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# A REVIEW ON METABOLIC ENGINEERING APPROACHES FOR ENRICHMENT AND PRODUCTION OF NEW SECONDARY METABOLITES IN *BASELLA* SPECIES

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## ABSTRACT

Plant metabolism represents a vast range of bioactive compounds with significant pharmacological properties. Secondary metabolites in plants are evolved as an emerging topic of research due to high structural complexity of a wide range of compounds and their economic value as a source of valuable drugs. Flavonoids, an important class of secondary metabolites accumulates in different plant tissues as potent antioxidant and their biosynthetic pathway is well characterized among different plant species. *Basella,* an important leafy vegetable are good source of naturally occurring bioactive compounds with high medicinal value. Metabolic engineering is an important method to identify the effects of pathway engineering in particular plant species which leads to significant changes in composition of natural products on an industrial scale. Recent advances in manipulation of the biosynthetic pathway through metabolic engineering allow increasing the concentration of the existing compound and by evaluating the role of structural and regulatory genes involved. Therefore, the present review highlights the progress towards the development of various metabolic engineering strategies available for flavonoid metabolism in *Basella* and the accumulated compounds which may serve as an important precursor of therapeutic drugs and food products in pharmaceutical and nutraceutical industries.

**KEYWORDS:** *Basella,* Flavonoids, Betalains, Biosynthetic Pathways, Metabolic engineering.

## INTRODUCTION

Plants are the richest source of bioactive compounds that have significant contribution towards human health. Approximately 300000 species of higher plants are available in nature. It is estimated that more than 50000 species of plants are being used for medicinal purposes. Natural products are the most important chemical entities in plants having vast structural diversity and possess a wide range of pharmacological properties which has been explored by mankind for their health benefits from ancient times. Compounds of plant origin are used as a precursor for preparation of novel drugs. Herbal medicines play a vital role in benefiting human health. According to World Health Organization (WHO), herbal medicines have been in regular use for more than 80% of the population worldwide for their primary health care needs. [16]

Natural products from plants can be categorized into primary and secondary metabolites. Primary metabolites are required for the growth and development of living organism including plants which consist of sugars, fatty acids, nucleic acids and amino acids. Secondary metabolites are important natural products, the absence of which does not result in immediate death, but in the long term their impairment may lead to malfunction and reduced survivability. Specific secondary metabolites are often restricted to a narrow set of species within a phylogenetic group. [9] Secondary metabolites are the important phytoconstituents in plants responsible for defence mechanism in plants against pathogen attack. They accumulate and synthesize in plants in response to various biotic and abiotic stress conditions associated with a specific plant family. Various plant families accumulate different classes of secondary metabolites for their defense mechanism. Solanaceae family plants accumulate terpenoids as their defensive compounds whereas Leguminous and Brassicaceae family plants accumulate flavonoids and glucosinolates respectively. [53]

The Shikimic acid pathway and the Malonic acid pathways are responsible for phenolic compound biosynthesis in plants. Most plant phenolics are synthesized by the shikimic pathway. The malonic acid pathway is predominant and significant in fungi and bacteria than higher plants. Shikimic acid is the important intermediate in the biosynthetic pathway. In most plant species, the important step is the conversion of phenylalanine to cinnamic acid by the elimination of an ammonia molecule. This reaction is catalyzed by the phenylalanine ammonia-lyase (PAL) enzyme. Phenylalanine ammonia lyase (PAL) is one of the best-studied enzymes of the phenylpropanoid pathway. PAL is an important regulatory enzyme of

the secondary metabolic pathways in plants. Various external and internal factors such as hormones, nutrient levels, light, fungal infection and wounding affects the activity of PAL.[36] The shikimic acid pathway, non-mevalonate (MEP) pathway and mevalonate (MVA) pathway produces a diverse range of compounds, such as the terpenoids, alkaloids, flavonoids and anthocyanins. These compounds have significant commercial value as pharmaceuticals, nutraceuticals, dyes, fragrances, flavours and pesticides due to their varying structural diversity. [51]

Flavonoids are considered as the important class of secondary metabolites widespread in the plant kingdom known to have a vast range of pharmacological properties. They are absorbed in the range of 280-315nm region and act as UV filters to protect photosynthetic tissues from internal damage. Some of their known functions are in flower pigmentation, plant defense against pathogens and legume nodulations. They play a crucial part as constitutive antifungal agents or as phytoalexins towards disease resistance. Isoflavonoids, flavans or flavanones are among the different classes of flavonoids known to be good antifungal agents in plants. Proanthocyanidins are known to provide defence against herbivores. They possess various pharmacological properties such as antioxidant, antimicrobial, anti-inflammatory, oestrogenic and anti-tumor activities. [6]

