

Floral Metabolism of Sugars and Amino Acids: Implications for Pollinators' Preferences and Seed and Fruit Set¹[OPEN]

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Primary metabolism in flowers sustains a plenitude of physiological and ecological functions related to floral development and plant reproduction. Carbohydrates and amino acids provide energy and precursors for the reactions of floral secondary metabolism, such as the molecules for color and scent, and constitute an important resource of food for the pollinators. Recent discoveries have advanced our understanding of the cycles of carbohydrate hydrolysis and resynthesis that regulate pollen development, pollen tube growth, and pollination as well as the composition of nectar. Pathways of de novo amino acid biosynthesis have been described in flowers, and the proteins that regulate pollen tube guidance and ultimately control fertilization are being progressively characterized. Finally, a novel field of research is emerging that investigates the chemical modification of sugars and amino acids by colonizing microorganisms and how these affect the pollinators' preferences for flowers. In this Update article, we provide an overview of the new discoveries and future directions concerning the study of the primary metabolism of flowers.

The chemistry of flowers is unique, as it sustains very diverse physiological functions such as flower development, the transition from flower to fruit, and the initial phases of seed set (O'Neill, 1997; Pélabon et al., 2015). In addition, floral metabolites fulfill relevant ecological roles, such as acting as signaling molecules in the chemical communication with animal pollinators (Borghi et al., 2017), providing protection against pests and colonizing microorganisms (Kessler and Baldwin, 2007; Nepi, 2014). Stunning displays of the metabolic resources of flowers are, for example, their colors and fragrance, which occur following the accumulation and emission of tinted and scented secondary metabolites, respectively (Grotewold, 2006; Tanaka et al., 2008; Khan and Giridhar, 2015). Perhaps less sensational, but equally as important, are the reactions of primary

metabolism. Indeed, these sustain the physiology of flowers, provide the chemical precursors, and meet the energy demand of floral secondary metabolism (Muhlemann et al., 2014). Moreover, sugars and amino acids also serve as a nutritional reward for the pollinators that feed on nectar and pollen (Pacini et al., 2006; Heil, 2011; Roy et al., 2017). Therefore, knowing how primary metabolites are imported or de novo synthesized in flowers, and how they are secreted and catabolized, is at the heart of floral biology, being relevant to understand the physiology of plant reproduction and the role that flowers play in the ecosystem. In this article, we aim to provide an overview of floral central metabolism. How primary metabolism sustains flower development, and fruit and seed set, also will be addressed. Finally, given the considerable proportion of primary metabolites that are channeled into nectar, we will discuss the chemical composition of nectar, the modifications that arise from fermentation processes by colonizing yeasts and bacteria, and the impact that these modifications have on pollinators' preferences for flowers.

ADVANCES

- The functional coupling of cwINV of the pollen tube and transmitting tissue supports tube elongation and successful fertilization.
- The sugar moiety of AGPs enables pollen response to LURE.
- Asparagine synthesis in flowers is supported by ASN1, which provides asparagine for embryo development.
- SWEET9 regulates nectar secretion.

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THE PATH OF SUGARS TOWARD THE FLOWER

As photosynthesis in flowers is confined to sepals and young petals, floral tissues rely heavily on the source-to-sink flow for the supply of carbon resources (Müller et al., 2010). Experiments performed in the early 1970s with cut carnation (*Dianthus caryophyllus*) flowers revealed that Suc is the sugar preferentially transported alongside the flower peduncle across the phloem (Ho and Nichols, 1975). However, it was not until much later that the transporter proteins (and the genes that encode them), regulating phloem loading and unloading, were discovered and described in detail.

It is now known that, in source tissues (mature leaves), Suc is loaded into the phloem by the coordinated activity of SUGARS WILL EVENTUALLY BE EXPORTED TRANSPORTER (SWEET) and SUCROSE UPTAKE CARRIER (SUC), the latter alternatively called SUCROSE UPTAKE TRANSPORTER (SUT) proteins; therefore, we will be referring to this family as SUC/SUT. SWEETs are efflux proteins of the plasma membrane, tonoplast, and Golgi that transport Suc and hexoses (Eom et al., 2015; Feng and Frommer, 2015), and SUC/SUTs are plasma membrane-localized proton-Suc symporters of the Major Facilitator Superfamily of cotransporters (Lalonde et al., 2004). In leaves of *Arabidopsis* (*Arabidopsis thaliana*), SWEET11 and SWEET12 are the proteins that export Suc from the parenchyma cells and provide it to SUT1 for phloem loading (Chen et al., 2012).

Flowers are strong sinks that draw Suc and nutrients toward them. The gradient that maintains this upward flow is generated in the flower by a series of phloem-unloading events and the postphloem distribution of photoassimilates to developing organs and tissues. Symplasmic and apoplastic retrieval of Suc have both been documented in flowers, and they reflect the pattern of floral development and the physiological needs of the newly formed tissues (Müller et al., 2010). Indeed, early studies conducted with symplasmic tracers revealed that the trafficking toward the apical meristem decreases after floral induction (Gisel et al., 2002), while the expression and activity of sugar transporters increase progressively while pollen matures and after fertilization has occurred (Gahrtz et al., 1996).

Symplasmic phloem unloading and postphloem transport requires functional plasmodesmata, and it occurs primarily in petals, along the filament that provides nutrients to the connective tissue of the anthers and across the funiculus (Imlau et al., 1999). A wave of symplasmic postphloem transport also has been observed in young flowers during ovule development (bud stages) and in mature flowers shortly after anthesis, and it seemingly mirrors the de novo formation of new plasmodesmata and morphological adjustments of preexisting connections (Werner et al., 2011).

Apoplastic phloem unloading and postphloem transport requires energy and the intervention of protein carriers to transport Suc across the plasma membrane. In flowers, this occurs in symplasmically isolated

tissues such as pollen grains and tubes as well as in developing embryos. The longstanding model of apoplastic phloem transport describes this flow as a two-step process in which Suc is initially unloaded from the sieve elements into the apoplasm and subsequently transported inside the sink cells. Proteins of the SWEET and SUC/SUT families that facilitate the upload of Suc in specific floral tissues have been characterized (see below), while carriers acting in the floral receptacle to export Suc from the cells of terminal phloem have not yet been identified. In this regard, SWEET efflux transporters, whose expression in floral tissues is well documented, are putative candidates (Lin et al., 2014; Eom et al., 2015; Fig. 1A).

Carbon is unloaded in target cells in the form of Suc or, alternatively, following its hydrolysis, as Fru and Glc. In the apoplastic space, the hydrolysis of Suc is catalyzed by invertases (INVs) ionically bound to the wall of the surrounding cells and, for this reason, named cwINVs (Ruan et al., 2010). These are enzymes of the β -fructofuranosidase family that catalyze the hydrolysis of terminal units of Fru from molecules of disaccharides and polysaccharides and contribute to creating the concentration gradient that allows Suc to be unloaded from the phloem and effectively partitioned among target floral tissues. In addition to cwINVs, flowers also express vacuolar invertases (VINs) and cytoplasmic invertases, the latter being *sensu stricto* Suc invertases (Ruan et al., 2010). Finally, plasma membrane SUGAR TRANSPORTER PROTEINs (STPs) also are expressed in flowers and mediate the uptake of the hexoses released in the apoplastic space (Büttner, 2010).

