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Molecular Mapping and Marker Assisted Selection for Development Edible Colour, β-carotene and Anthocyanin Bio-fortification in Cole and Root Crops

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Abstract

Vegetables are commonly called as protected food because of their protective effects against degenerative diseases such as cancer and cardiovascular disease. Inherent richness of vegetable crops for nutrients makes them suitable food material to address the major concerns of hunger and malnutrition in the developing countries Edible colours are natural pigment include anthocyanin, betalins, carotenoids, chlorophylls are responsible for Red, blue, Purple and orange colour in many vegetable crops. Conventional approaches like Single plant selection, Back cross, Pedigree selection and Bulk method already have been attempt but these are time consuming and difficulties in precise phenotyping of quality trait. Discovery of Molecular markers enhance efficiency of breeding, Marker assisted selection, Genome sequencing technology, RNA interference (RNAi) and genetic engineering play a significant role in reducing time and cost and development of edible colour rich vegetable cultivars and used for molecular tagging of desirable trait. Present Review has summarized the attempt and potential for improving Edible colour, β-carotene and Anthocyanin in roots and Brassica vegetable crops.

Keywords: Vegetable crops; Marker assisted selection; QTL mapping; β-carotene (Or) gene; Quality breeding; Anthocyanin (Pr) gene; Molecular marker

Introduction

Vegetables are immense potential to supplies all these mineral nutritional components which are note present in many other cereals crops. Some nutraceutical and edible colour nutrients such as anthocyanin, β-carotene, lycopene and glucosinolates have been reported to play significant role in health and reducing malnutritional problems in developing countries, their contents have been found more in beet root, cauliflower, carrot and broccoli, respectively [1]. Breeding and development of coloured vegetable are eye catching vegetables which are going to popularized in future years. Cole crops and root vegetables are immense value for their rich in nutrients and Neutraceutical and coloured pigment like Anthocyanin, β-carotene, Lycopene and others naturally occurring pigment which are responsible for Blue, purple, red and pink pigmentation in many vegetables crops. One of the most straightforward used breeding β-carotene in cauliflower, cabbage, knol khol, carrot and radish to fulfill the β-carotene requirement human nutrition and health benefit. Crop biofortification is an effective strategy to reduced malnutritional in developing country [2]. Gene introgression involves, selection of desirable targeted genes, and Back crossing is most commonly used for introducing desirable trait in recurrent parents. The (Or gene) a spontaneous semi dominant, single locus mutation responsible for β-carotene accumulation in cauliflower. Research work for introgression of "Or" gene in to Indian cauliflower was carried out at IARI, New Delhi at lead to development country first biofortified cauliflower cultivars "Pusa Beta-Kesari" are rich source of β-carotene (8-10 ppm) to reduced malnutritional problem in our country [3]. Anthocyanins are natural pigments belonging to the flavonoid family. These pigments play important ecological and metabolic functions in the plants. Improved anthocyanin concentration in vegetable has been made to increase their concentration in cauliflower, cabbage, and root vegetable crops like Purple carrot (200-350 mg), Red cabbage (MYB gene, 200-320 mg), Red radish (100-154 mg), and purple cauliflower (Pr gene, 20-60 mg).

Marker Assisted Selection used Introgression "(Or gene)" for High β-carotene Content in Cauliflower

Backcrossing has been widely used to incorporate one or few gene in to adopt elite cultivars. This method was first described in 1922 and was widely used between 1930s by F,N Briggs. The ('Or') Orange mutant of cauliflower is a spontaneous, semi dominant, single-locus mutation that lead to accumulate high level of β-carotene in plastids in curd of cauliflower. Li et al. reported that the 'Or' gene lead carotenoid accumulation in several tissues such as the curd, pith, leaf bases and shoot meristems, and cryptically in some cells of other organs, including the roots and developing fruits [4]. Li et al. reported that a total of 33 polymorphic AFLP fragments were identified to differentiate the wild-type and the 'Or' DNA bulks [5]. Li et al. reported that a total of 33 polymorphic AFLP fragments were identified to differentiate the wild-type and the 'Or' DNA bulks [5]. Harlan suggested that backcross method used, simply phenotyping of desirable trait, selection of desirable trait like cauliflower Curd colour), Carrot and root vegetables (Inner section of roots) etc., and Precise genotyping at early generation selection regarding desirable trait. Kalia et al. (Indian Agricultural Research Institute) started breeding work for introgression of "Or" mutant gene from using

