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Genetic control of glandular trichome development

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Abstract:

Plant glandular trichomes are epidermal secretory structures producing various specialized metabolites. These metabolites are involved in plant adaptation to its environment and many of them have remarkable properties exploited by fragrance, flavor and pharmaceutical industries. The identification of genes controlling glandular trichome development is of high interest to understand how plants produce specialized metabolites. Our knowledge about this developmental process is still limited, but genes controlling glandular trichome initiation and morphogenesis have recently been identified. In particular, R2R3-MYB and HD-ZIP IV transcription factors appear to play essential roles in glandular trichome initiation in *Artemisia annua* and tomato. In this review, we focus on the results obtained in these two species and we propose genetic regulation models integrating these data.

Glandular trichome density and agronomic performance

Scents, pigments, medicines… Life would be bleaker and more difficult for humans if plants were not producing such a wide variety of compounds. These chemicals are critical for the capacity of plants to adapt to their environment and to overcome the various challenges they are facing every day, like pollinator attraction or defense against pathogen attacks. Many of these compounds are produced by specialized secretory structures, for example **glandular trichomes** (see **Glossary**). Glandular trichomes are quite common as they can be found in approximately 30% of all vascular plants [1–3]. The development of these multicellular structures originating from the epidermis has been suggested as a model to study plant cell differentiation [4].

Compounds produced by plant glandular trichomes are exploited by industries that benefit from their various properties Essential oils are traditionally obtained from plant glandular trichomes by hydrodistillation or extraction with organic solvents. Chemical synthesis emerged as a preferred route for obtaining individual compounds that are naturally accumulated in glandular trichomes. In some cases, these approaches are combined to generate semi-synthetic products like the perfume ingredient ambroxide synthesized from sclareol extracted from *Salvia sclarea* [5,6]. More recently, plant genes have been exploited to engineer microbes producing specific compounds. For example, Ro et al**.** [7] reported the production of the arteminisin antimalarial drug precursor artemisinic acid in yeast. However, artemisinin world supply still mainly relies on extraction from *Artemisia annua* [8–10].

The amount of **specialized metabolites** produced by a plant is often tightly correlated to the density of glandular trichomes present at the surface of the epidermis [8,11–14]. Increasing glandular trichome density has recently emerged as a new plant breeding strategy to enhance the yield in compounds of interest for the pharmaceutical sector [8]. This strategy could also be used to breed crops with improved resistance to herbivores [2,15]. In some cases, decreased trichome density is also desirable in order to reduce the amount of compounds toxic to humans, like gossypol in cotton [16].

Engineering glandular trichome density or size requires reliable data about the genetic network controlling glandular trichome initiation and morphogenesis. Several lines of evidence indicate that glandular (and in general multicellular) trichome formation is probably controlled by a different network than the one controlling non-glandular trichome formation in *Arabidopsis thaliana* [4,17]. Compared to non-glandular trichome formation, our knowledge about genes involved in glandular trichome formation is limited. Nevertheless, recent studies have led to significant advances, which are summarized in this review.

What model(s) for the study of glandular trichome development?

As no glandular trichomes are found in *Arabidopsis thaliana*, research on glandular trichome development has been carried out on various other plant species. From the careful analysis of recent literature, three species emerge as main working materials: tomato (*Solanum lycopersicum*, Solanaceae), sweet wormwood (*Artemisia annua*, Asteraceae) and cucumber (*Cucumis sativus*, Cucurbitaceae). In tomato, glandular trichomes are essential for plant defense against herbivores [2,18–22]; in *Artemisia annua*, they produce the anti-malarial drug artemisinin [8,23,24]; and in cucumber, their size and number are important fruit quality traits [25–27]. Tomato and *Artemisia annua* have both already been suggested as good potential models for the study of glandular trichome development [12,24]. Tomato has long been established as a model plant in other fields of plant research; sequenced genome, reliable genetic tools and extensive genetic resources are available for *Solanum lycopersicum* and other related species [12]. Research efforts focusing on *Artemisia annua* are more recent, but genetic transformation protocols are available [28] and a draft assembly of the genome has been recently published [29].

Eight trichome types have been described on tomato leaves, among which four are glandular: type I, type IV, type VI and type VII. Type VI glandular trichomes are the most abundant ones and secrete mainly terpenoids, whereas type I and type IV are involved in acyl sugar biosynthesis [2,30] (**Figure 1**). Type I and type IV were previously suggested to be the same type according to the molecules they secrete [31], but are generally considered to be different according to their morphology and patterning [2,32]. *Artemisia annua* leaves display two types of trichomes: T-shaped non-glandular ones and glandular ones, which are able to accumulate artemisinin along with various other compounds [8,33,34] (**Figure 1**). Eight trichome types have been recently characterized on cucumber fruits including two glandular types: type I and type VI, type I being the most frequent of the two [35]. Type I glandular trichomes are also called bloom trichomes and are believed to be involved in fruit **cuticle** formation and in the secretion of mineral substances [35]. Given the fact that the genetic control of multicellular trichome development in cucumber fruits has been recently reviewed [26], this review focuses on recent discoveries concerning genes involved in glandular trichome development in tomato and *Artemisia annua*.