Genetic engineering in plants is done to increase the production of important metabolites or to form a new metabolite in plants. The function of enzymes involved in the accumulation of secondary metabolites makes the study of metabolic pathways simpler and easier. The use of genetic engineering in plant secondary metabolism is testing the reason being that similar enzymes functions in the same way and a small change in their configuration or structure leads to different catalytic activity. The recent advances in metabolomics study and emerging technologies provide an opportunity to separate and measure complex matrices such as plant tissues which will further help to assess the final consequences of any modifications of the metabolic pathways. Metabolic engineering improves the metabolite composition at the cellular levels thereby eliminating the undesired ones and enhances the production of existing secondary metabolites in plants. [1] The most common modification such as hydroxylation, decarboxylation, oxidation/reduction, and methylation. are intended to make small changes to the existing structural configuration to increase the functionality of each metabolite. The present review is aimed to understand the pathways predominant in *Basella* species and the possible methods or ways to study metabolic engineering of flavonoid pathway in *Basella*.

Further examples of enzymes involved in flavonoid biosynthetic pathways across different plant species are discussed to understand the mechanisms and applications of metabolic engineering.

### *Basella*: Nature, Occurrence and Distribution

*Basella* is an important leafy vegetable and medicinal herb contains various classes of bioactive compounds with significant pharmacological properties. These species of plants are succulent, branched with climbing growth habit.[7] These species have two important varieties known as *Basella rubra* Linn and *Basella alba* Linn belongs to the Chenopodiaceae family. They are commonly known as Ceylon spinach, Malabar spinach and Indian spinach. *Basella rubra* and *Basella alba*, are locally named as (Pui Shak) in West Bengal. They grow well in hot and humid climates. *B. alba* has round leaves, and green colour stems where as *B.rubra* has oval shaped leaves and red coloured stems.

### Ethnobotanical and Pharmacological properties

*B alba* is good source of vitamin A, vitamin C, vitamin B9 (folic acid), calcium, magnesium, iron.[7] They consists of vital anti-oxidants and essential amino acids such as arginine, isoleucine, leucine, lysine, threonine and tryptophan. [8] They are effective against anaemic patients and useful against various allergic reactions such as cold, cough and mild fever. [9] *Basella* fruit contains gomphrenin derivative which is a betalain pigment. Intake of *B. alba* as a part of regular diet has shown to improve vitamin A content in men and the leaf juice is used as demulcent in the case of dysentery. [10] The fruits of *Basella* plant species are deep red-violet in colour which are used as a dye and food colouring agent in the food industries. Stem and leaves of *B.alba* and *B.rubra* consist of mucilage which shows diuretic, laxative and antipyretic properties. The mucilage from leaves is also used as an external remedy for headaches. [11] Mucilage can be used as a thickener, water-retention agent, gelling agent and suspending agent. *B.alba* is widely used in Ayurveda system for the treatment of haemorrhages, skin diseases, sexual weakness, ulcers in children and pregnant women [7] Medicinal properties like anti-inflammatory, anti-cancer and anti-oxidant activity are reported for both species of *Basella.* [12] Fruit extracts of *B.rubra* were evaluated for their cytotoxic studies which showed 80% of the cell inhibition against Human cervical carcinoma cells of the cell line (SiHa). [13] Methanolic extract of *B.alba* were tested for androgenic activity in Male rats. The results showed that the plant extract significantly stimulated the testosterone and estradiol production in Leydig cells of rats. [14] Another study conducted on Wistar strain

albino rats to evaluate their haematological and biochemical parameters against an aqueous extract of *B.alba* leaves. The results revealed a significant increase in RBC count, WBC count, Hb concentration and platelet count which shows the importance of *B.alba* leaves to be taken as part of the regular diet for proper health management. [15] Ethanolic extract of *B.alba* showed good nephroprotective activity tested against GM-induced Wistar albino rats. The extract drastically decreased the levels of GM-induced elevated serum & urine levels of sodium, potassium, calcium, protein, creatinine, urea, uric acid and Y-glutamyl transferase enzyme activity. [16]