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Marker assisted backcross breeding (MABB) for introgression of Or gene in early maturity group (DC 98-1, DC-41-5, CC-14) and mid early maturity group (DC 309, CC-35, DC18-19) of tropical cauliflower breeding lines and BC2F2, generation he identified few breeding line which carry sufficient quantity of β-carotene (10-12 ppm) [6]. Fore ground selection or positive selection used to detect QTL for targeted gene, SSR markers was developed at the Division of Vegetable Science, IARI for hastening foreground and background selection in Indian cauliflower Or gene introgression programme in cauliflower [7]. Using Molecular marker that flanked (<5 cm) of targeted trait lead to minimized linkage drag and increased selection efficiency of targeted trait (Figure 1).

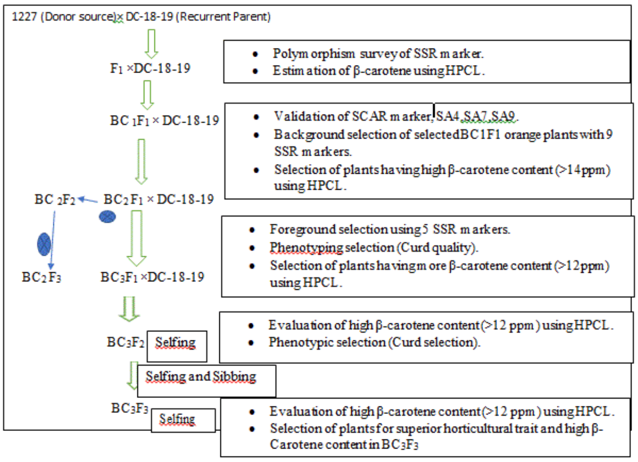

Figure 1: Schematic representation Marker assisted selection procedure for introgression “Or” gene rich β-Carotene in cauliflower [8].

Introgression Anthocyanin Rich “Pr” and β-carotene Rich “Or” Gene in other Vegetable Crops

IARI started breeding work for introgression of β-carotene (Or gene) and anthocyanin (Pr gene) rich genes into different cauliflower cultivars (Pusa Snowball K 1 and Pusa Snowball K 25) and desirable plant are selected and further advanced to next generation through marker-assisted backcross selection. Another work was initiated for introgression of β-carotene rich ‘Or’ gene into cabbage and broccoli at IARI, New Delhi and BC1 population of the introgressed β-carotene rich gene (Or) in cabbage (S 831) and broccoli (Pusa Broccoli KTS 1) was developed and plants are selected which are carrying β-carotene rich ‘Or’ gene are selected and further advanced to next generation through marker-assisted backcross selection. Introgression of “Pr” gene in Indian cauliflower accession lead to development “Pusa Purple cauliflower (KTCPF-1) having intense purple curd colour, and pigmentation in deep inside of bract of curd, average anthocyanin concentration range (43.7 mg/100 g) edible portion. Pusa Purple Broccoli having intense purple head colour has been released from IARI, New Delhi.

B-carotene accumulation is significantly reported in other vegetable crops like in Chinese cabbage, Cucumber and melons [9]. Yang et al. reported inheritance Orange flesh colour in F1 population was dominant nature in derived from crosses between XIS (Xishuangbanna gourd XIS; *Cucumis sativus* var.xishuangbannanesis and white fruited *Cucumis sativus* [9] (Figures 2 and 3).

