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Advances in Genetics and QTL Mapping In Carrot (Daucus Carota L.)

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Mini Review

Carrot (*Daucus carota* L., 2n = 2X = 18; 1C= 473Mb) is a cool weather crop grown in temperate and subtropical regions for its edible storage tap roots both for fresh as well as processed vegetable throughout the world and is most important of all the root crops [1-3]. It has been classified as 'poor man's gingeng' due to its rich β-carotene and tocopherol with health promoting properties [4-5]. It led to increasing awareness to the consumer consumption and nutritional industry to make natural products rich in carotenoids and anthocyanins [6]. Carrot has naturally wide genetic diversity due to its natural single locus mutations, natural hybridization of its wild relatives and ancestors followed by human selection [7-8]. The natural allele collection of carrot includes dominant alleles such as A (**α**-carotene accumulation), Io (intense orange xylem, which may be an allelic form of A), L_1 and L_2 (lycopene accumulation), O (orange xylem, which may also be an allelic form of A) as well as the recessives alleles y (yellow xylem) and rp (reduced pigmentation). The three dominant loci Y, Y_1, and Y_2 control differential distribution of **α** and **β**-carotene in xylem/phloem tissue. The Y_2 mutant controls low carotene content of the storage root xylem ("core") in high carotene orange backgrounds [8]. Laferriere et al. [9] demonstrated that in yellow × white cross, white colour was dominant over yellow which was controlled by a single dominant gene. Moreover, they reported that three dominant genes were responsible for the absence of pigmentation in white × orange crosses. Light orange colour has also been shown to be dominant to orange [10]. Kust [11] postulated three dominant alleles Y, Y_1 and Y_2 which prevented the formation of orange colour in root xylem tissue. Buish et al. (1979) characterized the effects of the series of Y alleles on carotenoid content in phloem and xylem. The Y and Y_2 allele were governing white pigmentation of root which was dominant to orange (yy).

Laferriere et al. [9] hypothesized that single major gene was governing white colour root of yellow × white crosses which three major genes were determining the white and orange. Further, research studies showed that two major genes in one cross and four major genes in orange × yellow cross. Simultaneously, Imam et al. [10] reported that lemon (light yellow) was dominant over light orange in lemon × light orange whereas light orange was dominant over orange in light orange × orange. Kust [11] described that dominant alleles of three genes (Y, Y_1 and Y_2) control the orange colour in the xylem which was epistatic to two pigment enhancing genes (IO, O). The orange colour of phloem was governed by pigment enhancing genes (IO, O) in equal or higher number without dominant alleles (Y-) and combination of these colour enhancing genes with three dominant allele (Y, Y_1 and Y_2). Furthermore, he suggested that the genetic constitution of white was recessive at all loci ($y_1y_1y_2y_2$ioiooo). β-carotene and anthocyanins impart orange/yellow and purple colour to carrot, respectively. The genetic control of carotene synthesis is complex which includes several enhancer genes and inhibitor genes. Kust [11] postulated the dominant (Y_2) locus control the carotene accumulation in carrot xylem core whereas the recessive (y_2/y_2) locus conditioned the β, and γ-carotene rich orange xylem core. Furthermore, the dominant heterozygous (Y_2-) possess xanthophyll-rich yellow white core. Therefore, Y, y and Y_2 loci were more influencing on the amount and distribution of α and β-carotene in which Y locus blocs the synthesis of α and β-carotene as well as xanthophylls whereas y, and Y_2 blocks synthesis of carotenes but not xanthophylls [12]. Umiel et al. [13] studied in F_2 populations of orange × red crosses that the inheritance of orange colour was governed by single dominant gene which is epistatic to red colour gene. Furthermore, on the basis biochemical analyses they proposed that 'A' gene for the accumulation of α-carotene were originated from orange parent and 'L' gene for lycopene formation were originated from the red parent.

Rhodes [14] in F_2 populations of red × light yellow cross observed segregation of 15: 1 ratio of yellow-orange to red colour. He suggested that two dominant genes conditioning the conversion of lycopene to α and β-carotene. The white or non-pigmentation of root were conditioned by recessive gene (rp) which controls the β-carotene synthesis. The mutant gene 'rp' had been identified and characterized, suggesting that it causes 93% reduction of to-

tal carotenoids [15]. Moreover, Koch et al. [11] postulated that 'rp' mutant produces more α-tocopherol which is provitamin-E.

The dominant gene of P_1 confers the purple colour of root which shows only partial variation on roots and it is hypostatic to P_2 gene influencing pigmentation in aerial parts of petiole, leaf and floral corolla [8]. Anthocyanin accumulation in the carrot phloem is controlled by P_1 locus, with purple (P_1) dominant to non-purple (p_1). Although, P_1 and Y_2 loci were unlinked in F_2 and BC populations to Eastern carrot germplasm [8,16]. The purple petiole of Tender Sweet was conditioned by single dominant gene 'G', dominant to green 'g' [17]. Purple colour of root was governed by two complimentary loci in F_1 progeny of two non-purple inbreeds [9]. The sugar condition of carrot was governed by single dominant gene (Rs). These genes express more reducing sugars of glucose and fructose in Rs/- plants. In contrast, rs/rs type plants produces more sucrose accumulation [18]. Broad sense heritability estimates have been determined for total dissolved solids 40-45% [19]. The rs/rs mutant plants has carrot invertase enzyme which knockout the function enzyme [20]. The heritability of nematode resistance (*Meloidogyne javanica*) was evaluated using the open-pollinated cultivars Brasília as resistant, and Kuronan as tolerant [21].

