SURVEY OF PLANT PIGMENTS: MOLECULAR AND ENVIRONMENTAL DETERMINANTS OF PLANT COLORS

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It is difficult to estimate the importance of plant pigments in plant biology. Chlorophylls are the most important pigments, as they are required for photosynthesis. Carotenoids are also necessary for their functions in photosynthesis. Other plant pigments such as flavonoids play a crucial role in the interaction between plants and animals as visual signals for pollination and seed scattering. Studies related to plant pigmentation are one of the oldest areas of work in plant science. The first publication about carotenoids appeared in the early nineteenth century, and the term "chlorophyll" was first used in 1818 (Davies, 2004). Since then, the biochemical structure of plant pigments has been revealed, as have the biosynthetic pathways for the major pigments that provide a useful variety of colors to blossoms and other plant organs. There is widespread interest in the application of molecular methods to improve our knowledge of gene regulation mechanisms and changes in plant pigment content. Genetic modification has been used to alter pigment production in transgenic plants. This review focuses on flower pigmentation, its biochemistry and biology. It presents a general overview of the major plant pigment groups as well as rarer plant dyes and their diversity and function in generating the range of colors observed in plants.

Key words: Flower and fruit colors, co-pigmentation, plant dyes, pigment groups.

INTRODUCTION

Plants owe their appearance in part to colorful substances consisting of biochromes, which either absorb or reflect light of varying wavelengths. The absorbed light is dissipated in the pigment, and the reflected light is visible as color. The colors are also the result of a mix of residual wavelengths that are reflected. The human eye is capable of seeing light within the range of 400–700 nm (Fig. 1), which corresponds to the colors of the rainbow identified by Newton: violet, indigo, blue, green, yellow, orange and red (Heldt, 2005).

The secrets of plant pigmentation are among the oldest interests of botanists. The first publications on the subject of carotenoids came in the beginning of the nineteenth century, and the term "chlorophyll" was first used in 1818 (Davies, 2004). The variety of colors and easy identification of mutations resulted in flowers becoming one of the most popular genetic research subjects, starting with Mendel's pioneering experiments on pigment inheritance in pea and leading to the identification of mobile colored elements called transposons in corn, for which Barbara McClintock received the 1983 Nobel Prize for Physiology and Medicine (McClintock, 1983). Research into pigments has led to many breakthrough discoveries in the field of molecular biology; for example, the first transcription factor was identified when anthocyanins were studied, and the first isolation of cDNA in plants came from anthocyanin research (Holton et al., 1993).

Pigments responsible for the appearance of colors in higher plants are classified in several groups: chlorophylls, carotenoids (carotenes, xanthophylls), flavonoids (chalcones, anthocyanins, flavones, flavonols) and betalains (betaxanthin, betacyanin) (Tab. 1).

In prokaryotic (Cyanobacteria) and eukaryotic cells (cyanelles, red algae) there are water-soluble photosynthetic pigments called phycobilins, which are found in the cytoplasm or in the stroma of the chloroplast (Marsac, 2003). There are two classes of phycobilins – phycocyanin and phycoerythrin (Tab. 1). The bluish pigment phycocyanin is found in cyanobacteria and gives them the common name "blue-green algae", and the reddish phycoerythrin is found only in red algae, *Rhodophyta* (Beale, 2003). These pigments are covalently bound to the proteins forming phycobiliproteins and are organized into

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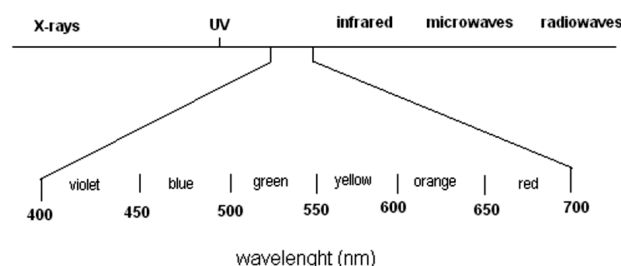
Fig. 1. Visible spectrum of electromagnetic radiation.

a supramolecular complex called the phycobilisome (Beale, 2003). Two major phycobilins are phyco-cyanobilin and phycoerythrobilin, which absorb light at 620 nm and 560 nm, respectively (Mimuro, 2002).

Numerous data on known pigments have shown the richness and variety of plant colors. So far approximately 600 carotenoids, 7,000 flavonoids and more than 500 anthocyanins have been identified (Davies, 2004).

