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A Foundation for Provitamin A Biofortification of Maize: Genome-Wide Association and Genomic Prediction Models of Carotenoid Levels

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ABSTRACT Efforts are underway for development of crops with improved levels of provitamin A carotenoids to help combat dietary vitamin A deficiency. As a global staple crop with considerable variation in kernel carotenoid composition, maize (*Zea mays* L.) could have a widespread impact. We performed a genome-wide association study (GWAS) of quantified seed carotenoids across a panel of maize inbreds ranging from light yellow to dark orange in grain color to identify some of the key genes controlling maize grain carotenoid composition. Significant associations at the genome-wide level were detected within the coding regions of *zep1* and *lut1*, carotenoid biosynthetic genes not previously shown to impact grain carotenoid composition in association studies, as well as within previously associated *lcyE* and *crtRB1* genes. We leveraged existing biochemical and genomic information to identify 58 *a priori* candidate genes relevant to the biosynthesis and retention of carotenoids in maize to test in a pathway-level analysis. This revealed *dxs2* and *lut5*, genes not previously associated with kernel carotenoids. In genomic prediction models, use of markers that targeted a small set of quantitative trait loci associated with carotenoid levels in prior linkage studies were as effective as genome-wide markers for predicting carotenoid traits. Based on GWAS, pathway-level analysis, and genomic prediction studies, we outline a flexible strategy involving use of a small number of genes that can be selected for rapid conversion of elite white grain germplasm, with minimal amounts of carotenoids, to orange grain versions containing high levels of provitamin A.

CAROTENOIDS are a group of >700 lipophilic yellow, orange, and red pigments primarily produced by photosynthetic organisms and also by certain fungi and bacteria

(Britton 1995a; Khoo *et al.* 2011). The length and number of conjugated double bonds in the carotenoid molecule determines its spectral absorption properties (color). There are two generalized classes of carotenoids: carotenes, which are cyclic or acyclic hydrocarbons, and xanthophylls, which are carotenes to which various oxygen functional groups have been added. Carotenoids serve a variety of functions in plants including acting as antioxidants, photoprotectants, accessory pigments for light harvesting, and substrates for production of volatile compounds in flowers, fruit, and seed (Goff and Klee 2006; Moise *et al.* 2014). Specific xanthophylls are precursors for biosynthesis of the plant hormone abscisic acid, which is essential for seed dormancy and response to environmental stresses (Kermode 2005).

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The most important and best-defined function of carotenoids in animals is as a dietary source of provitamin A. Provitamin A carotenoids are a small subset of the 700 carotenoids that are distinguished by having unhydroxylated β -rings. Provitamin A carotenoids can be converted by oxidative cleavage in the body to retinol, or vitamin A, which is stored in the liver (Stahl and Sies 2005; Combs 2012). Vitamin A (retinol) is involved in immune function and synthesis of various retinoic acid hormones and is converted to retinal, the primary light-absorbing pigment in the eye. Vitamin A deficiency can result in night blindness and increased susceptibility to infections and can eventually result in death (Combs 2012). It is estimated that 250,000–500,000 children become blind every year as a result of vitamin A deficiency and that half of these die within 1 year of losing their eyesight (<http://www.who.int/nutrition/topics/vad/en/>). The health benefits of vitamin A have prompted nutritional interventions including those promoting increased consumption of plant-based carotenoids, notably by the HarvestPlus maize biofortification program for Africa (<http://www.harvestplus.org>; Nestel *et al.* 2006; Tanumihardjo *et al.* 2008). In addition to provitamin A activities, all carotenoids are antioxidants and are generally considered nutritionally beneficial in the human diet and important for maintenance of optimal health (Jerome-Morais *et al.* 2011; Sen and Chakraborty 2011). As an example, specific isomers of the nonprovitamin A carotenoids, lutein and zeaxanthin, are present at high levels in the fovea of the eye where they are associated with prevention of age-related macular degeneration (Krinsky *et al.* 2003; Abdel-Aal *et al.* 2013), a leading cause of irreversible blindness in elderly populations of Western societies (Friedman *et al.* 2004).

Carotenoids are essential to many aspects of animal health, yet animals do not synthesize carotenoids, with the exception of the pea aphid (Moran and Jarvik 2010), and therefore must obtain them from their diet to meet minimal nutritional requirements. The most abundant provitamin A carotenoids in plant-based foods are β -carotene (two retinyl groups), β -cryptoxanthin (one retinyl group), and α -carotene (one retinyl group), but in most plant tissues they are substrates for hydroxylation reactions that produce the dihydroxyxanthophylls lutein and zeaxanthin (Figure 1)—the most prevalent carotenoids in vegetative and seed tissues (Howitt and Pogson 2006; Cazzonelli and Pogson 2010). The carotenoid biosynthetic pathway is conserved in plants and has been best characterized in the model dicot *Arabidopsis thaliana* (Dellapenna and Pogson 2006; Kim *et al.* 2009; Cuttriss *et al.* 2011) in which the molecular basis of these hydroxylation steps is well understood. The committed step of the carotenoid pathway is formation of phytoene from geranylgeranyl diphosphate (GGPP) by phytoene synthase (PSY) (Figure 1). A subsequent key branch point occurs at the level of lycopene cyclization. Lycopene β -cyclase activity at both ends of the molecule produces β -carotene, while addition of one β -ring and one ϵ -ring by lycopene ϵ -cyclase produces α -carotene. Hydroxylation of one β -carotene ring produces β -cryptoxanthin followed by hydroxylation of the

other β -ring to produce zeaxanthin. Similarly, hydroxylation of the β -ring of α -carotene produces zeinoxanthin, and subsequent hydroxylation of the ϵ -ring yields lutein.

Maize (*Zea mays* L.) grain exhibits considerable phenotypic variation for carotenoid profiles (Harjes *et al.* 2008; Berardo *et al.* 2009; Burt *et al.* 2011), including some of the highest carotenoid concentrations for cereal crops (Abdel-Aal *et al.* 2013). Biochemical characterization of maize endosperm color mutants and transposon tagging helped to identify some maize-specific homologs of carotenoid pathway genes cloned in bacteria and model plant species. The first was *phytoene synthase* (*y1*), for which mutant alleles were shown to result in white endosperm grain (Buckner *et al.* 1990). White endosperm grain resulting from the recessive *y1* allele provides negligible amounts of carotenoids compared to yellow and orange endosperm grain (Egesel *et al.* 2003; Howe and Tanumihardjo 2006; Burt *et al.* 2011). Subsequently, *phytoene desaturase* (*pds1*) (Li *et al.* 1996) and ζ -*carotene desaturase* (*zds1*) (Matthews *et al.* 2003) were cloned. The first quantitative trait loci (QTL) mapping study of maize grain carotenoids showed that some of the identified QTL were in proximity to two of three carotenoid biosynthetic genes that had been cloned at the time, *y1* and *zds1* (Wong *et al.* 2004). The finding of possible QTL association with carotenoid biosynthetic genes prompted efforts to identify and characterize alleles of genes in the carotenoid biosynthetic pathway that may be associated with quantitative levels of carotenoids. These alleles could then be selected with robust and inexpensive PCR-based assays for marker-assisted selection (MAS) efforts for desirable carotenoids, as opposed to high-performance liquid chromatography (HPLC), which is considerably more expensive and technically challenging to deploy in breeding programs.

Advances in genomics and bioinformatics resulted in the identification of additional genes in the maize carotenoid biosynthetic pathway (Wurtzel *et al.* 2012). This enabled discovery of an association of *lycopene ϵ -cyclase* (*lcyE*) with the ratio of α - to β -branch carotenoids (Harjes *et al.* 2008) and of *β -carotene hydroxylase 1* (*crtRB1*) with β -carotene concentration and conversion (Yan *et al.* 2010). *lcyE* and *crtRB1* alleles with substantially reduced transcript levels increased accumulation of β -branch carotenoids and decreased hydroxylation of β -carotene, respectively, resulting in higher provitamin A levels in maize kernels (Harjes *et al.* 2008; Yan *et al.* 2010). Genetic variation in *crtRB3* has been associated with α -carotene levels in maize (Zhou *et al.* 2012) and with favorable alleles of *y1* associated with higher total carotenoid content (Z. Fu *et al.* 2013).

Several candidate genes from the carotenoid biosynthetic pathway lie within QTL intervals associated with visual scores of relative orange endosperm color intensity (Chandler *et al.* 2013). Darker orange color in maize grain is associated with higher total carotenoids but does not necessarily result in higher provitamin A concentrations (Harjes *et al.* 2008; Burt *et al.* 2011). These results suggest

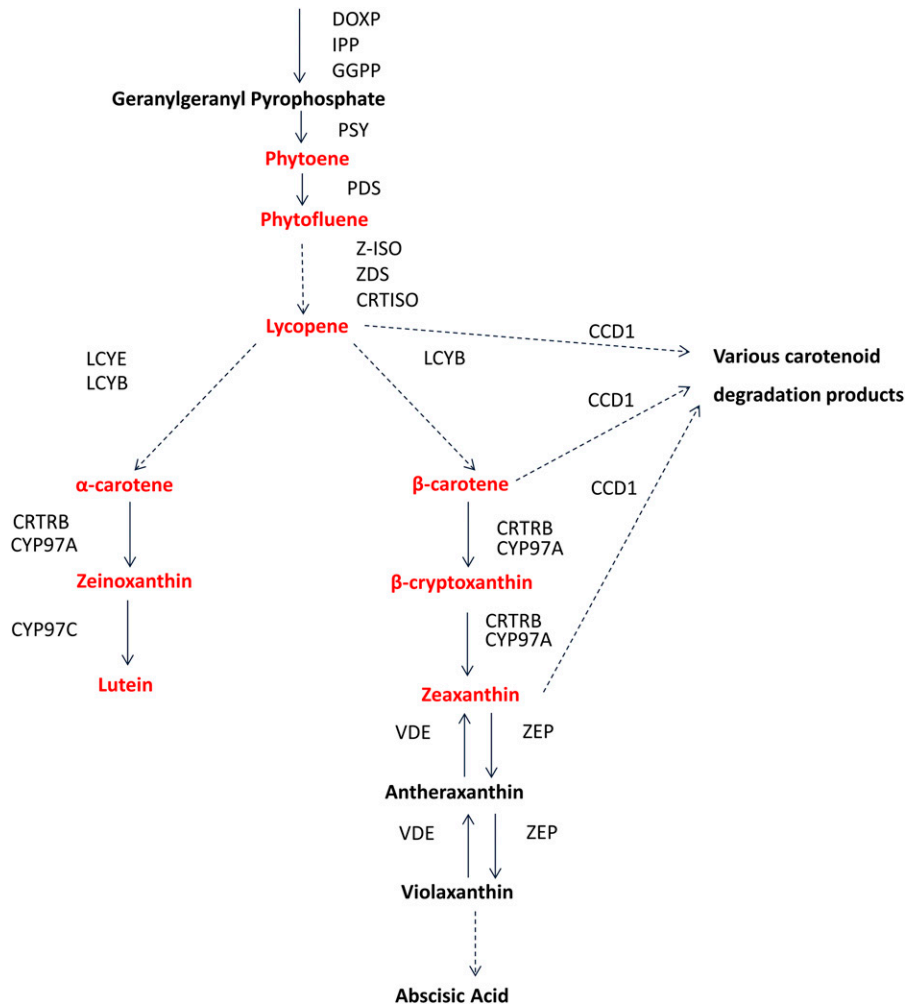


Figure 1 Carotenoid biosynthesis and degradation pathways. Compounds derived from this pathway are diagrammed as nodes in boldface type, with compounds measured in this study shown in red type. Enzymes known to be involved in the conversion of these compounds are adjacent to node connectors. Solid arrows represent single reactions; dashed arrows represent two or more reactions. Note that for some steps maize contains multiple paralogs for a reaction. Note that, in *Arabidopsis*, the CCD class of enzymes has been shown to degrade additional carotenoid compounds (Gonzalez-Jorge *et al.* 2013). DOXP, 1-deoxy-D-xylulose 5-phosphate synthase; IPP, isopentenyl pyrophosphate synthase; GGPP, geranylgeranyl pyrophosphate synthase; PSY, phytoene synthase; PDS, phytoene desaturase; Z-ISO, ζ -carotene isomerase; ZDS, ζ -carotene desaturase; CRTISO, carotenoid isomerase; LCYE, lycopene ϵ -cyclase; LCYB, lycopene β -cyclase; CYP97A, β -carotene hydroxylase (P450); CYP97C, ϵ -carotene hydroxylase (P450); CRTRB, β -carotene hydroxylase; VDE, violaxanthin de-epoxidase; ZEP, zeaxanthin epoxidase; CCD1, carotenoid cleavage dioxygenase 1.

that selection of visibly darker orange grain to increase synthesis and retention of total carotenoids needs to be combined with MAS for favorable QTL alleles of carotenoid biosynthetic genes such as *crtRB1* to increase provitamin A carotenoid levels. Selection for orange color has important ramifications, given that people in most Sub-Saharan African countries generally prefer to eat maize dishes that are prepared from white grain, in part because yellow maize grain is considered suitable only for animal consumption. Thus much of the maize grain grown for human consumption in Africa has white endosperm that provides inadequate levels of provitamin A carotenoids (Pfeiffer and McClafferty 2007; Stevens and Winter-Nelson 2008). Consequently, HarvestPlus has developed an integrated outreach, education, and consumer acceptance strategy in parallel with the breeding efforts to address vitamin A deficiency. This program uses darker orange endosperm color maize grain with elevated provitamin A carotenoids to distinguish maize varieties having elevated provitamin A carotenoids from white grain and yellow feed grain. The approach of using orange grain, which essentially has not been grown in Africa previously and thus is new to the consumer, appears initially to be effective in gaining acceptance in Zimbabwe (Muzhingi *et al.* 2008) and Zambia (Meenakshi 2010).

Maize carotenoids are a promising model system for the continued exploration of quantitative variation in a biochemical pathway, and the fundamental knowledge obtained can be directly applied in maize provitamin A biofortification breeding programs. A genome-wide association study (GWAS) of these phenotypes is a powerful approach that can be used to identify additional key genes and favorable alleles that affect carotenoid levels in maize grain. Furthermore, given that many of the genes in the carotenoid pathway have been well characterized, pathway-level association analysis serves as a potentially useful complement to GWAS that allows less stringent significance thresholds because fewer hypothesis tests are conducted (Califano *et al.* 2012). Various pathway-based association approaches have been pursued in human genetics, typically defining a pathway as a set of genes grouped together based on function or network analysis and testing its association with a disease phenotype (Lantieri *et al.* 2009; Wang *et al.* 2010). Alternatively, nontargeted metabolite profiling approaches can be used in combination with GWAS to dissect kernel phenotypes, as utilized in several recent maize studies (Riedelsheimer *et al.* 2012; J. Fu *et al.* 2013; Wen *et al.* 2014). In contrast, our targeted analysis of maize

grain carotenoids takes advantage of the genetic basis of a well-characterized biosynthetic pathway. Thus, as shown for the tocochromanol biosynthetic pathway in maize (Lipka *et al.* 2013), readjustment of the multiple testing problem to account only for the markers within or near these *a priori* candidate pathway genes is a viable approach to identify weaker-effect and relatively rare alleles contributing to carotenoid phenotypic variation.

The potential application of association results in breeding can be assessed by using marker data to predict grain carotenoid levels in statistical models commonly applied in genomic selection (GS). Previous work has suggested that GS approaches can accelerate the breeding cycle, enhancing genetic gain per unit of time by enabling selection of lines that show favorable genomic signatures for traits of interest but have not been phenotyped (Meuwissen *et al.* 2001; Lorenz *et al.* 2011). The statistical models and marker densities optimizing prediction of carotenoid levels have not been tested and are especially in question, given that the traits are likely oligogenic in genetic architecture but have been only partially characterized in maize grain (Wong *et al.* 2004; Chander *et al.* 2008; Kandianis *et al.* 2013). Information regarding *a priori* candidate pathway genes, QTL, or the combination thereof can be used to generate marker sets that more directly target the carotenoid phenotypes of interest, potentially achieving higher prediction accuracies than genome-wide models for these traits (Rutkoski *et al.* 2012). Importantly, the relative prediction accuracies of models built on marker sets with different levels of genome coverage, or that differ in the genes they target, provide a metric for the relative gains that each marker set could be expected to confer in a selection program.

We sought in this study to determine the controllers of natural variation for carotenoid content in grain and to develop a prediction model that can be used for biofortification of maize. Therefore, we conducted (i) a GWAS and a pathway-level analysis to identify novel genes responsible for quantitative variation of grain carotenoid levels in a maize inbred panel and (ii) genome-wide, pathway-level, and carotenoid QTL-targeted prediction studies to determine the model parameterizations and extent of marker density needed to accurately predict maize grain carotenoid levels. The results of this study will also be used to develop efficient strategies to convert locally adapted maize germplasm with white grain to orange, high provitamin A grain throughout Sub-Saharan Africa.

Materials and Methods

Germplasm

The 281-member maize inbred association panel that represents a significant portion of maize allelic diversity (Flint-Garcia *et al.* 2005) was grown in West Lafayette, IN, at Purdue University's Agronomy Center for Research and Education during the 2009 and 2010 growing seasons. The inbred association panel was grown in a field design

and grain samples were produced as described previously (Chandler *et al.* 2013; Lipka *et al.* 2013). Because of poor agronomic performance or late maturity of some lines, high-quality grain samples were obtained from only a total of 252 lines.

Carotenoid extraction and quantification

The general procedure used for extraction of lipid-soluble compounds from maize kernels for HPLC has been previously described (Lipka *et al.* 2013), except that 1 mg of β -apo-8'-carotenal was added per milliliter of extraction buffer as an internal recovery control. Twenty microliters of maize seed extract were injected onto a C₃₀ YMC column (3 μ m, 100 \times 3 mm, Waters Inc., Wilmington, MA) at 30° and a flow rate of 0.8 ml/min. HPLC mobile phases were buffer A (methyl *tert*-butyl ether) and buffer B (methanol: H₂O) (90:10, v:v). Carotenoids were resolved using the following gradient: 0–12 min: 100% B to 60% B; 12–17.5 min: 60% B to 22.5% B; 17.5–19.5 min: 22.5% B to 100% B; 19.5–21 min held at 100% B, for re-equilibration. Carotenoid spectra were collected from 200 to 600 nm using a photo-diode-array detector model SPD-M20A (Shimadzu, Kyoto, Japan). Individual carotenoids were identified by a combination of their order of elution in the chromatogram, retention times, characteristic spectral peaks, and additional fine spectral characteristics (Britton 1995b).

Carotenoid levels were quantified at 450 nm relative to five-point standard curves for purified all *trans* lutein, zeaxanthin or β -carotene standards except for ζ -carotene and phytofluene, which were done at 400 and 350 nm, respectively. Antheraxanthin, zeinoxanthin, and α -carotene were quantified using the lutein curve; zeaxanthin using the zeaxanthin curve; and lycopene, tetrahydrolycopene, β -cryptoxanthin, β -carotene, and δ -carotene using the β -carotene curve. Relative phytofluene and ζ -carotene levels were estimated from the β -carotene curve. While the major carotenoid species in most samples were in the all *trans* configuration, the system used was able to resolve one or more *cis* isomers for zeinoxanthin, α - and β -carotenes, lutein, zeaxanthin, tetrahydrolycopene, β -cryptoxanthin, phytofluene, and ζ -carotene. When *cis* isomers were present for a given carotenoid, these were quantified using the corresponding curve for their all *trans* isomers, and the values for all isomers for the carotenoid were summed.

Phenotypic data analysis

Nine carotenoid compounds were measured in grain samples from a 252-line subset of the 281-line association panel (Table 1). In addition, a series of 15 sums, ratios, and proportions were calculated from the measured values of these nine compounds. The additional derivative traits may reveal biochemical and genetic relationships not detectable from the measured carotenoids or provide information relevant to future biofortification efforts. The peak signal from a GWAS for white vs. nonwhite (yellow/orange) kernel color in this panel of 252 inbreds was a single nucleotide polymorphism (SNP) located 1141 bp upstream of the *y1*

Table 1 List of 24 grain carotenoid traits that were analyzed

Traits listed in Table 2	Traits listed in Table S5
β-Carotene ^a	Phytofluene ^a
β-Cryptoxanthin ^a	ζ-Carotene ^a
Zeaxanthin ^a	Tetrahydrolycopene ^a
α-Carotene ^a	Total β-xanthophylls ^b
Zeinoxanthin ^a	Total α-xanthophylls ^b
Lutein ^a	Provitamin A ^c /total carotenoids ^b
Acyclic and monocyclic carotenes ^b	Acyclic carotenes/cyclic carotenes ^b
Total carotenoids ^b	β-Carotene/(β-cryptoxanthin + zeaxanthin) ^b
β-Carotenoids/α-carotenoids ^b	Total carotenes/total xanthophylls ^b
β-Xanthophylls/α-xanthophylls ^b	
β-Carotene/β-cryptoxanthin ^b	
β-Cryptoxanthin/zeaxanthin ^b	
α-Carotene/zeinoxanthin ^b	
Zeinoxanthin/lutein ^b	
Provitamin A ^{b,c}	

The means of the BLUP values and heritability estimates for the 15 traits listed in the left column are reported in Table 2 and the values and estimates for the remaining traits are listed in Table S5.

^a Individual carotenoid compound measured by HPLC.

^b Derivative carotenoid trait.

^c Provitamin A is calculated as the sum of β-carotene, 1/2 α-carotene, and 1/2 β-cryptoxanthin.

transcription start site showing a P -value of 4.17×10^{-31} (Supporting Information, Figure S1). The white inbreds are homozygous for the recessive allele of *y1* (Emerson 1921; Buckner *et al.* 1990) and do not produce measurable carotenoids in the endosperm. To adjust for this, the white inbreds were excluded from further analysis. White endosperm lines were identified and excluded based on very low carotenoid levels determined by HPLC and confirmation with grain color descriptors in the GRIN database (<http://www.ars-grin.gov>). Consequently, a total of 201 lines with a range from light-yellow to dark-orange kernel color and adequate amounts of mature grain for analysis were used.

A total of 48, 117, 112, 15, 10, 5, 2, and 2 samples had phytofluene, tetrahydrolycopene, ζ-carotene, α-carotene, β-carotene, zeinoxanthin, β-cryptoxanthin, and lutein values, respectively, that were below the HPLC detection threshold. For these samples, uniform random variables between 0 and the minimum detected value were generated to approximate the compound values. This approach is similar to the one described in Lubin *et al.* (2004). Outliers were removed from all traits using SAS version 9.3 (SAS Institute 2012) following examination of the Studentized deleted residuals obtained from mixed linear models fitted for each trait with the line and field explanatory variables set as random effects (Kutner 2005).

For each of the 24 carotenoid traits, a best linear unbiased predictor (BLUP) for each line (Table S1) was obtained by fitting a mixed linear model across all environments in ASREML version 3.0 (Gilmour 2009). The model-fitting procedure has been previously described (Chandler

et al. 2013). The variance component estimates from these models were used to calculate heritabilities (\hat{h}_l^2) on a line mean basis (Holland *et al.* 2003; Hung *et al.* 2012), and standard errors of the heritability estimates were calculated using the delta method (Holland *et al.* 2003). To assess the relationship between carotenoid BLUPs, Pearson's correlation coefficient (r) was calculated. Finally, the Box-Cox procedure (Box and Cox 1964) was conducted on BLUPs of each trait to find the optimal transformation that corrected for unequal error variances and non-normality of error terms. This procedure is critical for preventing violations of the statistical assumptions made for the models used in GWAS and genomic prediction.

Genome-wide association study

We conducted a GWAS for each of the 24 carotenoid grain traits in the 201 lines with light-yellow to dark-orange kernel color. The SNP markers used in the GWAS have been previously described (Lipka *et al.* 2013). The genotyping-by-sequencing marker data set (partially imputed genotypes; January 10, 2012, version) is available for download from the Panzea database (http://www.panzea.org/dynamic/derivative_data/genotypes/Maize282_GBS_genos_imputed_20120110.zip). After removal of monomorphic and low-quality SNPs, a total of 462,702 SNPs were available for the 201-member association panel. Additionally, seven indels and one SNP (*lcyE* SNP216) located within or close to the coding regions of four carotenoid biosynthetic pathway and degradation genes (*y1*, *lcyE*, *crtRB1*, and *ccd1*) that had been previously analyzed were included (Harjes *et al.* 2008; Yan *et al.* 2010; Z. Fu *et al.* 2013; Kandianis *et al.* 2013) (Table S2). Prior to the GWAS, all missing SNP genotypes were conservatively imputed with the major allele.

The procedure for the GWAS has been previously described (Lipka *et al.* 2013). Briefly, the BLUPs of each carotenoid trait (Table S1) were used to test for an association at the 284,180 SNPs with minor allele frequencies (MAFs) ≥ 0.05 in the panel. Similarly, unified mixed linear models were fitted to each of the aforementioned seven indel markers (Table S2) using PROC MIXED in SAS version 9.3. To account for multiple allelic states, indels were analyzed as class explanatory variables in PROC MIXED. All unified mixed linear models included principal components (Price *et al.* 2006) and a kinship matrix (Loiselle *et al.* 1995) that were calculated from a subset of 34,368 non-industry SNPs from the Illumina MaizeSNP50 BeadChip. For each carotenoid trait, the Bayesian information criterion (Schwarz 1978) was implemented to determine the optimal number of principal components to include in the model as covariates. The amount of phenotypic variation explained by the model was estimated using a likelihood-ratio-based R^2 statistic, denoted R^2_{LR} (Sun *et al.* 2010). The Benjamini and Hochberg (1995) procedure was used to adjust for the multiple testing problem by controlling the false-discovery rate (FDR) at 5 and 10%.

A multi-locus mixed model (MLMM) procedure (Segura *et al.* 2012) was conducted to clarify the signals from major-effect loci identified in GWAS. This method employs

a stepwise mixed-model regression procedure with forward selection and backward elimination. The variance components of the model are re-estimated at each step. Because it is possible to have multiple polymorphisms in the optimal model, the MLM approach allows for an exhaustive search of the model space. All markers on the same chromosome of a major-effect locus were considered for inclusion as explanatory variables in the optimal model. The extended Bayesian information criterion (Chen and Chen 2008) was used to determine the optimal model. To examine the influence of polymorphisms identified through MLM on our results, GWAS was conducted again with these polymorphisms included as covariates in the unified mixed linear model.

Pathway-level analysis

We performed an analysis that used prior knowledge relevant to the biosynthesis and degradation of carotenoids to identify a subset of candidate genes. These genes encode isoprenoid and carotenoid biosynthetic pathway enzymes and carotenoid degradation enzymes, and all either have been shown to influence carotenoid phenotypes in previous work or were identified through homology with carotenoid, isoprenoid, and degradation-related genes in *Arabidopsis* (Dellapenna and Pogson 2006; Moise *et al.* 2014). A total of 37 genes related to carotenoid biosynthesis and degradation and 21 genes related to prenyl group synthesis were used to identify regions in the B73 Refgen_v2 genome to be used in the analysis (Table S3). The genes involved in isoprenoid synthesis were chosen because these compounds are in precursor pathways to carotenoids (Dellapenna and Pogson 2006; Cuttriss *et al.* 2011). The degradation enzymes were included on the basis of reported rates of degradation for one or more carotenoids (Vallabhaneni *et al.* 2010). Ultimately, the association results for 7408 SNP markers and 7 indels located within ± 250 kb of these 58 genes were considered in what we term the pathway-level analysis. For each trait, the unadjusted *P*-values of these markers were corrected for the multiple testing problem by using the Benjamini–Hochberg procedure (Benjamini and Hochberg 1995) to control the FDR at 5%.

Linkage disequilibrium analysis

The procedure used for calculating linkage disequilibrium (LD) has been previously described (Lipka *et al.* 2013). Briefly, the squared allele-frequency correlations (r^2) were calculated in TASSEL version 3.0 (Bradbury *et al.* 2007). Only markers with <10% missing data and MAF ≥ 0.05 were considered for estimating LD. To ensure accurate estimation of LD, the markers were not imputed prior to LD analysis.

Carotenoid prediction

To assess the ability of markers to predict carotenoid levels among the 201 lines, we examined the prediction accuracy of three statistical models commonly used in genomic selection and prediction approaches: ridge regression best linear unbiased prediction (RR-BLUP) (Meuwissen *et al.* 2001), least

absolute shrinkage and selection operator (LASSO) (Tibshirani 1996), and elastic net analysis (Zou and Hastie 2005) (Table S4). The RR-BLUP method was conducted using the rrBLUP R package (Endelman 2011), while the other two methods were conducted in the glmnet R package (Friedman *et al.* 2010). The same 24 carotenoid traits tested in a GWAS were included in the prediction analyses.

Each statistical model was tested with three different data sets that varied in marker scope: genome-wide, pathway-level, and carotenoid QTL-targeted. The genome-wide data set consisted of the 284,180 SNP markers and seven indels used for GWAS, whereas the pathway-level data set included the 7408 SNP markers and seven indels within ± 250 kb of the 58 candidate genes from the pathway-level analysis. The carotenoid QTL-targeted data set included 944 SNP markers and seven indels within ± 250 kb of eight key candidate genes underlying QTL associated with carotenoid biosynthesis and retention. These genes are considered important for selecting for individual carotenoids, higher total carotenoids, and higher provitamin A based on their function in the carotenoid pathway and previous results. The eight candidate genes, *y1*, *zds1*, *lcyE*, *crtRB3*, *lut1*, *crtRB1*, *zep1*, and *ccd1*, are all in chromosome regions associated with QTL for carotenoids (Wong *et al.* 2004; Chander *et al.* 2008; Zhou *et al.* 2012; Chandler *et al.* 2013; Kandianis *et al.* 2013). Six of eight genes were also associated with QTL for intensity of orange color, *crtRB3* and *lut1* being the exceptions (Chandler *et al.* 2013). A darker orange color is associated with higher total carotenoids, particularly lutein and zeaxanthin in maize (Pfeiffer and McClafferty 2007; Burt *et al.* 2011).

The full complement of 201 lines was used to generate the marker sets for prediction analyses, regardless of whether or not all 201 lines were phenotyped for a particular trait. The prediction accuracy of each model was assessed using the approach described in Resende *et al.* (2012). Briefly, the data were randomized into five folds for cross-validation. To enable a direct comparison between RR-BLUP, LASSO, and elastic net, the same fold assignments were used throughout this study. For each model, the correlations between observed and predicted trait values were standardized by dividing the average correlation estimates across the five folds by the square root of the heritability on a line mean basis estimated for that trait in the 201 lines.

Results

Phenotypic variation

Phenotypic variation for grain carotenoid content and composition was assessed in an association panel of 201 maize inbreds with kernel color ranging from light yellow to dark orange. Of the nine carotenoid compounds measured via HPLC in grain samples, the most abundant was zeaxanthin, and the least abundant was tetrahydrocyclopentadiene (Table 2 and Table S5). The strongest Pearson's correlation among the nine carotenoid compounds was between β -cryptoxanthin and zeaxanthin ($r_p = 0.63$), and the lowest

Table 2 Summary statistics of 15 grain carotenoid traits

Trait	No. of lines	BLUPs			Heritabilities	
		Mean	SD ^a	Range	Estimate	SE ^b
β-Carotene	199	1.31	0.61	0.31–3.27	0.82	0.035
β-Cryptoxanthin	199	1.44	1.05	0.13–5.17	0.95	0.009
Zeaxanthin	196	12.90	6.86	1.44–32.40	0.94	0.008
α-Carotene	201	1.24	0.38	0.45–2.65	0.25	0.049
Zeinoxanthin	198	0.82	0.82	0.12–5.29	0.88	0.016
Lutein	200	11.16	4.73	1.23–23.93	0.94	0.011
Acyclic and monocyclic carotenes	200	5.54	1.05	3.39–8.92	0.57	0.060
Total carotenoids	201	32.66	10.66	9.55–62.96	0.91	0.013
β-Carotenoids/α-carotenoids	190	1.92	1.17	0–7.87	0.98	0.002
β-Xanthophylls/α-xanthophylls	196	1.74	1.23	0.45–6.37	0.83	0.022
β-Carotene/β-cryptoxanthin	198	1.13	0.49	0.51–3.06	0.89	0.029
β-Cryptoxanthin/zeaxanthin	196	0.12	0.05	0.04–0.39	0.90	0.021
α-Carotene/zeinoxanthin	196	2.57	1.62	0.52–8.88	0.90	0.019
Zeinoxanthin/Lutein	195	0.10	0.06	0.03–0.42	0.89	0.023
Provitamin A ^c	199	2.68	1.01	0.81–5.55	0.80	0.033

Means and ranges (μg/g) for untransformed BLUPs of 15 carotenoid traits evaluated on a maize inbred association panel and estimated heritability on a line mean basis in two summer environments in West Lafayette, Indiana, across 2 years.

^a SD, standard deviation.

^b SE, standard error.

^c Provitamin A is calculated as the sum of β-carotene, 1/2 α-carotene, and 1/2 β-cryptoxanthin.

correlations were between β-cryptoxanthin and α-carotene; zeinoxanthin and zeaxanthin; and zeaxanthin and ζ-carotene ($r_p < 0.01$) (Table S6). As expected, compounds tended to be highly correlated with their corresponding precursor compounds in the carotenoid biosynthetic pathway. The average heritability on a line mean basis for the nine carotenoid compounds and the 15 sums, ratios, and proportions was 0.80, with a range from 0.98 for the ratio of β-branch to α-branch carotenoids to 0.25 for α-carotene. The relatively lower heritability of α-carotene may be related to technical limitations for reliable separation of it from other more abundant carotenes that overlap in elution on the HPLC system. Overall, the high heritabilities for carotenoids suggest that variation for these compounds in maize grain is largely influenced by genetic rather than environmental effects (Table S7).

Average quantities of the provitamin A carotenoids, α-carotene, β-carotene, and β-cryptoxanthin, were low relative to lutein and zeaxanthin (Table 2). The three provitamin A compounds, respectively, composed ~23, 49, and 27% of the average provitamin A concentration of 2.68 μg/g present in this panel. The heritabilities of β-carotene and β-cryptoxanthin, the more predominant provitamin A compounds, were high: 0.82 and 0.95, respectively. High heritabilities were also observed for the ratios of β-branch to α-branch carotenoids (0.98) and β-carotene to β-cryptoxanthin (0.89). Because higher heritability traits are more responsive to selection than low heritability traits, these high heritabilities indicate that selection for the more predominant provitamin A compounds should be effective.

Genome-wide association study

The genetic basis of variation for carotenoids in maize grain was dissected in the 201-member panel using 462,703 genome-wide SNPs and seven indels. Unified mixed linear

models (Yu *et al.* 2006) that accounted for population structure and familial relatedness were fitted to a subset of 284, 180 SNPs with MAF ≥ 0.05 and the seven indels. A total of 24 unique SNPs and two indels were significantly associated with one or more carotenoid traits at a genome-wide FDR of 5% (Table S8A, Figure S2). Because the statistical power from an association panel of 201 inbreds is limited, generally only capable of repeatedly detecting large-effect QTL (Long and Langley 1999), we searched for relatively smaller-effect QTL at a genome-wide FDR of 10%. Under this less conservative criterion, an additional 11 SNPs and one indel were significantly associated with at least one carotenoid trait (Table S8A). Most of the additional SNPs identified at 10% FDR were located in the same vicinity of the significant polymorphisms detected at 5% FDR.

Peak associations significant at 5% FDR for zeaxanthin, total β-xanthophylls, and β-xanthophylls/α-xanthophylls were found at two SNPs within the gene encoding zeaxanthin epoxidase (*zep1*, GRMZM2G127139) on chromosome 2 (uncorrected *P*-values 4.82×10^{-8} to 2.22×10^{-9}). Zeaxanthin epoxidase carries out a two-step reaction that produces violaxanthin from zeaxanthin through the intermediate antheraxanthin (Figure 1). Weaker associations were detected for zeaxanthin and β-xanthophylls/α-xanthophylls with five SNPs located ~26 kb downstream of *zep1* (*P*-values 7.57×10^{-6} to 1.19×10^{-6}) in the vicinity of a gene encoding a eukaryotic aspartyl protease (GRMZM2G062559). To better clarify the signals of association in this 1.2-Mb genomic interval, the MLM procedure (Segura *et al.* 2012) was conducted on a chromosome-wide basis for all three zeaxanthin-related traits. The resultant optimal model for two of the three traits, zeaxanthin and total β-xanthophylls, included peak SNP S2_44448432 located within *zep1*. No SNP was selected by MLM for the third trait, β-xanthophylls/α-xanthophylls.

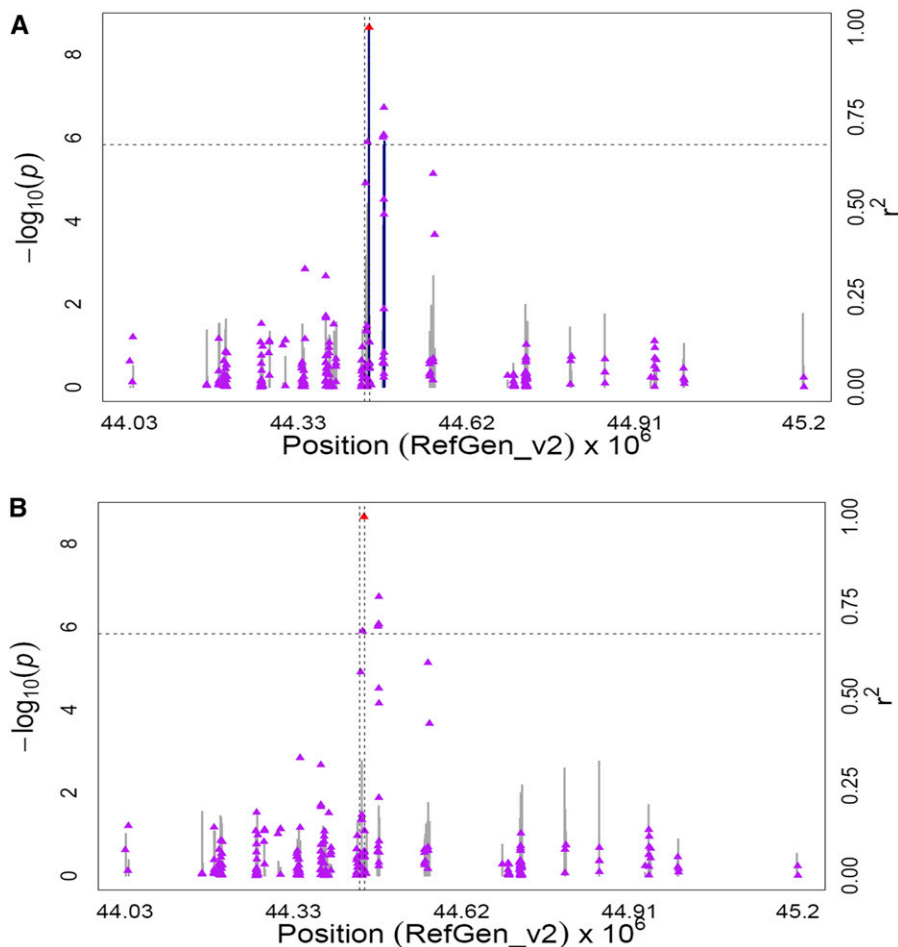


Figure 2 GWAS for zeaxanthin content in maize grain. (A) Scatter plot of association results from a unified mixed model analysis of zeaxanthin and LD estimates (r^2) across the *zep1* chromosome region. Negative \log_{10} -transformed P -values (left y-axis) from a GWAS for zeaxanthin and r^2 values (right y-axis) are plotted against physical position (B73 RefGen_v2) for a 1.2-Mb region on chromosome 2 that encompasses *zep1*. The blue vertical lines are $-\log_{10}$ P -values for SNPs that are statistically significant for zeaxanthin at 5% FDR, while the gray vertical lines are $-\log_{10}$ P -values for SNPs that are nonsignificant at 5% FDR. Triangles are the r^2 values of each SNP relative to the peak SNP (indicated in red) at 44,448,432 bp. The black horizontal dashed line indicates the $-\log_{10}$ P -value of the least statistically significant SNP at 5% FDR. The black vertical dashed lines indicate the start and stop positions of *zep1* (GRMZM2G127139). (B) Scatter plot of association results from a conditional unified mixed model analysis of zeaxanthin and LD estimates (r^2) across the *zep1* chromosome region, as in A. The peak SNP from the unconditional GWAS (S2_44448432; 44,448,432 bp) was included as a covariate in the unified mixed model to control for the *zep1* effect.

When GWAS was conducted with SNP S2_44448432 as a covariate for all three traits, the remaining signals on chromosome 2 were no longer significant (Figure 2, Figure S3, Figure S4, Table S8B).

The *lut1* gene (GRMZM2G143202) on chromosome 1 contains an intronic SNP (ss196425306; 86,844,203 bp) that was significantly associated with α -carotene/zeinoxanthin, zeinoxanthin, and zeinoxanthin/lutein (P -values 8.95×10^{-8} to 3.47×10^{-10}). The *lut1* gene encodes CYP97C, a cytochrome P450-type monooxygenase responsible for hydroxylating the ϵ -ring of zeinoxanthin to yield lutein (Tian *et al.* 2004; Quinlan *et al.* 2012). The only other statistically significant SNP (ss196425308; 86,945,134 bp) in this region was located ~ 100 kb downstream of *lut1* and was in perfect LD ($r^2 = 1$) with the peak SNP (ss196425306) in *lut1*. To further resolve the signals in the *lut1* region, the MLM procedure was run on these three carotenoid traits, with all SNPs on chromosome 1 considered for inclusion into the optimal models. All optimal models contained only the peak GWAS SNP in the *lut1* intron (Figure 3, Figure S5, Figure S6, Table S8C).

A cluster of association signals was detected in an 11-Mb region surrounding the *lcyE* gene (GRMZM2G012966) on chromosome 8, involving 16 markers at 10% FDR and six traits: lutein, zeaxanthin, total α -xanthophylls, total β -xanthophylls,

β -xanthophylls/ α -xanthophylls, and β -carotenoids/ α -carotenoids. *lcyE* encodes lycopene ϵ -cyclase, the committed step toward α -carotene biosynthesis whose activity influences flux between the α - and β -branches of the carotenoid pathway (Cunningham *et al.* 1996). The most significant associations in this region were from nine markers within ± 3 kb of the *lcyE*-coding region (P -values 8.99×10^{-7} to 5.05×10^{-16}). The MLM procedure with all chromosome 8 SNPs produced optimal models for lutein, total α -xanthophylls, β -xanthophylls/ α -xanthophylls, and β -carotenoids/ α -carotenoids with two *lcyE* polymorphisms, S8_138882897 and *lcyE* SNP216. When GWAS was conducted for these four traits using these two *lcyE* polymorphisms as covariates, the signals from remaining polymorphisms in the 11-Mb region surrounding *lcyE* disappeared (Figure 4, Figure S7, Figure S8, Figure S9, Figure S10, Figure S11, Table S8D). The optimal MLM for zeaxanthin and total β -xanthophylls also included one SNP (S8_171705574; 171,705,574 bp) located within a gene encoding a 3-hydroxyacyl-CoA dehydrogenase (GRMZM2G106250). When GWAS was performed using S8_171705574 as a covariate, the signal associated with 3-hydroxyacyl-CoA dehydrogenase disappeared, but the signals in the *lcyE* region remained (Figure 5, Figure S12, Table S8E).

A significant association at 5% FDR was identified between zeaxanthin and an insertion in the 3' end (3'TE

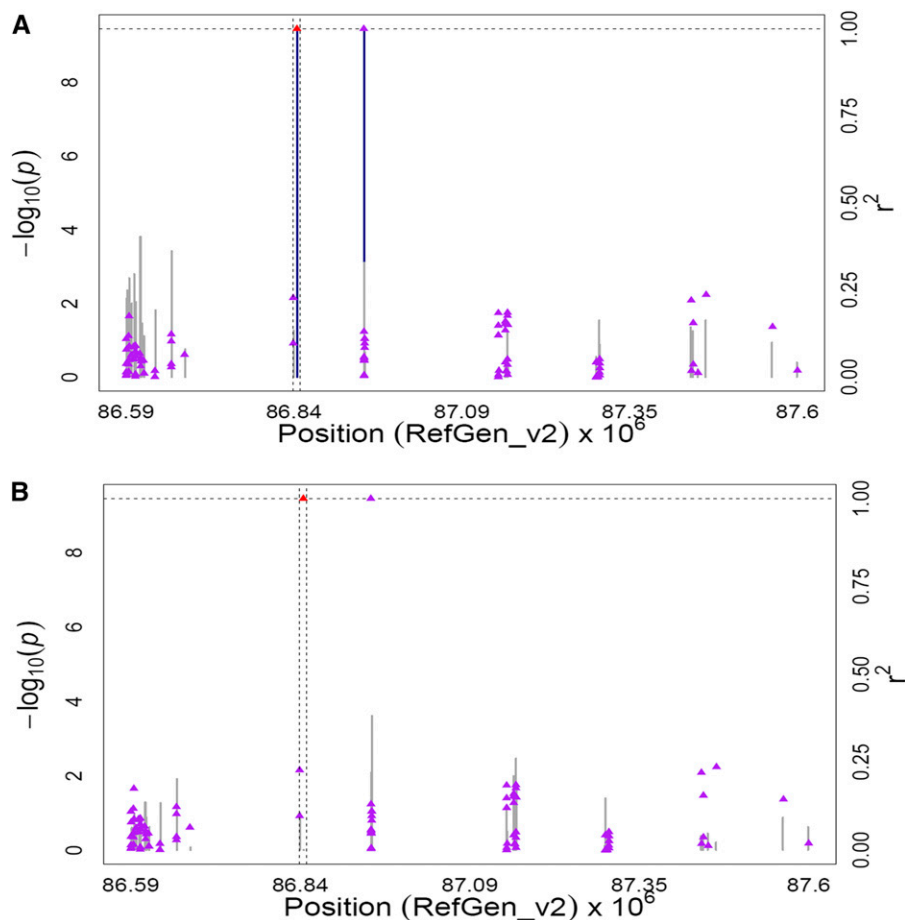


Figure 3 GWAS for the ratio of α -carotene to zeinoxanthin content in maize grain. (A) Scatter plot of association results from a unified mixed model analysis of the ratio of α -carotene to zeinoxanthin and LD estimates (r^2) across the *lut1* chromosome region. Negative \log_{10} -transformed P -values (left y -axis) from a GWAS for the ratio of α -carotene to zeinoxanthin and r^2 values (right y -axis) are plotted against physical position (B73 RefGen_v2) for a 1-Mb region on chromosome 1 that encompasses *lut1*. The blue vertical lines are $-\log_{10}$ P -values for SNPs that are statistically significant for the ratio of α -carotene to zeinoxanthin at 5% FDR, while the gray vertical lines are $-\log_{10}$ P -values for SNPs that are nonsignificant at 5% FDR. Triangles are the r^2 values of each SNP relative to the peak SNP (indicated in red) at 86,844,203 bp. The black horizontal dashed line indicates the $-\log_{10}$ P -value of the least statistically significant SNP at 5% FDR. The black vertical dashed lines indicate the start and stop positions of *lut1* (GRMZM2G14322.) (B) Scatter plot of association results from a conditional unified mixed model analysis of the ratio of α -carotene to zeinoxanthin and LD estimates (r^2) across the *lut1* chromosome region, as in A. The peak SNP from the unconditional GWAS (ss196425306; 86,844,203 bp) was included as a covariate in the unified mixed model to control for the *lut1* effect.

indel marker) of the *crtRB1* gene (GRMZM2G152135) on chromosome 10 (P -value 1.11×10^{-6}). At 10% FDR, signals for β -carotene/ $(\beta$ -cryptoxanthin+zeaxanthin) were detected by *crtRB1* InDel4, a coding region indel, and SNP ss196501627, with P -values of 2.23×10^{-7} and 3.51×10^{-7} , respectively. *crtRB1* encodes a nonheme dioxygenase that hydroxylates β -rings of carotenoids. Significant associations with β -carotene, ratios of β -carotene/ β -cryptoxanthin and β -carotene/ β -cryptoxanthin+zeaxanthin, and total carotenoid content were previously reported for *crtRB1* (Yan *et al.* 2010). The MLMM analysis produced an optimal model that contained only *crtRB1* InDel4, which, when included as a covariate in GWAS, removed other signals in the region (Figure 6, Figure S13, Figure S14, Table S8F).

The *zep1*, *lut1*, *lcyE*, and *crtRB1* genes were the only carotenoid biosynthetic genes identified in the GWAS with peak signals located within or adjacent to their coding regions. To simultaneously account for the potential confounding effects of these moderate-to-strong association signals (Platt *et al.* 2010), a more stringent conditional analysis was conducted. Inclusion of peak polymorphisms for each of the genes individually eliminated signals for that gene, but signals for the other three genes remained (Table S8, B–D and F). When polymorphisms tagging all four genes were simultaneously included as covariates in the GWAS model, however,

only two SNPs remained statistically significant at 5% FDR (Table S8G). The first of these SNPs—S7_13843351 (chromosome 7; 13,843,351 bp; associated with β -cryptoxanthin at P -value 4.86×10^{-8})—lies within GRMZM2G001938, an exostosin family protein. The second SNP—S8_171705574 (chromosome 8; 171,705,574 bp; associated with zeaxanthin at P -value 1.54×10^{-7})—lies in the putative 3-hydroxyacyl-CoA dehydrogenase (GRMZM2G106250). This gene was also found to be associated with zeaxanthin in the MLMM analysis of chromosome 8 presented above.

Pathway-level analysis

The large number of markers used for GWAS requires a very conservative adjustment for the multiple testing problem, permitting detection of only the strongest association signals. To assess weaker association signals, we performed a pathway-level analysis with a set of 58 *a priori* metabolic genes that are potentially involved in the genetic control of natural variation for carotenoid synthesis or degradation. The FDR procedure was conducted on a subset of 7408 SNPs and seven indels located within ± 250 kb of these 58 candidate genes tested for all 24 carotenoid traits, and a total of 38 SNPs and three indels were significant at 5% FDR (Table S9). Seven SNPs were in the vicinity of three genes involved in plastidic synthesis of isopentenyl pyrophosphate (IPP): *IPP*

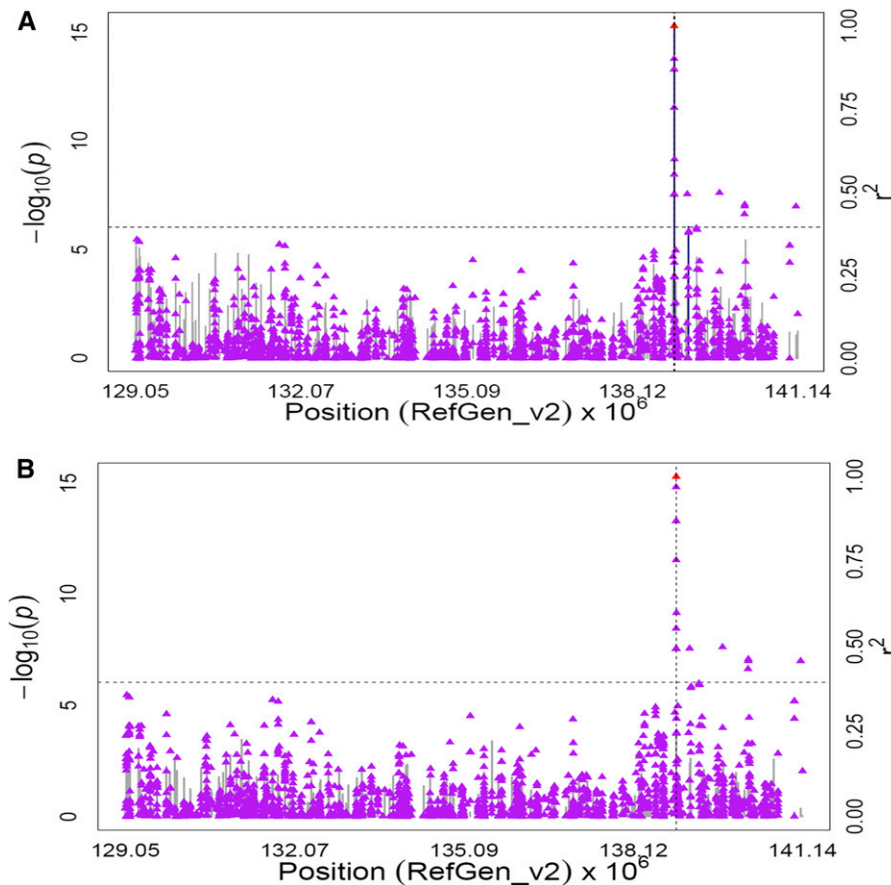


Figure 4 GWAS for the ratio of β -xanthophylls to α -xanthophylls content in maize grain. Scatter plot of association results from a unified mixed model analysis of the ratio of β -xanthophylls to α -xanthophylls and LD estimates (r^2) across the *lcyE* chromosome region. Negative \log_{10} -transformed P -values (left y -axis) from a GWAS for the ratio of β -xanthophylls to α -xanthophylls and r^2 values (right y -axis) are plotted against physical position (B73 RefGen_v2) for a 12-Mb region on chromosome 8 that encompasses *lcyE*. The blue vertical lines are $-\log_{10}$ P -values for SNPs that are statistically significant for the ratio of β -xanthophylls to α -xanthophylls at 5% FDR, while the gray vertical lines are $-\log_{10}$ P -values for SNPs that are nonsignificant at 5% FDR. Triangles are the r^2 values of each SNP relative to the peak SNP (indicated in red) at 138,883,206 bp. The black horizontal dashed line indicates the $-\log_{10}$ P -value of the least statistically significant SNP at 5% FDR. The black vertical dashed lines indicate the start and stop positions of *lcyE* (GRMZM2G12966). (B) Scatter plot of association results from a conditional unified mixed model analysis of the ratio of β -xanthophylls to α -xanthophylls and LD estimates (r^2) across the *lcyE* chromosome region, as in A. The two SNPs (*lcyE* SNP216 and S_138882897) from the optimal MLMM model were included as covariates in the unified mixed model to control for the *lcyE* effect.

isomerase 3 (*ippi3*, GRMZM2G133082), *1-deoxy-D-xylulose 5-phosphate synthase 2* (*dxs2*, GRMZM2G493395), and *geranylgeranyl pyrophosphate synthase 2* (*ggps2*, GRMZM2G102550). The remaining markers were within ± 250 kb of eight carotenoid biosynthetic pathway genes: β -carotene hydroxylase 6 (*hyd6*, GRMZM2G090051), *CYP97A* β -ring hydroxylase (*lut5*, GRMZM5G837869), *carotenoid isomerase 3* (*crti3*, GRMZM2G144273), ζ -carotene desaturase (*zds1*, GRMZM2G454952), *zep1*, *lut1*, *lcyE*, and *crtRB1*.

To account for the signals from *zep1*, *lut1*, *lcyE*, and *crtRB1*, an additional pathway-level analysis was performed as per GWAS using models with covariate markers of each gene individually and one model accounting for all four genes (Table S9, B–G). When a SNP tagging *zep1* or *lut1* was used as a covariate, signals in the vicinity of *hyd6* and *ippi3* were eliminated. When two markers tagging *lcyE* were used as covariates, no significant SNPs were detected in the regions of *crti3*, *ippi3*, or *zds1*. When *crtRB1* InDel4 was used as a covariate, signal was lost for *ggps2* and *zds1*. When covariates from *zep1*, *lut1*, *lcyE*, and *crtRB1* were placed into the model, the only significant signals remaining were from markers within ± 250 kb of *dxs2* (GRMZM2G493395) and *lut5* (GRMZM5G837869).

Prediction of carotenoid levels

We assessed the potential of genomic selection as a method for breeding maize grain with higher levels of carotenoids.

Specifically, the predictive abilities of marker data sets with three different levels of coverage—genome-wide (284,180 SNP markers and seven indels); 58 pathway-level genes (7408 SNP markers and seven indels); and eight candidate genes (*y1*, *zds1*, *lcyE*, *crtRB3*, *lut1*, *crtRB1*, *zep1*, and *ccd1*) underlying QTL associated with carotenoid levels in prior linkage population studies (944 SNP markers and seven indels)—were assessed and compared. These marker sets were tested in three types of linear regression models commonly used for genomic selection and prediction: RR-BLUP, LASSO, and elastic net analysis. While previous studies have shown that these approaches produce similar prediction accuracies (Riedelsheimer *et al.* 2012), it was useful to test multiple statistical models in this study, given the potential oligogenic architecture of carotenoid levels in maize grain (Wong *et al.* 2004; Chander *et al.* 2008; Kandianis *et al.* 2013).

We performed prediction analyses for 24 traits in total (Table 1): 15 traits expected to be of most interest to breeders (Table 2) and 9 traits capturing additional compounds, sums, ratios, and proportions (Table S5). Results for the two sets of traits (Table S10) showed equivalent trends; thus we will focus our reporting on the 15 highest-priority traits for breeding (Figure 7). We observed no consistent differences in predictive ability across the three statistical approaches (Table S10). Notably, there were no differences observed across the three marker sets for each of the traits tested; inclusion of more markers beyond those

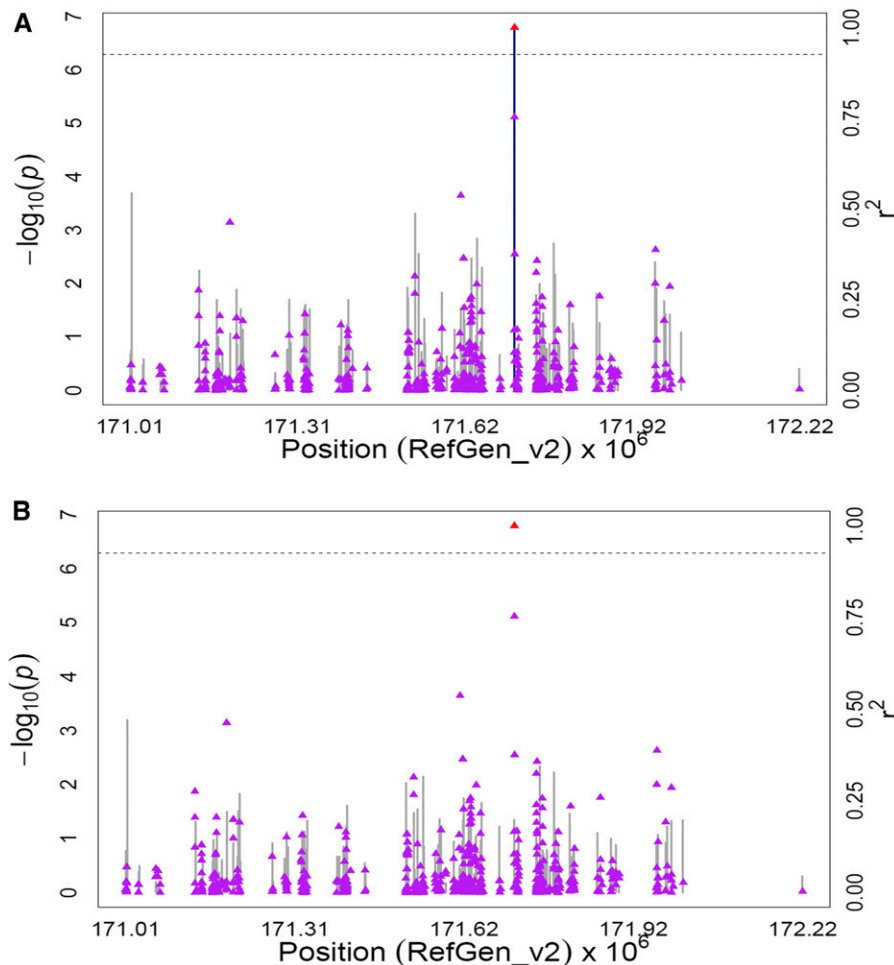


Figure 5 GWAS for total β -xanthophylls content in maize grain. (A) Scatter plot of association results from a unified mixed model analysis of total β -xanthophylls and LD estimates (r^2) across the surrounding chromosome region. Negative \log_{10} -transformed P -values (left y -axis) from a GWAS for total β -xanthophylls and r^2 values (right y -axis) are plotted against physical position (B73 RefGen_v2) for a 1.2-Mb region on chromosome 8. The blue vertical lines are $-\log_{10}$ P -values for SNPs that are statistically significant for total β -xanthophylls at 5% FDR, while the gray vertical lines are $-\log_{10}$ P -values for SNPs that are nonsignificant at 5% FDR. Triangles are the r^2 values of each SNP relative to the peak SNP (indicated in red) at 171,705,574 bp. The black horizontal dashed line indicates the $-\log_{10}$ P -value of the least statistically significant SNP at 5% FDR. (B) Scatter plot of association results from a conditional unified mixed model analysis of total β -xanthophyll and LD estimates (r^2) across the 1.2-Mb chromosome region, as in A. The peak SNP from the unconditional GWAS (S8_171705574; 171,705,574 bp) was included as a covariate in the unified mixed model to control for the novel effect detected on chromosome 8.

within ± 250 kb of eight candidate genes underlying maize grain carotenoid QTL did not confer additional predictive ability. Additionally, we determined that the carotenoid QTL-targeted marker set yielded substantially better prediction accuracies than marker sets generated from eight 500-kb regions selected at random throughout the genome (2.765-fold mean difference; paired $t = 10.68$, d.f. = 23, P -value = 1.09×10^{-10}) (Table S11). The carotenoid QTL-targeted marker set also outperformed markers within ± 250 kb of eight genes randomly selected from the other 50 *a priori* candidate genes represented in the pathway-level prediction set (2.709-fold mean difference; paired $t = 10.21$, d.f. = 23, P -value = 2.59×10^{-10}).

On average, we obtained a prediction accuracy of 0.43 across the 15 traits, with the highest prediction accuracies (averaged across the three marker sets and three models tested) for β -xanthophylls/ α -xanthophylls (0.71), β -carotenoids/ α -carotenoids (0.59), zeaxanthin (0.52), lutein (0.51), α -carotene/zeinoxanthin (0.51), zeinoxanthin (0.49), β -cryptoxanthin (0.44), and zeinoxanthin/lutein (0.43) (Table 3, Figure 7). We found a weak but significant positive relationship between trait heritabilities and unstandardized prediction correlations ($r_{sp} = 0.57$, P -value = 0.026). This relationship was no longer significant at a significance level of $\alpha = 0.05$

when α -carotene, the least heritable trait ($\hat{h}_i^2 = 0.25$), was excluded ($r_{sp} = 0.49$, P -value = 0.079). In contrast, standardized prediction accuracies for the 15 traits were observed to scale consistently with the number of significant marker associations observed in GWAS ($r_{sp} = 0.91$, P -value = 2.2×10^{-6}) (Table 3). The eight traits with prediction accuracies above or at the mean had at least one significant marker association in a GWAS at a genome-wide FDR of 10%. Given that the standardized prediction accuracies were also strongly positively correlated with the partial r^2 value of the most significantly associated marker for a given trait ($r_{sp} = 0.85$, P -value = 6.9×10^{-5}) and strongly negatively correlated with the P -values of that marker ($r_{sp} = -0.94$, P -value = 2.09×10^{-7}), these results also suggest that effect size of associated markers is an important factor driving prediction accuracy.

Discussion

Provitamin A biofortification efforts are strengthened by association studies that further characterize the underlying genetic basis of variation for maize grain carotenoids and thus provide more loci that can be used in different combinations in MAS and GS programs. Four major-effect loci were

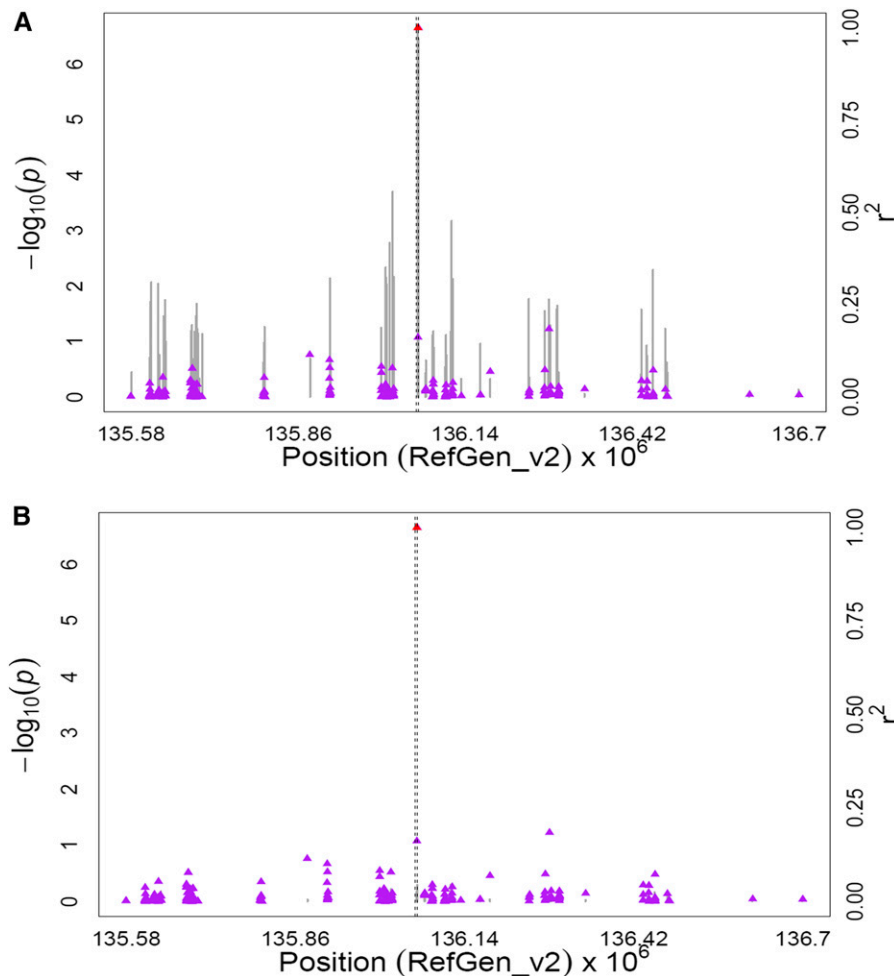


Figure 6 GWAS for the ratio of β -carotene to β -cryptoxanthin plus zeaxanthin content in maize grain. (A) Scatter plot of association results from a unified mixed model analysis of the ratio of β -carotene to β -cryptoxanthin plus zeaxanthin and LD estimates (r^2) across the *crtRB1* chromosome region. Negative \log_{10} -transformed P -values (left y-axis) from a GWAS for the ratio of β -carotene to β -cryptoxanthin plus zeaxanthin and r^2 values (right y-axis) are plotted against physical position (B73 RefGen_v2) for a 1.2-Mb region on chromosome 10 that encompasses *crtRB1*. The vertical lines are $-\log_{10} P$ -values for all tested SNPs in this region. Triangles are the r^2 values of each SNP relative to the peak polymorphism (indicated in red) at 136,059,748 bp. The black vertical dashed lines indicate the start and stop positions of *crtRB1* (GRMZM2G152135). (B) Scatter plot of association results from a conditional unified mixed model analysis of the ratio of β -carotene to β -cryptoxanthin plus zeaxanthin and LD estimates (r^2) across the *crtRB1* chromosome region, as in A. The peak polymorphism from the unconditional GWAS (*crtRB1* InDel4; 136,059,748 bp) was included as a covariate in the unified mixed model to control for the *crtRB1* effect.

identified in GWAS, the previously reported associations of *lcyE* and *crtRB1* with maize grain carotenoids, and notably new associations with *zep1* and *lut1*. MLMs and covariate analyses were used to distinguish and eliminate noncausal variation in LD with putative causal variants. We also demonstrated higher genetic mapping resolution with genome-wide SNP markers than previous QTL studies in biparental mapping populations that identified candidate genes associated with levels of carotenoids and orange kernel color in maize grain (Wong *et al.* 2004; Chander *et al.* 2008; Chandler *et al.* 2013; Kandianis *et al.* 2013).

A series of prediction analyses was used to compare the relative usefulness of the full set of GWAS markers with a pathway-level set of markers and with a smaller carotenoid QTL-targeted marker set. Alleles or haplotypes with effect estimates falling below the conservative detection thresholds applied in GWAS are fitted in genomic selection and prediction models in addition to more strongly associated loci. This increased genome coverage compared to traditional MAS may prove an effective selection strategy for maize grain carotenoid traits, including provitamin A.

