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1 **The genetic control of nectary development**

2

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11

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13 **Abstract**

14 Nectar is the most important reward offered by flowering plants to pollinators for pollination  
15 services. Since pollinator decline has emerged as a major threat for agriculture, and the food  
16 demand is growing globally, studying nectar gland is of utmost importance. Although the  
17 genetic mechanisms that control the development of angiosperm flowers have been quite  
18 well understood for many years, the development, the maturation of nectar gland and the  
19 secretion of nectar in synchrony with the maturation of the sexual organs appears to be one  
20 of the flower's best kept secrets. Here we review key findings controlling these processes.  
21 We also raise key questions that need to be addressed to develop crop ecological functions  
22 that take into consideration pollinators' needs.

23 **Nectar and Food Security**

24 Can we imagine the world without chocolate, coffee or vanilla ice cream, three goods  
25 derived from crops that depend on pollinators! In fact, animal pollinators are vital for life on  
26 earth. Pollinators have co-evolved with flowering plants for millions of years, ensuring their  
27 reproduction and keeping biodiversity and ecosystems alive. Insect pollinators are also a key  
28 to agriculture, contributing to the production of most fruits and vegetables necessary for  
29 healthy human diets. Over the last decades, there is mounting evidence of pollinator decline  
30 all over the world and consequences in many agricultural areas could be a major threat [1,2].

31 This is severe, knowing that yields of 87 out of 115 (76%) leading global food crops and 35%  
32 of global production depend on animal-mediated pollination [2,3]. From the economical  
33 perspective, the pollination services provided by insect pollinators have an estimated value  
34 of \$29 billion in the US alone [4,5] and \$153 billion per year [3] worldwide, equivalent to  
35 9.5% of the total world agricultural food production [6].

36

37 In agriculture, the main insect pollinators, by far, are bees. Unfortunately, honeybee colonies  
38 have decreased by 25% to 50% in Europe [7,8] and elsewhere [9,10]. Wild bees are also  
39 declining, with one tenth of the species extinct or in danger of extinction in Europe [11,12].  
40 While pollinator's declines leading to large scale losses of agricultural productivity or local  
41 and temporal pollination crisis remain in debate, all the specialists in the field recognize the  
42 importance of pollination services, supporting continued research and monitoring of  
43 pollinator biodiversity [1,11]. From a breeding point of view, one central issue is whether the  
44 cultivated varieties have been selected to reward pollinator services. To our knowledge, the  
45 answer is clearly no. Plant domestication and genetic selection have enhanced yield and  
46 improved nutritional values of harvested food and feed [13–15]. But crop ecological  
47 functions, such as plant-pollinator interaction, have been largely ignored. This is perplexing,  
48 knowing that half of the habitable land is used for agriculture [16]. Plant breeding also led to  
49 a reduction in genetic diversity with high risk of losing traits beneficial to pollinators.

50 Nectar and pollen are the main rewards to pollinators. Floral nectar is produced by  
51 specialized glands, called nectaries, in a process that is under complex developmental  
52 control. Since bees prefer flowers with larger rewards, usually in the form of pollen and  
53 nectar [17], investigating nectar related traits including nectary development and nectar  
54 secretion will be key to develop “pollinator friendly” cultivars that not only increase yield  
55 and its stability but also contribute to the rewarding and the preservation of the bees [18].  
56 Investigation of nectaries as they develop and mature holds great potential to identify novel  
57 targets to improve crop-pollinators interactions. In this review we will not address the  
58 question of the pollinators themselves as this has been reviewed elsewhere [19]. We review  
59 the roles and interactions of key genes controlling nectary development and recent inspiring  
60 findings regarding the gene networks regulating nectary maturation and nectar secretion, in  
61 various plant species. We also discuss how domestication and crop selection could impact

62 crop pollinator interactions and how new findings could help to breed varieties with  
63 ecological functions for the benefit of pollinators and food security.

#### 64 **Nectaries in flowering plants**

65 Nectaries are secretory structures that produce nectar, a carbohydrate rich solution  
66 composed mainly of sugars, [20,21] which connects the plants with their pollinators and  
67 defenders. Specifically, floral nectar is produced to attract pollinators, whereas extrafloral  
68 nectar acts to defend plants indirectly [22]. As nectaries are highly variable in their  
69 morphologies, anatomies and locations, they are defined based on their shared function: the  
70 secretion of nectar [23]. Although there have been reports of nectaries in ferns [24], and in  
71 Gnetales [25], nectaries are most widespread in angiosperms, in which there is a conserved  
72 floral organ patterning. Nevertheless, nectaries seem to play by their own rules, as they can  
73 be found in various floral and extrafloral positions [26]. In basal angiosperms, nectaries are  
74 usually associated with the perianth [27], whereas in eudicots they are associated with  
75 carpels and stamens. For example, in *Brassicaceae*, the nectaries are found at the base of  
76 the stamens [28], in the *Solanaceae*, at the base of the gynoecium, and in *Malvaceae*, they  
77 are found on the abaxial side of the involucre bracts as well as the adaxial side of sepals  
78 [22].