Quantitative trait loci (QTL) mapping

The recent development of molecular marker technologies and the use of these markers in detecting and mapping quantitative trait loci has become a powerful approach for studying the genetic and phenotypic basis of complex traits [22-25]. If individual genetic components associated with a complex trait can be identified, then research can focus on the function of each locus independently without the confounding effects of other segregating loci [25].

Bradeen et al. [26] identified six AFLP markers linked to Y_2 locus with distance of 2.8cM and 15.8cM from 103 F_2 populations of a cross B9304 × YC7262 which segregated for core colour by using bulked segregate analysis [27]. Subsequently, Vivek et al. [28] identified a single AFLP marker from the Y_2 locus and assigned on linkage group B with size of 2.2cM by using these population. Moreover, they have identified horticulturally important QTLs through segregation analysis. Santos et al. [29] revealed that 287AFLP markers were mapped by using 160F_2 population of Brasilia × HCM with character of medium orange and high carotene line, respectively. These markers were associated with α and β-carotene, lycopene, and the precursor's ζ-carotene and phytoene QTL conditioning each of the traits measured were detected. In total, 8, 3, 1, 4 and 5QTLs were detected for α-carotene, β-carotene, lycopene and the precursors ζ-carotene and phytoene accounting for 40%, 20%, 7.2%, 16.3% and 28%, respectively of total phenotypic variation whereas QTLs of Brasilia and HCM showed 3.7 to 13.2% total variation. Twenty major QTL have been identified for orange carrots which control carotenoids content [30]. Buish et al. [12] reported that these QTLs, two major genes (Y and Y_2) and clusters of genes were involved in common carotenoid biosynthetic pathway from the population of orange carrots (yyy_2y_2) and white carrots (YYY_2Y_2) with yellow and pale orange colour.

Santos et al. [29] identified that SNP based markers were closely linked to the Y_2 gene whereas as described by Bradeen and Simon (1998) also maps to this region. They considered ZDS2 and ZEP as candidate gene for Y_2 QTL. Phenotype of yellow (xanthophyll) and the orange (α- and β-carotene) were conditioned by Y_2 [12]. They revealed that Y_2 gene were derived from yellow segregates of B493 × QAL population. Furthermore, since it maps to this important region, Y_2may be responsible for at least some of the QTL effect observed by Santos et al. [31]. Santos et al. [29] identified quantitative trait loci (QTLs) for total carotenoids affecting the concentration of carotenoids in the ranges from 15.8, 21.7, 26.4, 37.7, and 44.2% of the total phenotypic variance for lycopene, α-carotene, β-carotene, ζ-carotene and phytoene, respectively. Just et al. [32] reported that 22 genes which involved in the carotenoid biosynthesis and metabolism, provided gene-specific codominant polymorphisms for eight of the nine linkage groups. One major QTL has been identified for β-carotene, total carotene and lycopene accumulation in the F_2 population of P50006 and HCM A.C. by using sequence related amplified polymorphism (SRAP) markers which showed 12.79%, 12.87%, and 14.61% of total phenotypic variations. The SRAP marker were tightly linked in 9 linkage groups in the size of 502.9cM with a mean interval of 5.5cM. The genetic variability of these three QTLs was due to additive genetic variance. In addition, a pair of epistasis QTL for β-carotene and lycopene accumulation showed 15.1% and 6.5% of total phenotypic variation, respectively. These SRAP markers linked to these QTLs could be used in selection or QTL pyramiding for high carotenoids and lycopene content in carrot breeding [33].

All five anthocyanin glycosides as well as root total pigment estimate (RTPE) differed quantitatively in the F_2 population. For the purpose of QTL analysis, a high resolution gene-derived SNP-based linkage map of carrot was constructed with 894 markers covering 635.1cM with a 1.3cM map resolution. A total of 15 significant QTL for all anthocyanin pigments and for RTPE mapped to six chromosomes. Eight QTL with the largest phenotypic effects mapped to two regions of chromosome 3 with co-localized QTL for several anthocyanin glycosides [34,35]. Ellison et al. [36] developed two closely linked codominant markers, 4135Apol$_1$ and 4144ApeKI, to more accurately select y_2y_2 plants with increased β-carotene accumulation. These markers have been tested not only within the mapping population, but also in a group of unrelated genetic materials, and have proven to be very accurate in predicting orange and no orange phenotypes. Further they identified that the single large effect QTL on the distal arm of chromosome 7 overlapped with the previously identified β-carotene accumulation QTL, Y_2. Fine mapping efforts reduced the genomic region of interest to 650kb including 72 genes [36].

This review briefly discussed about genetics and QTL mapping approaches in carrot for different carotenoids such as α-carotene,

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β-carotene, γ-carotene, lutein and different anthocyanin pigments. It will help the carrot breeders to understand the genetic mechanism of different traits, application of molecular markers and to map the targeted nutritional content. Furthermore, recent development of genomic resources and genome sequence data will help the carrot improvement efforts and help to identify additional candidate genes underlying different carrot nutritional accumulation in carrots.

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