ARTICLE ADDENDUM

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Hormone-mediated promotion of trichome initiation in plants is conserved but utilizes species- and trichome-specific regulatory mechanisms

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Plant trichome initiation is steered by diverse developmental and environmental cues, through molecular mechanisms that remain elusive in most plant species. Using a robust experimental method to investigate the molecular mechanisms by which phytohormones modulate leaf trichome formation, we verified the effect of jasmonates, cytokinins and gibberellins in Arabidopsis (*Arabidopsis thaliana*). All three phytohormones promoted Arabidopsis trichome initiation, but caused divergent effects on trichome maturation and other leaf parameters. Molecular analysis indicated that the phytohormones mediated trichome initiation by the transcriptional regulation of the components of the TRANSPARENT TESTA GLABRA1 (TTG1) activator/inhibitor complex. In this addendum, we additionally studied the effects of jasmonates, cytokinins and gibberellins on leaf trichome formation in a representative set of plant species, spanning the angiosperm lineage and covering different trichome types. We found that the general ability of the three phytohormones to impinge on trichome initiation is conserved across angiosperms, but that within a particular plant species distinct regulatory networks might be activated to steer the formation of the various trichome types.

Plants have evolved an incredible diversity of epidermal protuberances, ranging from simple unicellular protrusions to specialized multicellular trichomes that protect them from biotic and abiotic stresses. The molecular mechanisms that determine trichome formation have been studied extensively in Arabidopsis, in which trichome patterns have been shown to be generated by the action of an activator-inhibitor complex. Within this complex, R2R3-type MYB proteins bind to bHLH proteins.[1-4] The bHLH proteins, in turn, interact with the *TTG1*-encoded WD40 protein,[5] to form the WD40-bHLH-MYB regulatory complex that can stimulate protodermal cells to differentiate and develop into trichomes.[1,6,7] R1-type MYB proteins, lacking a transcription activation domain, can compete with the R2R3-type MYB proteins for binding with the bHLH proteins and inhibit trichome formation in the cells surrounding the trichome initial cell.[6,7] The development of the diverse types of trichomes found in other plant species might be regulated by still unidentified functional orthologs of the Arabidopsis genes or might involve distinct protein complexes. Phylogenetic analysis of the R2R3-MYB transcription factor family[8] together with some experimental findings in transgenic plants overexpressing heterologous R2R3-MYB or bHLH factors,[8,9] suggested that trichomes in Arabidopsis, and possibly other Rosids, develop through a transcriptional regulatory network that differs from that regulating trichome formation in Asterids.

Phytohormones are known to modulate epidermal differentiation programs, as demonstrated in Arabidopsis[4,10-13] and tomato (*Solanum lycopersicon*).[14-16] Hence, the existence of distinct regulatory