Glandular trichome morphology and development

Glandular trichome morphology has been described in a large number of plant species and this abundant literature highlights their tremendous diversity of shape and size [12]. Nevertheless, a common organization scheme shared by most glandular trichomes arises from the description of their structure. Glandular trichomes are usually multicellular and composed of 3 parts: a base, a stalk and a gland [3]. The gland is responsible for the secretion of specialized metabolites, the stalk is the structure bearing the gland and the base connects the stalk to surrounding epidermal cells. Each of these 3 parts can be unicellular or multicellular and cells can be more or less elongated. This variability in cell number and shape accounts for a large part of the high morphological diversity found among glandular trichomes and also for their wide variety of sizes. For example, 10-celled glandular trichomes of *Artemisia annua* have a biseriate structure of only 40-50 µm long, whereas tomato type I glandular trichomes are 2-3 mm long with a long stalk [2,12,36] (**Figure 1**). Secreted metabolites often accumulate in a storage cavity. This storage cavity can be subcuticular: in that case, molecules secreted at the top of gland cells accumulate under the cuticle which is gradually pushed away from the cell wall, as seen in *Artemisia annua* glandular trichomes [33]. The storage cavity can also be intercellular, as seen in type VI glandular trichomes of tomato [37,38]. The size of the storage cavity has an impact on glandular trichome shape. For example, in cultivated tomato, type VI glandular trichome glands have a four-leaf clover shape due to the small size of the storage cavity, whereas in the wild tomato species *Solanum habrochaites*, a larger storage cavity is responsible for their spherical shape [37]. An abscission zone between the stalk and the gland, allowing quick separation of the gland from the rest of the trichome, has been described in tomato type VI glandular trichomes [37].

Given their common organization scheme, glandular trichomes must share common key developmental events (**Figure 2**). A number of studies have attempted to describe the different steps of glandular trichome development in various plant species [33,37,39]. Trichome initiation occurs when an epidermal cell acquires a trichome identity according to signals received from surrounding cells. This cell then undergoes tightly controlled cell divisions; the number and the orientation of these divisions and the extent of cell elongation contribute to shape various trichome morphologies. These developmental steps are common to glandular and non-glandular trichomes. Additionally, in glandular trichomes one or more cells differentiate into gland cells. The acquisition of the secretory activity implies a profound remodeling of cell ultrastructure [40] and the activation of specialized metabolism pathways, for example terpenoid biosynthesis in *Artemisia annua* glandular trichomes [41–43]. All these developmental events are critical for glandular trichome patterning, morphogenesis and differentiation, but their genetic control remains poorly understood [39]. However, a certain number of genes involved in glandular trichome initiation have been recently characterized in *Artemisia annua* and tomato, along with several genes involved in glandular trichome morphogenesis.

Genes controlling glandular trichome initiation

Transcription factors

Several transcription factors involved in glandular trichome initiation have been identified both in tomato and *Artemisia annua*. The majority of them belong to two transcription factor subfamilies: the **R2R3-MYB** subfamily and the **HD-ZIP IV** subfamily.

Different members of the R2R3-MYB subfamily have been shown to regulate specialized metabolism [44] or epidermal cell fate, for example *MIXTA* and *MIXTA*-like genes [45]. The first *MIXTA* gene was characterized in snapdragon (*Antirrhinum majus*) and controls the differentiation of conical epidermal cells from flat epidermal cells [45]. In *Artemisia annua* and tomato, three members of the R2R3-MYB subfamily have been characterized as positive regulators of glandular trichome initiation: *AaMYB1* and *AaMIXTA1* in *Artemisia annua* [24,46] and *SlMX1* in tomato [47,48] (**Figure 3, Key Figure**). Indeed, *Artemisia annua* plants overexpressing *AaMYB1* show an increase in glandular trichome density [46]. Moreover, down-regulation of *AaMIXTA1* in *Artemisia annua* [24] and *SlMX1* in tomato [47,48] decreases glandular trichome density, while their upregulation increases glandular trichome density [24]. *AaMIXTA1* and *SlMX1* are both *MIXTA*-like genes [24,47] whereas *AaMYB1* belongs to another clade of the R2R3-MYB subfamily [46].

