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Biology, abiotic factors and biotechnology influencing flower colour

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Abstract

Flower colour is an important feature that adds aesthetic value to flowering plants as a key element in consumer choice. Colour expression is determined by a combination of several factors: type, quantity and stability of pigments, pH of the cells, copigmentation, and translocation of the pigments themselves from the production site. Various factors, such as light, temperature, sugars, fertilisers and metals, can also influence its expressions. The pigments most responsible for the colour of flowers are flavonoids and carotenoids. Intervening on this character through technical-agronomic interventions, or by modifying the pigment composition in plants through traditional breeding or, more recently, acting on the biosynthetic pathway through genetic engineering, is a highly relevant goal. The technical, genetic and biochemical knowledge presented in this review provides the basis for increasing the chances of success.

Keywords: Flowers Colour; Ornamental Plants; Pigmentation; Abiotic Factors; Flower Biotechnology; Sugars

1. Introduction

The colour of flowers is a fundamental quality aspect for technicians in the sector and a significant element of evaluation during the purchasing process. Therefore, it is essential to understand the main factors influencing its expression to identify techniques that can improve or preserve its quality as much as possible. Flower colour is mainly determined by the quantity and stability of pigments in the tissue, the pH of the vacuolar cells and the translocation of pigments from the production site [1,2]. The primary pigments that characterise the colour of flowers are flavonoids, carotenoids, betalains and, to some extent, chlorophyll. In addition, various external factors can influence the expression of colours, such as light, temperature, sugars and metals. However, the commercial value of ornamental plants is not only determined by their quality but also by the variety of colours and flowers available, with continuous genetic improvement activity in search of new products that can be brought to market [3,4,5]. Only a narrow spectrum of colours is available for some species of flowers, while in some species, some colours are absent. The introduction of new colours through genetic engineering techniques could have a vast influence on the world of floriculture, and indeed this area of research has attracted the attention of many researchers in recent years [6,7,8]. This review will present various chemical and biochemical aspects that underlie flower colour and the possible techniques that can be applied to improve and preserve its quality.

2. Pigmentation influencing flower colour

Among the pigments that influence flower colouration, flavonoids and betalains are located within the vacuolar cells, are not chemically related and have never been found together. Chlorophyll and carotenoids, on the other hand, are chemically related and located within the plastids found in the cytoplasm of the cells [9]. Flavonoids generally affect pink, red, orange, and scarlet, providing intensity to most white and cream colours. Carotenoids alone determine yellow, orange and red colourations. In many important ornamental species, carotenoids help extend the colour range of

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flavonoids by producing yellow, orange and dark brown colours in combination with them. Betalains determine the yellow, orange, red and purple colours in the flower tissues of only a few plant species. Each type of pigment results from a different sequence of biochemical reactions, and the production of each pigment is independent of the other pigments [10,11,12,13]. In most cases, a defect in the biosynthetic pathway of flavonoids does not affect that of carotenoids and chlorophyll. As for white flowers, they are generally believed to lack at least some pigments, but their absence in the petals could be due to factors as diverse as the malfunction of genes involved in colour transcription, their non-expression or an error in the translocation mechanism [14,15]. Flavonoids are phenylpropanoid compounds that are generally water-soluble and stored in the vacuole. The key enzyme in flavonoid biosynthesis, chalcone synthase (CHS), catalyses the multi-stage condensation of three acetate units, forming a chalcone from which all flavonoid structures originate. Research has focused on flavonoid biosynthesis, one of the most significant and characterised aspects of secondary plant metabolism [16,17]. The research of molecular biologists has complemented years of genetic and biochemical research into the enzymatic steps. The genes encoding most enzymes involved in anthocyanin biosynthesis have been identified and cloned, and knowledge of the factors regulating this process is also well-advanced [18,19]. The most common flavonoids include chalcones and anthocyanins, the most widespread and important pigments. In the petal tissues, the anthocyanins are specifically located in the vacuoles of the epidermal cells. Anthocyanins deprived of sugar and acyl radicals are called anthocyanidins. There are six main anthocyanidins, and three commonly identified: pelargonidin, which produces orange, pink and red colours; cyanidin, which produces red and mauve colours; and delphinidin, which produces purple, blue and dark blue colours.

