MAIZE HarvestPlus: BIOFORTIFYING MAIZE WITH PROVITAMIN A CAROTENOIDS

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Introduction

The HarvestPlus partners, including CIMMYT, IITA, ZARI (Zambian Agricultural Research Institute), and many others are developing biofortified maize to help alleviate malnutrition, which afflicts nearly half of the world's population. Micronutrient malnutrition, caused by inadequate consumption or utilization of iron, zinc or vitamin A, compromises the health of many populations. While the preferred solution is for all to consume balanced, healthy diets, the reality is that many do not currently enjoy access to such diets. Furthermore, recent global trends, such as the "food crisis" of 2008 in South Asia, suggest that as food prices increase, consumption of nutrient rich meats and vegetables may decrease among poor consumers who are driven to consume relatively more of cheaper, staple grains.

Biofortification of staple food grains can be an effective way to provide essential nutrients to consumers whose diets rely heavily on these grains. Other effective strategies include supplements and fortification of foods, but these are costly interventions that require repeated or continuous investments, whereas biofortified crops could be produced and regularly consumed, as long as appropriate varieties are available. The development and promotion of biofortified maize varieties has been an important effort at CIMMYT for more than 30 years (Gunaratna et al., 2010; Krivanek et al., 2007), and HarvestPlus and partners have been working for about 8 years on provitamin A bioforotification of maize.

Micronutrient Biofortification of Maize

Pixley et al. (2007) reported about the opportunities and strategies for biofortified maize during the ninth Asian regional maize workshop held in Beijing, 2005. Since then, excellent review papers have been published by Ortiz-Monasterio et al. (2007) and Pfeiffer and McClafferty (2007a, 2007b). Therefore, the objective of this paper is to report selected highlights of recent progress within the HarvestPlus maize team.

Conventional breeding to increase iron concentration has been mostly discontinued within the maize HarvestPlus team because of limited natural variation found for this trait. However, collaborative research with the USDA Soil and Plant Nutrition Laboratory at Cornell University, USA, has found significant genetic variation for bioavailability of iron in maize, opening a possible strategy which we thus far have not pursued due to prioritization of efforts as well as complexity and cost of the bioavailability assay (Pixley et al., 2011). Selection and breeding to increase zinc concentration in grain is progressing, and genotypes have been found or developed with zinc levels that exceed the target level (about 40 parts per million (ppm)) set by HarvestPlus. Exciting potential exists to enhance the bioefficacy of both provitamin A carotenoids and Zn in maize (or more broadly, in the diet) by increasing concentrations of both simultaneously; Tanumihardjo et al. (2010) have discussed the interrelated nature of these nutrients in processes related to macular protection, membrane transport and retinol formation, which impacts on their utilization by humans. One important complication applies to both iron and zinc: Because these minerals can not be synthesized by plants, they must be taken-up from the soil and translocated within the plant to the grain. This implies that the soil content of iron or zinc, and soil characteristics such as pH may be limiting factors, and even "efficient" genotypes may have low concentrations of these minerals when grown on certain soils.

Most of the maize biofortification work of HarvestPlus concerns breeding for increased concentrations of provitamin A carotenoids. As proof of concept, three cycles of intra-population S1 recurrent selection were completed at CIMMYT for three African open-pollinated varieties (OPVs), 'Obatanpa', 'SAM4' and 'ZM301'. In each selection cycle, the best S_1 lines with desirable agronomic traits and higher levels of provitamin A content were selected and inter-crossed to form the new cycle of selection. A trial consisting of the original cross (i.e. the cross or "backcross zero" between the OPV and a source of high provitamin A concentration) and the F2 for each cycle of selection of each of the three populations was evaluated for agronomic performance at more than 10 sites and for provitamin A concentrations at three sites. Results of analysis of controlled-pollination grain samples harvested from the three sites and analyzed at three laboratories showed a realized gain of 0.9 μg/g of total provitamin A per selection cycle in the three populations. The attainment of significant gains from S_1 recurrent selection across sites and laboratories provides evidence that these traits are heritable and selection gains are possible.

CIMMYT's maize biofortification program is primarily devoted to inbred line and hybrid development, while developing OPVs as a spin-off of this work. The general breeding strategy is to cross elite lines from our breeding programs in Africa, Mexico and elsewhere with source lines that have 10-20 μg/g of provitamin A carotenoids. These F1's are backcrossed to the elite line, and BC1F1 kernels are visually selected for intense orange or yellow color. Experience has convinced us that BC1-derived lines are unlikely to have more than about 5 μg/g of provitamin A, so we now routinely perform a second cross ("2nd dose"), BC1 by provitamin A source line. Best hybrids from this program have been obtained with ≈8 μg/g of provitamin A.