The main pigment groups are located at various sites in plant organs. Flavonoids appear in almost all tissues; carotenoids, for example, are present in leaves, roots, seeds, fruits and flowers. Some pigments such as anthocyanins or chlorophylls have a specific cellular or subcellular location. Anthocyanins are usually found in epidermal cells of flower petals, whereas chlorophylls and carotenoids are in plastids in subepidermal photosynthetic cells of leaves. Like anthocyanins, betalains are water-soluble and appear in vacuoles (Davies, 2004).

Anthropocentrism leads us to believe that flowers were created for our pleasure, to delight our eyes with colorful tones, and for artists as an inspiration for creation in the different arts. However, neither the brilliant colors of plants nor their intoxicating aromas were designed for people. All the characteristics that form the beauty of plants are actually information addressed to animals, insects, birds and bats, which visit plants in search of food. In these almost discourteous visits, they bring grains of dust effecting the pollination and thus the continuation of the species. For this reason, plants must be visible from a distance for airborne pollinators, and must stand out from their surroundings. Plants therefore developed, through evolution, a set of features (pigmentation, fragrance) that can be compared to aggressive advertising campaigns effectively targeting pollinators. The first plants, which appeared in the Mesozoic era, were probably cream-colored and only with time developed sharper colors, increasing the concentration of various pigments (Raven, 2005).
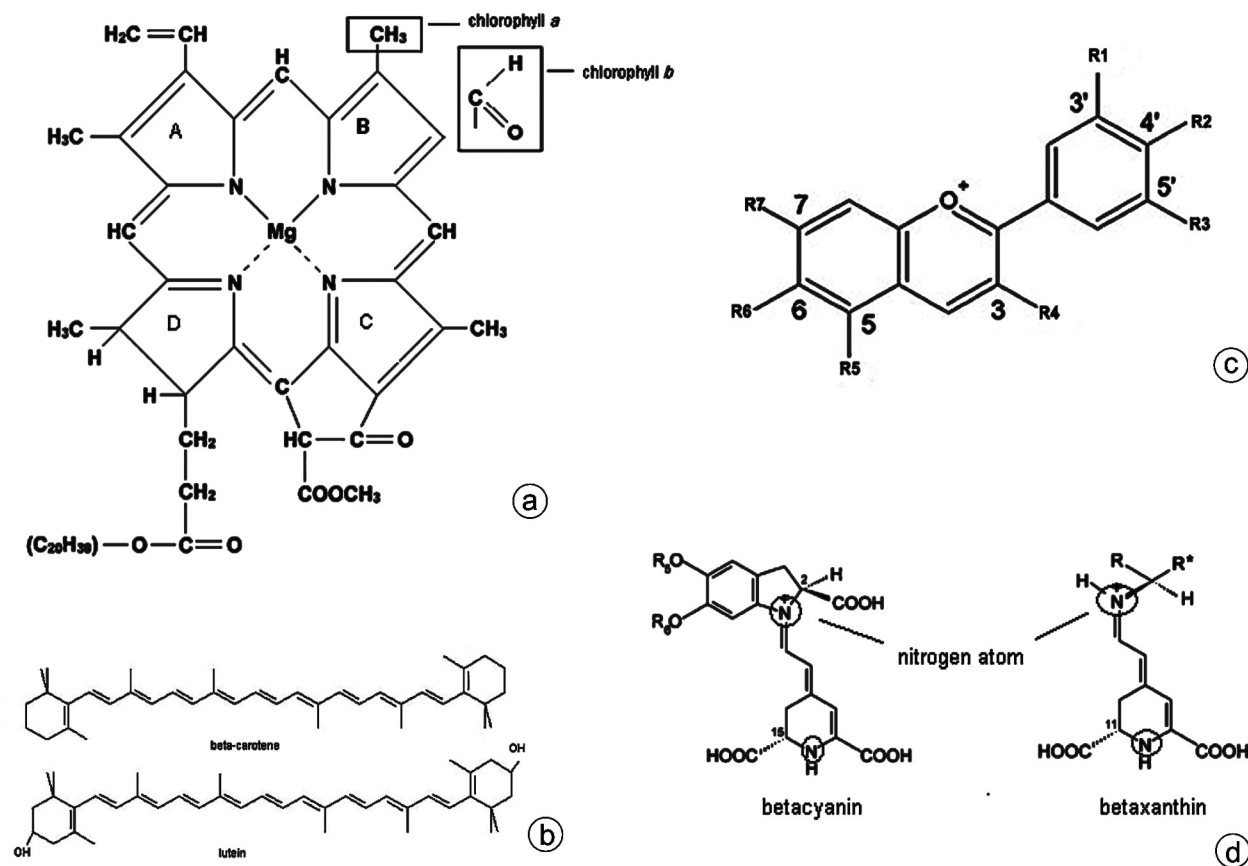
GREEN – CHLOROPHYLLS

