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1 **Genome wide association study of *Arabidopsis* seed** 2 **mucilage layers at a regional scale**

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10

11 **ABSTRACT**

12 The myxospermous species *Arabidopsis thaliana* extrudes a polysaccharidic mucilage from the seed
13 coat epidermis during imbibition. The whole seed mucilage can be divided into a seed-adherent layer
14 and a fully soluble layer, both layers presenting natural genetic variations. The adherent mucilage is
15 variable in size and composition, while the soluble mucilage is variable in composition and physical
16 properties. Studies reporting both the genetic architecture and the putative selective agents acting on this
17 natural genetic variation are scarce. In this study, we set up a Genome Wide Association study (GWAS)
18 based on 424 natural accessions collected from 166 natural populations of *A. thaliana* located south-
19 west of France and previously characterized for a very important number of abiotic and biotic factors.
20 We identified an extensive genetic variation for both mucilage layers. The adherent mucilage was
21 mainly related to precipitation and temperature whereas the non-adherent mucilage was unrelated to any
22 environmental factors. By combining a hierarchical Bayesian model with a local score approach, we
23 identified 55 and 28 candidate genes, corresponding to 26 and 10 QTLs for the adherent and non-
24 adherent mucilages, respectively. Putative or characterized function and expression data available in the
25 literature were used to filter the candidate genes. Only one gene among our set of candidate genes was
26 already described as a seed mucilage actor, leaving a large set of new candidates putatively implicated
27 in seed mucilage synthesis or release. The present study lay out foundation to understand the influence
28 of regional ecological factors acting on seed mucilage in *A. thaliana*.

29 **Keywords**

30 *Arabidopsis thaliana*, Environmental factor, GWAS, Mucilage, Natural accessions

31 **1 Introduction**

32 Seed mucilage is a polysaccharidic hydrogel extruded around seeds upon imbibition by the outermost
33 epidermal cell layer of the seed coat in myxospermous species (Phan and Burton, 2018). Seed mucilage
34 can help plant development from germination until seedling establishment in biotic and abiotic stress
35 conditions (Viudes et al., 2020). Depending on plant species, the interaction between seed mucilage and

1 biotic stresses can be direct, e.g. escaping ant predation by sticking to soil (Pan et al., 2021) or facilitating
2 passage through the digestive tract of birds (Kreitschitz et al., 2021), or indirect, e.g. mobilizing soil
3 bacteria that limit the development of plant pathogen fungi, which lead to reduced disease symptoms
4 and a better plant development (Meschke and Schrepf, 2010). Concerning abiotic stresses, seed
5 mucilage is known to influence or to rely on water around the seed by regulating the incoming flow, and
6 by changing soil water retention capacity (Deng et al., 2014; Saez-Aguayo et al., 2014; Teixeira et al.,
7 2020). The ecological function of seed mucilage was historically studied in several plant species. In
8 contrast, the genetics and development of seed mucilage was nearly exclusively studied in the model
9 plant *Arabidopsis thaliana*.

10 In *A. thaliana*, around one hundred genes are participating to mucilage secretory cell formation and
11 mucilage biosynthesis and release (Viudes et al., 2021). A large majority of these genes have been
12 characterized through classical forward and reverse genetic studies. First of these were major regulators
13 or main actors of mucilage establishment that lead to drastic phenotypes when mutated (Western, 2001).
14 Progressively, the newly discovered genes had more subtle roles for myxospermy, displaying more
15 discrete phenotypes such as specific polysaccharide proportions or decorations when mutated.

16 Evidence of natural diversity for seed mucilage was first shown with the characterisation of an *A.*
17 *thaliana* natural genotype, originated from Tajikistan, which is not able to extrude mucilage (Macquet
18 et al., 2007). Among 22 *A. thaliana* populations originating from central Asia and Scandinavia, seven
19 were reported to be fully defective for mucilage release (Saez-Aguayo et al., 2014). Total seed mucilage
20 can have great polysaccharide composition changes among natural populations, regarding mannose,
21 galactose, galacturonic acid and rhamnose monosaccharide contents (Voiniciuc et al., 2016). The *A.*
22 *thaliana* mucilage has two distinct layers, the inner layer is highly adherent to the seed thanks to
23 cellulosic microfibrils, and the outer layer is fully soluble (Tsai et al., 2017). The volume of adherent
24 mucilage was shown to be also variable among natural populations probably linked to the observed
25 cellulose crystalline structure changes (Voiniciuc et al., 2016). The non-adherent mucilage exhibits also
26 variable compositions and physical properties (Poulain et al., 2019). Interestingly, the intra-species loss
27 of myxospermy can occur several times due to independent genetic mutations (Saez-Aguayo et al.,
28 2014). Two independent Genome-Wide Association Studies (GWAS), based on *A. thaliana* adherent
29 and non-adherent seed mucilage phenotyping, highlighted 21 and 11 significant SNPs, respectively,
30 supporting the polygenic architecture of myxospermy (Fabrissin et al., 2019; Voiniciuc et al., 2016).
31 Due to the lack of availability of accurate environmental data for many *A. thaliana* natural populations,
32 natural genetic variation in mucilage characteristics has so far never been associated with environmental
33 data through a statistical approach.

34 Here, we aimed at using GWAS to further investigate the genetics underlying natural genetic variation
35 of *A. thaliana* myxospermy. Our hypothesis was that this approach without *a priori* will highlight new

1 candidate genes that were not yet linked to seed mucilage. The quantitative and independent
2 phenotyping of both mucilage layers should increase the efficiency of GWAS and also provide the
3 opportunity to study if a correlation exists between the two layers of mucilage. In addition, the
4 availability of climatic conditions and soil composition for the studied populations created a unique
5 opportunity to address the potential ecological role of myxospermy in *A. thaliana*.

6 **2 Material and methods**

7 **2.1 Plant material**

8 Our study was based on 168 natural populations of *A. thaliana* identified in spring 2014 and located in
9 the Midi-Pyrénées region (south-west of France) (Bartoli et al., 2018; Frachon et al., 2018). The average
10 distance between these populations is ~100 km. These populations have been characterized for six non-
11 correlated variables retrieved from the ClimateEU database (Frachon et al., 2018), 13 chemical and 1
12 physical soil properties with soil samples collected *in situ* in late autumn 2015 and late winter
13 2015 (Frachon et al., 2019), leaf and root bacterial microbiota and pathobiota in late autumn 2015 and
14 late winter 2015 (Bartoli et al., 2018), and plant communities in late spring 2015 (Frachon et al., 2019).
15 In addition, based on a Pool-Seq approach with an average of 15.3 plants per population, the 168
16 populations have been whole-genome sequenced with the Illumina technology, leading to the
17 identification of 1,638,649 SNPs (Frachon et al., 2018). For the purpose of this study, we used 166 out
18 of the 168 initial populations, each being composed of one to three accessions, thereby bringing the total
19 of phenotyped seed lots to 424. Differences in the maternal effects among the 424 seed lots were reduced
20 by growing one plant of each accession for one generation with the following steps: (i) for each
21 accession, several seeds were sown in one 7 x 7 x 6 cm plastic pot (Soparco®, Condé-sur-Huisne,
22 France) filled with damp standard culture soil (PROVEEN MOTTE 20, Soprimex®, L'Isle-d'Abeau,
23 France) on November 1st 2016, (ii) seeds were stratified at 4°C for four days in order to break primary
24 dormancy and promote germination, (iii) pots were transferred to greenhouse conditions (22°C, 16 h
25 photoperiod) on November 4th 2016, (iv) seedlings were thinned to one on November 25th 2016, (v) pots
26 were placed on a field station at the INRAE campus of Castanet-Tolosan (France) on December 5th 2021
27 in order to promote flowering through natural vernalization, (vi) at the onset of flowering, plants were
28 transferred in a greenhouse mimicking the outdoor conditions (no additional light and heating) but
29 protecting plants from rainfall, and (vii) plants were separated from each other by aratubes in order to
30 avoid cross-pollination among accessions. All seed lots were harvested during a 3-week period from
31 late April to early May 2017 and were then stored at 4°C until the set-up of the phenotyping experiments.

32 **2.2 Phenotyping experiments**

33 Given the high number of accessions, the experiments were performed sequentially with several
34 experimental batches distributed along a few months and randomly selected. The number of accessions
35 in each batch was dependent on the phenotypic traits. In order to consider micro-environmental

1 variations among the experimental batches, the same Columbia (Col-0) seed lot was used as an internal
2 control that was systematically included in all experimental batches in order to normalize the results.

3 *2.2.1 Adherent mucilage and seed area phenotyping*

4 A total of 44 experimental batches, including Col-0 as a control and 11 different accessions, were set up
5 during over 6 months. Because the adherent mucilage is strongly attached to the seed, we used a
6 previously described protocol that allows comparing adherent mucilage area among accessions in the
7 most standardized manner (Francoz et al., 2019). Briefly, to remove the non-adherent mucilage, 50 to
8 100 seeds per accession were vigorously shaken at 250 rpm in 1.6 ml of 0.01 M Tris-HCl pH 7.5
9 contained in a 2 ml tube. The remaining adherent layer of mucilage was then stained with a 0.02 % (w/v)
10 ruthenium red solution in Tris-HCl. The seeds were rinsed in 0.01 M Tris-HCl pH 7.5, transferred in
11 24-well microplates containing 0.01 M Tris-HCl pH 7.5 and imaged using an Epson perfection V100
12 photo scanner at 6400 dpi resolution. The measurements of seed area including or not the adherent
13 mucilage was realized with imageJ using a previously described macro (Francoz et al., 2019). The
14 adherent mucilage area was calculated for each seed by subtracting the seed area alone from the seed
15 area with the adherent mucilage. For each experimental batch, raw data for adherent mucilage area and
16 seed area of each accession were standardized with the corresponding means of adherent mucilage and
17 seed areas of Col-0.

18 *2.2.2 Non-adherent mucilage area phenotyping*

19 A total of 98 batches of experiments including Col-0 as a control and three or six different accessions,
20 were set up over 6 months. The non-adherent mucilage is totally soluble in water and quickly released
21 upon seed imbibition. To estimate its area, we used the MuSeeQ protocol (Miart et al., 2018). Briefly,
22 5 to 10 dry seeds by accession were individually and synchronously disposed on perfectly flat 0.6 %
23 (w/v) agarose media containing 0.00004 % (w/v) of toluidine blue O polychromatic stain (Sigma-
24 Aldrich, Saint-Quentin-Fallavier, France). Upon the contact with the aqueous media, the non-adherent
25 mucilage is released, spreading around the seed on the flat surface, and is simultaneously stained in pink
26 contrasting with the blue colour of the media. Top-view pictures were taken 24 h after seed deposition
27 with a camera (Canon DS 126271). The non-adherent mucilage area was measured for each seed using
28 imageJ with a previously described macro (Miart et al., 2018). For each experimental batch, raw data
29 for non-adherent mucilage area of each accession were standardized with the corresponding mean of
30 Col-0 non-adherent mucilage area.