Members of the HD-ZIP IV subfamily of transcription factors are known to be involved in epidermal cell differentiation in plants, including cuticle biosynthesis and patterning of trichomes and stomata [49,50]. In *Artemisia annua*, two HD-ZIP IV transcription factors, namely AaHD1 and AaHD8, have recently been shown to positively regulate glandular trichome initiation (**Figure 3**). Overexpression of *AaHD1* [34] or *AaHD8* [51] increases glandular trichome density, whereas downregulation of any of the two genes has the opposite effect. *AaHD8* acts upstream of *AaHD1* by directly promoting its expression [51]. The closest homolog of *AaHD8* in tomato is *CUTIN DEFICIENT 2* (*SlCD2*) [51]. A loss-of-function mutation in *SlCD2* is responsible for the phenotype of the *sticky peel* mutant of tomato, which displays a lower number of glandular trichomes (especially type VI) [50]. Therefore, the function of *AaHD8/SlCD2* in positive regulation of glandular trichome initiation seems to be conserved between tomato and *Artemisia annua*. Another HD-ZIP IV transcription factor, WOOLLY (Wo), appears to be an important regulator of glandular trichome initiation in tomato (**Figure 3**). Dominant point mutations in the C-terminus part of *Wo* are responsible for the phenotype of *woolly* mutants, which show dramatically increased trichome density [49,52]. According to the first characterization of *Wo*, type I glandular trichome density is increased in plants carrying dominant *woolly* mutations and reduced in *Wo*-RNAi plants, suggesting that *Wo* enhances type I glandular trichome initiation [49]. However, a recent re-analysis of the phenotype of *woolly* mutants indicates instead that *woolly* mutants show a higher density of type III and type V non-glandular trichomes and a lower density of type IV glandular trichomes in adult leaves [32]. According to this study, the effect of the dominant *woolly* point mutation is different depending on leaf developmental stage: indeed, a higher density of type IV glandular trichomes was observed in juvenile leaves of *woolly* mutants, whereas it was lower in adult leaves compared to the wild-type [32].

Two other transcription factors involved in glandular trichome initiation and belonging neither to the R2R3-MYB subfamily, nor to the HD-ZIP IV subfamily, have also been recently characterized in tomato: the C2H2 zinc-finger protein HAIR (SlH) [53] and the bHLH protein MYELOCYTOMATOSIS-RELATED 1 (SlMYC1) [54] (**Figure 3**). Downregulation of *SlMYC1* by RNAi or missense mutations in *SlH* reduce type VI or type I glandular trichome density, respectively. Moreover, type VI glandular trichomes are absent in *myc1* knockout mutants and *SlH* knockout leads to a hair-absent phenotype [53,54]. These results indicate that *SlMYC1* positively regulates the initiation of type VI glandular trichomes and that *SlH* is a key positive regulator of the initiation of all glandular trichome types. Interestingly, type VI glandular trichomes have smaller glands and shorter stalks in *SlMYC1*-RNAi plants, suggesting that *SlMYC1* is also an important regulator of later steps of type VI glandular trichome morphogenesis in tomato [54].

Cyclins

The induction of cell divisions in early steps of glandular trichome development requires not only transcription factors, but also cell cycle regulators like cyclins. The tomato gene *SlCycB2* encodes a B-type cyclin, which is a type of cyclin promoting the G2/M transition [55]. Type I glandular trichome density is reduced in *SlCycB2*-RNAi plants, highlighting an involvement of *SlCycB2* in glandular trichome initiation [49,55] (**Figure 3**). SlCycB2 may promote a shift from endoreduplication to mitosis in epidermal cells, thereby inducing the first cell divisions of type I glandular trichome development [49]. However, *SlCycB2* overexpression does not seem to be an efficient way to increase glandular trichome density because it inhibits the initiation of type I and type VI glandular trichomes [55]. *SlCycB2* expression is upregulated in *Wo*-overexpressing plants, downregulated in *Wo*-RNAi plants and upregulated in *SlMX1*-overexpressing plants, suggesting that *SlCycB2* expression may be positively regulated by *Wo* and *SlMX1* [47,49] (**Figure 3**).

Regulatory complexes

An important output of recent efforts in dissecting the genetic network underlying glandular trichome development was the identification of 3 complexes controlling glandular trichome initiation in tomato and *Artemisia annua*. In *Artemisia annua*, the expression of the positive regulator *AaHD1* was recently shown to be enhanced by a complex formed by two transcription factors: the HD-ZIP IV protein AaHD8 and the R2R3-MYB MIXTA-like protein AaMIXTA1 [51] (**Figure 3**). In tomato, SlCD2 may interact with a MIXTA-like transcription factor, maybe SlMX1, to form a complex similar to AaHD8-AaMIXTA1, but this remains to be demonstrated. A direct interaction between the C2H2 zinc-finger protein SlH and the HD-ZIP IV transcription factor Wo was recently detected, suggesting that SlH and Wo act as a heterodimer to induce type I glandular trichome formation [53] (**Figure 3**). Moreover, Wo and the B-type cyclin SlCycB2 physically interact, supporting the hypothesis that these two proteins also act together to induce type I glandular trichome formation [49] (**Figure 3**). An H-Wo-CycB2 complex may be involved in the positive regulation of type I glandular trichome initiation in tomato, but has not been detected yet [53].

