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Genetic determinism of prickles in rose

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Key message: The genetic determinism of prickle in rose is complex, with a major locus on LG3 that controls the absence/presence of prickles on the rose stem.

Declarations

Author Contributions

Contributions

NN Zhou designed the projects and obtained funding, planned and performed experiments, collected and analyzed data, and drafted the manuscript. F. Foucher and L. Hibrand-Saint Oyant were responsible for piloting and supervising the project, and for revising the manuscript. KX Tang contributed to designing the project and obtaining funding. J. Jeauffre led the qPCR experiment and analyzed data. T. Thouroude prepared the plant cuttings of F₁ individuals in the greenhouse. D. Lopez Arias modified the genetic map and helped to develop the R/qtl script.

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31 **Abstract**

32 Rose is one of the major ornamental plants. The selection of glabrous cultivars is an
33 important breeding target but remains a difficult task due to our limited genetic
34 knowledge. Our objective was to understand the genetic and molecular determinism of
35 prickles. Using a segregating diploid rose F₁ population, we detected two types of
36 prickles (glandular and non-glandular) in the progeny. We scored the number of non-
37 glandular prickles on the floral and main stems for three years. We performed QTL
38 analysis and detected four prickle loci on LG1, 3, 4 and 6. We determined the credible
39 interval on the reference genome. The QTL on LG3 is a major locus that controls the
40 presence of prickles, and three QTLs (LG3, 4 and 1) may be responsible for prickle
41 density. We further revealed that glabrous hybrids are caused by the combination of the
42 two recessive alleles from both parents. In order to test if rose prickles could originate
43 from a 'trichome-like structure', we used a candidate approach to characterize rose gene
44 homologues known in *Arabidopsis*, involved in trichome initiation. Four of these
45 homologues were located within the overlapping credible interval of the detected QTLs.
46 Transcript accumulation analysis weakly supports the involvement of trichome
47 homologous genes, in the molecular control of prickle initiation. Our studies provide
48 strong evidence for a complex genetic determinism of stem prickle and could help to
49 establish guidelines for glabrous rose breeding. New insights into the relationship
50 between prickles and trichomes constitute valuable information for reverse genetic

51 research on prickles.

52 **Keywords**

53 Trichome, QTL, *ZFP5*, *GIS2*, *MYB61*, *MYC1*

54 **Introduction**

55 Rose is the major ornamental plant worldwide with a wide diversity, diverse application
56 forms and an extensive cultivated area. Roses are sold as cut flowers, garden plants, in
57 pots, for essential oil, flower tea and culinary purposes. In past centuries, with the
58 continuous efforts of breeders, more than 33,000 varieties of roses were created (Young
59 2007). However, most of these varieties have persistent prickles on the stem. Prickles
60 can protect against herbivores by deterring them from eating the stem (Ronel and Lev-
61 Yadun 2012; Burns 2014). Furthermore, prickles can be desirable in roses when they
62 are used in hedges to protect properties (as was the case in Reunion Island during the
63 19th century). However, garden roses without prickles are often desirable. Cut roses
64 with prickles are more difficult to handle, harvest and transport and also constitute
65 safety hazards for consumers and workers. Retailers commonly remove prickles from
66 stems prior to sale. Removing the prickles increases labor costs and causes mechanical
67 damage to the stems, which affects vase life and ornamental value. Although a strong
68 market demand to develop roses without prickles exists (Nobbs 1984; Debener 1999;
69 Canli 2003; Canli and Skirvin 2003; Canli and Kazaz 2009), relatively little is known
70 about the genetic and molecular bases of prickle initiation and development.

71 In plants, prickles are described as outgrowths of the epidermis and subjacent layers

72 that lack vasculature, and mainly consist of lignin, suberin, cellulose and hemicellulose
73 (Asano et al. 2008; Li et al. 2012). In rose and raspberry, it was thought that prickles
74 were modified glandular trichomes that differentiate at the time of lignification into
75 their final prickle morphologies (Kellogg et al. 2011).

76 Until recently, only a few studies had been published about the molecular regulation of
77 prickle development, but great progress has been made in trichome initiation and
78 development, especially in *Arabidopsis*. Several transcription factors (TFs) such as
79 MYB, bHLH, WD40, WRKY and C2H2 zinc finger families' proteins have been
80 identified as being involved in trichome initiation and development (reviewed in
81 Balkunde et al. (2010), Pattanaik et al. (2014), Ma et al. (2016a), Huchelmann et al.
82 (2017) and Chopra et al. (2019)). A trimeric activator complex consisting of MYB
83 (GLABRA1) - bHLH (GLABROUS3/ENHANCER OF GL3) - WDR
84 (TRANSPARENT TESTA GL1) plays a key role in trichome development (Zhang 2003;
85 Kirik et al. 2005; Patra et al. 2013). This trimeric complex finely regulates the temporal
86 and spatial expression of *GLABRA2* (*GL2*) and *TRANSPARENT TESTA GL2* (*TTG2*),
87 determining the fate and pattern of trichome precursor cells (Rerie et al. 1994; Ishida et
88 al. 2008). The bHLH family genes, *MYC1* and *TT8*, belong to the same clade as *GL3*.
89 *AtMYC1* acts as a positive regulator of trichome initiation (Symonds et al. 2011; Zhao
90 et al. 2012), and *AtTT8* controls trichome development on leaf margins (Maes et al.
91 2008). AaMYB1 and its orthologue AtMYB61, belonging to the R2R3MYB subfamily,
92 were thought to affect terpene metabolism and trichome development in *A. annua* and
93 *A. thaliana*, respectively (Matías-Hernández et al. 2017).

The active TTG1 trimeric complex can be repressed by R3 MYB subfamily genes: *TRY/CPC/TCL1* act as negative regulators by competing with *GL1* for binding to *GL3* (Wang et al. 2008; Wester et al. 2009; Wang and Chen 2014). The active TTG1 complex, in interaction with *TTG2*, regulates the expression of the R3 MYB inhibitors that move to the neighboring cells where they repress trichome initiation (Pesch and Hülskamp 2004; Pesch et al. 2014).

Different growth regulators positively affect trichome initiation, such as GA3, cytokinin and jasmonic acid (Traw and Bergelson 2003), through the activation of *GL1* (Gan et al. 2006). Different C2H2 zinc-finger proteins such as *GLABROUS*, *INFLORESCENCE STEM* (*GIS*), *GIS2*, *GIS3*, *ZINC FINGER PROTEIN5*, 6 and 8 (Gan et al. 2006; Gan et al. 2007) include GA and cytokinin signaling pathways (Zhou et al. 2013). The novel transcription factor *TRP* interacts with *ZFP5* and negatively regulates trichome initiation through the gibberellic acid pathway (Kim et al. 2018).

In diploid rose, the presence of prickles on the stem was assumed to be controlled by a single dominant gene (Debener 1999; Shupert et al. 2007) located on linkage group 3, LG3 (Linde et al. 2006). Furthermore, two QTLs were detected on LG3 with the scoring of prickle density (Crespel et al. 2002). Using two F₁ progenies, Hibrand-Saint Oyant et al. (2018) also identified a large QTL (or two neighboring QTLs) on LG3 (between position 31 Mb - 46.5 MB corresponding to the end of the chromosome 3) and a significant association between position 31 and 32.4 Mb using a GWAS approach. In tetraploid roses, three QTLs were identified in relation to the number of prickles on the stem: two located on LG2 and one on LG3 (Koning-Boucoiran et al. 2009). Using the

same K5 population with the same phenotype data but a new genetic map, different QTLs were detected on LG3, 4 and 6 and on LG2 (one year) (Bourke et al. 2018). Recently, a WRKY transcription factor, homologous to Arabidopsis *TTG2*, was located close to a QTL controlling prickles density, and the gene transcripts are differentially accumulated between prickly and prickless roses (Hibrand-Saint Oyant et al. 2018). In this study, our objectives were to decipher the genetic determinism of stem prickles in rose and to characterize candidate genes involved in prickles initiation and development. First, we defined the different types of prickles on the stem and studied them separately. Using an F₁ progeny, we detected QTLs and their position in the rose genome sequence. We further analyzed how the alleles of the major QTLs affect the presence of prickles. We identified putative candidate genes (homologues of genes involved in trichome initiation and development in Arabidopsis) and studied their transcript accumulation. That study suggested that prickles and trichomes may carry two different genetic pathways, providing new insights into the relationship between prickles and trichomes.

Materials and methods

Plant material

A progeny of 151 diploid F₁ hybrids obtained from a cross between the female *Rosa chinensis* ‘Old Blush’ (OB) × the male *R. x wichurana* (RW) was used for map construction (described in Hibrand-Saint Oyant et al. 2018) and QTL analysis. The plants were grown in a field and managed by the Horticulture Experimental Unit (INRAE, Angers, France). The plants were pruned each December. In the following

spring, new stems developed from the axillary buds from the old pruned stems, and are referred to as “floral stems” since they develop flowers. Later, new stems arise from the base of the plant and are referred to as “main stems”. They remain vegetative in once-flowering individuals and may become floral in continuous-flowering individuals.

Phenotypic data collection and analyses

To score prickly density, we selected three independent floral and main stems for each F₁ progeny and the two parents. The prickly numbers were counted for each selected stem on four internodes (located in the middle of the stem) for three years (2016, 2017 and 2018).

Statistical analysis and visualization were performed using R version 3.2.3. We visualized the frequency distribution and Q-Q plot using the *hist*, *legend*, *qqnorm* and *qqline* functions. We performed mixed-factorial ANOVA analysis with *aov*. A *shapiro.test* was used to test the normality of the original data and the ANOVA residuals. When the null hypothesis was negated, *kruskal.test* was used to test if there was any significant difference between the replicate shoots, years and the type of stem variance. *pairwise.wilcox.test* with *p.adjust.method = BH* was used to calculate pairwise comparisons between group levels with corrections for multiple testing. We displayed the distribution of prickly density with a boxplot to compare the difference between the variance using the *ggplot2* and *ggpubr* packages.

Variance components were estimated with the restricted maximum likelihood (REML) method using the ‘*sommer*’ package. Phenotype variance components of prickles density were obtained using the following model:

$$P_{ijlr} = \mu + G_i + S_l + Y_{(l)j} + GS_{il} + GY_{ij} + \varepsilon_{ijlr},$$

where P_{ijlr} is the phenotypic value of a trait counted on a triplicate stem r of the stem type l of the individual i in the year j , μ is the overall mean, G_i is the random effect of genotype i , S_l is the random effect of stem type l , $Y_{(l)j}$ is the random effect of year j nested in stem type l , GS_{il} is the random interaction between genotype i and stem type l , GY_{ij} is the random interaction between genotype i and year j , and ε_{ijlr} is the random residual error.

The phenotypic variance (σ_P^2) of stem prickles was divided into the variance of genotypic effect (σ_G^2), genotype \times year interaction (σ_{GY}^2), genotype \times stem type interaction (σ_{GS}^2), and the residual error variance (σ_E^2).

Narrow-sense heritability (h^2) was calculated as follows:

$$h^2 = \sigma_G^2 / (\sigma_G^2 + \sigma_{GY}^2 / y + \sigma_{GS}^2 / s + \sigma_E^2 / ysr)$$

where y is the number of years, r is the number of replication shoots per individual, and s is the number of stem types (PF and PM).

Genotypic data

The genetic determinism was conducted using the genetic map previously obtained by Hibrand-Saint Oyant et al. (2018) and modified by Lopez-Arias et al. (in prep).

QTL Analysis

In this study, we performed QTL detection for prickles on the floral (PF) and main (PM)

stems from data scored in 2016, 2017, 2018 (referred to as PF2016, PF2017, PF2018, PM2016, PM2017 and PM2018, respectively). QTL analyses were carried out using the R/qtl in R version 3.2.3. Based on the non-normal phenotype distribution data, single QTL analysis and LOD scores were calculated using the ‘scanone’ function with non-parametric model (*model="np", ties.random = FALSE, method = "em"*) and the two-part model (*model="2part", upper = FALSE*) (Boyartchuk et al. 2001).

In the non-parametric model, the genome-wide and chromosome-wide significance thresholds of LOD scores were estimated by permutations tests (*n.perm = 1000, n.cluster = 20*). The Bayesian credible interval was computed with 0.95 and 0.99 coverage probabilities. When QTLs for different traits had overlapping 0.95 credible intervals, they were declared to be a potentially “common QTL (cQTL)” (Kawamura et al. 2011). The percent of variance explained by each QTL was calculated by ‘makeqtl’ and ‘fitqtl’ with a ‘normal’ model.

In the two-part model, the phenotype was separated into two parts: first, the trait value was considered as without (0) or with (1) prickles; if it had prickles, the trait value above zero was assumed to be normally distributed. Three LOD scores for each genomic position were calculated: $LOD(p)$ and $LOD(\mu)$ were calculated for binary traits (0 or 1) and non-zero phenotype quantitative traits (> 0), respectively; $LOD(p, \mu)$ is simply the sum of the LOD scores from the two separate analyses (Broman 2003). The genome-wide significance thresholds of three LOD hypotheses were also estimated by 1000 permutation tests and summarized by a 0.05 alpha threshold. The percent of variance explained was calculated by ‘makeqtl’ and ‘fitqtl’ with ‘binary’ and ‘normal’

models for binary(p) and quantitative(μ) traits.

Selection of rose candidate genes involved in prickly density

Proteins involved in trichome initiation and development were selected in *A. thaliana* from the TAIR database (<https://www.arabidopsis.org>) with searching terms GL1, MYB82, MYB61, CPC, TRY, GL3, TT8, MYC1, TTG1, TTG2, ZFP5, ZFP1, GIS2, GIS3, GL2. Rose homologues were searched using BLASTp in the *Rosa chinensis* Genome v1.0 (Hibrand-Saint Oyant et al. 2018). In addition, we also searched the transcription factors (TF) belonging to the bHLH, WD40, R2R3MYB, C2H2 and WRKY families in rose and which were located on the major cQTL interval of LG3. Using Geneious 9.1.7, 'Multiple Align' was performed for the family gene sequences. Conserved domains were used to build phylogenetic trees using the 'Geneious Tree Builder' tool with the Jukes-Cantor genetic distance model and the UPGMA tree build method. The rose candidate genes were named according to the following nomenclature corresponding to *Rc* (for *Rosa chinensis*) added to the corresponding gene name in Arabidopsis, e.g., *RcTTG2* for the rose *TTG2* homologue.

Gene expression analysis

Primers were designed using Primer Premier 5.0 software. To ensure the specificity of the primers, forward and reverse primers were designed in the last exon and in the beginning (first 100 bp) of the 3'UTR. Primer length was between 18 and 25 bp, product length was between 70 and 200 bp, GC content was between 40% and 60%, and the annealing temperature was 58~65 °C. Primers are listed in Supplementary Table 1.

