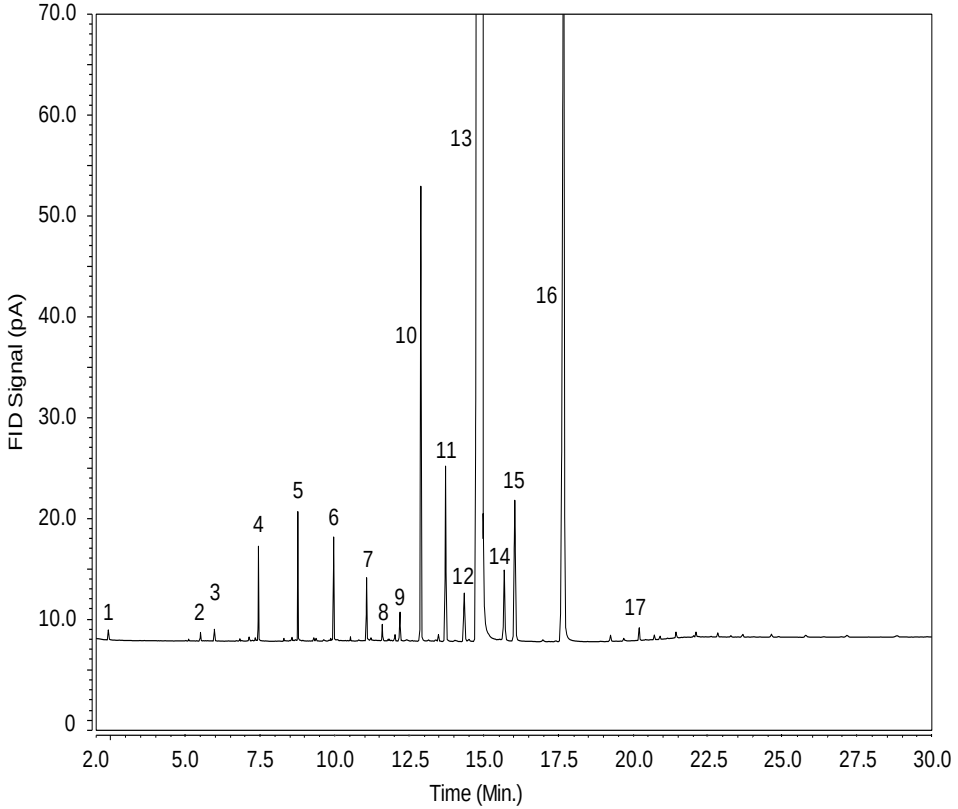


Fraction 5
4:1 v/v
HD

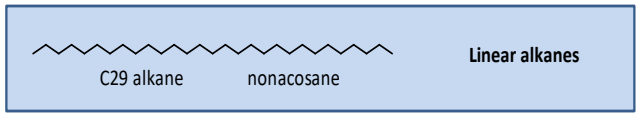
Claire Stevens CO-OP 2019

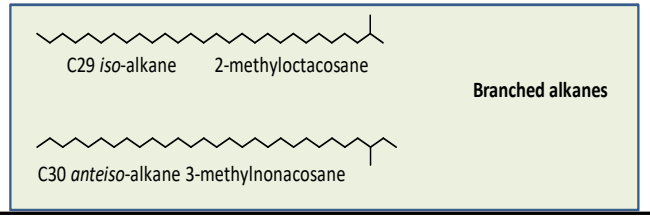
H=Hexane
D=Diethyl-ether

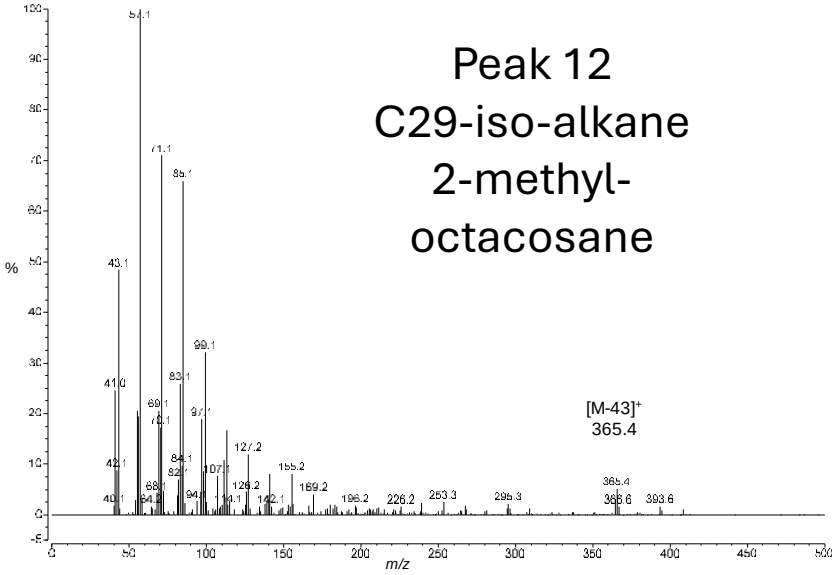
Brassica napus leaf wax (DH12075). Alkane fraction (Fraction 1).



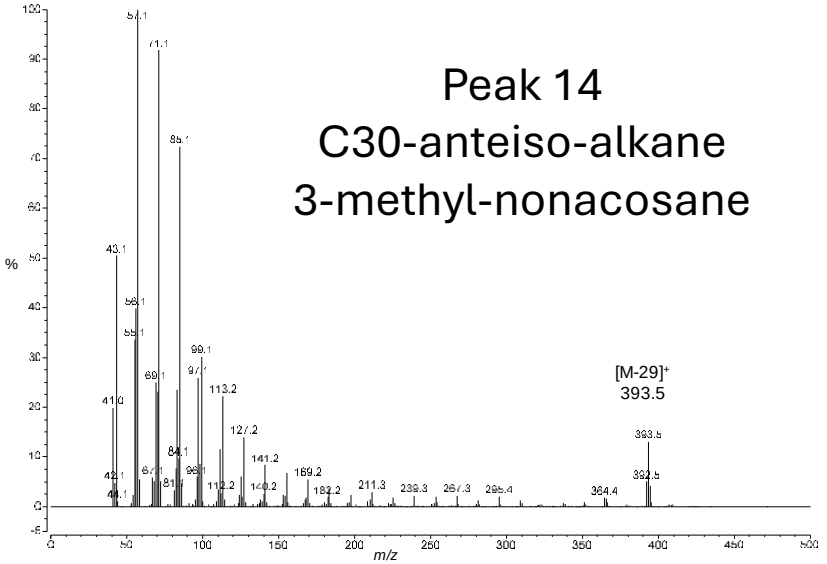
Peak	Identity	% of total alkanes
1	Unknown	0.02%
2	Unknown	0.02%
3	C16 alkane	0.02%
4	C18 alkane	0.14%
5	C20 alkane	0.20%
6	C22 alkane	0.15%
7	C24 alkane	0.11%
8	C25 alkane	0.03%
9	C26 alkane	0.06%
10	C27 alkane	1.11%
11	C28 alkane	0.53%
12	C29 *iso*-alkane	0.20%
13	C29 alkane	90.38%
14	C30 *anteiso*-alkane	0.31%
15	C30 alkane	0.66%
16	C31 alkane	5.99%
17	C33 alkane	0.03%







