HarvestPlus

Progress Report
May 2004 – April 2005

1. Update on Administrative and Collaborative Arrangements and Funding

Additions to the Program Management Team

- J.V. Meenakshi (India), Impact and Policy Coordinator, Appointment date: July, 2004, based at IFPRI – United States
- Kwasi Ampofo (Ghana), Reaching and Engaging End User Coordinator, Appointment date: March 2005, Based at CIAT – Uganda.
- Wolfgang Pfeiffer (Germany), Plant Breeding Coordinator, Appointment date: April 2005, Based at CIAT – Colombia.

Funding

Total funding for 2005 is $11.5 million, about 10% less than the $12.7 million proposed in the Biofortification Challenge Program proposal.

If donors continue with their presently stated policies, during 2003-2007 $29.5 million in funding would come from "new sources" (Gates Foundation + USAID/Health) and $18.8 million would come from "traditional sources" (World Bank + USAID/EGAT + DfID + DANIDA + SIDA + Austria).

2. Research Activities and Workplan Updates

2.1. Food Science and Human Nutrition

Retention Studies

Training course in carotenoid assays held at the University of Campinas, Brazil, for laboratory staff from Brazil, Colombia, Mozambique, Mexico, Nigeria, Peru, Philippines, Tanzania, and USA. A second training course was carried out in Brazil for technicians from national institutions working on maize, cassava, and sweetpotato.

An Inter-laboratory (15 labs) proficiency study for carotenoid analyses was completed under the direction of the University of Campinas. Studies on the variability in carotenoid retention after processing and cooking, underway for orange and yellow sweetpotato and cassava roots.

Procedures established for identifying appropriate mineral-free equipment for grinding grains and seeds for assay. Draft protocols for mineral sampling were developed for all crops and are being tested by the CG centers. Studies on mineral leaching in cooking and processing, underway for beans.

Bioavailability of Phase 1 Crops

The Nutrition Dept, Swiss Federal Institute of Technology (ETH), have identified collaborators in Rwanda for an isotopic test meal study to determine the effect of polyphenols and phytic acid on iron absorption from colored beans.

Efficacy study was initiated at CSIRO, Australia, to determine whether zinc from biofortified rice can improve zinc blood levels and both ameliorate gastrointestinal damage and improve gastrointestinal function in hospitalized children with acute diarrhea.

A HarvestPlus/IAEA competitive bid was released for second stable isotope study to determine the fractional zinc absorption for the high zinc wheat compared with normal wheat. This grant will be awarded to the University of Colorado, for work to be done in Pakistan.
Research is needed to develop a method to measure plant ferritin and to determine its bioavailability from Phase 1 crops. Calls for proposals were initiated by HarvestPlus/ International Atomic Energy Agency (IAEA). Grants will be awarded to the Center for BioIron, Children's Hospital Oakland Research Institute; Nutrition Dept, Iowa State University; Institute of Food Technology, UK.

Animal studies have begun at the Nutrition Dept, University of Wisconsin-Madison, to determine the bioefficacy of genetically improved maize

In collaboration with the IAEA, a competitive bid was issued for a stable isotope study to measure the bioefficacy of provitamin A carotenoids in sweetpotato cooked different ways. The grant will be awarded to the Dept of International Nutrition, U.C. Davis, for work to be done in Bangladesh.

Nutritional Breeding Objectives

A proof of concept study on inulin effect on bifidobacteria activity as an iron absorption enhancer was conducted at Cornell University. It showed a dose-response effect of inulin on hemoglobin repletion in anemic piglets. Additional studies will be carried out in 2005.

Colored beans contain chemical compounds in the bean seed coats that inhibit iron bioavailability. Whether the absence of these compounds results in the minerals in white beans being more bioavailable, or that white beans contain enhancing compounds, continues to be investigated through polyphenolics profiling of bean seed coats.

