Morphological and molecular studies for rose prickles provided new insights

Zhou NN[1,2*], Hibrand-Saint Oyant L[1*]& Foucher F[1*]
[1]Univ Angers, Institut Agro, INRAE, IRHS, SFR QUASAV, F-49000 Angers, France
[2]National Engineering Research Center for Ornamental Horticulture; Flower Research Institute (FRI), Yunnan Academy of Agricultural Sciences, Kunming 650231, China
[*]Corresponding author. Email: *ningning.zhou@aliyun.com* or *fabrice.foucher@inrae.fr*

Background

Prickles are sharp appendages of plants that was thought to be a defense against insect and mammalian herbivores [1,2]. In the agricultural production process, prickles are an undesirable trait because the crops with prickle are more difficult to handle, harvest, transport and also bring safety hazards for workers. Roses are among the most popular and economically important horticultural crops. In the *Rosa* genus, prickles are largely present with a great diversity in terms of form and density (Figure 1). With the recent development of genetic and genomic resources, rose can be a good model to study the molecular and genetic bases of prickle initiation and development [3]. This study focused on filling the gaps in prickle knowledge using morphological, genetics, and transcriptomics approaches. Our objective was to decipher the molecular and genetic control of the initiation and development of prickles on rose stems.

Figure 1: Prickle diversity on rose stem. Subg. *Rosa*: section *Cinnamomeae*. (a) *R. rugosa* scabrosa, (b) 'Marie Bugnet' (Hybrid *Rugosa*.), (c) *R. acicularis*, (d) *R. Rubella*, (e) *R. laxa retzius*, (f) *R. fraxinifolia*; section *Pimpinellifoliae* (g) *R. ecae*, (h) *R. omeiensis*, (i) *Rosa primula* (Les racines du vent), (g) *R. pimpinellifolia* King of the Scots, (k) *R. pimpinellifoli*a aïcha, (l) *R. foetida*. section *Bracteate* Theory (m) R. sherardi, (n) R. horrida, (o) R. scabriuscula, (p) *R. bracteate*; Subg. *Hesperhodos*: (q) *R. roxburghii*

hirtula. *Subg. Hulthemia* (r) *R. hultemia* persica. Subg. *Platyrhodon*: (s) *R. stellata*, (t) *R. minutifolia*. Credits: NN ZHOU, except r (Yuriy Danilevsky), s (Dave's Garden), t (Stan Shebs).

Methods

Plant materals

A diploid OW population, obtained from a cross between *Rosa chinensis* 'Old Blush' (OB) and *Rosa* × *wichurana* (RW), was grown in a field and managed by the Horticulture Experimental Unit (INRAE, Angers, France). F1 individuals used for morphological and transcriptomic studies were cut and managed in IRHS greenhouses in November 2017.

Rosa resources used for prickle investigation were planted in Loubert Rose Gardens (Rosiers sur Loire, France), INRAE (Angers, France) and Flower Research Institute (FRI, Kunming, China).

Morphological studies

To well understand the origin of prickles, their types, and their development, we investigated the prickle types in rose wild species, in parents and progeny of a F1 population (OW). We carried out a comprehensive anatomical study for two representative types of prickles, non-glandular (NGPs) and glandular (GPs) prickles. Based on these observations, we then performed a survey of prickle diversity on the genus *Rosa*, with a more precise observation on twelve representative genotypes of different Rosa sections [4].

Forward Genetic studies

To study the genetic determinism, we used 151 hybrids of OW population. Plants were scored for the number of prickles on the floral and main stems for three years. QTL analyses were carried out using the R/qtl in R version 3.2.3 using the non-parametric model and the two-part model [5].

Reverse genetics studies

As a certain homologies and resemblances exist between prickles and trichomes, in order to found putative candidate-gene for the identified QTLs, we looked for homologues of transcription factors (TFs) known to be involved in trichome initiation and development in *A. thaliana*. We annotated fifteen rose TFs and searched the genes that were co-located below the QTLs interval region and closely linked to the QTLs. Using RT-qPCR, we followed the transcript accumulation of these TFs genes in glabrous and prickly F1 hybrids at different developmental stages [5].