For the qPCR experimental design, we selected four contrasting once-flowering

223 individuals from the OW progeny for prickles density: two with no prickles (OW9067
224 and OW9068) and two with prickles (OW9137 and OW9071 with means of 2.5 and 4
225 prickles per internode on the main stem, respectively). The materials were sampled in
226 April 2018 in a greenhouse (three biological replicates). Stems were harvested at
227 different stages of prickles development for roses with prickles, and stems at the same
228 stages for roses without prickles (Supplementary Figure 1). Total RNA was extracted
229 using the NucleoSpin RNA Plus-XS kit for early stages (I and IIa) and using the
230 NucleoSpin RNA Plus-kit for later stages (IIb, IIc and III) according to the
231 manufacturer's instructions, with minus modifications (2%PVP40 in lysis buffet). The
232 purity of the RNA was checked on 1% agarose gel, and the concentration was measured
233 by an UV spectrophotometer. cDNA was obtained from 500 ng of total RNA using
234 iScript™ Reverse Transcription Supermix for RT-qPCR (Bio-Rad, Hercules)
235 accordant to the manufacturer's instructions. The purity and quality of the cDNA were
236 checked by performing PCR amplification with a blank and RW's DNA sample control,
237 and the concentration was measured with a UV spectrophotometer. RT-qPCR reactions
238 were performed using the soAdvanced™ Universal SYBR® Green Supermix (Bio-
239 Rad) on the CFX Connect Real-time PCR system (Bio-Rad). The gene efficiency was
240 evaluated with a serial dilution of the thirty cDNAs pooling (1:10, 25, 50, 100, 250,
241 500, 1000). A 1:25 dilution of each cDNA was used to analyse the expression pattern
242 of ten candidate genes and two reference genes *UBC* and *TCTP* (Randoux et al. 2012).
243 Data collection was performed using the Bio-Rad CFX Maestro1.1. Amplification
244 efficiency of the ten genes ranged from 90.5-104.1%. The reference genes *UBC* and

TCTP presented high expression stability in all the samples.

For the technical replicates, potential outliers were excluded from the analysis when the standard deviation (SD) of samples is higher than 0.5. Only seven technical replicates (seven out of 390) were excluded: CPC in PIIB (biological group A, C) and in NPIIC (group A), GIS2 in NPIIC (group C), NPIII (group B) and PIII (group B).

Normalized expression ($\Delta\Delta Cq$) was calculated using Bio-Rad Maestro1.1 software by applying the ‘*gene study*’ tool. The cluster analysis for sample and target genes with the mean value of normalized expression was performed using R software with the ‘*pheatmap*’ package. NP samples were used as controls to compare the normalized expression of genes between P and NP samples in the different stages. |Fold change (FC)| >2 and the Wilcoxon signed rank test (p-value < 0.05) as cut-off values in scatter plots were used to demonstrate the significant difference of normalized expression between P and NP samples. NPI was used as a control to visualize the relative normalized expression during stem development in prickly and glabrous stems.

Results

1-Type, distribution and genetic variability of stem prickles in OW progeny

Both parents of the F₁ progeny (‘Old Blush’ and *R. x wichurana*) present prickles on their stems (Figure 1a) (a mean of around ten prickles on four internodes). In the F₁ progeny, hybrids without prickles can be observed (14 out of 151; no prickles on the three stems scored over three years). These hybrids with glabrous stems (Figure 1b) are referred to as ‘prickless’ individuals (Figure 1c). Out of the 137 F₁ individuals with prickles (Figure 1b), nine hybrids were nearly prickless (prickle number on four

internodes < 1 for three scored years and two types of stems; Figure 1d, Supplementary Figure 2), and numerous stems were glabrous for some individuals, whereas other stems presented a few prickles (variable between the genotypes with unstable states between years and types of stems). Macroscopic analysis shows that parents that present prickles originated from a ‘non-glandular’ structure. These prickles are referred to as Non-Glandular Prickles (NGP). All the F_1 prickly individuals (137 out of 151) have NGP. However, some individuals with NGP prickles also present another type of prickle (27 out of 137). These prickles present a ‘glandular head’ structure and are referred to as Glandular Prickles (GP) (Figure 1b and 1c, Supplementary Figure 2). Since the presence of GP in the OW progeny is rare (27 and 12 out of 151 on flowers and main stems, respectively; Figure 1d) and very irregular, we decided to consider only NGPs in this study.

For the 151 F_1 progenies, the number of NGPs on four internodes of floral (PF) and main (PM) stems ranged from 0 to 52 and from 0 to 48, respectively (Supplementary Figure 2). Among them, OW9106 and OW9107 have a much higher prickle density (28 to 52 in the scored years) than the others. As for the two parents, OB and RW have an average of 11.1 and 8.7 on PF, and an average of 11.8 and 9.2 on PM, respectively (Figure 1a, Table 1). The ranges of NGP numbers in the F_1 hybrids were obviously beyond the values of the two parents, indicating a transgressive segregation.

The Shapiro-Wilk normality test and the Q-Q plot of original data ($W = (0.692\sim 0.936)$, $p < 2.96 \times 10^{-8}$) (Supplementary Figure 2) and variance residuals ($W = 0.88591$, p -value $< 2.2e^{-16}$) showed that the NGP densities on stems in the F_1 population were not

normally distributed. We tried to transform data (log10, SQRT, box-cox) to make them normal but without success. The Kruskal-Wallis test reveals a genotype effect, a year effect and a stem effect (Table 1). The high heritability ($h^2 \approx 0.97$) demonstrated that the genetic analyses of stem prickles of this population were reliable (Table 1).

2- QTL analysis

2.1 Non-parametric QTL analysis

For the female and male maps, strong QTLs were detected on LG3 for the two types of stems and for the three years ([Figure 2](#) and [Table 2](#)). The LOD scores are higher for the male map (between 8 and 11.5) and relatively lower for the female map (between 2.3 and 6.2). These QTLs explained between 6.65 to 37.4% of the phenotypic variance. The locations of these QTLs are very close. Indeed, on the female map, the marker at the peak of the QTLs is the same for both types of stems (Rh12GR_16570_782, 51.1 cM, located on the chr3 at 44,459,262 bp according to the *Rosa chinensis* Genome v1.0 (Hibrand-Saint Oyant et al. 2018)), except for PM2018 (Rh12GR_34665_95, 45.7 cM, located on chr3 at 41,401,120 bp). On the male map, for the two types of stems and for the three years, the marker with the highest LOD for the QTLs detected on LG3 is the same, Rh12GR_52506_1218 (42.6 cM on the LG3, 42,317,122 bp on Chr3), which is the terminal marker on the genetic map but not on the physical map.

Furthermore, if we consider the common 0.95 Bayesian credible interval of these QTLs on LG3 on the female and male maps, all intervals are overlapping (Table 2 and Figure 4). For the female map, the interval on LG3 was 40.38-53.75 cM, which corresponds to the interval 36,517,224-46,440,369 bp on the physical map of chr3 (Figure 4a), and

311 for the male map, the interval on LG3 was 37.69-42.55 cM, corresponding to the
 312 interval 41,648,024-42,317,122 bp on the physical map (Table 2, Figure 4b).
 313 On LG4, QTLs were only detected on the female map for the main stem for the three
 314 years (Figure 2, Table 2). The peak marker Rh12GR_60129_183 located at 30.6 cM,
 315 which is located on chr4 at 52,239,028 bp, explained 10.35 to 13.18% of the observed
 316 variance depending on the year of the phenotypic variance in the single QTL model.
 317 The common 0.95 credible interval on LG4 was 20.53-48.59 cM, which covered from
 318 46,189,407-56,107,784 bp on the physical map (Figure 4a, Table 2).
 319 On LG6, QTLs were only detected on the male map for three years for PM and for two
 320 years (2017 and 2018) for PF (Figure 2 and Table 2). For PM (2016, 2017 and 2018)
 321 and PF (2017), the peak marker is the same, Rh12GR_56601_1304 (29.7 cM, located
 322 on chr6 at 31,814,891 bp). For PF2018, the peak marker is Rh88_37299_454 (11.5 cM,
 323 located on chr6 at 5,410,244 bp). These QTLs explained between 5.28 and 8.45% of
 324 the observed variance. The common 0.95 credible interval was from 15.59 to 42.49 cM,
 325 which covered from 8,578,645 to 44,264,630 bp on the physical map (Figure 4b, Table
 326 2).
 327 On LG1, QTLs were only detected on the male map for PF for two years (2016 and
 328 2018), and explained 6.52 and 6.99% of phenotypic variance, respectively. The
 329 common 0.95 credible interval was at 12.78-44.11 cM, which covered from
 330 20,231,658-62,553,371 bp on the physical map (Figure 4b, Table 2).

We checked the interaction between OB3@Rh12GR_16570_782 and OB4@Rh12GR_60129_183, and between RW3@Rh12GR_52506_1218 and RW6@Rh12GR_56601_1304, and no significant interaction was detected.

2.2 Two-part QTL analysis

In order to extend the analysis even further, we performed a two-part QTL analysis to test the penetrance (presence/absence of prickles, $LOD(p)$ were calculated with binary traits) and the severity (density of prickles on stems with prickles, $LOD(\mu)$ were calculated with non-zero quantitative phenotype) of these QTLs.

For the hypothesis $LOD(p)$ on the female and male maps, we obtained a significant $LOD(p)$ on the LG3 for the two types of stem (PF and PM) and the three years (Figure 3, Supplementary Table 2). The marker with the highest LOD score on the OB map is the same: Rw35C24 (SSR marker) located at 44.4cM (Chr03: 40,215,502 bp). This QTL explained 13.38% to 16.72% of the variation. The peak marker on the RW map is also the same for PF and PM for the three years: Rh12GR_52506_1218 located at 42.6 cM (42,317,122 bp). This QTL explained 20.69 to 33.21% of the variation. These data suggested that the QTL detected on LG3 mainly controls the presence/absence of prickles. Moreover, the $LOD(p)$ on LG2 and LG6 for the male map were only significant in PF2016 and PM2016, respectively (Figure 3), and they showed a weak effect with an explanation of 1.80% and 2.70% of the variance, respectively (Supplementary Table 2).

For the $LOD(\mu)$ hypothesis, we detected a significant QTL on the female map on LG4 for PM (2016 and 2017) and PF (2016) (Figure 3, Supplementary Table 2). The QTLs

explained 9.02% to 9.88% of the observed phenotypic variances. Therefore, this QTL might be involved in the control of prickles density. On LG3, a significant QTL was detected on the male map for PM (2016, 2017, 2018) and PF (2016), suggesting that a QTL on LG3 might also control prickles density. This QTL is in the same region of the QTL detected for penetrance (Figure 3, Supplementary Table 2). On LG1, the LOD(μ) peaks in OB (PM2018) and in RW (PF2018) were higher than the genome-wide threshold (μ); these QTLs explained 6.66% and 7.80%, respectively, of prickles density variation.

2.3- The interaction of the LG3-QTL allele between OB and RW

Based on non-parametric and two-part methods, we identified QTLs for the presence of prickles on LG3 for the OB and RW maps in the same region. To further investigate how the alleles on these QTLs affect the presence of prickles, we visualized the number of prickles for each genotype in the hybrid population depending of the Mendelian distribution of the SNP markers at the LOD peak (Figure 5). The female and male alleles are referred to as *a,b* and *c,d*, respectively. The separation ratio *ac:ad:bc:cd* in offspring is 33:54:16:48, and was significantly different from the expected segregation of 1:1:1:1 (37.5 for each) with a *p-value* = 0.004 estimated by a chi-squared test (Figure 5).

For PF and PM in all three years, we clearly see that the *bd* allele combination in hybrids is correlated with no-prickle individuals or individuals with only a few prickles (less than two on four internodes), whereas *ac*, *ad* and *bc* genotypes present prickles (Figure 5). These results suggest a dominant/recessive model for this QTL with the *b* and *d*

alleles linked to the null or recessive alleles (prickless mutant) and the *a* and *c* alleles linked to the dominant alleles (prickles). For PM, a co-dominant effect can be detected since the phenotype for *ac* is significantly different from the one for *ad* and *bc* ($ac > ad$ and $ac > bc$, $p\text{-value} < 0.05$, except for PM2016 between *ac* and *ab*; Figure 5), even if the effect is weak (no large difference between the mean for *ac* and *ad/bc*). For PF, no co-dominant effect was detected.

We also observed some odd phenotypes. For instance, OW9067 and OW9068 (red dots) had no prickles and were grouped in the *ad* genotype, perhaps due to recombination between the marker and the prickle locus (Figure 5). For individuals with the *bd* genotype, six individuals (blue and green dots) always have prickles: OW9062, OW9021, OW9052 and OW9109 (blue dots) look like the usual prickle genotypes and are probably caused by recombination, but the two extreme exceptions, OW9106 and OW9107 (green dots) with the highest prickle density are not that easy to clarify. Moreover, some individuals exist with both prickly and glabrous stems in the same plant.

3- Candidate genes in the QTL interval region and gene expression analysis

3.1 Candidate gene characterization and location in rose.

Since it was proposed that prickles originate from a deformation of glandular trichomes in rose (Kellogg et al. 2011), we looked for rose homologues of transcription factors (TF) known to be involved in the molecular control of trichome initiation and development in Arabidopsis. The information from 15 TFs such as the bHLH (basic helix-loop-helix), C2H2 Zinc-Finger, MYB, WD40 repeat and WRKY families are

presented in Supplementary Table 3. For a more detailed annotation, we performed phylogenetic analyses on these protein families (Supplementary Figure 3).

Concerning the bHLH family (Supplementary Figure 3a), RC7G0190300, RC1G0342400 and RC6G0407800 showed strong similarity with GLABROUS3, MYC1 and TT8, respectively, where all of the proteins are in the same clade. They are referred to as RcGL3, RcMYC1 and RcTT8, respectively.

For the C2H2 family, RC3G0150000, RC4G0390900 and RC4G0476500 are closely related to GLABROUS INFLORESCENCE STEMS proteins (GIS, GIS2 and GIS3) and ZINC FINGER PROTEIN (ZFP5, 6 and 8). RC3G0150000 seems to be more closely related to GIS2, RC4G0390900 to GIS3 and RC4G0476500 to ZFP5. RC2G0415300 and RC6G0454700 are related to ZFP1 and ZFP3 and AT5G10970. They are referred to as RcZFP1-like1 and RcZFP1-like2, considering that they are closer to ZFP1 (Supplementary Figure 3b).

R2R3 MYB and R3 MYB belong to the MYB family (Supplementary Figure 3c). In the R2R3 MYB sub-family (blue sub-tree), RC7G0156100 is in the same clade as GLABROUS1, whereas RC2G0033100 and RC7G0261400 are more closely related to MYB82 and TT2, respectively. RC3G0322900 is in the same clade as MYB61, MYB50 and MYB86. In the R3 MYB sub-family (red sub-tree), RC2G0548400, RC1G0560100 and Chr1g0359121 (Raymond et al. 2018) are in the same clade of CPC, TRY, ETC1 and ETC3. RC1G0560100 and Chr1g0359121 are more closely related to TRY and CPC, and are referred to as RcTRY and RcCPC, respectively. (Supplementary Figure 3d).

419 In the WD40 family, RC1G0586100 showed a strong similarity to TRANSPARENT
420 TESTA GLABRA 1(TTG1), and RC3G0186600 and RC2G0693200 also belong to this
421 clade.