2.2. Plant Breeding

Breeding Phase 1 crops
The crop improvement activities for Phase 1 crops rice, wheat, maize, cassava, maize, beans, and sweetpotato focused i) on exploring the genetic variation for iron, zinc and ß-carotene in adapted and un-adapted germplasm using 'state of art' methodologies and transfer of the target traits into adapted genetic backgrounds; ii) applied breeding based on targeted crossed for micro-nutrients and the development, screening and testing of early segregating-, intermediate-and final germplasm products; iii) establishing the stability of micro-nutrient expression, i.e. GxE interactions, trough multi-location/multi-environment performance trials at CG centers, NARES breeding sites and on-farm trials in target areas; iv) establishing the inheritance of Fe, Zn, provitamins A; gene-discovery with the development of molecular markers/marker populations; v) seed dissemination of progenitor materials and advanced lines to collaborators; vi) capacity building and establishing productive research networks with NARES.

Rice

Micronutrient-dense rice employs a dual strategy considering conventional methods and transgenic approaches. Initially, the introduction of improved varieties will be targeted to Bangladesh, India, Indonesia, the Philippines, and Vietnam.

Initial germplasm screening and field evaluation for Fe and Zn in milled rice included 334 lines and was expanded to 500 accessions of Korean origin based on high iron content in tropical japonica germplasm. Screening in Bangladesh, Indonesia, India, and the Philippines is in progress.

Polished rice genotypes with more than 10 mg/kg iron and more than 30 mg/kg zinc were identified. Fe in novel germplasm sources represents a 300 – 400% increase in iron over polished commercial rice in the Philippines.

Wheat

The strategy centers on improving iron and zinc levels at CIMMYT and NARS in India and Pakistan using conventional methods and investigate a transgenic approach for high ß-carotene wheat. The initial target areas are the Indo-Gangetic plains of Pakistan and India with focus on the Eastern Plains; a region with high population densities and high micronutrient malnutrition.

International micronutrient trial deployed for planting in fall 2004 consisting of biofortified adapted lines with 80-100% increased zinc. Lines will be tested in the target regions in 2005. Thirty-two sets were distributed to India and Pakistan and additional sets deployed to China, Turkey and Mexico for planting on-station and on-farm. The trial will generate genotypic and GxE data on agronomic-, micronutrient- and end-use performance, adaptive pattern, and identify progenitors.

The development and distribution of high-micronutrient elite lines and versions of adapted varieties is fully operational and advanced lines display Fe and Zn levels of 47 mg/kg and 55 mg/kg, respectively. Crop improvement for high Fe and Zn in target NARS commenced. For Zn, strong evidence of transgressive segregation suggests potential to raise the target Zn level for wheat.

Maize

The project concentrates on increasing provitamins-A carotenoids (tentative target level of 15 µg/g ß-carotene) and attempts improving iron and zinc. Initial target countries are Brazil, Ethiopia, Ghana, Guatemala, and Zambia. Linkages with the private sector were established to gain access to advanced technologies and expertise and will be formalized during 2005.

Screening of 235 CIMMYT germplasm sources at Iowa State University revealed concentrations of provitamins >3 µg/g of and >4 µg/g of total provitamins-A (200-275% above the average). Similarly, high ß-carotene and ß-cryptoxanthin genotypes were identified in screening 293 genotypes in Zimbabwe. A total of 2000 samples was harvested in 2004 in Brazil, at CIMMYT, in Guatemala, Zambia, and Ethiopia and prepared for sample analysis.

Breeding efforts included crosses of elite southern African germplasm (inbred lines, populations, QPM) with high ß-carotene sources in 232 unique combinations and germplasm testing.

Cassava

The strategy for cassava is to identify and develop genotypes with high concentrations of provitamins-A in the roots in elite genetic backgrounds and then transfer/share the germplasm among collaborating NARS for further analysis during 2004-2006. CIAT coordinates overall activities for cassava biofortification with responsibility for research in Asia, Latin America, and the Caribbean, while IITA is responsible for cassava biofortification activities in Africa.

Germplasm improvement at CIAT identified new clones with the production of indexed vitroplants, established nurseries to develop new generations of clones, crosses to develop germplasm high in carotenoids and GxE studies. The maximum level of total carotenes from 2004 screening approached 15

µg/g (fresh weight basis). Inheritance studies on the synthesis and/or accumulation of carotenes in root tissue are underway.

