Prickle morphogenesis in rose is coupled with secondary metabolite accumulation and governed by canonical MBW transcriptional complex

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Abstract
Rose is an economically important flowering plant that holds an essential place in cut flower, medicinal, and aromatic industries. The presence of prickles, epidermal outgrowths resembling trichomes, on rose is highly undesirable as these make harvesting and transportation difficult. Attempts were made for generating rose varieties lacking prickles via breeding and natural selections; however, these approaches obtained only chimeric and genetically unstable prickle-less mutants. The alternative way to get rid of prickles is via genetic manipulations, but the molecular mechanisms of prickle initiation and development in rose are almost unexplored. Therefore, the present study was carried out to understand the morphological, molecular, and correlated metabolic changes underlining prickle morphogenesis in a prickle-bearing Rosa hybrida L. cv. "First Red (FR)". The histological and metabolomic analyses at three distinct stages of the prickle morphogenesis, namely, emerging tiny initiating prickles, partially greenish soft prickles, and brownish hard prickles, demonstrated a gradually increasing deposition of phenolic compounds and lignification with development. Corresponding RNAseq analysis revealed an upregulation of the genes involved in secondary metabolism, especially in the phenylpropanoid biosynthetic pathway. A set of genes encoding a transcriptional network similar to the one regulating epidermal cell differentiation leading to phenylpropanoid accumulation and trichome development, was also upregulated. Differential expression of this transcriptional network in prickle-less R. hybrida L. cv. "Himalayan Wonder" compared to prickly FR plants substantiated its involvement in prickle morphogenesis. The results collectively supported the proposition that prickles are evolved from trichomes and provided molecular clues towards engineering prickle-less roses.

Significance statement: Prickles, the vasculature less epidermal outgrowths resembling trichomes, are defense organs protecting plants against herbivory. Despite biological significance, the mechanism of prickle morphogenesis remains obscure. Here, we show that like trichomes, prickles accumulate secondary metabolites, especially lignin and flavonoids, during morphogenesis. Cognate transcriptome analysis
demonstrated that upregulation of a hormone-regulated transcriptional activation–inhibition network, known to govern trichome morphogenesis, likely triggers the differentiation of epidermal cells to outgrow into prickle.

**KEYWORDS**
flavonoids, prickle morphogenesis, prickles, *Rosa hybrida*, trichomes

# 1 | INTRODUCTION

Plants respond to environmental adversities, where each organ has a role in endurance through various mechanisms. Also, plants synthesize and store various secondary metabolites in the specific cells of different organs to combat adversities. A plethora of literature on the involvement of secondary metabolites in defense responses opened new avenues for investigating the underlying mechanisms. The defense system in plants differs in varied habitats; sharp and edgy spines, thorns, and prickles are the prominent defense armors for protection against insects and pests (Hanley et al., 2007). Among these defense armors, thorns and spines are derived from the lateral branch or leaf meristems and contain vasculature, whereas prickles are the epidermal or cortical tissue outgrowths lacking vasculature and resemble trichomes (Coyner et al., 2005; Poslusnzy & Fisher, 2000). The sharp and firm prickles store various metabolites and form a physical barrier protecting from herbivory (Gallenmuller et al., 2015; Hanley et al., 2007).

Many plants, especially from *Rosaceae*, *Araliaceae*, *Rutaceae*, *Leguminosae*, and *Solanaeae* families, have prickles on their stems, branches, leaves, and sometimes on fruits (Feng et al., 2015). Rose, a member of the *Rosaceae* family, is a globally cultivated commercial flower crop. The cultivars of the *Rosa hybrida*, a heterozygous tetraploid species that arose from complex interspecific crosses involving 10 wild species, are among the most cultivated roses. Almost 30,000–35,000 of these cultivars are currently being cultivated worldwide for their economic importance as cut flowers. The prickles on the stems and leaves affect yield, quality, harvesting, and transportation of roses. Although prickles are implicated in defense and protection, prickle-less roses are preferred for commercial purposes. Attempts have been made for generating desirable rose varieties lacking prickles via breeding and natural selections, where chimeric prickle-less mutants were obtained. However, these were genetically unstable (Rosu et al., 1995; Singh et al., 2016). The alternative way to get rid of prickles is via genetic manipulation; however, the molecular mechanisms of prickle initiation and development remain obscure.

Being epidermal outgrowth and having similar developmental and physiological features, prickles were proposed to be developed as an extended form of single-cell trichomes (Kellogg et al., 2011; Ma et al., 2016; Pandey et al., 2018). Accordingly, a study investigating the physiological relationship of prickles and trichomes in *Rubus idaeus* (raspberry), a member of the *Rosaceae* family, indicated that the prickles probably develop from the glandular trichomes (Kellogg et al., 2011). Like trichomes, prickles also accumulate secondary metabolites, especially phenylpropanoids, including lignin and suberin (Gallenmuller et al., 2015). However, in contrast to trichomes, prickles accumulate more lignin, where the lignification gradually increases with development and leads to their hardening (Asano et al., 2008). Although morphological and structural characteristics of trichomes on various organs of roses are studied (Wang et al., 2019), molecular aspects of their formation are apparently not explored. Since the trichome initiation and development is well established in model plants such as *Arabidopsis thaliana*, a relative analysis of trichomes versus prickles can offer clues to understand the process of prickle development.

