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Breeding for Colour Development in Vegetable Crops: Current Approaches and Achievements

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Colourful vegetables have enormous nutritional and medicinal value. The colour in vegetables helps in photosynthesis, pollination by attracting pollinators, nutrition content and have higher consumer preference. Pigments make nature colorful and likable. Plant pigments usually refer to four major well-known classes: chlorophylls, carotenoids, flavonoids, and betalains (Table 1.1). Each class may contain various numbers of chemical compounds that can be structurally categorized into distinct subgroups. Most pigments are colored. In general, the visible colors are the emission of certain wavelengths of light by colored pigments after they selectively absorb others specific to their molecules. The color spectra of pigments are illustrated with some examples of pigment rich fruits and vegetables. Absorbed light may be captured by a few capable pigments as energy to fuel plant photosynthesis and biochemical reactions. These colored pigments not only visually attract animals for flower pollination and seed dispersal but also function in critical biological processes for plants and play essential coevolutionary roles in ecosystems. The biological, ecological, and evolutionary importance of plant pigments and the derived colors cannot be overstated. On the other hand, many pigment-rich fruits and vegetables are critical in the human and animal diet. Some pigments are essential nutrients, and others may serve as nutraceuticals with additional medical benefits, including the prevention and treatment of certain diseases. Chlorophylls are the source of green in all land plants and green algae and function as the primary pigment to capture yellow and blue light for photosynthesis to power plant development and growth. Unlike chlorophylls, the other three are accessory pigments (generally with the absorbance spectrum complementary to chlorophylls) and secondary metabolites that possess much more diverse structures and functions in plants and offer more potential nutritional and health benefits in the diet. In this context, attention is only given to carotenoids, flavonoids, and betalains, with emphasis on the basic biological attributes and dietary benefits of each class.

Table: 1 Pigments in vegetable crops: Types & Distribution

Pigment class	Main subgroups	Typical colors	Examples
Carotenoids	Carotenes, lycopene and xanthophylls	Orange, yellow, red	Carrot, tomato, water melon, pepper, Leaf Vegetables
Flavonoids	Anthocyanins; flavonols	Purple, blue, red	Eggplant, red; Cabbage, onion
Betalains	β-cyanins and β-xanthins	Red,orange, yellow	Beet, Swiss Chard
Chlorophylls	a and b	Green	Any green plants

Carotenoids

Carotenoids are a large family of lipid-soluble tetraterpenoids with a basic 40-carbon polyene hydrocarbon chain structure. This family of over 600 members can be generally divided into two subgroups, carotenes ($C_{40}H_{56}$) and xanthophylls ($C_{40}H_{56}O_2$ or $C_{40}H_{56}O$, the oxygenated derivatives of carotenes), which differ in the terminal rings and oxygenation. For example, α-carotene, β-carotene, and lycopene are carotenes; lutein, zeaxanthin, and violaxanthin are xanthophylls. In plants, certain carotenoids function as complementary light-harvesting pigments to precisely absorb wavelengths of light not gathered by chlorophylls, the primary photosynthesis pigment. They also provide photo-protection against excess light damage to the photosynthetic reaction center by quenching excited species such as singlet oxygen and free radicals or by other carotenoid enabled mechanisms. In addition, carotenoids can be potent antioxidants in lipid formations due to the linearly conjugated carbon bonds that provide high reduction potential. Apocarotenoid hormones, including abscisic acid (ABA) and strigolactone, are produced at the extended steps following the carotenoid biosynthesis pathway, and may play a regulatory or signaling role in the pathway. Most genes and enzymes in the plant carotenoid biosynthesis pathway have been well characterized, and the transcriptional and post transcriptional regulations are being elucidated as well. This information facilitates more efficient selection of carotenoid-rich varieties through conventional breeding and large-scale biotechnological production of carotenoids via metabolic engineering in microorganisms.