*Basella* species are known to have good antimicrobial activities against various pathogenic organisms. Methanolic extract of *B.alba* was studied for their antimicrobial activity against (*Staphylococcus aureus, Micrococcus luteus, Bacillus subtilis and Pseudomonas aeruginosa*) and showed strong inhibition potential against all the bacterial strains.[17] Another study conducted on leaf ethanolic extract of *B.alba*, showed positive response against (*Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli*).[18] Antimicrobial activity of *B.alba* fruit extracts was tested against eight different microorganisms (*Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Lactobacillus, Klebsiella pneumonia, Aspergillus niger and Aspergillus fumigates*). The result showed highest activity against *Lactobacillus* and *Aspergillus fumigates* and moderate activity for all other microorganisms except *Klebsiella pneumonia*. [19] Aqueous, ethanolic and petroleum ether extracts of the leaves of *B.rubra* were tested against (*Staphylococcus aureus, Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Aspergillus niger* and *Vibrio cholera*). The antimicrobial activity of all the extracts was found to be higher against *Escherichia coli* and the mean zone of inhibition was also highest for *Escherichia coli*. No inhibition was shown against *Pseudomonas aeruginosa* for all the extracts. Therefore, the above studies reveal the potent antimicrobial activities of *Basella* species tested against various Gram (+ve) and Gram (-ve) organism and may lead to the discovery of new antibacterial drugs.

**Phytochemical composition**

Both species of *Basella* were analysed for their phytochemical composition. A recent study reported the presence of flavonols such as Myricetin, Quercetin, Luteolin, Apigenin and Kaempferol in the fruit extract of *B.rubra* along with phenolic compounds such as Generic acid, Sinapic acid, Ferulic acid, Coumaric acid and Chlorogenic acid.[13] The accumulation of

isovitexin (Apigenin glycoside) was shown in *B.rubra*.[20] Another study done on both species of *Basella* for the identification of flavonoids and it was found that Kaempferol is present in the shoot of both varieties in 1.4 and 3.6 mg/100 gram of fresh weight. [21] *B.alba* fruits were investigated for their bioactive compounds which show the accumulation of Gomphrenin-I, Betanidin-dihexose, Isobetanidin-hexose confirmed by HPLC and MS. [22]

**Pathways involved in *Basella* species**

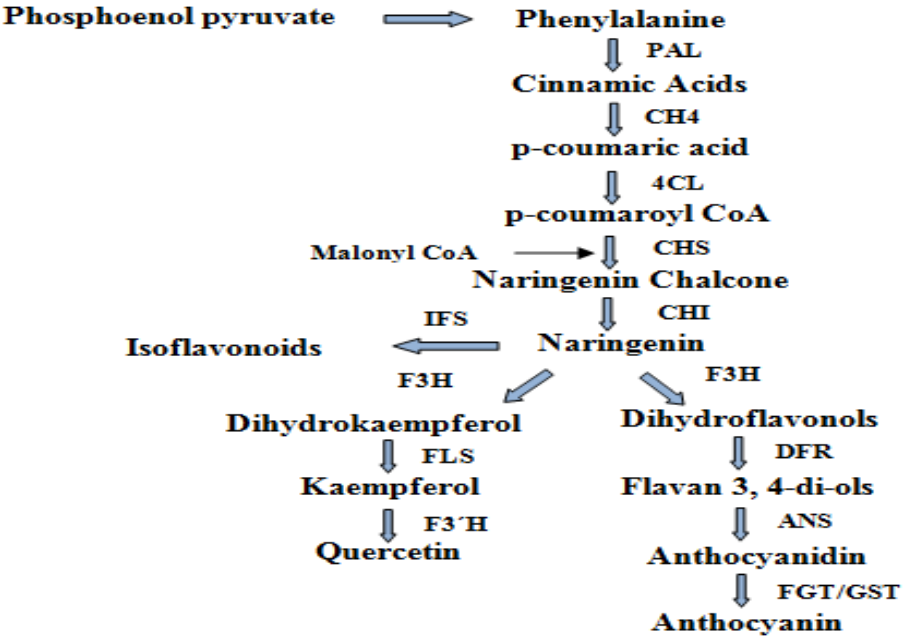


**Figure 1. Flavonoid pathway**

Flavonoids, have basic C6–C3–C6 ring configuration with enormous structural diversity and classified into different groups based on hydroxylation, methylation, glycosylation and acylation. Based on their structural configuration, they are classified into chalcones, flavones, flavonols and anthocyanins. They occur in epidermal cells of plant parts such as roots, stems, leaves, flowers, and fruits, in both glycosidic forms (glycosides) and non-glycosidic forms (aglycones). They are found in the form of water- soluble glycosides in epidermal cells of leaves but also occur in small amounts in epicuticular wax on the upper leaf surface. Flavonoids normally occur as glycosides and methylated derivatives in plants and are synthesized in the cytosol. The pathway is well understood and is conserved among seed plants. The upstream pathway leads to the formation of the core (flavylium ion), the basic skeleton of all flavonoids starting from the condensation of three molecules of malonyl-coA and one molecule of 4-coumaroyl coA to yield a tetraketide by chalcone synthase enzyme.[23] Chalcone synthase (CHS), a polyketide synthase, is the first committed enzyme in the

