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# Natural Genetic Variation in *Lycopene Epsilon Cyclase* Tapped for Maize Biofortification

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Dietary vitamin A deficiency causes eye disease in 40 million children each year and places 140 to 250 million at risk for health disorders. Many children in sub-Saharan Africa subsist on maize-based diets. Maize displays considerable natural variation for carotenoid composition, including vitamin A precursors α-carotene, β-carotene, and β-cryptoxanthin. Through association analysis, linkage mapping, expression analysis, and mutagenesis, we show that variation at the *lycopene epsilon cyclase* (*lcyE*) locus alters flux down α-carotene versus β-carotene branches of the carotenoid pathway. Four natural *lcyE* polymorphisms explained 58% of the variation in these two branches and a threefold difference in provitamin A compounds. Selection of favorable *lcyE* alleles with inexpensive molecular markers will now enable developing-country breeders to more effectively produce maize grain with higher provitamin A levels.

Maize is the dominant subsistence crop in much of sub-Saharan Africa and the Americas, where between 17 and 30% of children under age of 5 are vitamin A–deficient. This results in xerophthalmia (progressive blindness), increased infant morbidity and mortality, and depressed immunological responses (*1*). Vitamin A deficiency starts with inadequate provitamin A or vitamin A content or bioavailability in foods and is exacerbated by disease-induced malabsorption.

Diet diversification, food fortification, and supplementation (*2*–*4*) have all been used to combat dietary micronutrient deficiencies. Ideally, all children would have access to a varied diet rich in fruits and vegetables, but diet diversification is often limited by crop seasonality, expense, and low bioavailability of green leafy plant carotenoids (*5*, *6*). Poor infrastructure in developing countries has limited widespread use of direct vitamin supplementation. Perhaps the most feasible approach to eradicating death and disease caused by dietary deficiencies is biofortification, a process by which staple crops are purposefully bred for higher nutritional density (*7*, *8*). Although biofortified foods can potentially be an inexpensive, locally adaptable, and long-term solution to diet deficiencies, cultural preferences may limit their acceptance. This may be particularly true for those crops where transgenics are the only alternative to boost provitamin A content, given limited acceptance of genetically modified organisms in developing countries.

Carotenoids are derived from the isoprenoid biosynthetic pathway and are precursors of the plant hormone abscisic acid and of other apocarotenoids (*9*). The first committed step of this pathway [as recently revised (*10*)] is formation of phytoene from geranylgeranyl diphosphate by phytoene synthase (*y1*/*psy1*) (Fig. 1) (*11*). Recent studies in maize suggest that the *psy1* locus has been the target of a selective sweep following selection for endosperm-accumulating carotenoids and shift from white to yellow kernels (*12*). The first branch point of this pathway (Fig. 1) occurs at cyclization of lycopene where action of lycopene beta cyclase (LCYB) at both ends of linear lycopene produces a molecule with two β rings. Alternatively, the coaction of LCYB and lycopene epsilon cyclase (LCYE) generates a β,ε-carotene that is a precursor to lutein (*13*). Relative activities of LCYB and LCYE are hypothesized to regulate the proportion of carotenes directed to each branch of this pathway (*13*–*15*). Indeed, transgenic manipulations of LCYE expression in *Arabidopsis*, potato, and *Brassica* increase the pool of β ring–containing carotenes and xanthophylls (*13*, *16*–*18*).

Maize exhibits considerable natural variation for kernel carotenoids, with some lines accumulating as much as 66 μg/g. The pre-

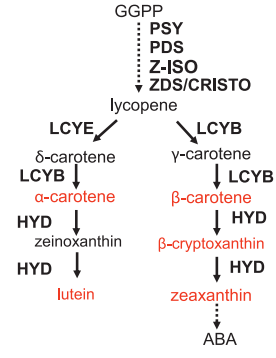


**Fig. 1.** Simplified carotenoid biosynthetic pathway in plants (*29*). Enzymatic reactions are represented by arrows, dashed lines represent multiple enzymatic steps. Substrates in red were evaluated in this study. Compounds: GGPP, geranylgeranyl diphosphate; ABA, abscisic acid. Enzymes: PSY, phytoene synthase; PDS, phytoene desaturase; Z-ISO, 15-*cis* zetacarotene isomerase; ZDS, zetacarotene desaturase; CRTISO, carotene isomerase; HYD, carotene hydroxylase enzymes, which include ε- and β-ring hydroxylases.

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dominant carotenoids in maize kernels, in decreasing order of concentration, are lutein, zeaxanthin, β-carotene, β-cryptoxanthin, and α-carotene. β-Carotene contains two provitamin A structures (two nonhydroxylated β-ionone rings) and β-cryptoxanthin and α-carotene one each (single nonhydroxylated β-ionone ring). Among lines included in our diverse maize panel, β-carotene levels reached 13.6 μg/g. However, most yellow maize grown and consumed throughout the world has only 0.5 to 1.5 μg/g β-carotene. Comparisons between β-carotene and total carotenoids with grain color (scaled according to shade of yellow) revealed poor correlations with low $R^2$ values (Fig. 2), which indicated that marker-assisted selection (MAS) may prove much more efficient than selection based on color alone.

To dissect the phenotypic diversity, we used an association-mapping approach that exploits the genetic diversity of maize to provide resolution within 2000 base pairs (bp) (*19*–*21*). In the context of plant breeding, this has the added advantage of identifying the most favorable allele within a diverse genetic background, which provides the necessary genotypic information to facilitate the design of efficient maize introgression and selection schemes throughout the world. We complemented the association mapping with linkage mapping to evaluate the effects in a genetically less complex background and with a mutagenesis program to isolate novel allelic variation within an elite near-isogenic background.

To evaluate functional diversity (Fig. 1), eight candidate genes representing select members of gene families encoding biosynthetic enzymes of the carotenoid pathway were sampled across a diverse panel of 288 maize lines, of which 204 were yellow. Subsets of yellow lines were grown in four different years and surveyed for whole-kernel carotenoids by high-performance liquid chromatography (HPLC). The yellow lines averaged 23 μg/g for total carotenoids (range 5.5 to 66.0 μg/g) and 1.7 μg/g for β-carotene (range 0.06 to 13.6 μg/g).

For association analysis, we used a mixed-model approach that controlled for complex population and pedigree relationships (*22*). Among our current sampling of candidate genes, *lycopene epsilon cyclase* (*lcyE*) (*14*) had the largest effect on partitioning the two branches of carotenoids and, consequently, on β-carotene and β-cryptoxanthin content. In maize, the single-copy *lcyE* gene consists of 10 exons spanning 3640 bp (Fig. 3). After initial association and screening for polymorphisms in key haplotypes, four regions were selected and scored across the entire panel. On the basis of the position of LCYE in the biochemical pathway, we predicted that the ratio of the sum of kernel carotenoids from each pathway branch would form the strongest association. Indeed, this was confirmed (Table 1), with the strength of the association confirming that *lcyE* plays a key role in controlling this ratio. Correspondingly, levels of predominant provitamin A compounds β-carotene and β-cryptoxanthin were also highly associated with *lcyE*.

Subsequent haplotype analysis revealed several probable causative polymorphisms for the ratio of α- and β-carotene branches for the 2003 field season (table S1). A large promoter indel and an amino acid substitution in exon 1 explain most of the variation ($R^2$ = 36%; $n$ = 135; $P$ = $1.27 \times 10^{-12}$) with a 5.2-fold effect. A second indel in the 3′ UTR also has a significant 3.3-fold effect and contributes to variation not explained by the promoter polymorphism (type III SS; $P = 1.9 \times 10^{-4}$). The fourth significant polymorphism at position 2238 in intron 4 was associated with a 2.5-fold effect (type III SS; $P$ = 0.0003). The overall, four-term model explains 58% of the variation ($P = 9.2 \times 10^{-17}$). These significant polymorphisms exhibit some linkage disequilibrium (LD), and only nine haplotypic classes exist in our sample, which limits full differentiation of the effects of each polymorphism. Overall, there is a ninefold difference between two of the more differentiated haplotype classes, and sixfold between two more common haplotypes (table S2). There was a threefold increase in the proportion of β-carotene and β-cryptoxanthin between the common haplotypes. Verification of these results was provided by

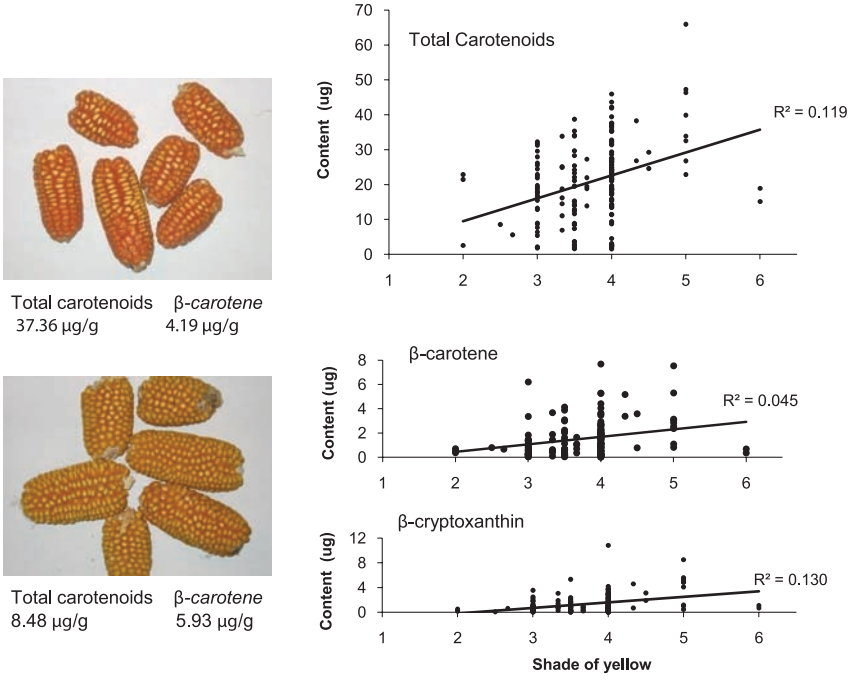


**Fig. 2.** Grain color and carotenoid content. The graphs depict the low correlation between visual grain color and total carotenoids, β-carotene, and β-cryptoxanthin in diverse inbreds. In these kernels, the shade of yellow ranges from white (score of 1) to dark orange (score of 6). White kernels were excluded from the analysis. The difficulty in visual selection for β-carotene content is further exemplified by the images on the left, where the yellow maize below has higher β-carotene than the orange variety above. These correlations are across the diverse panel of 228 maize inbreds; correlations for grain color and total carotenoids are higher when scored across segregating populations and narrow ranges of germ plasm, but correlations for β-carotene and β-cryptoxanthin remain low.

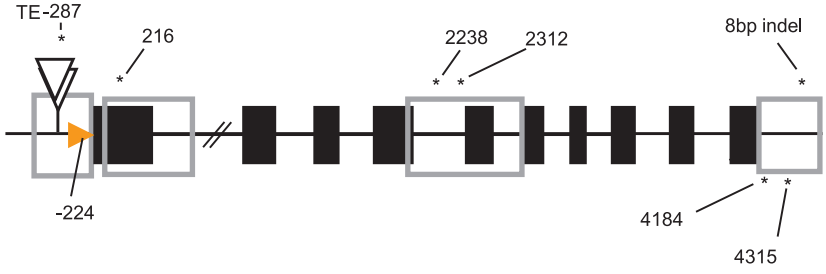


**Fig. 3.** Schematic diagram of the maize *lcyE*. Putative transcription start sites are depicted with orange arrow, translated exons as black squares, and the sampled regions as gray boxes. Polymorphisms that significantly associated with changes in flux between the lutein and zeaxanthin branches of the pathway are labeled with asterisks. The 5′ transposable element insertion(s) are represented by the white triangles. Positions relative to the sequence alignment are indicated numerically above the polymorphisms.

significant associations in subsequent field seasons (Table 1).

Expression analysis indicated that *lcyE* is preferentially expressed in the endosperm relative to the embryo (fig. S1). Expression profiling of kernels at 15 and 20 days after pollination (DAP) indicated expression levels correlated well with the ratio of carotenoids from each pathway branch, explaining 70 to 76% of the variance. Lines with transposon insertions near the start site had much lower expression levels [in 15 DAP and 20 DAP lower by a factor of 3.7 and 13, respectively (fig. S2)]. The 3′ indel may also have expression effects, but our statistical tests lacked the power to confirm this hypothesis. A quantitative trait locus (QTL) experiment that examined segregation of B73-Mo17 alleles in leaves found significant variation in the cis-regulation of *lcyE* expression, along with several other regions that also contribute to expression level control of *lcyE* (fig. S3).

In a previous study, three major QTL were identified for accumulation of carotenoids in maize (*23*). Two of these QTL colocalized with *y1* and *zeta carotene desaturase* (*zds*); the third QTL mapped to a region without a candidate gene. We mapped *lcyE* to chromosome 8 bin 5, near marker bnlg1599, and it colocalized with this previously undetermined QTL. This QTL showed significant effects for modification of the ratio of α to β branch carotenoids [logarithm of the odds ratio for linkage (or lod) score of 34.05; $R^2$ 54.4%] and explained 31.7% of the variation for lutein (lod 16.5). The magnitude of effects was not as large as in association or mutagenesis analysis. However, this biparental QTL population only segregated for the amino substitution (at codon 216) and a modest promoter polymorphism and does not segregate for the 3′ polymorphism. Notably, this QTL was not significant for total carotenoids, which further supports the conclusion that variation within *lcyE* gene underlies this QTL for carotenoid composition.

To confirm association and QTL results, mutagenesis induced by ethane methyl sulfonate (EMS) was conducted to isolate additional alleles of *lcyE*. Two $M_2$ ears of inbred Qx47 segregated for a distinct change in endosperm color from yellow to orange, with orange recessive to yellow (these color changes were apparent in the inbred isogenic background, but not in diverse breeding materials). HPLC analysis of orange and yellow kernels confirmed a shift in the zeaxanthin:lutein ratio in the direction of zeaxanthin. This orange endosperm mutation was backcrossed into the standard genetic inbred line B73, and *lcyE* was tested as a candidate gene, which revealed that the Qx47 *lcyE* haplotype cosegregates with orange endosperm and ratio of α-carotene versus β-carotene branch carotenoids (fig. S4).

The most favorable haplotype for higher β-carotene branch carotenoids included both the large promoter insertion and 3′ 8-bp insertion. In the diverse panel we tested, this haplotype occurs in 5% of temperate inbreds and 16% of tropical inbreds. MAS at this locus should be effective for several reasons: (i) The most favorable haplotype is found with at least modest frequency in different germ plasm sources and thus breeders can select donors from their relatively more adapted sources. (ii) The favorable haplotype has a large effect. (iii) Visual selection is ineffective for differentiating carotenoid composition and selecting provitamin A compounds. (iv) In comparison with HPLC analysis of carotenoids, polymerase chain reaction (PCR) scoring of the *lcyE* locus is much less expensive (costing perhaps 1/1000th that of HPLC) and more accessible to developing countries with greatest need for provitamin A.