31 **2.3 Statistical analyses**

32 *2.3.1 Natural genetic variation*

1 To estimate the extent of natural genetic variation for each mucilage trait, we run the following mixed
2 models using the PROC MIXED procedure in SAS v9.4 (SAS Institute Inc., Cary, North Carolina,
3 USA):

$$4 \quad \text{Model (1) } Y_{ijk} = \mu + \text{population}_i + \text{accession}_j(\text{population}_i) + \text{seed area}_k + \epsilon_{ijk}$$

5 Where ‘Y’ is the mucilage area of seeds, ‘ μ ’ is the overall mean of mucilage area, ‘population’ accounts
6 for genetic differences among the 166 populations, ‘accession(population)’ accounts for the mean
7 genetic difference among accessions within population, ‘seed area’ is a covariate to control for the
8 developmental effect of seed size on adherent mucilage area, and ‘ ϵ ’ is the residual term. The factor
9 ‘population’ was treated as a fixed effect, whereas the factor ‘accession’ was treated as a random effect.
10 Following Brachi et al. (2013), this model random term was tested with a likelihood ratio test (LRT)
11 with and without this effect.

12 For both phenotypic traits, we estimated the percentage of variance observed among and within
13 populations by running Model 1 with the VARCOMP procedure in SAS v9.4 (SAS Institute Inc., Cary,
14 North Carolina, USA). In addition, we estimated genotypic values of the 166 natural populations by
15 calculating least-square means (LSmeans) of each population after removing the
16 ‘accession(population)’ term from Model 1.

17 Spearman’s correlation coefficients (Spearman’s *rho*) were calculated to estimate relationships between
18 genetic differences among populations (i.e. genotypic values) and abiotic ecological variables. Resulting
19 *p*-values were corrected with an FDR (False Discovery Rate) correction.

20 *2.3.2 GWA mapping with local score analysis*

21 To detect QTLs associated with natural genetic variation of mucilage area related traits, we combined a
22 GWA mapping approach with a local score approach. This combination of approaches already allowed
23 the successful detection and cloning in *A. thaliana* of five QTLs in response to bacterial pathogens
24 (Aoun et al., 2020; Demirjian et al., 2022; Demirjian et al., 2023). First, we retrieved for each of the 166
25 populations the standardized allele frequencies for all the SNPs (see ‘Plant material’ subsection). These
26 standardized allele frequencies result from raw allele frequencies after accounting for the scaled
27 covariance of population allele frequencies, thereby making the phenotype-genotype associations robust
28 to complex demographic histories and allowing decreasing drastically the rate of false positives
29 (Frachon et al., 2019, 2018). Second, for each phenotypic trait, we estimated phenotype-genotype
30 associations across the genome by calculating Spearman correlation coefficients between standardized
31 allele frequencies of each SNP and genotypic values of the 166 populations, using the *cor.test* function
32 implemented under the *R* environment. Third, we implemented a local score approach on the set of *p*-
33 values associated with the Spearman’s *rho* values. The local score allows accumulating the statistical
34 signals from contiguous genetic markers in order to detect significant genomic regions associated with

1 phenotypic natural variation (Fariello et al., 2017). Through the p -values in a given QTL region, the
2 association signal will cumulate locally due to linkage disequilibrium between SNPs, which will then
3 increase the local score (Bonhomme et al., 2019). The tuning parameter ξ was fixed at 2 and significant
4 SNP-phenotype associations were identified by estimating a chromosome-wide significance threshold
5 for each chromosome (Bonhomme et al., 2019).

6 Based on a custom script described in Libourel et al. (2021), we retrieved all candidate genes underlying
7 QTLs by selecting all genes inside the QTL regions and also the first gene upstream and the first gene
8 downstream of these QTL regions. The TAIR 10 database (<https://www.arabidopsis.org/>) was used as a
9 reference.

10 **2.4 Transcriptomic profiling of candidate genes**

11 The expression profiles of the candidate genes were extracted from *A. thaliana* tissue-specific seed
12 development transcriptomic data (Belmonte et al., 2013). Whole transcriptomic data set used to decipher
13 the seed coat specific expression of the candidate genes are available in Supplementary Table S2.

14 **3 Results**

15 **3.1 Large genetic variation among and within natural populations for seed mucilage**

16 The sizes of adherent and non-adherent mucilage layers were measured for each accession belonging to
17 the 166 populations (Fig. 1). Although the two different mucilages exist in 3D, their quantifications
18 come from 2D images. Mucilage values are therefore expressed in surface unit. For adherent mucilage,
19 measured values correspond to orthogonal projections, which are coherent with the reality since the
20 seeds systematically lay down longitudinally in solution due to their oblong shape. Non-adherent
21 mucilage spreads on a flat surface and is consequently already a 2D object before the imaging.

22 For both mucilage layers, a large phenotypic variation was observed among the 166 populations (Fig.
23 1). The ratio of the adherent-mucilage area compared to Col-0 largely differed among the accessions
24 from south-west of France, with values ranging from 0.2 to more than 1.5 (Supplementary Fig. S1). In
25 comparison, this ratio for the non-adherent mucilage shows less variability among populations, with
26 values ranging from 0.5 to 1.5 (Supplementary Fig. S1). Note that the sizes of both mucilage layers are
27 positively correlated with seed size (Supplementary Table S1).

28 On average, a large and significant genetic variation was detected among accessions within populations
29 (Table 1), with the observation of populations presenting either no phenotypic or extensive variation
30 among accessions for adherent or non-adherent mucilage areas (Fig. 2). Overall, while 32.3 % and 13.8
31 % of phenotypic variance for adherent and non-adherent mucilage areas result from a genetic
32 differentiation among populations, respectively, a higher proportion of phenotypic variance result from
33 a genetic differentiation among accessions within populations, i.e. 42.7% and 34.1% for adherent or
34 non-adherent mucilage areas, respectively (Table 1).

1 No correlation was observed at the population level between the standardized adherent mucilage area
2 and standardized non-adherent mucilage area (Spearman's ρ 0.09, $P = 0.27$) (Fig. 3).

3 **3.2 Relationship between mucilage and environmental abiotic parameters**

4 Among population, variation of the standardized adherent mucilage area was significantly correlated
5 with several abiotic parameters collected from their native sites (Frachon et al., 2019, 2018) (Fig. 4).
6 The area of the adherent mucilage was negatively correlated with the mean of annual temperatures (Fig.
7 4a) and the mean of coldest month temperatures (Fig. 4b) and positively correlated with the precipitation
8 level during winter (Fig. 4c) and autumn (Fig. 4d). In contrast, variation of the non-adherent mucilage
9 area among populations was not correlated with any climate variables (Table 2, Supplementary Table
10 S1). In addition, while the adherent seed mucilage area was weakly associated with the total
11 concentration in nitrogen (positive relationship) and pH (negative relationship) (Supplementary Table
12 S1), no significant correlation was detected for the non-adherent mucilage with any of the variables
13 describing the agronomic properties of the soil of the native sites (Table 2, Supplementary Table S1).

14 **3.3 The phenotypic variation of the standardized areas of seed mucilage layers is highly polygenic**

15 GWA analyses revealed a polygenic architecture for both phenotypic traits, with the detection of 26
16 QTLs overlapping with 55 genes associated with the adherent mucilage area variability and 10 QTLs
17 overlapping with 28 genes associated with the non-adherent mucilage area variability (Fig. 5,
18 Supplementary Fig. S2 and S3). The 36 QTLs (corresponding to 83 ORFs) encompass 512 SNPs
19 distributed as following 198 in the coding regions (1 stop gained, 71 non synonymous mutations and
20 126 synonymous mutations), 177 in the non coding regions and 137 upstream or downstream of the
21 genes (Supplementary Table S4). Among these 36 QTLs, none were simultaneously associated with
22 both mucilage layers.

23 Some of the 83 genes identified in our study are members of multigenic families containing already
24 described myxospermy-related genes. For instance, we identified *GA2OX4* (*At1g47990*) and *SK19*
25 (*At2g03160*) for adherent mucilage (Fig. 6a), and *HB-2* (*At4g16780*), *SK41* (*At1g09840*), *CuAO α 2*
26 (*At1g31690*), and *CuAO α 3* (*At1g31710*) for non-adherent mucilage (Fig. 6b). These candidate genes
27 belong to four independent multigenic families containing gene members of the seed mucilage toolbox:
28 *GA3OX4* (*At1g80330*; (Kim et al., 2005)), *SK11/SK12* (*At4g34210/At4g34470*; (Li et al., 2018)), *HB-*
29 *25* (*At5g65410*; (Bueso et al., 2014)), and *CuAO α 1* (*At1g31670*, (Fabrissin et al., 2019)). Interestingly,
30 two multigenic families not previously shown to be involved in myxospermy were overrepresented,
31 such as the cytochrome P450 (CYP450) family with six genes (*At2g34500*, *At4g15380*, *At3g25180*,
32 *At4g15393*, *At2g34490*, *At1g13110*) and the serine carboxypeptidase like (SCPL) family with 4 genes
33 (*At2g23000*, *At2g22980*, *At2g22970*, *At2g22990*) (Fig. 6).

34 *SFP2* (*At5g27360*) is a candidate gene identified within the most significant QTL associated with the
35 adherent mucilage area (i.e. QTL 24, Fig. 5) with the presence of 42 significant SNPs within the gene

1 (Supplementary Table S3). Interestingly, *SFP2* is expressed during early seed development (Fig. 6a).
2 Worth of noting, QTL 24 also contains *SFP1* (*At5g27350*), the tandem duplicated of *SFP2*, , but
3 not expressed during seed development (Fig. 6a). For the non-adherent mucilage area, an interesting
4 polysaccharide-related candidate gene is *At1g13130*, an uncharacterized glycosyl hydrolase family 5
5 (GH5) found upstream of 22 significant SNPs within QTL 2 (Supplementary Table S3).

6 **4 Discussion**

7 In this study, we aimed at (i) exploring the natural genetic variation in myxospermy among 166 natural
8 populations of *A. thaliana* collected at a regional scale, (ii) identifying the ecological factors acting as
9 putative drivers of myxospermy, and (iii) applying recent methodologies in GWA mapping to unravel
10 the underlying genetic architecture.