The most eye-catching and widespread plant pigment is chlorophyll, which appears in leaves and other green parts of plants exposed to light. Chlorophyll is located, together with carotenoids, in chloroplasts, and there its physiological function is to absorb light energy and use it in photosynthesis. There are two kinds of chlorophyll in higher plants: green-blue chlorophyll a, and green-yellow chlorophyll b. Their amounts depend on the species of plant, light conditions, and the availability of minerals such as magnesium (Mg). Normal chlorophyll a content is 2–4 times that of chlorophyll b although spectrometry of acetone extracts of fresh mint, melissa and nettle leaves showed chlorophyll a/b ratios varying from 3:1 in melissa and nettles to 1:1 in mint (Dżugan, 2006). Plants growing in shade contain less chlorophyll a and more chlorophyll b. Replacing Mg with Fe ions gives a grey-brown chlorophyll product, and the presence of Zn and Cu ions increases the stability of the natural green color (Dżugan, 2006).

The chlorophyll molecule is a porphyrin derivative whose main skeleton is an arrangement of four pyrrole rings containing an Mg ion in the center. The presence of Mg in the center of a chlorophyll molecule plays an important role in absorption of light energy, and the pyrrole ring creates a structure with single and double bonds, which facilitates the

TABLE 1. Major pigments of plants and other organisms

Pigment group	Type	Localization	Color
Phycobilins	Phycocyanin Phycoerythrin	Cyanobacteria, Rhodophyta	Bluish Red
Chlorophylls	Chlorophyll a, b	All photosynthetic plants	Green
Carotenoids	Carotenes Xanthophylls	Plants, bacteria and some crustaceans	Yellow, orange, red
Flavonoids	Chalcones Flavonols Anthocyanins	Gymnosperms and angiosperms	Cream, pale yellow, pink, red, blue, black
Betalains	Betaxanthins Betacyanins	Caryophyllales, cactus and some fungi	Yellow, red

Fig. 2. The chemical structure of plant pigments. (**a**) Structure of the chlorophyll molecule. All plants, algae and cyanobacteria that photosynthesize contain chlorophyll *a*. A second kind of chlorophyll is chlorophyll *b*, which occurs only in green algae and in plants (Taiz and Zeiger 2006, modified), (**b**) Chemical structure of carotenoids, (**c**) Structure of the basic flavonoid skeleton with 15 carbons in two aromatic rings (A and B) connected by a three-carbon bridge, (**d**) Betalains: betacyanin and betaxanthin with nitrogen atom in the chromophore.

absorption of light photons. A phytol tail consisting of 20 carbon atoms is attached to this tetrapyrrole part of chlorophyll. The differences in structure between chlorophyll *a* and *b* are minimal and involve the various groups attached to the third carbon atom in the II pyrrole ring. Chlorophyll *a* attaches the CH_3 group, whereas chlorophyll *b* binds with CHO (Fig. 2a) (Heldt, 2005). Reverse conversion from chlorophyll *a* to chlorophyll *b* may occur in chloroplasts as a result of the activity of CAO oxygenases encoded by a class of genes identified in *Arabidopsis thaliana* and *Oryza sativa* (Espineda et al., 1999).

Removal of Mg from the chlorophyll molecule results in its conversion to pheophytin, grey-brown in color. Pheophytin accumulates in leaves during senescence of plants or as a result of damage caused by environmental pollution, such as acid rain (Dżugan, 2006).

The green pigment of plant leaves is due to the ability of chlorophyll to absorb light in the visible wavelength, primarily in the blue (420–460 nm) and red (650–700 nm) range, and reflection of green light (~550 nm). However, the physiological cause of plants' greenness is still not completely known. It is thought that green leaves adapted exceptionally well to varying light conditions, which prevail in many habitats. The success of green pigment results not only from chlorophyll's ability to absorb light under changing irradiance and maximal utilization of light energy in photosynthetic electron transport, but also from its ability to protect chloroplasts from light excess by dissipating it in the form of heat or fluorescence radiation (Nishio, 2000). Thus the greenness of plants is a result of complex reactions of chlorophyll biosynthesis. The final light-dependent reaction is catalyzed by NADPH-protochlorophyllide oxidoreductase (POR). In *Arabidopsis thaliana*, three POR isoforms occur: POR A, POR B and POR C (Beale, 2005). Angiosperms growing in darkness accumulate only small amounts of protochlorophyl-

lide, which is associated with NADPH and POR in a ternary complex, so these plants are not capable of chlorophyll synthesis. In these etiolated seedlings a large amount of oxidoreductase POR A proteins and protochlorophyllide, as well as POR A mRNA, are accumulated. Following exposure to light, the level of POR A falls rapidly as a result of proteolytic degradation. Rapid proteolysis of this isoform is the reason that plants growing in darkness do not contain chlorophyll. Yellow pigmentation is produced by carotenoids, which mask the green color of protochlorophyllide. The POR B isoform, responsible for chlorophyll synthesis, has been found in barley and *Arabidopsis thaliana* growing in light. The enzyme has been shown to be active only in light, probably as a result of its insensitivity to proteolytic hydrolysis (Lebedev et al., 1995; Tanaka and Tanaka, 2006).

