frontiers

*Supplementary Material*

# Dissecting the genetic basis for seed coat mucilage heteroxylan biosynthesis in *Plantago ovata* using gamma irradiation and infrared spectroscopy

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## 1 Supplementary Figures and Tables

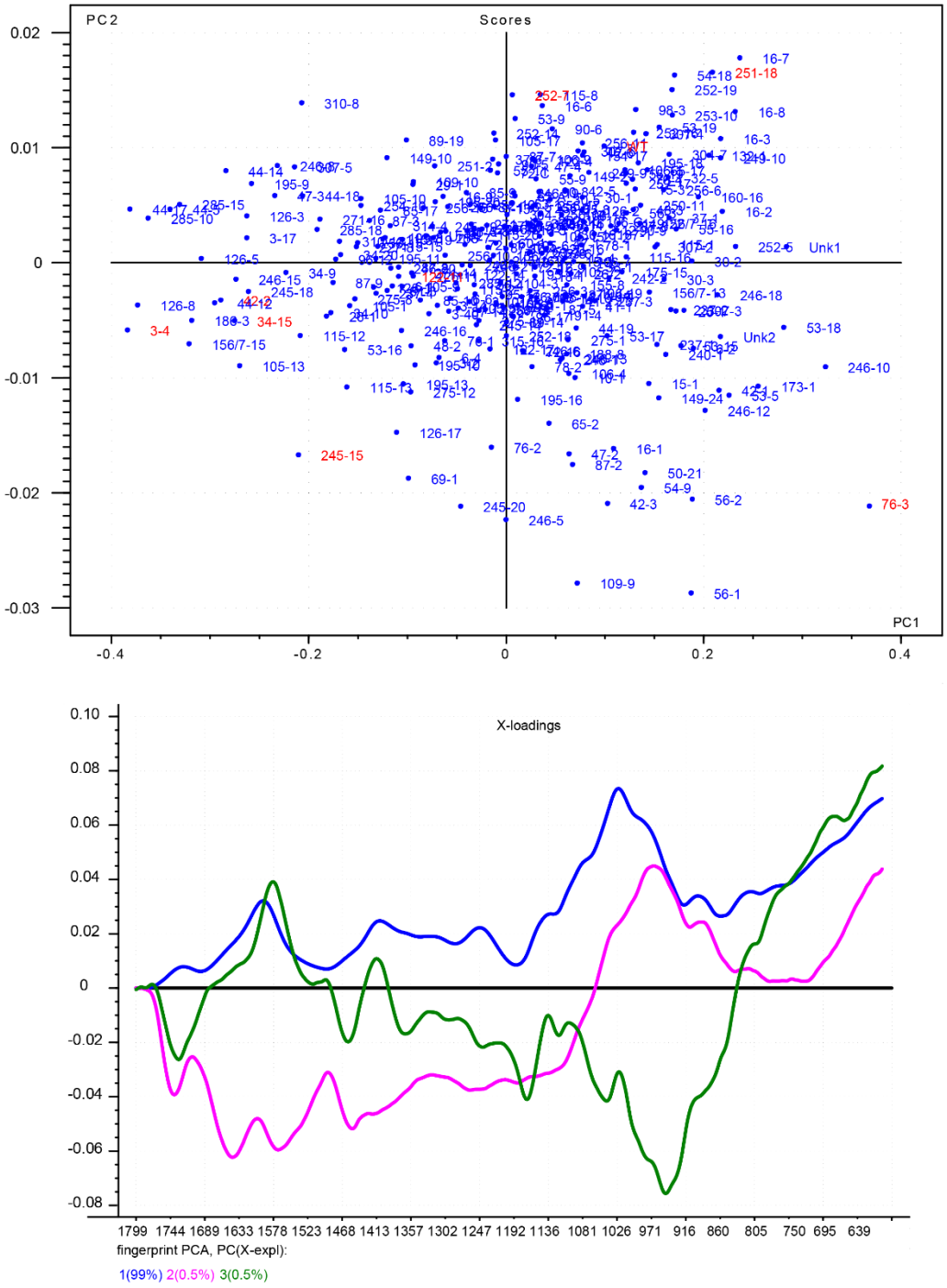


**Figure S1:** Principal Component Analysis of FTMIR aborbance readings derived from the fingerprint region of *P. ovata* seed samples. The upper panel shows the 2D PCA plot of normalised spectral data from 300 M3 lines including a wild-type (WT) control. Lines that were analysed in greater detail in this study are highlighted in red. The lower panel shows loadings for each principal component.

