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1 **Contrastive analysis of trichome distribution, morphology, structure and**  
2 **associated gene expression reveals formation factors in different trichome**  
3 **types of two commercial *Rosa* species.**

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13 **Abstract:** Trichomes are prevalent on the surfaces of many organs in *Rosa* and have an important impact on  
14 the edibility of the fruit. Here, the diversity, distribution, anatomical structure and genetic regulation of  
15 trichomes in *Rosa roxburghii* Tratt. (RR) and *R. sterilis* S. D. Shi (RS) are explored. RS and RR are important  
16 commercial crops in China due to their nutritional and medicinal values, however their consumption and  
17 utilization is limited, in part, by the abundance of trichomes on the fruit. **There are two main forms of trichomes**  
18 **in both germplasms, namely glandular and non-glandular trichomes.** Non-glandular flagellate and acicular  
19 trichomes are observed on the sepals, fruit, major leaf veins and pedicels of both germplasms, but non-glandular  
20 branched trichomes **are** present only on RS. Exfoliation of flagellate trichomes and lignification of acicular  
21 trichomes **occur** gradually on developing fruit. Capitate glandular and bowl-shaped glandular trichomes were  
22 abundant in RS, but were only observed on the major leaf veins of RR. Furthermore, some capitate glandular

23 trichomes on the pedicels were found to develop into prickles in RS. Transmission electron microscopy  
24 indicated that vacuolation and in the extraplasmic space occurred in the glandular cells of capitate trichomes at  
25 the late secretory stage. Prickles of RR and RS mainly consisted of lignin, suberin, cellulose and hemicellulose,  
26 however the ratios of these constituents varied between species. The expression levels of several well-known  
27 core trichome regulatory genes varied depending on the trichome types. The transcript abundance of *GL1*, *GL2*  
28 and *TTGI* was significantly higher in organs covered with acicular trichomes in both RR and RS, while the  
29 highest mRNA level of *TRY* was observed in glabrous organs, suggesting a negative effect of this gene on  
30 trichome formation. It was noteworthy that the negative regulatory factor *CPC* was only highly expressed in the  
31 leaf mesophyll of RR, where no glandular trichomes were present, but *GL3*, *PDF2-like* and *CPC* transcription  
32 factors co-regulated glandular trichome formation in RS. Expression of these genes peaked in the Fb3-stage  
33 buds of RR and B3-stage buds of RS, indicating different key phases for the regulation of trichome initiation.  
34 These data provide new insights into the genetic control of trichome formation in two *Rosa* species.

35 **Keywords** *Rosa*; trichome; morphology; prickle; gene expression

## 36 **1. Introduction**

37 Trichomes are present on the epidermal surfaces of leaves, stems, sarcocarps, fruits, seeds and other parts of  
38 many terrestrial plants (Chen et al., 2014). They originate from epidermal cells and are characterized by their  
39 morphology, including whether they are glandular or non-glandular, unicellular or multicellular, branched or  
40 non-branched (Werker, 2000). These structures can perform a range of functions for ecological interactions, such  
41 as protection against physical or biological stress (Karabourniotis et al., 2020). Furthermore, trichome structure  
42 has been used traditionally as an important factor in plant classification (Ma et al., 2016; Sajna et al., 2018).

43 The mechanisms of trichome initiation and development for unicellular trichomes have been extensively  
44 studied in *Arabidopsis* (Hülkamp, 2004; Schellmann and Hülkamp, 2005; Machado et al., 2009). Numerous

45 studies have identified a set of putative transcriptional regulators (comprising both positive and negative  
46 transcription factors) that control the process of epidermal cell development. The positive regulators include  
47 R2-R3 type MYB transcription factors **GLABRA1 (GL1)**, the basic helix-loop-helix (bHLH) protein  
48 **GLABRA3 (GL3)** and the WD-repeat protein **TRANSPARENT TESTA GLABRA1 (TTG1)** (Walker et al.,  
49 1999; Zhang et al., 2003). These three positive regulatory proteins together activate trichome differentiation by  
50 enhancing the expression of **GLABRA2 (GL2)** (Serna, 2004). Single-repeat R3 MYB transcription factors  
51 **CAPRICE (CPC)**, **TRIPTYCHON (TRY)**, and transcriptional enhancers of **TRY** and **CPC**, such as **ETC1 ETC2**  
52 **and ETC3**, competitively bind to bHLH factors when they move to the neighboring cells (Gan et al., 2011;  
53 Pesch and Hülskamp, 2011; Tominaga-Wada and Wada, 2017). However, unlike unicellular trichomes,  
54 relatively little is known about the development and regulatory networks of multicellular trichomes in plants.

55 *Rosa roxburghii* Tratt. (RR) is widely distributed in southwest and northwest China, whose fruits are  
56 valued for their high levels of ascorbic acid (AsA), superoxide dismutase activity and cancer-preventing effects.  
57 *R. sterilis* S. D. Shi (RS) similarly exhibits numerous nutritious and antioxidant activities. RR and RS fruits are  
58 being increasingly utilized as functional foods in the food industry to produce preserved foods and desserts, as  
59 well as in the beverage industry to produce wine, beer and soft drinks (He et al., 2016; Li et al., 2016). RR and  
60 RS belong to the **Rosaceae** family, and they have a very close genetic relationship based on RAPD and AFLP  
61 molecular markers (Wen and Deng, 2003; Wen et al., 2004). However, RR and RS differ in their appearance, the  
62 most significant of which are seed abortion and the differences in fruit shape, size and anatomy from RS to RR.

63 Trichomes are **present** on the buds, stems, pedicels and fruits of RR and RS, which **affect** the appearance  
64 and perceived quality of the fruits. However, to date, little is known about the formation or regulation of  
65 trichomes in these plants. Detailed analysis of trichome types and distribution in vegetative parts of *Rosa* have  
66 not been explored in previous studies (Caissard et al., 2006; Asano et al., 2008; Feng et al., 2015). However, He

67 et al., (1994) and Kellogg et al., (2010) explored the morphological diversity and anatomy in *Rosa* trichomes.  
68 We have also previously reported the types of trichome in two germplasms of *Rosa roxburghii* (Wang et al.,  
69 2019). However, the lack of structural studies and exploration of the molecular mechanisms on the development  
70 of trichomes have limited the elucidation of molecular pathways. As the current understanding of trichome  
71 distribution and development of RR and RS is insufficient, here we analyze the various forms of those structures  
72 on stems, sepal., fruit and pedicel in relation to the morphology, distribution, ultrastructure,  
73 and histochemistry of the trichomes. Furthermore, changes in relative expression levels of trichome-related  
74 genes (*TRY*, *CPC*, *GL1*, *GL2*, *GL3*, *TTG1*, *PDF2* and *PDF2-like*), in both developing buds and *in vitro* organs  
75 with different types of trichomes, were studied to provide opportunities for improving the germplasm of *Rosa*  
76 through genetic engineering technologies.

## 77 2. Materials and methods

### 78 2.1 Plant material

79 10-year old trees of *Rosa roxburghii* Tratt. (RR) and *R. sterilis* S. D. Shi (RS) were obtained from the fruit  
80 germplasm repository of Guizhou University, Guiyang, China (26°42.408'N, 106°67.353'E). Samples were  
81 collected at random and immediately frozen in liquid nitrogen and stored at -80 °C until processed.

### 82 2.2 Stereo microscopy

83 The floral bud, stems, leaves, pedicels, sepals and fruits RR and RS were examined using a  
84 stereomicroscope (Zeiss Stemi 508) to characterize the types and distribution of trichomes. Samples of floral  
85 bud were collected from adult trees (n=30) in early March 2018. The stems, pedicels and sepals were collected  
86 from the middle and upper canopy of adult trees (n=30) in middle-late April 2018, and in middle-late August,  
87 ripening fruits were obtained from both RR and RS.

### 88 2.3 Light microscopy

89 To investigate the anatomical structure and development of trichomes, the stems, young fruit and floral buds  
90 of differing developmental stages of both RR and RS were fixed in formalin, alcohol and acetic acid solution  
91 (FAA)(mixture of 50% ethanol, 5% acetic acid, 3.7% formaldehyde, and 41.3% water). Samples were embedded  
92 in paraffin wax and longitudinal and transverse sections (6-8  $\mu\text{m}$  thick) of stems and fruits were taken (Johansen,  
93 1940). Sections were stained with 0.05 % toluidine blue (pH 4.3) and 0.5 % fast green, then examined and  
94 photographed using a Leica light microscope.

## 95 2.4 Scanning Electron Microscopy

96 The floral buds, young fruits, pedicels, bearing branches, sepals and stems were harvested at various stages  
97 of growth from RR and RS and were immediately fixed in 3 % glutaraldehyde solution in 0.1 M phosphate  
98 buffer at pH 7.2, overnight at 4 °C. The plant material was dehydrated through a tert-butanol and ethanol  
99 mixture series (30, 50, 70, 90, and finally 100 % concentration three times) then dried via vacuum  
100 cryodesiccation. Samples were next sputter-coated with gold and viewed using S-3400N scanning electron  
101 microscope to examine the type and morphology of trichomes (Ma et al., 2016). The prickles and various  
102 trichome types were measured in terms of their total length and basal width from > 20 images per organ.

## 103 2.5 Transmission Electron Microscopy

104 Samples of flagellate, acicular and capitate trichomes, and young prickles, from RR and RS were fixed in  
105 3 % glutaraldehyde for 2 hours and washed thoroughly with 0.1 M phosphate buffer (pH 6.8). Afterwards, they  
106 are post-fixed with 1 % osmium tetroxide in phosphate buffer and subjected to following dehydration procedure  
107 in alcohol (50, 70, 80,90, and 100 %, each of 10 min), then two changes in 100 % acetone (each of 15 min), and  
108 embedded in epoxy resin. Ultrathin sections (80 nm) were transferred to formvar-coated grids and poststained  
109 with uranyl acetate and lead citrate (Ma et al., 2016). Observations were made with JEM-1200EX scanning  
110 transmission electron microscope at 120KV.

## 111 2.6 Flow cytometry analysis

112 Prickles from RR and RS were isolated and pooled as a sample and cells from the leaves of RR were used as  
113 a control. Nuclei from prickles or leaves were prepared and stained with 4',6-diamidino-2-phenylindole (DAPI)  
114 and a CyFlow Space (Sysmex, USA) analyzer was used to assess the relative DNA content in each sample  
115 (Galbraith et al., 2001).

## 116 2.7 Fourier transform infrared spectroscopy

117 Prickles from **ripe fruits and stems** of RR and RS were gently removed and pooled as a sample **in mid**  
118 **August**. The dry, milled lignin samples (sample and KBr tableting into a very thin film with a diameter of 13mm)  
119 were analyzed with a NICOLET iN10 MX Fourier transform infrared spectrometer, to investigate the IR bands  
120 and absorbance patterns of prickle and fruit spines, to assess the functional groups within the lignified cell wall  
121 (Li et al., 2012). Background spectra were taken in terms of every sample before getting sample spectra. Each  
122 spectrum was scanned 64 times across the range of 4000 to 600  $\text{cm}^{-1}$ , with a spectral resolution of 4  $\text{cm}^{-1}$ .