In *A. thaliana*, 30 genes encoding enzymes involved in chlorophyll synthesis have been identified. Mutations in those genes are usually lethal to plants (Beale, 2005). Chlorophyll synthesis mutants are often pale green, sensitive to light, and characterized by overproduction of protochlorophyllide (von Wettstein, 2000). These mutants are particularly helpful in understanding the regulation of chlorophyll synthesis and its integration with plant growth and development. Of particular interest is a corn mutant with mutations in the gene encoding uroporphyrinogen III decarboxylase. This mutation has characteristic necrotic patches on the leaves due to the accumulation of phototoxic intermediates. This is the only case of natural porphyria in plants (Hu et al., 1998).

YELLOW, ORANGE AND RED – CAROTENOIDS

Carotenoids are a large family of terpenoid pigments (more than 600 compounds) found in photosynthetic bacteria, algae, fungi, and cells of higher plants and animals. These pigments can be divided into two groups: orange-red carotenes, which are unsaturated hydrocarbons; and yellow-orange xanthophylls, which contain additional oxygen within their molecules (Fig. 2b).

In higher plants they are involved in light harvesting in photosynthesis and protection against excessive light, participating in energy and electron transfer. In light-harvesting reactions, carotenoids act as accessory pigments and transfer the excitation energy to chlorophyll *b*. However, the important function of these pigments is photoprotection of the reaction center (RC) of photo system II from photo-oxidative damage by either quenching triplet chlorophyll or quenching singlet oxygen (Young et al., 1997). The role of these pigments in photoprotection is based on fluorescence-quenching mechanisms. The excess energy is dissipated as heat; it is a feature of the interconversion between two carotenoids, zeaxanthin and violaxanthin, in the xanthophyll cycle. Under excess light, the violaxanthin is biochemically transformed (de-epoxidated) into zeaxanthin via intermediate antheraxanthin. In darkness or under low photon flux density, the process is reversed and violaxanthin content increases in plant leaves. The quenching mechanism is complex and not entirely understood in molecular detail (Horton and Ruban, 2005). Carotenoids are also present in chromoplasts, giving them a characteristic yellow-red-orange color. This pigmentation determines the color of some plants (roses and marigolds), fruits (tomatoes), roots (carrot) and seeds (red peppers). The unusual blue color of some carotenoids is the result of interaction between certain carotenoids with apoproteins such as astaxanthin with crustacyanin, which give the blue pigmentation of lobsters (Grotewold, 2006). β-carotene and lycopene are among the most common carotenoids in the plant kingdom, giving the characteristic colors to carrots and tomatoes; the most frequently represented xanthophylls are zeaxanthin, violaxanthin and lutein. Besides antenna functions, some xanthophylls (violaxanthin, lutein) are responsible for the yellow color of autumn leaves (xanthos = yellow, phyll = leaf) (Davies, 2004).

Carotenoids absorb light of 400–500 nm wavelength, which accounts for their orange coloring. In addition to their role in energy transfer to chlorophylls, carotenoids also have a key function in chloroplast protection under excess light conditions. By dissipating the excess energy they prevent reactive oxygen species production, photo-oxidation and damage to the photosynthetic system (Britton, 1995; Niyogi, 2000).

An important application of biotechnology is improvement of the dietary value of crops to supplement the local diet with nutrients that do not occur naturally in it. Carotenoids are an important part of the diet, comprising a source of vitamin A and antioxidants. One of the earliest genetic modifications was the introduction of β-carotene, a vitamin A precursor, into rice, which does not contain vitamin A or any of its biochemical precursors. Rice lines genetically modified with genes encoding phytoene synthase and lycopene β-cyclase originating from *Narcissus pseudonarcissus* and *Erwinia uredovora* were produced. The transformed lines produced β-carotene manifested phenotypically as orange pigmentation of rice grains, giving rise to the name "Golden Rice" (Giuliano et al., 2000; Al-Babili and Beyer, 2005).