Significant SNPs associated with zeaxanthin and total β -xanthophylls were identified in the coding region of *zep1*, which fits well with the activity of the encoded enzyme in

converting zeaxanthin to violaxanthin via antheraxanthin (Hieber *et al.* 2000). In the *zep1* region, QTL have been identified for levels of β -branch carotenoids, zeaxanthin, β -cryptoxanthin, and β -carotene (Kandianis *et al.* 2013) and for degree of orange color (Chandler *et al.* 2013), a trait associated with higher levels of zeaxanthin (Pfeiffer and McClafferty 2007). These linkage studies provide independent support for our association results for *zep1*.

A SNP in the *lut1*-coding region was associated through GWAS with α -carotene/zeinoxanthin, zeinoxanthin/lutein, and zeinoxanthin, again consistent with the enzymatic activity of *lut1* in forming lutein by hydroxylation of the ϵ -ring of zeinoxanthin (Tian *et al.* 2004; Quinlan *et al.* 2012). A QTL for lutein was reported near the *lut1* region in a low-resolution biparental mapping population (Chander *et al.* 2008). Pathway-level analysis with covariates for *lcyE* detected two additional SNPs \sim 240 kb upstream of the *lut1* start codon that were also associated with the ratio of zeinoxanthin to lutein. However, it may be difficult to determine whether or not these additional signals indicate an enhancer element upstream of *lut1* because this region is part of the chromosome 1 pericentromeric region (Gore *et al.* 2009). Substantially larger association panels that better exploit the recombinational history of maize, such as the

Model and Marker Set Comparison

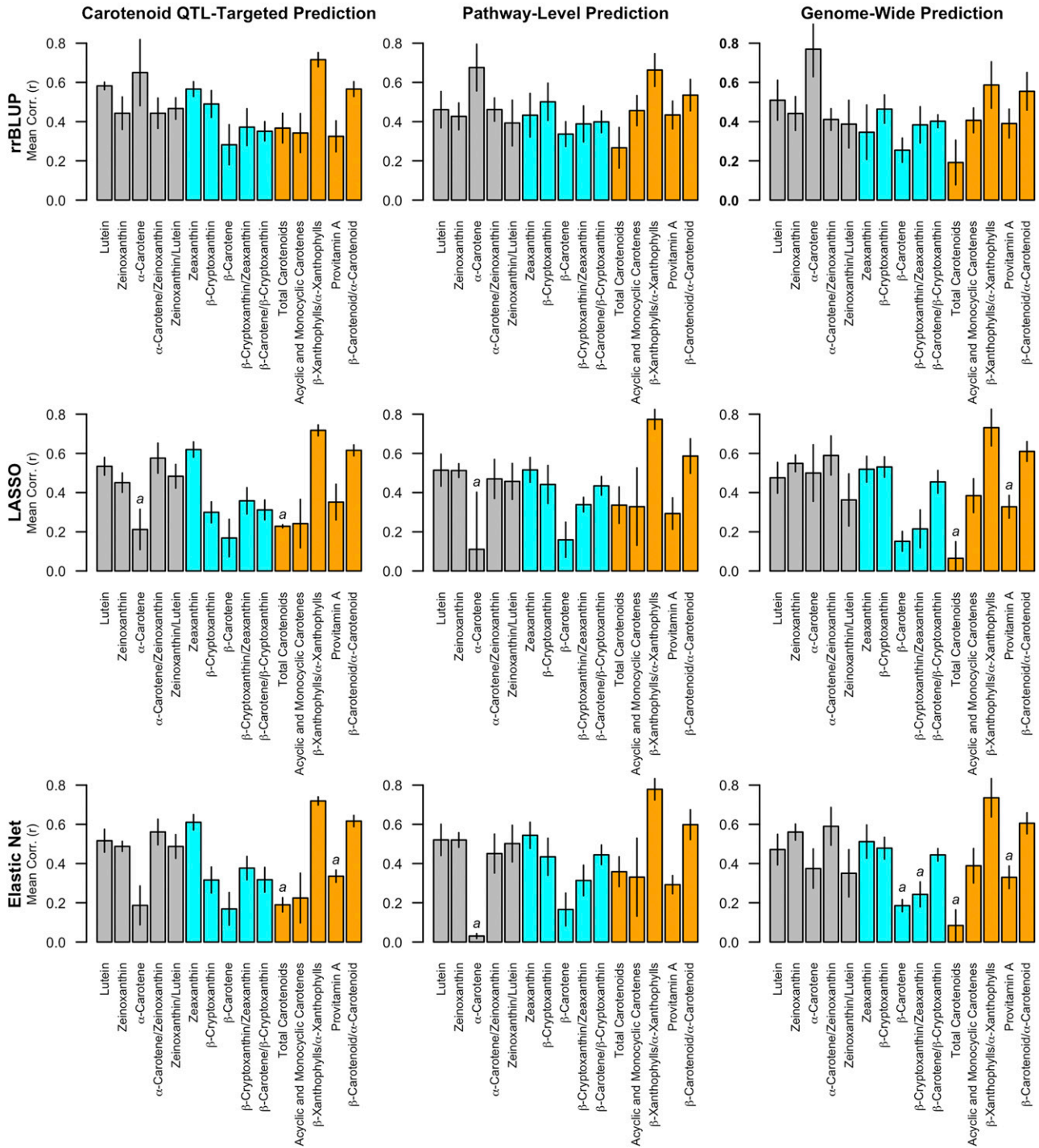


Figure 7 Comparison of genomic prediction methods and marker sets for 15 grain carotenoid traits. Three prediction methods—RR-BLUP, LASSO, and elastic net analysis—were tested using three marker sets as predictors: carotenoid QTL-targeted prediction (the 944 markers and seven indels within ± 250 kb of 8 *a priori* candidate genes), pathway-level prediction (the 7408 markers and seven indels within ± 250 kb of 58 *a priori* candidate genes), and genome-wide prediction (all 284,180 markers and 7 indels used in genome-wide association studies). Standardized average correlations resulting from the fivefold cross-validation are reported. A superscript “a” (a) indicates that no markers were selected in one or two of the five folds or in three of the five folds in one case (α -carotene using the Pathway-Level Prediction marker set in eNet.)

Table 3 Mean prediction accuracies and significant marker associations for 15 grain carotenoid traits

Trait	Mean prediction accuracy	Significant marker associations (10% FDR)	Partial r^2 of most significant marker	P -value of most significant marker	Significant marker associations within ± 3 kb of a candidate gene	
					Total	Per candidate gene
β -Xanthophylls/ α -xanthophylls	0.714	24	0.14	5.05E-16	13	<i>zep1</i> (2), <i>lcyE</i> (11)
β -Carotenoids/ α -carotenoids	0.587	4	0.17	2.08E-09	3	<i>lcyE</i> (3)
Zeaxanthin	0.518	11	0.19	2.22E-09	4	<i>zep1</i> (2), <i>lcyE</i> , <i>crtRB1</i>
Lutein	0.509	3	0.34	6.28E-09	2	<i>lcyE</i> (2)
α -Carotene/zeinoxanthin	0.506	3	0.19	3.31E-10	1	<i>lut1</i>
Zeinoxanthin	0.488	4	0.14	8.95E-08	1	<i>lut1</i>
β -Cryptoxanthin	0.439	1	0.13	1.66E-07	0	—
Zeinoxanthin/lutein	0.432	3	0.15	4.97E-08	1	<i>lut1</i>
β -Carotene/ β -cryptoxanthin	0.395	0	0.12	5.38E-07	0	—
α -Carotene	0.390	0	0.1	4.93E-06	0	—
Acyclic and monocyclic carotenes	0.345	0	0.1	5.72E-06	0	—
Provitamin A	0.342	0	0.1	5.81E-06	0	—
β -Cryptoxanthin/zeaxanthin	0.332	0	0.1	3.41E-06	0	—
Total carotenoids	0.231	0	0.11	5.80E-06	0	—
β -Carotene	0.208	0	0.09	1.46E-05	0	—

Mean prediction accuracies, significant marker associations, and the partial r^2 and P -values of the most significant marker of each trait from a GWAS for the 15 priority grain carotenoid traits. Mean prediction accuracies were obtained by averaging across RR-BLUP, LASSO, and elastic net analysis prediction methods and carotenoid QTL-targeted, pathway-level, and genome-wide marker sets. A 10% FDR threshold was used to determine significance. A full list of significant marker associations detected for each trait in GWAS without covariates, including those located within ± 3 kb of a candidate gene, can be found in Table S8A.

Ames diversity panel (Romay *et al.* 2013), are needed to provide more statistical power and precision in the *lut1* interval.

Significant SNPs associated with zeaxanthin and total β -xanthophylls were identified in the coding regions of *lcyE* and a gene encoding a *3-hydroxyacyl-CoA dehydrogenase*. Given that allelic variation in *lcyE* influences relative flux into the α - and β -branches of the carotenoid pathway (Harjes *et al.* 2008), this is a logical candidate gene for influencing levels of zeaxanthin and total β -xanthophylls. Although the *3-hydroxyacyl-CoA dehydrogenase* gene does not have a known function in the carotenoid pathway or in regulating the pathway, when a SNP in the *3-hydroxyacyl-CoA dehydrogenase*-coding region (S8_171705574) was used as a covariate in GWAS, the signal in the *lcyE* region was still present for zeaxanthin and total β -xanthophylls. Determining whether there is a true association of *3-hydroxyacyl-CoA dehydrogenase* with levels of zeaxanthin and total β -xanthophylls, or if the presence of these associations is due to long-range LD with *lcyE* or another gene on chromosome 8, merits further investigation. Again, this two-gene region could be better resolved in a larger association panel.

The *crtRB1* gene showed a relatively weak signal in GWAS with no significant SNPs at 5% FDR and only one significant SNP associated with the ratio of β -carotene to β -cryptoxanthin+zeaxanthin at a genome-wide FDR of 10%. The inclusion of two indel markers for *crtRB1* revealed signals between the 3' TE indel marker and zeaxanthin and total β -xanthophylls and between the InDel4 marker and ratio of β -carotene to β -cryptoxanthin+zeaxanthin. There was only one SNP in our data set within the coding region of *crtRB1* and, as a result, the SNPs did not capture the relevant var-

iation described in Yan *et al.* (2010). Notably, the detection of a significant association with the two indel markers showed that the contribution of the *crtRB1* gene was similar to that previously reported.

The analysis of a pathway-level, 58 *a priori* candidate gene set revealed additional weaker signals within ± 250 kb of 7 of these candidate genes. However, when covariates identified from MLM analysis as tagging the signals of *zep1*, *lut1*, *lcyE*, and *crtRB1* were added to the model, polymorphisms in the vicinity of 5 of these candidate genes lost significance and only *dxs2* and *lut5* remained significant. These results suggest that *dxs2* and *lut5* should be further investigated, as they logically could affect carotenoid traits. The gene regions and polymorphisms that were or were not significant depended on the analysis performed: GWAS, pathway-level analysis, MLM, and covariate analysis. The polymorphisms significant in one or more of these analyses should be evaluated in much larger association and linkage panels that provide greater genetic diversity, power, and precision. The pathway-level analysis that we performed was designed in part to minimize the multiple hypothesis testing penalty (Califano *et al.* 2012). Other statistical methodologies that consider all significant loci from GWAS, along with transcriptional and protein interaction networks, have the potential to identify genes outside of the pathway that affect carotenoid accumulation (Baranzini *et al.* 2009; Chan *et al.* 2011) as well as polymorphisms surrounding these gene regions that may be useful in selection programs for higher levels of provitamin A, total carotenoids, and orange grain color.

To evaluate the relative gains to be expected from conducting genomic selection for carotenoid traits in maize grain, we

tested multiple prediction methods and marker sets. The RR-BLUP method assigns equal variance to all included markers (Meuwissen *et al.* 2001). This approach is optimal for complex traits having many underlying QTL of small effect. Given that carotenoid traits are likely largely explained by a small number of moderate- to large-effect loci (Wong *et al.* 2004; Chander *et al.* 2008; Kandianis *et al.* 2013), we hypothesized that a variable selection method that shrinks the variance explained by noncontributing markers to near or equal to zero, such as LASSO or elastic net analysis, would show higher predictive ability. While no differences were found among the three statistical approaches used in this study, we recommend continued model comparison for carotenoid traits in future analyses that employ larger maize populations with higher marker densities (Gore *et al.* 2009; McMullen *et al.* 2009; Chia *et al.* 2012; Romay *et al.* 2013).

Across the 15 traits tested, the three statistical approaches achieved a wide range of mean prediction accuracies: from 0.21 for β -carotene to 0.71 for β -xanthophylls/ α -xanthophylls (Table 3). Standard errors were generally equivalent in size across the statistical methods and marker sets tested (Table S10). Notably, the seven traits showing below-average prediction accuracy also showed no significant marker associations in GWAS (Table 3). This result, along with the strong positive correlation observed between prediction accuracy and the partial r^2 value of the most strongly associated marker for each trait, suggests that markers in strong LD with causative variants of at least moderate effect likely contributed to higher prediction accuracy of particular carotenoid traits in maize grain. Additionally, the comparable predictive abilities observed between the eight-gene QTL-targeted set and the larger candidate gene and genome-wide marker sets supports the hypothesis that density of marker coverage in carotenoid candidate gene regions was the primary driver in determining relative and absolute predictive power for carotenoid traits in this panel.

Most notably, linear regression models into which only the 944 SNP markers and seven indels within ± 250 kb of the eight candidate genes in the carotenoid QTL-targeted data set were input were generally as predictive as models trained with all 284,180 genome-wide SNP markers and seven indels included (Figure 7). A similar result was reported in Rutkoski *et al.* (2012) for another oligogenic trait, deoxynivalenol levels in wheat: the addition of genome-wide markers was found to decrease prediction accuracies compared to a model containing only markers associated with QTL. This key finding of our study—that a more targeted approach based on ~ 300 -fold fewer markers was equally predictive as genome-wide coverage—suggests that QTL-targeted approaches will be effective for favorably modifying and improving carotenoid composition in maize grain. However, continued prediction analyses in panels with larger sample size and greater genetic diversity, as well as studies in breeding populations, are needed to further examine whether more extensive genome coverage affords higher prediction accuracies relative to the carotenoid QTL-targeted prediction sets due to

increased power to detect weaker QTL effects and rarer alleles in a larger panel or population.

In the panel we studied, many of the most significant SNP associations are related to known carotenoid genes. Given these results and the likely oligogenic nature of maize grain carotenoid traits, it was logical to confine pathway-level prediction efforts to genes within the biochemical pathway. Recent efforts have made use of transcriptional networks to identify groups of genes showing subthreshold associations with phenotypes of interest (Baranzini *et al.* 2009; Chan *et al.* 2011). Additionally, an experimental study of general combining ability in hybrid maize found use of metabolite profiles as predictor variables in prediction models, although without the use of network analysis, to achieve prediction accuracies similar to models based on SNP marker data, but did not observe further gains when the two types of data were combined (Riedelsheimer *et al.* 2012). Our understanding of the genetics underlying maize carotenoid levels may benefit from the integration of network analysis and prediction approaches. Targeted or nontargeted gene expression and metabolite profiling approaches could feasibly be used together, particularly in larger panels, to identify transcriptional and metabolite networks that exhibit associations with carotenoid phenotypes but may not be represented in pathway-level analyses. The constituents of these networks could then be combined as additional predictor variables in models for potential further gains in accuracy.

Prior to our study, the best-characterized genes for provitamin A biofortification in maize grain were *lycE* and *crtRB1* (Harjes *et al.* 2008; Yan *et al.* 2010; Burt *et al.* 2011; Babu *et al.* 2013). Prior to these findings, breeding programs for developing countries with vitamin A deficiency performed selection based on HPLC analysis to directly measure carotenoid levels in maize kernels. These efforts had achieved only 6–8 $\mu\text{g/g}$ provitamin A in their experimental maize breeding materials (<http://www.harvestplus.org>; Pixley *et al.* 2013). This was only half of the HarvestPlus biofortification initial target level of 15 $\mu\text{g/g}$, and only small incremental gains of provitamin A levels were achieved during cycles of selection. MAS for a favorable *crtRB1* allele has resulted in rapidly increasing provitamin A content to >20 $\mu\text{g/g}$ in maize grain from experimental lines soon to be released (Azmach *et al.* 2013; Pixley *et al.* 2013).

Despite the excellent progress in breeding for higher levels of provitamin A, even higher levels are needed to account for postharvest degradation, which can result in a 70% reduction in provitamin A content in a 4- to 6-month storage period. Furthermore, in the second phase of HarvestPlus, higher target levels of provitamin A will be set so that smaller, more attainable quantities of maize grain can be consumed in a day to provide a beneficial level of provitamin A. This will broaden the impact of high provitamin A maize intervention programs. Thus genetic research that enables continual increases in levels of provitamin A is needed. To this end, use of GWAS and pathway-level gene sets with covariate analysis has revealed additional potentially useful genes.

For maize provitamin A biofortification to be effective in Africa, breeders are faced with the challenge of converting white maize germplasm that has had no direct selection for alleles in the carotenoid pathway to germplasm that has a dark-orange endosperm, high total carotenoids, and high provitamin A. In addition to the two genes already tapped for biofortification efforts, our GWAS results demonstrate the substantial contribution of two new genes, *zep1* and *lut1*, to carotenoid variation in maize grain. The improved knowledge of the associated effects of these two genes may lead to better prediction and selection of carotenoid levels in breeding populations, particularly for xanthophylls, total carotenoids, and the color orange, given the roles of *zep1* and *lut1* in the biosynthetic pathway. Zeaxanthin and lutein are the most predominant carotenoid compounds in maize, and accessions with darker orange kernels generally have higher levels of these two compounds (Pfeiffer and McClafferty 2007; Burt *et al.* 2011).

Although the four genes detected in GWAS—*zep1*, *lut1*, *lcyE*, and *crtRB1*—are clearly important, they may not be sufficient for efficient breeding in all contexts and genetic backgrounds. We propose that favorable alleles at *y1*, *zds1*, *lcyE*, *crtRB3*, *lut1*, *crtRB1*, *zep1*, and *ccd1* could be selected for rapid conversion endeavors. Our prediction analyses show that these eight genes are at least as effective for predicting carotenoid levels as a genome-wide set of predictors. While simultaneously selecting for eight genes would be resource-intensive, testing for the presence or absence of specific favorable alleles at *y1*, *zds1*, *crtRB3*, *zep1*, *lut1*, and *ccd1* in addition to *lcyE* and *crtRB1* in the elite adapted white-grain germplasm to be improved and the respective orange-grain donor germplasm should help breeders design effective MAS conversion strategies. We hypothesize that, in lines that have yellow or orange endosperm color or in lines already in selection programs for provitamin A, fewer genes will need to be selected. While *crtRB1* has been shown to be very useful for improving β -carotene, current objectives include selecting for the color orange. This eight gene set is proposed to meet this need. Future breeding objectives will also include (i) increasing the β -cryptoxanthin component of provitamin A since studies have shown that β -cryptoxanthin appears to be twice as bioavailable as β -carotene (Davis *et al.* 2008; Burri *et al.* 2011; Turner *et al.* 2013) and (ii) selecting for higher zeaxanthin and lutein levels for prevention of macular degeneration. The *zep1* and *lut1* genes should be especially useful in selection programs designed to meet these targets.

Use of the genes or subsets of genes in the carotenoid prediction sets could have a transformational effect on maize in Sub-Saharan Africa, starting with Ethiopia and Zimbabwe, the next HarvestPlus target countries. The rapid, cost-effective development of high-yielding, locally adapted germplasm with high provitamin A and total carotenoids and dark-orange kernel color could effectively create a new widespread biofortified grain crop. Consumption of this grain will provide essential provitamin A carotenoids and

a broad carotenoid profile exhibiting an array of nutritional attributes.

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GENETICS

Supporting Information

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A Foundation for Provitamin A Biofortification of Maize: Genome-Wide Association and Genomic Prediction Models of Carotenoid Levels

Brenda F. Owens, Alexander E. Lipka, Maria Magallanes-Lundback, Tyler Tiede, Christine H. Diepenbrock, Catherine B. Kandianis, Eunha Kim, Jason Cepela, Maria Mateos-Hernandez, C. Robin Buell, Edward S. Buckler, Dean DellaPenna, Michael A. Gore, and Torbert Rocheford

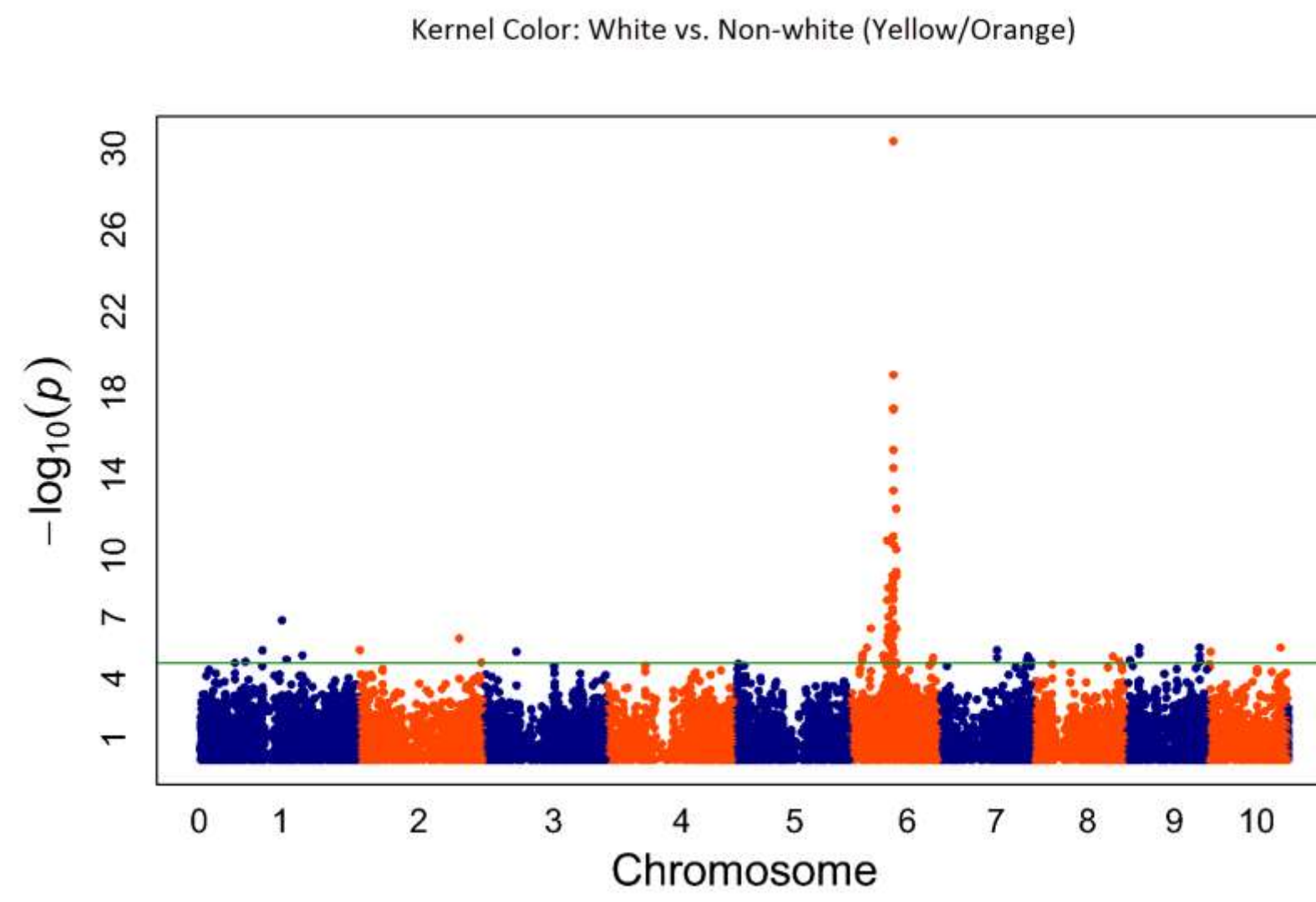
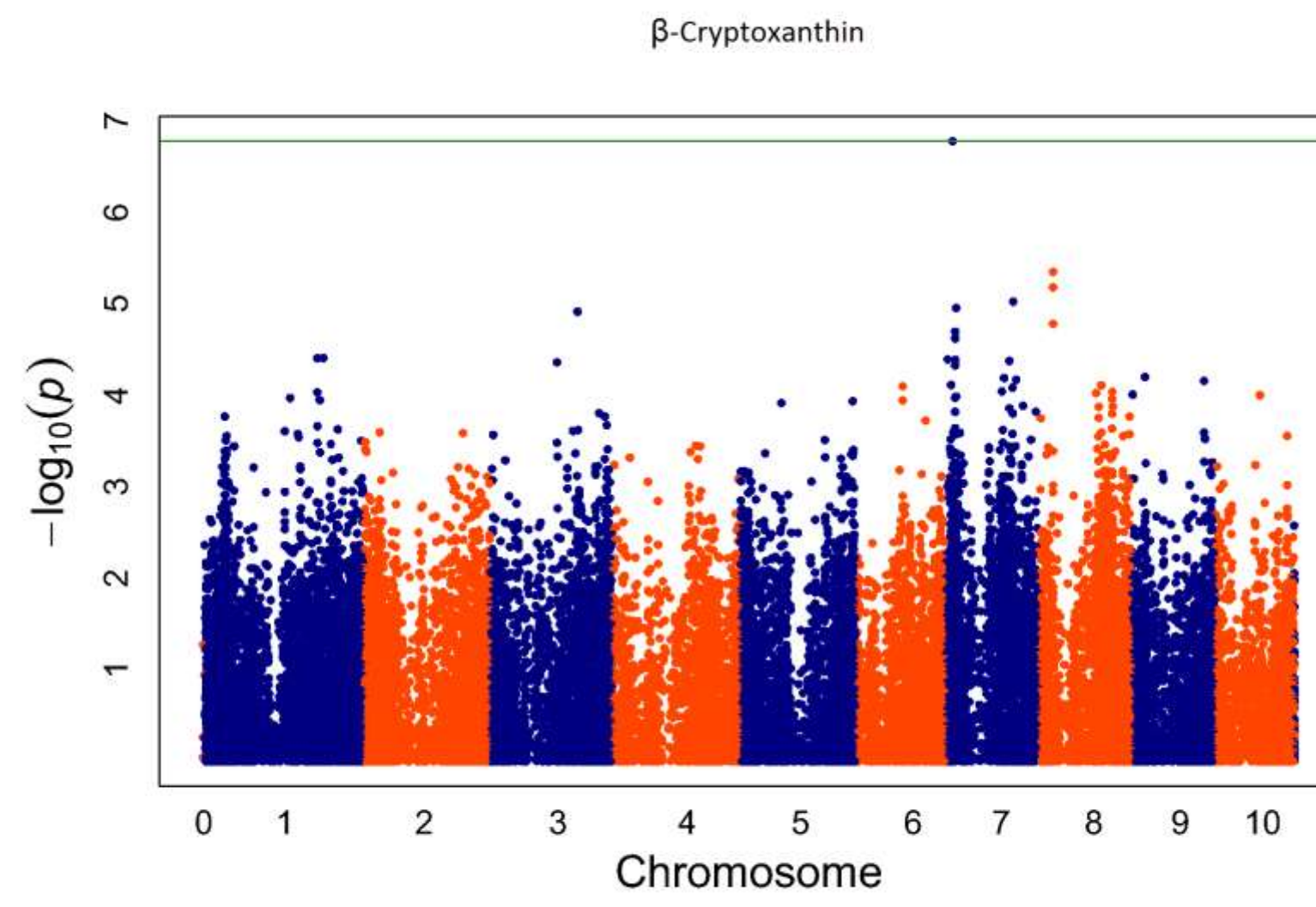
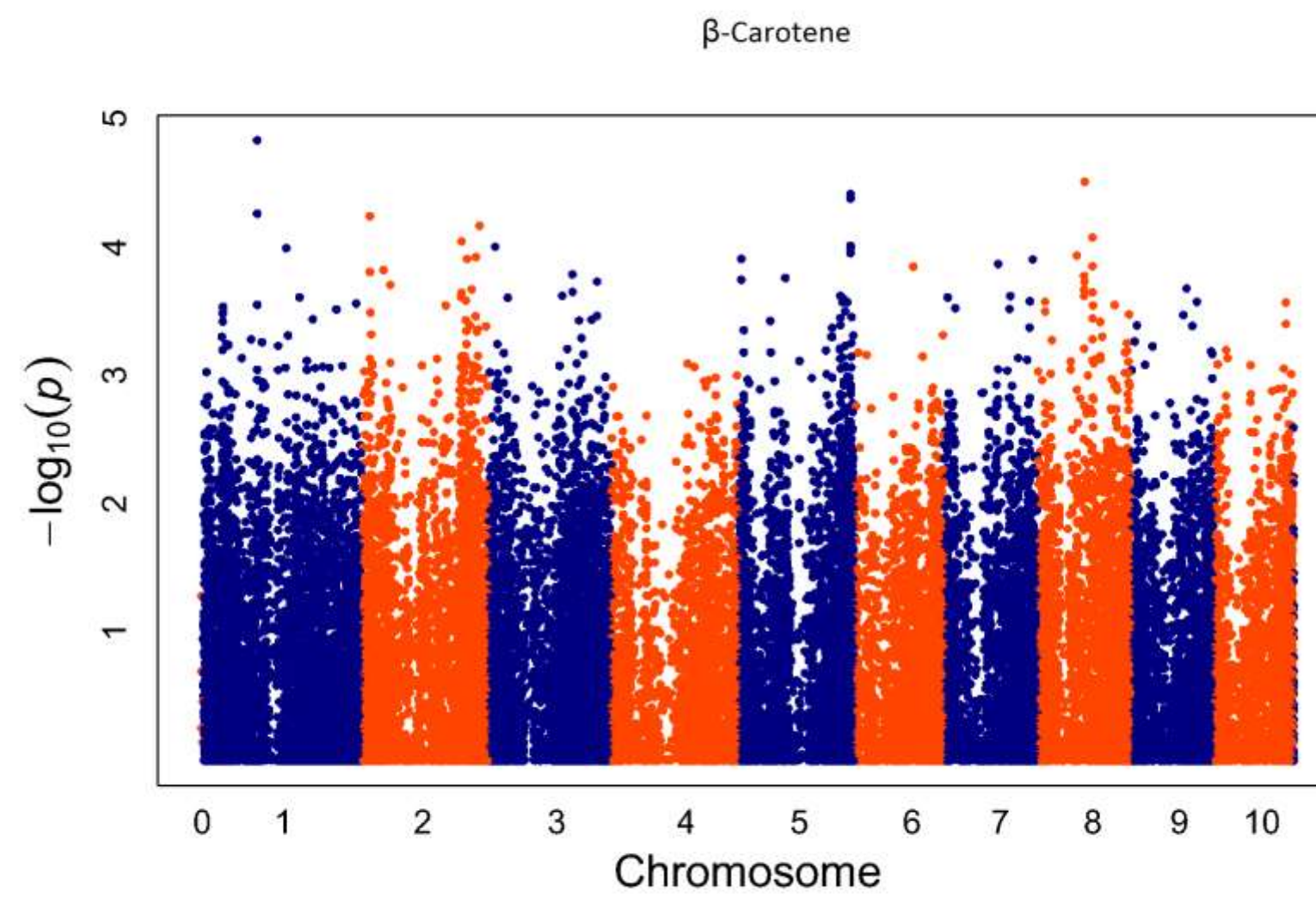
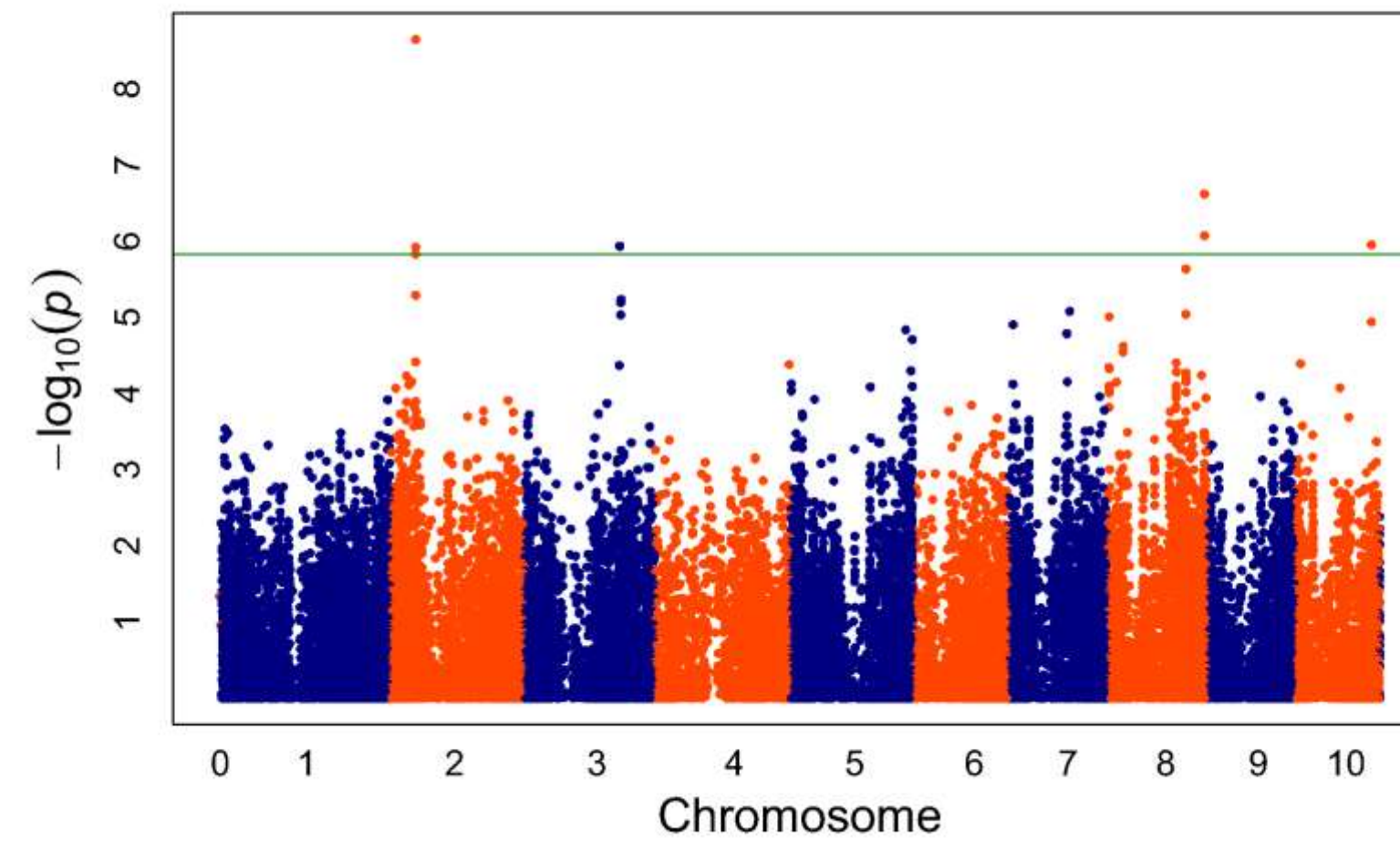


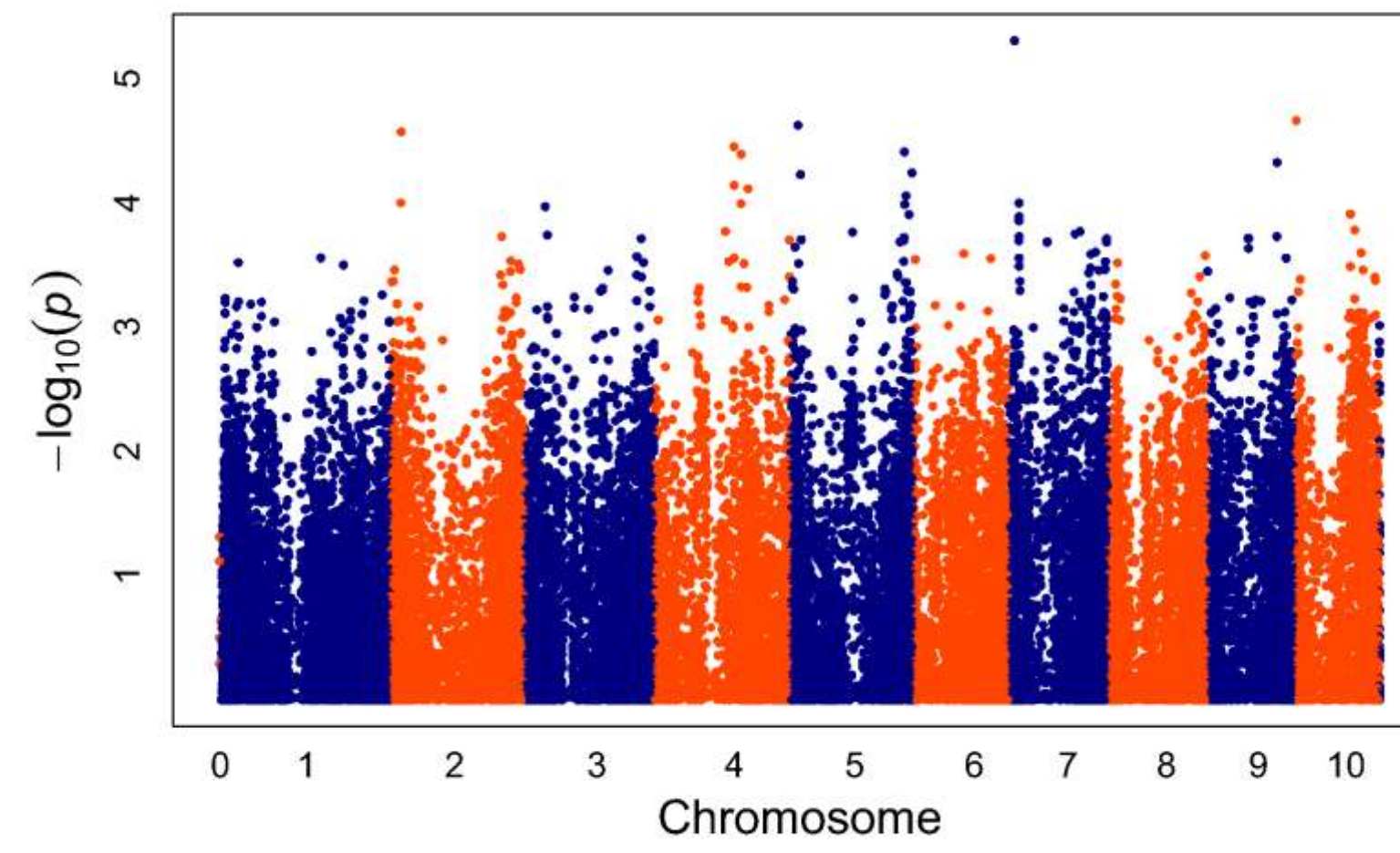
Figure S1 Genome-wide association study (GWAS) of a binary kernel color trait: white vs. non-white. The non-white class included maize inbred lines ranging from yellow to dark orange in kernel color. Scatter plot of association results from a unified mixed model analysis of the kernel color trait. Negative \log_{10} -transformed P -values (y-axis) from GWAS are plotted against physical position (B73 RefGen_v2) on each of 10 chromosomes (x-axis). Chromosomes are alternately colored. The horizontal green line indicates the $-\log_{10} P$ -value of the least statistically significant SNP at 5% false discovery rate.



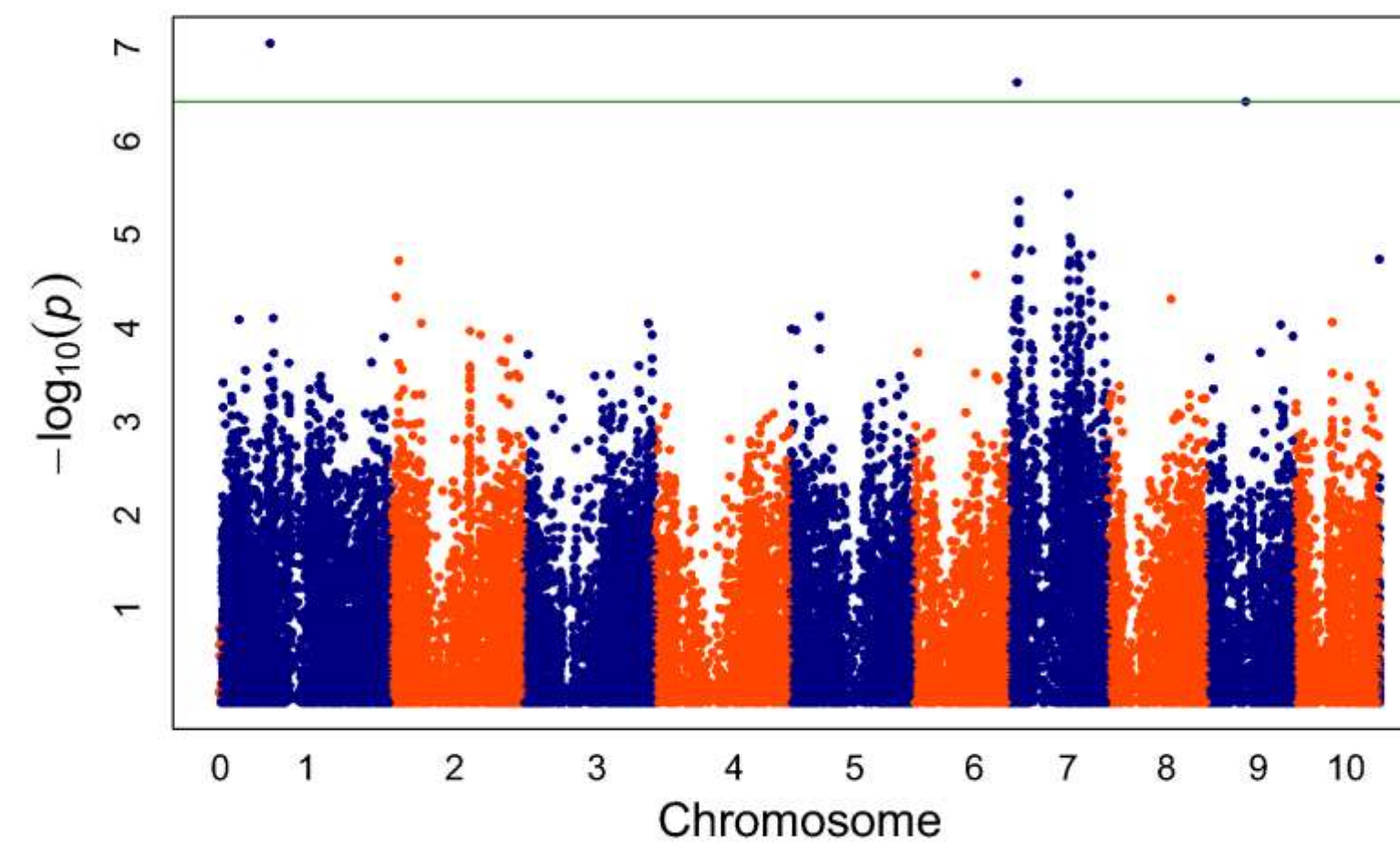
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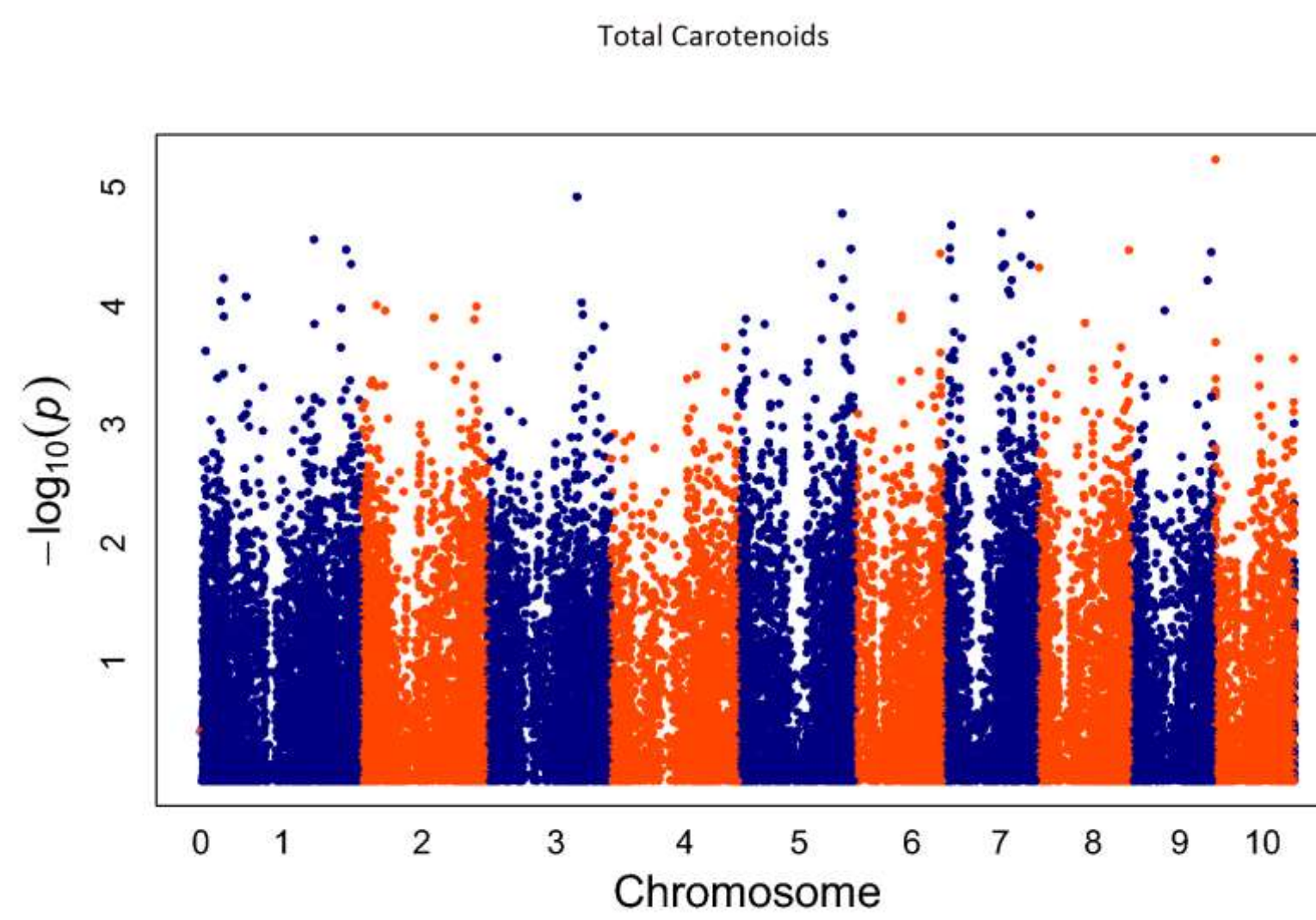
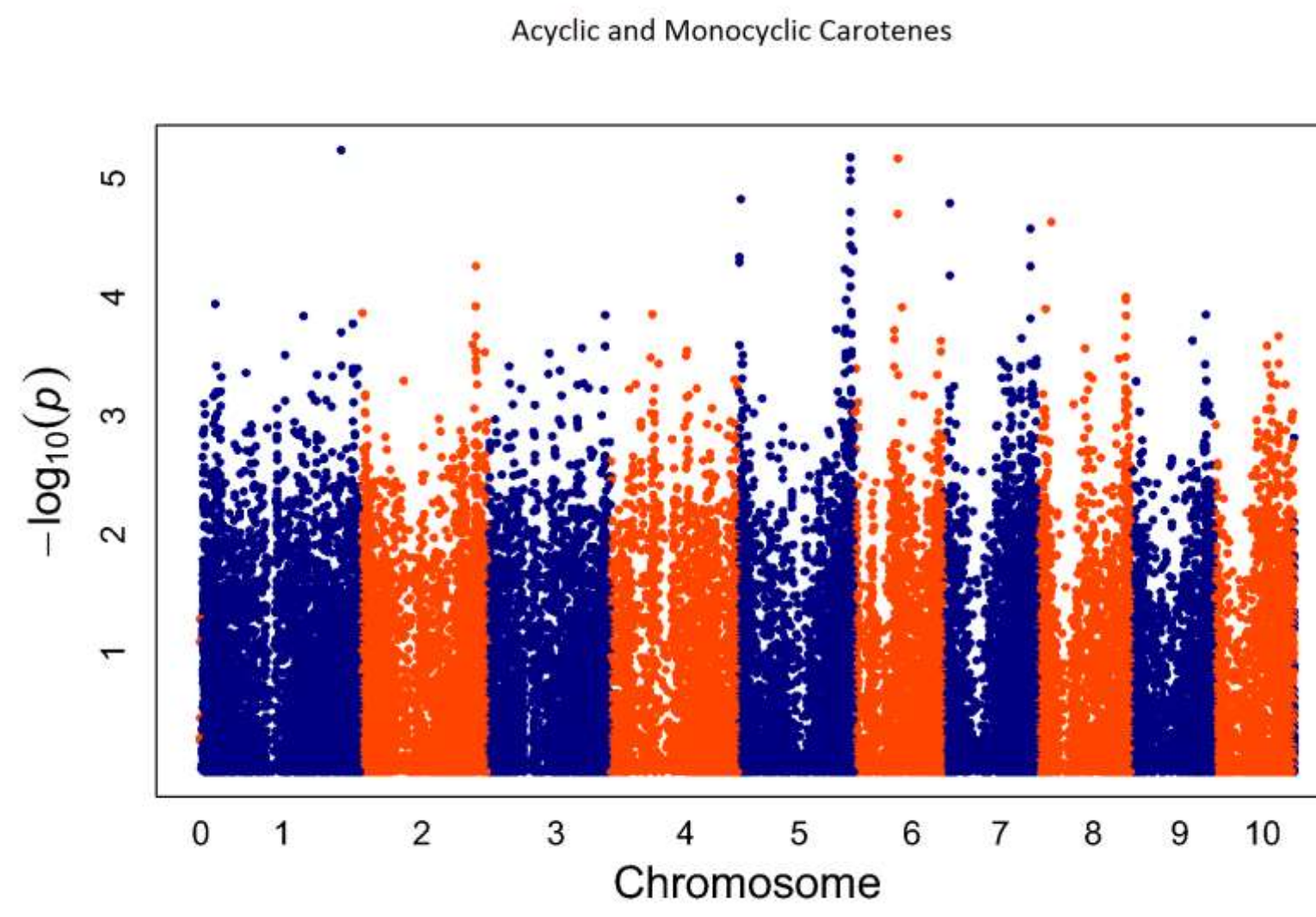
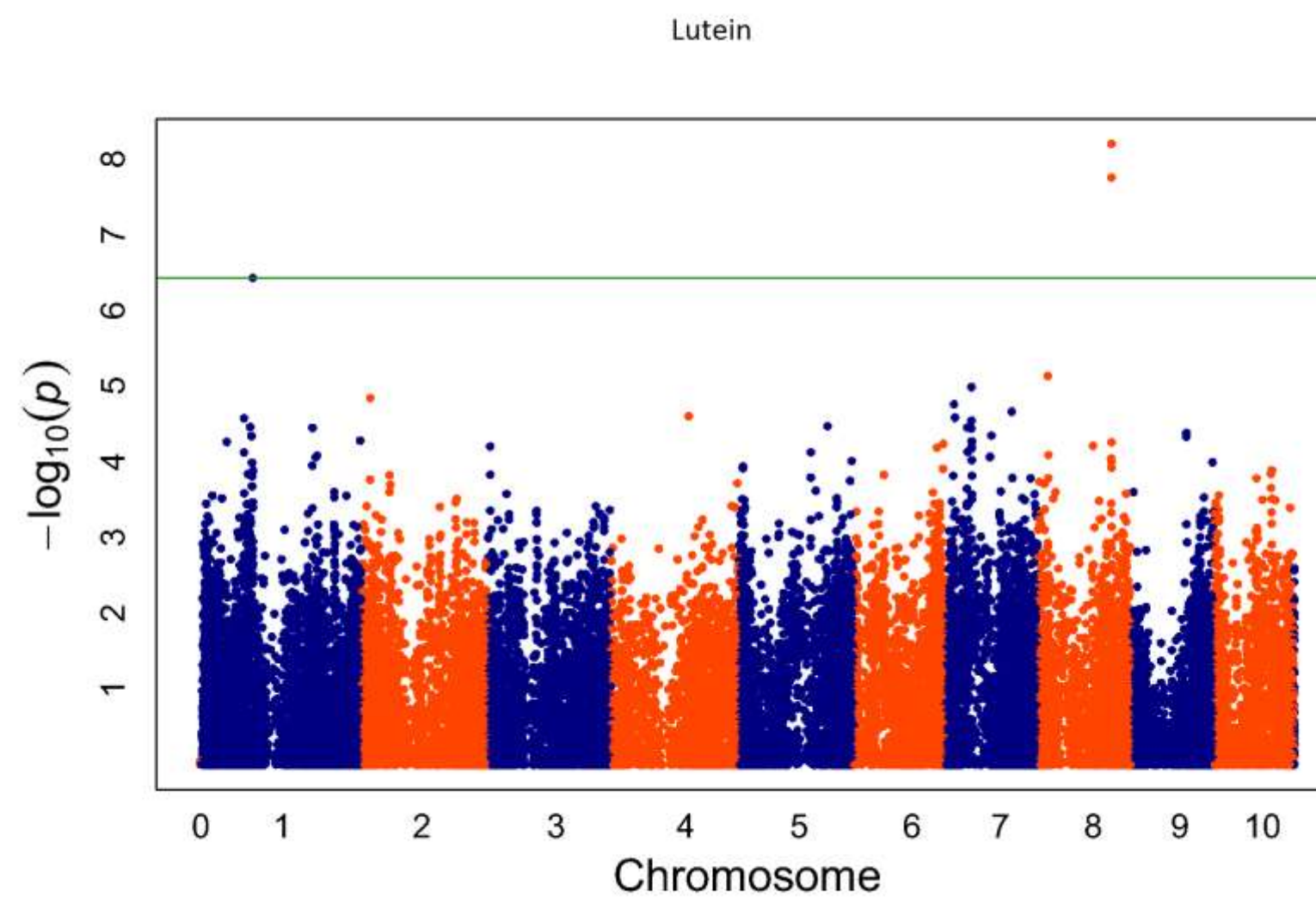


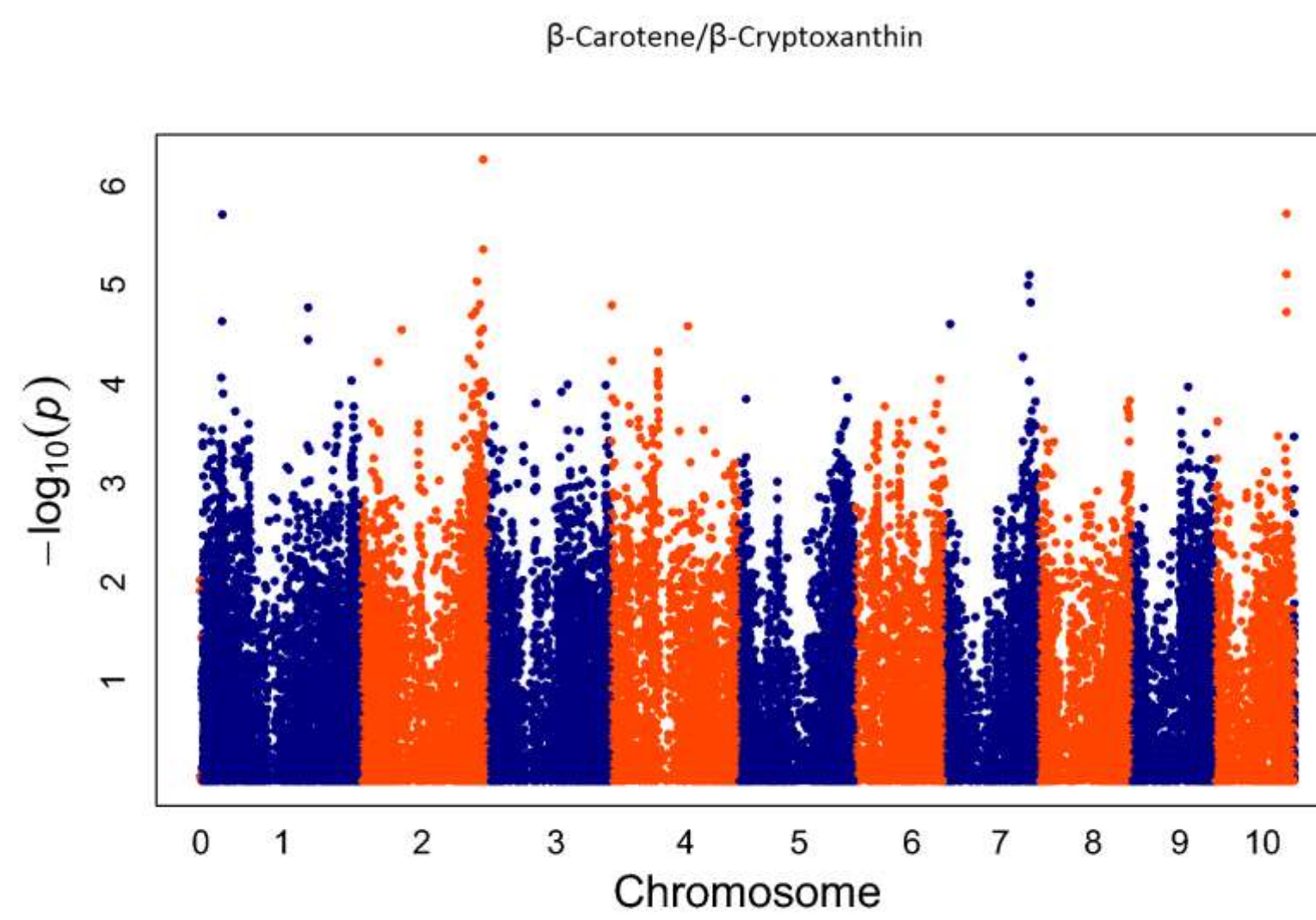
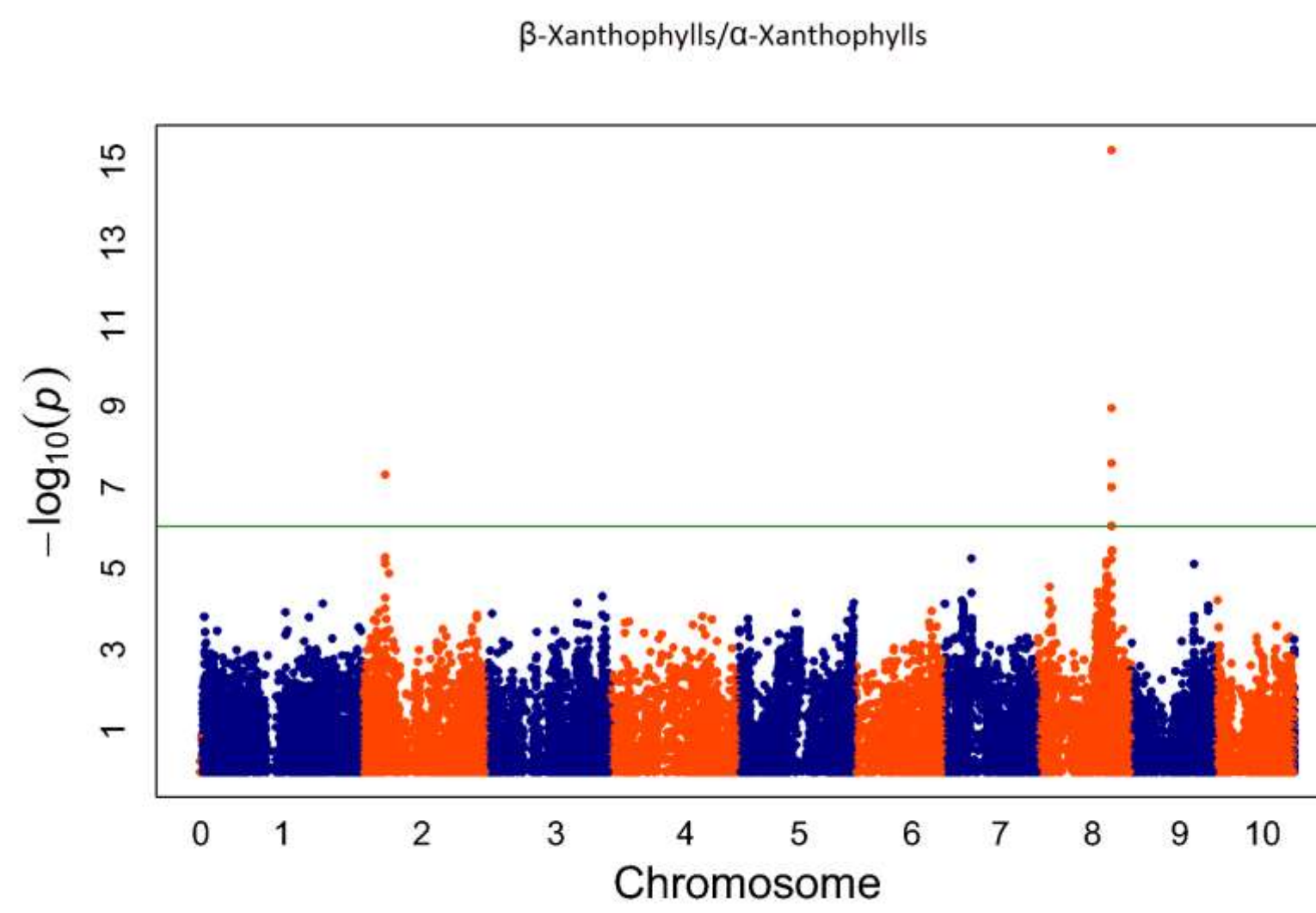
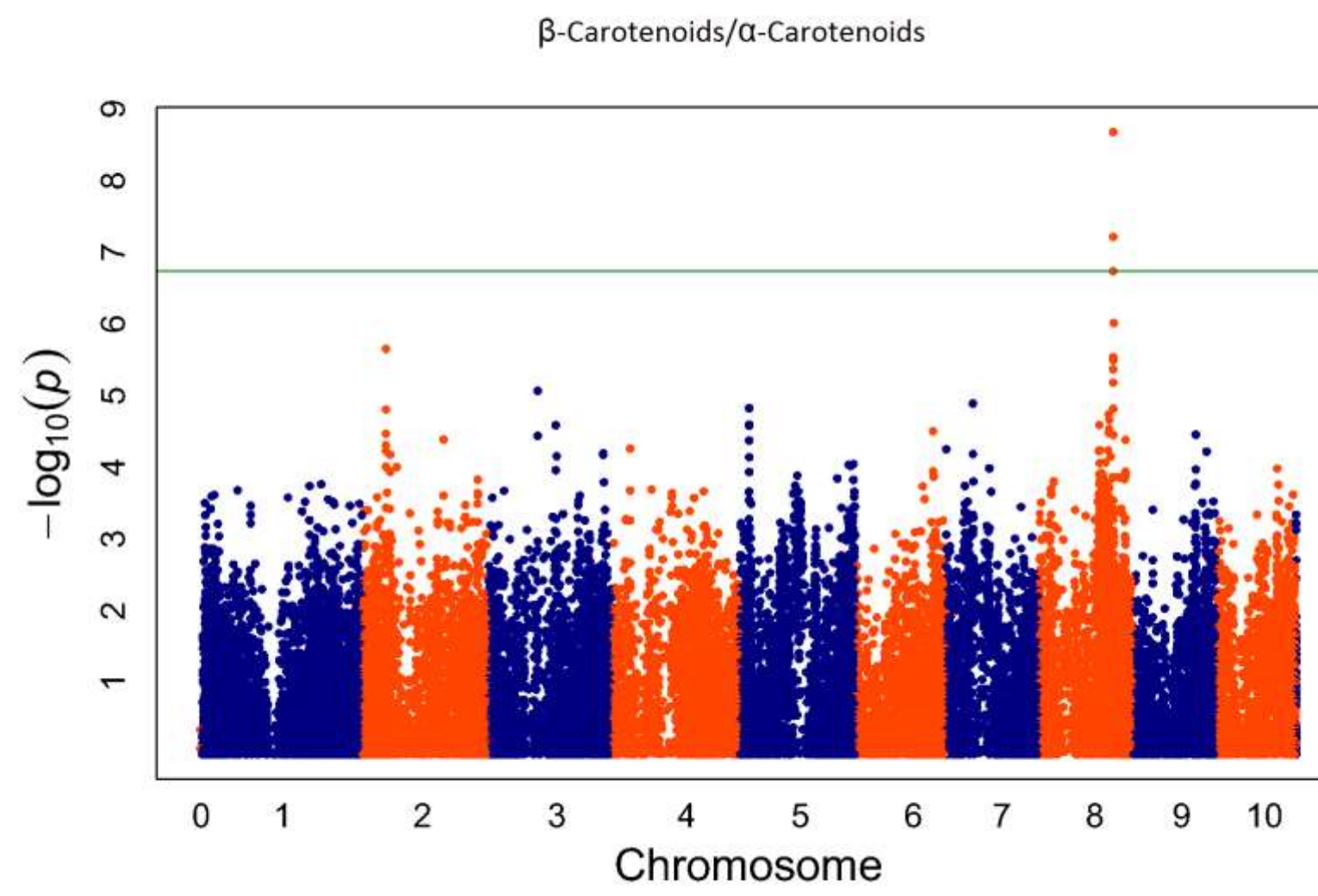
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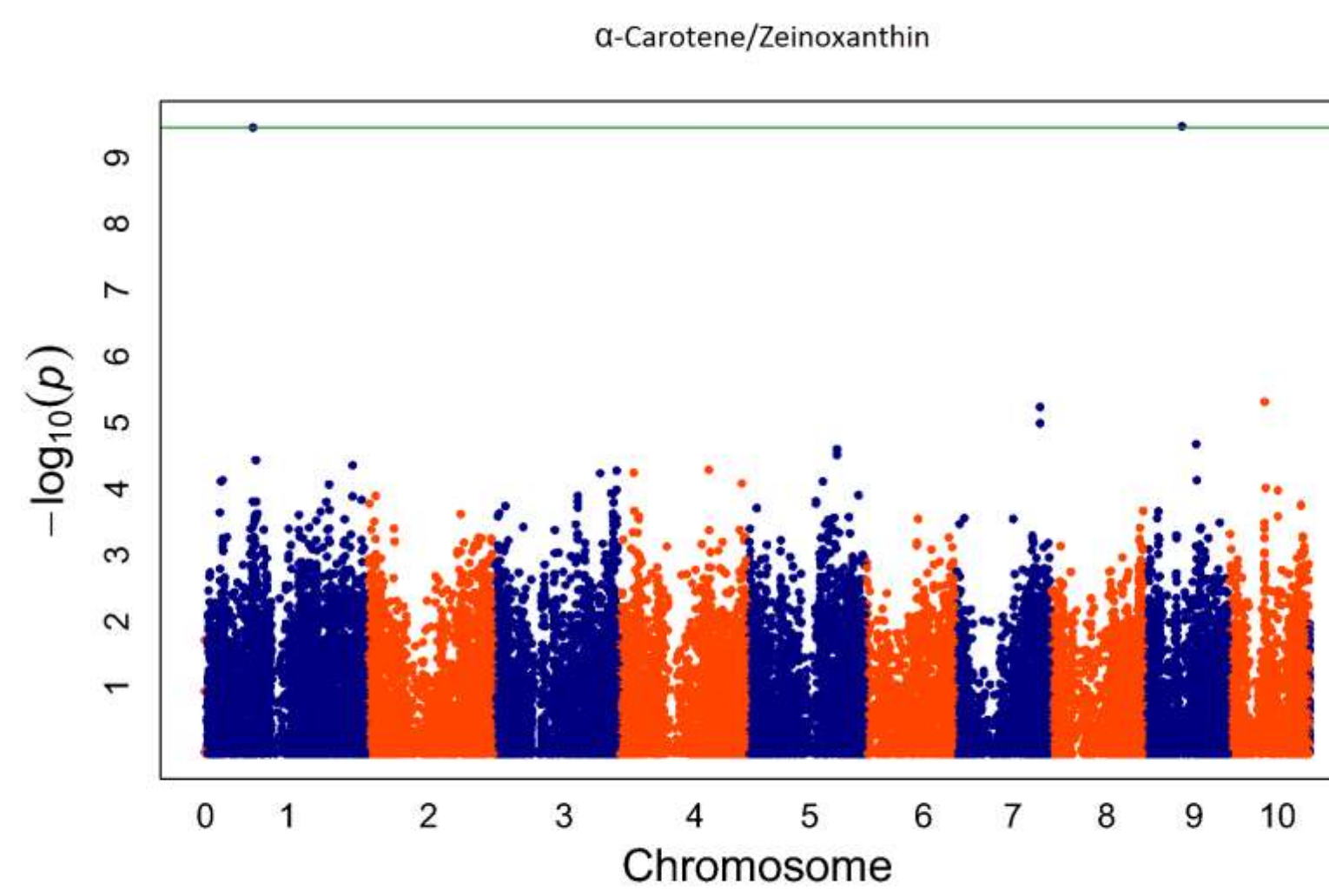
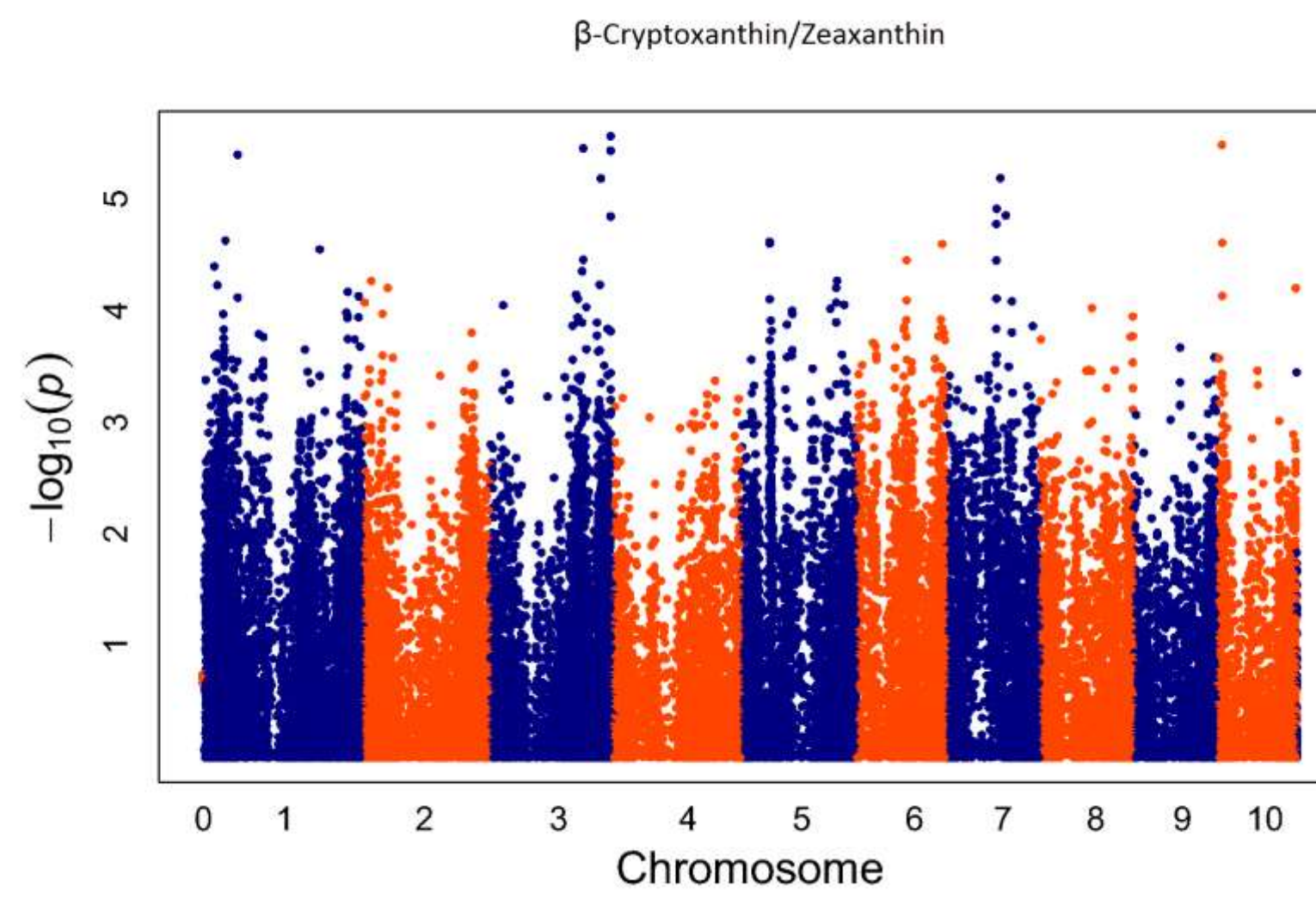