A high rate of sugar import is required to meet the respiratory demand of floral tissues, and indeed, high rates of respiration have commonly been documented in floral organs (Dickinson, 1965; Hew and Yip, 1991; Karapanos et al., 2010). Rates of respiration are exceptionally high in flowers of plants that carry out thermogenesis, such as the Araceae family including skunk cabbage (*Symplocarpus foetidus*) and voodoo lily (*Amorphophallus* spp.), which produce a raceme type of unbranched inflorescence known as the spadix (Plaxton and Podestá, 2006). On anthesis, the plentitude of mitochondria of the spadix contributes to enormous respiration rates via the alternative oxidase pathway (Seymour, 1999), which releases free energy as heat, being equivalent to a 10°C to 25°C increase in tissue temperature. This process serves two functions. First, it supports the extreme growth rates of the spadix (Dancer and ap Rees, 1989). Second, it is thought that the temperature aids in the production and volatilization of floral scents (Seymour, 1999), a process that also is aided by the high content of organic acids of the spadix. This striking specific example aside, little is known concerning the relative activities of organic acid metabolism in these tissues (Borghi et al., 2017); hence, the sections below focus on sugars and amino acids for which our spatial understanding is far more developed.

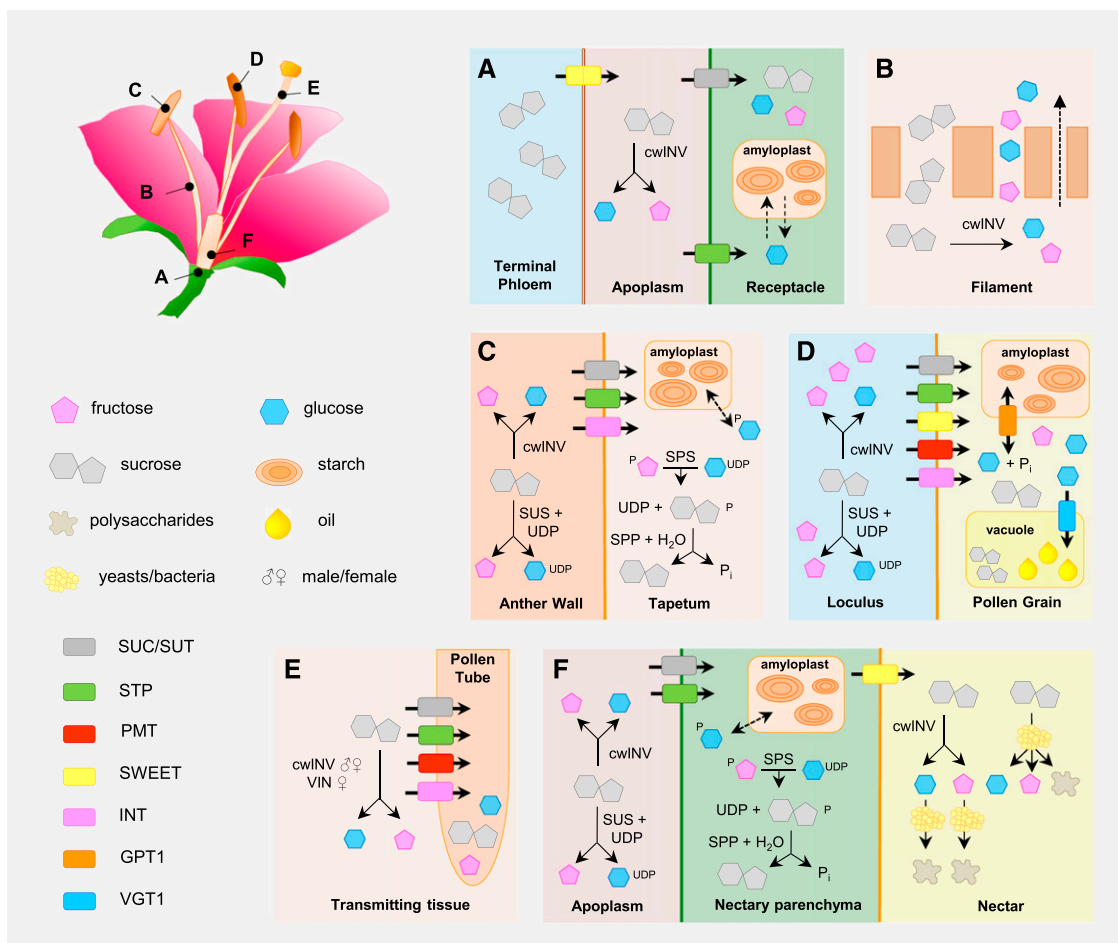


Figure 1. Sugar partitioning in floral tissues. A, Unloading of Suc from the terminal phloem: SWEET proteins are likely involved in the process. Cell wall invertase (cwINV) enzymes in the apoplastic space hydrolyze Suc to Glc and Fru. SUC TRANSPORTERS (SUTs), alternatively called SUC CARRIERS (SUCs), and STPs carry Suc and hexose across the plasma membrane of the receptacle cells where carbohydrates are stored as transitory starch. B, In the receptacle, Suc and hexoses are symplasmically transferred to the anthers. C, cwINVs and Suc synthases (SUS) hydrolyze Suc to hexoses, which are transported in the tapetum by SUC/SUT and STP transporters. In the tapetum while it develops, carbohydrates are stored as transitory starch, which is later converted to Suc by the combined activity of Suc phosphate synthases (SPS) and Suc phosphate phosphatase (SPP). D, Pollen grains are apoplastically isolated and take up Suc and hexoses via SUC/SUT and STP proteins. In developing pollen grains, Glc-6-P is transported across the membrane of amyloplasts by GLUCOSE PHOSPHATE TRANSPORTER1 (GPT1). Later during development, Glc released by the amyloplasts is internalized in the vacuole by the activity of VACUOLAR GLUCOSE TRANSPORTER1 (VGT1) and utilized for Suc synthesis. Depending upon the species, droplets of lipids also may accumulate in mature pollen grains. E, Pollen tube elongation is fueled by sugars secreted by the transmitting tissue. Hexoses are released in the transmitting tissue by the combined activity of cwINV of pollen grain (♂) and stigma (♀) and by stigma-specific VINs. F, SUC/SUT and STP in the cells of the nectary parenchyma take up Suc and hexoses that are stored as starch granules. Before anthesis, transitory starch is converted to Suc that is exported by SWEET proteins to the apoplastic space for nectar secretion. Here, cwINVs hydrolyze Suc to Fru and Glc. Alternatively, fermentation reactions carried out by colonizing yeasts and bacteria modify the sugar composition of nectar by releasing hexoses. Sometimes, the production of a mucous matrix of polysaccharides also has been observed.

CARBOHYDRATE PARTITIONING AND METABOLISM IN FLORAL ORGANS

The paragraphs that follow provide the description of primary metabolism organized by floral organs. Sepals that are similar to leaves in their anatomy and metabolism are not discussed, and we refer to reviews on leaf metabolism instead (Plaxton and Podestá, 2006; Smith and Stitt, 2007).