pathway. This tetraketide further undergoes cyclization to form naringenin-chalcone. The above reaction is catalyzed by the enzyme chalcone-flavanone isomerase. The next step in flavonoid biosynthesis is catalyzed by chalcone isomerase (CHI) which converts naringenin-chalcone to (2S)-5, 7, 4'-trihydroxy flavanone. Naringenin is considered to be an important intermediate in flavonoid biosynthesis which gives rise to different classes of flavonoids. The chalcone isomerase (CHI) enzyme is divided into two types: Type1 and Type2. Type1 (CHI) is responsible for the conversion of Isoliquiritigenin to Liquiritigenin because it is available in both legume and non-legume plants. The next step is the conversion of flavanone Naringenin to Dihydrokaempferol catalyzed by the enzyme flavanone-3'-hydroxylase. Flavonols are another class of flavonoids that diverges from the flavonone branch pathway. Dihydroflavonols are biosynthetic intermediates in the formation of flavonols. Flavonoid- 3-hydroxylase (F3'H) and flavonoid 3', 5' hydroxylase (F3'5'H), which are P450 enzymes, catalyze the hydroxylation of dihydrokaempferol (DHK) to form (2R,3R) dihydroquercetin and dihydromyricetin, respectively. Dihydrokaempferol is converted to kaempferol by flavonol synthase which further undergoes glycosylation at the 3rd position to yield different conjugates. Flavonol synthase introduces double bond between C-2 and C-3 positions to produce various structures such as Myricetin, Quercetin and Kaempferol. Quercetin further undergoes methylation and glycosylation at 3, 4, and 7 positions by the action of enzymes flavonol 3-O-glucosyltransferase and flavonol-7-O-glucosyl transferase to produce different derivatives. The enzymes catalyzing these steps have been isolated from Arabidopsis. The enzyme flavonoid 3', 5'-hydroxylase plays a vital role in the formation of anthocyanins which influence flower color. F3'H and F3'5'H determine the hydroxylation pattern of the B-ring of flavonoids and anthocyanins, and are necessary for cyanidin and delphinidin production, respectively. F3'H and F3'5'H catalyze the hydroxylation of flavanones, flavonols and flavones. Anthocyanidin synthase (ANS, also called leucoanthocyanidin dioxygenase), is responsible for the synthesis of colored anthocyanidins. The genes encoding the enzymes involved in flavonoid biosynthetic pathway has been isolated and characterized from flowers of different plant species. Some of the examples includes petunia, snapdragon (*Antirrhinum majus*), gentian (*Gentiana triflora*), torenia (*Torenia hybrida*), morning glories, and other tissues of Maize, Perilla and Arabidopsis.

Flavonols, an important class of flavonoids are known to have potent dietary antioxidant properties. Kaempferol and Quercetin are well-known flavonols with significant pharmacological properties and considered as main targets for metabolic engineering in

plants. Studies were done to evaluate the gene expression of the enzymes involved in the production of flavonols. Naringenin-chalcone is known to accumulate in low levels in the peels of tomato fruits. Constitutive expression of petunia chalcone isomerase (CHI) led to the formation of naringenin, and further converts naringenin to flavonol glycosides, only in the peel. Co-expression of two maize transcription factors, Lc and C1, led to the formation of kaempferol in the fruit. This results in the activation of all the structural genes (except for CHI) necessary for the formation of kaempferol and related anthocyanins.[24]

**Betalains**

Betalains are a nitrogen containing pigments synthesized in vacuoles. They show accumulation in leaf, stem, root, fruit, inflorescence/flower, petiole, bract and seed grains They show brilliant colour in fruits or flowers of different plant species mostly belonging to the family Caryophyllales. Apart from Caryophyllales, they are prevalent in families such as Aizoaceae, Amaranthus, Cactaceae, Didiereaceae. Betalains are an important class of phyto-compound found in red beet (*Beta vulgaris*) used as a natural colorant.[13] The color of betalain does not depend on pH and is more stable in comparison to anthocyanins. Betalains are known to have antioxidant and radical scavenging properties. [25] They are classified into red colored betacyanins and yellow colored betaxanthins. Over 50 molecular species of betacyanins and several betaxanthins are known to exist across different plant species. [26]

