Morphological and Structural Characters of Trichomes on Various Organs of *Rosa roxburghii*

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Abstract. *Rosa roxburghii* Tratt (Rosaceae) of various organ surfaces are widely existing trichomes. Certain varieties have fruits that are thickly covered with macroscopic trichomes. *R. roxburghii* Tratt (RR) and *R. roxburghii* Tratt. f. *esetosa* Ku (RRE) are important commercial horticultural crops in China because of their nutritional and medicinal values. RRE is generally considered a smooth-fruit variant that arose from RR. Despite their economic importance, the morphological and anatomic features of organ trichomes have not been explored in detail for these two rose germplasms. In this research, we investigated the distribution, morphology, and structure of trichomes distributed on the stem, pedicel, fruit, sepal, and marginal lobule sepals (MLS) of RR as well as RRE. This was accomplished using scanning electron microscopy (SEM). There are various shapes of trichomes distributed on the surfaces of stems, pedicels, fruits, and sepals of the two germplasms. Binate prickles arose on the stem nodes in both germplasms, but acicular trichomes, papillary trichomes, and ribbon trichomes were present only on the surfaces of pedicels in RR. Likewise, flagelliform trichomes were present only on the surfaces of pedicels in RRE. Furthermore, a transection of stems shows that thorns in the two germplasms are composed of epidermis, meristematic layer, and parenchyma cells. The trichome epidermis and meristematic layer in stems of RR are composed of round cells, whereas RRE exhibits square cells in the same layers. Trichomes on the fruit of RR were macroscopic and of single flagelliform and acicular shape. RRE exhibited polymorphic trichomes of flagelliform, triangular, capitate glandular, and elliptic glandular shapes on the pericarp. On the surfaces of RR sepals, there are thick macroscopic acicular trichomes. In contrast, RRE sepals presented flagelliform trichomes and capitate glandular trichomes. It is interesting that no trichomes were found on the surfaces of the MLS in the two germplasms; however, stomata were densely packed on the MLS of RRE when compared with RR. For RR, the trichomes on both sepal and fruit are composed of an epidermis layer and parenchyma cells; however, the epidermis cells of sepal trichomes are polygon-shaped, in contrast to the round epidermis cells in fruit. These results suggest that the two rose germplasms are good candidates for understanding the trichome ontogeny in the genus and for further breeding of the smooth organ trait in this rose species.

Trichomes usually root in epidermal cells and present everywhere at all times in many plant families. They exhibit a high level of multiplicity in morphology, cellular struc-

ture, and function (Uphof, 1962). Because the morphological and mechanical features (size, shape, density, and orientation) of trichomes influence many aspects of plant physiology and ecology, their functions are diverse (Wagner et al., 2004). Besides their role in mechanical defense against biotic and abiotic stresses, trichomes also play a role in the chemical defense of plants by producing a variety of metabolites (Wagner et al., 2004; Werker, 2000). These trichomes show unicellular or multicellular, glandular or nonglandular, and branched or unbranched

(Levin, 1973; Werker, 2000; Yang and Ye, 2013). It is observed that trichomes are widely distributed on all organs in angiosperms (Wagner et al., 2004; Werker, 2000). These special structures can defend themselves from the attack of herbivorous large mammals, mitigate insect and pathogen attacks, increase light reflectance, contain temperature, and reduce water loss (Levin, 1973; Wagner et al., 2004). Glandular trichomes, whose principal function may be to produce pest- or pollinator-interactive phytochemicals that are stored or volatilized at the plant surface, are the location of the biosynthesis and excretion of secondary metabolites (Keene and Wagner, 1985; Kelsey et al., 1984; Venditti et al., 2014; Wagner et al., 2004) and antipathogenic proteins (Shepherd et al., 2005) in many plants. Plant trichomes are specialized unicellular or multicellular structures that cover most surfaces of most plants. The morphology of these epidermal protuberances varies greatly depending on the tissue and the germplasms. Therefore, they have often been used in plant classification (Wagner, 1991). For model plant *Arabidopsis thaliana*, unicellular trichome development has been studied widely. This research has been particularly intense in trichome formation on leaves (Hüllskamp, 2004; Ishida et al., 2008; Pesch and Hüllskamp, 2009). Several regulators that function in distinct developmental paths were identified with classical molecular genetic approaches, for example, trichome initiation/formation, endo-reduplication, branch structure, and growth aspect (Chen et al., 2014; Schwab et al., 2000; Szymanski et al., 2000). Recently, cucumber has aroused much research interest as a model plant with multicellular trichomes (Chen et al., 2014; Cui et al., 2016; Li et al., 2015; Liu et al., 2016a; Pan et al., 2015; Wang et al., 2016; Zhao et al., 2015).