Peak 12
C29-iso-alkane
2-methyl-
octacosane

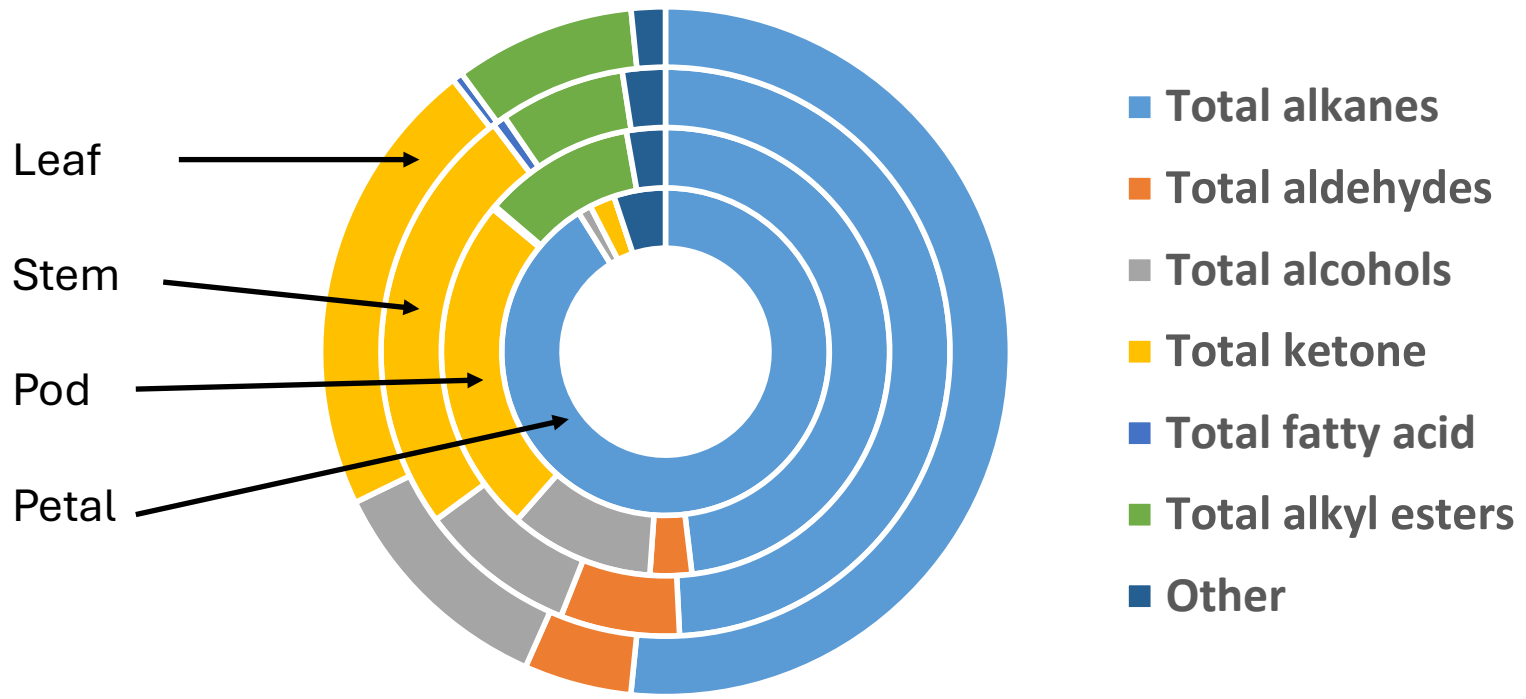


Peak 14
C30-anteiso-alkane
3-methyl-nonacosane

B. napus wax components identified (so far).

Class	Chain length	Name		Class	Chain length	Name
Alkanes	C16	Hexadecane		**Free fatty acids**	C16	Palmitic acid
	C18	Octadecane			C18	Stearic acid
	C20	Eicosane			C28	Octacosanoic acid
	C22	Docosane			C30	Triacontanoic acid
	C24	Tetracosane		**Alkyl esters**	C36	Multiple isomers
	C25	Pentacosane			C38	
	C26	Hexacosane			C40	
	C27	Heptacosane			C41	
	C28	Octacosane			C42	
	C29 *iso*	2-Methyl octacosane			C43	
	C29	Nonacosane			C44	
	C30 *anteiso*	3-Methyl nonacosane			C45	
	C30	Triacontane			C46	
	C31	Hentriacontane			C47	
	C33	Tritriacontane			C48	
Aldehydes	C26	Hexacosanal		**Triterpenes**	α-amyrin	
	C28	Octacosanal			β-amyrin	
	C30	Triacontanal				
	C32	Dotriacontanal				
Ketone	C29	Nonacosan-15-one				
Secondary alcohol	C29	Nonacosan-15-ol				
Primary alcohols	C22	Docosan-1-ol				
	C24	Tetracosan-1-ol				
	C26 *iso*	24-Methyl-pentacosan-1-ol				
	C26	Hexacosan-1-ol				
	C27 *anteiso*	24-methyl-hexacosan-1-ol				
	C27	Heptacosan-1-ol				
	C28 *iso*	26-Methyl-heptacosan-1-ol				
	C28	Octacosan-1-ol				
	C29 *anteiso*	26-Methyl-octacosan-1-ol				
	C30	Triacontan-1-ol				