An approach that empowers local breeder involvement through inexpensive visual selection for darker yellow to orange kernels to enhance flux into carotenoid pathway, and also incorporates MAS for *lcyE*, should result in increased levels of provitamin A compounds. To expedite creation of improved germ plasm globally, we provide information on PCR-based markers (fig. S5). Donor inbreds and improved breeding lines derived at the International Maize and Wheat Improvement Center (CIMMYT) from synthetics of diverse panel inbreds with higher β-carotene are available by contacting T. R.R. This will facilitate selection worldwide of the most favorable *lcyE* alleles, which we have begun in our program. We are screening tropical breeding germ plasm collections in collaboration with CIMMYT.

To date, MAS for natural variation has been limited by resolution and scope (germ plasm diversity). Alleles have generally been characterized in the limited genetic background and resolution of biparental QTL studies, leaving in question their relevance to broader germ plasm (*24*), particularly for germ plasm outside of the temperate United States. As a result, the primary use for MAS is backcross breeding of transgenic traits. In contrast, the association mapping approach used here allows for rapid generation of selectable markers based on performance of diverse germ plasm. This provides markers more relevant in a broad genetic background, and that enables breeders to search for favorable alleles in their locally adapted germ plasm sources.

In ongoing studies, we are attempting to identify alleles for other genes in the pathway that increase total carotenoids and that slow the conversion of β-carotene to β-cryptoxanthin and zeaxanthin, to exploit more fully the natural genetic variation potential in provitamin A biofortification of maize. These results will then be further incorporated in breeding efforts to create a healthier maize crop for the world's poorest people.

Although the genetic results and strategy presented here are encouraging, they need to be placed in context as part of an overall biofortification effort encompassing breeding infrastructure, seed distribution, societal acceptance,



**Table 1.** *lcyE* associations across seasons. Association results for significant polymorphisms identified in the four regions sampled along the *lcyE* gene. Each polymorphism is labeled numerically by its position on the alignment relative to the exon 1 start codon. Followed by the favorable allele (bold)/unfavorable allele at the site. An initial scan for association using both β-carotene and the ratio of the two pathway branches was conducted using the mixed model incorporating population structure and kinship. Subsequently a simpler general linear model (GLM) was used to evaluate data sets from additional years, including population structure (Q), given the oligogenic behavior of the trait the change in flux estimates for 2003 do not include Q. Avg., average; n.c., nonconvergence; n.s., not significant.

| | | *lcyE* association (*P*), mixed model of | | *lcyE* association as a ratio across environments (GLM) (*P*) | | | | Fold change in flux |
|---|---|---|---|---|---|---|---|---|
| | | β-Carotene/all | Ratio of branches | | | | | |
| Environment (year) | | (2003) | (2003) | (2002) | (2003) | (2004) | (2005) | (2003) |
| Avg. observation no. | | (157) | (154) | (44) | (156) | (154) | (156) | |
| Polymorphic site | | | | | | | | |
| 5′ TE | **1+4**/2/3 | $5.42 \times 10^{-4}$ | $3.96 \times 10^{-11}$ | 0.024 | $8.05 \times 10^{-11}$ | 0.008 | $8.61 \times 10^{-9}$ | 6.5 |
| 216 | **G** / T | n.c. | $1.35 \times 10^{-10}$ | 0.059 | $1.24 \times 10^{-10}$ | 0.003 | $2.93 \times 10^{-10}$ | 2.8 |
| 2238 | **G** / T | $1.22 \times 10^{-4}$ | $1.69 \times 10^{-9}$ | 0.008 | $2.12 \times 10^{-10}$ | 0.023 | $1.08 \times 10^{-9}$ | 2.7 |
| 2312 | **A** / T | $1.70 \times 10^{-3}$ | n.c. | n.s. | $6.84 \times 10^{-4}$ | 0.026 | 0.005 | 2.9 |
| 4184 | **G** / A | $3.06 \times 10^{-4}$ | n.c. | $8.87 \times 10^{-4}$ | $2.23 \times 10^{-10}$ | 0.019 | $1.13 \times 10^{-8}$ | 2.6 |
| 4315 | **C** / G | $1.84 \times 10^{-4}$ | $7.01 \times 10^{-10}$ | 0.012 | $3.07 \times 10^{-9}$ | $5.75 \times 10^{-4}$ | $6.79 \times 10^{-7}$ | 2.6 |
| 3′Indel | **8**/0 | $4.80 \times 10^{-3}$ | $2.75 \times 10^{-9}$ | n.s. | $1.46 \times 10^{-8}$ | $8.97 \times 10^{-4}$ | $4.13 \times 10^{-6}$ | 3.5 |

dietary habits, and nutritional impact. Information now available on some of these issues is encouraging. Results from an animal model for human vitamin A metabolism indicated vitamin A activity of provitamin A in orange maize was greater than assumed by a factor of about four (*25*). A successful intervention to introduce β-carotene–rich, orange sweet potato in Mozambique, where only white sweet potato was previously cultivated, suggests that orange-colored staple foods can be acceptable, and their regular consumption results in improved vitamin A status (*26*). Related follow-up acceptance studies of yellow and orange maize in Mozambique and Zimbabwe are in progress with initial results encouraging (*27*). The dietary habits of many Africans, in which maize is consumed for all three meals a day, indicates that maize is a good target for biofortification (*28*). The recent positive nutritional and acceptance results will need to be coordinated with comprehensive breeding and seed distribution efforts to realize the potential of provitamin A–biofortified maize, as, for example, is coordinated by the HarvestPlus Global Challenge Program.

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### Supporting Online Material

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References

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# Dual Positive and Negative Regulation of Wingless Signaling by Adenomatous Polyposis Coli

**Carter M. Takacs, Jason R. Baird, Edward G. Hughes, Sierra S. Kent, Hassina Benchabane, Raehum Paik, Yashi Ahmed***

**The evolutionarily conserved Wnt/Wingless signal transduction pathway directs cell proliferation, cell fate, and cell death during development in metazoans and is inappropriately activated in several types of cancer. The majority of colorectal carcinomas contain truncating mutations in the adenomatous polyposis coli (APC) tumor suppressor, a negative regulator of Wnt/Wingless signaling. Here, we demonstrate that *Drosophila* Apc homologs also have an activating role in both physiological and ectopic Wingless signaling. The Apc amino terminus is important for its activating function, whereas the β-catenin binding sites are dispensable. Apc likely promotes Wingless transduction through down-regulation of Axin, a negative regulator of Wingless signaling. Given the evolutionary conservation of APC in Wnt signal transduction, an activating role may also be present in vertebrates with relevance to development and cancer.**

The Wnt/Wingless (Wg) secreted proteins activate a signal transduction cascade that directs growth and differentiation in many tissues during animal development [reviewed in (*1*)]. Activation of target genes in response to the Wnt/Wg signal is dependent on the transcriptional activator β-catenin/Armadillo (Arm). In the absence of Wnt, four factors—APC, Axin, glycogen synthase kinase-3/Zeste white 3, and casein kinase 1—target β-catenin for phosphorylation and subsequent proteasomal degradation (*2*–*8*). Axin acts as a scaffold to facilitate β-catenin phosphorylation by binding β-catenin, APC, and the two kinases. Wnt-dependent down-regulation of Axin is important for β-catenin–mediated transcriptional activation (*9*–*11*). Mutational inactivation of negative regulatory components in the pathway and the resultant inappropriate activation of Wnt signaling is associated with the development of several types of cancer. The majority of colorectal adenomas and carcinomas contain mutations that eliminate the carboxy-terminal half of APC (*1*).

The Wnt/Wg signaling pathway shows considerable conservation among metazoans. Two APC homologs exist in humans, mice, and fruit flies, and the negative regulatory role of APC in Wnt signaling is conserved from flies to mammals (*12*–*15*). *Drosophila* Apc1 and Apc2 are ubiquitously expressed, and in most cells act redundantly to negatively regulate Wg signaling (*16*, *17*). However, in retinal photoreceptors, Apc2 activity is low enough that inactivation of Apc1 singly suffices to constitutively activate Wg signaling (*13*, *16*). In response, all photoreceptors undergo apoptosis (*13*) (Fig. 1, A and B) and before their deaths some photoreceptors adopt an aberrant cell fate, as indicated by ectopic expression of *homothorax* and *Rhodopsin 3* (*18*, *19*) (fig. S1, A to F).

To identify genes that promote Wg signaling, we performed a genetic screen for suppressors of photoreceptor apoptosis in the $Apc1^{Q8}$ null mutant (Methods). We found that apoptosis is suppressed by null and hypomorphic *Apc2* alleles (Fig. 1C; fig. S2, A to C; fig. S3, A to H; and table S1). Ectopic expression of *homothorax* and *Rhodopsin 3* is also suppressed, indicating that suppression of Wg signaling is not restricted to apoptosis (fig. S1, G to L, and fig. S4, A to F). Further, ectopic Wg signaling resulting from Arm overexpression is also partially suppressed by reduction of Apc2 (fig. S5, A to C). These data indicate that in addition to its well-established negative regulatory role, Apc2 also has an activating role in ectopic Wg signaling.

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# Supporting Online Material for

## Natural Genetic Variation in *Lycopene Epsilon Cyclase* Tapped for Maize Biofortification

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**This PDF file includes**

# Supporting Online Material

## MATERIALS AND METHODS

**Germplasm Evaluation:** A diverse association panel of 282 lines(S*1*) was phenotyped using HPLC analysis for carotenoid content and genotyped using SSR and SNP markers. The inbreds were grown in one-row plots in a randomized complete block design in Champaign-Urbana, Illinois during the summers of 2002, 2003, 2004, and 2005. HPLC analysis was performed on balanced bulks of a few to several self-pollinated ears of seed from each plot.

**Carotenoid Quantification**: Quantification of carotenoids was accomplished by standard regression with external standards (S*2, 3*). Evaluations were done on dry kernels, and values are quantified relative dry weight. The HPLC system consisted of a Waters Alliance 2690 separation module (system includes solvent delivery system, inline degasser, column heater, and sample cooler) attached to a Waters 996 Photodiode Array detector (PDA) and this system was managed by Waters Millennium 2001 (v 3.2). The column used for quantification was a reverse phase YMC carotenoid C30 column (5 μm, 4.6 x 100 mm) connected to a YMC C30 filter insert that acted as a guard column. The mobile phase was Acetonitrile, methanol, methylene chloride (75:20:5 v/v/v) with triethylamine (.05% of total volume) and BHT, mixed by the Alliance 2690 solvent delivery system to help reduce composition differences between runs. Samples were loaded into amber glass vials with 50 μl limited volume inserts (Alltech and Associates, Deerfield, IL). All samples were run immediately after extraction. Samples were stored at 4ºC if they were unable to be loaded into the HPLC immediately. Flow rate was 1.8 ml min.-1, with each run taking 20 – 23 minutes. To control fluctuations in retention time due to temperature differences, column temperature was set at 30ºC. Sample temperature was set at 4ºC to control degradation of samples by heat. Carotenoids were detected at 450 nm, and were quantified by external standards as described previously (S*2, 3*).

The ratio of the two branches was evaluated as natural logarithm of (α-carotene + lutein) / (β-carotene + β-cryptoxanthin + zeaxanthin). Evaluations of individual compounds generally compared the proportion of individual compound relative to the total carotenoid levels.

**Mutagenesis**: EMS pollen mutagenesis was conducted following the protocol of Neuffer(S*4*) on the DuPont inbred line Qx47. Carotenoid analysis of maize kernels was conducted by Craft Technologies, Inc (Wilson, NC, USA). Two self-pollinated $M_2$ ears derived from the EMS pollen mutagenesis of the inbred line Qx47 segregated for a distinct change in endosperm color from yellow to orange. These putative mutants were designated #26 and #28. $M_2$ kernels of each type were grown and self-pollinated. All of the orange $M_2$ kernels gave uniformly orange $M_3$ ears whereas yellow $M_2$ kernels produced both uniformly yellow and yellow/orange segregating ears, indicating dominance of yellow over orange. The #28 putative mutant was backcrossed 4 times into the public inbred B73 and selfed. Orange kernels were crossed to yellow kernels from the last backcross, and a 1:1 yellow:orange segregation was observed on 50% of the ears, again consistent with a single, Mendelian recessive gene. A SNP identified in *lcyE* between B73 and Qx47, which confers a *HinfI* restriction site in Qx47 and not in B73, allowed a co-segregation analysis on the backcross population described above. Following 4 backcrosses to B73, all of the orange kernels (n=47) were homozygous for the Qx47 (mutant #28) *lcyE* haplotype, and all of the yellow kernels (n=48) were heterozygous.

**Genotyping**: Candidate genes were selected based on previous characterization in maize or by homology with tomato, Arabidopsis, or rice genes (S*5, 6*). Given the ancient duplication in the

maize genome, active site homology was used to select homologs. Robust PCR primers for the diverse germplasm were designed based on MAGI (Maize Assembled Genomic Islands)(S*7*) or the TIGR Maize Database(S*8*) and refined by prescreening on a diverse panel of 32 lines (Genbank numbers: BV709178-BV709204, BV709242-BV709256, BV709298-BV709330, BV709398-BV709439, BV709481-BV709506, BV727024-BV727770). The selected amplicons with an average span of 500bp were then sequenced across the full panel of maize lines. One to two regions were sampled from each candidate gene. Given the rapid LD decay in maize we cannot rule out genes that showed no evidence of association (ie. we may have sampled the wrong region or paralogue). Once the *lcyE* association was identified the gene was sequenced from 12 diverse haplotypes. Three more regions were sampled across entire panel based on putative functional SNPs suggested by sequencing. Promoter haplotypes with multiple TE were scored by amplicon length, using PCR markers provided for maker assisted selection of these alleles.