Rosa roxburghii Tratt (RR) (Rosaceae) is a perennial rosebush native to China. Its cultivation area has gradually expanded, especially in Guizhou Province, because of its high nutritional and medical value (Liu et al., 2012; van Rensburg et al., 2005). To date, the economic cultivation area of this germplasm in China involves at least 50,000 ha. There are many drugs, health care products, cosmetics, and functional foods that use the plant (Lu et al., 2016). RR presents trichomes that are widely distributed on leaves, stems, branches, sepals, pedicels, and fruits. The trichomes on the fruits are commonly called “prickles.” These prickles negatively affect the appearance and perceived quality of the fruits. The prickles also hinder the production of processed products. *R. roxburghii* Tratt f. *esetosa* Ku (RRE) is a new germplasm without macroscopic prickles on the fruit, and it is generally considered to be a variant of RR (He et al., 1994). RRE is important for the cultivation of fresh food, as it is easy to harvest and process. RRE and RR represent good candidates for the systematic study of trichome formation in this genus.

In rosaceous plants, detailed study on the type and distribution of the vegetative organ trichomes has been conducted only in *Rubus*

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and Rosas (Coyner et al., 2005; Feng et al., 2015; Finn et al., 2008; Kellogg et al., 2011). These studies did not present clear and detailed anatomic structures or the distribution of trichomes, especially in different developmental stages of trichomes. Very little is known about the structure, ontogeny, and function of trichomes in *R. roxburghii* as well as their pertinence with other trichome types. Therefore, the aims of the present study were to study trichomes of *R. roxburghii* and to analyze the differences between RR and RRE trichomes. We explain the structure, formation, and development of RR and RRE trichomes to lay the theoretical foundation for further research in the future.

Materials and Methods

Plant materials. Ten-year-old plants of RR ‘Guinong 5’ and RRE were grown on yellow soil (pH 6.3–6.5) in the fruit germplasm repository of Guizhou University, Guiyang, China (lat. 26°42.408’N, long. 106°67.353’E), with conventional water management and fertilizer application. The stems, pedicels, fruits, sepals, and MLS were collected from the middle and upper canopy of 20 individual trees of both genotypes, respectively, in middle-late Apr. 2017. The samples of different organs were properly sized and quickly fixed in 3% glutaraldehyde solution for SEM observation after photographing with a digital camera. Samples of ripening fruits from both RR and RRE in middle-late Aug. 2017 were just used for photographing by digital camera.

SEM. These samples of the appropriate size were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer with a pH of 7.2 for 12 h. The fixed samples were dehydrated, subjected to a tert-butanol and ethanol mixture series (30%, 50%, 70%, 90%, and 100%), and vacuum cryodesiccation. Samples were directly placed on stubs with double-sided tape and sputter-coated with a gold thin film (Robards, 1978). The S-3400N scanning electron microscope was used to examine the types and morphology of trichomes. We have 108 clear pictures, and chose some representative pictures for the results analysis. The classification and terminology used in the description of the trichomes were based on Theobald et al. (1950), Payne (1978), and Hewson (1988).