Petals are different



Little change in 56 years of canola breeding

Variety	Year of Canadian registration	Total alkyl esters	Total alkanes	Total aldehydes	Total primary alcohol	Total free fatty acids	C29 ketone	C29 alcohol-2
Target	1966	6.24	58.33	4.26	2.06	0.49	17.07	10.67
Oro	1968	6.75	57.47	4.13	1.72	0.39	19.16	10.35
Midas	1973	6.40	57.95	4.21	1.74	0.36	17.88	10.82
Tower	1974	6.76	55.27	4.39	1.94	0.39	19.11	11.43
Westar	1982	6.67	56.00	4.88	2.09	0.49	17.95	11.10
Profit	1989	6.37	56.54	4.95	1.89	0.54	18.68	10.09
AC Excel	1990	5.40	57.34	5.06	2.18	0.47	17.93	10.89
AC Elect	1992	5.63	57.42	5.22	2.25	0.38	17.44	10.84
Defender	1994	6.74	54.21	5.39	2.26	0.56	19.60	10.51
46A65	1996	5.61	55.33	4.24	2.15	0.48	19.89	11.41
Q2	1998	5.97	56.36	4.28	1.88	0.31	19.24	11.35
SP BANNER	2002	4.99	57.33	4.16	2.08	0.33	19.41	11.32
InVigor 5440	2007	6.20	56.97	4.09	1.65	0.50	17.76	11.76
46H75	2011	5.12	54.83	5.07	2.49	0.54	19.75	11.37
74-44BL	2012	5.63	**60.40**	4.91	1.72	0.41	**15.14**	10.99
InVigor L252	2013	5.20	58.02	4.75	1.94	0.31	18.62	10.50
45H76	2014	5.99	54.64	4.04	1.87	0.45	20.13	11.65
PV 200 CL	2015	5.82	52.95	4.60	2.74	0.52	20.52	11.18
PV533G	2015	6.28	54.79	5.65	2.09	0.56	17.69	11.26
InVigor L233P	2016	5.47	56.59	5.45	2.66	0.55	17.70	10.70
75-42CR	2019	5.03	**63.69**	4.98	1.83	0.39	**13.24**	10.34
Argentine		6.78	56.40	4.38	2.00	0.50	18.28	10.82
H151797		5.31	57.50	4.94	2.07	0.38	18.28	10.78
NAM-0		6.14	57.15	4.10	1.78	0.45	17.69	11.94
YN04-C1213		**3.50**	56.27	4.48	2.63	0.58	19.09	12.29

Limited chemical diversity in *B. napus*.