Genes involved in hormonal signaling

Glandular trichome initiation is known to be regulated by plant hormones in various plant species [56]. In particular, **jasmonates** (JA) are able to induce glandular trichome initiation in tomato [56] and *Artemisia annua* [57]. Consistently, proteins involved in JA signaling have recently been shown to impact glandular trichome initiation in both species. In *Artemisia annua*, the transcriptional activity of the positive regulator AaHD1 is repressed by a direct interaction with AaJAZ8, which belongs to the **JAZ** family of JA signaling repressors [34] (**Figure 3**). In tomato, the overexpression of the JA signaling repressor *SlJAZ2* decreases glandular trichome density, indicating that *SlJAZ2* is a negative regulator of glandular trichome initiation [58]. The expression of *Wo* and *SlCycB2* is strongly repressed in plants overexpressing *SlJAZ2*, suggesting that *SlJAZ2* inhibits glandular trichome development by downregulating the positive regulators *Wo* and *SlCycB2* [58] (**Figure 3**). The induction of glandular trichome initiation by JA in *Artemisia annua* and tomato is probably operated through the JA-triggered degradation of the repressors AaJAZ8 and SlJAZ2 by the proteasome, respectively. Consistently, SlJAZ2 was shown to directly interact with the F-box protein SlCOI1 in a yeast two-hybrid screen [59] and SlCOI is itself a positive regulator of glandular trichome development [60]. In addition to jasmonate signaling, other hormonal signaling pathways control glandular trichome development. For instance, two genes involved in auxin signaling are required for correct glandular trichome initiation in tomato: *SlARF3* [61] and *SlIAA15* [62].

Genes controlling glandular trichome morphogenesis

Cytoskeleton regulators

Various cellular components are at play to define glandular trichome shape, which is essential for its correct functioning. Indeed, the alteration of type VI glandular trichome morphology caused by the *hairless* mutation in tomato leads to impaired synthesis of defense metabolites and decreased resistance to herbivores [63]. The mutation responsible for the observed bending and swelling of type VI glandular trichomes has been located in the gene encoding the SRA1 subunit of the WAVE regulatory complex [63]. This complex is highly conserved among eukaryotes and controls actin filament nucleation and polymerization [63]. Therefore, actin cytoskeleton remodeling seems to play a critical role in glandular trichome morphogenesis. This hypothesis is reinforced by the recent analysis of the tomato *inquieta* mutant [64]. Glandular trichomes of this mutant display similar morphological defects as glandular trichomes of the *hairless* mutant. This phenotype has been associated with a mutation in the homolog of the *ARPC2A* gene of *Arabidopsis thaliana*, which is another important actor of actin cytoskeleton polymerization [64].

Cuticle deposition regulators

Several studies have highlighted a tight link between cuticle deposition and non-glandular trichome development in *Arabidopsis thaliana*, with many genes involved in both processes [24]. Similarly, the correct accumulation of cuticle may be crucial for glandular trichome development in tomato and *Artemisia annua*. In *Artemisia annua*, downregulation of the AP2/ERF transcription factor gene *TRICHOME and ARTEMISININ REGULATOR 1* (*AaTAR1*) by RNAi leads to an altered cuticular wax deposition and an increase in cuticle permeability [65]. Interestingly, glandular trichomes of *AaTAR1*-RNAi plants have an abnormal morphology: the top of the gland is swollen and gland cell number is reduced [65]. In tomato, downregulation of the R2R3-MYB transcription factor gene *SlMX1* by RNAi decreases cuticle deposition along with trichome density, whereas the opposite is observed in lines overexpressing *SlMX1* [47,48]. Likewise, the *sticky peel* mutant, which carries a mutation in the HD-ZIP IV transcription factor gene *SlCD2*, is impaired in cutin accumulation and displays a lower glandular trichome density at the same time [50]. These examples highlight a link between cuticle formation and glandular trichome initiation in tomato and *Artemisia annua*, but it is unclear whether cuticle deposition is necessary for proper glandular trichome morphogenesis, or whether these two processes are simply co-regulated. The analysis of glandular trichome morphology in plants harboring mutations in cuticle biosynthesis genes could help to answer this question.

Concluding remarks and future perspectives

Glandular trichome initiation is a developmental process impacting glandular trichome density and specialized metabolite yield [12]. Transcription factors belonging to R2R3-MYB and HD-ZIP IV subfamilies appear to play prominent roles in the regulation of this process in tomato [32,47–50,52] and *Artemisia annua* [24,34,46,51]. These transcription factors have been shown to be involved in regulatory complexes: a cyclin/HD-ZIP IV complex [49] and a C2H2/HD-ZIP IV complex [53] were discovered in tomato, and a R2R3-MYB/HD-ZIP IV complex [51] was identified in *Artemisia annua*. More investigations are needed to determine whether similar complexes are operating in both species (see **Outstanding Questions**). In the later steps of glandular trichome development, the actin cytoskeleton [63,64] and the cuticle [47,48,50,65] seem to be critical for correct glandular trichome morphogenesis. The recent identification of quantitative trait loci (QTLs) controlling the shape of type VI glandular trichomes in tomato represents a precious information for the future characterization of more regulators of this process [66].