**Transcript profiling:** Maize inbreds used for LCYE transcript profiles were grown at the Lehman College field station, Bronx, New York, and developmentally staged endosperms dissected and stored at -80ºC until use. *RNA Isolation and Reverse transcription:* Total RNA was isolated using the RNeasy Plant Mini Kit (Qiagen Sciences, Maryland), and DNase I-treated (Invitrogen, Carlsbad, CA) prior to first strand cDNA synthesis using oligo (dT) as a primer and SuperscriptTM III RT (Invitrogen, Carlsbad, CA). 1ul of 50µM oligo $(dT)_{20}$, and 1ul of 10mM dNTP mix were mixed with 8µl of DNase I treated total RNA (~ 1 µg) and incubated at 65ºC for 5min, and left on ice for at least 1 min. 10µl of cDNA synthesis mix (2µl of 10X RT buffer, 4µl of 25mM MgCl2, 2µl of 0.1M DTT, 1µl of RNaseOUT™ (40 U/µl), 1ul of Superscript™ III RT (200 U/µl) was added and incubated for 50min at 50ºC and reactions terminated at 85ºC for 5min. Samples were collected by brief centrifugation and 1µl of RNase H added and incubated for 20min at 37ºC. *Quantitative RT-PCR:* cDNA samples were amplified on the MyIQ Single-Color Real-Time PCR detection system (Bio-Rad, Hercules, CA), using iQTM SYBR Green Supermix (Bio-Rad, Hercules, CA). 2µl (5ng/µl) of cDNA; 15µl of 2X iQTM SYBR Green Supermix; 11µl of Nuclease-Free water; 1µl (20ρm/µl) of each primer were used in a 30µl reaction volume. Thermal cycling conditions included an initial incubation at 94°C for 10s, followed by 35 cycles of 95°C for 10s, 58°C for 35s, and 72°C 10s. Melt curve analysis was performed verify primer specificity, and PCR products were confirmed by sequencing. The relative quantity of the transcripts was calculated by using the comparative threshold cycle (CT) method. Actin mRNA was amplified simultaneously for normalization between samples. Primers were designed to flank introns (LcyE: TTTACGTGCAAATGCAGTCAA, TGACTCTGAAGCTAGAGAAAG; Actin: CGATTGAGCATGGCATTGTCA, CCCACTAGCGTACAACGAA).

*eQTL in maize leaf tissue:* Experimental design, plant material, and array methods are described in detail in the Maize Oligonucleotide Array project database, which is publicly available from www.maizearray.org; this work is Study ID 21, Investigator Stapleton. Array element median spot value from the maizearray spreadsheet page was log-transformed and averaged within treatment group (UV and control). The mean values were merged with marker data for the recombinant inbred lines; the marker data was retrieved from MaizeGDB (www.maizegdb.org); with Iowa combined markers and Genoplante markers merged into the list for a total of 4751 markers. SAS Proc GLM was used to test for significant marker association with expression level for genes TM00018798 and TM00050085 and for treatment interaction for each gene.

**Statistical Analysis**: Association analysis was conducted using a mixed model incorporating kinship and population structure(S9) as implemented in TASSEL (www.maizegenetics.net)(S*10*) and SAS/STAT (Version 9.1, SAS Institute Inc., Cary, NC, USA). This approach simultaneously accounts for the multiple levels of relatedness based on random genetic markers that are used to establish population structure and a kinship matrix. The method has very good control of Type I error rates when a trait is polygenic. When examining the ratio of the two branches, we used a standard GLM model as this trait is either a mono- or oligo-genic trait.

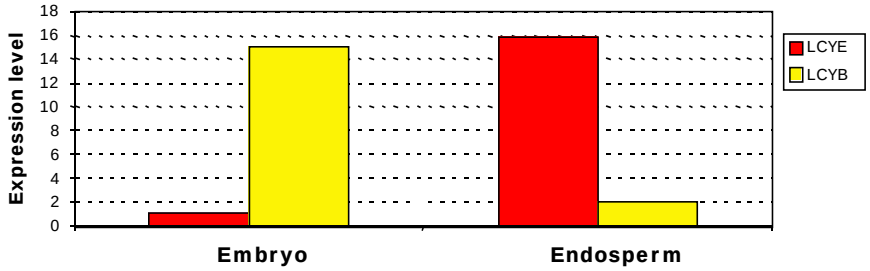
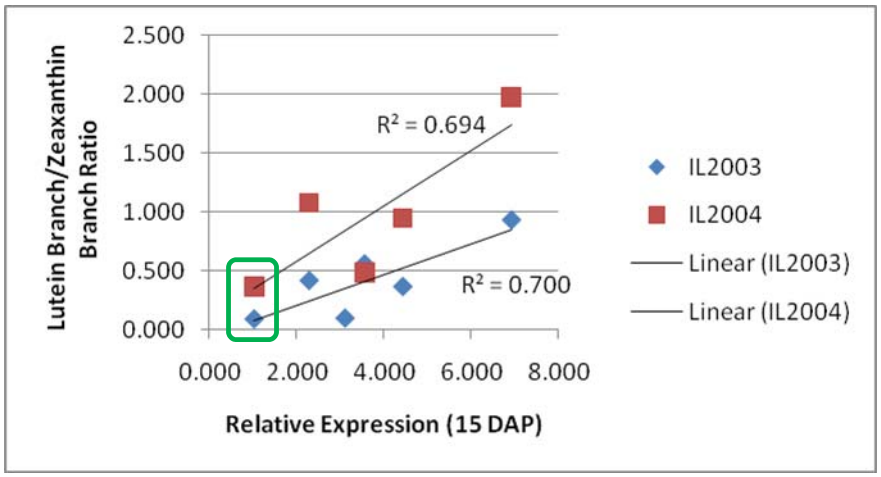
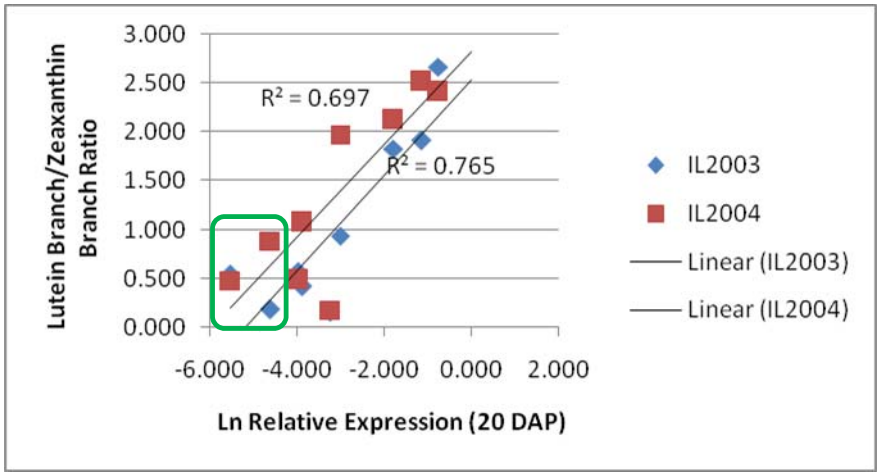



Figure S1. Relative gene expression of LCYB and LCYE in W22 maize seeds by quantitative RT-PCR.

mRNA transcript levels




- Figure S2. LCYE Quantitative RT-PCR for endosperm at 15 days after pollination (top) and 20 days after pollination (bottom). Samples surrounded by green box have transposon insertions near the start site.

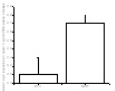
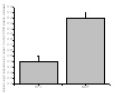
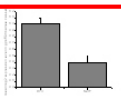
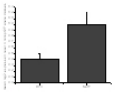
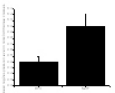
| marker | P value | bin | expression levels |
|---|---|---|---|
| gpm106a | 0.00003185 | 2.03 |  |
| umc1316 | 0.0000481 | 8.05 |  |
| gpm591b | 0.00010654 | 8.05 |  |
| mmp127 | 0.000106426 | 7.03 |  |
| mmc0411 | 0.000109265 | 7.03 |  |

Figure S3. Significant markers at a FDR of 10% are shown; P values are from SAS GLM analysis. Expression levels of probe TM00050085 designed from (*lcyE* sequence based AZM_584852) in IBM (B73 x Mo17) mapping lines were calculated by averaging the expression levels by allele at each of these marker loci. Probe TM00050085 was used to estimate expression. The second most significant marker is umc1316, which is on the same physical contig as *lcyE*. Although these lines differ for a significant promoter haplotypes, they do not segregate for the large transposon promoter differences.

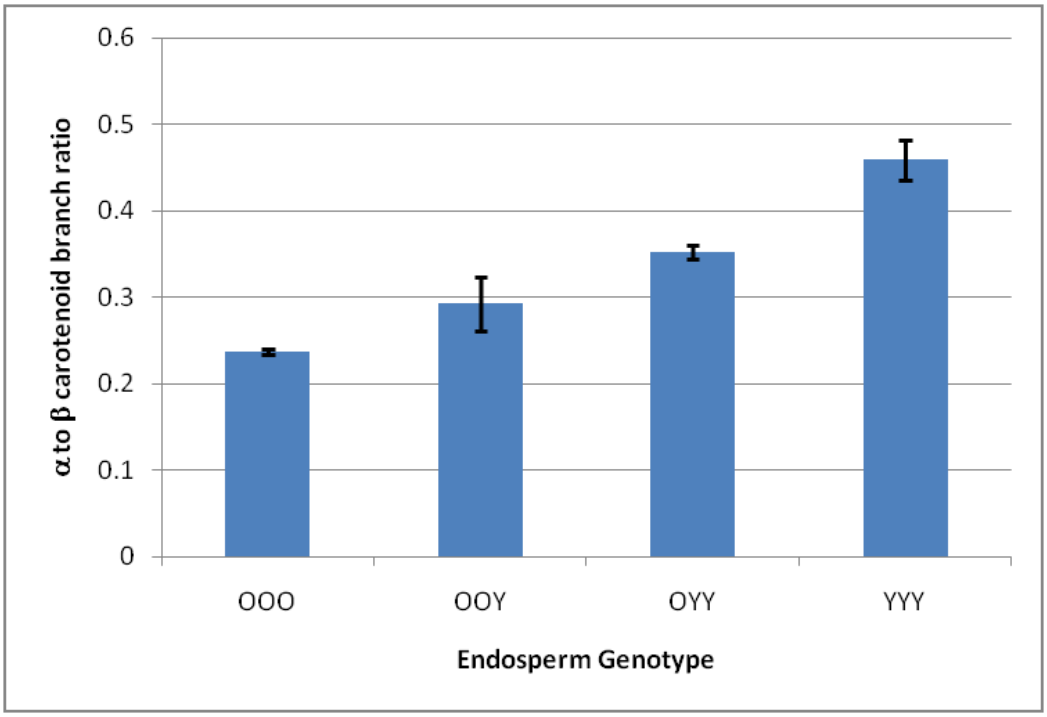
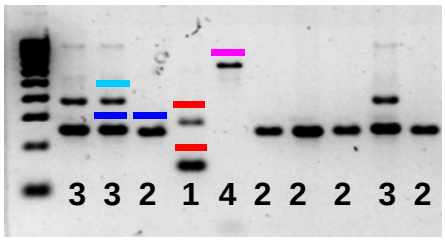
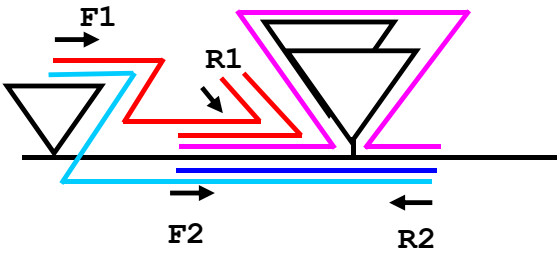


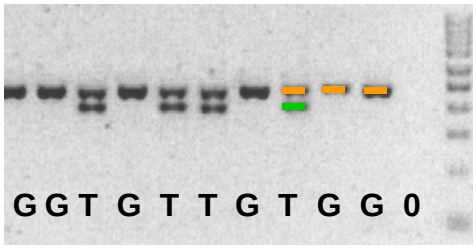

Figure S4. Comparison of ( and ( carotenoid levels for the EMS mutagenized *lcyE* allele (Orange allele is the #28 EMS mutation of the Qx47 allele; Y is wild-type Qx47 allele). This is the average of four samples for each endosperm genotype (endosperm tissue is triploid). The error bars are standard errors of the mean.

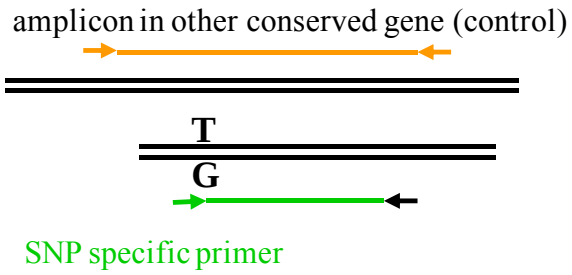
## *lcyE* PCR assay 5'indels / TE



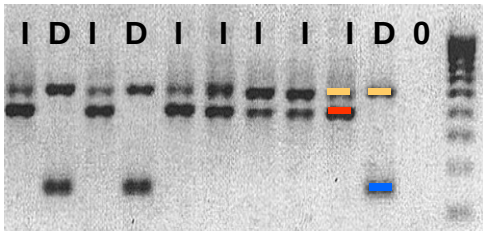




## *lcyE* PCR assay SNP216 (dominant)




## *lcyE* PCR assay 3'indel (codominant)



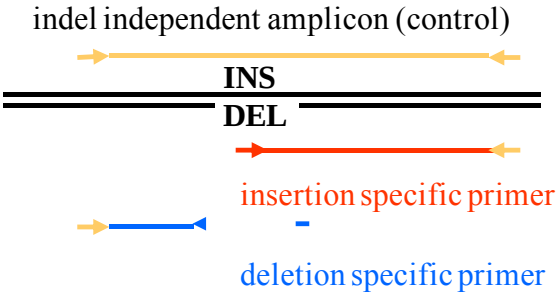
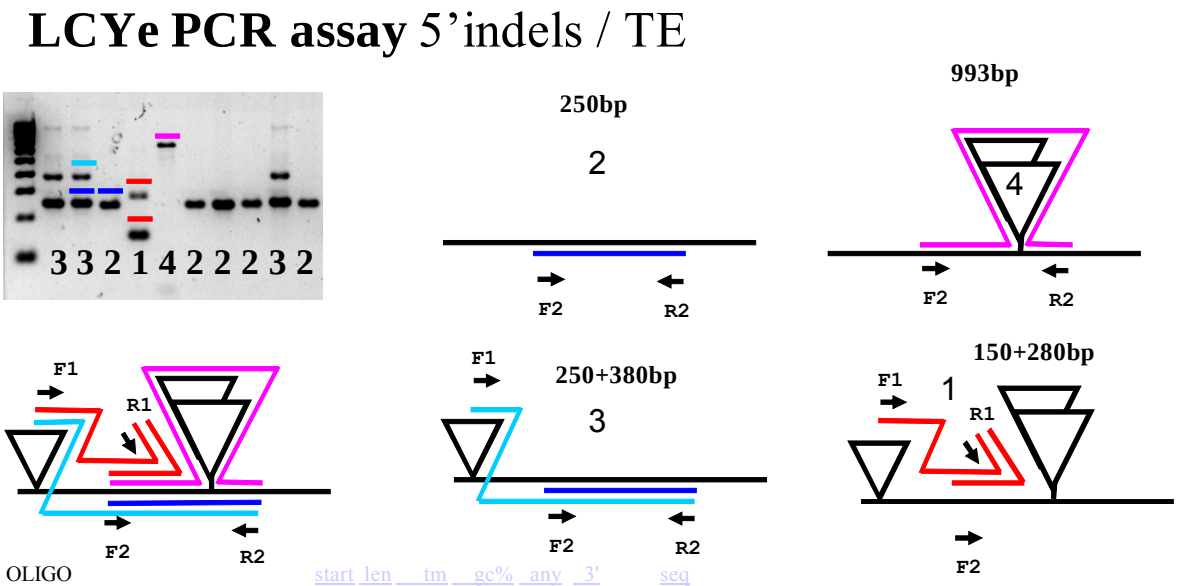




Figure S5. PCR assay for target polymorphisms. PCR assay for key associated polymorphisms haplotypes observed across the inbred line panel and diagram of primer annealing positions, black triangles represent indels (not to scale), colored lines represent the PCR amplicons corresponding to bands on gel identified by color. Assays are designed to incorporate the use of all indicated primers in a single reaction; the haplotype assignment (listed in each depicted lane) will result from one to multiple amplicons. For the 5' indels, class 1 and class 4 both share the promoter transposon; they were statistically the same in their effect and were fused for analysis.