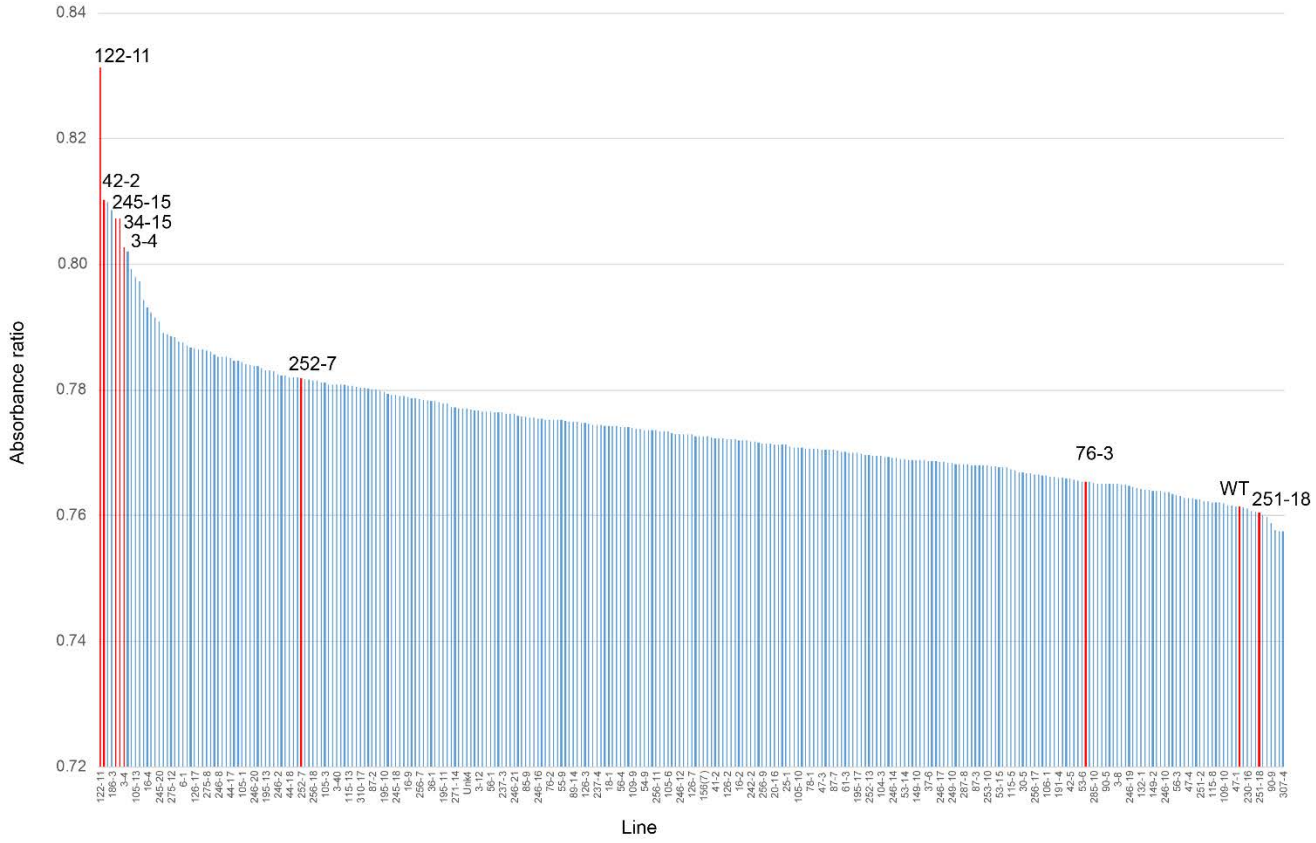


**Figure S2:** Absorbance ratio values of 1070cm-1/1040cm-1 from 300 *P.ovata* M3 lines and WT, sorted from largest to smallest. The same analysis was carried out for multiple xylan-associated ratios based on the normalised FTMIR data, with the aim of identifying lines showing distinct differences in heteroxylan composition/structure compared to WT. This data is shown as an example; other wavelength ratios showed distinct distribution patterns, but outlier lines were often conserved (See Table S1). The red bars indicate putative mutant candidates based on PCA analysis (see Fig S1 and S3).

