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► **To cite this version:**

Camille Chalvin, Stéphanie Drevensek, Michel Dron, Abdelhafid Bendahmane, Adnane Boualem. Genetic Control of Glandular Trichome Development. Trends in Plant Science, 2020, 25 (5), pp.477-487. 10.1016/j.tplants.2019.12.025 . hal-03217221

HAL Id: hal-03217221

<https://hal.inrae.fr/hal-03217221>

Submitted on 22 Aug 2022

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1 **Genetic control of glandular trichome development**

2

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11

12 **Keywords:** glandular trichome; development; transcription factor; specialized metabolism;

13 *Artemisia annua* (sweet wormwood); *Solanum lycopersicum* (tomato).

14

15 **Abstract:**

16 Plant glandular trichomes are epidermal secretory structures producing various specialized

17 metabolites. These metabolites are involved in plant adaptation to its environment and many

18 of them have remarkable properties exploited by fragrance, flavor and pharmaceutical

19 industries. The identification of genes controlling glandular trichome development is of high

20 interest to understand how plants produce specialized metabolites. Our knowledge about this

21 developmental process is still limited, but genes controlling glandular trichome initiation and

22 morphogenesis have recently been identified. In particular, R2R3-MYB and HD-ZIP IV

23 transcription factors appear to play essential roles in glandular trichome initiation in *Artemisia*

24 *annua* and tomato. In this review, we focus on the results obtained in these two species and

25 we propose genetic regulation models integrating these data.

26 **Glandular trichome density and agronomic performance**

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

36 Compounds produced by plant glandular trichomes are exploited by industries that benefit
37 from their various properties. Essential oils are traditionally obtained from plant glandular trichomes
38 by hydrodistillation or extraction with organic solvents. Chemical synthesis emerged as a preferred
39 route for obtaining individual compounds that are naturally accumulated in glandular trichomes. In
40 some cases, these approaches are combined to generate semi-synthetic products like the perfume
41 ingredient ambroxide synthesized from sclareol extracted from *Salvia sclarea* [5,6]. More recently,
42 plant genes have been exploited to engineer microbes producing specific compounds. For example, Ro
43 et al. [7] reported the production of the artemisinin antimalarial drug precursor artemisinic acid in
44 yeast. However, artemisinin world supply still mainly relies on extraction from *Artemisia annua* [8–
45 10].

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

51 decreased trichome density is also desirable in order to reduce the amount of compounds toxic
52 to humans, like gossypol in cotton [16].

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

60

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

62 As no glandular trichomes are found in *Arabidopsis thaliana*, research on glandular trichome
63 development has been carried out on various other plant species. From the careful analysis of
64 recent literature, three species emerge as main working materials: tomato (*Solanum*
65 *lycopersicum*, Solanaceae), sweet wormwood (*Artemisia annua*, Asteraceae) and cucumber
66 (*Cucumis sativus*, Cucurbitaceae). In tomato, glandular trichomes are essential for plant
67 defense against herbivores [2,18–22]; in *Artemisia annua*, they produce the anti-malarial drug
68 artemisinin [8,23,24]; and in cucumber, their size and number are important fruit quality traits
69 [25–27]. Tomato and *Artemisia annua* have both already been suggested as good potential
70 models for the study of glandular trichome development [12,24]. Tomato has long been
71 established as a model plant in other fields of plant research; sequenced genome, reliable
72 genetic tools and extensive genetic resources are available for *Solanum lycopersicum* and
73 other related species [12]. Research efforts focusing on *Artemisia annua* are more recent, but
74 genetic transformation protocols are available [28] and a draft assembly of the genome has
75 been recently published [29].