## 123 2.8 Total RNA extraction and qRT-PCR

124 Tissue samples were collected at varying stages of flower development: three samples were taken on a  
125 weekly basis from buds before shooting (**B1-3**), seven samples were taken through bud development during  
126 shooting every three days and finally samples were collected upon inflorescence (**Fb1-7**) (Fig. 1). Pericarp was  
127 sampled by gently pulling a razor over the young fruit and extracting a tissue sample of no more than 1 mm in  
128 thickness. Sarcocarp was sampled in an identical fashion, but here the tissue thickness can be up to 2 mm. Total  
129 RNA was extracted using a TRIzol RNA Purification Kit (TaKaRa, China). cDNA synthesis was performed  
130 according to the PrimeScript RT reagent Kit with gDNA Eraser protocol (TaKaRa, Kyoto, Japan). To identify  
131 **genes** of interest, sequences were compared **between** trichome related genes in *Arabidopsis* and a transcriptome

132 database for RR and RS (unpublished), which was built by our group. Ubiquitin (*UBQ*) was used as reference  
133 gene to normalize the expression data. Primers used for qRT-PCR were designed with Primer Premier 6  
134 software (Table 1). Quantitative RT-PCR (qRT-PCR) was performed on an ABI ViiA 7 DX system (Applied  
135 Biosystems) using SYBR Premix Ex Taq (TaKaRa). The relative gene expression level was calculated  
136 according to the  $2^{-\Delta\Delta CT}$  method (Livak et al., 2001). Each experiment was performed in triplicate, and each time  
137 the experiment included three biological replicates.

### 138 **3 Results**

#### 139 **3.1 Morphology and structure of trichomes**

140 Trichomes are abundant on the surfaces of both vegetative and reproductive organs of RR and RS,  
141 including the floral bud, stem, leaf, sepal., and fruit (Fig. 2). However, there are differences in the types of  
142 trichomes present on these organs. Broadly, the trichomes present on the surfaces of RR and RS could be split  
143 into two main categories: glandular trichomes and multicellular non-glandular trichomes. In RR, the glandular  
144 trichomes were only observed on the abaxial side of leaves (Fig. 2D). The prickles on the stem of RR are  
145 opposite and solitary on RS (Fig. 2C, 2I).

146 Scanning electron microscopy identified non-glandular trichomes including flagellate, acicular and  
147 branched forms, of varying size and distribution. Flagellate, multicellular and uniseriate trichomes with the  
148 distal end of the terminal cells delicate and greatly elongated (Fig. 3A, 3D, 3I). The flagellate trichomes were  
149 abundant on the surfaces of immature sepals and pedicels in RR and RS. The acicular trichomes, non-secretory  
150 multicellular trichomes were unbranched (Fig. 3A, 3B, 3E,3H) and exhibited a higher variation in density and  
151 size in different organs. The acicular trichomes on immature sepals of RR have a wider base than those of RS  
152 (Fig. 3A, 3B).



153 Acicular trichomes were present on the immature pedicel of RR, but not in RS. However, the fruit bearing stem  
154 and immature stem surfaces of RR did not have trichomes. Unlike RR, RS had branched trichomes with six  
155 arms on its sepals (Fig. 3C). Two types glandular trichomes, capitate (Fig. 3B, 3F, 3G, 3J, 3O, 3P) and  
156 bowl-shaped glandular trichomes (Fig. 3Q), cover the epidermis of RS. The capitate glandular trichomes are  
157 multicellular, non-vascularized and composed of both epidermis and subjacent layers. They consist of glandular  
158 heads with multiple layers of epidermal cells and long stalks. The head of the glandular trichome has a wrinkled  
159 surface and the accumulation of a secretion was observed in the apical region of the head (Fig. 3G, 3K, 3P).  
160 Stomata were occasionally observed on the upper region of glandular heads (Fig. 3G, 3Q). The capitate  
161 glandular trichomes are distributed on the surface of many organs in RS but predominantly present on the sepals,  
162 immature pericarp and immature pedicel (Fig. 3B, 3F, 3J), and only small number were found on the immature  
163 stems (Fig. 3O). Bowl-shaped glandular trichomes were multicellular, non-vascularized, disc-shaped  
164 (patelliform) with a concave surface, and two mastoid structures in the center of the surface (Fig. 3Q). These  
165 were only observed on immature stems.

166 The types of trichomes observed and their distribution are provided in Table 2. Statistical analysis showed  
167 that the flagellate trichomes of RR and RS were statistically different in the size ( $P < 0.05$ ). The acicular  
168 trichomes of RR are larger than RS on pericarp ( $P < 0.05$ ), which were no difference in sepals. The capitate as  
169 trichomes of RR on the leaf abaxial were significantly larger than those found on all organs of RS ( $P < 0.05$ ),  
170 well as of capitate trichomes head width ( $P < 0.05$ ). The trichomes present on pedicels were characterized  
171 by having a larger size than trichomes found on all other organs of RS. It was noted that not only the length of  
172 prickles but also the basal width of prickles are larger in RR. Prickles reached a final height of  $5.04 \pm 0.36$  mm  
173 and base width of  $5.08 \pm 0.26$  mm in RR and final height of  $3.18 \pm 0.09$  mm and base width of  $3.939 \pm 0.44$  mm  
174 in RS ( $P < 0.05$ ).

175 Acicular trichomes on the fruit of RR first developed in an erect manner, growing outwards, perpendicular  
176 to the fruit surface (Fig. 4A) and as they developed, they began to curve downwards, finally becoming sharp,  
177 pointed hooks with a broad base (Fig. 4B). Acicular trichomes on fruit of RS are widely spaced and small in size  
178 (Fig. 4D-E). Unlike acicular trichomes, capitate glandular trichomes grow both in length and in basal width, and  
179 fully mature to prickles (Fig. 4E, 4G). However, not all capitate glandular trichomes develop to prickles and  
180 there are still many capitate glandular trichomes on the mature fruit and pedicel (Fig. 4E, 4G). The capitate  
181 glandular trichomes of RS have faint yellow heads when young and wine-red when mature.

182 Longitudinally sliced tissue structure of matured prickles of RR and RS were studied using light  
183 microscopy (Fig. 5). Initial observations the prickles of RR and RS indicate that they are multicellular and  
184 non-vascularized. The surface of prickles was cornified and epidermal cells had thickened cell walls and  
185 enlarged vacuoles and were densely arranged. Prickles of RS consist predominantly of epidermal tissue (Fig.  
186 5F), whereas prickles of RR are composed cells of both epidermal and cortical origin (Fig. 5C). Interestingly, a  
187 layer was observed which was comparable to the abscission layer of deciduous leaves in RS, however the  
188 prickles of RR did not exhibit this layer.

189 The ultrastructure of trichomes was explored using transmission electron microscopy (TEM). Acicular  
190 trichomes appeared highly vacuolated, with the cytoplasm restricted to a narrow peripheral band around the  
191 interior of the cell walls. The cells of acicular trichomes on the sepals of RR had different forms, but all were  
192 surrounded by a relatively thick cell wall (Fig. 6A, 6B). The epidermal cells were loosely arranged, and the  
193 intercellular spaces were large. The small amount of peripheral cytoplasm and plastids were aggregated in the  
194 corners of the cells, surrounding large vacuoles. The acicular trichomes on fruits of RR had a similar cellular  
195 morphology (Fig. 6C, 6D), the cell of acicular trichomes on fruits closely arranged and a few Golgi apparatus  
196 and mitochondria were found. In mature acicular trichomes of RS sepals (Fig. 6E, 6F), the most striking

197 ultrastructural features are the closely arranged cells, the high density of the cytoplasm, and the large size of the  
198 nuclei, which also have prominent nucleoli. Plastids were present too, of varying size and shape, having electron  
199 dense stroma and occasional starch grains. Vesicle-like structures of different sizes with an irregular outline  
200 were also located in cytoplasm; some of these were translucent whilst others contained an osmiophilic core (Fig.  
201 6G, 6H).

202 The flagellate trichomes of RR epidermal cells are long and closely arranged (Fig. 7A). They have thick  
203 cell walls with a dense cytoplasm, vacuoles of varying size, large nuclei with prominent nucleoli, extensive  
204 rough endoplasmic reticulum, and a zigzag-shaped structure. Various plastids had electron dense stroma and  
205 occasional starch grains (Fig. 7B). Compared to the flagellate trichomes of RR, the epidermal cells of RS are  
206 elliptic and plastids are ameboid or cup-shaped, many being electron-dense and occasionally containing starch  
207 granules. Golgi stacks and rough endoplasmic reticulum occur infrequently (Fig. 7C, 7D).

208 The cells of young prickles in RR consist of densely arranged elliptic cells with a thick cell wall. Within  
209 the cells, there are varying degrees of vacuolation and osmiophilic secretory material is localized adjacent to the  
210 outer periclinal cell wall (Fig. 8A, 8B). Except the nucleolus, no other organelles were observed. The structure  
211 of prickles in RS is broadly like those in RR (Fig. 8C, 8D).

212 Glandular trichomes on RS were selected to further study the structure of the capitate trichomes, whereby  
213 ultra-thin sections sepal., fruit, pedicel, and stem were examined (Fig. 9). The capitate trichomes have common  
214 and distinguishing features in all four organs. The glandular cells have large vacuoles which occupy the  
215 majority of the cellular space, with cytoplasm located only in the peripheral region of the cells and numerous,  
216 densely stained droplets of secretory product were observed in the periplasmic space. The glandular  
217 cells contain starch granules and have plasmodesmata, although these are not as clearly visible in cells from the  
218 sepal (Fig. 9A, 9B). The glandular cells from the sepal are markedly different, containing other organs and the

219 cells are polygonal in shape and loosely arranged.

220 The FTIR spectra of prickles from fruit are shown in Fig.10 (A, B). The spectra of RR and RS are largely  
221 similar, and the peaks predominantly ranged from 800-1800  $\text{cm}^{-1}$ . The main peaks, which are the stretching  
222 vibrations of -OH and suberin, are at 3403 and 2918  $\text{cm}^{-1}$ . The bands at 1452, 1383, 1250, 1157 and 1105  $\text{cm}^{-1}$   
223 are indicative of lignin and cellulose. However, between RR and RS, the relative intensity of peaks are different.  
224 The prickles on stems of RR and RS are mainly composed of lignin, suberin, cellulose and hemicellulose, but  
225 that the chemical composition of the prickle of stems is different (Fig. 10C, 10D). **The FTIR absorbance was**  
226 **notably higher in the prickles from stems of RS than RR.** Furthermore, the two species varied in the peak at  
227 1379  $\text{cm}^{-1}$ ; in RS this peak is intense but in RR it appears as little more than a shoulder.

