The Genetic Control of Nectary Development

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Abstract

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

Nectar and Food Security

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

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

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

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

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

Nectaries in flowering plants

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

Models for studying nectary development

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

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

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

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

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

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

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

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

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

The last year marked two decades of the publication of a paper identifying CRABS CLAW, the only example of a single gene required for nectary gland development in arabidopsis, as no morphological or molecular signs of nectaries are observed in *crc* mutants [26]. In addition, CRC is also implicated in FM determinacy and carpel development [47]. As a member of the YABBY protein family, CRC is characterized by a C_2C_2 zinc finger domain located at N-terminus and a helix-loop-helix motif (YABBY domain) at the C terminus which is similar to the high mobility group (HMG) box motif [46]. Restricted to nectaries and carpels by the action of the floral-meristem identity genes *AP1*, *LFY* and *UFO* [46], *CRC* expression commences before the nectaries emerge and continues until after anthesis. Already from stage 6 of flower development on, *CRC* expression occupies an almost continuous ring of

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

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

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

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

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

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

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

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

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

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

Hormone signaling and nectary development

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

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

Hormone signaling and nectar secretion

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

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

Nectar synthesis and secretion

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

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

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

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

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

A recent RNA-seq analysis demonstrated that CRC together with SUPERMAN (SUP), a gene that encodes a C_2C_2-type zinc-finger protein involved in FM termination, coordinate hormone-, stress-, and metabolic gene expression in stamen development [96]. This global transcriptomic study identified and selected 263 differentially expressed genes in the crc mutant which could help us better understand its roles in other biological processes [96]. Moreover, it seems that genes involved in auxin and gibberellin signaling might play significant roles in nectary development and further research using candidate gene approaches as well as 'OMICS' analyses are required to validate this hypothesis.

Concluding Remarks and Future Perspectives

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

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

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

Gene name	Function	Expression in *arf6 arf8* 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 [a]Data are based on Affymetrix ATH1 gene chip array [76]