79

#### 80 **Models for studying nectary development**

81 Due to the lack of an ideal single model for nectary biology, nectary development has been  
82 studied on a variety of species. The majority of studies on transcriptional and hormonal  
83 regulation of nectary biology were done using *Arabidopsis thaliana*, an outstanding model in  
84 terms of genetic and genomic resources [29]. However, in this case, biochemical aspects of  
85 nectary function were limited due to the rather small nectary size (~100 microns wide and  
86 deep), and extremely low volumes of nectar [29]. On the other hand, owing to much larger  
87 flowers and nectar volumes, excellent studies on nectary metabolomics were conducted  
88 using tobacco [30] and *Cucurbita pepo* [31–33]. Moreover, some of the bee visitation  
89 experiments with respect to different floral traits were done using *Vicia faba* [15], while  
90 *Sinningia speciosa* served as a useful tool for studying co-evolution of the flower shape and  
91 pollinator visitation [34].

92

93 When it comes to studying nectary biology, species bearing unisexual flowers such as the  
94 cucurbits (Figure 1) have particular advantages. First, they offer the opportunity to study  
95 synchronization of the nectar secretion with the maturation of the sexual organ. Second,  
96 they are practical to dissociate gland development from the development of the sexual  
97 organs. Third, they are attractive owing to the relatively large size and volumes of the  
98 nectary gland which facilitates their manipulation and makes them suitable for biochemical  
99 analyses. Forth, the wide spread of sex determination morphs in the Cucurbitaceae plant  
100 family compel for insect-mediated fertilization [35,36]. Nevertheless, each of these different  
101 models has its advantages, and combined together they allow a systems approach for a  
102 comprehensive understanding of the co-evolution of nectary biology and plant pollinators.

103

#### 104 **The nectary development and the ABC(E) genes in *Arabidopsis thaliana***

105 In arabidopsis, floral nectary development begins at the base of the stamens in the third  
106 **whorl** around stage 9, approximately 3.5 days before anthesis [37], comprising a receptacle  
107 tissue with six glands on the abaxial side of the stamens [26]. From the onset of nectary  
108 development, there are two distinct nectary cell types: an outer epidermal layer and an  
109 inner starch granule-containing parenchymal tissue [26].

110 Despite great variation in morphology and size, floral organ order is conserved across  
111 angiosperms [38]. Four concentric floral whorls are specified by the synchronous overlapping  
112 actions of various transcription factors, commonly referred to as the ABC(E) genes.  
113 Specifically, the A genes specify sepals, the A and B genes together specify petals, the B and  
114 C genes together specify stamens, and the C genes specify carpels [37,39,40]. Since  
115 mutations in single A-, B- and C-class homeotic mutants still develop nectaries, Baum and  
116 colleagues initially proposed a model which suggested that the arabidopsis nectary is an  
117 ABC-independent structure associated with the third whorl [26]. Later on, it was proposed  
118 that B-, C- and E- (SEPALATA) functions are redundantly required for nectary development  
119 [41,42].

120 Here, we will review to what extent could the ABC(E) genes affect the sizes and positions of  
121 nectaries. Firstly, both arabidopsis and petunia plants that lack C lineage genes, do not  
122 develop nectaries [42]. In addition, nectary gland development is reduced in the B- (*pi-1*,  
123 *ap3*) and the C- (*ag-1*) class mutants, based on the absence of nectary tissue in *pi ag* and *ap3*

124 *ag* flowers of arabidopsis [41] (Table 1). However, the lack of nectary tissue in these mutants  
125 may be due to the completely different organization of the floral whorls, rather than due to  
126 a direct effect of the B- function genes [42]. Nevertheless, the nectaries of the *ap3* and *pi*  
127 mutants show changes in size and morphology and thus our understanding of the role of the  
128 B- class genes in nectary development remains incomplete. Secondly, mutations in the E-  
129 class genes (*SEP*), which are required for B and C gene activity [43–45], also result in a failure  
130 of nectary development. In addition to the regulation by B- and C- MADS box genes, two  
131 other genes, *LEAFY* (*LFY*) and *UFO*, which regulate both the homeotic genes and the  
132 formation of the third whorl, also affect nectary development [26]. In both *lfy* and *ufo* single  
133 mutants, nectaries were rarely found, whereas in *lfy ufo* double mutants, no nectaries  
134 develop [46], (Table 1). Furthermore, the experiment with the superman (*sup-1*) mutant, in  
135 which the third whorl is repeated multiple times, showed that nectaries are associated with  
136 each of the third whorls [26].