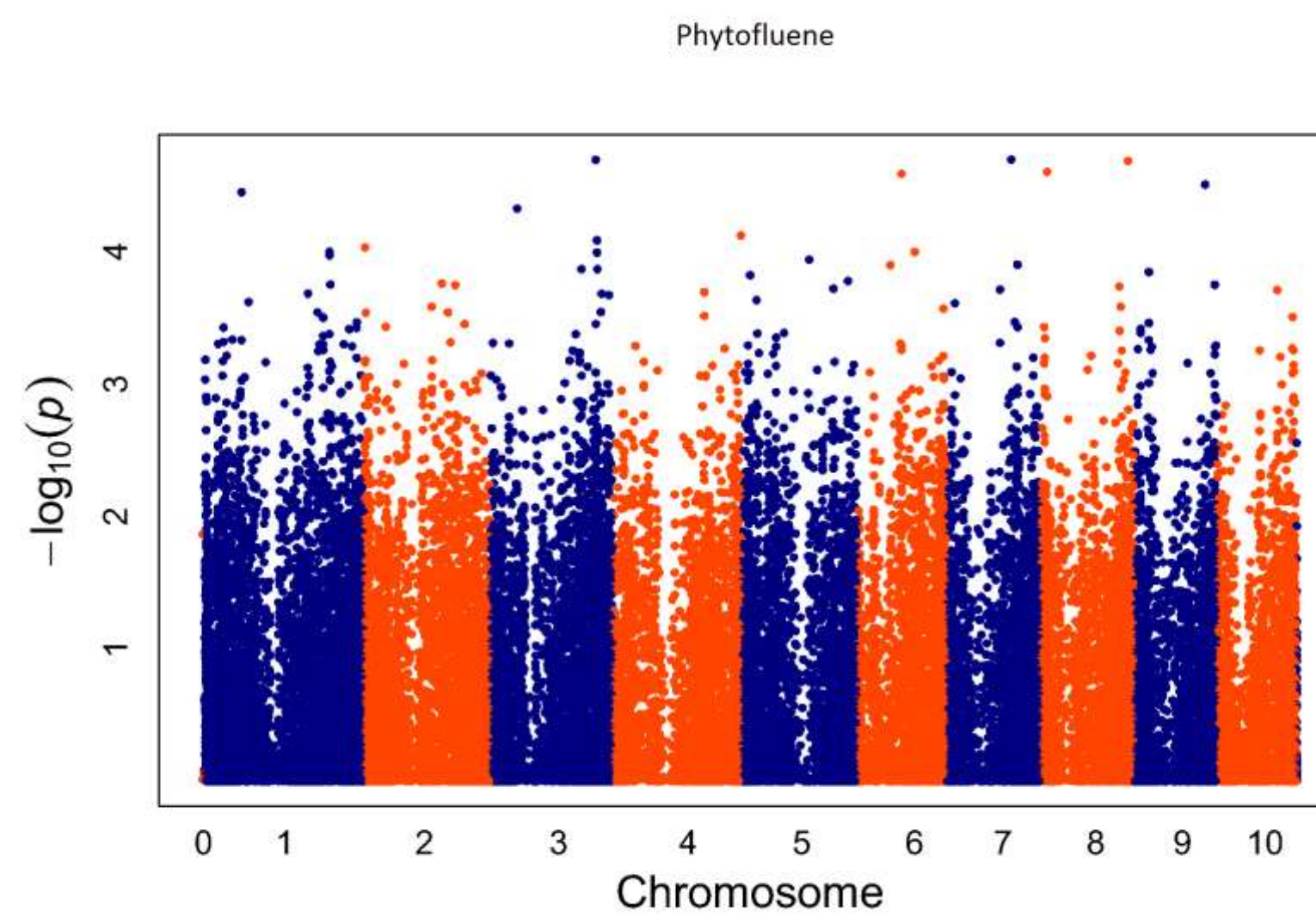
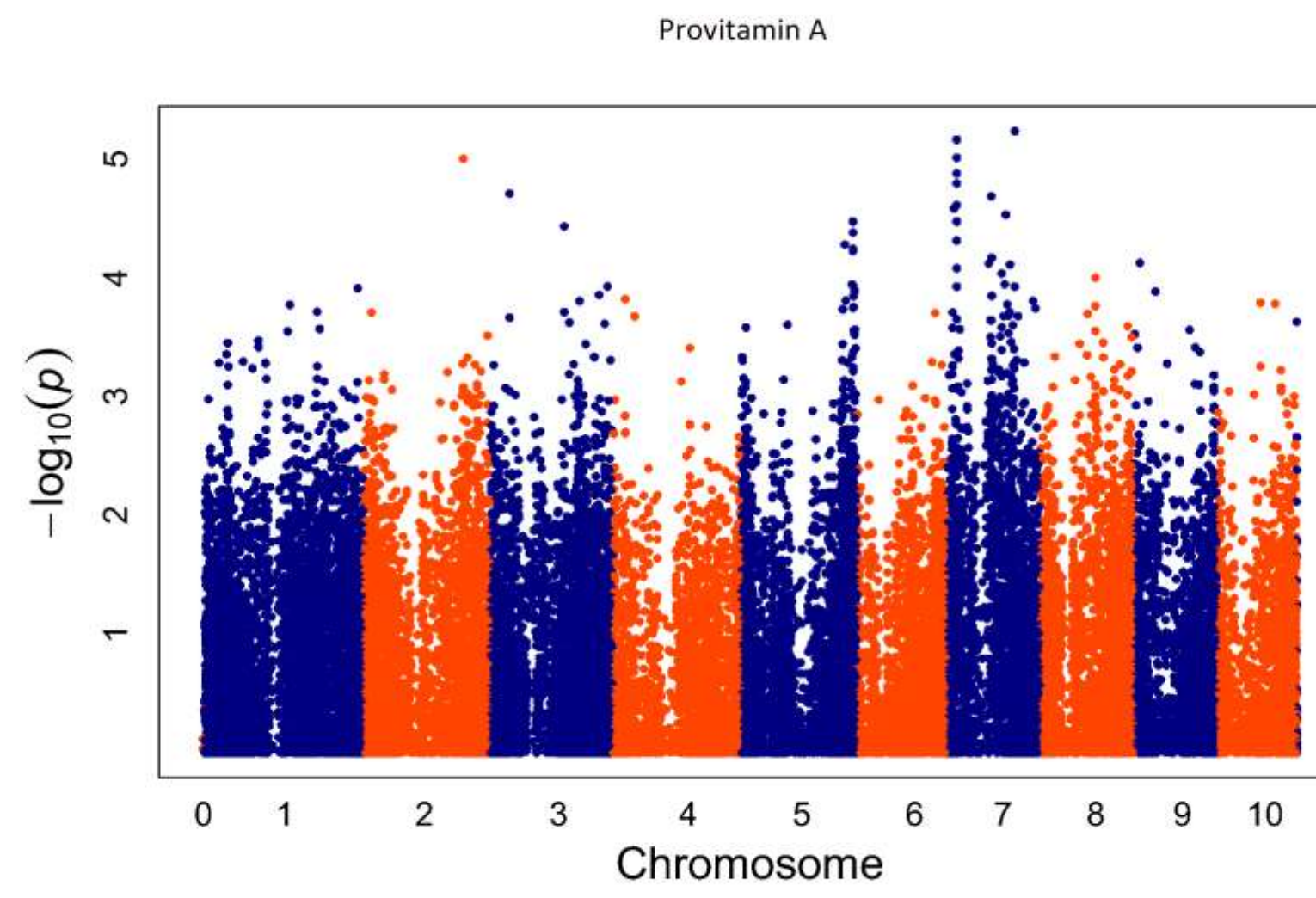
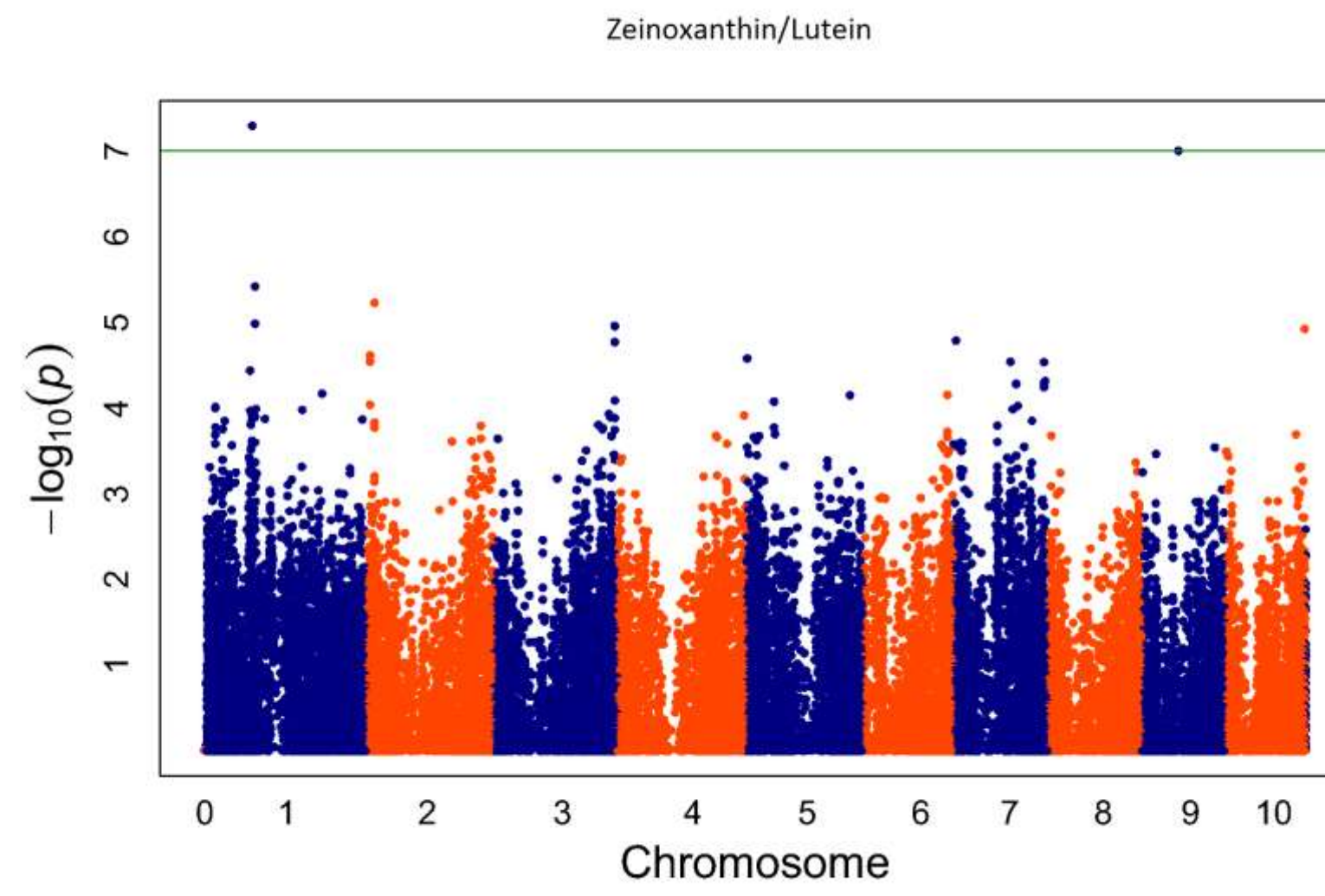
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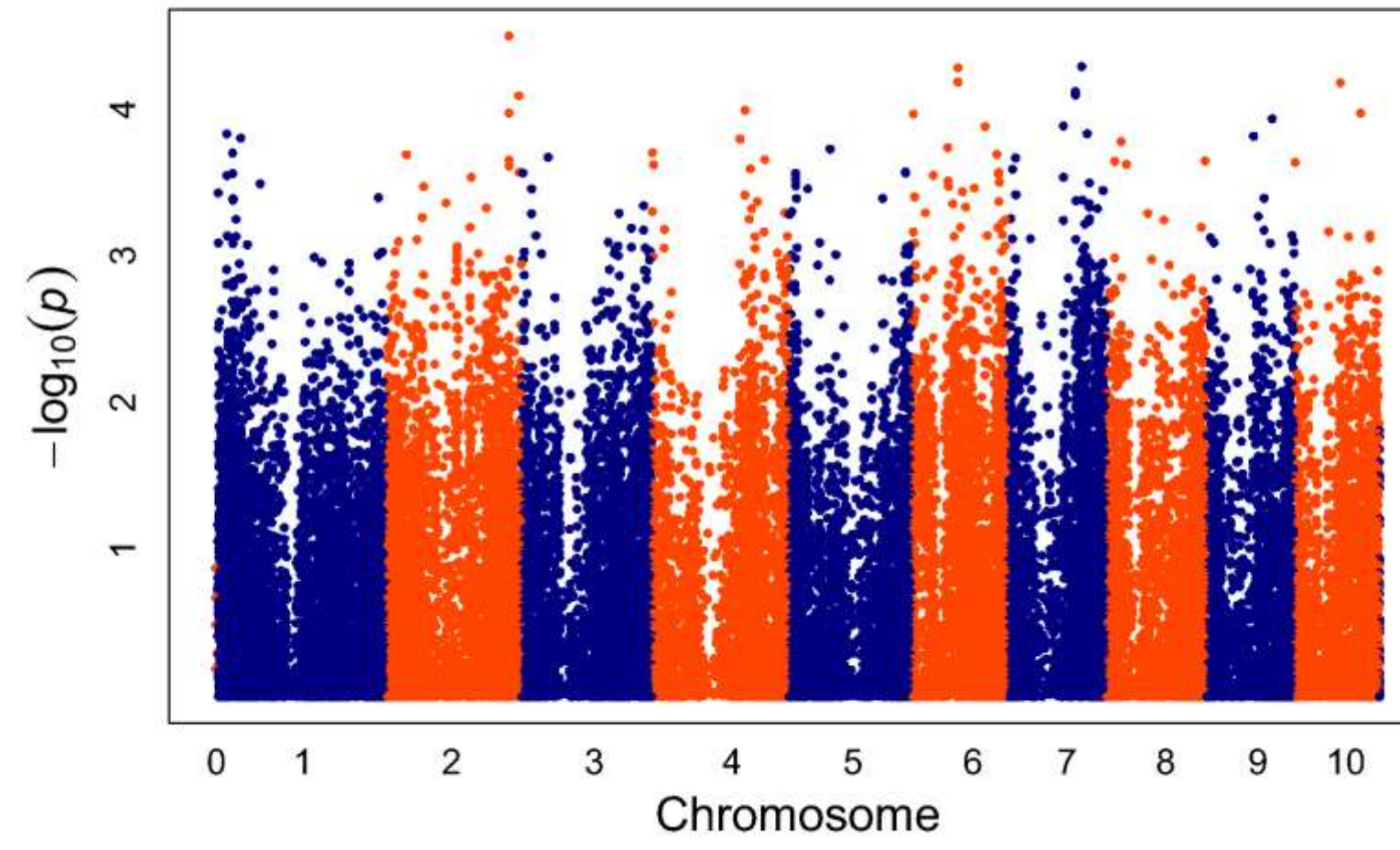




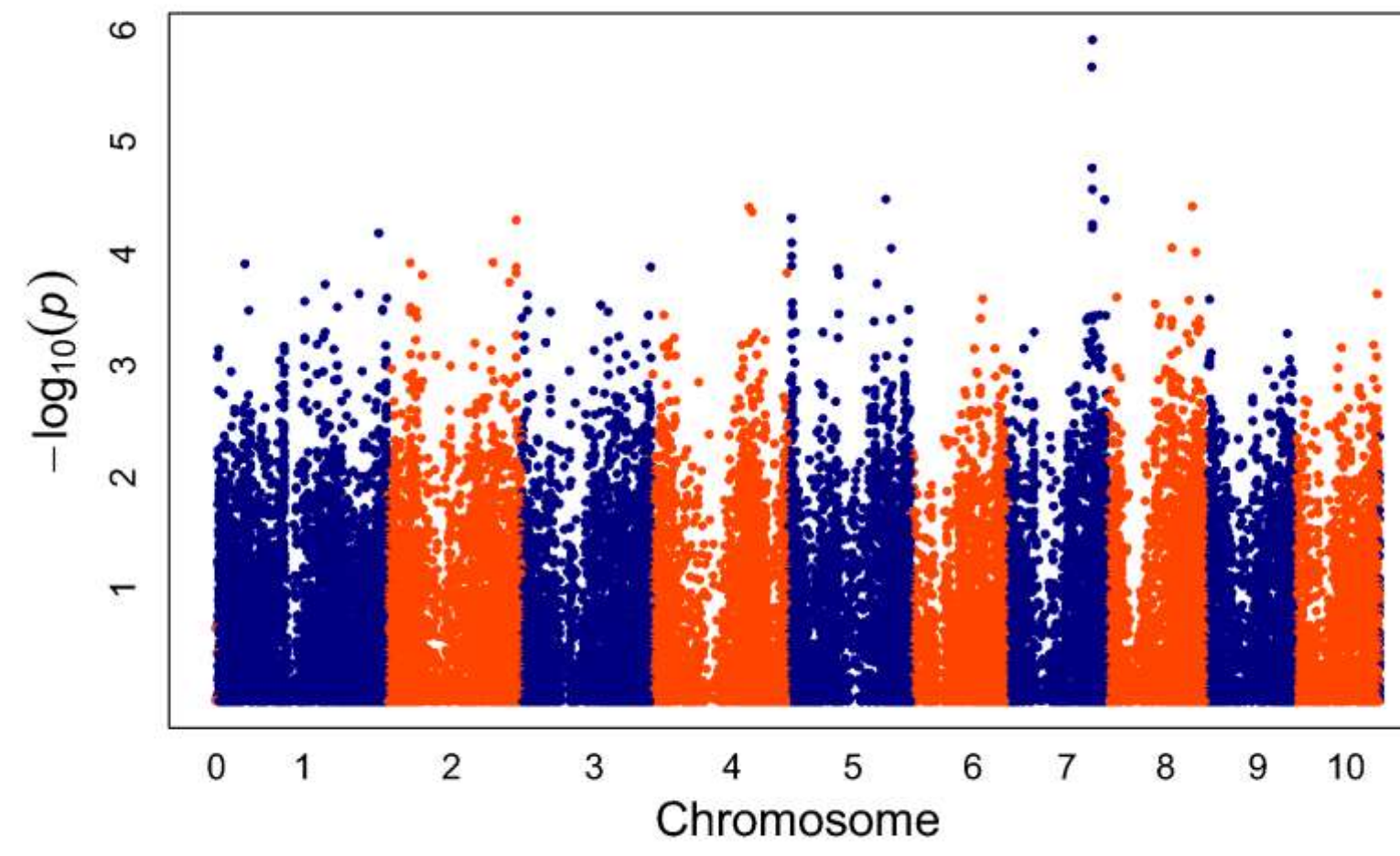




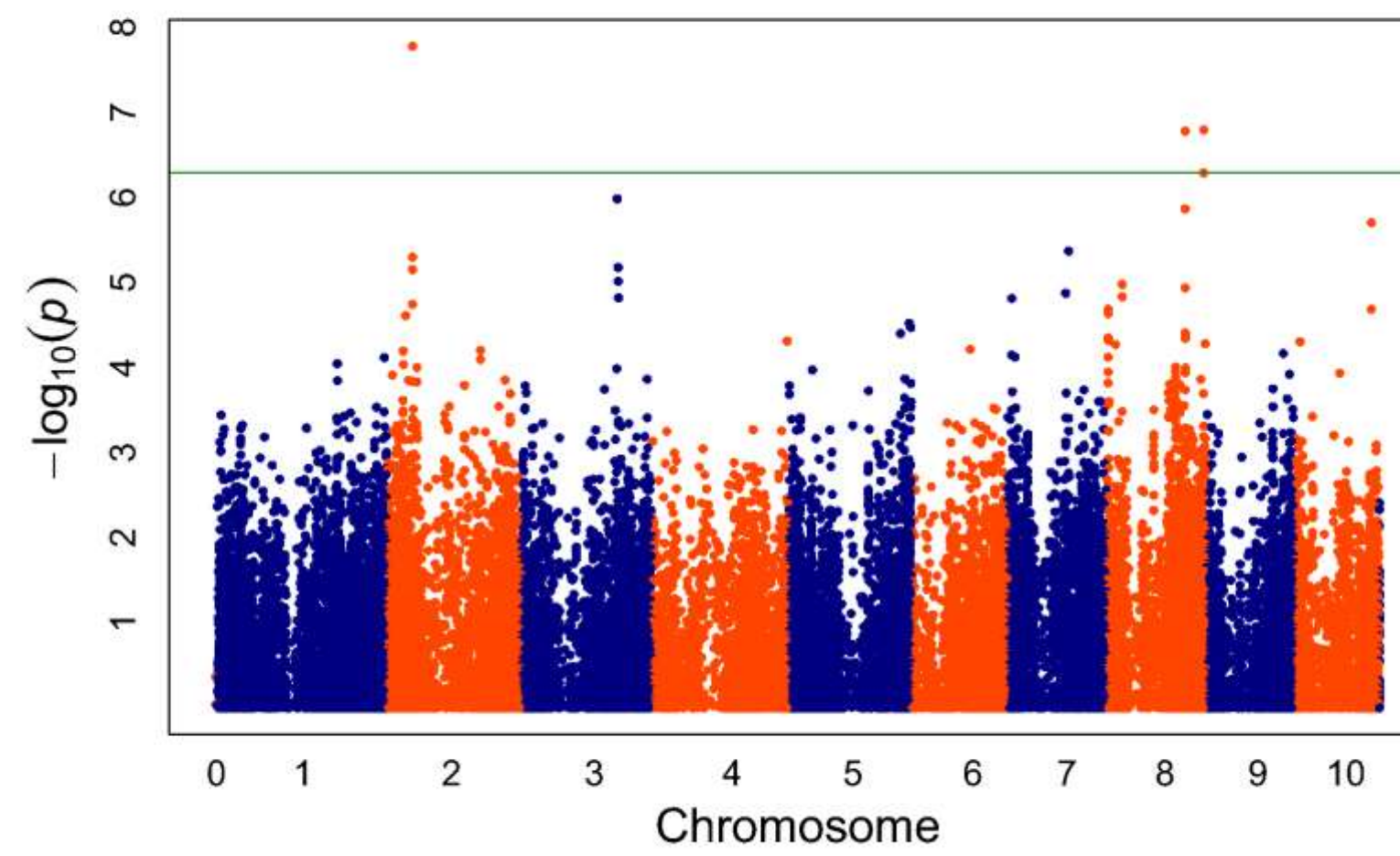
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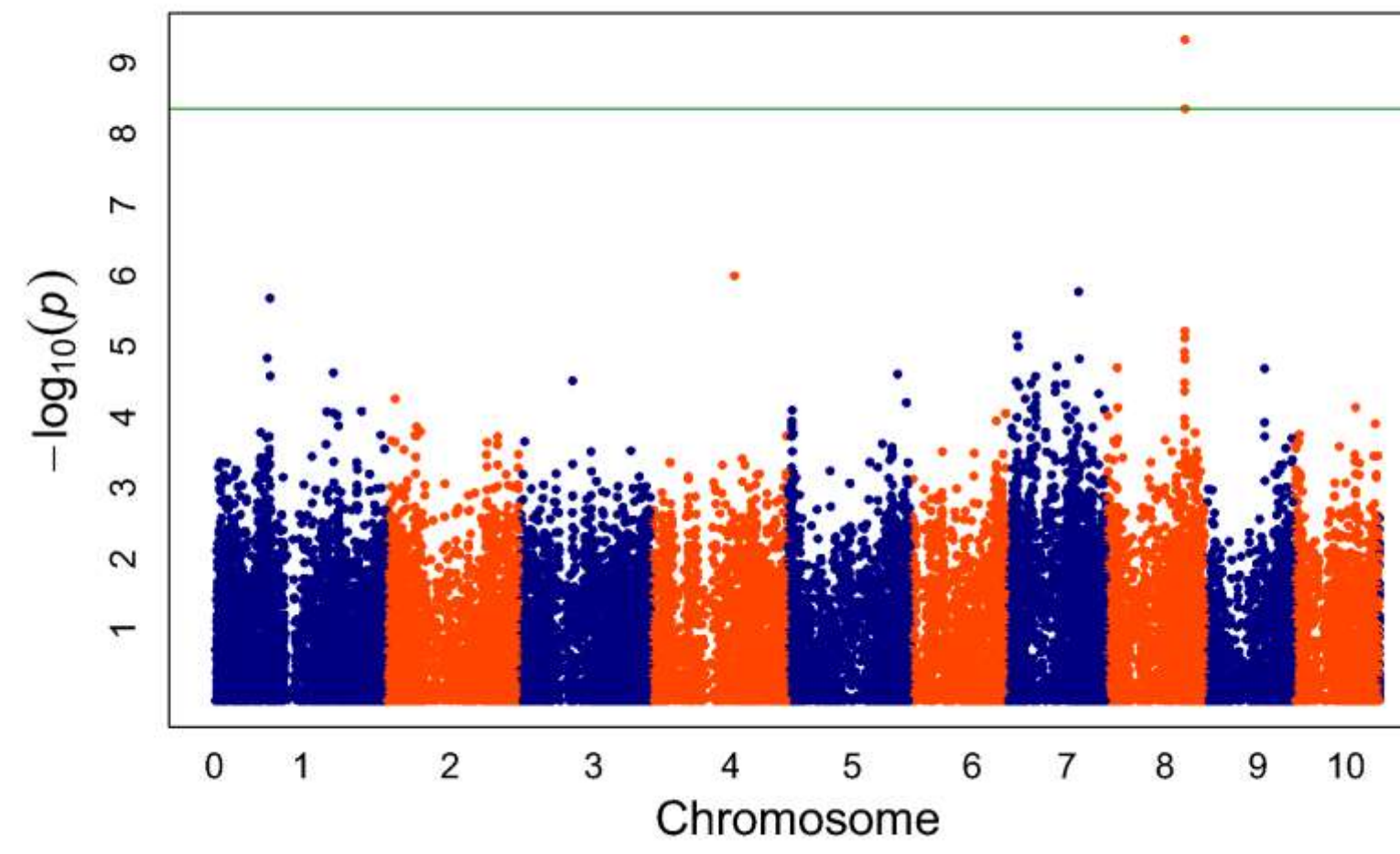
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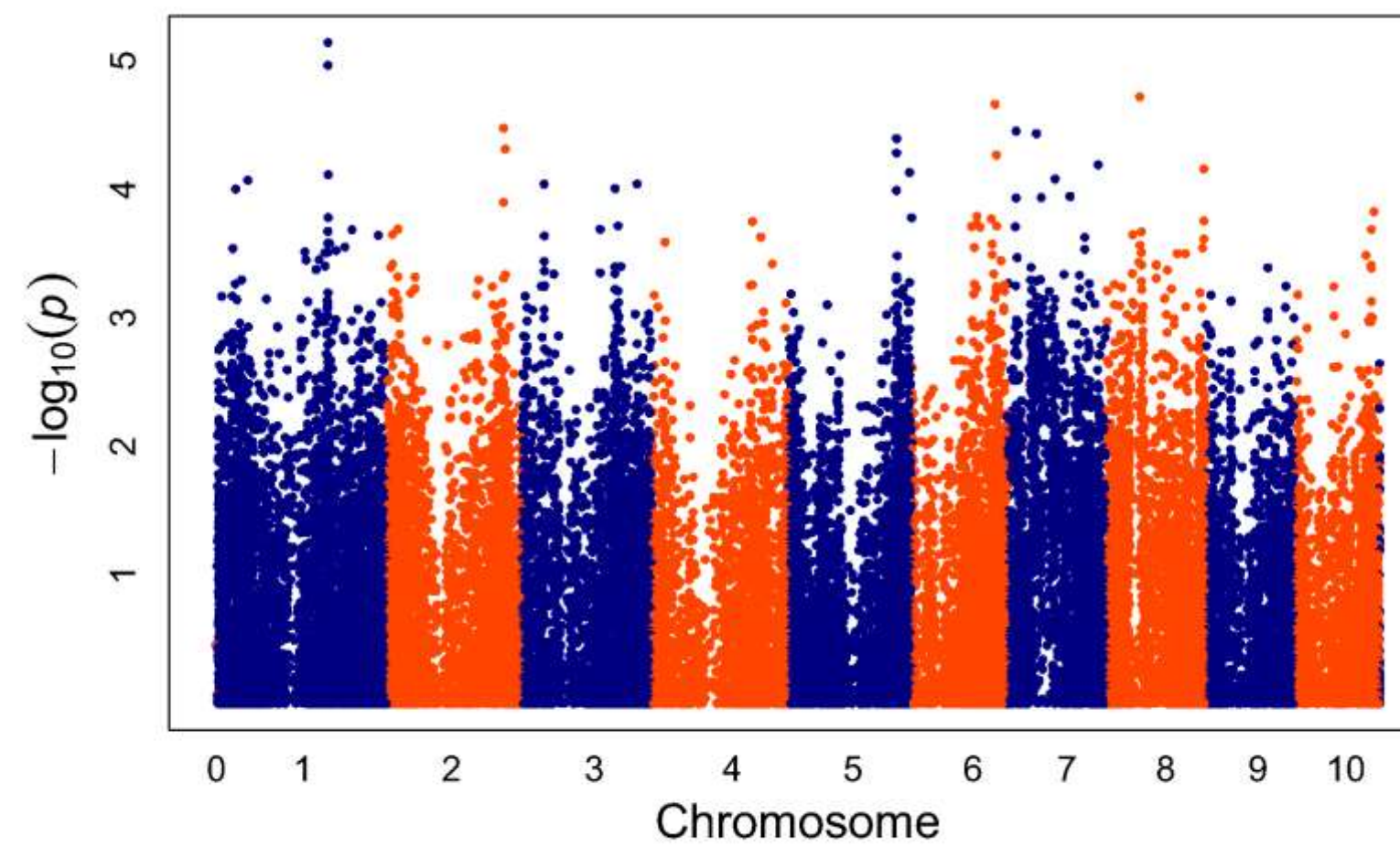
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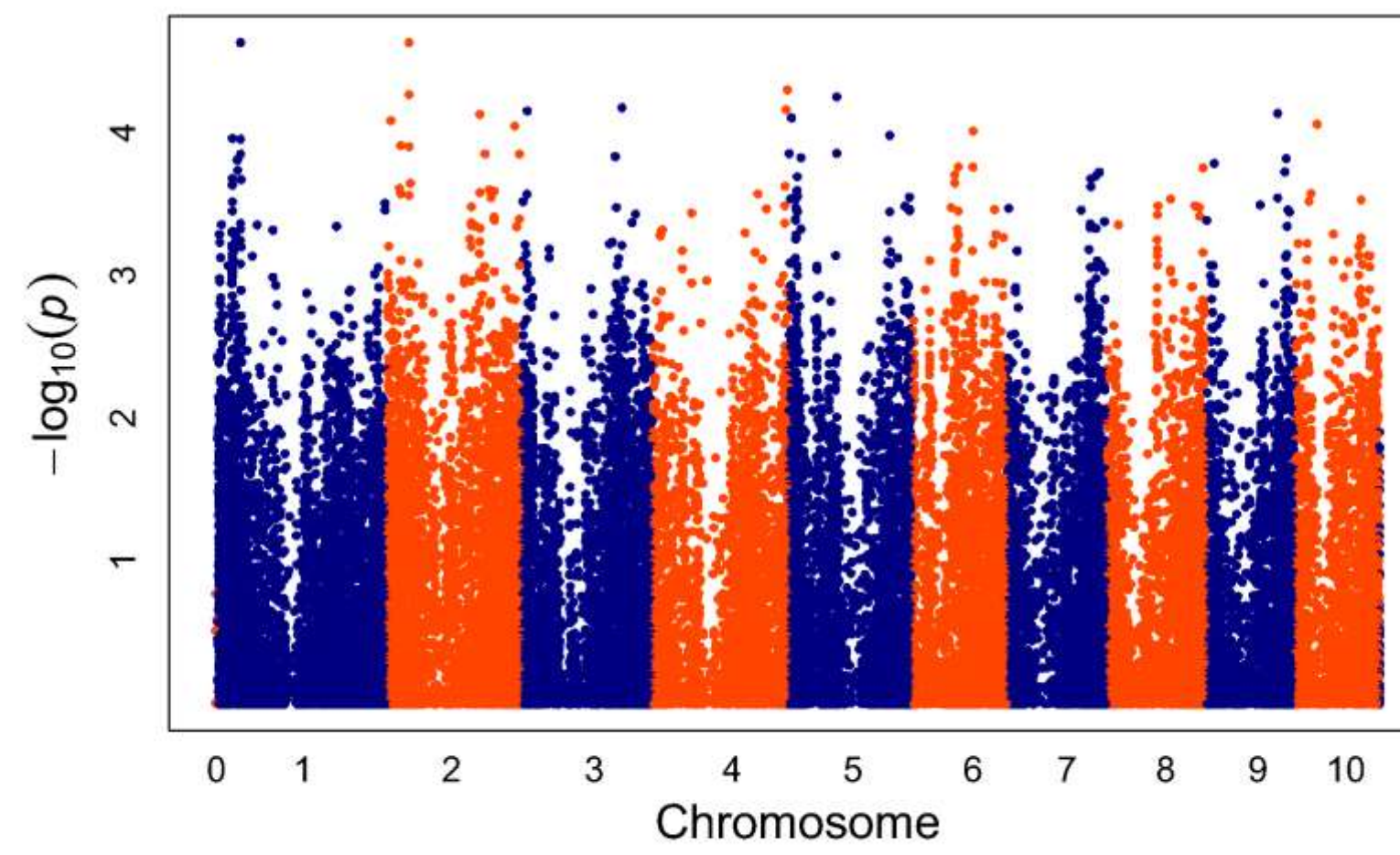
Total α -Xanthophylls



Provitamin A/Total Carotenoids



Acyclic Carotenenes/Cyclic Carotenenes



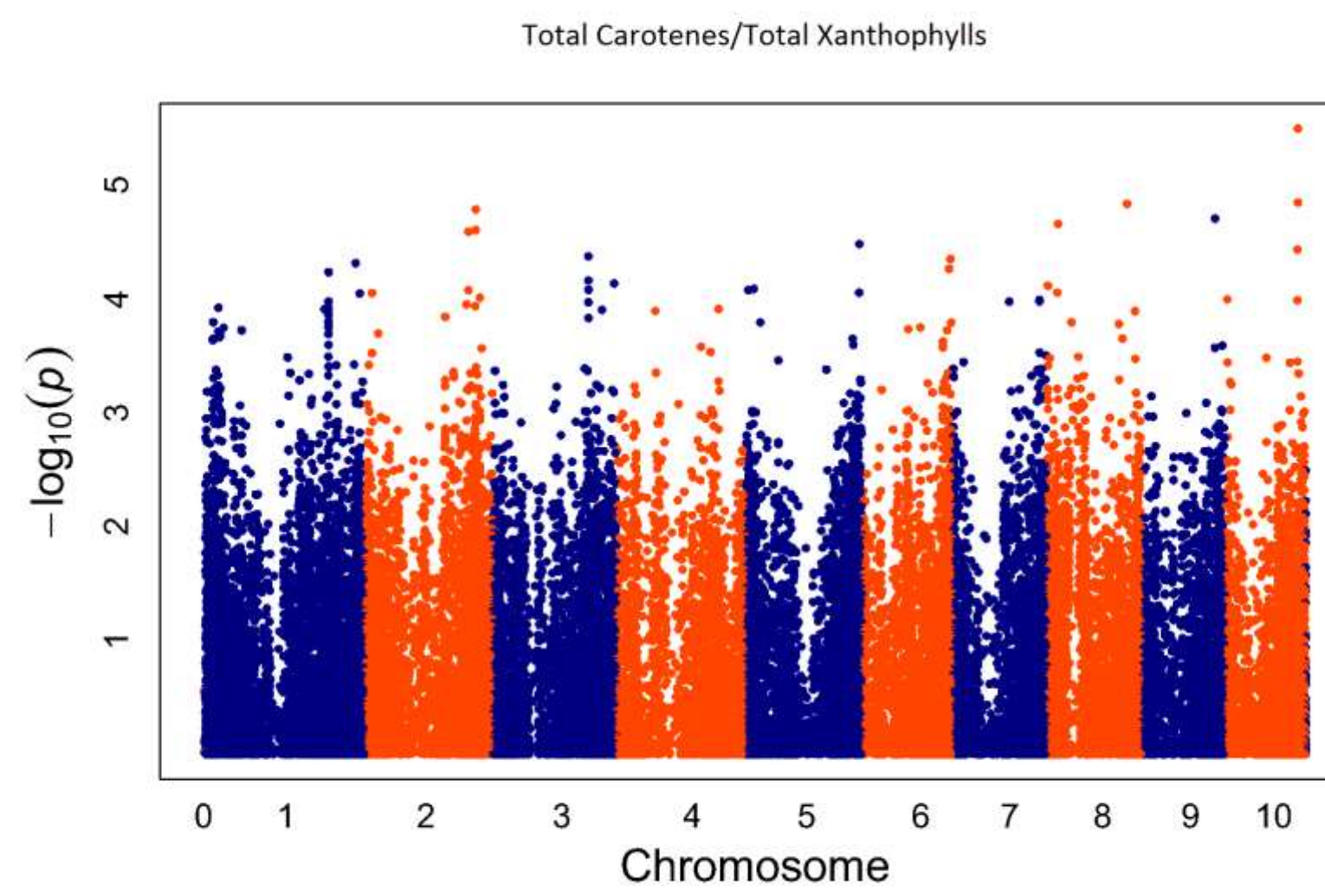
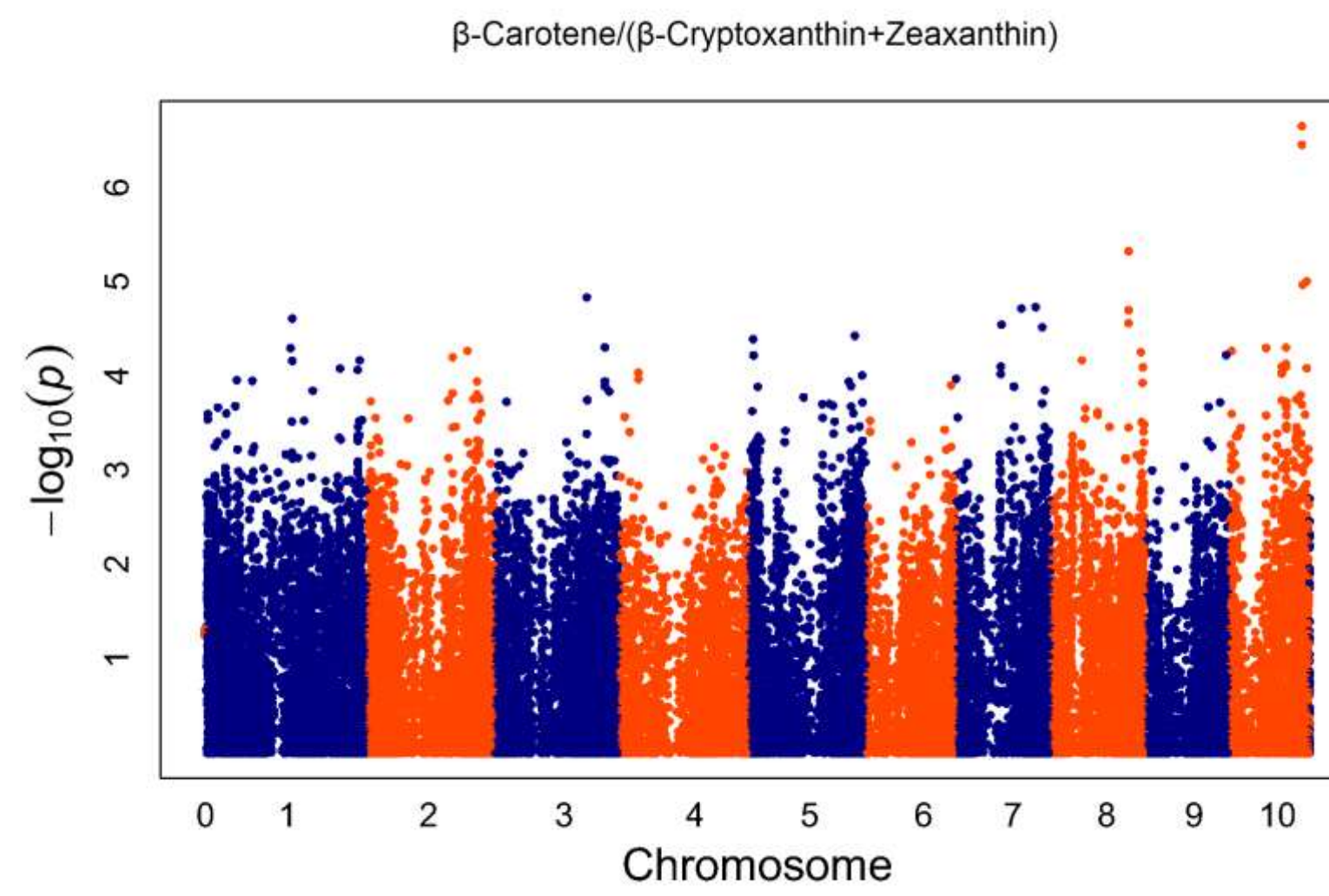


Figure S2 Genome-wide association study (GWAS) of 24 carotenoid grain traits. Scatter plots of association results from a unified mixed model analysis of each carotenoid grain trait. Negative \log_{10} -transformed P -values (y-axis) from GWAS are plotted against physical position (B73 RefGen_v2) on each of 10 chromosomes (x-axis). Chromosomes are alternatingly colored. The horizontal green line indicates the $-\log_{10} P$ -value of the least statistically significant SNP at 5% false discovery rate (FDR). Additional information for all statistically significant markers at 5% and 10% FDR are provided in Table S8 (A).

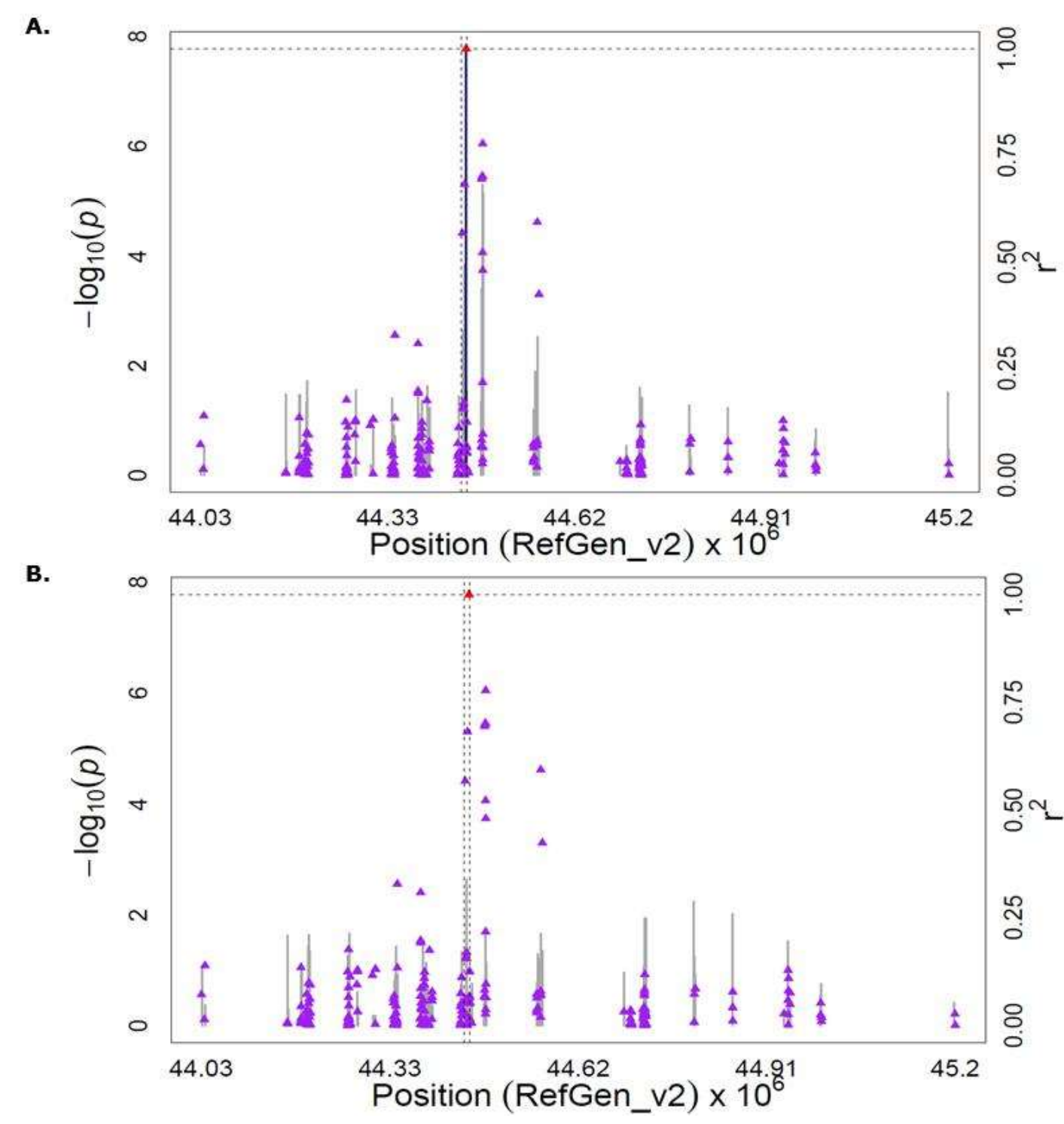


Figure S3 Genome-wide association study (GWAS) for total β -xanthophylls content in maize grain. (A) Scatter plot of association results from a unified mixed model analysis of total β -xanthophylls and linkage disequilibrium (LD) estimates (r^2) across the *zep1* chromosome region. Negative \log_{10} -transformed P -values (left y-axis) from a GWAS for total β -xanthophylls and r^2 values (right y-axis) are plotted against physical position (B73 RefGen_v2) for a 1.2 Mb region on chromosome 2 that encompasses *zep1*. The blue vertical lines are $-\log_{10} P$ -values for SNPs that are statistically significant for total β -xanthophylls at 5% false discovery rate (FDR), while the gray vertical lines are $-\log_{10} P$ -values for SNPs that are non-significant at 5% FDR. Triangles are the r^2 values of each SNP relative to the peak SNP (indicated in red) at 44,448,432 bp. The black horizontal dashed line indicates the $-\log_{10} P$ -value of the least statistically significant SNP at 5% FDR. The black vertical dashed lines indicate the start and stop positions of *zep1* (GRMZM2G127139). (B) Scatter plot of association results from a conditional unified mixed model analysis of total β -xanthophylls and LD estimates (r^2) across the *zep1* chromosome region, as in (A). The peak SNP from the unconditional GWAS (S2_44448432; 44,448,432 bp) was included as a covariate in the unified mixed model to control for the *zep1* effect.

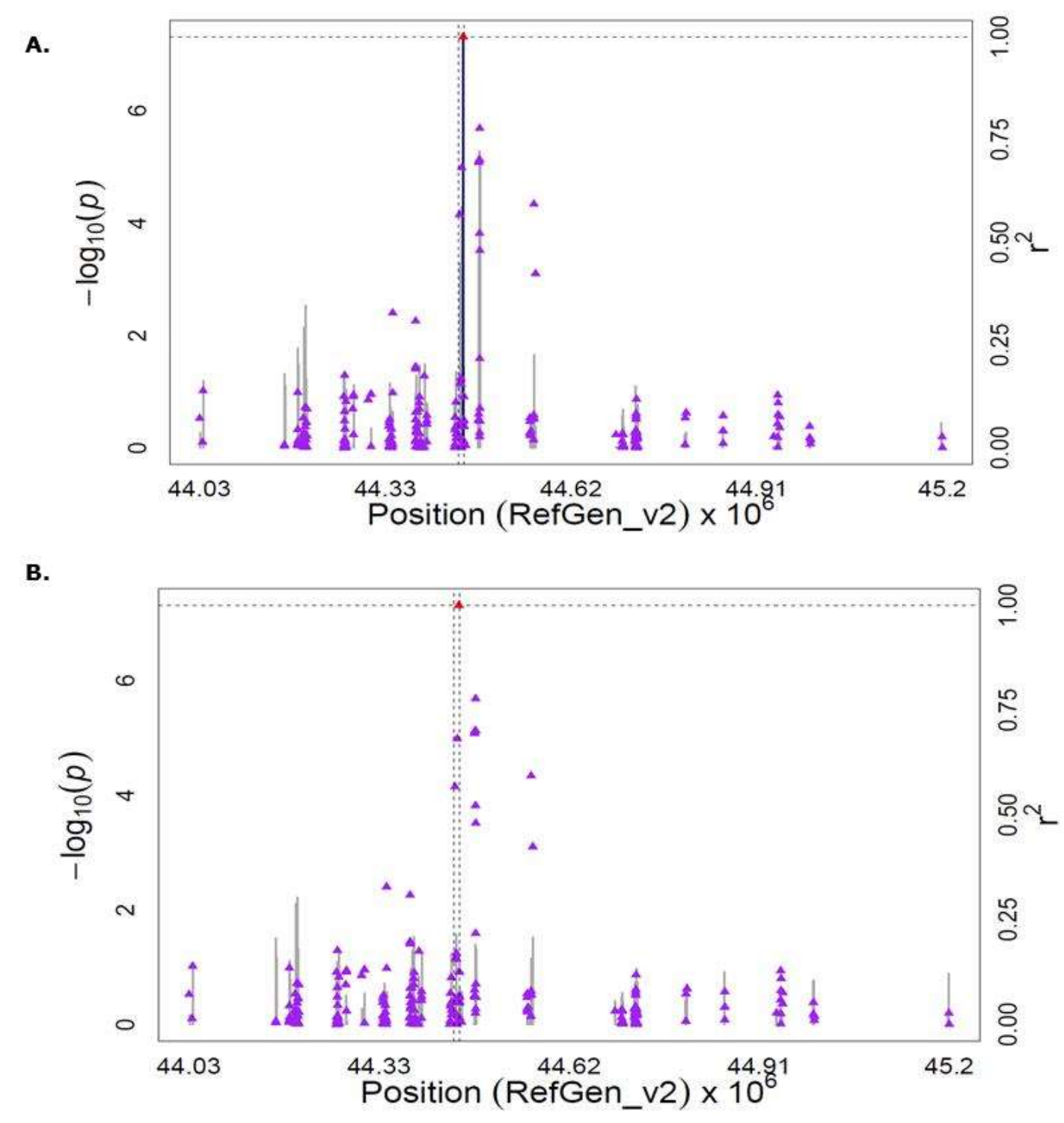


Figure S4 Genome-wide association study (GWAS) for the ratio of total β -xanthophylls to total α -xanthophylls content in maize grain. (A) Scatter plot of association results from a unified mixed model analysis of the ratio of total β -xanthophylls to total α -xanthophylls and linkage disequilibrium (LD) estimates (r^2) across the *zep1* chromosome region. Negative \log_{10} -transformed P -values (left y-axis) from a GWAS for the ratio of total β -xanthophylls to total α -xanthophylls and r^2 values (right y-axis) are plotted against physical position (B73 RefGen_v2) for a 1.2 Mb region on chromosome 2 that encompasses *zep1*. The blue vertical lines are $-\log_{10}$ P -values for SNPs that are statistically significant for the ratio of total β -xanthophylls to total α -xanthophylls at 5% false discovery rate (FDR), while the gray vertical lines are $-\log_{10}$ P -values for SNPs that are non-significant at 5% FDR. Triangles are the r^2 values of each SNP relative to the peak SNP (indicated in red) at 44,448,432 bp. The black horizontal dashed line indicates the $-\log_{10}$ P -value of the least statistically significant SNP at 5% FDR. The black vertical dashed lines indicate the start and stop positions of *zep1* (GRMZM2G127139). (B) Scatter plot of association results from a conditional unified mixed model analysis of the ratio of total β -xanthophylls to total α -xanthophylls and LD estimates (r^2) across the *zep1* chromosome region, as in (A). The peak SNP from the unconditional GWAS (S2_44448432; 44,448,432 bp) was included as a covariate in the unified mixed model to control for the *zep1* effect.

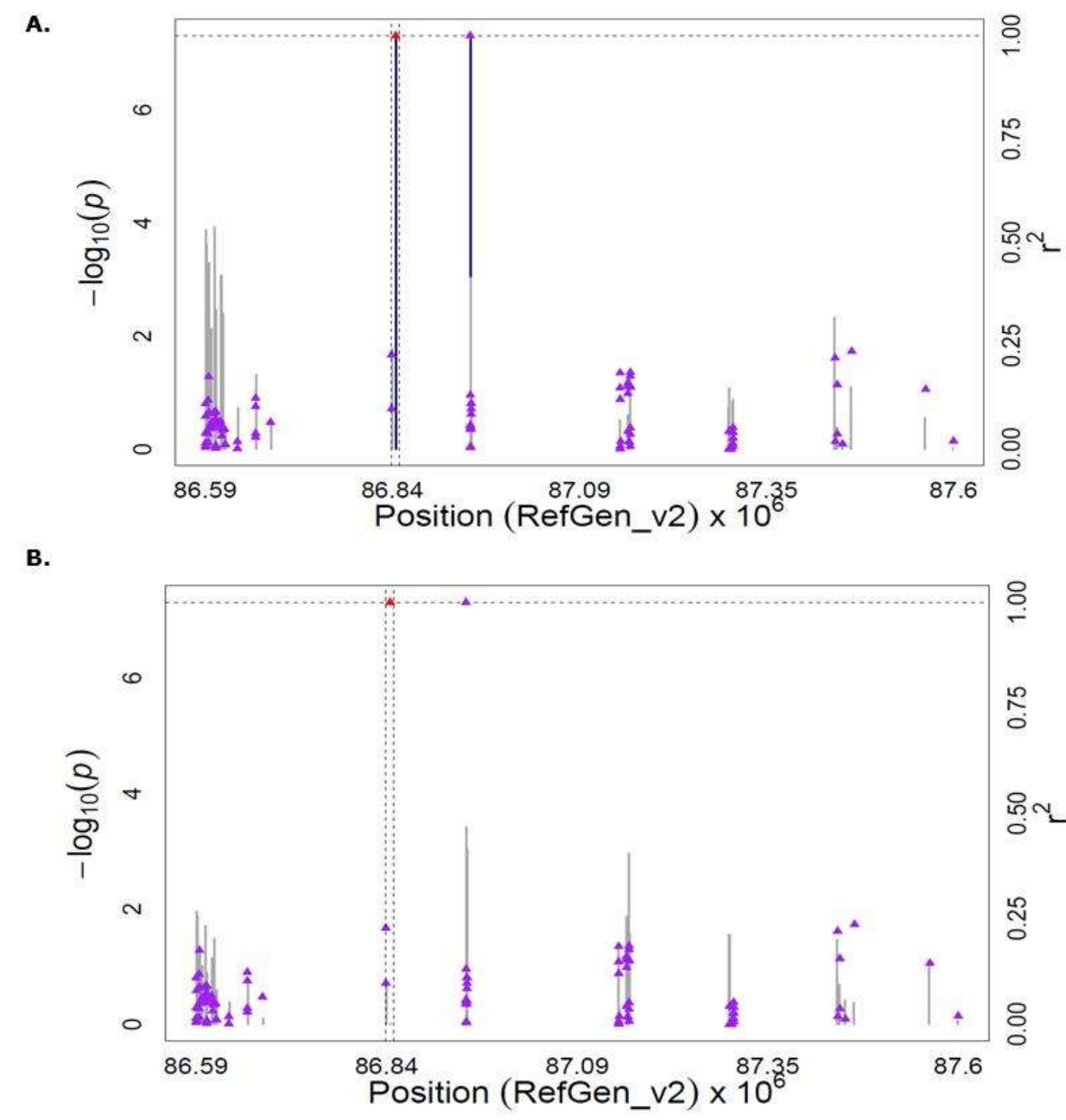


Figure S5 Genome-wide association study (GWAS) for the ratio of zeinoxanthin to lutein content in maize grain. (A) Scatter plot of association results from a unified mixed model analysis of the ratio of zeinoxanthin to lutein and linkage disequilibrium (LD) estimates (r^2) across the *lut1* chromosome region. Negative \log_{10} -transformed P -values (left y-axis) from a GWAS for the ratio of zeinoxanthin to lutein and r^2 values (right y-axis) are plotted against physical position (B73 RefGen_v2) for a 1 Mb region on chromosome 1 that encompasses *lut1*. The blue vertical lines are $-\log_{10} P$ -values for SNPs that are statistically significant for the ratio of zeinoxanthin to lutein at 5% false discovery rate (FDR), while the gray vertical lines are $-\log_{10} P$ -values for SNPs that are non-significant at 5% FDR. Triangles are the r^2 values of each SNP relative to the peak SNP (indicated in red) at 86,844,203 bp. The black horizontal dashed line indicates the $-\log_{10} P$ -value of the least statistically significant SNP at 5% FDR. The black vertical dashed lines indicate the start and stop positions of *lut1* (GRMZM2G14322.) (B) Scatter plot of association results from a conditional unified mixed model analysis of the ratio of zeinoxanthin to lutein and LD estimates (r^2) across the *lut1* chromosome region, as in (A). The peak SNP from the unconditional GWAS (ss196425306; 86,844,203 bp) was included as a covariate in the unified mixed model to control for the *lut1* effect.

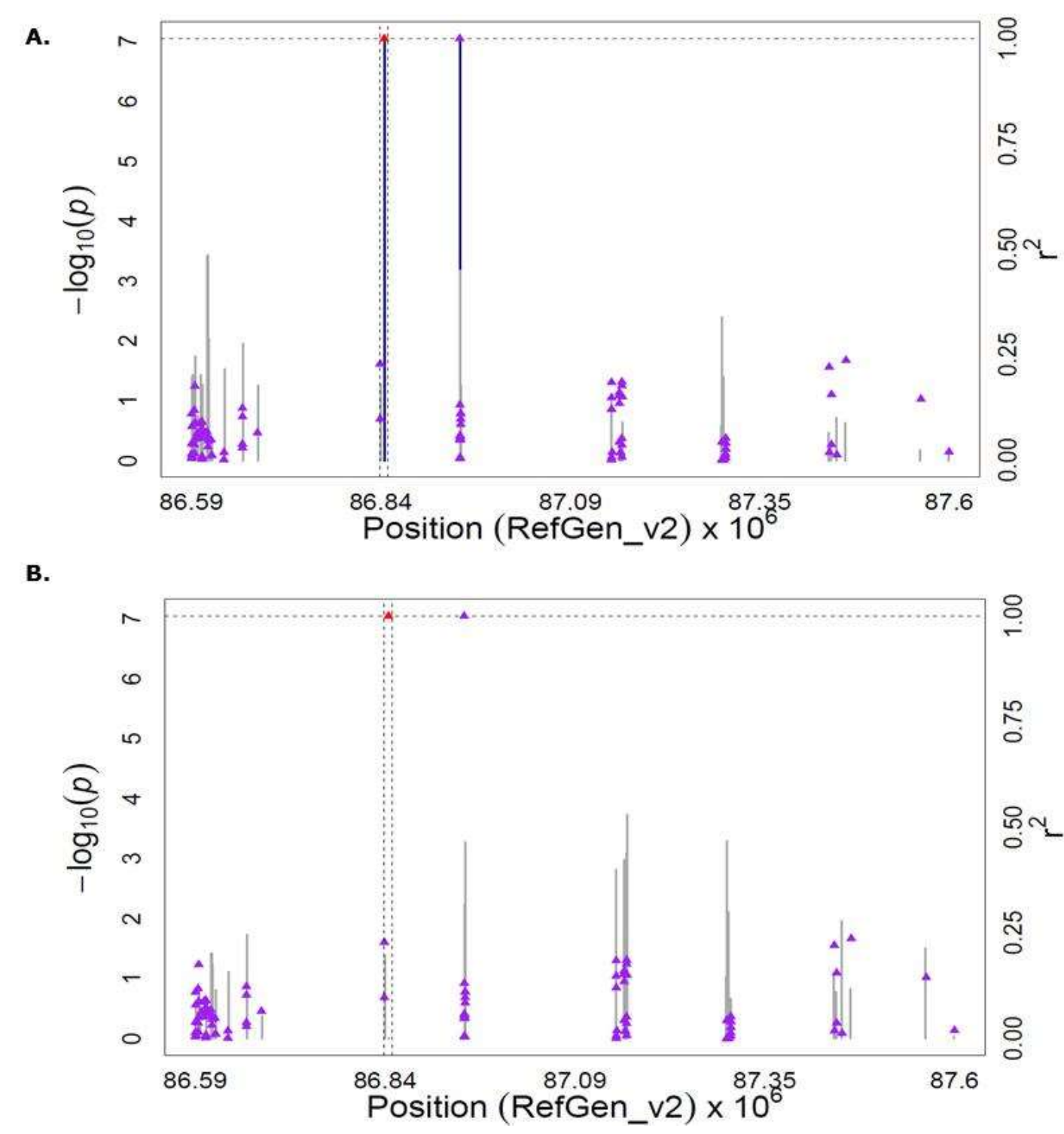


Figure S6 Genome-wide association study (GWAS) for zeinoxanthin content in maize grain. (A) Scatter plot of association results from a unified mixed model analysis of zeinoxanthin and linkage disequilibrium (LD) estimates (r^2) across the *lut1* chromosome region. Negative \log_{10} -transformed P -values (left y-axis) from a GWAS for zeinoxanthin and r^2 values (right y-axis) are plotted against physical position (B73 RefGen_v2) for a 1 Mb region on chromosome 1 that encompasses *lut1*. The blue vertical lines are $-\log_{10} P$ -values for SNPs that are statistically significant for zeinoxanthin at 5% false discovery rate (FDR), while the gray vertical lines are $-\log_{10} P$ -values for SNPs that are non-significant at 5% FDR. Triangles are the r^2 values of each SNP relative to the peak SNP (indicated in red) at 86,844,203 bp. The black horizontal dashed line indicates the $-\log_{10} P$ -value of the least statistically significant SNP at 5% FDR. The black vertical dashed lines indicate the start and stop positions of *lut1* (GRMZM2G14322.) (B) Scatter plot of association results from a conditional unified mixed model analysis of zeinoxanthin and LD estimates (r^2) across the *lut1* chromosome region, as in (A). The peak SNP from the unconditional GWAS (ss196425306; 86,844,203 bp) was included as a covariate in the unified mixed model to control for the *lut1* effect.