Transcriptomic studies

To mine new regulators of prickle initiation and development, we used a mixed pool of F1 individuals with or without NGPs, we presented the transcriptomic changes during prickle initiation and

development by comparing the transcriptome of rose stems with and without prickles and a detailed time-course transcriptomic analysis of prickle development. We developed two unusual transcriptomic analyses methods to optimize the candidate genes pool. The precisely detailed analyses allow us to detect important regulators of prickle initiation and development. We highlighted the best potential regulators of prickle initiation by combining the transcriptomic results with genetic studies [5].

Results

Based on the prickle investigation in 110 roses, we proposed the first categorization of prickles in the *Rosa* genus. They are mainly divided into two categories: NGPs and GPs, and sub-categories were defined based on the presence/absence of hairs and branches. We demonstrated that NGPs and GPs are both originated from multiple cells of the ground meristem beneath the protoderm (Figure 2d and h). For GPs, the gland cells are originated from the protoderm of GP at the early developmental stage (Figure 2i and g). Our conclusions clearly demonstrate that prickles are not modified trichomes (which are originated from the protoderm). These conclusions are different from the previous studies, which reported that prickles were originated from epidermis [6–8] and were modified from glandular trichomes [9,10], or induced from the signals of glandular trichomes [11]. These results have been accepted to publish in Horticulture research [4].

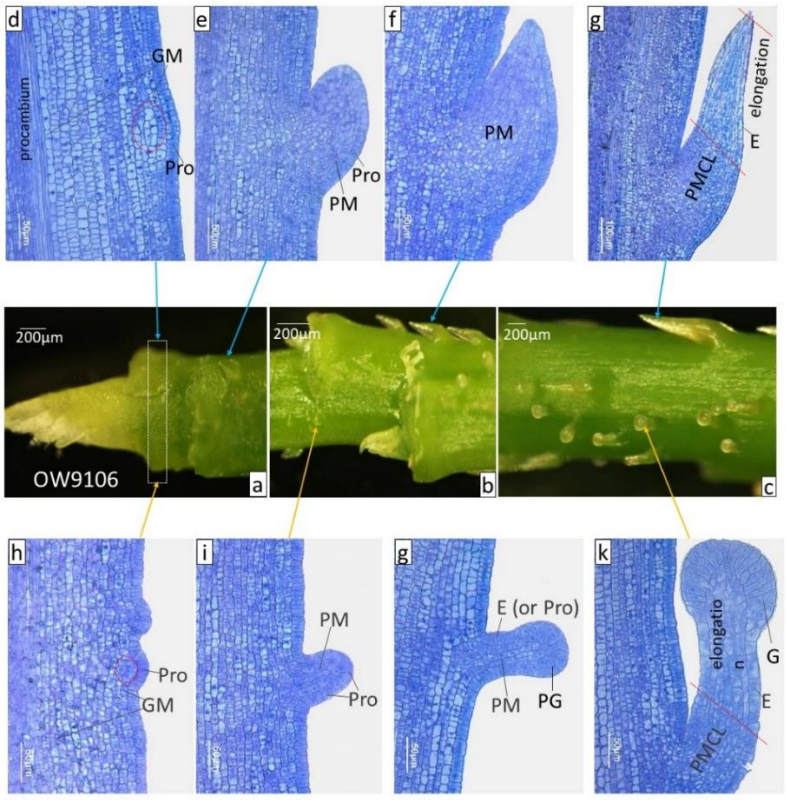

Figure 2: Anatomy of non-glandular (NGPs) and glandular (GPs) prickles in OW9106. (a-c) Macroscopic pictures of the different stages of GP and NGP on the stem (leaves and leaf primordia were removed); Anatomy of stage I (d-f), IIa (g) of non-glandular prickle; Anatomy of stage I (h-g), IIa (k) of glandular prickle. White dotted frame represents the first internode; I: prickle initiation; Pro: protoderm; GM: ground meristem; PM: prickle meristem; PMCL: prickle meristematic cell like; E: epidermis; PG: precursor gland; G: gland.