137 In conclusion, using genetic analyses with floral homeotic mutants, it has been  
138 demonstrated that arabidopsis nectary is a third whorl structure [26] whose development  
139 requires a C-class gene: either *AG*, or in its absence, ectopically expressed *SHP* genes are also  
140 sufficient. Nevertheless, as different eudicot species have nectaries in different locations, the  
141 outline for arabidopsis will not necessarily be the same in other species that display  
142 nectaries at different positions.

### 143 ***CRABS CLAW* (*CRC*) and the ABC genes in nectary development**

144 The last year marked two decades of the publication of a paper identifying *CRABS CLAW*, the  
145 only example of a single gene required for nectary gland development in arabidopsis, as no  
146 morphological or molecular signs of nectaries are observed in *crc* mutants [26]. In addition,  
147 *CRC* is also implicated in FM determinacy and carpel development [47]. As a member of the  
148 *YABBY* protein family, *CRC* is characterized by a C<sub>2</sub>C<sub>2</sub> zinc finger domain located at N-  
149 terminus and a helix-loop-helix motif (*YABBY* domain) at the C terminus which is similar to  
150 the high mobility group (HMG) box motif [46]. Restricted to nectaries and carpels by the  
151 action of the floral-meristem identity genes *AP1*, *LFY* and *UFO* [46], *CRC* expression  
152 commences before the nectaries emerge and continues until after anthesis. Already from  
153 stage 6 of flower development on, *CRC* expression occupies an almost continuous ring of

154 receptacle cells between the stamen and sepal primordia, including regions where nectaries  
155 will develop, suggesting that *CRC* plays a role in the early specification of cells that will  
156 become nectaries [46]. Using the ABC homeotic mutants, Baum and colleagues (2001)  
157 demonstrated that *CRC* mRNA expression is negatively controlled by A and B functions in the  
158 outer and the third whorl respectively, but can occur independently of C function outside  
159 the third whorl. Although ectopic expression of *CRC* is not sufficient to induce ectopic  
160 nectaries, *CRC* is necessary for nectary development in various genetic backgrounds,  
161 indicating that it is one of the key genes directing nectary development in arabidopsis [26].

162

163 More recently, Gross et al. (2018) demonstrated that *CRC* forms homodimers and  
164 heterodimers with *INO*, a member of the same protein family via the YABBY domain.  
165 However, this interaction should not control the nectary development, but may control  
166 other functions such as petal and sepal development and leaf structure as the two genes are  
167 co-expressed in these tissues. Furthermore, their work showed that *CRC* has two distinct  
168 functions: 1) it is involved in floral meristem termination via transcriptional repression, and  
169 2) it acts as a transcriptional activator in nectary development and carpel fusion and growth  
170 control [48].

171

## 172 ***CRC* and the ABC(E) genes: things get complicated**

173 The next question was how does *CRC* fit into the ABC(E) floral genes puzzle. Lee and  
174 colleagues (2005) identified the *CRC* promoter region that is necessary and sufficient for  
175 proper *CRC* expression [49]. They found it harbors two CArG [CC(A/T)6GG] boxes, known  
176 binding sites for MADS box proteins. This section will review how MADS box proteins  
177 *AGAMOUS*, *SHATTERPROOF1/2*, *PISTILLATA* and *SEPALLATA1/2/3* regulate *CRC* expression  
178 and nectary development. Firstly, a study by Wuest and colleagues has shown that TFs AP3  
179 and PI directly suppress the expression of *CRC* [50]. Secondly, mutations in the *SEP* genes,  
180 which are redundantly required to specify petals, stamens and carpels [43–45,51,52], result  
181 in a failure of nectary development despite having a third whorl, and are therefore required  
182 for *CRC* activation in the third whorl. Thirdly, in the absence of B- and C-class gene activities,  
183 *SHATTERPROOF1* (*SHP1*) and *SHP2*, which encode proteins similar to *AG*, might rescue  
184 nectary development, if they are ectopically expressed, as in an A-class *ap2* mutant

185 background [42,49,53,54]. However, *ag shp1 shp2* triple mutants do not develop nectaries,  
186 while nectaries still develop in *ag* and in *shp1 shp2* mutants (Table 1), suggesting that  
187 SHP1/2 may not need to be in an *ap2* background to be functional [42].

188 All these experiments led to a conclusion that the C- and E- class gene activities are  
189 redundantly required for *CRC* activation and nectary development. The lack of nectary  
190 formation in BC double mutants, but their presence in B and C single mutants, would suggest  
191 redundancy of these proteins in the complex with the SEP proteins [49]. Nevertheless, as the  
192 architecture of these mutants is highly modified, it remains to be determined whether the  
193 lack of nectaries is a direct effect. Finally, bearing in mind that the function and the  
194 expression domain of the C- lineage genes and *CRC* is much broader than the nectary  
195 development, the restriction of nectaries at the base of carpels in petunia, and at the base of  
196 stamens in arabidopsis must depend on the presence of additional local genetic factors [42].  
197 Moreover, it is important to note that the genetic analyses were carried out in eudicots, and  
198 thus extrapolations from this data should be limited to these taxa. Furthermore, *CRC*'s DNA  
199 binding motif and target genes in developing nectaries still need to be identified.

