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

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Maize is the dominant subsistence crop in much of sub-Saharan Africa and the Americas, where 17 to 30 percent of children under age 5 are vitamin A deficient. Diet diversification, food fortification, and supplementation have all been used to combat dietary micronutrient deficiencies. However, diet diversification is often limited by crop seasonality, expense, and low bioavailability of green leafy plant carotenoids. In addition, poor infrastructure has limited widespread use of direct vitamin supplementation. Perhaps the most feasible approach, therefore, is biofortification, a process by which staple crops are purposefully bred for higher nutritional density. Biofortified foods could potentially be an inexpensive, locally adaptable, and long-term solution to diet deficiencies.

Maize exhibits considerable natural variation for kernel carotenoids, with some lines accumulating as much as 66 µg/g. (Most yellow maize grown throughout the world has only 0.5–1.5 µg/g). Carotenoids are derived from the isoprenoid biosynthetic pathway, and a "fork" in this road determines which carotenoid is produced.

Intuitively, one way to determine which maize has more of the preferred β-carotene among total carotenoids would be grain color—the more yellow the more β-carotene. However, this was not borne out by this study. The authors suggest marker-assisted selection may prove more efficient than selection based on color alone.

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To dissect the phenotypic diversity, the authors used an association-mapping approach that exploits the genetic diversity of maize to provide resolution within 2000 base pairs. This was complemented 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, 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. The yellow lines averaged 23 µg/g for total carotenoids and 1.7 µg/g for β-carotene.

For association analysis, the authors used a mixed model approach that controlled for complex population and pedigree relationships. Among their sampling of candidate genes, *lycopene epsilon cyclase (lcyE)* had the largest effect on partitioning the two branches of carotenoids and, consequently, on β-carotene and β-cryptoxanthin content. Subsequent haplotype analysis revealed four probable causative polymorphisms for the ratio of α- and β-carotene branches, and these explained 58 percent of the variation.

From this work, the authors drew four conclusions: 1) The most favorable haplotype is found with at least modest frequency in different germplasm sources and thus breeders can select donors from their relatively more adapted sources; 2) The favorable haplotype has a large effect; 3) Visual selection is ineffective for differentiating carotenoid composition and selecting provitamin A compounds; and 4) in comparison with HPLC (high-performance liquid chromatography) analysis of carotenoids, polymerase chain reaction scoring of the *lcyE* locus is much less expensive 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 marker-assisted selection for *lcyE*, should result in increased levels of provitamin A compounds.

To date, marker-assisted selection for natural variation has been limited by resolution and germplasm diversity. Alleles have generally been characterized in the limited genetic background and resolution of biparental QTL (quantitative trait loci) studies, leaving in question their relevance to broader germplasm. As a result, the primary use for marker-assisted selection 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 germplasm. This provides markers more relevant in a broad genetic background and enables breeders to search for favorable alleles in their locally adapted germplasm sources.

Although these genetic results and strategy are encouraging, they need to be part of an overall biofortification effort encompassing breeding infrastructure, seed distribution, societal acceptance, dietary habits, and nutritional impact. The dietary habits of many Africans, who consume maize for all three meals a day, indicate that maize is a good target for biofortification. 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.

HarvestPlus is an international research program that seeks to reduce micronutrient malnutrition by breeding nutrient-dense staple food crops. HarvestPlus Abstracts summarize key findings from research conducted by HarvestPlus collaborators around the world.