228 To determine whether endoreduplication contributed to the enlarged RR and RS trichome cells, we  
229 measured the DNA content of mature fruit prickles using flow cytometry. As shown in Fig. 11, both RR and RS  
230 displayed a peak of DNA content at around 200. Surprisingly, no difference in nuclear ploidy levels was  
231 observed between fruit prickles and leaf tissue (2C), suggesting that RR and RS prickle cells did not undergo  
232 any endoreduplication.

### 233 **3.2 Trichome-related gene expression in organs with different trichome types.**

234 Above we report the distribution and structure of different types of trichomes, homogenous or  
235 heterogenous in their distribution over various organs, providing a basis for the exploration of regulatory  
236 mechanisms governing trichome formation and differentiation at specific developmental stages. Based upon  
237 previous findings about trichome formation factors in other plants, eight trichome-related genes were studied to  
238 assess their expression levels in organs with different types of trichomes according to quantitative PCR.  
239 Trichome-related genes expression displayed broadly consistent patterns in different trichomes types in  
240 some organs of RR and RS (Fig. 12). The expression levels of *GL2*, *GL3*, *TTG1* and *PDF2* is known to be

241 positively related to trichome formation, and here expression was higher in sepal II with acicular trichomes than  
242 that in sepal I, which was covered with flagellate trichomes in RR, however, the expression levels of *GL2* and  
243 *PDF2-like* was significantly higher in sepal I. Furthermore, the gene which is negatively associated with  
244 trichome formation, namely *TRY*, was significantly higher in sepal I, in which no acicular trichomes were  
245 observed. Compared to sepal II, which had visible acicular trichomes and glandular trichomes in RS, the  
246 expression of *GL1*, *GL2*, *TTG1* and *PDF2* was significantly higher in sepal I, and the surface of sepal I was  
247 covered with flagellate and branched trichomes. *CPC*, a gene that has been known to function in suppressing  
248 trichome initiation was significantly higher expressed in sepal I, compared to sepal II. Comparing the hairless  
249 sarcocarp and pericarp, of which both were covered with acicular trichomes in RR, the expression levels of *GL1*,  
250 *GL2* and *TTG1* were higher in the pericarp than the sarcocarp. The *TRY* gene also exhibits opposite trends with  
251 the positive regulatory factors. The qPCR data presented here demonstrates that the expression levels of *GL1*,  
252 *GL2*, *GL3*, *TTG1* and *PDF2-like* were significantly higher in pericarp with acicular and glandular trichomes  
253 than in sarcocarp of RS. In RR, the expression levels of *GL1*, *GL2*, and *TTG1* were significantly higher in the  
254 pedicel, with acicular and flagellate trichomes, than in the smooth bearing branch. In RS, the expression levels  
255 of *GL1*, *GL2*, *GL3*, *PDF2* and *PDF2-like* genes were significantly higher in the pedicel with flagellate and  
256 glandular trichomes than in smooth bearing branch. Conversely, expression levels of *CPC* and *TRY* were  
257 significantly higher in bearing branch than in the pedicel. The expression levels of *GL1*, *GL2* and *GL3* were  
258 significantly higher in main veins, with profuse acicular and glandular trichomes, as compared to the mesophyll  
259 layer, which has no trichomes. Moreover, *CPC*, the gene that has been known to play negative roles in trichome  
260 formation, was highly expressed in mesophyll and lowly expressed in main veins. Overall, the expression level  
261 of *GL1*, *GL2* and *TTG1* which are positively regulating trichomes formation are higher in covered with acicular  
262 trichomes organs in RR, whereas the *TRY* inhibits trichome formation are expressed higher in lack of trichomes

263 Organs, and *CPC* only high expressed in mesophyll and higher expression level in in main vein or other  
264 trichomes organs, which might be associated with the glandular trichomes formation. Our results show that *GLI*  
265 and *GL2* were expressed at higher levels in acicular trichome organs of RS. Moreover, *GL3* and *PDF2-like* were  
266 very high expressed and *CPC* showed the lowest expression in densely glandular trichome organs which maybe  
267 shows that these genes significant linked the presence/absence and types of trichomes on the surface of RS.

### 268 **3.3 Expression profiles of trichome-related genes during bud development**

269 Expression of trichome-related genes in RR and RS were investigated during different stages of bud and  
270 floral bud development through qPCR (Fig. 13). Trichome-related genes correlated with bud growth and  
271 development. In RS, the total expression of *GLI*, *GL2*, *GL3*, *PDF2-like*, *CPC* and *TRY* follow a very similar  
272 trend through bud development, and the expression levels peaked in development stage B3. Although *TTG1* and  
273 *PDF2* have the same trend in bud development of RS, they do not peak at this stage. At the beginning of  
274 inflorescence, the positive regulatory genes and the negative regulatory genes showed an increasing trend and  
275 reached a peak on B3 in RS, while the trend of RR were slightly different. *GL3*, *TTG1*, *PDF2*, *PDF2-like* and  
276 *TRY* showed increased expression during bud development of RR, but did not reach a maximum value and  
277 begin to decrease again, except with *PDF2*. The relative expression levels of *GLI* and *CPC* gradually decreased  
278 in RR during inflorescence. During inflorescence, the mRNA levels of positive regulatory genes *GL3*, *TTG1*,  
279 *PDF2*, *PDF2-like* and the negative regulatory gene *CPC* initially increased before decreasing in RS. However,  
280 the time required to reach peak was different; *TTG1*, *PDF2-like* and *CPC* were Fb3 and the rest were Fb4. In  
281 RR, trichome-related genes exhibited a similar pattern, in which initially expression increased then a subsequent  
282 decrease were observed, and the peak of almost all genes occurred in stage Fb3.

## 283 **4 Discussion**

284 Trichomes are hair-like structures that commonly present on the surface of branches, roots, leaves and

285 sepals (Chen et al., 2014; Haratym et al., 2015), and indeed some fruits are also thickly covered with trichomes.  
286 The surface of fruits of RR and RS are one such example, being densely covered with trichomes as seen in  
287 many species of the *Rosa* family. These trichomes have implications for fruit quality, cultivation, and processing  
288 (Singh et al., 2020). Here, two morphologically distinct trichome types were observed, namely glandular and  
289 non-glandular trichomes, which have been previously reported in the genus *Rosa*. (Wang et al., 2019). The  
290 external and internal features of trichome morphology and subcellular features of two main type trichomes and  
291 capitate glandular morphotype were analyzed together here for the first time in RR and RS.

292 Non-glandular trichomes of RR and RS were mainly flagellate and acicular trichomes. These are typical  
293 types of non-glandular trichomes from other *Rosa* species, including *Rosa. roxburghii* Tratt f. *esetosa* Ku (Wang  
294 et al., 2019) and *Rosa hybrida* L. 'Radtko' (Kellogg et al., 2011). Acicular trichomes had a homogeneous  
295 distribution across sepals and fruit of RR and RS, being especially prevalent on the surface of RR fruit. Acicular  
296 trichomes are longer than other trichomes; their sharp tips can serve as a mechanical barrier and a defense  
297 mechanism for the plant (Fahn, 1952). It has been shown in *Withania somnifera* (L.) Dunal  
298 (Solanaceae) by Munien et al., (2015) that trichome density is closely related to insect pest resistance. Acicular  
299 trichomes of RR contained a multicellular basal pedestal, which serves to support the trichome and to provide a  
300 point of attachment that “anchors” the trichome to the epidermal surface (Fig. 3A, 4B, 5A). Our results found a  
301 difference in the structure of acicular trichomes in RR and RS (Fig. 4B, 4E). Acicular trichomes of RS do not  
302 have as large a basal pedestal as RR which may be the reason for shedding easily from the surface of fruit with  
303 the maturing (Gallenmüller et al., 2015).

304 Flagellate trichomes are mainly distributed on the surfaces of sepals and pedicels in both species and  
305 flagellate trichomes of RS are longer than RR. Flagellate trichomes were more abundant in RR and were only  
306 present on immature organs (Fig. 3A, 3I). This phenomenon may be due to the mature organ’s establishment of

307 phytochemical defense mechanism, by which point they no longer need the protection of flagellate trichomes  
308 (Munien et al.,2015). Plastids were observed in the cells of flagellate trichomes, which likely contributed to the  
309 silver-white coloration on the sepal's surface of RR and RS (Fig. 7A, 7D).

310 Compared with RR, RS had more abundant non-glandular trichome types. In addition to acicular and  
311 flagellate trichomes, there are branching trichomes which have not been observed in other *Rosa* species.  
312 Non-glandular trichomes have been shown to function in defense against insect herbivores including  
313 influencing oviposition, protection against UV light and low temperature, and facilitating seed dispersal  
314 (Karabourniotis, et al., 2020).

315 Our results showed that the greatest differences between trichomes of RR and RS were found in glandular  
316 trichomes. The glandular trichomes of RS were located on the surface of all above ground organs, however, in  
317 RR limited numbers of glandular trichomes could only be found on the abaxial side of leaves. This may indicate  
318 that some genes regulating glandular trichomes on RS have different regulatory pathways with RR, which is in  
319 agreement with the hypothesis suggested by Wen et al., (2004), whereby RS was suggested to originate from a  
320 mutant of male-sterile *R. kweichonensis*. RS carries both capitate and bowl-shaped glandular trichomes, but  
321 capitate glandular trichomes were most common. Capitate glandular trichomes were predominantly present on  
322 the pedicel and fruits, with sparse distribution on the bearing branch. Capitate glandular trichomes were  
323 multicellular, non-vascularized, thick stalked and had large head cells which were composed of 16-34  
324 thin-walled cells, with a small number of pores visible in the center of the head (Fig. 3G, 3J, 3K). Stomata are  
325 frequently observed in the early developmental stages of glandular trichomes, corroborating previous work in  
326 *Vitis davidii* which showed that stomata on glandular trichomes form in the period of rapid cell proliferation and  
327 elongation of the upper region epidermal cells (Ma et al., 2016). The development of stomata was not observed  
328 in RS, however this may be a result of variance in the sampling period. The head of capitate trichomes often



329 secrete sticky mucilage to trap insects which can avoid the invasion of insects (Nogueira et al., 2013; Uzelac et  
330 al., 2017). Similarly, capitate glandular trichomes can secrete substances via pore-like openings on each of the  
331 multicellular head cells based on our observation (Fig. 3G). The nucleus is surrounded by plastids containing  
332 starch grains and plastoglobuli, and the cytoplasm contains osmiophilic droplets, mitochondria and endoplasmic  
333 reticulum, all of which, in combination, are considered to be evidence of secretory activity in a range of  
334 angiosperm taxa (Haratym et al., 2017). However, we observed the ultrastructure of the head of capitate  
335 trichome in RS and did not see evidence for such secretion. It may be the case that these trichomes are only  
336 secretory at specific developmental stages.

