Non-glandular prickle formation is associated with development and secondary metabolism-related genes in *Rosa multiflora*

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Introduction

Roses are among the most economically important ornamental plants worldwide. But prickles on the stem and leaves cause difficulties during cultivation, harvest and transportation, thus are an undesirable horticultural character. However, little is known about the molecular mechanisms of prickle development. Therefore, we develop *Rosa multiflora* as a model plant to study prickle formation. Our study provides novel insights into the molecular network underlying the regulation of prickle morphogenesis in *R. multiflora*, and the identified candidates might be applied to the genetic improvement of roses.

Results

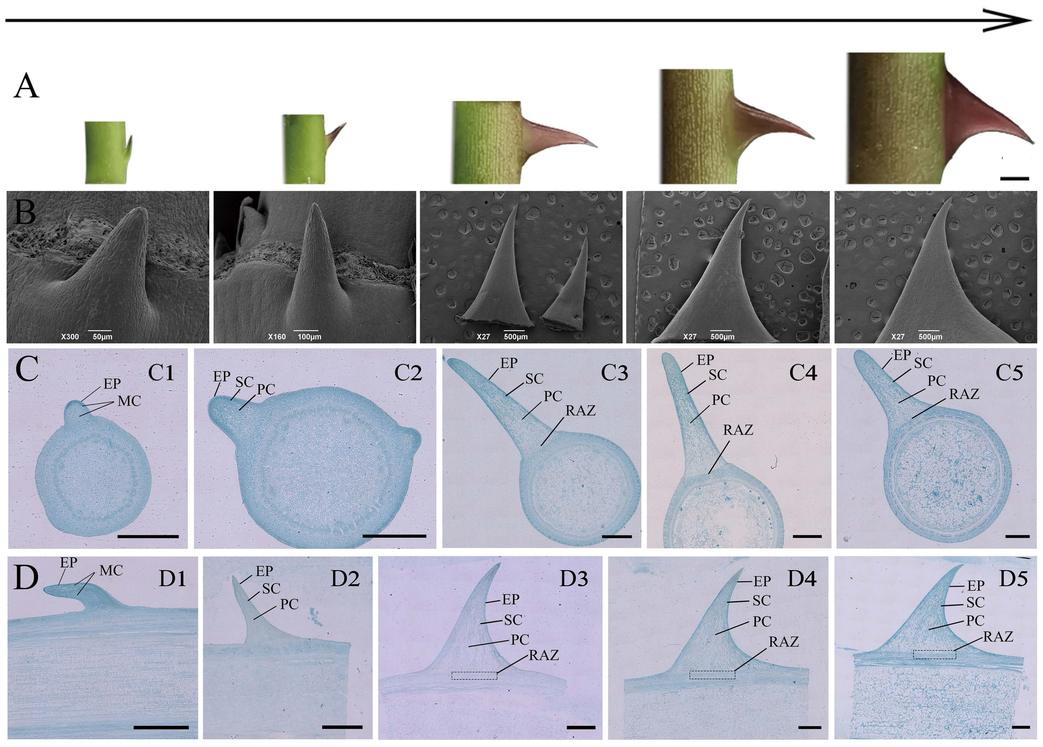

Fig. 1 Structure of prickles from different internodes on developing stems of *R. multiflora*. (A) Light micrographs. (B) Scanning electron micrographs. (C)Transverse section. (D) Longitudinal section. Scale bars = 1 mm. EP, Epidermis; MC, Meristematic Cell; PC, Parenchyma Cell; RAZ, Resembling Abscission Zone; SC, Sclerenchyma Cell. Black long arrow indicates developmental progression of the prickle.