422 In the WRKY family, as previously shown by Hibrand-Saint Oyant et al. (2018),
423 RC3G0244800 shows a strong similarity with AtTTG2 (TESTATRANSPARENT
424 GLABRA2), whereas RC3G0309600 and RC3G0309700 seem to be more closely
425 related to WRKY54 and WRKY70, RC3G0392200 to WRKY74, and RC3G0414600
426 appears to be related to WRKY34 and WRKY2.

427 We then located these rose homologue genes on the rose genome and looked for co-
428 location between these genes and the QTLs previously described (Figure 4A and B).
429 Concerning the QTLs on LG3 (male and female map, Figure 4a and b), the most
430 interesting TF among the detected genes was *RcMYB61* (*RC3G0322900*, at Chr03:
431 39,896,892-39,899,077) located in the cQTL interval (36.517-46.440 Mb) for the
432 female map (Figure 4A). As previously described (Hibrand-Saint Oyant et al. 2018), a
433 homologue of *TTG2*, a WRKY transcription factor (*RC3G0244800*), is also located in
434 the credible interval. *RcGIS2* (*RC3G015000*), a *GIS2* homologue is also located on LG3
435 but not in the cQTL interval. In addition to the candidate TFs, we also scanned the other
436 TFs co-located in the cQTL interval on LG3 of the female map. There are four bHLH,
437 two C2H2, three R2R3MYB and seven WD40 transcription factors (Supplementary
438 Figure 3, in blue) located under the cQTL.

439 Concerning the cQTL interval on LG4, *RcGIS3* is positioned at Chr04: 50,315,805 -
440 50,317,009 (1.21 Kb), and near the peak marker Rh12GR_55601_1304 (52,239,028

kb) on the female map (Figure 4A). *RC4G0476500*, a ZFP5 homologue, is also located on the female LG4 but not in the QTL interval.

Concerning the QTL on the male LG1, *RcMYC1*, *RcTRY* and *RcCPC*, which are positioned at 44,468,298-44,473,643 bp, 47,708,966-47,709,896 bp and 62,070,383-62,072,848 bp, respectively, are located in the cQTL region (20.232Mb-62.553Mb) of PF (2016, 2018) on the male LG1. The gene *RC1G0586100* (*RcTTG1*) is also located on LG1 but outside this interval.

For the male LG6, *RC6G0407800*, a homologue of *TT8*, is not located in the cQTL credible interval, and no studied gene was detected below this QTL.

3.2 Candidate gene expression in glabrous and prickly roses.

Based on the positional approach, we identified ten interesting candidate genes, six within the QTL interval and the other four outside of QTL but near the credible interval (Figure 4). In order to obtain more information about these genes, we studied their transcript accumulation by RT-qPCR in tissues from prickly (P) and prickless (NP) stems at different developmental stages: I, IIa, IIb, IIc, III (Supplementary Figure 1).

The cluster analysis of gene expression clearly showed that all the samples can be divided into two main groups: PI, NPI, PIIa, NPIIa, PIIb, NPIIb were gathered into one group, and PIIc, NPIIc, PIII, NPIII into another group (Figure 6a). At the sup-group level, PI and NPI, PIIa and NPIIa, PIIb and NPIIb, PIIc and NPIIc were clustered together, respectively. At the same stem developmental stage, prickly and glabrous samples (P and NP) behave similarly, suggesting no major difference of transcript accumulation between prickly and glabrous samples; the observed differences are more

463 closely related to stem development.

464 To extend the analysis even further, we used NP as a control to compare the normalized
465 expression of genes between P and NP samples in the different stages (Figure 6b). In
466 stage I, two genes are differentially expressed: *RcMYB61* and *RcGIS2* were down-
467 regulated in prickly stems, with a significant p-value = 4.1×10^{-5} and 2.9×10^{-4} (Figure 6b),
468 respectively. In stage IIa, only *RcZFP5* was significantly differentially expressed
469 between P and NP, with a p-value = 0.0056 and FC = -5.7606 (Figure 6b). A different
470 pattern is observed in stage IIb where *RcZFP5* expression was up-regulated with FC =
471 8.2240 and a p-value = 0.0025. In addition, the transcripts of *RcMYC1*, *RcCPC* and
472 *RcGIS2* were also significantly accumulated (p-value = 4.1×10^{-5} , 0.0048, 0.0012,
473 respectively) in stage IIb. In stage IIc, no significant change in gene expression was
474 detected. In stage III, the *RcGIS2* transcript is differentially accumulated with FC = -
475 4.908 and a p-value = 0.043. The same pattern is observed for *RcMYB61* with a p-
476 value = 4.9×10^{-4} .

477 We followed the transcript accumulation during stem development in prickly and
478 glabrous stems (NPI as a control; Figure 6c). All the studied genes are regulated
479 between the different samples. For instance, *RcMYB61* is up-regulated and *RcMYC1* is
480 down-regulated between the different stages. For *RcZFP5*, we observed a delay in the
481 decrease of transcript accumulation, with a decrease in stage IIa for glabrous stems and
482 in stage IIb for stems with prickles (Figure 6c).

483 Discussion

484 *Two types of prickles are present in the OW progeny, originating from different*

structures.

A good understanding of prickles morphology is required to serve as the foundation for genetic and molecular studies. We identified two different types of prickles in our population: it appears that GP and NGP originate from glandular and non-glandular structures, respectively. This conclusion is different from previous studies in rose, which reported that prickles were extensions or modifications of glandular trichomes (Kellogg et al. 2011), and in other species (Ma et al. 2016b; Pandey et al. 2018). Asano et al. (2008) observed two types of prickles in the cultivated rose ‘Laura’, described as large size and small size prickles. The large size prickles look similar to NGPs in our study. The small size prickles, referred to as acicles (Asano et al. 2008), are more closely related to the glandular prickles (GP) we observed since they have a glandular head that accompanies them throughout their lifetime. The difference between these two types of prickles is also related to their segregation in the OW population (Figure 1d), demonstrating that different genetic determinisms are involved. In this study, since only a few F₁ individuals had GPs, we cannot perform a genetic analysis on GPs, we concentrated our analysis on NGPs.

A complex genetic determinism for prickles in rose

Prickles on stems exhibited transgressive segregation in diploid OW, the same as for the tetraploid K5 population (Koning-Boucoiran et al. 2012; Gitonga et al. 2014; Bourke et al. 2018), supporting the hypothesis that multiple loci may be responsible for this trait.

Using the ‘non- parametric’ QTL approach, we detected a stable QTL on LG3 in the three different years for both types of stems (PM and PF) on both the male and female genetic maps. We also demonstrated that this QTL mainly controls the presence/absence of prickles (Figure 3) using the ‘two-part’ QTL method. Interestingly, for PM in males, the QTL on LG3 may also be involved in regulating prickles density (severity in the two-part QTL analysis; Figure 3). A similar phenomenon was observed for the petal number with a locus on LG3 that controls the difference between simple and double petals, and a variance of the petal number that exists within the double petal flower is controlled by another locus (Roman et al. 2015; Hibrand-Saint Oyant et al. 2018).

We further enhanced the description of QTLs on LG3 that affect the presence/absence of prickles. A significantly distorted segregation was observed at the peak marker position. That unusual segregation ratio might be explained by the presence of a self-incompatibility locus (Hibrand-Saint Oyant et al. 2018) near the peak marker for this QTL. On the basis of the phenotype-genotype relationship (Figure 5), we proposed that the *PRICKLE* alleles on this QTL are both heterozygous (np/P) in OB and RW, and that the presence of prickles is controlled by a dominant allele (np/P or P/P), and that the glabrous stem in the progeny is due to the combination of the two recessive alleles coming from both parents (np/np). These results are important for breeders who need to combine recessive alleles to obtain glabrous roses, an allelic combination that can be difficult in tetraploid roses. Development of specific molecular markers of the recessive allele may be useful for breeders. However, it should be noted that the actual markers

used (peak of the QTL) are only closely linked to the *PRICKLE* locus and few recombinants are observed in the progeny. Furthermore, the phenotype of the individuals with the two recessive alleles (*bd* phenotype; Figure 5) are not stable and some of the hybrids were regularly seen to develop some prickles on parts of the stems. Indeed, this phenomenon is widespread in roses. Rose breeders have reported that glabrous mutants have either been unstable for the prickless trait (Nobbs 1984; Rosu et al. 1995), or reverted to the prickly character after a freezing winter or other environmental stresses (Nobbs 1984; Oliver 1986; Druitt and Shoup 1991; Canli 2003). Taken together, we assumed that a single major locus on LG3 controlled the absence/presence of stem prickles. Further investigations are necessary to more closely identify molecular markers (for molecular assisted breeding) and the mechanisms behind the instability.

In the *ac*, *ad* and *bc* genotypes, each genotype has a continuous quantitative trait, indicating that there are other loci responsible for prickle density variance. Other QTLs affecting quantitative traits were detected on LG4 in OB and on LG1 and 3 in RW (Two-part QTL analysis; Figure 3). The LG4 QTL has a strong effect on PM but a weak effect on PF. For the QTL on LG1, it only had a weak effect on PF and on PM in 2018. Those three loci are related to the density of prickles, indicating that there are multiple genes responsible for the density trait, and that those genes have a different effect on the different stems.

Detected QTLs are conserved in the Rosa genus and the Rosideae subfamily

Thanks to the link between genetic maps and reference genome sequences (Hibrand-

Saint Oyant et al. 2018), we were able to compare our results with previous genetic studies by associating genetic map markers.

A QTL was previously detected on LG3 in different diploid and tetraploid populations (Crespel et al. 2002; Linde et al. 2006; Koning-Boucoiran et al. 2012; Hibrand-Saint Oyant et al. 2018 ; Bourke et al. 2018), which is consistent with our results: a strong QTL on Chr3 with a high LOD value was detected in all of the environments (across and between years and types of stems). This demonstrated that Chr3 QTL is a robust QTL detected independently of ploidy and the environment, and is present in various genetic backgrounds.

Recently, three QTLs on LG3, 4 and 6 were detected in the tetraploid K5 population with a high density of SNPs genetic map (Bourke et al. 2018). Interestingly, the QTLs identified from the diploid (OW) were almost identical to tetraploid (K5) populations (LG3, 4 and 6), with the slight difference that we also detected a weak QTL on LG1, which was only significant in males for two of the years. This slight difference might be due to the genetic background of the parents of the K5 and OW populations. In fact, in K5 populations, one parent is prickly and the other glabrous, whereas in OW populations, both parents have prickles. Bourke et al. (2018) reported that two SNP markers, K7826_576 (located on the Chr3: 37,706,920 pb) and K5629_995 (located on the Chr4: 57,791,999 bp) are linked to the stem prickle trait. When compared with our results, K7826_576 is located within our Chr3 cQTL interval region (36,517,224-46,440,369 bp; Figure 4), and K5629_995 is very close to our Chr4 QTL interval (46,189,407-56,107,784 bp). These results suggest that QTLs detected on LG3 and 4

could be similar between OW and K5 progenies.

In *Rosaceae*, the genetic determinism of prickle was studied in raspberry (*Rubus idaeus*), where two QTLs were detected on LG4 and 6 (Molina-Bravo et al. 2014). Using synteny viewer tools (<https://www.rosaceae.org/synview/search>; Jung et al. 2014), we checked the synteny. The region where the QTL is located on LG6 in *R. occidentalis* (position 6,028Mb) is syntenic with a region on rose chromosome 2 (position 42,330 Mb), where no QTL for prickle density was detected in our study. The region where the QTL 4 is located (position 0.101 Mb) is syntenic with the region on rose chromosome 4 (position 58,768 Mb), very close to the main QTL we detected on this chromosome (Table 2). These results could suggest that the two QTLs in rose and raspberry might be syntenic and share a common evolutionary history. In another publication, Graham et al. (2006) identified the gene *H* that controls cane pubescence. The locus is mapped on LG2, which is syntenic with the rose LG6 where one of the QTLs is located, detected in *R. x wichurana*. However, no precise location is available to allow us to assume a possible common origin.

Candidate gene below the QTL interval region

Prickles are assumed to originate from a ‘trichome-like structure’. In order to find a putative candidate gene for the identified QTLs, we looked for homologue genes known to be involved in trichome initiation and development in Arabidopsis. We annotated 15 rose TFs that, based on similarity, can be involved in trichome development in rose: RcGL1, RcMYB82, RcMYB61, RcCPC, RcTRY, RcGL3, RcTT8, RcMYC1, RcTTG1, RcTTG2, RcZFP5, RcGIS3, RcGIS2, RcZFP1 and RcGL2 (Table 3). Among them, a

few were below the detected QTLs: RcMYB61 and RcTTG2 below the QTL on LG3; RcGIS3 below the QTL on LG4; and RcCPC, RcTRY and RcMYC1 below the QTL on LG1. ZFP5 (Chr04: 57,125,905 bp) is out of the QTL interval on LG4 in OW, but close to the peak LOD marker K5629_995 of QTL in the K5 population (Chr04: 57,791,999 bp) (Bourke et al. 2018). These genes are good candidates for the detected QTLs.

Candidate genes transcript expression in glabrous and prickly F₁ individuals

We quantified ten TF gene transcripts in glabrous and prickly F₁ individuals in different developmental stages using RT-qPCR. Surprisingly, minor differences were observed between glabrous and prickly samples, with the main differences occurring between developmental stages (as demonstrated by the heatmap analysis, Figure 6a). Based on transcript accumulation, this suggests that these homologues, known to be involved in trichome initiation and development in Arabidopsis, are not implicated in prickly initiation in rose, leading to the hypothesis that the two processes (trichome initiation and prickly initiation) might involve different gene pathways. The candidate gene approach may not be appropriate and a non-a priori approach such as a transcriptomic analysis could be done between individuals with and without prickles.

Nevertheless, some differences in transcript accumulation are observed between candidate genes. In the early stage (stage I), only *RcMYB61* and *RcGIS2* are slightly more highly accumulated in glabrous stems. However, GIS2 and MYB61 are positive regulators of trichome initiation (Gan et al. 2006), which is difficult to reconcile with an increase in transcript accumulation in glabrous stems (Figure 6). Negative feedback

regulation during prickle initiation can explain this point, as regularly observed in trichome initiation (Pattanaik et al. 2014) or, perhaps, differences are not at the transcriptional level. It could be interesting to sequence the genes in the two parents to see if a mutation can explain the phenotype.

RcZFP5 may also be an interesting candidate gene. This gene showed a different regulation between glabrous and prickly stems. At stage IIa, *RcZFP5* shows a strong down-regulation in glabrous tissue, whereas this down-regulation is observed later at stage IIc in tissues with prickles (Figure 6C). Furthermore, this gene is close to the QTL on LG4. Its early repression in glabrous stems might explain why no prickles developed. In *A. thaliana*, *ZFP5* controls trichome initiation through GA signaling (Zhou et al. 2011). These data (concerning *ZFP5* and *MYB61*) might suggest an implication of GA in prickle development. However, this hypothesis needs to be functionally validated in rose.

Conclusion

Prickle structure is an undesirable trait, not only in rose but in most crops in general. We identified a complex genetic determinism with a major locus on LG3 that controls the presence of prickles and a few QTLs that control prickle density. Further studies are necessary to develop markers for breeding selection and to identify the molecular bases. Using a candidate gene approach, we proposed different hypotheses concerning the gene involved in prickle initiation in rose. Approaches such as transcriptomics may help to identify new key regulators of prickle initiation and development in rose.