337         During observation of the pedicel of RS (Fig. 3J, 4F, 5E), we initially only saw capitate glandular and  
338 flagellate trichomes from tissues harvested in April. As time progressed, prickles were observed on the surfaces  
339 of RS pedicels, however our sampling schedule did not allow us to see the early developmental stages of these  
340 prickles. Morphologically, there is no difference between glandular trichomes and prickles, but prickles are  
341 more robust (Coyner et al., 2005). We surmise that the prickles undergo early developmental stages like other  
342 capitate glandular trichomes (Fig. 3J) and once some capitate glandular trichomes have reached the  
343 post-secretory phase (Fig. 3G), the heads are shed and they increase in both height and basal width, thus  
344 developing into prickles (Fig.4G). Therefore, it is reasonable to presume that the prickles of RS are homologous  
345 to capitate glandular trichomes. Rose and raspberry prickles progress through the four stages: (I) prickle is  
346 defined simply as a mass of proliferating cells, (II) a stalk emerges from the epidermis at the base of the cell  
347 mass and lifting it distally from the stem, (III) the distal cell mass falls off the stalk, followed by cell expansion  
348 in the stalk, (IV) the prickle is lignified and growth halted (Kellogg et al., 2011). This progress is similar with  
349 RS. However, it is important to note that only a small subset of glandular trichomes complete this final stage of  
350 development and form prickles in RS (Fig. 1F, G, 4E, 4G), this differs from *Vitis Davidii*, where all form

351 prickles (Ma et al., 2016). The glandular trichome head may send signals to the epidermal and/or cortical organs  
352 that cause a proliferation of cells, allowing a prickle to develop (Kellogg, 2011).

353 Prickles lack internal vascular material and result from multiple cellular divisions of the epidermis (Fig. 5C,  
354 5F). The dense cytoplasm had begun to degenerate, with reduced numbers of organelles visible and was located  
355 only in the peripheral region of the cells (Fig. 8). A layer was observed which resembled an abscission zone at  
356 the base of prickles of RS, where they meet the epidermis (Fig. 5F). Both mature and young prickles could be  
357 broken off with little force at this layer, suggesting this abscission zone fully forms early on in the prickle's  
358 development. The prickles of RR do not have such a zone (Fig. 5C), but it was previously reported in the  
359 prickles of *Rosa hybrida* cv. "Laura" and "Queen Elizabeth" (Asano et al., 2008, Li et al., 2012).

360 Analysis by FTIR showed that prickles on the fruit surface and stem of RR and RS  
361 consisted mainly of cellulose, hemicellulose, cork and lignin, but there were significant differences in the  
362 relative abundance of these compounds. These findings were similar to previous studies on wood (Asano et al.,  
363 2008; Le et al., 2017). Li et al., 2012 studied the anatomical structure and chemical composition in prickles of  
364 *Rosa hybrida*, and found that differences in chemical composition of prickles maybe related to the strength with  
365 which the prickle adheres to the stem. The concentration of cellulose, hemicellulose, cork and lignin in prickles  
366 were higher in RS than RR, both on the fruit surface and stem. This difference may be one of the reasons why  
367 prickles on RS are more weakly adhered than those on RR when the fruit is ripe.

368 Genetic analysis in *Arabidopsis* has established a regulatory pathway that controls trichome initiation  
369 (Hülkamp and Schnittger, 1998; Hülkamp, 2004). The active WD40-bHLH-MYB complex activates trichome  
370 differentiation by directly inducing the expression of a homeodomain-leucine zipper (HD-ZIP) IV gene  
371 *GLABRA2* (*GL2*) (Schellmann and Hülkamp 2005). Although it is possible that the regulation pathway of  
372 unicellular trichomes is different from that of multicellular trichome, many regulatory factors have the same

373 effect under different regulation pathways. For instance, Over-expression of *CsTRY*, *PaTRY*, *PaCPC-like1*,  
374 *PaCPC-like2* and *PaCPC-like3* in *Arabidopsis* resulted in glabrous phenotype (Tang et al., 2012; Zhang et al.,  
375 2019). *CsGL3*, *CsGL1*, *MICT* (*Micro-trichome*), *TBH* (*Tiny branched hair*), and *TRIL* (*Trichome-less*) encode  
376 HD-Zip proteins with different subfamilies; genetic and molecular analyses have revealed that these  
377 transcription factors are responsible for the differentiation of epidermal cells and the development of  
378 multicellular trichomes in cucumber (Liu et al., 2016). Though qRT-PCR analysis of trichome-related genes in  
379 different organs which covered with different types trichomes, we found that most of these trichome-related  
380 genes showed broadly consistent patterns of expression in RR and RS. This observation may partly explain the  
381 different types trichome in different organs. Based on the expression of trichome-related genes in different  
382 organs with different types of trichomes in RR and RS, we have proposed that there are differences in the  
383 regulation of trichomes between these two germplasms. Our results indicate that *GL1* and *GL2* affect acicular  
384 trichome development in RR and RS, and that *TTG1* and *TRY* acts only in RR. The described regulatory  
385 changes may cause the abundance of acicular trichomes and glandular trichomes in RS, and the repression of  
386 some trichome-related genes. The *PDF2* of *Arabidopsis* has not been shown to be involved in trichome  
387 formation but type I trichome formation is controlled by *PDF2* in tomato (Yang et al., 2011). However, the  
388 expression of *PDF2-like* was higher in the pedicel and pericarp, which were covered with glandular trichomes;  
389 such diverse expression patterns suggest that *PDF2-like* may be regulating glandular trichomes in RS and a  
390 different regulatory pathway occurs in RR. *CPC* plays an important role in the regulation of non-glandular and  
391 glandular trichomes, as indicated in plant species including tomato, poplar, and London plane (Tominaga-Wada  
392 et al., 2013; Zhang et al., 2019). However, here the expression level of *CPC* in tissues without glandular  
393 trichomes are significantly higher than those with. This finding suggested that *CPC* mainly regulates the  
394 formation of glandular trichomes in RR and RS.

395 During three stages of bud and seven stages of floral bud development, all these genes responded to the  
396 growth stages, thereby potentially eliciting influence on trichome initiation. However, the expression patterns  
397 were inconsistent between RR and RS. In this study, we found maximum expression of *GL1*, *GL3*, *TTG1*, *PDF2*,  
398 *CPC* and *TRY* at the Fb3 stage of floral bud development in RR, but all of these genes had the highest  
399 expression at the B3 stage of bud development in RS. Since different types of trichomes were present in RR  
400 compared to RS, we reasoned that the Fb3 stage is a critical trichome developmental period of RR, whereas the  
401 B3 stage is for RS. The transcripts of *GL1*, *GL2*, *GL3*, *PDF2-like* and *CPC* were expressed highest in the B3  
402 stage of bud and floral bud development in RS, which may be due to the range of developmental stages of buds,  
403 and how the glandular and acicular trichomes were clustered on newly growing shoots and leaves. We observed  
404 that most trichome related genes have only one peak in Fb3 of floral bud development of RR, whereas the *GL1*,  
405 *GL2* and *TRY* had three peaks in Fb1, Fb3 and Fb5. This may suggest that these genes play vital roles in  
406 acicular trichome development and formation in floral buds of RR. These trichome-related genes are highly  
407 expressed in young leaf primordia and then progressively decrease as trichomes begin to initiate (Kirik et al.,  
408 2005). However, here the expression of these genes peaked at different developmental stages in different organs  
409 and correlated with the formation of different types of trichome, suggesting not only a role in trichome initiation,  
410 but also formation.

411 The types, distribution and micromorphology of trichomes in RR and RS were studied and putative  
412 regulatory genes identified. The data presented here provides a basis for creating new *Rosa* varieties with  
413 desired trichome growth and density through breeding and genetic engineering and helps to understand the  
414 complex network of regulatory interactions controlling the development of multicellular trichomes. This is the  
415 first report of quantitative analysis of trichome-related gene expression during bud development across different

416 trichomes types and organs. Future work should focus on validating a set of reference genes related to trichome  
417 initiation and growth, and to determine the precise function of these genes in *Rosa*.

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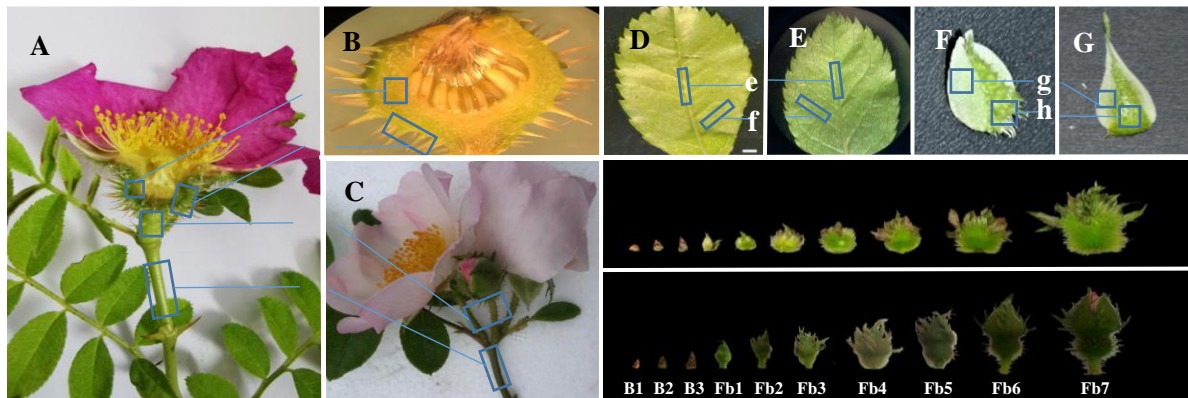
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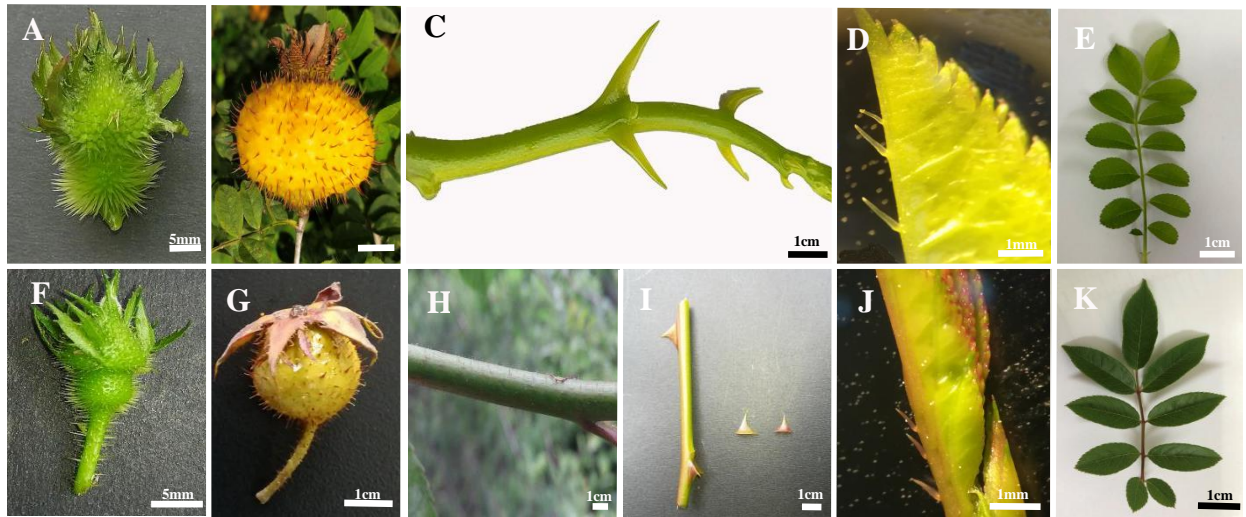
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516 **FIGURES:**



517 **Fig.1** The sampling site of *R. roxburghii* Tratt. (A, D, F, H) and *R. sterilis* S. D. Shi (B, C, E, G, I) for RNA extraction. (a) Sarcocarp;

518 (b) Pericarp; (c) Pedicel; (d) Bearing branch; (e) Vein; (f) Mesophyll; (g) Sepal I; (h) Sepal II.