11 The whole study is based on seed mucilage area variation. The main concern was the slight positive
12 correlation of seed size with the size of both mucilage layers, (Supplementary Table S1). If the cells
13 keep a proportional size in the seed, it is coherent to have larger mucilage secretory cells in larger seeds
14 (not evaluated here) and, consequently, more abundant mucilage. Nonetheless, extensive genetic
15 variation was detected for both mucilage traits after correcting for the effect of seed size variation, using
16 seed size as a covariate (Table 1).

17 Adherent and non-adherent mucilage have different polysaccharide compositions that provide structure
18 only to the former (Macquet et al., 207b). According to the hypothesis that for an equal amount of
19 synthesized mucilage, the adherent and non-adherent mucilage areas are dependent on their structural
20 polysaccharide content, the two layers of mucilage should be negatively correlated. However, adherent
21 mucilage and non-adherent mucilage were uncorrelated in this study (Fig. 3), thereby contradicting this
22 hypothesis. Consequently, the differences of mucilage area among accessions could be due to
23 differences in total amount of synthesized mucilage and/or in a change in mucilage composition. Then,
24 in this scenario, we have more likely measured a value that depends on a combination of mucilage
25 amount and composition. Note that the experimental estimation of the area of the non-adherent mucilage
26 could also reflect its ability to spread on agarose, then its structure, rather than its total amount.

27 Variation among populations in adherent mucilage is negatively correlated with temperature. It was
28 recently demonstrated that the thick adherent seed mucilage of *Lepidium perfoliatum*, another
29 myxospermous Brassicaceae species, prevents the germination under relatively low temperatures (10°C
30 and 15°C) and increases germination under higher temperatures (25°C and 30°C), in particular in
31 presence of a long light time exposure (Zhou et al., 2021). This suggests a role of the adherent mucilage
32 in germination inhibition under cold temperature until optimal conditions are obtained. The observed
33 correlation between adherent mucilage and temperature and precipitation could therefore be indirect,
34 through germination regulation. Indeed, *A. thaliana* seed mucilage was often assumed to favor
35 germination by providing more water to the embryo (Arsovski et al., 2009; Penfield, 2001). However,

1 24 h after imbibition, *A. thaliana* embryos contain less water in mucilaginous seeds than in non-
2 mucilaginous seeds (Saez-Aguayo et al., 2014). In *L. perfoliatum*, seed mucilage was shown to increase
3 drastically germination in optimal water content whereas no impact was found under osmotic stress
4 (Zhou et al., 2021). The absence of correlation between soil composition and non-adherent mucilage
5 might be explained by the variability of soil composition at the micro-scale level and also because the
6 non-adherent mucilage will rapidly spread into the ground during the first rain and thus could not
7 influence directly the seed development. However, as the non-adherent mucilage spreads into the
8 ground, it is surprising that physical parameters such as soil water holding capacity are not correlated.
9 Therefore, the fact that mucilage phenotypes are rather related to temperature and precipitation than soil
10 properties suggests a possible physiological importance of seed mucilage during underground seedling
11 development rather than during germination.

12 Fewer QTLs were found for the non-adherent mucilage than for the adherent mucilage, which is in
13 agreement with the estimates of inter-population variance (13.8 % for non-adherent mucilage compared
14 to 32.3 % for adherent mucilage). In *A. thaliana*, about one hundred genes have previously been
15 characterized for their implication in seed mucilage biosynthesis and release (Viudes et al., 2021). Our
16 GWA mapping approach allowed identifying 82 new candidate genes and only one belonging to the list
17 of previously validated candidate genes. The 100 genes already characterized from previous studies
18 together with our GWA analyses highlight the extreme polygenic character for mucilage traits. Our
19 GWAS revealed genes inducing weak phenotypes more difficult to identify with regular genetic
20 approach (reverse and forward genetics), which can in part explain the small overlap between the two
21 sets of identified genes.

22 The gene already known for its implication in seed mucilage is *CuAOα1* (*At1g31670*) encoding a copper
23 amine oxidase that was identified with GWAS on non-adherent mucilage (Supplementary Fig. S3 and
24 Table S3). The *cuaoa1* mutant has less rhamnogalacturonan I (RGI) pectin domain in its non-adherent
25 mucilage (Fabrissin et al., 2019). Interestingly, *CuAOα1* has been identified with two other genes in a
26 previous GWAS on mucilage, which did not reveal any other previously characterized myxospermy-
27 related genes (Fabrissin et al., 2019; Voiniciuc et al., 2016) illustrating the pioneer capacity of this
28 approach. In addition to its expression in seeds that is restricted to the seed coat, *CuAOα1* is expressed
29 in root tips (Klepikova et al., 2016), especially in the lateral root cap and it is induced by high nitrate
30 concentration (Gifford et al., 2008). During the lateral root cap cell formation, a pectinaceous mucilage
31 accumulates inside the sixth cell layer (c6) below the quiescent center to, *in fine* extrude root mucilage
32 around the c7 (Maeda et al., 2019). Because *CuAOα1* seems to be involved in RGI production in seed
33 mucilage (Fabrissin et al., 2019), it would be interesting to investigate as well its putative role in root
34 mucilage. Although a putative link between root mucilage and non-adherent mucilage remains elusive,
35 there is an interesting parallel between their developmental and ecological roles.

1 The identification of new putative candidate genes is also well supported by their spatio-temporal
2 expression. Among the 83 candidates, 40 are expressed in the seed coat along the Col-0 seed
3 development kinetics (Belmonte et al., 2013) (Fig. 6). Previously characterized functions, associated
4 with seed development expression data, provide interesting elements for some candidates. Intriguingly,
5 several candidate genes are related to the secondary metabolism. *IMS2/MAM3* (*At5g23020*), with a role
6 in glucosinolate biosynthesis (Petersen et al., 2019), is located upstream of 10 significant SNPs within
7 the adherent mucilage QTL 21 (Supplementary Table S3) and expressed only at the mature green stage,
8 just before seed drying (Fig. 5 and Fig. 6a). The *CYP450* gene, *CYP71A2* (*At2g34490*), contains the 17
9 significant SNPs of the non-adherent mucilage QTL 8 and shows a seed coat-specific expression
10 increasing along seed development (Fig. 6b) and extending to endosperm at mature green stage
11 (Supplementary Table S2). *CYP450* genes can be implicated in seed suberin and cutin biosynthesis
12 (Renard et al., 2020) and are often implicated in secondary metabolite decoration (Schuler and Werck-
13 Reichhart, 2003). *SCPL11* (*At2g22970*), *SNG1* (*At2g22990*), and *SCPL10* (*At2g23000*), localized within
14 non-adherent QTLs 6 and 7 (Fig. 5), have a seed coat specific expression. These three genes are serine
15 carboxypeptidase-like proteins that belong to a large multigenic family implicated in secondary
16 metabolism. *SCPL19/SNG2* (*At5g09640*) has a role in sinapoylcholine formation in *A. thaliana* seeds
17 (Shirley et al., 2001). Although a direct link between *A. thaliana* seed mucilage and secondary
18 metabolism remains elusive, a regulatory network was identified between seed mucilage, seed pigments
19 and seed secondary metabolites (Lepiniec et al., 2006; Salem et al., 2017; Viudes et al., 2020). *SFPI*,
20 the paralog of *SFP2* that is located within QTL 24, encodes a monosaccharide transporter during leaves
21 senescence (Quirino et al., 2001). The involvement of *SFP2* in sugar transport related to seed adherent
22 mucilage polysaccharides remains to be demonstrated. *At1g13130* found upstream of 22 significant
23 SNPs is a GH5 whose substrate specificity is unknown. However, GH5 family members have glycosyl
24 hydrolase activities on various substrates (<http://www.cazy.org/GH5.html>) including polysaccharides
25 found in the mucilage secretory cells (MSCs) such as cellulose, xylan or mannan and might be
26 implicated in mucilage polysaccharide hydrolysis. *At1g13130* is expressed sharply at heart and linear
27 cotyledon stages (Fig. 6a), co-occurring partially with cellulose fibrils formation since *CESA5*
28 (*At5g09870*) (main cellulose synthase involved in seed mucilage) is highly expressed at the linear
29 cotyledon stage (Sullivan et al., 2011).

30 Additionally, in other species than *A. thaliana*, seed and root mucilages were shown to change soil
31 physical properties, which in turn influence water retention and water availability (Ahmed et al., 2016;
32 Deng et al., 2014), and induce modification of the rhizospheric microbial communities (Sasse et al.,
33 2018). If the seed mucilage is also implicated in biotic interactions, it may explain the association of
34 both seed mucilage layers with several candidate genes implicated in secondary metabolism identified
35 in our GWAS, knowing that plant secondary metabolites play crucial roles during plant-microbes
36 interactions (Kessler and Kalske, 2018).

1 Conclusion

2 The seed mucilage was highly variable among the Midi-Pyrénées regional populations for both layers.
3 The detected QTLs underlying this morphological variability, contain one previously characterized gene
4 and 82 putative new seed mucilage actors. Considering the 100 genes already characterized in previous
5 studies, this highlights the extreme polygenic character of this complex trait in *A. thaliana*. The
6 candidate gene *CuAOa1* (*Atlg31670*) identified in our study for the non-adherent mucilage, was
7 previously shown to be involved in non-adherent mucilage, thereby validating the significance of our
8 approach. New candidate genes coming from our study await functional validation. The correlation of
9 adherent seed mucilage area with temperature and precipitations seems to be important and the tempting
10 indirect link of this correlation with germination will be interesting to validate through germination
11 assays using seed with or without mucilage. Altogether this study shed light on putative new genetic
12 actors and on the ecological role of seed mucilage.

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18

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4

5

6 **Table 1** Global statistics for the used GWAS model shows that variance is surprisingly more explained
7 by morphological variability between the 424 accessions than between the 166 populations. *F*
8 stands for a F-test, LTR for likelihood ratio test, *P* for the P-value and *R*² for coefficient of
9 determination ("R squared")

10 **Table 2** Spearman correlation across every populations between measured traits and their harvest site
11 environmental parameters. Indicated values are correlation coefficients that are significant after
12 FDR correction.

13

14 **Fig. 1.** Measurement of adherent (**a**) and non-adherent mucilage (**b**) of each accession belonging to the
15 166 natural populations and source picture examples illustrating the extreme area values. Each
16 individual boxplot represents the measured area distribution (μm^2) of the adherent mucilage within
17 a population. Each population contains one to three accession. For each accession 50 to 100 seeds
18 or 5 to 10 seeds are phenotyped for adherent and non-adherent mucilage respectively. Boxplots are
19 ordered by their mean value. The images correspond to the extreme values to highlight the existing
20 contrast. Measurement were realized on such images after vigorous shaking and ruthenium red
21 staining or 24 h after dry seed deposition on a Toluidine blue agarose media for adherent and non-
22 adherent mucilage respectively.