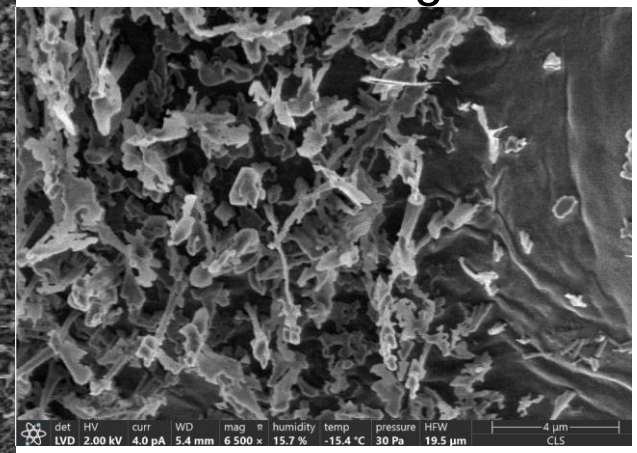
More diversity in *B.carinata*

Plant Genome Resources Canada (PGRC) Brassica carinata diversity collection 2022

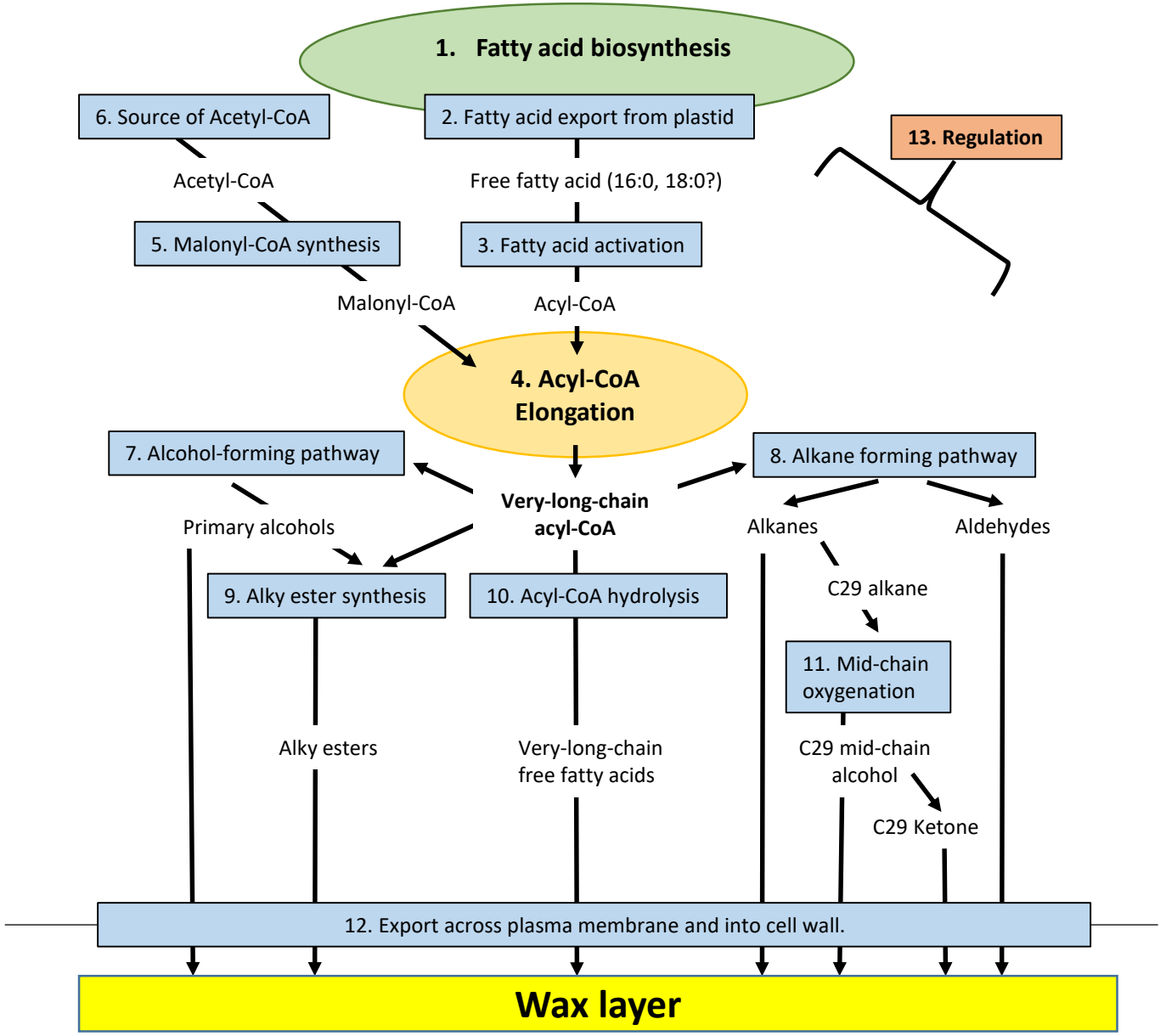
Environmental SEM with cooled stage

B. napus DH12075
Leaf lower surface

Controlled environment grown.