519 **Fig.2.** The macroscopic depiction of *R. roxburghii* Tratt. (A-E) and *R. sterilis* S. D. Shi (F-K) organs. (A) Floral bud; (B) Ripe fruit;  
 520 (C) Stem; (D) Leaf abaxial; (E) Leaf adaxial; (F) Floral bud; (G) Ripe fruit; (H) Immature stem; (I) Stem; (J) Leaf abaxial; (K) Leaf  
 521 adaxial.

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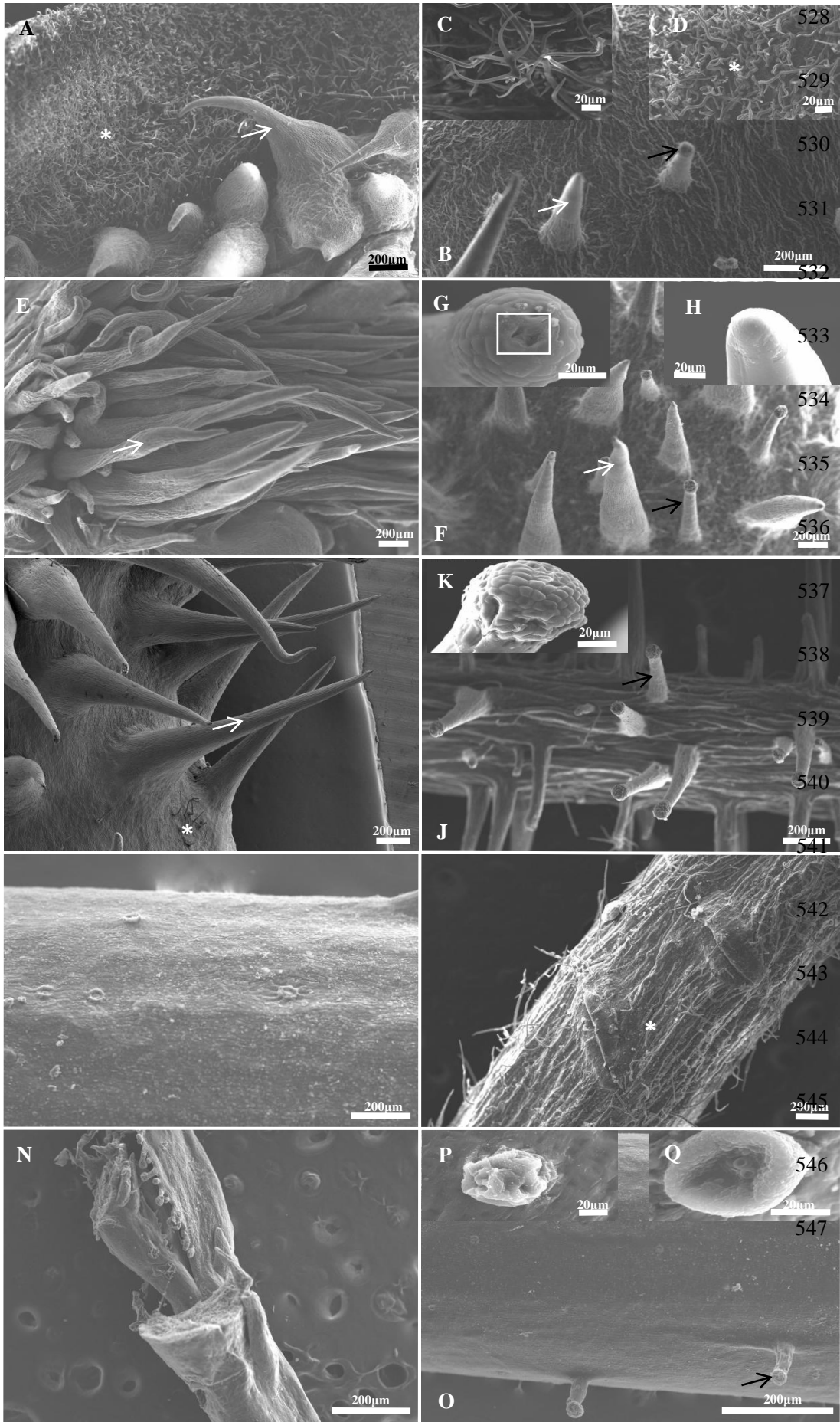
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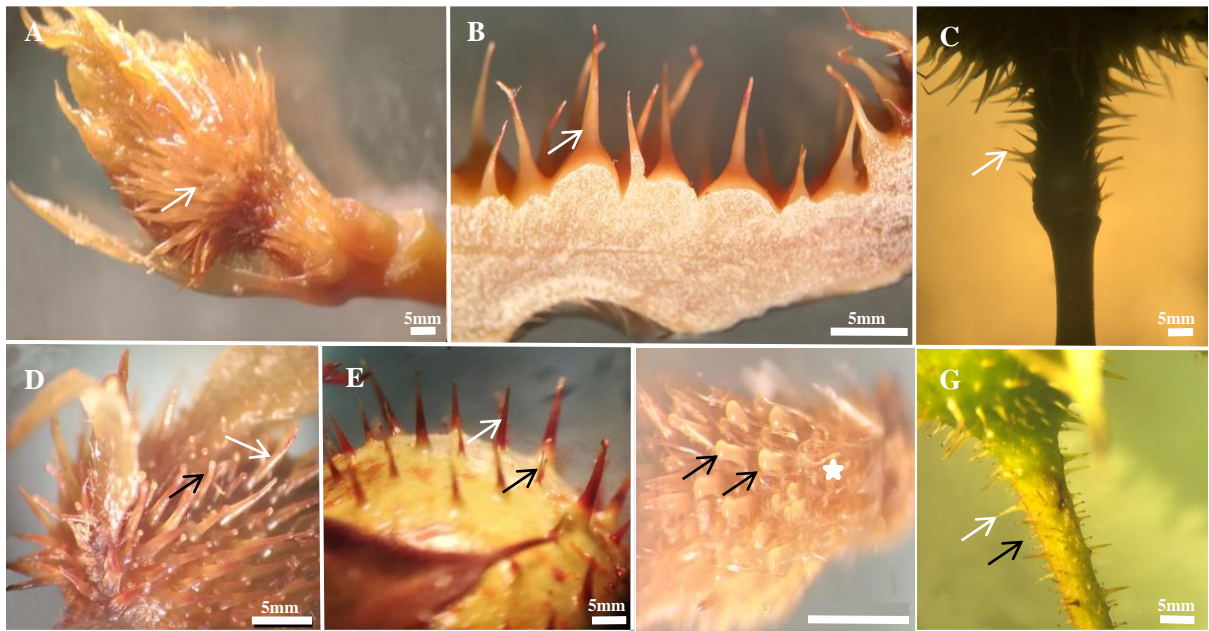
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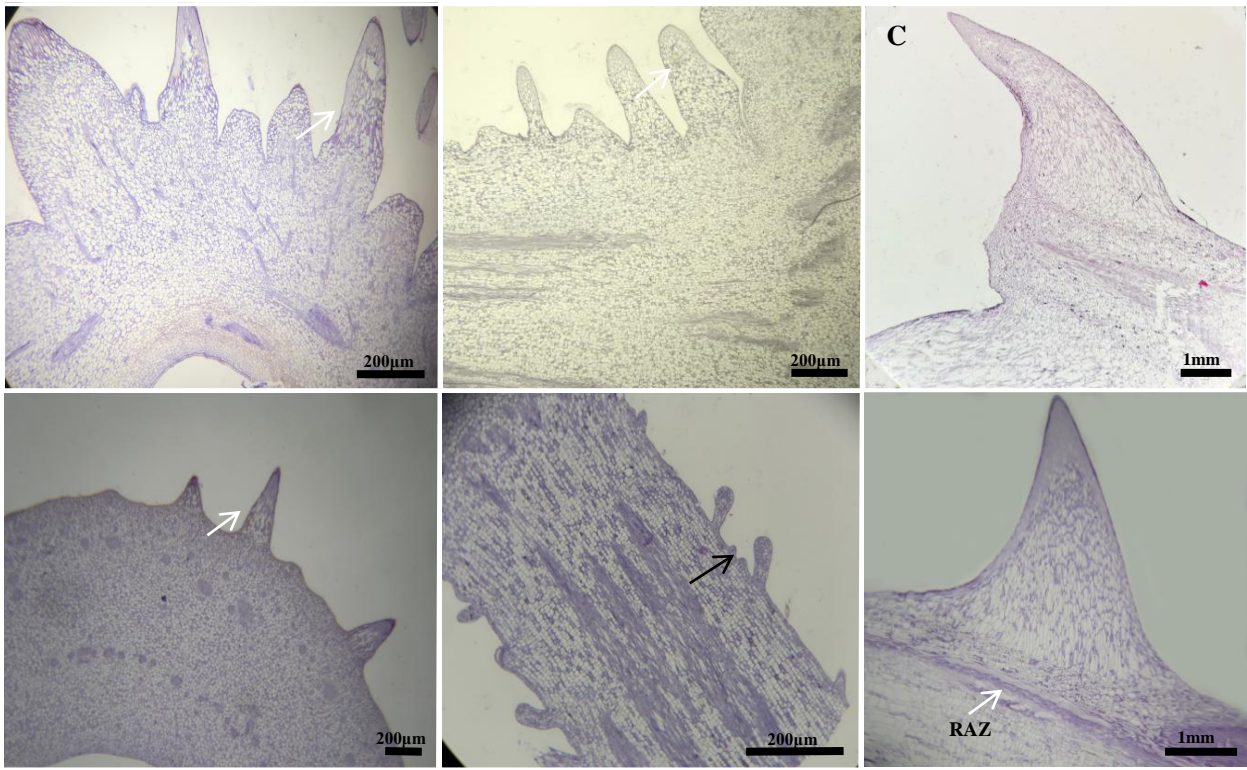