23

24 **Fig. 2.** The populations can contain either homogeneous or contrasted accessions for each mucilage
25 layers. Example of adherent (**a**) and non-adherent (**b**) mucilage standardized area values for four
26 population examples containing three homogeneous (left side) and three contrasted (right side)
27 accessions, respectively.

28

29 **Fig. 3.** The two mucilage areas are not correlated at the population level. Scatter plots of the mean value
30 of each population for the standardized adherent mucilage versus standardized non-adherent
31 mucilage areas. The black line corresponds to the linear model with its confidence interval in grey.
32 Each dot corresponds to one population containing data from its accessions. *rho* is the spearman
33 correlation coefficient.

34

35 **Fig. 4.** The adherent mucilage area is negatively correlated with temperature and positively with
36 precipitation. Graphs show the relationship between the standardized adherent mucilage area and
37 the mean annual temperature (**a**), mean coldest month temperature (**b**), winter precipitation (**c**) and
38 autumn precipitation (**d**). The Spearman correlation coefficient is indicated above each graph. The
39 black line is the linear model with its confidence interval in grey. These four environmental
40 parameters are the only ones to remain significant (P-value < 0.001) after FDR correction of P-
41 values among the tested parameters (Supplementary Table S1). Each dot corresponds to one
42 population containing data from its accessions.

43

44 **Fig. 5.** Highlight of genome QTLs explaining the morphological variability of adherent and non-
45 adherent seed mucilage standardized area. Manhattan plots representing the Lindey process value
46 for each SNP among the genome of *A. thaliana*, highlights 26 QTLs for adherent (**a**) and 10 QTLs

1 for non-adherent **(b)** seed mucilage, respectively. The threshold used for QTL selection is
2 represented by the dotted line at Lindey value of 5. Everything below is considered as background
3 noise.

4
5 **Fig. 6.** About 50% of the 83 candidate genes from the GWAS are expressed in the seed coat during seed
6 development. Fifty five and 28 selected genes are present within 26 and 10 QTLs highlighted by
7 the GWAS for adherent **(a)** and non-adherent **(b)** mucilage layers, respectively. These are
8 confronted to previously published transcriptomic data of the laser-captured seed coat during six
9 seed developmental stages (Belmonte et al., 2013). No significant values below the threshold of
10 “45” are in grey, low expressed genes are in green, highly expressed genes are in red. Note that
11 ten genes were not present in the tissue arrays. Number of significant SNPs found within the gene
12 region are indicated, genes with no value were located downstream or upstream of significant
13 SNPs. **(c)** Mucilage secretory cell (MSC) morphology for every seed developmental stage
14 available in transcriptomic (adapted from Francoz et al., 2019).

15
16 **Supplementary Fig. S1.** Relatively low variation of the three parameters measured on the Col-0
17 standard included in each batch and repartition of the observed variability of the studied natural
18 populations phenotypes after normalization with the internal Col-0 standard. **(a)** Boxplot showing
19 mean values of seed area and adherent mucilage area of the Col-0 seeds used as a standard in the
20 44 individual experimental batches during the phenotyping of studied populations. The areas are
21 expressed in μm^2 . **(b)** Boxplot showing mean values of non-adherent mucilage areas of the Col-0
22 seeds used as a standard in the 98 batches of experiment during the phenotyping of studied
23 populations. The areas are expressed in mm^2 . **(c)** Violin plot showing mean values of seed, adherent
24 mucilage, and non-adherent mucilage area normalized with the Col-standard (y axis) for each of
25 the 424 accessions. Area values are expressed relatively to the mean area of Col-0 seeds (set to 1)
26 included in each respective experimental batch. The x axis wideness of violin plot shows the
27 relative frequency of accessions for the corresponding value displayed on the y axis.

28 **Supplementary Fig. S2.** Zoom on significant QTLs related to the genetic variability of adherent
29 mucilage. Each peak is delimited by the two vertical dot lines with the first and last significant
30 SNP contained in it. Each SNP is plotted with its $-\log_{10}$ of the P-value and the colored line
31 represents the Lindley process evolution.

32 **Supplementary Fig. S3.** Zoom on significant QTLs related to the genetic variability of non-adherent
33 mucilage. Each peak is delimited by the two vertical dot lines with the first and last significant
34 SNP contained in it. Each SNP is plotted with its $-\log_{10}$ of the P-value and the colored line
35 represents the Lindley process evolution.

36
37 **Supplementary Table S1** Spearman correlation matrix across every population between measured traits
38 and their harvested site environmental parameters. Indicated values in the upper half are spearman
39 correlation coefficient and values in the down part are the corresponding p-values after an FDR
40 correction.

41
42 **Supplementary Table S2.** Expression of every candidate genes in the different seed tissues during six
43 seed developmental stages (Belmonte et al., 2013). No significant values below the threshold of
44 “45” are in grey, low expressed genes are in green, highly expressed genes are in red. Note that 10
45 genes had no spot ID in the tissue arrays.

46
47 **Supplementary Table S3** Distribution and number of significant SNPs in each QTLs. The Table shows

1 the number of SNPs identified with the GWAS performed on the adherent mucilage (left) and non-
2 adherent mucilage (right) and ID of the gene overlapping with each SNP, or found within a 2 kb
3 upstream and downstream region.

4

5

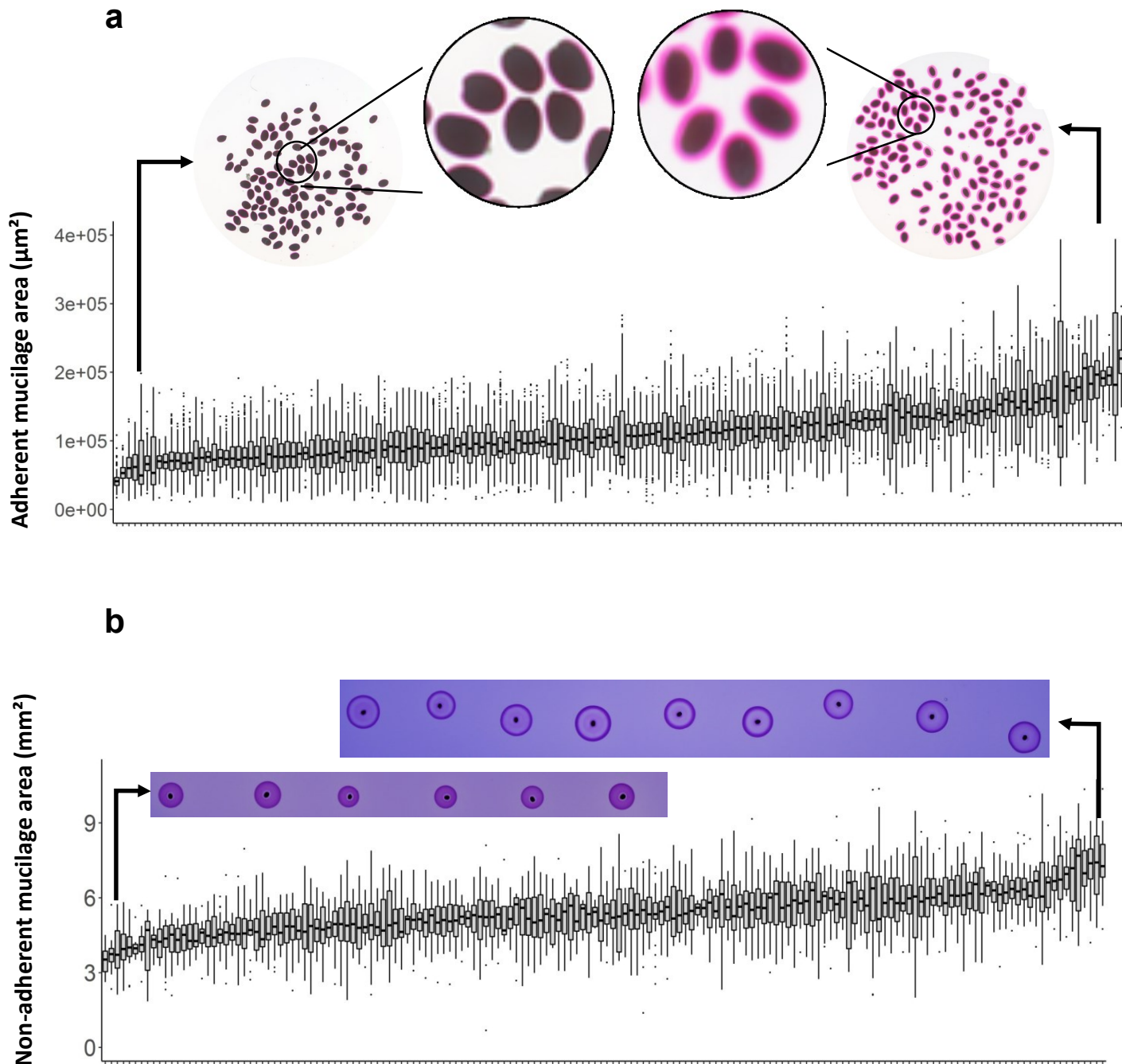


Fig. 1. Measurement of **adherent (a)** and **non-adherent mucilage (b)** of each accession belonging to the 166 natural populations and source picture examples illustrating the extreme area values. Each individual boxplot represents the measured area distribution (μm^2) of the adherent mucilage within a population. Each population contain one to three accession. For each accession 50 to 100 seeds or 5 to 10 seeds are phenotyped for adherent and non-adherent mucilage respectively. Boxplots are ordered by their mean value. The images correspond to the extreme values to highlight the existing contrast. Measurement were realized on such images after vigorous shaking and ruthenium red staining or 24 h after dry seed deposition on a Toluidine blue agarose media for adherent and non-adherent mucilage respectively.