Results

Trichome distribution. The trichomes nearly distributed on all organs observed of RR and RRE, except the MLS (Table 1). There are a large number trichomes on the surfaces of stems, pedicels, fruits, and sepals in RR and RRE (Fig. 1A–J). RRE presents an apparently glabrous phenotype with the naked eye (Fig. 1B, E, F, and H) and SEM revealed that all trichomes in this genotype were small and stunted (Figs. 2B, C, 3D–K, and 5E–Q).

Stem. Prickles were observed on the stem surfaces of RR and RRE, and binate prickles

arose on the stem nodes in both germplasms (Fig. 1I and J).

Pedicel. Nonglandular trichomes were observed on the pedicel surfaces of RR. These trichomes have different shapes, including a type of papilla tuber, a species of ribbon trichome, and a acicular trichome (Fig. 2A); however, there were flagelliform trichomes only on the surfaces of early-stage RRE pedicels (Fig. 2B and C).

Fruit. On the pericarp surfaces of flower buds in RR, early-stage trichomes are fleshy, top passivated, and have a wide base, and belong to acicular trichome (Fig. 3A and B). The middle stage of trichome development is marked by elongation, a slender apex slender, and a small base. This trichome type is referred to as “flagelliform,” and they belong to the nonglandular trichome category (Fig. 3C and D). There are four different trichome types on the pericarp surfaces of RRE flower buds. Triangular trichomes (indicated with arrows) have an appearance is similar to triangle (Fig. 3E–G). Flagelliform trichomes exhibit single, uniserate, multicellular, and pointed varieties (Fig. 3H, I, and P). The bases of flagelliform trichomes have a papilla tuber (Fig. 3M, N, and Q). Capitate glandular trichomes have a short stalk as well as a round head (Fig. 3J–L). Elliptic glandular trichome (indicated by arrows) exhibit a fissure (Fig. 3O).

Sepal. Two different trichomes were present on the surface of sepals in RR, including acicular trichomes and flagelliform trichomes. The basal acicular trichomes are enlarged (Fig. 4A). Flagelliform trichomes were composed of single, uniserate, multicellular, and pointed types. They were also unbranched (Fig. 4B and C). However, there is another trichome architecture on the surface of sepals in RRE. These trichomes are subdivided into two subtypes: capitate glandular trichomes have a short stalk and round multicellular head (Fig. 4D, E, and H–J), and nonglandular trichomes are also unbranched, and they are similar to flagelliform trichomes in RR (Fig. 4F, G, and K).

MLS. No trichomes were observed on the surfaces of the MLS in the two germplasms; however, stomata were densely arranged on the MLS of RRE (Fig. 5C and D). This stomata pattern was not observed in RR (Fig. 5A and B).

Prickle transection of stem. Using SEM, we have defined the internal structure of prickle transections for stems of RR and RRE. The stem prickle of the epidermis in RR is composed of approximately one to three layers of cells. The cells are small, round, and tightly packed. The cell wall is thick, and the cell cavity is large. The cells of the 8 to 10 layers proximate to the inner epidermis belong to meristematic layer. The meristematic layer cells are small, round, tightly arranged, and possess a thick cell wall. This results in a small amount of intercellular space. The volumes of the inward cells are larger than the cells of the epidermis, the arrangement of the cells is loose, the cell walls appear thin, there are no secondary walls, and the intercellular space is

Table 1. The trichome types and distribution in various organs of *Rosa roxburghii*.

Specific name	Organ	Prickle	Nonglandular trichome				Glandular trichome	
			Acicular trichome	Flagelliform trichome	Ribbon trichome	Triangular trichome	Papillary trichome	Elliptic trichome
<i>R. roxburghii</i> Tratt (RR)	Stem	*						
	Pedicel							
	Fruit		*	*			*	
	Sepal		*					
<i>R. roxburghii</i> Tratt. f. <i>esetosa</i> Ku (RRE)	Marginal lobule sepals							
	Stem	*						
	Pedicel			*				*
	Fruit			*				*
	Sepal							
	Marginal lobule sepals							

— indicates absent; * indicates present.