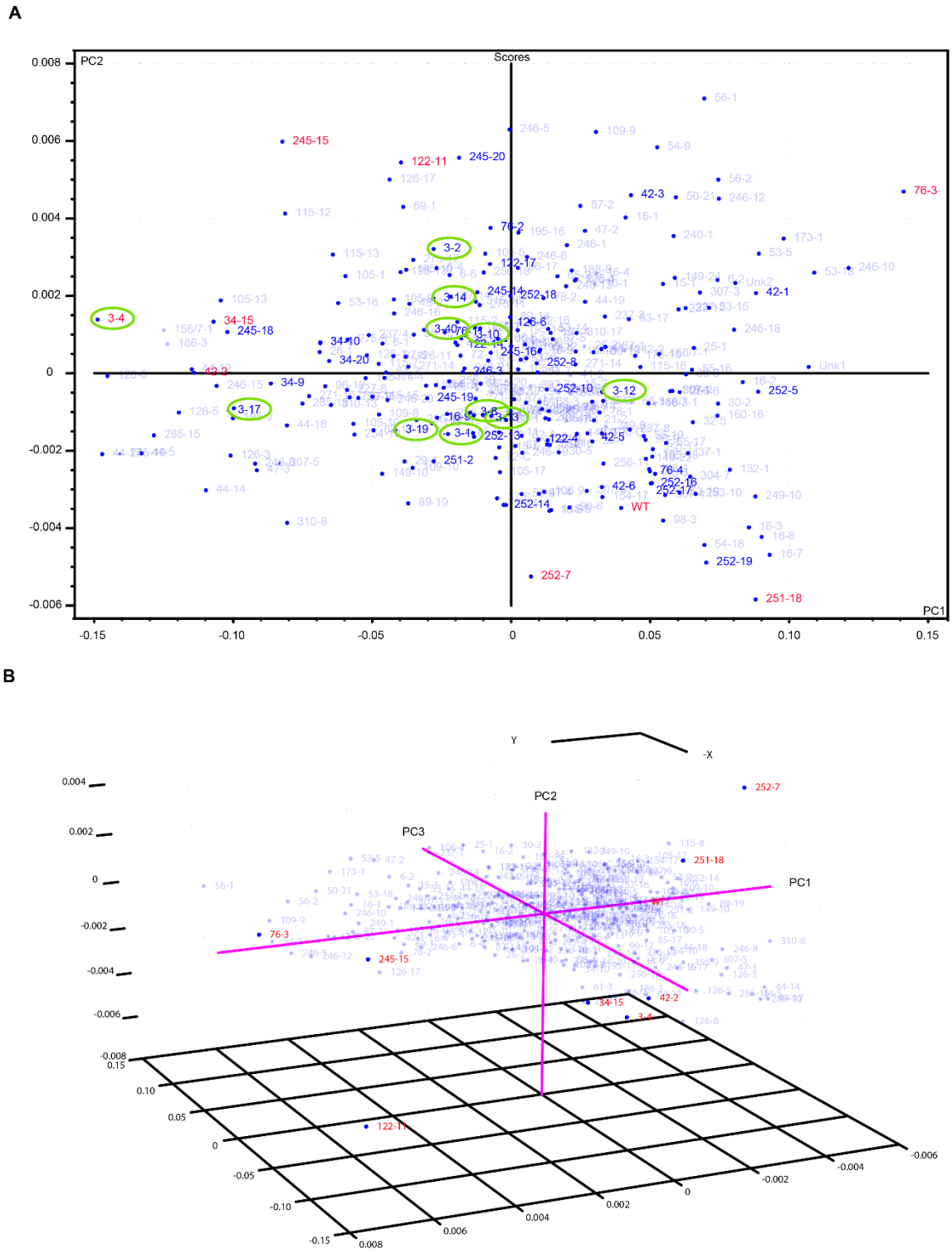


**Figure S3:** Principal Component Analysis of FTMIR aborbance readings derived from xylan-associated wavelengths of *P. ovata* seed samples. The upper panel shows the 2D PCA plot of normalised spectral data from 300 M3 lines including a wild-type (WT) control. Lines that were analysed in greater detail in this study are highlighted in red. Sister plants of line 3-4 are indicated with a green ring. The lower panel shows the 3D PCA plot of the same data.