**Biosynthesis of betalains**

The biosynthetic pathways of betalains and the role of enzymes and genes involved are less understood than flavonoid pathway in plants. Many studies in the recent past have been carried out to understand the complexity of the structures and enzymatic reactions in the betalain pathway. Tyrosine is the main precursor or starting compound for betalain biosynthesis.[27] Another study reported tyroamine is responsible for betalains production in plants.[28] Tyrosine-based pathway for betalain biosynthesis is well known and has been studied in detail across various plant species. The first step in the biosynthesis of betalains is the hydroxylation of tyrosine catalyzed by tyrosinase enzyme having hydroxylase activity which produces di-hydroxyphenylalanine (DOPA). Tyrosinase exhibits oxygenase and oxidase activity. Tyrosinase stimulates betalain production in the presence of Cu2+. [13] Betalains can be synthesized by two independent ways from DOPA. At first, DOPA is catalyzed by DOPA dioxygenase to produce betalamic acid which further is converted to betaxanthin in conjugation with amino acids and amines. Betalamic acid is the chromophore

molecule for both betaxanthin and betacyanins. In another way, DOPA is catalyzed by the enzyme DOPA oxidase and polyphenol oxidase to form cyclo DOPA (cDOPA) which gives rise to betanidin. cDOPA and its derivatives are necessary to produce betacyanin. Betanidin is further converted to betanin by betanidin-5-glucosyl transferase. Further modification of (cDOPA) with sugar molecules gives rise to various glycosyl moieties. [28]

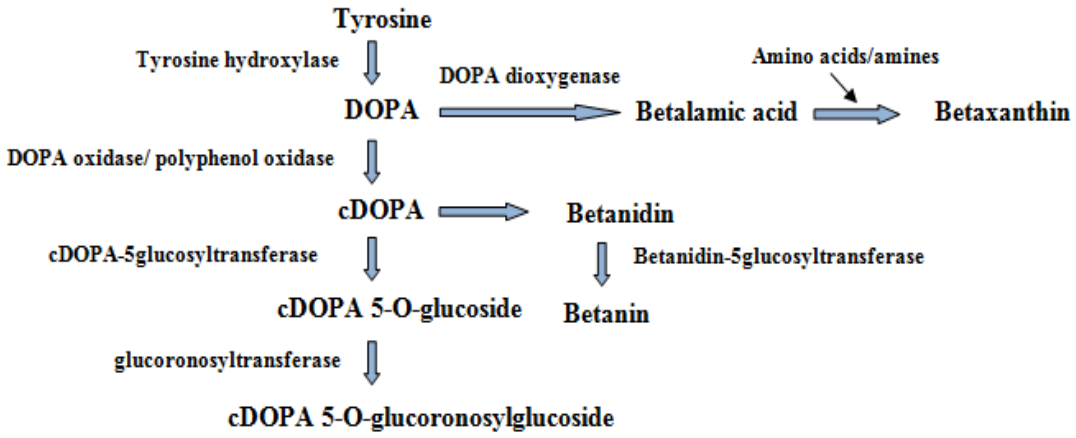


**Figure 2. Betalain pathway**

Eight different species of betalain producing plants has been studied in detail to identify the genes encoding DOPA-dioxygenase and glucosyltransferase. Tyrosinase, an important enzyme in the pathway has been characterized and purified from betalain producing plants and the gene encoding it has been cloned from the fungus *A.muscaria*. [25] The two possible routes of betalain biosynthesis are evidenced from studies across different plant species. A study done from the culture cells of livingstone daisy *(Dorothenthus bellidiformis)* confirms the glucosyltransferase activity of betanidin. [29] Another study on cultured cells of livingstone daisy showed that the enzymes involved in glycosylation of betanidin (UDP-glucose:betanidin 5-O-GT and UDP-glucose:betanidin 6-O-GT) were purified and characterized. [30] The presence of cDOPA-5-O-glucoside in young beet plants [31] and root peels of red beet has been observed. [32] External factors are also responsible for betalain accumulation in plants. A study reported the activation of biosynthetic genes in response to herbivore stress which finally leads to the accumulation of betalain in *A.hyphochondriacus*.[13] Although the above studies reports the accumulation of betalain related compounds but genes and enzymes involved in betalain biosynthesis has been partly characterized in limited number of species.[26] Therefore, flavonoid pathway was choosen as an appropriate pathway to study metabolic engineering in *Basella* plant species.