Wax biosynthesis in Arabidopsis



TRANSCRIPTOME ANALYSIS Petiole and Petiole Epidermis

TRANSCRIPTOME ANALYSIS Top 100 highly expressed genes (epidermis)

Rank	*Brassica napus* gene ID (DH12075)	Arabidopsis Ortholog	Arabidopsis Annotation	Process
1	BnaN17g49910	AT4G30270.1	MERI5B, MERI-5, XTH24, SEN4 | xyloglucan endotransglucosylase/hydrolase 24 | chr4:14819445-14820448	Cell wall
2	BnaN03g51590	AT4G30270.1	MERI5B, MERI-5, XTH24, SEN4 | xyloglucan endotransglucosylase/hydrolase 24 | chr4:14819445-14820448	Cell wall
3	BnaN13g16540	AT5G59310.1	LTP4 | lipid transfer protein 4 | chr5:23925296-23925772	Lipid transport
4	BnaN04g25160	AT2G38540.1	LP1, LTP1, ATLTP1 | lipid transfer protein 1 | chr2:16130418-16130893	Lipid transport
5	BnaN02g10550		No Arabidopsis Ortholog	
6	BnaN02g10540		No Arabidopsis Ortholog	
7	BnaN01g12010	AT4G21960.1	PRXR1 | Peroxidase superfamily protein | chr4:11646613-11648312	Cell wall/metabolism
8	BnaN03g38740	AT2G05520.1	GRP-3, ATGRP-3, GRP3, ATGRP3 | glycine-rich protein 3 | chr2:2026217-2026882	Cell division
9	BnaN13g48730	AT2G05520.4	GRP-3, ATGRP-3 | glycine-rich protein 3 | chr2:2026217-2026882	Cell division
10	BnaN05g06710	AT2G38530.1	LTP2, LP2, cdf3 | lipid transfer protein 2 | chr2:16128481-16128948	Lipid transport
11	BnaN03g13740	AT5G59320.1	LTP3 | lipid transfer protein 3 | chr5:23929051-23929492	Lipid transport
12	BnaN16g42310		No Arabidopsis Ortholog	
13	BnaN11g14650	AT4G21960.1	PRXR1 | Peroxidase superfamily protein | chr4:11646613-11648312	Cell wall/metabolism
14	BnaN08g19780	AT4G38770.1	PRP4, ATPRP4 | proline-rich protein 4 | chr4:18097009-18098448	Cell wall
15	BnaN06g28880	AT5G25460.1	Protein of unknown function, DUF642 | chr5:8863430-8865394	Unknown
16	BnaN12g24530		No Arabidopsis Ortholog	
17	BnaN14g07880	AT2G38530.1	LTP2, LP2, cdf3 | lipid transfer protein 2 | chr2:16128481-16128948	Lipid transport
18	BnaN12g11700		No Arabidopsis Ortholog	
19	BnaN17g36630	AT5G25460.1	Protein of unknown function, DUF642 | chr5:8863430-8865394	Unknown
20	Bna00208bs0030	AT2G34420.1	LHB1B2, LHCB1.5 | photosystem II light harvesting complex gene B1B2 | chr2:14522716-14523513	Photosynthesis