Fig. 1. The macroscopic depiction of *R. roxburghii* Tratt (RR) and *R. roxburghii* Tratt. f. *esetosa* Ku (RRE) organs. (A, C, D) The flower bud of RR. (B, E, F) The flower bud of RRE. (G) The fruit of RR. (H) The fruit of RRE. (I) Prickle on the stem of RR. (J) Prickle on the stem of RRE. SL, sepal; ft, fruit; pl, pedicel; ls, marginal lobule sepals. Scale bars = 5 mm (A–J).

Fig. 2. Scanning electron microscope images of pedicels of *R. roxburghii* Tratt (RR) and *R. roxburghii* Tratt. f. *esetosa* Ku (RRE). (A) Nonglandular trichomes (arrows) of RR at an early stage. (B, C) Nonglandular trichomes of RRE at an early stage. Scale bar = 200 μ m (A–C).

large. These cells can be characterized as parenchyma cells (Fig. 6A and B). The stem prickles transection for RRE shows that the epidermis is composed of approximately one to three layers of cells. The cells are small, square, and tightly arranged. The cell wall is thick, and the cell cavity is large. The 8 to 10 layers of cells proximate to the epidermis are smaller, cell walls are thicker, and the cells exhibit a tight arrangement. These cells belong to the meristematic layer. The inward cells are parenchyma cells, and they are larger, arranged loosely, and exhibit thin cell walls without secondary walls. In addition, there is a large intercellular space (Fig. 6C and D).

Prickle transection of fruit. We also studied the trichomes transection of pericarp and

sepals in RR. The trichomes transection of the epidermis in sepals is composed of approximately one to four layers of cells. The cells are small and polygon-shaped, and they are tightly arranged. The cell wall is thick, and the cell cavity also is small. The cells of inward again five to seven layers are parenchyma cells. The cells are larger, they are loosely arranged, and their cell walls are thin. There is no secondary wall of parenchyma cells, and the intercellular space is large (Fig. 7A and B). The trichomes transection of the epidermis in pericarp is composed of approximately one to two layers of cells. The cells are small and square, and they are tightly arranged. The cell wall is thick, and the cell cavity is small. The inward cells

are parenchyma cells, which gradually become larger. Their arrangement is loose, their cell walls are thin, and no secondary wall can be observed (Fig. 7C).

Discussion

Among the two germplasms investigated in this study, we encountered two trichome morphotypes on the plant organs (stem, pedicel, fruit, sepal, and MLS) (Fig. 1A–J). The trichome types in RR and RRE are documented: nonglandular and glandular. Nonglandular trichomes include the ribbon, acicular, flagelliform, and triangular trichome types (Figs. 2A–C, 3A–I, and 4A–C, F, G, K). Glandular trichomes include the capitate and elliptic glandular trichome types (Figs. 3J, K, L, O, and 4H–J). These trichomes may perform different functions in plant physiology and ecology with various morphological, mechanical, and phytochemical characteristics (Kortekamp and Zyprian, 1999; Wagner et al., 2004). Because variable morphological features of trichomes are found, it is difficult to determine the exact trichome types that are being referred to in the literature (Theobald et al., 1979). The typical trichome types of RR are ribbon trichomes, flagelliform trichomes, and acicular trichomes (Figs. 2A, 3A–D, and 4A–C). The typical trichome types in RRE are triangular trichomes, capitate glandular, flagelliform trichomes, and elliptic glandular trichomes (Figs. 3E–Q and 4D–K). It is interesting that no trichomes were observed on the surfaces of the MLS in the two germplasms. Stomata were densely arranged on the MLS of RRE, but this characteristic was not observed in RR (Fig. 5A–D). The structure of the stem prickles transection in the two germplasms is composed of epidermis, meristematic layer, and parenchyma cells (Fig. 6A–D). The trichome transections of both pericarp and sepals in RR exhibit epidermis and parenchyma cells. The epidermis cells of sepal trichomes are polygon-shaped, and fruit trichomes exhibit round epidermis cells (Fig. 7A–C).