All floral organs, with the exception of sepals, depend upon sugar import in a measure that correlates with their growth and stage of development and are sustained mainly by postphloem Suc delivery and only to a minor extent by carbohydrates produced via floral carbon fixation (Aschan and Pfanz, 2003). With regard to metabolism, petals are mixotrophic: young green petals are able to perform photosynthesis, but they

progressively lose the ability to fix carbon as they mature and ultimately become fully heterotrophic (Thomas et al., 2003). For example, the corolla of young *Nicotiana tabacum* flowers contains chlorophylls and an active Rubisco enzyme, which both contribute to carbon acquisition. However, at the onset of anthesis, the rate of photosynthesis decreases progressively while pigments accumulate gradually from the distal part of the perianth inward (Müller et al., 2010). Indeed, a biochemical reprogramming occurring at (or around) anthesis has been documented in numerous plant species. For example, in petals of *Antirrhinum majus* (snapdragon), chlorophyll content decreases at anthesis (Muhlemann et al., 2012), and similarly, in *Arabidopsis*, the dismantling of the photosynthetic apparatus, which follows the conversion of chloroplasts to chromoplasts, starts soon after the flowers open (Wagstaff et al., 2009). This shift from autotrophic to heterotrophic metabolism is transcriptionally regulated and marks the moment when flower secondary metabolism becomes predominant (Muhlemann et al., 2012; Tsanakas et al., 2014). As glycolysis and the pentose phosphate pathway are transcriptionally down-regulated and floral secondary metabolic pathways are up-regulated, the concentrations of metabolic intermediates of the tricarboxylic acid cycle, such as fumarate and malate, also decline, while the content of volatile organic compound precursors, such as the aromatic amino acids Phe and Tyr, increases progressively (Muhlemann et al., 2012). Indeed, the physiological function of petals is to attract animal pollinators to flowers, for which pigments and scented molecules are synthesized. Therefore, the transcriptional coregulation of primary and secondary metabolism ensures that the energy requirements and precursors for the biosynthesis of secondary metabolites are in place when this switch occurs.

Petals also store carbohydrates that serve an important function during flower opening. For this, reserve polysaccharides (starch and/or fructans) are accumulated gradually during petal development but degraded rapidly at the onset of anthesis to generate the osmotic potential that leads to cellular water influx and, finally, to flower opening (Bialeski, 1993; Collier, 1997; van Doorn and van Meeteren, 2003; van Doorn and Kamdee, 2014). As plasmodesmata are functional in young petals, symplasmic postphloem Suc is arguably the prime supplier of these reserves, although Suc carriers also may contribute. In this regard, it is worth mentioning that the proton-monosaccharide symporter *SUT13* that transports Glc, Fru, and other D-hexoses is highly expressed in the vasculature of young emerging petals (Nørholm et al., 2006) and, therefore, also may participate in hexose unloading in target cells.

Androecium and gynoecium are fully heterotrophic; that is, their metabolism and growth are entirely supported by the carbon resources synthesized in source tissues.

In the androecium (stamen), photoassimilates are delivered to the anthers through the filament, a duct of vascular tissue that symplasmically connects the

anthers to the flower (Mascarenhas, 1989). Carbon unloaded from the filament is stored as granules of starch and soluble sugars (primarily Suc) in the tapetum, a layer of nutritive cells that develops between the anther wall and the sporogenous tissue (Goldberg et al., 1993). In certain plant species, such as tomato (*Solanum lycopersicum*), Suc is partially hydrolyzed to Glc and Fru while being transported along the filament, so that hexoses are readily available as they reach the anther wall (Pressman et al., 2012; Fig. 1B). Otherwise, Suc synthase in the tapetum cleaves Suc before uptake, while in the cytoplasm of the same cells, Suc phosphate synthase and Suc phosphate phosphatase resynthesize it once again (Fig. 1C; Pacini et al., 1985; Hedhly et al., 2016). Due to the presence of this futile cycle of Suc breakdown and resynthesis (Geigenberger and Stitt, 1991; Nguyen-Quoc and Foyer, 2001), it has been proposed that the tapetum has a buffering function that regulates sugar availability in the whole anther (Castro and Clément, 2007). As anthers develop, sugar resources are mobilized and exported to the locular fluid that surrounds and nourishes the pollen grains while the tapetum slowly degenerates (Pacini et al., 1985). Pollen grains are symplasmically isolated from the surrounding tissues and rely upon plasma membrane transporters for sugar intake. During the course of evolution, this complete dependence on apoplasmic transport gave rise to myriad pollen-specific sugar carriers that mediate pollen sugar uptake during development, germination, and pollen tube growth (Table I provides an overview of sugar transporters characterized in *Arabidopsis* and their site[s] of floral expression). Immature pollen grains utilize sugars for cell wall synthesis as well as to build up the carbon reserves that will be utilized during germination (Hafidh et al., 2016).

In the initial phase of development, cwINVs bound to the wall of pollen grains and Suc synthase enzymes hydrolyze Suc from the locular fluid (Goetz et al., 2001; Persia et al., 2008; Hirsche et al., 2009; Le Roy et al., 2013; Carrizo García et al., 2015), while STP transporters facilitate the uptake of the units of Glc that are released in these reactions (Truernit et al., 1996). Fru, which also is released from the breakdown of Suc, is internalized primarily by POLYOL/MONOSACCHARIDE TRANSPORTERS1 (PMT1; Klepek et al., 2010) and, to a minor extent, by STPs (Hedhly et al., 2016). However, the Fru-to-Glc ratio in the apoplasmic space that surrounds the wall of pollen grains is above 2, implying a preferential uptake of Glc (Pressman et al., 2012). Nonhydrolyzed Suc is most probably internalized by SWEET8 (Fig. 1D; Chen et al., 2012). During pollen maturation, the GLUCOSE PHOSPHATE TRANSPORTER1 transports Glc-6-P across the outer membrane of amyloplasts for starch biosynthesis (Pacini et al., 1985; Niewiadowski et al., 2005; Castro and Clément, 2007). Alternatively, carbohydrates are utilized for the synthesis of the pollen cell wall. At the beginning of their development, pollen grains of all plant species accumulate starch in amyloplasts, which is later

Table 1. Overview of the sugar transporters expressed in flowers of *Arabidopsis*, organ and tissue of expression, transported substrate, and associated mutant phenotype

INT2, INOSITOL TRANSPORTER; n/a, not available; PMT, POLYOL/MONOSACCHARIDE TRANSPORTER; RPG, RUPTURED POLLEN GRAIN; VEX, VEGETATIVE CELL EXPRESSED1; VGT, VACUOLAR GLUCOSE TRANSPORTER; WT, wild type.