Carotenoids present in leaves, together with anthocyanins, are responsible for the beautiful colors of autumn. One hypothesis (Hamilton and Brown, 2001) suggests that the colors of leaves have the same significance as colored lines painted on the face of a native American: strength and readiness to fight. Trees that quickly change their foliage color to yellow, purple and gold are demonstrating their

vitality and strong metabolic processes. This is a signal for insects that the tree is strong and that in case of attack it may attack the parasite with a harmful substance produced as a result of the metabolic process (Lee and Gould, 2002). Another suggestion, the resorption protection hypothesis, is that anthocyanins produced during senescence help to photoprotect plants from excess light and can affect a plant's ability to resorb important foliar nutrients such as nitrogen and phosphorus. In this case, anthocyanins serve as a barrier cream in leaves by shielding the photosynthetic apparatus from potentially harmful light levels (Hoch et al., 2003).

RED, BLUE AND PURPLE – ANTHOCYANINS AND BETALAINS

Flavonoids

Flavonoids are chemical substances of the polyphenol group whose primary skeleton comprises 15 carbon atoms arranged in two phenyl rings, A and B, joined by a three-carbon bridge (Fig. 2c).

So far more than 7,000 derivatives containing this structure have been found. In higher plants these compounds are divided into several classes differing in the number and location of hydroxyl groups on rings A and B, the degree of oxidation of ring C, and the presence of dimeric structures, that is, repetition of the C6-C3-C6 structure. The flavonoids include anthocyanins, chalcones, aurones, flavones and flavonols. They all are water soluble, located in vacuoles often as glycoconjugates, and absorb visible light in the 280–315 nm range. Flavonoids are biochemically active substances with varied functions: they are responsible for the color of flower petals and fruits, they create a UV protective filter, they serve as antioxidants, and they have antibacterial and antiviral activity. They also control gene transcription for proteins involved in auxin transport and give important visual signals to insect and animal pollinators (Davies, 2004; Winkel-Shirley, 2002). The names of many known anthocyanins reflect their history and originate from the names of the families in which they were first discovered (e.g., *Centaurea, Delphinium, Pelargonium*). In 1913, Willstätter and Everest identified the first anthocyanin in the blue cornflower, and since then the structures of over 600 different anthocyanins have been identified (Winkel-Shirley, 2006). As early as 1664 the violet pigment in the pansy (*Viola* sp.), belonging to the anthocyanins, was discovered, and was used as the first natural pH indicator.

The rich variety of flavonoids and their role in providing color to plants are one of the oldest botanical research areas. Anthocyanins are the most common pigments of the flavonoid group, which often give a bright red, red, blue or violet color to plant

TABLE 2. The most common anthocyanidins and their colors

Anthocyanidyn	Type of ring substituent (R1-R7)	Color
Pelargonidin	R2,4,5,7,-OH; R1, 3, 6 -H	orange red
Cyanidin	R1,2,4,5,7-OH; R 3, 6-H	purplish red
Delphinidin	R1,2,3,4,5,7-OH; R6 -H	blue
Malvidin	R2,4,5,7,-OH; R1,3-OCH$_3$	purple violet
Peonidin	R2,4,5,7,-OH; R1, OCH$_3$	rose
Petunidin	R1,2,4,5,7,-OH; R3-OCH$_3$	purple violet

petals, fruits and stems. The key to this variety of color is the degree of oxidation of the central chromophore group, anthocyanidin, as well as the number and type of attachments to the main skeleton. Anthocyanins are glycosides that have sugars attached to the anthocyanidin. In the structure of anthocyanins, 17 anthocyanidins have been distinguished, the most common of which are cyanidin, delphinidin, pelargonidin, peonidin, malvidin and petunidin. Anthocyanin color is influenced by many factors. One of them is the number of hydroxyl and methoxyl groups. If many OH groups are present, the color is blue. The presence of many OCH$_3$ groups shifts the color towards red (Tab. 2) (Winkel-Shirley, 2002; Grotewold, 2006). A characteristic property of these pigments is the change of tone with environmental factors (Davies, 2004). In this respect, anthocyanins are true chameleons in the pigment world. In acidic pH, anthocyanins take a red color, and in alkali pH, blue. Anthocyan color also depends on the presence of metal ions such as aluminum (Al), iron (Fe) and magnesium (Mg); the intense blue color of the dayflower (*Commelina communis*) is brought about by the anthocyan complex with two Mg atoms. The co-occurrence of anthocyanins in association with other pigments such as carotenoids and flavonoids also has a significant effect on petal pigmentation (Griesbach, 2005; Grotewold, 2006). Flavones usually have a yellowish or cream color, and also absorb ultraviolet, so they can provide protection against UV radiation. In such complex structures, anthocyanins take on more intense and stable colors.