76 Eight trichome types have been described on tomato leaves, among which four are glandular:
77 type I, type IV, type VI and type VII. Type VI glandular trichomes are the most abundant
78 ones and secrete mainly terpenoids, whereas type I and type IV are involved in acyl sugar
79 biosynthesis [2,30] (**Figure 1**). Type I and type IV were previously suggested to be the same
80 type according to the molecules they secrete [31], but are generally considered to be different
81 according to their morphology and patterning [2,32]. *Artemisia annua* leaves display two
82 types of trichomes: T-shaped non-glandular ones and glandular ones, which are able to
83 accumulate artemisinin along with various other compounds [8,33,34] (**Figure 1**). Eight
84 trichome types have been recently characterized on cucumber fruits including two glandular
85 types: type I and type VI, type I being the most frequent of the two [35]. Type I glandular
86 trichomes are also called bloom trichomes and are believed to be involved in fruit **cuticle**
87 formation and in the secretion of mineral substances [35]. Given the fact that the genetic
88 control of multicellular trichome development in cucumber fruits has been recently reviewed
89 [26], this review focuses on recent discoveries concerning genes involved in glandular
90 trichome development in tomato and *Artemisia annua*.

91

92 **Glandular trichome morphology and development**

93 Glandular trichome morphology has been described in a large number of plant species and
94 this abundant literature highlights their tremendous diversity of shape and size [12].
95 Nevertheless, a common organization scheme shared by most glandular trichomes arises from
96 the description of their structure. Glandular trichomes are usually multicellular and composed
97 of 3 parts: a base, a stalk and a gland [3]. The gland is responsible for the secretion of
98 specialized metabolites, the stalk is the structure bearing the gland and the base connects the
99 stalk to surrounding epidermal cells. Each of these 3 parts can be unicellular or multicellular
100 and cells can be more or less elongated. This variability in cell number and shape accounts for

101 a large part of the high morphological diversity found among glandular trichomes and also for
102 their wide variety of sizes. For example, 10-celled glandular trichomes of *Artemisia annua*
103 have a biseriate structure of only 40-50 μm long, whereas tomato type I glandular trichomes
104 are 2-3 mm long with a long stalk [2,12,36] (**Figure 1**). Secreted metabolites often
105 accumulate in a storage cavity. This storage cavity can be subcuticular: in that case, molecules
106 secreted at the top of gland cells accumulate under the cuticle which is gradually pushed away
107 from the cell wall, as seen in *Artemisia annua* glandular trichomes [33]. The storage cavity
108 can also be intercellular, as seen in type VI glandular trichomes of tomato [37,38]. The size of
109 the storage cavity has an impact on glandular trichome shape. For example, in cultivated
110 tomato, type VI glandular trichome glands have a four-leaf clover shape due to the small size
111 of the storage cavity, whereas in the wild tomato species *Solanum habrochaites*, a larger
112 storage cavity is responsible for their spherical shape [37]. An abscission zone between the
113 stalk and the gland, allowing quick separation of the gland from the rest of the trichome, has
114 been described in tomato type VI glandular trichomes [37].

115 Given their common organization scheme, glandular trichomes must share common key
116 developmental events (**Figure 2**). A number of studies have attempted to describe the
117 different steps of glandular trichome development in various plant species [33,37,39].
118 Trichome initiation occurs when an epidermal cell acquires a trichome identity according to
119 signals received from surrounding cells. This cell then undergoes tightly controlled cell
120 divisions; the number and the orientation of these divisions and the extent of cell elongation
121 contribute to shape various trichome morphologies. These developmental steps are common
122 to glandular and non-glandular trichomes. Additionally, in glandular trichomes one or more
123 cells differentiate into gland cells. The acquisition of the secretory activity implies a profound
124 remodeling of cell ultrastructure [40] and the activation of specialized metabolism pathways,
125 for example terpenoid biosynthesis in *Artemisia annua* glandular trichomes [41–43]. All these

126 developmental events are critical for glandular trichome patterning, morphogenesis and
127 differentiation, but their genetic control remains poorly understood [39]. However, a certain
128 number of genes involved in glandular trichome initiation have been recently characterized in
129 *Artemisia annua* and tomato, along with several genes involved in glandular trichome
130 morphogenesis.