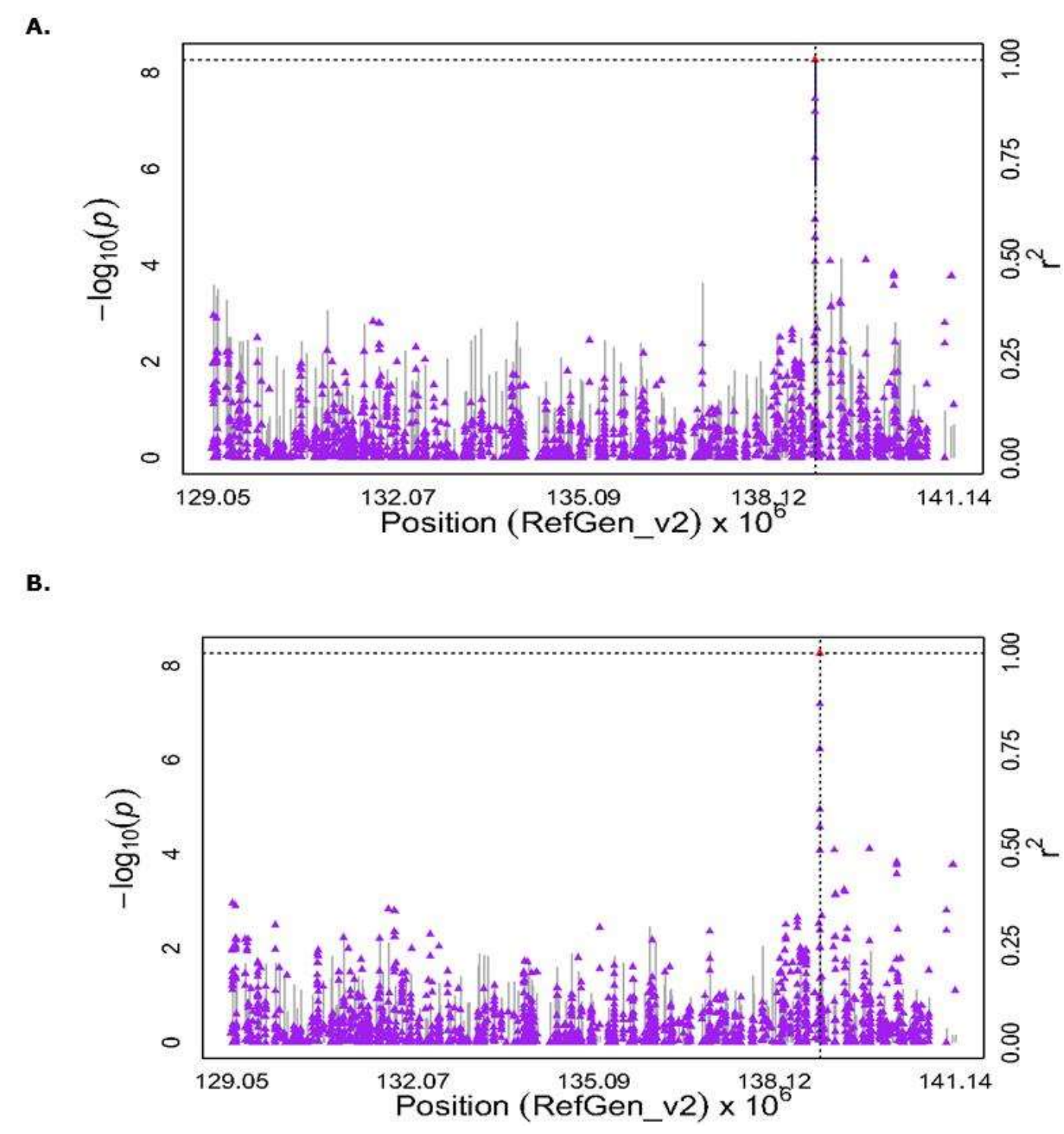


Figure S7 Genome-wide association study (GWAS) for zeaxanthin content in maize grain. Scatter plot of association results from a unified mixed model analysis of zeaxanthin and linkage disequilibrium (LD) estimates (r^2) across the *lcyE* chromosome region. Negative \log_{10} -transformed P -values (left y-axis) from a GWAS for zeaxanthin and r^2 values (right y-axis) are plotted against physical position (B73 RefGen_v2) for a 12 Mb region on chromosome 8 that encompasses *lcyE*. The blue vertical lines are $-\log_{10} P$ -values for SNPs that are statistically significant for zeaxanthin at 5% false discovery rate (FDR), while the gray vertical lines are $-\log_{10} P$ -values for SNPs that are non-significant at 5% FDR. Triangles are the r^2 values of each SNP relative to the peak SNP (indicated in red) at 138,883,206 bp. The black horizontal dashed line indicates the $-\log_{10} P$ -value of the least statistically significant SNP at 5% FDR. The black vertical dashed lines indicate the start and stop positions of *lcyE* (GRMZM2G12966). (B) Scatter plot of association results from a conditional unified mixed model analysis of zeaxanthin and LD estimates (r^2) across the *lcyE* chromosome region, as in (A). The two SNPs (*lcyE* SNP216 and S8_138882897) from the optimal multi-locus mixed model (MLMM) model were included as covariates in the unified mixed model to control for the *lcyE* effect.

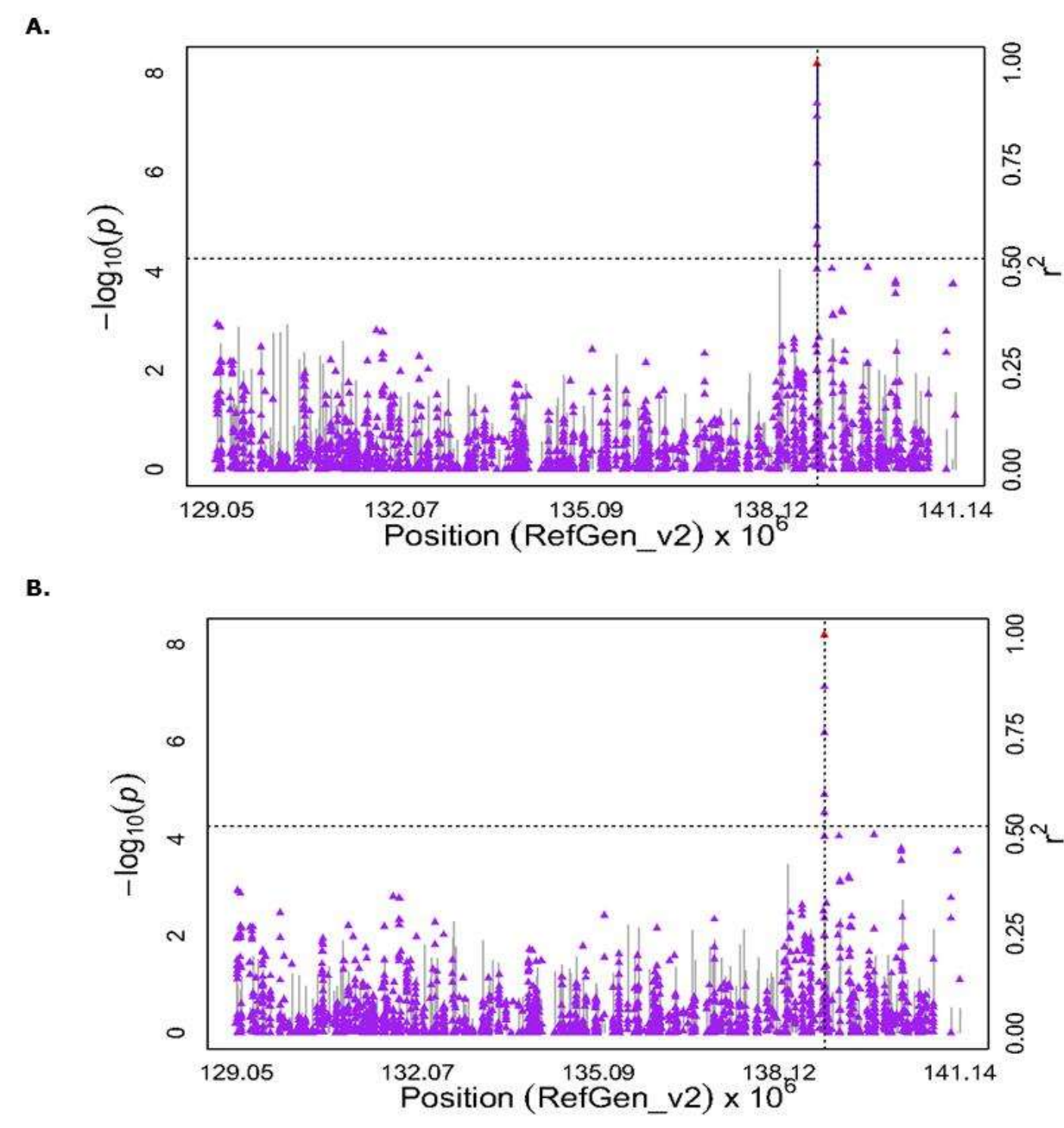


Figure S8 Genome-wide association study (GWAS) for lutein content in maize grain. Scatter plot of association results from a unified mixed model analysis of lutein and linkage disequilibrium (LD) estimates (r^2) across the *lcyE* chromosome region. Negative \log_{10} -transformed P -values (left y-axis) from a GWAS for lutein and r^2 values (right y-axis) are plotted against physical position (B73 RefGen_v2) for a 12 Mb region on chromosome 8 that encompasses *lcyE*. The blue vertical lines are $-\log_{10} P$ -values for SNPs that are statistically significant for lutein at 5% false discovery rate (FDR), while the gray vertical lines are $-\log_{10} P$ -values for SNPs that are non-significant at 5% FDR. Triangles are the r^2 values of each SNP relative to the peak SNP (indicated in red) at 138,883,206 bp. The black horizontal dashed line indicates the $-\log_{10} P$ -value of the least statistically significant SNP at 5% FDR. The black vertical dashed lines indicate the start and stop positions of *lcyE* (GRMZM2G12966). (B) Scatter plot of association results from a conditional unified mixed model analysis of lutein and LD estimates (r^2) across the *lcyE* chromosome region, as in (A). The two SNPs (*lcyE* SNP216 and S8_138882897) from the optimal multi-locus mixed model (MLMM) model were included as covariates in the unified mixed model to control for the *lcyE* effect.

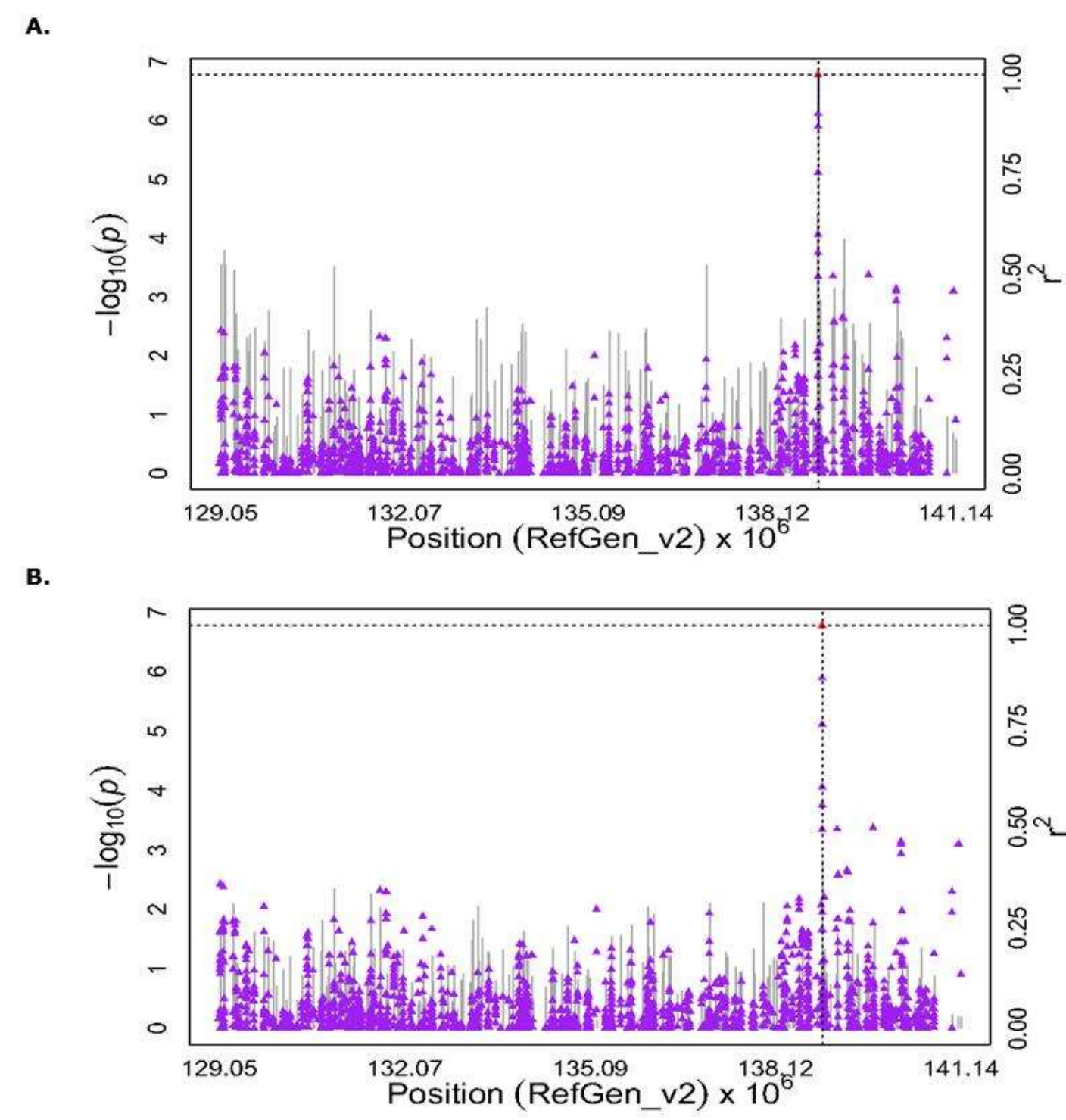


Figure S9 Genome-wide association study (GWAS) for total β -xanthophyll content in maize grain. Scatter plot of association results from a unified mixed model analysis of total β -xanthophyll and linkage disequilibrium (LD) estimates (r^2) across the *lcyE* chromosome region. Negative \log_{10} -transformed P -values (left y-axis) from a GWAS for total β -xanthophyll and r^2 values (right y-axis) are plotted against physical position (B73 RefGen_v2) for a 12 Mb region on chromosome 8 that encompasses *lcyE*. The blue vertical lines are $-\log_{10} P$ -values for SNPs that are statistically significant for total β -xanthophyll at 5% false discovery rate (FDR), while the gray vertical lines are $-\log_{10} P$ -values for SNPs that are non-significant at 5% FDR. Triangles are the r^2 values of each SNP relative to the peak SNP (indicated in red) at 138,883,206 bp. The black horizontal dashed line indicates the $-\log_{10} P$ -value of the least statistically significant SNP at 5% FDR. The black vertical dashed lines indicate the start and stop positions of *lcyE* (GRMZM2G12966). (B) Scatter plot of association results from a conditional unified mixed model analysis of total β -xanthophyll and LD estimates (r^2) across the *lcyE* chromosome region, as in (A). The two SNPs (*lcyE* SNP216 and S8_138882897) from the optimal multi-locus mixed model (MLMM) model were included as covariates in the unified mixed model to control for the *lcyE* effect.

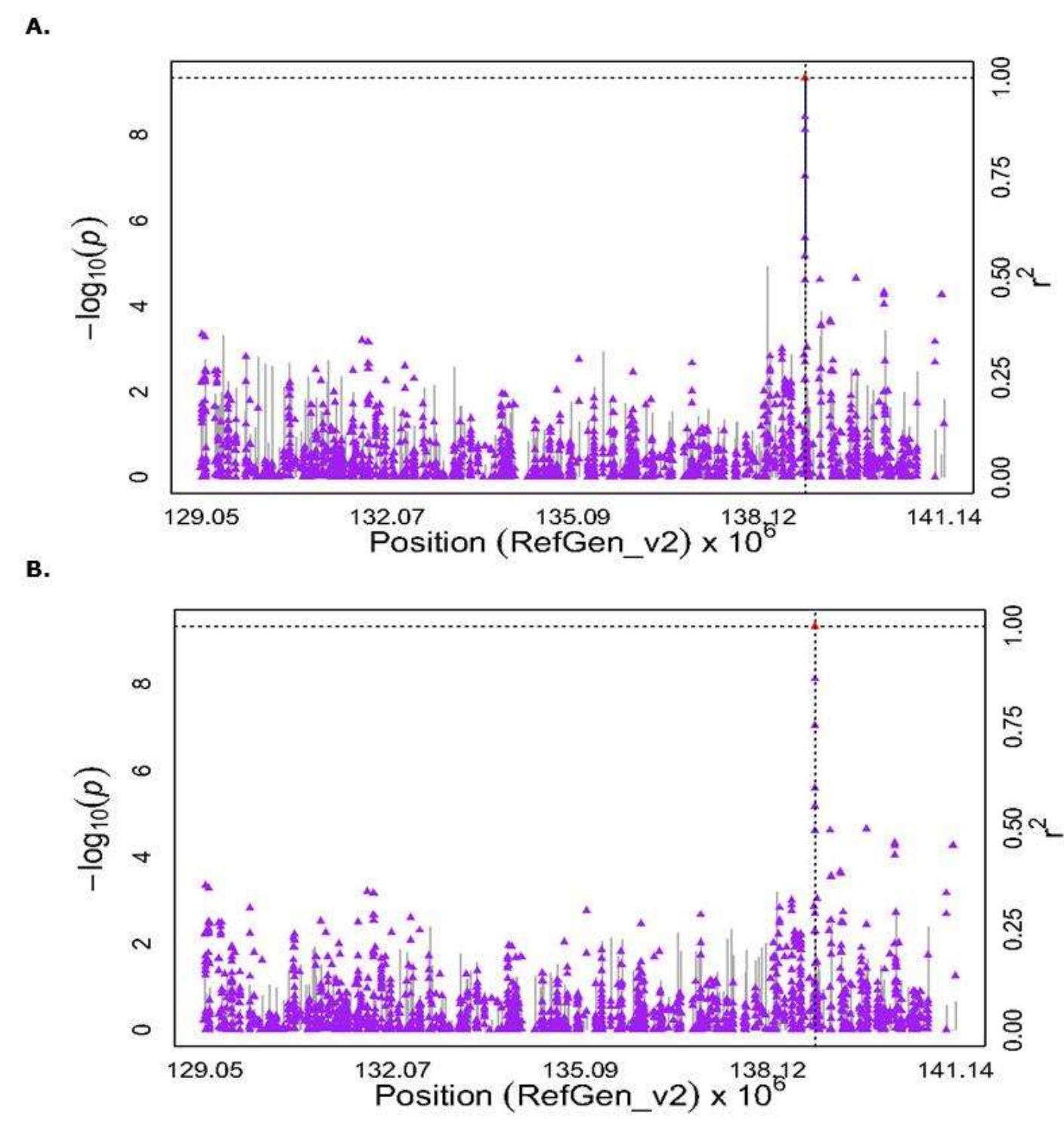


Figure S10 Genome-wide association study (GWAS) for total α -xanthophyll content in maize grain. Scatter plot of association results from a unified mixed model analysis of total α -xanthophyll and linkage disequilibrium (LD) estimates (r^2) across the *lcyE* chromosome region. Negative \log_{10} -transformed P -values (left y-axis) from a GWAS for total α -xanthophyll and r^2 values (right y-axis) are plotted against physical position (B73 RefGen_v2) for a 12 Mb region on chromosome 8 that encompasses *lcyE*. The blue vertical lines are $-\log_{10} P$ -values for SNPs that are statistically significant for total α -xanthophyll at 5% false discovery rate (FDR), while the gray vertical lines are $-\log_{10} P$ -values for SNPs that are non-significant at 5% FDR. Triangles are the r^2 values of each SNP relative to the peak SNP (indicated in red) at 138,883,206 bp. The black horizontal dashed line indicates the $-\log_{10} P$ -value of the least statistically significant SNP at 5% FDR. The black vertical dashed lines indicate the start and stop positions of *lcyE* (GRMZM2G12966). (B) Scatter plot of association results from a conditional unified mixed model analysis of total α -xanthophyll and LD estimates (r^2) across the *lcyE* chromosome region, as in (A). The two SNPs (*lcyE* SNP216 and S8_138882897) from the optimal multi-locus mixed model (MLMM) model were included as covariates in the unified mixed model to control for the *lcyE* effect.

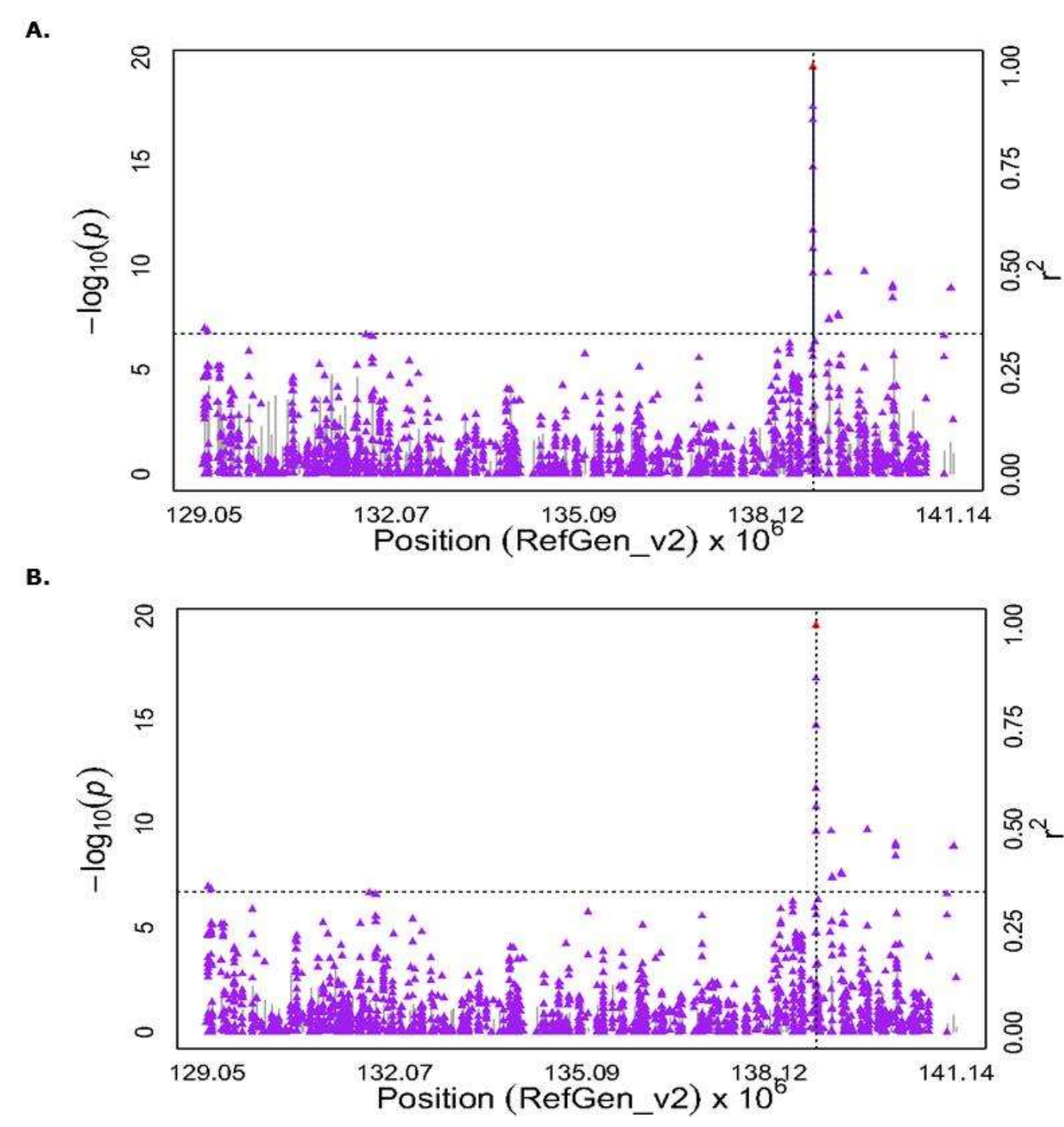


Figure S11 Genome-wide association study (GWAS) for the ratio of β -carotenoid to α -carotenoid content in maize grain. Scatter plot of association results from a unified mixed model analysis of the ratio of β -carotenoid to α -carotenoid content and linkage disequilibrium (LD) estimates (r^2) across the *lcyE* chromosome region. Negative \log_{10} -transformed P -values (left y-axis) from a GWAS for the ratio of β -carotenoid to α -carotenoid content and r^2 values (right y-axis) are plotted against physical position (B73 RefGen_v2) for a 12 Mb region on chromosome 8 that encompasses *lcyE*. The blue vertical lines are $-\log_{10} P$ -values for SNPs that are statistically significant for the ratio of β -carotenoid to α -carotenoid content at 5% false discovery rate (FDR), while the gray vertical lines are $-\log_{10} P$ -values for SNPs that are non-significant at 5% FDR. Triangles are the r^2 values of each SNP relative to the peak SNP (indicated in red) at 138,883,206 bp. The black horizontal dashed line indicates the $-\log_{10} P$ -value of the least statistically significant SNP at 5% FDR. The black vertical dashed lines indicate the start and stop positions of *lcyE* (GRMZM2G12966). (B) Scatter plot of association results from a conditional unified mixed model analysis of the ratio of β -carotenoid to α -carotenoid content and LD estimates (r^2) across the *lcyE* chromosome region, as in (A). The two SNPs (*lcyE* SNP216 and S8_138882897) from the optimal multi-locus mixed model (MLMM) model were included as covariates in the unified mixed model to control for the *lcyE* effect.

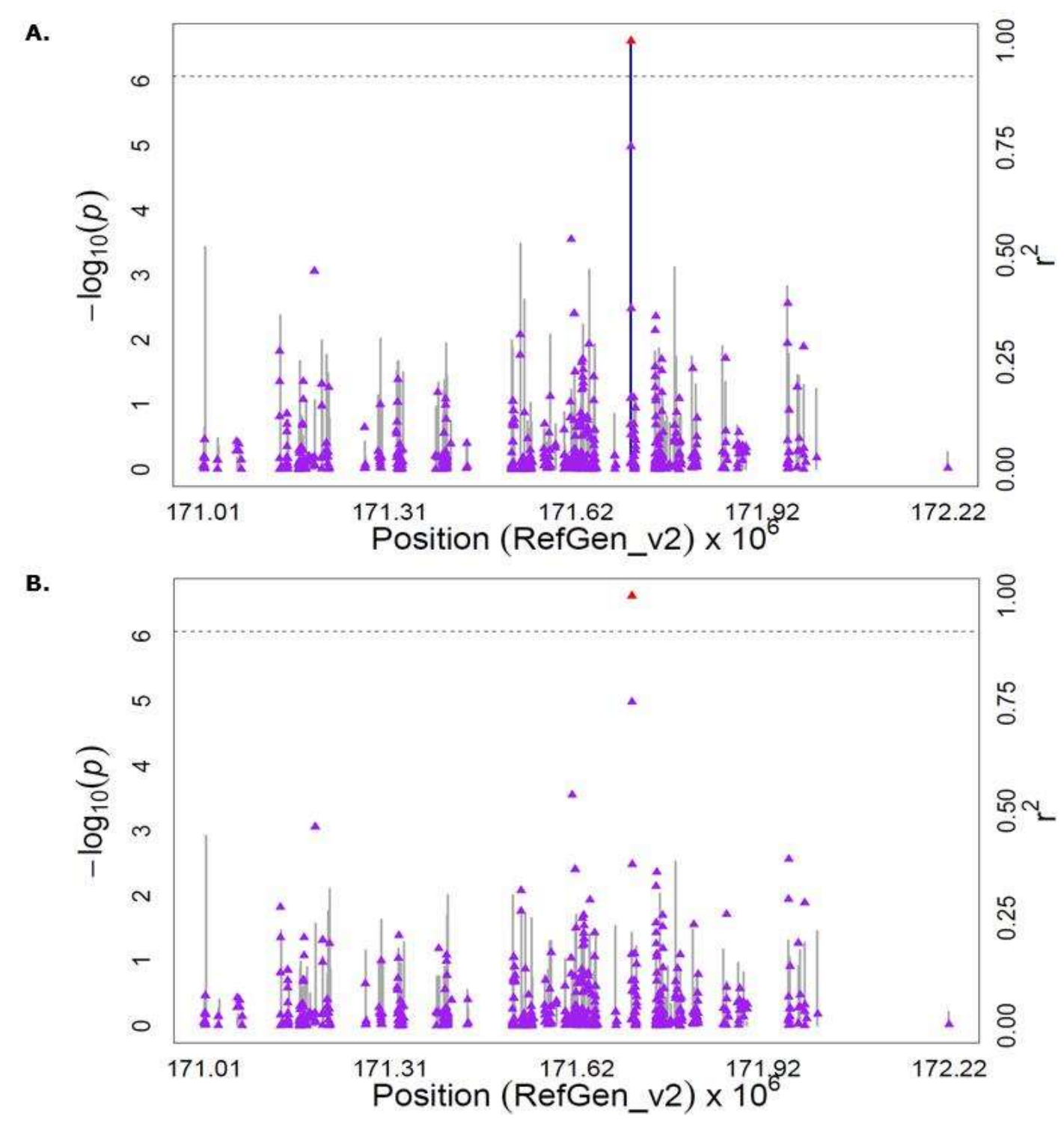


Figure S12 Genome-wide association study (GWAS) for zeaxanthin content in maize grain. (A) Scatter plot of association results from a unified mixed model analysis of zeaxanthin and linkage disequilibrium (LD) estimates (r^2) across the surrounding chromosome region. Negative \log_{10} -transformed P -values (left y-axis) from a GWAS for zeaxanthin and r^2 values (right y-axis) are plotted against physical position (B73 RefGen_v2) for a 1.2 Mb region on chromosome 8. The blue vertical lines are $-\log_{10} P$ -values for SNPs that are statistically significant for zeaxanthin at 5% false discovery rate (FDR), while the gray vertical lines are $-\log_{10} P$ -values for SNPs that are non-significant at 5% FDR. Triangles are the r^2 values of each SNP relative to the peak SNP (indicated in red) at 171,705,574 bp. The black horizontal dashed line indicates the $-\log_{10} P$ -value of the least statistically significant SNP at 5% FDR. (B) Scatter plot of association results from a conditional unified mixed model analysis of zeaxanthin and LD estimates (r^2) across the 1.2 Mb chromosome region, as in (A). The peak SNP from the unconditional GWAS (S8_171705574; 171,705,574 bp) was included as a covariate in the unified mixed model to control for the novel effect detected on chromosome 8.

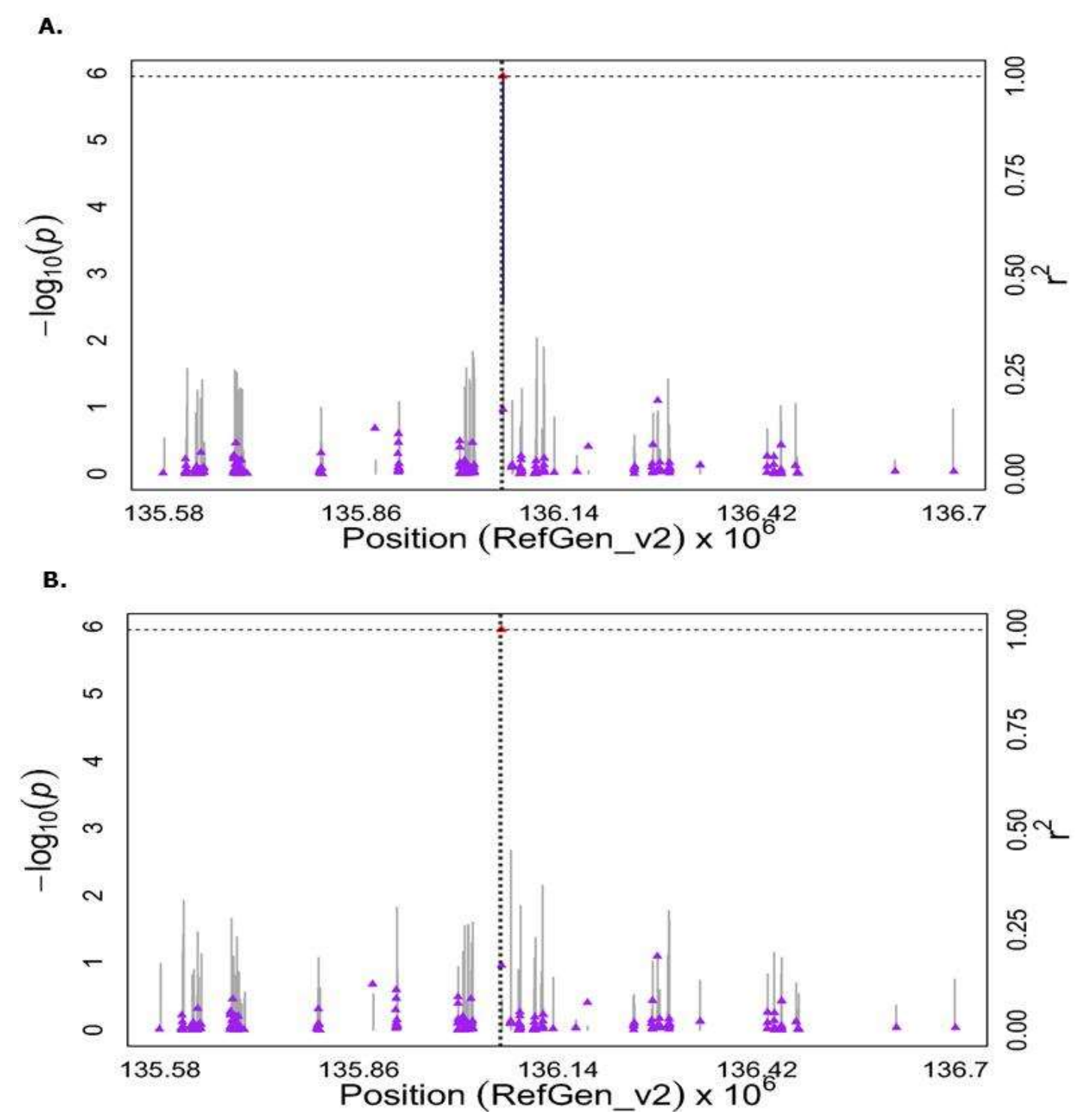


Figure S13 Genome-wide association study (GWAS) for zeaxanthin content in maize grain. (A) Scatter plot of association results from a unified mixed model analysis of zeaxanthin and linkage disequilibrium (LD) estimates (r^2) across the *cr1RB1* chromosome region. Negative \log_{10} -transformed P -values (left y-axis) from a GWAS of zeaxanthin and r^2 values (right y-axis) are plotted against physical position (B73 RefGen_v2) for a 1.2 Mb region on chromosome 10 that encompasses *cr1RB1*. The blue vertical lines are $-\log_{10} P$ -values for SNPs that are statistically significant for zeaxanthin at 5% false discovery rate (FDR), while the gray vertical lines are $-\log_{10} P$ -values for SNPs that are non-significant at 5% FDR. Triangles are the r^2 values of each SNP relative to the peak polymorphism (indicated in red) at 136,059,748 bp. The black horizontal dashed line indicates the $-\log_{10} P$ -value of the least statistically significant SNP at 5% FDR. The black vertical dashed lines indicate the start and stop positions of *cr1RB1* (GRMZM2G152135). (B) Scatter plot of association results from a conditional unified mixed model analysis of zeaxanthin and LD estimates (r^2) across the *cr1RB1* chromosome region, as in (A). The peak polymorphism from the unconditional GWAS (*cr1RB1* InDel4; 136,059,748 bp) was included as a covariate in the unified mixed model to control for the *cr1RB1* effect.

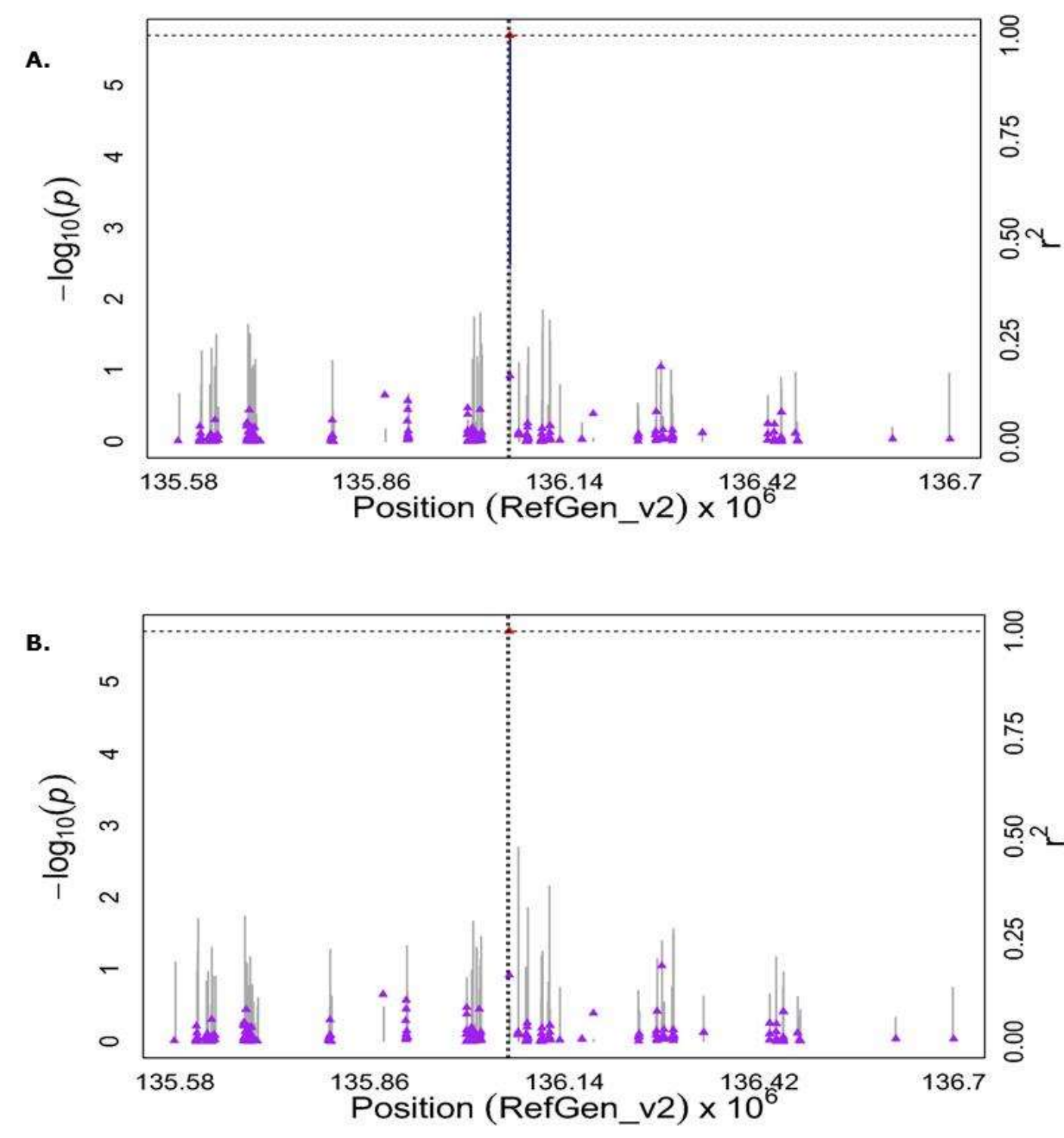


Figure S14 Genome-wide association study (GWAS) for total β -xanthophyll content in maize grain. (A) Scatter plot of association results from a unified mixed model analysis of total β -xanthophyll content and linkage disequilibrium (LD) estimates (r^2) across the *crTRB1* chromosome region. Negative \log_{10} -transformed P -values (left y-axis) from a GWAS of total β -xanthophyll content and r^2 values (right y-axis) are plotted against physical position (B73 RefGen_v2) for a 1.2 Mb region on chromosome 10 that encompasses *crTRB1*. The blue vertical lines are $-\log_{10} P$ -values for SNPs that are statistically significant for total β -xanthophyll content at 5% false discovery rate (FDR), while the gray vertical lines are $-\log_{10} P$ -values for SNPs that are non-significant at 5% FDR. Triangles are the r^2 values of each SNP relative to the peak polymorphism (indicated in red) at 136,059,748 bp. The black horizontal dashed line indicates the $-\log_{10} P$ -value of the least statistically significant SNP at 5% FDR. The black vertical dashed lines indicate the start and stop positions of *crTRB1* (GRMZM2G152135). (B) Scatter plot of association results from a conditional unified mixed model analysis of total β -xanthophyll and LD estimates (r^2) across the *crTRB1* chromosome region, as in (A). The peak polymorphism from the unconditional GWAS (*crTRB1* InDel4; 136,059,748 bp) was included as a covariate in the unified mixed model to control for the *crTRB1* effect.

Table S1 Best Linear Unbiased Predictor (BLUP) Values. BLUP values for the 24 carotenoid traits used for the genome-wide association study, pathway-level analysis, and genomic prediction for 201 inbred maize lines.

Table S1 is available for download as an Excel file at <http://www.genetics.org/lookup/suppl/doi:10.1534/genetics.114.169979/-/DC1>.

Table S2 Coordinates for Additional Insertion-Deletion (Indel) and Single-Nucleotide Polymorphism (SNP) Markers

Gene-Specific Assay	PCR-Based Marker	Primer Sequence (5'-3' direction)	Specific to PCR Primer			Coordinates Spanning Longest Amplicon Length ^b		
			Chr	Start AGP2	StopAGP2	Chr	Start AGP2	StopAGP2
<i>y1</i> Indel388	PSY-8F	TGAAACAAACAAAGCCAGCA	chr6	82,016,321	82,016,340			
	PSY-9R	GCCTCTCCTCTTTCGCGTA	chr6	82,017,115	82,017,134			
	PSY-10F	GAAACAAACAAAGCCAGCAG	chr6	82,016,322	82,016,341			
	PSY-11R	CTCCGGCCTCTCTTCT	chr6	82,017,121	82,017,139	chr6	82,016,321	82,017,139
<i>lcyE</i> 5'TE	lcyE-TE103PF-F1	CGTAGCAAGCCATTATTTT	chr8	138,882,291	138,882,313			
	lcyE-TE103PF-R1	CGGTATGGTTTTGGTATACGG	chr8	°				
	lcyE-ZG111R1-F1	AAGCATCCGACAAAATAACAG	chr8	138,882,423	138,882,444			
	lcyE-TE105PR-R1	GAGAGGGAGACGACGAGACAC	chr8	138,882,649	138,882,670	chr8	138,882,291	138,882,670
<i>lcyE</i> SNP216	lcyE-SNP216-F1	GCGGCACTGGGGTGGAT	chr8	138,883,009	138,883,026			
	lcyE-SNP216-R1	TGAAGTACGGCTGCAGACAACG	chr8	138,883,381	138,883,403	chr8	138,883,009	138,883,403
<i>lcyE</i> 3'	lcyE-3'indl-F1	GTACGTGTTTCATCTCCGTACCC	chr8	°				
	lcyE-3'indl-R1	CTTGGTGAACGCATTCTGTTGG	chr8	°				
	lcyE-3'indl-F2	GGACCGAAGCAACCAACTG	chr8	°				
	lcyE-3'indl-R2	GGCGAAATGGGTACGGCC	chr8	°		chr8	138,889,812	138,892,812
<i>ccd1</i> 5'	CCD1-WC-L1	CCGTGCTCGGACAGAATAGT	chr8	°				
	CCD1-B73-rev-L1	CTCACACGTGTCAACGCC	chr9	152,093,059	152,093,042			
	CCD1-ALL-R1	GTGGTTTCGGTGGCTGTC	chr9	152,092,686	152,092,700	chr9	152,092,686	152,093,042
<i>crRB1</i> 5'TE	crRB1 H1UF	TTAGAGCCTCGACCCTCTGTG	chr10	136,061,212	136,061,232			
	crRB1 H1UR	AATCCCTTCCATGTTACGC	chr10	136,060,416	136,060,435	chr10	136,060,416	136,061,232
<i>crRB1</i> InDel4	crRB1 D4F	ACCGTACGTGCTTCGTGCC	chr10	136,059,806	136,059,787			
	crRB1 D4R	CTCCGGCCTCTTCTC	chr10	136,059,690	136,059,708	chr10	136,059,690	136,059,806
<i>crRB1</i> 3'TE	crRB1 65F	ACACCACATGGACAAGTTCG	chr10	°				
	crRB1 62R	CACTCTGGCCATGAACAC	chr10	°				
	crRB1 66R	ACAGCAATACAGGGACCAG	chr10	°		chr10	136,060,219	136,063,219

RefGen_v2 Coordinates and PCR primer sequences for the additional seven indel markers and one SNP marker within and near the coding regions of one carotenoid degradation gene and three carotenoid biosynthetic pathway genes. Amplification protocols for gene-specific PCR-based marker sets are listed in: Fu et al. 2013b for *y1*; Harjes et al. 2008 for *lcyE*; Kandianis et al. for *ccd1*; Yan et al. 2010 for *crRB1*.

^aRefGen_v2 Coordinates are not available

^bPhysical distance encompassing all possible primer combinations

Table S3 Genomic Information for the 58 *a priori* Candidate Genes (A)

<i>a priori</i> candidate gene pathway	MaizeGDB Name	MaizeGDB Full Name	MaizeGDB Synonym(s)	RefGen_v2 Gene Model ID	Annotated Gene Function	RefGen_v2 Chromosome	RefGen_v2 ORF Start bp	RefGen_v2 ORF Stop bp
carotenoid_synthesis_and_degradation	ao1	aldehyde oxidase1	ao1, cl1856_2b, aldehyde oxidase1	GRMZM2G141535	Aldehyde oxidase and xanthine dehydrogenase	1	286,448,581	286,456,365
carotenoid_synthesis_and_degradation	ao2	aldehyde oxidase2	ao2	GRMZM5G899851	Aldehyde oxidase and xanthine dehydrogenase	5	4,588,532	4,592,775
carotenoid_synthesis_and_degradation	ao3	aldehyde oxidase3	ao3, TMR51, aldehyde oxidase3, GRMZM2G124260, rs131175362, ss196414838, pzb01403, IDP2436	GRMZM2G019799	Aldehyde oxidase and xanthine dehydrogenase	1	286,358,278	286,366,211
carotenoid_synthesis_and_degradation	ao4	aldehyde oxidase4	ao4	GRMZM2G141473	Aldehyde oxidase and xanthine dehydrogenase	1	286,506,118	286,513,080
carotenoid_synthesis_and_degradation	ao5	aldehyde oxidase5	ao5	GRMZM2G406830	Aldehyde oxidase and xanthine dehydrogenase	7	7,446,258	7,451,594
carotenoid_synthesis_and_degradation	ccd7	carotenoid cleavage dioxygenase7	ccd7	GRMZM2G158657	*carotenoid cleavage dioxygenase7	2	19,458,968	19,461,625
carotenoid_synthesis_and_degradation	ccd8	carotenoid cleavage dioxygenase8	ccd8, ccd8a, Zmccd8	GRMZM2G446858	*carotenoid cleavage dioxygenase8	3	197,015,856	197,019,350
Prenyl_Group_Synthesis	chph1	chlorophyllase1	chph1	GRMZM2G170734	chlorophyllase, chloroplast	7	62,130,993	62,132,323
Prenyl_Group_Synthesis	cmk1	4-diphosphocytidyl-2-C-methyl-D-erythritol kinase1	cmk1, umc2169, 4-diphosphocytidyl-2-C-methyl-D-erythritol kinase1, cdp-me kinase1, cdpmek1	GRMZM5G859195	4-diphosphocytidyl-2-C-methyl-D-erythritol kinase	3	187,922,271	187,927,591
carotenoid_synthesis_and_degradation	crt1	carotene isomerase1	crt1, carotenoid isomerase1, CRTISO1	GRMZM2G108457	carotenoid isomerase	4	200,869,070	200,873,710
carotenoid_synthesis_and_degradation	crt2	carotene isomerase2	crt2, carotenoid isomerase2, CRTISO2	GRMZM2G106531	carotenoid isomerase	2	226,366,352	226,371,341
carotenoid_synthesis_and_degradation	crt3	carotene isomerase3	crt3, CRTISO3	GRMZM2G144273	carotenoid isomerase	5	1,333,304	1,341,577
carotenoid_synthesis_and_degradation	cyp13	cytochrome P450 13	cyp13, Cytochrome P450, CYP97A16, lutein5, lut5, CYP97A	GRMZM5G837869	CYP97A3, Cytochrome P450 beta-ring hydroxylase	5	215,827,224	215,831,730
carotenoid_synthesis_and_degradation	cyp14	cytochrome P450 14	cyp14, CYP97C, lutein1, lut1, cytochrome P450-type monooxygenase CYP97C1	GRMZM2G143202	CYP97A3, Cytochrome P450 epsilon-ring hydroxylase	1	86,838,334	86,848,726
carotenoid_synthesis_and_degradation	cyp15	cytochrome P450 15	cyp15, CYP97B, cytochrome P450-type monooxygenase CYP97B3	GRMZM2G010221	CYP97B, cytochrome P450	4	235,724,340	235,728,875
Prenyl_Group_Synthesis	dmes1	4-Diphosphocytidyl-2C-methyl-D-erythritol synthase1	dmes1, si618008b02(470), si618008b02f	GRMZM5G856881	2-C-methyl-D-erythritol 4-phosphate cytidyltransferase	3	170,115,790	170,118,780
Prenyl_Group_Synthesis	dmes2	4-Diphosphocytidyl-2C-methyl-D-erythritol synthase2	dmes2	GRMZM2G172032	2-C-methyl-D-erythritol 4-phosphate cytidyltransferase	8	164,748,939	164,752,371
Prenyl_Group_Synthesis	dxr1	deoxy xylulose reductoisomerase1	dxr1, IDP154, deoxy xylulose reductoisomerase1, 1-deoxy-D-xylulose 5-phosphate reductoisomerase1, CL389_1(210), CL389_1b	GRMZM2G056975	1-deoxy-D-xylulose 5-phosphate reductoisomerase	3	30,226,804	30,233,358
Prenyl_Group_Synthesis	dxr2	deoxy xylulose reductoisomerase2	dxr2	GRMZM2G036290	1-deoxy-D-xylulose 5-phosphate reductoisomerase	8	8,094,442	8,101,055
Prenyl_Group_Synthesis	dxs1	deoxy xylulose synthase1	dxs1, PZA02247, CL392_1, AY110050, deoxy xylulose synthase1	GRMZM2G137151	1-deoxy-D-xylulose 5-phosphate synthase	6	146,378,393	146,382,661
Prenyl_Group_Synthesis	dxs2	deoxy xylulose synthase2	dxs2, CL732_-1, deoxy xylulose synthase2	GRMZM2G493395	1-deoxy-D-xylulose 5-phosphate synthase	7	14,077,852	14,081,075
Prenyl_Group_Synthesis	dxs3	deoxy xylulose synthase3	dxs3, pco071268	GRMZM2G173641	1-deoxy-D-xylulose 5-phosphate synthase	9	20,462,059	20,467,072
Prenyl_Group_Synthesis	ggh1	geranylgeranyl hydrogenase1	ggh1	GRMZM2G105644	geranylgeranyl reductase	5	206,890,298	206,892,838
Prenyl_Group_Synthesis	ggh2	geranylgeranyl hydrogenase2	ggh2	GRMZM2G419111	geranylgeranyl reductase	3	40,062,008	40,064,270
Prenyl_Group_Synthesis	ggps1	geranylgeranyl pyrophosphate synthase1	ggps1, GGPPS1, ggpps1	AC194970.5_FG001	geranylgeranyl pyrophosphate synthase	2	207,236,995	207,238,335
Prenyl_Group_Synthesis	ggps2	geranylgeranyl pyrophosphate synthase2	ggps2, GGPPS2, ggpps2	GRMZM2G102550	geranylgeranyl pyrophosphate synthase	7	160,531,537	160,533,586
Prenyl_Group_Synthesis	ggps3	geranylgeranyl pyrophosphate synthase3	ggps3, GGPPS3, ggpps3	GRMZM2G058404	geranylgeranyl pyrophosphate synthase	8	6,358,798	6,360,117
Prenyl_Group_Synthesis	hds1	hydroxymethylbutenyl diphosphate synthase1	hds1, 4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase1, Hydroxymethylbutenyl diphosphate synthase1	GRMZM2G137409	4-hydroxy-3-methylbut-2-enyl diphosphate synthase	5	182,124,005	182,130,631
carotenoid_synthesis_and_degradation	hyd3	hydroxylase3	hyd3, crtRB1, beta-carotene hydroxylase 1, CrR-B1, bch2	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	10	136,057,100	136,060,219
carotenoid_synthesis_and_degradation	hyd4	hydroxylase4	hyd4, bch1, crtRB3, HYD1, beta-carotene hydroxylase homolog, BCH1	GRMZM2G164318	Beta-carotene hydroxylase (non-heme dioxygenase type)	2	15,865,938	15,868,219
carotenoid_synthesis_and_degradation	hyd5	hydroxylase5	hyd5, crtRB5	GRMZM2G382534	Beta-carotene hydroxylase (non-heme dioxygenase type)	9	153,692,212	153,694,576
carotenoid_synthesis_and_degradation	hyd6	hydroxylase6	hyd6, crtRB2, beta-carotene hydroxylase homolog	GRMZM2G090051	Beta-carotene hydroxylase (non-heme dioxygenase type)	1	5,380,152	5,382,574
carotenoid_synthesis_and_degradation	hyd7	hydroxylase7	hyd7, crtRB4, hydroxylase7	GRMZM2G163683	Beta-carotene hydroxylase (non-heme dioxygenase type)	4	236,023,117	236,025,051
carotenoid_synthesis_and_degradation	hyd8	hydroxylase8	hyd8	GRMZM5G826824	Beta-carotene hydroxylase (non-heme dioxygenase type)	1	6,353,416	6,354,652
Prenyl_Group_Synthesis	ippi1	isopentenyl pyrophosphate isomerase1	ippi1, isopentenyl diphosphate isomerase1, isopentenyl pyrophosphate isomerase1	GRMZM2G108285	isopentenyl pyrophosphate isomerase	7	155,559,747	155,562,921
Prenyl_Group_Synthesis	ippi2	isopentenyl pyrophosphate isomerase2	ippi2, isopentenyl pyrophosphate isomerase2, isopentenyl diphosphate isomerase2	GRMZM2G145029	isopentenyl pyrophosphate isomerase	8	104,659,886	104,663,941
Prenyl_Group_Synthesis	ippi3	isopentenyl pyrophosphate isomerase3	ippi3, isopentenyl pyrophosphate isomerase3, isopentenyl diphosphate isomerase3	GRMZM2G133082	isopentenyl pyrophosphate isomerase	6	147,131,116	147,136,679
Prenyl_Group_Synthesis	lw1	lemon white1	lw1, luteus17, zebra crossbands7, zb7, zb*-N101, zb*-101, isPH, hydroxymethylbutenyl diphosphate reductase1, hmdr1, hdr1, blt1, blotchedN43, 4-hydroxy-3-methylbut-2-enyl diphosphate reductase1,	GRMZM2G027059	4-hydroxy-3-methylbut-2-enyl diphosphate reductase	1	272,936,836	272,940,502

			I17, lemon white1					
carotenoid_synthesis_and_degradation	lyce1	lycopene epsilon cyclase1	lyce1, lcyE1, lycE, lcyE, Lcy-E, lycopene epsilon cyclase1	GRMZM2G012966	lycopene epsilon-cyclase	8	138,882,594	138,889,812
Prenyl_Group_Synthesis	mecs1	2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase1	mecs1	GRMZM5G835542	2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase	4	155,830,779	155,832,786
Prenyl_Group_Synthesis	mecs2	2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase2	mecs2	AC209374.4_FG002	2-C-methyl-D-erythritol 2,4-cyclodiphosphate synthase	5	196,279,295	196,281,037
carotenoid_synthesis_and_degradation	nced2	nine-cis-epoxycarotenoid dioxygenase2	nced2, NCED2, 9-cis-epoxycarotenoid dioxygenase5a, NCED5a, vp14 homolog	GRMZM2G407181	*9-cis-epoxycarotenoid dioxygenase5a	1	174,524,887	174,527,795
carotenoid_synthesis_and_degradation	nced3	nine-cis-epoxycarotenoid dioxygenase3	nced3, vp14 homolog, 9-cis-epoxycarotenoid dioxygenase5b, NCED5b, NCED3	GRMZM5G858784	*9-cis-epoxycarotenoid dioxygenase5b	3	87,344,791	87,346,554
carotenoid_synthesis_and_degradation	nced4	nine-cis-epoxycarotenoid dioxygenase4	nced4, NCED9a, vp14 homolog, 9-cis-epoxycarotenoid dioxygenase9a	GRMZM2G408158	*9-cis-epoxycarotenoid dioxygenase9a	2	234,574,835	234,576,854
carotenoid_synthesis_and_degradation	nced5	nine-cis-epoxycarotenoid dioxygenase5	nced5, 9-cis-epoxycarotenoid dioxygenase9b, NCED9b, vp14 homolog	GRMZM2G417954	*9-cis-epoxycarotenoid dioxygenase9b	7	5,976,197	5,978,481
carotenoid_synthesis_and_degradation	nced6	nine-cis-epoxycarotenoid dioxygenase6	nced6, NCED6, Carotenoid cleavage dioxygenase4a, CCD4a	GRMZM2G110192	*carotenoid cleavage dioxygenase4a	4	159,724,032	159,726,475
carotenoid_synthesis_and_degradation	nced7	nine-cis-epoxycarotenoid dioxygenase7	nced7, NCED9c, vp14 homolog, 9-cis-epoxycarotenoid dioxygenase9c	GRMZM2G330848	*9-cis-epoxycarotenoid dioxygenase9c	7	175,861,745	175,863,458
carotenoid_synthesis_and_degradation	nced8	nine-cis-epoxycarotenoid dioxygenase8	nced8, NCED5, Carotenoid cleavage dioxygenase4b, CCD4b	GRMZM2G150363	*carotenoid cleavage dioxygenase4b	5	200,687,176	200,689,579
carotenoid_synthesis_and_degradation	nced9	nine-cis-epoxycarotenoid dioxygenase9	nced9, NCED9d, 9-cis-epoxycarotenoid dioxygenase9d	GRMZM5G838285	*9-cis-epoxycarotenoid dioxygenase9d	5	16,850,172	16,851,977
carotenoid_synthesis_and_degradation	ps1	pink scutellum1	ps1, ps*-Mu85-3061-21, lycb1, lcyb1, lcyb, lycB, vp7, ps*-8205, pink scutellum1, lyc1, ps*-85-3288-28	GRMZM5G849107	lycopene beta-cyclase	5	100,700,176	100,702,026
carotenoid_synthesis_and_degradation	psy2	phytoene synthase2	psy2, csu572, pco131047(641), PCO131047b, phytoene synthase2	GRMZM2G149317	phytoene synthase	8	168,273,042	168,276,092
carotenoid_synthesis_and_degradation	vde1	violaxanthin de-epoxidase1	vde1, si605018d09, VDE, violaxanthin de-epoxidase1	GRMZM2G027219	violaxanthin de-epoxidase	2	74,086,504	74,089,290
carotenoid_synthesis_and_degradation	vp14	viviparous14	vp14, NCED1, nine-cis-epoxycarotenoid dioxygenase1, NCED1 homolog, siu95953a(82), siu95953a, viviparous14, umc1218, ufg4	GRMZM2G014392	*9-cis-epoxycarotenoid dioxygenase1	1	250,892,567	250,895,242
carotenoid_synthesis_and_degradation	vp5	viviparous5	vp5, viviparous5, MAGI_109001, PZB00718, MAGI_22938, umc1070, phytoene desaturase, L39266, pds*-L39266, PZB00648, PZA02069, CL1803_1, phytoene desaturase, pds1, vp5-8419, y-vp*-8419, y-vp*-83-3101-36, y-vp*-85-3101-36, vp5-83-3101-36	GRMZM2G410515	phytoene desaturase	1	17,660,941	17,667,054
carotenoid_synthesis_and_degradation	wc1	white cap1	wc1, ccd1, ZmCCD1, PCO084517, AY106323, IDP700, white cap1	GRMZM2G057243	*carotenoid cleavage dioxygenase1	9	152,086,899	152,092,882
carotenoid_synthesis_and_degradation	y1	yellow endosperm1	y1, y1ssr, rs131175743, rs130328408, y4, yellow endosperm1, white1, pb1, Psy1	GRMZM2G300348	phytoene synthase	6	82,017,148	82,021,007
carotenoid_synthesis_and_degradation	zds1	zeta carotene desaturase1	zds1, zeta carotene desaturase candidate, cl78_1(541), CL78_1	GRMZM2G454952	zeta-carotene desaturase	7	17,470,585	17,479,020
carotenoid_synthesis_and_degradation	zep1	zeaxanthin epoxidase1	zep1, fha5, TMR41	GRMZM2G127139	zeaxanthin epoxidase	2	44,440,299	44,449,237

Genomic information for the 58 *a priori* candidate genes involved in the biosynthesis of isoprenoids and carotenoids, as well as the degradation of carotenoids.

*Carotenoid cleavage enzymes fall into to major phyletic groups, the carotenoid cleavage dioxygenases (which generally have broad substrate specificity) and the NCED clade, which are involved in ABA synthesis and highly specific for 9-cis-epoxycarotenoids. Note that with the exception of ZmCCD1 and ZmNCED1 (vp14) maize carotenoid cleavage family members have not had their biochemical activities determined. Nomenclature of other maize family members is relative to their most closely related sequence in Arabidopsis but this does not necessarily imply a corresponding biochemical activity for the maize enzyme. Nomenclature is as listed in maizeGDB v3.

Table S3 Genomic Information for the 8 *a priori* Candidate Genes (B)

<i>a priori</i> candidate gene pathway	MaizeGDB Name	MaizeGDB Full Name	MaizeGDB Synonym(s)	RefGen_v2 Gene Model ID	Annotated Gene Function	RefGen_v2 Chromosome	RefGen_v2 ORF Start bp	RefGen_v2 ORF Stop bp
carotenoid_synthesis_and_degradation	cyp14	cytochrome P450 14	cyp14, CYP97C, lutein1, lut1, cytochrome P450-type monooxygenase CYP97C1	GRMZM2G143202	CYP97A3, Cytochrome P450 epsilon-ring hydroxylase	1	86838334	86848726
carotenoid_synthesis_and_degradation	hyd4	hydroxylase4	hyd4, bch1, crtRB3, HYD1, beta-carotene hydroxylase homolog, BCH1	GRMZM2G164318	Beta-carotene hydroxylase (non-heme dioxygenase type)	2	15865938	15868219
carotenoid_synthesis_and_degradation	zep1	zeaxanthin epoxidase1	zep1, fha5, TMR41	GRMZM2G127139	zeaxanthin epoxidase	2	44440299	44449237
carotenoid_synthesis_and_degradation	y1	yellow endosperm1	y1, y1ssr, rs131175743, rs130328408, y4, yellow endosperm1, white1, pb1, Psy1	GRMZM2G300348	phytoene synthase	6	82017148	82021007
carotenoid_synthesis_and_degradation	zds1	zeta carotene desaturase1	zds1, zeta carotene desaturase candidate, cl78_1(541), CL78_1	GRMZM2G454952	zeta-carotene desaturase	7	17470585	17479020
carotenoid_synthesis_and_degradation	lyce1	lycopene epsilon cyclase1	lyce1, lcye1, lycE, lcyE, LCY-E, lycopene epsilon cyclase1	GRMZM2G012966	lycopene epsilon-cyclase	8	138882594	138889812
carotenoid_synthesis_and_degradation	wc1	white cap1	wc1, ccd1, ZmCCD1, PCO084517, AY106323, IDP700, white cap1	GRMZM2G057243	*carotenoid cleavage dioxygenase1	9	152086899	152092882
carotenoid_synthesis_and_degradation	hyd3	hydroxylase3	hyd3, crtRB1, beta-carotene hydroxylase 1, CrtR-B1, bch2	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	10	136057100	136060219

Genomic information for the eight candidate genes that are hypothesized to be critical for marker-assisted selection for orange-colored maize kernels with high total carotenoid and provitamin A levels.

*Carotenoid cleavage enzymes fall into to major phyletic groups, the carotenoid cleavage dioxygenases (which generally have broad substrate specificity) and the NCED clade, which are involved in ABA synthesis and highly specific for 9-cis-epoxycarotenoids. Note that with the exception of ZmCCD1 and ZmNCED1 (vp14) maize carotenoid cleavage family membes have not had their biochemical activities determined. Nomenclature of other maize family members is relative to their most closely related sequencein Arabidopsis but this does not necessarily imply a corresponding biochemical activity for the maize enzyme. Nomenclature is as listed in maizeGDB v3.

Table S4 Genomic Prediction Model Specifications

GP Method	Parameters	Script details
RR-BLUP ^a	K = "RR"	Kinship.blup function in RR-BLUP R package : Jeff Endelman, 2011
LASSO ^b	$\alpha = 1$	cv.glmnet and predict functions in GLMNet R package : Jerome Friedman, Trevor Hastie, Rob Tibshirani, 2009
Elastic net	$\alpha = 0.8$	

^aRR-BLUP, Ridge regression best linear unbiased prediction

^bLASSO, Least absolute shrinkage and selection operator

^cGLMNet, Lasso and elastic net regularized generalized linear models

Table S5 BLUPs and Heritabilities for 9 Carotenoid Traits

Trait	No. Lines	BLUPs			Heritabilities	
		Mean	S.D. ^a	Range	Estimate	S.E. ^b
Phytofluene	199	0.90	0.42	0.20 - 2.22	0.65	0.058
ζ-Carotene	200	0.62	0.25	0.28 - 1.61	0.45	0.067
Tetrahydrolycopene	197	0.24	0.07	-0.06 - 0.52	0.60	0.067
Total β-Xanthophylls	195	14.10	7.62	0.89 - 35.95	0.96	0.006
Total α-Xanthophylls	200	12.07	5.40	1.50- 28.02	0.91	0.013
Provitamin A ^c /Total Carotenoids	199	0.07	0.03	0.02 - 0.20	0.86	0.023
Acyclic Carotenes/Cyclic Carotenes	190	0.08	0.06	0 - 0.30	0.74	0.028
β-Carotene/(β-Cryptoxanthin+Zeaxanthin)	196	0.10	0.06	0.03 - 0.38	0.93	0.015
Total Carotenes/Total Xanthophylls	190	0.15	0.05	0 - 0.32	0.62	0.056

Means and ranges (μg/g) for untransformed best linear unbiased predictors (BLUPs) of an additional 9 carotenoid grain traits evaluated on a maize inbred association panel, and estimated heritability on a line mean basis in two summer environments, in West Lafayette, IN, across two years.

^aS.D., Standard deviation.

^bS.E., Standard error.

^cProvitamin A is calculated as the sum of β-carotene, ½ α-carotene and ½ β-cryptoxanthin.

