From flowers to seeds: how the metabolism of flowers frames plant reproduction

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Flowers are characterized by a plenitude of primary and secondary metabolites and flower-specific biosynthetic pathways that all concur to promote plant reproduction and the initial stages of embryo development. The floral secondary metabolites of flowers contribute to scent and colour, which are used by flowers to attract pollinators. Besides, many metabolites responsible for the conferral of colour also serve as photo-protectants towards the damaging effects of UV solar radiation. The whole metabolism of flowers is sustained by a network of primary metabolites that provide metabolic precursors for the biosynthesis of secondary metabolites and support flower development. Moreover, many primary metabolites are channelled into nectar, the food of pollinators. However, this complex metabolic network is susceptible to environmental constraints such as heat and drought, which can hamper plant reproduction by destabilizing the whole metabolism of flowers. Here, we provide a short overview of the different metabolic pathways of flowers and how they support pollination and fertilization.

Floral pigments: biosynthesis and function

Colour in flowers is obtained through the synthesis and accumulation of pigments. Still, not all flowers accumulate pigments, and not all pigments are visible to our eyes. Indeed, there are flowers that accumulate considerable amounts of different pigments, yet they look entirely white. While leaves and roots also synthesize and accumulate pigments, flowers exceed all other plant organs for variety and amount. To facilitate discussion about these numerous (in the order of a few hundreds of thousands) compounds the following classification based on common chemical properties and biosynthetic pathways is often utilized.

Flavonoids, including their chemically modified derivatives, are one of the better-studied classes of flower pigments. Their biosynthetic pathway is very well characterized, and the genes and transcription factors regulating their expression are also well known in many plant species. The amino acid phenylalanine (Phe) is the precursor of all flavonoids, whose synthesis occurs in the cell's cytoplasm, and then accumulated in the vacuole. Their colour varies from white and yellow (flavonols) to red, purple and blue (anthocyanins) and it changes depending upon the pH (Figure 1A and B). In flowers, flavonoids are primarily found in petals and pollen, where they confer protection to UV radiation

in addition to luring pollinators (Figure 2 provides an overview of floral parts and their names).

Carotenoids also constitute a large class of floral pigments. They are synthesized in the chloroplast along the isoprenoid pathway, which produces volatile compounds in addition to carotenoids. In flowers, carotenoids are often found in petals (Figure 1C) and the tapetum (the tissue that gives origin to pollen grains), where they are stored in special plastids called chromoplasts. In addition to flowers, other plant organs accumulating large amounts of carotenoids are fruits (e.g., tomatoes) and roots (e.g., carrots).

Betalains is a small class of flower pigments found only in the plants of the order of Caryophyllales, which includes, among others, cacti, carnations, beets and amaranth. Betalains are synthesized in the cytoplasm starting from tyrosine (Tyr) and later transported to the vacuole where they are stored. Betalains vary in colour from yellow to purple and deep red (Figure 1D).

Flowers can accumulate pigments from only two of these chemical groups, with betalains and flavonoids being mutually exclusive. Still, the combination of different classes of carotenoids with flavonoids, or betalains, results in an astonishing array of colour combinations that serve, among others, to attract pollinators. Indeed, the different combinations of flower colours often mirror the animals' visual acuity, preferences and instinct. Moreover, given that insects and humans perceive colour differently, assembling

Figure 1. Photographs of flowers of (A) white and (B) red petunia, (C) tomato and (D) succulent plant whose colour is obtained with the accumulation of pigments of the classes of flavonoids, anthocyanins, carotenoids and betalains, respectively

pigments from different chemical classes can result in attractive combinations for the animal pollinators which sometimes we cannot even see. Floral pigments also protect gametes from the damaging effects of UV radiation, with flavonoids having a principal function as phytochemical sunscreens. Therefore, any impairment in the biosynthesis or accumulation of floral pigments may result in reduced plant fitness either because animal-mediated pollination is less efficient or because gametes are less viable, with the second scenario having detrimental consequences for the reproductive success of both selfing and outcrossing species.

Finally, it is relevant to mention here that in flowers of certain species, colour is obtained with microscopic structures that bend light wavelengths at different angles. These colours that do not depend on the presence of pigments are called "structural colours" to differentiate them from "chemical colours" obtained via synthesis and accumulation of chemical compounds.

