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Variability of carotenoid biosynthesis in orange colored *Capsicum* spp.

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ABSTRACT

Pepper, *Capsicum* spp., is a worldwide crop valued for heat, nutrition, and rich pigment content. Carotenoids, the largest group of plant pigments, function as antioxidants and as vitamin A precursors. The most abundant carotenoids in ripe pepper fruits are β-carotene, capsanthin, and capsorubin. In this study, the carotenoid composition of orange fruited *Capsicum* lines was defined along with the allelic variability of the biosynthetic enzymes. The carotenoid chemical profiles present in seven orange pepper varieties were determined using a novel UPLC method. The orange appearance of the fruit was due either to the accumulation of β-carotene, or in two cases, due to only the accumulation of red and yellow carotenoids. Four carotenoid biosynthetic genes, *Psy*, *Lcyb*, *CrtZ-2*, and *Ccs* were cloned and sequenced from these cultivars. This data tested the hypothesis that different alleles for specific carotenoid biosynthetic enzymes are associated with specific carotenoid profiles in orange peppers. While the coding regions within *Psy* and *CrtZ-2* did not change in any of the lines, the genomic sequence contained introns not previously reported. *Lcyb* and *Ccs* contained no introns but did exhibit polymorphisms resulting in amino acid changes; a new *Ccs* variant was found. When selectively breeding for high provitamin A levels, phenotypic recurrent selection based on fruit color is not sufficient, carotenoid chemical composition should also be conducted. Based on these results, specific alleles are candidate molecular markers for selection of orange pepper lines with high β-carotene and therefore high provitamin A levels.

1. Introduction

Colored vegetables can contribute required and recommended amounts of carotenoids that are important antioxidants or provitamin A compounds in the human diet. According to the World Health Organization, vitamin A deficiency is a public health problem causing blindness in an estimated 250,000–500,000 children per year [1]. High β-carotene peppers, *Capsicum* spp., could be a solution in the battle to fight vitamin A deficiency. Humans are able to convert some provitamin A carotenoids like β-carotene into vitamin A using intestinal mono-oxygenases; 6 μg of β-carotene are converted to 1 μg of retinol (vitamin A) [2]. In addition, β-carotene is an efficient radical scavenger quenching free radicals before they damage cells [3,4].

Carotenoids are responsible for a variety of colors ranging from yellow to red, as is apparent in fully mature ripe *Capsicum* fruits. Carotenoids are lipid soluble compounds derived from the isoprenoid pathway and share a 40-carbon isoprene backbone with a variety of ring structures at one or both ends, which in the case of pepper fruits are stored in the chromoplasts [5]. The carotenoid pathway begins with the rate-limiting step, the synthesis of phytoene by phytoene synthase [6]. Several desaturation reactions convert phytoene to the orange colored β-carotene, and then β-carotene is oxygenated to form xanthophylls like β-cryptoxanthin, zeaxanthin, and antheraxanthin (Fig. 1). *Capsicum* species uniquely have capsanthin–capsorubin synthase (CCS) that synthesizes two red pigments, capsanthin and capsorubin [7,8].

Capsicums are one of the oldest and most popular vegetables and spices in the world [9]. In the United States, consumption of colored *Capsicum* fruits has increased dramatically over the last two decades [10]. Pepper fruits range in color starting at green, ivory, or yellow and ripening to variety of colors including brown, orange, red, violet, or yellow mature stages [11]. Due to the range of colors in *Capsicum* fruits, and the importance of carotenoid accumulation in ripe *Capsicum* fruits, studies have documented carotenoid levels in many varieties [5,12]. Not all orange colored organs in plants are due to accumulations in β-carotene; for example, the orange color in carrots (*Daucus carota* L.) is due to an accumulation of β-carotene, while the orange color in papaya (*Carica papaya* L.) is due to a diluted concentration of the red carotenoid lycopene [13].

Expression levels of the carotenoid biosynthetic genes are directly linked to high levels of total carotenoid accumulation in *Capsicum* [14,15], however regulation of carotenoid biosynthesis is not understood. The biosynthetic steps for the conversion of the C40