200 More recently, *CRC* was reported to bind promoter regions of *3-KETOACYL-COA SYNTHASE 7*  
201 and *15* (*KCS7* and *KCS15*), two genes that are involved in the synthesis of fatty acids [55],  
202 which are then used as signaling molecules or in cuticular wax synthesis [56]. This report  
203 sheds some new light on our understanding of *CRC* which seems to control other important  
204 biological processes.

205

## 206 **Functional conservation of *CRC* in flowering plants**

207 What do we know on the identified regulators of nectary development in flowering plants?  
208 Most of the rosid and asterid species have their nectaries associated either with stamens or  
209 carpels [57]. Bearing in mind that both positions occupy the C-function domain and the fact  
210 that *CRC* expression in nectaries has been shown to be conserved in a number of higher  
211 eudicot species [58], Morel and colleagues suggested that floral nectary development in  
212 rosids and asterids generally occurs via the C-lineage/*CRC* module [42]. Since all C-lineage  
213 genes from petunia (*pMADS3* and *FBP6*) and arabidopsis (*AG*, *SHP1* and *SHP2*) are able to  
214 activate *CRC* expression, this suggests that C-lineage gene dependent *CRC* activation already  
215 existed before the split between rosids and asterids. This could further suggest a common



216 evolutionary origin for nectary development in the two major core eudicot lineages, at least  
217 for species in which the nectaries are associated with the reproductive organs, and thus  
218 residing within the classical C-function expression domain [42].

219 Interestingly, outside of the flower, *CRC* expression was also detected in extrafloral nectaries  
220 of the rosid species *Capparis flexuosa*, as well as in nectaries that develop from the midvein  
221 of leaves and on the involucre bracts in *Gossypium hirsutum* [58]. On the other hand, in  
222 basal eudicot species, no evidence of *CRC* expression in nectaries was found in *Aquilegia*  
223 *formosa* and *Epimedium sagittatum*, [58,59]. Nevertheless, Min and colleagues filled this gap  
224 by demonstrating that in those two genera, nectary development is controlled by  
225 the *STYLISH*-like genes instead, which encode a group of plant specific TFs that are required  
226 for carpel fusion and the correct development of the style and stigma in arabidopsis [60,61].  
227 In addition, their work showed that the expression of the *STY1* homologs is closely  
228 associated with nectaries in the divergent members of both the *Ranunculaceae* and  
229 *Berberidaceae*, both basal eudicots [60]. All these examples show that nectary development  
230 may require *CRC* function, but its activation may not necessarily depend on C-lineage genes,  
231 or that nectary development can even occur independently of *CRC* [42], which is in line with  
232 the hypothesis that nectaries evolved multiple times independently [42,62].

233

234 While the involvement of *CRC* in carpel development was present in the ancestral  
235 angiosperms [63], its involvement in nectary development, at least on present data, may be  
236 restricted to the eudicots. [58,59]. Finally, the observation that nectaries are absent from  
237 the flowers of ANITA (*Amborella*, *Nymphaeales*, *Illiciaceae*, *Trimeniaceae*,  
238 *Austrobaileyaceae*) grade angiosperms [64] may suggest that nectaries evolved after the  
239 separation of the ANITA clades from the remaining lineage [63].

240

#### 241 ***BLADE ON PETIOLE (BOP)* gene and nectary development**

242 Similar to *CRC*, *BLADE ON PETIOLE1* and *BOP2* play an important role in nectary development  
243 in arabidopsis. *BOP1* and *BOP2* are part of the NPR1 (NON-EXPRESSOR OF PR1) protein  
244 family, which is characterized by a series of conserved cysteines and two protein-protein  
245 interaction domains [51]. *BOP1/2* are expressed in undifferentiated cells at the base of  
246 developing lateral organs and are needed to repress indeterminate growth and promote

247 differentiation in the proximal regions of lateral organs [51,65–67]. Localized to the cytosol  
248 and nucleus [65,68], BOP1/2 can form homo- and heterodimers [69] and interact with the  
249 TGA transcription factor, PERIANTHIA (PAN) [70] (Figure 2), mutations of which affect the  
250 floral organ number in the first three whorls [71]. Unlike the *crc* mutant which completely  
251 lacks nectaries, in *bop1/bop2* double mutant, nectaries are not entirely absent but rather  
252 reduced in size and do not differentiate key nectary features such as parenchymal and  
253 secretory tissue [72]. Similarly to *CRC*, *BOP* is expressed very early in nectary development  
254 and may be controlling other downstream elements in conjunction with *CRC* [51]. Moreover,  
255 phenotyping of *bop 1 bop 2 pan3* triple mutants revealed that BOP1/2 and PAN function in  
256 the same genetic pathway and have a joint role in abaxial patterning of the floral meristem,  
257 as no additive or synergistic increase in patterning defects was observed in these mutants  
258 [70]. Taken together, it has been proposed that, once induced by an appropriate signal, BOP  
259 proteins may interact in the nucleus with TGA transcription factors, such as PAN, to regulate  
260 the transcription of floral patterning genes. The relationship between *BOP1/2* and other  
261 floral homeotic genes such as *AG* is, however, lacking. Analysis of mutants, involving for  
262 instance *bop1/bop2* and *ag*, could answer whether or not other floral homeotic genes feed  
263 into the *BOP1/2*-dependent pathway of nectary development.