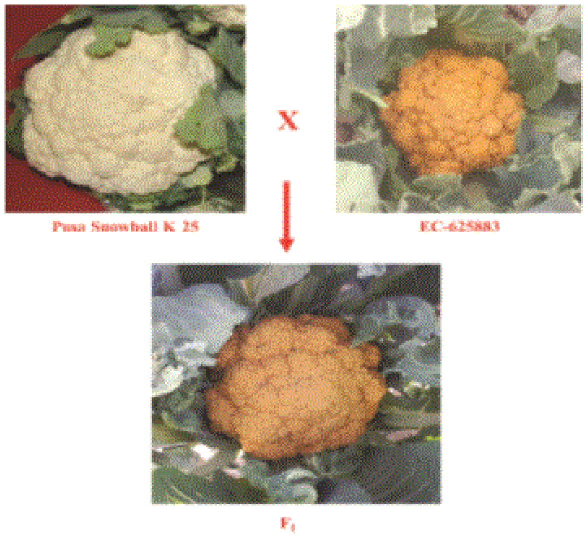

Figure 2: Introgression “Or” gene Indian cauliflower Cultivar Pusa Snowball K-25 (White curd) from EC-625883(orange curd coloured) at IARI, New Delhi.

Figure 3: First Biofortified cauliflowerVariety “Pusa Beta-Kesari” released for sowing October-November, Semi blanching, Orange curd coloured (8-10 ppm β-carotene content), Curd weight 1.250 kg at IARI, New Delhi.

Molecular Mapping and Development and Use of Molecular Markers for Two Gain-of-function Colour Gene Mutations in Cauliflower

Galli et al. develop the molecular markers for early detection of colours in cauliflower [10]. The different colours in cauliflowers might be due to mutations in chromosome. For early detection of colours at seedlings stage they developed molecular marker with the help of DNA transposans and retrotransposans markers were identify (Figures 4-6).

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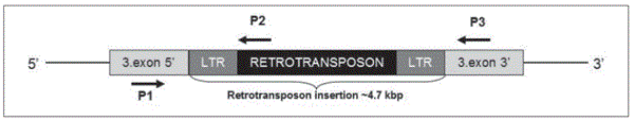

Figure 4: Schematic diagram of the third exon of the mutant or gene.

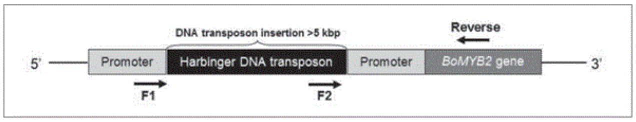

Figure 5: Schematic diagram of the promoter region of the mutant BoMYB2 gene.

Figure 6: Curd of orange, purple and white cauliflowers phenotype of the homozygous or mutant in the fields.

Anthocyanin in Cole Crops: Genetics, Breeding and Molecular Approaches

Anthocyanin are colour pigment are responsible for red blue and purple pigmentation in several vegetable crops. Cauliflower white curd are predominantly grown but some extent green headed are more popular in Lazio and Marche and purple colour are traditionally grown in silicy [11]. Interest of coloured cauliflower is increased with increasing health beneficially an anthocyanin is one among them responsible for anticancer, anti-oxidant and protective against other degenerative disease. Violet and Purple pigmentation in cauliflower are regulated by several transcriptional factor. The major tissue specific transcriptional activation gene like BoMYB2 leading increased the expression of Both BobHLH1 and BobHLH2 gene tend to form a complex regulation factor MYB-bHLH-WD40 (MBW),which activate several gene responsible for anthocyanin pigmentation in several parts of plants like curd, leaf bases etc. [11]. Among anthocyanin pigmentation one cyanidin-3-(6-p-coumaryl)-sophoroside-5-glucosid were mainly responsible colour development in cauliflower and one major concern stability of anthocyanin pigment during processing, bleaching, cooking, freezing Could be necessary for processing attributes.