Floral scent: biosynthesis and function

The scent of flowers is composed of a blend of lipophilic and slightly polar molecules with low vapour pressure, hence highly volatiles also at ambient temperature, a characteristic that facilitates scent diffusion in the surrounding environment. As such, pollinators can easily locate a flower by following its fragrance. These molecules altogether are referred to as volatile organic compounds (VOCs), although they belong to distinct chemical classes as we describe here.

Terpenes, and their oxygenated forms, the terpenoids, are the largest class of VOCs emitted by plants. They are all composed of one or multiple units of a five-carbon compound called isoprene (Figure 3). Two major pathways contribute to the synthesis of terpenes, the mevalonate (MVA) pathway in the cytosol and the methylerythritol 4-phosphate (MEP) pathway in the plastid. In addition, enzymes of the class of phosphatases also contribute to the synthesis of small terpenoids in the cytosol, while enzymes located in the endoplasmic reticulum and mitochondria are responsible for additional chemical modifications. Typically, we perceive terpenoids as a pleasant smell, with the molecules responsible for the scent of lemons and jasmine flowers being the most commonly utilized terpenoids also in the industry of food and cosmetics.

Benzenoids, phenylpropanoids and phenylpropanoid-related compounds are synthesized from the amino acids Phe and tyrosine (Tyr) which are produced along the shikimate and chorismate pathways. VOCs included in these groups are the molecules
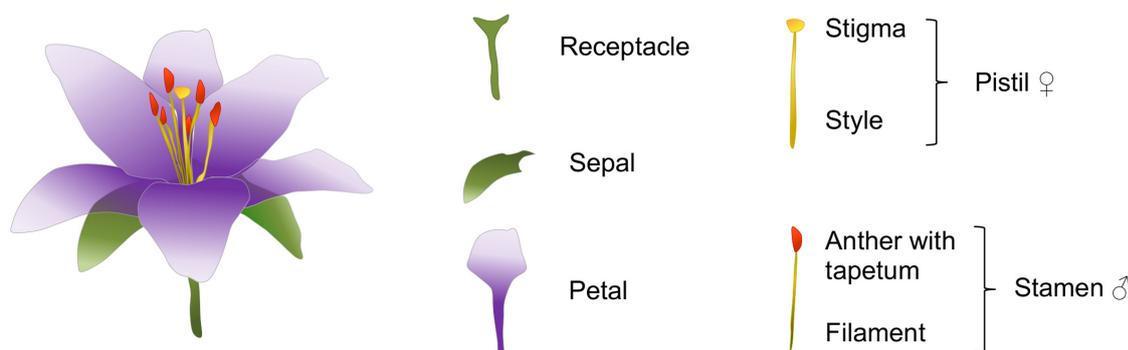
Figure 2. Schematic representation of a flower and flower parts
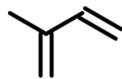
Figure 3. Molecular structure of isoprene, the basic unit of terpene and terpenoids

conferring the typical smell to petunia flowers, such as benzaldehyde and phenylacetaldehyde, or the eugenol, typically extracted from bay leaves and cinnamon. Many benzoic acid derivatives serve as defensive compounds and may, or may not, be volatile.

Green leaf volatiles (GLVs) are small aliphatic compounds obtained by the cleavage of fatty acids of the plasma membrane and the endomembrane system by the activity of lipoxygenase enzymes. The large majority of these compounds are released by leaves after mechanical damage, for example, when leaves are chewed by herbivores. Indeed, GLVs constitute an indirect mechanism of plant defence that attract predators of herbivores. Although GLVs are mainly released by leaves, flowers also emit a wide range of compounds belonging to this class.

Finally, flowers may also emit N- and S-containing compounds that are most likely derived from amino acids catabolism. For example, S-containing compounds confer a typical pungent smell to flowers of plants of the cabbage family, while N-containing compounds are typically emitted by flowers that smell like rotten meat, which beetles and flies pollinate.

All the compounds described above may also undergo chemical modifications such as acylation, methylation and hydroxylation, which contribute to modify volatility or olfactory properties.