For the genetic determinism study, we performed QTL analysis for these two types of prickles. However, for the GPs, we were unable to detect any significant QTLs since the individuals presenting GPs were not numerous enough in the OW population. Thus, we focused on exploring genetic determinism and the gene network for the NGPs, the most common type of prickles on rose stems. We detected four prickle loci on LG1, 3, 4 and 6 and determined their credible interval on the 'Old blush' reference genome [12]. We further detected that one QTL on LG3 is a major locus that controls the presence of prickles, and three QTLs (LG3, 4 and 1) may be responsible for prickle density (Figure 3). We also revealed that glabrous hybrids are caused by the combination of the two recessive alleles from both parents. The results have been published in journals Nature Plants[12] and theoretical and Applied Genetics (TAG) [11].

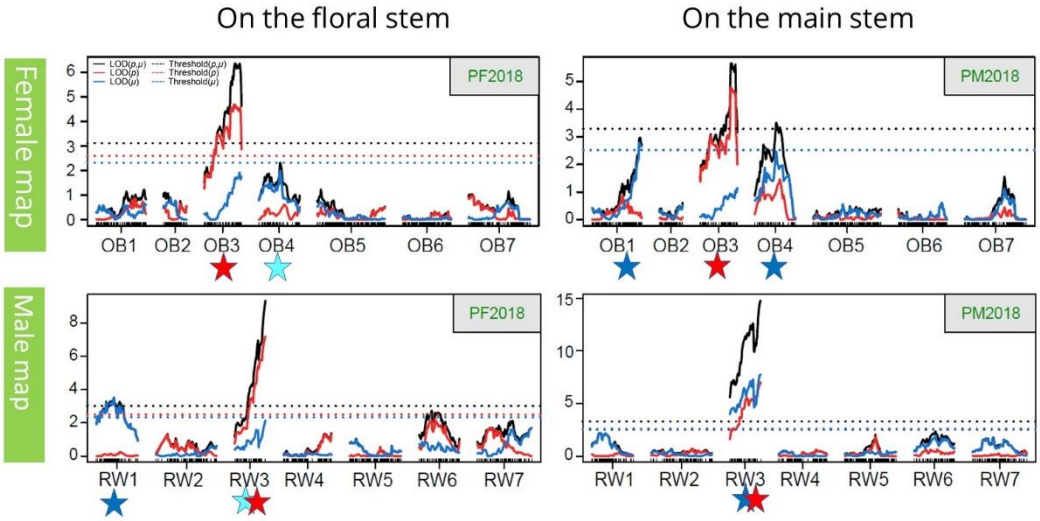

Figure 3: LOD curves of the QTL scan for the NGPs on the floral (FM) and main (PM) stems in (**a)** female (OB) and (**b)** male (RW) maps calculated using the two-part approach. The LOD (p) value (penetrance) is in red, the LOD (μ) value (severity) is in blue, and the LOD (p, μ) value is in black. The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

By studying the transcript accumulation of ten homologues TF in glabrous and prickly F1 hybrids at different developmental stages (Figure 4a), we find that only minor differences exist between glabrous and prickle samples of each stage (Figure 4b). The main differences were observed between the

development stages not only in prickle samples, but also in glabrous samples (Figure 4c). These results have been published in TAG [5]. Therefore, according to anatomic and transcriptomic evidences, we suggested that non-glandular prickles and non-glandular trichomes have different genetic pathways controlling their initiation [4]. This conclusion is different from the current hypothesis: rose NGPs and *A. thaliana* unicellular non-glandular trichomes (NGTs) share the same genetic pathway for their initiation [13,14].