Table S6 Correlation Matrix for Untransformed BLUPs of the 24 Carotenoid Traits

	β -carotene	β -cryptoxanthin	Zeaxanthin	α -carotene	Zeinoxanthin	Lutein	Acyclic and Monocyclic Carotenes	Total Carotenoids	β -Carotenoids / α -Carotenoids	β -Xanthophylls/ α -Xanthophylls	β -Carotene/ β -Cryptoxanthin	β -Cryptoxanthin / Zeaxanthin	α -Carotene/Zeaxanthin	Zeinoxanthin/Lutein	Phytofluene	ζ -Carotene	Tetrahydrocyclopene	Total β -Xanthophylls	Total α -Xanthophylls	Provitamin A*	Provitamin A* / Total Carotenoids	Acyclic Carotenes / Cyclic Carotenes	β -Carotene / (β -Cryptoxanthin + Zeaxanthin)	Total Carotenes / Total Xanthophylls
β -carotene		0.43	0.38	0.37	0.03	-0.02	0.55	0.43	0.28	0.26	0.31	0.18	<0.01	0.06	0.05	-0.09	-0.05	0.40	0.01	0.89	0.74	-0.18	0.53	0.35
β -cryptoxanthin	<0.01		0.63	<0.01	0.25	-0.17	0.16	0.58	0.54	0.60	-0.38	0.51	-0.34	0.38	-0.08	-0.02	-0.12	0.73	-0.09	0.71	0.42	-0.23	-0.07	-0.17
Zeaxanthin	<0.01	<0.01		-0.06	<0.01	-0.09	0.24	0.71	0.65	0.71	-0.23	-0.07	-0.10	0.05	0.09	<0.01	-0.19	0.99	-0.10	0.51	0.10	-0.13	-0.27	-0.22
α -carotene	<0.01	0.95	0.38		0.34	0.53	0.62	0.38	-0.33	-0.32	0.24	0.20	0.11	0.16	0.13	0.19	-0.03	-0.05	0.58	0.44	0.27	-0.02	0.43	0.34
Zeinoxanthin	0.67	<0.01	0.99	<0.01		0.35	0.22	0.31	-0.19	-0.20	-0.16	0.42	-0.48	0.70	0.03	0.08	0.03	0.04	0.49	0.23	0.06	-0.08	0.01	-0.04
Lutein	0.80	0.01	0.20	<0.01	<0.01		0.36	0.40	-0.56	-0.57	0.02	0.04	0.02	<0.01	0.24	0.18	0.01	-0.09	0.97	0.03	-0.26	0.05	0.10	0.11
Acyclic and Monocyclic Carotenes	<0.01	0.02	<0.01	<0.01	<0.01	<0.01		0.62	-0.01	-0.06	0.16	0.13	0.03	0.15	0.56	0.50	0.02	0.24	0.38	0.57	0.33	0.32	0.37	0.44
Total Carotenoids	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01		0.25	0.22	-0.21	0.20	-0.18	0.24	0.27	0.30	-0.12	0.72	0.45	0.62	0.11	-0.02	-0.08	-0.02
β -Carotenoids/ α -Carotenoids	<0.01	<0.01	<0.01	<0.01	0.01	<0.01	0.93	<0.01		0.97	-0.17	0.04	-0.10	0.06	-0.15	-0.06	-0.04	0.65	-0.55	0.36	0.29	-0.13	-0.14	-0.16
β -Xanthophylls/ α -Xanthophylls	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.40	<0.01	<0.01		-0.20	<0.01	-0.08	-0.03	-0.09	-0.08	-0.14	0.71	-0.56	0.35	0.22	-0.11	-0.20	-0.19
β -Carotene/ β -Cryptoxanthin	<0.01	<0.01	<0.01	<0.01	0.03	0.74	0.02	<0.01	0.01	<0.01		-0.27	0.25	-0.20	-0.08	-0.13	0.11	-0.27	-0.01	0.08	0.27	<0.01	0.57	0.31
β -Cryptoxanthin/Zeaxanthin	0.01	<0.01	0.35	<0.01	<0.01	0.55	0.07	<0.01	0.56	0.98	<0.01		-0.35	0.48	-0.04	0.03	-0.01	0.03	0.16	0.36	0.41	-0.06	0.15	0.13
α -Carotene/Zeaxanthin	0.97	<0.01	0.17	0.10	<0.01	0.80	0.62	0.01	0.16	0.25	<0.01	<0.01		-0.48	0.06	-0.02	-0.01	-0.14	-0.08	-0.14	-0.07	0.03	0.10	0.20
Zeinoxanthin/Lutein	0.38	<0.01	0.51	0.03	<0.01	0.95	0.03	<0.01	0.43	0.64	<0.01	<0.01	<0.01		-0.03	0.08	0.02	0.08	0.14	0.24	0.24	-0.09	-0.01	-0.07
Phytofluene	0.50	0.24	0.21	0.07	0.70	<0.01	<0.01	<0.01	0.03	0.20	0.25	0.60	0.41	0.64		0.42	-0.10	0.07	0.25	-0.02	-0.14	0.48	0.05	0.29
ζ -Carotene	0.21	0.75	0.98	0.01	0.28	0.01	<0.01	<0.01	0.39	0.26	0.07	0.63	0.76	0.26	<0.01		-0.16	<0.01	0.20	-0.07	-0.15	0.42	-0.03	0.20
Tetrahydrocyclopene	0.45	0.08	0.01	0.67	0.67	0.90	0.79	0.08	0.59	0.05	0.14	0.87	0.93	0.73	0.16	0.02		-0.19	-0.02	-0.09	0.04	0.08	0.07	0.07
Total β -Xanthophylls	<0.01	<0.01	<0.01	0.49	0.62	0.19	<0.01	<0.01	<0.01	<0.01	<0.01	0.66	0.05	0.23	0.34	0.96	0.01		-0.08	0.57	0.15	-0.15	-0.25	-0.21
Total α -Xanthophylls	0.91	0.20	0.18	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.90	0.03	0.25	0.04	<0.01	<0.01	0.73	0.25		0.09	-0.22	0.07	0.10	0.11
Provitamin A*	<0.01	<0.01	<0.01	<0.01	<0.01	0.64	<0.01	<0.01	<0.01	<0.01	0.23	<0.01	0.05	<0.01	0.80	0.33	0.19	<0.01	0.19		0.74	-0.25	0.39	0.16
Provitamin A* / Total Carotenoids	<0.01	<0.01	0.18	<0.01	0.39	<0.01	<0.01	0.12	<0.01	<0.01	<0.01	<0.01	0.30	<0.01	0.04	0.03	0.58	0.03	<0.01	<0.01		-0.34	0.63	0.31
Acyclic Carotenes / Cyclic Carotenes	0.01	<0.01	0.06	0.81	0.28	0.50	<0.01	0.81	0.07	0.11	0.95	0.40	0.62	0.19	<0.01	<0.01	0.27	0.03	0.29	<0.01	<0.01		-0.10	0.39
β -Carotene / (β -Cryptoxanthin + Zeaxanthin)	<0.01	0.36	<0.01	<0.01	0.88	0.17	<0.01	0.25	0.04	0.01	<0.01	0.03	0.14	0.85	0.47	0.65	0.35	<0.01	0.16	<0.01	<0.01	0.16		0.66
Total Carotenes / Total Xanthophylls	<0.01	0.02	<0.01	<0.01	0.61	0.12	<0.01	0.80	0.02	0.01	<0.01	0.07	<0.01	0.31	<0.01	<0.01	0.32	<0.01	0.11	0.02	<0.01	<0.01	<0.01	

Pearson correlation coefficients are presented in the upper triangle, and the *P*-values for the significance of associations are in the lower triangle.

*Provitamin A is calculated as the sum of β -carotene, $\frac{1}{2}$ α -carotene and $\frac{1}{2}$ β -cryptoxanthin.

Table S7 Variance Component Estimates from Mixed Linear Models Fitted to 24 Grain Carotenoid Traits

	Trait	Genetic Variance Component	Environmental Variance Component	GxE ^a Variance Component
15 priority traits	Lutein	24.8787	1.5973	1.5368
	Zeinoxanthin	2.0509	0.2717	0.0286
	α-Carotene	0.2446	0.7396	0.0317
	α-Carotene/Zeinoxanthin	4.0856	0.4429	1.3267
	Zeinoxanthin/Lutein	0.0137	0.0018	0.0142
	Zeaxanthin	58.1510	3.6651	0.0000
	β-Cryptoxanthin	1.4692	0.0741	0.0691
	β-Carotene	0.5365	0.1189	0.1785
	β-Cryptoxanthin/Zeaxanthin	0.0099	0.0011	0.0015
	β-Carotene/β-Cryptoxanthin	0.6497	0.0792	0.5125
	Total Carotenoids	129.6260	12.7090	0.0000
	Acyclic and Monocyclic Carotenes	2.3351	1.7549	0.4304
	β-Xanthophylls/α-Xanthophylls	25.9938	5.3756	0.0000
	Provitamin A	1.5758	0.3941	0.2488
	β-Carotenoids/α-Carotenoids	1.9802	0.0337	0.0000
9 additional traits	ζ-Carotene	0.1592	0.1985	0.0697
	Phytofluene	0.2827	0.1545	0.1323
	Tetrahydrolycopene	0.0157	0.0103	0.0223
	Total β-Xanthophylls	74.3102	3.3002	0.0000
	Total α-Xanthophylls	35.2877	3.3811	0.0000
	Provitamin A/Total Carotenoids	0.0017	0.0003	0.0001
	β-Carotene/(β-Cryptoxanthin+Zeaxanthin)	0.0086	0.0007	0.0005
	Acyclic Carotenes/Cyclic Carotenes	0.0047	0.0017	0.0000
	Total Carotenoids/Total Xanthophylls	0.0053	0.0002	0.0031

Variance component estimates from mixed linear models fitted to each of the 24 maize grain traits. These mixed liner models included random effects accounting for genotype, environment, and their interaction.

^aGxE, The variance component accounting for the interaction between genotype and environment.

Table S8 Genome-wide Association Study Results with No Covariates (A)

<i>a priori</i> candidate gene pathway	RefGen_v2 Gene ID	Annotated gene containing associated SNP or gene within 3kb of associated SNP	Trait	SNP ID	SNP Source	Chr	Position in RefGen_v2	P-value	FDR-Adjusted P-value	Minor Allele Frequency (MAF)	Sample Size	MAF Tropical (8% of 201 Lines)	MAF Temperate (92% of 201 Lines)	R-square_LR from Model without SNP	R-square_LR from Model with SNP	Effect Size	Lambda from Box-Cox Procedure	Back-Transformed Effect Estimates
Carotenoid Synthesis and Degradation	GRMZM2G143202	lut1	α -Carotene/Zeinoxanthin	ss196425306	55K	1	86,844,203	3.47E-10	3.36E-05	0.31	196	0.40	0.28	0.17	0.35	0.06	-0.25	-0.22
Carotenoid Synthesis and Degradation	GRMZM2G143202	lut1	Zeinoxanthin/Lutein	ss196425306	55K	1	86,844,203	4.97E-08	7.19E-03	0.29	195	0.40	0.28	0.09	0.24	-0.19	-0.35	0.84
Carotenoid Synthesis and Degradation	GRMZM2G143202	lut1	Zeinoxanthin	ss196425306	55K	1	86,844,203	8.95E-08	1.30E-02	0.30	198	0.40	0.28	0.10	0.24	-0.11	-0.25	0.62
			α -Carotene/Zeinoxanthin	ss196425308	55K	1	86,945,134	3.47E-10	3.36E-05	0.31	196	0.40	0.27	0.17	0.35	0.06	-0.25	-0.22
			Zeinoxanthin/Lutein	ss196425308	55K	1	86,945,134	4.97E-08	7.19E-03	0.29	195	0.40	0.27	0.09	0.24	-0.19	-0.35	0.84
			Zeinoxanthin	ss196425308	55K	1	86,945,134	8.95E-08	1.30E-02	0.30	198	0.40	0.27	0.10	0.24	-0.11	-0.25	0.62
			Lutein	S1_96310268	GBS	1	96,310,268	3.71E-07	3.61E-02	0.17	200	0.06	0.21	0.17	0.28	1.19	0.80	1.67
Carotenoid Synthesis and Degradation	GRMZM2G127139	zep1	Zeaxanthin	S2_44448432	GBS	2	44,448,432	2.22E-09	3.22E-04	0.11	196	0.29	0.09	0.05	0.24	-0.34	0.35	-0.69
Carotenoid Synthesis and Degradation	GRMZM2G127139	zep1	Total β -Xanthophylls	S2_44448432	GBS	2	44,448,432	1.66E-08	2.41E-03	0.11	195	0.29	0.09	0.05	0.22	-0.43	0.40	-0.76
Carotenoid Synthesis and Degradation	GRMZM2G127139	zep1	β -Xanthophylls/ α -Xanthophylls	S2_44448432	GBS	2	44,448,432	4.82E-08	2.80E-03	0.11	196	0.29	0.09	0.15	0.29	0.13	-0.40	-0.26
Carotenoid Synthesis and Degradation	GRMZM2G127139	zep1	Zeaxanthin	S2_44448438	GBS	2	44,448,438	2.22E-09	3.22E-04	0.11	196	0.29	0.09	0.05	0.24	0.34	0.35	1.31
Carotenoid Synthesis and Degradation	GRMZM2G127139	zep1	Total β -Xanthophylls	S2_44448438	GBS	2	44,448,438	1.66E-08	2.41E-03	0.11	195	0.29	0.09	0.05	0.22	0.43	0.40	1.46
Carotenoid Synthesis and Degradation	GRMZM2G127139	zep1	β -Xanthophylls/ α -Xanthophylls	S2_44448438	GBS	2	44,448,438	4.82E-08	2.80E-03	0.11	196	0.29	0.09	0.15	0.29	-0.13	-0.40	0.42
			Zeaxanthin	S2_44473748	GBS	2	44,473,748	1.47E-06	4.27E-02	0.14	196	0.25	0.12	0.05	0.17	0.24	0.35	0.86
			β -Xanthophylls/ α -Xanthophylls	S2_44473748	GBS	2	44,473,748	5.21E-06	9.00E-02	0.14	196	0.25	0.12	0.15	0.24	-0.09	-0.40	0.28
			Zeaxanthin	S2_44473758	GBS	2	44,473,758	1.47E-06	4.27E-02	0.14	196	0.24	0.12	0.05	0.17	-0.24	0.35	-0.55
			β -Xanthophylls/ α -Xanthophylls	S2_44473758	GBS	2	44,473,758	5.21E-06	9.00E-02	0.14	196	0.24	0.12	0.15	0.24	0.09	-0.40	-0.20
			Zeaxanthin	S2_44473801	GBS	2	44,473,801	1.47E-06	4.27E-02	0.14	196	0.24	0.12	0.05	0.17	0.24	0.35	0.86
			β -Xanthophylls/ α -Xanthophylls	S2_44473801	GBS	2	44,473,801	5.21E-06	9.00E-02	0.14	196	0.24	0.12	0.15	0.24	-0.09	-0.40	0.28
			β -Xanthophylls/ α -Xanthophylls	S2_44474139	GBS	2	44,474,139	7.57E-06	9.64E-02	0.14	196	0.29	0.13	0.15	0.24	0.09	-0.40	-0.20
			Zeaxanthin	S2_44474308	GBS	2	44,474,308	1.19E-06	4.27E-02	0.21	196	0.38	0.28	0.05	0.17	0.21	0.35	0.73
			β -Xanthophylls/ α -Xanthophylls	S2_44474308	GBS	2	44,474,308	7.03E-06	9.64E-02	0.21	196	0.38	0.28	0.15	0.24	-0.08	-0.40	0.24
			Zeaxanthin	S3_169734997	GBS	3	169,734,997	1.16E-06	4.27E-02	0.06	196	0.25	0.07	0.05	0.17	-0.42	0.35	-0.79
			Total β -Xanthophylls	S3_169734997	GBS	3	169,734,997	1.04E-06	5.00E-02	0.06	195	0.25	0.07	0.05	0.18	-0.58	0.40	-0.89
			Total α -Xanthophylls	ss196456701	55K	4	146,977,283	1.00E-06	9.76E-02	0.12	200	0.38	0.08	0.10	0.22	-0.87	0.70	-0.95
			β -Cryptoxanthin	S7_13843351	GBS	7	13,843,351	1.66E-07	4.84E-02	0.15	199	0.10	0.16	0.11	0.24	-0.04	0.10	-0.33
			Zeinoxanthin	S7_15282645	GBS	7	15,282,645	2.34E-07	2.27E-02	0.17	198	0.42	0.20	0.10	0.23	-0.12	-0.25	0.65
			β -Xanthophylls/ α -Xanthophylls	ss196477160	55K	7	51,472,566	5.57E-06	9.00E-02	0.43	196	0.43	0.43	0.15	0.24	0.07	-0.40	-0.16
			β -Xanthophylls/ α -Xanthophylls	S8_129072699	GBS	8	129,072,699	6.48E-06	9.40E-02	0.36	196	0.50	0.35	0.15	0.24	0.07	-0.40	-0.15
			β -Xanthophylls/ α -Xanthophylls	S8_129124626	GBS	8	129,124,626	8.20E-06	9.91E-02	0.29	196	0.27	0.31	0.15	0.24	-0.07	-0.40	0.20
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	Total α -Xanthophylls	<i>lycE</i> 5'TE	Additional Markers	8	138,882,481	4.37E-09	6.39E-04	NA	NA	NA	NA	.10	.43	NA	0.70	NA
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	Lutein	<i>lycE</i> 5'TE	Additional Markers	8	138,882,481	1.75E-08	2.56E-03	NA	NA	NA	NA	.11	.49	NA	0.80	NA
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β -Xanthophylls/ α -Xanthophylls	ss196504160	55K	8	138,882,711	1.11E-09	1.61E-04	0.35	196	0.48	0.34	0.15	0.33	0.11	-0.40	-0.22

Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β -Carotenoids/ α -Carotenoids	ss196504160	55K	8	138,882,711	2.08E-09	6.00E-04	0.36	190	0.48	0.34	0.18	0.35	0.12	-0.85	-0.12
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β -Xanthophylls/ α -Xanthophylls	S8_138882711	GBS	8	138,882,711	8.85E-07	2.37E-02	0.28	196	0.41	0.30	0.15	0.26	-0.09	-0.40	0.25
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	Total β -Xanthophylls	ss196504160	55K	8	138,882,711	1.36E-06	5.61E-02	0.35	195	0.48	0.34	0.05	0.18	-0.26	0.40	-0.53
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	Zeaxanthin	ss196504160	55K	8	138,882,711	2.30E-06	6.05E-02	0.35	196	0.48	0.34	0.05	0.16	-0.19	0.35	-0.45
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β -Xanthophylls/ α -Xanthophylls	S8_138882747	GBS	8	138,882,747	8.85E-07	2.37E-02	0.28	196	0.41	0.30	0.15	0.26	-0.09	-0.40	0.25
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β -Xanthophylls/ α -Xanthophylls	S8_138882751	GBS	8	138,882,751	8.85E-07	2.37E-02	0.28	196	0.41	0.30	0.15	0.26	-0.09	-0.40	0.25
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β -Xanthophylls/ α -Xanthophylls	S8_138882798	GBS	8	138,882,798	8.99E-07	2.37E-02	0.31	196	0.21	0.36	0.15	0.26	-0.08	-0.40	0.25
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β -Xanthophylls/ α -Xanthophylls	S8_138882897	GBS	8	138,882,897	9.76E-08	4.72E-03	0.43	196	0.12	0.44	0.15	0.28	-0.08	-0.40	0.25
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β -Carotenoids/ α -Carotenoids	S8_138882897	GBS	8	138,882,897	6.03E-08	8.70E-03	0.44	190	0.12	0.44	0.18	0.32	-0.10	-0.85	0.13
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β -Xanthophylls/ α -Xanthophylls	S8_138883026	GBS	8	138,883,026	5.90E-06	9.00E-02	0.40	196	0.18	0.48	0.15	0.24	0.07	-0.40	-0.16
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β -Xanthophylls/ α -Xanthophylls	S8_138883056	GBS	8	138,883,056	5.90E-06	9.00E-02	0.40	196	0.18	0.48	0.15	0.24	-0.07	-0.40	0.21
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β -Xanthophylls/ α -Xanthophylls	<i>lycE</i> SNP216	Additional Markers	8	138,883,206	5.05E-16	1.46E-10	NA	NA	NA	NA	.10	.24	NA	-0.40	NA
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	Total α -Xanthophylls	<i>lycE</i> SNP216	Additional Markers	8	138,883,206	4.62E-10	1.35E-04	NA	NA	NA	NA	.10	.40	NA	0.70	NA
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	Lutein	<i>lycE</i> SNP216	Additional Markers	8	138,883,206	6.28E-09	1.84E-03	NA	NA	NA	NA	.11	.45	NA	0.80	NA
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	Total β -Xanthophylls	<i>lycE</i> SNP216	Additional Markers	8	138,883,206	1.65E-07	1.20E-02	NA	NA	NA	NA	.06	.30	NA	0.40	NA
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β -Xanthophylls/ α -Xanthophylls	PZB00665.1	4K	8	138,886,137	3.82E-06	8.52E-02	0.35	196	0.05	0.38	0.15	0.25	0.08	-0.40	-0.17
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β -Xanthophylls/ α -Xanthophylls	S8_138888278	GBS	8	138,888,278	2.52E-08	2.44E-03	0.47	196	0.19	0.42	0.15	0.30	-0.09	-0.40	0.28
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β -Carotenoids/ α -Carotenoids	S8_138888278	GBS	8	138,888,278	1.82E-07	1.75E-02	0.47	190	0.19	0.42	0.18	0.31	-0.10	-0.85	0.13
			β -Xanthophylls/ α -Xanthophylls	ss196508843	55K	8	139,143,878	8.80E-07	2.37E-02	0.29	196	0.38	0.26	0.15	0.26	0.08	-0.40	-0.18
			β -Carotenoids/ α -Carotenoids	S8_140192724	GBS	8	140,192,724	9.64E-07	6.95E-02	0.34	190	0.19	0.28	0.18	0.29	-0.09	-0.85	0.12
			β -Xanthophylls/ α -Xanthophylls	S8_140192724	GBS	8	140,192,724	3.49E-06	8.43E-02	0.33	196	0.19	0.28	0.15	0.25	-0.08	-0.40	0.22
			Total β -Xanthophylls	S8_171705545	GBS	8	171,705,545	5.15E-07	2.98E-02	0.10	195	0.14	0.14	0.05	0.19	-0.40	0.40	-0.72

			Zeaxanthin	S8_171705545	GBS	8	171,705,545	8.40E-07	4.27E-02	0.11	196	0.14	0.14	0.05	0.17	-0.28	0.35	-0.61
			Total β-Xanthophylls	S8_171705574	GBS	8	171,705,574	1.61E-07	1.20E-02	0.10	195	0.25	0.13	0.05	0.20	-0.42	0.40	-0.74
			Zeaxanthin	S8_171705574	GBS	8	171,705,574	2.39E-07	2.31E-02	0.11	196	0.25	0.13	0.05	0.19	-0.29	0.35	-0.63
			α-Carotene/Zeaxanthin	ss196491114	55K	9	69,215,031	3.31E-10	3.36E-05	0.31	196	0.37	0.29	0.17	0.35	-0.06	-0.25	0.30
			Zeinoxanthin/Lutein	ss196491114	55K	9	69,215,031	9.80E-08	9.46E-03	0.28	195	0.37	0.29	0.09	0.23	0.19	-0.35	-0.39
			Zeinoxanthin	ss196491114	55K	9	69,215,031	3.76E-07	2.74E-02	0.30	198	0.37	0.29	0.10	0.23	0.11	-0.25	-0.34
			β-Xanthophylls/α-Xanthophylls	ss196493105	55K	9	118,437,281	7.64E-06	9.64E-02	0.29	196	0.19	0.42	0.15	0.24	-0.09	-0.40	0.25
Carotenoid Synthesis and Degradation	GRMZM2G152135	crtRB1	β-Carotene/(β-Cryptoxanthin+Zeaxanthin)	crtRB1 InDel4	Additional Markers	10	136,059,748	2.23E-07	5.10E-02	NA	NA	NA	NA	.06	.11	NA	-0.25	NA
Carotenoid Synthesis and Degradation	GRMZM2G152135	crtRB1	β-Carotene/(β-Cryptoxanthin+Zeaxanthin)	ss196501627	55K	10	136,060,033	3.51E-07	5.10E-02	0.19	196	0.00	0.22	0.04	0.18	0.12	-0.25	-0.36
Carotenoid Synthesis and Degradation	GRMZM2G152135	crtRB1	Zeaxanthin	crtRB1 3'TE	Additional Markers	10	136,061,719	1.11E-06	4.27E-02	NA	NA	NA	NA	.05	.17	NA	0.35	NA
Carotenoid Synthesis and Degradation	GRMZM2G152135	crtRB1	Total β-Xanthophylls	crtRB1 3'TE	Additional Markers	10	136,061,719	1.97E-06	7.13E-02	NA	NA	NA	NA	.06	.18	NA	0.40	NA

Statistically significant results from genome-wide association studies on 24 grain carotenoid traits without any markers tagging peak GWAS signals included as covariates. Markers (Column E) that were significantly associated with the indicated trait (Column D) at 5% false discovery rate (FDR) are demarcated with boldface font and those significant only at 10% FDR without boldface font.

Table S8 Genome-wide Association Study Results with Covariate from *zep1* (B)

<i>a priori</i> candidate gene pathway	RefGen_v2 Gene ID	Annotated gene containing associated SNP or gene within 3kb of associated SNP	Trait	SNP ID	SNP Source	Chr	Position in RefGen_v2	P-value	FDR-Adjusted P-value	Minor Allele Frequency (MAF)	Sample Size	MAF Tropical (8% of 201 Lines)	MAF Temperate (92% of 201 Lines)	R-square_LR from Model without SNP	R-square_LR from Model with SNP	Effect Size	Lambda from Box-Cox Procedure	Back-Transformed Effect Estimates
Carotenoid Synthesis and Degradation	GRMZM2G143202	lut1	α -Carotene/Zeaxanthin	ss196425306	55K	1	86,844,203	3.47E-10	3.36E-05	0.31	196	0.40	0.28	0.17	0.35	0.06	-0.25	-0.22
Carotenoid Synthesis and Degradation	GRMZM2G143202	lut1	Zeaxanthin/Lutein	ss196425306	55K	1	86,844,203	4.97E-08	7.19E-03	0.29	195	0.40	0.28	0.09	0.24	-0.19	-0.35	0.84
Carotenoid Synthesis and Degradation	GRMZM2G143202	lut1	Zeaxanthin	ss196425306	55K	1	86,844,203	8.95E-08	1.30E-02	0.30	198	0.40	0.28	0.10	0.24	-0.11	-0.25	0.62
			α -Carotene/Zeaxanthin	ss196425308	55K	1	86,945,134	3.47E-10	3.36E-05	0.31	196	0.40	0.27	0.17	0.35	0.06	-0.25	-0.22
			Zeaxanthin/Lutein	ss196425308	55K	1	86,945,134	4.97E-08	7.19E-03	0.29	195	0.40	0.27	0.09	0.24	-0.19	-0.35	0.84
			Zeaxanthin	ss196425308	55K	1	86,945,134	8.95E-08	1.30E-02	0.30	198	0.40	0.27	0.10	0.24	-0.11	-0.25	0.62
			Lutein	S1_96310268	GBS	1	96,310,268	3.30E-07	3.22E-02	0.17	200	0.06	0.21	0.12	0.24	1.23	0.80	1.73
			β -Carotene/ β -Cryptoxanthin	S2_228979822	GBS	2	228,979,822	5.38E-07	7.85E-02	0.08	198	0.30	0.05	0.10	0.22	-0.17	-0.70	0.31
			Total α -Xanthophylls	ss196456701	55K	4	146,977,283	1.00E-06	9.76E-02	0.12	200	0.38	0.08	0.10	0.22	-0.87	0.70	-0.95
			β -Cryptoxanthin	S7_13843351	GBS	7	13,843,351	1.66E-07	4.84E-02	0.15	199	0.10	0.16	0.11	0.24	-0.04	0.10	-0.33
			Zeaxanthin	S7_15282645	GBS	7	15,282,645	2.34E-07	2.27E-02	0.17	198	0.42	0.20	0.10	0.23	-0.12	-0.25	0.65
			β -Xanthophylls/ α -Xanthophylls	ss196477156	55K	7	51,471,492	1.02E-05	7.84E-02	0.40	196	0.43	0.39	0.29	0.37	0.06	-0.40	-0.14
			β -Xanthophylls/ α -Xanthophylls	ss196477160	55K	7	51,472,566	1.06E-06	2.56E-02	0.43	196	0.43	0.43	0.29	0.38	0.07	-0.40	-0.16
			β -Xanthophylls/ α -Xanthophylls	ss196477229	55K	7	51,645,966	1.04E-05	7.84E-02	0.49	196	0.43	0.49	0.29	0.37	-0.06	-0.40	0.18
			β -Xanthophylls/ α -Xanthophylls	ss196477237	55K	7	51,806,575	1.04E-05	7.84E-02	0.49	196	0.43	0.50	0.29	0.37	0.06	-0.40	-0.14
			β -Xanthophylls/ α -Xanthophylls	ss196477251	55K	7	51,981,502	1.04E-05	7.84E-02	0.49	196	0.43	0.50	0.29	0.37	0.06	-0.40	-0.14
			β -Xanthophylls/ α -Xanthophylls	ss196477253	55K	7	51,997,363	1.04E-05	7.84E-02	0.49	196	0.43	0.49	0.29	0.37	0.06	-0.40	-0.14
			β -Xanthophylls/ α -Xanthophylls	ss196477265	55K	7	52,248,496	1.04E-05	7.84E-02	0.49	196	0.43	0.48	0.29	0.37	-0.06	-0.40	0.18
			β -Xanthophylls/ α -Xanthophylls	ss196477269	55K	7	52,290,305	1.04E-05	7.84E-02	0.49	196	0.43	0.50	0.29	0.37	-0.06	-0.40	0.18
			Total β -Xanthophylls	S8_27117706	GBS	8	27,117,706	1.52E-06	6.30E-02	0.17	195	0.25	0.17	0.22	0.32	-0.29	0.40	-0.57
			Zeaxanthin	S8_27117706	GBS	8	27,117,706	2.00E-06	8.27E-02	0.17	196	0.25	0.17	0.24	0.33	-0.21	0.35	-0.48
			β -Xanthophylls/ α -Xanthophylls	PZB01094.1	4K	8	27,117,892	5.82E-06	7.04E-02	0.21	196	0.29	0.21	0.29	0.37	-0.08	-0.40	0.22
			Total β -Xanthophylls	S8_27118357	GBS	8	27,118,357	1.86E-06	6.74E-02	0.19	195	0.48	0.18	0.22	0.32	-0.27	0.40	-0.55
			Zeaxanthin	S8_27118357	GBS	8	27,118,357	2.91E-06	9.36E-02	0.19	196	0.48	0.18	0.24	0.33	-0.19	0.35	-0.46
			β -Xanthophylls/ α -Xanthophylls	S8_111289041	GBS	8	111,289,041	3.06E-06	4.94E-02	0.40	196	0.05	0.34	0.29	0.38	0.07	-0.40	-0.16
			β -Carotenoids/ α -Carotenoids	S8_112713556	GBS	8	112,713,556	2.23E-06	4.28E-02	0.18	189	0.27	0.17	0.29	0.38	0.10	-0.85	-0.11
			β -Xanthophylls/ α -Xanthophylls	ss196516758	55K	8	112,713,556	4.09E-06	5.65E-02	0.20	196	0.45	0.17	0.29	0.37	-0.08	-0.40	0.24
			β -Carotenoids/ α -Carotenoids	ss196516758	55K	8	112,713,556	4.34E-06	7.81E-02	0.21	189	0.45	0.17	0.29	0.37	-0.09	-0.85	0.12
			β -Xanthophylls/ α -Xanthophylls	S8_112713556	GBS	8	112,713,556	8.41E-06	7.84E-02	0.17	196	0.27	0.17	0.29	0.37	0.08	-0.40	-0.18
			β -Xanthophylls/ α -Xanthophylls	S8_123811152	GBS	8	123,811,152	1.02E-05	7.84E-02	0.18	196	0.47	0.19	0.29	0.37	0.07	-0.40	-0.16
			β -Carotenoids/ α -Carotenoids	S8_123811152	GBS	8	123,811,152	5.18E-06	8.78E-02	0.19	189	0.47	0.19	0.29	0.37	0.09	-0.85	-0.09
			β -Xanthophylls/ α -Xanthophylls	ss196516738	55K	8	124,488,144	1.43E-06	3.19E-02	0.20	196	0.00	0.24	0.29	0.38	-0.09	-0.40	0.28
			β -Carotenoids/ α -Carotenoids	ss196516738	55K	8	124,488,144	6.21E-06	9.94E-02	0.21	189	0.00	0.24	0.29	0.37	-0.10	-0.85	0.14
			β -Xanthophylls/ α -Xanthophylls	S8_129072699	GBS	8	129,072,699	2.05E-06	3.88E-02	0.36	196	0.50	0.35	0.29	0.38	0.07	-0.40	-0.15
			β -Xanthophylls/ α -Xanthophylls	S8_129080393	GBS	8	129,080,393	5.19E-06	6.55E-02	0.47	196	0.21	0.38	0.29	0.37	-0.06	-0.40	0.17
			β -Xanthophylls/ α -Xanthophylls	S8_129080428	GBS	8	129,080,428	1.46E-05	9.64E-02	0.39	196	0.21	0.49	0.29	0.36	-0.06	-0.40	0.16
			β -Xanthophylls/ α -Xanthophylls	S8_129122614	GBS	8	129,122,614	8.88E-06	7.84E-02	0.47	196	0.20	0.39	0.29	0.37	-0.06	-0.40	0.16
			β -Xanthophylls/ α -Xanthophylls	S8_129122646	GBS	8	129,122,646	8.88E-06	7.84E-02	0.47	196	0.20	0.39	0.29	0.37	-0.06	-0.40	0.16

			β -Xanthophylls/ α -Xanthophylls	S8_129124046	GBS	8	129,124,046	3.63E-06	5.55E-02	0.34	196	0.50	0.35	0.29	0.37	0.06	-0.40	-0.14
			β-Xanthophylls/α-Xanthophylls	S8_129124626	GBS	8	129,124,626	2.42E-06	4.13E-02	0.29	196	0.27	0.31	0.29	0.38	-0.07	-0.40	0.19
			β -Xanthophylls/ α -Xanthophylls	S8_129135865	GBS	8	129,135,865	1.45E-05	9.64E-02	0.32	196	0.47	0.34	0.29	0.36	0.06	-0.40	-0.14
			β -Xanthophylls/ α -Xanthophylls	S8_129137347	GBS	8	129,137,347	6.90E-06	7.84E-02	0.41	196	0.15	0.48	0.29	0.37	0.06	-0.40	-0.14
			β -Xanthophylls/ α -Xanthophylls	ss196512938	55K	8	129,291,444	1.08E-05	7.84E-02	0.34	196	0.14	0.36	0.29	0.37	-0.06	-0.40	0.18
			β -Xanthophylls/ α -Xanthophylls	S8_129313857	GBS	8	129,313,857	1.36E-05	9.41E-02	0.29	196	0.05	0.34	0.29	0.36	-0.07	-0.40	0.19
			β -Xanthophylls/ α -Xanthophylls	S8_130513659	GBS	8	130,513,659	1.31E-05	9.27E-02	0.14	196	0.33	0.14	0.29	0.36	-0.08	-0.40	0.23
			β -Xanthophylls/ α -Xanthophylls	S8_130930928	GBS	8	130,930,928	4.37E-06	5.76E-02	0.09	196	0.42	0.05	0.29	0.37	0.11	-0.40	-0.24
			β-Carotenoids/α-Carotenoids	ss196486757	55K	8	131,113,149	2.15E-06	4.28E-02	0.41	189	0.33	0.44	0.29	0.38	-0.08	-0.85	0.10
			β -Xanthophylls/ α -Xanthophylls	ss196486757	55K	8	131,113,149	7.62E-06	7.84E-02	0.42	196	0.33	0.44	0.29	0.37	-0.06	-0.40	0.17
			β -Xanthophylls/ α -Xanthophylls	ss196486759	55K	8	131,124,166	1.06E-05	7.84E-02	0.34	196	0.19	0.36	0.29	0.37	-0.06	-0.40	0.17
			β-Carotenoids/α-Carotenoids	S8_131533827	GBS	8	131,533,827	8.54E-07	3.51E-02	0.30	189	0.13	0.37	0.29	0.39	0.09	-0.85	-0.10
			β -Xanthophylls/ α -Xanthophylls	S8_131533827	GBS	8	131,533,827	3.86E-06	5.60E-02	0.29	196	0.13	0.37	0.29	0.37	0.07	-0.40	-0.16
			Zeaxanthin	S8_137047040	GBS	8	137,047,040	2.68E-06	9.36E-02	0.32	196	0.15	0.36	0.24	0.33	0.17	0.35	0.55
			β-Xanthophylls/α-Xanthophylls	ss196501608	55K	8	138,514,315	2.05E-06	3.88E-02	0.41	196	0.40	0.44	0.29	0.38	-0.07	-0.40	0.19
			β-Carotenoids/α-Carotenoids	ss196501608	55K	8	138,514,315	2.06E-06	4.28E-02	0.41	189	0.40	0.44	0.29	0.38	-0.08	-0.85	0.10
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β -Xanthophylls/ α -Xanthophylls	<i>lycE</i> 5'TE	Additional Markers	8	138,882,481	7.24E-14	2.10E-08	NA	196	NA	NA	.21	.30	NA	-0.40	NA
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	Total α -Xanthophylls	<i>lycE</i> 5'TE	Additional Markers	8	138,882,481	4.85E-09	7.09E-04	NA	200	NA	NA	.09	.43	NA	0.70	NA
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	Lutein	<i>lycE</i> 5'TE	Additional Markers	8	138,882,481	1.92E-08	2.81E-03	NA	200	NA	NA	.11	.49	NA	0.80	NA
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	Zeaxanthin	<i>lycE</i> 5'TE	Additional Markers	8	138,882,481	1.19E-07	1.30E-02	NA	196	NA	NA	.24	.38	NA	0.35	NA
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	Total β -Xanthophylls	<i>lycE</i> 5'TE	Additional Markers	8	138,882,481	3.22E-07	2.33E-02	NA	195	NA	NA	.22	.40	NA	0.40	NA
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β -Xanthophylls/ α -Xanthophylls	ss196504160	55K	8	138,882,711	1.32E-10	1.91E-05	0.35	196	0.48	0.34	0.29	0.46	0.10	-0.40	-0.22
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β -Carotenoids/ α -Carotenoids	ss196504160	55K	8	138,882,711	4.23E-10	1.22E-04	0.37	189	0.48	0.34	0.29	0.45	0.11	-0.85	-0.12
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β -Xanthophylls/ α -Xanthophylls	S8_138882711	GBS	8	138,882,711	2.59E-07	7.52E-03	0.28	196	0.41	0.30	0.29	0.40	-0.08	-0.40	0.24
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	Zeaxanthin	ss196504160	55K	8	138,882,711	1.37E-07	1.30E-02	0.35	196	0.48	0.34	0.24	0.36	-0.19	0.35	-0.45
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	Total β -Xanthophylls	ss196504160	55K	8	138,882,711	1.19E-07	1.73E-02	0.35	195	0.48	0.34	0.22	0.35	-0.27	0.40	-0.54
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β -Carotenoids/ α -Carotenoids	S8_138882711	GBS	8	138,882,711	1.85E-06	4.28E-02	0.29	189	0.41	0.30	0.29	0.38	-0.09	-0.85	0.11
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β -Xanthophylls/ α -Xanthophylls	S8_138882747	GBS	8	138,882,747	2.59E-07	7.52E-03	0.28	196	0.41	0.30	0.29	0.40	-0.08	-0.40	0.24
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β -Carotenoids/ α -Carotenoids	S8_138882747	GBS	8	138,882,747	1.85E-06	4.28E-02	0.29	189	0.41	0.30	0.29	0.38	-0.09	-0.85	0.11
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β -Xanthophylls/ α -Xanthophylls	S8_138882751	GBS	8	138,882,751	2.59E-07	7.52E-03	0.28	196	0.41	0.30	0.29	0.40	-0.08	-0.40	0.24
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β -Carotenoids/ α -Carotenoids	S8_138882751	GBS	8	138,882,751	1.85E-06	4.28E-02	0.29	189	0.41	0.30	0.29	0.38	-0.09	-0.85	0.11
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β -Xanthophylls/ α -Xanthophylls	S8_138882798	GBS	8	138,882,798	8.75E-08	4.68E-03	0.31	196	0.21	0.36	0.29	0.40	-0.08	-0.40	0.25
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β -Carotenoids/ α -Carotenoids	S8_138882798	GBS	8	138,882,798	4.77E-07	2.29E-02	0.31	189	0.21	0.36	0.29	0.39	-0.09	-0.85	0.12

Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β -Xanthophylls/ α -Xanthophylls	S8_138882897	GBS	8	138,882,897	1.52E-08	1.10E-03	0.43	196	0.12	0.44	0.29	0.42	-0.08	-0.40	0.24
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β -Carotenoids/ α -Carotenoids	S8_138882897	GBS	8	138,882,897	1.27E-08	1.83E-03	0.43	189	0.12	0.44	0.29	0.42	-0.10	-0.85	0.13
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β -Xanthophylls/ α -Xanthophylls	S8_138883026	GBS	8	138,883,026	1.13E-07	4.68E-03	0.40	196	0.18	0.48	0.29	0.40	0.08	-0.40	-0.17
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β -Carotenoids/ α -Carotenoids	S8_138883026	GBS	8	138,883,026	2.13E-07	1.23E-02	0.40	189	0.18	0.48	0.29	0.40	0.09	-0.85	-0.10
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β -Xanthophylls/ α -Xanthophylls	S8_138883056	GBS	8	138,883,056	1.13E-07	4.68E-03	0.40	196	0.18	0.48	0.29	0.40	-0.08	-0.40	0.23
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β -Carotenoids/ α -Carotenoids	S8_138883056	GBS	8	138,883,056	2.13E-07	1.23E-02	0.40	189	0.18	0.48	0.29	0.40	-0.09	-0.85	0.12
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	Total α -Xanthophylls	<i>lycE</i> SNP216	Additional Markers	8	138,883,206	4.71E-10	1.38E-04	NA	200	NA	NA	.09	.40	NA	0.70	NA
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	Zeaxanthin	<i>lycE</i> SNP216	Additional Markers	8	138,883,206	1.57E-09	4.54E-04	NA	196	NA	NA	.24	.39	NA	0.35	NA
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	Total β -Xanthophylls	<i>lycE</i> SNP216	Additional Markers	8	138,883,206	5.83E-09	1.69E-03	NA	195	NA	NA	.22	.40	NA	0.40	NA
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	Lutein	<i>lycE</i> SNP216	Additional Markers	8	138,883,206	6.78E-09	1.98E-03	NA	200	NA	NA	.11	.45	NA	0.80	NA
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β -Xanthophylls/ α -Xanthophylls	PZB00665.1	4K	8	138,886,137	1.03E-05	7.84E-02	0.35	196	0.05	0.38	0.29	0.37	0.07	-0.40	-0.15
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β -Xanthophylls/ α -Xanthophylls	S8_138888278	GBS	8	138,888,278	2.25E-09	2.17E-04	0.47	196	0.19	0.42	0.29	0.44	-0.09	-0.40	0.28
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β -Carotenoids/ α -Carotenoids	S8_138888278	GBS	8	138,888,278	3.13E-08	3.01E-03	0.47	189	0.19	0.42	0.29	0.41	-0.10	-0.85	0.13
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	Zeaxanthin	S8_138888278	GBS	8	138,888,278	3.36E-06	9.74E-02	0.46	196	0.19	0.42	0.24	0.33	0.16	0.35	0.53
			β -Xanthophylls/ α -Xanthophylls	ss196508843	55K	8	139,143,878	4.62E-07	1.22E-02	0.29	196	0.38	0.26	0.29	0.39	0.08	-0.40	-0.17
			β -Carotenoids/ α -Carotenoids	ss196508843	55K	8	139,143,878	1.95E-06	4.28E-02	0.31	189	0.38	0.26	0.29	0.38	0.08	-0.85	-0.09
			β -Xanthophylls/ α -Xanthophylls	S8_140192724	GBS	8	140,192,724	2.14E-06	3.88E-02	0.33	196	0.19	0.28	0.29	0.38	-0.07	-0.40	0.20
			β -Carotenoids/ α -Carotenoids	S8_140192724	GBS	8	140,192,724	1.45E-06	4.28E-02	0.34	189	0.19	0.28	0.29	0.38	-0.08	-0.85	0.11
			Total β -Xanthophylls	S8_171705545	GBS	8	171,705,545	7.56E-07	3.65E-02	0.10	195	0.14	0.14	0.22	0.33	-0.36	0.40	-0.67
			Zeaxanthin	S8_171705545	GBS	8	171,705,545	1.04E-06	5.03E-02	0.11	196	0.14	0.14	0.24	0.34	-0.25	0.35	-0.56
			Zeaxanthin	S8_171705574	GBS	8	171,705,574	2.84E-07	1.64E-02	0.11	196	0.25	0.13	0.24	0.35	-0.26	0.35	-0.58
			Total β -Xanthophylls	S8_171705574	GBS	8	171,705,574	2.31E-07	2.23E-02	0.10	195	0.25	0.13	0.22	0.34	-0.38	0.40	-0.69
			α -Carotene/Zeaxanthin	ss196491114	55K	9	69,215,031	3.31E-10	3.36E-05	0.31	196	0.37	0.29	0.17	0.35	-0.06	-0.25	0.30
			Zeinoxanthin/Lutein	ss196491114	55K	9	69,215,031	9.80E-08	9.46E-03	0.28	195	0.37	0.29	0.09	0.23	0.19	-0.35	-0.39
			Zeinoxanthin	ss196491114	55K	9	69,215,031	3.76E-07	2.74E-02	0.30	198	0.37	0.29	0.10	0.23	0.11	-0.25	-0.34
Carotenoid Synthesis and Degradation	GRMZM2G152135	crtRB1	β -Carotene/(β -Cryptoxanthin+Zeaxanthin)	<i>crtRB1</i> InDel4	Additional Markers	10	136,059,748	2.14E-07	5.10E-02	NA	196	NA	NA	.04	.09	NA	-0.25	NA
Carotenoid Synthesis and Degradation	GRMZM2G152135	crtRB1	β -Carotene/ β -Cryptoxanthin	<i>crtRB1</i> InDel4	Additional Markers	10	136,059,748	5.29E-07	7.85E-02	NA	198	NA	NA	.07	.07	NA	-0.70	NA
Carotenoid Synthesis and Degradation	GRMZM2G152135	crtRB1	β -Carotene/(β -Cryptoxanthin+Zeaxanthin)	ss196501627	55K	10	136,060,033	3.51E-07	5.10E-02	0.19	196	0.00	0.22	0.04	0.18	0.12	-0.25	-0.36
Carotenoid Synthesis and Degradation	GRMZM2G152135	crtRB1	Zeaxanthin	<i>crtRB1</i> 3'TE	Additional Markers	10	136,061,719	1.79E-07	1.30E-02	NA	196	NA	NA	.24	.31	NA	0.35	NA
Carotenoid Synthesis and Degradation	GRMZM2G152135	crtRB1	Total β -Xanthophylls	<i>crtRB1</i> 3'TE	Additional Markers	10	136,061,719	7.09E-07	3.65E-02	NA	195	NA	NA	.22	.29	NA	0.40	NA
Carotenoid Synthesis and Degradation	GRMZM2G152135	crtRB1	Total Carotenes/Total Xanthophylls	<i>crtRB1</i> 3'TE	Additional Markers	10	136,061,719	1.89E-07	5.43E-02	NA	188	NA	NA	.05	.15	NA	-0.55	NA

Statistically significant results from genome-wide association studies on 24 grain carotenoid traits with the peak SNP tagging the GWAS signal from *zep1* included as a covariate. Markers (Column E) that were significantly associated with the indicated trait (Column D) at 5% false discovery rate (FDR) are demarcated with boldface font and those significant only at 10% FDR without boldface font. Note that addition of the significant marker *crtRB1* InDel4 did not improve the partial R-square value of the model for the β -Carotene/ β -Cryptoxanthin trait due to taxa that had missing data for the marker state.

Table S8 Genome-wide Association Study Results with Covariate from *lut1* (C)

<i>a priori</i> candidate gene pathway	RefGen_v2 Gene ID	Annotated gene containing associated SNP or gene within 3kb of associated SNP	Trait	SNP ID	SNP Source	Chr	Position in RefGen_v2	P-value	FDR-Adjusted P-value	Minor Allele Frequency (MAF)	Sample Size	MAF Tropical (8% of 201 Lines)	MAF Temperate (92% of 201 Lines)	R-square_LR from Model without SNP	R-square_LR from Model with SNP	Effect Size	Lambda from Box-Cox Procedure	Back-Transformed Effect Estimates
			Lutein	S1_96310268	GBS	1	96,310,268	3.32E-07	3.23E-02	0.17	200	0.06	0.21	0.12	0.25	1.23	0.80	1.73
Carotenoid Synthesis and Degradation	GRMZM2G127139	zep1	Zeaxanthin	S2_44448432	GBS	2	44,448,432	2.06E-09	2.98E-04	0.11	196	0.29	0.09	0.04	0.24	-0.34	0.35	-0.69
Carotenoid Synthesis and Degradation	GRMZM2G127139	zep1	Total β -Xanthophylls	S2_44448432	GBS	2	44,448,432	1.57E-08	2.28E-03	0.11	195	0.29	0.09	0.05	0.22	-0.43	0.40	-0.76
Carotenoid Synthesis and Degradation	GRMZM2G127139	zep1	β -Xanthophylls/ α -Xanthophylls	S2_44448432	GBS	2	44,448,432	5.29E-07	1.28E-02	0.11	196	0.29	0.09	0.11	0.24	0.12	-0.40	-0.24
Carotenoid Synthesis and Degradation	GRMZM2G127139	zep1	β -Carotenoids/ α -Carotenoids	S2_44448432	GBS	2	44,448,432	9.29E-07	4.88E-02	0.12	189	0.29	0.09	0.20	0.31	0.13	-0.85	-0.13
Carotenoid Synthesis and Degradation	GRMZM2G127139	zep1	Zeaxanthin	S2_44448438	GBS	2	44,448,438	2.06E-09	2.98E-04	0.11	196	0.29	0.09	0.04	0.24	0.34	0.35	1.31
Carotenoid Synthesis and Degradation	GRMZM2G127139	zep1	Total β -Xanthophylls	S2_44448438	GBS	2	44,448,438	1.57E-08	2.28E-03	0.11	195	0.29	0.09	0.05	0.22	0.43	0.40	1.46
Carotenoid Synthesis and Degradation	GRMZM2G127139	zep1	β -Xanthophylls/ α -Xanthophylls	S2_44448438	GBS	2	44,448,438	5.29E-07	1.28E-02	0.11	196	0.29	0.09	0.11	0.24	-0.12	-0.40	0.37
Carotenoid Synthesis and Degradation	GRMZM2G127139	zep1	β -Carotenoids/ α -Carotenoids	S2_44448438	GBS	2	44,448,438	9.29E-07	4.88E-02	0.12	189	0.29	0.09	0.20	0.31	-0.13	-0.85	0.17
			Zeaxanthin	S2_44473748	GBS	2	44,473,748	1.32E-06	3.82E-02	0.14	196	0.25	0.12	0.04	0.17	0.24	0.35	0.86
			β -Xanthophylls/ α -Xanthophylls	S2_44473748	GBS	2	44,473,748	1.65E-05	9.94E-02	0.14	196	0.25	0.12	0.11	0.20	-0.09	-0.40	0.27
			Zeaxanthin	S2_44473758	GBS	2	44,473,758	1.32E-06	3.82E-02	0.14	196	0.24	0.12	0.04	0.17	-0.24	0.35	-0.55
			β -Xanthophylls/ α -Xanthophylls	S2_44473758	GBS	2	44,473,758	1.65E-05	9.94E-02	0.14	196	0.24	0.12	0.11	0.20	0.09	-0.40	-0.19
			Zeaxanthin	S2_44473801	GBS	2	44,473,801	1.32E-06	3.82E-02	0.14	196	0.24	0.12	0.04	0.17	0.24	0.35	0.86
			β -Xanthophylls/ α -Xanthophylls	S2_44473801	GBS	2	44,473,801	1.65E-05	9.94E-02	0.14	196	0.24	0.12	0.11	0.20	-0.09	-0.40	0.27
			Zeaxanthin	S2_44474308	GBS	2	44,474,308	1.09E-06	3.82E-02	0.21	196	0.38	0.28	0.04	0.17	0.21	0.35	0.73
			β -Xanthophylls/ α -Xanthophylls	S2_44474308	GBS	2	44,474,308	1.55E-05	9.94E-02	0.21	196	0.38	0.28	0.11	0.20	-0.08	-0.40	0.23
			Zeaxanthin	S3_169734997	GBS	3	169,734,997	9.39E-07	3.82E-02	0.06	196	0.25	0.07	0.04	0.17	-0.42	0.35	-0.79
			Total β -Xanthophylls	S3_169734997	GBS	3	169,734,997	7.99E-07	3.85E-02	0.06	195	0.25	0.07	0.05	0.18	-0.58	0.40	-0.89
			β -Xanthophylls/ α -Xanthophylls	S3_169734997	GBS	3	169,734,997	1.53E-05	9.94E-02	0.06	196	0.25	0.07	0.11	0.20	0.16	-0.40	-0.31
			Zeaxanthin	S3_172380629	GBS	3	172,380,629	3.84E-06	9.28E-02	0.05	196	0.15	0.05	0.04	0.16	-0.42	0.35	-0.78
			β -Xanthophylls/ α -Xanthophylls	ss196415633	55K	3	216,418,000	1.11E-05	8.55E-02	0.07	196	0.33	0.04	0.11	0.21	0.12	-0.40	-0.25
			β -Cryptoxanthin	S7_13843351	GBS	7	13,843,351	3.40E-08	9.94E-03	0.15	199	0.10	0.16	0.15	0.29	-0.04	0.10	-0.34
			Zeinoxanthin	S7_15282645	GBS	7	15,282,645	3.40E-07	9.89E-02	0.17	198	0.42	0.20	0.25	0.36	-0.11	-0.25	0.57
			β -Xanthophylls/ α -Xanthophylls	ss196477160	55K	7	51,472,566	1.69E-05	9.94E-02	0.43	196	0.43	0.43	0.11	0.20	0.07	-0.40	-0.15
			β -Xanthophylls/ α -Xanthophylls	S8_21700838	GBS	8	21,700,838	7.67E-06	7.92E-02	0.45	196	0.17	0.38	0.11	0.21	-0.07	-0.40	0.19
			β -Xanthophylls/ α -Xanthophylls	PZD00025.1	4K	8	22,245,644	8.90E-06	7.92E-02	0.15	196	0.24	0.14	0.11	0.21	0.10	-0.40	-0.21
			β -Xanthophylls/ α -Xanthophylls	S8_27118357	GBS	8	27,118,357	1.09E-05	8.55E-02	0.19	196	0.48	0.18	0.11	0.21	0.08	-0.40	-0.18
			β -Xanthophylls/ α -Xanthophylls	ss196485943	55K	8	113,211,580	9.06E-06	7.92E-02	0.30	196	0.29	0.25	0.11	0.21	-0.07	-0.40	0.21
			β -Xanthophylls/ α -Xanthophylls	ss196485947	55K	8	113,283,478	5.25E-06	6.35E-02	0.10	196	0.38	0.07	0.11	0.21	-0.13	-0.40	0.40
			β -Xanthophylls/ α -Xanthophylls	S8_113350643	GBS	8	113,350,643	1.17E-05	8.67E-02	0.10	196	0.47	0.09	0.11	0.21	0.12	-0.40	-0.24
			β -Xanthophylls/ α -Xanthophylls	ss196503032	55K	8	120,970,129	1.37E-05	9.94E-02	0.40	196	0.00	0.45	0.11	0.20	-0.07	-0.40	0.19
			β -Xanthophylls/ α -Xanthophylls	ss196503028	55K	8	120,970,146	1.73E-05	9.94E-02	0.39	196	0.00	0.44	0.11	0.20	0.07	-0.40	-0.15
			β -Xanthophylls/ α -Xanthophylls	ss196486295	55K	8	121,437,490	1.78E-05	9.94E-02	0.33	196	0.00	0.36	0.11	0.20	-0.07	-0.40	0.19
			β -Xanthophylls/ α -Xanthophylls	ss196486297	55K	8	121,437,794	1.78E-05	9.94E-02	0.33	196	0.00	0.36	0.11	0.20	-0.07	-0.40	0.19
			β -Xanthophylls/ α -Xanthophylls	ss196516738	55K	8	124,488,144	5.06E-06	6.35E-02	0.20	196	0.00	0.24	0.11	0.21	-0.09	-0.40	0.27
			β -Xanthophylls/ α -Xanthophylls	S8_128602581	GBS	8	128,602,581	1.57E-05	9.94E-02	0.36	196	0.18	0.47	0.11	0.20	-0.07	-0.40	0.19

			Xanthophylls															
			β-Xanthophylls/α-Xanthophylls	S8_128947357	GBS	8	128,947,357	1.60E-05	9.94E-02	0.31	196	0.37	0.36	0.11	0.20	0.07	-0.40	-0.16
			β-Xanthophylls/α-Xanthophylls	S8_129072699	GBS	8	129,072,699	1.41E-06	2.55E-02	0.36	196	0.50	0.35	0.11	0.23	0.07	-0.40	-0.16
			β-Xanthophylls/α-Xanthophylls	S8_129075429	GBS	8	129,075,429	7.91E-06	7.92E-02	0.42	196	0.50	0.44	0.11	0.21	0.07	-0.40	-0.15
			β-Xanthophylls/α-Xanthophylls	S8_129080393	GBS	8	129,080,393	6.40E-06	7.43E-02	0.47	196	0.21	0.38	0.11	0.21	-0.07	-0.40	0.18
			β-Xanthophylls/α-Xanthophylls	S8_129122614	GBS	8	129,122,614	8.84E-06	7.92E-02	0.47	196	0.20	0.39	0.11	0.21	-0.06	-0.40	0.18
			β-Xanthophylls/α-Xanthophylls	S8_129122646	GBS	8	129,122,646	8.84E-06	7.92E-02	0.47	196	0.20	0.39	0.11	0.21	-0.06	-0.40	0.18
			β-Xanthophylls/α-Xanthophylls	S8_129124046	GBS	8	129,124,046	3.27E-06	4.75E-02	0.34	196	0.50	0.35	0.11	0.22	0.07	-0.40	-0.16
			β-Xanthophylls/α-Xanthophylls	S8_129124626	GBS	8	129,124,626	1.85E-06	2.83E-02	0.29	196	0.27	0.31	0.11	0.22	-0.08	-0.40	0.22
			β-Xanthophylls/α-Xanthophylls	S8_129137347	GBS	8	129,137,347	4.09E-06	5.39E-02	0.41	196	0.15	0.48	0.11	0.22	0.07	-0.40	-0.15
			β-Xanthophylls/α-Xanthophylls	ss196512938	55K	8	129,291,444	9.28E-06	7.92E-02	0.34	196	0.14	0.36	0.11	0.21	-0.07	-0.40	0.20
			β-Xanthophylls/α-Xanthophylls	S8_129313857	GBS	8	129,313,857	1.49E-05	9.94E-02	0.29	196	0.05	0.34	0.11	0.20	-0.07	-0.40	0.20
			β-Xanthophylls/α-Xanthophylls	ss196486728	55K	8	130,510,859	8.61E-06	7.92E-02	0.17	196	0.33	0.16	0.11	0.21	0.08	-0.40	-0.18
			β-Xanthophylls/α-Xanthophylls	S8_130513659	GBS	8	130,513,659	7.07E-06	7.88E-02	0.14	196	0.33	0.14	0.11	0.21	-0.09	-0.40	0.27
			β-Xanthophylls/α-Xanthophylls	S8_130930928	GBS	8	130,930,928	1.69E-06	2.73E-02	0.09	196	0.42	0.05	0.11	0.22	0.13	-0.40	-0.26
			β-Xanthophylls/α-Xanthophylls	ss196486759	55K	8	131,124,166	1.12E-05	8.55E-02	0.34	196	0.19	0.36	0.11	0.21	-0.07	-0.40	0.19
			β-Xanthophylls/α-Xanthophylls	S8_131533827	GBS	8	131,533,827	1.02E-05	8.47E-02	0.29	196	0.13	0.37	0.11	0.21	0.07	-0.40	-0.16
Carotenoid Synthesis and Degradation	GRMZM2G012966	IcyE	Total α-Xanthophylls	IcyE 5'TE	Additional Markers	8	138,882,481	4.97E-09	7.26E-04	NA	200	NA	NA	.09	.43	NA	0.70	NA
Carotenoid Synthesis and Degradation	GRMZM2G012966	IcyE	Lutein	IcyE 5'TE	Additional Markers	8	138,882,481	2.24E-08	3.27E-03	NA	200	NA	NA	.12	.49	NA	0.80	NA
Carotenoid Synthesis and Degradation	GRMZM2G012966	IcyE	β-Xanthophylls/α-Xanthophylls	ss196504160	55K	8	138,882,711	4.03E-10	5.84E-05	0.35	196	0.48	0.34	0.11	0.31	0.11	-0.40	-0.23
Carotenoid Synthesis and Degradation	GRMZM2G012966	IcyE	β-Carotenoids/α-Carotenoids	ss196504160	55K	8	138,882,711	2.75E-09	7.92E-04	0.37	189	0.48	0.34	0.20	0.36	0.12	-0.85	-0.12
Carotenoid Synthesis and Degradation	GRMZM2G012966	IcyE	β-Xanthophylls/α-Xanthophylls	S8_138882711	GBS	8	138,882,711	3.23E-07	1.04E-02	0.28	196	0.41	0.30	0.11	0.24	-0.09	-0.40	0.26
Carotenoid Synthesis and Degradation	GRMZM2G012966	IcyE	β-Carotenoids/α-Carotenoids	S8_138882711	GBS	8	138,882,711	1.71E-06	5.46E-02	0.29	189	0.41	0.30	0.20	0.30	-0.09	-0.85	0.12
Carotenoid Synthesis and Degradation	GRMZM2G012966	IcyE	Total β-Xanthophylls	ss196504160	55K	8	138,882,711	1.77E-06	6.40E-02	0.35	195	0.48	0.34	0.05	0.17	-0.26	0.40	-0.53
Carotenoid Synthesis and Degradation	GRMZM2G012966	IcyE	Zeaxanthin	ss196504160	55K	8	138,882,711	2.80E-06	7.37E-02	0.35	196	0.48	0.34	0.04	0.16	-0.19	0.35	-0.44
Carotenoid Synthesis and Degradation	GRMZM2G012966	IcyE	β-Xanthophylls/α-Xanthophylls	S8_138882747	GBS	8	138,882,747	3.23E-07	1.04E-02	0.28	196	0.41	0.30	0.11	0.24	-0.09	-0.40	0.26
Carotenoid Synthesis and Degradation	GRMZM2G012966	IcyE	β-Carotenoids/α-Carotenoids	S8_138882747	GBS	8	138,882,747	1.71E-06	5.46E-02	0.29	189	0.41	0.30	0.20	0.30	-0.09	-0.85	0.12
Carotenoid Synthesis and Degradation	GRMZM2G012966	IcyE	β-Xanthophylls/α-Xanthophylls	S8_138882751	GBS	8	138,882,751	3.23E-07	1.04E-02	0.28	196	0.41	0.30	0.11	0.24	-0.09	-0.40	0.26
Carotenoid Synthesis and Degradation	GRMZM2G012966	IcyE	β-Carotenoids/α-Carotenoids	S8_138882751	GBS	8	138,882,751	1.71E-06	5.46E-02	0.29	189	0.41	0.30	0.20	0.30	-0.09	-0.85	0.12
Carotenoid Synthesis and Degradation	GRMZM2G012966	IcyE	β-Xanthophylls/α-Xanthophylls	S8_138882798	GBS	8	138,882,798	2.65E-07	1.04E-02	0.31	196	0.21	0.36	0.11	0.24	-0.09	-0.40	0.26
Carotenoid Synthesis and Degradation	GRMZM2G012966	IcyE	β-Carotenoids/α-Carotenoids	S8_138882798	GBS	8	138,882,798	2.59E-06	6.88E-02	0.31	189	0.21	0.36	0.20	0.30	-0.09	-0.85	0.12
Carotenoid Synthesis and Degradation	GRMZM2G012966	IcyE	β-Xanthophylls/α-Xanthophylls	S8_138882897	GBS	8	138,882,897	1.55E-08	1.12E-03	0.43	196	0.12	0.44	0.11	0.27	-0.09	-0.40	0.26

Carotenoid Synthesis and Degradation	GRMZM2G012966	lcyE	β -Carotenoids/ α -Carotenoids	S8_138882897	GBS	8	138,882,897	6.73E-08	9.69E-03	0.43	189	0.12	0.44	0.20	0.33	-0.10	-0.85	0.13
Carotenoid Synthesis and Degradation	GRMZM2G012966	lcyE	β -Xanthophylls/ α -Xanthophylls	S8_138883026	GBS	8	138,883,026	1.04E-06	2.02E-02	0.40	196	0.18	0.48	0.11	0.23	0.08	-0.40	-0.17
Carotenoid Synthesis and Degradation	GRMZM2G012966	lcyE	β -Xanthophylls/ α -Xanthophylls	S8_138883056	GBS	8	138,883,056	1.04E-06	2.02E-02	0.40	196	0.18	0.48	0.11	0.23	-0.08	-0.40	0.22
Carotenoid Synthesis and Degradation	GRMZM2G012966	lcyE	β -Xanthophylls/ α -Xanthophylls	<i>lcyE</i> SNP216	Additional Markers	8	138,883,206	7.15E-16	2.07E-10	NA	196	NA	NA	.08	.22	NA	-0.40	NA
Carotenoid Synthesis and Degradation	GRMZM2G012966	lcyE	Total α -Xanthophylls	<i>lcyE</i> SNP216	Additional Markers	8	138,883,206	5.37E-10	1.57E-04	NA	200	NA	NA	.09	.40	NA	0.70	NA
Carotenoid Synthesis and Degradation	GRMZM2G012966	lcyE	Lutein	<i>lcyE</i> SNP216	Additional Markers	8	138,883,206	6.44E-09	1.88E-03	NA	200	NA	NA	.12	.45	NA	0.80	NA
Carotenoid Synthesis and Degradation	GRMZM2G012966	lcyE	Total β -Xanthophylls	<i>lcyE</i> SNP216	Additional Markers	8	138,883,206	1.64E-07	1.19E-02	NA	195	NA	NA	.05	.29	NA	0.40	NA
Carotenoid Synthesis and Degradation	GRMZM2G012966	lcyE	β -Xanthophylls/ α -Xanthophylls	PZB00665.1	4K	8	138,886,137	5.28E-07	1.28E-02	0.35	196	0.05	0.38	0.11	0.24	0.08	-0.40	-0.18
Carotenoid Synthesis and Degradation	GRMZM2G012966	lcyE	β -Xanthophylls/ α -Xanthophylls	S8_138888278	GBS	8	138,888,278	2.98E-09	2.88E-04	0.47	196	0.19	0.42	0.11	0.29	-0.10	-0.40	0.30
Carotenoid Synthesis and Degradation	GRMZM2G012966	lcyE	β -Carotenoids/ α -Carotenoids	S8_138888278	GBS	8	138,888,278	1.80E-07	1.73E-02	0.47	189	0.19	0.42	0.20	0.32	-0.10	-0.85	0.13
			β -Xanthophylls/ α -Xanthophylls	ss196508843	55K	8	139,143,878	1.33E-07	7.74E-03	0.29	196	0.38	0.26	0.11	0.25	0.09	-0.40	-0.19
			β -Carotenoids/ α -Carotenoids	ss196508843	55K	8	139,143,878	1.02E-06	4.88E-02	0.31	189	0.38	0.26	0.20	0.31	0.09	-0.85	-0.10
			β -Xanthophylls/ α -Xanthophylls	ss196507132	55K	8	139,300,073	3.68E-06	5.09E-02	0.39	196	0.18	0.49	0.11	0.22	-0.07	-0.40	0.22
			β -Xanthophylls/ α -Xanthophylls	S8_140192724	GBS	8	140,192,724	6.19E-07	1.38E-02	0.33	196	0.19	0.28	0.11	0.23	-0.08	-0.40	0.24
			β -Carotenoids/ α -Carotenoids	S8_140192724	GBS	8	140,192,724	2.63E-06	6.88E-02	0.34	189	0.19	0.28	0.20	0.30	-0.09	-0.85	0.11
			Total β -Xanthophylls	S8_171705545	GBS	8	171,705,545	3.98E-07	2.30E-02	0.10	195	0.14	0.14	0.05	0.18	-0.40	0.40	-0.72
			Zeaxanthin	S8_171705545	GBS	8	171,705,545	6.85E-07	3.82E-02	0.11	196	0.14	0.14	0.04	0.17	-0.28	0.35	-0.61
			Total β -Xanthophylls	S8_171705574	GBS	8	171,705,574	1.27E-07	1.19E-02	0.10	195	0.25	0.13	0.05	0.20	-0.42	0.40	-0.74
			Zeaxanthin	S8_171705574	GBS	8	171,705,574	1.98E-07	1.91E-02	0.11	196	0.25	0.13	0.04	0.19	-0.30	0.35	-0.63
			β -Xanthophylls/ α -Xanthophylls	ss196493105	55K	9	118,437,281	1.54E-06	2.63E-02	0.29	196	0.19	0.42	0.11	0.22	-0.09	-0.40	0.28
Carotenoid Synthesis and Degradation	GRMZM2G152135	crtRB1	β -Carotene/(β -Cryptoxanthin+Zeaxanthin)	<i>crtRB1</i> InDel4	Additional Markers	10	136,059,748	1.72E-07	5.00E-02	NA	196	NA	NA	.03	.08	NA	-0.25	NA
Carotenoid Synthesis and Degradation	GRMZM2G152135	crtRB1	β -Carotene/(β -Cryptoxanthin+Zeaxanthin)	ss196501627	55K	10	136,060,033	3.67E-07	5.33E-02	0.19	196	0.00	0.22	0.04	0.18	0.12	-0.25	-0.36
Carotenoid Synthesis and Degradation	GRMZM2G152135	crtRB1	Total Carotenes/Total Xanthophylls	<i>crtRB1</i> 3'TE	Additional Markers	10	136,061,719	1.33E-07	3.82E-02	NA	188	NA	NA	.02	.14	NA	-0.55	NA
Carotenoid Synthesis and Degradation	GRMZM2G152135	crtRB1	Zeaxanthin	<i>crtRB1</i> 3'TE	Additional Markers	10	136,061,719	1.12E-06	3.82E-02	NA	196	NA	NA	.04	.15	NA	0.35	NA
Carotenoid Synthesis and Degradation	GRMZM2G152135	crtRB1	Total β -Xanthophylls	<i>crtRB1</i> 3'TE	Additional Markers	10	136,061,719	1.77E-06	6.40E-02	NA	195	NA	NA	.05	.17	NA	0.40	NA

Statistically significant results from genome-wide association studies on 24 grain carotenoid traits with the peak SNP tagging the GWAS signal from *lut1* included as a covariate. Markers (Column E) that were significantly associated with the indicated trait (Column D) at 5% false discovery rate (FDR) are demarcated with boldface font and those significant only at 10% FDR without boldface font.