#### 264 **Hormone signaling and nectary development**

265 Hormone action is often mediated by transcription factors such as the auxin response  
266 factors (ARFs), some of which are microRNA (miRNA) regulated. For example, AUXIN  
267 RESPONSE FACTOR 6 (ARF6) and ARF8, which are the cleavage targets of the  
268 microRNA *miR167* [73], act redundantly to promote and coordinate maturation of nectary,  
269 petal, stamen and gynoecium [74,75]. In *arf6/arf8* double mutants, nectaries are very small  
270 and only detectable in a fraction of flowers, indicating that auxin signaling pathways are  
271 required for proper nectary growth and function [76,77]. In addition, ARF6 and ARF8 have  
272 been shown to activate jasmonate biosynthesis, which in turn activates MYB21 and MYB24  
273 which are also expressed in nectaries. However, unlike ARF6 and ARF8, morphological and  
274 gene expression analyses showed that MYB21 and MYB24 only affect nectary gene  
275 expression, but not nectary formation [73]. Specifically, the *myb21/myb24* flowers have  
276 reduced expression of arabidopsis terpene synthase genes *TPS11* and *TPS21*. In addition,  
277 MYB21 was shown to promote the production of volatile sesquiterpenes, and together with

278 MYB24, to mediate secondary jasmonate responses in stamens, which may attract  
279 pollinators and/or repel pathogens [73]. MYB21 also feeds back negatively on expression of  
280 jasmonate biosynthesis pathway genes to decrease flower JA level, which also correlates  
281 with termination of growth after the flowers have opened [73]. However, analysis of  
282 jasmonate insensitive mutant *coi1-1* revealed no phenotypic alteration of nectaries,  
283 excluding the possibility that JA regulates nectary development[76–78].

284

### 285 **Hormone signaling and nectar secretion**

286 The role of hormonal signaling in nectary development has not been studied in detail.  
287 Nevertheless, auxin, gibberellin and JA have been reported to play important roles in  
288 regulating nectar production [79–81]. For example, *PIN6*, that encodes an auxin efflux  
289 transporter family protein, is a nectary-enriched gene whose expression is positively  
290 correlated with total nectar production [79]. Moreover, plants with the knocked-out  
291 *GA2OX6*, a gene encoding the enzyme that catalyzes inactivation of bioactive GAs, have  
292 elevated levels of bioactive GAs, which, leads to decreased expression of genes involved in  
293 nectar production, including *PIN6* [80]. In addition, there are nine other nectary-enriched  
294 genes whose expression were reported to depend on GAs signaling [80] (Table 2).

295

296 According to the current model of nectar secretion (reviewed in [82]), (Figure 3), GAs  
297 endogenous to nectaries negatively regulate nectar production [80], whereas GAs from  
298 other floral tissues (developing stamens) seem to indirectly regulate nectary function  
299 through induction of JA-mediated responses, which likely diffuse to nectaries to induce auxin  
300 production in a positive feedback loop [73]. As a result, auxin may induce ARF6/8 expression  
301 and lead to the expression of the MYB21/MYB24 genes which are required for nectary  
302 maturation and function [73]. Moreover, jasmonate insensitive tobacco plants with silenced  
303 *NtCOI1* gene have nectarless phenotype and were reported to act upstream of MYB305 [83],  
304 a gene which plays a critical role in starch metabolism and nectar production [30].  
305 Interestingly, ectopic expression of MYB305 in tobacco leaves was able to induce expression  
306 of the *nec1* [84], the only reported gene that controls the development of extrafloral  
307 nectaries in cotton [85]. In sum, JA, in interaction with other hormones, plays a central role  
308 in the coordination of the maturation of the nectaries, stamen, gynoecium and petals, all to  
309 attract pollinators, when the flower is competent for reproduction.