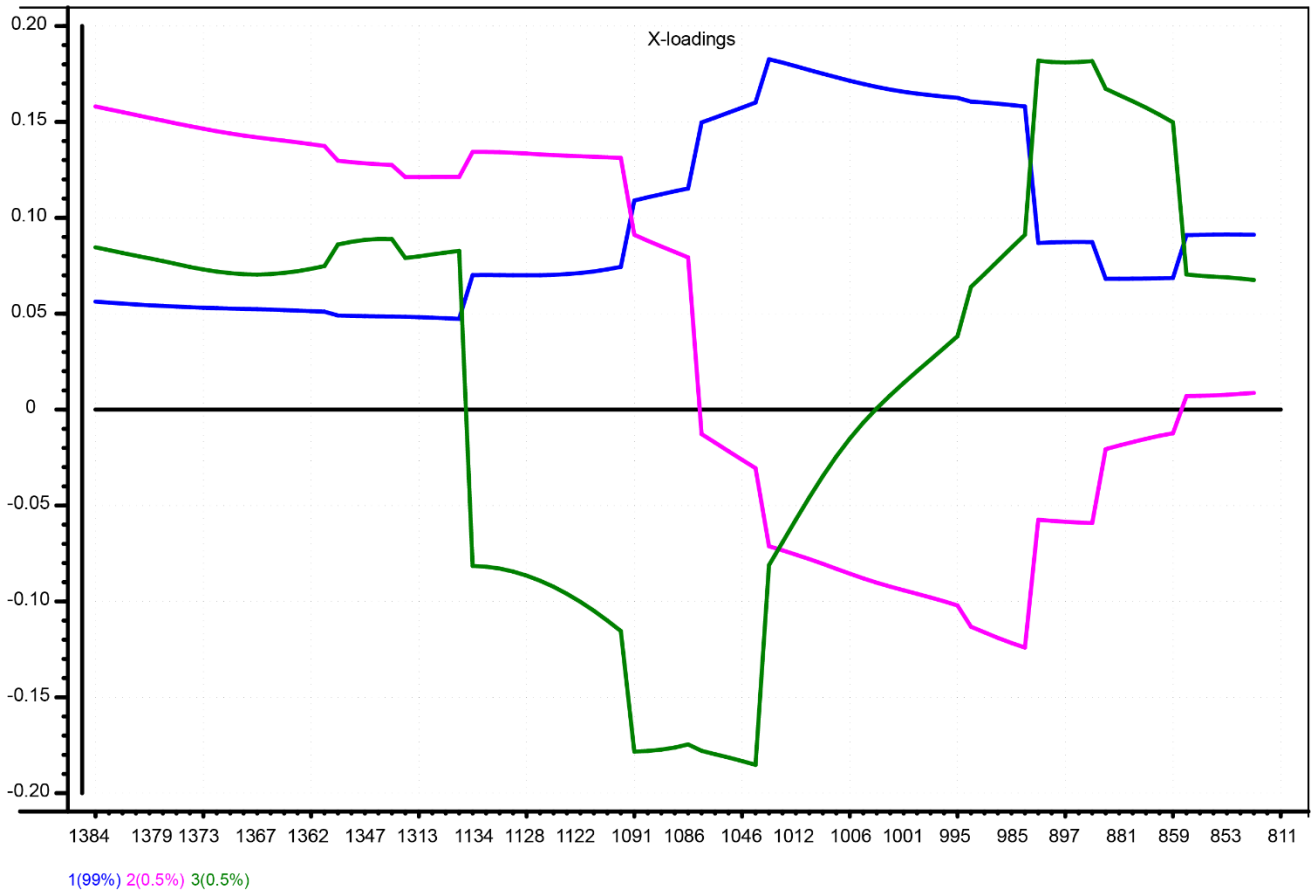


**Figure S4:** PCA loadings derived from xylan-associated wavelengths of *P. ovata* seed samples. The PCA plots are shown in Fig S3.