By contrast, peonidin, petunidin, and malvidin are present only in certain plants [20,21,22]. Therefore, a sufficient level of stability is achieved through pigmentation, i.e. the formation of molecular complexes with substances called pigments. There are two types of copigments: flavonols (kaempferol, myricetin, and quercitin) and flavones (apigenin, triacetin, and luteolin). These compounds are directly responsible for the colour of flowers only in very few cases, while through pigmentation, flavones and flavonols can more easily influence the perception of colour [23].

Carotenoids are a ubiquitous group of plant pigments that differ significantly from flavonoids in structure and compartmentalisation. Pigments derived from isoprenoids are usually hydrophobic, fat-soluble pigments with a structure typically based on forty carbon atoms. While their primary function is to protect photosynthetic tissues from photo-oxidation, carotenoids can also result in colour pigmentations in organs such as fruits and flowers. In the latter, carotenoids are synthesised and stored in chromoplasts, specialised plastids differentiated from chloroplasts or non-photosynthetic plastids [24]. Two common groups of carotenoids associated with flower colour are the carotenes and their oxygenated derivatives, the xanthophylls, associated with the colours orange and yellow, respectively. More than six hundred naturally occurring carotenoids have been isolated and identified. Some ornamental species are very rich in carotenoids, both in quantity and in diversity: for example, in Narcissus, the level of beta-carotene can reach 16.5% of the dry weight in the flower crown, while in Rosa, 75 different carotenoids have been identified [25,26,27]. As can be seen in Figure 1, the presence of different carotenoids influences the colouration and intensity of colours in Echinopsis flowers.

Betalains, nitrogenous vacuolar pigments with betalamic acid as a chromophore, produce colours such as yellow, orange, red, and violet. Information on betalains is currently scarce, but much research is currently being conducted in this field, not least because studying the enzymatic processes that lead to the production of new flower colours is an attractive goal [28,29]. A list of possible pigments influencing the various colourings has been made in Table 1.

Figure 1 Pigments and copigments in particular the presence of carotenoids, influence the manifestation of flowers colour

Table 1 Pigments responsible for the colour of flowers

Colour	Pigment influencing colour
White, cream	Flavones, flavonols
Yellow	Carotenoids, flavonoids
Orange	Carotenoids, pelargonidins
Scarlet	Pelargonidin, cyaniding
Brown	Cyanidine
Magenta	Cyanidine
Pink	Peonidine
Mauve, violet	Delphinidin
Blue	Cyanidine, Delphinidin
Purple	Delphinidin
Green	Chlorophylls

3. Abiotic factors influencing flower colouration

3.1. Light influence

Anthocyanin biosynthesis requires light. Increasing the colour intensity of some fruits using artificial light in the post-harvest period has been a model for many researchers to investigate. Flowers open at low light intensities, like roses, petunias, and carnations, have faded colours. A reaction mediated by UV photoreceptors, cryptochromes, phytochromes and sugar production by leaves or stems causes poor pigmentation when light intensity is low. Some authors have shown that localised photomorphogenic responses in flowers mediate anthocyanin production; shading the flowers have been found to reduce anthocyanin biosynthesis [30]. In Antirrhinum majus, on the other hand, an increase in anthocyanin content was found with increasing light intensity. Furthermore, it was shown that using different types of light, the anthocyanin content was higher with fluorescent and blue light and lowered with red light and incandescent lamps. Fluorescent lamps provided the best efficiency for good flower colouration at wavelengths of 470-600 nm [31,32,33].

3.2. Temperature influence on flower colour

Many researchers have considered the effects of temperature on anthocyanin synthesis and fruit colour development during post-harvest storage. There has been evidence that low temperatures increase the transcription level of genes whose products are either key enzymes of the general phenylpropanoid biosynthetic pathway, like phenylalanine ammonia-lyase (PAL), or genes responsible for enzymes involved in flavonoids and anthocyanin biosynthesis [34]. Indeed, one of the factors responsible for the lower anthocyanin concentration in plants at high temperatures is reduced biosynthesis. However, temperatures can influence the synthesis and stability of anthocyanins [35]. The pigments' concentration decreases at high temperatures due to decreased synthesis and increased degradation. Some scientists studied the combined effect of high temperatures and increased metal concentrations on the accumulation of anthocyanins in flowers, finding that high temperatures caused discolouration of the petals. In addition, a decrease in the activity of the PAL and CHI enzymes that synthesise anthocyanins was found. On other plants, lower temperature regimes also resulted in more intense flower colouration than higher temperatures [36,37].