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Figure legends

Figure 1 Different types of prickles on the OW progeny stem and their distribution. **(a)**

Stem prickles in the female ‘Old Blush’ (OB) and the male *R. x wichurana* (RW); NGPs:

non-glandular prickles. **(b)** Stem prickles in F₁ progeny. Glabrous: no prickles

whatsoever on the recorded stems in the three years. **(c)** Macroscopic photos of the

terminal part of the stems with different types of prickles (number of offspring); GPs:

glandular prickles. **(d)** The distribution and Q-Q plot of NGPs and GPs in the F₁

progeny in 2018; PF: prickles on the floral stem; PM: prickles on the main stem.

Figure 2 LOD curves of the QTL scan for the NGPs on the floral (FM) and main (PM)

stems in **(a)** female (OB) and **(b)** male map (RW) calculated with a non-parametric

model for the three years (2016, 2017 and 2018, with red, blue and green lines,

respectively). The LOD threshold value is represented by a dotted line in red, blue and green for 2016, 2017 and 2018, respectively.

Figure 3 LOD curves of the QTL scan for the NGPs on the floral (FM) and main (PM) stems in (a) female (OB) and (b) male (RW) maps calculated using the two-part approach. The LOD (p) value (penetrance) is in red, the LOD (μ) value (severity) is in blue, and the LOD (p, μ) value is in black. The dotted line represents the LOD threshold. QTLs above threshold value are indicated by stars: red for penetrance, blue for severity.

Figure 4 Common QTLs (cQTLs) and candidate genes in (a) the female linkage groups 3 and 4, and (b) the male linkage groups 1, 3 and 6. Areas highlighted in pink, blue and yellow on the linkage groups represent the 0.95 Bayesian interval of cQTL for specific PF, PM and both, respectively. Bars and lines on the right of each chromosome represent 0.95 and 0.99 Bayesian intervals of the QTL with a different color for NGPs on the floral stem and the main stem (pink and blue, respectively). The red markers are the peak of the QTL. Brown markers are SSR markers and black markers are SNP markers.

Figure 5 The interaction of the different alleles of the LG3 QTL between OB and RW. Genotype: *ac*, *ad*, *bc*, *bd* (number of individuals), *a/b* and *c/d* alleles belong to females and males, respectively. For the phenotype, the mean values of prickly density for PF and PM for the three years are presented. Some individuals are highlighted with green dots (OW9106 and OW9107), blue dots (OW9062, OW9021, OW9052 and OW9109) and red dots (OW9067 and OW9068). The asterisk indicates that the difference is significant with a p-value of less than 0.05

Figure 6 Transcript accumulation of candidate genes followed by qPCR during prickle development. **(a)** A heatmap of samples and genes. **(b)** The scatter plot of the candidate genes' normalized expression in prickle and glabrous individuals in different stages (as defined in Supplementary Figure 1). The red and green lines represent a two-fold change in the accumulation with an increase or a decrease, respectively. Gene transcripts differentially accumulated (p -value < 0.05) are represented by red or green dots for up- or down-accumulation, respectively. **(c)** Transcript accumulation in the different stages of prickle (P) and glabrous (NP) stems with NPI as a control.

Electronic Supplementary Material

Supplementary Figure 1 Stem development and sampling stages in (a) non-glandular prickles (NGP, OW9137), (b) glandular prickles (GP, OW9106), and (c) glabrous stems (NP, OW9068). For the glabrous stems, the developmental stages correspond to the stages for the stem with prickles.

Supplementary Figure 2 Frequency distribution and Q-Q plot of non-glandular prickles on four internodes in the OW population for floral stems (PF) and the main stem (PM) for the three years (2016, 2017 and 2018).

Supplementary Figure 3 Phylogenetic analysis of the transcription factor family involved in trichome initiation and development: (a) bHLH, (b) C2H2 Zinc-Finger, (c) MYB: R3MYB (red sub-tree) and R2R3MYB (blue sub-tree), and (d) WD40. The rose genes homologues of genes involved in trichome initiation and development are in red.

For *A. thaliana*, the protein name corresponds to the TAIR database (<https://www.arabidopsis.org/>), and for rose, to the reference genome of the haploid of

‘Old Blush’ (Hibrand-Saint Oyant et al. (2018)), except for Chr1g0359121 and Chr2g0138951 (Raymond et al. 2018).

Supplementary Table 1 Primer sequences of candidate genes for qPCR

Supplementary Table 2 Summary of QTLs for NGPs using the two-part QTL model in OW progeny

Supplementary Table 3 Summary of the rose homologous genes known in *A. thaliana* to be involved in trichome initiation

Supplementary Table 4 Prickle number on four internodes of two types of stems for the three years in OW progeny.

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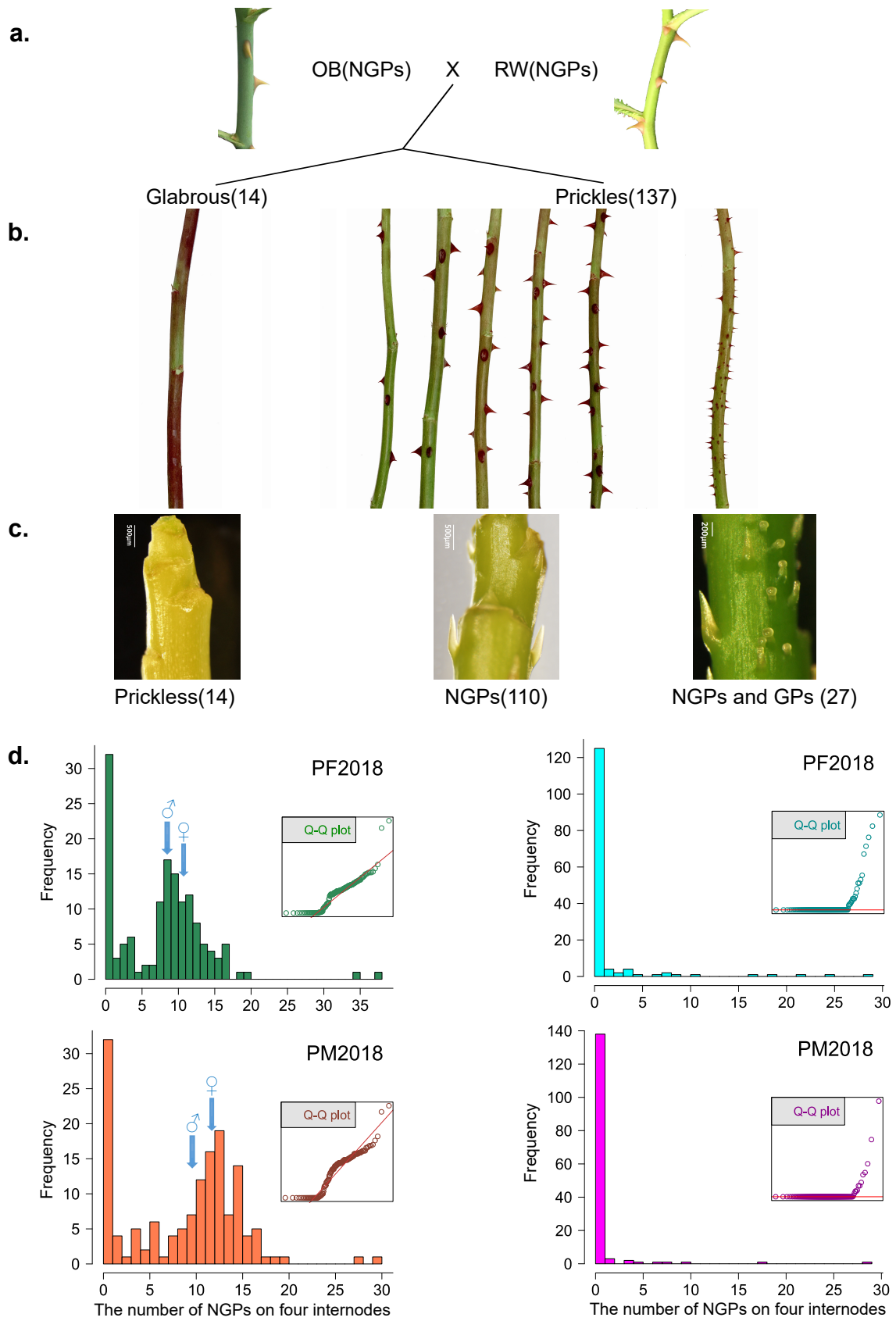


Figure 1

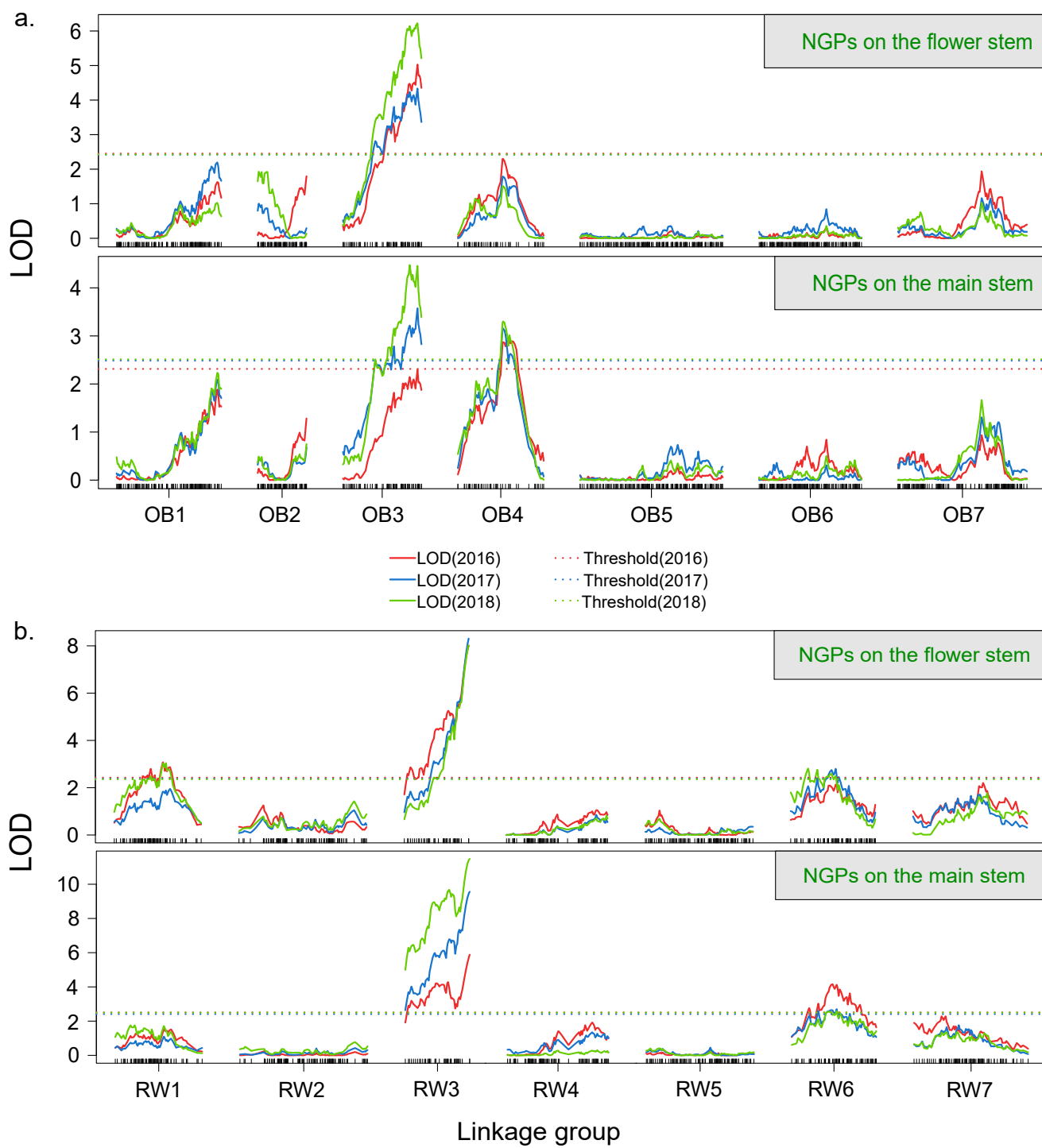


Figure 2

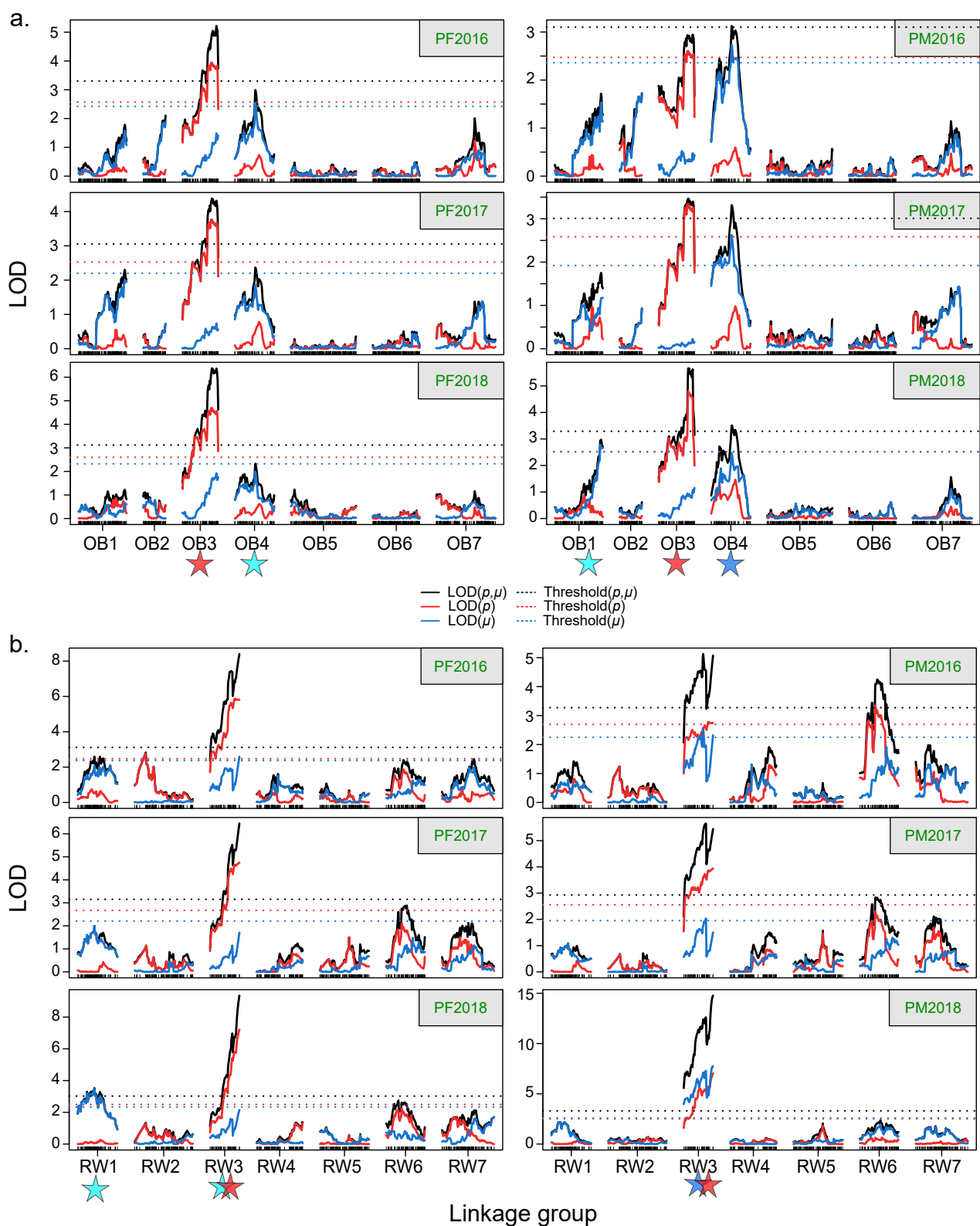


Figure 3

a.