131

132 **Genes controlling glandular trichome initiation**

133 *Transcription factors*

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

137 Different members of the R2R3-MYB subfamily have been shown to regulate specialized
138 metabolism [44] or epidermal cell fate, for example *MIXTA* and *MIXTA*-like genes [45]. The
139 first *MIXTA* gene was characterized in snapdragon (*Antirrhinum majus*) and controls the
140 differentiation of conical epidermal cells from flat epidermal cells [45]. In *Artemisia annua*
141 and tomato, three members of the R2R3-MYB subfamily have been characterized as positive
142 regulators of glandular trichome initiation: *AaMYB1* and *AaMIXTA1* in *Artemisia annua*
143 [24,46] and *SIMX1* in tomato [47,48] (**Figure 3, Key Figure**). Indeed, *Artemisia annua* plants
144 overexpressing *AaMYB1* show an increase in glandular trichome density [46]. Moreover,
145 down-regulation of *AaMIXTA1* in *Artemisia annua* [24] and *SIMX1* in tomato [47,48]
146 decreases glandular trichome density, while their upregulation increases glandular trichome
147 density [24]. *AaMIXTA1* and *SIMX1* are both *MIXTA*-like genes [24,47] whereas *AaMYB1*
148 belongs to another clade of the R2R3-MYB subfamily [46].

149 Members of the HD-ZIP IV subfamily of transcription factors are known to be involved in
150 epidermal cell differentiation in plants, including cuticle biosynthesis and patterning of

151 trichomes and stomata [49,50]. In *Artemisia annua*, two HD-ZIP IV transcription factors,
152 namely AaHD1 and AaHD8, have recently been shown to positively regulate glandular
153 trichome initiation (**Figure 3**). Overexpression of *AaHD1* [34] or *AaHD8* [51] increases
154 glandular trichome density, whereas downregulation of any of the two genes has the opposite
155 effect. *AaHD8* acts upstream of *AaHD1* by directly promoting its expression [51]. The closest
156 homolog of *AaHD8* in tomato is *CUTIN DEFICIENT 2 (SICD2)* [51]. A loss-of-function
157 mutation in *SICD2* is responsible for the phenotype of the *sticky peel* mutant of tomato, which
158 displays a lower number of glandular trichomes (especially type VI) [50]. Therefore, the
159 function of *AaHD8/SICD2* in positive regulation of glandular trichome initiation seems to be
160 conserved between tomato and *Artemisia annua*. Another HD-ZIP IV transcription factor,
161 WOOLLY (*Wo*), appears to be an important regulator of glandular trichome initiation in
162 tomato (**Figure 3**). Dominant point mutations in the C-terminus part of *Wo* are responsible for
163 the phenotype of *woolly* mutants, which show dramatically increased trichome density
164 [49,52]. According to the first characterization of *Wo*, type I glandular trichome density is
165 increased in plants carrying dominant *woolly* mutations and reduced in *Wo*-RNAi plants,
166 suggesting that *Wo* enhances type I glandular trichome initiation [49]. However, a recent re-
167 analysis of the phenotype of *woolly* mutants indicates instead that *woolly* mutants show a
168 higher density of type III and type V non-glandular trichomes and a lower density of type IV
169 glandular trichomes in adult leaves [32]. According to this study, the effect of the dominant
170 *woolly* point mutation is different depending on leaf developmental stage: indeed, a higher
171 density of type IV glandular trichomes was observed in juvenile leaves of *woolly* mutants,
172 whereas it was lower in adult leaves compared to the wild-type [32].

173 Two other transcription factors involved in glandular trichome initiation and belonging
174 neither to the R2R3-MYB subfamily, nor to the HD-ZIP IV subfamily, have also been
175 recently characterized in tomato: the C2H2 zinc-finger protein HAIR (*SIH*) [53] and the

176 bHLH protein MYELOCYTOMATOSIS-RELATED 1 (SIMYC1) [54] (**Figure 3**).
177 Downregulation of *SIMYC1* by RNAi or missense mutations in *SIH* reduce type VI or type I
178 glandular trichome density, respectively. Moreover, type VI glandular trichomes are absent in
179 *myc1* knockout mutants and *SIH* knockout leads to a hair-absent phenotype [53,54]. These
180 results indicate that *SIMYC1* positively regulates the initiation of type VI glandular trichomes
181 and that *SIH* is a key positive regulator of the initiation of all glandular trichome types.
182 Interestingly, type VI glandular trichomes have smaller glands and shorter stalks in *SIMYC1*-
183 RNAi plants, suggesting that *SIMYC1* is also an important regulator of later steps of type VI
184 glandular trichome morphogenesis in tomato [54].