Figure S6. Details for the lcyE 5' Indel PCR for Marker-Assisted Breeding



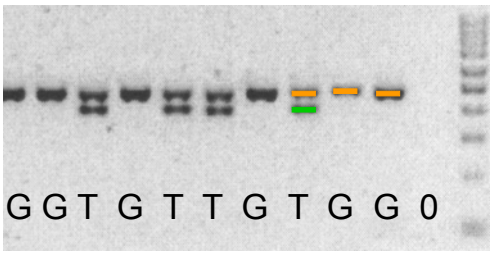

| OLIGO | start | len | tm | gc% | any | 3' | seq | |
|---|---|---|---|---|---|---|---|---|
| **TE103PF F-1** LEFT PRIMER | 149 | 23 | 61.63 | 39.13 | 6.00 | 2.00 | CGCTAGCAAGCCCATTATTTTTA | (F1) |
| **TE103PR R-1** RIGHT PRIMER | 428 | 22 | 60.35 | 45.45 | 6.00 | 2.00 | CGGTATGGTTTTTGGTATACGG | (R1) |
| **TE105PR** R1 RIGHT PRIMER | 306 | 21 | 61.40 | 61.9 | 3.00 | 1.00 | GAGAGGGAGACGACGAGACAC | (R2) |
| **ZGt111204-976R(1) F1** LEFT | 1299 | 22 | 60.36 | 40.91 | 3.00 | 1.00 | AAGCATCCGACCAAAATAACAG | (F2) |

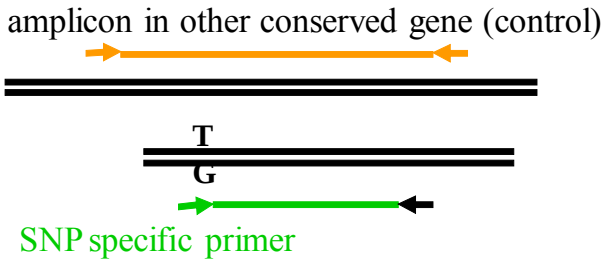
Note: There are cases in weak PCR reactions, when the 250+380bp class will be confused with the 250bp class . Essentially the 380bp band is not amplified.
On a functional note: 993bp class and 150+280bp class are functionally similar as the close insertion is most important. These are collapsed together for many of the haplotype statistical analyses. 150+280bp is rare. These haplotypes increase B-carotene and zeaxanthin.

Figure S7. Details for the lcyE SNP216 PCR for Marker-Assisted Breeding

# LCYe PCR assay SNP216

dominant marker




| OLIGO | start | len | tm | gc% | any | 3' | seq |
|---|---|---|---|---|---|---|---|
| **S216-L1** LEFT PRIMER | 480 | 18 | 70.24 | 72.22 | 3.00 | 2.00 | GCGGCAGTGGGCGTGGAT |
| **S216-R1** RIGHT PRIMER | 874 | 23 | 68.70 | 56.52 | 6.00 | 2.00 | TGAAGTACGGCTGCAGGACAACG |

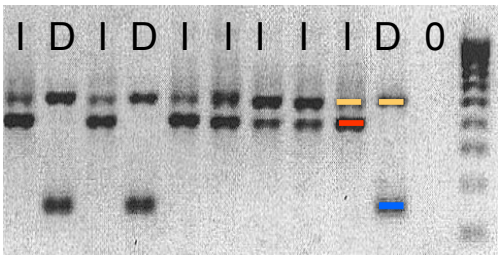
PRODUCT SIZE: 395

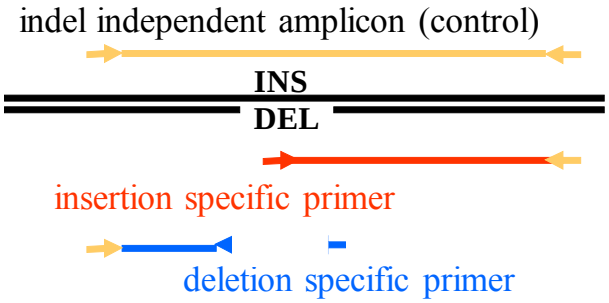
Upper band is a control PCR product from a separate gene. This just insures DNA quality is sufficient for PCR.

Figure S8: Details for the lcyE 3'Indel PCR for Marker-Assisted Breeding

# **LCYe PCR assay** 3'indel

## co-dominant marker




| OLIGO | start | len | tm | gc% | any | 3' | seq |
|---|---|---|---|---|---|---|---|
| **3pINDL-L1** LEFT PRIMER | 377 | 24 | 66.85 | 58.33 | 5.00 | 0.00 | GTACGTCGTTCATCTCCCGTACCC |
| **3pINDL-R1** RIGHT PRIMER | 775 | 23 | 65.91 | 47.83 | 4.00 | 0.00 | CTTGGTGAACGCATTTCTGTTGG |
| PRODUCT SIZE: 399 | | | | | | | |
| **3pINDL-L2** LEFT PRIMER | 273 | 19 | 64.00 | 63.16 | 4.00 | 2.00 | GGACCGGAACAGCCAACTG |
| **3pINDL-R2** RIGHT PRIMER | 416 | 18 | 65.82 | 66.67 | 4.00 | 4.00 | GGCGAAATGGGTACGGCC |
| PRODUCT SIZE: 144 | | | | | | | |
| **3pINDL-L2** LEFT PRIMER | | | | | | | |
| **3pINDL-R1** RIGHT PRIMER | | | | | | | |
| PRODUCT SIZE: 502 | | | | | | | |

I = 0bp reference=399+502bp; D=8bp deletion=144+502bp
D class increases B-carotene and zeaxanthin branch.

**Table S1**. Haplotype estimated effects. Count of haplotypic classes and estimated effects on the ratio of the pathway braches ln(lutein + α-carotene) / (zeaxanthin + β-carotene + β-cryptoxanthin)

| Full haplotype | | | | | | LSMEAN | STDERR |
|---|---|---|---|---|---|---|---|
| 5'TE | 216 | 2238 | 3'INDEL | *N* | ln(ratio) | | ratio |
| 1+4 | G | G | 0 | 4 | -0.632 | 0.278 | 0.532 |
| 1+4 | G | G | 8 | 6 | -1.2 | 0.227 | 0.301 |
| 2 | C | G | 0 | 2 | 0.134 | 0.394 | 1.144 |
| 2 | C | T | 0 | 2 | 1.019 | 0.394 | 2.77 |
| 2 | G | G | 0 | 21 | -0.11 | 0.122 | 0.896 |
| 2 | G | G | 8 | 9 | -0.952 | 0.186 | 0.386 |
| 2 | G | T | 0 | 10 | 0.539 | 0.176 | 1.714 |
| 3 | C | G | 0 | 23 | 0.473 | 0.116 | 1.604 |
| 3 | C | T | 0 | 38 | 0.766 | 0.09 | 2.152 |

Maximum change in flux 9.2-fold. ANOVA $R^2$ = 58%; $N$ = 115; $P$ =9.1x10$^{-17}$

| Two-indel haplotype | | | | LSMEAN | STDERR |
|---|---|---|---|---|---|
| 5'TE | 3'INDEL | *N* | ln(ratio) | | ratio |
| 1+4 | 0 | 5 | -0.984 | 0.275 | 0.374 |
| 1+4 | 8 | 8 | -1.127 | 0.217 | 0.324 |
| 2 | 0 | 47 | 0.03 | 0.09 | 1.03 |
| 2 | 8 | 21 | -0.792 | 0.134 | 0.453 |
| 3 | 0 | 69 | 0.645 | 0.074 | 1.906 |
| 3 | 8 | 1 | -0.103 | 0.614 | 0.902 |

Maximum change in flux 5.9 fold
ANOVA $R^2$ = 50%; $N$ = 151; $P$ = 1.6x10$^{-20}$