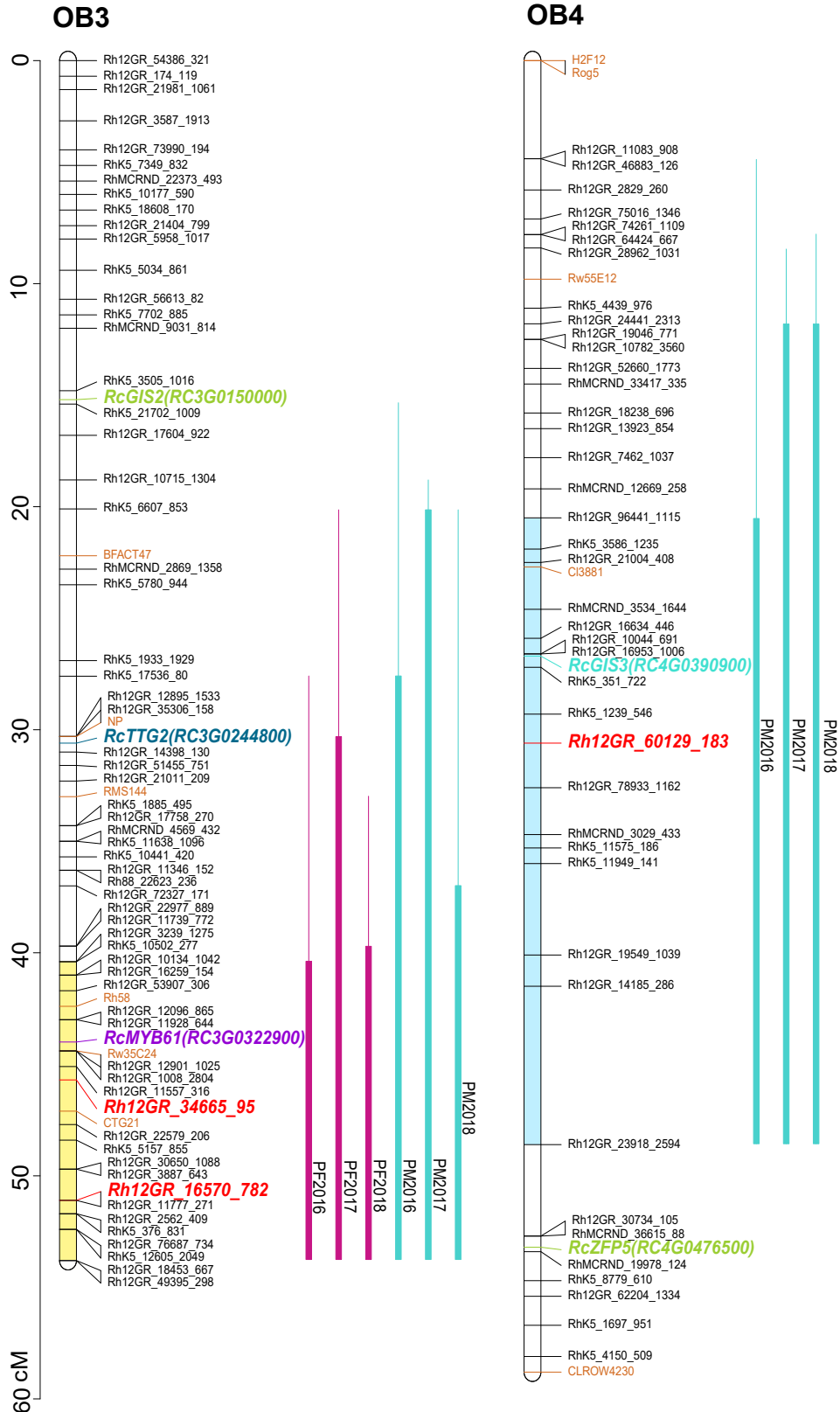


Figure 4

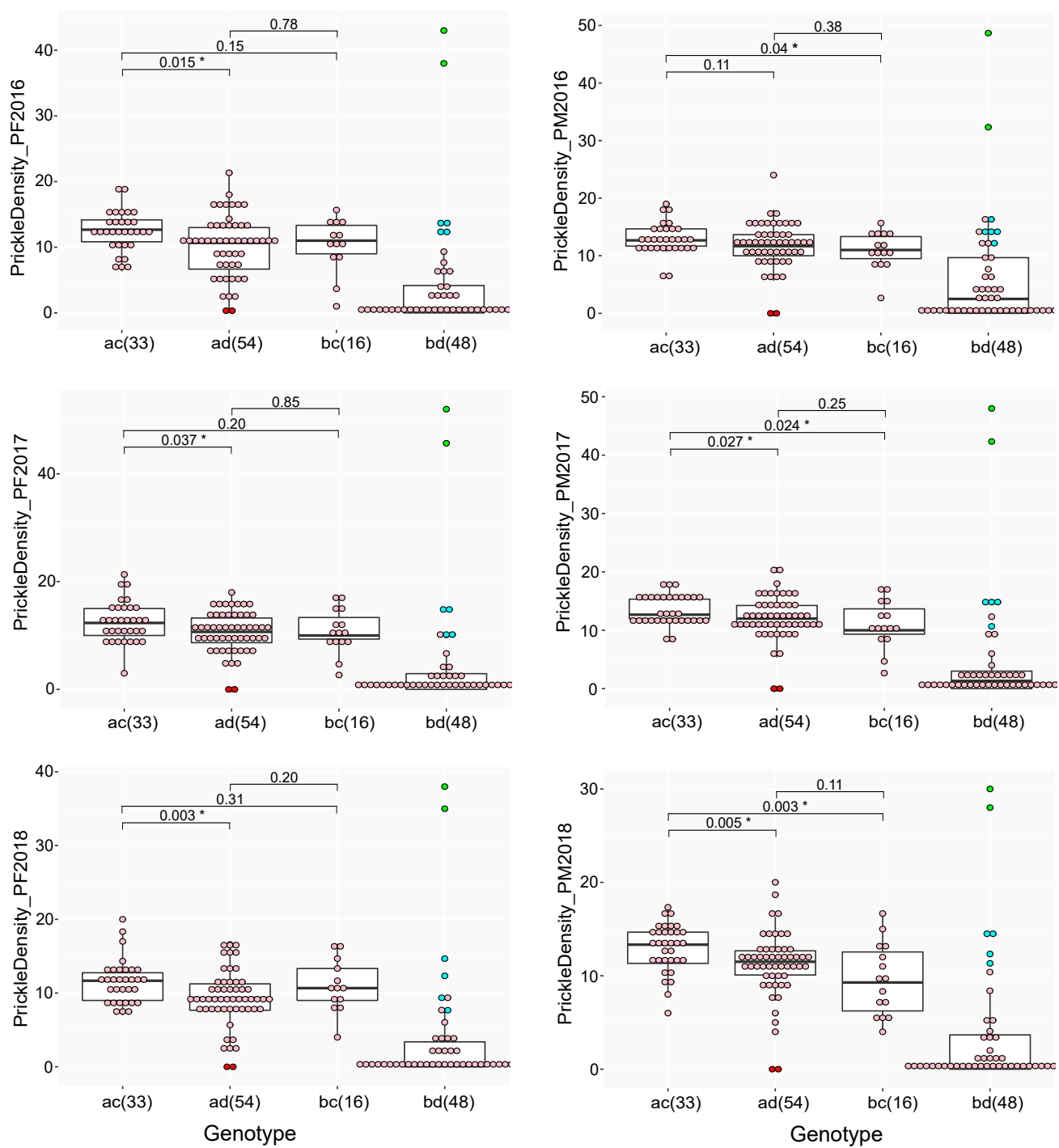
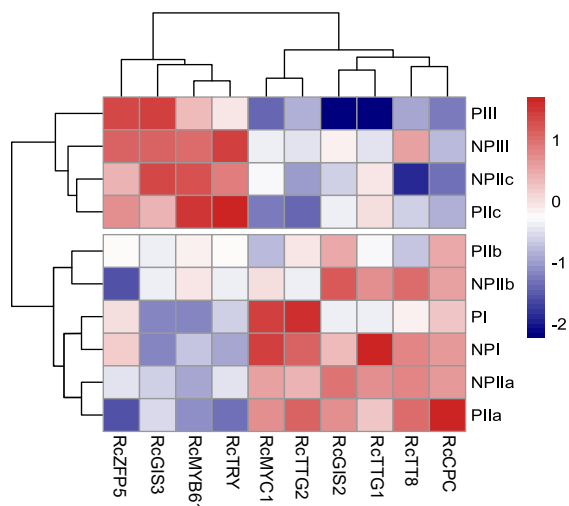
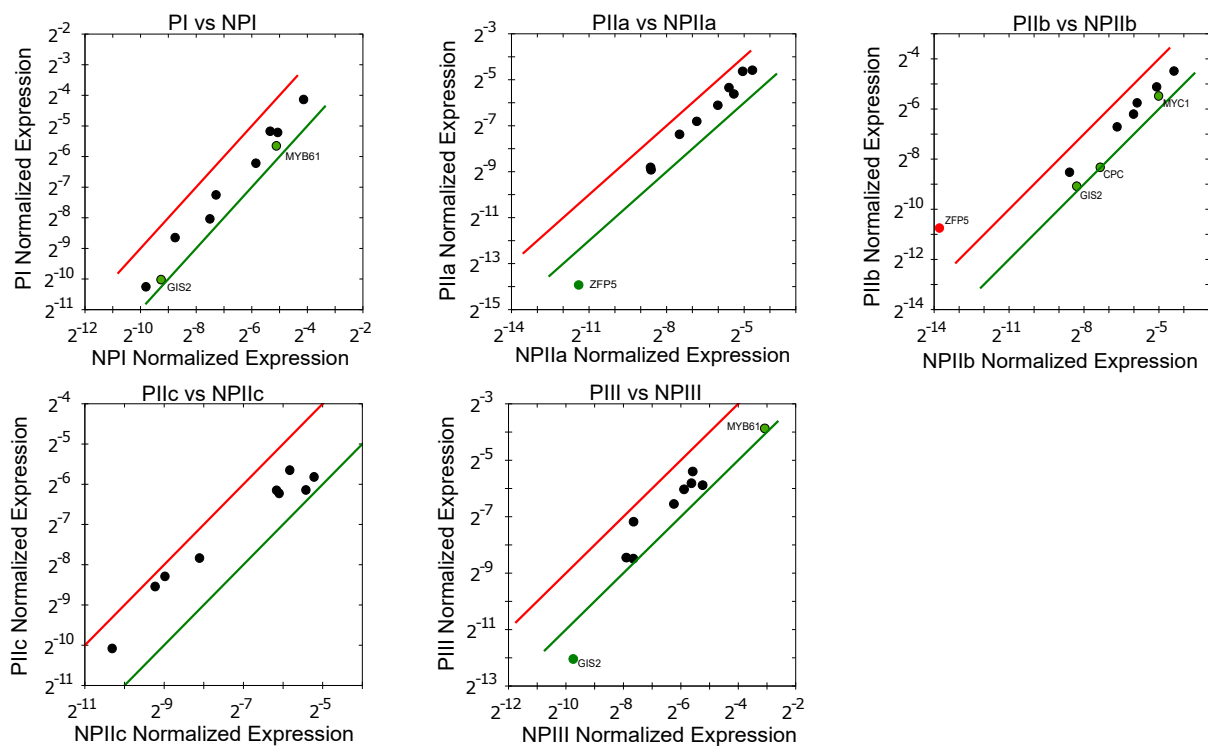


Figure 5

a.



b.



c.

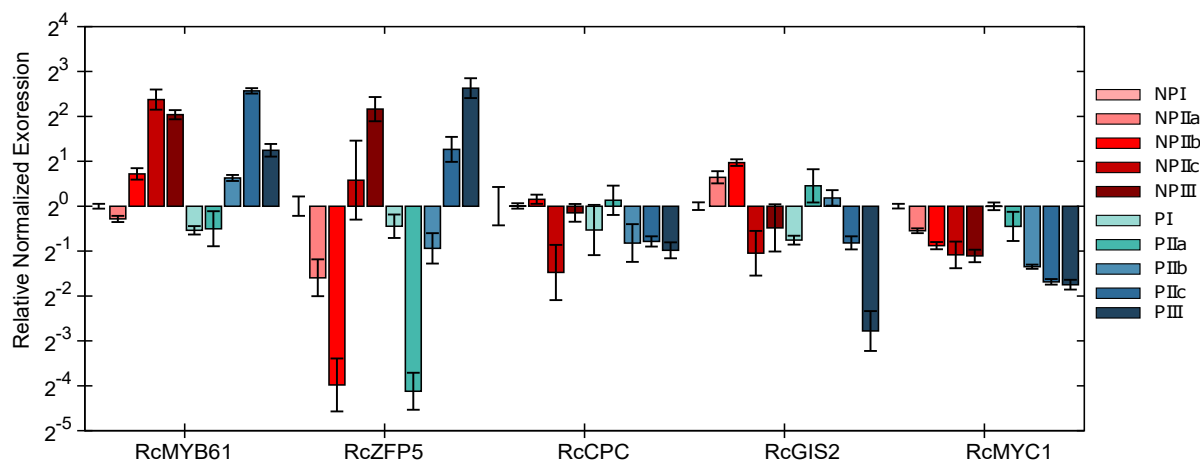


Figure 6

Table 1 Mean, median and range values for prickles on 4 internodes, phenotypic variance components and the trait heritability

| Trait | Year | OB Mean±SD | RW Mean±SD | F1 | | | | | Kruskal-Wallis test (p-value) | | | | Variance components | | | |
|-------|------|---------------|---------------|--------|--------|------|--------|--------|-------------------------------|-------------------|---------|-------------|---------------------|-----------------|-----------------|------------------|
| | | | | range | 1st Qu | Mean | median | 3rd Qu | Genotype | Replicate shoots | Year | Stem type | σ^2_G | SE _G | σ^2_{GY} | SE _{GY} |
| PF | 2016 | 11.3±1.3 | 8.9±0.8 | 0 - 43 | 2.4 | 8.5 | 9.8 | 12.7 | | | | | | | | |
| | 2017 | 11.6±1.1 | 8.9±0.9 | 0 - 52 | 2.7 | 9.0 | 9.7 | 13.0 | <2.20E-16*** | 0.96 ^N | 0.06** | | | | | |
| | 2018 | 10.6±1.0 | 8.4±0.5 | 0 - 38 | 2.7 | 7.9 | 9.0 | 11.3 | | | | 4.93E-08*** | 41.41 | 4.98 | 1.50 | 0.26 |
| PM | 2016 | 11.9±1.6 | 9.3±1.2 | 0 - 48 | 6.7 | 10.2 | 11.7 | 13.3 | | | | | | | | |
| | 2017 | 11.8±1.4 | 9.2±1.1 | 0 - 47 | 3.0 | 10.2 | 11.3 | 14.0 | <2.20E-16*** | 0.81 ^N | 0.04*** | | | | | |
| | 2018 | 11.7±1.6 | 9.1±1.1 | 0 - 30 | 3.5 | 8.8 | 10.8 | 13.0 | | | | | | | | |