185

186 *Cyclins*

187 The induction of cell divisions in early steps of glandular trichome development requires not
188 only transcription factors, but also cell cycle regulators like cyclins. The tomato gene *SlCycB2*
189 encodes a B-type cyclin, which is a type of cyclin promoting the G2/M transition [55]. Type I
190 glandular trichome density is reduced in *SlCycB2*-RNAi plants, highlighting an involvement
191 of *SlCycB2* in glandular trichome initiation [49,55] (**Figure 3**). *SlCycB2* may promote a shift
192 from endoreduplication to mitosis in epidermal cells, thereby inducing the first cell divisions
193 of type I glandular trichome development [49]. However, *SlCycB2* overexpression does not
194 seem to be an efficient way to increase glandular trichome density because it inhibits the
195 initiation of type I and type VI glandular trichomes [55]. *SlCycB2* expression is upregulated in
196 *Wo*-overexpressing plants, downregulated in *Wo*-RNAi plants and upregulated in *SIMX1*-
197 overexpressing plants, suggesting that *SlCycB2* expression may be positively regulated by *Wo*
198 and *SIMX1* [47,49] (**Figure 3**).

199

200 *Regulatory complexes*

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

215

216 *Genes involved in hormonal signaling*

217 Glandular trichome initiation is known to be regulated by plant hormones in various plant
218 species [56]. In particular, **jasmonates** (JA) are able to induce glandular trichome initiation in
219 tomato [56] and *Artemisia annua* [57]. Consistently, proteins involved in JA signaling have
220 recently been shown to impact glandular trichome initiation in both species. In *Artemisia*
221 *annua*, the transcriptional activity of the positive regulator *AaHD1* is repressed by a direct
222 interaction with *AaJAZ8*, which belongs to the **JAZ** family of JA signaling repressors [34]
223 (**Figure 3**). In tomato, the overexpression of the JA signaling repressor *SlJAZ2* decreases
224 glandular trichome density, indicating that *SlJAZ2* is a negative regulator of glandular
225 trichome initiation [58]. The expression of *Wo* and *SlCycB2* is strongly repressed in plants

226 overexpressing *SIJAZ2*, suggesting that *SIJAZ2* inhibits glandular trichome development by
227 downregulating the positive regulators *Wo* and *SICycB2* [58] (**Figure 3**). The induction of
228 glandular trichome initiation by JA in *Artemisia annua* and tomato is probably operated
229 through the JA-triggered degradation of the repressors AaJAZ8 and SIJAZ2 by the
230 proteasome, respectively. Consistently, SIJAZ2 was shown to directly interact with the F-box
231 protein SICOI1 in a yeast two-hybrid screen [59] and SICOI is itself a positive regulator of
232 glandular trichome development [60]. In addition to jasmonate signaling, other hormonal
233 signaling pathways control glandular trichome development. For instance, two genes involved
234 in auxin signaling are required for correct glandular trichome initiation in tomato: *SIARF3*
235 [61] and *SIIAA15* [62].

236

237 **Genes controlling glandular trichome morphogenesis**

238 *Cytoskeleton regulators*

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

250 mutation in the homolog of the *ARPC2A* gene of *Arabidopsis thaliana*, which is another
251 important actor of actin cytoskeleton polymerization [64].

252

253 *Cuticle deposition regulators*

254 Several studies have highlighted a tight link between cuticle deposition and non-glandular
255 trichome development in *Arabidopsis thaliana*, with many genes involved in both processes
256 [24]. Similarly, the correct accumulation of cuticle may be crucial for glandular trichome
257 development in tomato and *Artemisia annua*. In *Artemisia annua*, downregulation of the
258 AP2/ERF transcription factor gene *TRICHOME* and *ARTEMISININ REGULATOR 1*
259 (*AaTARI*) by RNAi leads to an altered cuticular wax deposition and an increase in cuticle
260 permeability [65]. Interestingly, glandular trichomes of *AaTARI*-RNAi plants have an
261 abnormal morphology: the top of the gland is swollen and gland cell number is reduced [65].
262 In tomato, downregulation of the R2R3-MYB transcription factor gene *SIMX1* by RNAi
263 decreases cuticle deposition along with trichome density, whereas the opposite is observed in
264 lines overexpressing *SIMX1* [47,48]. Likewise, the *sticky peel* mutant, which carries a
265 mutation in the HD-ZIP IV transcription factor gene *SICD2*, is impaired in cutin accumulation
266 and displays a lower glandular trichome density at the same time [50]. These examples
267 highlight a link between cuticle formation and glandular trichome initiation in tomato and
268 *Artemisia annua*, but it is unclear whether cuticle deposition is necessary for proper glandular
269 trichome morphogenesis, or whether these two processes are simply co-regulated. The
270 analysis of glandular trichome morphology in plants harboring mutations in cuticle
271 biosynthesis genes could help to answer this question.