Name	Arabidopsis gene	Arabidopsis gene ID	*B.napus* potential homologues (DH12075)	Epidermis	Whole petiole
VLC-aldehyde decarbonylase	CER1	AT1G02205	BnaN18g53870	65828	108
			Bna01708s0010	30069	0
			Bna25797s0010	0	0
			Bna01708s0020	0	0
	CER1-Like	AT2G37700	BnaN04g24680	2376	184
			BnaN14g55470	6230	779
			BnaN14g55480	3616	369
VLC-acyl-CoA reductase	CER3/WAX2	AT5G57800	BnaN03g10750	0	0
			BnaN13g13230	0	0
			BnaN02g11420	15400	334
			BnaN10g12700	7457	420
			BnaN03g10770	0	0
			BnaN12g12900	9414	216
			BnaN19g43290	9618	585

Regulation

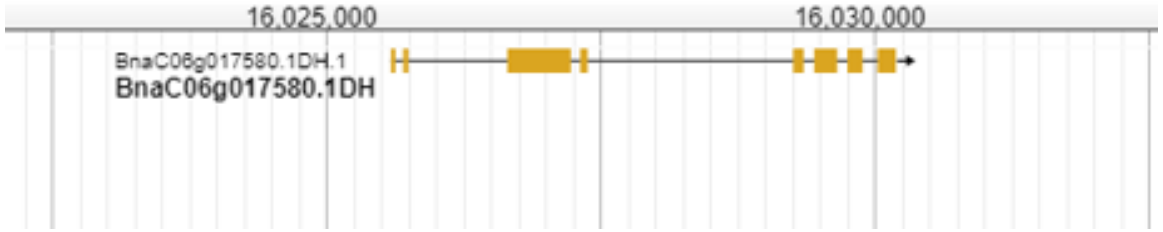
Arabidopsis gene	Arabidopsis gene ID	*B.napus* potential homologues (DH12075)	Epidermis	Whole petiole
DEWAX	AT5G61590	BnaN19g07370	4863	2657
		BnaN17g39310	9135	3841
		BnaN03g41000	8420	2890
		BnaN09g06640	6187	4960
DEWAX2	AT5G07580	BnaN19g59730	4529	8799
		BnaN02g02250	1228	1642
		BnaN12g02040	1204	0
SHINE1/WIN1	AT1G15360	BnaN18g21930	302	0
		BnaN08g26410	0	0
		BnaN15g11960	352	0
		BnaN09g51740	0	0
		BnaN06g10230	619	0
		BnaN18g46610	0	0
GCN5	AT3G54610	BnaN04g04650	508	454
		BnaN04g04690	333	555
		BnaN14g33530	289	594
		BnaN09g39580	0	0
		BnaN14g33490	451	511
		BnaN19g17570	2000	1100
MYB96	AT5G62470	Bna15446s0010	8550	484
		BnaN02g40410	8815	510
		BnaN19g07610	5401	369
		BnaN09g06900	6315	453
		BnaN13g56450	1458	171
		BnaN06g22560	1152	255

Anomalies from automated genome annotation

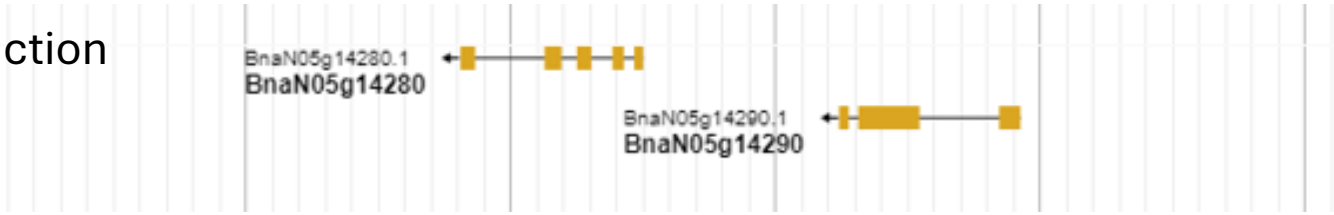
Name	Arabidopsis gene	Arabidopsis gene ID	*B.napus* potential homologues (DH12075)	Epidermis	Whole petiole
WSD1-like O-acyl transferase	WSD1	AT5G37300	BnaN04g28050	0	0
			BnaN05g14280	10090	0
			BnaN05g14290	6551	0
			BnaN16g14670	14321	0

Tandem duplication?