Table S8 Genome-wide Association Study Results with Covariates for *lcyE* (D)

<i>a priori</i> candidate gene pathway	RefGen_v2 Gene ID	Annotated gene containing associated SNP or gene within 3kb of associated SNP	Trait	SNP ID	SNP Source	Chr	Position in RefGen_v2	P-value	FDR-Adjusted P-value	Minor Allele Frequency (MAF)	Sample Size	MAF Tropical (8% of 201 Lines)	MAF Temperate (92% of 201 Lines)	R-square_LR from Model without SNP	R-square_LR from Model with SNP	Effect Size	Lambda from Box-Cox Procedure	Back-Transformed Effect Estimates
			Zeaxanthin	S1_2940079	GBS	1	2,940,079	3.37E-06	9.80E-02	0.05	176	0.50	0.03	0.18	0.29	-0.37	0.35	-0.74
Carotenoid Synthesis and Degradation	GRMZM2G143202	lut1	α -Carotene/Zeaxanthin	ss196425306	55K	1	86,844,203	7.23E-10	7.03E-05	0.33	178	0.40	0.28	0.16	0.37	0.07	-0.25	-0.22
Carotenoid Synthesis and Degradation	GRMZM2G143202	lut1	Zeinoxanthin	ss196425306	55K	1	86,844,203	4.28E-08	4.15E-03	0.32	178	0.40	0.28	0.10	0.27	-0.12	-0.25	0.67
Carotenoid Synthesis and Degradation	GRMZM2G143202	lut1	Zeinoxanthin/Lutein	ss196425306	55K	1	86,844,203	4.98E-08	4.80E-03	0.31	175	0.40	0.28	0.08	0.25	-0.21	-0.35	0.93
			α -Carotene/Zeaxanthin	ss196425308	55K	1	86,945,134	7.23E-10	7.03E-05	0.33	178	0.40	0.27	0.16	0.37	0.07	-0.25	-0.22
			Zeinoxanthin	ss196425308	55K	1	86,945,134	4.28E-08	4.15E-03	0.32	178	0.40	0.27	0.10	0.27	-0.12	-0.25	0.67
			Zeinoxanthin/Lutein	ss196425308	55K	1	86,945,134	4.98E-08	4.80E-03	0.31	175	0.40	0.27	0.08	0.25	-0.21	-0.35	0.93
Carotenoid Synthesis and Degradation	GRMZM2G127139	zep1	Zeaxanthin	S2_44448432	GBS	2	44,448,432	7.43E-09	1.08E-03	0.11	176	0.29	0.09	0.18	0.36	-0.32	0.35	-0.67
Carotenoid Synthesis and Degradation	GRMZM2G127139	zep1	Total β -Xanthophylls	S2_44448432	GBS	2	44,448,432	8.43E-08	1.22E-02	0.11	175	0.29	0.09	0.20	0.34	-0.40	0.40	-0.73
Carotenoid Synthesis and Degradation	GRMZM2G127139	zep1	β -Xanthophylls/ α -Xanthophylls	S2_44448432	GBS	2	44,448,432	5.10E-07	7.40E-02	0.11	176	0.29	0.09	0.45	0.54	0.10	-0.40	-0.22
Carotenoid Synthesis and Degradation	GRMZM2G127139	zep1	Zeaxanthin	S2_44448438	GBS	2	44,448,438	7.43E-09	1.08E-03	0.11	176	0.29	0.09	0.18	0.36	0.32	0.35	1.22
Carotenoid Synthesis and Degradation	GRMZM2G127139	zep1	Total β -Xanthophylls	S2_44448438	GBS	2	44,448,438	8.43E-08	1.22E-02	0.11	175	0.29	0.09	0.20	0.34	0.40	0.40	1.34
Carotenoid Synthesis and Degradation	GRMZM2G127139	zep1	β -Xanthophylls/ α -Xanthophylls	S2_44448438	GBS	2	44,448,438	5.10E-07	7.40E-02	0.11	176	0.29	0.09	0.45	0.54	-0.10	-0.40	0.32
			Zeaxanthin	S5_216074707	GBS	5	216,074,707	2.89E-06	9.80E-02	0.14	176	0.17	0.17	0.18	0.29	-0.23	0.35	-0.52
			β -Cryptoxanthin	S7_13843351	GBS	7	13,843,351	1.40E-07	4.09E-02	0.16	179	0.10	0.16	0.19	0.33	-0.04	0.10	-0.33
			Zeinoxanthin	S7_15282645	GBS	7	15,282,645	9.69E-07	7.05E-02	0.18	178	0.42	0.20	0.10	0.23	-0.12	-0.25	0.65
			Total β -Xanthophylls	ss196478758	55K	7	103,626,333	6.37E-07	4.60E-02	0.41	175	0.19	0.44	0.20	0.32	-0.24	0.40	-0.50
			Zeaxanthin	ss196478758	55K	7	103,626,333	7.39E-07	5.35E-02	0.41	176	0.19	0.44	0.18	0.31	-0.18	0.35	-0.42
			Zeinoxanthin	S7_107111687	GBS	7	107,111,687	1.48E-06	7.19E-02	0.16	178	0.23	0.25	0.10	0.23	0.12	-0.25	-0.36
			Zeinoxanthin	S7_107111713	GBS	7	107,111,713	1.48E-06	7.19E-02	0.16	178	0.23	0.25	0.10	0.23	0.12	-0.25	-0.36
			Total β -Xanthophylls	S7_108788777	GBS	7	108,788,777	1.08E-06	6.23E-02	0.49	175	0.29	0.43	0.20	0.32	-0.23	0.40	-0.47
			Zeaxanthin	S7_108788777	GBS	7	108,788,777	3.13E-06	9.80E-02	0.49	176	0.29	0.43	0.18	0.29	-0.16	0.35	-0.39
			Total Carotenoids	S7_121184182	GBS	7	121,184,182	9.65E-07	9.10E-02	0.08	181	0.14	0.08	0.06	0.19	-1.41	0.65	-1.26
			Total Carotenoids	S7_121184311	GBS	7	121,184,311	8.11E-07	9.10E-02	0.08	181	0.13	0.09	0.06	0.19	-1.42	0.65	-1.27
			Total Carotenoids	S7_121185458	GBS	7	121,185,458	1.37E-06	9.66E-02	0.06	181	0.13	0.07	0.06	0.19	-1.55	0.65	-1.40
			Total β -Xanthophylls	S7_121185500	GBS	7	121,185,500	1.99E-07	1.91E-02	0.09	175	0.19	0.09	0.20	0.33	0.40	0.40	1.33
			Zeaxanthin	S7_121185500	GBS	7	121,185,500	4.13E-07	3.99E-02	0.09	176	0.19	0.09	0.18	0.31	0.29	0.35	1.08
			Total Carotenoids	S7_121185500	GBS	7	121,185,500	3.67E-07	9.10E-02	0.08	181	0.19	0.09	0.06	0.20	1.42	0.65	2.88
			Total β -Xanthophylls	S8_171705574	GBS	8	171,705,574	1.57E-06	7.28E-02	0.11	175	0.25	0.13	0.20	0.31	-0.36	0.40	-0.68
			Zeaxanthin	S8_171705574	GBS	8	171,705,574	1.50E-06	7.77E-02	0.11	176	0.25	0.13	0.18	0.30	-0.26	0.35	-0.58
			α -Carotene/Zeaxanthin	ss196491114	55K	9	69,215,031	7.23E-10	7.03E-05	0.33	178	0.37	0.29	0.16	0.37	-0.07	-0.25	0.31
			Zeinoxanthin	ss196491114	55K	9	69,215,031	4.28E-08	4.15E-03	0.32	178	0.37	0.29	0.10	0.27	0.12	-0.25	-0.37
			Zeinoxanthin/Lutein	ss196491114	55K	9	69,215,031	4.98E-08	4.80E-03	0.31	175	0.37	0.29	0.08	0.25	0.21	-0.35	-0.41
Carotenoid Synthesis and Degradation	GRMZM2G152135	crtRB1	Total Carotenenes/Total Xanthophylls	<i>crtRB1</i> InDel4	Additional Markers	10	136,059,748	3.98E-08	5.72E-03	NA	188	NA	NA	.06	.19	NA	-0.55	NA
Carotenoid Synthesis and Degradation	GRMZM2G152135	crtRB1	Zeaxanthin	<i>crtRB1</i> InDel4	Additional Markers	10	136,059,748	3.38E-06	9.80E-02	NA	196	NA	NA	.24	.32	NA	0.35	NA
Carotenoid Synthesis and Degradation	GRMZM2G152135	crtRB1	β -Carotene/(β -Cryptoxanthin+Zeaxanthin)	<i>crtRB1</i> 3'TE	Additional Markers	10	136,061,719	2.13E-10	6.21E-05	NA	196	NA	NA	.04	.10	NA	-0.25	NA
Carotenoid Synthesis and Degradation	GRMZM2G152135	crtRB1	Total Carotenenes/Total Xanthophylls	<i>crtRB1</i> 3'TE	Additional Markers	10	136,061,719	3.30E-09	9.49E-04	NA	188	NA	NA	.06	.22	NA	-0.55	NA
Carotenoid Synthesis and Degradation	GRMZM2G152135	crtRB1	Total β -Xanthophylls	<i>crtRB1</i> 3'TE	Additional Markers	10	136,061,719	1.76E-06	7.28E-02	NA	195	NA	NA	.29	.38	NA	0.40	NA
Carotenoid Synthesis and Degradation	GRMZM2G152135	crtRB1	Zeaxanthin	<i>crtRB1</i> 3'TE	Additional Markers	10	136,061,719	1.61E-06	7.77E-02	NA	196	NA	NA	.24	.33	NA	0.35	NA

Statistically significant results from genome-wide association studies on 24 grain carotenoid traits with the markers tagging *lcyE* identified in the multi-locus mixed model included as covariates. Markers (Column E) that were significantly associated with the indicated trait (Column D) at 5% false discovery rate (FDR) are

Table S8 Genome-wide Association Study Results with S8_171705574 Covariate (E)

<i>a priori</i> candidate gene pathway	RefGen_v2 Gene ID	Annotated gene containing associated SNP or gene within 3kb of associated SNP	Trait	SNP ID	SNP Source	Chr	Position in RefGen_v2	P-value	FDR- Adjusted P-value	Minor Allele Frequency (MAF)	Sample Size	MAF Tropical (8% of 201 Lines)	MAF Temperate (92% of 201 Lines)	R-square_LR from Model without SNP	R-square_LR from Model with SNP	Effect Size	Lambda from Box- Cox Procedure	Back- Transformed Effect Estimates
Carotenoid Synthesis and Degradation	GRMZM2G143202	lut1	α -Carotene/Zeinoxanthin	ss196425306	55K	1	86,844,203	5.23E-10	5.06E-05	0.31	196	0.40	0.28	0.17	0.35	0.06	-0.25	-0.22
Carotenoid Synthesis and Degradation	GRMZM2G143202	lut1	Zeinoxanthin/Lutein	ss196425306	55K	1	86,844,203	4.34E-08	6.28E-03	0.29	195	0.40	0.28	0.09	0.24	-0.19	-0.35	0.84
Carotenoid Synthesis and Degradation	GRMZM2G143202	lut1	Zeinoxanthin	ss196425306	55K	1	86,844,203	7.31E-08	1.06E-02	0.30	198	0.40	0.28	0.10	0.25	-0.11	-0.25	0.62
			α -Carotene/Zeinoxanthin	ss196425308	55K	1	86,945,134	5.23E-10	5.06E-05	0.31	196	0.40	0.27	0.17	0.35	0.06	-0.25	-0.22
			Zeinoxanthin/Lutein	ss196425308	55K	1	86,945,134	4.34E-08	6.28E-03	0.29	195	0.40	0.27	0.09	0.24	-0.19	-0.35	0.84
			Zeinoxanthin	ss196425308	55K	1	86,945,134	7.31E-08	1.06E-02	0.30	198	0.40	0.27	0.10	0.25	-0.11	-0.25	0.62
			Lutein	S1_96310268	GBS	1	96,310,268	5.53E-07	5.39E-02	0.17	200	0.06	0.21	0.13	0.25	1.21	0.80	1.70
Carotenoid Synthesis and Degradation	GRMZM2G127139	zep1	Zeaxanthin	S2_44448432	GBS	2	44,448,432	2.15E-09	3.11E-04	0.11	196	0.29	0.09	0.19	0.35	-0.32	0.35	-0.66
Carotenoid Synthesis and Degradation	GRMZM2G127139	zep1	Total β -Xanthophylls	S2_44448432	GBS	2	44,448,432	1.75E-08	2.54E-03	0.11	195	0.29	0.09	0.20	0.34	-0.40	0.40	-0.72
Carotenoid Synthesis and Degradation	GRMZM2G127139	zep1	β -Xanthophylls/ α - Xanthophylls	S2_44448432	GBS	2	44,448,432	1.31E-07	7.61E-03	0.11	196	0.29	0.09	0.19	0.31	0.12	-0.40	-0.25
Carotenoid Synthesis and Degradation	GRMZM2G127139	zep1	Zeaxanthin	S2_44448438	GBS	2	44,448,438	2.15E-09	3.11E-04	0.11	196	0.29	0.09	0.19	0.35	0.32	0.35	1.19
Carotenoid Synthesis and Degradation	GRMZM2G127139	zep1	Total β -Xanthophylls	S2_44448438	GBS	2	44,448,438	1.75E-08	2.54E-03	0.11	195	0.29	0.09	0.20	0.34	0.40	0.40	1.32
Carotenoid Synthesis and Degradation	GRMZM2G127139	zep1	β -Xanthophylls/ α - Xanthophylls	S2_44448438	GBS	2	44,448,438	1.31E-07	7.61E-03	0.11	196	0.29	0.09	0.19	0.31	-0.12	-0.40	0.39
			Zeaxanthin	S2_44473748	GBS	2	44,473,748	1.72E-07	9.98E-03	0.14	196	0.25	0.12	0.19	0.31	0.24	0.35	0.87
			Total β -Xanthophylls	S2_44473748	GBS	2	44,473,748	5.64E-07	3.26E-02	0.14	195	0.25	0.12	0.20	0.31	0.32	0.40	0.99
			β -Xanthophylls/ α -Xanthophylls	S2_44473748	GBS	2	44,473,748	2.75E-06	5.71E-02	0.14	196	0.25	0.12	0.19	0.28	-0.10	-0.40	0.28
			Zeaxanthin	S2_44473758	GBS	2	44,473,758	1.72E-07	9.98E-03	0.14	196	0.24	0.12	0.19	0.31	-0.24	0.35	-0.55
			Total β -Xanthophylls	S2_44473758	GBS	2	44,473,758	5.64E-07	3.26E-02	0.14	195	0.24	0.12	0.20	0.31	-0.32	0.40	-0.61
			β -Xanthophylls/ α -Xanthophylls	S2_44473758	GBS	2	44,473,758	2.75E-06	5.71E-02	0.14	196	0.24	0.12	0.19	0.28	0.10	-0.40	-0.20
			Zeaxanthin	S2_44473801	GBS	2	44,473,801	1.72E-07	9.98E-03	0.14	196	0.24	0.12	0.19	0.31	0.24	0.35	0.87
			Total β -Xanthophylls	S2_44473801	GBS	2	44,473,801	5.64E-07	3.26E-02	0.14	195	0.24	0.12	0.20	0.31	0.32	0.40	0.99
			β -Xanthophylls/ α -Xanthophylls	S2_44473801	GBS	2	44,473,801	2.75E-06	5.71E-02	0.14	196	0.24	0.12	0.19	0.28	-0.10	-0.40	0.28
			Zeaxanthin	S2_44474139	GBS	2	44,474,139	9.65E-07	4.36E-02	0.14	196	0.29	0.13	0.19	0.29	-0.23	0.35	-0.52
			β -Xanthophylls/ α -Xanthophylls	S2_44474139	GBS	2	44,474,139	4.99E-06	8.52E-02	0.14	196	0.29	0.13	0.19	0.28	0.09	-0.40	-0.20
			Zeaxanthin	S2_44474308	GBS	2	44,474,308	1.09E-06	4.36E-02	0.21	196	0.38	0.28	0.19	0.29	0.20	0.35	0.67
			Total α -Xanthophylls	ss196456701	55K	4	146,977,283	7.48E-07	7.29E-02	0.12	200	0.38	0.08	0.11	0.23	-0.89	0.70	-0.95
			β -Cryptoxanthin	S7_13843351	GBS	7	13,843,351	2.78E-07	8.12E-02	0.15	199	0.10	0.16	0.17	0.29	-0.04	0.10	-0.32
			Zeinoxanthin	S7_15282645	GBS	7	15,282,645	1.18E-07	1.14E-02	0.17	198	0.42	0.20	0.10	0.24	-0.12	-0.25	0.69
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	Total α -Xanthophylls	<i>lycE</i> 5'TE	Additional Markers	8	138,882,481	3.99E-09	5.83E-04	NA	200	NA	NA	.10	.43	NA	0.70	NA
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	Lutein	<i>lycE</i> 5'TE	Additional Markers	8	138,882,481	1.52E-08	2.22E-03	NA	200	NA	NA	.11	.49	NA	0.80	NA
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β -Xanthophylls/ α - Xanthophylls	ss196504160	55K	8	138,882,711	3.69E-09	5.35E-04	0.35	196	0.48	0.34	0.19	0.35	0.10	-0.40	-0.21
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β -Carotenoids/ α -Carotenoids	ss196504160	55K	8	138,882,711	4.67E-09	1.34E-03	0.37	189	0.48	0.34	0.22	0.38	0.11	-0.85	-0.12
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β -Xanthophylls/ α - Xanthophylls	S8_138882711	GBS	8	138,882,711	1.58E-06	4.57E-02	0.28	196	0.41	0.30	0.19	0.29	-0.08	-0.40	0.24
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β -Xanthophylls/ α - Xanthophylls	S8_138882747	GBS	8	138,882,747	1.58E-06	4.57E-02	0.28	196	0.41	0.30	0.19	0.29	-0.08	-0.40	0.24
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β -Xanthophylls/ α - Xanthophylls	S8_138882751	GBS	8	138,882,751	1.58E-06	4.57E-02	0.28	196	0.41	0.30	0.19	0.29	-0.08	-0.40	0.24
Carotenoid Synthesis and	GRMZM2G012966	lycE	β -Xanthophylls/ α -Xanthophylls	S8_138882798	GBS	8	138,882,798	2.66E-06	5.71E-02	0.31	196	0.21	0.36	0.19	0.28	-0.08	-0.40	0.23

Degradation																		
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β-Xanthophylls/α-Xanthophylls	S8_138882897	GBS	8	138,882,897	2.15E-07	1.04E-02	0.43	196	0.12	0.44	0.19	0.31	-0.08	-0.40	0.23
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β-Carotenoids/α-Carotenoids	S8_138882897	GBS	8	138,882,897	1.14E-07	1.64E-02	0.43	189	0.12	0.44	0.22	0.35	-0.09	-0.85	0.12
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β-Xanthophylls/α-Xanthophylls	lycE SNP216	Additonal Markers	8	138,883,206	2.78E-15	8.06E-10	NA	196	NA	NA	.13	.26	NA	-0.40	NA
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	Total α-Xanthophylls	lycE SNP216	Additonal Markers	8	138,883,206	3.24E-10	9.48E-05	NA	200	NA	NA	.10	.40	NA	0.70	NA
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	Lutein	lycE SNP216	Additonal Markers	8	138,883,206	4.75E-09	1.39E-03	NA	200	NA	NA	.11	.45	NA	0.80	NA
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β-Xanthophylls/α-Xanthophylls	PZB00665.1	4K	8	138,886,137	1.47E-06	4.57E-02	0.35	196	0.05	0.38	0.19	0.29	0.08	-0.40	-0.18
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β-Xanthophylls/α-Xanthophylls	S8_138888278	GBS	8	138,888,278	1.58E-08	1.53E-03	0.47	196	0.19	0.42	0.19	0.33	-0.09	-0.40	0.28
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β-Carotenoids/α-Carotenoids	S8_138888278	GBS	8	138,888,278	2.43E-07	2.33E-02	0.47	189	0.19	0.42	0.22	0.34	-0.10	-0.85	0.13
			β-Xanthophylls/α-Xanthophylls	ss196508843	55K	8	139,143,878	3.32E-06	6.02E-02	0.29	196	0.38	0.26	0.19	0.28	0.08	-0.40	-0.17
			β-Xanthophylls/α-Xanthophylls	S8_140192724	GBS	8	140,192,724	2.98E-06	5.77E-02	0.33	196	0.19	0.28	0.19	0.28	-0.08	-0.40	0.22
			β-Carotenoids/α-Carotenoids	S8_140192724	GBS	8	140,192,724	1.29E-06	9.31E-02	0.34	189	0.19	0.28	0.22	0.32	-0.09	-0.85	0.11
			α-Carotene/Zeaxanthin	ss196491114	55K	9	69,215,031	5.01E-10	5.06E-05	0.31	196	0.37	0.29	0.17	0.35	-0.06	-0.25	0.30
			Zeaxanthin/Lutein	ss196491114	55K	9	69,215,031	8.52E-08	8.23E-03	0.28	195	0.37	0.29	0.09	0.24	0.19	-0.35	-0.39
			Zeaxanthin	ss196491114	55K	9	69,215,031	3.07E-07	2.23E-02	0.30	198	0.37	0.29	0.10	0.23	0.11	-0.25	-0.34
Carotenoid Synthesis and Degradation	GRMZM2G152135	crtRB1	β-Carotene/(β-Cryptoxanthin+Zeaxanthin)	crtRB1 InDel4	Additonal Markers	10	136,059,748	2.87E-07	5.79E-02	NA	196	NA	NA	.04	.08	NA	-0.25	NA
Carotenoid Synthesis and Degradation	GRMZM2G152135	crtRB1	β-Carotene/(β-Cryptoxanthin+Zeaxanthin)	ss196501627	55K	10	136,060,033	3.99E-07	5.79E-02	0.19	196	0.00	0.22	0.04	0.18	0.12	-0.25	-0.36
Carotenoid Synthesis and Degradation	GRMZM2G152135	crtRB1	Zeaxanthin	crtRB1 3'TE	Additonal Markers	10	136,061,719	1.20E-06	4.36E-02	NA	196	NA	NA	.17	.27	NA	0.35	NA

Statistically significant results from genome-wide association studies on 24 grain carotenoid traits with SNP S8_171705574 included as a covariate. Markers (Column E) that were significantly associated with the indicated trait (Column D) at 5% false discovery rate (FDR) are demarcated with boldface font and those significant only at 10% FDR without boldface font.

Table S8 Genome-wide Association Study Results with Covariate for *crTRB1* (F)

<i>a priori</i> candidate gene pathway	RefGen_v2 Gene ID	Annotated gene containing associated SNP or gene within 3kb of associated SNP	Trait	SNP ID	SNP Source	Chr	Position in RefGen_v2	P-value	FDR-Adjusted P-value	Minor Allele Frequency (MAF)	Sample Size	MAF Tropical (8% of 201 Lines)	MAF Temperate (92% of 201 Lines)	R-square_LR from Model without SNP	R-square_LR from Model with SNP	Effect Size	Lambda from Box-Cox Procedure	Back-Transformed Effect Estimates
Carotenoid Synthesis and Degradation	GRMZM2G143202	lut1	α -Carotene/Zeaxanthin	ss196425306	55K	1	86,844,203	8.94E-10	8.59E-05	0.32	190	0.40	0.28	0.17	0.36	0.06	-0.25	-0.21
Carotenoid Synthesis and Degradation	GRMZM2G143202	lut1	Zeaxanthin/Lutein	ss196425306	55K	1	86,844,203	5.87E-08	8.43E-03	0.30	189	0.40	0.28	0.09	0.25	-0.19	-0.35	0.85
Carotenoid Synthesis and Degradation	GRMZM2G143202	lut1	Zeaxanthin	ss196425306	55K	1	86,844,203	1.21E-07	1.74E-02	0.31	192	0.40	0.28	0.10	0.24	-0.11	-0.25	0.62
			α -Carotene/Zeaxanthin	ss196425308	55K	1	86,945,134	8.94E-10	8.59E-05	0.32	190	0.40	0.27	0.17	0.36	0.06	-0.25	-0.21
			Zeaxanthin/Lutein	ss196425308	55K	1	86,945,134	5.87E-08	8.43E-03	0.30	189	0.40	0.27	0.09	0.25	-0.19	-0.35	0.85
			Zeaxanthin	ss196425308	55K	1	86,945,134	1.21E-07	1.74E-02	0.31	192	0.40	0.27	0.10	0.24	-0.11	-0.25	0.62
			Lutein	S1_96310268	GBS	1	96,310,268	2.24E-07	3.24E-02	0.17	194	0.06	0.21	0.12	0.26	1.28	0.80	1.81
Carotenoid Synthesis and Degradation	GRMZM2G127139	zep1	Zeaxanthin	S2_44448432	GBS	2	44,448,432	2.71E-09	3.89E-04	0.11	190	0.29	0.09	0.12	0.30	-0.32	0.35	-0.67
Carotenoid Synthesis and Degradation	GRMZM2G127139	zep1	Total β -Xanthophylls	S2_44448432	GBS	2	44,448,432	2.34E-08	3.35E-03	0.11	189	0.29	0.09	0.12	0.28	-0.41	0.40	-0.74
Carotenoid Synthesis and Degradation	GRMZM2G127139	zep1	β -Xanthophylls/ α -Xanthophylls	S2_44448432	GBS	2	44,448,432	8.21E-08	7.61E-03	0.11	190	0.29	0.09	0.18	0.31	0.13	-0.40	-0.26
Carotenoid Synthesis and Degradation	GRMZM2G127139	zep1	Zeaxanthin	S2_44448438	GBS	2	44,448,438	2.71E-09	3.89E-04	0.11	190	0.29	0.09	0.12	0.30	0.32	0.35	1.22
Carotenoid Synthesis and Degradation	GRMZM2G127139	zep1	Total β -Xanthophylls	S2_44448438	GBS	2	44,448,438	2.34E-08	3.35E-03	0.11	189	0.29	0.09	0.12	0.28	0.41	0.40	1.37
Carotenoid Synthesis and Degradation	GRMZM2G127139	zep1	β -Xanthophylls/ α -Xanthophylls	S2_44448438	GBS	2	44,448,438	8.21E-08	7.61E-03	0.11	190	0.29	0.09	0.18	0.31	-0.13	-0.40	0.41
			Zeaxanthin	S2_44473748	GBS	2	44,473,748	3.89E-07	1.60E-02	0.13	190	0.25	0.12	0.12	0.25	0.25	0.35	0.89
			Total β -Xanthophylls	S2_44473748	GBS	2	44,473,748	1.41E-06	4.49E-02	0.13	189	0.25	0.12	0.12	0.24	0.32	0.40	1.01
			β -Xanthophylls/ α -Xanthophylls	S2_44473748	GBS	2	44,473,748	1.76E-06	6.32E-02	0.13	190	0.25	0.12	0.18	0.28	-0.10	-0.40	0.31
			Zeaxanthin	S2_44473758	GBS	2	44,473,758	3.89E-07	1.60E-02	0.13	190	0.24	0.12	0.12	0.25	-0.25	0.35	-0.56
			Total β -Xanthophylls	S2_44473758	GBS	2	44,473,758	1.41E-06	4.49E-02	0.13	189	0.24	0.12	0.12	0.24	-0.32	0.40	-0.62
			β -Xanthophylls/ α -Xanthophylls	S2_44473758	GBS	2	44,473,758	1.76E-06	5.05E-02	0.13	190	0.24	0.12	0.18	0.28	0.10	-0.40	-0.21
			Zeaxanthin	S2_44473801	GBS	2	44,473,801	3.89E-07	1.60E-02	0.13	190	0.24	0.12	0.12	0.25	0.25	0.35	0.89
			Total β -Xanthophylls	S2_44473801	GBS	2	44,473,801	1.41E-06	4.49E-02	0.13	189	0.24	0.12	0.12	0.24	0.32	0.40	1.01
			β -Xanthophylls/ α -Xanthophylls	S2_44473801	GBS	2	44,473,801	1.76E-06	5.05E-02	0.13	190	0.24	0.12	0.18	0.28	-0.10	-0.40	0.31
			Zeaxanthin	S2_44474139	GBS	2	44,474,139	1.48E-06	4.26E-02	0.14	190	0.29	0.13	0.12	0.24	-0.23	0.35	-0.53
			β -Xanthophylls/ α -Xanthophylls	S2_44474139	GBS	2	44,474,139	2.48E-06	6.49E-02	0.14	190	0.29	0.13	0.18	0.28	0.10	-0.40	-0.21
			Zeaxanthin	S2_44474308	GBS	2	44,474,308	1.63E-06	4.26E-02	0.21	190	0.38	0.28	0.12	0.24	0.20	0.35	0.68
			Zeaxanthin	S3_169734997	GBS	3	169,734,997	1.04E-06	3.33E-02	0.06	190	0.25	0.07	0.12	0.24	-0.39	0.35	-0.75
			Total β -Xanthophylls	S3_169734997	GBS	3	169,734,997	8.48E-07	4.06E-02	0.06	189	0.25	0.07	0.12	0.24	-0.54	0.40	-0.86
			Zeaxanthin	S3_172380629	GBS	3	172,380,629	3.87E-06	9.20E-02	0.05	190	0.15	0.05	0.12	0.23	-0.38	0.35	-0.75
			β -Cryptoxanthin	S7_13843351	GBS	7	13,843,351	1.77E-07	5.11E-02	0.16	193	0.10	0.16	0.13	0.27	-0.04	0.10	-0.33
			Zeaxanthin	S7_15282645	GBS	7	15,282,645	2.70E-07	2.59E-02	0.18	192	0.42	0.20	0.10	0.23	-0.12	-0.25	0.66
			Zeaxanthin	S8_2511818	GBS	8	2,511,818	4.16E-06	9.20E-02	0.05	190	0.45	0.11	0.12	0.23	-0.33	0.35	-0.69
			β -Xanthophylls/ α -Xanthophylls	PZD00025.1	4K	8	22,245,644	2.97E-06	7.12E-02	0.14	190	0.24	0.14	0.18	0.28	0.10	-0.40	-0.21
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β -Xanthophylls/ α -Xanthophylls	<i>lycE</i> 5'TE	Additional Markers	8	138,882,481	2.43E-13	3.49E-08	NA	196	NA	NA	.04	.24	NA	NA	NA
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β -Carotenoids/ α -Carotenoids	<i>lycE</i> 5'TE	Additional Markers	8	138,882,481	1.35E-13	3.86E-08	NA	189	NA	NA	.01	.28	NA	NA	NA
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β -Xanthophylls/ α -Xanthophylls	ss196504160	55K	8	138,882,711	6.86E-09	6.58E-04	0.35	190	0.48	0.34	0.18	0.34	0.10	-0.40	-0.21
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β -Carotenoids/ α -Carotenoids	ss196504160	55K	8	138,882,711	7.10E-09	1.01E-03	0.36	184	0.48	0.34	0.20	0.37	0.11	-0.85	-0.12
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β -Carotenoids/ α -Carotenoids	S8_138882897	GBS	8	138,882,897	1.28E-07	1.22E-02	0.43	184	0.12	0.44	0.20	0.34	-0.10	-0.85	0.13
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β -Xanthophylls/ α -Xanthophylls	S8_138882897	GBS	8	138,882,897	4.03E-07	1.66E-02	0.43	190	0.12	0.44	0.18	0.30	-0.08	-0.40	0.24
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	Lutein	<i>lycE</i> SNP216	Additional Markers	8	138,883,206	1.08E-09	3.12E-04	NA	200	NA	NA	.12	.49	NA	NA	NA

Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	Zeaxanthin	lycE SNP216	Additional Markers	8	138,883,206	1.02E-06	3.33E-02	NA	196	NA	NA	.17	.34	NA	NA	NA
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	Total β-Xanthophylls	lycE SNP216	Additional Markers	8	138,883,206	8.01E-07	4.06E-02	NA	195	NA	NA	.18	.39	NA	NA	NA
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	Total α-Xanthophylls	lycE SNP216	Additional Markers	8	138,883,206	1.39E-10	4.03E-05	NA	200	NA	NA	.09	.42	NA	NA	NA
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β-Xanthophylls/α-Xanthophylls	lycE SNP216	Additional Markers	8	138,883,206	1.86E-14	5.35E-09	NA	196	NA	NA	.04	.25	NA	NA	NA
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β-Xanthophylls/α-Xanthophylls	S8_138888278	GBS	8	138,888,278	1.06E-07	5.07E-03	0.47	190	0.19	0.42	0.18	0.31	-0.09	-0.40	0.27
Carotenoid Synthesis and Degradation	GRMZM2G012966	lycE	β-Carotenoids/α-Carotenoids	S8_138888278	GBS	8	138,888,278	3.97E-07	2.83E-02	0.47	184	0.19	0.42	0.20	0.32	-0.10	-0.85	0.13
			β-Xanthophylls/α-Xanthophylls	S8_140192724	GBS	8	140,192,724	3.36E-06	7.44E-02	0.34	190	0.19	0.28	0.18	0.28	-0.08	-0.40	0.22
			β-Carotenoids/α-Carotenoids	S8_140192724	GBS	8	140,192,724	1.27E-06	7.22E-02	0.35	184	0.19	0.28	0.20	0.31	-0.09	-0.85	0.12
			Zeaxanthin	S8_171705545	GBS	8	171,705,545	2.95E-07	1.60E-02	0.11	190	0.14	0.14	0.12	0.25	-0.28	0.35	-0.61
			Total β-Xanthophylls	S8_171705545	GBS	8	171,705,545	2.80E-07	2.01E-02	0.10	189	0.14	0.14	0.12	0.26	-0.40	0.40	-0.72
			Zeaxanthin	S8_171705574	GBS	8	171,705,574	9.09E-08	8.71E-03	0.11	190	0.25	0.13	0.12	0.27	-0.29	0.35	-0.63
			Total β-Xanthophylls	S8_171705574	GBS	8	171,705,574	9.66E-08	9.24E-03	0.10	189	0.25	0.13	0.12	0.27	-0.41	0.40	-0.74
			α-Carotene/Zeaxanthin	ss196491114	55K	9	69,215,031	8.69E-10	8.59E-05	0.32	190	0.37	0.29	0.17	0.36	-0.06	-0.25	0.29
			Zeinoxanthin/Lutein	ss196491114	55K	9	69,215,031	1.13E-07	1.08E-02	0.29	189	0.37	0.29	0.09	0.24	0.19	-0.35	-0.39
			Zeinoxanthin	ss196491114	55K	9	69,215,031	4.90E-07	3.53E-02	0.30	192	0.37	0.29	0.10	0.23	0.11	-0.25	-0.34

Statistically significant results from genome-wide association studies on 24 grain carotenoid traits with the peak marker tagging the GWAS signal from *crtRB1* included as a covariate. Markers (Column E) that were significantly associated with the indicated trait (Column D) at 5% false discovery rate (FDR) are demarcated with boldface font and those significant only at 10% FDR without boldface font.

Table S8 Genome-wide Association Study Results with Covariates for *lut1*, *zep1*, *lycE* and *crtRB1* (G)

<i>a priori</i> candidate gene pathway	RefGen_v2 Gene ID	Annotated gene containing associated SNP or gene within 3kb of associated SNP	Trait	SNP ID	SNP Source	Chr	Position in RefGen_v2	P-value	FDR-Adjusted P-value	Minor Allele Frequency (MAF)	Sample Size	MAF Tropical (8% of 201 Lines)	MAF Temperate (92% of 201 Lines)	R-square_LR from Model without SNP	R-square_LR from Model with SNP	Effect Size	Lambda from Box-Cox Procedure	Back-Transformed Effect Estimates
			β-Cryptoxanthin	S7_13843351	GBS	7	13,843,351	4.86E-08	1.42E-02	0.16	177	0.10	0.16	0.24	0.39	-0.04	0.10	-0.33
			Total Carotenoids	S7_121184182	GBS	7	121,184,182	3.30E-07	9.66E-02	0.08	179	0.14	0.08	0.16	0.29	-1.41	0.65	-1.26
			Total β-Xanthophylls	S7_121185500	GBS	7	121,185,500	5.90E-07	8.50E-02	0.09	173	0.19	0.09	0.41	0.51	0.34	0.40	1.07
			Zeaxanthin	S8_171705574	GBS	8	171,705,574	1.54E-07	4.44E-02	0.11	174	0.25	0.13	0.42	0.53	-0.25	0.35	-0.56
			Total β-Xanthophylls	S8_171705574	GBS	8	171,705,574	3.92E-07	8.50E-02	0.10	173	0.25	0.13	0.41	0.51	-0.34	0.40	-0.65

Statistically significant results from genome-wide association studies on 24 grain carotenoid traits with markers tagging the signals at *lut1*, *zep1*, *lycE*, and *crtRB1* included as covariates. Markers (Column E) that were significantly associated with the indicated trait (Column D) at 5% false discovery rate (FDR) are demarcated with boldface font and those significant only at 10% FDR without boldface font.

Table S9 Results from the Pathway-level Analysis with No Covariates (A)

SNP ID	<i>a priori</i> candidate gene pathway	RefGen_v2 Gene ID	Annotated Gene Function	Trait	SNP Source	Chromosome	Position	Gene ORF start	Gene ORF End	Distance from Gene ORF Start	Distance from Gene ORF Finish	P-value	FDR Adjusted P-value	Minor Allele Frequency (MAF)	MAF Tropical (8% of 201 Lines)	MAF Temperate (92% of 201 Lines)	Sample Size	R-square_LR from Model without SNP	R-square_LR from Model with SNP	Effect Size	Lambda from Box-Cox Procedure	Back-Transformed Effect Estimates
S1_5345354	Carotenoid Synthesis and Degradation	GRMZM2G090051	Beta-carotene hydroxylase (non-heme dioxygenase type)	β -Xanthophylls/ α -Xanthophylls	GBS	1	5,345,354	5,380,152	5,382,574	-34,798	-37,220	1.51E-04	4.96E-02	0.09	0.32	0.08	196	0.15	0.21	0.1	-0.4	-0.2
ss196425306	Carotenoid Synthesis and Degradation	GRMZM2G143202	CYP97A3, Cytochrome P450 epsilon-ring hydroxylase	Zeinoxanthin	55K	1	86,844,203	86,838,334	86,848,726	5,869	-4,523	8.95E-08	3.41E-04	0.3	0.4	0.28	198	0.1	0.24	-0.11	-0.25	0.62
ss196425306	Carotenoid Synthesis and Degradation	GRMZM2G143202	CYP97A3, Cytochrome P450 epsilon-ring hydroxylase	α -Carotene/Zeaxanthin	55K	1	86,844,203	86,838,334	86,848,726	5,869	-4,523	3.47E-10	1.32E-06	0.31	0.4	0.28	196	0.17	0.35	0.06	-0.25	-0.22
ss196425306	Carotenoid Synthesis and Degradation	GRMZM2G143202	CYP97A3, Cytochrome P450 epsilon-ring hydroxylase	Zeinoxanthin/Lutein	55K	1	86,844,203	86,838,334	86,848,726	5,869	-4,523	4.97E-08	1.88E-04	0.29	0.4	0.28	195	0.09	0.24	-0.19	-0.35	0.84
ss196425308	Carotenoid Synthesis and Degradation	GRMZM2G143202	CYP97A3, Cytochrome P450 epsilon-ring hydroxylase	Zeinoxanthin	55K	1	86,945,134	86,838,334	86,848,726	106,800	96,408	8.95E-08	3.41E-04	0.3	0.4	0.27	198	0.1	0.24	-0.11	-0.25	0.62
ss196425308	Carotenoid Synthesis and Degradation	GRMZM2G143202	CYP97A3, Cytochrome P450 epsilon-ring hydroxylase	α -Carotene/Zeaxanthin	55K	1	86,945,134	86,838,334	86,848,726	106,800	96,408	3.47E-10	1.32E-06	0.31	0.4	0.27	196	0.17	0.35	0.06	-0.25	-0.22
ss196425308	Carotenoid Synthesis and Degradation	GRMZM2G143202	CYP97A3, Cytochrome P450 epsilon-ring hydroxylase	Zeinoxanthin/Lutein	55K	1	86,945,134	86,838,334	86,848,726	106,800	96,408	4.97E-08	1.88E-04	0.29	0.4	0.27	195	0.09	0.24	-0.19	-0.35	0.84
S2_44445965	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeaxanthin	GBS	2	44,445,965	44,440,299	44,449,237	5,666	-3,272	3.84E-05	2.24E-02	0.18	0.33	0.26	196	0.05	0.13	-0.19	0.35	-0.45
S2_44445965	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Xanthophylls/ α -Xanthophylls	GBS	2	44,445,965	44,440,299	44,449,237	5,666	-3,272	5.13E-05	2.05E-02	0.18	0.33	0.26	196	0.15	0.22	0.08	-0.4	-0.17
S2_44445965	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Carotenoids/ α -Carotenoids	GBS	2	44,445,965	44,440,299	44,449,237	5,666	-3,272	3.36E-05	1.80E-02	0.18	0.33	0.26	190	0.18	0.26	0.09	-0.85	-0.1
S2_44448432	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeaxanthin	GBS	2	44,448,432	44,440,299	44,449,237	8,133	-805	2.22E-09	8.42E-06	0.11	0.29	0.09	196	0.05	0.24	-0.34	0.35	-0.69
S2_44448432	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Total β -Xanthophylls	GBS	2	44,448,432	44,440,299	44,449,237	8,133	-805	1.66E-08	6.30E-05	0.11	0.29	0.09	195	0.05	0.22	-0.43	0.4	-0.76
S2_44448432	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Xanthophylls/ α -Xanthophylls	GBS	2	44,448,432	44,440,299	44,449,237	8,133	-805	4.82E-08	7.29E-05	0.11	0.29	0.09	196	0.15	0.29	0.13	-0.4	-0.26
S2_44448432	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Carotenoids/ α -Carotenoids	GBS	2	44,448,432	44,440,299	44,449,237	8,133	-805	2.20E-06	3.31E-03	0.12	0.29	0.09	190	0.18	0.28	0.12	-0.85	-0.13
S2_44448438	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeaxanthin	GBS	2	44,448,438	44,440,299	44,449,237	8,139	-799	2.22E-09	8.42E-06	0.11	0.29	0.09	196	0.05	0.24	0.34	0.35	1.31
S2_44448438	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Total β -Xanthophylls	GBS	2	44,448,438	44,440,299	44,449,237	8,139	-799	1.66E-08	6.30E-05	0.11	0.29	0.09	195	0.05	0.22	0.43	0.4	1.46
S2_44448438	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Xanthophylls/ α -Xanthophylls	GBS	2	44,448,438	44,440,299	44,449,237	8,139	-799	4.82E-08	7.29E-05	0.11	0.29	0.09	196	0.15	0.29	-0.13	-0.4	0.42
S2_44448438	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Carotenoids/ α -Carotenoids	GBS	2	44,448,438	44,440,299	44,449,237	8,139	-799	2.20E-06	3.31E-03	0.12	0.29	0.09	190	0.18	0.28	-0.12	-0.85	0.17
S2_44472618	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeaxanthin	GBS	2	44,472,618	44,440,299	44,449,237	32,319	23,381	1.26E-04	4.16E-02	0.13	0.38	0.15	196	0.05	0.12	-0.2	0.35	-0.47
S2_44473748	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeaxanthin	GBS	2	44,473,748	44,440,299	44,449,237	33,449	24,511	1.47E-06	1.59E-03	0.14	0.25	0.12	196	0.05	0.17	0.24	0.35	0.86
S2_44473748	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Total β -Xanthophylls	GBS	2	44,473,748	44,440,299	44,449,237	33,449	24,511	5.02E-06	4.74E-03	0.14	0.25	0.12	195	0.05	0.16	0.31	0.4	0.97
S2_44473748	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Xanthophylls/ α -Xanthophylls	GBS	2	44,473,748	44,440,299	44,449,237	33,449	24,511	5.21E-06	2.79E-03	0.14	0.25	0.12	196	0.15	0.24	-0.09	-0.4	0.28
S2_44473748	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Carotenoids/ α -Carotenoids	GBS	2	44,473,748	44,440,299	44,449,237	33,449	24,511	4.89E-05	2.16E-02	0.14	0.25	0.12	190	0.18	0.25	-0.09	-0.85	0.12
S2_44473758	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeaxanthin	GBS	2	44,473,758	44,440,299	44,449,237	33,459	24,521	1.47E-06	1.59E-03	0.14	0.24	0.12	196	0.05	0.17	-0.24	0.35	-0.55
S2_44473758	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Total β -Xanthophylls	GBS	2	44,473,758	44,440,299	44,449,237	33,459	24,521	5.02E-06	4.74E-03	0.14	0.24	0.12	195	0.05	0.16	-0.31	0.4	-0.6
S2_44473758	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Xanthophylls/ α -Xanthophylls	GBS	2	44,473,758	44,440,299	44,449,237	33,459	24,521	5.21E-06	2.79E-03	0.14	0.24	0.12	196	0.15	0.24	0.09	-0.4	-0.2
S2_44473758	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Carotenoids/ α -Carotenoids	GBS	2	44,473,758	44,440,299	44,449,237	33,459	24,521	4.89E-05	2.16E-02	0.14	0.24	0.12	190	0.18	0.25	0.09	-0.85	-0.1
S2_44473801	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeaxanthin	GBS	2	44,473,801	44,440,299	44,449,237	33,502	24,564	1.47E-06	1.59E-03	0.14	0.24	0.12	196	0.05	0.17	0.24	0.35	0.86
S2_44473801	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Total β -Xanthophylls	GBS	2	44,473,801	44,440,299	44,449,237	33,502	24,564	5.02E-06	4.74E-03	0.14	0.24	0.12	195	0.05	0.16	0.31	0.4	0.97
S2_44473801	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Xanthophylls/ α -Xanthophylls	GBS	2	44,473,801	44,440,299	44,449,237	33,502	24,564	5.21E-06	2.79E-03	0.14	0.24	0.12	196	0.15	0.24	-0.09	-0.4	0.28
S2_44473801	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Carotenoids/ α -Carotenoids	GBS	2	44,473,801	44,440,299	44,449,237	33,502	24,564	4.89E-05	2.16E-02	0.14	0.24	0.12	190	0.18	0.25	-0.09	-0.85	0.12
S2_44473994	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Xanthophylls/ α -Xanthophylls	GBS	2	44,473,994	44,440,299	44,449,237	33,695	24,757	9.40E-05	3.56E-02	0.17	0.35	0.16	196	0.15	0.22	-0.07	-0.4	0.21
S2_44473994	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Carotenoids/ α -Carotenoids	GBS	2	44,473,994	44,440,299	44,449,237	33,695	24,757	9.75E-05	3.86E-02	0.18	0.35	0.16	190	0.18	0.25	-0.08	-0.85	0.11
S2_44474139	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeaxanthin	GBS	2	44,474,139	44,440,299	44,449,237	33,840	24,902	5.10E-06	4.29E-03	0.14	0.29	0.13	196	0.05	0.16	-0.23	0.35	-0.52

S2_44474139	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Total β -Xanthophylls	GBS	2	44,474,139	44,440,299	44,449,237	33,840	24,902	1.80E-05	1.24E-02	0.14	0.29	0.13	195	0.05	0.15	-0.29	0.4	-0.57
S2_44474139	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Xanthophylls/ α -Xanthophylls	GBS	2	44,474,139	44,440,299	44,449,237	33,840	24,902	7.57E-06	3.18E-03	0.14	0.29	0.13	196	0.15	0.24	0.09	-0.4	-0.2
S2_44474139	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Carotenoids/ α -Carotenoids	GBS	2	44,474,139	44,440,299	44,449,237	33,840	24,902	5.81E-05	2.43E-02	0.15	0.29	0.13	190	0.18	0.25	0.09	-0.85	-0.1
S2_44474308	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeaxanthin	GBS	2	44,474,308	44,440,299	44,449,237	34,009	25,071	1.19E-06	1.59E-03	0.21	0.38	0.28	196	0.05	0.17	0.21	0.35	0.73
S2_44474308	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Total β -Xanthophylls	GBS	2	44,474,308	44,440,299	44,449,237	34,009	25,071	7.04E-06	5.92E-03	0.22	0.38	0.28	195	0.05	0.16	0.26	0.4	0.79
S2_44474308	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Xanthophylls/ α -Xanthophylls	GBS	2	44,474,308	44,440,299	44,449,237	34,009	25,071	7.03E-06	3.13E-03	0.21	0.38	0.28	196	0.15	0.24	-0.08	-0.4	0.24
S2_44474308	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Carotenoids/ α -Carotenoids	GBS	2	44,474,308	44,440,299	44,449,237	34,009	25,071	1.55E-05	8.96E-03	0.22	0.38	0.28	190	0.18	0.26	-0.09	-0.85	0.11
S5_1315672	Carotenoid Synthesis and Degradation	GRMZM2G144273	carotenoid isomerase	Zeaxanthin	GBS	5	1,315,672	1,333,304	1,341,577	-17,632	-25,905	7.43E-05	2.96E-02	0.29	0.17	0.36	196	0.05	0.13	0.17	0.35	0.56
S5_1315682	Carotenoid Synthesis and Degradation	GRMZM2G144273	carotenoid isomerase	Zeaxanthin	GBS	5	1,315,682	1,333,304	1,341,577	-17,622	-25,895	9.26E-05	3.29E-02	0.28	0.06	0.35	196	0.05	0.13	0.17	0.35	0.56
S5_215994270	Carotenoid Synthesis and Degradation	GRMZM5G837869	CYP97A3, Cytochrome P450 beta-ring hydroxylase	Zeaxanthin	GBS	5	215,994,270	215,827,224	215,831,730	167,046	162,540	8.03E-05	3.04E-02	0.07	0.05	0.07	196	0.05	0.13	-0.28	0.35	-0.62
S5_216074707	Carotenoid Synthesis and Degradation	GRMZM5G837869	CYP97A3, Cytochrome P450 beta-ring hydroxylase	Zeaxanthin	GBS	5	216,074,707	215,827,224	215,831,730	247,483	242,977	1.95E-05	1.23E-02	0.13	0.17	0.17	196	0.05	0.14	-0.22	0.35	-0.5
S5_216074707	Carotenoid Synthesis and Degradation	GRMZM5G837869	CYP97A3, Cytochrome P450 beta-ring hydroxylase	Total β -Xanthophylls	GBS	5	216,074,707	215,827,224	215,831,730	247,483	242,977	3.36E-05	1.91E-02	0.13	0.17	0.17	195	0.05	0.14	-0.28	0.4	-0.57
S6_146970803	Prenyl Group Synthesis	GRMZM2G133082	isopentenyl pyrophosphate isomerase	β -Carotenoids/ α -Carotenoids	GBS	6	146,970,803	147,131,116	147,136,679	-160,313	-165,876	1.34E-04	4.87E-02	0.25	0.07	0.33	190	0.18	0.24	0.07	-0.85	-0.07
S7_13843351	Prenyl Group Synthesis	GRMZM2G493395	1-deoxy-D-xylulose 5-phosphate synthase	Zeaxanthin	GBS	7	13,843,351	14,077,852	14,081,075	-234,501	-237,724	1.38E-04	4.35E-02	0.15	0.1	0.16	196	0.05	0.12	-0.18	0.35	-0.43
S7_13843351	Prenyl Group Synthesis	GRMZM2G493395	1-deoxy-D-xylulose 5-phosphate synthase	β -Cryptoxanthin	GBS	7	13,843,351	14,077,852	14,081,075	-234,501	-237,724	1.66E-07	1.27E-03	0.15	0.1	0.16	199	0.11	0.24	-0.04	0.1	-0.33
S7_13843351	Prenyl Group Synthesis	GRMZM2G493395	1-deoxy-D-xylulose 5-phosphate synthase	Total β -Xanthophylls	GBS	7	13,843,351	14,077,852	14,081,075	-234,501	-237,724	7.52E-05	2.99E-02	0.15	0.1	0.16	195	0.05	0.13	-0.25	0.4	-0.52
S7_13980028	Prenyl Group Synthesis	GRMZM2G493395	1-deoxy-D-xylulose 5-phosphate synthase	Zeinoxanthin	GBS	7	13,980,028	14,077,852	14,081,075	-97,824	-101,047	1.58E-05	4.01E-02	0.41	0.4	0.5	198	0.1	0.19	-0.08	-0.25	0.38
ss196475750	Carotenoid Synthesis and Degradation	GRMZM2G454952	zeta-carotene desaturase	Total α -Xanthophylls	55K	7	17,254,696	17,470,585	17,479,020	-215,889	-224,324	3.14E-05	4.01E-02	0.11	0.19	0.1	200	0.1	0.19	0.7	0.7	1.13
S7_160777986	Prenyl Group Synthesis	GRMZM2G102550	geranylgeranyl pyrophosphate synthase	β -Carotene/(β -Cryptoxanthin+Zeaxanthin)	GBS	7	160,777,986	160,531,537	160,533,586	246,449	244,400	3.04E-05	4.61E-02	0.07	0.08	0.1	196	0.04	0.13	0.14	-0.25	-0.41
S7_160778001	Prenyl Group Synthesis	GRMZM2G102550	geranylgeranyl pyrophosphate synthase	β -Carotene/(β -Cryptoxanthin+Zeaxanthin)	GBS	7	160,778,001	160,531,537	160,533,586	246,464	244,415	3.04E-05	4.61E-02	0.07	0.08	0.1	196	0.04	0.13	0.14	-0.25	-0.41
S7_160778016	Prenyl Group Synthesis	GRMZM2G102550	geranylgeranyl pyrophosphate synthase	β -Carotene/(β -Cryptoxanthin+Zeaxanthin)	GBS	7	160,778,016	160,531,537	160,533,586	246,479	244,430	3.04E-05	4.61E-02	0.07	0.08	0.1	196	0.04	0.13	-0.14	-0.25	0.83
S7_160779488	Prenyl Group Synthesis	GRMZM2G102550	geranylgeranyl pyrophosphate synthase	β -Carotene/ β -Cryptoxanthin	GBS	7	160,779,488	160,531,537	160,533,586	247,951	245,902	7.90E-06	2.01E-02	0.12	0.26	0.12	198	0.1	0.19	0.12	-0.7	-0.15
ss196487098	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Zeaxanthin	55K	8	138,861,176	138,882,594	138,889,812	-21,418	-28,636	9.56E-05	3.29E-02	0.32	0.29	0.33	196	0.05	0.13	0.16	0.35	0.52
ss196487098	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total β -Xanthophylls	55K	8	138,861,176	138,882,594	138,889,812	-21,418	-28,636	3.92E-05	1.91E-02	0.32	0.29	0.33	195	0.05	0.14	0.23	0.4	0.67
ss196487098	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Xanthophylls/ α -Xanthophylls	55K	8	138,861,176	138,882,594	138,889,812	-21,418	-28,636	1.25E-04	4.52E-02	0.32	0.29	0.33	196	0.15	0.22	-0.07	-0.4	0.19
ss196487098	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Carotenoids/ α -Carotenoids	55K	8	138,861,176	138,882,594	138,889,812	-21,418	-28,636	1.36E-04	4.87E-02	0.32	0.29	0.33	190	0.18	0.24	-0.07	-0.85	0.09
<i>lcyE</i> 5'TE	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Lutein	Additional Markers	8	138,882,481	138,882,594	138,889,812	-114	-7,332	1.75E-08	6.69E-05	NA	NA	NA	200	0.11	0.49	NA	0.8	NA
<i>lcyE</i> 5'TE	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Zeaxanthin	Additional Markers	8	138,882,481	138,882,594	138,889,812	-114	-7,332	9.09E-06	6.88E-03	NA	NA	NA	196	0.05	0.25	NA	0.35	NA
<i>lcyE</i> 5'TE	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total β -Xanthophylls	Additional Markers	8	138,882,481	138,882,594	138,889,812	-114	-7,332	1.16E-05	8.76E-03	NA	NA	NA	195	0.06	0.3	NA	0.4	NA
<i>lcyE</i> 5'TE	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total α -Xanthophylls	Additional Markers	8	138,882,481	138,882,594	138,889,812	-114	-7,332	4.37E-09	1.67E-05	NA	NA	NA	200	0.11	0.49	NA	0.7	NA
S8_138882711	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Zeaxanthin	GBS	8	138,882,711	138,882,594	138,889,812	117	-7,101	5.31E-05	2.51E-02	0.28	0.41	0.3	196	0.05	0.13	0.16	0.35	0.55
S8_138882711	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total β -Xanthophylls	GBS	8	138,882,711	138,882,594	138,889,812	117	-7,101	4.54E-05	1.91E-02	0.28	0.41	0.3	195	0.05	0.14	0.23	0.4	0.66
S8_138882711	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Xanthophylls/ α -Xanthophylls	GBS	8	138,882,711	138,882,594	138,889,812	117	-7,101	8.85E-07	6.80E-04	0.28	0.41	0.3	196	0.15	0.26	-0.09	-0.4	0.25
S8_138882711	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Carotenoids/ α -Carotenoids	GBS	8	138,882,711	138,882,594	138,889,812	117	-7,101	4.27E-06	3.57E-03	0.28	0.41	0.3	190	0.18	0.28	-0.09	-0.85	0.12
ss196504160	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Zeaxanthin	55K	8	138,882,711	138,882,594	138,889,812	117	-7,101	2.30E-06	2.17E-03	0.35	0.48	0.34	196	0.05	0.16	-0.19	0.35	-0.45
ss196504160	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total β -Xanthophylls	55K	8	138,882,711	138,882,594	138,889,812	117	-7,101	1.36E-06	2.57E-03	0.35	0.48	0.34	195	0.05	0.18	-0.26	0.4	-0.53
ss196504160	Carotenoid Synthesis	GRMZM2G012966	lycopene epsilon-cyclase	Total α -	55K	8	138,882,711	138,882,594	138,889,812	117	-7,101	1.50E-05	2.30E-02	0.36	0.48	0.34	200	0.1	0.19	0.55	0.7	0.87

	and Degradation			Xanthophylls																		
ss196504160	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Xanthophylls/ α -Xanthophylls	55K	8	138,882,711	138,882,594	138,889,812	117	-7,101	1.11E-09	4.19E-06	0.35	0.48	0.34	196	0.15	0.33	0.11	-0.4	-0.22
ss196504160	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Carotenoids/ α -Carotenoids	55K	8	138,882,711	138,882,594	138,889,812	117	-7,101	2.08E-09	1.56E-05	0.36	0.48	0.34	190	0.18	0.35	0.12	-0.85	-0.12
S8_138882747	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Zeaxanthin	GBS	8	138,882,747	138,882,594	138,889,812	153	-7,065	5.31E-05	2.51E-02	0.28	0.41	0.3	196	0.05	0.13	0.16	0.35	0.55
S8_138882747	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total β -Xanthophylls	GBS	8	138,882,747	138,882,594	138,889,812	153	-7,065	4.54E-05	1.91E-02	0.28	0.41	0.3	195	0.05	0.14	0.23	0.4	0.66
S8_138882747	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Xanthophylls/ α -Xanthophylls	GBS	8	138,882,747	138,882,594	138,889,812	153	-7,065	8.85E-07	6.80E-04	0.28	0.41	0.3	196	0.15	0.26	-0.09	-0.4	0.25
S8_138882747	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Carotenoids/ α -Carotenoids	GBS	8	138,882,747	138,882,594	138,889,812	153	-7,065	4.27E-06	3.57E-03	0.28	0.41	0.3	190	0.18	0.28	-0.09	-0.85	0.12
S8_138882751	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Zeaxanthin	GBS	8	138,882,751	138,882,594	138,889,812	157	-7,061	5.31E-05	2.51E-02	0.28	0.41	0.3	196	0.05	0.13	0.16	0.35	0.55
S8_138882751	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total β -Xanthophylls	GBS	8	138,882,751	138,882,594	138,889,812	157	-7,061	4.54E-05	1.91E-02	0.28	0.41	0.3	195	0.05	0.14	0.23	0.4	0.66
S8_138882751	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Xanthophylls/ α -Xanthophylls	GBS	8	138,882,751	138,882,594	138,889,812	157	-7,061	8.85E-07	6.80E-04	0.28	0.41	0.3	196	0.15	0.26	-0.09	-0.4	0.25
S8_138882751	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Carotenoids/ α -Carotenoids	GBS	8	138,882,751	138,882,594	138,889,812	157	-7,061	4.27E-06	3.57E-03	0.28	0.41	0.3	190	0.18	0.28	-0.09	-0.85	0.12
S8_138882798	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Xanthophylls/ α -Xanthophylls	GBS	8	138,882,798	138,882,594	138,889,812	204	-7,014	8.99E-07	6.80E-04	0.31	0.21	0.36	196	0.15	0.26	-0.08	-0.4	0.25
S8_138882798	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Carotenoids/ α -Carotenoids	GBS	8	138,882,798	138,882,594	138,889,812	204	-7,014	2.87E-06	3.57E-03	0.31	0.21	0.36	190	0.18	0.28	-0.09	-0.85	0.12
S8_138882897	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Zeaxanthin	GBS	8	138,882,897	138,882,594	138,889,812	303	-6,915	6.07E-05	2.71E-02	0.44	0.12	0.44	196	0.05	0.13	0.15	0.35	0.48
S8_138882897	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total β -Xanthophylls	GBS	8	138,882,897	138,882,594	138,889,812	303	-6,915	1.15E-04	4.34E-02	0.44	0.12	0.44	195	0.05	0.13	0.19	0.4	0.55
S8_138882897	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total α -Xanthophylls	GBS	8	138,882,897	138,882,594	138,889,812	303	-6,915	7.60E-06	1.45E-02	0.43	0.12	0.44	200	0.1	0.2	-0.52	0.7	-0.65
S8_138882897	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Xanthophylls/ α -Xanthophylls	GBS	8	138,882,897	138,882,594	138,889,812	303	-6,915	9.76E-08	1.23E-04	0.43	0.12	0.44	196	0.15	0.28	-0.08	-0.4	0.25
S8_138882897	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Carotenoids/ α -Carotenoids	GBS	8	138,882,897	138,882,594	138,889,812	303	-6,915	6.03E-08	2.27E-04	0.44	0.12	0.44	190	0.18	0.32	-0.1	-0.85	0.13
S8_138883026	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Xanthophylls/ α -Xanthophylls	GBS	8	138,883,026	138,882,594	138,889,812	432	-6,786	5.90E-06	2.79E-03	0.4	0.18	0.48	196	0.15	0.24	0.07	-0.4	-0.16
S8_138883026	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Carotenoids/ α -Carotenoids	GBS	8	138,883,026	138,882,594	138,889,812	432	-6,786	6.51E-06	4.45E-03	0.4	0.18	0.48	190	0.18	0.27	0.08	-0.85	-0.09
S8_138883056	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Xanthophylls/ α -Xanthophylls	GBS	8	138,883,056	138,882,594	138,889,812	462	-6,756	5.90E-06	2.79E-03	0.4	0.18	0.48	196	0.15	0.24	-0.07	-0.4	0.21
S8_138883056	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Carotenoids/ α -Carotenoids	GBS	8	138,883,056	138,882,594	138,889,812	462	-6,756	6.51E-06	4.45E-03	0.4	0.18	0.48	190	0.18	0.27	-0.08	-0.85	0.11
lcyE SNP216	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Lutein	Additional Markers	8	138,883,206	138,882,594	138,889,812	612	-6,606	6.28E-09	4.80E-05	NA	NA	NA	200	0.11	0.45	NA	0.8	NA
lcyE SNP216	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total β -Xanthophylls	Additional Markers	8	138,883,206	138,882,594	138,889,812	612	-6,606	1.65E-07	4.17E-04	NA	NA	NA	195	0.06	0.3	NA	0.4	NA
lcyE SNP216	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total α -Xanthophylls	Additional Markers	8	138,883,206	138,882,594	138,889,812	612	-6,606	4.62E-10	3.53E-06	NA	NA	NA	200	0.11	0.45	NA	0.7	NA
lcyE SNP216	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Xanthophylls/ α -Xanthophylls	Additional Markers	8	138,883,206	138,882,594	138,889,812	612	-6,606	5.05E-16	3.82E-12	NA	NA	NA	196	0.1	0.24	NA	-0.4	NA
PZB00665.1	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Xanthophylls/ α -Xanthophylls	4K	8	138,886,137	138,882,594	138,889,812	3,543	-3,675	3.82E-06	2.63E-03	0.35	0.05	0.38	196	0.15	0.25	0.08	-0.4	-0.17
PZB00665.1	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Carotenoids/ α -Carotenoids	4K	8	138,886,137	138,882,594	138,889,812	3,543	-3,675	1.51E-05	8.96E-03	0.35	0.05	0.38	190	0.18	0.26	0.08	-0.85	-0.09
S8_138888278	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Zeaxanthin	GBS	8	138,888,278	138,882,594	138,889,812	5,684	-1,534	6.99E-05	2.94E-02	0.46	0.19	0.42	196	0.05	0.13	0.15	0.35	0.49
S8_138888278	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total β -Xanthophylls	GBS	8	138,888,278	138,882,594	138,889,812	5,684	-1,534	4.20E-05	1.91E-02	0.47	0.19	0.42	195	0.05	0.14	0.21	0.4	0.62
S8_138888278	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total α -Xanthophylls	GBS	8	138,888,278	138,882,594	138,889,812	5,684	-1,534	6.09E-06	1.45E-02	0.47	0.19	0.42	200	0.1	0.2	-0.55	0.7	-0.68
S8_138888278	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Xanthophylls/ α -Xanthophylls	GBS	8	138,888,278	138,882,594	138,889,812	5,684	-1,534	2.52E-08	6.36E-05	0.47	0.19	0.42	196	0.15	0.3	-0.09	-0.4	0.28
S8_138888278	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Carotenoids/ α -Carotenoids	GBS	8	138,888,278	138,882,594	138,889,812	5,684	-1,534	1.82E-07	4.56E-04	0.47	0.19	0.42	190	0.18	0.31	-0.1	-0.85	0.13
crtRB1 InDel4	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	Zeaxanthin	Additional Markers	10	136,059,748	136,057,100	136,060,219	2,648	-471	1.14E-05	7.88E-03	NA	NA	NA	196	0.05	0.13	NA	0.35	NA
crtRB1 InDel4	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	Total β -Xanthophylls	Additional Markers	10	136,059,748	136,057,100	136,060,219	2,648	-471	2.06E-05	1.30E-02	NA	NA	NA	195	0.06	0.13	NA	0.4	NA
crtRB1 InDel4	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	β -Carotene/ β -Cryptoxanthin	Additional Markers	10	136,059,748	136,057,100	136,060,219	2,648	-471	1.90E-06	1.45E-02	NA	NA	NA	198	0.09	0.08	NA	-0.7	NA
crtRB1 InDel4	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	β -Carotene/(β -Cryptoxanthin+Zeaxanthin)	Additional Markers	10	136,059,748	136,057,100	136,060,219	2,648	-471	2.23E-07	1.33E-03	NA	NA	NA	196	0.06	0.11	NA	-0.25	NA

<i>crtRB1</i> InDel4	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	Total Carotenes/Total Xanthophylls	Additional Markers	10	136,059,748	136,057,100	136,060,219	2,648	-471	3.15E-06	2.36E-02	NA	NA	NA	188	0.04	0.11	NA	-0.55	NA
ss196501627	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	β -Carotene/ β -Cryptoxanthin	55K	10	136,060,033	136,057,100	136,060,219	2,933	-186	7.72E-06	2.01E-02	0.18	0	0.22	198	0.1	0.19	0.11	-0.7	-0.14
ss196501627	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	β -Carotene/(β -Cryptoxanthin+Zeaxanthin)	55K	10	136,060,033	136,057,100	136,060,219	2,933	-186	3.51E-07	1.33E-03	0.19	0	0.22	196	0.04	0.18	0.12	-0.25	-0.36
<i>crtRB1</i> 3'TE	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	Zeaxanthin	Additional Markers	10	136,061,719	136,057,100	136,060,219	4,619	1,500	1.11E-06	1.59E-03	NA	NA	NA	196	0.05	0.17	NA	0.35	NA
<i>crtRB1</i> 3'TE	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	Total β -Xanthophylls	Additional Markers	10	136,061,719	136,057,100	136,060,219	4,619	1,500	1.97E-06	2.98E-03	NA	NA	NA	195	0.06	0.18	NA	0.4	NA
<i>crtRB1</i> 3'TE	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	β -Xanthophylls/ α -Xanthophylls	Additional Markers	10	136,061,719	136,057,100	136,060,219	4,619	1,500	1.51E-04	4.96E-02	NA	NA	NA	196	0.1	0.03	NA	-0.4	NA
<i>crtRB1</i> 3'TE	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	β -Carotene/ β -Cryptoxanthin	Additional Markers	10	136,061,719	136,057,100	136,060,219	4,619	1,500	1.86E-05	3.55E-02	NA	NA	NA	198	0.09	0.07	NA	-0.7	NA

Statistically significant results from a pathway-level analysis of 58 *a priori* candidate genes from the carotenoid biosynthesis, carotenoid degradation, and isoprenoid biosynthetic pathways on 24 grain carotenoid traits without any markers tagging the peak GWAS signals included as covariates. All markers (Column A) proximal to *a priori* candidate genes (Column B) found to be significantly associated with the indicated trait (Column E) at 5% FDR are shown.