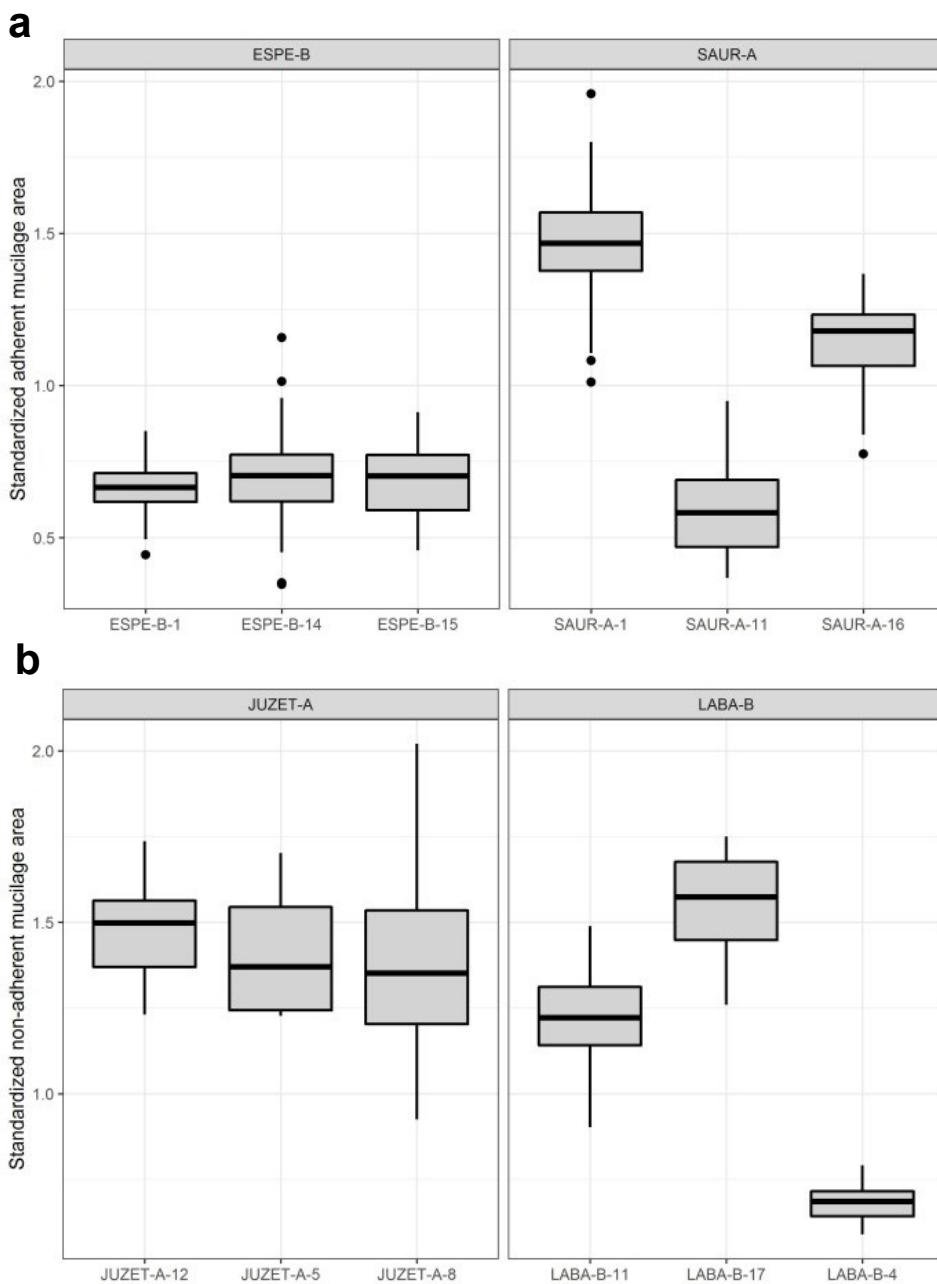


Fig. 2. The populations can contain either homogeneous or contrasted accessions for both mucilage layers. Example of adherent (**a**) and non-adherent (**b**) mucilage standardized area values for four population examples containing three homogeneous (left side) and three contrasted (right side) accessions, respectively.

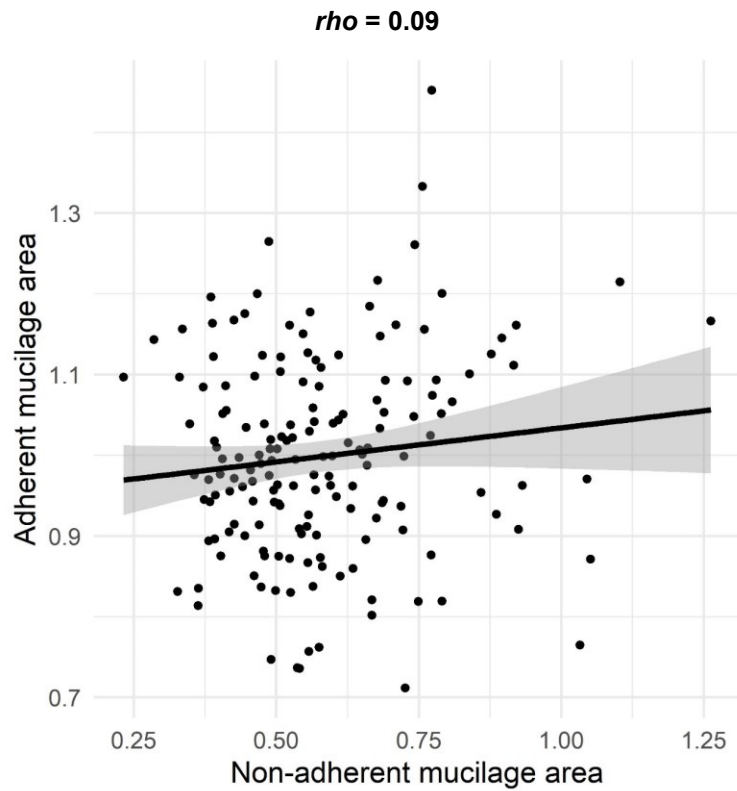


Fig. 3. The two mucilage areas are not correlated at the population level. Scatter plots of the mean value of each population for standardized adherent mucilage versus non-adherent mucilage areas. The black line corresponds to the linear model with its confidence interval in grey. **Each dot corresponds to one population containing data from its accessions.** ρ is the Spearman correlation coefficient.

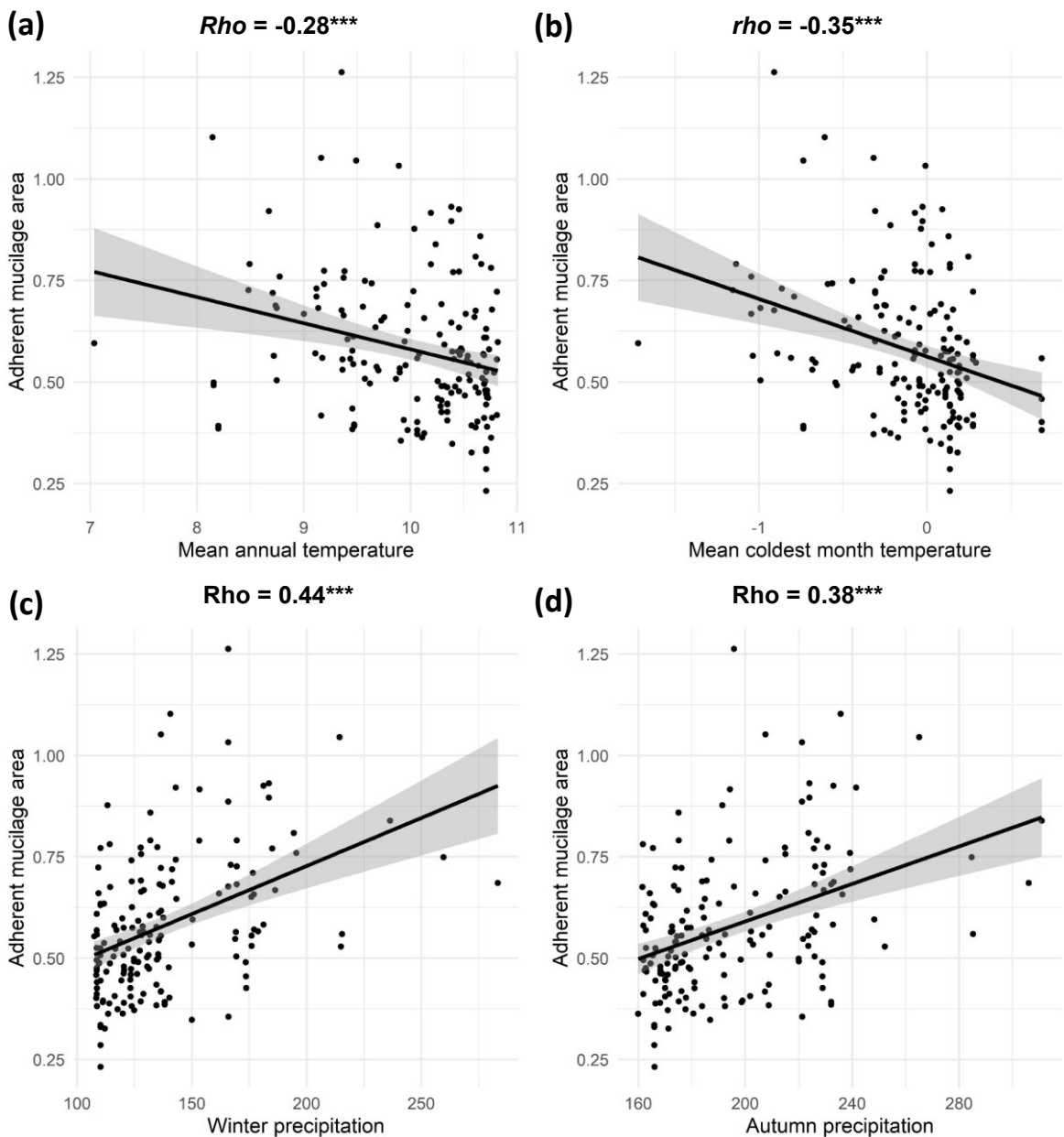


Fig. 4. The adherent mucilage area is negatively correlated with temperature and positively with precipitation. Graphs show the relationship between the standardized adherent mucilage area and the mean annual temperature (a) mean coldest month temperature (b) winter precipitation (c) and autumn precipitation (d). Spearman correlation coefficient is indicated upon each graph. Black line is the linear model with its confidence interval in grey. This four environmental parameters are the only one to remain significant (P -value < 0.001) after FDR correction of P -values among the tested parameters (Supplementary Table S1). Each dot corresponds to one population containing data from its accessions.