The MBW transcriptional activator–inhibitor complex comprising of MYB, bHLH, and the WD40 protein determines the fate of the epidermal cells to form trichomes. Within this complex, the R2R3-type MYB GLABRA 1 (GL1), bind to bHLHs GLABRA 3 (GL3) or ENHANCER OF GLABRA 3 (EGL3) and WD 40 repeat protein TRANSPARENT TESTA GLABRA 1 (TTG1) to constitute MBW activator complex. The MBW activator complex (GL1-GL3/EGL3-TTG1) induces the expression of Homeo Domain-Leucine Zipper transcription factor (TF) GLABRA 2 (GL2) and WRKY TF TRANSPARENT TESTA GLABRA 2 (TTG2), which initiate the cell transition to differentiation into trichomes and induces phenylpropanoid accumulation (Huang et al., 2019; Kirik et al., 2005; Payne et al., 2000; Walker et al., 1999; Zhao et al., 2008). Another R2R3 protein, MYB23, acts redundantly to GL1 to initiate trichome morphogenesis (Kirik et al., 2005). Besides, bHLH TF TRANSPARENT TESTA 8 (TT8 or bHLH42) and R2R3-MYB TFs, MYB123 [also called TRANSPARENT TESTA 2 (TT2)] and MYB75 [also referred to as PRODUCTION OF ANTHOCYANIN PIGMENT 1 (PAP1)], are also associated with this complex and promote phenylpropanoids accumulation and trichome development (Johnson et al., 2002; Kirik et al., 2005; Payne et al., 2000; Walker et al., 1999; Zhao et al., 2008). MBW complex also induces the expression of repressor genes encoding single repeat R3-type MYBs such as TRIPTYCHON (TRY), CAPRICE (CPC), and TRICHOMELESS1 (TCL1), which can move to the adjacent cells and assemble into an inhibitor complex or inhibit the expression of MBW genes preventing trichome formation (Fambrini & Pugliesi, 2019; Pesch et al., 2015). Given the resemblance of prickles with trichomes, some studies provided preliminary indications for the involvement of MBW complex in prickle morphogenesis. In *Rosa rugosa*, the TTG1 expression was much higher in the variety with denser prickles compared to a less-prickly variety, implicating it with prickle initiation and development (Feng et al., 2015).
Despite preliminary efforts, the mechanisms of prickle initiation and development remain unexplored. Hence, the present study was conducted to investigate the mechanism of prickle development in prickle-bearing cultivar of *R. hybrida* L. cv. "First Red (FR)," and the findings were validated in a prickle-less cultivar *R. hybrida* L. cv. "Himalayan Wonder (HW)," selected previously at our institute. The results revealed the distinct morphological and anatomical features together with metabolite accumulation and cognate gene expression at different stages of prickle morphogenesis. In-depth analysis of differential gene expression during prickle morphogenesis retrieved the upregulation of MBW complex and associated genes known to be involved in phenylpropanoid accumulation and trichome development. The validation of the expression of this network in the prickle-less cultivar HW compared to prickly FR plants demonstrated the involvement of MBW complex in prickle morphogenesis, suggesting that prickles are evolved from trichomes.

### EXPERIMENTAL PROCEDURES

#### 2.1 Plant material and sample collection

Plants of prickle-bearing *R. hybrida* cv. "FR" and prickle-less *R. hybrida* cv. "HW" were maintained in the poly house at CSIR-Institute of Himalayan Bioresource Technology, Palampur, Himachal Pradesh, India (Figure 1a). In case of prickly FR plants, samples in three biological replicates for each prickle developmental stage, namely, initiating prickle together with bark (IP), soft prickle (SP), hard prickle (HP), stem, and leaf, were collected from three independent and healthy plants. For prickle-less variety HW, the bark tissue corresponding to the initiating prickles in FR plants beneath the pedicel was collected in triplicate. Unless specified, samples are immediately frozen in liquid nitrogen and stored at −80°C until further use.

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**FIGURE 1** Stages of prickle morphogenesis in *R. hybrida* cv. "FR".  
(a) A healthy rose plant in the flowering stage depicting three stages of prickles, initiating prickle (IP), soft prickle (SP), and hard prickle (HP). (b–d) Transverse section of bark (b), SP (c), and HP (d). Ep, Epidermis; Cp, cortical parenchyma; Xy, xylem; Ph, phloem; Me, medulla. Scale bar = 100 μM. Oval indicates the area of lignin deposition. Red brace in (b) shows the region where prickle is initiating. (e) Protein-free cell wall (PFCW) content in prickles. (f) Lignin content in prickles. (g) Total phenolics content (TPC) in prickles, leaves, and stems. Data points represent the mean ± standard deviation (SD) of three independent biological replicates. Lowercase letters above each bar indicate statistically significant differences between mean values at each genotype (*p* < .05, one-way ANOVA with Tukey’s post hoc HSD test).
2.2 | Microtomy and histological staining

Microtomy-based histological analysis was performed as described previously (Shafi et al., 2015). Briefly, fresh cuttings of stems and prickles were fixed in FAA [1:1:18— formaldehyde: glacial acetic acid (50%): ethanol] at room temperature (RT). Dehydration was performed in a series of tertiary butyl alcohol series and embedded in paraffin (58–60°C). Sections (20 µm) were cut using a Finesse microtome (Finesse, USA) and stained in safranin (0.1%) for 3 h, followed by 30 s of counterstain by fast green (0.1%) in clove oil. Stained sections were mounted on a glass slide by DPX (a mixture of distyrene, plasticizer, and xylene) covering slip and visualized under a fluorescence microscope (Zeiss Axiovert 100 M inverted microscope). Observed sections showed deposition for lignified and suberized cell walls, and tannins in red, whereas non-lignified walls of phloem and parenchyma were green in colour (Ma et al., 1993).

2.3 | Quantification of lignin content

Lignin content at the different developmental stages of prickles was estimated as described previously (Iiyama & Wallis, 1990). Briefly, 300-mg tissue was homogenized in 50 mM phosphate buffer (pH 7.0) and centrifuged at 1,400 g for 5 min at RT, followed by three washes in 1% Triton x 100 (v/v) in 1 M NaCl (pH 7.0). The resulting pellet was again washed with 1 M NaCl, distilled water, and absolute acetone. The washed pellet was dried at 60°C for 24 h, lyophilized, and was used as the PFCW fraction. The PFCW content was measured on the basis of fresh weight of the tissue (mg/g of FW). Twenty milligram of PFCW fraction was incubated with 0.5 ml acetyl bromide (25% v/v in acetic acid), at 70°C for 30 min in a screw-cap tube. Tubes were immediately cooled on ice and mixed with solubilization buffer (0.9 ml of 2 M NaOH and 0.1 ml of 0.5 M hydroxylamine HCl). Tubes were centrifuged at 1,400 g for 5 min, supernatant was collected, and the absorbance was recorded at 280 nm using a Biospectrometer (Eppendorf, Germany). Lignin content was quantified using a standard curve prepared using 1 mg/ml lignin (Sigma-Aldrich, USA; Figure S1a).

2.4 | Estimation of total phenolics

The quantification of total phenolics content (TPC) was performed using Folin–Ciocalteau reagent (Singleton et al., 1999). Briefly, 0.5 ml of the methanolic extracts (10 mg/ml) of IP containing bark, SP, and HP were mixed with 2.5 ml of Folin–Ciocalteau reagent and 2.5 ml of 7.5% NaHCO₃ in a 2 ml tube. The tubes were incubated at 45°C for 45 min. After cooling at RT, absorbance was recorded at 765 nm. The TPC was measured as gallic acid equivalent (mg/g) using gallic acid as a standard (Figure S1b).