272

273 **Concluding remarks and future perspectives**

274 Glandular trichome initiation is a developmental process impacting glandular trichome
275 density and specialized metabolite yield [12]. Transcription factors belonging to R2R3-MYB
276 and HD-ZIP IV subfamilies appear to play prominent roles in the regulation of this process in
277 tomato [32,47–50,52] and *Artemisia annua* [24,34,46,51]. These transcription factors have
278 been shown to be involved in regulatory complexes: a cyclin/HD-ZIP IV complex [49] and a
279 C2H2/HD-ZIP IV complex [53] were discovered in tomato, and a R2R3-MYB/HD-ZIP IV
280 complex [51] was identified in *Artemisia annua*. More investigations are needed to determine
281 whether similar complexes are operating in both species (see **Outstanding Questions**). In the
282 later steps of glandular trichome development, the actin cytoskeleton [63,64] and the cuticle
283 [47,48,50,65] seem to be critical for correct glandular trichome morphogenesis. The recent
284 identification of quantitative trait loci (QTLs) controlling the shape of type VI glandular
285 trichomes in tomato represents a precious information for the future characterization of more
286 regulators of this process [66].

287 Almost all genes recently shown to be involved in glandular trichome development in tomato
288 and *Artemisia annua* also impact the development of non-glandular trichomes
289 [24,32,34,46,47,51,53,55,62,63,65]. *SIMYC1* is the only gene identified so far which seems to
290 affect only glandular trichome development [54]. It would be of high interest to identify other
291 genes specifically controlling glandular trichome development, in particular genes involved in
292 the acquisition of the secretory activity. In tomato, such genes may be found among *SIMYC1*
293 targets, which remain to be identified. Alternatively, a comparative study of type IV and type
294 V trichomes could provide information concerning glandular cell differentiation, because
295 these two trichome types are morphologically very similar, except for the apical cell which is
296 glandular in type IV and non-glandular in type V trichomes [2]. Laser microdissection could
297 be a powerful tool for comparative analyses of glandular trichome types. It is not easy to
298 isolate protruding organs with this technique, but it has already been performed successfully

299 on *Artemisia annua* to compare glandular and non-glandular trichomes [41]. In *Artemisia*
300 *annua*, given the fact that HD-ZIP transcription factors act only as dimers, AaHD1 may
301 interact with distinct HD-ZIP transcription factors to induce glandular or non-glandular
302 trichome initiation. Therefore, the study of AaHD1 interactors may lead to the identification
303 of regulators specific to glandular trichome initiation [34].

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

320

321 **Acknowledgement**

322 The authors acknowledge the Plant Biology and Breeding department of INRA, the French
323 National Research Agency (ANR) and the grants Program LabEx Saclay Plant Sciences-SPS
324 (ANR-10-LABX-40-SPS) for their support.

325 The authors would like to thanks Eva Heripre for her support in SEM imaging (MSSMat
326 Laboratory, CentraleSupélec), and Pascal Audigier, Florie Vion, Shirley Partel and Holger
327 Ornstrup for the the plant care.

328

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330

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490

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

493 The ectopic expression of the tomato gene *Wo^v*, a strong allele of *Wo*, induces multicellular
494 trichome formation in tobacco (*Nicotiana tabacum*) and potato (*Solanum tuberosum*), and
495 tobacco homologs of *Wo* and *SlCycB2* are upregulated in *Wo^v*-overexpressing tobacco plants
496 [52]. Moreover, the overexpression of the tobacco gene *NtCycB2* in tomato led to a phenotype
497 comparable to the overexpression of *SlCycB2* [55]. The ectopic expression of the tomato *Hair*
498 gene in tobacco also triggers trichome formation [53]. Similarly, the ectopic expression of
499 pepper (*Capsicum annuum*) or tobacco orthologues of *SlH* in tomato plants induces trichome
500 formation [53]. Taken together, these results support the idea that the function of at least *H*
501 and *CycB2*, and probably *Wo*, is conserved among *Solanaceae* species.