Table 1 Global statistics for the used GWAS model shows that variance is surprisingly more explained by morphological variability between the 424 accessions than between the 166 populations. *F* stands for a F-test, LTR for likelihood ratio test, *P* for the P-value and *R*² for coefficient of determination ("R squared")

Terms	Adherent mucilage area			Non adherent mucilage area		
	<i>F</i> or LRT	<i>P</i>	<i>R</i> ²	<i>F</i> or LRT	<i>P</i>	<i>R</i> ²
Population	2,9	3,2E-14	32,3	1,82	1,1E-05	13,8
Accession (Population)	30514,8	1,0E-16	42,7	642,3	1,0E-16	34,1
Seed area	13811,8	1,0E-32	-	ne	ne	ne
Residuals			25,0			52,1

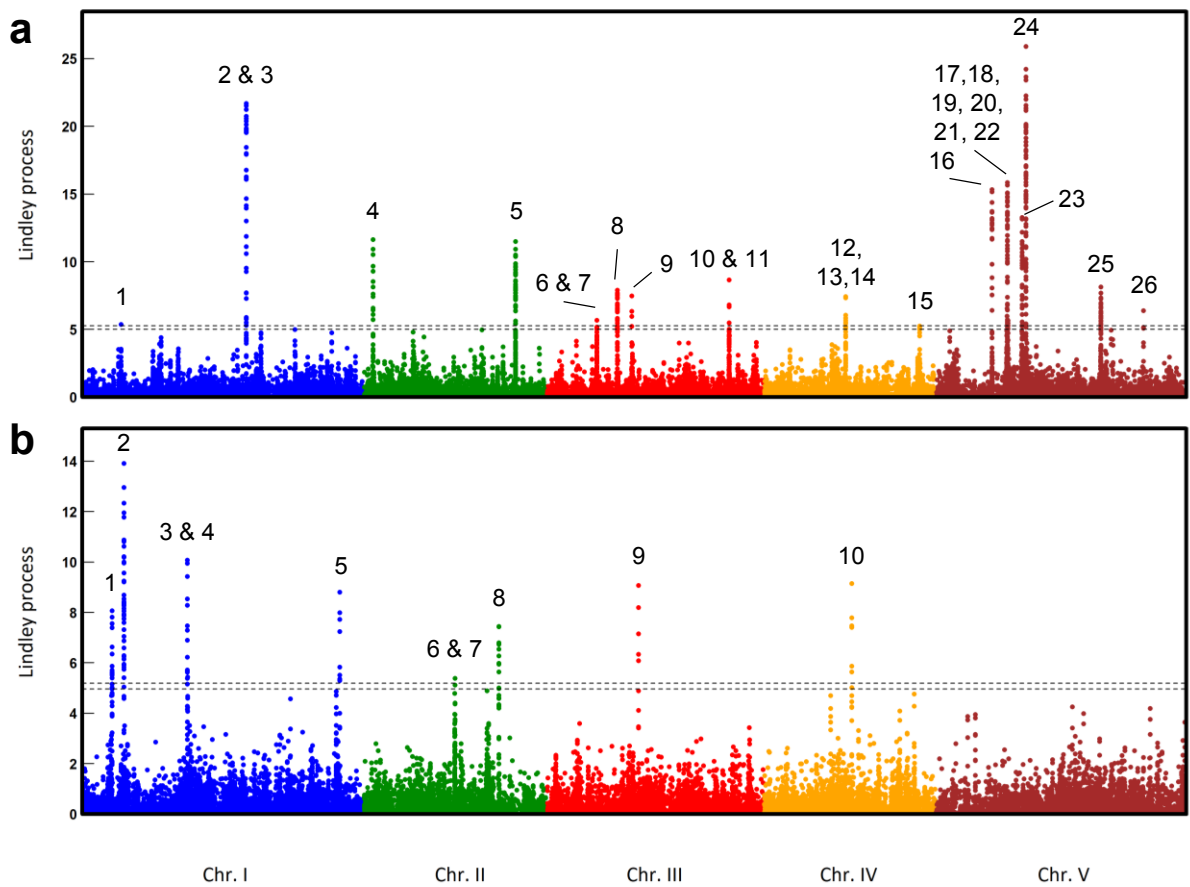


Fig. 5. Highlight of genome QTLs explaining the morphological variability of adherent and non-adherent seed mucilage layers. Manhattan plots representing the Lindley process value for each SNP among the genome of *A. thaliana*, highlights 26 QTLs for adherent **(a)** and 10 QTLs for non-adherent **(b)** seed mucilage. The threshold used for QTL selection is represented by the dotted line at Lindley value of 5. Everything below is considered as background noise.

a			Preglobular	Globular	Heart	Linear cotyledon	Bending cotyledon	Mature green
AGI code	Number of SNPs	Gene name						
At5g44080			1194	1158	898	1019	965	617
At5g22880	5	<i>HTB2</i>	736	807	217	184	94	112
At4g15390	13		11	6	5	473	727	109
At3g21610			295	487	276	340	423	370
At5g26360		<i>CCT3</i>	374	349	244	257	282	136
At1g12270		<i>HOP1</i>	344	205	228	234	107	162
At4g35260		<i>IDH1</i>	326	193	319	216	234	196
At5g27360	42	<i>SFP2</i>	213	301	276	94	42	27
At3g53110		<i>LOS4</i>	237	273	157	96	103	173
At3g21630		<i>CERK1</i>	195	87	241	83	116	107
At2g03150	14	<i>RSA1</i>	238	186	69	96	128	120
At5g44070	14	<i>PCS1</i>	124	229	121	121	193	192
At5g18200			142	124	223	93	75	83
At5g22875			149	204	184	157	148	181
At3g15940	8	<i>UGT</i>	67	75	97	158	196	143
At5g55060			64	85	105	80	89	171
At2g39130	17		65	88	110	142	84	65
At5g23020		<i>IMS2</i>	4	9	9	9	89	126
At4g35240			45	119	37	28	51	92
At3g53090	12	<i>UPL7</i>	26	48	29	32	31	96
At2g39110	9	<i>PBL38</i>	24	25	31	41	23	85
At1g47980			2	13	1	31	25	79
At2g03140			60	63	36	17	67	73
At3g25170	5	<i>RALFL26</i>	15	69	4	61	11	18
At2g39140		<i>SVR1</i>	17	48	16	22	26	15
At1g12290		<i>CC-NBS-LRR</i>	16	45	22	22	32	48
At2g39120	22	<i>WTF9</i>	45	9	35	27	20	25
At3g15950	5	<i>NAI2</i>	16	11	7	7	5	40
At1g47990	4	<i>GA2OX4</i>	2	2	1	2	15	38
At5g18210			6	18	19	14	34	12
At3g15930			15	6	2	7	15	32
At4g35250	7	<i>HCF244</i>	13	16	18	17	30	10
At1g12280	2	<i>SUMM2</i>	5	23	14	7	10	29
At2g39100			17	21	13	26	10	21
At5g27350		<i>SFP1</i>	10	8	10	10	15	25
At3g53100	3		22	25	19	10	9	11
At3g25180		<i>CYP82G1</i>	4	3	7	6	15	23
At4g15380	3	<i>CYP75A4</i>	0	6	7	23	15	17
At3g21620	26		5	11	2	19	14	21
At5g22870			0	3	8	4	8	19
At5g22900		<i>CHX3</i>	10	1	5	1	1	17
At5g22890	3	<i>STOP2</i>	2	11	2	3	4	15
At5g22860			9	4	2	4	5	14
At3g53080			5	1	2	10	9	12
At5g26340		<i>STP13</i>	4	11	5	1	1	9
At3g25165		<i>RALFL25</i>	3	11	2	1	6	10
At4g15370		<i>BARS1</i>	2	2	6	3	10	3
At1g48000		<i>MYB112</i>	1	1	5	3	3	9
At2g03160		<i>ASK19</i>	6	6	1	5	3	7
At5g44065								
At5g55056								
At3g15960								
At4g15393		<i>CYP72A5</i>						
At5g23027								
At5g27370		<i>DMP10</i>						

b			Preglobular	Globular	Heart	Linear cotyledon	Bending cotyledon	Mature green
AGI code	Number of SNPs	Gene name						
At2g34480		<i>RPL18AB</i>	947	761	654	832	924	532
At2g22970		<i>SCPL11</i>	675	361	258	92	56	40
At2g34490	17	<i>CYP71A2</i>	17	52	154	101	527	463
At1g09830		<i>PUR2</i>	283	362	361	231	238	172
At1g31670		<i>CuAOα1</i>	1	17	5	4	18	354
At2g22990		<i>SNG1</i>	245	299	81	55	22	43
At1g31330		<i>GH5</i>	35	21	172	66	6	18
At1g13120	22	<i>GLE1</i>	118	72	105	78	139	102
At1g09840	21	<i>SK41</i>	116	70	76	39	47	43
At4g16800			42	59	103	59	46	72
At2g23000		<i>SCPL10</i>	78	89	96	24	50	8
At1g31760		<i>SWIB5</i>	82	51	48	69	93	53
At1g09850		<i>XBCP3</i>	40	51	79	45	37	62
At1g31690	15	<i>CuAOα2</i>	18	17	15	14	22	39
At1g73880	8	<i>UGT89B1</i>	16	35	14	14	14	26
At1g13110		<i>CYP71B7</i>	22	29	18	34	28	32
At1g31710		<i>CuAOα3</i>	8	16	6	3	16	27
At4g16790	6	<i>HRGP</i>	4	12	18	23	24	21
At1g31770	5		10	11	4	7	6	23
At2g34500		<i>CYP71A1</i>	2	22	1	10	9	5
At4g16780		<i>HB-2</i>	3	1	8	9	17	6
At3g26880			2	3	0	1	3	15
At3g26870			8	2	2	9	13	10
At2g22980	2	<i>SCPL13</i>	7	11	2	1	5	11
At1g73880	8	<i>UGT89B1</i>						
At1g73875		<i>CCR4E</i>						
At1g31772		<i>DEFL</i>						
At1g73885								

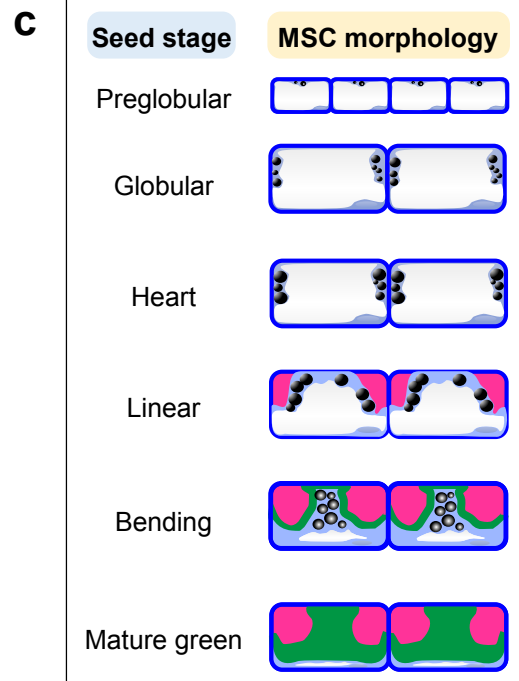


Fig. 6. About 50% of the 83 candidate genes from the GWAS are expressed in the seed coat during seed development. Fifty five and 28 selected genes are present within 26 and 10 QTLs highlighted by the GWAS for adherent (a) and non-adherent (b) mucilage layers, respectively. These are confronted to previously published transcriptomic data of the isolated seed coat during six seed developmental stages (Belmonte et al., 2013). No significant values below the threshold of “45” are in grey, low expressed genes are in green, highly expressed genes are in red. Note that ten genes were not present in the tissue arrays. Number of significant SNPs found within the gene region are indicated, genes with no value were located downstream or upstream of significant SNPs. (c) Mucilage secretory cell (MSC) morphology for every seed developmental stage available in transcriptomic (adapted from Francoz et al., 2019).