626

Figure 1

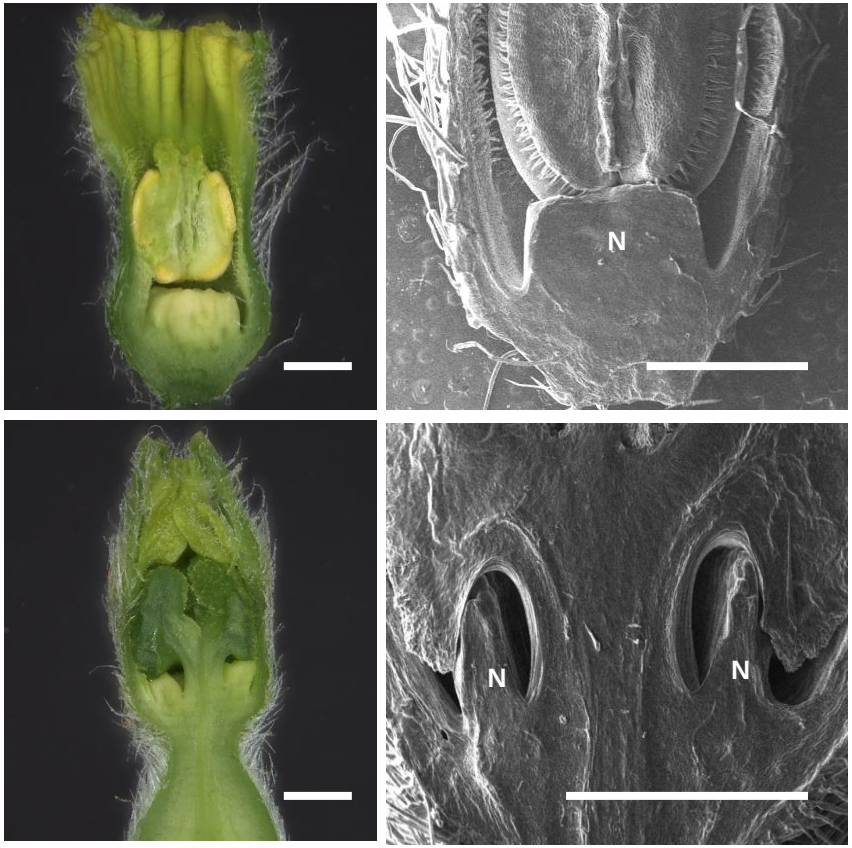


Figure 2

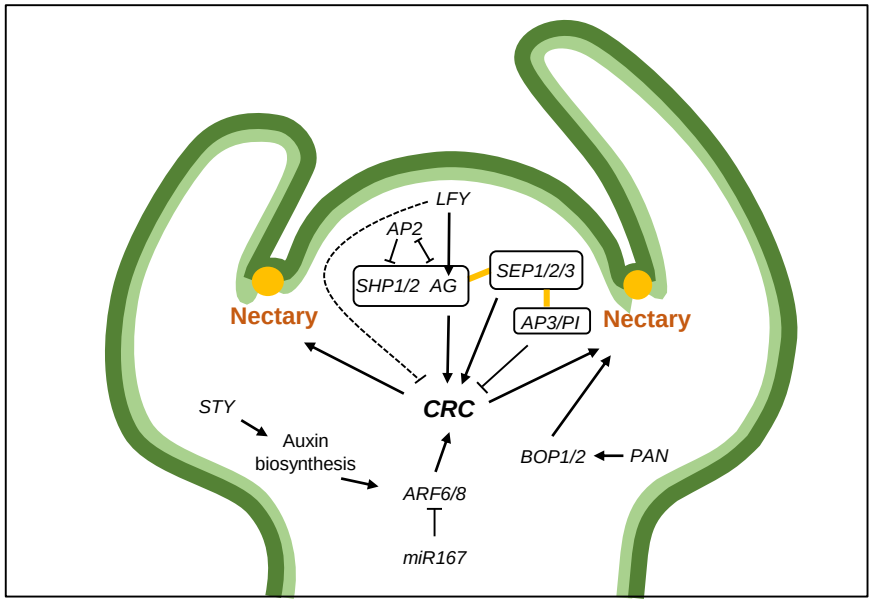
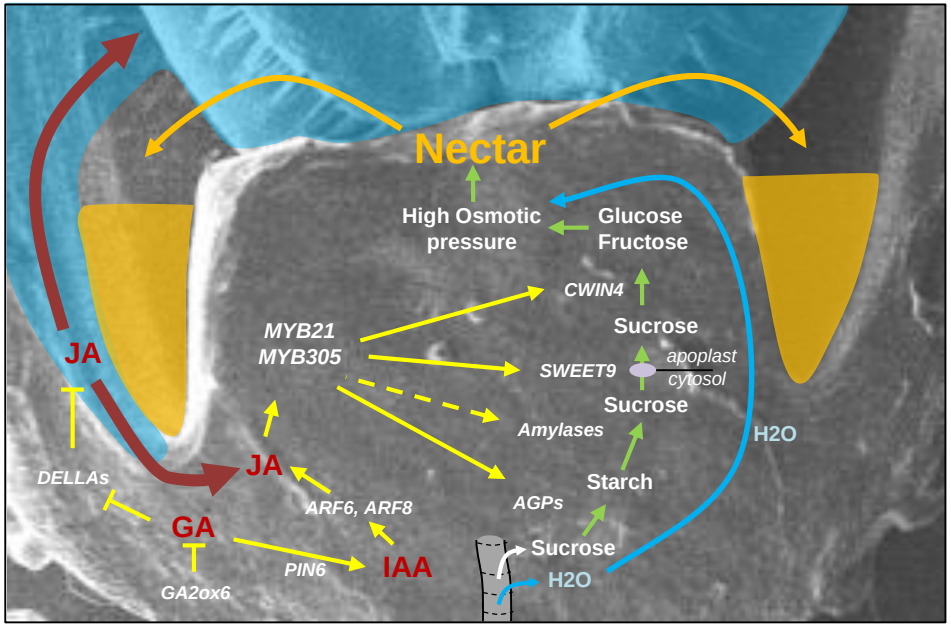

LFY
AP2
SHP1/2 AG
SEP1/2/3
AP3/PI
Nectary
Nectary
CRC
STY
Auxin
biosynthesis
ARF6/8
miR167
BOP1/2
PAN

Figure 3


Nectar
High Osmotic pressure
Glucose
Fructose
CWIN4
Sucrose
MYB21
MYB305
SWEET9
apoplast
cytosol
Sucrose
Amylases
H2O
JA
JA
DELLAs
ARF6, ARF8
AGPs
Starch
GA
IAA
PIN6
Sucrose
GA2ox6
H2O