The application of electron microscopy in research on plant petals revealed the presence of spherical bodies known as anthocyanic vacuolar inclusions (AVIs) in some taxa, for example *Lisianthius, Dianthus caryophyllus, Salvia, Antirrhinum, Eustoma* and *Delphinium*. These structures may be formed by anthocyanins attached by H-bonding to the protein matrix, and their con-

centration intensifies petal color, especially in areas rich in AVIs (Markham et al., 2000). Recent electron microscopy studies of the AVIs of lisianthus (*Eustoma grandifolium*) and carnation (*Dianthus carryophyllus*) report that AVIs are compact, irregular bodies formed from ER-derived vesicles in epidermal cells in different regions of the petal, and are not surrounded by membranes. Based on the subcellular evidence, it is suggested that anthocyanins may be first packed into prevacuolar compartments (PVCs) localized in the vicinity of their biosynthesis sites. Subsequently these PVCs develop and merge with the central vacuole, where anthocyanins are finally sequestered (Zhang et al., 2006).

Many genes associated with the biosynthesis of various anthocyanins have been identified. The first plants serving as color models in genetic experiments were corn, snapdragon and petunia, and work on these subjects led to the isolation of many structural and regulatory genes coding key proteins involved in the biosynthesis of anthocyanins and their sub-cellular organisation. Recognition of the complete *Arabidopsis thaliana* genome enriched our knowledge of the mechanisms regulating the expression of genes typically active in petal epidermal cells and which may react to environmental factors such as wavelength and quality of light (Winkel-Shirley, 2001; Grotewold, 2006). Synthesis of anthocyanins may also be induced by many other stress factors such as cold, high temperatures, salinity and deficiency of minerals, particularly nitrogen and phosphorus, which is usually manifested by purple pigmentation of stems and leaves. This shows that flavonoid synthesis is one of the general plant reactions to stresses (Winkel-Shirley, 2002). The most common environmental factors influencing pigment color is soil pH. It can affect pH within the cell compartments and in this way can change the subcellular accumulation of flavonoids or other cellular components that interact with flavonoid pigments (Verweij et al., 2008). Mutations in glucosyltransferase genes (*GTs*) changed the location of flavonoids from vacuole to cytoplasm, resulting in flower yellowing. Some of the genes regulating vacuole pH also controlled anthocyan biosynthesis in the petunia (Davies, 2004; Verweij et al., 2008).

Research on anthocyanins in dicotyledons provides evidence that plant coloration involves two levels of genetic regulation of flavonoid synthesis. The first comprises genes encoding the enzymes involved in early steps of flavonoid synthesis: chalcone synthase (*CHS*) and chalcone isomerase (*CHI*). The second involves regulation of the expression of genes involved in the late stages of anthocyan production, such as *DFR* (dihydroflavonol reductase) or *LDOX/ANS* (leucoanthocyanidyn dioxygenase/anthocyanidins synthase) (Winkel-Shirley, 2002; Davies, 2004). Mutation of *CHS* in both corn and petunia

produced white instead of yellow pollen in wild plants (Winkel-Shirley, 2002). In the case of *DFR*, for example, genetic modifications using antisense gene structures for nucleotide sequencing of later stages in flavonoid synthesis changed the flower pigmentation from white to pink in petunia and from red to pink in gerbera (Davies, 2004). Genetic engineering has created the possibility to use these genes to transform plant species and strains to attain the required color.

<div align="center">Blue</div>

Growers of decorative plants have been trying for centuries to obtain a blue rose. In this genus blue flower color is not found in nature, as its petal cells do not accumulate delphinidins or any delphinidin derivatives, due to the lack of a key enzyme involved in their synthesis: flavonoid 3',5'- hydroxylase. Roses do accumulate large amounts of pelargonidins, cyanidins and carotenoids. Apart from this, the pH in the vacuoles of rose petal epidermal cells ranges between 3.69 and 5.78. At such a low pH, anthocyanins have a red or pink tone. One strain of rose, "Rhapsody in Blue," contains a small amount of the blue pigment rosacyanin in AVIs. Color changes in this rose from purple to blue progressing with age are caused by increasing accumulation of anthocyanins in vesicle-like AVI structures. However, the molecular mechanism of this phenomenon is not yet clear (Katsumoto et al., 2007).

Scientists from Florigene in Australia transformed plants with the gene coding flavonoid hydroxylase enzyme, vital for the synthesis of blue derivative delphinidins, to grow blue roses and carnations. The first transgenic rose with the flavonoid hydroxylase gene originating from the pansy, the petals of which contain delphinidin, was created in 1992 (http://www.florigene.com). The next achievement of the scientists at Florigene was successful transformation of selected rose strains with a construct of two genes: the gene encoding F3'5' H from the pansy and the *DFR* gene from the iris. This transformation caused greater delphinidin accumulation in genetically modified roses. The ability to pass on the blue color became a fully inherent trait, which became apparent in successive generations.

