Abstract of the Thesis

Carotenoids are a wide-spread group of lipid-soluble pigments with high structural and functional diversities that are synthesized in bacteria, fungi, algae and plants, and accumulated in animals where they may undergo further modifications. Dietary carotenoids play important roles in human health - particularly α-carotene and β-carotene are precursors of vitamin A, the latter is very essential to prevent blindness, xerophthalmia, premature death and other degenerative disorders such as cancer, cardiovascular disease. With the increase in consumer awareness about the health benefits offered by carotenoids from natural sources, the demand for synthetic ones is tapering. Green leafy vegetables form the important dietary source of carotenoids, among which coriander foliage is a rich source, containing about 5 to 15 mg/100 g FW, composed mainly of β-Carotene and Lutein. Coriander is also rich in other vitamins such as folates and ascorbic acid, health promoting anti-oxidants, minerals (zinc, manganese, iron) and other biomolecules of nutraceutical importance. For these reasons, the present study focused on the evaluation of different coriander cultivars, elucidation of the chemical nature of carotenoids and the regulation of their biosynthesis.

Knowledge about the amount of variability present in the genomic composition and the quantitative heritability of qualitative traits are essential for realizing desirable qualities in plant-food commodities. To achieve this, the genetic relatedness and variations among 11 commercially cultivated Coriandrum sativum L. were established using molecular markers such as RAPD, ISSR and ITS markers. Coriander showed very high diversity where some reached maturity much faster and rapidly accumulated nutritional metabolites than in others, indicating a close relationship between genetic diversity with nutritional metabolism. In the present study, using 22 RAPD primers, 9 ISSR and ITS1 and ITS4 primers, gene amplification was done by PCR method. This resulted in totally 269 bands of which 196 are polymorphic bands. RAPD and ISSR analysis revealed 71% and 77% polymorphism respectively. Dendrograms generated using UPGMA showed 4 distinct clusters with complete separation of the highest carotenoids producing variety GS4 Multicut from all other cultivars in both RAPD and ISSR analyses. The variety “Mysore local” and the low carotenoid yielding variety Mahak grouped in different clusters, showing genetic distinctiveness. Even though no sequence variation was observed in ITS region, variations were observed in the 26S rRNA region. A group of 5 cultivars showed highly diverse relatedness from other cultivars. Genetically distinct cultivars are useful for breeding high yielding carotenoid-rich varieties of coriander. In the present study, both RAPD and ISSR based molecular markers provided enough information to distinguish different cultivars with many advantages.

The availability of carotenoid from vegetables depends on their maturity, levels of carotenoid isomers, and food processing methods. To obtain data in this regards, growth characteristics and carotenoids accumulation in 11 varieties of commercially
important coriander (*Coriandrum sativum* L.) were studied. In all varieties, β-carotene content was higher in foliage at mature stage than at seedling stage. Variety GS4 Multicut was found to produce highest biomass 6.18 g/plant, total carotenoids (217.50 mg/100 g DW) β-carotene (73.64 mg/100 g DW) and lutein (130.26 mg/100 g DW) at pre-flowering stage. Among xanthophylls, Lutein was the predominant carotenoid which was followed by very low levels of violaxanthin and neoxanthin. Lycopene and zeaxanthin were not detected, probably due to the complete utilization of these for the synthesis of downstream carotenoids such as β-carotene and lutein.

Since carotenoids are potent antioxidants, the free radical scavenging activity of coriander carotenoids also checked. The total carotenoid fraction showed a strong antioxidant activity in case of hydroxyl radical induced DNA degradation with an IC₅₀ value of 14.29 µg/ml. The extract of coriander also rendered strong protection to DNA against pro-oxidation by Fenton’s reagent, and showed good activity in DPPH assay. Such activities were much higher than standard gallic acid, except for DPPH method. The *trans*-β-carotene is more bioavailable than the *cis* form and normally after processing there is a possibility of *cis*-β-carotene formation (which is stable). Therefore, the stability of carotenoids after processing was also determined. Among several methods tested, microwave drying showed better retention of both carotenoids and chlorophylls in coriander foliage, which also helped to retain the intactness of β-carotene as well as enhanced extractability of pigments.

No study has been conducted on the characterization of carotenoids and their enhancement by elicitation in coriander. This study demonstrated that foliar-application of elicitors, methyl jasmonate (MeJa) and salicylic acid (SA), differentially elicited total carotenoids, β-carotene, lutein, chlorophylls, total phenolics and chlorogenic acid in coriander, GS4 Multicut and Mahak, the cultivars with high and low carotenoids respectively. Carotenoids and total phenolics increased 6.8 and 3 folds respectively when treated with MeJa (10 µmol/L), whereas SA (500 µmol/L) showed 5.4 and 3.5 folds of respective compounds. These treatments also enhanced levels of β-carotene, lutein, chlorophylls and chlorogenic acid. These observations indicate that precise elicitation is a novel natural method for significant enhancement of important compounds in coriander.

To elucidate the molecular mechanism of carotenoid accumulation, transcriptional expression profile of ten carotenoid pathway genes, under the influence of MeJa was determined. In coriander, a significant difference in the degree of expression of genes after treatment is correlated with carotenoid content at different times. Foliar application of methyl jasmonate at low concentration (10 µmol/L) increased the expression level of *PDS, ZDS, CHXE* and *LCYE*. However, a higher concentration (500 µmol/L) decreased the expression of *CHXE* and *CCD1* after third day, which completely vanished on twelfth day. Regulation of carotenoid biosynthetic genes was studied using inhibitors that specifically inactivate certain biosynthetic pathway enzymes, targeting three different regulatory points. For this, fosmidomycin, which inhibits DXR enzyme in the MEP pathway (non-mevalonate)
pathway, norflurazon, which hamper the PDS gene and amitrol, which interrupts the lycopene cyclization were used. Both fosmidomycin and norflurazon reduced total carotenoids, β-carotene and lutein, whereas, amitrol treatment affected mainly β-carotene, although lutein and total carotenoids were found to reduce at later periods. Fosmidomycin reduced the expression of PDS, ZDS, LCYE, whereas, IPI, CHX and PSY remained unaffected. Norflurazon treatment, highly down regulated PDS and ZDS and LCYE, although the expression of BCHY remained unchanged. Foliage lost all pigments after 12-15 days and appeared bleached. In coriander, lutein is the predominant carotenoid in leaves followed by β-carotene. Therefore, data about the regulation of lycopene cyclases which controls α- and β- branches becomes essential. After amitrol treatment, along with β- and ε-cyclases, unexpectedly PDS was also down regulated after three days. Apart from suppression of carotenoids, chlorophylls also disappeared, revealing a direct metabolic association of chlorophylls with carotenoids. The set of data generated here is novel and useful in understanding the mechanisms of regulation of carotenoids in coriander. The current study also provides a platform for the in-depth research on the regulation of carotenoid genes in foliar vegetables, having applications in their genetic improvement program as well as in other food crops.