Broccoli (*Brassica oleracea*) has immense nutritive values and anthocyanin pigment are responsible for purple, violet and blue pigmentation. Yu et al. identified and mapped two loci responsible for purple pigmentation at sepal in broccoli and also reported that purple pigmentation can be induced by cold treatment [12]. Rahim et al. identified several candidate gene like (BoPAL, BoDFR, BoMYB114, BoTT8, BoMYC1.1, BoMYC1.2, and BoTTG1) responsible for anthocyanin biosynthesis in different tissues [13]. Among several anthocyanin derivative one cyanidin 3-O-diglucoside-5-O-glucoside derivatives are predominant Acylated anthocyanin pigment responsible for purple colour on different plants part like curd and leaves bases etc. [14]. Cabbage (*Brassica oleracea*) red and purple colour due to presence of Anthocyanin pigment as for broccoli and cauliflower. The extensive research have been identified several structural candidate gene like (CHS, F3H, F30H, DFR, LDOX, and GST) responsible for anthocyanin biosynthesis for expression of red pigmentation in different plant parts [15]. Jin et al. reported that expression and synthesis of anthocyanin pigmentation was regulated several plants hormones like ABA (abscisic acid) and Ethylene and proposed several putative candidate gene which regulated ABA biosynthesis like BoNCED2.1, BoNCED2.2 and two Ethylene-biosynthesis genes (likes BoACS11, BoACO4 were expressed and lead for purple colour pigmentation in various plant parts like curd, leaves [16].

Genetic Inheritance of Root Colour and QTL Mapping in Different Root Crops

Carrot

Carrot (*Daucus carota* L) is an important root vegetable crop. It has rich sources of β-carotene, lycopene, lutein and anthocyanin in orange, red, yellow and black colour of carrot respectively. The important varieties of carrot grown in different parts of India are Pusa Kulfi, Pusa Kesar, Pusa Asita, Pusa Kulfi, Pusa Nayanjyothi, Pusa Vasuda, Pusa Payasa, Pusa Yamdagni, Pusa Meghali, Pusa Nayanjyothi, Pusa Rudira, Zeno, Early Nantes, Nantes, Nantes Half Long, Imperator and Chantenay [17]. Kust reported three dominant alleles-Y, Y1, Y2-which prevented the formation of orange colour in root xylem tissue and found Orange carrots are yyy2y2, while white carrots are YYY2Y2, with yellow and pale orange colour in root. Freeman reported that when dominant allele (Rs) is present, the carrot root will accumulate the reducing sugars fructose and glucose but when recessive alleles (rsrs) present them the carrot root will accumulate primarily sucrose. Vivek reported that a single AFLP marker located 2.2 cm from the Y2 locus, assigning the locus to one end of linkage group B (β-Carotene). Single dominant gene (P1) are responsible for anthocyanin accumulation in carrot roots and Yildiz genetically mapped P1 and several anthocyanin biosynthetic genes responsible for anthocyanin pigmentation in carrot. Orange colour carrot dur to presence of carotenoids and Four carotenoids viz., β-carotene, α-carotene, γ-carotene and β-cryptoxanthin have vitamin A activity required for human health. Simon et al. reported the carotenoid content in dark orange coloured carrot ranges from 130-150 ppm [18]. Red colour carrot due to presence of lycopene and most suited for Halwa preparation in North India during winter months (November-January). Carrot having purple varieties includes solid purple carrots, often referred to black carrot due to presence of anthocyanins pigment in purple carrots (sometimes referred to as black carrot), they are important derivatives of cyanidin, pelargonidin and peonidin glycosides are reported. The Anthocyanin content in black carrot (Pusa Asita) ranges 300-350 mg/100 g. Multicolour carrots are developed by combining various pigment together like purple orange (anthocyanins+β-carotene), purple white (anthocyanins+lutein) and purple red (anthocyanins+lycopene) combination provides the potential anticancer activity. Sara et al. reported that the purple-orange-red cultivar that contains approximately 40 ppm carotenes and 62 ppm lycopene respectively. Santos et al. reported that SNP based markers were closely linked to the Y2 gene whereas as described by Bradeen [18]. They considered ZDS2 and ZEP as candidate gene for Y2 QTL (Figures 7-10).