Almost all genes recently shown to be involved in glandular trichome development in tomato and *Artemisia annua* also impact the development of non-glandular trichomes [24,32,34,46,47,51,53,55,62,63,65]. *SlMYC1* is the only gene identified so far which seems to affect only glandular trichome development [54]. It would be of high interest to identify other genes specifically controlling glandular trichome development, in particular genes involved in the acquisition of the secretory activity. In tomato, such genes may be found among SlMYC1 targets, which remain to be identified. Alternatively, a comparative study of type IV and type V trichomes could provide information concerning glandular cell differentiation, because these two trichome types are morphologically very similar, except for the apical cell which is glandular in type IV and non-glandular in type V trichomes [2]. Laser microdissection could be a powerful tool for comparative analyses of glandular trichome types. It is not easy to isolate protruding organs with this technique, but it has already been performed successfully on *Artemisia annua* to compare glandular and non-glandular trichomes [41]. In *Artemisia annua*, given the fact that HD-ZIP transcription factors act only as dimers, AaHD1 may interact with distinct HD-ZIP transcription factors to induce glandular or non-glandular trichome initiation. Therefore, the study of AaHD1 interactors may lead to the identification of regulators specific to glandular trichome initiation [34].

A better understanding of glandular trichome development will open exciting avenues for the targeted improvement of agronomical traits. For example, tomato lines with more type IV trichomes or bigger type VI trichome secretory cavities could produce more acylsugars and terpenes, respectively, and thus show better resistance to herbivores [32,66]. The yield of high value-added compounds produced in plant glandular trichomes could also be increased, with benefits for perfume and pharmaceutical industries. Results obtained on *Artemisia annua*, which produces the anti-malarial drug artemisinin, are encouraging. The overexpression of *AaMIXTA1*, *AaHD1* or *AaHD8* significantly enhanced artemisinin production, without any adverse effect on plant growth and fitness [24,34,51]. The highest increase was observed with the overexpression of *AaMIXTA1*, which doubled artemisinin content [24]. Knowing to what extent gene networks controlling glandular trichome development are conserved among the plant kingdom (**Box 1**) will be critical to develop plant breeding strategies based on glandular trichome phenotype in other plant species. Targeted mutagenesis approaches like **CRISPR-Cas9** or **TILLING** could be used to investigate whether the functions of already identified genes are conserved or not, and to characterize new regulators and actors of glandular trichome development.

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Box 1. Are glandular trichome development regulators conserved in different plant lineages?

The ectopic expression of the tomato gene *Wo*^{v}, a strong allele of *Wo*, induces multicellular trichome formation in tobacco (*Nicotiana tabacum*) and potato (*Solanum tuberosum*), and tobacco homologs of *Wo* and *SlCycB2* are upregulated in *Wo*^{v}-overexpressing tobacco plants [52]. Moreover, the overexpression of the tobacco gene *NtCycB2* in tomato led to a phenotype comparable to the overexpression of *SlCycB2* [55]. The ectopic expression of the tomato *Hair* gene in tobacco also triggers trichome formation [53]. Similarly, the ectopic expression of pepper (*Capsicum annuum*) or tobacco orthologues of *SlH* in tomato plants induces trichome formation [53]. Taken together, these results support the idea that the function of at least *H* and *CycB2*, and probably *Wo*, is conserved among *Solanaceae* species.

In *Arabidopsis thaliana*, the R2R3-MYB transcription factor GLABRA 1 (AtGL1) interacts with bHLH and WD40 proteins to form a MYB-bHLH-WD40 complex [67]. This complex induces the expression of the HD-ZIP IV transcription factor GLABRA 2 (AtGL2), which positively regulates non-glandular trichome initiation. Besides, single repeat R3-MYB transcription factors repress non-glandular trichome initiation [67]. The *Artemisia annua* gene *AaMYB1* enhances *AtGL1* and *AtGL2* expression and induces non-glandular trichome initiation when ectopically expressed in *Arabidopsis thaliana* [46]. The orthologue of *AaMYB1* in *Arabidopsis thaliana* is *AtMYB61*. Non-glandular trichome density is reduced in *myb61* mutants, indicating that *AtMYB61* positively regulates non-glandular trichome initiation in *Arabidopsis thaliana* [46]. Another study shows that a functional orthologue of the R3-MYB gene *AtTRY* may be present in tomato: indeed, *SlTRY* is able to inhibit trichome initiation when expressed in *Arabidopsis thaliana* [68]. Taken together, these results suggest that several genes may have a conserved function in the regulation of trichome initiation in *Arabidopsis thaliana*, *Artemisia annua* and tomato.