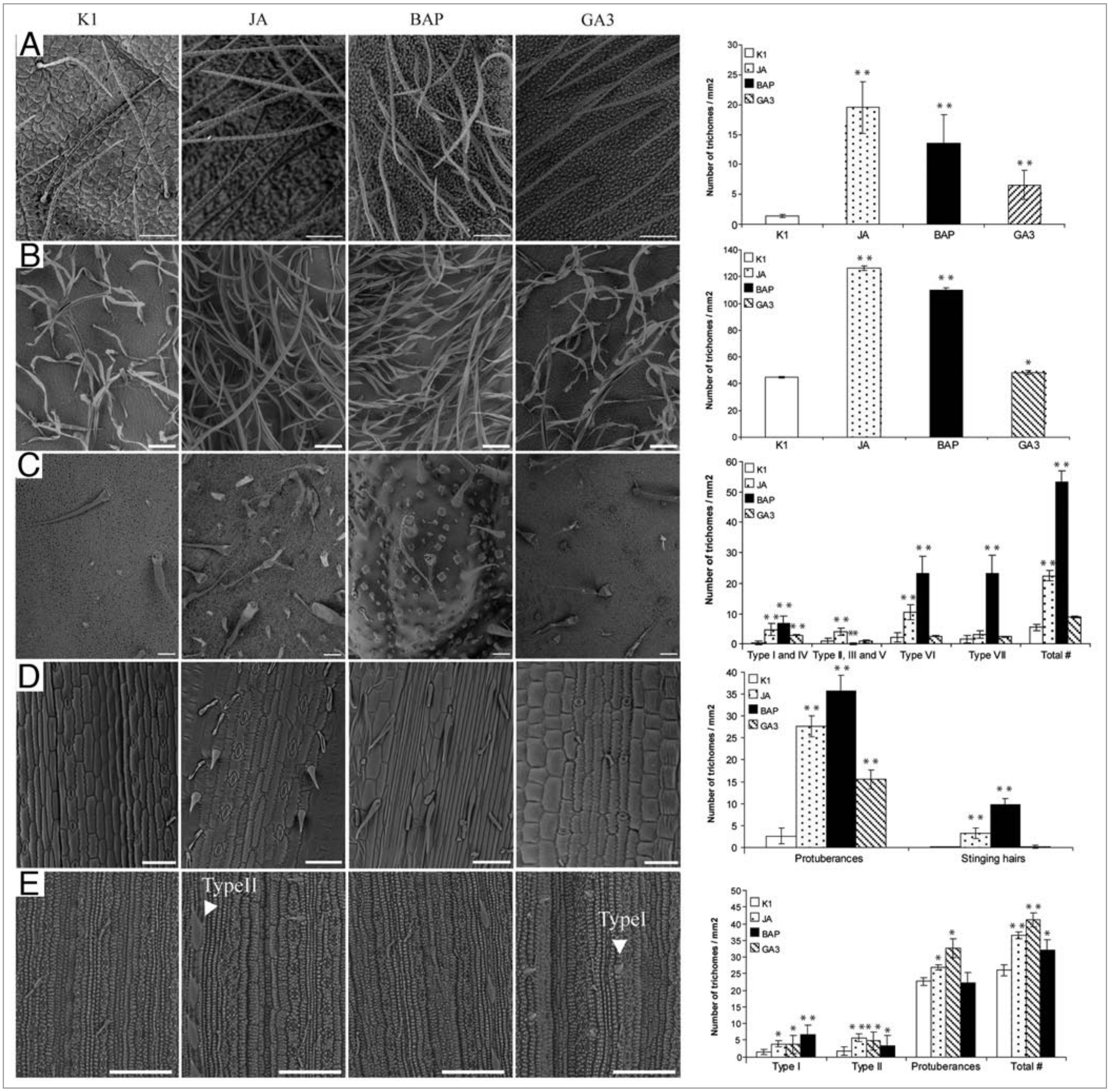

Figure 1. Phytohormonal effects on glandular and filamentous trichome formation. Scanning electron microscope (SEM) images of elicited leaves (left) and graphic overviews of the number of trichomes (right) on leaves of *M. truncatula* (A), poplar (B), tomato (C), maize (D) and rice (E). Individual seedlings were grown in separate containers and mock-treated (K1) or induced with JA (5 μM), BAP (1 μM) or GA_3 (1 μM) for 3 to 4 weeks after the first four leaves had been formed. Detached leaves were mounted on SEM stubs and examined with a Tabletop TM-1000 (Hitachi High Technologies America, Tokyo, Japan). The total trichome number per leaf lamina was counted with Photoshop7 on digitized images of scans of the full abaxial leaf surface. Scale bar = 100 μm. Error bars represent the standard error (n = 6 for A–C and n = 4 for D–E). Statistical significance was determined by Student t test (**$p < 0.01$, *$p < 0.05$).

networks might also be reflected by the conservation of the perception of trichome regulatory cues. Therefore, we designed a robust experimental method[4] to evaluate whether the capability of phytohormones to modulate epidermal differentiation programs was conserved across the plant kingdom and across different trichome types. In particular, the effects were studied of cytokinin (6-benzylaminopurine [BAP]), gibberellin (GA_3) and jasmonic acid (JA) on trichome formation, first in the Rosid Arabidopsis,[4] and subsequently in three other dicots, the Rosids *Medicago truncatula* and poplar (*Populus trichocarpa*) and the Asterid tomato, and two monocots, rice (*Oryza sativa*) and maize (*Zea mays*) (**Fig. 1**).

