

Supplementary Materials

Table S1 List of Wild Type Canola Varieties

Country of Origin	Variety Name	Accession Number
Bangladesh	BAU-M/50	PI 432392
Canada	BO-63	Ames 15651
Canada, Ontario	Jet Neuf	PI 649127
Canada, Saskatchewan	Golden	PI 649126
China	Gan You no. 4	PI 436557
Denmark, Fyn	Viking	PI 601266
France	Tantal	PI 383422
France	France 11	PI 469799
Germany	Laura	PI 458952
Japan	Norin 3	PI 469954
Korea, South	Aomori-1	PI 469726
Netherlands	Arco C10-2	Ames 15650
New Zealand	77-58	PI 458965
Poland	Poland 5	PI 470007
Poland, Wroclaw	Ames 2778	Ames 2778
Soviet Union, Former	Russia 6	PI 470022
Sweden	Comet	PI 649130
Sweden, Malmohus	Topas	PI 601201
United States, California	Siberian	Ames 26626
United States, Oregon	77-70	PI 458979

Table S2 PCR amplification of SU canola and Clearfield canola DNA with *CruA* and SU canola specific primer sets. DNA isolated from three SU canola events, C1511, C5507, and 40K, and three Clearfield canola varieties, 5545 CL, CS220 CL and 2022 CL were challenged with primers specific for SU canola or for the *CruA* gene, 200 ng DNA per reaction. PCR conditions as described in Methods. For all conditions except water control, n=3. ND indicates that no amplification was detected. NA indicates “not applicable”, because no amplification was observed.

Sample ID	Ct		Ave Ct		Std Dev Ct		Rel Std Dev Ct (%)	
	SU Canola Primers	CruA Primers	SU Canola Primers	CruA Primers	SU Canola Primers	CruA Primers	SU Canola Primers	CruA Primers
5545 CL		20.7839	ND	20.8573	NA	0.0660	NA	0.32
5545 CL		20.8499						
5545 CL		20.8060						
C5507	23.7372	20.9056	23.7098	21.0294	0.0345	0.1436	0.15	0.68
C5507	23.7211	20.9412						
C5507	23.6710	20.9296						
CS2200 CL		21.2058	ND	21.1852	NA	0.0291	NA	0.14
CS2200 CL		21.1646						
CS2200 CL		21.3600						
2022 CL		21.0245	ND	20.9552	NA	0.0887	NA	0.42
2022 CL		20.9790						
2022 CL		21.0462						
C1511	23.3823	20.8426	23.0039	20.7361	0.5469	0.1827	2.38	0.88
C1511	23.4061	20.8837						
C1511	23.4181	20.8706						
40K	22.4625	20.4839	22.3650	20.5499	0.0912	0.0595	0.41	0.29
40K	22.3506	20.5995						
40K	22.2818	20.5663						
water								
water								

Table S3. Quantitative PCR amplification of DNA from SU canola, event 40K, Clearfield canola variety 5545 CL and 20 varieties of wild type canola with CruA and SU canola-specific primer sets. PCR conditions are as described in Methods, with 300 ng input DNA per reaction for each variety except that the concentration of 40K DNA in the reactions labeled 40K-10, 40K-1 and 40K-0.1 were 300, 30 and 3 ng DNA 40K DNA per reaction, respectively, with total DNA adjusted to 300 ng for these reactions with DNA from canola variety 5454 CL. The n = 4 for all wild-type varieties. ND indicates that no amplification was observed. N/A indicates “Not Applicable”.