2.5 | HPTLC- and UPLC-PDA-based profiling of secondary metabolites

Qualitative and quantitative analyses of secondary metabolites were carried out using an HPTLC and UPLC-PDA, as described previously (Kumar et al., 2015). Briefly, 0.5 g freshly collected tissue of the bark containing IP, SP, and HP was ground to a fine powder in liquid nitrogen. The powders were resuspended separately in 20-ml 100% (v/v) methanol, sonicated in a bath sonicator at 45°C for 30 min each for three consecutive extractions, and filtered through Whatman No. 1 filter paper. The filtrates were dried at 45 ± 5°C under reduced pressure in a rotary evaporator and weighed for calculating the extractive yield. The extractive yield was calculated as follows: EY (final weight of dry filtrate/initial weight of tissue) x 100 (Figure S2a). The dried filtrates were resuspended in 100% (v/v) methanol at a 10 mg/ml concentration and passed through a 0.22 µm PVDF membrane-based filter before performing HPTLC and UPLC-PDA.

For qualitative analysis, samples were analyzed on an HPTLC system (CAMAG, Switzerland). Catechin (1 mg/ml) was used as a standard for HPTLC. An automated TLC sampler applied the samples and the standard on a pre-coated silica gel plate as 6-mm-wide bands under nitrogen gas flow. Chromatography was carried out in an automated developing chamber pre-saturated with 20 ml of mobile phase ethyl acetate-methanol-formic acid-water (20:4:2:1, v/v/v/v) for 30 min at RT (25 ± 2°C) and 72% ± 2% relative humidity. The length of the chromatogram run was 80 mm from the base. The plates were then air-dried and visualized at 254 nm, 366 nm, and white light for HPTLC fingerprints in the normal phase. Qualitative evaluation of the plate was performed at 254 nm, with 4 × 0.45 mm width, 20 mm/s scanning speed, and 100-µm step 1 data resolution. The CAMAG video documentation system, coupled with Reprostar 3 was used for imaging the thin-layer chromatograms. Image acquisition, data processing, and analysis were made on WinCATS 1.4.2 planar software (CAMAG, Switzerland).

For quantitative analysis of the metabolites, samples were analyzed in an UPLC-PDA system (Waters, USA) using a standard cocktail of 12 different metabolites. The standard metabolites, namely gallic acid, catechin, caffeic acid, syringic acid, rutin, hyperoside, isoquercetin, luteolin, quercetin, cinnamic acid, epigallocatechin, and kaempferol (Sigma-Aldrich, USA) were dissolved in methanol. An equal amount of each standard solution (1 mg/ml) was mixed and serially diluted in methanol to prepare standard regression equations (Table S8). The chromatographic separations were carried out on Waters BEH C18 (2.1 mm × 100 mm, 1.7 µm) column at 24°C on Acquity UPLC hyphenated to Q-TOF micro mass system (Waters, USA). Separation was performed with a gradient of mobile phase A containing 0.1% formic acid in water and mobile phase B containing 0.1% formic acid in acetonitrile. The gradient started from 0 to 0.3 min with 5% of B, 0.3 to 10 min with 5%–22% of B, 10 to 10.5 min with 22%–28% of B, 10.5 to 16.0 min with 28%
of isotropic B, and 16 to 17 min with 28%–90% of B, 17 to 18 min with 90% isotropic B, 18 to 18.5 min 90%–95% of B (initial) and 18.5 to 20 min for initial conditioning. The flow rate was 240 µl/min, with an injection volume of 1 µl. The run time of the method was 20 min. The chromatograms were analyzed at 270 nm. Mass spectrometry was done using an electrospray ionization method with positive ion mode, capillary voltage 3.2 kV, cone voltage 30 V, source block temperature 80°C, desolvation temperature 220°C, cone gas flow 50 L/h, and desolvation gas flow 400 L/h. Nitrogen was used as the drying gas. The resulting mass spectra were analyzed in mass Lynx v4.1 software (Waters, USA).

2.6 | RNA-sequencing (RNA-seq), reference-based annotation, and bioinformatic analysis

Total RNA was isolated from three independent biological replicates of IP, SP, and HP, using the method described by Gha...analyzer 2100 system (Agilent Technologies, USA). RNA samples with RNA integrity (RIN) ≥ 8 were used for constructing libraries using TruSeq RNA stranded sample prep kit (Illumina, USA) as per the manufacturer’s instructions (Figure S3a,b and Table S1). The libraries were pooled at 400-pmol concentration, denatured, and sequenced on an Illumina NovaSeq 6000 system (Illumina) to generate 101-bp paired-end reads.

The raw sequencing reads were demultiplexed, followed by generation of fastq files using the bcl2fastq converter tool and filtration using NGSQC tool kit v2.3.3 (Patel & Jain, 2012). The clean reads obtained were mapped onto R. chinensis cv. Old Blush Homozygous Genome v2.0 (OBDH v2.0, https://www.roseta... Genome v2.0 (which) genome using CLCBio genomics workbench v12.0.3 (Qiagen Arhus, USA). Initial gene expression tracks were obtained by CLCBio RNA-seq analysis tool with default parameters including “genome annotated with gene only” option. This RNAseq analysis tool uses the multifactorial statistics based on a negative binomial generalized linear model (GLM). Reads per Kilobase of transcript, per Million mapped reads (RPKM), was used to get the expression levels. The transcripts having RPKM < 1 and the transcripts which are not present in at least two replicates were filtered out. For remaining transcripts, RPKM values were used for differential expression analysis between two groups in three stages, i.e., HP versus SP (A), HP versus IP (B), and SP versus IP (C), to get statistical comparison table using default parameters (min absolute fold change > 1.5 and maximum p < .05). These tables were used for computing uniquely and commonly DEGs. Due to the lack of complete annotations in rose, the corresponding annotations of common DEGs were extracted from The Arabidopsis Information Resource (TAIR) database (www.arabidopsis.org) using KEGG Orthology-Based Annotation System (Xie et al., 2011). GO analysis was done using AgriGO (Tian et al., 2017) with a significance of p < .05. KEGG (Kanehisa & Goto, 2000) was used for pathway enrichment studies. Detailed mining of transcription factors (TFs) was done using PlantRegMap (Tian et al., 2020). The detailed flowchart of reference-based RNA-seq data analysis is given in Figure S3c.