Table S9 Results from the Pathway-Level Analysis with S2_44448432 as Covariate Tagging *zep1* (B)

SNP ID	<i>a priori</i> candidate gene pathway		Annotated Gene Function	Trait	SNP Source	Chromosome	Position	Gene ORF start	Gene ORF End	Distance from Gene ORF Start	Distance from Gene ORF Finish	P-value	FDR Adjusted P-value	Minor Allele Frequency (MAF)	MAF Tropical (8% of 201 Lines)	MAF Temperate (92% of 201 Lines)	Sample Size	R- square_LR from Model without SNP	R- square_LR from Model with SNP	Effect Size	Lambda from Box-Cox Procedur e	Back- Transformed Effect Estimates
ss196425306	Carotenoid Synthesis and Degradation	GRMZM2G143202	CYP97A3, Cytochrome P450 epsilon-ring hydroxylase	Zeinoxanthin	55K	1	86,844,203	86,838,334	86,848,726	5,869	-4,523	8.95E-08	3.41E-04	0.3	0.4	0.28	198	0.1	0.24	-0.11	-0.25	0.62
ss196425306	Carotenoid Synthesis and Degradation	GRMZM2G143202	CYP97A3, Cytochrome P450 epsilon-ring hydroxylase	α -Carotene/Zeaxanthin	55K	1	86,844,203	86,838,334	86,848,726	5,869	-4,523	3.47E-10	1.32E-06	0.31	0.4	0.28	196	0.17	0.35	0.06	-0.25	-0.22
ss196425306	Carotenoid Synthesis and Degradation	GRMZM2G143202	CYP97A3, Cytochrome P450 epsilon-ring hydroxylase	Zeinoxanthin/Lutein	55K	1	86,844,203	86,838,334	86,848,726	5,869	-4,523	4.97E-08	1.88E-04	0.29	0.4	0.28	195	0.09	0.24	-0.19	-0.35	0.84
ss196425308	Carotenoid Synthesis and Degradation	GRMZM2G143202	CYP97A3, Cytochrome P450 epsilon-ring hydroxylase	Zeinoxanthin	55K	1	86,945,134	86,838,334	86,848,726	106,800	96,408	8.95E-08	3.41E-04	0.3	0.4	0.27	198	0.1	0.24	-0.11	-0.25	0.62
ss196425308	Carotenoid Synthesis and Degradation	GRMZM2G143202	CYP97A3, Cytochrome P450 epsilon-ring hydroxylase	α -Carotene/Zeaxanthin	55K	1	86,945,134	86,838,334	86,848,726	106,800	96,408	3.47E-10	1.32E-06	0.31	0.4	0.27	196	0.17	0.35	0.06	-0.25	-0.22
ss196425308	Carotenoid Synthesis and Degradation	GRMZM2G143202	CYP97A3, Cytochrome P450 epsilon-ring hydroxylase	Zeinoxanthin/Lutein	55K	1	86,945,134	86,838,334	86,848,726	106,800	96,408	4.97E-08	1.88E-04	0.29	0.4	0.27	195	0.09	0.24	-0.19	-0.35	0.84
S5_1315672	Carotenoid Synthesis and Degradation	GRMZM2G144273	carotenoid isomerase	Zeaxanthin	GBS	5	1,315,672	1,333,304	1,341,577	-17,632	-25,905	9.61E-06	7.11E-03	0.29	0.17	0.36	196	0.24	0.32	0.17	0.35	0.57
S5_1315672	Carotenoid Synthesis and Degradation	GRMZM2G144273	carotenoid isomerase	Total β -Xanthophylls	GBS	5	1,315,672	1,333,304	1,341,577	-17,632	-25,905	3.44E-05	1.73E-02	0.29	0.17	0.36	195	0.22	0.3	0.22	0.4	0.65
S5_1315682	Carotenoid Synthesis and Degradation	GRMZM2G144273	carotenoid isomerase	Zeaxanthin	GBS	5	1,315,682	1,333,304	1,341,577	-17,622	-25,895	1.74E-05	1.10E-02	0.28	0.06	0.35	196	0.24	0.32	0.17	0.35	0.56
S5_1315682	Carotenoid Synthesis and Degradation	GRMZM2G144273	carotenoid isomerase	Total β -Xanthophylls	GBS	5	1,315,682	1,333,304	1,341,577	-17,622	-25,895	6.11E-05	2.89E-02	0.28	0.06	0.35	195	0.22	0.29	0.22	0.4	0.63
S5_216074707	Carotenoid Synthesis and Degradation	GRMZM5G837869	CYP97A3, Cytochrome P450 beta-ring hydroxylase	Zeaxanthin	GBS	5	216,074,707	215,827,224	215,831,730	247,483	242,977	8.60E-05	3.43E-02	0.13	0.17	0.17	196	0.24	0.3	-0.18	0.35	-0.43
S7_13843351	Prenyl Group Synthesis	GRMZM2G493395	1-deoxy-D-xylulose 5-phosphate synthase	Zeaxanthin	GBS	7	13,843,351	14,077,852	14,081,075	-234,501	-237,724	4.69E-05	2.15E-02	0.15	0.1	0.16	196	0.24	0.31	-0.17	0.35	-0.42
S7_13843351	Prenyl Group Synthesis	GRMZM2G493395	1-deoxy-D-xylulose 5-phosphate synthase	β -Cryptoxanthin	GBS	7	13,843,351	14,077,852	14,081,075	-234,501	-237,724	1.66E-07	1.27E-03	0.15	0.1	0.16	199	0.11	0.24	-0.04	0.1	-0.33
S7_13843351	Prenyl Group Synthesis	GRMZM2G493395	1-deoxy-D-xylulose 5-phosphate synthase	Total β -Xanthophylls	GBS	7	13,843,351	14,077,852	14,081,075	-234,501	-237,724	2.74E-05	1.68E-02	0.15	0.1	0.16	195	0.22	0.3	-0.25	0.4	-0.51
S7_13980028	Prenyl Group Synthesis	GRMZM2G493395	1-deoxy-D-xylulose 5-phosphate synthase	Zeinoxanthin	GBS	7	13,980,028	14,077,852	14,081,075	-97,824	-101,047	1.58E-05	4.01E-02	0.41	0.4	0.5	198	0.1	0.19	-0.08	-0.25	0.38
ss196475750	Carotenoid Synthesis and Degradation	GRMZM2G454952	zeta-carotene desaturase	Total α -Xanthophylls	55K	7	17,254,696	17,470,585	17,479,020	-215,889	-224,324	3.14E-05	4.00E-02	0.11	0.19	0.1	200	0.1	0.19	0.7	0.7	1.13
S7_160777986	Prenyl Group Synthesis	GRMZM2G102550	geranylgeranyl pyrophosphate synthase	β -Carotene/ β -Cryptoxanthin+Zeaxanthin)	GBS	7	160,777,986	160,531,537	160,533,586	246,449	244,400	3.04E-05	4.61E-02	0.07	0.08	0.1	196	0.04	0.13	0.14	-0.25	-0.41
S7_160778001	Prenyl Group Synthesis	GRMZM2G102550	geranylgeranyl pyrophosphate synthase	β -Carotene/ β -Cryptoxanthin+Zeaxanthin)	GBS	7	160,778,001	160,531,537	160,533,586	246,464	244,415	3.04E-05	4.61E-02	0.07	0.08	0.1	196	0.04	0.13	0.14	-0.25	-0.41
S7_160778016	Prenyl Group Synthesis	GRMZM2G102550	geranylgeranyl pyrophosphate synthase	β -Carotene/ β -Cryptoxanthin+Zeaxanthin)	GBS	7	160,778,016	160,531,537	160,533,586	246,479	244,430	3.04E-05	4.61E-02	0.07	0.08	0.1	196	0.04	0.13	-0.14	-0.25	0.83
S7_160779488	Prenyl Group Synthesis	GRMZM2G102550	geranylgeranyl pyrophosphate synthase	β -Carotene/ β -Cryptoxanthin)	GBS	7	160,779,488	160,531,537	160,533,586	247,951	245,902	7.90E-06	2.01E-02	0.12	0.26	0.12	198	0.1	0.19	0.12	-0.7	-0.15
ss196487098	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Zeaxanthin	55K	8	138,861,176	138,882,594	138,889,812	-21,418	-28,636	4.82E-05	2.15E-02	0.32	0.29	0.33	196	0.24	0.31	0.15	0.35	0.49
ss196487098	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total β -Xanthophylls	55K	8	138,861,176	138,882,594	138,889,812	-21,418	-28,636	2.35E-05	1.62E-02	0.32	0.29	0.33	195	0.22	0.3	0.22	0.4	0.63
ss196487098	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Xanthophylls/ α -Xanthophylls	55K	8	138,861,176	138,882,594	138,889,812	-21,418	-28,636	6.92E-05	4.36E-02	0.32	0.29	0.33	196	0.29	0.35	-0.06	-0.4	0.18
<i>IcyE</i> 5'TE	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Lutein	Additional Markers	8	138,882,481	138,882,594	138,889,812	-114	-7,332	1.92E-08	7.34E-05	NA	NA	NA	200	0.11	0.49	NA	0.8	NA
<i>IcyE</i> 5'TE	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Zeaxanthin	Additional Markers	8	138,882,481	138,882,594	138,889,812	-114	-7,332	1.19E-07	3.39E-04	NA	NA	NA	196	0.24	0.38	NA	0.35	NA
<i>IcyE</i> 5'TE	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total β -Xanthophylls	Additional Markers	8	138,882,481	138,882,594	138,889,812	-114	-7,332	3.22E-07	8.11E-04	NA	NA	NA	195	0.22	0.4	NA	0.4	NA
<i>IcyE</i> 5'TE	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total α -Xanthophylls	Additional Markers	8	138,882,481	138,882,594	138,889,812	-114	-7,332	4.85E-09	1.85E-05	NA	NA	NA	200	0.09	0.43	NA	0.7	NA
<i>IcyE</i> 5'TE	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Xanthophylls/ α -Xanthophylls	Additional Markers	8	138,882,481	138,882,594	138,889,812	-114	-7,332	7.24E-14	5.48E-10	NA	NA	NA	196	0.21	0.3	NA	-0.4	NA
S8_138882711	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Zeaxanthin	GBS	8	138,882,711	138,882,594	138,889,812	117	-7,101	7.95E-06	6.69E-03	0.28	0.41	0.3	196	0.24	0.32	0.16	0.35	0.55
S8_138882711	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total β -Xanthophylls	GBS	8	138,882,711	138,882,594	138,889,812	117	-7,101	9.61E-06	9.09E-03	0.28	0.41	0.3	195	0.22	0.31	0.22	0.4	0.66
S8_138882711	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Xanthophylls/ α -Xanthophylls	GBS	8	138,882,711	138,882,594	138,889,812	117	-7,101	2.59E-07	1.96E-04	0.28	0.41	0.3	196	0.29	0.4	-0.08	-0.4	0.24
S8_138882711	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Carotenoids/ α -Carotenoids	GBS	8	138,882,711	138,882,594	138,889,812	117	-7,101	1.85E-06	1.54E-03	0.29	0.41	0.3	189	0.29	0.38	-0.09	-0.85	0.11
ss196504160	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Zeaxanthin	55K	8	138,882,711	138,882,594	138,889,812	117	-7,101	1.37E-07	3.39E-04	0.35	0.48	0.34	196	0.24	0.36	-0.19	0.35	-0.45
ss196504160	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total β -Xanthophylls	55K	8	138,882,711	138,882,594	138,889,812	117	-7,101	1.19E-07	4.52E-04	0.35	0.48	0.34	195	0.22	0.35	-0.27	0.4	-0.54
ss196504160	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total α -Xanthophylls	55K	8	138,882,711	138,882,594	138,889,812	117	-7,101	1.50E-05	2.30E-02	0.36	0.48	0.34	200	0.1	0.19	0.55	0.7	0.87
ss196504160	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Xanthophylls/ α -Xanthophylls	55K	8	138,882,711	138,882,594	138,889,812	117	-7,101	1.32E-10	4.98E-07	0.35	0.48	0.34	196	0.29	0.46	0.1	-0.4	-0.22
ss196504160	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Carotenoids/ α -Carotenoids	55K	8	138,882,711	138,882,594	138,889,812	117	-7,101	4.23E-10	3.17E-06	0.37	0.48	0.34	189	0.29	0.45	0.11	-0.85	-0.12
S8_138882747	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Zeaxanthin	GBS	8	138,882,747	138,882,594	138,889,812	153	-7,065	7.95E-06	6.69E-03	0.28	0.41	0.3	196	0.24	0.32	0.16	0.35	0.55
S8_138882747	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total β -Xanthophylls	GBS	8	138,882,747	138,882,594	138,889,812	153	-7,065	9.61E-06	9.09E-03	0.28	0.41	0.3	195	0.22	0.31	0.22	0.4	0.66
S8_138882747	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Xanthophylls/ α -Xanthophylls	GBS	8	138,882,747	138,882,594	138,889,812	153	-7,065	2.59E-07	1.96E-04	0.28	0.41	0.3	196	0.29	0.4	-0.08	-0.4	0.24
S8_138882747	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Carotenoids/ α -Carotenoids	GBS	8	138,882,747	138,882,594	138,889,812	153	-7,065	1.85E-06	1.54E-03	0.29	0.41	0.3	189	0.29	0.38	-0.09	-0.85	0.11
S8_138882751	Carotenoid Synthesis and Degradation	GRMZM2G012966	ly																			

S8_138882897	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Zeaxanthin	GBS	8	138,882,897	138,882,594	138,889,812	303	-6,915	5.03E-06	6.35E-03	0.44	0.12	0.44	196	0.24	0.33	0.15	0.35	0.49
S8_138882897	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total β -Xanthophylls	GBS	8	138,882,897	138,882,594	138,889,812	303	-6,915	1.65E-05	1.37E-02	0.44	0.12	0.44	195	0.22	0.3	0.2	0.4	0.56
S8_138882897	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total α -Xanthophylls	GBS	8	138,882,897	138,882,594	138,889,812	303	-6,915	7.60E-06	1.45E-02	0.43	0.12	0.44	200	0.1	0.2	-0.52	0.7	-0.65
S8_138882897	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Xanthophylls/ α -Xanthophylls	GBS	8	138,882,897	138,882,594	138,889,812	303	-6,915	1.52E-08	2.88E-05	0.43	0.12	0.44	196	0.29	0.42	-0.08	-0.4	0.24
S8_138882897	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Carotenoids/ α -Carotenoids	GBS	8	138,882,897	138,882,594	138,889,812	303	-6,915	1.27E-08	4.78E-05	0.43	0.12	0.44	189	0.29	0.42	-0.1	-0.85	0.13
S8_138883026	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Zeaxanthin	GBS	8	138,883,026	138,882,594	138,889,812	432	-6,786	2.20E-05	1.19E-02	0.4	0.18	0.48	196	0.24	0.31	-0.14	0.35	-0.36
S8_138883026	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total β -Xanthophylls	GBS	8	138,883,026	138,882,594	138,889,812	432	-6,786	3.10E-05	1.68E-02	0.4	0.18	0.48	195	0.22	0.3	-0.19	0.4	-0.42
S8_138883026	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Xanthophylls/ α -Xanthophylls	GBS	8	138,883,026	138,882,594	138,889,812	432	-6,786	1.13E-07	1.22E-04	0.4	0.18	0.48	196	0.29	0.4	0.08	-0.4	-0.17
S8_138883026	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Carotenoids/ α -Carotenoids	GBS	8	138,883,026	138,882,594	138,889,812	432	-6,786	2.13E-07	3.19E-04	0.4	0.18	0.48	189	0.29	0.4	0.09	-0.85	-0.1
S8_138883056	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Zeaxanthin	GBS	8	138,883,056	138,882,594	138,889,812	462	-6,756	2.20E-05	1.19E-02	0.4	0.18	0.48	196	0.24	0.31	0.14	0.35	0.47
S8_138883056	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total β -Xanthophylls	GBS	8	138,883,056	138,882,594	138,889,812	462	-6,756	3.10E-05	1.68E-02	0.4	0.18	0.48	195	0.22	0.3	0.19	0.4	0.56
S8_138883056	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Xanthophylls/ α -Xanthophylls	GBS	8	138,883,056	138,882,594	138,889,812	462	-6,756	1.13E-07	1.22E-04	0.4	0.18	0.48	196	0.29	0.4	-0.08	-0.4	0.23
S8_138883056	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Carotenoids/ α -Carotenoids	GBS	8	138,883,056	138,882,594	138,889,812	462	-6,756	2.13E-07	3.19E-04	0.4	0.18	0.48	189	0.29	0.4	-0.09	-0.85	0.12
<i>lcyE</i> SNP216	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Lutein	Additional Markers	8	138,883,206	138,882,594	138,889,812	612	-6,606	6.78E-09	5.18E-05	NA	NA	NA	200	0.11	0.45	NA	0.8	NA
<i>lcyE</i> SNP216	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Zeaxanthin	Additional Markers	8	138,883,206	138,882,594	138,889,812	612	-6,606	1.57E-09	1.19E-05	NA	NA	NA	196	0.24	0.39	NA	0.35	NA
<i>lcyE</i> SNP216	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total β -Xanthophylls	Additional Markers	8	138,883,206	138,882,594	138,889,812	612	-6,606	5.83E-09	4.41E-05	NA	NA	NA	195	0.22	0.4	NA	0.4	NA
<i>lcyE</i> SNP216	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total α -Xanthophylls	Additional Markers	8	138,883,206	138,882,594	138,889,812	612	-6,606	4.71E-10	3.60E-06	NA	NA	NA	200	0.09	0.4	NA	0.7	NA
PZB00665.1	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Xanthophylls/ α -Xanthophylls	4K	8	138,886,137	138,882,594	138,889,812	3,543	-3,675	1.03E-05	7.09E-03	0.35	0.05	0.38	196	0.29	0.37	0.07	-0.4	-0.15
PZB00665.1	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Carotenoids/ α -Carotenoids	4K	8	138,886,137	138,882,594	138,889,812	3,543	-3,675	2.05E-05	1.54E-02	0.35	0.05	0.38	189	0.29	0.36	0.08	-0.85	-0.08
S8_138888278	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Lutein	GBS	8	138,888,278	138,882,594	138,889,812	5,684	-1,534	8.74E-06	2.23E-02	0.47	0.19	0.42	200	0.12	0.21	-0.85	0.8	-0.91
S8_138888278	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Zeaxanthin	GBS	8	138,888,278	138,882,594	138,889,812	5,684	-1,534	3.36E-06	5.09E-03	0.46	0.19	0.42	196	0.24	0.33	0.16	0.35	0.53
S8_138888278	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total β -Xanthophylls	GBS	8	138,888,278	138,882,594	138,889,812	5,684	-1,534	3.28E-06	4.97E-03	0.47	0.19	0.42	195	0.22	0.32	0.22	0.4	0.65
S8_138888278	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total α -Xanthophylls	GBS	8	138,888,278	138,882,594	138,889,812	5,684	-1,534	6.09E-06	1.45E-02	0.47	0.19	0.42	200	0.1	0.2	-0.55	0.7	-0.68
S8_138888278	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Xanthophylls/ α -Xanthophylls	GBS	8	138,888,278	138,882,594	138,889,812	5,684	-1,534	2.25E-09	5.67E-06	0.47	0.19	0.42	196	0.29	0.44	-0.09	-0.4	0.28
S8_138888278	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Carotenoids/ α -Carotenoids	GBS	8	138,888,278	138,882,594	138,889,812	5,684	-1,534	3.13E-08	7.83E-05	0.47	0.19	0.42	189	0.29	0.41	-0.1	-0.85	0.13
<i>lcyE_3TE</i>	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Carotenoids/ α -Carotenoids	Additional Markers	8	138,891,312	138,882,594	138,889,812	8,718	1,500	3.31E-05	2.26E-02	NA	NA	NA	189	0.2	0.16	NA	-0.85	NA
<i>crtRB1</i> InDel4	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	Zeaxanthin	Additional Markers	10	136,059,748	136,057,100	136,060,219	2,648	-471	3.73E-05	1.88E-02	NA	NA	NA	196	0.24	0.26	NA	0.35	NA
<i>crtRB1</i> InDel4	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	Total β -Xanthophylls	Additional Markers	10	136,059,748	136,057,100	136,060,219	2,648	-471	7.75E-05	3.45E-02	NA	NA	NA	195	0.22	0.24	NA	0.4	NA
<i>crtRB1</i> InDel4	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	β -Carotene/ β -Cryptoxanthin	Additional Markers	10	136,059,748	136,057,100	136,060,219	2,648	-471	5.29E-07	4.03E-03	NA	NA	NA	198	0.07	0.07	NA	-0.7	NA
<i>crtRB1</i> InDel4	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	β -Carotene/ β -Cryptoxanthin+Zeaxanthin	Additional Markers	10	136,059,748	136,057,100	136,060,219	2,648	-471	2.14E-07	1.33E-03	NA	NA	NA	196	0.04	0.09	NA	-0.25	NA
<i>crtRB1</i> InDel4	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	Total Carotenes/Total Xanthophylls	Additional Markers	10	136,059,748	136,057,100	136,060,219	2,648	-471	7.68E-06	1.91E-02	NA	NA	NA	188	0.05	0.11	NA	-0.55	NA
ss196501627	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	Zeaxanthin	55K	10	136,060,033	136,057,100	136,060,219	2,933	-186	5.50E-05	2.31E-02	0.2	0	0.22	196	0.24	0.31	0.16	0.35	0.54
ss196501627	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	Total β -Xanthophylls	55K	10	136,060,033	136,057,100	136,060,219	2,933	-186	9.04E-05	3.80E-02	0.2	0	0.22	195	0.22	0.29	0.22	0.4	0.64
ss196501627	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	β -Carotene/ β -Cryptoxanthin	55K	10	136,060,033	136,057,100	136,060,219	2,933	-186	7.72E-06	2.01E-02	0.18	0	0.22	198	0.1	0.19	0.11	-0.7	-0.14
ss196501627	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	β -Carotene/ β -Cryptoxanthin+Zeaxanthin	55K	10	136,060,033	136,057,100	136,060,219	2,933	-186	3.51E-07	1.33E-03	0.19	0	0.22	196	0.04	0.18	0.12	-0.25	-0.36
ss196501627	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	Total Carotenes/Total Xanthophylls	55K	10	136,060,033	136,057,100	136,060,219	2,933	-186	6.15E-06	1.91E-02	0.19	0	0.22	188	0.07	0.17	0.19	-0.55	-0.27
<i>crtRB1</i> 3'TE	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	Zeaxanthin	Additional Markers	10	136,061,719	136,057,100	136,060,219	4,619	1,500	1.79E-07	3.39E-04	NA	NA	NA	196	0.24	0.31	NA	0.35	NA
<i>crtRB1</i> 3'TE	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	Total β -Xanthophylls	Additional Markers	10	136,061,719	136,057,100	136,060,219	4,619	1,500	7.09E-07	1.34E-03	NA	NA	NA	195	0.22	0.3	NA	0.4	NA
<i>crtRB1</i> 3'TE	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	β -Carotene/ β -Cryptoxanthin	Additional Markers	10	136,061,719	136,057,100	136,060,219	4,619	1,500	1.52E-05	2.90E-02	NA	NA	NA	198	0.07	0.05	NA	-0.7	NA
<i>crtRB1</i> 3'TE	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	Total Carotenes/Total Xanthophylls	Additional Markers	10	136,061,719	136,057,100	136,060,219	4,619	1,500	1.89E-07	1.41E-03	NA	NA	NA	188	0.05	0.15	NA	-0.55	NA

Statistically significant results from a pathway-level analysis of 58 *a priori* candidate genes from the carotenoid biosynthesis, carotenoid degradation, and isoprenoid biosynthetic pathways on 24 grain carotenoid traits with a SNP tagging the peak GWAS signals from *zep1* included as a covariate. All markers (Column A) proximal to *a priori* candidate genes (Column B) found to be significantly associated with the indicated trait (Column E) at 5% FDR are shown.

Table S9 Results from the Pathway-Level Analysis with ss196425306 as Covariate Tagging *lut1* (C)

SNP ID	<i>a priori</i> candidate gene pathway	RefGen_v2 Gene ID	Annotated Gene Function	Trait	SNP Source	Chromosome	Position	Gene ORF start	Gene ORF End	Distance from Gene ORF Start	Distance from Gene ORF Finish	P-value	FDR Adjusted P-value	Minor Allele Frequency (MAF)	MAF Tropical (8% of 201 Lines)	MAF Temperate (92% of 201 Lines)	Sample Size	R-square_LR from Model without SNP	R-square_LR from Model with SNP	Effect Size	Lambda from Box-Cox Procedure	Back-Transformed Effect Estimates
S2_44445965	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeaxanthin	GBS	2	44,445,965	44,440,299	44,449,237	5,666	-3,272	3.62E-05	2.11E-02	0.18	0.33	0.26	196	0.04	0.13	-0.19	0.35	-0.45
S2_44445965	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Total β -Xanthophylls	GBS	2	44,445,965	44,440,299	44,449,237	5,666	-3,272	1.39E-04	4.76E-02	0.18	0.33	0.26	195	0.05	0.12	-0.24	0.4	-0.49
S2_44445965	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeinoxanthin/Lutein	GBS	2	44,445,965	44,440,299	44,449,237	5,666	-3,272	2.27E-05	1.21E-02	0.18	0.33	0.26	189	0.2	0.28	0.09	-0.35	-0.22
S2_44445965	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Carotenoids/ α -Carotenoids	GBS	2	44,445,965	44,440,299	44,449,237	5,666	-3,272	2.27E-05	1.21E-02	0.18	0.33	0.26	189	0.2	0.28	0.09	-0.85	-0.1
S2_44448432	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeaxanthin	GBS	2	44,448,432	44,440,299	44,449,237	8,133	-805	2.06E-09	7.79E-06	0.11	0.29	0.09	196	0.04	0.24	-0.34	0.35	-0.69
S2_44448432	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Total β -Xanthophylls	GBS	2	44,448,432	44,440,299	44,449,237	8,133	-805	1.57E-08	5.95E-05	0.11	0.29	0.09	195	0.05	0.22	-0.43	0.4	-0.76
S2_44448432	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Xanthophylls/ α -Xanthophylls	GBS	2	44,448,432	44,440,299	44,449,237	8,133	-805	5.29E-07	3.64E-04	0.11	0.29	0.09	196	0.11	0.24	0.12	-0.4	-0.24
S2_44448432	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeinoxanthin/Lutein	GBS	2	44,448,432	44,440,299	44,449,237	8,133	-805	9.29E-07	1.39E-03	0.12	0.29	0.09	189	0.2	0.31	0.13	-0.35	-0.29
S2_44448432	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Carotenoids/ α -Carotenoids	GBS	2	44,448,432	44,440,299	44,449,237	8,133	-805	9.29E-07	1.39E-03	0.12	0.29	0.09	189	0.2	0.31	0.13	-0.85	-0.13
S2_44448438	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeaxanthin	GBS	2	44,448,438	44,440,299	44,449,237	8,139	-799	2.06E-09	7.79E-06	0.11	0.29	0.09	196	0.04	0.24	0.34	0.35	1.31
S2_44448438	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Total β -Xanthophylls	GBS	2	44,448,438	44,440,299	44,449,237	8,139	-799	1.57E-08	5.95E-05	0.11	0.29	0.09	195	0.05	0.22	0.43	0.4	1.46
S2_44448438	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Xanthophylls/ α -Xanthophylls	GBS	2	44,448,438	44,440,299	44,449,237	8,139	-799	5.29E-07	3.64E-04	0.11	0.29	0.09	196	0.11	0.24	-0.12	-0.4	0.37
S2_44448438	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeinoxanthin/Lutein	GBS	2	44,448,438	44,440,299	44,449,237	8,139	-799	9.29E-07	1.39E-03	0.12	0.29	0.09	189	0.2	0.31	-0.13	-0.35	0.47
S2_44448438	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Carotenoids/ α -Carotenoids	GBS	2	44,448,438	44,440,299	44,449,237	8,139	-799	9.29E-07	1.39E-03	0.12	0.29	0.09	189	0.2	0.31	-0.13	-0.85	0.17
S2_44472618	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeaxanthin	GBS	2	44,472,618	44,440,299	44,449,237	32,319	23,381	1.13E-04	3.72E-02	0.13	0.38	0.15	196	0.04	0.12	-0.2	0.35	-0.47
S2_44473748	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeaxanthin	GBS	2	44,473,748	44,440,299	44,449,237	33,449	24,511	1.32E-06	1.43E-03	0.14	0.25	0.12	196	0.04	0.17	0.24	0.35	0.86
S2_44473748	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Total β -Xanthophylls	GBS	2	44,473,748	44,440,299	44,449,237	33,449	24,511	4.49E-06	4.24E-03	0.14	0.25	0.12	195	0.05	0.16	0.31	0.4	0.97
S2_44473748	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Xanthophylls/ α -Xanthophylls	GBS	2	44,473,748	44,440,299	44,449,237	33,449	24,511	1.65E-05	7.34E-03	0.14	0.25	0.12	196	0.11	0.2	-0.09	-0.4	0.27
S2_44473748	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeinoxanthin/Lutein	GBS	2	44,473,748	44,440,299	44,449,237	33,449	24,511	4.35E-05	1.92E-02	0.14	0.25	0.12	189	0.2	0.27	-0.09	-0.35	0.33
S2_44473748	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Carotenoids/ α -Carotenoids	GBS	2	44,473,748	44,440,299	44,449,237	33,449	24,511	4.35E-05	1.92E-02	0.14	0.25	0.12	189	0.2	0.27	-0.09	-0.85	0.12
S2_44473758	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeaxanthin	GBS	2	44,473,758	44,440,299	44,449,237	33,459	24,521	1.32E-06	1.43E-03	0.14	0.24	0.12	196	0.04	0.17	-0.24	0.35	-0.55
S2_44473758	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Total β -Xanthophylls	GBS	2	44,473,758	44,440,299	44,449,237	33,459	24,521	4.49E-06	4.24E-03	0.14	0.24	0.12	195	0.05	0.16	-0.31	0.4	-0.61
S2_44473758	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Xanthophylls/ α -Xanthophylls	GBS	2	44,473,758	44,440,299	44,449,237	33,459	24,521	1.65E-05	7.34E-03	0.14	0.24	0.12	196	0.11	0.2	0.09	-0.4	-0.19
S2_44473758	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeinoxanthin/Lutein	GBS	2	44,473,758	44,440,299	44,449,237	33,459	24,521	4.35E-05	1.92E-02	0.14	0.24	0.12	189	0.2	0.27	0.09	-0.35	-0.23
S2_44473758	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Carotenoids/ α -Carotenoids	GBS	2	44,473,758	44,440,299	44,449,237	33,459	24,521	4.35E-05	1.92E-02	0.14	0.24	0.12	189	0.2	0.27	0.09	-0.85	-0.1
S2_44473801	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeaxanthin	GBS	2	44,473,801	44,440,299	44,449,237	33,502	24,564	1.32E-06	1.43E-03	0.14	0.24	0.12	196	0.04	0.17	0.24	0.35	0.86
S2_44473801	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Total β -Xanthophylls	GBS	2	44,473,801	44,440,299	44,449,237	33,502	24,564	4.49E-06	4.24E-03	0.14	0.24	0.12	195	0.05	0.16	0.31	0.4	0.97
S2_44473801	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Xanthophylls/ α -Xanthophylls	GBS	2	44,473,801	44,440,299	44,449,237	33,502	24,564	1.65E-05	7.34E-03	0.14	0.24	0.12	196	0.11	0.2	-0.09	-0.4	0.27
S2_44473801	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeinoxanthin/Lutein	GBS	2	44,473,801	44,440,299	44,449,237	33,502	24,564	4.35E-05	1.92E-02	0.14	0.24	0.12	189	0.2	0.27	-0.09	-0.35	0.33
S2_44473801	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Carotenoids/ α -Carotenoids	GBS	2	44,473,801	44,440,299	44,449,237	33,502	24,564	4.35E-05	1.92E-02	0.14	0.24	0.12	189	0.2	0.27	-0.09	-0.85	0.12
S2_44473994	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeinoxanthin/Lutein	GBS	2	44,473,994	44,440,299	44,449,237	33,695	24,757	9.15E-05	3.61E-02	0.18	0.35	0.16	189	0.2	0.26	-0.08	-0.35	0.28
S2_44473994	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Carotenoids/ α -Carotenoids	GBS	2	44,473,994	44,440,299	44,449,237	33,695	24,757	9.15E-05	3.61E-02	0.18	0.35	0.16	189	0.2	0.26	-0.08	-0.85	0.11
S2_44474139	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeaxanthin	GBS	2	44,474,139	44,440,299	44,449,237	33,840	24,902	4.65E-06	3.91E-03	0.14	0.29	0.13	196	0.04	0.15	-0.23	0.35	-0.52
S2_44474139	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Total β -Xanthophylls	GBS	2	44,474,139	44,440,299	44,449,237	33,840	24,902	1.65E-05	1.13E-02	0.14	0.29	0.13	195	0.05	0.14	-0.29	0.4	-0.57
S2_44474139	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Xanthophylls/ α -Xanthophylls	GBS	2	44,474,139	44,440,299	44,449,237	33,840	24,902	2.87E-05	1.14E-02	0.14	0.29	0.13	196	0.11	0.2	0.09	-0.4	-0.19
S2_44474139	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeinoxanthin/Lutein	GBS	2	44,474,139	44,440,299	44,449,237	33,840	24,902	4.62E-05	1.93E-02	0.15	0.29	0.13	189	0.2	0.27	0.09	-0.35	-0.22
S2_44474139	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Carotenoids/ α -Carotenoids	GBS	2	44,474,139	44,440,299	44,449,237	33,840	24,902	4.62E-05	1.93E-02	0.15	0.29	0.13	189	0.2	0.27	0.09	-0.85	-0.1
S2_44474308	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeaxanthin	GBS	2	44,474,308	44,440,299	44,449,237	34,009	25,071	1.09E-06	1.43E-03	0.21	0.38	0.28	196	0.04	0.17	0.21	0.35	0.73
S2_44474308	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Total β -Xanthophylls	GBS	2	44,474,308	44,440,299	44,449,237	34,009	25,071	6.51E-06	5.47E-03	0.22	0.38	0.28	195	0.05	0.15	0.26	0.4	0.8
S2_44474308	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Xanthophylls/ α -Xanthophylls	GBS	2	44,474,308	44,440,299	44,449,237	34,009	25,071	1.55E-05	7.34E-03	0.21	0.38	0.28	196	0.11	0.2	-0.08	-0.4	0.23
S2_44474308	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeinoxanthin/Lutein	GBS	2	44,474,308	44,440,299	44,449,237	34,009	25,071	1.55E-05	8.94E-03	0.22	0.38	0.28	189	0.2	0.28	-0.09	-0.35	0.29
S2_44474308	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Carotenoids/ α -Carotenoids	GBS	2	44,474,308	44,440,299	44,449,237	34,009	25,071	1.55E-05	8.94E-03	0.22	0.38	0.28	189	0.2	0.28	-0.09	-0.85	0.11
S5_1315672	Carotenoid Synthesis and Degradation	GRMZM2G144273	carotenoid isomerase	Zeaxanthin	GBS	5	1,315,672	1,333,304	1,341,577	-17,632	-25,905	6.46E-05	2.58E-02	0.29	0.17	0.36	196	0.04	0.13	0.17	0.35	0.57
S5_1315672	Carotenoid Synthesis and Degradation	GRMZM2G144273	carotenoid isomerase	Total β -Xanthophylls	GBS	5	1,315,672	1,333,304	1,341,577	-17,632	-25,905	1.38E-04	4.76E-02	0.29	0.17	0.36	195	0.05	0.12	0.22	0.4	0.64
S5_1315672	Carotenoid Synthesis and Degradation	GRMZM2G144273	carotenoid isomerase	β -Xanthophylls/ α -Xanthophylls	GBS	5	1,315,672	1,333,304	1,341,577	-17,632	-25,905	6.93E-05	2.42E-02	0.29	0.17	0.36	196	0.11	0.19	-0.07	-0.4	0.21
S5_1315682	Carotenoid Synthesis and Degradation	GRMZM2G144273	carotenoid isomerase	Zeaxanthin	GBS	5	1,315,682	1,333,304	1,341,577	-17,622	-25,895	8.09E-05	2.92E-02	0.								

S7_160778001	Prenyl Group Synthesis	GRMZM2G102550	geranylgeranyl pyrophosphate synthase	xanthin) β-Carotene/(β-Cryptoxanthin+Zea xanthin)	GBS	7	160,778,001	160,531,537	160,533,586	246,464	244,415	2.90E-05	4.40E-02	0.07	0.08	0.1	196	0.04	0.13	0.14	-0.25	-0.41
S7_160778016	Prenyl Group Synthesis	GRMZM2G102550	geranylgeranyl pyrophosphate synthase	β-Carotene/(β-Cryptoxanthin+Zea xanthin)	GBS	7	160,778,016	160,531,537	160,533,586	246,479	244,430	2.90E-05	4.40E-02	0.07	0.08	0.1	196	0.04	0.13	-0.14	-0.25	0.83
S7_160779488	Prenyl Group Synthesis	GRMZM2G102550	geranylgeranyl pyrophosphate synthase	β-Carotene/β-Cryptoxanthin	GBS	7	160,779,488	160,531,537	160,533,586	247,951	245,902	1.28E-05	2.45E-02	0.12	0.26	0.12	198	0.1	0.19	0.11	-0.7	-0.14
ss196487098	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Zeaxanthin	55K	8	138,861,176	138,882,594	138,889,812	-21,418	-28,636	8.50E-05	2.93E-02	0.32	0.29	0.33	196	0.04	0.12	0.16	0.35	0.53
ss196487098	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total β-Xanthophylls	55K	8	138,861,176	138,882,594	138,889,812	-21,418	-28,636	3.39E-05	1.80E-02	0.32	0.29	0.33	195	0.05	0.14	0.23	0.4	0.68
ss196487098	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β-Xanthophylls/α-Xanthophylls	55K	8	138,861,176	138,882,594	138,889,812	-21,418	-28,636	6.53E-05	2.42E-02	0.32	0.29	0.33	196	0.11	0.19	-0.07	-0.4	0.2
lcyE 5' TE	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Lutein	Additional Markers	8	138,882,481	138,882,594	138,889,812	-114	-7,332	2.24E-08	8.56E-05	NA	NA	NA	200	0.12	0.49	NA	0.8	NA
lcyE 5' TE	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Zeaxanthin	Additional Markers	8	138,882,481	138,882,594	138,889,812	-114	-7,332	9.13E-06	6.92E-03	NA	NA	NA	196	0.04	0.24	NA	0.35	NA
lcyE 5' TE	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total β-Xanthophylls	Additional Markers	8	138,882,481	138,882,594	138,889,812	-114	-7,332	1.04E-05	7.87E-03	NA	NA	NA	195	0.05	0.29	NA	0.4	NA
S8_138882711	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Zeaxanthin	GBS	8	138,882,711	138,882,594	138,889,812	117	-7,101	6.12E-05	2.58E-02	0.28	0.41	0.3	196	0.04	0.13	0.16	0.35	0.54
S8_138882711	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total β-Xanthophylls	GBS	8	138,882,711	138,882,594	138,889,812	117	-7,101	5.47E-05	2.30E-02	0.28	0.41	0.3	195	0.05	0.13	0.22	0.4	0.65
S8_138882711	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β-Xanthophylls/α-Xanthophylls	GBS	8	138,882,711	138,882,594	138,889,812	117	-7,101	3.23E-07	3.06E-04	0.28	0.41	0.3	196	0.11	0.24	-0.09	-0.4	0.26
S8_138882711	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Zeinoxanthin/Lutein	GBS	8	138,882,711	138,882,594	138,889,812	117	-7,101	1.71E-06	1.60E-03	0.29	0.41	0.3	189	0.2	0.3	-0.09	-0.35	0.32
S8_138882711	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β-Carotenoids/α-Carotenoids	GBS	8	138,882,711	138,882,594	138,889,812	117	-7,101	1.71E-06	1.60E-03	0.29	0.41	0.3	189	0.2	0.3	-0.09	-0.85	0.12
ss196504160	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Zeaxanthin	55K	8	138,882,711	138,882,594	138,889,812	117	-7,101	2.80E-06	2.65E-03	0.35	0.48	0.34	196	0.04	0.16	-0.19	0.35	-0.44
ss196504160	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total β-Xanthophylls	55K	8	138,882,711	138,882,594	138,889,812	117	-7,101	1.77E-06	2.68E-03	0.35	0.48	0.34	195	0.05	0.17	-0.26	0.4	-0.53
ss196504160	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β-Xanthophylls/α-Xanthophylls	55K	8	138,882,711	138,882,594	138,889,812	117	-7,101	4.03E-10	1.52E-06	0.35	0.48	0.34	196	0.11	0.31	0.11	-0.4	-0.23
ss196504160	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Zeinoxanthin/Lutein	55K	8	138,882,711	138,882,594	138,889,812	117	-7,101	2.75E-09	2.06E-05	0.37	0.48	0.34	189	0.2	0.36	0.12	-0.35	-0.27
ss196504160	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β-Carotenoids/α-Carotenoids	55K	8	138,882,711	138,882,594	138,889,812	117	-7,101	2.75E-09	2.06E-05	0.37	0.48	0.34	189	0.2	0.36	0.12	-0.85	-0.12
S8_138882747	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Zeaxanthin	GBS	8	138,882,747	138,882,594	138,889,812	153	-7,065	6.12E-05	2.58E-02	0.28	0.41	0.3	196	0.04	0.13	0.16	0.35	0.54
S8_138882747	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total β-Xanthophylls	GBS	8	138,882,747	138,882,594	138,889,812	153	-7,065	5.47E-05	2.30E-02	0.28	0.41	0.3	195	0.05	0.13	0.22	0.4	0.65
S8_138882747	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β-Xanthophylls/α-Xanthophylls	GBS	8	138,882,747	138,882,594	138,889,812	153	-7,065	3.23E-07	3.06E-04	0.28	0.41	0.3	196	0.11	0.24	-0.09	-0.4	0.26
S8_138882747	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Zeinoxanthin/Lutein	GBS	8	138,882,747	138,882,594	138,889,812	153	-7,065	1.71E-06	1.60E-03	0.29	0.41	0.3	189	0.2	0.3	-0.09	-0.35	0.32
S8_138882747	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β-Carotenoids/α-Carotenoids	GBS	8	138,882,747	138,882,594	138,889,812	153	-7,065	1.71E-06	1.60E-03	0.29	0.41	0.3	189	0.2	0.3	-0.09	-0.85	0.12
S8_138882751	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Zeaxanthin	GBS	8	138,882,751	138,882,594	138,889,812	157	-7,061	6.12E-05	2.58E-02	0.28	0.41	0.3	196	0.04	0.13	0.16	0.35	0.54
S8_138882751	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total β-Xanthophylls	GBS	8	138,882,751	138,882,594	138,889,812	157	-7,061	5.47E-05	2.30E-02	0.28	0.41	0.3	195	0.05	0.13	0.22	0.4	0.65
S8_138882751	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β-Xanthophylls/α-Xanthophylls	GBS	8	138,882,751	138,882,594	138,889,812	157	-7,061	3.23E-07	3.06E-04	0.28	0.41	0.3	196	0.11	0.24	-0.09	-0.4	0.26
S8_138882751	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Zeinoxanthin/Lutein	GBS	8	138,882,751	138,882,594	138,889,812	157	-7,061	1.71E-06	1.60E-03	0.29	0.41	0.3	189	0.2	0.3	-0.09	-0.35	0.32
S8_138882751	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β-Carotenoids/α-Carotenoids	GBS	8	138,882,751	138,882,594	138,889,812	157	-7,061	1.71E-06	1.60E-03	0.29	0.41	0.3	189	0.2	0.3	-0.09	-0.85	0.12
S8_138882798	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β-Xanthophylls/α-Xanthophylls	GBS	8	138,882,798	138,882,594	138,889,812	204	-7,014	2.65E-07	3.06E-04	0.31	0.21	0.36	196	0.11	0.24	-0.09	-0.4	0.26
S8_138882798	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Zeinoxanthin/Lutein	GBS	8	138,882,798	138,882,594	138,889,812	204	-7,014	2.59E-06	2.16E-03	0.31	0.21	0.36	189	0.2	0.3	-0.09	-0.35	0.31
S8_138882798	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β-Carotenoids/α-Carotenoids	GBS	8	138,882,798	138,882,594	138,889,812	204	-7,014	2.59E-06	2.16E-03	0.31	0.21	0.36	189	0.2	0.3	-0.09	-0.85	0.12
S8_138882897	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Lutein	GBS	8	138,882,897	138,882,594	138,889,812	303	-6,915	1.84E-05	3.52E-02	0.43	0.12	0.44	200	0.12	0.21	-0.78	0.8	-0.85
S8_138882897	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Zeaxanthin	GBS	8	138,882,897	138,882,594	138,889,812	303	-6,915	5.90E-05	2.58E-02	0.44	0.12	0.44	196	0.04	0.13	0.15	0.35	0.48
S8_138882897	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total β-Xanthophylls	GBS	8	138,882,897	138,882,594	138,889,812	303	-6,915	1.10E-04	4.17E-02	0.44	0.12	0.44	195	0.05	0.13	0.19	0.4	0.55
S8_138882897	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β-Xanthophylls/α-Xanthophylls	GBS	8	138,882,897	138,882,594	138,889,812	303	-6,915	1.55E-08	2.92E-05	0.43	0.12	0.44	196	0.11	0.27	-0.09	-0.4	0.26
S8_138882897	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Zeinoxanthin/Lutein	GBS	8	138,882,897	138,882,594	138,889,812	303	-6,915	6.73E-08	2.52E-04	0.43	0.12	0.44	189	0.2	0.33	-0.1	-0.35	0.34
S8_138882897	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β-Carotenoids/α-Carotenoids	GBS	8	138,882,897	138,882,594	138,889,812	303	-6,915	6.73E-08	2.52E-04	0.43	0.12	0.44	189	0.2	0.33	-0.1	-0.85	0.13
S8_138883026	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β-Xanthophylls/α-Xanthophylls	GBS	8	138,883,026	138,882,594	138,889,812	432	-6,786	1.04E-06	6.08E-04	0.4	0.18	0.48	196	0.11	0.23	0.08	-0.4	-0.17
S8_138883026	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Zeinoxanthin/Lutein	GBS	8	138,883,026	138,882,594	138,889,812	432	-6,786	6.84E-06	4.41E-03	0.4	0.18	0.48	189	0.2	0.29	0.08	-0.35	-0.2
S8_138883026	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β-Carotenoids/α-Carotenoids	GBS	8	138,883,026	138,882,594	138,889,812	432	-6,786	6.84E-06	4.41E-03	0.4	0.18	0.48	189	0.2	0.29	0.08	-0.85	-0.09
S8_138883056	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β-Xanthophylls/α-Xanthophylls	GBS	8	138,883,056	138,882,594	138,889,812	462	-6,756	1.04E-06	6.08E-04	0.4	0.18	0.48	196	0.11	0.23	-0.08	-0.4	0.22
S8_138883056	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Zeinoxanthin/Lutein	GBS	8	138,883,056	138,882,594	138,889,812	462	-6,756	6.84E-06	4.41E-03	0.4	0.18	0.48	189	0.2	0.29	-0.08	-0.35	0.28
S8_138883056	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β-Carotenoids/α-Carotenoids	GBS	8	138,883,056	138,882,594	138,889,812	462	-6,756	6.84E-06	4.41E-03	0.4	0.18	0.48	189	0.2	0.29	-0.08	-0.85	0.11
lcyE SNP216	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Lutein	Additional Markers	8	138,883,206	138,882,594	138,889,812	612	-6,606	6.44E-09	4.92E-05	NA	NA	NA	200	0.12	0.45	NA	0.8	NA
lcyE SNP216	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total β-Xanthophylls	Additional Markers	8	138,883,206	138,882,594	138,889,812	612	-6,606	1.64E-07	4.13E-04	NA	NA	NA	195	0.05	0.29	NA	0.4	NA
lcyE SNP216	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β-Xanthophylls/α-Xanthophylls	Additional Markers	8	138,883,206	138,882,594	138,889,812	612	-6,606	7.15E-16	5.41E-12	NA	NA	NA	196	0.08	0.22	NA	-0.4	NA
PZB00665.1	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β-Xanthophylls/α-Xanthophylls	4K	8	138,886,137	138,882,594	138,889,812	3,543	-3,675											

S8_138888278	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Zeaxanthin	GBS	8	138,888,278	138,882,594	138,889,812	5,684	-1,534	6.16E-05	2.58E-02	0.46	0.19	0.42	196	0.04	0.13	0.15	0.35	0.5
S8_138888278	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total β -Xanthophylls	GBS	8	138,888,278	138,882,594	138,889,812	5,684	-1,534	3.58E-05	1.80E-02	0.47	0.19	0.42	195	0.05	0.14	0.21	0.4	0.62
S8_138888278	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Xanthophylls/ α -Xanthophylls	GBS	8	138,888,278	138,882,594	138,889,812	5,684	-1,534	2.98E-09	7.52E-06	0.47	0.19	0.42	196	0.11	0.29	-0.1	-0.4	0.3
S8_138888278	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Zeinoxanthin/Lutein	GBS	8	138,888,278	138,882,594	138,889,812	5,684	-1,534	1.80E-07	4.50E-04	0.47	0.19	0.42	189	0.2	0.32	-0.1	-0.35	0.35
S8_138888278	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Carotenoids/ α -Carotenoids	GBS	8	138,888,278	138,882,594	138,889,812	5,684	-1,534	1.80E-07	4.50E-04	0.47	0.19	0.42	189	0.2	0.32	-0.1	-0.85	0.13
S8_138911758	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Xanthophylls/ α -Xanthophylls	GBS	8	138,911,758	138,882,594	138,889,812	29,164	21,946	8.16E-05	2.57E-02	0.06	0.16	0.05	196	0.11	0.19	-0.14	-0.4	0.46
S8_139136162	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Xanthophylls/ α -Xanthophylls	GBS	8	139,136,162	138,882,594	138,889,812	253,568	246,350	2.76E-05	1.14E-02	0.45	0	0.47	196	0.11	0.2	-0.07	-0.4	0.2
<i>crtRB1</i> InDel4	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	Zeaxanthin	Additonal Markers	10	136,059,748	136,057,100	136,060,219	2,648	-471	1.23E-05	8.47E-03	NA	NA	NA	196	0.04	0.11	NA	0.35	NA
<i>crtRB1</i> InDel4	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	Total β -Xanthophylls	Additonal Markers	10	136,059,748	136,057,100	136,060,219	2,648	-471	2.44E-05	1.42E-02	NA	NA	NA	195	0.05	0.11	NA	0.4	NA
<i>crtRB1</i> InDel4	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	β -Carotene/ β -Cryptoxanthin	Additonal Markers	10	136,059,748	136,057,100	136,060,219	2,648	-471	2.20E-06	1.67E-02	NA	NA	NA	198	0.08	0.09	NA	-0.7	NA
<i>crtRB1</i> InDel4	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	β -Carotene/(β -Cryptoxanthin+Zeaxanthin)	Additonal Markers	10	136,059,748	136,057,100	136,060,219	2,648	-471	1.72E-07	1.31E-03	NA	NA	NA	196	0.03	0.08	NA	-0.25	NA
<i>crtRB1</i> InDel4	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	Total Carotenes/Total Xanthophylls	Additonal Markers	10	136,059,748	136,057,100	136,060,219	2,648	-471	5.91E-06	2.21E-02	NA	NA	NA	188	0.02	0.09	NA	-0.55	NA
ss196501627	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	β -Carotene/ β -Cryptoxanthin	55K	10	136,060,033	136,057,100	136,060,219	2,933	-186	7.95E-06	2.02E-02	0.18	0	0.22	198	0.1	0.2	0.11	-0.7	-0.14
ss196501627	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	β -Carotene/(β -Cryptoxanthin+Zeaxanthin)	55K	10	136,060,033	136,057,100	136,060,219	2,933	-186	3.67E-07	1.39E-03	0.19	0	0.22	196	0.04	0.18	0.12	-0.25	-0.36
<i>crtRB1</i> 3'TE	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	Zeaxanthin	Additonal Markers	10	136,061,719	136,057,100	136,060,219	4,619	1,500	1.12E-06	1.43E-03	NA	NA	NA	196	0.04	0.15	NA	0.35	NA
<i>crtRB1</i> 3'TE	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	Total β -Xanthophylls	Additonal Markers	10	136,061,719	136,057,100	136,060,219	4,619	1,500	1.77E-06	2.68E-03	NA	NA	NA	195	0.05	0.17	NA	0.4	NA
<i>crtRB1</i> 3'TE	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	β -Xanthophylls/ α -Xanthophylls	Additonal Markers	10	136,061,719	136,057,100	136,060,219	4,619	1,500	1.00E-04	3.03E-02	NA	NA	NA	196	0.08	0.03	NA	-0.4	NA
<i>crtRB1</i> 3'TE	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	β -Carotene/ β -Cryptoxanthin	Additonal Markers	10	136,061,719	136,057,100	136,060,219	4,619	1,500	6.68E-06	2.02E-02	NA	NA	NA	198	0.08	0.09	NA	-0.7	NA
<i>crtRB1</i> 3'TE	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	Total Carotenes/Total Xanthophylls	Additonal Markers	10	136,061,719	136,057,100	136,060,219	4,619	1,500	1.33E-07	9.94E-04	NA	NA	NA	188	0.02	0.14	NA	-0.55	NA

Covariate Statistically significant results from a pathway-level analysis of 58 *a priori* candidate genes from the carotenoid biosynthesis, carotenoid degradation, and isoprenoid biosynthetic pathways on 24 grain carotenoid traits with a SNP tagging the peak GWAS signals from *lut1* included as a covariate. All markers (Column A) proximal to *a priori* candidate genes (Column B) found to be significantly associated with the indicated trait (Column E) at 5% FDR are shown.

Table S9 Results from the Pathway-Level Analysis with S8_138882897 and *lcyE* SNP216 as Covariates Tagging *lcyE* (D)

SNP ID	<i>a priori</i> candidate gene pathway	RefGen_v2 Gene ID	Annotated Gene Function	Trait	SNP Source	Chromosome	Position	Gene ORF start	Gene ORF End	Distance from Gene ORF Start	Distance from Gene ORF Finish	P-value	FDR Adjusted P-value	Minor Allele Frequency (MAF)	MAF Tropical (8% of 201 Lines)	MAF Temperate (92% of 201 Lines)	Sample Size	R-square_LR from Model without SNP	R-square_LR from Model with SNP	Effect Size	Lambda from Box-Cox Procedure	Back-Transformed Effect Estimates
ss196425293	Carotenoid Synthesis and Degradation	GRMZM2G143202	CYP97A3, Cytochrome P450 epsilon-ring hydroxylase	Zeinoxanthin/Lutein	55K	1	86,597,575	86,838,334	86,848,726	-240,759	-251,151	1.62E-05	3.05E-02	0.31	0.24	0.33	175	0.08	0.18	-0.16	-0.35	0.65
ss196501639	Carotenoid Synthesis and Degradation	GRMZM2G143202	CYP97A3, Cytochrome P450 epsilon-ring hydroxylase	Zeinoxanthin/Lutein	55K	1	86,597,631	86,838,334	86,848,726	-240,703	-251,095	7.61E-06	1.92E-02	0.31	0.24	0.33	175	0.08	0.19	-0.17	-0.35	0.7
ss196425306	Carotenoid Synthesis and Degradation	GRMZM2G143202	CYP97A3, Cytochrome P450 epsilon-ring hydroxylase	Zeinoxanthin	55K	1	86,844,203	86,838,334	86,848,726	5,869	-4,523	4.28E-08	1.63E-04	0.32	0.4	0.28	178	0.1	0.27	-0.12	-0.25	0.67
ss196425306	Carotenoid Synthesis and Degradation	GRMZM2G143202	CYP97A3, Cytochrome P450 epsilon-ring hydroxylase	α -Carotene/Zeaxanthin	55K	1	86,844,203	86,838,334	86,848,726	5,869	-4,523	7.23E-10	2.76E-06	0.33	0.4	0.28	178	0.16	0.37	0.07	-0.25	-0.22
ss196425306	Carotenoid Synthesis and Degradation	GRMZM2G143202	CYP97A3, Cytochrome P450 epsilon-ring hydroxylase	Zeinoxanthin/Lutein	55K	1	86,844,203	86,838,334	86,848,726	5,869	-4,523	4.98E-08	1.88E-04	0.31	0.4	0.28	175	0.08	0.25	-0.21	-0.35	0.93
ss196425308	Carotenoid Synthesis and Degradation	GRMZM2G143202	CYP97A3, Cytochrome P450 epsilon-ring hydroxylase	Zeinoxanthin	55K	1	86,945,134	86,838,334	86,848,726	106,800	96,408	4.28E-08	1.63E-04	0.32	0.4	0.27	178	0.1	0.27	-0.12	-0.25	0.67
ss196425308	Carotenoid Synthesis and Degradation	GRMZM2G143202	CYP97A3, Cytochrome P450 epsilon-ring hydroxylase	α -Carotene/Zeaxanthin	55K	1	86,945,134	86,838,334	86,848,726	106,800	96,408	7.23E-10	2.76E-06	0.33	0.4	0.27	178	0.16	0.37	0.07	-0.25	-0.22
ss196425308	Carotenoid Synthesis and Degradation	GRMZM2G143202	CYP97A3, Cytochrome P450 epsilon-ring hydroxylase	Zeinoxanthin/Lutein	55K	1	86,945,134	86,838,334	86,848,726	106,800	96,408	4.98E-08	1.88E-04	0.31	0.4	0.27	175	0.08	0.25	-0.21	-0.35	0.93
S2_44448432	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeaxanthin	GBS	2	44,448,432	44,440,299	44,449,237	8,133	-805	7.43E-09	2.81E-05	0.11	0.29	0.09	176	0.18	0.36	-0.32	0.35	-0.67
S2_44448432	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Total β -Xanthophylls	GBS	2	44,448,432	44,440,299	44,449,237	8,133	-805	8.43E-08	3.18E-04	0.11	0.29	0.09	175	0.2	0.34	-0.4	0.4	-0.73
S2_44448432	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Xanthophylls/ α -Xanthophylls	GBS	2	44,448,432	44,440,299	44,449,237	8,133	-805	5.10E-07	1.93E-03	0.11	0.29	0.09	176	0.45	0.54	0.1	-0.4	-0.22
S2_44448438	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeaxanthin	GBS	2	44,448,438	44,440,299	44,449,237	8,139	-799	7.43E-09	2.81E-05	0.11	0.29	0.09	176	0.18	0.36	0.32	0.35	1.22
S2_44448438	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Total β -Xanthophylls	GBS	2	44,448,438	44,440,299	44,449,237	8,139	-799	8.43E-08	3.18E-04	0.11	0.29	0.09	175	0.2	0.34	0.4	0.4	1.34
S2_44448438	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Xanthophylls/ α -Xanthophylls	GBS	2	44,448,438	44,440,299	44,449,237	8,139	-799	5.10E-07	1.93E-03	0.11	0.29	0.09	176	0.45	0.54	-0.1	-0.4	0.32
S2_44473748	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeaxanthin	GBS	2	44,473,748	44,440,299	44,449,237	33,449	24,511	1.91E-05	1.80E-02	0.14	0.25	0.12	176	0.18	0.27	0.21	0.35	0.7
S2_44473758	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeaxanthin	GBS	2	44,473,758	44,440,299	44,449,237	33,459	24,521	1.91E-05	1.80E-02	0.14	0.24	0.12	176	0.18	0.27	-0.21	0.35	-0.48
S2_44473801	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeaxanthin	GBS	2	44,473,801	44,440,299	44,449,237	33,502	24,564	1.91E-05	1.80E-02	0.14	0.24	0.12	176	0.18	0.27	0.21	0.35	0.7
S2_44474139	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeaxanthin	GBS	2	44,474,139	44,440,299	44,449,237	33,840	24,902	4.98E-05	4.19E-02	0.15	0.29	0.13	176	0.18	0.26	-0.19	0.35	-0.46
S2_44474308	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeaxanthin	GBS	2	44,474,308	44,440,299	44,449,237	34,009	25,071	5.83E-05	4.41E-02	0.21	0.38	0.28	176	0.18	0.26	0.17	0.35	0.57
S5_216074707	Carotenoid Synthesis and Degradation	GRMZM5G837869	CYP97A3, Cytochrome P450 beta-ring hydroxylase	Zeaxanthin	GBS	5	216,074,707	215,827,224	215,831,730	247,483	242,977	2.89E-06	5.11E-03	0.14	0.17	0.17	176	0.18	0.29	-0.23	0.35	-0.52
S5_216074707	Carotenoid Synthesis and Degradation	GRMZM5G837869	CYP97A3, Cytochrome P450 beta-ring hydroxylase	Total β -Xanthophylls	GBS	5	216,074,707	215,827,224	215,831,730	247,483	242,977	5.35E-06	8.08E-03	0.14	0.17	0.17	175	0.2	0.3	-0.29	0.4	-0.58
S7_13843351	Prenyl Group Synthesis	GRMZM2G493395	1-deoxy-D-xylulose 5-phosphate synthase	β -Cryptoxanthin	GBS	7	13,843,351	14,077,852	14,081,075	-234,501	-237,724	1.40E-07	1.07E-03	0.16	0.1	0.16	179	0.19	0.33	-0.04	0.1	-0.33
S7_13843351	Prenyl Group Synthesis	GRMZM2G493395	1-deoxy-D-xylulose 5-phosphate synthase	Total β -Xanthophylls	GBS	7	13,843,351	14,077,852	14,081,075	-234,501	-237,724	2.96E-05	3.72E-02	0.17	0.1	0.16	175	0.2	0.28	-0.25	0.4	-0.51
S7_160777986	Prenyl Group Synthesis	GRMZM2G102550	geranylgeranyl pyrophosphate synthase	β -Carotene/(β -Cryptoxanthin+Zeaxanthin)	GBS	7	160,777,986	160,531,537	160,533,586	246,449	244,400	3.46E-05	4.40E-02	0.07	0.08	0.1	178	0.04	0.14	0.15	-0.25	-0.43
S7_160778001	Prenyl Group Synthesis	GRMZM2G102550	geranylgeranyl pyrophosphate synthase	β -Carotene/(β -Cryptoxanthin+Zeaxanthin)	GBS	7	160,778,001	160,531,537	160,533,586	246,464	244,415	3.46E-05	4.40E-02	0.07	0.08	0.1	178	0.04	0.14	0.15	-0.25	-0.43
S7_160778016	Prenyl Group Synthesis	GRMZM2G102550	geranylgeranyl pyrophosphate synthase	β -Carotene/(β -Cryptoxanthin+Zeaxanthin)	GBS	7	160,778,016	160,531,537	160,533,586	246,479	244,430	3.46E-05	4.40E-02	0.07	0.08	0.1	178	0.04	0.14	-0.15	-0.25	0.93
S8_168167679	Carotenoid Synthesis and Degradation	GRMZM2G149317	phytoene synthase	β -Carotene/(β -Cryptoxanthin+Zeaxanthin)	GBS	8	168,167,679	168,273,042	168,276,092	-105,363	-108,413	1.27E-05	3.22E-02	0.11	0.05	0.12	178	0.04	0.15	-0.14	-0.25	0.81
<i>crtRB1</i> InDel4	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	Zeaxanthin	Additonal Markers	10	136,059,748	136,057,100	136,060,219	2,648	-471	3.38E-06	5.11E-03	NA	NA	NA	196	0.24	0.32	NA	0.35	NA
<i>crtRB1</i> InDel4	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	Total β -Xanthophylls	Additonal Markers	10	136,059,748	136,057,100	136,060,219	2,648	-471	4.93E-06	8.08E-03	NA	NA	NA	195	0.29	0.36	NA	0.4	NA
<i>crtRB1</i> InDel4	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	β -Carotene/ β -Cryptoxanthin	Additonal Markers	10	136,059,748	136,057,100	136,060,219	2,648	-471	6.88E-07	5.25E-03	NA	NA	NA	198	-0.06	0.03	NA	-0.7	NA
<i>crtRB1</i> InDel4	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	Total Carotenes/Total Xanthophylls	Additonal Markers	10	136,059,748	136,057,100	136,060,219	2,648	-471	3.98E-08	1.49E-04	NA	NA	NA	188	0.06	0.19	NA	-0.55	NA
ss196501627	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	β -Carotene/(β -Cryptoxanthin)	55K	10	136,060,033	136,057,100	136,060,219	2,933	-186	8.21E-06	2.41E-02	0.18	0	0.22	179	0.1	0.21	0.11	-0.7	-0.14
ss196501627	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	β -Carotene/(β -Cryptoxanthin+Zeaxanthin)	55K	10	136,060,033	136,057,100	136,060,219	2,933	-186	8.35E-07	3.18E-03	0.19	0	0.22	178	0.04	0.19	0.12	-0.25	-0.37
<i>crtRB1</i> 3'TE	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	Zeaxanthin	Additonal Markers	10	136,061,719	136,057,100	136,060,219	4,619	1,500	1.61E-06	4.06E-03	NA	NA	NA	196	0.24	0.33	NA	0.35	NA
<i>crtRB1</i> 3'TE	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	Total β -Xanthophylls	Additonal Markers	10	136,061,719	136,057,100	136,060,219	4,619	1,500	1.76E-06	4.44E-03	NA	NA	NA	195	0.29	0.38	NA	0.4	NA
<i>crtRB1</i> 3'TE	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	β -Carotene/ β -Cryptoxanthin	Additonal Markers	10	136,061,719	136,057,100	136,060,219	4,619	1,500	9.48E-06	2.41E-02	NA	NA	NA	198	-0.06	0.02	NA	-0.7	NA
<i>crtRB1</i> 3'TE	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	β -Carotene/(β -Cryptoxanthin+Zeaxanthin)	Additonal Markers	10	136,061,719	136,057,100	136,060,219	4,619	1,500	2.13E-10	1.62E-06	NA	NA	NA	196	0.04	0.1	NA	-0.25	NA
<i>crtRB1</i> 3'TE	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	Total Carotenes/Total Xanthophylls	Additonal Markers	10	136,061,719	136,057,100	136,060,219	4,619	1,500	3.30E-09	2.47E-05	NA	NA	NA	188	0.06	0.22	NA	-0.55	NA

Statistically significant results from a pathway-level analysis of 58 *a priori* candidate genes from the carotenoid biosynthesis, carotenoid degradation, and isoprenoid biosynthetic pathways on 24 grain carotenoid traits with the markers tagging *lcyE* identified in the multi-locus mixed model included as covariates. All markers (Column A) proximal to a *a priori* candidate genes (Column B) found to be significantly associated with the indicated trait (Column E) at 5% FDR are shown.