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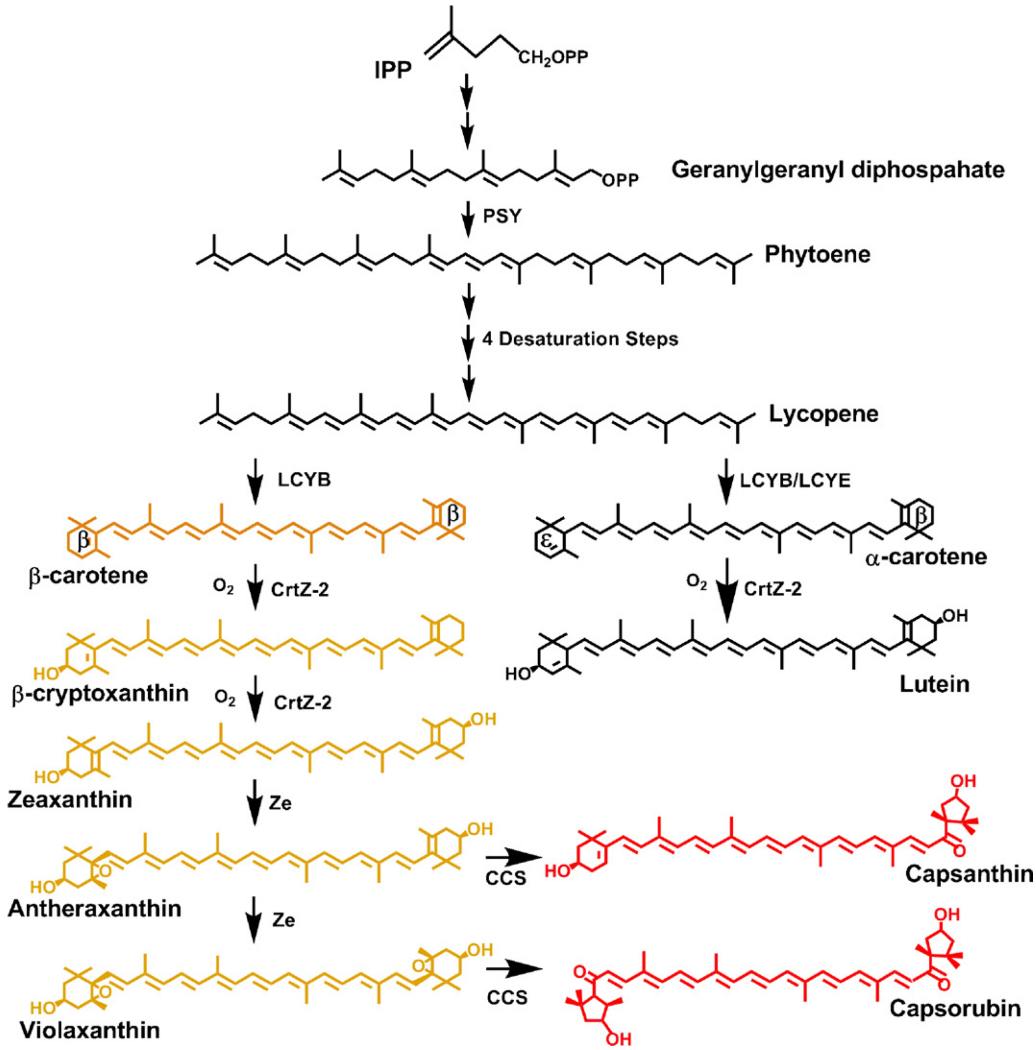

Fig. 1. Carotene and xanthophyll biosynthetic pathway in *Capsicum*.

terpenoid, phytoene, into carotenes and xanthophylls have been determined in *Capsicum* [5]. In peppers, there are at least 34 unesterified carotenoids that have been extracted and separated using high performance liquid chromatography (HPLC). These include β-carotene, β-cryptoxanthin, zeaxanthin, violaxanthin, capsanthin, and capsorubin [5]. The heredity of mature pepper fruit color nonetheless is still not fully understood. Hurtado-Hernandez and Smith [16] reported three independent loci determining fruit color and eight phenotypes in the F_2 segregation of a hybridization of a red and white pepper. This study showed that red color is dominant over white and yellow. Lefebvre et al. [17] and Huh et al. [18] characterized two loci, the phytoene synthase gene and the capsanthin–capsorubin gene. The third locus predicted by Hurtado-Hernandez has not been identified. The three-locus model offers multiple theories for the production of an orange phenotype in *Capsicum* fruits. U.S. Patent number 5,4440,069 [19] describes an orange dominant pepper where two orange peppers when hybridized produce orange, not red, F_1 fruits; *Capsicum annuum* cv. Valencia is a dominant orange pepper (S. Czaplewski, pers. comm.).

Phenotypic recurrent selection could increase abundance of a specific metabolite. It has allowed a 3-fold increase of petal color in red clover [20]. Similarly, phenotypic recurrent selection can be used to increase nutritive value of a crop if the phenotype is related to a metabolite associated with the nutritive value of the crop [21]. Therefore, the characterization of the orange phenotype is important to plant breeders wanting to increase the abundance of β-carotene in the fruit. For example, in *Capsicum*, if an orange fruit phenotype was associated with high β-carotene levels, selecting for a more provitamin A rich fruit could be accomplished quickly and efficiently.

In this study, we investigated the β-carotene content of red and orange fruited *Capsicum* varieties to examine the relationship between fruit color and β-carotene content. The genotypes selected included a variety of pod types, as there is a wide range of carotenoid levels among the different pod types [12]. From these results we expected to determine if orange fruit color is always associated with accumulations of β-carotene. A detailed study of seven orange pepper varieties, characterizing six carotenoids and DNA sequences of four carotenoid biosynthetic genes was performed to identify metabolic and genetic differences among *C. annuum* orange peppers.

2. Materials and methods

2.1. Plant material

Forty different genotypes from three species (*C. annuum* L., *Capsicum baccatum* L., and *Capsicum chinense* Jacq.) were extracted for carotenoid identification and quantification. Seven orange *C. annuum* cultivars (Valencia, NuMex Sunset, Fogo, Orange Grande,