2.7 | Reverse transcription-quantitative polymerase chain reaction

Total RNA was isolated from the three independent samples of bark, IP, SP, and HP in FR plants and bark tissue in HW plants, using the method as described by Gha...analyzer 2100 system (Thermo Life Sciences, USA). The RT-qPCR was performed using DyNAmo HS SYBR Green qPCR Kit (Applied Biosystems) on a StepOnePlus Real-Time PCR System (Applied Biosystems). The relative expression of target genes was calculated by the comparative 2-ΔΔCt method and normalized to the transcript levels of Ubiquitin C (UBC; Klie & Debener, 2011). The primers were designed by Primer Express v 3.0.1 (Applied Biosystems, USA) with default parameters and are listed in Table S7.

2.8 | In situ flavonoid staining and confocal laser scanning microscopy

Fluorescence induction using Naturstofreagenz A (NA) is an established method for flavonoids localization (Hutzler et al., 1998). Fresh samples of HP were cut into thin sections using a sharp scalpel and kept on a glass slide. A drop (100 µl) of sample buffer [100 mM, potassium phosphate pH 6.8, 1% NaCl (w/v)] was added onto the sections and covered with the coverslip. The sections were observed under confocal laser scanning microscopy (CLSM) at an excitation/emission wavelength of 488 nm/420–600 nm for observing autofluorescence. After that, a droplet of 0.1% NA in sample buffer (prepared freshly from a stock of 2.5% [w/v] NA in Ethanol) was added and incubated for 5 min. The fluorescence of NA-stained flavonoids was observed at an excitation/emission wavelength of 364 nm/385–660 nm.

2.9 | Statistical analysis

For all experiments, sample values are represented in the mean ± standard deviation (SD) of three independent biological replicates. Statistical significance among the differences between mean values at each sample was analyzed by one-way ANOVA with Tukey’s post hoc HSD test, using Prism5 software.

2.10 | NCBI data submission

The data are submitted to NCBI Gene bank with Bio project accession PRJNA624802 and SRA accessions SRR11528770, SRR11528771,
SRR11528772, SRR11528773, SRR11528774, SRR11528775, SRR11528776, SRR11528777, and SRR11528778.

3 | RESULTS

3.1 | Prickles exhibit distinct morphological and anatomical features during morphogenesis

Plants of the Rosaceae family have evolved prickles for their defense. R. hybrida L. cv. "FR" also has prickles that can be distinguished based on their morphology and position on the stem. Based on the morphology, prickles in the present work were divided into three types: tiny greener prickles called initiating prickles (IP); greenish, easy to break enlarged but less differentiated outgrowths referred to as soft prickles (SP); and the red to brownish, mature, and easy to break enlarged but less differentiated outgrowths comparatively smaller (+0.179 mm and 7.03 × 0.23 mm, respectively. The IP was comparatively smaller (-2 mm × 0.5 mm) and undetachable from the pedicel.

To address the pheno-morphic differences/similarities within the prickles, histological analysis was performed in bark of rose stem containing IP, SP, and HP (Figure 1b–d). The transverse section of the initial SP revealed that prickles initiate from the cortical parenchyma (Cp) and were surrounded by the epidermal lining (Ep). Protruding cells elongate from Cp and differentiate into a lignified and hardened layer towards the tip (Figure 1c). SP also has vascular features as depicted by xylem (Xy), phloem (Ph), and central part of the medulla (Me; Figure 1c). During the development, cells tend to stretch out, and the lignification started from the tip to Cp in HP (indicated by arrows, Figure 1d). On the contrary, the IP consists of a highly organized group of cortical cells having Ep, Cp, and well-defined vasculature (indicated by a circle, Figure 1b). The IP had a lower amount of lignin as compared to that in SP and HP, and hence, these are relatively softer compared to SP and HP.

3.2 | Secondary metabolites accumulate during prickle morphogenesis

Histological analysis revealed the anatomical differences and showed the specificity of the active constituents, such as lignin, between the different stages of prickle development. Given that lignin mainly accumulates in the cell walls, protein-free cell wall (PFCW) fraction was estimated in three developmental stages. Interestingly, SP showed the highest amounts of PFCW fractions (213.8 ± 11.2 mg/g of fresh weight), followed by HP (193.7 ± 9.7) and IP (99.18 ± 10.59; Figure 1e). Data on lignin content showed that, as observed in histological sections, HP showed the maximum lignin content (11.9 ± 1.1 mg/g of fresh weight), followed by SP (8.08 ± 0.72) and IP (10.31 ± 0.36; Figure 1f and Figure S1a). Lignins are the polymers of phenylpropane units (Ralph et al., 2004), in which a significant portion of the units are phenolic (Brunow & Lundquist, 2010). However, the phenol content of lignins differs considerably. To ascertain the nature and types of phenolic groups in the lignins, TPC was estimated during prickle developmental phases. Since IP had comparatively lesser lignin content, TPC was estimated only in SP and HP. TPC was also estimated in leaves and stem. As compared to leaf and stem (leaf: 43.47 ± 5.9, and stem: 52.02 ± 7.8 μg/mg of gallic acid equivalent), HP accumulates significantly higher content of total phenolics (90.44 ± 27.06; Figure 1g). Notably, the phenolic accumulation in SP was comparatively lesser (29.52 ± 5.6) among all the tissues analyzed (Figure 1g).

Accumulation of lignin and total phenolics in HP prompted us to investigate the presence of other plant metabolites during prickle development vis-à-vis leaf and stem tissues. There was no significant difference in the extractive yeild of metabolites in SP (6.73 ± 1.1%) and HP (6.71 ± 2.4%). However, the content was higher in leaf tissue (10.59 ± 1.4%) and the least in stem tissue (3.52 ± 0.18%; Figure S2a). Subsequent HPTLC profiles revealed a significant difference in the polyphenolic content in prickles, especially in HP, as compared to leaf and stem tissue (Figure S2b). HP accumulated a higher content of metabolites, seemingly phenylpropanoids such as flavonoids, which are implicated in stress and defense. Strikingly, HP also accumulates high catechin content, which was not reported until now. These observations directed the need for downstream molecular studies to delineate why and how these active metabolites accumulate during prickle development.