**Table S2.** *lcyE* gene structure. Based on maize contig ZmGSStuc11-12-04.976.1. Predicted exons are in yellow, predicted in other genes features in green, polymorphisms of interest in red (number next to them are the aligned sequence position used in the text), and primers to score key polymorphisms are in blue.

```
   1 AGCCAGATAA TTCAAAAACT CCATAAACTC CATGGTGGAC CTACTCCACG GTGGAGTTGA
  61 TGGAGTAGAG CTAAAAATGG TGGAGTAAGC AGTCCCAAAC ACCCTAAAAA AGTGATCAAT
 121 TGTCATTAAA ACATCTTAAA AGACCATATT GCCATTCAAC CAACCACCCT TCTCTCACTG
 181 GTACCTGGAC TAGGGCTGAA AAAACCCTCG AGGCTCGCGA GCTGGCTCGG GCTCGATCAG
 241 TCTAGGCTTG GCTCATATAG GTCTGAGTCG AGCCTACTTT TGTGGCTTGC TTAAACAGCG
 301 AGCCGAACTT GAGCTAGCCT AGTGATATGG CGCTATATTG TTATACTTTT ACAACATTAT
 361 TATGTTTTTG TTTTATTTTC TTATTTTTAT GGACTCAATA CTAGTTTAAA ATATGTTGTT
 421 GTCTTTTTGG AACATATTTT TTATTTTATA TATAGTGAAA ATATACATTT TATGCTCTAA
 481 ATAGGAGAGT GTGGTGTCTC GCGAGTCGGC TCGAGAGCGT GAACGAGCTC GAGCCGAGCC
 541 ATATCTCCCG GCTCGCAAGA GGACCGAGTA AGCCGAGCCT TGCTCGCTGG TTTACCGAGC
 601 CGCACCGTCG GCTCGGCTCG TCTCCGGCCG TAACCTGGAC AATGTAATAA AGGAGAGCAC
 661 ACACTGTAAA AGAAGTCGTT TAGTCTCTTT TAGTCACCTC TTGAGAGACT AGAGACTAAA
 721 ATGATTTAGG TGTCGTTTAG TTCACATATT TATAATGTGA TGGGTAACTG ATAACGTTAC
 781 ATCATGTTTG TTTAAATCCA ACCGCAGTCA GTTATCATAG TAGGAACTAA TATCAACCTA
 841 TTCAAACTTG TTACCGCTGG TACTCGGGTG TAAATCATTG CCGTTATCAT TTATGTTACA
 901 TTTCGTGAAC TAAACGACAC ATTAGTCTCC TTCTAGTCAT CTATTTAACA ATTTAGGATC
 961 TGAAACTAAC TAAAATACAT AGACTAAAAA TTAGTCCTCG AAACAATCAG AACCAGAGGC
1021 TGTCTCCAAT AGCTTTTCTA TCATATTTTT TATTTTAAAG TTTACTCTAT AAATAATGTA
1081 CTTTACACTA CAAAACGTTG TTTTACGTGA CCATATGTAC ACTCTCCTCA AAACAGCCTT
                                  TE103PF F-1 left primer
1141 GAACTACCAC CATCCATATG TGTAGGCGCT AGCAAGCCCA TTATTTTTAT TCTATCATTT
1201 ATCTCATCCA CTATTCAAAC TTTACTTTAC AAATATTATC ATGTATCCCT AAGCAGGGAA
                                  ZGt111204-976R(1) F1 Left primer
1261 GACATTCCAG CCCAATAAAA TAGCCGGAGC CAACCCAAAA GCATCCGACC AAAATAACAG
1321 CCGAGCCCAA TGCGTGAAAG CACCACGTCA CTGTCACTGA CACGCGGGAC GGGCAGCGCA
                                  Transposon insertion
1381 GCCGGAGAGG TGGAGAGTGG ACACT----- ---------- ----------
                                       TE103PR R-1 right primer
1441 ------ ---------- ---------- ---------- ---------- ----------
1501 ---------- ---------- ---------- ---------- ---------- ----------
1561 ---------- ---------- ---------- ---------- ---------- ----------
1621 ---------- ---------- ---------- ---------- ---------- ----------
1681 ---------- ---------- ---------- ---------- ---------- ----------
1741 ---------- ---------- ---------- ---------- ---------- ----------
1801 ---------- ---------- ---------- ---------- ---------- ----------
1861 ---------- ---------- ---------- ---------- ---------- ----------
1921 ---------- ---------- ---------- ---------- ---------- ----------
1981 ---------- ---------- ---------- ---------- ---------- ----------
2041 ---------- ---------- ---------- ---------- ---------- ----------
2101 ---------- ---------- ---------- ---------- ---------- ----------
2161 ---------- ---------- ---------- ---------- ---------- ----------
2221 ---------- ---------- ---------- ---------- ---------- ----------
2281 ---------- ---------- ---------- ---------- ---------- ----------
2341 ---CACGGCT CACGCCCCCG GCGCCCTCCT GAAAATGCGA AAACCATATC CACGCCGCGT
       →Potential Transcript Start
2401 GGGCCCTGTG CCAATCCAAA ACCGCCACGT GCCATATCCG GTGATACGCC TCCCCGGCCT
     TE105PR R1 right primer
2461 CTCGTGTCTC GTCGTCTCCC TCTCCTCCA CAACGGCATA GCTGCAGCAG CCAAGTCCTC
      →Potential Transcript Start
2521 TCCGCTCTCT TCGCCGCCAC CGCCGACAAA TAACCGCCGC AATTCGAAGC AAGAGAGCCG
                                                  Translation Start
2581 CACCAGGAGA AGATATAGTG AGAGTGAGAG CCCGAGGAGG GGGTGGCGCG ATGGGGCTCT
                                  1st exon
2641 CGGGCGCGAC GATCTCGGCG CCGCTCGGCT GCTGCGTGCT GCGGTGCGGG GCAGTTGGCG
2701 GAGGTAAGGC CCTGAAGGCG GACGCGGAGA GGTGGCGGCG GGCGGGATGG AGCCGACGCG
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2761 TTGGCGGACC GAAGGTGAGG TGCGTGGCGA CCGAGAAGCA CGACGAGACG GCGGCGGTCG
     S216-L1 left primer SNP216(T/G)
2821 GGGCGGCAGT GGGCGTGGAT TTCGCGGACG AGGAGGACTA CCGCAAGGGC GGCGGCGGCG
2881 AGCTGCTTTA CGTGCAAATG CAGTCAACCA AGCCCATGGA AAGCCAGTCC AAGATCGCTT
2941 CCAAGGTGAT TAGATTGCCA ACACTAATTT GCTAATCATA TTTGGAGTTT GTTCTAGTGA
3001 TCGTGATTAA CGTGTGAAGG AAAAAAATAA ATTATAGGTT TCGCCCCCCT TTATTGTGCT
3061 GGTATTTGGA GATTTATTTA CAGGATGGAA TCCAGAACAT CATGCACCTA CTGAATATTA
3121 CCTTTTCCGG TAGAAAAAAG AAAACATAAC CAATGTTTAG GGCACGACAA TACTGCACGT
                    S216-R1 right primer
3181 TTCAATATGA ACCACGTTGT CCTGCAGCCG TACTTCACCT GCGTTGGCTA CAGCTACAAG
3241 GATGTTCACT GGAAAGCAGG GTGATCGCCT AGCGGTAGGC AACGCGTTTC ACCTTCCTGG
3301 CTGGTTTTGC AGTCTAGATG TTTCGAGCCA GTAGATTGAC ATAGTTTGCC CGTTTGGAAA
3361 TGTATGTAAA TTAAATATTC TGGAAATGTA TTTCATGAGA AAATGGCAGT ACCTCATCAG
3421 GAACTGTCAT AACACTAAAT CTACTGTGGT TCGTGGGAAC AAAGACTGCT CACATACTTC
3481 TCTGGACTAT TCTACTTATA CGAACTACAG TGCCAGGCAC CACATTAACG ACCGTTATTT
3541 TTTTGTGCGC ACTAGTTCCT TACATTGTAT GAGTATGACA GTAGCTTGTT GCCTGATCAC
3601 ACAGTAATGT TGAGTTCTCT GGATTGTACA CGCATGAGCA TCATGTCATT TCCAGTCCAA
3661 TGTCACTGCT GACTTGCTAT AATTTGAGCA GTCAAGTGGA GTACTCTAGC TAGGCACAGG
3721 CAATTCCACA AAACCTGGAA TTTTGTATAT GACTGCACAG AACAGAAGAG CTTCCTTGTA
3781 TAGCGCTCTT TCTTTTTTTC CTTCTGTATC AATACAAGTT GTGCAGATCT CCTGCCGACC
3841 GTTAAAAAAA CCCGCAGAGC TTCAAATGGA GCCTTTGTGC CATTATTTTT TTTATACGGA
3901 ATACGTGGGC CATAAACTAT GCACCCCACC AGCGCTATCC TTATTTTTTC TTCATAAAAA
3961 AACTATTTTG CTTCAGGGCG AGGTAACCAA TGTTCAACTT TACATGGCAT CAGTCTTATT
4021 TTATATAAAA AAGTGCTAAC AGTCTATTCT GTCTTTGTAT CCTAAGGAAA GTTAACTGTG
                                  2nd exon
4081 GTCTTATGTT CTGTTTGCAG CTATCGCCCA TATCTGATGA AAATACAGTG CTTGATTTGG
4141 TTATCATTGG TTGTGGTCCA GCTGGTCTTT CTCTAGCTTC AGAGTCAGCT AAGAAAGGTC
4201 TCACTGTAGG TCTCATTGGG CCTGACCTTC CATTCACAAA TAACTATGGT GTGTGGGAGG
4261 ATGAATTTAA AGGTATTCTG TTATTTGCTT GTTGAATGCA GAGTGCCTGC ATAACATCTC
4321 TGTCAGTGTC AGCCCAACTT AACATGATGA TATATACTTT TTTCTCTCGT CTACTGTCCA
                                  3rd exon
4381 GATCTCGGTC TAGAGAGCTG TATCGAGCAC GTCTGGAAGG ATACTATTGT CTACCTAGAC
4441 AATAACAAGC CGATACTGAT TGGCCGTTCT TATGGCAGGG TGCACCGTGA CTTGCTCCAT
4501 GAGGAACTGC TGAAAAGGTA AATCTTCATG TGGTAGTTAG AATTGGGACT CAGAAGTGTC
4561 TTTTCCATGC ATGGTGGTAT ATAATATAAT TGACGAGTAG CAACACTTGA ATAATTCGCA
                                  4th exon
4621 GATGCTATGA AGCTGGCGTG ACATACCTGA ACTCCAAAGT GGACAAGATC ATAGAATCTC
4681 CAGATGGACA CAGAGTAGTC TGTTGTGACA AGGGTCGCGA GATAATTTGC AGGCTTGCCA
4741 TTGTTGCTTC GGGGGCAGCA TCTGGTAGGC TTCTAGAGTA TGAGGTTGGG GGTCCCCGTG
4801 TTTGCGTGCA GACTGCATAC GGAGTAGAAG TTGAGGTACA CCAAGACTCA AGAATCTGTT
          SNP T/G (2238)
4861 CATTTTTTTT TATTGCTTGC ACAAATTAGA CAGACCAGTT TGCTCAATAA GTCTCTAGTT
                          SNP T/A (2312)
4921 GAAATTTTCA ATATATCATT TATGTGCTAG ATCTTCGTTA TTTCAAAACT GTTTGAAAAT
                                  5th exon
4981 TTCAGGTGGA AAATAACCCA TATGATCCTA GCTTAATGGT TTTCATGGAC TACAGAGATT
5041 GTTTCAAAGA GGAATTCTCA CACACTGAAC AAGAAAATCC CACTTTCCTG TATGCTATGC
5101 CCATGTCACC CACACGAGTT TTCTTTGAGG TCGGTACAGA GTTTTTGTTA CCTCTGTGAT
                                  6th exon
5161 TAAGCAACCC GCGCTTCCCA TCAAATTCTC AAAATTTGTG ACTAAATACC TTATCACAGG
5221 AAACATGCTT AGCTTCTAAA GATGCTATGT CTTTTGATCT ACTTAAGAAG AGGCTGATGT
5281 ATCGGTTGAA CGCGATGGGA ATTCGTATCC TGAAAGTTTA TGAGGAGGTA AGAGGGTCTA
                                  7th exon
5341 CAATTCTTAA TTCTCACACA TTATATTCTG AAAATAAACT GAAATGTTCT CGGTTTAAGG
5401 AATGGTCCTA CATTCCTGTT GGGGGGTCCT TACCAAACGC AGATCAGAAG AGCCTTGCAT
5461 TCGGTGCTGC AGCAAGTATG GTGCATCCTG CAACAGGTAC ATACGAATCA TCAATTTTTT
                                  8th exon
5521 GCACGCCAGT TTCCTCCTAG CTTATATTGA CAACTTTTCG CAAAATACAT AGGGTACTCA
5581 GTGGTCAGAT CTTTGTCTGA GGCTCCAAGA TATGCTTCTG TGATATCGGA CATCTTAGGA
5641 AATCGAGTTC CTGCAGAATA TATGCTCGGA AATTCACAAA ATTACAGTCC ATCAATGCTT
5701 GGTAAGCATT TTTCTGGTAT TTATTCAAGT TGGTTGGTTC GGTTCTACTG TGTAAAGGTT
                                  9th exon
5761 CAGACAAGTT AGCGAAACTA TTATAATATG ATGTTTCAGC ATGGAGAACA CTGTGGCCTC
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5821 AAGAGAGGAA ACGCCAACGA TCCTTCTTCC TTTTCGGATT AGCGTTGATA ATCCAACTGA
5881 ATAATGAAGG CATACAAACA TTCTTCGAAG CCTTTTTCAG GGTGCCGAGA TGGTAGTCGC
5941 ACTTTTTACC TTGTCTCAGT TGGTCTTCAG AGAAATTCAG TGCGCTGAAG GCTACTACCT
10th exon
6001 CCATGAAGTT TTTGATAACC ACTATTTTTC CTTGAACAGG ATGTGGCGAG GATTTCTTGG
6061 CTCGACCCTT TCATCCGTCG ATCTCATACT ATTCTCATTC TACATGTTTG CGATAGCTCC
6121 AAATCAATTG CGAATGAACC TTGTCAGGCA TCTCCTCTCT GACCCAACTG GCTCATCCAT
6181 GATCAAGACC TACCTGACCT TATAAAACCA TTTGCACCAG GCTGCAAGAA CTCTTAGAAA
6241 CTGTACAGTT TTGTAGTTGT ACATAAGTTA GAGAGGATCT GGGGGGTTAC TTGGCGGCGG
6301 ATCTAGGGGT TAGCAGCAAT GCTATAATAC ACTGTAAATC TTTTATGGTT GCTATGGTGA
6361 TTGGATAGAG AAGCACACCG TGTTGTGCAC GATGGAAGAA TAATAAGAGA GATCAGGTGA
6421 TGGTCATGGT TCCTGCATT~ GGCCAATTTT AGGTTGCATT TGCTGTTTCA AGGCTTCTTA
6481 CATGTCCAAT CAATTACACC CTTATTTTAG GTTGCTCAAT GCCAATTTTC TTGTGGAACA
6541 ATATTTGCAA AAGCAAAAAA GGGAAAAACA TAATTGGATG TGCAAGAATA GTATGAGGCT
6601 GTCCTTGCTC CTGCGCCATG GCATTTAGCT GCAAGATGTT GGGGAAGGAG AAGACCTGTA
6661 GGAGACTAAG GTTGGAGGCA AGGGAGAGAA AGGAAGGAGG GCAGGAGGAG AGGCGGAACA
6721 ACCCGTCTCC CTCCATCTCT CTACCTTCTT CTCCTGCGCC CCTATCCATC GCTCGCACCA
SNP A/G_(4184)
6781 CCCACTGACC GGCCGGCGGC ATCCTTATTA CCATAACATC ACGGGACGGT GGCGCGATGC
6841 AAGGAGGCCC ACTGAGCCCG GATGAGTACT GGGTGATATC GCCGCCGGCG CTGCTGCACC
3pINDL-L2 left primer
6901 AGCCGGCGTC CACCATCGTC GTGGCCATCG ACCGGGACCG GAACAGCCAA CTGGCCGTGA
SNP C/G_(4315)
6961 AGTGGGTGGT GGACCACCTC CTCTCCGGCG CCTCTCATAT CGTCCTGCTC CACGTGGCCG
3pINDL-L1 left primer 3pINDL-R2 right primer
7021 TCCATTACCA CACGACCCGT ACGTCGTTCA TCTCCCGTAC --------CC ATTTCGCCAG
3’8bp InDel
7081 TGCAGCAGCA GCAGCTGAGC TCTGGTTTGC TTGGACATGC GCAGATGGGT TCGCCATGGT