Name	Gene No.	Organ (or Tissue) of Expression	Substrate	Mutant Phenotype	References
SUC1	At1g71880	Stamen, pollen, funiculus (ecotype specific)	Suc	No germination of pollen in the absence of Suc, lower anthocyanin accumulation	Sauer and Stolz (1994); Stadler et al. (1999); Sivitz et al. (2008); Feuerstein et al. (2010)
SUC2/ SUT1	At1g22710	Sepals, filament	Suc, maltose, α - and β -phenylglucoside	Plants are smaller than the WT when grown without Suc	Truernit and Sauer (1995)
SUC3/ SUT2	At2g02860	Carpel, pollen grain, and pollen tube	Suc, maltose	n/a	Meyer et al. (2000, 2004)
SUC4/ SUT4	At1g09960	Anthers, pistil, tonoplast	Suc (low-affinity transporter)	WT	Weise et al. (2000); Schneider et al. (2012)
SUC7/ SUT7	At1g66570	Pollen tube growth	Suc	Shorter pollen tube growth (ecotype-dependent phenotype)	Johnson et al. (2004); Sauer et al. (2004)
SUC8/ SUT8	At2g14670	Transmitting tissue	High-affinity Suc; maltose	n/a	Sauer et al. (2004)
SUC9/ SUT9	At5g06170	Transmitting tissue	High-affinity Suc; α - and β -glucosides, maltose	Early flowering in short days	Sauer et al. (2004); Sivitz et al. (2008)
STP1	At1g11260	Whole flower	Glc, Gal, Man, Xyl, 3- <i>O</i> -methylglucose, Fru (traces)	n/a	Sauer et al. (1990)
STP4	At3g19930	Anthers and pollen (expression increases during pollen development)	Glc, Gal, Man, Xyl, 3- <i>O</i> -methylglucose	n/a	Truernit et al. (1996)
STP2	At1g07340	Pollen grains	Glc, Gal, Man, Xyl, 3- <i>O</i> -methylglucose, Fru (traces)	n/a	Truernit et al. (1999)
STP6	At3g05960	Pollen (late stages of development)	Glc, Gal, Man, Xyl, 3- <i>O</i> -methylglucose, Fru, ribose	WT	Schneidereit et al. (2003)
STP9	At1g50310	Mature pollen, pollen tube	Glc, Gal, Man, Xyl	n/a	Schneidereit et al. (2003)
STP10	At3g19940	Pollen tube	Gal, Man, Glc	WT	Rottmann et al. (2016)
STP11	At5g23270	Pollen tube	Glc, Gal, Man, Xyl, 3- <i>O</i> -methylglucose, Fru, ribose	n/a	Schneidereit et al. (2005)
STP13	At5g26340	Sepals, vasculature of emerging petals	Glc, Fru, Gal, Man	WT	Bi et al. (2005); Nørholm et al. (2006)
SWEET1	At1g21460	Pollen grain, pollen tube, petals, sepals	Glc, Gal	n/a	Chen et al. (2010)
SWEET2	At3g14770	Sepal, petals, stamen	2-Deoxyglucose	n/a	Chen et al. (2015a)
SWEET3	At5g53190	Stamen	2-Deoxyglucose	n/a	Chen et al. (2015a)
SWEET4	At3g28007	Whole flower, stamen	2-Deoxyglucose	n/a	Chen et al. (2015a)
SWEET5/ VEX	At5g62850	Pollen (vegetative cells)	Glc	n/a	Engel et al. (2005)
SWEET7	At4g10850	Stamen	Glc	n/a	Chen et al. (2015a)
SWEET8/ RPG1	At5g40260	Pollen grain, pollen tube, tapetum	Glc	Male sterility, abnormal microspore membrane, defective exine pattern formation	Guan et al. (2008); Sun et al. (2013)
SWEET9	At2g39060	Nectary	Suc	No nectar secretion	Ge et al. (2000); Lin et al. (2014)
SWEET13/ RPG2	At5g50800	Tapetum, tetrad	Suc	Male sterility, abnormal microspore membrane, defective exine pattern formation	Sun et al. (2013)
SWEET14	At4g25010	Stamen	Suc	n/a	Chen et al. (2015a)

(Table continues on following page.)

Table 1. (Continued from previous page.)

Name	Gene No.	Organ (or Tissue) of Expression	Substrate	Mutant Phenotype	References
VGT1	At3g03090	Pollen (vacuolar transporter)	Glc, Fru	Late bolting and flowering	Aluri and Büttner (2007)
INT2	At1g30220	Tapetum	Myoinositol and several inositol epimers	WT	Schneider et al. (2007)
INT4	At4g16480	Pollen, pollen tube, transmitting tissue	Myoinositol and several inositol epimers	n/a	Schneider et al. (2006)
PMT1	At2g16120	Germinating pollen grains, pollen tube	Xylitol, Fru	WT	Klepek et al. (2010)
PMT2	At2g16130	Mature pollen grains, pollen tube	Xylitol, Fru	WT	Klepek et al. (2010)
PMT5/ PLT5	At3g18830	Peduncle, sepals, stigma, anthers, abscission zone	Ribose, arabinose, Xyl, Glc, Man, Gal, Fru, rhamnose, Fuc, sorbitol, erythiol, xylitol, inositol	n/a	Klepek et al. (2005); Reinders et al. (2005)

partially or completely converted to Suc, other sugar polymers, and sometimes lipids that are either stored in small vacuoles or deposited freely in the cytoplasm (Fig. 1D). VACUOLAR GLUCOSE TRANSPORTER1 may be involved in this process, as it moves Glc across the tonoplast and is highly expressed in pollen grains (Aluri and Büttner, 2007). The presence of starch in mature pollen negatively correlates with pollen longevity and is used as an indicator of pollination type (Franchi et al., 2011). In fact, starchy short-lived pollen is partially hydrated and readily collected for dispersal by animal pollinators. This type of pollen is referred to as recalcitrant pollen, since it does not require dormancy and quickly germinates when deposited on a permissive stigma (Franchi et al., 2011). Conversely, pollen grains rich in Suc are dry and long-lived and are produced by plant species well adapted to arid climates (Hoekstra et al., 1989; Franchi et al., 1996; Nepi et al., 2001). Once pollen is mature, the anther wall breaks and pollen grains are released. The rupture of the anther wall also depends upon sugar metabolism and it is attained by raising the osmotic potential of the cells at the junction between the anthers and filament, a process presumably promoted by the activities of VIN (Wang and Ruan, 2016) and SUC1 (Singh and Knox, 1984; Stadler et al., 1999).

Pollination is characterized by an intense sugar turnover. In fact, once the pollen grain reaches the stigma and rehydrates, sugar resources are mobilized and the pollen tube emerges and starts to elongate inside the transmitting tissue of the style (Rounds et al., 2011). As ovules are usually borne at the base of the carpel, frequently one to a few centimeters from the stigma, rehydrated pollen grains rapidly grow a long tube to deliver sperm for the process of fertilization. Tube elongation is supported by a rapid and intense deposition of plasma membrane and new cell wall material at the tip of the tube, for which sugars provide building blocks (Konar and Linskens, 1966) and energy (Ylstra et al., 1998). Pollen germinates in a sugar-rich