**Emerging tools for identifying metabolic engineering targets**

The plant system should respond to a stable transformation to undergo the process of metabolic engineering. Metabolic enzymes in plants can act on a number of different substrates. The substrate specificity exhibited by these biosynthetic enzymes provides a unique platform for evolutionary innovations. [33] Plant metabolic engineering can also be used to increase plant performance, for example by increasing resistance to pathogens or tolerance to abiotic stress conditions.[34] The defense compounds play a crucial role in the secondary metabolism of plants. They are of the different types such as phytoanticipins which are constitutively expressed and phytoalexins which are synthesized either of infection or stress (biotic and abiotic) conditions. [35] Therefore, the regulations of secondary metabolism in plants are partly dependent on external stress signals. The approach towards metabolic engineering in plants to enhance natural product yield depends on the desired outcome. Flavors, aromas, and pigments are popular targets for plant metabolic engineering. [24] For food crops, to increase the ability of a plant to adapt to varying environmental conditions (e.g. pests, temperature, drought, UV) conditions or to enhance the nutritional value of a food product, it is recommended to increase the production of an entire class of compounds. Increased yield of a single product is achieved through targeting specific compounds within a biosynthetic pathway to explore their significant biological applications. Upregulation of specific pathway genes, competing pathway genes, transcription factors, as well as the introduction of heterologous genes can be a suitable approach in this case. But to implement these strategies, at least, partial characterization of the biosynthetic pathway for the desired compounds is required. The intermediates of the pathway should be known to identify the enzymes involved. But the problem arises during the isolation of the enzymes which is affected by low levels of the enzymes and to find out the substrate for measuring activity. As the biosynthetic pathway consists of different compartments, the intermediates need to be reallocated in the proper compartment. Reallocation is a complex phenomenon in plant cells which usually involves the process of diffusion. Storage is a key aspect of secondary metabolites production. Secondary metabolites are stored in the vacuoles. A basic and broad understanding of the desired biosynthetic pathway is necessary to undergo genetic modification. The key is the step by step approach for elucidating the pathways. One of the best examples to understand the metabolic engineering strategies is the expression of a (*Narcissus pseudonarcissus*) phytoene synthase enzyme in the rice endosperm which shows the accumulation of colorless carotene phytoene. It shows the capability of rice to express genes in the β-carotene, which requires three plant enzymes. Another classical example is the

production of Golden rice achieved by the expression of a bacterial carotene desaturase from (*Erwinia uredovora*) along with phytoene synthase and lycopene- β- cyclise from (*Narcissus pseudonarcissus*) in the rice endosperm. This expression resulted in the accumulation of yellow coloured β-carotene. [5] Although the secondary metabolism in plants is vast and extensively studied but very few of the pathways in plants are elucidated completely regarding intermediates, enzymes and genes. One has to clearly understand the flux in the metabolite pathway for doing metabolic engineering of plant secondary metabolism. The engineering mechanism in plants can be done either by increasing or decreasing the flux through a pathway. Enzymes of the metabolic pathway regulate flux. Decreasing the flux helps to eliminate toxic or undesired compounds and the pathways that compete or interferes with the pathway of interest. The pathway that leads to catabolism might also interfere with the increased levels of desired compounds. Further to decrease a flux, it is desirable to decrease the level of the protein of interest by an antisense or RNAi approach. Selecting targets for metabolic engineering in the pathways is an important phase of the process to increase the level of a compound. It helps to identify possible sites of modification by overcoming limiting steps. Engineering long pathways require extensive studies to elucidate the pathway as only a few genes of plant secondary metabolite pathways are known. Microbial genes can be used to produce certain reactions in plants for which the encoding plant genes are not known yet. One such example of a microbial gene over expression is the production of salicylate in plants. [36] Over expression of regulatory genes is another approach which can be used to induce series of genes of a secondary metabolite pathway. [37] This can be explained by an example in which metabolic engineering of the signal transduction pathway was done to induce a pathway of interest, e.g. over expression of a transcription factor. [38] Using inducible promoters to modify a constitutive pathway into an inducible one is another possibility for metabolic engineering. This method has the advantage of separating growth and secondary metabolite production, to avoid competition between the two processes for the energy use and precursor pools in the plant cells.