502

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

515 that several genes may have a conserved function in the regulation of trichome initiation in
516 *Arabidopsis thaliana*, *Artemisia annua* and tomato.

517 However, *AtGL1* does not have any impact on glandular trichome development when
518 expressed in tobacco, and the expression of *AmMIXTA*, a gene of *Antirrhinum majus* closely
519 related to *AaMIXTA1* and *SIMX1*, could not rescue the phenotype of the *gll* mutant of
520 *Arabidopsis thaliana* [4]. Moreover, the closest homolog of the tomato HD-ZIP IV
521 transcription factor *Wo* in *Arabidopsis thaliana* is not *AtGL2* but *PROTODERMAL FACTOR*
522 *2 (AtPDF2)*, a gene involved in shoot epidermal cell differentiation but not in trichome
523 initiation [4,49]. Although some regulators involved in early steps of trichome development
524 may be conserved, other regulators appear to have evolved independently.

525

526

527

528 **Glossary**

529

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

532 **Glandular trichome:** Plant epidermal outgrowth that synthesizes, stores and emits
533 specialized metabolites.

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

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

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

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

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

548

549

550

551 **Figure legends.**

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

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

560

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

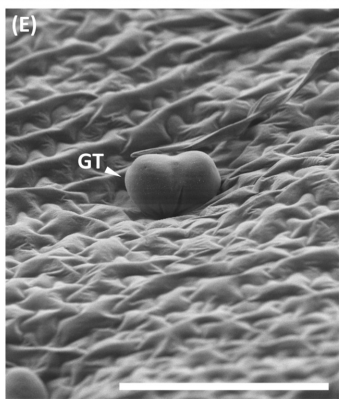
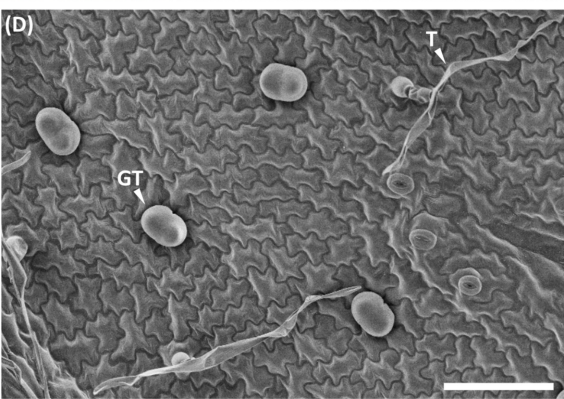
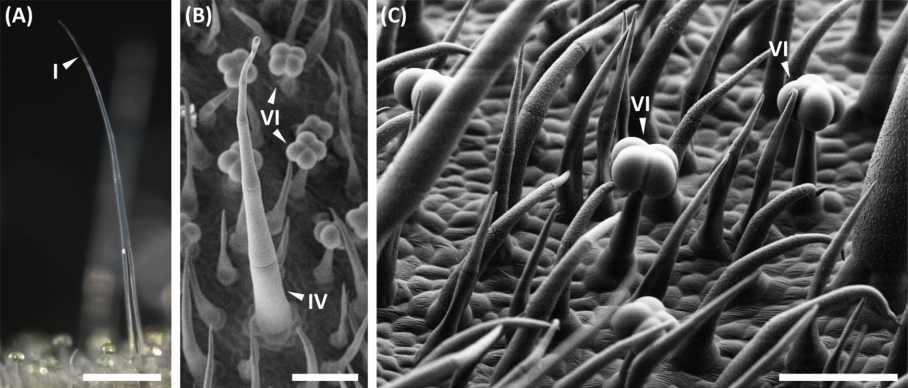
562 (A,B) Scanning electron microscopy (SEM) analysis of the *Salvia sclarea* calyx surface.
563 Different glandular trichome developmental stages (A) and a typical mature glandular
564 trichome (B) are shown. (C) Schematic illustration of the main steps of the glandular trichome
565 development. Briefly,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