A first generation or set of provitamin A biofortified maize hybrids was tested for two years (2008-2009 and 2009-2010) in multiple locations in Zambia, Zimbabwe and Mexico. Eight hybrids were preliminarily identified as agronomically competitive with currently popular commercial hybrids, and five of these were submitted by ZARI for inclusion in the Zambian national performance trials, as part of pre-release requirements, during summer 2010-2011. Seed of these hybrids is being multiplied for use in farmer-participatory trials and demonstration plots during 2011-2012. Various acceptability trials, including preparation and tasting of popular foods, have been and will continue to be conducted for these hybrids. It is hoped that one or more hybrids will be suitable for release, commercialization and promotion in Zambia by 2013.

The use of marker-assisted selection (MAS) in breeding for provitamin A concentration in maize became possible after Harjes et al. (2008) published results of association mapping research, and PCR-based markers were subsequently developed to enable selection for favorable alleles of *LycE* (lycopene epsilon cyclase), a crucial gene that governs partitioning of alpha and beta carotene branches in the carotenoid biosynthetic pathway. More recently, collaborative research between CIMMYT, the University of Illinois, and Cornell University, reported important allelic variation and developed useful markers for alleles of a second crucial gene that is involved in the conversion of beta carotene into beta cryptoxanthin in the downstream carotenoid pathway, *CrtR-B1* (carotene beta-hydroxylase 1) (Yan et al., 2010). A large-scale allele mining effort is underway at CIMMYT , which has identified a number of lines with favorable allele constitution at either or both the loci in diverse tropical/sub-tropical germplasm backgrounds.

The effectiveness of these markers for selection in tropical and sub-tropical adapted germplasm was validated at CIMMYT using several crosses with diverse genetic backgrounds, including seven segregating for favorable and unfavorable alleles of both genes. Seeds of 400 plants derived from each population were analyzed for carotenoid composition and genotyped using markers linked to the most important alleles of the two key genes to determine their effect on provitamin A content. The results of analysis of the nine genotypic classes in the six crosses showed that these alleles had strong effects, ranging from 43% to 258% increase (average 150% increase, i.e. 250% of the homozygous unfavorable genotype) for provitamin A concentration in all crosses. These markers are currently being used at CIMMYT to enrich provitamin A concentrations in tropical and sub-tropical maize. The markers are also currently being validated at IITA using diverse inbred lines with contrasting provitamin A content.

We are now using MAS to develop better source lines, combining both of these favorable alleles together with above-average concentrations of provitamin A. Use of MAS promises to increase the efficiency and effectiveness of breeding programs for provitamin A, and we expect to soon have hybrids with 10-15 μg/g of provitamin A.

Challenges and opportunities

An important concern in this work relates to the post-harvest retention or loss of provitamin A carotenoids. Both enzymatic and non-enzymatic (oxidative) reactions can result in deterioration of provitamin A carotenoids. Preliminary results suggests that losses are greatest, and may range from 25-50% during the first 4-8 weeks after harvest, and that storage form (i.e. unshelled with or without husks, shelled grain, milled flour, etc.) and conditions (primarily temperature) significantly influence the extent of losses. Of course, cooking also results in some loss of provitamin A carotenoids, but these losses seem to be more consistent and predictable than those caused by other factors mentioned above. The total expected losses from harvest to consumers' plates have been taken into account in setting the breeding targets for biofortification, but improved understanding of retention/losses, as well as other important determinants of bioefficacy and bioeffectiveness, may require periodic adjustments to these target levels.

The greatest challenges in this work are to develop biofortified cultivars that combine: 1) meaningfully enhanced levels of one or more nutrient, 2) competitive agronomic performance (e.g. yield, stress tolerance), 3) farmer acceptance, and 4) consumer acceptance. Data and evidence to date indicate that the technical goals can be achieved, at least in developing agronomically competitive maize with enhanced levels of provitamins A. At the same time, participatory variety selection programs, education and marketing efforts must be combined in a coordinated strategy that paves the way for adoption and consumption of biofortified maize varieties.

The greatest opportunity is to realize the potential of biofortification: Simultaneously increasing productivity and nutritional value of the staple food of millions of consumers, some of whom suffer from micronutrient malnutrition.

For further information, or to request seed of experimental biofortified germplasm, the reader is invited to contact the corresponding author of this manuscript.

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