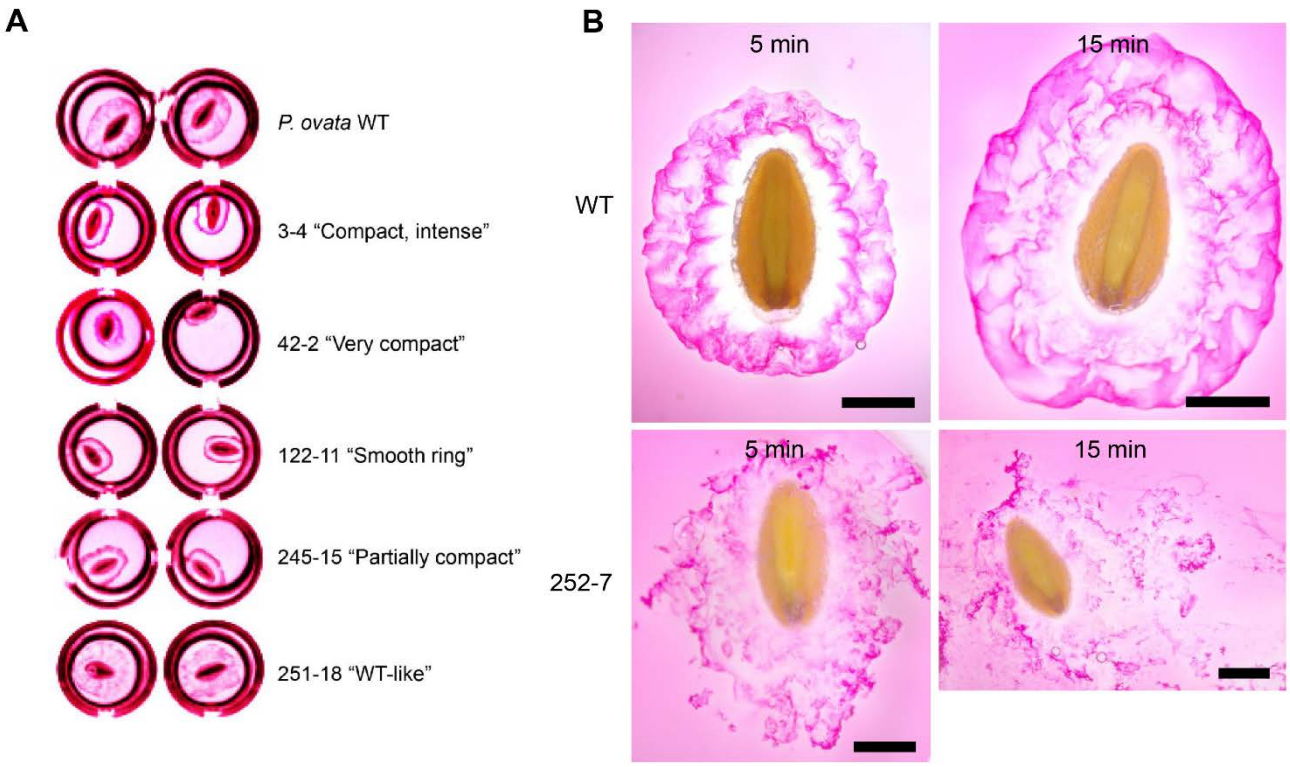


**Figure S5:** Ruthenium red staining of *P. ovata* seeds from different phenotypic classes. (**A**) Distinct mucilage phenotypes as they appear in microtitre plate assays. (**B**) Comparison of mucilage release and adhesion in *P. ovata* WT and 252-7 over time after RR-staining on slides. Scale bar = 1mm.

