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Genetic dissection of carotenoids in maize kernels using high-density single nucleotide polymorphism markers in a recombinant inbred line population

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ABSTRACT

Carotenoids are antioxidants and vitamin A precursors that have important roles in human health. Hence, improving the carotenoid contents in maize kernels is a priority objective for breeders in order to obtain nutritional biofortification outcomes. In the current study, the genetic architecture of carotenoids in maize kernels was explored using a recombinant inbred line (RIL) population derived from a cross between inbred lines By804 and B73. A total of 81 QTLs were detected by using a high-density bin map and a simple sequence repeat (SSR)-based linkage map, with one to seven QTLs for each trait explaining 4.21%–47.53% of the phenotypic variation. A comparison of the QTL mapping efficiency between the two linkage maps revealed that the high-density bin map had higher resolution. In the current study 46 additional QTLs were identified, with 16 being common with previous studies and 14 newly identified. Among the results, 29.6% (24/81) of QTLs explained >10% of the phenotypic variation in the RIL population, and 70.4% (57/81) explained ≤10%. These results suggest that a few large-effect QTLs, together with a variable number of minor-effect QTLs, contributed to most of the genetic components of carotenoids in maize kernels.

1. Introduction

Carotenoids are one of the largest groups of isoprenoid molecules synthesized by plants and many microorganisms [1]. More than 500 specific carotenoids have been isolated and characterized, but only 24 generally occur in human foodstuffs. The principal carotenoids in foods are β-carotene, β-cryptoxanthin, lycopene, lutein, and violaxanthin [2]. Dietary carotenoids are documented to act as health-promoting phytonutrients for humans [3]. Carotenoids with unsubstituted β-ionone end groups are precursors of vitamin A, which plays essential roles in immune responses, vision, and cellular growth [4,5]. Extreme vitamin A deficiency (VAD) may result in corneal ulceration, night blindness, xerophthalmia, sensitivity to infections, and even death, particularly in children less than five years of age (http://www.who.int/nutrition/topics/vad/en/). VAD is a major world health problem in developing countries, especially sub-Saharan Africa and Southeast Asia. Maize, one of the most important world

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crops, naturally accumulates carotenoids in the edible seed endosperm [6]. Hence, improving provitamin A in maize kernels is an effective way to address VAD.

Maize carotenoids are a suitable model system for investigation of quantitative variation in a biochemical pathway [7]. In the last few decades, maize homologs encoding enzymes involved in the carotenoid biosynthetic pathway steps have been well described. These include phytoene synthase (PSY1, PSY2) [8,9], phytoene desaturase (PDS) [10,11], ζ-carotene desaturase (ZDS) [10], carotenoid Z-isomerase (Z-ISO) [12], carotenoid cleavage dioxygenase 1 (CCD1) [13], lycopene β-cyclase (LCYB) [14], lycopene ε-cyclase (LCYE) [15], β-carotene hydroxylase 1 (crtRB1) [11,16], and zeaxanthin epoxidase (ZEP1) [16]. Other genes encoding enzymes in the maize carotenoid biosynthetic pathway have also been cloned and mapped using homologies in model plant species including 9-*cis*-epoxycarotenoid dioxygenase (NCED), carotenoid isomerase (CRTISO), deoxyxylulose 5-phosphate synthase (DXS), deoxyxylulose 5-phosphate reductoisomerase (DXR), hydroxymethylbutenyl 4-diphosphate reductase (HDR), hydroxymethylbutenyl 4-diphosphate synthase (HDS), geranyl geranyl pyrophosphate synthase (GGPPS), and isopentyl pyrophosphate isomerase (IPPI) [6]. Among these genes, natural variants of *PSY1*, *LCYE*, *crtRB1*, and *crtRB3* were mined by pathway-driven association analysis [15,17–19]. *PSY1* was strongly associated with quantitative variation in carotenoid content and composition [17]. *LCYE* and *crtRB1* were associated with the ratio of α to β-branch carotenoids and β-carotene concentration and conversion, respectively, leading to higher provitamin A levels in maize kernels [15,19]. *crtRB3* affected the accumulation of α-carotene in maize kernels [18]. Although the carotenoid metabolic pathway is clear, little is known about the regulation of carotenoid biosynthesis and accumulation in maize.