environment (Labarca et al., 1970) that boosts glycolysis (Carrizo García et al., 2015) to produce abundant pyruvate to be flown through the tricarboxylic acid cycle for ATP production. It has been discovered that aerobic fermentation also supports pollen tube growth. For this, a series of cytosolic reactions collectively referred to as the pyruvate dehydrogenase (PDH) bypass convert pyruvate to acetyl-CoA, which is then transported to mitochondria (tricarboxylic acid cycle) and glyoxysomes (glyoxylate pathway) or utilized for the synthesis of lipids (Gass et al., 2005; Rounds et al., 2011). The PDH bypass confers the evolutionary advantage of fast growth to pollen grains that germinate onto permissive stigmas so that wild-type pollen outcompetes PDH bypass-defective mutants. This extensive uptake of sugars in elongating pollen tubes is mediated by SUC/SUT transporters and the activity of STPs, PMTs, and cwINVs (Mascarenhas, 1970; Singh and Knox, 1984; Truernit et al., 1996; Ylstra et al., 1998; Stadler et al., 1999; Schneidereit et al., 2003, 2005; Scholz-Starke et al., 2003; Büttner, 2010; Klepek et al., 2010). Recently, it was found that the combined activities of cwINVs on the cell wall of pollen tubes and VINs in the cells of the transmitting tissue of the style also promote proper tube elongation (Fig. 1E). Indeed, VINs in the female tissues show a wave of activation that follows closely the growth of the pollen tube, so that sugar resources are rapidly made available as the tip of the tube moves forward along the style (Goetz et al., 2017). The discovery of this mutual interaction adds one more tile to the mosaic of molecular mechanisms that regulate the long-known phenomenon of the appearance of random elongation trajectories and slow growth rates observed in pollen grains germinated *in vitro*.

The gynoecium (pistil) is composed of one or more carpels, each consisting of a stigma, style, and ovary. The metabolic activity of each part of the pistil specifies its physiological function, correlates with flower development, and is subject to transcriptional regulation. Indeed, an analysis of transcript levels in pollinated and

unpollinated pistils of *Arabidopsis* revealed that pollination, precisely the transfer of pollen grains to a compatible stigma, triggers the metabolic switch that assists flower fertilization (Boavida et al., 2011). While flowers develop, the pistil sympatrically receives Suc from source tissues and stores it as transitory starch at the base of the carpel and alongside the style up to the base of the stigma (Hedhly et al., 2016). It is accepted that Glc-6-P/phosphate antiporter is responsible for carrying Glc across the outer membrane of plastids to support the progressive enlargement of starch granules (Dresselhaus and Franklin-Tong, 2013).

At the onset of anthesis, when flowers open, the starch that had accumulated at the base of the stigma is mobilized gradually to support the metabolic activity of the papillae. The papillae are cells with finger-like projections located atop the stigma whose function is to synthesize and release a multitude of different secretions, including mucilages, pectins (Edlund et al., 2004), arabinogalactan proteins (AGPs; Lee et al., 2008), and oils (Konar and Linskens, 1966), which combine to facilitate adhesion, hydration, and germination of pollen grains. Flowers with dry stigmas still secrete polymers in the cleft underneath the cuticle (Clarke et al., 1977). The material secreted by the papillae forms the foot that anchors the pollen grain and creates the hydrophilic microenvironment that attracts water and germinating factors toward the pollen. Cytological examinations of stigmas from diverse plant species revealed the presence of cells with dense cytoplasm, numerous mitochondria, endoplasmic reticulum, and Golgi vesicles, which are notable signs of an intense metabolic activity (Rosen and Thomas, 1970; Labarca and Loewus, 1972; Elleman et al., 1992). Direct evidence of stigma active metabolism has been provided by experiments performed with radioactively labeled sugars and amino acids. Indeed, when detached styles of lily (*Lilium longiflorum*) flowers were provided with labeled Glc, inositol, and Pro, radioactivity was revealed in the sugars (Gal, rhamnose, and arabinose) secreted by the stigma as well as in the carbohydrates that composed the cell wall of pollen grains germinated on top of those (Labarca et al., 1970). Therefore, the stigma not only provides a solid surface where pollen grains can land and adhere but also actively supports pollen germination and the initial phases of pollen tube protrusion. The stigmatic secretion of pectinases and expansins that loosen the cell wall of the grain and ease the protrusion of the tube also has been reported (Boavida et al., 2011).

The style is sympatrically connected to the flower; however, the strong transcriptional signal of *SUC8* and *SUC9* transporter genes measured in the cells of the transmitting tissue (Sauer et al., 2004) suggests that postphloem uptake of Suc is achieved both sympatrically and apoplasmically. The style fulfills similar secreting activity to the stigma (Rosen and Thomas, 1970; Sassen, 1974) and, in particular, it releases large amounts of AGPs in the extracellular matrix through which pollen tubes elongate. AGPs are proteoglycans

made of a short chain of dipeptide motifs and a large carbohydrate component that constitutes up to 90% of the total AGP molecule (Ellis et al., 2010; Knoch et al., 2014). Given that transitory starch stored along the style also is mobilized at anthesis, it most probably provides the backbones for the synthesis of the carbohydrate component of the AGP proteins, although this has not been proven directly. The sugar moiety of the AGPs triggers signaling responses that guide the pollen tube toward the ovule (Dresselhaus and Franklin-Tong, 2013; Jiao et al., 2017). Internalization of AGP carbohydrates by the pollen tube followed by carbohydrate relocation in the callus that pushes the nuclei forward in the direction of pollen tube growth also has been documented (Lee et al., 2009).

Sympatric connections with the ovule are present in the bundle sheet of funiculus and chalaza (Imlau et al., 1999; Werner et al., 2011), where transitory starch also accumulates (Hedhly et al., 2016). However, after fertilization, the developing embryo remains sympatrically isolated from the mother tissues, and further transport of photoassimilates is attained with specific transporters and carriers (Stadler et al., 2005; Werner et al., 2011; Chen et al., 2015b). Here, the expression of *SUC1*, *SUC8*, and *SUC9* genes has been detected (Sauer et al., 2004; Feuerstein et al., 2010), although the differential expression of the *SUC1* transporter observed in *Arabidopsis* ecotypes suggests functional redundancy. In the ovule, the synergid cells that surround the egg also are metabolically very active. Analysis of transcripts isolated from the synergids of *Torenia fournieri* revealed that 30% of the transcripts encode Cys-rich proteins of the secretory pathway. These include short-distance signaling peptides involved in sporophytic guidance, such as LURE and LORELEI that guide the pollen tube out the transmitting tissue inside the micropyle to fertilize the ovule (Jones-Rhoades et al., 2007; Capron et al., 2008; Okuda et al., 2009; Higashiyama and Yang, 2017). After double fertilization, large amounts of sugars are transported to the endosperm, where they accumulate as storage reserves for the embryo and are converted to starch, lipids, or proteins. As double fertilization marks the beginning of seed and fruit development, we refer to the numerous reviews that have been written on the topic (Weber et al., 1997; Chen et al., 2015b; Sosso et al., 2015). Nevertheless, the energy resources still present in the flower (petals, carpel, and filament) are promptly mobilized and repurposed. Indeed, experiments performed with radioactively labeled Suc applied to senescing daylily (*Heimerocallis* spp.) flowers revealed that up to 50% of the total Suc is rapidly and extensively retrotranslocated to young developing buds at a rate of 25 cm per hour (Bialeski, 1995; Sklensky and Davies, 2011). Reabsorption of unutilized nectar also has been observed; however, further research on this topic is clearly warranted.

Finally, it must be remembered that most of the transporters mediating sugar partitioning in flowers

have been identified and characterized in studies conducted in model plants such as *Arabidopsis*, *N. tabacum*, and *Petunia hybrida*. While these studies provide a general understanding of the processes occurring in model flowers, different taxa may have evolved slightly different sets of transporters and enzymes to distribute sugars among tissues and cells.