3.3 | Reference-based transcriptomic profiling uncovered the gene expression changes during prickle morphogenesis

Histology and metabolite analysis provided potential insights into the prickle formation and hardening. To further dissect the mechanism of prickle morphogenesis, RNA-seq-based transcriptome analysis was performed (Figure S3a–c and Table S1). Triplicate samples of the three prickle development stages generated a total of 905.25 million (M) raw reads and 90.45 Giga bases (GB). Quality control filtering of raw reads generated about 811.2 M reads (~99.6%) with high-quality data of 78.06 GB (Figure 2a and Table 1). Since R. hybrida genome is not available, the R. chinensis cv “Old Blush Double Haploid (OBDH)” v2.0 genome (Table S2) was referred for annotation. All the replicates of IP (97%), SP (97.43%), and HP (96.3%) were significantly mapped with a high fraction of genic regions (0.97), suggesting a good quality mapping (Table S3). Principal component analysis of the individual filtered read files showed distant grouping of the reads from individual replicates, indicating high confidence in the biological data points (Figure 2b).

Expression of different genes was analyzed in three stages of prickle development and was compared as follows: comparison A (HP versus SP), comparison B (HP versus IP), and comparison
C (SP versus IP). These comparisons generated a list of 16,318; 11,507; and 13,332 differentially expressed genes (DEGs) in comparisons A, B, and C, respectively (Table 2a and Data S1–S3). A total of 7,841 genes were upregulated, whereas 8,477 were downregulated in HP compared to those in SP. Compared to IP, 5,326 genes exhibited upregulation and 6,181 shown downregulation in SP. Similarly, 6,318 genes were upregulated, whereas 7,014 were downregulated in HP as compared to those in IP (Table 2a). Gene Ontology (GO) analysis revealed significant enrichment of genes involved in secondary metabolite and cell wall components biosynthesis among the genes upregulated in HP as compared to both in IP and in SP (Figure S4a–d). On the contrary, genes involved in cell division, DNA replication/repair, organelle fission histone methylations, and structural development symbolizing prickle morphogenesis were upregulated in SP as compared to those in IP (Figure S4e,f).

A total of 5,605 genes were commonly differentially expressed between comparisons A and C, whereas 4,355 genes were similar in comparisons A and B. Uniquely expressing genes were higher in comparison A (5,256 genes), among other two comparisons B (1,256 genes) and C (1,831 genes; Figure 2c and Data S4). To further analyze the DEGs, commonly DEGs were extracted in three conditions. By applying the statistical significance threshold of p value and false discovery rate (<.05) in CLCBio workbench, a set of 3,832 genes was obtained, which was commonly differentially expressed in all the three conditions (Figure 2c and Table 2b).
3.4 Genes related to secondary metabolite biosynthesis were upregulated during prickle morphogenesis

Comparative gene expression analysis is a proven way to understand molecular changes occurring during various biological processes. A similar approach was applied to elucidate molecular events taking place during prickle morphogenesis. Since the complete annotation of rose genome was not available, orthology-based method was used to extract the corresponding annotation of common DEGs from the Arabidopsis genome (TAIR; Figure S3c and Data S4). Out of 3,832 commonly DEGs, annotation for 2,715 genes was found in TAIR using KOBAS v1.2 (Data S4). The GO analysis of these 2,715 genes using AgriGO (Tian et al., 2017) revealed a significant enrichment of genes involved in phenylpropanoid metabolic process, cell wall biogenesis, mitotic cell cycle, cell division, secondary metabolic process, microtubule motor activity, and polymer cytoskeleton fiber synthesis (Figure 2d and Figure S5a–c and Table S4). GO analysis indicated various biological processes accentuating in prickle morphogenesis, including secondary metabolites biosynthesis, cell division and enlargement, cell wall biogenesis, and hardening process. Besides, genes involved in response to stress stimuli were also differentially expressed, suggesting a role of prickles in stress management. As observed in GO analysis, pathway enrichment analysis using KEGG also showed overrepresentation of the genes encoding enzymes involved in phenylpropanoid biosynthetic pathway, plant-pathogen interactions, glutathione metabolism, cell cycle, and hormone signal transduction (Table S5). Besides, the KEGG-orthology analysis revealed enrichment of long-chain acyl-CoA synthetases, MYB-transcription factors, and Laccases (Table S6).

To gain further insights, the common DEGs were analyzed to dissect the genes showing gradual upregulation and downregulation with prickle morphogenesis. A set of 76 genes showing a gradual

| Table 2 | Differential gene expression in threeprickle morphotypes, IP, SP, and HP |
|---------|---------------------------------------------------------------------|
| Comparison | Upregulated | Downregulated |
| (a) Total DEGs in three stages of prickle development. | | |
| A (HP versus SP) | 7,841 | 8,477 |
| B (HP versus IP) | 5,326 | 6,181 |
| C (SP versus IP) | 6,318 | 7,014 |
| (b) Common DEGs in three stages of prickle development. | | |
| A (HP versus SP) | 1,884 | 1,948 |
| B (HP versus IP) | 1,341 | 2,491 |
| C (SP versus IP) | 1,271 | 2,561 |

*Fold change ≥ 1.5 for upregulation and ≤ 1.5 for downregulation. FDR and p-value cut-off ≤ .05.

**Figure 3** Genes related to phenylpropanoid biosynthesis and the developmental processes were differentially expressed during prickle morphogenesis. (a and b) Heatmap showing the expression pattern of upregulated (a) and downregulated (b) genes with prickle morphogenesis. A total of 77 genes were upregulated, and 28 genes were downregulated gradually with prickle development. Z score for both (a) and (b) is shown below (b). (c) Relative expression levels of selected genes involved in phenylpropanoid biosynthesis were analyzed by RT-qPCR. UBC was used as an internal control. Values represent the mean ± SD of three independent biological replicates. Lowercase letters above each bar indicate statistically significant differences between mean values at each genotype (p < .05, one-way ANOVA with Tukey’s post hoc HSD test).
increase and 28 genes exhibiting a gradual decline were obtained during transition from IP to HP (Figure 3a,b). In silico functional analysis of genes showing gradual upregulation revealed enrichment of genes involved in phenylpropanoid biosynthesis, especially those involved in lignin and flavonoid biosynthesis (Figure 3a,b; Figures S6a and S7). Expression of selected genes of the flavonoid biosynthesis pathway, namely, PHENYLALANINE AMMONIA-LYASE 1 (PAL1), CINNAMATE 4-HYDROXYLASE (C4H), HYDROXY CINNAMOYL-CoA SHIKIMATE/QUINATE HYDROXYCINNAMOYL TRANSFERASE (HCT), O-METHYL TRANSFERASE 1 (OMT1), CHALCONE SYNTHASE (CHS/TT4), FLAVONOL SYNTHASE 1 (FLS1), CHALCONE ISOMERASE LIKE (CHIL), and ALIPHATIC SUBERIN FERULOYL TRANSFERASE (ASFT/RWP1), were validated using reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Data showed a positive correlation between expression observed in the RNA-seq and the RT-qPCR data (Figure 3c). Elevated expression of flavonoid and lignin biosynthesis genes in SP and HP suggested their cognate accumulation during later stages of prickle development. It also appears that initially, lignin deposition in the prickles was slow, as evident from histological and chemical data (Figure 1b–g), and the relative expression of cognate genes in IP and SP (Figure 3a,c); this results in a soft and hollow structure in the early stages. During maturation, accumulated phenolics act as precursors for lignin polymerization leading to hardening of the prickles. The increased expression of TT4, involved in the synthesis of chalcone scaffold that acts as the backbone for all kinds of flavonoids, suggests the accumulation of flavonoids during the maturation. Similarly, upregulation of RWP1 expression in HP perhaps activates pathways that resulted in formation of pectin polymers required for the organization and biogenesis of primary and secondary cell walls. Collectively, higher expression of phenylpropanoids biosynthetic genes could lead to a higher accumulation of lignin and flavonoids in HP.