Usually, 20–60 compounds are blended together to form a flower scent, with differences depending upon species, physiological status and age of flowers and the preferences of animals that pollinate them. Indeed, flowers pollinated by mammals or birds, which make large use of visual cues to locate flowers, usually emit little or no scent. Conversely, insect-pollinated flowers usually emit abundant and different classes of volatiles. Among insect-pollinated flowers, plant species visited by nocturnal moths emit VOCs at night from white or pale-yellow flowers which glow in the moonshine. Conversely, flowers pollinated by diurnal insects emit VOCs in the late morning or mid-day when pollinators are actively foraging for nectar and pollen. In flowers of all species, VOCs emission halts soon after pollination, as floral metabolic recourses are channelled towards the formation of seeds and fruits. Indeed, synthesis and emission of VOCs are well regulated at the level of transcription by a battery of transcription factors that integrate across pathways of colour and scent production, as well as transporters that accumulate molecular precursors of VOCs in specific cells and regulate the final rate of VOCs emission.

Nectar

In obligatory outcrossing species, which strictly need pollinators for reproductive success, colour and scent frame plant reproduction as these metabolites function as signals for the attraction of pollinators, which, in their turn, act as carriers for pollens by transporting it from one flower to another. However, insect pollinators reach out to flowers to gather food from nectar and pollen, whose presence is signalled by scent and colour. Nectar is a sugar-based water solution, predominantly composed by sucrose, fructose and glucose which are present in different proportion depending upon the species. Minor amounts of other metabolites and vitamins are also present in nectar. Pollen, instead, is a primary source of amino acids or lipids.

Integration of floral traits

The metabolic traits described previously, that are colour, scent and metabolic composition of nectar, are integrated to optimize the pollination needs of a given species (Figure 4). We have already mentioned that insect-pollinated flowers typically emit a blend of multiple VOCs, a trait found in flowers with a particular shape and colour, which also meet the needs of those pollinators. For example, bee-pollinated flowers are usually blue and violet, butterfly-pollinated flowers show a wide array of colours, and sexually deceptive develop flowers that resemble for shape, colour, and scent the female counterpart of their male pollinators. Conversely, plants that self-pollinate do not invest in colour and fragrance, which would not find use in those flowers, but instead develop small flowers typically with no scent and a few pigments. Optimized integration of metabolic and developmental pathways is needed to achieve this level of perfection, for which transcription factors and plant growth regulators play relevant roles.

Flower primary metabolism

Now that we described the compounds responsible for the production of colour and scent, we will focus on the overall metabolism of flowers which supports the synthesis of the compounds described earlier and the processes that provide for the initial stages of flower and seed set. It is said that flowers primarily receive carbohydrates and amino acids from other organs, for example, leaves and roots, where these metabolites are synthesized. However, sepals and the elongating stems
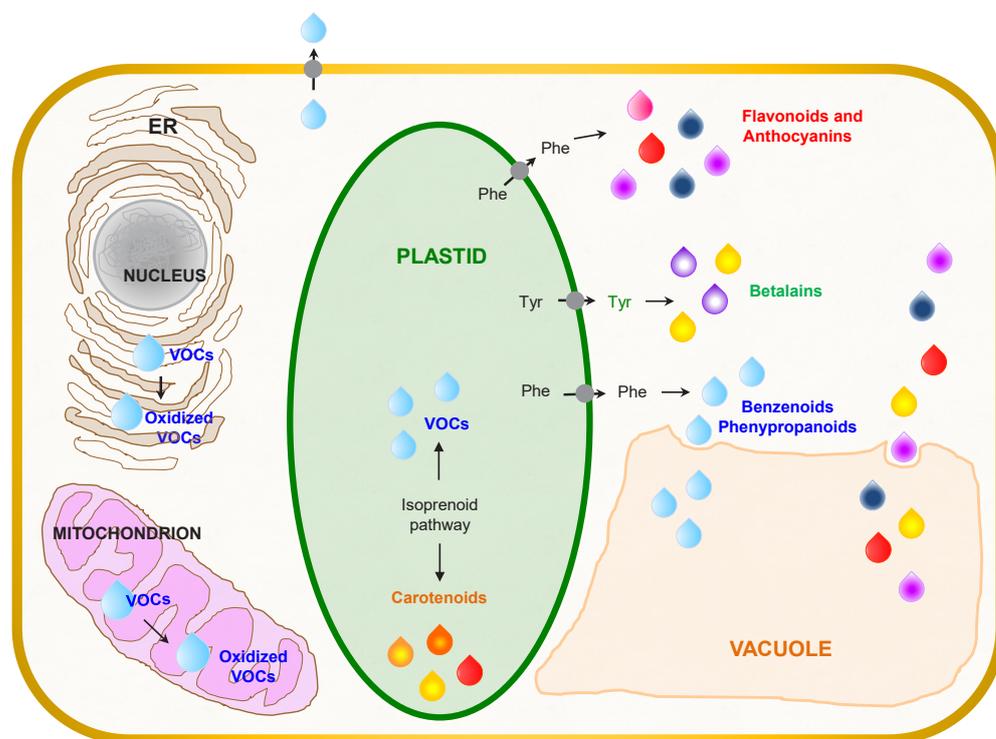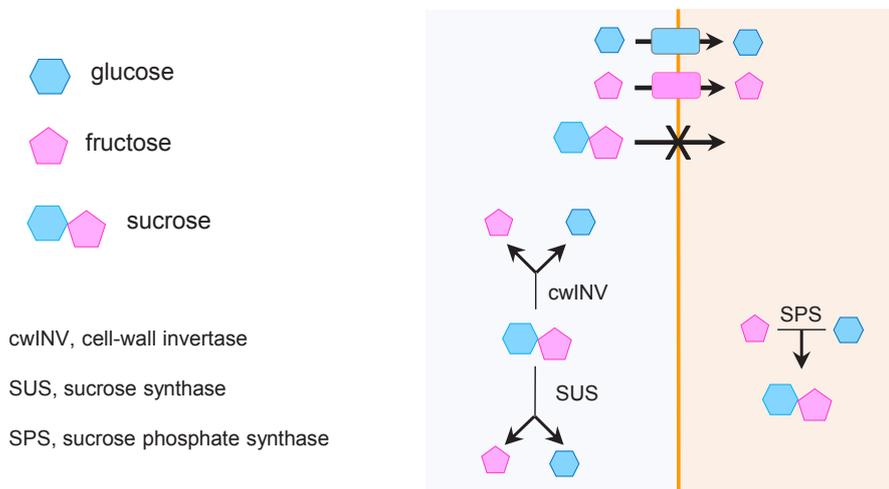
Figure 4. Schematic representation of a hypothetical cell of a petal and major pathways for the biosynthesis of pigments and volatile organic compounds (VOCs)