**Approach towards metabolic engineering study in *Basella* species**

The studies conducted on *Basella* species [13, 20, 21, 22] reported the presence of phenolics, flavonoids and betacyanins from different parts of *B.alba* and *B.rubra* which shows the occurrence of phenolic, flavonoid and betacyanin pathway in *Basella*. The above studies show the potential of these plant species to treat various human ailments because the compounds identified possess a wide range of biological activities with significant

pharmacological properties. The application of various methods of metabolic engineering are explained with suitable examples in different plant species to get a better understanding and detailed analysis of the process to be implemented for the pathways and enzymes involved in *Basella*. Among all the three pathways known to occur in *Basella*, the genes of flavonoid pathway have been extensively studied across various plant species. Therefore, this study focussed particularly to understand the complexity of genes and their role towards the synthesis of flavonoids in *Basella* and further the methods by which one can increase the production of existing compounds or synthesis of new compounds to enrich the significance of the plant species as a potential source in food and pharmaceutical industries.

Flavonoid studies across different plant species have generated a vast knowledge about the pathway which enabled modern tools for successful metabolic engineering of the flavonoid pathway. Therefore, engineering of flavonoid biosynthesis in plants has significant scientific and economical importance. Their wide occurrence, complex structural configuration and diverse functions made them a suitable system for chemical, genetic and enzymological studies. Metabolic engineering has provided a means to improve flavonoid composition as well as content. Successful strategies for flavonoid engineering in plants can be achieved by addressing these four key challenges.

- Firstly, the introduction of single or multiple genes of the metabolic pathway can resolve the structural challenge. Over expression of the rate-limiting enzymes, may often increase flavonoid accumulation.
- Transcriptional factors play a major role in overcoming the regulation issue. In plants, identification of metabolite-specific transcription factors and the over expression of these factors to co-ordinately activate the entire pathway has led to the successful engineering of metabolic pathways.
- Metabolic flux challenges can be countered with the whole genome or targeted transcriptomic and metabolomic analysis. Based on pathway modelling and flux analysis, over-expression of target genes or suppression of competitive metabolic pathways has allowed redirection of metabolic fluxes, increasing the yield of the desired products.
- Finally, storage is an important challenge needs to be tightly controlled in the plant. Specific transporters of secondary metabolites play important roles in systematic metabolic engineering. These transporters can move the products to the proper sub-cellular location or extracellular space for sequestration. They allow high-level accumulation without toxicity to the cells or the establishment of metabolic equilibration

in favour of biosynthesis by removing the products away from the biosynthetic enzymes.[39]

Major eatable food crops have less accumulation of flavonoids in specific tissues or lack important class of flavonoid. Engineering of flavonoid pathways can enhance the nutritional value of the edible part of the plant. So, the enzymes responsible for various modifications of core flavonoid structure such as methylation, prenylation, hydroxylation and acylation needs to be identified and characterized for successful engineering of the flavonoid pathway. Many of the structural and some of the regulatory genes of the flavonoid pathway has been cloned from several model plants systems such as Maize, Antirrhinum, Tobacco, Petunia and Arabidopsis [40] and have been expressed in genetically modified micro-organisms. [41] The first study on genetic modification of the flavonoid biosynthetic pathway was reported in 1987. The study was done on anthocyanin biosynthesis in petunia by modifying a gene from maize which showed the accumulation of pelargonidin type anthocyanin. [42] Another study conducted on Petunia reported the silencing of the endogenous homologous gene by an additional chalcone synthase (CHS) copy resulted in the loss of flower colour. [43] Similar studies were reported in Petunia. [44, 45, 46] CHS is the key and most extensively modified enzyme in the flavonoid pathway. Another example of genetic modification in anthocyanin biosynthesis was observed in Rose flower with the production of blue flower that led to delphinidin derivatives. [47] Metabolic engineering not only modify flavonoid content but also their composition in food plants.[48] The above statement can be explained by a study conducted on tomatoes whose flavonoid content was enriched which reduced cardiovascular risk markers in experimental animals and showed a positive effect on health.[49] Dicot plants have been studied and examined for metabolic engineering of flavonoids. It has been observed that in most dicot plants, the structural genes are regulated as two sets i.e. early genes and late genes. *Arabidopsis thaliana* consists of several traits which make it useful model for understanding the genetic patterns of the flowering plants. A study shows that flavonoid production under the influence of light, first results in the production of transcripts of CHS, CHI, F3H, and FLS in Arabidopsis. In the later stage, DFR and ANS are activated. [50] The study conducted in Antirrhinum and Petunia was similar, although F3H belongs to the set of "late" genes in Antirrhinum.[51] Tomato fruit peel accumulates low levels of kaempferol and quercetin. The introduction and over-expression of the regulatory genes Lc and C1 of maize led to an increase in kaempferol levels formation up to 60% in the flesh of the fruits. The introduction of CHI gene from Petunia resulted in an increase of up to 70% in quercetin

formation in the peels. In potato, expression of Lc and C1 genes led to significantly increased levels of kaempferol accumulation in the potato tubers. [52]