In case of genes gradually downregulating with prickle morphogenesis, the functional ontologies, including response to stimulus, response to stress, and developmental processes, were overrepresented (Figure S6b). Among these genes, a set of developmental genes were quite interesting in terms of their potential role in prickle initiation. These developmental genes include TRANSPARENT TESTA GLABRA 1 (TTG1), ABL INTERACTOR-LIKE PROTEIN 3 (ABIL3), AGAMOUS-LIKE MADS-BOX PROTEIN (AGL12), BRASSINOSTEROID INSENSITIVE 1 (BRI1), RECEPTOR-LIKE PROTEIN KINASE 7 (RLK7), MYB DOMAIN PROTEIN 62 (MYB62), ARGinine DECARboxYLAse 2 (ADC2), CYTOCHROME P450, FAMILY 78, SUBFAMILY A, POLYPEPTIDE 6 (CYP78A6), DELTA 1-PYRROLINE-5-CARboxYLATE SYNTHASE 2 (P5C2), and POLYOL/MONOSACCHARIDE TRANSPORTER 5 (PMT5; Figure 3b).

Many of these genes seem to have a role during the transition phase, where one of the epidermal cells differentiates to form a prickle. Among these genes, TTG1 encodes a WD40 repeat protein and a core member of the MBW activator complex, which activates trichome initiation and patterning (Zhao et al., 2008). Similarly, ABIL3 is also associated with cell differentiation and microtubule-mediated elongation during root development and trichome morphogenesis (Jorgens et al., 2010). The AGL12 acts as a transcription activator that regulates root development by controlling cell proliferation and cellular differentiation in the root meristem (Tapia-Lopez et al., 2008); it might have a role in the activation of the epidermal cell differentiation for prickle initiation. BRI1 is a brassinosteroid receptor that regulates a signaling cascade involved in plant development, including expression of light- and stress-regulated genes, promotion of cell elongation, leaf senescence, and flowering (Li & Chory, 1997).

Higher expression of BRI1 suggests the possible involvement of brassinosteroids in the transition phase and initial development of prickles. Likewise, the MYB62 is a transcription factor that determines root architecture and secondary roots development through sensing phosphate by repressing gibberellin acid (GA) biosynthesis and signaling (Devaiah et al., 2009). According to its known function, it is very likely that MYB62 regulates phosphate and GA responses to promote cell differentiation and elongation during prickle initiation. Other genes, ADC2, RLK7, P5C2, CYP78A6, and PMT5, probably play roles in oxidative stress tolerance and cell wall remodeling during the initial phase of prickle morphogenesis.

### 3.5 Targeted metabolic profiling revealed the accumulation of specific flavonoids during prickle development

Transcriptome analysis corroborated the accumulation of phenolic metabolites, especially flavonoids, in addition to lignin, during the prickle morphogenesis. This result prompted us to identify and quantify the cognate flavonoids in the prickles. Metaboloite analysis confirmed the accumulation of catechin and revealed the presence of other flavonoids, mainly glycosidic derivatives of quercetin, namely, rutin, hyperoside, and isoquercetin, in rose prickles (Figure 4a and Table 3). HP accumulated twofold and sixfold higher catechin (64.6 ± 4.5 µg/mg of extract) as compared to SP and IP, respectively (Figure 4a). Isoquercetin and rutin were found to be the highest in IP, followed by in HP and SP of rose. On the contrary, the hyperoside was present only in SP and HP, whereas it was absent in IP. The varying levels of flavonoids suggest their possible roles during different stages of prickle development.

Metabolite profiling identified and quantified the specific flavonoids accumulated in prickles during development. Their tissue-specific localization was further assessed using microscopic analysis. NA, a chemical that makes the flavonoids to fluoresce explicitly, was used to visualize the flavonoids in the HP. Accordingly, the transverse and the longitudinal sections of HP were prepared and stained with NA. The resulting flavonoid fluorescence was visualized under the confocal microscope (Figure 4bi–viii). NA staining of the flavonoids revealed that almost every cell in the epidermis of HP accumulated flavonoids in an organized manner (Figure 4bii–iv,vi–viii). The longitudinal sections showed that the flavonoids are distributed uniformly in the secondary cell wall and intercellular spaces (Figure 4biv,vii). In addition, the lining of epidermal cells in the mature prickles showed a high accumulation of flavonoids. Surprisingly, the
A similar accumulation and patterning of flavonoids were observed in IP and SP also (Figure S8a).

Although flavonoids are mainly implicated in protection against stress conditions (Biasutto & Zoratti, 2014; Liang et al., 2016; Yamaji & Ichihara, 2012), these are also crucial for developmental processes, including nodulation and pollen fertility. Therefore, flavonoid biosynthetic genes exhibit an increased expression upon exposure to stresses as well as upon sensing developmental cues. Higher expression of genes associated with flavonoid biosynthetic vis-à-vis selective accumulation of flavonoids in the prickles suggests their role in the development of prickles, in addition to defense.