Well	Canola Variety	Ct CruA Primer Set	Mean Ct CruA Primer Set	Std Dev Ct CruA Primer Set	Ct SU Canola Primer Set	Mean Ct SU Canola Primer Set	Std Dev Ct SU Canola Primer Set
A1	40K-10	19.876	19.904	0.04	25.356	25.343	0.02
A2	40K-10	19.933			25.330		
A3	40K-1	20.099	20.202	0.15	28.191	28.608	0.59
A4	40K-1	20.305			29.026		
A5	40K-0.1	20.221	20.239	0.02	33.433	33.094	0.48
A6	40K-0.1	20.256			32.755		
A7	No DNA	34.360	34.963	0.85	ND	ND	N/A
A8	No DNA	35.565			ND		
B1	5545 CL	20.877	20.852	0.09	ND	ND	N/A
B2	5546 CL	20.822			ND		
B3	5547 CL	20.741			ND		
B4	5548 CL	20.966			ND		
B5	Variety 1	21.254	21.193	0.04	ND	ND	N/A
B6	Variety 1	21.173			ND		
B7	Variety 1	21.161			ND		
B8	Variety 1	21.185			ND		
B9	Variety 2	19.641	19.530	0.15	ND	ND	N/A
B10	Variety 2	19.627			ND		
B11	Variety 2	19.531			ND		
B12	Variety 2	19.321			ND		
C1	Variety 3	20.154	20.231	0.07	ND	ND	N/A
C2	Variety 3	20.314			ND		
C3	Variety 3	20.194			ND		
C4	Variety 3	20.264			ND		
C5	Variety 4	19.452	19.444	0.02	ND	ND	N/A
C6	Variety 4	19.429			ND		
C7	Variety 4	19.424			ND		

C8	Variety 4	19.472			ND		
C9	Variety 5	19.371	19.306	0.10	ND	ND	N/A
C10	Variety 5	19.412			ND		
C11	Variety 5	19.237			ND		
C12	Variety 5	19.205			ND		
D1	Variety 6	18.744	18.823	0.09	ND	ND	N/A
D2	Variety 6	18.745			ND		
D3	Variety 6	18.892			ND		
D4	Variety 6	18.911			ND		
D5	Variety 7	19.273	19.242	0.04	ND	ND	N/A
D6	Variety 7	19.236			ND		
D7	Variety 7	19.191			ND		
D8	Variety 7	19.267			ND		
D9	Variety 8	19.191	19.157	0.09	ND	ND	N/A
D10	Variety 8	19.254			ND		
D11	Variety 8	19.147			ND		
D12	Variety 8	19.034			ND		
E1	Variety 9	19.217	19.358	0.11	ND	ND	N/A
E2	Variety 9	19.375			ND		
E3	Variety 9	19.368			ND		
E4	Variety 9	19.472			ND		
E5	Variety 10	19.673	19.471	0.14	ND	ND	N/A
E6	Variety 10	19.390			ND		
E7	Variety 10	19.349			ND		
E8	Variety 10	19.472			ND		
E9	Variety 11	19.561	19.459	0.15	ND	ND	N/A
E10	Variety 11	19.598			ND		
E11	Variety 11	19.417			ND		
E12	Variety 11	19.262			ND		
F1	Variety 12	19.116	19.166	0.07	ND	ND	N/A
F2	Variety 12	19.096			ND		
F3	Variety 12	19.203			ND		
F4	Variety 12	19.250			41.542		
F5	Variety 13	19.454	19.408	0.04	ND	ND	N/A
F6	Variety 13	19.405			ND		
F7	Variety 13	19.407			ND		
F8	Variety 13	19.367			ND		
F9	Variety 14	19.078	19.051	0.09	ND	ND	N/A
F10	Variety 14	19.129			ND		
F11	Variety 14	19.082			ND		
F12	Variety 14	18.915			ND		

G1	Variety 15	19.624	19.570	0.05	ND	ND	N/A
G2	Variety 15	19.501			43.841		
G3	Variety 15	19.556			ND		
G4	Variety 15	19.598			41.406		
G5	Variety 16	20.468	20.211	0.23	ND	ND	N/A
G6	Variety 16	20.205			ND		
G7	Variety 16	20.258			ND		
G8	Variety 16	19.912			ND		
G9	Variety 17	21.231	21.174	0.04	ND	ND	N/A
G10	Variety 17	21.180			ND		
G11	Variety 17	21.150			ND		
G12	Variety 17	21.134			ND		
H1	Variety 18	19.064	18.990	0.10	ND	ND	N/A
H2	Variety 18	19.078			ND		
H3	Variety 18	18.934			ND		
H4	Variety 18	18.883			ND		
H5	Variety 19	23.654	23.744	0.06	ND	ND	N/A
H6	Variety 19	23.753			ND		
H7	Variety 19	23.796			ND		
H8	Variety 19	23.772			ND		
H9	Variety 20	18.286	18.224	0.07	ND	ND	N/A
H10	Variety 20	18.146			ND		
H11	Variety 20	18.284			ND		
H12	Variety 20	18.178			ND		

Table S4a. Real time quantitative PCR analysis of SU canola DNA, variety 40K, mixed at 5 concentrations with Clearfield canola variety 5545 CL. Total DNA was 300 ng/reaction and n = 12. PCR conditions, as described in Methods using the SU canola-specific primers and probe.