PF: prickles on the floral stem

PM: prickles on the main stem

1st Qu: First quartile(Q1) means 25% observations are below this quantity (approx)

3rd Qu: Third quartile(Q3) means 75% observations are below this quantity (approx)

σ^2_G : Variance components of genotype, genotype x year interaction (σ^2_{GY}), genotype x stem interaction (σ^2_{GS}), and the residual error (σ^2_E)

SE: the standard error

h^2 : Narrow sense heritability

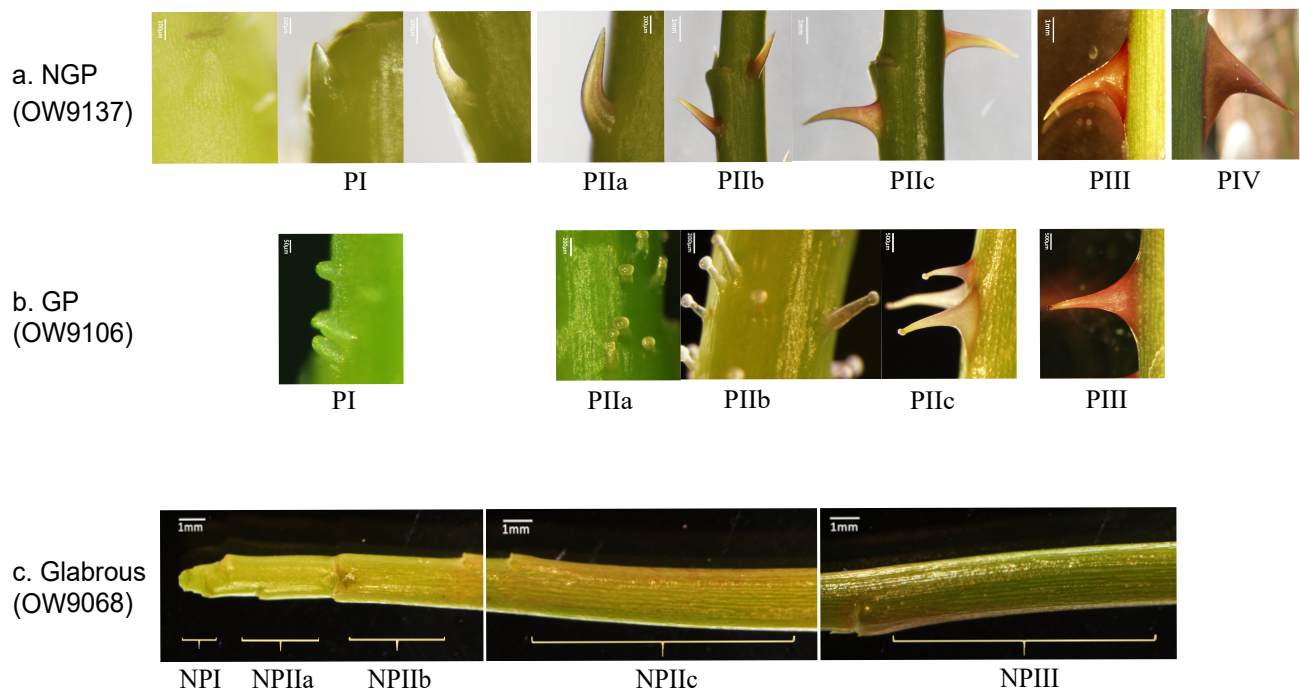
| Component in σ^2_P | | | | $h^2(\%)$ | SE |
|---------------------------|------------------|--------------|-----------------|-----------|----|
| σ^2_{GS} | SE _{GS} | σ^2_E | SE _E | | |

1.27 0.27 9.13 0.29 96.66 0.0048

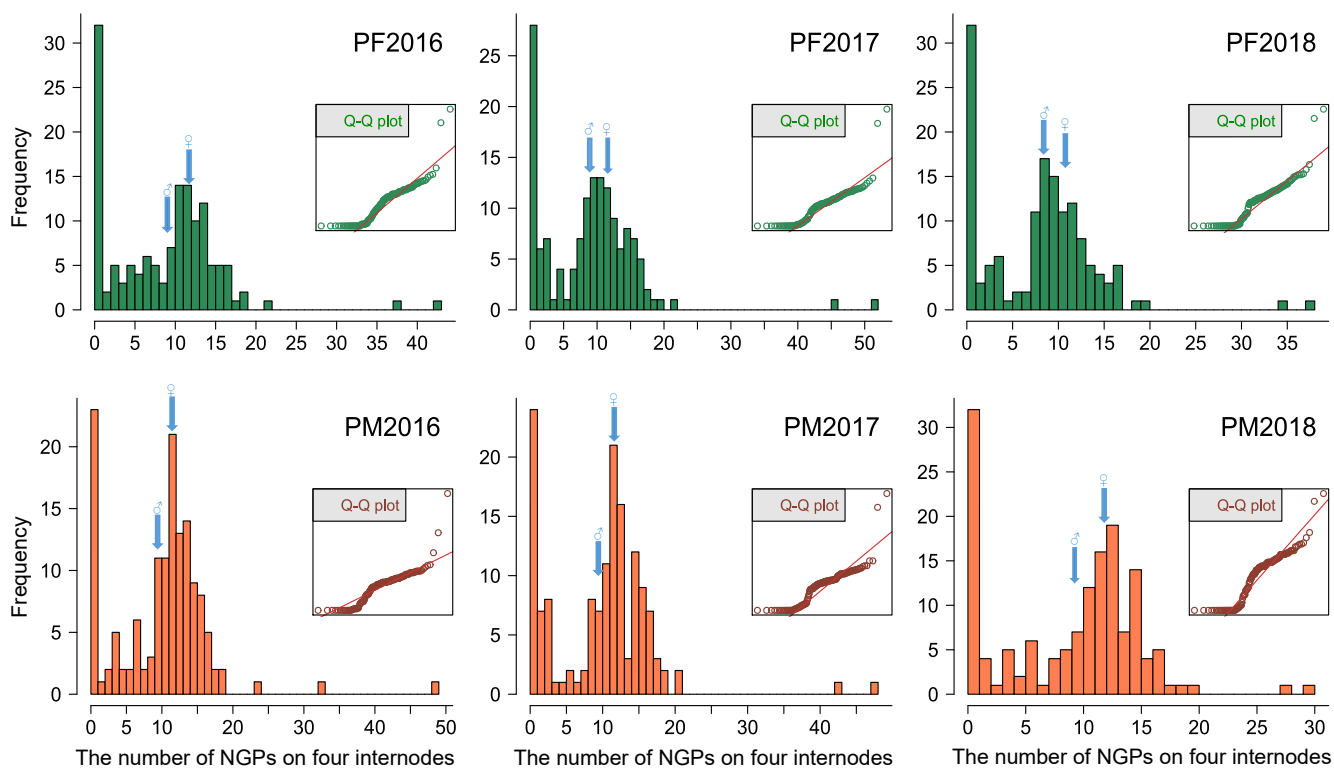
Table 2 Summary of QTLs for NGP with non-parametric model in OW progeny

| QTL | Phenotyping | PT ^a | QTL Characteristics | | | | | 0.95 bayes interval | | 0.99 bayes interval | |
|-----|-------------|-----------------|---------------------|-------------------------|-------------------|-------------------|-----------------|---------------------|---------------------|---------------------|---------------------|
| | | | LOD | LG@positon ^b | r(%) ^c | MM ^d | bp ^e | cM ^f | bp | cM | bp |
| 1a | PF2016 | 2.45 | 5.03 | OB3@51.1 | 11.74 | Rh12GR_16570_782 | 44459262 | 40.38 - 53.75 | 36517224 - 46440369 | 27.59 - 53.75 | 27934327 - 46440369 |
| | PF2017 | 2.42 | 4.33 | OB3@51.1 | 6.92 | Rh12GR_16570_782 | 44459262 | 30.31 - 53.75 | 33612066 - 46440369 | 20.14 - 53.75 | 24888779 - 46440369 |
| | PF2018 | 2.43 | 6.22 | OB3@51.1 | 14.84 | Rh12GR_16570_782 | 44459262 | 39.71 - 53.75 | 36517224 - 46440369 | 32.98 - 53.75 | 33612066 - 46440369 |
| | PM2016 | 2.31 | 2.31 | OB3@51.1 | 7.29 | Rh12GR_16570_782 | 44459262 | 27.59 - 53.75 | 27934327 - 46440369 | 15.43 - 53.75 | 23585838 - 46440369 |
| | PM2017 | 2.49 | 3.58 | OB3@51.1 | 6.65 | Rh12GR_16570_782 | 44459262 | 20.14 - 53.75 | 24888779 - 46474274 | 18.8 - 53.75 | 24000000 - 46440369 |
| | PM2018 | 2.51 | 4.48 | OB3@45.7 | 11.78 | Rh12GR_34665_95 | 41401120 | 36.99 - 53.75 | 35014990 - 46440369 | 20.14 - 53.75 | 24888779 - 46440369 |
| 1b | PF2016 | 2.42 | 8 | RW3@42.6 | 20.05 | Rh12GR_52506_1218 | 42317122 | 37.69 - 42.55 | 41648024 - 42317122 | 35.67 - 42.55 | 40854291 - 42317122 |
| | PF2017 | 2.38 | 8.31 | RW3@42.6 | 12.94 | Rh12GR_52506_1218 | 42317122 | 37.69 - 42.55 | 41648024 - 42317122 | 37.69 - 42.55 | 41648024 - 42317122 |
| | PF2018 | 2.36 | 8.03 | RW3@42.6 | 21.45 | Rh12GR_52506_1218 | 42317122 | 37.69 - 42.55 | 41648024 - 42317122 | 37.69 - 42.55 | 41648024 - 42317122 |
| | PM2016 | 2.51 | 5.88 | RW3@42.6 | 12.76 | Rh12GR_52506_1218 | 42317122 | 16.8 - 42.55 | 32557591 - 42317122 | 9.44 - 42.55 | 16767733 - 42317122 |
| | PM2017 | 2.41 | 9.56 | RW3@42.6 | 12.12 | Rh12GR_52506_1218 | 42317122 | 37.69 - 42.55 | 41648024 - 42317122 | 35.67 - 42.55 | 40854291 - 42317122 |
| | PM2018 | 2.5 | 11.5 | RW3@42.6 | 37.40 | Rh12GR_52506_1218 | 42317122 | 28.3 - 42.55 | 36807925 - 42317122 | 16.8 - 42.55 | 32557591 - 42317122 |
| 2 | PM2016 | 2.31 | 2.89 | OB4@30.6 | 11.64 | Rh12GR_60129_183 | 52239028 | 20.53 - 48.59 | 46189407 - 56107784 | 5.77 - 48.59 | 33319795 - 56107784 |
| | PM2017 | 2.49 | 3.15 | OB4@30.6 | 13.18 | Rh12GR_60129_183 | 52239028 | 11.81 - 48.59 | 36731337 - 56107784 | 4.43 - 48.59 | 30431277 - 56107784 |
| | PM2018 | 2.51 | 3.3 | OB4@30.6 | 10.35 | Rh12GR_60129_183 | 52239028 | 11.81 - 48.59 | 36731337 - 56107784 | 7.78 - 48.59 | 34803638 - 56107784 |
| 3 | PF2017 | 2.38 | 2.8 | RW6@29.7 | 6.73 | Rh12GR_56601_1304 | 31814891 | 8.11 - 42.49 | 4339433 - 44264630 | 0 - 48.52 | 1518964 - 48810479 |
| | PF2018 | 2.36 | 2.81 | RW6@11.5 | 6.67 | Rh88_37299_454 | 5410244 | 6.74 - 44.11 | 7764439 - 62612495 | 1.34 - 54.05 | 1461254 - 64122872 |
| | PM2016 | 2.51 | 4.16 | RW6@29.7 | 8.45 | Rh12GR_56601_1304 | 31814891 | 0 - 38.43 | 1518964 - 48896977 | 0 - 42.49 | 1518964 - 44264630 |
| | PM2017 | 2.41 | 2.7 | RW6@29.7 | 7.07 | Rh12GR_56601_1304 | 31814891 | 15.59 - 45.19 | 8578645 - 45439915 | 8.11 - 49.9 | 4339433 - 41715319 |
| | PM2018 | 2.5 | 2.58 | RW6@29.7 | 5.28 | Rh12GR_56601_1304 | 31814891 | 4.21 - 48.53 | 3340353 - 48180089 | 0 - 53.21 | 1518964 - 52689670 |
| 4 | PF2016 | 2.42 | 3.08 | RW1@34.2 | 6.52 | RhK5_3678_875 | 59006755 | 12.78 - 44.11 | 20231658 - 62553371 | 6.74 - 54.05 | 7764439 - 64122872 |
| | PF2018 | 2.36 | 3.04 | RW1@34.2 | 6.99 | RhK5_3678_875 | 59006755 | 6.74 - 44.11 | 7764439 - 62553371 | 1.34 - 54.05 | 3670420 - 64122872 |