3.6 | Expression of transcription factors network supported the similarities between prickle- and trichome development

Expression of various TFs was analyzed in the RNAseq data to gain insights into the flavonoid accumulation and prickle development. The resulting analysis revealed differential expression of a subset of 42 TFs, dominated mainly by the MYBs (Figure 5a). Many of these TFs, including MYBs, are known to be involved in the regulation of metabolites biosynthesis, including phenolics, and these are implicated in the regulation of trichome initiation and development (Fridman et al., 2005; Gershenzon & Dudareva, 2007; Romani et al., 2000; Weinhold & Baldwin, 2011; Xie et al., 2008). Interestingly, genes constituting the MBW activation–inhibition complex were also falling in this subset (Figure 5a).

Global transcriptome analysis indicated the highest expression of the above set of transcriptional network encoding genes at initial development stage, indicating their functional role in prickle initiation and morphogenesis. If indeed these genes are involved, one would expect their basal or no expression in the bark tissue adjacent to initiating prickles. Therefore, the expression of these genes was analyzed by RT-qPCR in IP, SP, and HP as compared to the bark tissue close to the initiating prickles. The expression of MBW activation–inhibition complex genes was significantly higher in prickles than in the bark tissue (Figure 5b). Compared to bark tissue having no outgrowth, the central components of MBW complex, including GL1, GL3, and TTG1, and the downstream TTG2 were highly expressed in the IP (Figure 5b). Besides, MYB23, which functions redundantly to GL1 during trichome initiation, was also upregulated in IP. Likewise, other MYBs, including MYB42, MYB61, MYB75, MYB82, and MYB85, involved in accumulation of phenylpropanoids and other secondary metabolites, also showed a higher expression at IP and were reduced significantly after that (Figure 5a,b). Interestingly, MYB123 (TT2) and bHLH (TT8) involved in phenylpropanoid biosynthesis exhibited a gradual increase until SP (Figure 5a,b), suggesting their involvement in the initiation of the hardening and lignification process. Similarly, MYB43, involved in secondary cell wall biogenesis and lignin biosynthesis, was significantly upregulated in HP (Figure 5a,b), corroborating the lignification and hardening of prickles during maturation.

Besides R2R3 MYBs, expression of R3 MYBs, including TRY, TCL1, and CPC, was also upregulated in IP (Figure 5a,b). Expression of these R3 MYBs is controlled by MBW activator complex, after which these proteins move to adjacent cells (Kurata et al., 2005;
Tominaga-Wada & Wada, 2018). In the adjacent cells, TRY competes with GL1 to constitute MBW inhibitor complex, whereas TCL1 and CPC directly bind to the promoters of GL1 and GL2, respectively, to repress their expression, thereby promoting the formation of inhibitor complex and inhibiting prickle initiation (Kurata et al., 2005; Lee & Schiefelbein, 2002; Wang et al., 2007). Higher expression of these R3 MYBs in IP suggests that these proteins move from differentiating prickle forming cells to the adjacent cells, thereby repressing any outgrowth.

GA, JA, and cytokinins are implicated in cell differentiation regulation, and it is shown that these hormones regulate the expression of some of the key MBW genes by triggering or repressing the expression of transcription activators and inhibitors, respectively. In this context, MYC1, a JA-responsive bHLH protein that together with GL1 induces the expression of flavonoid pathway genes (Hichri et al., 2010), was also upregulated in IP (Figure 5a,b). Similarly, GLABROUS INFLORESCENCE STEMS 2 (GIS2), encoding a cytokinin-induced C2H2 transcription factor that induces the expression of GL1, showed a higher expression at IP as compared to the bark tissue (Figure 5a,b). Uregulation of hormones-responsive TFs indicated the role of these hormones in regulating the expression of MBW complex and in prickle morphogenesis.

A recent study analyzed the prickle development transcriptome comparing prickly and prickle-less Solanum viarum Dunal (Pandey et al., 2018). This study also showed the upregulation of genes encoding enzymes involved in secondary metabolites biosynthesis and development-related transcription factors in prickles. Comparative analysis suggested that coupling of secondary metabolites accumulation with the epidermal outgrowth is a general phenomenon which is conserved across the species.

**TABLE 3** UPLC-PDA-based quantification of phenylpropanoids identified in prickle morphotypes and bark tissue of rose (represented in µg/mg of MeOH extract, ND-not detected)

| Flavonoids                        | MW      | RT (min) | Structure | UPLC-based estimation (µg/mg extract) |
|-----------------------------------|---------|----------|-----------|--------------------------------------|
| Catechin                          | 290.26  | 5.61     |           |                                      |
| Rutin (quercetin-3-rutinoside)    | 610.52  | 9.3      |           | 0.3 ± 0.05                           |
| Hyperoside (quercetin-3-O-galactoside) | 464.38  | 9.61     |           | ND                                   |
| Isoquercetin (quercetin-3-O-monoglucoside) | 464.05  | 9.77     |           | 1.03 ± 0.19                          |

Abbreviations: MW, molecular weight; RT, retention time.

### 3.7 Expression pattern of MBW activator/inhibitor complex and associated genes in prickle-less mutant reinforced that prickles evolve from trichomes

The increased expression of MBW complex during early development stages suggested its role in prickle initiation and development; however, this evidence was not enough. To further corroborate this result, a prickle-less cultivar of *R. hybrida*, namely, HW, was investigated (Figure 6a). The expression of the MBW activator/inhibitor complex and associated genes was analyzed using RT-qPCR (Figure 6b). For this analysis, bark tissue with initial prickles close to pedicel in prickle-bearing plants and the bark tissue from the same region in prickle-less plants were used (Figure 6a). Data showed that the MBW activator complex and associated protein-encoding genes were upregulated in prickle-bearing plants, whereas these remained unaffected in the prickle-less mutant (Figure 6b).

Similarly, the R3 MYB TRY also showed upregulation in prickly-tissue (Figure 6b). On the contrary, TCL1 and CPC were upregulated by almost 7-fold and 30-fold, respectively, in prickle-less plants (Figure 6b). Since TRY moves from differentiating tissue to adjacent cells, its higher expression in prickly plants is justified (Figure 6b). The CPC and TCL1 expressed distinctively (Figure 6b), possibly because these genes can express independent of MBW complex (Yu et al., 2010). The SQUAMOSA-PROMOTER BINDING PROTEIN-LIKE (SPL) can directly bind to the promoters of TCL1 and CPC to derive their MBW-independent expression (Yu et al., 2010). Higher TCL1 and CPC expression suggested that some other factors, such as SPL, induce their expression to repress the epidermal cell differentiation in prickle-less plants. Nonetheless, as indicated by the...
expression of transcription factors implicated in promoting or inhibiting trichome initiation and patterning, these are likely involved in prickle morphogenesis.