310

### 311 **Nectar synthesis and secretion**

312 A recent study on *Cucurbita pepo* nectary identified key genes in nectar synthesis and  
313 secretion during starch synthesis (*STARCH BRANCHING ENZYME* - *CpSBE2*), starch  
314 degradation (*BETA AMYLASE* - *CpBAM1*), sucrose synthesis (*SUCROSE PHOSPHATE SYNTHASE*  
315 - *CpSPS*), sucrose export (*CpSWEET9*) and sucrose hydrolysis (*CELL WALL INVERTASE4* –  
316 *CpCWIN4*) [31]. A common theme in nectar synthesis and secretion in different species is the  
317 transformation of the starch breakdown products into sucrose by the action of the SPSs and  
318 sucrose phosphate phosphatases (SPP), after which sucrose is exported from the nectary  
319 cells in a concentration dependent manner via uniporter SWEET9 (Figure 3). However, the  
320 final step of sucrose hydrolysis by CWIN4 seems to be species-specific and it might play  
321 different roles. For example, in the hexose-rich nectar of arabidopsis, CWIN4 generates a  
322 concentration gradient to drive sugar export, while in the sucrose-rich nectar of *C. pepo* [33]  
323 its role is likely in dictating the final nectar quality [31].

324

325 In a study on ornamental tobacco, in which flower development is divided into 12 stages  
326 [86], nectary starch degradation (20% by mass) was shown to rapidly produce a large  
327 amount of glucose between stage S9 of flower development, characterized by enlarging of  
328 the corolla tube, and S12 (anthesis) doubling the physiological cellular osmolarity (~300  
329 Osm). This increase in cellular osmolarity will lead to a dramatic decline in the water  
330 potential, triggering influx of water from the phloem via sieve elements. As a result, the  
331 increased hydrostatic pressure within nectary causes nectar to exude through nectary pores  
332 [87]. This report suggested that two processes, starch degradation and rapid sugar influx, are  
333 determinants of sugar composition in floral nectar [87].

334

### 335 **Nectary transcriptome: There is more to nectaries than TFs, but not much more**

336 Global transcriptomics analyses of nectaries have been studied in several species [31,88–91].  
337 The first report of a nectary transcriptome study was performed by Kram and colleagues  
338 (2009), who identified 270 genes preferentially expressed in arabidopsis nectaries [88].  
339 Interestingly, the short list of the nectary-enriched genes studied by Reeves and colleagues  
340 showed that 18 genes were underrepresented in *arf6-2 arf8-3* mutants [73]. Among them  
341 were *CRC*[46]; *YABBY5*, encoding a protein closely related to *CRC*; *CWIN4*, encoding a cell

342 wall invertase required for nectary sink strength and nectar production [92]; *SWEET9*,  
343 encoding a nectary-specific glucose transporter [93,94]; and *JMT* encoding S-adenosyl-L-  
344 methionine jasmonic acid carboxyl methyltransferase, which makes the volatile compound  
345 methyl jasmonate [95]. Interestingly, each of these genes was underrepresented in both  
346 *arf6-2 arf8-3* and *myb21-5 myb24-5* flowers, except for *CRC* which was underrepresented in  
347 *arf6-2 arf8-3* flowers only, suggesting the requirement of auxin signaling for CRC-mediated  
348 pathways.

349

350 A recent RNA-seq analysis demonstrated that CRC together with SUPERMAN (SUP), a gene  
351 that encodes a C<sub>2</sub>C<sub>2</sub>-type zinc-finger protein involved in FM termination, coordinate  
352 hormone-, stress-, and metabolic gene expression in stamen development [96]. This global  
353 transcriptomic study identified and selected 263 differentially expressed genes in the *crc*  
354 mutant which could help us better understand its roles in other biological processes [96].  
355 Moreover, it seems that genes involved in auxin and gibberellin signaling might play  
356 significant roles in nectary development and further research using candidate gene  
357 approaches as well as 'OMICS' analyses are required to validate this hypothesis.

### 358 **Concluding Remarks and Future Perspectives**

359 Despite the central role of nectar glands in the interaction between flowering plants and  
360 pollinators and the numerous investigations, we have only scratched the surface of  
361 molecular mechanisms controlling their development. The knowledge is so limited today  
362 that no breeding program can be conceived to favour the preservation of pollinators and to  
363 improve fruit set. With the recent development of single cell omics technologies,  
364 metabolome profiling, precise phenotyping and low cost of genome sequencing we can  
365 foresee projects that tackle the interaction of plant and pollinators at the flower level, to  
366 identify key genes controlling nectary development and nectar metabolism and secretion, as  
367 well as at the population level, to bring new insights on the heritability and the variability of  
368 the traits. Phenotyping of cultivated accessions, land races and related wild species for  
369 pollinator foraging activities will also permit to investigate whether domestication have  
370 filtered, in or out, certain nectar-related traits. Moreover, evo-devo analyses of the  
371 identified genes shaping plant-pollinators interactions could help better understand the role

372 and the relationship of the controlled phenotypes in the context of the co-evolution of the  
373 plant with the pollinators.

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583  
584

585 **Glossary**

586 **Nectar:** a sugar-rich, phloem derived solution which contains products of primary and  
587 secondary metabolism.

588 **Nectary:** Secretory nectar-producing organ formed anywhere on the plant apart from the  
589 root.