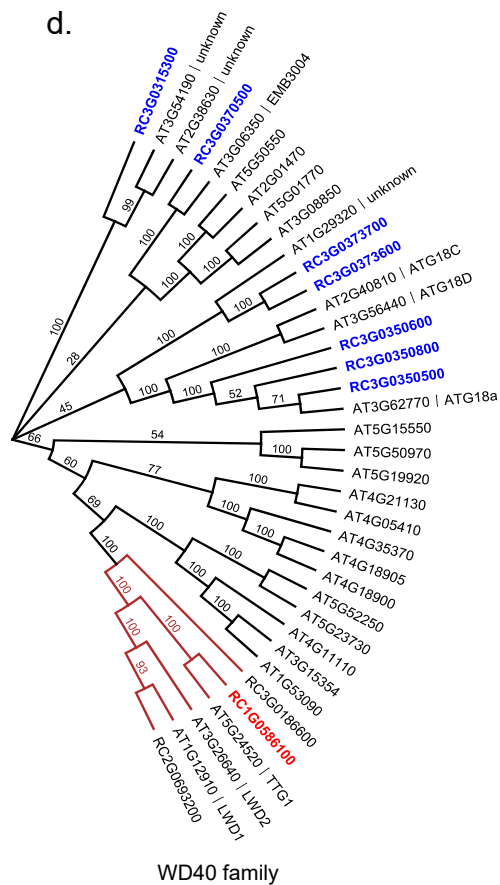
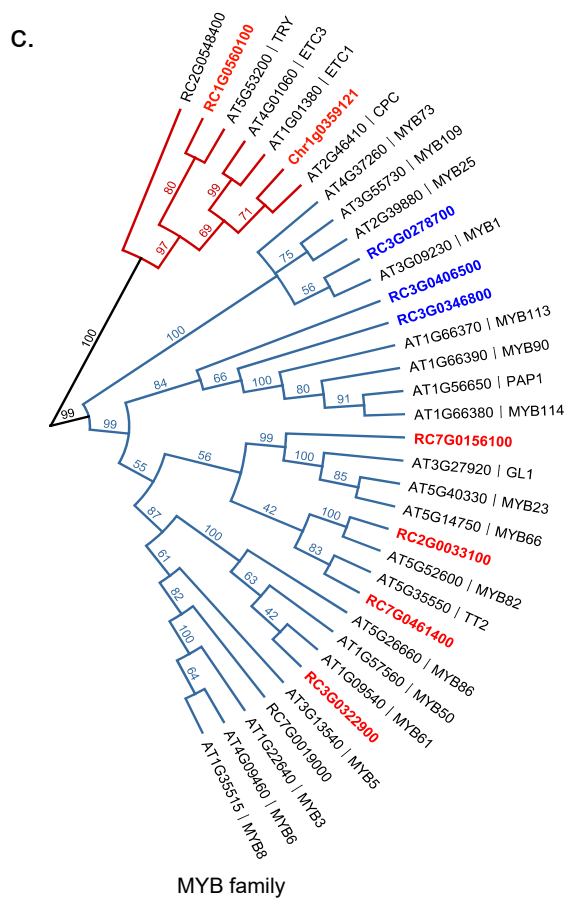
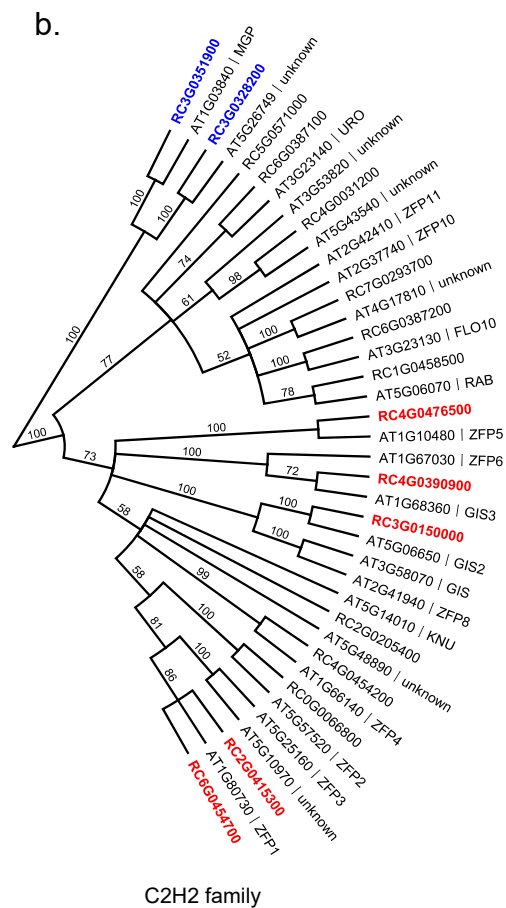
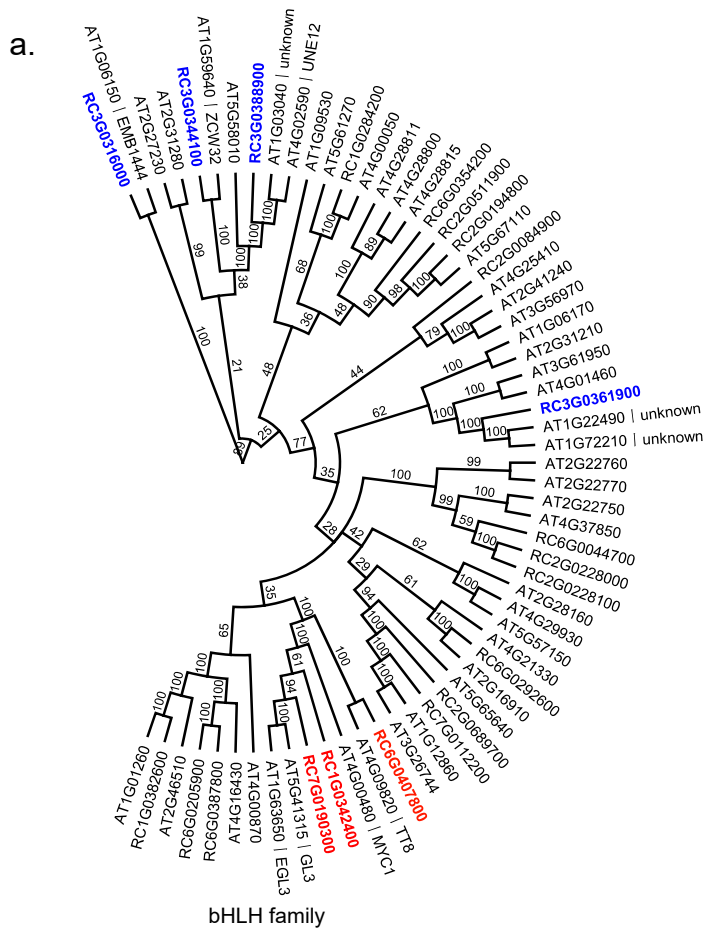
^aPT genome-wild LOD significance threshold was defined by a permutation test. ^bLG@positon chromosomal linkage group, using the separate map (OB and RW) numbering of (Hibrand-Saint Oyant et al., 2018) @ peak location in cM. ^cr(%) Percentage of explanation. ^dClosest molecular marker (MM) associated. ^elocation in base pair (bp) on the *Rosa chinensis* Genome v1.0 (Hibrand-Saint Oyant et al., 2018). ^fcentiMorgan position of QTL peak.



Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3

Supplementary Table 1. Primer sequences of candidate genes for qPCR

| Primer name | sequence of primers |
|-------------|---------------------------------|
| RcMYC1-1-F | 5' CCACCCTCAATGATGTTCTC 3' |
| RcMYC1-1-R | 5' TTCTGGCGTCTCAACACTTAC 3' |
| RcTT8-1-F | 5' AGAGAGCGATGGATTGTTGG 3' |
| RcTT8-1-R | 5' GCCCTCTTCACTTCTGTAATGG 3' |
| RcGIS2-1-F | 5' CTGGTGACTCCGTTGTTCG 3' |
| RcGIS2-1-R | 5' TCCCTAAGATGGATGGATTGA 3' |
| RcGIS3-1-F | 5' GGCCATCGTTGAGTAGGTTC 3' |
| RcGIS3-1-R | 5' GGAGTCAGAGGCTGAGTTGC 3' |
| RcTRY-1-F | 5' GGAAAGCAGAAGAAATAGAGAGG 3' |
| RcTRY-1-R | 5' CTACTACTGACAAGGAAAACCAATG 3' |
| RcTTG1-1-F | 5' TCCAATGTCAATGTACTCGGC 3' |
| RcTTG1-1-R | 5' CCTCCTCAAACCTTCAACAGC 3' |
| RcTTG2-1-F | 5' CCTCAAACCCAGGAGCATC 3' |
| RcTTG2-1-R | 5' CAACAGCTTGATCCCTGAGAG 3' |
| RcCPC-F | 5' GACATTGTGAGGTGTTTGCTGAG 3' |
| RcCPC-R | 5' AATCCGCTGAAAGTTCGACG 3' |
| RcMYB61-F | 5' GGATCTTCAGAGACTCGCTGTAGC 3' |
| RcMYB61-R | 5' CAAGCCCTCCTCTCACATTCAT 3' |
| RcZFP5-F | 5' CAGGAGAAAGCAGACCAGTGAT 3' |
| RcZFP5-R | 5' GGCAAGCCAATCCCTAACTG 3' |

Supplementary Table 2. Summary of QTLs for NGP with two-part QTL model in OW progeny

| Trait | Phenotyping | PT ^a | QTL Characteristics | | | |
|----------------|-------------|-----------------|-------------------------|-------------------|-----------------------|-------------------|
| | | | LG@positon ^b | MM ^c | bd ^d | r(%) ^e |
| Binary(p) | PF2016 | 2.57 | OB3@44.4 | Rw35C24 | 40,215,502 | 15.93 |
| | PF2017 | 2.53 | OB3@44.4 | Rw35C24 | 40,215,502 | 15.18 |
| | PF2018 | 2.6 | OB3@44.4 | Rw35C24 | 40,215,502 | 16.12 |
| | PM2016 | 2.47 | OB3@44.4 | Rw35C24 | 40,215,502 | 14.76 |
| | PM2017 | 2.58 | OB3@44.4 | Rw35C24 | 40,215,502 | 13.38 |
| | PM2018 | 2.51 | OB3@44.4 | Rw35C24 | 40,215,502 | 16.72 |
| | PF2016 | 2.67 | RW3@42.6 | Rh12GR_52506_1218 | 42,317,122 | 29.31 |
| | PF2017 | 2.59 | RW3@42.6 | Rh12GR_52506_1218 | 42,317,122 | 30.33 |
| | PF2018 | 2.57 | RW3@42.6 | Rh12GR_52506_1218 | 42,317,122 | 28.72 |
| | PM2016 | 2.54 | RW3@42.6 | Rh12GR_52506_1218 | 42,317,122 | 20.69 |
| | PM2017 | 2.56 | RW3@42.6 | Rh12GR_52506_1218 | 42,317,122 | 26.84 |
| | PM2018 | 2.59 | RW3@42.6 | Rh12GR_52506_1218 | 42,317,122 | 33.21 |
| | PF2016 | 2.67 | RW2@16.2 | CTG356 | 1,674,220 | 1.80 |
| | PM2016 | 2.47 | RW6@22.3 | RhMCRND_12897_444 | 17,698,816 | 2.70 |
| | PF2016 | 2.42 | OB4@30.6 | Rh12GR_60129_183 | 52,239,028 | 9.02 |
| | PM2016 | 2.36 | OB4@30.6 | Rh12GR_60129_183 | 52,239,028 | 9.26 |
| | PM2017 | 1.92 | OB4@30.6 | Rh12GR_60129_183 | 52,239,028 | 9.88 |
| | PM2018 | 2.8 | OB1@67.7 | Rh12GR_62822_144 | 7388536 and 7,633,108 | 6.66 |
| Quntitative(μ) | PF2016 | 2.39 | RW3@42.6 | Rh12GR_52506_1218 | 42,317,122 | 20.98 |
| | PM2016 | 2.26 | RW3@28.3 | Rh12GR_78941_279 | 36,727,828 | 14.23 |
| | PM2017 | 1.88 | RW3@32.3 | Rh88_36897_190 | 38,554,327 | 12.61 |
| | PM2018 | 2.52 | RW3@42.6 | Rh12GR_52506_1218 | 42,317,122 | 38.64 |
| | PF2018 | 2.57 | RW1@24.1 | Rh88_6034_211 | 45,638,457 | 7.80 |

^aPT genome-wide LOD significance threshold was defined by a permutation test. ^bLG@positon chromosomal linkage group, using the separate map (OB and RW) numbering of (Hibrand-Saint Oyant et al., 2018) @ peak location in cM. ^cClosest molecular marker (MM) associated. ^dlocation in base pair (bp) on the *Rosa chinesis* Genome v1.0 (Hibrand-Saint Oyant et al., 2018). ^er(%) Percentage of explanation.

Supplementary Table 3 Summary the rose homologies genes know in *A. thaliana* to be involved in trichome initiation

| Family | AT genes | Function description | Mutant vs WT | Rose gene name | Rose geneID | Genome location |
|-----------|-----------------|--|---|----------------|--------------|------------------------------------|
| R2R3MYB | AT3G27920/GL1 | Interacts with JAZ and DELLA proteins to regulate trichome initiation | No trichome | RcGL1 | RC7G0156100 | Chr07:11958961..11961286 (2.33 Kb) |
| | AT5G52600/MYB82 | MYB82 and GL1 can form homodimers and heterodimers at R2R3-MYB domains. At least one of the two introns in MYB82 is essential to the protein's trichome developmental function | | RcMYB82 | RC2G0033100 | Chr02:2470719..2472719 (2 Kb) |
| | AT1G09540/MYB61 | Affects trichome initiation, root development and stomatal aperture | Fewer trichome | RcMYB61 | RC3G0322900 | Chr03:39896892..39899077(2.18kb) |
| R3MYB | AT2G46410/CPC | Positive regulator of hair-cell differentiation. Preferentially transcribed in hairless cells. | Increase density | RcCPC | Chr1g0359121 | Chr01:47708266..47710558bp(2.32kb) |
| | AT5G53200/TRY | Involved in trichome branching | cluster phenotype | RcTRY | RC1G0560100 | Chr01:62070383..62072848(2.47 Kb) |
| bHLH | AT5G41315/GL3 | Encodes a basic helix loop helix domain protein that interacts with GL1 in trichome development. GL3 interacts with JAZ and DELLA proteins to regulate trichome initiation. | Fewer trichome | RcGL3 | RC7G0190300 | Chr07:15536877..15543259 (6.38 Kb) |
| | AT4G09820/TT8 | TT8 is a regulation factor that acts in a concerted action with TT1, PAP1 and TTG1 on the regulation of flavonoid pathways, Also important for marginal trichome development. | Fewer trichome | RcTT8 | RC6G0407800 | Chr06:52002793..52009528 (6.74 Kb) |
| | AT4G00480/MYC1 | MYC-related protein with a basic helix-loop-helix motif at the C-terminus and a region similar to the maize B/R family at the N-terminus | Fewer trichome | RcMYC1 | RC1G0342400 | Chr01:44468298..44473643 (5.35 Kb) |
| WD40 | AT5G24520/TTG1 | Involved in trichome and root hair development. Controls epidermal cell fate specification. | No trichome | RcTTG1 | RC1G0586100 | Chr01:63982095..63985616 (3.52 Kb) |
| WRKY | AT2G37260 /TTG2 | Trichome development | Trichome clusters and a reduced trichome number | RcTTG2 | RC3G0244800 | Chr03:33397852..33403551 (5.7 Kb) |
| C2H2 | AT1G10480/ZFP5 | Acts downstream of ZFP6 in regulating trichome development by integrating GA and cytokinin signaling. | | RcZFP5 | RC4G0476500 | Chr04:57125905..57127513 (1.61 Kb) |
| | AT1G68360/GIS3 | GIS3 is involved in trichome initiation and development downstream of GA and cytokinin signaling. | | RcGIS3 | RC4G0390900 | Chr04:50315805..50317009 (1.21 Kb) |
| | AT1G80730/ZFP1 | Expressed at high levels in the shoot apex, including the apical meristem, developing leaves and the developing vascular system | | RcZFP1-like1 | RC2G0415300 | Chr02:47908413..47909551 (1.14 Kb) |
| | | | | RcZFP1-like2 | RC6G0454700 | Chr06:55856328..55858302 (1.98 Kb) |
| | AT5G06650/GIS2 | Regulates trichome formation on inflorescence stems; is also influenced by cytokinins | | RcGIS2 | RC3G0150000 | Chr03:23331984..23333173 (1.19 Kb) |
| HD-ZIP IV | AT1G79840/GL2 | A homeodomain protein affects epidermal cell identity including trichomes, root hairs and seed coat | | RcGL2 | RC2G0467100 | Chr02:54366345..54368366 (2.02 Kb) |
| | | | | | RC2G0467200 | Chr02:54367536..54371324 (3.79 Kb) |
| | | | | | Chr2g0138951 | Che02:54366345..54371324(5.81 Kb) |