3.3. Cellular pH

Many factors are essential in determining the final colour in the plant tissue, among them the level of oxygenation and the pH of the vacuole. Colour variations in anthocyanidins, for example, are caused by the pH in the vacuole, which affects the secondary structure of anthocyanins and thus affects the colour of flowers. Differences in the pH of the v-acuole can therefore result in flowers containing the same anthocyanins having different colours. For example, a drop in pH causes a shift towards the red cationic form of anthocyanins, while an increase in pH causes a shift towards the blue quinoidal forms. The pH of vacuolar extracts of petal cells is usually slightly acidic, but the range can vary from 2.5 to 7.5.

Furthermore, pH can also vary between cultivars within a species and even at different stages of development of a single flower, such as in Ipomea flowers where pH variation results in a change in flower colour from the moment of opening to the actual opening [38]. Anthocyanidins with an ortho-dihydroxyl system (cyanidins, delphinidins, petunidins) can also form stable complex forms with certain metals (molybdenum, iron, tin, aluminium, titanium, chromium, uranium and lead). During senescence, the colour of the petals can be modified by varying the pH of the vacuoles if the pigments are mainly anthocyanins. Treating cut flowers with sugars delays the pH increase and prevents the blue colouration in flowers [39].

3.4. Sugars: energy for flowers

Sugars play an important role in maintaining the quality of cut flowers, as the amount of sugars contained in cut stems is limited. Sucrose is the most important photosynthetically assimilated form of carbon transported in plants and is the primary carbon source for petal growth. It is well known that continuous post-harvest treatments with sucrose in the storage water improve flowers' opening and prolong many species' pot life. However, in some cases, these treatments also increase the anthocyanin concentration in the petals and, thus, the appearance of stronger colouration [40]. The stimulation of anthocyanin expression in cut flowers by sucrose appears to be determined by the expression of genes involved in their biosynthesis. Sugars are not only thought to act as a specific signal for the activation of gene expression of anthocyanin biosynthesis but rather as a source of carbohydrate metabolism, specifically the phosphorylation of hexoses, on which the induction of anthocyanin synthesis is dependent [41].

Furthermore, it has been shown that signal transduction related to sugar phosphorylation must interact with the gibberellin signal to induce gene expression and anthocyanin accumulation in corollas. An improvement in the colour intensity of Lilium tepals was found following repeated treatment with sucrose. At the same time, adding sugars to the storage water did not affect the longevity and size of the flowers but increased the intensity of the colouration. The positive effect of sucrose on flower colouration was demonstrated in Anthirrinum, where the anthocyanin content was higher after adding sugar to the storage water.

3.5. Metals and pigmentation

Some metals can chelate with anthocyanins containing an ortho-dihydroxyl group to form intensely coloured and stable metal complexes in a pH range where anthocyanins alone would be colourless. In flowers, however, metals are only present in small amounts and not in all plants, so they are unlikely to affect the colour of flowers, except for iron and aluminium, especially those containing anthocyanidins, which do not form metal chelates. In flowers, the main effect of metals on pigments is a change in colour [42]. Magnesium treatment on plants, for example, increased the concentration of anthocyanins in the organs they accumulated, decreasing the rate of anthocyanin degradation. A product commonly used in the post-harvest treatment of various cut species is silver thiosulphate (STS). In some research, flowers pre-treated with STS and sucrose were found to have a more intense colour.

3.6. Effect of nutrition and growth regulators on flower colour

In order to improve fruit and flower colouration, good results can be achieved by adjusting the NPK administration with fertilisation. On flowering plants, positive effects on gladiolus following nitrogen and potassium fertilisation on the colour of flower spikes of different cultivars are shown. On geranium, phosphorus deficiency stress resulted in a darker colour of the purple rings on the leaves of this species [43]. A reddish or purple colouration of the leaves of phosphorus-deficient plants was shown to be due to increased anthocyanin production. Some authors have found a beneficial effect on the quality and intensity of flower colour using controlled-release fertilisers compared to traditional water-soluble fertilisers [44]. On Anthirrinum flowers, benzyl-adenine (BA), indoleacetic acid (IAA), abscisic acid (ABA), and gibberellic acid (GA) were studied [43]. GA and IAA decreased in anthocyanin content, while BA and ABA showed a slight increase in anthocyanin content at low concentrations.