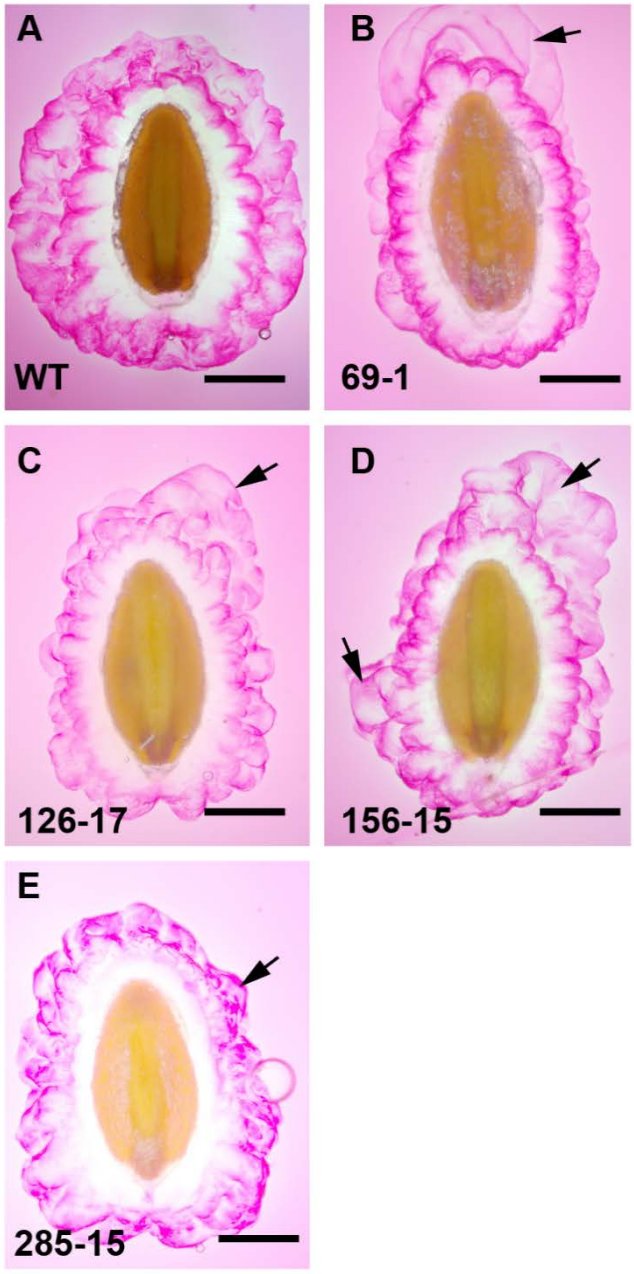


**Figure S6:** Ruthenium red staining of *P. ovata* seeds from M3 lines showing an "irregular bump" phenotype, as indicated by arrows. Bar = 1mm.

**Table S1**: Primers used for semi-quantitative PCR in this study

| Name | Fwd Primer | Rev Primer | Product Size |
|---|---|---|---|
| *PoGAP* | TGCCTTGAGCAAGAACTTTGT | AACTGAGTGCATGTGGACGAT | 100 |
| *PoHisH4* | GCTGTCACCTATACCGAGCAC | TCAGCAGAAACAGAAATCCAGA | 208 |
| *PoAGO1* | TAGGTTTACTGGTGCCACGTC | CAACACAACACAGGCATTTGA | 131 |
| *PoAGO5* | GAACATACGCATTGAGGCACT | AAACACAACTCGCCAAAACAT | 152 |
| *PoCesA3* | GGAAATCCTAGACGACCGAGT | ACAAAAGACACGACCGACAAC | 102 |
| *PoCesA4* | GCAAACTGGTCCAATTCTCAA | TCAAACACAGACTTCGAGCAA | 107 |
| *PoCesA1* | CCTTATGAACAGTGGGGGAAT | TCATACACATCAAGGCCAACA | 101 |
| *PoCesA9* | TTGCTGTGGGTTCGTATCAA | CAGCAATACCCTCGGTTTGT | 178 |
| *PoCslA* | TTTGTTTTCCGGCTAAGGAAT | CAGCAGTAGGAAGGAGTCACG | 139 |
| *PoCslG2* | ATCCTGAAAGGCCATGTTTCT | GGAATAAGGACAAGCCCGTAG | 223 |
| *PoUXS_1* | TCTGTCGCCTTGGTGATATTT | AGCTCGCTGAGACTGTGAAAG | 112 |
| *PoUXS_2* | TTCTCCTGGATTTCCGAGATT | TTGATGACGGTCGTGTTGTTA | 182 |
| *PoUXS_3* | TACAACAGATGGGATGCAGTG | GGACTTCCTCTCATGGTGACA | 140 |
| *PoUAM* | CAGTTGACCCCTACTTCACCA | AACAATCCCTATCCCGTTCAC | 214 |
| *PoGT61_2* | CGTGCTTTCTTCTCGACTCAC | CCAACATGGTCTTTCTTCCAA | 146 |
| *PoGT61_8* | GGGCAAACTGTATCCAAGTGA | TCCATGGCCTTAACTAACGTG | 153 |
| *PoGT61_7* | TGGCTTTCAGAATGGTTTCTC | GGGAAGTACCCAATGGACAGT | 139 |
| *PoGT61_6* | GAATATGAAAGGGGTGCATCA | TTCACTGGCCACTTGTGAAAT | 295 |
| *PoGT61_1* | AAACTAATTGGGGGCAATGAC | AATGTACCTCCCGTTGGAAAC | 179 |
| *PoXylT* | TTCCAGGCAGAGCTGAAACTA | CACGGCTTAGCAATGAAGAAG | 202 |
| *PoIRX10_3* | TTCCAAATTAGCAGCCACATC | TTCCAAATTAGCAGCCACATC | 275 |
| *PoIRX10_4* | TATTTCCCCGATTAGGTCTGG | AAAATTTGAACGGACCTGGAT | 160 |