7141 TGAGACCACG CAGGGTGCGC TGGAGGCTGA AATGAAGGAG ATCTTTGTCC CCTACAGAGG
7201 ATTCTTCAAC CGGAATGGGG TAAATGTA

**Table S3.** Association for other candidate genes with *lcyE* effects included as a covariate. NC is noncovergence of the mixed model often caused by non-normal trait distributions or low frequency SNPs. Please note this table includes over 600 tests, and these *P* values have not been multiple test corrected. Except for a few false very significant results ($P < 0.002$) most of these are false positives. For contrast *LcyE*'s initial association was $P = 2.23 \times 10^{-10}$.

Since the completion of the study of the *LcyE*, we surveyed the numerous hydroxylase paralogues in maize and we have found an additional important modifier.

| Gene | Site in amp | α–carotene | β–carotene | β-crypto xanthin | ln(ratio β–carotene to ALL) | Ln(ratio alpha to beta branch) | Lutein | Total carotenoids | Zeaxanthin |
|---|---|---|---|---|---|---|---|---|---|
| DXS | 28 | 0.2371 | 0.1519 | 0.0563 | 0.4102 | 0.7845 | 0.2285 | 0.0593 | 0.1241 |
| DXS | 60 | 0.8879 | 0.2813 | 0.5883 | 0.0333 | 0.5988 | 0.8708 | 0.5704 | 0.6461 |
| DXS | 76 | 0.81 | 0.1171 | 0.9992 | 0.0173 | 0.4242 | 0.701 | 0.1109 | 0.5923 |
| DXS | 77 | 0.1778 | 0.0077 | 0.0245 | 0.6387 | 0.1216 | 0.1762 | 0.0439 | 0.0202 |
| DXS | 78 | 0.1778 | 0.0077 | 0.0245 | 0.6655 | 0.1216 | 0.1762 | 0.023 | 0.0202 |
| DXS | 230 | 0.5205 | 0.1184 | 0.3269 | 0.1333 | 0.7315 | 0.1121 | 0.0124 | 0.5997 |
| DXS | 254 | 0.2202 | 0.0116 | 0.0354 | 0.2741 | 0.6124 | 0.1412 | 0.0115 | NC |
| DXS | 278 | 0.8048 | 0.8835 | 0.6287 | 0.7257 | 0.9462 | 0.4561 | 0.6865 | 0.6827 |
| DXS | 355 | 0.6518 | 0.4944 | 0.7252 | 0.0189 | 0.511 | 0.3273 | 0.4035 | 0.9212 |
| DXS | 455 | 0.3151 | 0.0065 | 0.1349 | 0.4172 | 0.1577 | 0.174 | 0.0769 | 0.1702 |
| DXS | 483 | 0.2233 | 0.0096 | 0.0231 | 0.378 | 0.3398 | 0.3018 | 0.0174 | 0.025 |
| DXS | 486 | 0.6477 | 0.2652 | 0.2275 | 0.3596 | 0.2886 | 0.4687 | 0.9709 | 0.4079 |
| DXS | 498 | 0.7345 | 0.241 | 0.2017 | 0.3759 | 0.2002 | 0.3535 | 0.9389 | 0.3472 |
| DXS | 500 | 0.7345 | 0.241 | 0.2017 | 0.3759 | 0.2002 | 0.3535 | 0.9389 | 0.3472 |
| DXS | 501 | 0.7345 | 0.241 | 0.2017 | 0.3759 | 0.2002 | 0.3534 | 0.9389 | 0.3472 |
| DXS | 550 | 0.2669 | 0.037 | 0.2126 | 0.4405 | 0.4883 | 0.2648 | 0.5178 | 0.6628 |
| IPPI | 8 | 0.8112 | 0.084 | 0.2897 | 0.1898 | 0.764 | 0.3348 | 0.0459 | 0.1987 |
| IPPI | 139 | 0.9833 | 0.9171 | 0.8982 | 0.1041 | 0.8642 | 0.2773 | 0.4831 | 0.7651 |
| IPPI | 179 | 0.3325 | 0.7327 | 0.4663 | 0.4661 | 0.3537 | 0.5148 | 0.875 | 0.5798 |
| IPPI | 282 | 0.829 | 0.6559 | 0.7676 | 0.0278 | 0.734 | 0.0789 | 0.3568 | 0.8519 |
| IPPI | 318 | 0.8327 | 0.5699 | 0.7606 | 0.0203 | 0.6756 | 0.0457 | 0.3189 | 0.8909 |
| IPPI | 353 | 0.8159 | 0.4442 | 0.0205 | 0.1233 | 0.5828 | 0.0272 | 0.0043 | 0.0441 |
| IPPI | 363 | 0.8159 | 0.4442 | 0.0205 | 0.1233 | 0.5828 | 0.0272 | 0.0043 | 0.0441 |
| IPPI | 443 | 0.6911 | 0.4921 | 0.0288 | 0.2649 | 0.4581 | 0.0261 | 0.0042 | 0.0421 |
| IPPI | 464 | 0.7282 | 0.5847 | 0.0345 | 0.2229 | 0.486 | 0.0179 | 0.0038 | 0.0405 |
| GGPR | 286 | 0.9369 | 0.9273 | 0.4883 | 0.747 | 0.4076 | 0.796 | 0.6812 | 0.8748 |
| GGPR | 376 | 0.57 | 0.8065 | 0.5194 | 0.5828 | 0.2386 | 0.8719 | 0.6522 | 0.9112 |
| GGPR | 412 | 0.8061 | 0.9808 | 0.7428 | 0.9891 | 0.1495 | 0.8592 | 0.5688 | 0.7711 |
| CYC3 -Y1 linked | 23 | 0.6393 | 0.24 | 0.0042 | 0.631 | 0.0435 | 0.8876 | 0.055 | 0.088 |
| CYC3 -Y1 linked | 26 | 0.5911 | 0.074 | 0.0061 | 0.5947 | 0.7579 | 0.7681 | 0.0519 | 0.0217 |
| CYC3 -Y1 linked | 54 | 0.6134 | 0.1812 | 0.0016 | 0.6302 | 0.0435 | 0.7837 | 0.0496 | 0.0409 |
| CYC3 -Y1 linked | 88 | 0.5287 | 0.4183 | 0.0043 | NC | 0.1816 | 0.5562 | 0.242 | 0.5195 |
| CYC3 -Y1 linked | 91 | 0.6349 | 0.791 | 0.5006 | 0.6004 | 0.3798 | 0.6268 | 0.6101 | 0.593 |
| CYC3 -Y1 linked | 180 | 0.3863 | 0.0188 | 0.001 | 0.8401 | 0.364 | 0.6895 | 0.0149 | 0.0039 |
| CYC3 -Y1 linked | 321 | 0.4263 | 0.1398 | 0.0017 | 0.7642 | 0.0293 | 0.5794 | 0.0213 | 0.0352 |
| CYC3 -Y1 linked | 362 | 0.4695 | 0.1514 | 0.0016 | 0.7254 | 0.0271 | 0.5959 | 0.0201 | 0.0294 |
| PDS | 18 | 0.8802 | 0.8974 | 0.1835 | NC | 0.253 | 0.2656 | 0.8978 | 0.2143 |
| PDS | 79 | 0.4025 | 0.9908 | 0.6043 | NC | 0.8083 | 0.8773 | 0.8363 | NC |
| PDS | 127 | 0.2148 | 0.991 | 0.8985 | NC | 0.9135 | 0.3852 | 0.7841 | NC |
| PDS | 131 | 0.3594 | 0.672 | 0.2522 | 0.172 | 0.8956 | 0.9504 | 0.5738 | 0.4989 |
| PDS | 133 | 0.0092 | 0.6048 | 0.3693 | 0.3684 | 0.5978 | 0.7748 | 0.9983 | NC |
| PDS | 139 | 0.1092 | 0.4423 | 0.5733 | 0.0066 | 0.6981 | 0.4126 | 0.7815 | NC |
| PDS | 152 | 0.3321 | 0.7198 | 0.2701 | 0.1269 | 0.7553 | 0.9353 | 0.5587 | NC |
| PDS | 228 | 0.0572 | 0.1975 | 0.4745 | 0.071 | 0.304 | 0.0357 | 0.4489 | NC |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| PDS | 234 | 0.0063 | 0.4809 | 0.355 | 0.2519 | 0.9464 | 0.7728 | 0.8827 | 0.8871 |
| PDS | 272 | 0.6654 | 0.8195 | 0.297 | 0.1222 | 0.2284 | 0.2233 | 0.9346 | NC |
| PDS | 287 | 0.1897 | 0.968 | 0.8924 | 0.1841 | 0.9935 | 0.4181 | 0.8318 | NC |
| ZDS | 1 | 0.642 | 0.2672 | 0.5328 | 0.1934 | 0.1161 | 0.0333 | 0.5945 | NC |
| ZDS | 2 | 0.642 | 0.2672 | 0.5328 | 0.1934 | 0.1161 | 0.0333 | 0.5945 | NC |
| ZDS | 11 | 0.8396 | 0.1983 | 0.5624 | 0.0465 | 0.0467 | 0.0018 | 0.2332 | NC |
| ZDS | 18 | 0.1503 | 0.0322 | 0.0075 | 0.3016 | 0.2737 | 0.1594 | 0.2094 | 0.0394 |
| ZDS | 66 | 0.6081 | 0.6857 | 0.8158 | 0.9189 | 0.1213 | 0.7664 | 0.4856 | 0.2952 |
| ZDS | 171 | 0.9719 | 0.9492 | 0.834 | 0.5235 | 0.5321 | 0.4577 | 0.8066 | 0.5402 |
| ZDS | 184 | 0.9518 | 0.9468 | 0.7323 | 0.5556 | 0.5147 | 0.4024 | 0.769 | NC |
| ZDS | 232 | 0.378 | 0.7053 | 0.4137 | 0.4004 | 0.7075 | 0.4239 | 0.5496 | 0.8488 |
| ZDS | 240 | 0.2529 | 0.632 | 0.3714 | 0.4677 | 0.0312 | 0.0255 | 0.3853 | 0.9601 |
| ZDS | 246 | 0.2529 | 0.632 | 0.3714 | 0.4677 | 0.0312 | 0.0255 | 0.3853 | 0.96 |
| ZDS | 295 | 0.7837 | 0.0702 | 0.0524 | 0.243 | 0.0024 | 0.0989 | 0.4511 | 0.0358 |
| ZDS | 298 | 0.9557 | 0.0748 | 0.0233 | 0.4399 | 0.0034 | 0.316 | 0.2071 | 0.0141 |
| ZDS | 308 | 0.3108 | 0.6138 | 0.2585 | 0.4899 | 0.0511 | 0.0266 | 0.6494 | 0.5288 |
| ZDS | 319 | 0.4409 | 0.0592 | 0.0348 | 0.2822 | NC | 0.2883 | 0.3085 | 9.75E-04 |
| ZDS | 335 | 0.8568 | 0.243 | 0.6598 | 0.0271 | 0.0669 | 0.0016 | 0.1915 | 0.928 |
| ZDS | 336 | 0.3157 | 0.2312 | 0.1889 | 0.1852 | 0.5988 | 0.2959 | 0.6522 | 0.921 |
| ZDS | 353 | 0.3307 | 0.1081 | 0.0757 | 0.2991 | 0.0595 | 0.96 | 0.9059 | 0.0455 |
| ZDS | 361 | 0.5256 | 0.9609 | 0.4308 | 0.2515 | 0.8792 | 0.0876 | 0.1758 | 0.384 |
| ZDS | 367 | 0.8731 | 0.2602 | 0.6681 | 0.029 | 0.0643 | 0.0017 | 0.1963 | 0.9075 |
| ZDS | 385 | 0.7028 | 0.2944 | 0.5314 | 0.1223 | 0.1051 | 0.0117 | 0.3432 | 0.7657 |
| ZDS | 392 | 0.3479 | 0.5354 | 0.2189 | 0.3847 | 0.1203 | 0.827 | 0.2506 | 0.112 |
| ZDS | 393 | 0.8993 | 0.2839 | 0.6536 | 0.0373 | 0.0772 | 0.0032 | 0.2291 | 0.8479 |
| LCYB | 245 | 0.0113 | 0.7164 | 0.8482 | 0.5514 | 0.0977 | 0.0193 | 0.1451 | 0.5793 |
| ZEP1 | 4 | 0.9198 | 0.8331 | 0.8092 | 0.2878 | 0.4009 | 0.4954 | 0.9906 | NC |
| ZEP1 | 22 | 0.3307 | 0.6742 | 0.7683 | 0.9957 | 0.1559 | 0.325 | 0.862 | NC |
| ZEP1 | 30 | 0.6661 | 0.1045 | 0.3337 | 0.6447 | 0.3068 | 0.7685 | 0.2967 | NC |
| ZEP1 | 287 | 0.2799 | 0.7669 | 0.6654 | 0.1363 | 0.3472 | 0.6085 | 0.9097 | 0.7806 |
| ZEP1 | 290 | 0.025 | 0.1072 | 0.6579 | 0.0229 | 0.7588 | 0.0736 | 0.0294 | 0.4187 |
| ZEP1 | 318 | 0.2602 | 0.5188 | 0.5674 | 0.7964 | 0.1841 | 0.6976 | 0.5644 | 0.2024 |
| ZEP1 | 391 | 0.4151 | 0.2918 | 0.8485 | 0.6222 | 0.625 | 0.7273 | 0.8793 | 0.3292 |

**Table S4.** HPLC carotenoid values for the 2003 field in IL.

| Taxa | Lutein | Zeaxanthin | β-cryptoxanthin | α–Carotene | β–Carotene | Total Carotenoid |
|---|---|---|---|---|---|---|
| 3811 | 13.28 | 18.14 | 2.18 | 0.07 | 2.71 | 36.38 |
| 4226 | 16.9 | 8.01 | 0.68 | 0.15 | 0.34 | 26.08 |
| 4722 | 5.77 | 14.59 | 3.01 | 0.09 | 1.99 | 25.45 |
| A214N | 13.74 | 8.43 | 0.71 | 0.25 | 0.5 | 23.63 |
| A239 | 13.93 | 13.26 | 2.92 | 0.44 | 2.39 | 32.94 |
| A272 | 5.72 | 43.97 | 8.52 | 0.2 | 7.54 | 65.95 |
| A556 | 14.5 | 4.26 | 0.76 | 0.79 | 1.75 | 22.06 |
| A6 | 7.59 | 22.94 | 5.5 | 0.07 | 2.99 | 39.09 |
| A619 | 10.03 | 10.05 | 2.46 | 0.41 | 6.11 | 29.06 |
| A632 | 6.87 | 10.23 | 1.11 | 0.05 | 0.69 | 18.95 |
| A634 | 6.04 | 2.27 | 0.21 | 0.1 | 0.08 | 8.7 |
| A635 | 20.55 | 7.01 | 0.66 | 0.25 | 0.92 | 29.39 |
| A641 | 8.29 | 5.97 | 3.57 | 0.34 | 1.1 | 19.27 |
| A654 | 5.24 | 0.76 | 0.16 | 0.05 | 0.23 | 6.44 |
| A659 | 12.68 | 8.05 | 0.76 | 0.42 | 0.62 | 22.53 |
| A661 | 11.28 | 4.86 | 0.27 | 0.11 | 0.06 | 16.58 |
| A679 | 9.11 | 2.51 | 0.58 | 0.8 | 0.57 | 13.57 |
| A680 | 11.4 | 2.82 | 0.47 | 0.41 | 0.24 | 15.34 |
| B10 | 27 | 8.42 | 1.36 | 0.79 | 1.87 | 39.44 |
| B103 | 8.34 | 6.52 | 1.07 | 0.09 | 0.25 | 16.27 |
| B104 | 19.62 | 5.92 | 0.82 | 0.21 | 0.87 | 27.44 |
| B105 | 21.73 | 6.05 | 0.99 | 0.68 | 1.84 | 31.29 |
| B109 | 16.67 | 5.3 | 1.16 | 1.1 | 0.61 | 24.84 |
| B115 | 20.48 | 6.41 | 0.67 | 0.34 | 2.22 | 30.12 |
| B14A | 7.64 | 4.49 | 0.6 | 0.23 | 0.49 | 13.45 |
| B164 | 11.84 | 5.53 | 0.33 | 0.11 | 0.81 | 18.62 |
| B2 | 12.79 | 5.66 | 0.55 | 0.24 | 0.5 | 19.74 |
| B37 | 17.07 | 4.57 | 0.9 | 0.57 | 1.15 | 24.26 |
| B46 | 11.06 | 9.34 | 1.78 | 0.48 | 1.46 | 24.12 |
| B52 | 6.69 | 4.06 | 0.29 | 0.11 | 0.26 | 11.41 |
| B57 | 21.49 | 2.45 | 1.12 | 1.39 | 0.74 | 27.19 |
| B64 | 1.4 | 13.47 | 2.65 | 0.05 | 2.2 | 19.77 |
| B68 | 8.61 | 5.05 | 0.29 | 0.17 | 0.19 | 14.31 |
| B73 | 11.75 | 4.79 | 1.27 | 0.91 | 0.57 | 19.29 |
| B73HTRHM | 12.88 | 3.93 | 0.67 | 0.54 | 0.3 | 18.32 |
| B75 | 15.29 | 3.23 | 0.61 | 1.07 | 1.86 | 22.06 |
| B76 | 31.28 | 5.72 | 1.18 | 1.11 | 1.28 | 40.57 |
| B77 | 8.19 | 5.95 | 1.23 | 0.61 | 8.46 | 24.44 |
| B79 | 18.47 | 2.77 | 0.47 | 0.34 | 0.83 | 22.88 |
| B84 | 12.15 | 4.18 | 0.33 | 0.22 | 0.49 | 17.37 |