Nonglandular trichomes. The organs of RR are covered by ribbon trichomes, acicular trichomes, and flagelliform trichomes (Figs. 2A, 3A–D, and 4A–C). There are two types of nonglandular trichomes in RRE: triangular trichomes and flagelliform trichomes (Figs. 3E–I, M, P, and 4F, G, K). Ribbon trichomes, acicular trichomes, and flagelliform trichomes are common in other plants (e.g., Chen et al., 2007; Ickert-Bond et al., 2015; Liu et al., 2013, 2016; Ma et al., 2016; Moore, 1991; Wen et al., 2013), but reports of triangular trichomes are rare. Compared with the trichomes found on organs of fruits in RR, the triangular trichomes are a special nonglandular trichome type in RRE (Fig. 3E–G). However, there are no triangular trichomes in RR. The reason for this difference may be because of a trichome-related mutation. All types of trichomes found may do duty as a mechanical barrier against severe spring temperature, herbivores, and pathogens

Fig. 3. Scanning electron microscope images of outer pericarp of surface in *R. roxburghii* Tratt (RR) and *R. roxburghii* Tratt. f. *esetosa* Ku (RRE). (A, B) Nonglandular trichomes in early-stage RR. (C, D) Nonglandular trichomes in mid stage of RR. (E, F, G) Triangular trichomes (arrows) on the pericarp of RRE. (H, I, P) Flagelliform trichomes of RRE. (J, K, L) Capitate glandular trichome (arrows) of RRE. (M, N, Q) Tuberculate processes (arrows) of RRE. (O) Elliptic glandular trichome (arrows) and have a gully of RRE. Scale bars = 200 μ m (A, C, D, F, H, I, K, M, P), 20 μ m (B, E, G, L, N, O, Q), and 10 μ m (J).

Fig. 4. Scanning electron microscope images of morphology on the sepals of *R. roxburghii* Tratt (RR) and *R. roxburghii* Tratt. f. *esetosa* Ku (RRE). (A) Acicular trichomes of RR. (B, C) Flagelliform trichomes of RR. (D, E, H, I, J) Capitate glandular trichomes (arrows) of RRE. (F, G, K) Nonglandular trichomes of RRE. Scale bars = 200 μ m (A–D, G, K), 100 μ m (E, I), and 10 μ m (F, H, J).

(Kortekamp and Zyprian, 1999; Werker, 2000), as well as restrict insect activities on the surfaces of flower buds (Ma et al., 2016; Wagner et al., 2004). They may also serve as photo-protective against light stress (Liakopoulos et al., 2006). Ribbon trichomes of young plant organs in RR and RRE may serve as a significant defense mechanism against abiotic and biotic stresses. These trichomes may also assist in decreasing water loss and facilitate acclimation to xeric environments (Wagner et al., 2004). We conclude that the ribbon trichome of RR may be a morphological synapomorphy for the Rosaceae clade, and this hypothesis should be experimented with in conjunction with a fully resolved phylogeny of the Rosaceae clade in the future.

Acicular trichomes and flagelliform trichomes are commonly called simple trichomes. Simple trichomes differ in size, density, cell number, length, color, and distribution. Obviously, simple trichomes of RRE were longer and denser than RR (Figs. 2A–C and 4A–C, F, G). Simple trichomes on the two germplasms of *R. roxburghii* may protect plant organs against damage. This is the case in other germplasms of *R. roxburghii*. In many germplasms, a dense indumentum composed of simple trichomes serves as a filter protecting plant tissues against hurt from ultraviolet-B radiation and as a deterrent to insect activity and so on (Karabourniotis et al., 1995; Levin, 1973;

Liakoura et al., 1997; Manetas, 2003; Yan et al., 2012). Short simple trichomes have no effect on the oviposition of leafhoppers, but they did reduce the *Anagrus* spp. parasitism rate. In contrast, long simple trichomes could make a difference of the leafhopper oviposition on different herbaceous plants (Pavan and Picotti, 2009).