**Table S2:** Absorbance ratio ranking of selected putative *P. ovata* mutants. The top row shows the absorbance ratios. Red coloration indicates the wild-type and internal mutant controls

| Line | 1164/1048 | 1110/1040 | 1070/1040 | 990/1380 | 895/1460 |
|---|---|---|---|---|---|
| *P. ovata* | 238 | 294 | 290 | 19 | 64 |
| 3-4 | 1 | 3 | 7 | 301 | 298 |
| 3-12 | 181 | 79 | 97 | 78 | 57 |
| 6-4 | 108 | 19 | 14 | 268 | 231 |
| 6-6 | 293 | 82 | 57 | 211 | 139 |
| 10-1 | 11 | 50 | 125 | 251 | 284 |
| 16-4 | 71 | 22 | 13 | 153 | 132 |
| 34-15 | 77 | 12 | 6 | 271 | 174 |
| 34-9 | 121 | 14 | 15 | 259 | 242 |
| 42-2 | 47 | 5 | 2 | 250 | 196 |
| 42-3 | 6 | 20 | 30 | 276 | 291 |
| 44-12 | 8 | 9 | 3 | 297 | 294 |
| 44-14 | 182 | 107 | 50 | 3 | 2 |
| 44-17 | 41 | 17 | 34 | 269 | 4 |
| 44-5 | 117 | 37 | 54 | 158 | 125 |
| 50-21 | 4 | 13 | 47 | 280 | 295 |
| 55-9 | 73 | 165 | 118 | 73 | 175 |
| 56-1 | 7 | 42 | 100 | 281 | 293 |
| 56-2 | 14 | 26 | 21 | 239 | 287 |
| 65-2 | 30 | 93 | 171 | 240 | 290 |
| 69-1 | 26 | 27 | 33 | 292 | 296 |
| 105-13 | 13 | 8 | 10 | 296 | 272 |
| 105-17 | 195 | 213 | 203 | 57 | 30 |
| 105-5 | 110 | 10 | 9 | 107 | 203 |
| 109-9 | 5 | 64 | 136 | 290 | 299 |
| 115-12 | 18 | 16 | 72 | 294 | 230 |
| 115-7 | 144 | 61 | 86 | 147 | 9 |
| 122-11 | 9 | 1 | 1 | 137 | 137 |
| 126-11 | 258 | 54 | 44 | 222 | 130 |
| 126-17 | 66 | 18 | 25 | 286 | 228 |
| 126-5 | 12 | 21 | 11 | 260 | 269 |
| 126-7 | 261 | 110 | 151 | 167 | 106 |
| 126-8 | 2 | 2 | 20 | 299 | 301 |
| 156-15 | 3 | 6 | 12 | 298 | 300 |
| 156-16 | 299 | 270 | 154 | 37 | 39 |
| 186-3 | 48 | 72 | 4 | 291 | 128 |
| 195-12 | 281 | 174 | 122 | 101 | 164 |
| 195-13 | 17 | 15 | 43 | 287 | 283 |
| 230-16 | 298 | 223 | 292 | 107 | 144 |
| 245-15 | 15 | 4 | 5 | 300 | 292 |
| 245-18 | 28 | 31 | 76 | 295 | 248 |
| 245-20 | 20 | 23 | 16 | 293 | 289 |
| 246-15 | 59 | 141 | 111 | 288 | 285 |
| 246-20 | 127 | 191 | 40 | 149 | 179 |
| 246-5 | 16 | 11 | 8 | 289 | 297 |
| 246-8 | 234 | 53 | 31 | 44 | 8 |
| 252-7 | 125 | 98 | 52 | 4 | 192 |
| 275-1 | 270 | 146 | 83 | 230 | 146 |
| 275-12 | 10 | 7 | 19 | 285 | 288 |
| 285-15 | 35 | 29 | 24 | 261 | 98 |
| 304-7 | 72 | 63 | 48 | 118 | 226 |
| 307-5 | 206 | 194 | 101 | 22 | 5 |
| 310-8 | 229 | 216 | 138 | 5 | 1 |
| U-4 | 106 | 60 | 94 | 146 | 77 |