Bean

HarvestPlus biofortified bean research focus is on increasing the concentration of iron and zinc in agronomically superior varieties by conventional methods. The project has entered plans for wide scale deployment with the two bean varieties, Gofta and Maharagi Soja that consistently exhibit high iron and/or zinc. The DR of Congo is increasing the seed production of Maharagi Soja and 1500 kg seed of Gofta were produced in Ethiopia. In addition, 38 promising lines have been distributed to Uganda, Ethiopia, Malawi, DR Congo, Kenya, Rwanda, and Tanzania for agronomic evaluation. Forty farmers in Kisii district, Kenya, started evaluating micronutrient rich bean lines on farm and on-farm trials were conducted in three environments in Colombia.

Mineral analyses for 1036 landraces/accessions from Rwanda and 800 new collections from DR Congo and Uganda commenced in 2004.

242 hybrid combinations were created in 2004 including 112 crosses made for climbers and bush between CIAT lines or African cultivars and high iron sources. Interspecific progeny with *P. coccineus* express high iron and zinc and were used as parents for 1st time. The F2 nursery with more than 3000 families yielded 600 drought tolerant selections for further evaluation for disease resistance and mineral analysis. Combinations of drought tolerance with high iron concentration have been confirmed in advanced germplasm.

Sweetpotato

Africa's sweetpotato cultivars are predominantly white or yellow-fleshed varieties that contain insignificant amounts of provitamins-A. Orange-fleshed varieties are believed to be one of the least expensive, rich, year-round sources of provitamins-A available to the poor. 100 g of boiled OFSP can provide more than 100% of the recommended daily allowance for vitamin A for a preschool child and more than the RDA even for adults. To encourage the adoption of OFSP varieties by farmers and consumers, the texture of the OFSP varieties must conform to sensory preferences and the preference of adults for varieties with high dry-matter content addressed in breeding.

About 40 varieties of sweetpotato that are high in both provitamin A and dry matter have been introduced to Sub-Saharan Africa. Virus indexed materials are produced for more than 30 local varieties collected in several countries prior to their regional distribution. High dry matter OFSP varieties obtained from CIP headquarters in Lima, Peru, are currently being evaluated for adaptability to different agro-ecologies in Uganda, Kenya, Ethiopia, Rwanda, Madagascar and Tanzania. More than 10 varieties are being tested in national and regional multi-location GxE evaluation trials across different agro-ecological areas in selected countries to identify at least 4 outstanding OFSP regional varieties for dissemination during 2005. On-farm trials on the adaptability and acceptability studies of OFSP varieties through farmer participatory research are conducted in Ghana, Tanzania, Kenya, Zambia, Rwanda, and Ethiopia.

Crossing in Uganda, South Africa, CIP-Lima and other countries generated in excess of 120,000 seeds for population development of OFSP with high dry matter, ß-carotene and resistance to sweetpotato virus diseases. Over 3 million virus free vines have been multiplied and distributed to establish secondary multiplication nurseries in different regions of Tanzania, South Africa and South Africa. At least nine OFSP varieties have been officially pre-released Uganda (2), South Africa (4) and Ghana (3).

Phase 2 Crops

Many of the world's poor depend on the HarvestPlus Phase 2 crops as their primary staple. These crops include millet, sorghum, potato, pigeon pea, lentil, banana/plantain, barley, cowpea, groundnut, and yams. Pre-breeding studies, germplasm screening, and quantification of GxE are research activities common for Phase 2 crops. Several Phase 2 crops expanded research to the full range of activities described earlier for Phase I crops; one example is potato with plans to distribute germplasm under a 'Fast Track' scheme. In addition, secondary data are being collected to determine the location and consumption patterns of malnourished populations that consume Phase 2 crops.

2.3. Biotechnology and Nutritional Genomics

The biotechnology- nutritional genomics component of HarvestPlus focuses on three distinct sets of activities including:

Quantitative traits Loci (QTL) for seed carotenoids in model species to better understand the fundamental processes affecting carotenoid synthesis and accumulation in seeds. In 2004, the analysis of the RIL population grown in Houston for carotenoid QTLs was completed. A major focus of the work is to genetically identify and physically isolate quantitative trait loci (QTL) in Arabidopsis thaliana that impact carotenoid levels in seed and understand the molecular and biochemical mechanism(s) involved. To date four major QTL that impact total carotenoids or specific carotenoids in Arabidopsis seed were identified and fine mapping was initiated for specific loci..