FLORAL AMINO ACIDS: SYNTHESIS AND FUNCTION

The assimilation of inorganic nitrogen (N) into organic compounds takes place primarily in the root, where ammonium is initially incorporated into Gln, Asn, Glu, and Asp (Coruzzi, 2003; Näsholm et al., 2009). Then, amino acids and small peptides are loaded into the vasculature of xylem and phloem and transported to sink organs. In flowers, symplasmic and apoplasmic partitioning of organic N follows the routes described previously for sugar distribution. Similarly, the transport of amino acids and small peptides across the plasma membrane of symplasmically isolated tissue requires the assistance of protein carriers. In fact, numerous amino acid transporters are expressed in pollen, which is symplasmically isolated from the remaining part of the flower (Foster et al., 2008). Current knowledge on amino acid transporters (Rentsch et al., 2007; Tegeder, 2012) and their expression and function in flowers (Tegeder and Rentsch, 2010) has been compiled in recent reviews. Therefore, we refer to those for a detailed description of floral transporters and provide an overview of the sites of their expression in Figure 2. In this section, we focus on the more recent discoveries regarding the synthesis and physiological functions of amino acids that take place in flowers.

In flowers, amino acids are utilized as building blocks for the synthesis of enzymes and structural proteins as well as precursors of N-containing secondary metabolites and signaling molecules. Despite the common belief that amino acids are transported exclusively to flowers from roots and leaves, evidence has emerged that their *de novo* synthesis also occurs in floral tissues. For example, studies conducted in petals of *P. hybrida* initially aimed to characterize the enzymatic reactions for the production of benzenoid volatile compounds brought about the identification of the enzymes prephenate amino transferase and arogenate dehydratase of the Phe biosynthetic pathway. The plastidial CATION AMINO ACID PHENYLALANINE TRANSPORTER also was identified in these studies (Maeda et al., 2010, 2011; Maeda and Dudareva, 2012; Widhalm et al., 2015), as well as the cytosolic Phe biosynthetic pathway (Yoo et al., 2013). Phylogenetic studies revealed that these enzymes and transporters also are present in plants that produce negligible amounts of aromatic volatile compounds, where they may provide the Phe that is incorporated into floral enzymes and structural proteins.

Recently, it was shown that the Asn biosynthetic pathway also is active in flowers. Indeed, *ASPARAGINE*

SYNTHETASE1 (ASN1), encoding for the enzyme that transfers amide N from Gln to Asn, releasing Asn and Glu, displays a high level of expression in flowers. *ASN1* transcription increases throughout the lifespan of *Arabidopsis* flowers, starting from the stages that precede anthesis up to pollination and early embryo growth (Le et al., 2010). Concomitantly, genes of the Asn metabolism also are up-regulated and floral Asn content also rises (Gaufichon et al., 2017). Having a high N:C ratio (2:4), Asn is an efficient carrier and storage compound of N. Thus, enhancing the supply of Asn during flower development is an effective strategy to store reserves that will be used to generate energy during ovule maturation and embryo growth (Gaufichon et al., 2017). In developing pollen grains, hydrolysis of Asn by asparaginase A1 and B1 (*ASPGA1* and *ASPG1*) releases ammonium, which is later reassimilated into Gln, the precursor for the synthesis of other amino acids (Ivanov et al., 2012; Guan et al., 2015).

De novo synthesis of Pro also occurs in flowers. Pro is found as a free amino acid in pollen grains and nectar and as a component of proteins of the pollen coat (Lamport et al., 2011; Biancucci et al., 2015). Free Pro in pollen grains is thought to act as an osmotic protectant toward desiccation (Chiang and Dandekar, 1995), while Pro in nectar may serve as a propellant for the lift phase of the flight of insect pollinators (Carter et al., 2006). In addition to the transcriptional activation of the floral Pro transporter (Schwacke et al., 1999), high levels of Pro in flower are obtained via up-regulation of the pyrroline-5-carboxylate synthetase enzyme, which catalyzes the conversion of Glu to pyrroline-5-carboxylate (Kavi Kishor et al., 2015). Interestingly, Pro is the most abundant amino acid in nectar, regardless of the evolutionary distance between plant species (Carter et al., 2006; Nepi et al., 2012). This suggests that Pro may have a relevant ecological role for rewarding animal pollinators (Carter et al., 2006).

As we mentioned briefly above, amino acids and small peptides are synthesized in large amounts by the cells of the transmitting tissue and ovule. These include numerous small secreted proteins of unknown function (Jones-Rhoades et al., 2007) and Cys-rich proteins such as the LUREs, which are involved in the female gametophytic guidance of pollen tube attraction (Okuda et al., 2009; Takeuchi and Higashiyama, 2012; Higashiyama and Yang, 2017). γ -Amino butyric acid (GABA), a non-proteinogenic amino acid that contributes to pollen tube growth and attraction, also is produced in the cells of the transmitting tissue. GABA is synthesized in the cytosol by the activity of GABA Glu decarboxylase and degraded in the mitochondria by the activity of the GABA transaminase (Fait et al., 2008), the latter being encoded by the *POLLEN-PISTIL INCOMPATIBILITY2 (POP2)* gene. The coordinated activity of Glu decarboxylase and transaminase along the pistil creates a gradient of GABA concentration that guides the pollen tube toward the ovule. The disruption of this gradient observed in the *pop2* mutants negatively influences the success of fertilization (Palanivelu et al., 2003).