| C49 | 17.16 | 1.41 | 1.06 | 2.03 | 1.51 | 23.17 |
| C49A | 6.95 | 9.19 | 1.33 | 0.1 | 0.53 | 18.1 |
| CH70130 | 6.27 | 6.09 | 1.33 | 0.13 | 1.25 | 15.07 |
| CH9 | 9.53 | 6.08 | 1.51 | 0.11 | 1.54 | 18.77 |

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|---|---|---|---|---|---|---|
| CI21E | 4.15 | 5.12 | 0.44 | 0.11 | 0.24 | 10.06 |
| CI31A | 18.36 | 0.89 | 0.56 | 0.46 | 0.82 | 21.09 |
| CI3A | 8.79 | 17.34 | 2.31 | 0.27 | 2.95 | 31.66 |
| CI7 | 6.36 | 2.77 | 0.84 | 0.87 | 13.63 | 24.47 |
| CI90C | 16.38 | 3.92 | 0.35 | 0.12 | 0.72 | 21.49 |
| CI91B | 19.24 | 12.34 | 1.69 | 0.27 | 1.82 | 35.36 |
| CM105 | 8.81 | 3.78 | 0.48 | 0.51 | 2.07 | 15.65 |
| CM174 | 9.56 | 4.47 | 0.73 | 0.44 | 1.87 | 17.07 |
| CM7 | 15.26 | 4.63 | 0.99 | 0.98 | 1.96 | 23.82 |
| CML323 | 3.83 | 18.03 | 5.26 | 0.12 | 5.31 | 32.55 |
| CML328 | 3.35 | 23.56 | 10.84 | 0.51 | 7.7 | 45.96 |
| CMV3 | 14.76 | 1.92 | 0.66 | 0.85 | 2 | 20.19 |
| CO125 | 9.87 | 0.82 | 0.25 | 0.34 | 0.15 | 11.43 |
| D940Y | 16 | 8.72 | 1.19 | 0.23 | 1.2 | 27.34 |
| DE1 | 12.69 | 8.6 | 0.95 | 0.1 | 1.16 | 23.5 |
| DE3 | 13.85 | 2.4 | 0.7 | 1.52 | 13.34 | 31.81 |
| DE811 | 4.26 | 22.89 | 3.01 | 0.07 | 2.14 | 32.37 |
| EP1 | 12.13 | 11.41 | 1.93 | 0.14 | 0.8 | 26.41 |
| F2 | 8.47 | 3.91 | 0.45 | 0.16 | 0.27 | 13.26 |
| F2834T | 5.15 | 6.9 | 0.85 | 0.02 | 0.93 | 13.85 |
| F44 | 5.51 | 8.88 | 1.55 | 0.14 | 1.08 | 17.16 |
| F6 | 15.04 | 13.52 | 0.79 | 0.21 | 0.98 | 30.54 |
| GT112 | 6.11 | 4.74 | 0.29 | 0.32 | 1.39 | 12.85 |
| H100 | 11.12 | 8.17 | 0.88 | 0.14 | 0.48 | 20.79 |
| H49 | 22.01 | 10.94 | 0.47 | 0.24 | 1.3 | 34.96 |
| H84 | 8.44 | 2.38 | 0.31 | 0.31 | 2.72 | 14.16 |
| H91 | 7.21 | 3.33 | 0.18 | 0.06 | 0.18 | 10.96 |
| H99 | 25.39 | 8.52 | 0.6 | 1.42 | 1.69 | 37.62 |
| HY | 7.22 | 3.28 | 0.28 | 0.21 | 0.06 | 11.05 |
| K148 | 15.23 | 1.06 | 0.64 | 0.42 | 3.65 | 21 |
| K4 | 7.87 | 3.5 | 1.8 | 0.22 | 5.29 | 18.68 |
| KI11 | 7.77 | 22.84 | 2.47 | 0.12 | 4.06 | 37.26 |
| KI21 | 13 | 20.41 | 1.62 | 0.29 | 2.28 | 37.6 |
| KI3 | 2.28 | 23.61 | 4.79 | 0.09 | 2.32 | 33.09 |
| KI43 | 12.6 | 21.41 | 2.9 | 0.6 | 4.42 | 41.93 |
| KI44 | 10.63 | 20.31 | 3.37 | 0.21 | 1.56 | 36.08 |
| KUI2007 | 7.09 | 31.18 | 4.86 | 0.08 | 3.15 | 46.36 |
| L317 | 8.38 | 5.62 | 0.7 | 0.09 | 0.37 | 15.16 |
| L578 | 4.61 | 4.43 | 0.45 | 0.03 | 0.57 | 10.09 |
| M14 | 31.33 | 8.79 | 1.06 | 0.68 | 1.8 | 43.66 |
| MEF15655 | 6.38 | 6.27 | 1.84 | 0.25 | 0.55 | 15.29 |
| MO17 | 17.24 | 14.25 | 1.18 | 0.14 | 1.1 | 33.91 |
| MO44 | 12.13 | 5.83 | 0.42 | 0.12 | 0.56 | 19.06 |
| MO45 | 17.32 | 12.57 | 3.07 | 0.31 | 2.25 | 35.52 |
| MO46 | 17.51 | 6.98 | 1.1 | 0.73 | 0.98 | 27.3 |
| MO47 | 11.35 | 9.72 | 1.82 | 0.14 | 1.46 | 24.49 |
| MOG | 5.56 | 12.18 | 3.13 | 0.16 | 3.59 | 24.62 |
| MS1334 | 21.68 | 2.87 | 0.7 | 0.37 | 0.4 | 26.02 |
| MS153 | 19.66 | 5.24 | 0.6 | 0.14 | 1.66 | 27.3 |
| MS71 | 8.91 | 3.04 | 0.22 | 0.06 | 0.24 | 12.47 |

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|---|---|---|---|---|---|---|
| MT42 | 10.26 | 6.86 | 0.68 | 0.76 | 1.92 | 20.48 |
| N192 | 10.71 | 5.98 | 1.01 | 0.57 | 0.52 | 18.79 |
| N28HT | 22.9 | 9.17 | 0.9 | 0.31 | 0.58 | 33.86 |
| N6 | 9.5 | 5.84 | 1.59 | 0.65 | 3.01 | 20.59 |
| N7A | 24.48 | 6.18 | 1.48 | 1.34 | 0.64 | 34.12 |
| NC222 | 14.83 | 7.88 | 2.19 | 0.63 | 4.15 | 29.68 |
| NC230 | 7.26 | 13.69 | 1.94 | 0.36 | 3.47 | 26.72 |
| NC232 | 4.26 | 6.77 | 1.03 | 0.1 | 2.12 | 14.28 |
| NC236 | 5.02 | 6.68 | 1.01 | 0.18 | 2.25 | 15.14 |
| NC238 | 11.81 | 5.67 | 0.72 | 0.21 | 2.09 | 20.5 |
| NC250 | 9.98 | 3.17 | 0.33 | 0.08 | 0.37 | 13.93 |
| NC258 | 11.52 | 6.88 | 0.18 | 0.01 | 0.17 | 18.76 |
| NC260 | 6.84 | 10.1 | 1.72 | 0.06 | 1.59 | 20.31 |
| NC262 | 4.89 | 13.66 | 1.4 | 0.09 | 1.46 | 21.5 |
| NC264 | 13.63 | 6.07 | 0.32 | 0.08 | 0.68 | 20.78 |
| NC268 | 10.03 | 12.56 | 3.79 | 0.29 | 2.03 | 28.7 |
| NC290A | 9.06 | 16.95 | 2.87 | 0.35 | 3.58 | 32.81 |
| NC292 | 13.88 | 6.29 | 1.67 | 0.77 | 0.71 | 23.32 |
| NC294 | 17.62 | 4.86 | 0.95 | 0.98 | 3.9 | 28.31 |
| NC298 | 18.1 | 18.32 | 2.83 | 0.22 | 2.76 | 42.23 |
| NC300 | 0.7 | 1.04 | 3.08 | 0.23 | 1.9 | 6.95 |
| NC302 | 2.37 | 17.91 | 2.11 | 0.08 | 1.56 | 24.03 |
| NC306 | 7.49 | 9.59 | 2.14 | 0.13 | 1.07 | 20.42 |
| NC308 | 12.9 | 8.2 | 2.17 | 0.46 | 1.33 | 25.06 |
| NC310 | 3.47 | 1.69 | 0.17 | 0.05 | 0.07 | 5.45 |
| NC312 | 3.49 | 3.23 | 0.42 | 0.08 | 0.18 | 7.4 |
| NC314 | 5.4 | 7.26 | 0.9 | 0.08 | 0.34 | 13.98 |
| NC318 | 3.99 | 3.85 | 0.28 | 0.05 | 0.34 | 8.51 |
| NC320 | 2.75 | 6.93 | 0.78 | 0.01 | 0.64 | 11.11 |
| NC322 | 4.59 | 3.89 | 0.2 | 0.03 | 0.19 | 8.9 |
| NC324 | 5.76 | 5.36 | 0.37 | 0.05 | 0.12 | 11.66 |
| NC326 | 11.71 | 4 | 0.59 | 0.39 | 0.24 | 16.93 |
| NC328 | 5.72 | 1.53 | 0.15 | 0.07 | 0.24 | 7.71 |
| NC330 | 12.9 | 4.04 | 0.97 | 0.55 | 0.33 | 18.79 |
| NC332 | 4.03 | 4.2 | 0.24 | 0.04 | 0.25 | 8.76 |
| NC334 | 3.82 | 4.32 | 0.26 | 0.04 | 0.21 | 8.65 |
| NC338 | 3.64 | 0.38 | 0.63 | 0.28 | 0.68 | 5.61 |
| NC342 | 11.21 | 8.7 | 0.92 | 0.17 | 1.02 | 22.02 |
| NC344 | 12.39 | 7.609 | 0.394 | 0.04 | 0.321 | 20.754 |
| NC350 | 2.655 | 1.488 | 2.356 | 0.44 | 2.46 | 9.399 |
| NC354 | 8.528 | 19.195 | 4.606 | 0.78 | 5.188 | 38.297 |
| NC356 | 8.366 | 31.93 | 4.124 | 0.3 | 2.561 | 47.281 |
| NC358 | 17.172 | 8.319 | 1.921 | 1.09 | 0.787 | 29.289 |
| NC360 | 9.259 | 15.615 | 1.407 | 0.11 | 0.807 | 27.198 |
| NC362 | 14.37 | 13.661 | 2.4 | 0.36 | 3.105 | 33.896 |
| NC364 | 12.523 | 12.586 | 2.358 | 0.35 | 2.707 | 30.524 |
| NC366 | 11.024 | 14.843 | 3.189 | 0.6 | 2.594 | 32.25 |
| NC368 | 10.557 | 7.91 | 1.65 | 0.34 | 0.651 | 21.108 |
| NC370 | 1.751 | 7.807 | 0.504 | 0.09 | 0.735 | 10.887 |
| NC372 | 11.161 | 2.849 | 0.595 | 0.43 | 0.322 | 15.357 |

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|---|---|---|---|---|---|---|
| ND246 | 16.061 | 4.164 | 0.239 | 0.43 | 1.106 | 22 |
| OH40B | 27.309 | 4.319 | 0.824 | 0.88 | 2.051 | 35.383 |
| OH43 | 24.9 | 7.017 | 0.781 | 0.33 | 2.254 | 35.282 |
| OH43E | 8.603 | 8.389 | 2.21 | 0.43 | 5.267 | 24.899 |
| OH7B | 17.856 | 10.097 | 1.062 | 0.5 | 0.812 | 30.327 |
| OS420 | 14.614 | 7.573 | 1.018 | 0.76 | 2.851 | 26.816 |
| PA762 | 16.139 | 6.407 | 0.941 | 1.08 | 3.383 | 27.95 |
| PA875 | 11.807 | 3.789 | 0.348 | 0.25 | 0.889 | 17.083 |
| PA880 | 12.29 | 4.774 | 0.324 | 0.09 | 0.288 | 17.766 |
| PA91 | 10.193 | 11.076 | 1.897 | 0.52 | 1.457 | 25.143 |
| R109B | 5.797 | 5.759 | 1.85 | 1.43 | 0.658 | 15.494 |
| R168 | 10.005 | 4.586 | 0.358 | 0.12 | 0.308 | 15.377 |
| R177 | 20.075 | 7.118 | 1.951 | 0.59 | 1.008 | 30.742 |
| R229 | 15.967 | 6.084 | 1.5 | 1.06 | 1.123 | 25.734 |
| R4 | 24.006 | 5.072 | 1.063 | 1.36 | 0.779 | 32.28 |
| SC213R | 11.008 | 8.481 | 1.42 | 0.32 | 3.696 | 24.925 |
| SC357 | 13.368 | 11.892 | 0.839 | 0.1 | 0.866 | 27.065 |
| SC55 | 2.087 | 0.142 | 0.601 | 0.87 | 7.294 | 10.994 |
| SD40 | 9.87 | 12.062 | 1.775 | 0.07 | 1.648 | 25.425 |
| SD44 | 13.917 | 5.12 | 0.662 | 0.22 | 1.953 | 21.872 |
| T232 | 5.649 | 10.786 | 3.783 | 0.35 | 1.12 | 21.688 |
| T234 | 7.004 | 0.479 | 0.096 | 0.12 | 0.808 | 8.507 |
| T8 | 8.102 | 8.779 | 0.85 | 0.03 | 1.045 | 18.806 |
| TX303 | 15.947 | 6.24 | 0.712 | 0.55 | 3.396 | 26.845 |
| TZI18 | 14.396 | 21.598 | 3.466 | 0.36 | 2.36 | 42.18 |
| TZI25 | 9.871 | 10.168 | 3.695 | 0.56 | 1.985 | 26.279 |
| VA102 | 9.849 | 18.319 | 4.193 | 0.62 | 2.299 | 35.28 |
| VA14 | 16.62 | 5 | 0.544 | 0.19 | 0.544 | 22.898 |
| VA17 | 13.972 | 6.386 | 0.856 | 0.25 | 0.673 | 22.137 |
| VA22 | 4.926 | 3.29 | 0.457 | 0.12 | 0.19 | 8.983 |
| VA26 | 14.739 | 4.316 | 0.332 | 0.38 | 1.619 | 21.386 |
| VA35 | 11.156 | 15.022 | 2.809 | 0.26 | 1.792 | 31.039 |
| VA59 | 6.848 | 9.391 | 1 | 0.13 | 0.327 | 17.696 |
| VA85 | 17.559 | 2.486 | 2.002 | 1.04 | 1.135 | 24.222 |
| VA99 | 6.185 | 16.012 | 2.407 | 0.19 | 2.637 | 27.431 |
| VAW6 | 7.172 | 1.488 | 0.265 | 0.14 | 1.83 | 10.895 |
| W182B | 10.734 | 6.967 | 0.963 | 0.11 | 0.721 | 19.495 |
| W22 | 8.853 | 2.587 | 0.299 | 0.13 | 0.223 | 12.092 |
| W401 | 7.589 | 1.162 | 0.509 | 1.02 | 0.344 | 10.624 |
| W64A | 10.091 | 3.765 | 0.3 | 0.26 | 0.114 | 14.53 |
| WD | 11.751 | 17.299 | 5.354 | 1.35 | 3.007 | 38.761 |
| WF9 | 10.415 | 4.496 | 0.76 | 0.15 | 0.408 | 16.229 |
| YU796NS | 14.324 | 2.716 | 0.727 | 0.32 | 1.481 | 19.568 |

**Table S5.** PCR scores for the diverse maize association panel.

| LINE | 5' | S216 | 3' |
|---|---|---|---|
| 3316 | 250 | Control | 399+502 |
| 3811 | 250:993 | Control | 144+502 |
| 4226 | 250+380 | 395+Control | 399+502 |
| 4722 | 250 | Control | 399+502 |
| A188 | 250 | Control | 399+502 |
| A214N | 250+380 | 395+Control | 399+502 |
| A239 | 250 | Control | 399+502 |
| A272 | 250+380 | 395+Control | 144+502 |
| A4415 | 993 | Control | 399+502 |
| A554 | 250 | Control | 399+502 |
| A556 | 250+380 | 395+Control | 399+502 |
| A6 | 993 | Control | 399+502 |
| A619 | 250 | Control | 144+502 |
| A632 | 250 | Control | 399+502 |
| A634 | 250+380 | 395+Control | 399+502 |
| A635 | 250+380 | 395+Control | 399+502 |
| A641 | 250+380 | 395+Control | 399+502 |
| A654 | 250+380 | 395+Control | 399+502 |
| A659 | 250+380 | 395+Control | 399+502 |
| A661 | 250+380 | 395+Control | 399+502 |
| A679 | 250+380 | 395+Control | 399+502 |
| A680 | 250+380 | 395+Control | 399+502 |
| A682 | 250+380 | 395+Control | 399+502 |
| AB28A | 250 | Null | 399+502 |
| B10 | 250+380 | 395+Control | 399+502 |
| B103 | 250+380 | 395+Control | 399+502 |
| B104 | 250+380 | 395+Control | 399+502 |
| B105 | 250+380 | 395+Control | 399+502 |
| B109 | 250+380 | 395+Control | 399+502 |
| B115 | 250+380 | 395+Control | 399+502 |
| B14A | 250+380 | 395+Control | 399+502 |
| B164 | 250+380 | 395+Control | 399+502 |
| B2 | 250+380 | 395+Control | 399+502 |
| B37 | 250 | Control | 399+502 |
| B46 | 250+380 | 395+Control | 399+502 |
| B52 | 250+380 | 395+Control | 399+502 |
| B57 | 250+380 | 395+Control | 399+502 |
| B64 | 250 | Control | 144+502 |
| B68 | 250+380 | 395+Control | 399+502 |