The nonglandular trichomes of representatives of Rosaceae have hardly been described in detail (e.g., Kellogg et al., 2011), and there has been no research on nonglandular trichomes of germplasms in *R. roxburghii*. Nonglandular trichomes have traditionally been described according to their density and overall appearance instead of their structure. For example, “villose leaves” have been used as a description for *Anemopaegma velutinum*, referring to a hairy cover of thick, straight, long, and soft trichomes (Bureau and Schumann, 1897). However, no information is helpful on the anatomy and developmental sequence of nonglandular trichomes in Rosaceae or other plant groups, such as Lamiaceae (Naidu and Shah, 1981). Nonglandular trichomes have generally been regarded as morphologically homogeneous structures. In general, the variable number of cells and variation in cell size, as well as the variable patterns of the trichome morphotype distribution on different plant parts, show the importance for more detailed morpho-evolutionary studies of trichomes.

Glandular trichomes. Glandular trichomes may show uni- or multiseriate stalks and uni- or multicellular glandular heads. They can originate from the epidermis or from both the epidermis and next layers (Werker, 2000). Some types of glandular trichomes in many germplasms are solely originated from the epidermis, and there are particularly prominent glandular trichomes in Asteraceae, Cucurbitaceae, Cannabaceae, Lamiaceae, and Solanaceae (Akers et al., 1978; Ascensão and Pais, 1998; Celep et al., 2014; Kolb and Müller, 2004; Nielsen et al., 1991; Tissier, 2012; Werker et al., 1985). In RR, glandular trichomes were not found on all organs (Figs. 2A, 3A–D, 4A–C, and 5A, B). In RRE, there were capitate glandular trichomes and elliptic glandular trichomes (Figs. 3J–L, O, and 4D, E, H–J). The reason for the distinction in the two germplasms of *R. roxburghii* may be that the trichome is not entirely developed. The two types of glandular trichomes also present significant variation in morphology and cell architecture. Capitate glandular trichomes have a short stalk as well as a round head, and elliptic glandular trichomes present a fissure. Although the two trichome types present a notable distinction in morphology, these two trichome types may both be derived from the epidermis or the epidermis and subjacent layers. We need further study to elucidate the root of the glandular trichomes in RRE. However, all capitate glandular trichomes of RRE, which were examined in our study, have no stomata at the top of trichomes (Figs. 3J and 4H, J). This pattern is similar to the capitate glandular trichomes on the abaxial leaves of *Salvia quezelii*

Fig. 5. Scanning electron microscope images of marginal lobule sepals (MLS) in *R. roxburghii* Tratt (RR) and *R. roxburghii* Tratt. f. *esetosa* Ku (RRE). (A, B) MLS of RR. (C, D) MLS of RRE. Scale bars = 200 μ m (A–D).

Fig. 6. Scanning electron microscope images of prickles of stem in *R. roxburghii* Tratt (RR) and *R. roxburghii* Tratt. f. *esetosa* Ku (RRE). (A, B) Prickle transection of stem in RR. (C, D) Stem prickles transection of stem in RRE. EP = epidermis; ML = meristematic layer; PC = parenchyma cell. Scale bars = 50 μ m (A, B, C, D).