548 **Fig. 3.** SEM of trichomes of *R. roxburghii* Tratt. and *R. sterilis* S. D. Shi (A) flagellate trichomes (stars) and acicular trichomes  
 549 (white arrows) on immature sepals of *Rosa roxburghii* Tratt.; (B-D): Trichomes on immature sepals of *Rosa sterilis* S. D. Shi. (B)  
 550 Capitulate glandular trichomes (black arrows) and acicular trichomes (white arrows); (C) Branch trichomes; (D) Flagellate trichomes  
 551 (stars); (E) Acicular trichomes (white arrows) on immature pericarp of RR; (F-H): Trichomes on immature pericarp of RS; (F)  
 552 Acicular trichomes (white arrows) and capitulate glandular trichomes (black arrows); (G) Capitulate glandular trichomes with an  
 553 obvious stoma (drawing districts) on the top of glandular head; (H) Acicular trichomes local magnification map; (I) Trichomes on  
 554 immature pedicel of RR; (J-K) Trichomes on immature pedicel of RS; (J) Capitulate glandular trichomes (black arrows); (K)  
 555 Capitulate glandular trichomes local magnification map; (L) Bearing branch of RR; (M) Bearing branch of RS with flagellate  
 556 trichomes (stars); (N) Immature stem of RR; (O-Q) Immature stem of RS; (O) Capitulate glandular trichomes (black arrows); (P)  
 557 Capitulate glandular trichomes local magnification map; (Q) Bowl-shaped glandular trichomes.

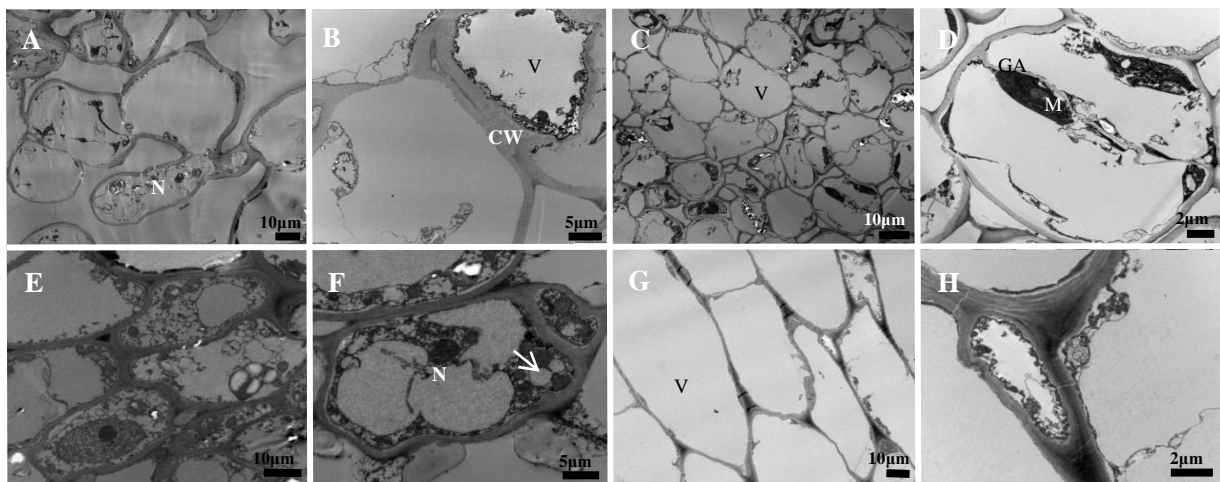


558 **Fig.4.** Development of trichomes of *R. roxburghii* Tratt. (A-C) and *R. sterilis* S. D. Shi (D-G). (A) Acicular trichomes (white arrow)  
 559 on floral bud; (B) Acicular trichomes (white arrow) on fruit; (C) Acicular trichomes (white arrow) on younger pedicel; (D)  
 560 Capitulate glandular trichomes (black arrow) and acicular trichomes (white arrow) on floral bud; (E) Capitulate glandular trichomes  
 561 (black arrow) and acicular trichomes (white arrow) on fruit; (F) Capitulate glandular trichomes (black arrow) and flagellate

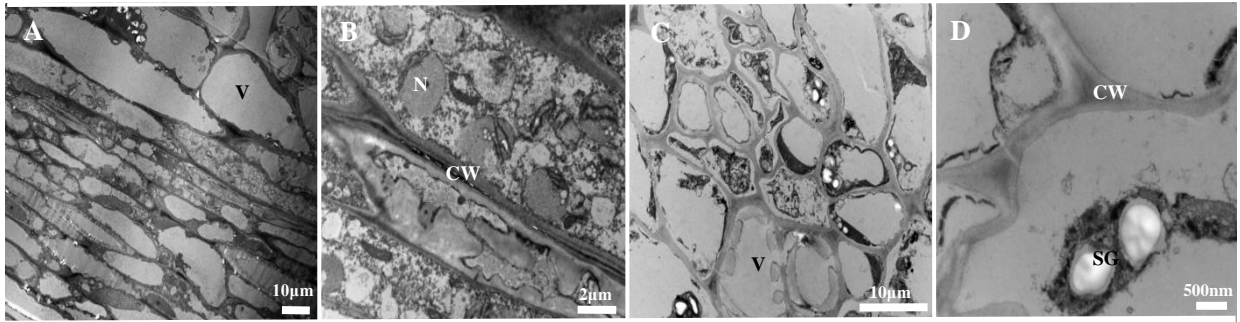
562 trichomes (stars) on younger pedicel; (G) Capitate glandular trichomes (black arrow) and acicular trichomes (white arrow) on  
 563 mature pedicel.



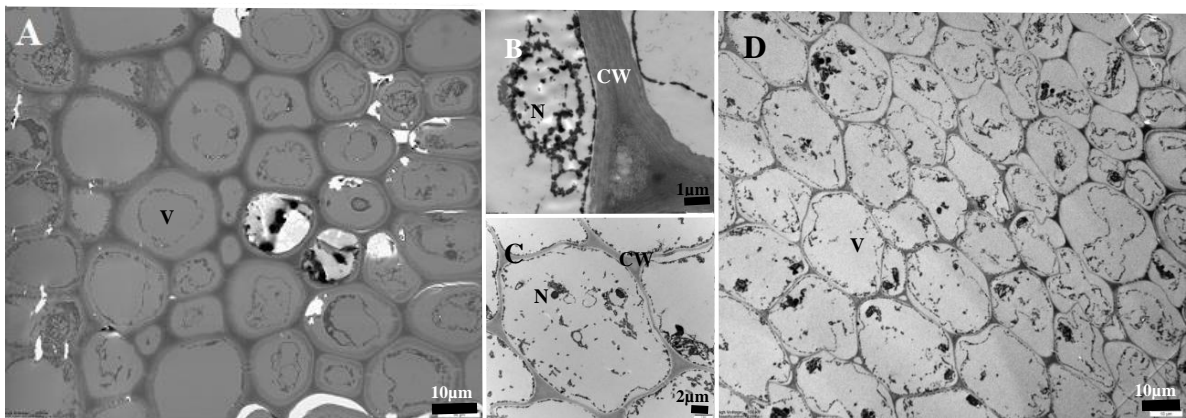
564 **Fig.5.** Light microscopy images of acicular trichomes and prickles of *R. roxburghii* Tratt. (A-C) and *R. sterilis* S. D. Shi (D-F) (A)  
 565 Acicular trichomes (white arrows) on the fruit; ( B) Acicular trichomes (white arrows) on the pedicel; (C) Prickle on the stem; (D)  
 566 Acicular trichomes (white arrows) on the fruit; ( E) Capitate glandular trichomes (black arrows) on younger pedicel; (F) Prickle on  
 567 the stem (RAZ: resembling abscission zone).



568 **Fig. 6.** Transmission electronic microscopic images of acicular trichomes of *R. roxburghii* Tratt. (A-D) and *R. sterilis* S. D. Shi  
 569 (E-H). (A) Acicular trichomes of sepals; (B) A local magnification map; (C) Acicular trichomes on fruit; (D) C local magnification  
 570 map; (E) Acicular trichomes of sepals; (F) E local magnification map; (G) Acicular trichomes on fruit; (H) G local magnification  
 571 map. N: Nucleus; CW: cell wall; V: vacuole; GA: Golgi body; M: Mitochondria.



572  
 573 **Fig. 7.** Sepals with flagellate trichomes of *Rosa roxburghii* Tratt. (A-B) and *Rosa sterilis* S. D. Shi (C-D) viewed by transmission  
 574 electronic microscopic. Dense thready material (arrow) N: Nucleus; CW: cell wall; V: vacuole; SG:starch grains.