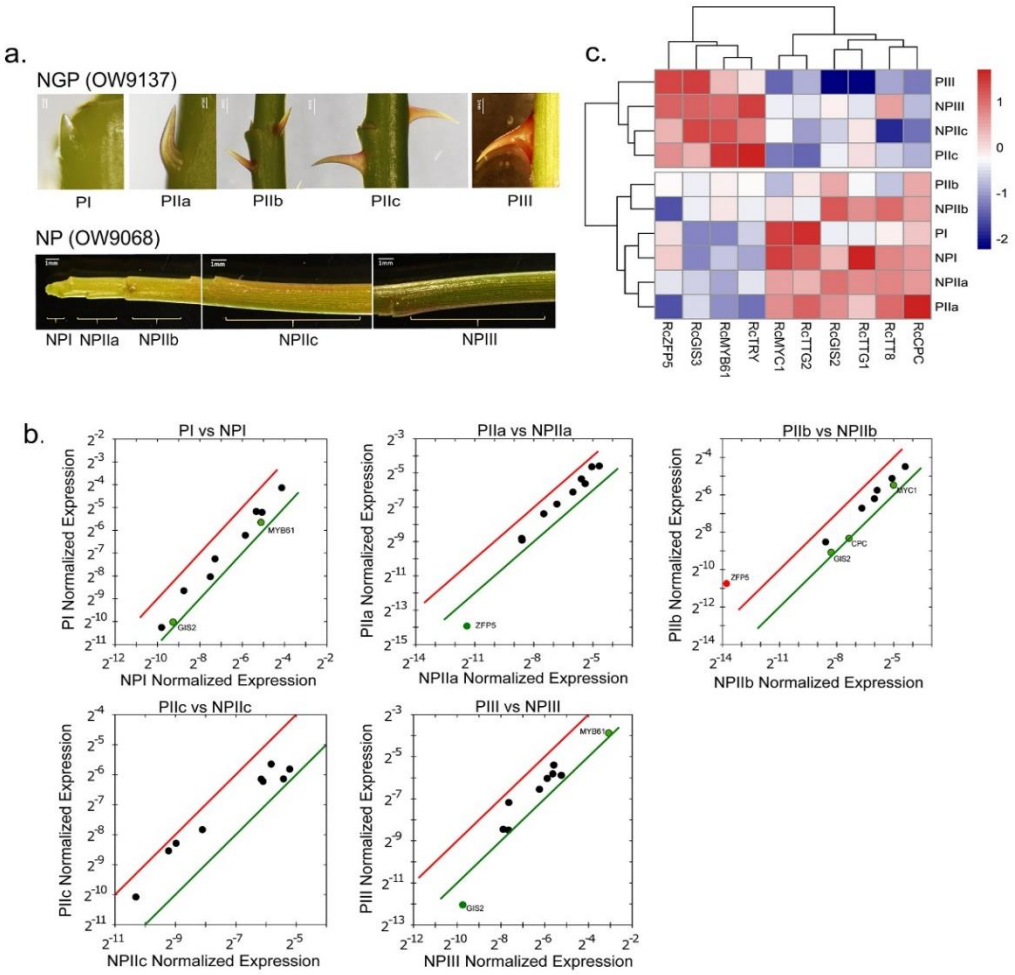

Figure 4: Transcript accumulation of candidate genes followed by qPCR in glabrous and prickly F1 hybrids at different developmental stages. **(a)** Stem development and sampling stages in non-glandular prickles (NGP, OW9137) and glabrous stems (NP, OW9068). **(b)**The scatter plot of the candidate genes' normalized expression in prickle and glabrous individuals in different stages (as defined in Figure 1a). 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)** A heatmap showed the relative expression of ten candidate genes in all the samples. [5]

Using the transcriptomic approach, we detected 2118 candidate genes may positively and negatively involve in prickle initiation. By the unusual methods developed in this study, we provided a narrow range of candidate genes pool (660) as the priority candidates for prickle initiation only. We further mined identified ten best candidates using an integrative approach. We also provided evidences to support our new insight of NGPs and NGTs have different gene pathways. The results have not published yet.

