

Figure S1: Phenotyping of the 3 selected RILs with contrasting mucilage release and for the analyze of the shapes of the seed by ImageJ.

- a. Mucilage released of the three RILs. Bar = 1,5cm.
- b. Seed size of the three RILs. Bar = 5 mm.

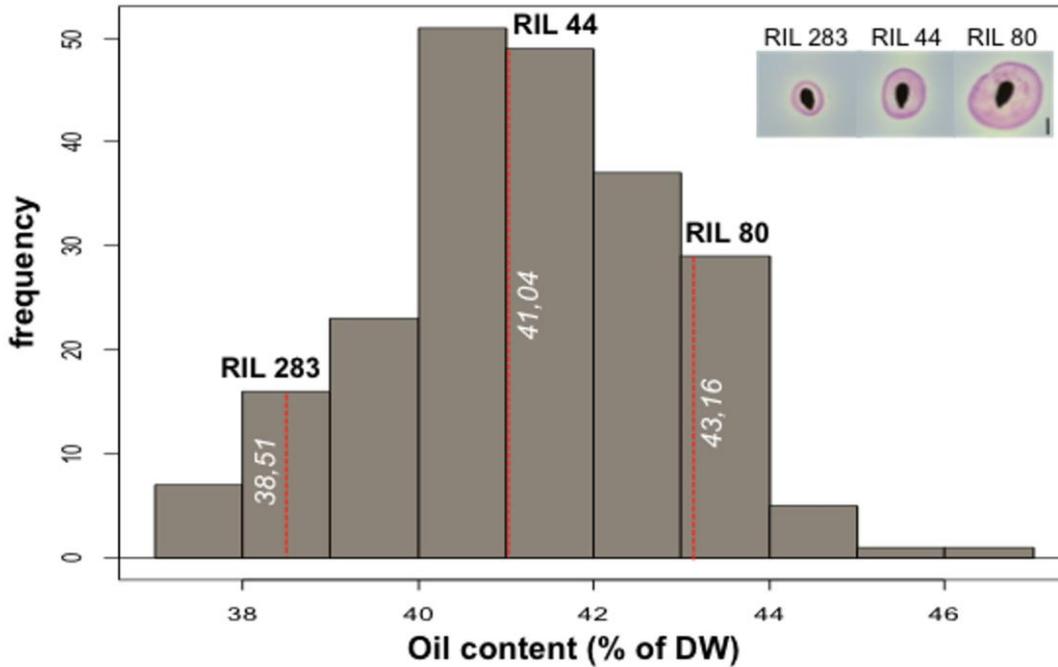


Figure S2: Identification of the 3 selected RILs displaying contrasting phenotypes for seed oil content and mucilage release.

Frequency histogram of the recombinant inbred lines (RILs) population screened for seed oil content. Quantification was performed in mature seeds from 186 field-growth RILs (at F10 generation) produced by crossing between Oliver and Viking cultivars. Red dashed lines show seed oil contents found for the RILs selected in this study (283, 44 and 80) for which mucilage phenotypes are shown in the pictures at the top right. Bar = 3 mm.

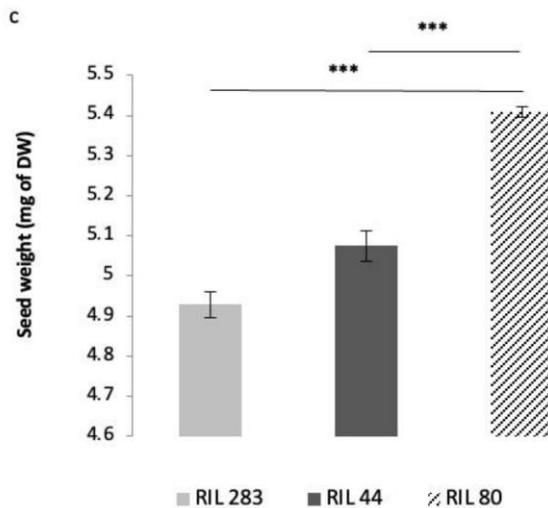


Figure S3: Seed traits description of the selected RILs.

a. Seed coat colour of the three selected RILs. Bar = 8 mm.

b. Relative size of a representative flax seed embryo at 10 DPA. The green embryo can be seen through the transparent seed coat. Bar = 1 mm.

c. Seed weight of the three selected RILs. Error bars represent +/- SE. Kruskal-Wallis *H*-test followed by a Mann-Whitney *U*-test, ****P*<0.001. Data are means of three biological replicates.