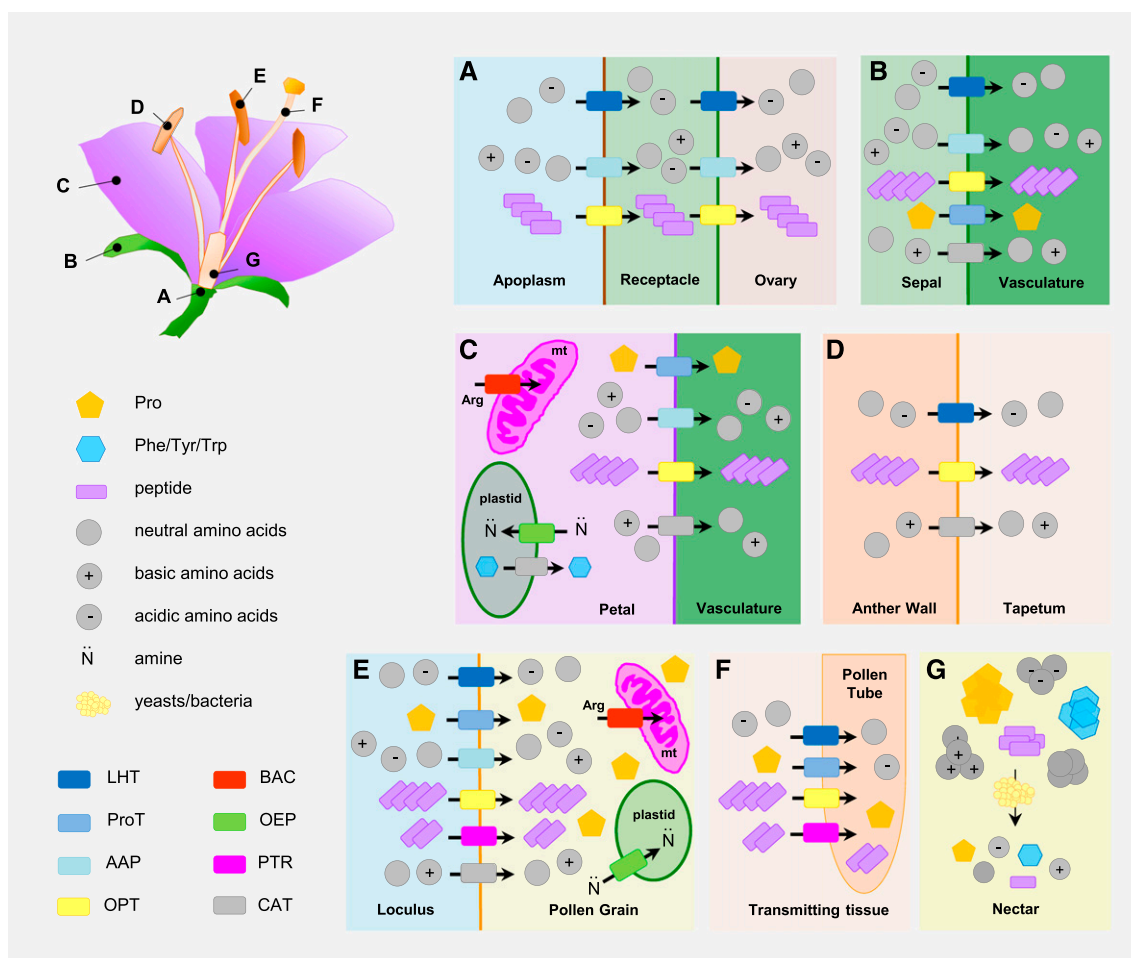


Figure 2. Partitioning of amino acids, amides and peptides in floral tissues. A, In the floral receptacle, transporters of the families of Lys His Type (LHT), Amino Acid Permease (AAP), and Oligo-Peptide Transporters (OPT) mediate the uptake of neutral (gray circles) and acidic (gray circles with a minus sign) amino acids, neutral and basic (gray circles with a plus sign) amino acids, and tetrapeptides and pentapeptides (purple rectangles), respectively. Expression of the same set of transporters at the base of the ovary suggests that they also mediate the uptake of amino acids and peptides in the ovary. B, In sepals, LHT, AAP, OPT, Proline Transporter (ProT), and Cationic Amino Acid Transporter (CAT), the latter mediating the transport of both neutral and basic amino acids, are expressed in the cells surrounding the vasculature, where they presumably mediate xylem-phloem exchange and/or retrieval of leaked organic nitrogen. C, In petals, plastidial CATs export Phe, Tyr, and Trp to the cytoplasm, while Outer Envelope Plastidial Protein (OEP) imports amines (N) in the stroma. Mitochondrial Basic Amino Acid Carriers (BAC) import Arg in the mitochondrion (mt). The expression of LHT, AAP, OPT, ProT, and CAT also was detected in the cells surrounding the petal vasculature. D to F, Apoplasmically isolated tissues such as the cells of the tapetum (D), developing pollen grains (E), and pollen tubes (F) express a vast majority of different transporters. PTR-Like Peptide Transporter (PTR) in pollen grains and pollen tubes mediates the uptake of dipeptides and tripeptides. G, In nectar, the pool of amino acids and peptides is depleted by colonizing yeasts and bacteria.

CHEMICAL MODIFICATIONS OF NECTAR BY COLONIZING MICROORGANISMS

A large proportion of floral primary metabolites is channeled into the nectar, which is a water-based secretion of sugars, amino acids, lipids, and secondary metabolites including volatile organic compounds. The ecological function of nectar is to reward animal pollinators; therefore, its chemical composition differs among plant species, as it evolved to enhance floral visitation by pollinators and reduce the losses imposed by nectar thieves and florivores (Nicolson and

Thornburg, 2007). In the last decade, research on nectar had seen a renaissance brought about by targeted nectary transcriptomics and metabolomics (Kram et al., 2009; Noutsos et al., 2015) and the discovery of the mechanisms regulating nectar secretion, such as transporters (Lin et al., 2014), hormones (Wiesen et al., 2016), and transcription factors (Liu et al., 2009). These recent discoveries on nectar biology have been compiled in exhaustive reviews (Nepi, 2017; Roy et al., 2017). Hence, we exclusively focus discussion here on the chemical modifications of

nectar primary metabolites that are induced by floral microbes.

The inoculation of floral nectar with yeasts, bacteria, and fungi takes place during floral visitation by pollinators and florivores (Belisle et al., 2012; Anderson et al., 2013; Aleklett et al., 2014). Given this horizontal transfer from the animals to the flowers, the epiphytic communities of microbes of the anthosphere differ from the microbial species of the rhizosphere and phyllosphere. Moreover, in a single flower, organ-specific species of microbes grow separately on sepals, petals, and nectaries (Junker et al., 2011; Aleklett et al., 2014). The microbial communities that colonize the anthosphere depend upon the combinatorial effects of plant genotype and the ability of the microbes to competitively exploit the carbon and nitrogen resources present in floral tissues and exudates (Junker and Keller, 2015; Pozo et al., 2016). Without any doubt, nectar offers an ideal habitat to support microbial growth, with sugars providing the substrate for microbial fermentation and amino acids contributing the required nitrogen. That microorganisms can modify the composition of nectar became evident when the uniformity of nectar sugar content from plants grown in a glasshouse was contrasted with the variability of field-grown plants of the same species (Canto et al., 2007).

Yeasts are the most common group of microbes found in flowers, with individuals of the genus *Metschnikowia* being predominant (Pozo et al., 2016). Following yeast inoculation, Suc-dominant nectar shows a shift toward Fru or Glc that is proportional to the rate of microbial growth (Herrera et al., 2008; de Vega and Herrera, 2013). This implies that yeast cells hydrolyze Suc to Fru and Glc but then preferentially utilize one of the two hexoses. Lastly, highly concentrated Suc-dominant nectar (typically dry nectar) prevents yeast growth (Schaeffer et al., 2015).

Floral bacteria received far less attention than yeasts, until pyrosequencing and culturing techniques made it possible to identify the strains that grow on flowers (Fridman et al., 2012; Junker and Keller, 2015). Similarly to yeast, bacterial growth also correlates negatively with total sugar content and/or Suc content in nectar but usually correlates positively with the proportion of the monosaccharide Fru (Vannette et al., 2012; Lenaerts et al., 2016; Vannette and Fukami, 2016). In addition, bacterial isolates of the genus *Acinetobacter* can convert Suc into a mucous matrix of polysaccharides (Fridman et al., 2012; Fig. 1F).