Conclusion

We proposed first classification of prickles in *Rosa* genus: prickles are mainly divided into NGPs and GPs, and sub-categories were defined based on the presence/absence of hairs (trichomes) and branches. The non-glandular prickle (NGP) is the major type in roses. NGP and GP are both originated from multiple cells of the ground meristem under the protoderm. For GP, only the gland cells come from the epidermis (or protoderm). Since NGP and GP have their own developmental processes, I suggested NGP is not modified from glandular structure. The different segregations of GP and NGP in OW population indicated that they do not share a common genetic basis. The NGP genetic determinism in rose is complex, with a major locus on LG3 that controls the absence/presence of prickles on the rose stem, and several QTL that controls the prickle density (three QTL on LG4, 3, 1 have been detected in OW population). Further studies are necessary to develop markers for breeding selection and to identify the molecular bases. With anatomic and transcriptomic evidences, we suggested that non-glandular prickles and non-glandular trichomes have different genetic pathways controlling their initiation. Approaches such as transcriptomics may help to identify new key regulators of prickle initiation and development in rose.

Funding

These works were supported by funding from the National Natural Science Foundation of China (31760585) and the China Scholarship Council ([2017]3109).

References

1 Cooper SM, Owen-Smith N. Effects of plant spinescence on large mammalian herbivores. *Oecologia* 1986; **68**: 446–455.

2 Kariyat RR, Hardison SB, De Moraes CM, Mescher MC. Plant spines deter herbivory by restricting caterpillar movement. *Biology Letters* 2017; **13**: 20170176.

3 Zhou NN. *Genetics and genomics of prickles on rose stem. PhD thesis*. 2021.

4 Zhou NN, Simonneau F, Thouroude T, Hibrand-Saint Oyant L, Foucher F. Morphological studies for rose prickles provided new insights. *Horticulture research* 2021; Accepted to publish.

5 Zhou NN, Tang KX, Jeauffre J *et al.* Genetic determinism of prickles in rose. *Theor Appl Genet* 2020. doi:10.1007/s00122-020-03652-7.

6 Peiterson AK. *Blackberries of New England - Genetic status of the plants. Bull. 218 Vermont Agricultural Experiment Station*. 1921.

7 Esau K. Anatomy of Seed Plants. *Soil Science* 1960; **90**: 149.

8 Coyner MA, Skirvin RM, Norton MA, Otterbacher AG. Thornlessness in blackberries. *Small Fruits Review* 2005; **4**: 83–106.

9 Kellogg AA, Branaman TJ, Jones NM, Little CZ, Swanson J-D. Morphological studies of developing *Rubus* prickles suggest that they are modified glandular trichomes. *Botany* 2011; **89**: 217–226.

10 Khadgi A, Weber CA. Morphological Characterization of Prickled and Prickle-free Rubus Using Scanning Electron Microscopy. *HortScience* 2020; **55**: 676–683.

11 Pandey S, Goel R, Bhardwaj A, Asif MH, Sawant SV, Misra P. Transcriptome analysis provides insight into prickle development and its link to defense and secondary metabolism in *Solanum viarum* Dunal. *Sci Rep* 2018; **8**: 1–12.

12 Hibrand-Saint Oyant L, Ruttink T, Hamama L *et al.* A high-quality genome sequence of *Rosa chinensis* to elucidate ornamental traits. *Nature Plants* 2018; **4**: 473.

13 Swarnkar MK, Kumar P, Dogra V, Kumar S. Prickle morphogenesis in rose is coupled with secondary metabolite accumulation and governed by canonical MBW transcriptional complex. *Plant Direct* 2021; **5**: e00325.

14 Zhong M-C, Jiang X-D, Yang G-Q *et al.* A genomic link in China roses: and they all lived prickly but water deficient ever after? *National Science Review* 2021; nwab092.