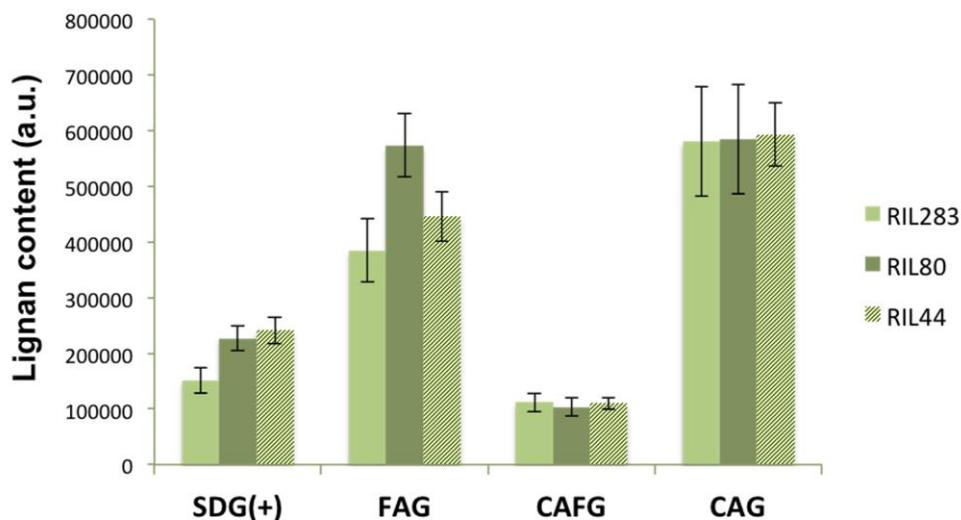


Figure S4: HPLC-UV analysis of the lignan content of the SDG-HMG complex.

The quantification of the main lignan content was performed on mature seed from selected RILs. Error bars represent +/- SE (n = 10 to 18 biological replicates). Kruskal-Wallis *H*-test followed by a Mann-Whitney *U*-test, **P*<0.05.

SDG(+), Secoisolariciresinol diglucoside; FAG, ferulic acid glucoside; CAFG, caffeic acid glucoside; CAG, coumaric acid glucoside.