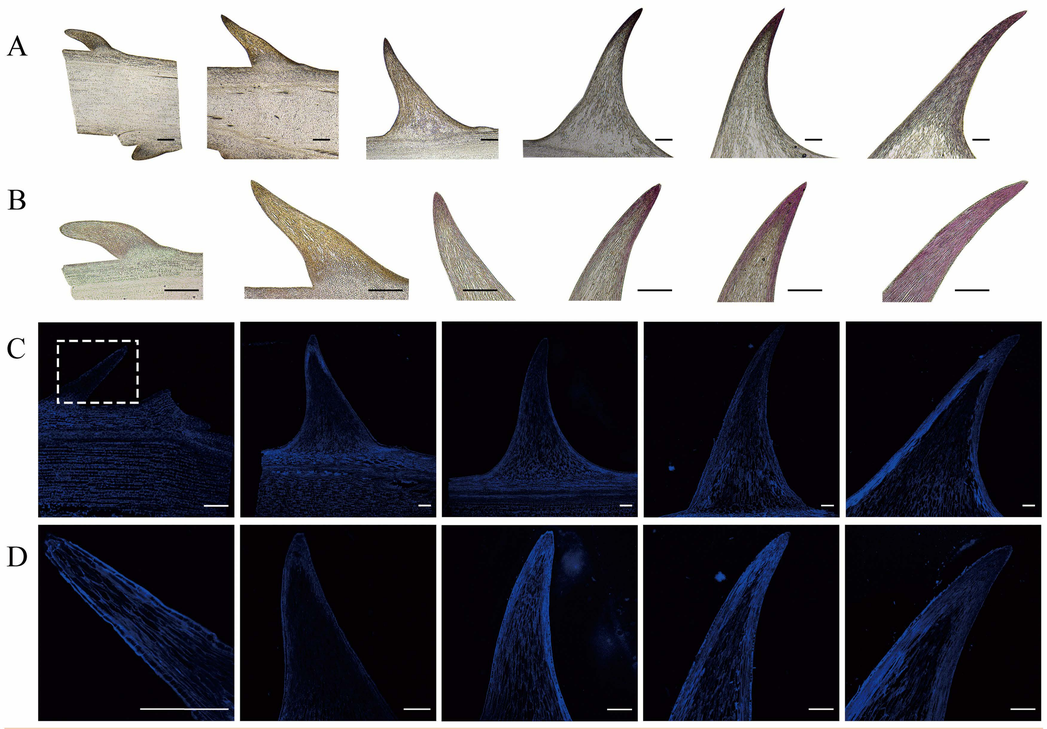

Fig. 2 Histochemical localization of prickles from different internodes on a developing stem of *R. multiflora*. (A-B) Lignin deposition of the whole prickle and the enlarged prickle tip. Scale bars = 200 μm. (C-D) Cellulose deposition of the whole prickle and the enlarged prickle tip. Scale bars = 500 μm, Scale bars = 200 μm, respectively.

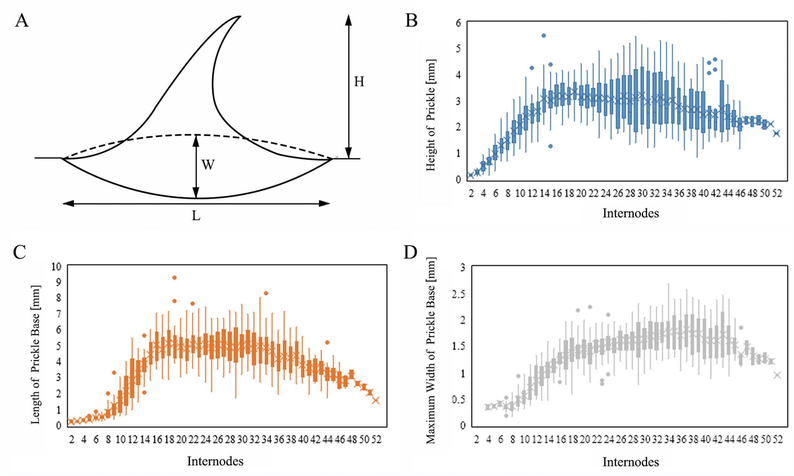

Fig. 3 Analysis of prickle morphology. (A) Diagram of prickle geometry where: H, Height; L, Length of prickle base; w, maximum width of prickle base. (B-D) Morphological indices of prickle height, length, and maximum width of prickle base.

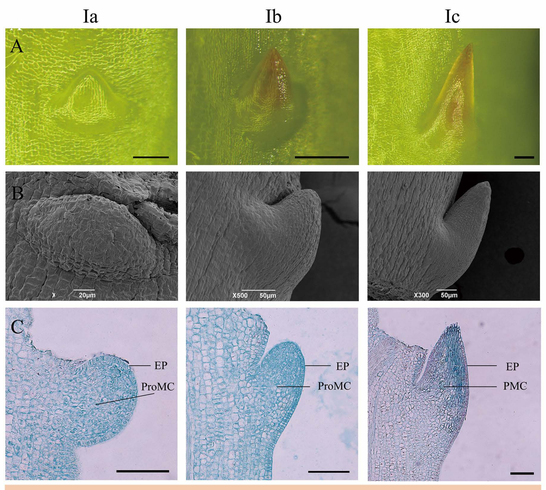

Fig. 4 Prickle primordium observation in the early (Ia), intermediate (Ib), and late stages (Ic). (A) Light micrographs. (B) Scanning electron micrographs. (C) Micrographs of paraffin section. Scale bars = 100 μm. EP, Epidermis; PMC, Primary Meristem. Cell; ProMC, Promeristem Cell.