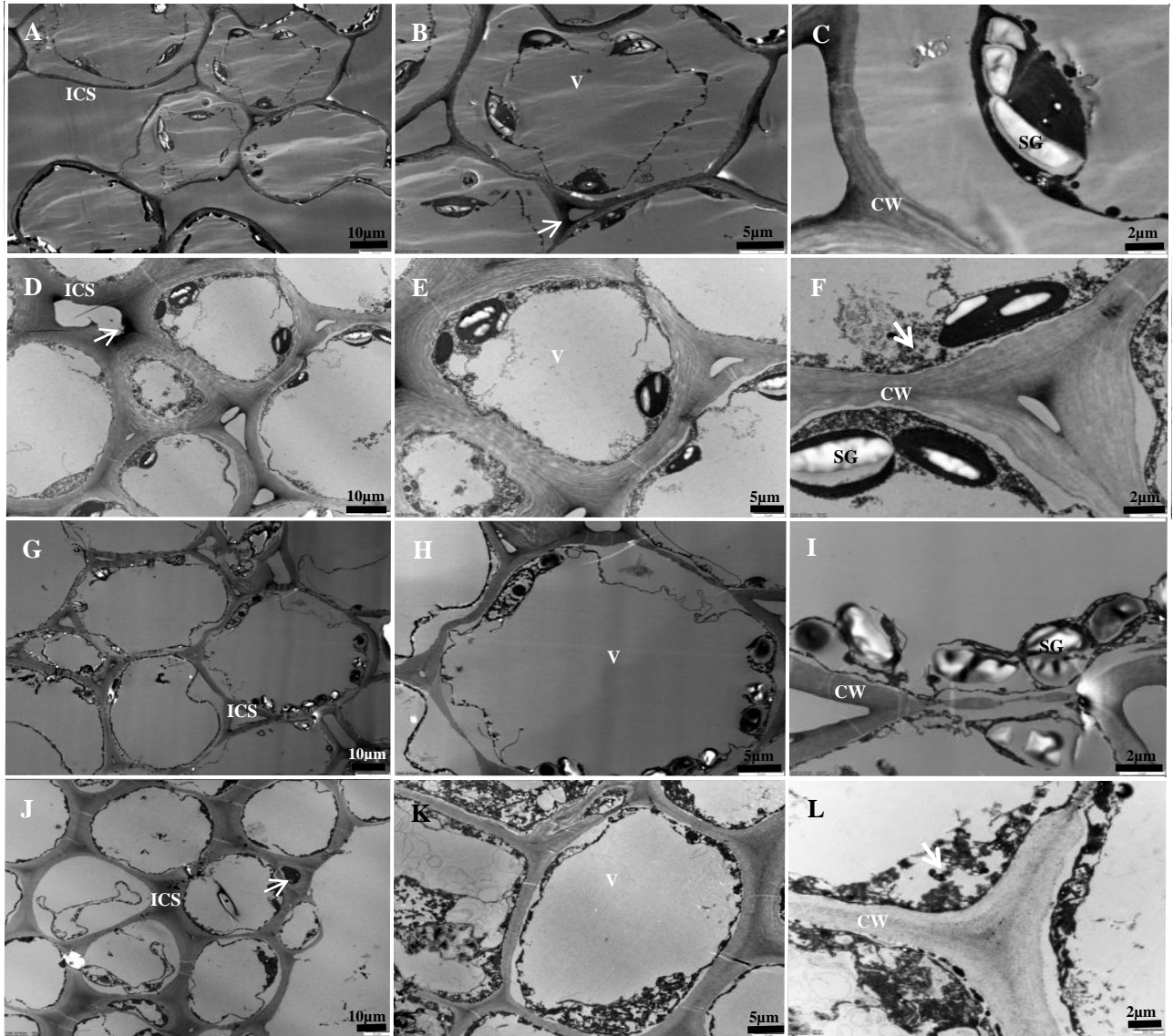
575 **Fig. 8.** Transmission electronic microscopic images of young prickles of *Rosa roxburghii* Tratt. (A-B) and *Rosa sterilis* S. D. Shi  
 576 (C-D). CW: cell wall; V: vacuole.

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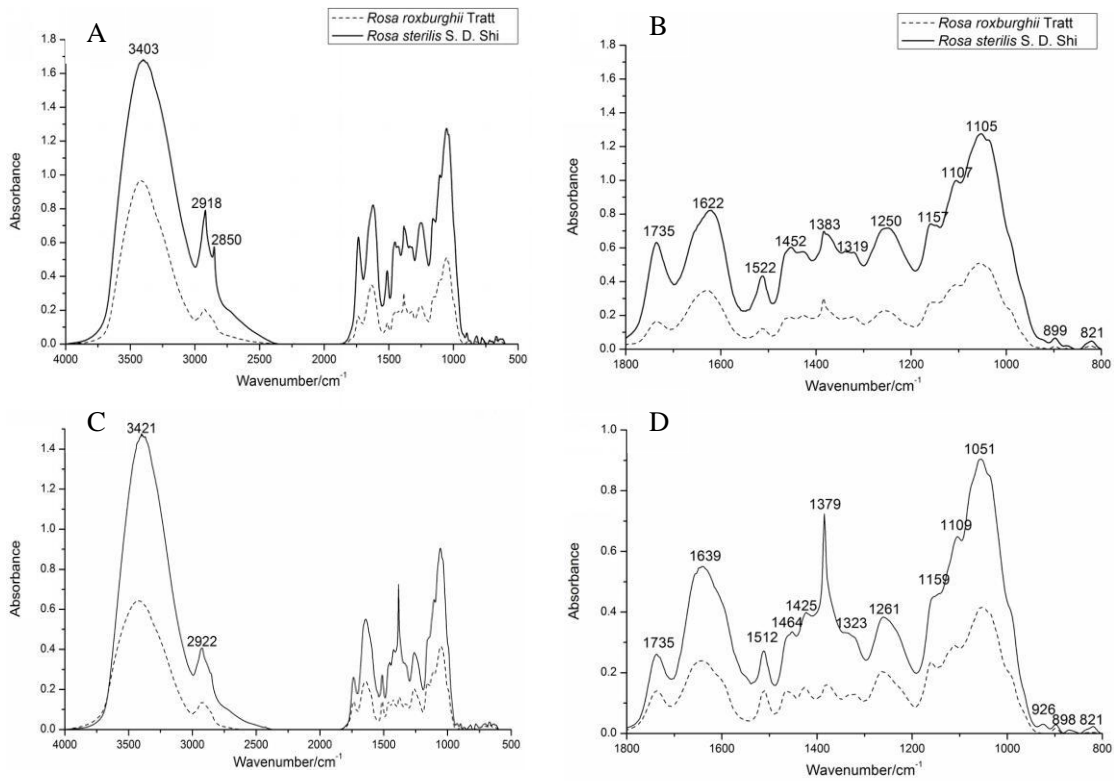
580



582 **Fig. 9.** Ultrastructure of the head of the capitate glandular trichomes in *Rosa. sterilis* S. D. Shi (A) Capitate glandular trichomes of  
 583 sepals; (B-C) A local magnification map (arrow indicates stained secretory product); (D) Capitate glandular trichomes of pedicel  
 584 (arrow indicates stained secretory product); (E-F) D local magnification map (arrow indicate stained secretory product); (G)  
 585 Capitate glandular trichomes of fruits; (H-I) G local magnification map; (J) Capitate glandular trichomes of stems (arrow indicates  
 586 stained secretory product); (K-L) J local magnification map (arrow indicates dense granular material) N: Nucleus; CW: cell wall;  
 587 ICS: intercellular spaces; V: vacuole; SG: starch grains.

588

589



590

591 **Fig.10.** Fourier transform infrared spectra of prickles of fruit and stem in RR and RS. (A) Fourier transform infrared spectra of

592 prickles on fruit in *Rosa roxburghii* Tratt. and *Rosa sterilis* S. D. Shi; (B) A Partial enlargement map; (C) Fourier transform infrared

593 spectra of prickles on stem in RR and RS; (D) C Partial enlargement map.