(Celep et al., 2014). Terpenes, phenolics, alkaloids, and lipophilic compounds can be secreted by glandular trichomes, or other substances that deter or poison herbivores and pathogens (Levin 1973; Tissier 2012; Werker 2000). These glandular trichomes can also secrete resin to provide a protective layer of the developing tissues against cold temperatures (Lapinjoki et al., 1991). Secreted mucilage from *Drosera* serve as an adhesive trap for insects (Outenreath and Dauwaldert, 1986). This trichome type may serve as a defense mechanism against insects. The composition of mucilage and secondary metabolites of RRE will be explored in the future. It has been reported that the extraplasmic and intercellular spaces can be the site of the accumulation secretions from glandular trichomes in *Nicotiana tabacum* (Akers et al.,

1978). Extracellular spaces also show the preservation of secretions in other glandular trichomes (Ascensao et al., 1997; Gravano et al., 1998; Turner et al., 2000; Uzelac et al., 2017; Vermeer and Peterson, 1979). In the secretory cells of glandular trichomes in *Passiflora foetida*, the formation of secretions shows no difference of the developmental process of prickles on raspberries and roses (Kellogg et al., 2011). Furthermore, trichomes act as the precursors to prickles that have been found in many other species (Delbrouck, 1875; Leelavathi and Ramayya, 1983).

Internal structure of trichomes. The framework of epidermis, meristem, and parenchyma cells for prickles is proposed because the anatomic characteristics of prickles are similar to those of epidermis, meristem,

and parenchyma cells of leaves, stems, and other organs. The structure of stem prickles in two germplasms of *R. roxburghii* is composed of epidermis, a meristematic layer, and parenchyma cells, but there is a major distinction between epidermis cells and meristematic cells in the two varieties (Fig. 6A–D). The epidermis and meristematic layer of RR present round cells, but the epidermis and meristematic layer in RRE exhibit square cells. These cellular distinctions might lead to the different appearance of prickles in RR and RRE (Fig. 6B and D). The parenchyma cells are similar between the two germplasms. The determination of the specific functions of the previously mentioned structures still requires further study. The anatomic results show that the *R. roxburghii* epidermis does not exhibit obvious vascular tissue. This is similar to the reports of the predecessors of the *Rose* cultivars ‘Laura’ and ‘Shortcake’ (Asano et al., 2008; Kellogg et al., 2011).

Trichome initiation in RR and RRE. The results show that there are flagelliform trichomes on the fruits of RR (Fig. 3C and D). The flagelliform trichomes of RR are similar to the multicellular trichomes in fruits of wild-type cucumber. These flagelliform trichomes belong to the simple trichome type (Liu et al., 2016a). Four trichome types were found in RRE: triangular trichomes, capitate glandular trichomes, elliptic glandular trichomes, and flagelliform trichomes (Figs. 2C, D, 3E–Q, and 4D–K). Capitate glandular trichomes in RRE were similar to type I trichomes found in three trichome-related mutants of cucumber, reported as MICT, TBH, and CsGL1 (Chen et al., 2014; Li et al., 2015; Zhao et al., 2015). These trichomes have a small papillar-shaped head. Furthermore, the trichome initiation and development in RR was controlled by two allelic genes, in which two dominant genes made the fruit prickles stiff, one dominant gene made fruit prickles soft, and two pairs of recessive genes made fruits similar to those found in RRE (Gao and Luo, 1994). In cucumber, there were also two allelic genes. However, the main difference is that recessive genes override the effect of dominant genes (Liu et al., 2016b). The two genes (*Csa3M748220* and *Csa6M514870*) that referred to multicellular trichome development in cucumber were not found in the transcriptional data in RR (Yan et al., 2015). The trichome development positive regulator pathway model in RR may be different from that in cucumber. The genetic determinants of these traits require further study. Our current knowledge of the trichomes relevant gene regulatory networks is primarily restricted to the unicellular trichomes of the model plant *Arabidopsis*. Not much is known about control the development of multicellular trichomes in RR. Future studies should focus on the analysis of diverse expression data generated by RNA-sequencing that may show new information for identifying assumed key transcription factors of multicellular trichome development in RR.

Fig. 7. Scanning electron microscope images of trichomes transection of pericarp and sepals in *R. roxburghii* Tratt (RR). (A, B) Trichomes transection of sepals. (C) Trichomes transection of pericarp. EP = epidermis; PC = parenchyma cell. Scale bars = 50 μ m (A–C).

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