B. napus WSD1 gene prediction
C genome copy



B. napus WSD1 gene prediction
A genome copy



Function and potential for crop improvement

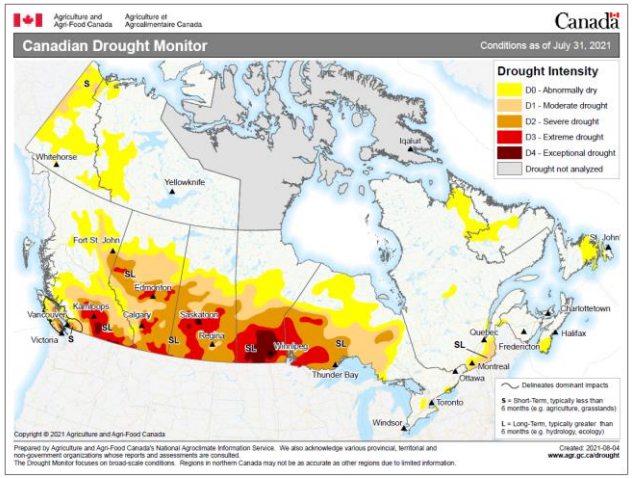


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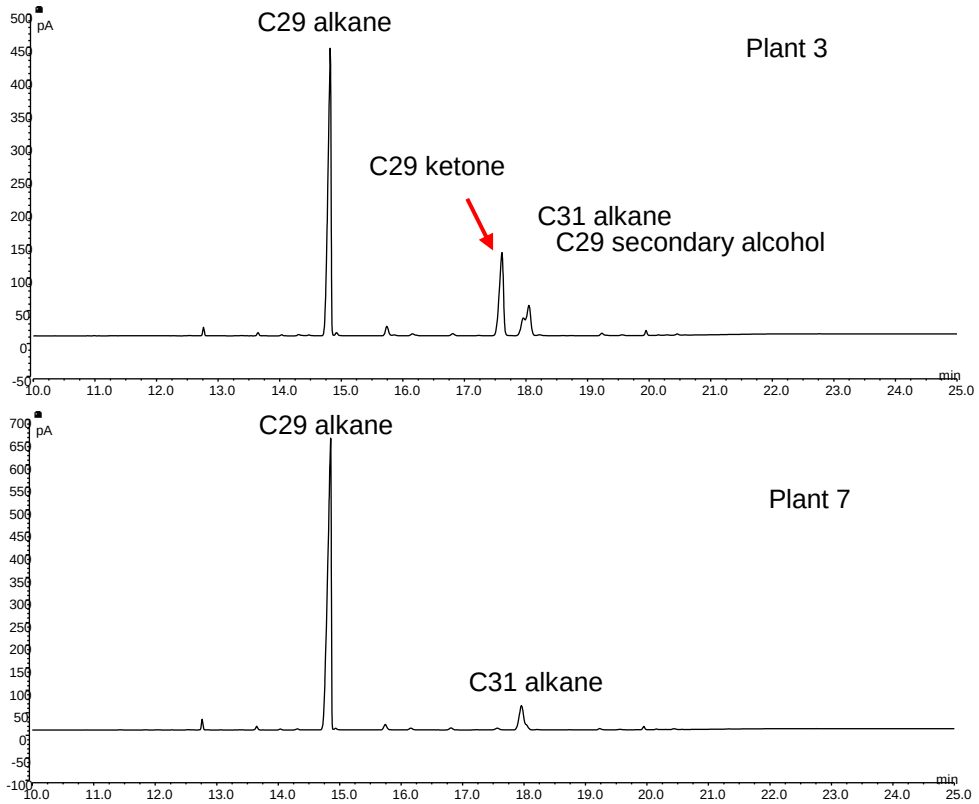
Image from Canola Council of Canada

Variant composition for functional studies



12 October 2021

Thank you.