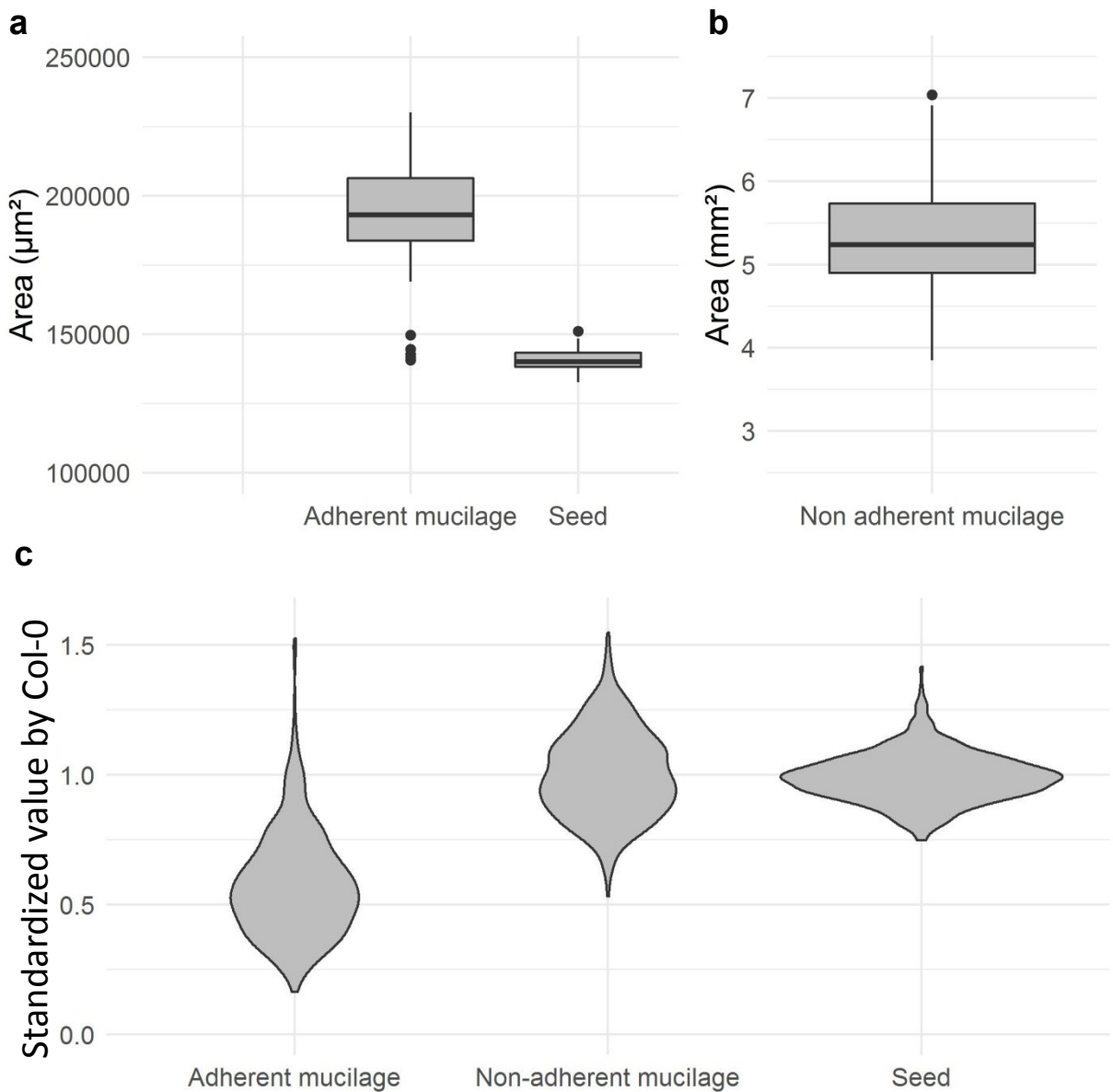


Fig. S1. Relatively low variation of the three parameters measured on the Col-0 standard included in each batch and repartition of the observed variability of the studied natural populations phenotypes after normalization with the internal Col-0 standard. **(a)** Boxplot showing mean values of seed area and adherent mucilage area of the Col-0 seeds used as a standard in the 44 individual experimental batches during the phenotyping of studied populations. The areas are expressed in μm^2 . **(b)** Boxplot showing mean values of non-adherent mucilage areas of the Col-0 seeds used as a standard in the 98 batches of experiment during the phenotyping of studied populations. The areas are expressed in mm^2 . **(c)** Violin plot showing mean values of seed, adherent mucilage, and non-adherent mucilage area normalized with the Col-standard (y axis) for each of the 424 accessions. Area values are expressed relatively to the mean area of Col-0 seeds (set to 1) included in each respective experimental batch. The x axis wideness of violin plot shows the relative frequency of accessions for the corresponding value displayed on the y axis.

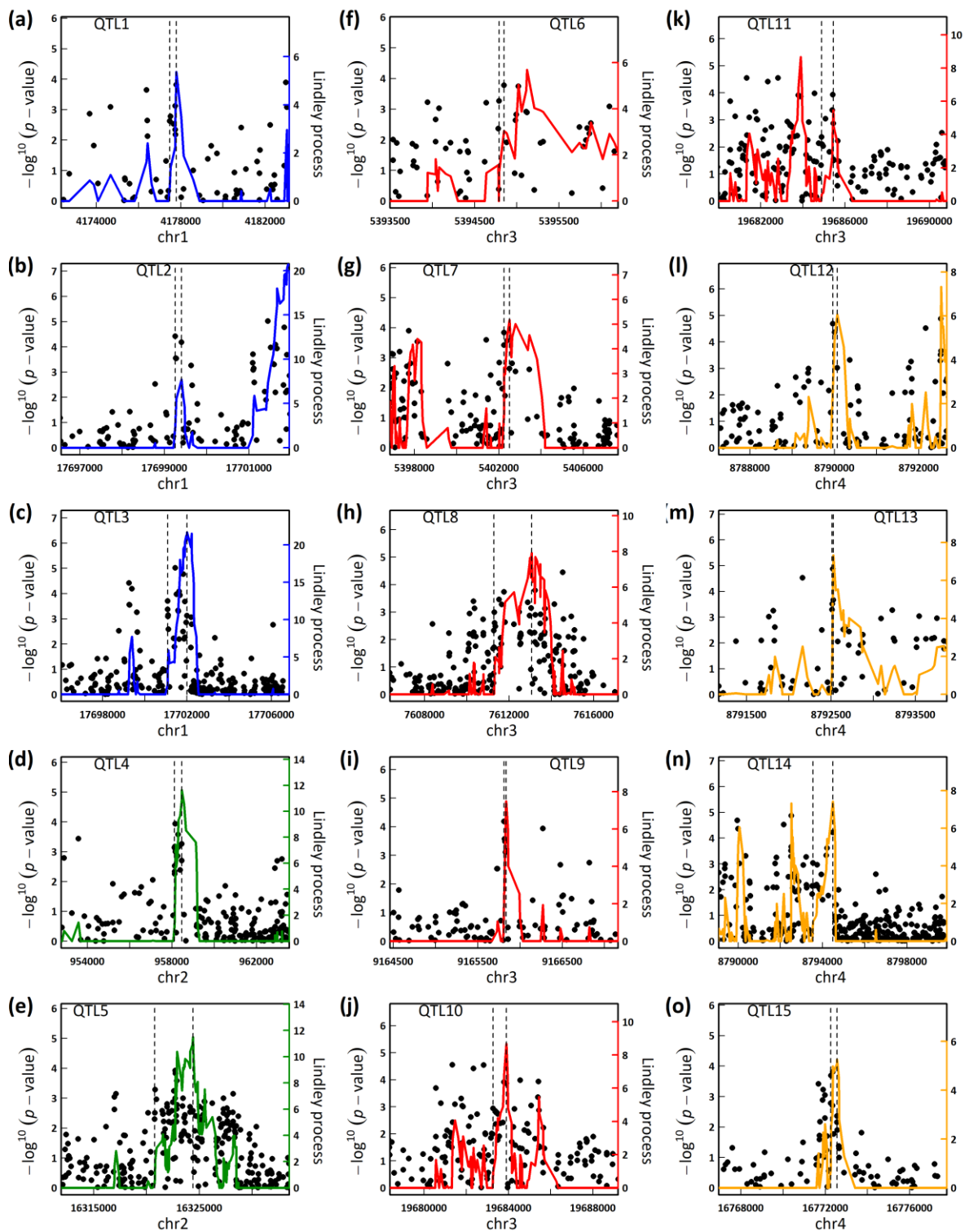


Fig. S2. Zoom on significant QTLs related to the genetic variability of adherent mucilage. Each pic is delimited by the two vertical dot lines with the first and last significant SNP contain in it. Each SNP is plotted with its $-\log_{10}$ of the P-value and the colored line represent the Lindley process evolution.