Supplementary Table 4 Prickle number on four internodes of two types of stems for three years in OW progeny

| Indiciduals | PF2016 | PF2017 | PF2018 | PM2016 | PM2017 | PM2018 |
|-------------|--------|--------|--------|--------|--------|--------|
| Ow9001 | 0.67 | 5 | 2.67 | 10 | 12.67 | 10.33 |
| Ow9003 | 0 | 0 | 0 | 14 | 12.33 | 8.33 |
| Ow9004 | 2 | 3 | 0.33 | 3 | 3 | 0 |
| Ow9005 | 10.67 | 12 | 11.67 | 10.67 | 12.33 | 11 |
| Ow9006 | 3.67 | 4 | 9 | 3.67 | 4 | 0 |
| Ow9007 | 3.67 | 4.67 | 8.33 | 10 | 4.67 | 5.67 |
| Ow9008 | 6 | 0.67 | 0 | 6 | 2.67 | 1.33 |
| Ow9009 | 7 | 4.33 | 2 | 7 | 5.33 | 12.33 |
| Ow9010 | 11.67 | 11 | 7.67 | 11.67 | 15.67 | 12.67 |
| Ow9011 | 11 | 11.67 | 9 | 11 | 11.67 | 11.67 |
| Ow9012 | 11.67 | 14.67 | 11.67 | 15 | 17 | 14.33 |
| Ow9013 | 1 | 2.67 | 4 | 2.67 | 2.67 | 4 |
| Ow9014 | 2.33 | 10 | 0.67 | 9.67 | 10 | 2 |
| Ow9016 | NA | NA | NA | 8.33 | NA | NA |
| Ow9017 | 15.67 | 17 | 14.33 | 15.67 | 18.33 | 14.67 |
| Ow9018 | 9.67 | 8.67 | 11.33 | 9.67 | 8.67 | 10 |
| Ow9019 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ow9021 | 13.67 | 15.33 | 14.67 | 13.67 | 15.33 | 14.33 |
| Ow9022 | 9.67 | 10 | 7.67 | 11.33 | 9.67 | 9.33 |
| Ow9023 | 15.33 | 15.67 | 13.67 | 15.33 | 15.67 | 14.33 |
| Ow9024 | 16 | 13 | 11.33 | 16 | 16.67 | 16.33 |
| Ow9025 | 14 | 13 | 11.67 | 14 | 13 | 13.67 |
| Ow9027 | 13 | 8.67 | 8.33 | 13 | 8.67 | 9 |
| Ow9029 | 10 | 10 | 7.67 | 10 | 10 | 8.33 |
| Ow9030 | 10.67 | 10.67 | 11.67 | 12 | 11 | 11.33 |
| Ow9031 | 12.33 | 12.33 | 8.33 | 12.33 | 12.33 | 15.33 |
| Ow9032 | 9.33 | 6.67 | 3.33 | 14.33 | 2 | 0.33 |
| Ow9033 | 12.33 | 11 | 9 | 12.33 | 12 | 13.33 |
| Ow9034 | 15.67 | 17.33 | 13.33 | 15.67 | 17.33 | 15 |
| Ow9035 | 14 | 15 | 16.67 | 14 | 15 | 5 |
| Ow9036 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ow9037 | 0 | 0.67 | 2 | 0.33 | 0.67 | 1 |
| Ow9038 | 0 | 0 | 0 | 7.67 | 2.33 | 5.33 |
| Ow9039 | 10.33 | 13 | 10.33 | 13.67 | 12 | 14.33 |
| Ow9040 | 11.67 | 11.67 | 9 | 11.67 | 12 | 10.33 |
| Ow9041 | 5.67 | 6.67 | 8.67 | 5.67 | 6.67 | 10.33 |
| Ow9042 | 11.67 | 9.67 | 13 | 11.67 | 12.67 | 13.67 |
| Ow9044 | 0.67 | 1 | 0.67 | 3.33 | 1 | 0 |
| Ow9045 | 3.33 | 4.33 | 4.33 | 3.33 | 2.67 | 3.67 |
| Ow9046 | 0 | 0.33 | 4 | 12.67 | 0.33 | 0.33 |
| Ow9047 | 4.33 | 2 | 6 | 4.33 | 2 | 0 |
| Ow9049 | 1 | 0 | 4 | 1 | 0 | 0 |
| Ow9050 | 14 | 15.33 | 16 | 14 | 15.33 | 13.33 |
| Ow9051 | 0.67 | 0.33 | 0 | 0.67 | 0.33 | 0.33 |
| Ow9052 | 13.67 | 14.33 | 12.33 | 16.33 | 14.33 | 12.33 |
| Ow9054 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ow9055 | 6.67 | 6.67 | 9.33 | 6.67 | 11 | 11.67 |

| | | | | | | |
|--------|-------|-------|-------|-------|-------|-------|
| Ow9056 | NA | 0 | 0 | 0 | 0 | 0 |
| Ow9057 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ow9058 | NA | 0 | 0 | 0 | 0 | 0 |
| Ow9059 | 0.33 | 1.33 | 0.33 | 0.33 | 1.33 | 0.67 |
| Ow9060 | 1 | 1.33 | 2.67 | 1 | 1.33 | 1 |
| Ow9061 | 12.67 | 12 | 9.33 | 11.33 | 12 | 12.33 |
| Ow9062 | 12.67 | 9.67 | 7.67 | 14.67 | 14.33 | 14.67 |
| Ow9065 | 2 | 1.67 | 0 | 3.67 | 8.67 | 4 |
| Ow9066 | 2.33 | 6.33 | 3.67 | 10.67 | 10 | 11 |
| Ow9067 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ow9068 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ow9069 | 14.33 | 14.67 | 11 | 14.33 | 14.67 | 13 |
| Ow9071 | 14 | 21.33 | 12.67 | 18.67 | 16 | 13.67 |
| Ow9072 | 16.67 | 16.67 | 13 | 16.67 | 16.67 | 12.33 |
| Ow9074 | 11.67 | 11 | 10.33 | 11.67 | 11 | 5.67 |
| Ow9075 | 11.67 | 14 | 9.33 | 11.67 | 14 | 12.67 |
| Ow9076 | 2.67 | 3 | 1.67 | 11.67 | 3 | 0 |
| Ow9077 | 10.67 | 9.67 | 10.33 | 10.67 | 15 | 14.33 |
| Ow9078 | 0.67 | 0 | 0 | 0.67 | 0 | 0 |
| Ow9079 | 11.67 | 8.67 | 7.67 | 11.67 | 12 | 12 |
| Ow9080 | 12.67 | 10.33 | 9.67 | 13.33 | 16.33 | 12.33 |
| Ow9081 | 13 | 13 | 12.33 | 13 | 15 | 15.33 |
| Ow9082 | 9.33 | 12 | 11.33 | 9.33 | 12 | 11.33 |
| Ow9083 | 12 | 9 | 9 | 12 | 9 | 12 |
| Ow9084 | 14.33 | 14 | 13.67 | 15.67 | 20.33 | 17 |
| Ow9085 | 12 | 3 | 10 | 12 | 12 | 13 |
| Ow9087 | 14.33 | 15 | 12.67 | 14.33 | 15.33 | 14.67 |
| Ow9088 | 10.33 | 11.33 | 10.33 | 12.33 | 11.33 | 11 |
| Ow9089 | 13.33 | 13.67 | 13 | 13.33 | 15 | 15.33 |
| Ow9091 | 0.67 | 1 | 0.67 | 0.67 | 1 | 0 |
| Ow9092 | 8 | 7.33 | 8.33 | 15.67 | 12 | 6 |
| Ow9095 | 11 | 11.33 | 7.33 | 11 | 11.33 | 12.33 |
| Ow9096 | 5.67 | 10.67 | 9.33 | 9.67 | 10.67 | 11 |
| Ow9098 | 2.67 | 10.67 | 9.33 | 9.33 | 11 | 11.33 |
| Ow9099 | 13.33 | 12 | 14.67 | 13.33 | 12.67 | 9.33 |
| Ow9100 | 6.67 | 8 | 8.33 | 6.67 | 8 | 9.33 |
| Ow9101 | 15 | 16 | 11.33 | 15 | 15 | 17.33 |
| Ow9103 | 15 | 8.67 | 11 | 15 | 15 | 14.33 |
| Ow9104 | 10.67 | 12.33 | 9 | 13 | 11.67 | 13 |
| Ow9105 | 12.67 | 12 | 8 | 12.67 | 12.33 | 12 |
| Ow9106 | 43 | 45.67 | 38 | 48.67 | 48 | 30 |
| Ow9107 | 38 | 52 | 35 | 32.33 | 42.33 | 29 |
| Ow9109 | 12 | 10.67 | 9.67 | 12 | 10.67 | 11.33 |
| Ow9111 | 6 | 2.67 | 0.33 | 16.33 | 2 | 10.33 |
| Ow9113 | 15.67 | 14.33 | 13.33 | 15.67 | 18.33 | 13.33 |
| Ow9115 | 6.67 | 0.33 | 0.33 | 6.67 | 6 | 1.33 |
| Ow9116 | 12 | 12.67 | 12 | 12 | 12.67 | 11.33 |
| Ow9117 | 13.33 | 14 | 15 | 15 | 16 | 14.33 |
| Ow9119 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ow9120 | 8 | 10 | 9.33 | 8 | 10 | 7 |
| Ow9121 | 4.67 | 5.33 | 5.67 | 11.67 | 10.33 | 9.67 |

| | | | | | | |
|--------|-------|-------|-------|-------|-------|-------|
| Ow9122 | 9 | 9.67 | 10.67 | 9 | 9.67 | 7.33 |
| Ow9123 | 11.67 | 7.67 | 7.67 | 10 | 9 | 9 |
| Ow9124 | 5 | 10 | 9.67 | 12.67 | 12.67 | 12.67 |
| Ow9125 | 7.67 | 9 | 7 | 11.33 | 11.67 | 9.33 |
| Ow9126 | 9.67 | 15 | 10 | 24 | 20.33 | 12.33 |
| Ow9127 | 13.33 | 11.67 | 8.67 | 13.33 | 11.33 | 11 |
| Ow9128 | 0 | 1.33 | 0 | 0 | 1.33 | 1.33 |
| Ow9129 | 5 | 10.67 | 11.33 | 12 | 11 | 11.33 |
| Ow9132 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ow9133 | 0.67 | 1.67 | 0 | 0.67 | 1.67 | 0 |
| Ow9134 | 18 | 13.67 | 16.67 | 18 | 13.67 | 18.67 |
| Ow9137 | 5 | 7.67 | 3.67 | 8.33 | 11 | 8 |
| Ow9138 | 10.67 | 10 | 8 | 15 | 15.67 | 12.33 |
| Ow9139 | 18.67 | 20 | 20 | 17.33 | 17.33 | 15 |
| Ow9140 | 6.67 | 9 | 9 | 6.67 | 11.67 | 10.67 |
| Ow9142 | 10.67 | 10.33 | 9.67 | 10.67 | 10.67 | 12.67 |
| Ow9143 | 16.67 | 12 | 9.33 | 13 | 12.33 | 9.33 |
| Ow9144 | 21.33 | 15 | 16 | 16 | 14.33 | 14.67 |
| Ow9147 | 16.33 | 18 | 13.67 | 16.33 | 18 | 11.33 |
| Ow9148 | NA | 16 | 16 | NA | 16 | 8 |
| Ow9149 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ow9150 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ow9151 | NA | 12.33 | 12 | NA | 12.33 | 13.33 |
| Ow9152 | 13.33 | 13.33 | 16.33 | 13.33 | 15 | 20 |
| Ow9153 | 0 | 2.33 | 2.33 | 3.67 | 2.33 | 3 |
| Ow9154 | 16.33 | 15.33 | 15.33 | 16.33 | 15 | 12.33 |
| Ow9155 | 8 | 9 | 9 | 11.67 | 11.67 | 10.33 |
| Ow9156 | 8.33 | 8 | 7.67 | 11.67 | 11.67 | 10.67 |
| Ow9158 | NA | 9 | 3 | NA | 8.67 | 4 |
| Ow9159 | 9.67 | 10 | 10.67 | 11 | 12.33 | 11.33 |
| Ow9160 | 17 | 15.33 | 10 | 12 | 12.33 | 14.33 |
| Ow9161 | 10.33 | 9.67 | 10 | 10.33 | 12 | 7.33 |
| Ow9163 | 0.67 | 2.33 | 0 | 0.67 | 2.33 | 0.67 |
| Ow9166 | 0 | 0 | 0 | 2 | 0 | 0 |
| Ow9167 | 11 | 9.67 | 10 | 11 | 9.67 | 10 |
| Ow9168 | 6.33 | 9 | 7 | 6.33 | 9 | 6 |
| Ow9169 | 10.67 | 10.67 | 11 | 10.67 | 10.67 | 11.33 |
| Ow9171 | 0 | 0.67 | 0 | 0 | 0.67 | 0 |
| Ow9172 | 13.33 | 16.33 | 17 | 13.33 | 16.33 | 15.33 |
| Ow9173 | NA | 16.67 | 16.67 | NA | 16.67 | 16.67 |
| Ow9174 | NA | 8 | 8 | 13.33 | 8 | 5.33 |
| Ow9175 | 11.67 | 9 | 8.33 | 13.33 | 14 | 12.33 |
| Ow9178 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ow9179 | 12.67 | 13 | 12.33 | 12.67 | 12.67 | 16.33 |
| Ow9180 | 11 | 10.33 | 10.67 | 11 | 11.33 | 12.33 |
| Ow9181 | 0 | 0 | 0 | 0 | 0 | 0 |
| Ow9182 | 7.67 | NA | 7.67 | 9.67 | NA | 3.67 |
| Ow9185 | 12.67 | 11.33 | 11.67 | 12.67 | 11.33 | 11.67 |
| Ow9186 | 0 | NA | 2.67 | 4.67 | NA | 2 |
| Ow9190 | 11.67 | 16.33 | 10.67 | 11.67 | 13 | 13 |
| Ow9191 | 2.67 | 7.33 | 7.67 | 9.67 | 9 | 8.67 |

| | | | | | | |
|--------|------|-------|-------|-------|-------|-------|
| Ow9192 | 8.33 | 10.33 | 9 | 12.67 | 11.33 | 11.33 |
| Ow9197 | 19 | 19 | 18.33 | 19 | 16.33 | 17 |