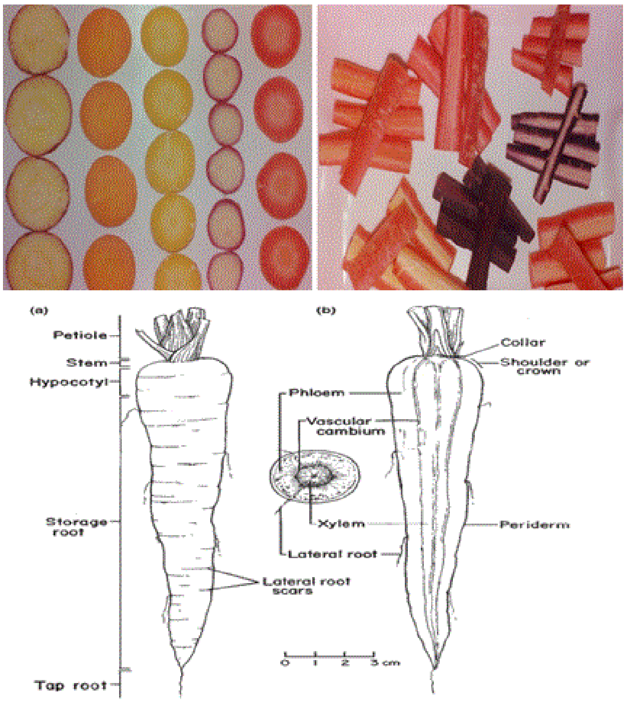

Figure 7: Carrot Botony and Root Morphology Phloem, Xylem, Vascular Cambium, Periderm. Source: Carrot and umbelliferae vegetable crops [19].

Figure 8: Pigment power converted in to Nutrient rich varietal wealth in carrot, source: Root epidermis colour: Purple colour of root epidermis is dominant over yellow colour. Root phloem colour: Orange colour of phloem governed by single dominant gene over purple phloem colour. Root xylem colour: Monogenic controlled. Red colour: Pigment rich Varietal wealth in Carrot.

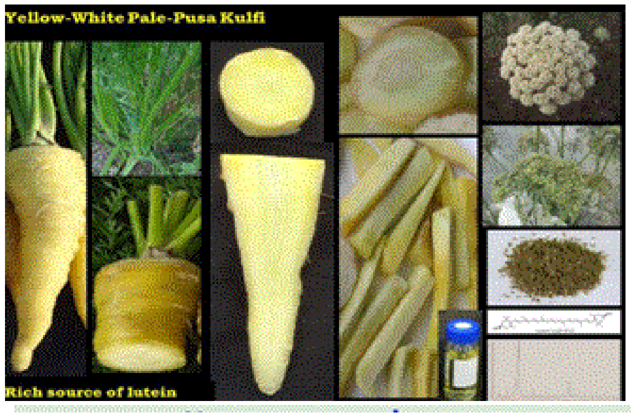

Figure 9: Lutein rich white pale mustard coloured epidermal, phloem and xylem carrot roots [17].

Figure 10: Anthocyanin rich purple coloured epidermal, phloem and xylem carrot roots [17].

Radish

Radish is also important root vegetable, having rich source of Vitamins and minerals. The root colour of radish is Digenic and governed by complementary gene action. Pink flesh is richer Vit-c content than white flesh. Purple or pink pigmentation in radish due to presence of Anthocyanin. Keeping view importance of colour rich pigment in human nutrition several multiple colour rich cultivars like Pusa Sagarika (First purplish flesh coloured radish was developed at IARI, New Delhi), Pusa Jamuni (Purple flesh radish cultivar, high anthocyanins and ascorbic acid content), Pusa Gulabi (Pink flesh radish cultivar, high carotenoids and Anthocyanins content), Pusa Hriday (Pink fleshed), Kashi Lohit (Attractive red colour, suitable for salad, excellent source of Anti-oxidant) are developed for Ninch market. Betalains are widely used as natural colourants for many centuries, but their attractiveness for use as colourants of foods has increased recently due to their reportedly high anti-oxidative, free radical scavenging activities and concerns about the use of various synthetic alternatives eg: Beet root (Figure 11).

Figure 11: Pigment rich varietal wealth in Radish.

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