However, *AtGL1* does not have any impact on glandular trichome development when expressed in tobacco, and the expression of *AmMIXTA*, a gene of *Antirrhinum majus* closely related to *AaMIXTA1* and *SlMX1*, could not rescue the phenotype of the *gl1* mutant of *Arabidopsis thaliana* [4]. Moreover, the closest homolog of the tomato HD-ZIP IV transcription factor *Wo* in *Arabidopsis thaliana* is not *AtGL2* but *PROTODERMAL FACTOR 2* (*AtPDF2*), a gene involved in shoot epidermal cell differentiation but not in trichome initiation [4,49]. Although some regulators involved in early steps of trichome development may be conserved, other regulators appear to have evolved independently.

Glossary

Cuticle: Hydrophobic protective film produced by plant epidermal cells and covering plant epidermis.

Glandular trichome: Plant epidermal outgrowth that synthetizes, stores and emits specialized metabolites.

HD-ZIP IV transcription factor: HD-ZIP transcription factors are plant-specific and possess a homeodomain (HD) DNA-binding domain and a leucine-zipper (ZIP) dimerization motif. Members of the HD-ZIP IV subfamily also have a START domain.

Jasmonates: Phytohormones regulating plant stress response and development. For example, they induce the production of specialized metabolites upon herbivore feeding or attack by a necrotrophic pathogen, and also play a role in primary root growth and flower development.

JAZ repressor: Protein possessing a ZIM domain and involved in the negative regulation of jasmonate signaling.

R2R3-MYB transcription factor: Transcription factor possessing two DNA-binding MYELOBLASTOSIS-RELATED (MYB) domain repeats. This subfamily of MYB transcription factors is specific to the plant kingdom.

Specialized metabolite: Compound which is not essential for plant growth and development, but critical for plant adaptation to its environment. Specialized metabolites are also known as secondary metabolites.

Figure legends.

Figure 1. Glandular trichomes of tomato (*Solanum lycopersicum*) and sweet wormwood (*Artemisia annua*).

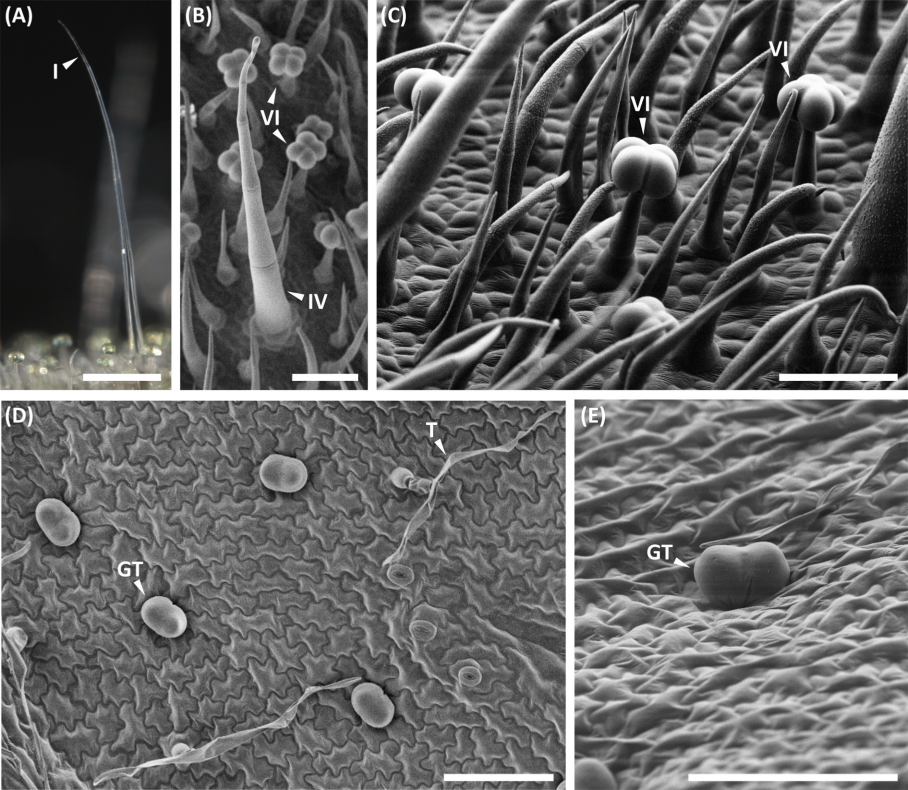
(A) Trichomes of a tomato stem observed with a zoom stereomicroscope. (B,C) Trichomes of a tomato leaf (adaxial face) observed with a scanning electron microscope. (D,E) Trichomes of an *Artemisia annua* leaf (adaxial face) observed with a scanning electron microscope. Scale bars: (A) 500 µm and (B-E) 100 µm. Abbreviations: I, type I glandular trichome; IV, type IV glandular trichome; VI, type VI glandular trichome; GT, glandular trichome; T, T-shaped trichome.

Figure 2. Glandular trichome initiation and development in *Salvia sclarea*, clary sage.

(A,B) Scanning electron microscopy (SEM) analysis of the *Salvia sclarea* calyx surface. Different glandular trichome developmental stages (A) and a typical mature glandular trichome (B) are shown. (C) Schematic illustration of the main steps of the glandular trichome development. Briefly, an epidermal pavement cell becomes determinated for glandular trichome initiation (the initial cell). The initial cell enlarges and enters the mitosis process. After several cell divisions, it gives rise to a multicellular stalk and glandular head. Scale bars: (A,B) 20 µm.