of many flowers also contribute to these resources. Once the metabolites reach the flower receptacle (i.e., the tissue to which all the different parts of a flower are attached), they have to be distributed to the other flower tissues. To warrant that proper allocation of resources to flower tissues is achieved timely and effectively, many redundant transporters with different degrees of affinities for the metabolites of the same chemical class are expressed in flowers. Thus, should a defective transporter appear due to a genetic mutation or developmental constraints, the presence of proteins with similar affinities will ensure that allocation of metabolites is still possible and pollination and fertilization attained. Indeed, proper transport of carbohydrates to anthers and ovary is needed to sustain male and female gametes' development. Gradients of sugars also support the elongation of pollen tubes from the stigma to the ovule. Finally, soon after fertilization, carbohydrates are required to support the development of embryos and seeds. However, when the external pressure on flower metabolism is strong enough, biosynthetic pathways are halted or heavily impaired. This happens, for example, when heat and drought occur at the time of flowering.

Pollen and pistil are both susceptible to environmental stresses, with invertase and sucrose synthase enzymes being the primary targets. These enzymes break down sucrose into the monosaccharides glucose and fructose (Figure 5), which are then transported inside the cells and utilized as sources of energy or metabolic precursors to synthesize other compounds. Once heat and drought stress impair these proteins functionality, the whole flower's metabolism is negatively impacted.

More recently, scientists have started noticing that flower secondary metabolism is also altered when plants are exposed to heat and drought at the time of flowering. Indeed, the chemical composition of the floral bouquet emitted by flowers after exposure to stress, the secretion of nectar, and the composition of floral pigments are all affected. As researchers continue to investigate the impact of abiotic stresses on flower metabolism, they will gain further knowledge of the consequences of altered flower metabolism on crop production and the interaction between flowers and pollinators. Gaining this knowledge is needed to implement practices that warrant plant reproduction success under stress and minimize climate change effects on crop yield. ■

Figure 5. Schematic representation of the principal mechanism of sucrose uploading and partitioning in floral tissues. Cell wall invertase enzymes hydrolyse sucrose to glucose and fructose, which can be transported across the plasma membrane by specific transporters. Inside the cell, sucrose phosphate synthases use glucose and fructose to synthesize sucrose.

Further readings

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