Metal homeostasis genes identified and characterized and gene specific markers developed. Result from this activity will help breeders with identified markers related to genes that contribute to the delivery of iron and zinc to seeds. Close collaboration was established between the nutritional genomics team, CIMMYT, and Sabanic University-Turkey) for metal-related genes in wheat. PCR based products were generated on several wheat parental lines using publicly available ESTs for metal-related genes in wheat. The genotype-specific products are now being sequenced to identify polymorphisms between parents and will be used to develop markers for screening segregating populations to map wheat metal-related genes.

Technologies to improve the Golden Rice strategy. Because of possible co-suppression phenomena that may occur during later generations it is desirable to have a Golden Rice version that relies on bacterial genes only. Early evidence suggests better pathway effectiveness, most likely because of improved protein-protein interaction between the two bacterial proteins.

2.4 Impact and Policy

Ex ante models

Models for analyzing the health burden of iron and zinc deficiency, thereby enabling the quantification, of the potential benefits of biofortification have been developed. The framework used is Disability-Adjusted Life Years (DALYs). The countries for which the estimates are being computed include: Bangladesh, Brazil, Democratic Republic of Congo, Ethiopia, Honduras, India, Nigeria, Pakistan, Philippines and Uganda.

Ex post analyses

The next set of impact analyses (to be implemented in 2005 and beyond) have been modified and developed to take into account the 'fast tracking' of some of the Phase 1 crops. Emphasis will be placed on consumption surveys in countries that are the focus of the micronutrient-dense sweet potato, beans and wheat varieties. Impact is working closely with the proposed 'Reaching End User' component of

HarvestPlus to ensure rigorous monitoring and evaluation systems and also to participate in the development and implementation of the diagnostic studies being planned under that component.

2.5 Reaching End Users

In the original proposal, it was an important oversight to postpone collaborative interaction with institutions/implementers related to "Reaching and Engaging End-users" (that is, moving the biofortified varieties from the research station to consumers) until after nutritionally-improved varieties had been tested and proven for their potential to improve micronutrient status and ready for distribution. Additional funding is needed (and is being sought) for these activities, especially in view of "fast-track" opportunities in particular for disseminating orange-flesh sweet potatoes, and to some extent for high-iron beans and high-zinc wheat as well.

The HarvestPlus "Reaching and Engaging End-Users" meeting held in May, 2004, in Rome was quite useful in highlighting the range of complexities involved in transferring both "invisible" products (e.g. the iron and zinc content of grain) and "visible" products (e.g. beta-carotene in roots and tubers) to the intended beneficiaries. Relevant interventions include participatory breeding and participatory varietal selection, a clear understanding the marketing chain and marketing channels, convincing "enablers" such as influential agriculture and health officials to support the biofortification strategy, and nutrition communication.

3. New Initiatives

HarvestPlus China

A meeting of about a dozen Chinese scientists and the HarvestPlus program director was convened in May, 2004, at the Chinese Academy of Agricultural Sciences, to discuss the feasibility of formation of a HarvestPlus China program, similar to HarvestPlus, to be funded by the Chinese government and other donors. The idea was received positively and a follow-up two-day meeting involving 45 Chinese scientists and representatives of HarvestPlus was held in Beijing in November to plan organizational and research activities for 2005. HarvestPlus agreed to provide $350,000 to fund research proposals under a competitive bidding process to stimulate interest in the biofortification strategy in China. Seven of sixteen proposals submitted were selected for funding in April 2005. The selected proposals will provide $350,000 in counterpart funding from Chinese sources.

Latin America

A large research activity specifically directed to developing biofortified crops for Latin America was initiated by CIAT. The research planned for this regional activity draws on lessons learned from HarvestPlus and will feed into the work currently underway by the HarvestPlus research program. A grant of $16 million over six years from CIDA witll fund this work

4. Communications In Reach/Outreach

The Hub is the program's collaborative intranet platform where members can participate in discussions related to areas of expertise, view and store documents, select presentation and collaboration tools and work with other members of the HarvestPlus team. Membership = 125.

A technical monograph series, abstract series, and seminar series were initiated. HarvestPlus held meetings to strengthen and coordinate intra-program activities as well as present the program to key stakeholders in research and development.