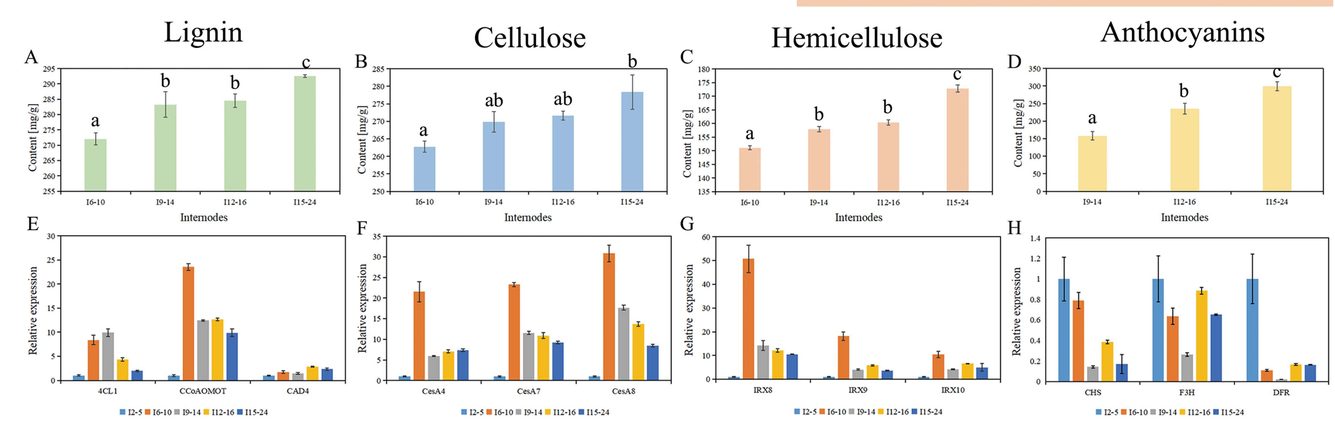

Fig. 5 Chemical content determination and biosynthesis-related gene expression analysis of prickles from different internodes on developing stem of *R. multiflora*. I2-5, I6-10, I9-14, I12-16, I15-24 means prickles of internode 2-5, internode 6-10, internode 9-14, internode 12-16, internode 15-24.

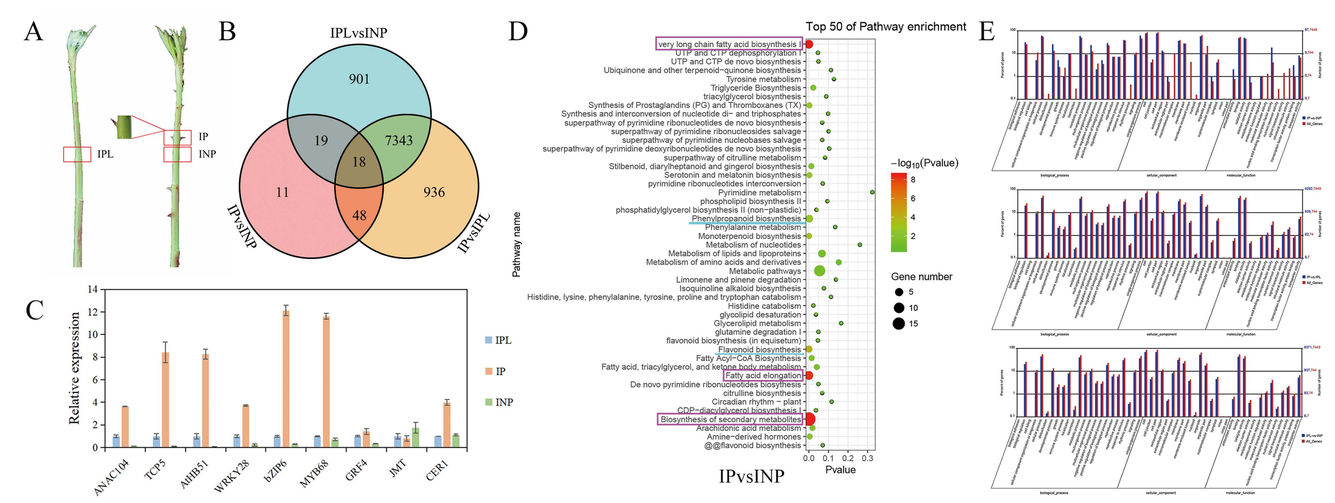

Fig. 6 Comparative transcriptome analysis of prickly *R. multiflora* plant and its prickleless plant. (A) Sampling schematic of RNA-seq. (B) Venn diagram of commonly and uniquely expressed DEGs. (C)Validation of transcriptomic sequencing data by qRT-PCR. (D) KEGG pathway enrichment analysis. (E) WEGO analysis of DEGs. IP, internode segment with a prickle at the stem top of a *R. multiflora* plant; INP, non-prickle internode segment at the stem top of a *R. multiflora* plant; IPL, internode segment at the stem top of a *R. multiflora* 'inermis' No.1 prickleless plant.

Conclusions

R. multiflora prickles were identified as non-glandular ones, and their developmental process were divided into five stages (initiation, elongation, transition, deposition, and maturation), which was accompanied by the accumulation of secondary metabolites such as lignin, cellulose and anthocyanins. Comparative transcriptome analysis identified key pathways as well as development and secondary metabolism -related genes potentially involved in prickle formation.

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