Although the chemical modifications of nectar by yeasts and bacteria look very similar, the effects on pollinators' preferences frequently diverge. The presence of bacterial colonies on floral nectar often has negative effects on pollination success (Vannette et al., 2012) and insect visitation rate (Junker et al., 2014). Conversely, nectar inoculated with yeasts results in increased seed set of bumblebee- and hummingbird-pollinated flowers (Vannette et al., 2012) as well as enhanced pollen donation via an increment in the pollinator foraging rate (Schaeffer and Irwin, 2014). Nectar modifications

of the amino acid content represent a small and under-investigated field of research. However, early observations suggest that bacteria may cause a reduction of specific amino acids such as Thr and Val (Lenaerts et al., 2016).

PRIMARY METABOLITES AND POLLINATORS' PREFERENCES: IMPLICATIONS FOR SEED AND FRUIT SET

Seeds and fruits develop following a successful process of plant reproduction, which ultimately depends upon the proper development of the stamen and pistil coupled to successful pollination and fertilization events. Since floral primary metabolism is deeply intertwined with flower development, aberrant floral metabolism can form a prezygotic barrier that prevents mating and fertilization. For example, mutations that impair the metabolic processes of pollen and egg maturation, or that interfere with pollen germination, tube growth, and guidance (both in the pollen and in the transmitting tissue), impede pollination and fertilization. As detrimental as these mutations seem to be, they are favorably exploited in agriculture, for example, in the generation of male-sterile plants for hybrid breeding (Goetz et al., 2001; Hirsche et al., 2009). Neither as equally well exploited nor understood is the influence that primary metabolism of nectar and pollen has on pollinators' preferences. Animal-pollinated plants depend upon insects, birds, and mammals as go-betweens for pollen transfer. Therefore, their flowers produce signals (color and scent) to attract pollinators and nectar and pollen rewards to preserve floral fidelity. However, as animal pollinators display preferences for quality and quantity of the reward, their choice to pollinate, or not pollinate, a flower affects fertilization and ultimately may reduce seed and fruit set (Hanley et al., 2008; Carruthers et al., 2017). Insect preferences for flower color and odor have been largely investigated, and a few studies describe the link between plant genotype and animal behavioral response (Bradshaw and Schemske, 2003; Hoballah et al., 2007; Klahre et al., 2011; Owen and Bradshaw, 2011; Yuan et al., 2013; Amrad et al., 2016; Sheehan et al., 2016). Instead, traditional single-gene studies to ascertain pollinators' responses to changes in floral primary metabolism have been delayed by the late discovery of the *SWEET9* transporter as a mediator of nectar secretion (Lin et al., 2014), and so far, no transporter has been identified that controls the secretion of amino acids in floral nectar (Zhou et al., 2016).

However, a growing body of evidence shows that primary metabolites of pollen and nectar are shaping the interaction between plants and their pollinators through pollination syndromes (Johnson et al., 2006; Weiner et al., 2010). That pollinators' choices can affect nectar chemical composition has been evident since the early studies of Baker (1977), who observed that nectars are richer in amino acids if they are the only source of

OUTSTANDING QUESTIONS

- In which measure is floral central metabolism self-sustained or supported by carbon and nitrogen fluxes from source tissues?
- How is floral central metabolism transcriptionally and posttranscriptionally regulated?
- What is the influence of environmental cues on floral central metabolism and how does this influence flower-pollinator interactions and ultimately fruit and seed yield?
- What regulates the secretion of amino acids and proteins into nectar?
- How do developmental changes in the metabolism of one floral organ influence the metabolism of others?

protein-building material of the pollinators that feed on those. A similar conclusion was reached in more recent studies, which showed that pollinators' preference had the most important effect on amino acid content in nectar, while taxonomic plant groups had a weakly significant effect (Petanidou et al., 2006). For example, Phe- and GABA-enriched nectars are commonly represented in plants pollinated by long-tongued bees and flies independently of their taxonomic group. As GABA functions as an inhibitory neurotransmitter of insects' nervous systems, GABA-rich nectar may be preferred by pollinators for its calming effect (Nepi et al., 2012). The volume of secreted nectar also varies in response to pollinators' preferences, and this happens more rapidly than initially thought. In fact, nectarless plants among bumblebee-pollinated plants appear in a time as short as 10 generations (Gervasi and Schiestl, 2017). Pollen chemistry may have a weaker influence on insect preferences, as polylectic bees, which collect pollen from plants of different species, appear to disregard the chemical composition of pollen despite the beneficial effect that blends of amino acids and polypeptide have on colony fitness (Vanderplanck et al., 2014). However, this may not hold true for oligolectic bees, for which pollen chemistry drives evolutionary host shifts (Vanderplanck et al., 2017). These studies and many others present in the literature demonstrate that pollen and nectar chemistry influence pollinators' choices but that the interaction is specific between a plant and its pollinators. In addition, it should be considered that very little is known about the transcriptional and posttranscriptional regulation of floral primary metabolism and how metabolism changes in

response to environmental fluctuations (e.g. temperature and rainfall), which, ultimately, also may affect pollination and fertilization success (Gallagher and Campbell, 2017).

CONCLUSION AND PERSPECTIVE

The current knowledge on flower central metabolism is largely inferred from experiments performed in leaves, and it is certainly skewed by the generally accepted prejudice that flowers are entirely heterotrophic. However, we have seen that cycles of carbohydrate hydrolysis and resynthesis are functional and activated in flowers and they are the routes for the biosynthesis of amino acids and peptides. Therefore, there are many unsolved issues concerning floral central metabolism and the implications for flower development, pollinators' choices, and seed and fruit set (see Outstanding Questions). The investigations on floral metabolism have certainly been made difficult by the redundant number of transporters of sugars and amino acids that, on one side, warrant pollination and fertilization success to the plant but, on the other side, add complexity to a deep understanding of single-gene function. In addition, studies performed on knockout mutants focused on the leaf or root phenotypes and only rarely on flower physiology. Largely underinvestigated is the measure by which floral metabolism depends on *de novo* biosynthesis versus intake of sugars and amino acids. There also is a lack of knowledge concerning the spatial distribution of metabolites in floral tissues and their physiological and ecological significance. In addition, analysis of metabolites often is conducted on individual flowers (or inflorescences) that are regarded as integral floral units, despite the fact that, in no other plant organ as much as in flowers, tissue compartmentalization underlies function. In addition, floral metabolism dramatically changes multiple times in response to development and, presumably, to environmental cues (Lauxmann et al., 2016). Recent advances in analytical techniques that couple metabolomics with histological imaging can now be employed to shed light on this topic (Dong et al., 2016). Techniques of rapid sequencing coupled with genome-wide association studies and quantitative trait locus analysis promise to speed the discovery rate of genes and the network of genes that control plant-pollinator interactions (Clare et al., 2013; Sedio, 2017). Moreover, with the use of the CRISPR/Cas9 technology, the link between a plant genotype, floral chemotype, and pollinator response will likely be possible in nonmodel organisms.

In conclusion, floral biology has become an intense area of study for scientists with expertise in the most disparate disciplines encompassing plant developmental biology, molecular biology, metabolomics and genomics, entomology, and behavioral biology. As described above, recent advances, in particular in understanding sugar transport and pollen tube growth, and in better characterizing the chemical composition

of nectar provide a firm foundation to comprehend the link between floral metabolism and function. That said, it is our belief that today, as never before, a more holistic and collaborative approach is demanded to shed light on the mechanisms that control floral metabolism and the implications for flower development and plant-pollinator interactions.

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