590 **Whorl:** a concentric ring of floral organs.

591 **Transcription factor (TF):** a molecule that binds to DNA-regulatory sequence to modulate  
592 the rate of gene transcription.

593 **CRABS CLAW (CRC):** a putative TF which controls processes such as carpel development, floral  
594 meristem termination, and floral nectary formation.

595 **YABBY (YAB):** the gene family named after the crabs claw-like appearance of the apically unfused  
596 carpels of the *crc-1* mutant.

597

598 **Figure 1.** Transversely dissected flower of melon (*Cucumis melo*) prior to anthesis using  
599 binocular (left) and scanning electron microscopy (SEM, right). Nectary develops as a single  
600 dome-shaped structure at the base of the male flower (upper panel), while it completely  
601 encircles the stigmatic tissue of the female flower (lower panel). Scale bar represents 2 mm.

602

603 **Figure 2.** Model of genetic control of nectary development. In arabidopsis, *CRC* is activated  
604 by a combination of the C- (*AG*) and E- (*SEP*) class gene activities. In the absence of the *AG*  
605 gene activity, *SHATTERPROOF1/2* can be sufficient to activate *CRC* (Proposed by Lee et al.  
606 2005), while the B functions *AP3/PI* repress *CRC* [50]. *BOP1/2* genes control nectary size [51],  
607 and they function in the same genetic pathway with *PAN*. In basal eudicots, the *STY* genes  
608 play the key role in nectary formation [60] through the auxin biosynthesis pathway [97].

609 **Figure 3.** Model of nectar secretion at anthesis (S12). Hormones GA, auxin and JA regulate  
610 nectar secretion. GAs endogenous to nectaries repress nectar production [80], while GA  
611 outside of the nectary may induce JA production in stamen filaments which leads to auxin

612 production in a positive feedback loop [76]. In turn, IAA may trigger ARF expression and lead  
613 to the expression of the MYB21/MYB305, which are required for nectary maturation and  
614 transcription of the downstream genes in starch metabolism [reviewed in [4]. Upon starch  
615 breakdown, the cellular osmotic pressure rises and triggers the influx of water from the  
616 phloem. As a result, the increased hydrostatic pressure within nectary causes nectar to  
617 exude through nectary pores [87].

618

619 **Table 1.** The effects of floral homeotic mutants on nectary phenotype in *Arabidopsis thaliana*

Gene (Mutant)	Function	Nectary phenotype	Refs.
<i>lfy-6</i>	Master regulator of flower meristem identity	Sometimes present in lateral domain, reduced in size, do not possess stomata	[26]
<i>ufo-2</i>	Regulator of floral meristem identity	Reduced in size, do not possess stomata. More glands present than in <i>lfy-6</i>	[26]
<i>lfy-6 ufo-2</i>		No nectaries	[26]
<i>pi-1</i>	B	Nectaries reduced in size; LN normal, MN not always present	[26]
<i>ag-1</i>	C	Disk shaped; nectaries develop between the 2 <sup>nd</sup> and the 3 <sup>rd</sup> , or outside the 3 <sup>rd</sup> whorl	[98]
<i>ap3</i>	B	Most flowers have both LN and MN but without stomata	[98]
<i>sep 1/2/3</i>	E	No nectaries	[41]
<i>shp1 shp2</i>	C	Normal	[42]
<i>pi-1 ag-1</i>	B C	No nectaries	[26], [98]
<i>ap3-3 ag-3</i>	B C	No nectaries	[98]
<i>ap2-2 pi-1</i>	A B	Nectaries develop interior of the lateral first whorl organs	[99]
<i>ag shp1 shp2</i>	C	No nectaries	[42]
<i>ap2 pi ag shp1 shp2</i>	A B C	No nectaries	[49]

620 LN – lateral nectaries; MN – median nectaries.

621

622

623 **Table 2.** Selected genes with altered expression in nectaries of *arf6-2 arf8-3* mutants relative  
 624 to the wild type<sup>a</sup>

Gene name	Function	Expression in <i>arf6 arf8</i> compared to Col-0 at stage 12	Dependence on additional hormonal signaling	Locus	Refs.
CRC	transcription factor	Down regulated	unknown	AT1G69180	[73]
YABBY5	transcription factor YABBY family protein	Down regulated	unknown	AT2G26580	[73]
CWIN4	beta-fructosidase, putative expressed protein	Down regulated	unknown	AT2G36190	[73]
SWEET9	nodulin MtN3 family protein	Down regulated	unknown	AT2G39060	[73]
TPS11	terpene synthase/cyclase family protein	Down regulated	DELLA-repressed	AT5G44630	[73], [100]
	cytochrome P450 family protein	Down regulated	DELLA-repressed	AT5G44620	[73], [100]
	copper-binding family protein	Down regulated	DELLA-repressed	AT5G24580	[73], [100]
JMT	S-adenosyl-L-methionine:jasmonic acid carboxyl methyltransferase	Down regulated	DELLA-repressed	AT1G19640	[73], [100]
	3-hydroxyisobutyryl-coenzyme A hydrolase, putative	unchanged	DELLA-repressed	AT2G30650	[73], [100]
	a putative zinc finger protein	Down regulated	GA-repressed	AT1G32540	[73], [100]
	unknown expressed protein	Down regulated	GA-induced	AT3G60780	[73], [100]
SAUR66	auxin-responsive protein	Down regulated	GA-induced		[73], [100]
	strictosidine synthase family protein	Down regulated	GA-induced	AT1G74020	[73]