Overexpression of the *F3'5'H* gene alone was not enough to create the blue pigmentation of carnations. However, transformation of the white carnation with both *F3'5'H* from the pansy and *DRF* from the petunia resulted in the production of a blue pigment named "Moondust" (Katsumoto et al., 2007; http://www.florigene.com/research).

The first anthocyan isolated from plants was cyanidin, originating from the cornflower petal. The same pigment was later identified in the rose; the difference in pigmentation between the flowers of

those two species was explained by the difference in their petal pH. The crystallographic structure of pigment isolated from the cornflower shows it to be a large complex composed of six cyanidin molecules chelated with Fe^{3+} and Mg^{2+} and six flavones associated with Ca^{2+}. This new supermolecule, named protocyanin, may be the challenge awaiting breeders looking for a source of blue pigment for many decorative plant strains (Shiono et al., 2005).

Betalains

Betalains substitute anthocyanins in most species of Caryophyllales. They have also been found in certain fungi (*Amanita, Hygrocybe*) and cactii. Since betalains derive from tyrosine, an amino acid common to all organisms, the presence of nitrogen in their molecule is characteristic. Betalains differ structurally in the sidechain groups, which may be amino acids or amines (Fig. 2d), and are divided into two groups: betaxanthins (yellow; e.g., indicaxanthin in cactii) and betacyanins (red to violet; e.g., amaranthin in *Amaranthus caudatus*). Sometimes they form complexes with tyrosine or glycine (e.g., portulacaxanthin II and III identified in *Portulaca grandifolia*). The most well known betalains, betanin and vulgaxanthin, were isolated from beetroot. Betalains are present also in fungi in the fruiting body (musca-aurin). The toadstool contains, instead of betacyanin, betalamic acid-derived compounds: musca-purpurin and musca-flavin, which give its cap its characteristic red color (Davies, 2004).

Spanish researchers made an interesting observation in a very original flower known as Mirabilis (*Mirabilis jalapa*), whose petals display fluorescence. The petals of this plant open in the late evening or overcast days in order to be more noticeable to insects through their fluorescence. This plant's petals contain two types of pigment: one of them emits fluorescence and the other is an antifluorescent pigment. Some petals, yellow in color, contain betaxanthin and emit green fluorescent light, and other parts of flowers contain both yellow betaxanthin and violet betacyanin pigments. The violet pigment absorbs green light emitted during fluorescence. The combination of these two pigments can create green patterns which have been suggested to act as a guide for insects and bats sensitive to this color (Gandía-Herrero et al., 2005).

ACHROMATIC COLORS: BLACK AND WHITE

Gardeners and breeders of decorative plants have been trying for hundreds of years to breed and select for black flowers. Over many generations they have produced blossoms of dark purple, dark violet or dark chocolate. A species of tulip, *Tulipa julia*, has visible dark stains on the external side of its petals,

Fig. 3. *Lisianthius nigrescens*, the blackest flower in the world.

which are caused by a very high concentration of anthocyanins (co-pigmentation). Looking deeper into various black petals, we can see the various shades of purple, blue and red. "Queen of the Night," considered the blackest strain of tulip, owes its dark violet color to a high concentration of delphinidin, which in specific light conditions gives a distinctly black color. In tulips with high delphinidin content and other associated pigments (cyanidin and pelargonidin), the color may in fact appear to be black. The black pansy has a high concentration the delphinidin derivative 5-O-glucoside-3-O [4-p-coumaroylrhamnosyl (1,6)glucoside], commonly known as violanin (Markham et al., 2004).

Black flowers are a true rarity and are considered an extravagance among breeders, often unattainable. Black-flowered species have been collected in the wild, most recently by the American botanists Rob Nicholson and Melvin Shemluck along with their Mexican colleague Raul Rivera. They relocated a population of *Lisianthius nigrescens* in Oaxaca, Mexico, based on a locale given by a 1939 herbarium specimen made by the legendary Richard Schultes. Known as *la flor de muerte* (the flower of death) in Oaxaca, this trio managed to collect plant and floral specimens and then shared these with pigment chemists and pollination biology specialists. The results of the interdisciplinary team showed that *L. nigrescens* contains two types of pigments belonging to the anthocyanins, in unprecedented quantities. These compounds accounted for as much as 24% of the flower's dry mass (Nicholson, 1999). The petals of *L. nigrescens* absorb light across the whole visible wavelength, and are also capable of absorbing UV, so these plants truly are the blackest flowers in the world (Fig. 3).