In Arabidopsis, which develops trichomes of the unicellular, filamentous type only, all three phytohormones provoked a seemingly common effect on cell patterning by stimulating trichome initiation, resulting in increased trichome numbers and densities, but nonetheless caused distinct effects on trichome (ultra)structure and maturation, and leaf pavement cell formation and anthocyanin biosynthesis, for instance.[4] The phytohormonal control of trichome promotion in Arabidopsis was mediated, at least in part, by transcriptional regulation of the established TTG1 complex and depended on the R2R3-MYB factor GLABRA1.[4] Furthermore, profound functional specialization of the bHLH factors and a resultant differential molecular regulation of trichome initiation on leaf lamina and leaf margins were exposed. Trichome formation on leaf lamina relied entirely on the bHLH factors GLABRA3 and ENHANCER OF GLABRA3, whereas the close homolog TRANSPARENT TESTA8 was essential for marginal trichome development.[4]

Contrary to Arabidopsis, the Rosids *M. truncatula* and poplar develop trichomes of the multicellular, filamentous type only. Nonetheless, for the latter two species, a trichome elicitation pattern was observed comparable to that of Arabidopsis; all three hormones also increased the number of trichomes per leaf (**Fig. 1A and B**); pointing toward conserved regulatory networks across the Rosid lineage that respond similarly to hormonal cues.

The Asterid tomato is an excellent example of a plant species that produces several types of trichomes, which are categorized as types I to VII.[17] The formation of glandular trichomes with a unicellular head (types I and IV) was promoted by all phytohormones (**Fig. 1C**). Promotion of the different multicellular head-type glands differed: type VI was stimulated by JA and BAP, but not by GA_3, and type VII by BAP only (**Fig. 1C**). The number of filamentous trichomes (types II, III and V) increased only a little after treatment with JA and not by BAP or GA_3 (**Fig. 1C**). Hence, in tomato, each hormone was capable of stimulating trichome initiation, with distinct effects on the different types, but none promoted all types concomitantly.

Also in monocots, different response patterns were observed for the various trichome types. Both in maize and rice, two main types of trichomes can be distinguished, the simple protuberances and stinging hairs (**Fig. 1D and E**). In maize, emergence of protuberances increased with all phytohormones, but stinging hair formation only with JA and BAP (**Fig. 1D**). Conversely, only JA and BAP enhanced protuberance formation in rice, but all three phytohormones influenced the two stinging hair types (**Fig. 1E**). Hence, also in monocots, all phytohormones were capable of promoting trichome initiation and, as in the Asterid lineage, with distinct and species-bound specificities for the different types.

In conclusion, we have demonstrated that the phytohormones BAP, GA_3 and JA can impinge on the developmental programs that determine epidermal differentiation in plant species spanning the angiosperm lineage. This finding points toward the existence of conserved signaling networks across this lineage that respond similarly to developmental and environmental cues. Since we have shown that in Arabidopsis the phytohormonal control of trichome formation depends on and is mediated by the transcriptional regulation of the WD40-bHLH-MYB complex,[4] the presented study provides the rationale for an alternative, transcriptomics-based strategy to discover trichome regulatory genes in plant species in which mutant screens are not as straightforward as in Arabidopsis. Within such a gene-discovery program, the phytohormonal trichome promotion patterns can be key to unravel the genetic control of trichome formation.

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