569

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

572 (A) In *Artemisia annua*, the R2R3-MYB MIXTA1/HD-ZIP IV HD8 complex activates HD1
573 to induce the glandular trichome initiation. The JA signaling repressor JAZ8 represses HD1
574 transcriptional activity, thereby inhibiting glandular trichome initiation. In the presence of JA,
575 JAZ8 is degraded by the proteasome system, leading to the release of HD1 and the glandular

576 trichome initiation. In addition, MYB1 also induced the initiation of glandular trichomes. (B)
577 In *Solanum lycopersicum*, the HD-ZIP IV transcription factor WOOLLY interacts with the B-
578 type cyclin CycB2 and with the C2H2 zinc-finger protein HAIR to initiate the glandular
579 trichome development. A HAIR-WOOLLY-CycB2 complex may exist, but has not been
580 detected yet. The bHLH transcription factor MYC1, the R2R3-MYB transcription factor MX1
581 and the HD-ZIP IV transcription factor CD2 also participate to the glandular trichome
582 initiation. CD2 is the closest tomato homolog of the transcription factor HD8 of *Artemisia*
583 *annua*. Like HD8, CD2 may interact with a MIXTA-like protein, maybe MX1. MX1 and
584 WOOLLY both induce the expression of *CycB2*. Like in *Artemisia annua*, the JA signaling
585 repressor JAZ2 inhibits the expression of *WOOLLY* and *CycB2* expression. . In the presence
586 of JA, JAZ2 is degraded leading to the initiation of glandular trichomes. Abbreviations: HD,
587 HOMEODOMAIN PROTEIN; HD-ZIP IV, HOMEODOMAIN LEUCINE ZIPPER IV; JAZ,
588 JASMONATE ZIM DOMAIN PROTEIN; bHLH, basic HELIX LOOP HELIX; MX1,
589 MIXTA-like 1; CD2, CUTIN DEFICIENT 2