Figure 3. Simplified model of glandular trichome initiation in sweet wormwood (*Artemisia annua*) and tomato (*Solanum lycopersicum*).

(A) In *Artemisia annua*, the R2R3-MYB MIXTA1/HD-ZIP IV HD8 complex activates HD1 to induce the glandular trichome initiation. The JA signaling repressor JAZ8 represses HD1 transcriptional activity, thereby inhibiting glandular trichome initiation. In the presence of JA, JAZ8 is degraded by the proteasome system, leading to the release of HD1 and the glandular trichome initiation. In addition, MYB1 also induced the initiation of glandular trichomes. (B) In *Solanum lycopersicum,* the HD-ZIP IV transcription factor WOOLLY interacts with the B-type cyclin CycB2 and with the C2H2 zinc-finger protein HAIR to initiate the glandular trichome development. A HAIR-WOOLLY-CycB2 complex may exist, but has not been detected yet. The bHLH transcription factor MYC1, the R2R3-MYB transcription factor MX1 and the HD-ZIP IV transcription factor CD2 also participate to the glandular trichome initiation. CD2 is the closest tomato homolog of the transcription factor HD8 of *Artemisia annua*. Like HD8, CD2 may interact with a MIXTA-like protein, maybe MX1. MX1 and WOOLLY both induce the expression of *CycB2*.Like in *Artemisia annua,* the JA signaling repressor JAZ2 inhibits the expression of *WOOLLY* and *CycB2* expression. . In the presence of JA, JAZ2 is degraded leading to the initiation of glandular trichomes. Abbreviations: HD, HOMEODOMAIN PROTEIN; HD-ZIP IV, HOMEODOMAIN LEUCINE ZIPPER IV; JAZ, JASMONATE ZIM DOMAIN PROTEIN; bHLH, basic HELIX LOOP HELIX; MX1, MIXTA-like 1; CD2, CUTIN DEFICIENT 2


(A)
I
(B)
VI
IV
(C)
VI
VI
(D)
T
GT
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GT

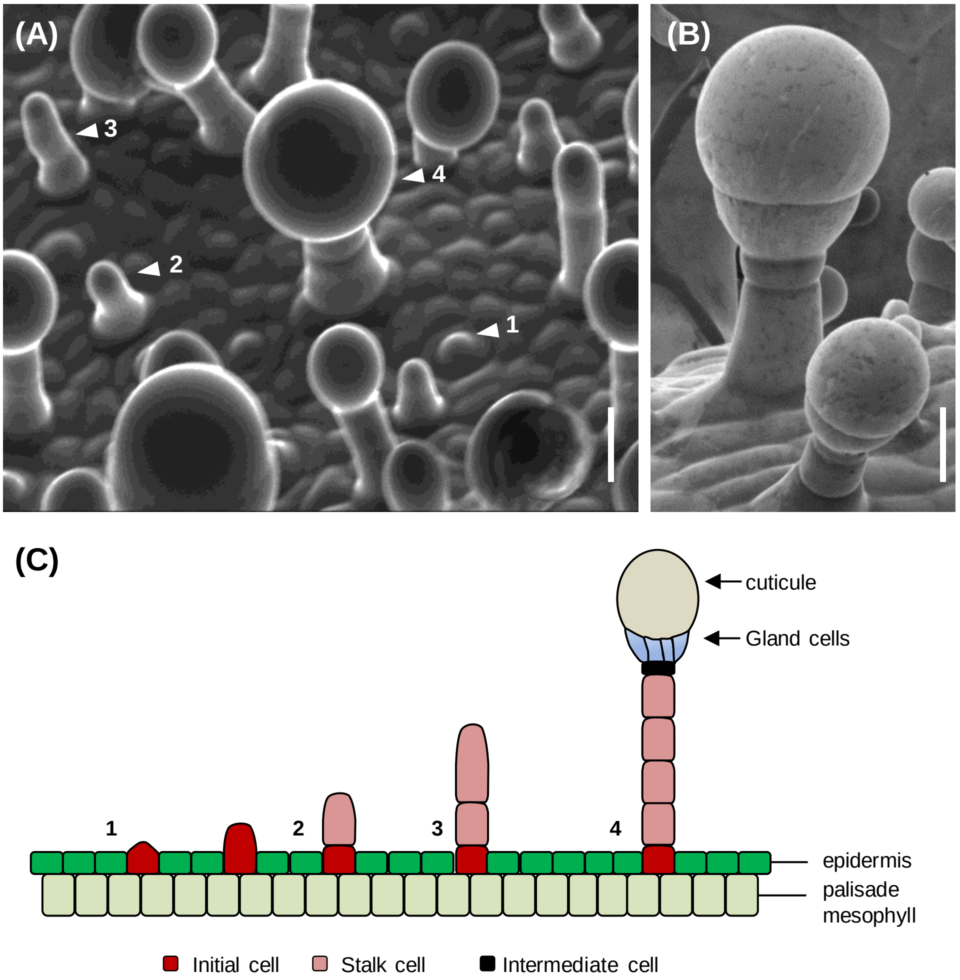
(A)
3
4
2
1
(B)
(C)
cuticule
Gland cells
1
2
3
4
epidermis
palisade
mesophyll
🟥 Initial cell
🟥 Stalk cell
■ Intermediate cell