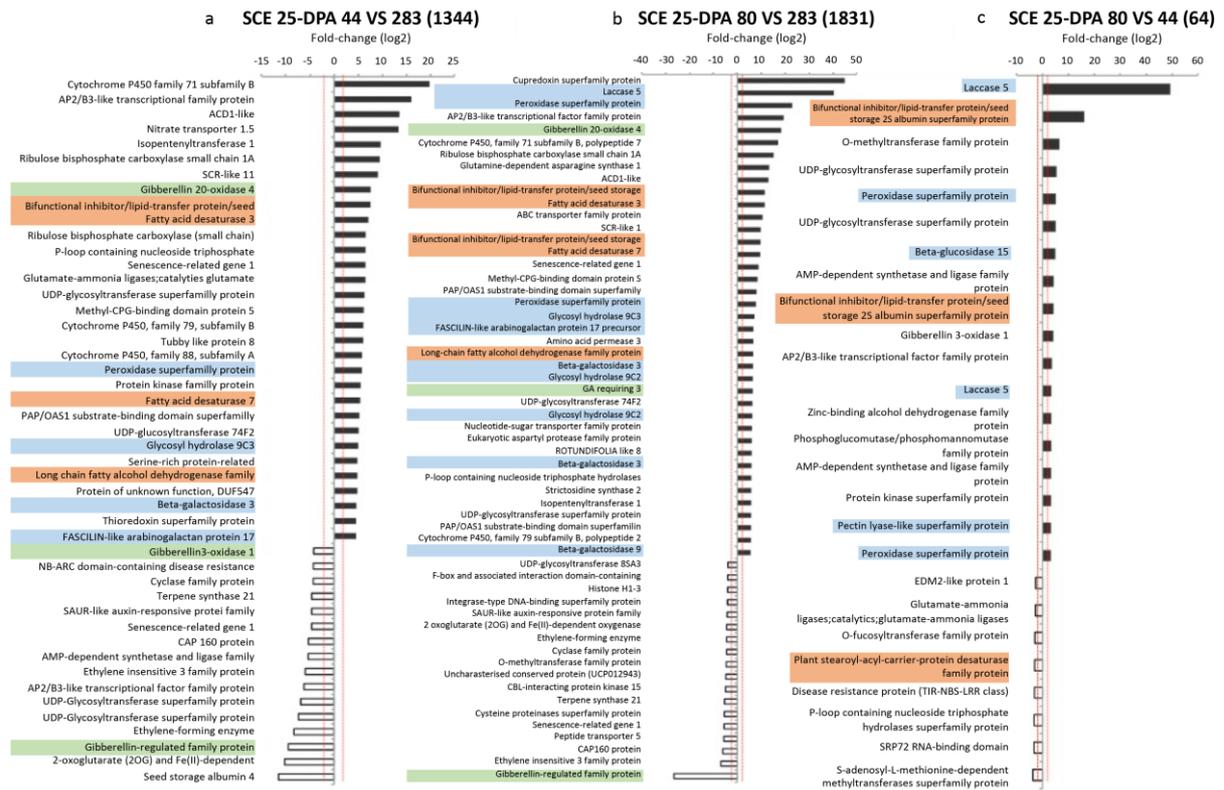


Figure S5: Clustering of the most discriminant genes in pairwise comparisons analysis of RILs in the mid-stage of seed-coat development.

The three clusters correspond to the pairwise comparisons between RILs 44 and 283 (a), RILs 80 and 283 (b) and RILs 80 and 44 (c). Each number associated to the cluster header represents the number of unique gene and overlapping genes for the corresponding pairwise comparison. Genes highlighted in blue are potentially involved in the cell wall and mucilage-related processes. Genes highlighted in red are involved in the seed coat oil and FA metabolisms, while genes highlighted in green correspond to those involved in the GA metabolism. Red dashed lines are positive (>2) and negative (<-2) fold-change thresholds.