Flavonoids

Flavonoids are a huge family (over 9000 members) of water-soluble polyphenolic compounds with a basic 15-carbon benzo-γ-pyrone (C6–C3–C6) skeleton. Numerous combinations of several substitution groups diversify the chemical structures, properties, and biological functions, and result in at least nine major subgroups: anthocyanins (anthocyanidins), condensed tannins (proanthocyanidins), flavonols, flavones, flavandiols, isoflavonoids, chalcones, aurones, and phlobaphenes. In plants, these colored and colorless flavonoids are synthesized in the complex flavonoid biosynthesis pathway and play much more diverse biological roles, compared to carotenoids. Flavonoids, particularly anthocyanins, are high in many colored fruits and vegetables, such as blueberry (*Vaccinium* spp.), blackberry (*Rubus* spp.), raspberry (*Rubus idaeus*), strawberry (*Fragaria ananassa*), grape (*Vitis vinifera*), sweet cherry (*Prunus avium*), plum (*Prunus domestica*), peach (*Prunus persica*), pomegranate (*Punica granatum*), red cabbage (*Brassica oleracea* var. *capitata*), and eggplant (*Solanum melongena*).

Betalains

Betalains are a class of water-soluble indole-derived glycoside pigments that are found only in the order Caryophyllales (e.g., beets, cacti, and amaranths) and never coexist in plants with anthocyanins. In other words, betalains substitute for anthocyanins that are completely absent in the Caryophyllales plants. Betalains differ from anthocyanins in the chemical structures and some properties, but share similarities to anthocyanins in the color spectra, biological functions, and other properties. For example, betalains contain nitrogen but anthocyanins do not. Similarly, betalains are also localized in vacuoles and reportedly offer potent antioxidant capacity and strong chemoprevention function. Betalains can be structurally divided into betacyanins and betaxanthins that color flowers, fruits, and sometimes vegetative organs primarily into yellow, red, or violet. Betanin, with the molecular formula $C_{24}H_{27}N_2O_{13}$, is an important food colorant produced by beetroot. Compared to those many with carotenoids and flavonoids, betalains-containing fruits and vegetables are rather limited and less well known, but include beet (Beta vulgaris), Swiss chard (Beta vulgaris spp. cicla), dragon fruit (Hylocereus undatus), and cactus pear (Opuntia ficus-indica).

Breeding Approaches For Colour Improvement In Vegetable Crops

- Selection
- Mutagenesis
- Hybridization
- Interspecific hybridization
- Somaclonal Variation
- Genetic engineering

Selection

Most of the colour varieties are developed by this method. Selected on the basis of phenotype. Carrot cream colour variety Pusa Kulfi content high lutein and Pusa Ashita have high anthocyanin are the two Classical example.

Mutagenesis

Mutagenesis is a process by which the genetic information of an organism is changed by the production of a mutation. It may occur spontaneously in nature, or as a result of exposure to mutagens. It can also be achieved experimentally using laboratory procedures.

Carotenoid pathway mutants in Pepper & Tomato

Species	Mutant name	Phenotype
Tomato	r (yellow flesh)	Yellow fruit color
	delta	Orange fruit color
	tangerine	Orange fruit color
	Beta	Orange fruit color
Pepper	y (yellow)	Yellow fruit color
	c2	Yellow fruit color

Hybridization

The mating or crossing of two plants or lines of dissimilar genotype is known as hybridization. In plants, crossing is done by placing pollen grains from one genotype i.e., male parent, on the stigma of flowers of other genotype i.e., female parent. It is essential to prevent self-pollination as well as chance cross- pollination in the flowers of female parent used for crossing. The seeds as well as progenies resulting from hybridization are called hybrid or F_1. The progeny of F_1 obtained from selfing or intermating of F_1 plants, and the subsequent generations are known as segregating generations.

High-Carotene Cucumber Germplasm Early Orange Mass 400, Early Orange Mass 402, Late Orange Mass 404. Developed by crosses between U.S. pickling cucumber lines (*Cucumis* sativus L. var. *sativus*) and the orange-fruited Xishuangbannan cucumber (*C. sativus* L. var. *xishuangbannanesis* Qi et Yuan).