To compare, *Eustoma grandiflorum*, another member of the Gentianaceae family, contains 1.4% anthocyanins in its violet petals. Large vacuoles within the flower cells of this species are filled with anthocyanin inclusions, bodies in which concentrated anthocyanins seem to be present almost as a powder (Marhkam et al., 2004).

An example of another very dark pigment is the dark-brown flower *Gastrolobium melanopetalum*,

which is being grown at the Botanic Garden of Smith College in Northampton, Massachusetts, along with *Lisianthius nigrescens*. Research on these Mexican and Australian plants is still in progress, and is inspiring researchers to seek out genes responsible for the production of large amounts of anthocyanin pigments. If it were to become possible to transform cultivated roses and tulips with such pigments, the dreams of breeders and gardeners might just come true.

Black olives contain phenyl elements, which account not only for their bitter taste but also for their dark color. The black color is given by cyanidin 3-rutinoside and cyanidin 3-glucoside, which comprise 90% of all anthocyanins contained in olives. Under the influence of fermentation during processing, however, these pigments are lost and the color is maintained by its substitution with another more stable pigment, the nature of which remains unknown (Piga et al., 2005).

The "Pino noir" strain of black grape, from which pink champagne is made, owes its color to several anthocyanins, 3-glucosides, 3-acetylglucosides and 3-coumaroylglucosides of malvinidin, delphinidin and cyanidin (Bridle and Garcia-Viguera, 1997). Also, genetic testing has revealed that in the small leaves and fruit epidermis of *Vitis vinfera* there is a high level of mRNA-encoding gene *F3'5'H*, which correlates with the storage of large amounts of anthocyanins based on delphinidin by these organs (Yakushiji et al., 2006).

Black leaves may appear during the creation of an abscission zone or where leaves are attacked by a pathogen or parasite. Healthy black leaves do occur, however, though extremely rarely. An example of a plant with very dark leaves is *Ophiopogon planiscapus* 'Nigrescens,' which contains an unusually high amount of chlorophyll *a* and *b*, and anthocyanins in surface layers of mesophyll (Hatier and Gould, 2007).

White pigmentation is triggered by the same effect we see in snowflakes – nothing more than blisters of air filling large spaces between cells in flower petals. When we submerge such a flower in alcohol, the white pigment disappears as the liquid fills the free space.

Cream and yellow pigments are brought about by chalcones and flavones, commonly seen in plants of the Asteraceae and Fabaceae families. Another white pigment, appearing in thin-walled cork cells of the birch tree, is betulin (Davies, 2004; Grotewold, 2006).

It might seem that the flower world is rich in white, but this is only as seen through our eyes. Generally speaking, white flowers are strong UV-absorbers, and they reflect with approximately equal strength all other wavelengths, stimulating our receptors of all types. White flowers in the eyes of an insect are very rare. Insects have the ability to detect UV, and to them the flowers are chromatic. Thus, white dye has many colors for them (Kevan et al., 1996).

An extreme white is observed in petunia into which an introduced additional copy of the gene encoding for chalcone synthase (CHS) has triggered an effect known as gene silencing – instead of becoming darker, the flowers become white or else mosaic with white and colored petals (van Der Krol et al., 1990).

Research on flower pigments sometimes yields unexpected discoveries for different fields in biology. Gene silencing and siRNA (small interfering RNA) have become subjects of interest for many researchers, opening new doors in molecular biology for research into plant color properties.

SUMMARY

Pigments that give color to angiosperm flowers are generally common among vascular plants. The pigmentation is generated by the electronic structure of the dyes interacting with sunlight in plant tissue. The primary pigments occurring in plants are chlorophylls and carotenoids, accumulated in plastids, and anthocyanins and betalains, which are dissolved in vacuolar sap. Flavonoids and carotenoids are ubiquitously distributed in plant pigments. Each of these groups of compounds indicate their presence by giving flowers, fruits and leaves different shades of green, red, violet, yellow and orange. Different pigments display an ability to absorb varied wavelengths of visible light. Different types of pigment are marked with their maximum absorption, which is an important ecological feature for various organisms living in environments of diverse light availability. Flavonoids, together with anthocyanins, confer a wide spectrum of color to flowers and fruits, including yellow, scarlet, red, pink, violet and blue. Carotenoids furnish flowers and fruits with distinct colors from yellow to red, and are essential components for photosynthesis.

Among the few strains of dark flowers, only a few closely approach the color black. A naturally occurring, almost black plant from the Gentianaceae family is *Lisianthius nigrescens*.

The intensive colors of some flowers, fruits and leaves are due to a combination of various pigments, often in complex structures called co-pigments, which give more stable colors.

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