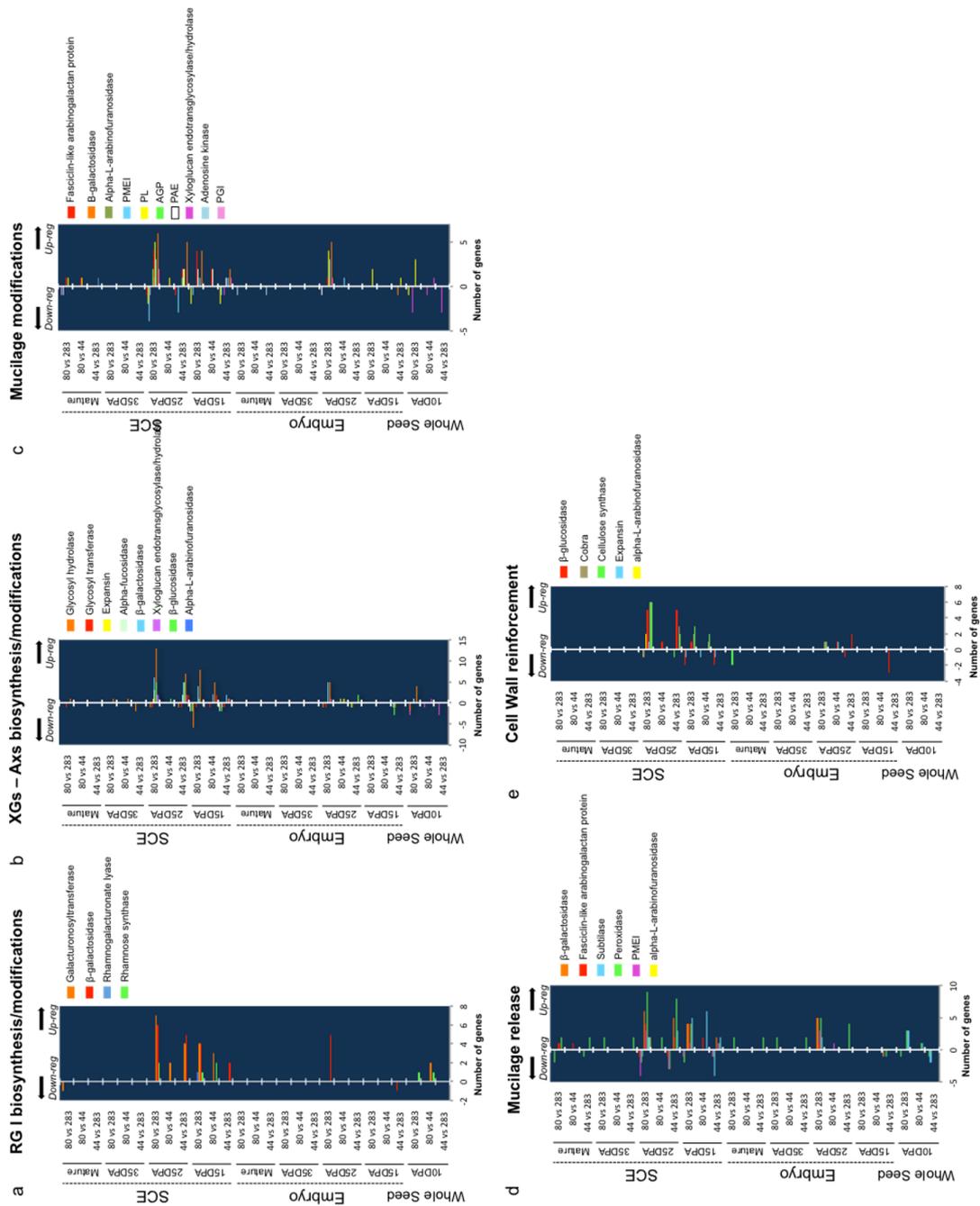


Figure S6: Analyses of the distribution of the flax genes between the 3 RILs according to their respective functions in the mucilage-related events, across tissues and during seed development. The histogram shows the number of unique genes from pairwise comparison analysis (three-ways ANOVA) of the three RILs. Genes are classified in five different categories according to their roles based on literature.

Table S1: Discriminating metabolites identified by 1D ¹H NMR in the three RILs.

<i>Compounds</i>	<i>Integrated area</i>		<i>Chemical shift δH and multiplicity on 1H spectrum (coupling constant J) of the peak used for calculation of the areas</i>
L-Valine	1.012	1.0	CH ₃ , 1,011 ppm, d (7 Hz)
L-Alanine	1.502	1.475	CH ₃ , 1,49 ppm, d (7.23 Hz)
Linamarin	4.572	4.56	CH, 4.57 ppm, d (7.81 Hz)
Neolinustatine	4.483	4.47	CH, 4.48 ppm, d (7.84 Hz)
Linustatine	4.45	4.442	CH, 4.45 ppm, d (7.71 Hz)
L-Asparagine	2.95	2.932	CH ₂ , 2.95 ppm, dd (16.92; 4,03 Hz)
Ethanolamine	3.128	3.1	CH ₂ , 3.12 ppm, m
Choline	3.221	3.2125	CH ₃ , 3.22 ppm, s
Raffinose D(+)	4.96	4.92	CH, 4.94 ppm, d (3.71 Hz)
Sucrose	4.165	4.148	CH, 4.16 ppm, d (8.61 Hz)
L-Tryptophane	7.29	7.274	CH, 7.28 ppm, s
Trigonelline	9.17	9.14	CH, 9.155 ppm, s
Formic acid	8.485	8.46	CH, 8.47 ppm, s
L(+)-Tartaric-acid	4.335	4.312	CH, 4.32 ppm, s
L-Aspartic-acid	2.679	2.652	CH ₂ , 2.67 ppm, dd (17.3; 8.90 Hz)
L-Glutamic-acid	2.436	2.397	CH ₂ , 2.41 ppm, m
L-Glutamine	2.482	2.437	CH ₂ , 2.46 ppm, m
D(+)-Glucose	3.185	3.175	CH, 3.20 ppm, dd (9.30; 7.93 Hz)
Glycerol	3.518	3.511	CH ₂ , 3.515, m
Acetate	1.96	1.949	CH ₃ , 1,955 ppm, s