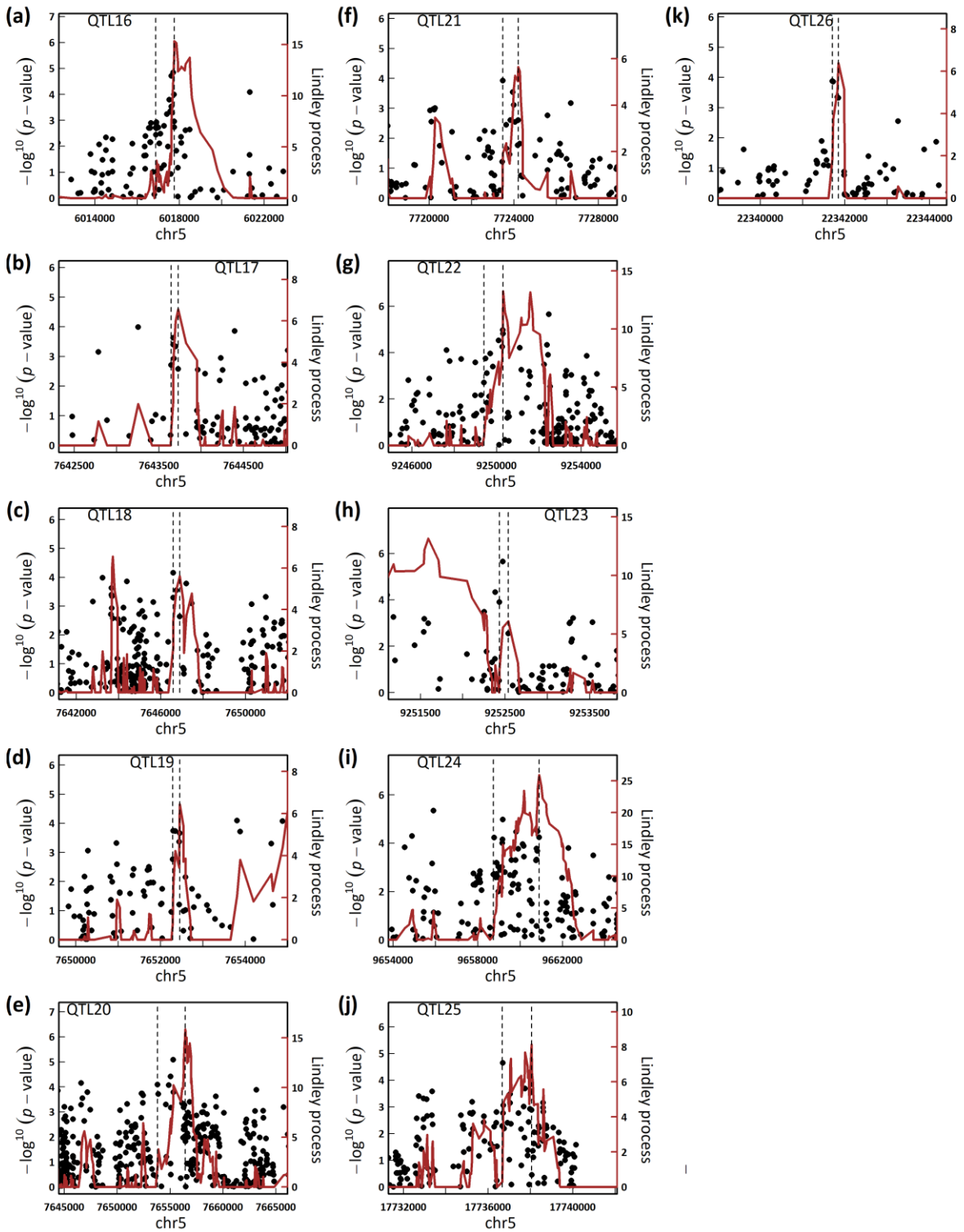


Fig. S2 (following). Zoom on significant QTLs related to the genetic variability of adherent mucilage. Each pic is delimited by the two vertical dot lines with the first and last significant SNP contain in it. Each SNP is plotted with its $-\log_{10}$ of the P-value and the colored line represent the Lindley process evolution.

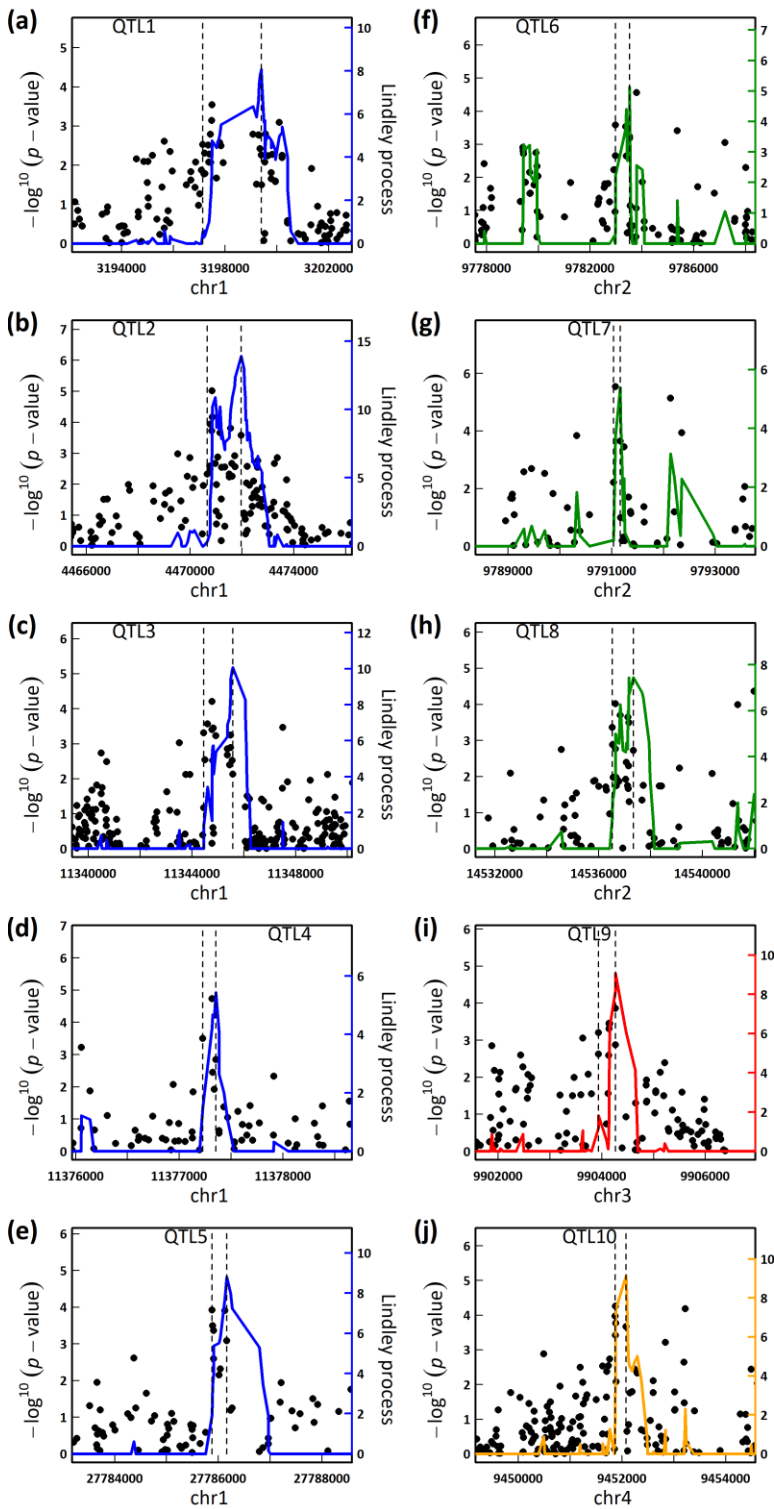


Fig. S3. Zoom on significant QTLs related to the genetic variability of non-adherent mucilage. Each pic is delimited by the two vertical dot lines with the first and last significant SNP contained in it. Each SNP is plotted with its $-\log_{10}$ of the P-value and the colored line represent the Lindley process evolution.

Table S3 Distribution and number of significant SNPs in each QTLs. The table shows the number of SNPs identified with the GWAS performed on adherent mucilage (left) and non-adherent mucilage (right) and the ID of the gene overlapping with each SNP, or found within a 2 kb upstream and downstream region.

Adherent mucilage					Non-adherent mucilage				
QTLs	SNPs	Overlap ID	Upstream ID	Downstream ID	QTLs	SNPs	Overlap ID	Upstream ID	Downstream ID
QTL1	2	AT1G12280	AT1G12270	AT1G12290	QTL1	21	AT1G09840	AT1G09830	AT1G09850
	5	NA	AT1G12280	AT1G12290	QTL2	22	AT1G13120	AT1G13110	AT1G13130
QTL2	4	AT1G47990	AT1G47980	AT1G48000	QTL3	15	AT1G31690	AT1G31670	AT1G31710
QTL3	23	NA	AT1G47990	AT1G48000	QTL4	5	AT1G31770	AT1G31760	AT1G31772
QTL4	14	AT2G03150	AT2G03140	AT2G03160	QTL5	8	AT1G73880	AT1G73875	AT1G73885
QTL5	9	AT2G39110	AT2G39100	AT2G39120	QTL6	2	AT2G22980	AT2G22970	AT2G22990
	1	NA	AT2G39110	AT2G39120		4	NA	AT2G22980	AT2G22990
	22	AT2G39120	AT2G39110	AT2G39130	QTL7	3	NA	AT2G22990	AT2G23000
	17	AT2G39130	AT2G39120	AT2G39140	QTL8	17	AT2G34490	AT2G34480	AT2G34500
QTL6	8	AT3G15940	AT3G15930	AT3G15950	QTL9	10	NA	AT3G26870	AT3G26880
QTL7	5	AT3G15950	AT3G15940	AT3G15960	QTL10	6	AT4G16790	AT4G16780	AT4G16800
QTL8	26	AT3G21620	AT3G21610	AT3G21630					
QTL9	5	AT3G25170	AT3G25165	AT3G25180					
	12	AT3G53090	AT3G53080	AT3G53100					
QTL11	5	AT3G53090	AT3G53080	AT3G53110					
	3	AT3G53100	AT3G53090	AT3G53110					
QTL12	3	AT4G15380	AT4G15370	AT4G15390					
QTL13	4	NA	AT4G15380	AT4G15390					
QTL14	13	AT4G15390	AT4G15380	AT4G15393					
	1	NA	AT4G15390	AT4G15393					
QTL15	7	AT4G35250	AT4G35240	AT4G35260					
QTL16	20	NA	AT5G18200	AT5G18210					
QTL17	6	NA	AT5G22860	AT5G22870					
QTL18	4	NA	AT5G22860	AT5G22870					
QTL19	5	AT5G22880	AT5G22875	AT5G22890					
	3	AT5G22890	AT5G22880	AT5G22900					
QTL20	16	NA	AT5G22890	AT5G22900					
	10	NA	AT5G23020	AT5G23027					
QTL22	14	NA	AT5G26340	AT5G26360					
QTL23	3	NA	AT5G26340	AT5G26360					
QTL24	42	AT5G27360	AT5G27350	AT5G27370					
QTL25	14	AT5G44070	AT5G44065	AT5G44080					
	5	NA	AT5G44070	AT5G44080					
QTL26	4	NA	AT5G55056	AT5G55060					