Flavonoid intermediates which don't exist naturally in the target plant can be tested which reflects the capability of internal enzymes that whether they can convert the flavonoid intermediates to the desired flavonoids in the target plant system. A study reported that the gene encoding FNS II has been cloned from snapdragon and torenia for improving the flavone formation to enrich the nutritional value.[53] The role of transcriptional factors in controlling pigmentation pattern through the regulation mechanism of structural genes of the flavonoid-anthocyanin pathway has been identified in many plants.[40] Transcriptional regulation of structural biosynthetic genes has evolved as a major mechanism in plants. Specific transcription factors interact with promoter regions of target genes, thus regulating the initiation rate of m-RNA synthesis. [54] Various factors such as tissue type, internal signals (e.g. hormones) and external signals (e.g. microbial elicitors, UV-irradiation) are considered to be important for controlling regulatory genes responsible. [55] The use of transcription factors for metabolic engineering in plant systems is found to be more effective when large numbers of genes are known to be associated with domestic use of the crop plants. In this situation, nearly 70% of the genes are transcriptionally regulated while more than 20 % of the genes encode enzymes. At the cellular level, enzymes are localised to different but specific compartments. An important challenge continues to be establishing in which cells particular metabolites accumulate and within a cell in which compartment or compartments. This aspect of metabolism is particularly relevant when multiple cell types are part of a single metabolic pathway.[34] In this review, efforts are made to look at recent examples of metabolic engineering of flavonoids in different plant species. The secondary metabolites known to be present in *Basella* are known to be good antioxidant along with other beneficial properties such as anticancer, anti-inflammatory etc. The methods discussed here can be implemented for metabolic engineering in *Basella* plant species. These examples will certainly provide a unique opportunity to understand the genetics of the flavonoid pathway in *Basella* species, which is not entirely explored and further the way of enhancing its pharmacological properties and phytochemical composition with the synthesis of new compounds. Though metabolic engineering methods significantly improves the production of secondary metabolites in plants but the complexity of the pathways and localisation of the precursors and intermediates between the compartments makes the process a bit tedious. Metabolic pathways show high degree of connectivity when metabolites are involved in two or more

pathways. Therefore, the introduction of large number of input genes has the potential to give unexpected effects. [56] Plant metabolic engineering is full of promise, but success cases continue to be rare. In the last few years, unique analytical methods have been developed that allows the identification and sensitive detection of molecular masses of secondary metabolites in various complex mixtures of plant systems. [34]

## CONCLUSION AND FUTURE PROSPECTS

The flavonoid pathway is extensively studied and well characterized in plants. Metabolic engineering provides unique opportunity to explore new tools for the illustrative study of flavonoid pathway and simultaneously identifies the role of genes which helps to enhance the productivity and increased levels of existing compounds in a specific plant species. Plants have regulatory systems for correlating metabolic activities of the pathway. A particular metabolic pathway can be controlled either by over-expressing and or suppressing several enzymes or through the use of transcriptional regulators which can control several endogenous genes. Specific genes and part of the pathway that is engineered results in the accumulation of various coloured pathway intermediates. Enzymes of the metabolic pathway are localised in different compartments specifically at the cellular level. To improve metabolic efficiencies in any of the plant systems, four factors i.e. structural, regulatory, metabolic flux and storage are considered most for better outcomes of the employed method. The present review highlights various aspects of plant metabolic modelling in context of understanding predicting and modifying complex flavonoid metabolism. Metabolic engineering of flavonoid pathway in *Basella* could open a new avenue for the systematic and detailed phytochemical analysis of the flavonoid composition and further can increase the nutritional value by the formation of new secondary metabolites. But to implement these metabolically engineered systems, it is necessary to have the regulatory guidelines. In the forthcoming years, it will be a tremendous challenge to gather and understand useful and updated information about regulation at all levels of genes, enzymes, compartmentation, transport and accumulation. This will open the way for successful strategies for altering the accumulation of individual compounds.

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