Table S9 Results from the Pathway-Level Analysis with S8_171705574 Covariate (E)

SNP ID	<i>a priori</i> candidate gene pathway	RefGen_v2 Gene ID	Annotated Gene Function	Trait	SNP Source	Chromosome	Position	Gene ORF start	Gene ORF End	Distance from Gene ORF Start	Distance from Gene ORF Finish	P-value	FDR Adjusted P-value	Minor Allele Frequency (MAF)	MAF Tropical (8% of 201 Lines)	MAF Temperate (92% of 201 Lines)	Sample Size	R-square_LR from Model without SNP	R-square_LR from Model with SNP	Effect Size	Lambda from Box-Cox Procedure	Back-Transformed Effect Estimates
ss196425306	Carotenoid Synthesis and Degradation	GRMZM2G143202	CYP97A3, Cytochrome P450 epsilon-ring hydroxylase	Zeinoxanthin	55K	1	86,844,203	86,838,334	86,848,726	5,869	-4,523	7.31E-08	2.78E-04	0.3	0.4	0.28	198	0.1	0.25	-0.11	-0.25	0.62
ss196425306	Carotenoid Synthesis and Degradation	GRMZM2G143202	CYP97A3, Cytochrome P450 epsilon-ring hydroxylase	α -Carotene/Zeaxanthin	55K	1	86,844,203	86,838,334	86,848,726	5,869	-4,523	5.23E-10	1.99E-06	0.31	0.4	0.28	196	0.17	0.35	0.06	-0.25	-0.22
ss196425306	Carotenoid Synthesis and Degradation	GRMZM2G143202	CYP97A3, Cytochrome P450 epsilon-ring hydroxylase	Zeinoxanthin/Lutein	55K	1	86,844,203	86,838,334	86,848,726	5,869	-4,523	4.34E-08	1.64E-04	0.29	0.4	0.28	195	0.09	0.24	-0.19	-0.35	0.84
ss196425308	Carotenoid Synthesis and Degradation	GRMZM2G143202	CYP97A3, Cytochrome P450 epsilon-ring hydroxylase	Zeinoxanthin	55K	1	86,945,134	86,838,334	86,848,726	106,800	96,408	7.31E-08	2.78E-04	0.3	0.4	0.27	198	0.1	0.25	-0.11	-0.25	0.62
ss196425308	Carotenoid Synthesis and Degradation	GRMZM2G143202	CYP97A3, Cytochrome P450 epsilon-ring hydroxylase	α -Carotene/Zeaxanthin	55K	1	86,945,134	86,838,334	86,848,726	106,800	96,408	5.23E-10	1.99E-06	0.31	0.4	0.27	196	0.17	0.35	0.06	-0.25	-0.22
ss196425308	Carotenoid Synthesis and Degradation	GRMZM2G143202	CYP97A3, Cytochrome P450 epsilon-ring hydroxylase	Zeinoxanthin/Lutein	55K	1	86,945,134	86,838,334	86,848,726	106,800	96,408	4.34E-08	1.64E-04	0.29	0.4	0.27	195	0.09	0.24	-0.19	-0.35	0.84
S2_44445965	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeaxanthin	GBS	2	44,445,965	44,440,299	44,449,237	5,666	-3,272	9.02E-05	4.24E-02	0.18	0.33	0.26	196	0.19	0.25	-0.17	0.35	-0.41
S2_44445965	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Xanthophylls/ α -Xanthophylls	GBS	2	44,445,965	44,440,299	44,449,237	5,666	-3,272	1.38E-04	4.75E-02	0.18	0.33	0.26	196	0.19	0.25	0.07	-0.4	-0.16
S2_44445965	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Carotenoids/ α -Carotenoids	GBS	2	44,445,965	44,440,299	44,449,237	5,666	-3,272	8.85E-05	3.49E-02	0.18	0.33	0.26	189	0.22	0.29	0.08	-0.85	-0.09
S2_44448432	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeaxanthin	GBS	2	44,448,432	44,440,299	44,449,237	8,133	-805	2.15E-09	8.14E-06	0.11	0.29	0.09	196	0.19	0.35	-0.32	0.35	-0.66
S2_44448432	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Total β -Xanthophylls	GBS	2	44,448,432	44,440,299	44,449,237	8,133	-805	1.75E-08	6.64E-05	0.11	0.29	0.09	195	0.2	0.34	-0.4	0.4	-0.72
S2_44448432	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Xanthophylls/ α -Xanthophylls	GBS	2	44,448,432	44,440,299	44,449,237	8,133	-805	1.31E-07	1.98E-04	0.11	0.29	0.09	196	0.19	0.31	0.12	-0.4	-0.25
S2_44448432	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Carotenoids/ α -Carotenoids	GBS	2	44,448,432	44,440,299	44,449,237	8,133	-805	6.74E-06	7.02E-03	0.12	0.29	0.09	189	0.22	0.31	0.11	-0.85	-0.12
S2_44448438	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeaxanthin	GBS	2	44,448,438	44,440,299	44,449,237	8,139	-799	2.15E-09	8.14E-06	0.11	0.29	0.09	196	0.19	0.35	0.32	0.35	1.19
S2_44448438	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Total β -Xanthophylls	GBS	2	44,448,438	44,440,299	44,449,237	8,139	-799	1.75E-08	6.64E-05	0.11	0.29	0.09	195	0.2	0.34	0.4	0.4	1.32
S2_44448438	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Xanthophylls/ α -Xanthophylls	GBS	2	44,448,438	44,440,299	44,449,237	8,139	-799	1.31E-07	1.98E-04	0.11	0.29	0.09	196	0.19	0.31	-0.12	-0.4	0.39
S2_44448438	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Carotenoids/ α -Carotenoids	GBS	2	44,448,438	44,440,299	44,449,237	8,139	-799	6.74E-06	7.02E-03	0.12	0.29	0.09	189	0.22	0.31	-0.11	-0.85	0.15
S2_44473748	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeaxanthin	GBS	2	44,473,748	44,440,299	44,449,237	33,449	24,511	1.72E-07	2.61E-04	0.14	0.25	0.12	196	0.19	0.31	0.24	0.35	0.87
S2_44473748	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Total β -Xanthophylls	GBS	2	44,473,748	44,440,299	44,449,237	33,449	24,511	5.64E-07	8.53E-04	0.14	0.25	0.12	195	0.2	0.31	0.32	0.4	0.99
S2_44473748	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Xanthophylls/ α -Xanthophylls	GBS	2	44,473,748	44,440,299	44,449,237	33,449	24,511	2.75E-06	1.49E-03	0.14	0.25	0.12	196	0.19	0.28	-0.1	-0.4	0.28
S2_44473748	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Carotenoids/ α -Carotenoids	GBS	2	44,473,748	44,440,299	44,449,237	33,449	24,511	5.19E-05	2.43E-02	0.14	0.25	0.12	189	0.22	0.29	-0.09	-0.85	0.12
S2_44473758	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeaxanthin	GBS	2	44,473,758	44,440,299	44,449,237	33,459	24,521	1.72E-07	2.61E-04	0.14	0.24	0.12	196	0.19	0.31	-0.24	0.35	-0.55
S2_44473758	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Total β -Xanthophylls	GBS	2	44,473,758	44,440,299	44,449,237	33,459	24,521	5.64E-07	8.53E-04	0.14	0.24	0.12	195	0.2	0.31	-0.32	0.4	-0.61
S2_44473758	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Xanthophylls/ α -Xanthophylls	GBS	2	44,473,758	44,440,299	44,449,237	33,459	24,521	2.75E-06	1.49E-03	0.14	0.24	0.12	196	0.19	0.28	0.1	-0.4	-0.2
S2_44473758	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Carotenoids/ α -Carotenoids	GBS	2	44,473,758	44,440,299	44,449,237	33,459	24,521	5.19E-05	2.43E-02	0.14	0.24	0.12	189	0.22	0.29	0.09	-0.85	-0.1
S2_44473801	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeaxanthin	GBS	2	44,473,801	44,440,299	44,449,237	33,502	24,564	1.72E-07	2.61E-04	0.14	0.24	0.12	196	0.19	0.31	0.24	0.35	0.87
S2_44473801	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Total β -Xanthophylls	GBS	2	44,473,801	44,440,299	44,449,237	33,502	24,564	5.64E-07	8.53E-04	0.14	0.24	0.12	195	0.2	0.31	0.32	0.4	0.99
S2_44473801	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Xanthophylls/ α -Xanthophylls	GBS	2	44,473,801	44,440,299	44,449,237	33,502	24,564	2.75E-06	1.49E-03	0.14	0.24	0.12	196	0.19	0.28	-0.1	-0.4	0.28
S2_44473801	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Carotenoids/ α -Carotenoids	GBS	2	44,473,801	44,440,299	44,449,237	33,502	24,564	5.19E-05	2.43E-02	0.14	0.24	0.12	189	0.22	0.29	-0.09	-0.85	0.12
S2_44473994	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeaxanthin	GBS	2	44,473,994	44,440,299	44,449,237	33,695	24,757	5.18E-05	3.02E-02	0.17	0.35	0.16	196	0.19	0.26	0.17	0.35	0.57
S2_44473994	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Xanthophylls/ α -Xanthophylls	GBS	2	44,473,994	44,440,299	44,449,237	33,695	24,757	9.35E-05	3.54E-02	0.17	0.35	0.16	196	0.19	0.25	-0.07	-0.4	0.21
S2_44473994	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Carotenoids/ α -Carotenoids	GBS	2	44,473,994	44,440,299	44,449,237	33,695	24,757	8.38E-05	3.49E-02	0.18	0.35	0.16	189	0.22	0.29	-0.08	-0.85	0.11
S2_44474139	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeaxanthin	GBS	2	44,474,139	44,440,299	44,449,237	33,840	24,902	9.65E-07	1.14E-03	0.14	0.29	0.13	196	0.19	0.29	-0.23	0.35	-0.52
S2_44474139	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Total β -Xanthophylls	GBS	2	44,474,139	44,440,299	44,449,237	33,840	24,902	3.38E-06	4.26E-03	0.14	0.29	0.13	195	0.2	0.29	-0.29	0.4	-0.57
S2_44474139	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Xanthophylls/ α -Xanthophylls	GBS	2	44,474,139	44,440,299	44,449,237	33,840	24,902	4.99E-06	2.52E-03	0.14	0.29	0.13	196	0.19	0.28	0.09	-0.4	-0.2
S2_44474139	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β -Carotenoids/ α -Carotenoids	GBS	2	44,474,139	44,440,299	44,449,237	33,840	24,902	7.20E-05	3.18E-02	0.15	0.29	0.13	189	0.22	0.29	0.09	-0.85	-0.1
S2_44474308	Carotenoid Synthesis and	GRMZM2G127139	zeaxanthin epoxidase	Zeaxanthin	GBS	2	44,474,308	44,440,299	44,449,237	34,009	25,071	1.09E-06	1.14E-03	0.21	0.38	0.28	196	0.19	0.29	0.2	0.35	0.67

S2_44474308	Degradation Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Total β-Xanthophylls	GBS	2	44,474,308	44,440,299	44,449,237	34,009	25,071	6.38E-06	5.36E-03	0.22	0.38	0.28	195	0.2	0.29	0.24	0.4	0.73
S2_44474308	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β-Xanthophylls/α-Xanthophylls	GBS	2	44,474,308	44,440,299	44,449,237	34,009	25,071	1.04E-05	4.58E-03	0.21	0.38	0.28	196	0.19	0.27	-0.08	-0.4	0.22
S2_44474308	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β-Carotenoids/α-Carotenoids	GBS	2	44,474,308	44,440,299	44,449,237	34,009	25,071	3.06E-05	1.77E-02	0.22	0.38	0.28	189	0.22	0.3	-0.08	-0.85	0.11
S5_1315672	Carotenoid Synthesis and Degradation	GRMZM2G144273	carotenoid isomerase	Zeaxanthin	GBS	5	1,315,672	1,333,304	1,341,577	-17,632	-25,905	7.31E-05	3.95E-02	0.29	0.17	0.36	196	0.19	0.26	0.16	0.35	0.52
ss196471946	Carotenoid Synthesis and Degradation	GRMZM2G300348	phytoene synthase	Acyclic and Monocyclic Carotenes	55K	6	82,019,955	82,017,148	82,021,007	2,807	-1,052	4.82E-06	3.68E-02	0.18	0.1	0.19	200	0.05	0.16	0.01	-0.15	-0.07
S7_13843351	Prenyl Group Synthesis	GRMZM2G493395	1-deoxy-D-xylulose 5-phosphate synthase	β-Cryptoxanthin	GBS	7	13,843,351	14,077,852	14,081,075	-234,501	-237,724	2.78E-07	2.12E-03	0.15	0.1	0.16	199	0.17	0.29	-0.04	0.1	-0.32
S7_13843351	Prenyl Group Synthesis	GRMZM2G493395	1-deoxy-D-xylulose 5-phosphate synthase	Total β-Xanthophylls	GBS	7	13,843,351	14,077,852	14,081,075	-234,501	-237,724	1.12E-04	4.97E-02	0.15	0.1	0.16	195	0.2	0.26	-0.23	0.4	-0.48
S7_13980028	Prenyl Group Synthesis	GRMZM2G493395	1-deoxy-D-xylulose 5-phosphate synthase	Zeinoxanthin	GBS	7	13,980,028	14,077,852	14,081,075	-97,824	-101,047	1.08E-05	2.75E-02	0.41	0.4	0.5	198	0.1	0.2	-0.08	-0.25	0.39
S7_16077798_6	Prenyl Group Synthesis	GRMZM2G102550	geranylgeranyl pyrophosphate synthase	β-Carotene/(β-Cryptoxanthin+Zeaxanthin)	GBS	7	160,777,986	160,531,537	160,533,586	246,449	244,400	3.16E-05	4.80E-02	0.07	0.08	0.1	196	0.04	0.13	0.14	-0.25	-0.41
S7_16077800_1	Prenyl Group Synthesis	GRMZM2G102550	geranylgeranyl pyrophosphate synthase	β-Carotene/(β-Cryptoxanthin+Zeaxanthin)	GBS	7	160,778,001	160,531,537	160,533,586	246,464	244,415	3.16E-05	4.80E-02	0.07	0.08	0.1	196	0.04	0.13	0.14	-0.25	-0.41
S7_16077801_6	Prenyl Group Synthesis	GRMZM2G102550	geranylgeranyl pyrophosphate synthase	β-Carotene/(β-Cryptoxanthin+Zeaxanthin)	GBS	7	160,778,016	160,531,537	160,533,586	246,479	244,430	3.16E-05	4.80E-02	0.07	0.08	0.1	196	0.04	0.13	-0.14	-0.25	0.83
S7_16077948_8	Prenyl Group Synthesis	GRMZM2G102550	geranylgeranyl pyrophosphate synthase	β-Carotene/β-Cryptoxanthin	GBS	7	160,779,488	160,531,537	160,533,586	247,951	245,902	1.08E-05	2.74E-02	0.12	0.26	0.12	198	0.1	0.19	0.12	-0.7	-0.15
S8_13864894_0	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β-Carotenoids/α-Carotenoids	GBS	8	138,648,940	138,882,594	138,889,812	-233,654	-240,872	1.31E-04	4.67E-02	0.19	0.28	0.25	189	0.22	0.28	-0.08	-0.85	0.1
ss196487098	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Zeaxanthin	55K	8	138,861,176	138,882,594	138,889,812	-21,418	-28,636	3.89E-05	2.46E-02	0.32	0.29	0.33	196	0.19	0.26	0.16	0.35	0.51
ss196487098	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total β-Xanthophylls	55K	8	138,861,176	138,882,594	138,889,812	-21,418	-28,636	1.53E-05	1.16E-02	0.32	0.29	0.33	195	0.2	0.28	0.22	0.4	0.65
ss196487098	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β-Xanthophylls/α-Xanthophylls	55K	8	138,861,176	138,882,594	138,889,812	-21,418	-28,636	8.49E-05	3.38E-02	0.32	0.29	0.33	196	0.19	0.25	-0.07	-0.4	0.19
ss196487098	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β-Carotenoids/α-Carotenoids	55K	8	138,861,176	138,882,594	138,889,812	-21,418	-28,636	9.59E-05	3.60E-02	0.32	0.29	0.33	189	0.22	0.29	-0.07	-0.85	0.1
lcyE 5' TE	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Lutein	Additional Markers	8	138,882,481	138,882,594	138,889,812	-114	-7,332	1.52E-08	5.81E-05	NA	NA	NA	200	0.11	0.49	NA	0.8	NA
lcyE 5' TE	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total α-Xanthophylls	Additional Markers	8	138,882,481	138,882,594	138,889,812	-114	-7,332	3.99E-09	1.52E-05	NA	NA	NA	200	0.1	0.43	NA	0.7	NA
S8_13888271_1	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Zeaxanthin	GBS	8	138,882,711	138,882,594	138,889,812	117	-7,101	1.01E-04	4.24E-02	0.28	0.41	0.3	196	0.19	0.25	0.15	0.35	0.48
S8_13888271_1	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total β-Xanthophylls	GBS	8	138,882,711	138,882,594	138,889,812	117	-7,101	9.91E-05	4.69E-02	0.28	0.41	0.3	195	0.2	0.26	0.2	0.4	0.57
S8_13888271_1	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β-Xanthophylls/α-Xanthophylls	GBS	8	138,882,711	138,882,594	138,889,812	117	-7,101	1.58E-06	1.19E-03	0.28	0.41	0.3	196	0.19	0.29	-0.08	-0.4	0.24
S8_13888271_1	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β-Carotenoids/α-Carotenoids	GBS	8	138,882,711	138,882,594	138,889,812	117	-7,101	1.03E-05	7.02E-03	0.29	0.41	0.3	189	0.22	0.3	-0.08	-0.85	0.11
ss196504160	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Lutein	55K	8	138,882,711	138,882,594	138,889,812	117	-7,101	2.40E-05	3.67E-02	0.36	0.48	0.34	200	0.13	0.22	0.84	0.8	1.15
ss196504160	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Zeaxanthin	55K	8	138,882,711	138,882,594	138,889,812	117	-7,101	8.11E-06	6.15E-03	0.35	0.48	0.34	196	0.19	0.28	-0.17	0.35	-0.4
ss196504160	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total β-Xanthophylls	55K	8	138,882,711	138,882,594	138,889,812	117	-7,101	6.36E-06	5.36E-03	0.35	0.48	0.34	195	0.2	0.29	-0.23	0.4	-0.47
ss196504160	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total α-Xanthophylls	55K	8	138,882,711	138,882,594	138,889,812	117	-7,101	9.40E-06	1.44E-02	0.36	0.48	0.34	200	0.11	0.21	0.56	0.7	0.9
ss196504160	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β-Xanthophylls/α-Xanthophylls	55K	8	138,882,711	138,882,594	138,889,812	117	-7,101	3.69E-09	1.40E-05	0.35	0.48	0.34	196	0.19	0.35	0.1	-0.4	-0.21
ss196504160	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β-Carotenoids/α-Carotenoids	55K	8	138,882,711	138,882,594	138,889,812	117	-7,101	4.67E-09	3.50E-05	0.37	0.48	0.34	189	0.22	0.38	0.11	-0.85	-0.12
S8_13888274_7	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Zeaxanthin	GBS	8	138,882,747	138,882,594	138,889,812	153	-7,065	1.01E-04	4.24E-02	0.28	0.41	0.3	196	0.19	0.25	0.15	0.35	0.48
S8_13888274_7	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total β-Xanthophylls	GBS	8	138,882,747	138,882,594	138,889,812	153	-7,065	9.91E-05	4.69E-02	0.28	0.41	0.3	195	0.2	0.26	0.2	0.4	0.57
S8_13888274_7	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β-Xanthophylls/α-Xanthophylls	GBS	8	138,882,747	138,882,594	138,889,812	153	-7,065	1.58E-06	1.19E-03	0.28	0.41	0.3	196	0.19	0.29	-0.08	-0.4	0.24
S8_13888274_7	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β-Carotenoids/α-Carotenoids	GBS	8	138,882,747	138,882,594	138,889,812	153	-7,065	1.03E-05	7.02E-03	0.29	0.41	0.3	189	0.22	0.3	-0.08	-0.85	0.11
S8_13888275_1	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Zeaxanthin	GBS	8	138,882,751	138,882,594	138,889,812	157	-7,061	1.01E-04	4.24E-02	0.28	0.41	0.3	196	0.19	0.25	0.15	0.35	0.48
S8_13888275_1	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total β-Xanthophylls	GBS	8	138,882,751	138,882,594	138,889,812	157	-7,061	9.91E-05	4.69E-02	0.28	0.41	0.3	195	0.2	0.26	0.2	0.4	0.57
S8_13888275_1	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β-Xanthophylls/α-Xanthophylls	GBS	8	138,882,751	138,882,594	138,889,812	157	-7,061	1.58E-06	1.19E-03	0.28	0.41	0.3	196	0.19	0.29	-0.08	-0.4	0.24
S8_13888275_1	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β-Carotenoids/α-Carotenoids	GBS	8	138,882,751	138,882,594	138,889,812	157	-7,061	1.03E-05	7.02E-03	0.29	0.41	0.3	189	0.22	0.3	-0.08	-0.85	0.11
S8_13888279_8	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β-Xanthophylls/α-Xanthophylls	GBS	8	138,882,798	138,882,594	138,889,812	204	-7,014	2.66E-06	1.49E-03	0.31	0.21	0.36	196	0.19	0.28	-0.08	-0.4	0.23
S8_13888279_8	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β-Carotenoids/α-Carotenoids	GBS	8	138,882,798	138,882,594	138,889,812	204	-7,014	1.16E-05	7.22E-03	0.31	0.21	0.36	189	0.22	0.3	-0.08	-0.85	0.11
S8_13888289	Carotenoid	GRMZM2G012966	lycopene epsilon-cyclase	Lutein	GBS	8	138,882,897	138,882,594	138,889,812	303	-6,915	7.94E-06	1.52E-02	0.43	0.12	0.44	200	0.13	0.23	-0.82	0.8	-0.88

7	Synthesis and Degradation																					
S8_138882897	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total α -Xanthophylls	GBS	8	138,882,897	138,882,594	138,889,812	303	-6,915	3.85E-06	8.95E-03	0.43	0.12	0.44	200	0.11	0.21	-0.54	0.7	-0.67
S8_138882897	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Xanthophylls/ α -Xanthophylls	GBS	8	138,882,897	138,882,594	138,889,812	303	-6,915	2.15E-07	2.72E-04	0.43	0.12	0.44	196	0.19	0.31	-0.08	-0.4	0.23
S8_138882897	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Carotenoids/ α -Carotenoids	GBS	8	138,882,897	138,882,594	138,889,812	303	-6,915	1.14E-07	4.28E-04	0.43	0.12	0.44	189	0.22	0.35	-0.09	-0.85	0.12
S8_138883026	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Xanthophylls/ α -Xanthophylls	GBS	8	138,883,026	138,882,594	138,889,812	432	-6,786	1.09E-05	4.58E-03	0.4	0.18	0.48	196	0.19	0.27	0.07	-0.4	-0.15
S8_138883026	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Carotenoids/ α -Carotenoids	GBS	8	138,883,026	138,882,594	138,889,812	432	-6,786	1.01E-05	7.02E-03	0.4	0.18	0.48	189	0.22	0.31	0.08	-0.85	-0.09
S8_138883056	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Xanthophylls/ α -Xanthophylls	GBS	8	138,883,056	138,882,594	138,889,812	462	-6,756	1.09E-05	4.58E-03	0.4	0.18	0.48	196	0.19	0.27	-0.07	-0.4	0.2
S8_138883056	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Carotenoids/ α -Carotenoids	GBS	8	138,883,056	138,882,594	138,889,812	462	-6,756	1.01E-05	7.02E-03	0.4	0.18	0.48	189	0.22	0.31	-0.08	-0.85	0.1
lcyE SNP216	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Lutein	Additional Markers	8	138,883,206	138,882,594	138,889,812	612	-6,606	4.75E-09	3.63E-05	NA	NA	NA	200	0.11	0.45	NA	0.8	NA
lcyE SNP216	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Zeaxanthin	Additional Markers	8	138,883,206	138,882,594	138,889,812	612	-6,606	7.05E-06	5.93E-03	NA	NA	NA	196	0.17	0.35	NA	0.35	NA
lcyE SNP216	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total β -Xanthophylls	Additional Markers	8	138,883,206	138,882,594	138,889,812	612	-6,606	1.88E-05	1.29E-02	NA	NA	NA	195	0.18	0.39	NA	0.4	NA
lcyE SNP216	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total α -Xanthophylls	Additional Markers	8	138,883,206	138,882,594	138,889,812	612	-6,606	3.24E-10	2.48E-06	NA	NA	NA	200	0.1	0.4	NA	0.7	NA
lcyE SNP216	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Xanthophylls/ α -Xanthophylls	Additional Markers	8	138,883,206	138,882,594	138,889,812	612	-6,606	2.78E-15	2.10E-11	NA	NA	NA	196	0.13	0.26	NA	-0.4	NA
PZB00665.1	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Xanthophylls/ α -Xanthophylls	4K	8	138,886,137	138,882,594	138,889,812	3,543	-3,675	1.47E-06	1.19E-03	0.35	0.05	0.38	196	0.19	0.29	0.08	-0.4	-0.18
PZB00665.1	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Carotenoids/ α -Carotenoids	4K	8	138,886,137	138,882,594	138,889,812	3,543	-3,675	3.05E-06	5.72E-03	0.35	0.05	0.38	189	0.22	0.32	0.09	-0.85	-0.1
S8_138888278	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Lutein	GBS	8	138,888,278	138,882,594	138,889,812	5,684	-1,534	6.03E-06	1.52E-02	0.47	0.19	0.42	200	0.13	0.23	-0.87	0.8	-0.92
S8_138888278	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Zeaxanthin	GBS	8	138,888,278	138,882,594	138,889,812	5,684	-1,534	1.22E-04	4.86E-02	0.46	0.19	0.42	196	0.19	0.25	0.14	0.35	0.44
S8_138888278	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total β -Xanthophylls	GBS	8	138,888,278	138,882,594	138,889,812	5,684	-1,534	9.58E-05	4.69E-02	0.47	0.19	0.42	195	0.2	0.26	0.19	0.4	0.54
S8_138888278	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total α -Xanthophylls	GBS	8	138,888,278	138,882,594	138,889,812	5,684	-1,534	4.68E-06	8.95E-03	0.47	0.19	0.42	200	0.11	0.21	-0.56	0.7	-0.69
S8_138888278	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Xanthophylls/ α -Xanthophylls	GBS	8	138,888,278	138,882,594	138,889,812	5,684	-1,534	1.58E-08	4.00E-05	0.47	0.19	0.42	196	0.19	0.33	-0.09	-0.4	0.28
S8_138888278	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Carotenoids/ α -Carotenoids	GBS	8	138,888,278	138,882,594	138,889,812	5,684	-1,534	2.43E-07	6.08E-04	0.47	0.19	0.42	189	0.22	0.34	-0.1	-0.85	0.13
S8_139136162	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Xanthophylls/ α -Xanthophylls	GBS	8	139,136,162	138,882,594	138,889,812	253,568	246,350	1.24E-04	4.47E-02	0.45	0	0.47	196	0.19	0.25	-0.06	-0.4	0.18
crtrB1 InDel4	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	Zeaxanthin	Additional Markers	10	136,059,748	136,057,100	136,060,219	2,648	-471	2.43E-05	1.67E-02	NA	NA	NA	196	0.17	0.24	NA	0.35	NA
crtrB1 InDel4	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	Total β -Xanthophylls	Additional Markers	10	136,059,748	136,057,100	136,060,219	2,648	-471	4.22E-05	2.66E-02	NA	NA	NA	195	0.18	0.25	NA	0.4	NA
crtrB1 InDel4	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	β -Carotene/ β -Cryptoxanthin	Additional Markers	10	136,059,748	136,057,100	136,060,219	2,648	-471	3.17E-06	2.42E-02	NA	NA	NA	198	0.07	0.06	NA	-0.7	NA
crtrB1 InDel4	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	β -Carotene/(β -Cryptoxanthin+Zeaxanthin)	Additional Markers	10	136,059,748	136,057,100	136,060,219	2,648	-471	2.87E-07	1.51E-03	NA	NA	NA	196	0.04	0.08	NA	-0.25	NA
crtrB1 InDel4	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	Total Carotenes/Total Xanthophylls	Additional Markers	10	136,059,748	136,057,100	136,060,219	2,648	-471	4.43E-06	3.31E-02	NA	NA	NA	188	0.03	0.09	NA	-0.55	NA
ss196501627	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	β -Carotene/ β -Cryptoxanthin	55K	10	136,060,033	136,057,100	136,060,219	2,933	-186	8.30E-06	2.74E-02	0.18	0	0.22	198	0.1	0.2	0.11	-0.7	-0.14
ss196501627	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	β -Carotene/(β -Cryptoxanthin+Zeaxanthin)	55K	10	136,060,033	136,057,100	136,060,219	2,933	-186	3.99E-07	1.51E-03	0.19	0	0.22	196	0.04	0.18	0.12	-0.25	-0.36
crtrB1 3'TE	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	Zeaxanthin	Additional Markers	10	136,061,719	136,057,100	136,060,219	4,619	1,500	1.20E-06	1.14E-03	NA	NA	NA	196	0.17	0.27	NA	0.35	NA
crtrB1 3'TE	Carotenoid Synthesis and Degradation	GRMZM2G152135	Beta-carotene hydroxylase (non-heme dioxygenase type)	Total β -Xanthophylls	Additional Markers	10	136,061,719	136,057,100	136,060,219	4,619	1,500	4.42E-06	4.77E-03	NA	NA	NA	195	0.18	0.29	NA	0.4	NA

Statistically significant results from a pathway-level analysis of 58 *a priori* candidate genes from the carotenoid biosynthesis, carotenoid degradation, and isoprenoid biosynthetic pathways on 24 grain carotenoid traits with SNP S8 171705574 included as a covariate. All markers (Column A) proximal to *a priori* candidate genes (Column B) found to be significantly associated with the indicated trait (Column E) at 5% FDR are shown.

Table S9 Results from the Pathway-Level Analysis with crtRB1_3' TE as Covariate Tagging crtRB1 (F)

SNP ID	<i>a priori</i> candidate gene pathway	RefGen_v2 Gene ID	Annotated Gene Function	Trait	SNP Source	Chromosome	Position	Gene ORF start	Gene ORF End	Distance from Gene ORF Start	Distance from Gene ORF Finish	P-value	FDR Adjusted P-value	Minor Allele Frequency (MAF)	MAF Tropical (8% of 201 Lines)	MAF Temperate (92% of 201 Lines)	Sample Size	R-square_LR from Model without SNP	R-square_LR from Model with SNP	Effect Size	Lambda from Box-Cox Procedure	Back-Transformed Effect Estimates
S1_5345354	Carotenoid Synthesis and Degradation	GRMZM2G090051	Beta-carotene hydroxylase (non-heme dioxygenase type)	β-Xanthophylls/α-Xanthophylls	GBS	1	5,345,354	5,380,152	5,382,574	-34,798	-37,220	6.42E-05	2.25E-02	0.09	0.32	0.08	190	0.18	0.25	0.1	-0.4	-0.22
S1_5345457	Carotenoid Synthesis and Degradation	GRMZM2G090051	Beta-carotene hydroxylase (non-heme dioxygenase type)	β-Carotenoids/α-Carotenoids	GBS	1	5,345,457	5,380,152	5,382,574	-34,695	-37,117	1.46E-04	4.72E-02	0.06	0.21	0.07	184	0.2	0.27	0.14	-0.85	-0.14
S1_17695167	Carotenoid Synthesis and Degradation	GRMZM2G410515	phytoene desaturase	β-Carotenoids/α-Carotenoids	GBS	1	17,695,167	17,660,941	17,667,054	34,226	28,113	1.42E-04	4.72E-02	0.41	0.42	0.46	184	0.2	0.27	-0.07	-0.85	0.08
ss196425306	Carotenoid Synthesis and Degradation	GRMZM2G143202	CYP97A3, Cytochrome P450 epsilon-ring hydroxylase	Zeinoxanthin	55K	1	86,844,203	86,838,334	86,848,726	5,869	-4,523	1.21E-07	4.55E-04	0.31	0.4	0.28	192	0.1	0.24	-0.11	-0.25	0.62
ss196425306	Carotenoid Synthesis and Degradation	GRMZM2G143202	CYP97A3, Cytochrome P450 epsilon-ring hydroxylase	α-Carotene/Zeaxanthin	55K	1	86,844,203	86,838,334	86,848,726	5,869	-4,523	8.94E-10	3.37E-06	0.32	0.4	0.28	190	0.17	0.36	0.06	-0.25	-0.21
ss196425306	Carotenoid Synthesis and Degradation	GRMZM2G143202	CYP97A3, Cytochrome P450 epsilon-ring hydroxylase	Zeinoxanthin/Lutein	55K	1	86,844,203	86,838,334	86,848,726	5,869	-4,523	5.87E-08	2.20E-04	0.3	0.4	0.28	189	0.09	0.25	-0.19	-0.35	0.85
ss196425308	Carotenoid Synthesis and Degradation	GRMZM2G143202	CYP97A3, Cytochrome P450 epsilon-ring hydroxylase	Zeinoxanthin	55K	1	86,945,134	86,838,334	86,848,726	106,800	96,408	1.21E-07	4.55E-04	0.31	0.4	0.27	192	0.1	0.24	-0.11	-0.25	0.62
ss196425308	Carotenoid Synthesis and Degradation	GRMZM2G143202	CYP97A3, Cytochrome P450 epsilon-ring hydroxylase	α-Carotene/Zeaxanthin	55K	1	86,945,134	86,838,334	86,848,726	106,800	96,408	8.94E-10	3.37E-06	0.32	0.4	0.27	190	0.17	0.36	0.06	-0.25	-0.21
ss196425308	Carotenoid Synthesis and Degradation	GRMZM2G143202	CYP97A3, Cytochrome P450 epsilon-ring hydroxylase	Zeinoxanthin/Lutein	55K	1	86,945,134	86,838,334	86,848,726	106,800	96,408	5.87E-08	2.20E-04	0.3	0.4	0.27	189	0.09	0.25	-0.19	-0.35	0.85
S2_44445965	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeaxanthin	GBS	2	44,445,965	44,440,299	44,449,237	5,666	-3,272	8.13E-06	6.11E-03	0.17	0.33	0.26	190	0.12	0.22	-0.2	0.35	-0.47
S2_44445965	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Total β-Xanthophylls	GBS	2	44,445,965	44,440,299	44,449,237	5,666	-3,272	4.20E-05	2.71E-02	0.17	0.33	0.26	189	0.12	0.21	-0.25	0.4	-0.51
S2_44445965	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β-Xanthophylls/α-Xanthophylls	GBS	2	44,445,965	44,440,299	44,449,237	5,666	-3,272	2.43E-05	1.01E-02	0.17	0.33	0.26	190	0.18	0.26	0.08	-0.4	-0.18
S2_44445965	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β-Carotenoids/α-Carotenoids	GBS	2	44,445,965	44,440,299	44,449,237	5,666	-3,272	4.62E-05	2.11E-02	0.17	0.33	0.26	184	0.2	0.28	0.09	-0.85	-0.1
S2_44448432	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeaxanthin	GBS	2	44,448,432	44,440,299	44,449,237	8,133	-805	2.71E-09	1.02E-05	0.11	0.29	0.09	190	0.12	0.3	-0.32	0.35	-0.67
S2_44448432	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Total β-Xanthophylls	GBS	2	44,448,432	44,440,299	44,449,237	8,133	-805	2.34E-08	8.77E-05	0.11	0.29	0.09	189	0.12	0.28	-0.41	0.4	-0.74
S2_44448432	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β-Xanthophylls/α-Xanthophylls	GBS	2	44,448,432	44,440,299	44,449,237	8,133	-805	8.21E-08	1.23E-04	0.11	0.29	0.09	190	0.18	0.31	0.13	-0.4	-0.26
S2_44448432	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β-Carotenoids/α-Carotenoids	GBS	2	44,448,432	44,440,299	44,449,237	8,133	-805	6.76E-06	8.38E-03	0.11	0.29	0.09	184	0.2	0.3	0.12	-0.85	-0.12
S2_44448438	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeaxanthin	GBS	2	44,448,438	44,440,299	44,449,237	8,139	-799	2.71E-09	1.02E-05	0.11	0.29	0.09	190	0.12	0.3	0.32	0.35	1.22
S2_44448438	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Total β-Xanthophylls	GBS	2	44,448,438	44,440,299	44,449,237	8,139	-799	2.34E-08	8.77E-05	0.11	0.29	0.09	189	0.12	0.28	0.41	0.4	1.37
S2_44448438	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β-Xanthophylls/α-Xanthophylls	GBS	2	44,448,438	44,440,299	44,449,237	8,139	-799	8.21E-08	1.23E-04	0.11	0.29	0.09	190	0.18	0.31	-0.13	-0.4	0.41
S2_44448438	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β-Carotenoids/α-Carotenoids	GBS	2	44,448,438	44,440,299	44,449,237	8,139	-799	6.76E-06	8.38E-03	0.11	0.29	0.09	184	0.2	0.3	-0.12	-0.85	0.16
S2_44472618	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeaxanthin	GBS	2	44,472,618	44,440,299	44,449,237	32,319	23,381	3.07E-05	1.93E-02	0.12	0.38	0.15	190	0.12	0.21	-0.21	0.35	-0.49
S2_44472618	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β-Xanthophylls/α-Xanthophylls	GBS	2	44,472,618	44,440,299	44,449,237	32,319	23,381	8.92E-05	2.79E-02	0.12	0.38	0.15	190	0.18	0.25	0.08	-0.4	-0.18
S2_44473748	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeaxanthin	GBS	2	44,473,748	44,440,299	44,449,237	33,449	24,511	3.89E-07	5.85E-04	0.13	0.25	0.12	190	0.12	0.25	0.25	0.35	0.89
S2_44473748	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Total β-Xanthophylls	GBS	2	44,473,748	44,440,299	44,449,237	33,449	24,511	1.41E-06	1.76E-03	0.13	0.25	0.12	189	0.12	0.24	0.32	0.4	1.01
S2_44473748	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β-Xanthophylls/α-Xanthophylls	GBS	2	44,473,748	44,440,299	44,449,237	33,449	24,511	1.76E-06	1.32E-03	0.13	0.25	0.12	190	0.18	0.28	-0.1	-0.4	0.31
S2_44473748	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β-Carotenoids/α-Carotenoids	GBS	2	44,473,748	44,440,299	44,449,237	33,449	24,511	5.10E-05	2.11E-02	0.14	0.25	0.12	184	0.2	0.28	-0.1	-0.85	0.13
S2_44473758	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeaxanthin	GBS	2	44,473,758	44,440,299	44,449,237	33,459	24,521	3.89E-07	5.85E-04	0.13	0.24	0.12	190	0.12	0.25	-0.25	0.35	-0.56
S2_44473758	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Total β-Xanthophylls	GBS	2	44,473,758	44,440,299	44,449,237	33,459	24,521	1.41E-06	1.76E-03	0.13	0.24	0.12	189	0.12	0.24	-0.32	0.4	-0.62
S2_44473758	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β-Xanthophylls/α-Xanthophylls	GBS	2	44,473,758	44,440,299	44,449,237	33,459	24,521	1.76E-06	1.32E-03	0.13	0.24	0.12	190	0.18	0.28	0.1	-0.4	-0.21
S2_44473758	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β-Carotenoids/α-Carotenoids	GBS	2	44,473,758	44,440,299	44,449,237	33,459	24,521	5.10E-05	2.11E-02	0.14	0.24	0.12	184	0.2	0.28	0.1	-0.85	-0.1
S2_44473801	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeaxanthin	GBS	2	44,473,801	44,440,299	44,449,237	33,502	24,564	3.89E-07	5.85E-04	0.13	0.24	0.12	190	0.12	0.25	0.25	0.35	0.89
S2_44473801	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Total β-Xanthophylls	GBS	2	44,473,801	44,440,299	44,449,237	33,502	24,564	1.41E-06	1.76E-03	0.13	0.24	0.12	189	0.12	0.24	0.32	0.4	1.01
S2_44473801	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β-Xanthophylls/α-Xanthophylls	GBS	2	44,473,801	44,440,299	44,449,237	33,502	24,564	1.76E-06	1.32E-03	0.13	0.24	0.12	190	0.18	0.28	-0.1	-0.4	0.31
S2_44473801	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β-Carotenoids/α-Carotenoids	GBS	2	44,473,801	44,440,299	44,449,237	33,502	24,564	5.10E-05	2.11E-02	0.14	0.24	0.12	184	0.2	0.28	-0.1	-0.85	0.13
S2_44473994	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeaxanthin	GBS	2	44,473,994	44,440,299	44,449,237	33,695	24,757	4.68E-05	2.51E-02	0.17	0.35	0.16	190	0.12	0.2	0.18	0.35	0.6
S2_44473994	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β-Xanthophylls/α-Xanthophylls	GBS	2	44,473,994	44,440,299	44,449,237	33,695	24,757	4.51E-05	1.78E-02	0.17	0.35	0.16	190	0.18	0.25	-0.08	-0.4	0.23
S2_44473994	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β-Carotenoids/α-Carotenoids	GBS	2	44,473,994	44,440,299	44,449,237	33,695	24,757	6.11E-05	2.24E-02	0.17	0.35	0.16	184	0.2	0.28	-0.09	-0.85	0.11
S2_44474139	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Zeaxanthin	GBS	2	44,474,139	44,440,299	44,449,237	33,840	24,902	1.48E-06	1.53E-03	0.14	0.29	0.13	190	0.12	0.24	-0.23	0.35	-0.53
S2_44474139	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	Total β-Xanthophylls	GBS	2	44,474,139	44,440,299	44,449,237	33,840	24,902	5.63E-06	6.03E-03	0.14	0.29	0.13	189	0.12	0.23	-0.3	0.4	-0.59
S2_44474139	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β-Xanthophylls/α-Xanthophylls	GBS	2	44,474,139	44,440,299	44,449,237	33,840	24,902	2.48E-06	1.70E-03	0.14	0.29	0.13	190	0.18	0.28	0.1	-0.4	-0.21
S2_44474139	Carotenoid Synthesis and Degradation	GRMZM2G127139	zeaxanthin epoxidase	β-Carotenoids/α-Carotenoids	GBS	2	44,474,139	44,440,299	44,449,237	33,840	24,902	5.82E-05	2.24E-02	0.14	0.29	0.13	184	0.2	0.28	0.09	-0.85	-0.1

S7_13843351	Prenyl Group Synthesis	GRMZM2G493395	1-deoxy-D-xylulose 5-phosphate synthase	Total β -Xanthophylls	GBS	7	13,843,351	14,077,852	14,081,075	-234,501	-237,724	5.39E-05	3.11E-02	0.16	0.1	0.16	189	0.12	0.2	-0.25	0.4	-0.51
<i>lcyE</i> 5'TE	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Zeaxanthin	Additional Markers	8	138,882,481	138,882,594	138,889,812	-114	-7,332	4.06E-05	2.35E-02	NA	NA	NA	196	0.17	0.34	NA	0.35	NA
<i>lcyE</i> 5'TE	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total β -Xanthophylls	Additional Markers	8	138,882,481	138,882,594	138,889,812	-114	-7,332	4.34E-05	2.71E-02	NA	NA	NA	195	0.18	0.38	NA	0.4	NA
<i>lcyE</i> 5'TE	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Xanthophylls/ α -Xanthophylls	Additional Markers	8	138,882,481	138,882,594	138,889,812	-114	-7,332	2.43E-13	9.12E-10	NA	NA	NA	196	0.04	0.24	NA	-0.4	NA
<i>lcyE</i> 5'TE	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Carotenoids/ α -Carotenoids	Additional Markers	8	138,882,481	138,882,594	138,889,812	-114	-7,332	1.35E-13	1.01E-09	NA	NA	NA	189	0.01	0.28	NA	-0.85	NA
S8_138882711	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Xanthophylls/ α -Xanthophylls	GBS	8	138,882,711	138,882,594	138,889,812	117	-7,101	8.52E-06	4.00E-03	0.28	0.41	0.3	190	0.18	0.27	-0.08	-0.4	0.22
S8_138882711	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Carotenoids/ α -Carotenoids	GBS	8	138,882,711	138,882,594	138,889,812	117	-7,101	1.86E-05	1.38E-02	0.28	0.41	0.3	184	0.2	0.29	-0.08	-0.85	0.11
ss196504160	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Lutein	55K	8	138,882,711	138,882,594	138,889,812	117	-7,101	1.54E-05	2.92E-02	0.36	0.48	0.34	194	0.12	0.21	0.88	0.8	1.2
ss196504160	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Zeaxanthin	55K	8	138,882,711	138,882,594	138,889,812	117	-7,101	1.43E-05	9.78E-03	0.35	0.48	0.34	190	0.12	0.21	-0.16	0.35	-0.4
ss196504160	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total β -Xanthophylls	55K	8	138,882,711	138,882,594	138,889,812	117	-7,101	9.27E-06	8.69E-03	0.35	0.48	0.34	189	0.12	0.22	-0.23	0.4	-0.48
ss196504160	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total α -Xanthophylls	55K	8	138,882,711	138,882,594	138,889,812	117	-7,101	6.32E-06	1.20E-02	0.36	0.48	0.34	194	0.11	0.21	0.59	0.7	0.94
ss196504160	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Xanthophylls/ α -Xanthophylls	55K	8	138,882,711	138,882,594	138,889,812	117	-7,101	6.86E-09	1.72E-05	0.35	0.48	0.34	190	0.18	0.34	0.1	-0.4	-0.21
ss196504160	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Carotenoids/ α -Carotenoids	55K	8	138,882,711	138,882,594	138,889,812	117	-7,101	7.10E-09	2.64E-05	0.36	0.48	0.34	184	0.2	0.37	0.11	-0.85	-0.12
S8_138882747	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Xanthophylls/ α -Xanthophylls	GBS	8	138,882,747	138,882,594	138,889,812	153	-7,065	8.52E-06	4.00E-03	0.28	0.41	0.3	190	0.18	0.27	-0.08	-0.4	0.22
S8_138882747	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Carotenoids/ α -Carotenoids	GBS	8	138,882,747	138,882,594	138,889,812	153	-7,065	1.86E-05	1.38E-02	0.28	0.41	0.3	184	0.2	0.29	-0.08	-0.85	0.11
S8_138882751	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Xanthophylls/ α -Xanthophylls	GBS	8	138,882,751	138,882,594	138,889,812	157	-7,061	8.52E-06	4.00E-03	0.28	0.41	0.3	190	0.18	0.27	-0.08	-0.4	0.22
S8_138882751	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Carotenoids/ α -Carotenoids	GBS	8	138,882,751	138,882,594	138,889,812	157	-7,061	1.86E-05	1.38E-02	0.28	0.41	0.3	184	0.2	0.29	-0.08	-0.85	0.11
S8_138882798	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Xanthophylls/ α -Xanthophylls	GBS	8	138,882,798	138,882,594	138,889,812	204	-7,014	7.06E-06	4.00E-03	0.31	0.21	0.36	190	0.18	0.27	-0.08	-0.4	0.22
S8_138882798	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Carotenoids/ α -Carotenoids	GBS	8	138,882,798	138,882,594	138,889,812	204	-7,014	1.13E-05	1.20E-02	0.31	0.21	0.36	184	0.2	0.29	-0.09	-0.85	0.11
S8_138882897	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Lutein	GBS	8	138,882,897	138,882,594	138,889,812	303	-6,915	8.91E-06	2.25E-02	0.43	0.12	0.44	194	0.12	0.22	-0.85	0.8	-0.9
S8_138882897	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total α -Xanthophylls	GBS	8	138,882,897	138,882,594	138,889,812	303	-6,915	4.25E-06	1.07E-02	0.43	0.12	0.44	194	0.11	0.21	-0.56	0.7	-0.69
S8_138882897	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Xanthophylls/ α -Xanthophylls	GBS	8	138,882,897	138,882,594	138,889,812	303	-6,915	4.03E-07	4.33E-04	0.43	0.12	0.44	190	0.18	0.3	-0.08	-0.4	0.24
S8_138882897	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Carotenoids/ α -Carotenoids	GBS	8	138,882,897	138,882,594	138,889,812	303	-6,915	1.28E-07	3.18E-04	0.43	0.12	0.44	184	0.2	0.34	-0.1	-0.85	0.13
S8_138883026	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Xanthophylls/ α -Xanthophylls	GBS	8	138,883,026	138,882,594	138,889,812	432	-6,786	6.58E-05	2.25E-02	0.39	0.18	0.48	190	0.18	0.25	0.07	-0.4	-0.15
S8_138883026	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Carotenoids/ α -Carotenoids	GBS	8	138,883,026	138,882,594	138,889,812	432	-6,786	2.77E-05	1.61E-02	0.4	0.18	0.48	184	0.2	0.28	0.08	-0.85	-0.09
S8_138883056	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Xanthophylls/ α -Xanthophylls	GBS	8	138,883,056	138,882,594	138,889,812	462	-6,756	6.58E-05	2.25E-02	0.39	0.18	0.48	190	0.18	0.25	-0.07	-0.4	0.18
S8_138883056	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Carotenoids/ α -Carotenoids	GBS	8	138,883,056	138,882,594	138,889,812	462	-6,756	2.77E-05	1.61E-02	0.4	0.18	0.48	184	0.2	0.28	-0.08	-0.85	0.1
<i>lcyE</i> SNP216	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Lutein	Additional Markers	8	138,883,206	138,882,594	138,889,812	612	-6,606	1.08E-09	8.17E-06	NA	NA	NA	200	0.12	0.49	NA	0.8	NA
<i>lcyE</i> SNP216	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Zeaxanthin	Additional Markers	8	138,883,206	138,882,594	138,889,812	612	-6,606	1.02E-06	1.28E-03	NA	NA	NA	196	0.17	0.34	NA	0.35	NA
<i>lcyE</i> SNP216	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total β -Xanthophylls	Additional Markers	8	138,883,206	138,882,594	138,889,812	612	-6,606	8.01E-07	1.76E-03	NA	NA	NA	195	0.18	0.39	NA	0.4	NA
<i>lcyE</i> SNP216	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total α -Xanthophylls	Additional Markers	8	138,883,206	138,882,594	138,889,812	612	-6,606	1.39E-10	1.05E-06	NA	NA	NA	200	0.09	0.42	NA	0.7	NA
<i>lcyE</i> SNP216	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Xanthophylls/ α -Xanthophylls	Additional Markers	8	138,883,206	138,882,594	138,889,812	612	-6,606	1.86E-14	1.40E-10	NA	NA	NA	196	0.04	0.25	NA	-0.4	NA
PZB00665.1	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Xanthophylls/ α -Xanthophylls	4K	8	138,886,137	138,882,594	138,889,812	3,543	-3,675	2.40E-05	1.01E-02	0.34	0.05	0.38	190	0.18	0.26	0.07	-0.4	-0.16
PZB00665.1	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Carotenoids/ α -Carotenoids	4K	8	138,886,137	138,882,594	138,889,812	3,543	-3,675	5.06E-05	2.11E-02	0.35	0.05	0.38	184	0.2	0.28	0.08	-0.85	-0.09
S8_138888278	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Lutein	GBS	8	138,888,278	138,882,594	138,889,812	5,684	-1,534	3.70E-06	1.40E-02	0.47	0.19	0.42	194	0.12	0.23	-0.91	0.8	-0.95
S8_138888278	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	Total α -Xanthophylls	GBS	8	138,888,278	138,882,594	138,889,812	5,684	-1,534	3.32E-06	1.07E-02	0.47	0.19	0.42	194	0.11	0.21	-0.59	0.7	-0.72
S8_138888278	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Xanthophylls/ α -Xanthophylls	GBS	8	138,888,278	138,882,594	138,889,812	5,684	-1,534	1.06E-07	1.33E-04	0.47	0.19	0.42	190	0.18	0.31	-0.09	-0.4	0.27
S8_138888278	Carotenoid Synthesis and Degradation	GRMZM2G012966	lycopene epsilon-cyclase	β -Carotenoids/ α -Carotenoids	GBS	8	138,888,278	138,882,594	138,889,812	5,684	-1,534	3.97E-07	7.38E-04	0.47	0.19	0.42	184	0.2	0.32	-0.1	-0.85	0.13

Statistically significant results from a pathway-level analysis of 58 *a priori* candidate genes from the carotenoid biosynthesis, carotenoid degradation, and isoprenoid biosynthetic pathways on 24 grain carotenoid traits the peak marker tagging the GWAS signal from *crtRB1* included as a covariate. All markers (Column A) proximal to *a priori* candidate genes (Column B) found to be significantly associated with the indicated trait (Column E) at 5% FDR are shown.

Table S9 Results from the Pathway-Level Analysis with Covariates Tagging *lut1*, *zep1*, *lcyE*, and *crtRB1* (G)

SNP ID	<i>a priori</i> candidate gene pathway	RefGen_v2 Gene ID	Annotated Gene Function	Trait	SNP Source	Chromosome	Position	Gene ORF start	Gene ORF End	Distance from Gene ORF Start	Distance from Gene ORF Finish	P-value	FDR Adjusted P-value	Minor Allele Frequency (MAF)	MAF Tropical (8% of 201 Lines)	MAF Temperate (92% of 201 Lines)	Sample Size	R-square_LR from Model without SNP	R-square_LR from Model with SNP	Effect Size	Lambda from Box-Cox Procedure	Back-Transformed Effect Estimates
S5_216074707	Carotenoid Synthesis and Degradation	GRMZM5G837869	CYP97A3, Cytochrome P450 beta-ring hydroxylase	Zeaxanthin	GBS	5	216,074,707	215,827,224	215,831,730	247,483	242,977	4.72E-06	2.93E-02	0.13	0.17	0.17	174	0.42	0.5	-0.19	0.35	-0.45
S5_216074707	Carotenoid Synthesis and Degradation	GRMZM5G837869	CYP97A3, Cytochrome P450 beta-ring hydroxylase	Total β-Xanthophylls	GBS	5	216,074,707	215,827,224	215,831,730	247,483	242,977	9.94E-06	3.74E-02	0.13	0.17	0.17	173	0.41	0.49	-0.25	0.4	-0.51
S7_13843351	Prenyl Group Synthesis	GRMZM2G493395	1-deoxy-D-xylulose 5-phosphate synthase	Zeaxanthin	GBS	7	13,843,351	14,077,852	14,081,075	-234,501	-237,724	7.77E-06	2.93E-02	0.17	0.1	0.16	174	0.42	0.5	-0.17	0.35	-0.41
S7_13843351	Prenyl Group Synthesis	GRMZM2G493395	1-deoxy-D-xylulose 5-phosphate synthase	β-Cryptoxanthin	GBS	7	13,843,351	14,077,852	14,081,075	-234,501	-237,724	4.86E-08	3.70E-04	0.16	0.1	0.16	177	0.24	0.39	-0.04	0.1	-0.33
S7_13843351	Prenyl Group Synthesis	GRMZM2G493395	1-deoxy-D-xylulose 5-phosphate synthase	Total β-Xanthophylls	GBS	7	13,843,351	14,077,852	14,081,075	-234,501	-237,724	2.44E-06	1.83E-02	0.17	0.1	0.16	173	0.41	0.5	-0.25	0.4	-0.51

Statistically significant results from a pathway-level analysis of 58 *a priori* candidate genes from the carotenoid biosynthesis, carotenoid degradation, and isoprenoid biosynthetic pathways on 24 grain carotenoid traits with markers tagging the signals at *lut1*, *zep1*, *lcyE*, and *crtRB1* included as covariates. All markers (Column A) proximal to *a priori* candidate genes (Column B) found to be significantly associated with the indicated trait (Column E) at 5% FDR are shown.

Table S10 Comparison of Genomic Prediction Methods for 24 Grain Carotenoid Traits using Three Marker Sets as Predictors

	Trait	Heritability	Carotenoid QTL-Targeted Prediction			Pathway-Level Prediction			Genome-Wide Prediction		
			RR-BLUP ^a	LASSO ^b	eNet ^c	RR-BLUP	LASSO	eNet	RR-BLUP	LASSO	eNet
15 priority traits	Lutein	0.94	0.582 (0.039)	0.534 (0.091)	0.516 (0.117)	0.461 (0.185)	0.514 (0.163)	0.521 (0.160)	0.509 (0.204)	0.476 (0.157)	0.471 (0.156)
	Zeinoxanthin	0.88	0.443 (0.166)	0.451 (0.099)	0.488 (0.051)	0.427 (0.136)	0.512 (0.070)	0.520 (0.075)	0.442 (0.172)	0.549 (0.085)	0.560 (0.082)
	α-Carotene	0.25	0.65 (0.338)	0.212 (0.206) ^d	0.187 (0.198)	0.676 (0.238)	0.111 (0.582) ^d	0.030 (0.025) ^d	0.769 (0.282)	0.500 (0.289)	0.374 (0.200)
	α-Carotene/Zeaxanthin	0.9	0.443 (0.155)	0.576 (0.153)	0.561 (0.13)	0.462 (0.118)	0.470 (0.199)	0.451 (0.199)	0.411 (0.110)	0.589 (0.200)	0.590 (0.193)
	Zeinoxanthin/Lutein	0.89	0.467 (0.11)	0.483 (0.121)	0.487 (0.121)	0.393 (0.233)	0.457 (0.183)	0.502 (0.187)	0.387 (0.243)	0.363 (0.266)	0.350 (0.241)
	Zeaxanthin	0.94	0.566 (0.076)	0.619 (0.078)	0.611 (0.079)	0.433 (0.223)	0.516 (0.127)	0.544 (0.134)	0.346 (0.278)	0.519 (0.132)	0.512 (0.170)
	β-Cryptoxanthin	0.95	0.49 (0.137)	0.3 (0.107)	0.316 (0.132)	0.501 (0.190)	0.441 (0.194)	0.434 (0.190)	0.464 (0.144)	0.530 (0.104)	0.478 (0.111)
	β-Carotene	0.82	0.282 (0.205)	0.168 (0.191)	0.169 (0.167)	0.336 (0.127)	0.159 (0.180)	0.166 (0.167)	0.254 (0.123)	0.152 (0.101)	0.185 (0.062) ^d
	β-Cryptoxanthin/Zeaxanthin	0.9	0.372 (0.188)	0.358 (0.134)	0.377 (0.119)	0.388 (0.184)	0.339 (0.074)	0.314 (0.155)	0.384 (0.185)	0.215 (0.193)	0.243 (0.127) ^d
	β-Carotene/β-Cryptoxanthin	0.89	0.351 (0.098)	0.312 (0.101)	0.317 (0.126)	0.399 (0.110)	0.434 (0.095)	0.444 (0.100)	0.402 (0.065)	0.455 (0.116)	0.444 (0.064)
	Total Carotenoids	0.91	0.367 (0.151)	0.228 (0.015) ^d	0.19 (0.072) ^d	0.267 (0.208)	0.336 (0.186)	0.358 (0.152)	0.191 (0.228)	0.065 (0.170) ^d	0.084 (0.160) ^d
	Acyclic and Monocyclic Carotenoids	0.57	0.342 (0.199)	0.242 (0.247)	0.224 (0.254)	0.456 (0.153)	0.328 (0.395)	0.331 (0.397)	0.407 (0.126)	0.384 (0.173)	0.389 (0.175)
	β-Xanthophylls/α-Xanthophylls	0.83	0.716 (0.073)	0.717 (0.056)	0.719 (0.042)	0.663 (0.166)	0.774 (0.101)	0.779 (0.108)	0.587 (0.237)	0.732 (0.187)	0.736 (0.195)
	Provitamin A	0.8	0.325 (0.158)	0.352 (0.182)	0.335 (0.062) ^d	0.434 (0.142)	0.293 (0.161)	0.292 (0.093)	0.390 (0.147)	0.328 (0.116) ^d	0.330 (0.115) ^d
	β-Carotenoids/α-Carotenoids	0.98	0.566 (0.076)	0.615 (0.056)	0.617 (0.058)	0.535 (0.161)	0.586 (0.176)	0.598 (0.152)	0.555 (0.192)	0.610 (0.101)	0.605 (0.107)
9 additional traits	ζ-Carotene	0.45	0.346 (0.073)	0.409 (0.2)	0.385 (0.241)	0.457 (0.247)	0.431 (0.111)	0.447 (0.123)	0.465 (0.205)	0.469 (0.124)	0.431 (0.111)
	Phytofluene	0.65	0.358 (0.273)	0.283 (0.16)	0.262 (0.161)	0.549 (0.150)	0.499 (0.185)	0.494 (0.182)	0.497 (0.149)	0.397 (0.086)	0.499 (0.185)
	Tetrahydrocyclopene	0.6	0.369 (0.122)	0.197 (0.096)	0.208 (0.102)	0.522 (0.148)	0.451 (0.154)	0.463 (0.162)	0.541 (0.151)	0.682 (0.132)	0.451 (0.154)
	Total β-Xanthophylls	0.96	0.41 (0.135)	0.446 (0.086)	0.449 (0.097)	0.396 (0.251)	0.554 (0.158)	0.559 (0.188)	0.313 (0.287)	0.428 (0.204)	0.554 (0.158)
	Total α-Xanthophylls	0.91	0.546 (0.09)	0.51 (0.102)	0.503 (0.107)	0.400 (0.210)	0.540 (0.119)	0.542 (0.120)	0.479 (0.226)	0.455 (0.159)	0.540 (0.119)
	Provitamin A/Total Carotenoids	0.86	0.335 (0.224)	0.281 (0.272)	0.275 (0.271)	0.286 (0.124)	-0.101 (0.17) ^d	-0.031 (0.15) ^d	0.321 (0.089)	-0.054 (0.119) ^d	0.065 (0.259) ^d
	β-Carotene/(β-Cryptoxanthin+Zeaxanthin)	0.93	0.308 (0.221)	0.428 (0.12)	0.411 (0.146)	0.251 (0.182)	0.361 (0.118)	0.355 (0.111)	0.290 (0.148)	0.396 (0.095)	0.361 (0.118)
	Acyclic Carotenoids/Cyclic Carotenoids	0.74	0.149 (0.106)	0.142 (0.09) ^d	0.221 (0.083) ^d	0.577 (0.155)	0.729 (0.150)	0.734 (0.156)	0.565 (0.219)	0.743 (0.198)	0.729 (0.150)
	Total Carotenoids/Total Xanthophylls	0.62	0.286 (0.367)	0.378 (0.28)	0.376 (0.273)	0.351 (0.088)	0.327 (0.098) ^d	0.310 (0.093) ^d	0.294 (0.059)	0.291 (0.121)	0.327 (0.098) ^d

The three marker sets tested were carotenoid quantitative trait loci (QTL)-targeted prediction (the 944 SNP markers and 7 indels within ± 250 kb of 8 *a priori* candidate genes), pathway-level prediction (the 7,408 SNP markers and 7 indels within ± 250 kb of 58 *a priori* candidate genes,) and genome-wide prediction (all 284,180 SNP markers and 7 indels used in our genome-wide association studies). Standardized average correlations resulting from the 5-fold cross-validation are reported, with standardized standard deviations in parentheses. Standardization was conducted as follows: Raw correlations were divided by the square root of a trait's broad-sense heritability to obtain standardized average correlations, also called prediction accuracy. Raw standard deviations were squared to obtain variance, divided by heritability, and the square root was taken to obtain standardized standard deviation.

^aRR-BLUP, Ridge Regression Best Linear Unbiased Prediction

^bLASSO, Least Absolute Shrinkage and Selection Operator

^ceNet, Elastic net

^dindicates that no markers were selected in one or two of the five folds, or in three of the five folds in one case (α-Carotene, Pathway-Level Prediction, eNet)

Table S11 Prediction Accuracies for 24 Grain Carotenoid Traits using the Carotenoid QTL-Targeted Prediction Marker Set Relative to Random Marker Sets

Trait	Mean Prediction Accuracy			Fold Difference in Prediction Accuracy	
	Carotenoid QTL-Targeted Prediction (CQTP)	Random Candidate Regions (Candidate)	Random Genomic Regions (Genomic)	CQTP: Candidate	CQTP: Genomic
Lutein	0.544	0.205	0.210	2.649	2.594
Zeinoxanthin	0.461	0.195	0.178	2.361	2.587
α-Carotene	0.350	0.176	0.174	1.985	2.006
α-Carotene/Zeaxanthin	0.527	0.221	0.196	2.388	2.687
Zeinoxanthin/Lutein	0.479	0.192	0.156	2.497	3.076
Zeaxanthin	0.599	0.132	0.133	4.527	4.492
β-Cryptoxanthin	0.369	0.189	0.195	1.954	1.889
β-Carotene	0.206	0.108	0.131	1.918	1.569
β-Cryptoxanthin/Zeaxanthin	0.369	0.155	0.166	2.375	2.221
β-Carotene/β-Cryptoxanthin	0.327	0.139	0.117	2.347	2.796
Total Carotenoids	0.262	0.111	0.080	2.362	3.255
Acyclic and Monocyclic Carotenes	0.269	0.177	0.153	1.518	1.756
β-Xanthophylls/α-Xanthophylls	0.717	0.189	0.209	3.796	3.437
Provitamin A	0.337	0.175	0.171	1.925	1.969
β-Carotenoids/α-Carotenoids	0.599	0.209	0.217	2.874	2.764
ζ-Carotene	0.380	0.124	0.107	3.071	3.558
Phytofluene	0.301	0.135	0.127	2.231	2.375
Tetrahydrocyclopene	0.258	0.156	0.157	1.656	1.648
Total β-Xanthophylls	0.435	0.092	0.109	4.720	3.981
Total α-Xanthophylls	0.520	0.156	0.191	3.324	2.725
Provitamin A/Total Carotenoids	0.297	0.149	0.136	1.993	2.190
β-Carotene/(β-Cryptoxanthin+Zeaxanthin)	0.382	0.148	0.120	2.575	3.194
Acyclic Carotenes/Cyclic Carotenes	0.171	0.046	0.066	3.716	2.586
Total Carotenes/Total Xanthophylls	0.347	0.081	0.069	4.256	4.997

The three marker sets tested were the carotenoid quantitative trait loci (QTL)-targeted prediction set (the 944 SNP markers and 7 indels within ± 250 kb of 8 *a priori* candidate genes), random candidate gene-targeted regions (markers within ± 250 kb of 8 other candidate genes in the pathway-level prediction set), and 8 random 500 kb genomic regions (selected from the markers used in our genome-wide association studies that did not overlap with the carotenoid QTL-targeted prediction set). Average raw correlations resulting from the 5-fold cross-validation were divided by the square root of a trait's broad-sense heritability to obtain prediction accuracies, which are reported as an average across the three genomic prediction methods tested: ridge-regression best linear unbiased prediction (RR-BLUP), least absolute shrinkage and selection operator (LASSO), and elastic net (eNET). For the random candidate region and random genomic region analyses, marker selection was conducted over 100 iterations with replacement, and prediction accuracies were averaged across these iterations. R scripts for these analyses are available upon request.