4. Biotechnology in flower colour improvement

Genetic improvement programmes through crossing and selection have long been carried out to produce new varieties and colour combinations. The application of traditional techniques, however, results in severe limitations due to the randomness, the unpredictability of obtaining new colours, and the waiting periods for obtaining results, which are longer or shorter depending on the reproductive cycle of the crops being studied. The biotechnological approach to directly modify the colour of flowers using molecular techniques, therefore, appears promising. Detailed knowledge of the flavonoid biosynthetic pathways is the primary condition for developing molecular techniques to modify the anthocyanin pattern. By knowing the enzymes, the flavonoid biosynthetic pathway can be followed at every step, starting with the precursors and ending with the production of anthocyanins [44]. All the enzymes involved in the process, except anthocyanidin synthase, have been determined, characterised, and their genes isolated. The colour of the flowers was changed to Gerbera, Rosa, Petunia and Dianthus. The first transgenic flower was obtained in 1987 in Petunia by inserting the gene encoding the DFR enzyme. The insertion of genes involved in the biosynthesis of flavonoids, mediated by *Agrobacterium tumefaciens*, has, in many cases, led to an alteration in the colour of the flowers without leading to changes in the characters present [45]. Some work on *Zantedeschia sp.* and Osteospermum has made it possible to characterise the biosynthetic pathways of anthocyanins responsible for the orange, red and blue colouration of flowers, showing that only the lack of one of these enzymes results in the formation of petals with different colours, up to and including white. The use of antisense sequences proved to be an efficient way of inhibiting the expression of specific plant genes [46,47]. The wide range of colours obtained was found to be dependent on higher, lower or even no expression of the enzyme. In orchids, molecular techniques made it possible to identify DNA fragments present only in mutant lines of white-flowered Dendrobium. Genetic engineering techniques have also enabled the development of new flower colour classes by inserting the gene for green fluorescent protein (GFP) extracted from jellyfish into plant genomes, resulting in new flower colour classes [48,49]. The characteristic of the GFP protein to generate fluorescence in the visible range in petals could also provide a new method of phenotypic or physiological monitoring and the possibility of discriminating transgenic plants. Genetic improvement and hybridisation work have resulted in new colours and fragrances in Echinopsis hybrids (Figure 2). Currently, studies have not only focused on colour intensity but also on the life of the flowers on the plant [50].

Figure 2 Hybridization and genetic engineering techniques allow Echinopsis hybrids with distinctive colours and shapes to be obtained

5. Conclusion

The colour of flowers is one of the fundamental characteristics for evaluating ornamental products. The quality of the flower colour, its preservation in the post-harvest stages and the variety of colours available are, in fact, essential for the economic success of a cut flower or flower pot species. The biochemical processes underlying pigment biosynthesis, external factors that can alter flower colour, genetic analyses of character control and gene transfer involved in the expression of flower tissue colour have been described in this review. To date, considerable knowledge is available on the biosynthetic process of flavonoids, which are the most common pigments directly responsible for most colours. In addition, many studies have been conducted on the external factors that modify floral colouration. As a result, various techniques and practical applications have been proposed to floriculturists to improve and preserve the quality of flower colour. The numerous studies on the biochemical and genetic aspects of flavonoid biosynthesis have made it possible to acquire the indispensable bases for the development of biomolecular strategies and techniques capable of intervening in the modification of flower colours, such as the insertion of genes not present in the gene pool of the species under examination, the insertion of genes capable of overcoming genetic blocks, or the insertion of exogenous DNA or RNA capable of interfering with the expression of the endogenous gene at the level of transcription or the synthesis of the protein product. Introducing these techniques in ornamental plants will make it possible to produce new flower colours otherwise unattainable in that species, a goal that has already yielded many results.

Compliance with ethical standards

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Disclosure of conflict of interest

The author declares no conflict of interest.

Statement of ethical approval

The present research work does not contain any studies performed on animals/humans subjects

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