| B73 | 250+380 | 395+Control | 399+502 |
| B73HTRHM | 250+380 | 395+Control | 399+502 |
| B75 | 250+380 | 395+Control | 399+502 |

| | | | |
|---|---|---|---|
| B76 | 250 | Control | 399+502 |
| B77 | 250 | Control | 144+502 |
| B79 | 250+380 | 395+Control | 399+502 |
| B84 | 250+380 | 395+Control | 399+502 |
| B97 | 250+380 | 395+Control | 399+502 |
| C103 | 250 | Control | 399+502 |
| C123 | 250 | Control | 399+502 |
| C49A | 250+380 | 395+Control | 399+502 |
| CH70130 | 250 | Control | 144+502 |
| CH9 | 250+380 | 395+Control | 399+502 |
| CI.7 | 250 | Control | 399+502 |
| CI1872 | 250 | Control | 144+502 |
| CI21E | 250 | Control | 144+502 |
| CI28A | 250+380 | 395+Control | 399+502 |
| CI31A | 250 | Control | 144+502 |
| CI3A | 250 | Control | 399+502 |
| CI64 | 993 | Control | 144+502 |
| CI66 | 250 | Control | 399+502 |
| CI90C | 250 | Control | 399+502 |
| CI91B | 250+380 | 395+Control | 399+502 |
| CM105 | 250+380 | 395+Control | 399+502 |
| CM174 | 250+380 | 395+Control | 399+502 |
| CM37 | 250+380 | 395+Control | 399+502 |
| CM7 | 250+380 | 395+Control | 399+502 |
| CML10 | 250:993 | Control | 399+502 |
| CML103 | Null | Control | 399+502 |
| CML108 | 250 | Control | 399+502 |
| CML11 | 993 | Control | 399+502 |
| CML14 | 250 | 395+Control | 399+502 |
| CML154Q | 993 | Control | 399+502 |
| CML157Q | 993 | Control | 399+502 |
| CML158Q | 993 | Control | 399+502 |
| CML218 | 250 | Control | 144+502 |
| CML220 | 993 | Control | 399+502 |
| CML228 | 150+280 | Control | 399+502 |
| CML238 | 250 | Control | 144+502 |
| CML247 | 250 | Control | 144+502 |
| CML254 | 250 | Control | 144+502 |
| CML258 | 993 | Control | 399+502 |
| CML261 | 250 | Control | 399+502 |
| CML264 | 993 | Control | 399+502 |
| CML277 | 250 | Control | 144+502 |
| CML281 | 250 | Control | 399+502 |
| CML287 | 993 | Control | 399+502 |

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|---|---|---|---|
| CML311 | 250 | Control | 399+502 |
| CML314 | 250 | 395+Control | 399+502 |
| CML321 | 250:993 | Control | 399+502 |
| CML322 | 993 | Control | 399+502 |
| CML323 | 250 | Control | 144+502 |
| CML328 | 150+280 | Control | 399+502 |
| CML331 | Null | Control | 399+502 |
| CML332 | Null | Control | 399+502 |
| CML333 | 250 | Control | 144+502 |
| CML341 | 993 | Control | 144+502 |
| CML38 | 250 | Control | 144+502 |
| CML45 | 250 | Control | 144+502 |
| CML5 | 993 | Control | 399+502 |
| CML52 | 250 | Control | 399+502 |
| CML61 | 250 | Control | 144+502 |
| CML69 | 250 | Control | 399+502 |
| CML77 | 250 | Control | 144+502 |
| CML91 | 250 | Control | 144+502 |
| CML92 | 250 | Control | 144+502 |
| CMV3 | 250+380 | Null | 399+502 |
| CO106 | 250 | 395+Control | 399+502 |
| CO125 | 250 | Control | 399+502 |
| CO255 | 250 | Control | 399+502 |
| D940Y | 250+380 | Control | 399+502 |
| DE1 | 250+380 | 395+Control | 399+502 |
| DE2 | 250+380 | 395+Control | 399+502 |
| DE3 | 250+380 | 395+Control | 399+502 |
| DE811 | 250 | Control | 399+502 |
| E2558W | 250+380 | 395+Control | 399+502 |
| EP1 | 250 | Control | 399+502 |
| F2834T | 250 | Control | 399+502 |
| F44 | 250 | Control | 399+502 |
| F6 | 250 | Control | 399+502 |
| F7 | 250 | Control | 399+502 |
| GA209 | 250 | Control | 399+502 |
| GT112 | 250 | Control | 399+502 |
| H105W | 250 | Control | 399+502 |
| H49 | 250+380 | 395+Control | 399+502 |
| H84 | 250 | Control | 399+502 |
| H91 | 250+380 | 395+Control | 399+502 |
| H95 | 250+380 | 395+Control | 399+502 |
| H99 | 250 | Control | 399+502 |
| HI27 | 250 | Control | 399+502 |
| HP301 | 250 | Control | 399+502 |

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|---|---|---|---|
| HY | 250+380 | 395+Control | 399+502 |
| I137TN | 993 | Control | 399+502 |
| I205 | 250+380 | 395+Control | 399+502 |
| I29 | 250 | Control | 399+502 |
| IA2132 | 250+380 | 395+Control | 399+502 |
| IA5125 | 250+380 | 395+Control | 399+502 |
| IDS28 | 250+380 | 395+Control | 399+502 |
| IDS69 | 250 | Control | 399+502 |
| IDS91 | 250 | Control | 399+502 |
| IL101 | 250 | Control | 399+502 |
| IL14H | 250+380 | 395+Control | 399+502 |
| IL677A | 993 | Control | 399+502 |
| K148 | 250 | Control | 399+502 |
| K4 | 250 | Control | 399+502 |
| K55 | 250 | Control | 399+502 |
| K64 | 993 | Control | 144+502 |
| KI11 | 250 | Control | 399+502 |
| KI14 | 250 | Control | 399+502 |
| KI2021 | 250 | Control | 399+502 |
| KI21 | 250 | Control | 399+502 |
| KI3 | 993 | Control | 144+502 |
| KI43 | 250 | Control | 144+502 |
| KI44 | 250 | Control | 144+502 |
| KY21 | 993 | Control | 144+502 |
| KY226 | 250 | Control | 399+502 |
| KY228 | 250:993 | Control | 399+502 |
| L317 | 250+380 | 395+Control | 399+502 |
| L578 | 250 | Control | 399+502 |
| M14 | 250 | Control | 399+502 |
| M162W | 250+380 | 395+Control | 399+502 |
| M37W | 250 | Control | 144+502 |
| MEF156552 | 250+380 | 395+Control | 399+502 |
| MO17 | 250 | Control | 399+502 |
| MO18W | 250+380 | 395+Control | 399+502 |
| MO1W | 250+380 | 395+Control | 399+502 |
| MO24W | 250 | Control | 399+502 |
| MO44 | 250 | Control | 399+502 |
| MO45 | 250+380 | 395+Control | 399+502 |
| MO46 | 250+380 | 395+Control | 399+502 |
| MO47 | 250+380 | 395+Control | 399+502 |
| MOG | 250:993 | Control | 144+502 |
| MP339 | 250 | Control | 144+502 |
| MS1334 | 250+380 | 395+Control | 399+502 |
| MS153 | 250+380 | 395+Control | 399+502 |

| | | | |
|---|---|---|---|
| MS71 | 250+380 | 395+Control | 399+502 |
| MT42 | 250 | 395+Control | 399+502 |
| N192 | 250+380 | 395+Control | 399+502 |
| N28HT | 250+380 | 395+Control | 399+502 |
| N6 | 250 | Control | 399+502 |
| N7A | 250+380 | 395+Control | 399+502 |
| NC222 | 250 | Control | 399+502 |
| NC230 | 250 | Control | 399+502 |
| NC232 | 993 | Control | 399+502 |
| NC236 | 250 | Control | 399+502 |
| NC238 | 250 | Control | 399+502 |
| NC250 | 250 | Control | 399+502 |
| NC258 | 250 | Control | 399+502 |
| NC260 | 250 | Control | 399+502 |
| NC262 | 250 | Control | 144+502 |
| NC264 | 250+380 | 395+Control | 399+502 |
| NC290A | 250 | Control | 144+502 |
| NC294 | 250+380 | 395+Control | 399+502 |
| NC296 | 993 | Control | 144+502 |
| NC296A | 993 | Control | 144+502 |
| NC298 | 250 | Control | 144+502 |
| NC300 | 250 | Control | 144+502 |
| NC302 | 250 | Control | 144+502 |
| NC304 | 993 | Control | 399+502 |
| NC306 | 250 | Control | 399+502 |
| NC310 | 250+380 | 395+Control | 399+502 |
| NC314 | 250 | Control | 399+502 |
| NC318 | 250+380 | Null | 144+502 |
| NC320 | 993 | Control | 144+502 |
| NC324 | 250 | Control | 399+502 |
| NC326 | 250+380 | 395+Control | 399+502 |
| NC328 | 250+380 | 395+Control | 399+502 |
| NC33 | 250 | Control | 399+502 |
| NC336 | 993 | Control | 144+502 |
| NC338 | 250 | Control | 144+502 |
| NC340 | 250 | Control | 144+502 |
| NC342 | 250 | Control | 399+502 |
| NC344 | 250:250+380 | Control | 399+502 |
| NC346 | 993 | Control | 144+502 |
| NC348 | 250 | Control | 144+502 |
| NC350 | 250 | Control | 144+502 |
| NC352 | 993 | Control | 144+502 |
| NC354 | 993 | Control | 399+502 |
| NC356 | 993 | Control | 144+502 |

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|---|---|---|---|
| NC358 | 250+380 | 395+Control | 399+502 |
| NC360 | 250 | Control | 144+502 |
| NC362 | 250 | Control | 144+502 |
| NC364 | 250 | Control | 144+502 |
| NC366 | 250 | Control | 399+502 |
| NC368 | 250+380 | 395+Control | 399+502 |
| ND246 | 250+380 | 395+Control | 399+502 |
| OH40B | 250+380 | 395+Control | 399+502 |
| OH43 | 250+380 | 395+Control | 399+502 |
| OH43E | 250 | Control | 144+502 |
| OH603 | 250+380 | 395+Control | 399+502 |
| OH7B | 250+380 | 395+Control | 399+502 |
| OS420 | 250 | Control | 399+502 |
| P39 | 250+380 | 395+Control | 399+502 |
| PA762 | 250+380 | 395+Control | 399+502 |
| PA875 | 250+380 | 395+Control | 399+502 |
| PA880 | 250+380 | 395+Control | 399+502 |
| PA91 | 993 | Control | 144+502 |
| R109B | 250+380 | 395+Control | 399+502 |
| R168 | 250+380 | 395+Control | 399+502 |
| R177 | 250+380 | 395+Control | 399+502 |
| R229 | 250+380 | 395+Control | 399+502 |
| R4 | 250:250+380 | 395+Control | 399+502 |
| SA24 | 250 | Control | 399+502 |
| SC213R | 250 | Control | 399+502 |
| SC357 | 250 | Control | 399+502 |
| SC55 | 250 | Control | 399+502 |
| SD40 | 250 | Control | 1,2 |
| SD44 | 250+380 | 395+Control | 399+502 |
| SG1533 | 250 | Control | 399+502 |
| SG18 | 250 | Control | 399+502 |
| T232 | 250 | Control | 144+502 |
| T234 | 250+380 | 395+Control | 399+502 |
| T8 | 250 | Control | 399+502 |
| TX303 | 250+380 | 395+Control | 399+502 |
| TX601 | 250 | Control | 144+502 |
| TZI10 | 250 | Control | 144+502 |
| TZI11 | 250 | Control | 399+502 |
| TZI16 | 993 | Control | 399+502 |
| TZI18 | 993 | Control | 144+502 |
| TZI25 | 150+280 | 395+Control | 399+502 |
| TZI8 | 250 | Control | 399+502 |
| TZI9 | 250 | Control | 144+502 |
| U267Y | 250 | Control | 144+502 |

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|---|---|---|---|
| VA102 | 250 | Control | 399+502 |
| VA14 | 250+380 | 395+Control | 399+502 |
| VA17 | 250+380 | 395+Control | 399+502 |
| VA22 | 250+380 | 395+Control | 399+502 |
| VA26 | 250+380 | 395+Control | 399+502 |
| VA35 | 250 | Control | 399+502 |
| VA59 | 250 | Control | 399+502 |
| VA85 | 250+380 | 395+Control | 399+502 |
| VA99 | 993 | Control | 144+502 |
| VAW6 | 250 | Control | 399+502 |
| W117HT | 250 | 395+Control | 399+502 |
| W153R | 250+380 | 395+Control | 399+502 |
| W182B | 250 | Control | 399+502 |
| W22 | 250+380 | 395+Control | 399+502 |
| W22 RR:STD | 250+380 | 395+Control | 399+502 |
| W64A | 250+380 | 395+Control | 399+502 |
| WD | 250 | Control | 399+502 |
| WF9 | 250+380 | 395+Control | 399+502 |
| YU796_NS | 250+380 | 395+Control | 399+502 |

Note: This table includes data for additional lines not used the analysis (e.g. white maize), but which could be useful for breeding efforts.

**Supplementary References**


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