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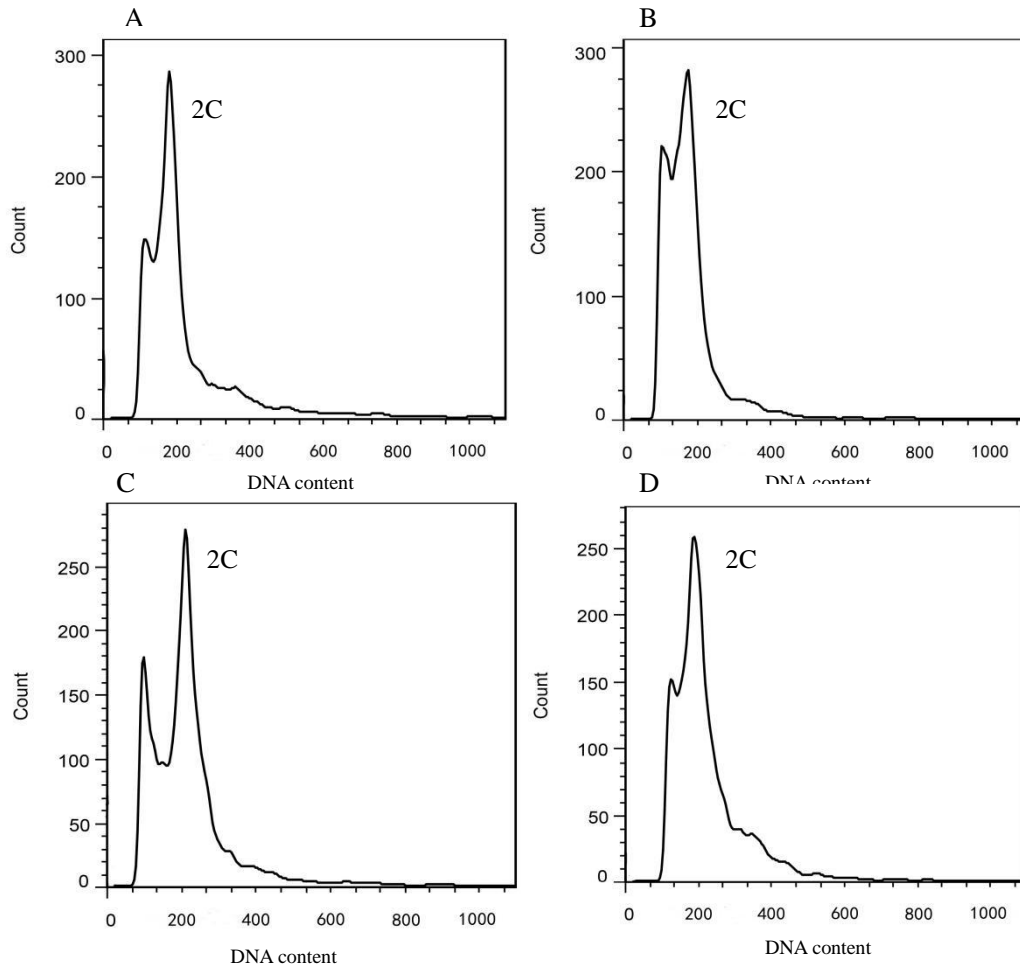
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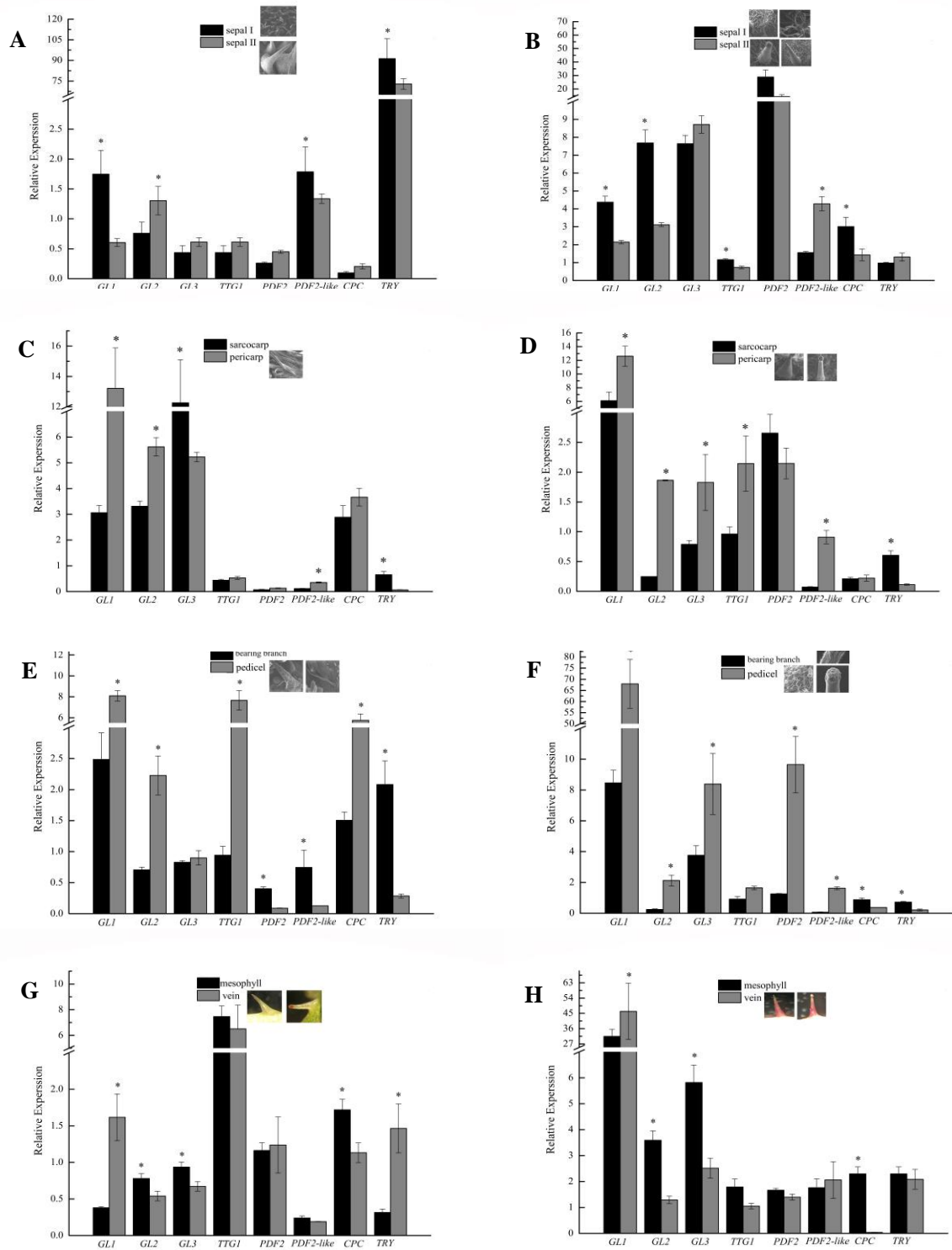
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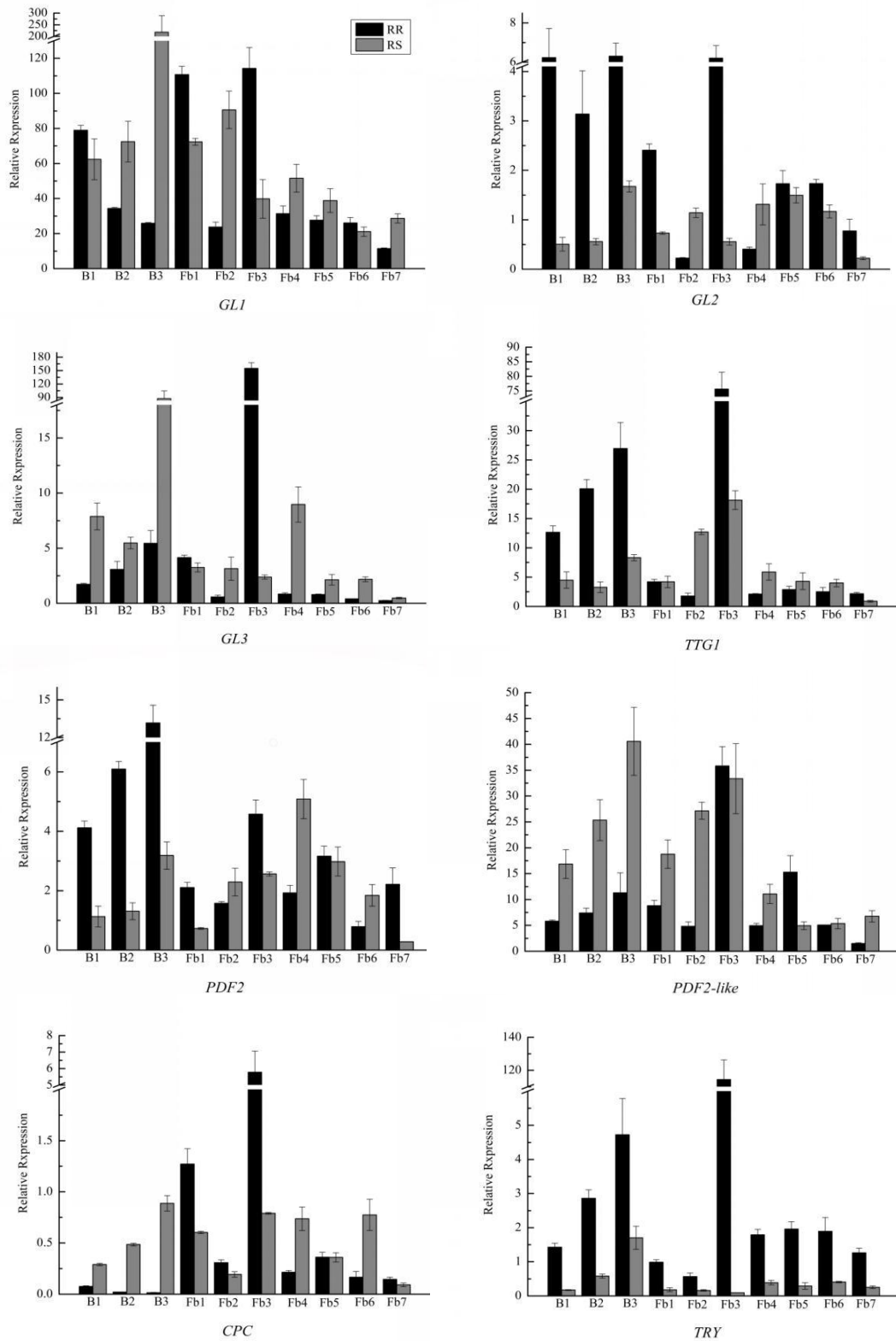




600  
 601 **Fig.11.** DNA ploidy analysis of *Rosa sterilis* S. D. Shi and *Rosa roxburghii* Tratt. (A) DNA ploidy map of leaves in RS, (B) DNA  
 602 ploidy map of fruit prickles in RS, (C) DNA ploidy map of leaves in RR, (D) DNA ploidy map of fruit prickles in RR.



603 **Fig.12.** qRT-PCR analysis of genes related to trichome development in different parts of *R. roxburghii* Tratt. (A, C, E, G) and *R.*  
 604 *sterilis* S. D. Shiat (B, D, F, H). Values are expressed as the means of at least three independent replicates  $\pm$  SD. \* = significant to  $p$   
 605  $< 0.05$ .



606  
 607 **Fig.13.** Expression pattern of trichome formation related genes in bud and floral bud of *R. roxburghii* Tratt. and *R. sterilis* S. D. Shi  
 608 at different development stages. Values are expressed as the means of at least three independent replicates  $\pm$  SD. Note: B: buds; Fb:  
 609 flora buds.

610 **Table 1:** qRT-PCR primer information of related genes

Gene name	Primer sequence5'-3'	Gene name	Primer sequence5'-3'
<i>GL1</i>	Forward: GCAGCAGCCACTACCTGATTC	<i>PDF2</i>	Forward: ACATGCTGCTTACAGTACCTCAC
	Reverse: CCAACACCACTATCTCGACTGATC		Reverse: AGACTCTGGATGTGCTATCAACTG
<i>GL2</i>	Forward: TAGCAATGGCGAAGGAGACAATAG	<i>PDF2-like.</i>	Forward: ATTGACTGTGGATGGAACAACCTGG
	Reverse: CACCAAGGACGCATCAATGTTATC		Reverse: TCTCCCCCTCATATCTGAGACCA
<i>GL3</i>	Forward: AGAGCTGGCAGTATATGGATGATG	<i>CPC</i>	Forward: TTCTTGCTCTAACCGAGACTTGAG
	Reverse: TCACCGTTAGGACCGGAGAC		Reverse: GGAAGATCAGCAGAGGAGATTGAG
<i>TTG1</i>	Forward: CACGCAATGGCATTCACTC	<i>TRY</i>	Forward: TTAAGGCAGAAGTGAGCAGTATGG
	Reverse: ATGGCGACGATTCTGATGGATAG		Reverse: ACGACTCGGTATGTGTCAITATCC
<i>UBQ</i>	Forward: ATGCAGATYTTTGTGAAGAC		
	Reverse: ACCACCACGRAGACGGAG		

611 **Table 2:** The trichome types, distribution and characteristics (mean ± SE) of *R. roxburghii* Tratt. and *R. sterilis* S. D. Shi Note:

612 "—" showed absent; values are means ± SD (n=30), different small letters in the same column meant significant difference at

613 P < 0.05 levels respectively.

Species	Organ	Non-glandular trichome				Glandular trichome	
		Flagellate trichomes (µm)	Acicular trichomes (µm)	Prickle (µm)	Prickle base width (µm)	Capitate glandular trichomes length (µm)	Capitate glandular trichomes head width (µm)
<i>Rosa roxburghii</i> Tratt.(RR)	Sepal	174.03±44.23c	499.82±148.34c	—	—	—	—
	Pericarp	—	1261.99±58.93a	—	—	—	—
	Pedicel	208.51±40.44c	841.27±67.42b	—	—	—	—
	Bearing branch	—	—	—	—	—	—
	Stem	—	—	5047.16±363.27a	5080.24±259.19a	—	—
<i>Rosa sterilis</i> S. D. Shi (RS)	Leaf adaxial	—	—	—	—	—	—
	Leaf abaxial	—	443±65.15cd	—	—	833.33 ±134.25a	110±8.47a
	Sepal	349.79±103.26b	489.53±167.69c	—	—	307.58±122.14c	68.96±6.93b
	Pericarp	—	577.28±149.01c	—	—	324.56±150.22c	70.07±7.02b
	Pedicel	451.28±112.51a	—	—	—	339.56±88.23c	100.63±4.88a
<i>Rosa sterilis</i> S. D. Shi (RS)	Bearing branch	221.34±45.21c	—	—	—	—	—
	Stem	—	—	3185.38±95.32b	3934.09±443.68b	185.84±23.64d	88.16±6.34b
	Leaf adaxial	—	—	—	—	—	—
	Leaf abaxial	—	351±26.76d	—	—	603.21±54.15b	76.21±6.87b

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