Declared Concentration	Log Conc of Replicates	DNA Conc of Replicates	Average DNA Conc	Std Dev of Average DNA Conc	Rel Std Deviation	Accuracy of Measured DNA Conc
%		% SU Canola DNA in total DNA	% SU Canola DNA in total DNA		%	% difference between measured conc and declared conc
10	0.804591	6.3766	8.6311	0.7583	8.79	13.69
	0.927165	8.4560				
	0.973636	9.4110				
	0.951302	8.9393				
	0.938817	8.6859				
	0.946058	8.8320				
	0.947224	8.8557				
	0.951552	8.9444				
	0.944200	8.7943				
	0.961706	9.1560				
	0.935072	8.6114				
	0.929967	8.5107				
1	-0.023572	0.9472	1.0110	0.0503	4.98	-1.10
	-0.010532	0.9760				
	0.007002	1.0163				
	-0.007064	0.9839				
	0.008223	1.0191				
	0.015408	1.0361				
	0.025618	1.0608				
	0.054278	1.1331				
	0.004477	1.0104				
	-0.001183	0.9973				
	-0.000323	0.9993				
	-0.020937	0.9529				
0.1	-0.990040	0.1023	0.1034	0.0115	11.15	-3.38
	-1.009683	0.0978				
	-0.936466	0.1158				

	-0.948757	0.1125				
	-1.076852	0.0838				
	-1.063867	0.0863				
	-1.034236	0.0924				
	-0.972478	0.1065				
	-0.947619	0.1128				
	-0.925202	0.1188				
	-0.996393	0.1008				
	-0.955859	0.1107				
0.05	-1.375018	0.0422	0.0465	0.0078	16.80	6.93
	-1.395826	0.0402				
	-1.362700	0.0434				
	-1.251057	0.0561				
	-1.332320	0.0465				
	-1.206167	0.0622				
	-1.304603	0.0496				
	-1.469348	0.0339				
	-1.387447	0.0410				
	-1.351574	0.0445				
	-1.265928	0.0542				
	-1.350631	0.0446				
0.01	-2.527344	0.0030	0.0043	0.0030	69.01	56.68
	-2.567878	0.0027				
	-2.263025	0.0055				
	-2.428935	0.0037				
	-2.798959	0.0016				
	-1.973487	0.0106				
	-2.211171	0.0061				
	-2.031167	0.0093				
	-2.811722	0.0015				
	-2.491970	0.0032				
	-2.643231	0.0023				
	-2.616819	0.0024				

S4b Definition of Limit of Quantitation and Calculation of Limit of Detection

Limit of Quantitation defined as the lowest concentration of analyte at which the Relative Standard Deviation is less than 25%.

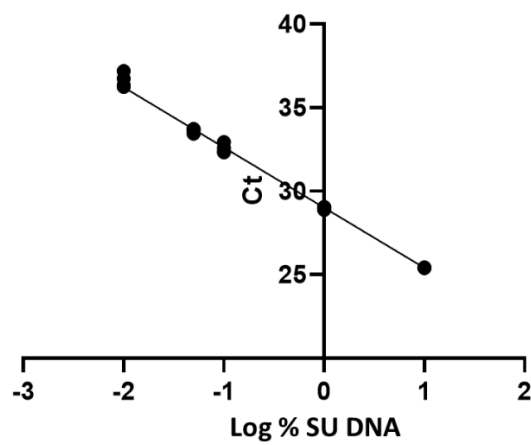
LOQ = 0.050 % SU Canola DNA in 300 ng Total DNA

Calculation of Relative Limit of Detection

$$\text{LOD} = (\text{SD})(t\text{-value}(\text{pValue}, n-1))$$

$$\text{LOD} = (0.008)(t\text{-value}(0.99, 12-1))$$

$$\text{LOD} = (0.008)(3.10580652) = 0.025$$



S5 AEA Validation Report (Separate Document, PDF)

See separate PDF