625 <sup>a</sup>Data are based on Affymetrix ATH1 gene chip array [76]

626

Figure 1

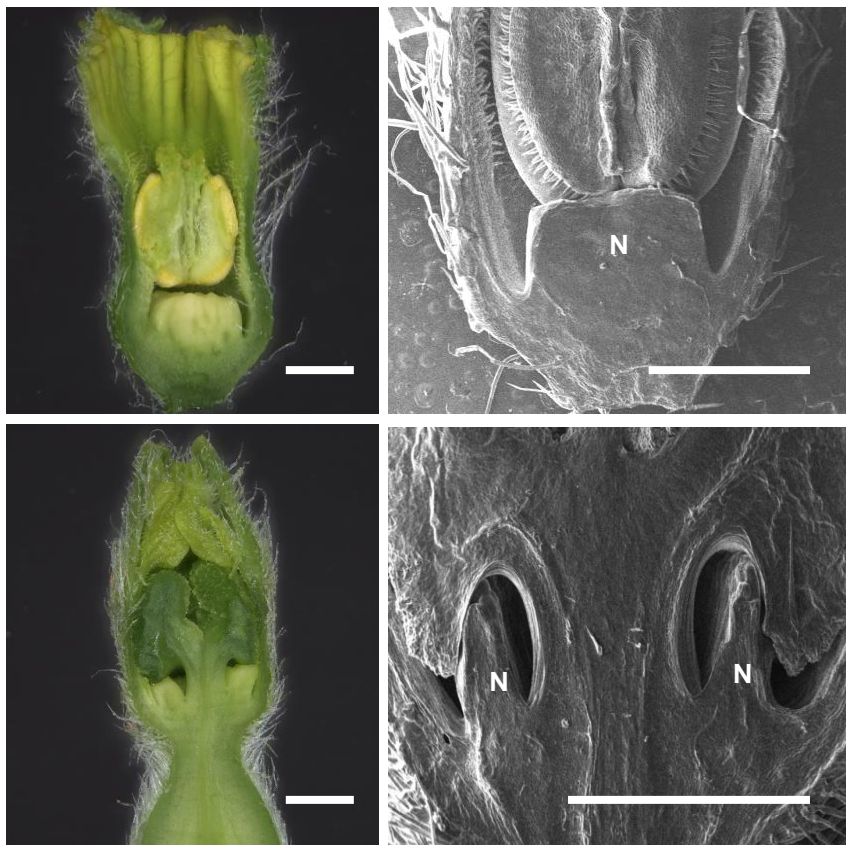


Figure 2

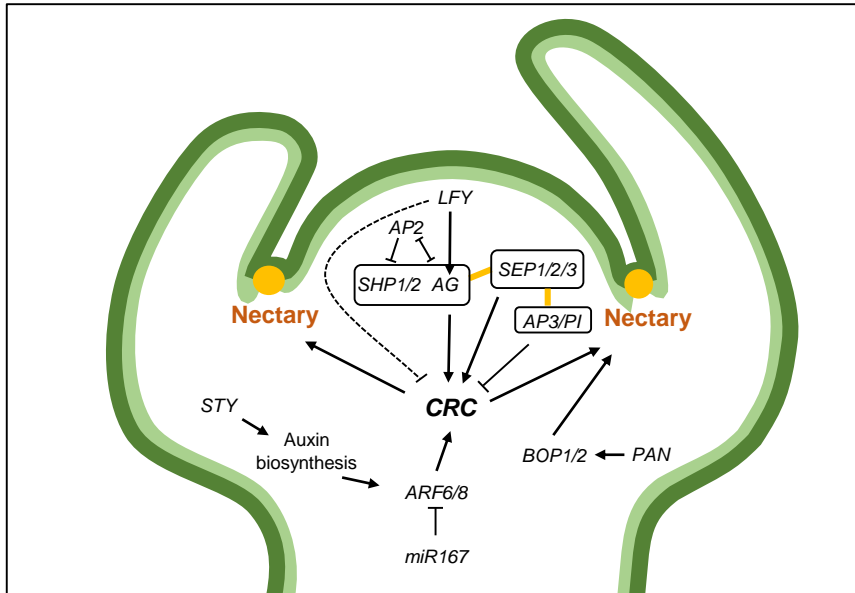




Figure 3