Figure 2

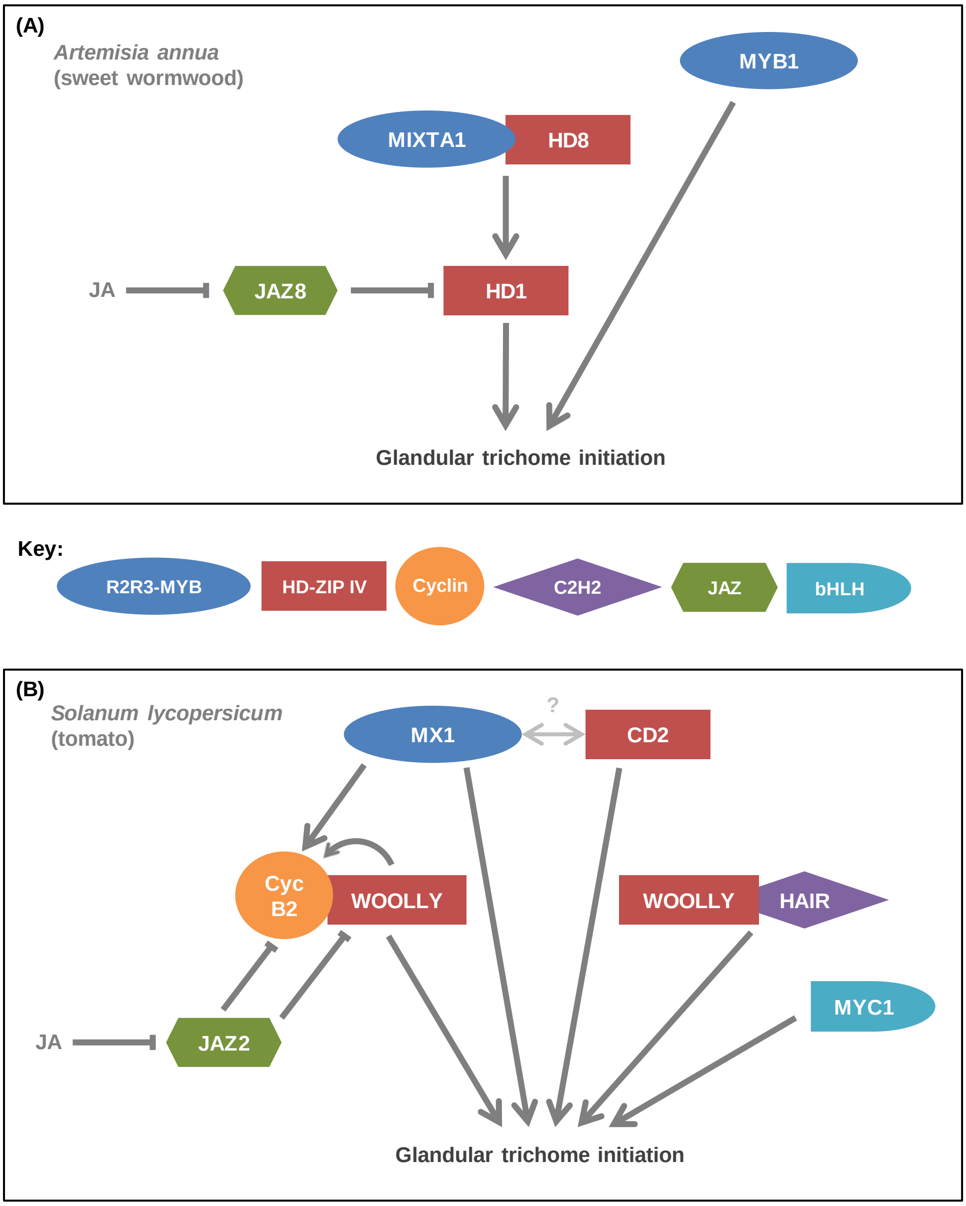
(A)
Artemisia annua
(sweet wormwood)
MYB1
MIXTA1
HD8
JA
JAZ8
HD1
Glandular trichome initiation
Key:
R2R3-MYB
HD-ZIP IV
Cyclin
C2H2
JAZ
bHLH
(B)
Solanum lycopersicum
(tomato)
MX1
?
CD2
Cyc B2
WOOLLY
WOOLLY
HAIR
MYC1
JA
JAZ2
Glandular trichome initiation

Figure 3