Interspecific hybridization

Interspecific hybridization is **the crossing of two species from the same genus**. This allows the exploitation of useful genes from wild, unimproved species for the benefit of the cultivated species. M. L. Tomes(1958) , bred Caro-Red, a Provitamin -A rich tomato variety. Develeped by cross between common tomatoes, *Lycopersicon esculentum* Mill., and the wild species, *L. hirsutum* Humb.Due to its orange colour it not got commercial acceptance and consumers preference.

Somaclonal Variation

Tissue culture derived plants show variation termed somaclonal variation. Chromosomal rearrangements are an important source of this variation A cultivar of sweet potato 'Scarlet' having higher yield and disease resistance characteristics similar to their parent but also have darker and more stable skin colour.

Edible colour rich varieties of vegetables

- **Carrot**

Pusa Rudhira (high lycopene), Pusa Meghali (high beta carotene), Pusa Ashita (high anthocyanin).

- **Radish**

Pusa Gulabi (anthocyanin), Pusa Jamuni (anthocyanin), Pusa Mridula (lycopene).

- **Tomato**

Pusa Rohini (lycopene), Pusa Uphar (lycopene).

- **Onion**

Pusa Ridhi (rich in antioxidant).

- **Amaranthus**

Pusa Lal Chaulai (anthocyanin).

- **Pumpkin**

Pusa vikas (beta carotene).

Case study-1

Anthocyanin enrichment of tomato (*Solanum lycopersicum* L.) fruit by metabolic engineering

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Objectives

- To enrich the anthocyanin content of the fruits of a cultivated tomato cultivar
- To enhance the antioxidant value of tomato fruits.

Material and method

- Two gene - *delila* and *rosea1* from *antirrhinum majus*
- Transformation of tomato with pGAntho construct was carried out by *Agrobacterium*-mediated gene transformation.

Gene cloning and vector construction

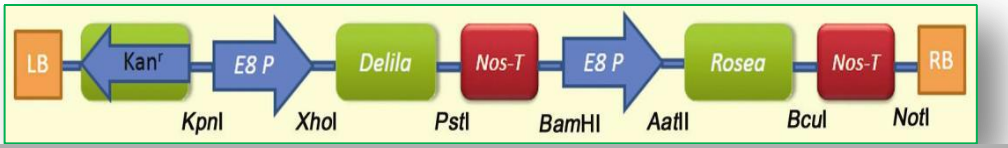

Figure1. Schematic diagram of the *pGAntho* gene construct in pGreen II.

Result

- Transgenic tomato plants accumulating high amounts (70–100 fold) of anthocyanin in the fruit were developed.

Forty-six primary tomato transformants were generated using *Agrobacterium*-mediated transformation using pGAntho binary vector. PCR screening using *Del*, *Ros*1 gene-specific primers confirmed the presence of the transgene in the primary transformants and two lines were selected for further analysis.

Estimation of anthocyanins:

Anthocyanin estimation revealed significantly ($P > 0.05$) higher accumulation of anthocyanins in the transgenic fruits compared to the WT, vector control and commercial control fruits. The average anthocyanin content of the transgenic fruit was 0.1 mg/g fresh weight, which was 70–100 fold higher than that of the control fruits (Figure: *a*).