**Table S3** FTMIR absorbance ratios for 37 putative *P.ovata* mucilage mutants representing 22 M1-derived families

| Name | Line | RR Phenotype | 1164/1048 | 1110/1040 | 1070/1040 | 990/1380 | 895/1460 |
|---|---|---|---|---|---|---|---|
| *rxm1* | **3-4** | Compact, intense | 0.435 | 0.610 | 0.803 | 1.818 | 1.840 |
| | **16-3** | WT-like, less | 0.347 | 0.518 | 0.767 | 2.292 | 2.244 |
| | **16-7** | WT-like, less | 0.337 | 0.513 | 0.761 | 2.423 | 2.413 |
| | **16-8** | WT-like, less | 0.328 | 0.518 | 0.764 | 2.419 | 2.395 |
| | **34-9** | Compact, intense | 0.345 | 0.557 | 0.792 | 2.187 | 2.216 |
| *rxm4* | **34-15** | Compact, intense | 0.351 | 0.559 | 0.807 | 2.169 | 2.324 |
| | **42-2** | Very compact | 0.359 | 0.576 | 0.810 | 2.198 | 2.294 |
| | **42-3** | Very compact | 0.393 | 0.553 | 0.786 | 2.153 | 1.977 |
| | **44-5** | Smooth ring | 0.346 | 0.543 | 0.782 | 2.278 | 2.373 |
| | **44-12** | Smooth ring | 0.389 | 0.567 | 0.810 | 1.996 | 1.926 |
| | **44-14** | Smooth ring | 0.341 | 0.532 | 0.782 | 2.456 | 2.632 |
| | **44-17** | Smooth ring | 0.361 | 0.555 | 0.785 | 2.170 | 2.602 |
| | **54-9** | WT-like | 0.347 | 0.542 | 0.774 | 2.194 | 2.141 |
| | **54-18** | WT-like | 0.336 | 0.515 | 0.765 | 2.456 | 2.511 |
| | **56-1** | WT-like | 0.390 | 0.542 | 0.777 | 2.128 | 1.940 |
| | **56-2** | WT-like | 0.378 | 0.548 | 0.788 | 2.208 | 2.056 |
| | **69-1** | WT-like, bumpy | 0.370 | 0.546 | 0.785 | 2.048 | 1.880 |
| | **76-3** | WT-like | 0.347 | 0.526 | 0.765 | 2.270 | 2.117 |
| | **105-13** | Compact | 0.379 | 0.567 | 0.798 | 2.012 | 2.129 |
| | **109-9** | WT-like | 0.398 | 0.539 | 0.774 | 2.056 | 1.834 |
| | **115-12** | Compact | 0.377 | 0.556 | 0.780 | 2.027 | 2.241 |
| *rxm2* | **122-11** | Smooth ring | 0.387 | 0.654 | 0.831 | 2.293 | 2.363 |
| | **126-5** | WT-like, bumpy | 0.386 | 0.553 | 0.797 | 2.187 | 2.142 |
| | **126-8** | WT-like, bumpy | 0.423 | 0.620 | 0.789 | 1.916 | 1.762 |
| | **126-17** | WT-like, bumpy | 0.354 | 0.555 | 0.787 | 2.109 | 2.245 |
| | **156-15** | WT-like, bumpy | 0.422 | 0.571 | 0.794 | 1.950 | 1.818 |
| *rxm3* | **245-15** | Partially compact | 0.378 | 0.582 | 0.807 | 1.913 | 1.966 |
| | **245-18** | Partially compact | 0.368 | 0.545 | 0.779 | 2.016 | 2.208 |
| | **245-20** | Partially compact | 0.373 | 0.551 | 0.791 | 2.043 | 2.043 |
| | **246-5** | Compact, slow | 0.378 | 0.562 | 0.802 | 2.071 | 1.873 |
| | **246-8** | Compact, slow | 0.336 | 0.540 | 0.785 | 2.369 | 2.553 |
| | **246-15** | Compact, slow | 0.356 | 0.528 | 0.776 | 2.078 | 2.074 |
| | **251-18** | WT-like | 0.354 | 0.519 | 0.760 | 2.426 | 2.400 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **252-7** | Dispersed | 0.345 | 0.533 | 0.782 | 2.445 | 2.298 |
| **285-15** | WT-like, bumpy | 0.364 | 0.546 | 0.787 | 2.185 | 2.399 |
| **307-5** | Compact | 0.338 | 0.522 | 0.776 | 2.391 | 2.589 |
| **310-8** | Compact | 0.336 | 0.520 | 0.774 | 2.448 | 2.712 |
| **WT** | WT-like | 0.335 | 0.509 | 0.761 | 2.393 | 2.433 |

**RR**- ruthenium red stain after 10 min imbibition, **WT** - wild-type, **compact** – RR-stained mucilage did not extend as far as WT, **intense** – appeared more heavily stained at the periphery of the mucilage compared to WT, **WT-like** – similar in appearance to WT, **WT-like, less** – similar to WT in plates and slides, but mucilage did not stain as intensely, **partially compact** – appeared compact in plates but only slightly reduced compared to WT on slides, **smooth ring** – the outer layer of mucilage stained in a perfectly smooth ring, **bumpy** – similar to WT but sporadic bumps appeared at the periphery where mucilage appears to spread faster, **dispersed** – appears punctate and disconnected from the seed, ***rxm*** – putative *reduced xylan in mucilage* mutant.