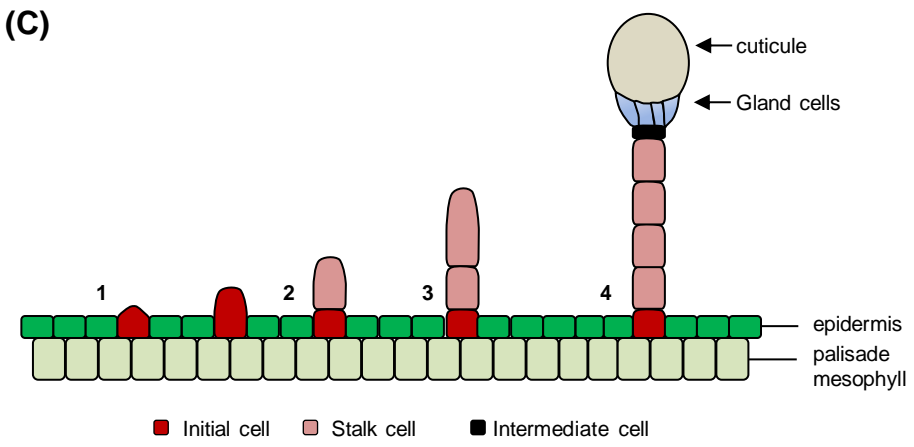
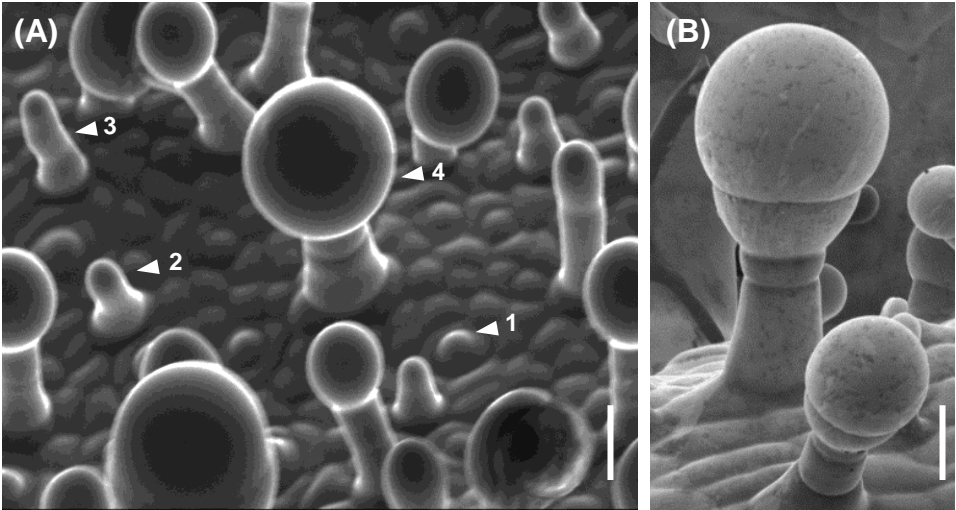
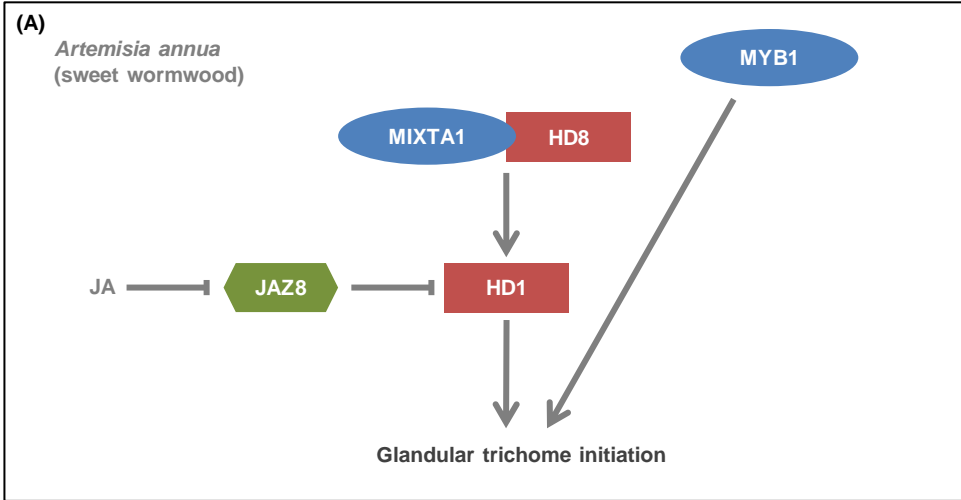


Figure 2



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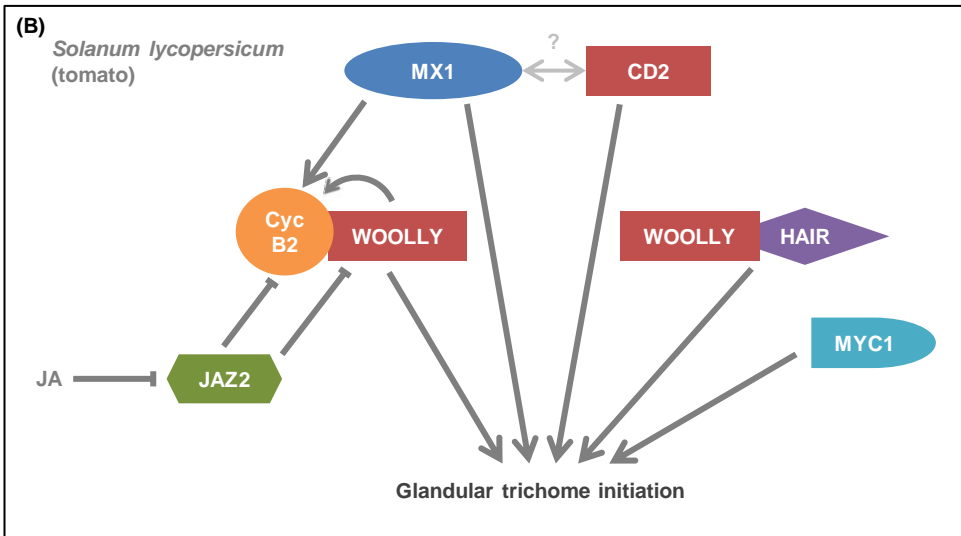


Figure 3