QTL mapping is widely used to determine genetic architecture of trait variation in many organisms [20]. Several plant species, including *Arabidopsis* [7,21], carrot [22], grape [23], and wheat [24], have been subjected to QTL analysis to dissect phenotypic variation in carotenoids. There are a few studies on QTL mapping for carotenoids in maize. Eighteen, 16, 18, and 23 loci for maize carotenoids were identified using 123 SSR markers in a W64a × A632 $F_{2:3}$ segregating population and one test-cross population with AE335, 201 molecular markers in a By804 × B73 recombinant inbred line (RIL) population, 109 SSR markers in a DEexp × CI7 $F_{2:3}$ population, and 117 SSR markers in a A619 × SC55 $F_{2:3}$ population [11,25,26], respectively. The limitation of these early studies was that the detection of QTLs was performed using low-density markers. However, low-density markers do not permit precise dissection of multiple linked genes controlling complex traits [27,28]. Large-scale analysis of markers that cover the entire genome and account for almost all potential recombinant events in a population had already improved knowledge of the modes of allele action related to maize starch and vitamin E [27,29]. Therefore, large-scale analysis of markers might be able to improve our understanding of the modes of allelic action in regard to carotenoids. Compared with SSR markers, more recently developed single nucleotide polymorphism (SNP) markers represent a better marker system because they are evenly distributed across the genome, co-dominant and accurate. Furthermore, they can be generated and analyzed in a high-throughput, cost-effective way.

The objectives of this study were to dissect the genetic architecture of carotenoids in maize kernels using a high-density bin map and an SSR-based linkage map in a By804 × B73 RIL population, and to compare the power and resolution of QTL mapping for carotenoids in maize kernels between two linkage maps.

2. Materials and methods

2.1. Genetic materials and phenotyping

A set of 178 RILs, derived from the cross of maize inbred lines By804 and B73, was used to dissect the genetic architecture of carotenoids in maize kernels. The procedures of cultivation, harvesting, sampling and carotenoid extraction was described in detail in a previous study [11]. Briefly, the RIL population and their parents, By804 and B73, were grown in a randomized complete block design with two replications at the Agronomy Farm, China Agricultural University, Beijing, during the summer seasons of 2004 and 2005. Each genotype was grown in a row length of 4.00 m, with 0.67 m between rows and a planting density of 45,000 plants ha^{-1}. More than six plants in each row were self-pollinated and the pollinated ears were harvested at maturity. Kernels from each harvested ear in each row were bulked separately and representative samples were taken to measure carotenoids by High Performance Liquid Chromatography (HPLC). Besides 5 measured traits, α-carotene (AC), β-carotene (BC), β-cryptoxanthin (BCRY), lutein (LUT), and zeaxanthin (ZEA), additional carotenoid-related traits including total carotenoids (TC = LUT + AC + BC + BCRY + ZEA), total provitamin A [TVA = BC + (BCRY + AC)/2], RATIO (LUT + AC)/(BC + BCRY + ZEA), AC/LUT, AC/TC, BC/TC, BC/BCRY, BCRY/ZEA, BCRY/TC, BC/TC, and ZEA/TC, were calculated for subsequent analysis.

2.2. Phenotypic data analysis

The linear mixed effect function "lmer" in the lme4 package of R version 3.1.1 was performed to obtain the Best Linear Unbiased Prediction (BLUP) value for carotenoids: $y_{ij} = \mu + f_j + e_i + \varepsilon_{ij}$, where μ is the grand mean of all environments, y_{ij} is the phenotypic value of individual j in environment i, f_i is the genetic effect, e_i is the effect of different environments and ε_{ij} is the random error. The grand mean was fitted as a fixed effect, and genotype and environment were measured as random effects. The variances of all carotenoid related traits were analyzed by the "aov" function in R version 3.1.1. The model for the variance analysis was $y = \mu + \alpha_g + \beta_e + \varepsilon$, where α_g was the effect of the gth line, β_e was the effect of the eth environment and ε is the error. All effects were considered to be random. These variance components were used to calculate broad-sense heritability as $h^2 = \sigma_g^2/(\sigma_g^2 + \sigma_e^2/n)$ [30], where σ_g^2 is the genetic variance, σ_e^2 is the residual error and n is the number of environments. Pearson correlation coefficients and *P*-values were calculated by the cor.test function in R version 3.1.1.

2.3. Genetic linkage maps

All 178 lines in the By804 × B73 RIL population, together with the parents, were genotyped using the Illumina MaizeSNP50