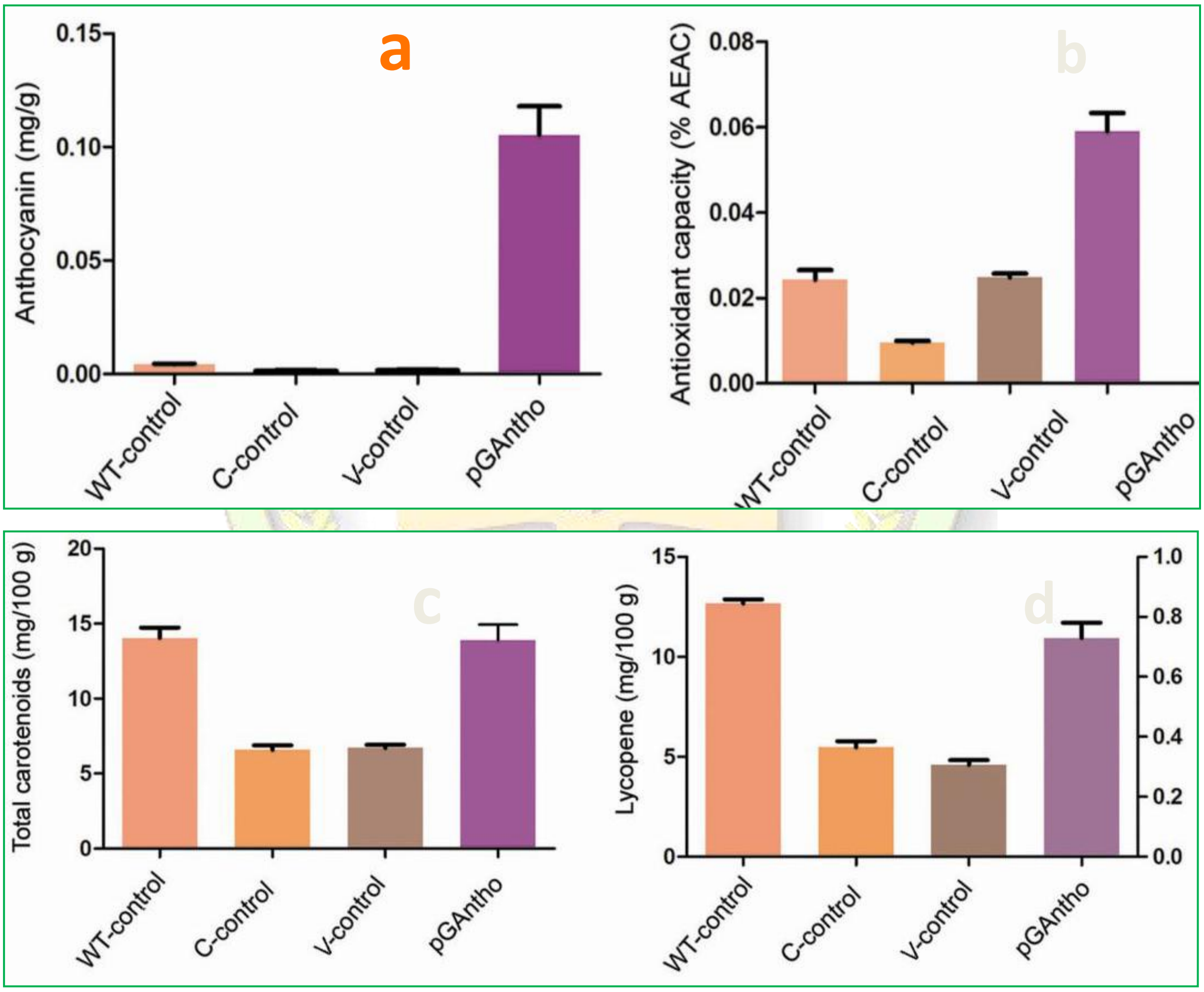

Figure 2. Biochemical analysis of tomato fruit. ***a***, Anthocyanin content; ***b***, Antioxidant capacity; ***c***, Total carotenoid content; ***d***, Lycopene content. Error bars represent standard error mean of triplicates. WT, Wild type; C, Commercial; V, vector and pGAntho, Transgenic tomato.

Estimation of antioxidants:

The antioxidant capacity was significantly higher ($P > 0.05$) in the transgenic fruits (0.0506% AEAC) than in the controls and was 6.2 times higher than that of the commercial control fruits and nearly double than that of the WT control fruits (Figure2 *b*).

Estimation of carotenoids:

The total carotenoid content of the transgenic fruits (13.89 mg/100 g) was on par with that of the WT control, while it was nearly two fold higher than that of commercial control fruits (Figure 2 *c*).

Estimation of lycopene:

The lycopene content of the transgenic fruits and WT control fruits did not differ significantly and was 10.93 mg and 12.65 mg per 100 g fruit respectively, whereas commercial control fruits had only half as much lycopene as the anthocyanin-rich fruits (Figure 2 *d*).

References

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