Similar lignification patterns in the bark tissue of prickly and prickle-less plants (Figure S8b) indicated that the epidermal cells were similar in these two types of plants. However, upon instigation, perhaps by hormones, the expression of MBW complex was induced, which promoted formation of outgrowth in prickly plants. On the other hand, epidermal cells of prickle-less plant remained undifferentiated. This could be due to either insensitiveness of epidermal cells to stimulus or that the MBW activator complex genes underwent epigenetic modifications, which silenced their expression. Further studies would unveil the reasons behind this phenomenon.

**Discussion**

The present study was aimed to understand morphological, histological as well as molecular changes occurring during the prickle morphogenesis in a domesticated cultivar of rose, *R. hybrida* cv. "FR," to deduce insights into the biological relevance of prickles and towards engineering prickle-less roses. Based on the morphological features, three distinct development stages IP, SP, and HP, were identified (Figure 1a–e) and investigated. Histological analysis showed that the prickles emerged as an outgrowth from the epidermis or cortical tissue (Figure 1c,d), similar to trichomes (Larkin et al., 1996). Some specific epidermal cells on the stem or leaf primordia, perhaps, underwent differentiation. These cells probably underwent active division followed by few rounds of endoreduplication (as suggested by
GO analysis of genes upregulated in SP versus IP, Figure S4f), leading to their expansion and protrusion out of the surface, thereby forming prickles (Figure 1d). During morphogenesis, the prickles accumulated secondary metabolites, especially flavonoids, and underwent lignification (Figure 1e–g).

Being epidermal outgrowths, prickles seem to resemble trichomes. Similar to trichomes, prickles showed a patterning and distribution of phenolics in the secondary cell wall and intercellular spaces (Figures 1c,d and 4bv–viii). These secondary metabolites underline the hardening of the prickles and implicate prickles in the defense against abiotic factors, insects, and pests. Accumulation of secondary metabolites such as catechin and quercetin indicates the involvement of prickles in protection against abiotic stresses as well (Liang et al., 2016). Besides protection against stress, these secondary metabolites are also crucial for the developmental processes (Janisch et al., 2006). Selective accumulation of specific flavonoids such as rutin and isoquercetin during the early stages of prickles morphogenesis (Figure 4b and Table 3) suggests their role in the initial transition phase, perhaps as antioxidants, aiding in the differentiation of epidermal cells to outgrow into prickles.

The MBW activator–inhibitor complex regulates the formation of trichomes, wherein a competition between the R2R3 type and the R3 type of MYB TFs determines the fate of the epidermal cells to form trichomes (Fambrini & Pugliesi, 2019; Payne et al., 2000; Pesch et al., 2015; Walker et al., 1999; Zhao et al., 2008). Many of these genes expressed differentially during prickle morphogenesis, where a significant upregulation of the activator complex was observed at the initial stages (Figure 5a,b). Besides, many genes required for cell differentiation and elongation during root and trichome development also showed a higher expression in the early phase. The increased expression of genes induced by the hormones mentioned above (Figure 5a,b), which directly regulate MBW complex, indicated that these hormones govern the prickle morphogenesis. Furthermore, the expression of MBW activator–inhibitor complex in prickly and prickle-less plants (Figure 6a,b) corroborated the resemblance of cell fate determination in trichomes and prickles.

A recent genetic study in R. chinensis “OB” also tested the involvement of MBW transcriptional complex in prickle development (Zhou et al., 2020). Many of the TFs constituting MBW complex were found to lie in the regions on chromosomes LG3, LG4, and LG5, which were suggested as the quantitative trait loci (QTLs) regulating...
In trichomes, the fate of the epidermal cells to undergo differentiation and accumulate secondary metabolites or to keep on dividing, is determined by phytohormones, especially GA, cytokinins, and JA, by regulating the expression of MBW complex (Fambrini & Pugliesi, 2019). Similarly, prickle initiation, patterning, and morphogenesis are also seem to be governed by these hormones (Figures 5, 6b and 7). Collectively, the results supported the concept that prickles are evolved from trichomes, and signified the importance of MBW complex and other development-related genes in prickles initiation and morphogenesis.

Although the initiation of trichomes and prickles seems similar, their later development probably is different. Prickles do not undergo branching (Figure 1b–d) and have more lignin deposition leading to their hardening, whereas trichomes undergo branching and are comparatively much softer. In addition, trichomes are generally a single cell structure that undergoes enlargement via endoreduplication, whereas prickles are multicellular, which seems to undergo both cell enlargement as well as cell division (Figures 1c–d and 7). Hormonal balance probably regulates these attributes and governs the formation of thick-walled and lignified pointed prickles.

Based on the findings of this study, it seems, differentiation of epidermal cells in the bark of rose is determined by MYB proteins, where R2R3 MYBs GL1 and MYB23 along with GL3 or EGL3 and TTG1 constitute a MBW activator complex to promote the expression of GL2 and TTG2, which trigger the differentiation of epidermal cells forming outgrowth (Figure 7). The same transcriptional complex would induce the expression of R3 MYBs such as TRY, CPC, and TCL1, protein of which move to the adjacent cells and prevent the formation of MBW activator complex and differentiation in these cells (Figure 7).

Collectively, data suggested that the prickles, sharp-pointed structures, are protective, non-vascular outgrowths of the epidermis resembling trichomes. The lignification and secondary metabolite accumulation seem to provide a physical barrier from herbivory and protect plants from environmental stresses. The prickles morphogenesis-transcriptome data and its validation in prickles-less cultivar supported resemblance of prickles with trichomes and provided molecular insights towards prickles initiation. The validation of genes identified to be involved in prickles morphogenesis using genetic and biochemical tools is necessary to corroborate the findings of this study. In addition, further analyses at developmental stages would help to decipher the molecular basis of how and when the prickles acquire distinctions compared to trichomes.

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CONFLICT OF INTEREST
The authors have declared no conflict of interest.
AUTHOR CONTRIBUTIONS
MKS performed experiments, analyzed transcriptome, and wrote the initial draft. PK performed metabolite profiling. VD designed experiments, analyzed data, and wrote the manuscript. SK conceptualized, designed and supervised the study and finalized the manuscript.

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**SUPPORTING INFORMATION** Additional Supporting Information may be found online in the Supporting Information section.

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