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Title: ***BioCassava Plus*****, Providing Complete Nutrition**

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Abstract:

BioCassava Plus is a multidisciplinary team of scientists brought together to develop cassava cultivars that provide complete nutrition for sub-Saharan Africans. *BioCassava Plus* will develop novel transgenic cassava germplasm with increased bioavailable levels of zinc, iron, protein and vitamins A and E. Effective delivery of the enhanced cassava will be achieved by linking optimal nutritional traits with improved post-harvest durability, reduced cyanogen content, and elevated viral disease resistance; farmer incentives that will facilitate adoption of *BioCassava Plus* cultivars. The *BioCassava Plus* program is distinguished by effective collaboration with African partners, and field and human feeding trials to demonstrate efficacy.

Key Words: Cassava, Manihot esculenta, human nutrition, germplasm, transgenic, BioCassava Plus

Malnutrition and Food Insecurity in Africa

The majority of countries in sub-Saharan Africa fail to produce consistently adequate amounts of nutritionally balanced foods to meet the needs of their populace. Reductions in the incidence of under-nutrition achieved in Latin America and Asia achieved by the Green Revolution have not been matched in Africa (Rosegrant et al. 2001). Sub-Saharan Africa remains the only region in the world where both the total number and percentage of malnourished children continues to increase.

Protein-energy malnutrition afflicts 30% of sub-Saharan Africa's children and contributes to 55% of childhood deaths in the region (Pelletier et al. 1995). Severe malnutrition, manifest as the potentially fatal kwashiorkor and marasmus, is seen in 10% of children in sub-Saharan Africa. Moderate malnutrition causes retarded physical and mental development and increased susceptibility to infection. The burden of moderate malnutrition on sub-Saharan Africa is evidenced by the prevalence of stunting in children under the age of five, estimated to be 42%, and exemplified by recent studies in Burkina Faso which show that malnourished children infected with malaria have two-fold higher morbidity rates then well-nourished children (Unicef, 2003; Muller et al., 2003).

Vitamin and mineral deficiencies have substantial deleterious impacts on human health and economic productivity. It is estimated that 50% of the population of sub-Saharan Africa suffers from iron deficiency, 33% from zinc deficiency and 90% of children from the region receive inadequate amounts of vitamin A (Micronutrient Initiative, 2004). These deficiencies manifest themselves as anemia, eye disease, increased susceptibility to infection, impaired neuropsychological development, and stunting (FAO). Vitamin and mineral deficiencies often overlap and interact, resulting in individuals who are recurrently ill, or limited in mental or physical capacity; thereby compounding the burden on families, health services, education systems, and local and national economies. Agricultural development strategies that provide sufficient and reliable sources of energy, protein and essential micronutrients are desperately needed in sub-Saharan Africa.

Cassava, The African Staple

Since its introduction into Africa in the 16th century, cultivation of the starchy root crop cassava (*Manihot esculenta*, Crantz), has expanded to more than 100 million ha, with average production now approximately 150 kg/yr of fresh roots per person (FAO database). Cassava and maize are the most important staple crops in sub-Saharan Africa. In excess of 200 million people in sub-Saharan Africa rely on cassava as their major source of dietary energy. An estimated 95% of all cassava in Africa is cultivated by resource-poor, subsistence farmers mostly for on-farm consumption, with surpluses traded in fresh and processed forms in local markets. Typical African cassava harvests yield 8-10 tons/ha fresh weight, but when grown under ideal conditions cassava can attain yields of 90 tons/ha (FAO database).

Several characteristics make cassava ideally suited to subsistence farming in Africa. A flexible harvest window allows cassava roots to be “stored” in the soil and serve as a reserve food source for up to 30 months following planting. The crop is also favored because of its robust tolerance to biotic and abiotic stress and ease of cultivation. When and where other crops fail, cassava can be relied upon as a source of food. It can be grown successfully on infertile soils and can tolerate drought for up to 90 days by dropping its leaves and utilizing stored energy within its root system to re-establish the leaf canopy when water once again becomes available. This contrasts with maize, which is poorly adapted to drought stress and is subject to large fluctuations in yield depending on availability of water. The cultivation of cassava requires less labor than other staple crops. Cassava is typically planted at the start of the rainy season and harvested 12-18 months later. Once the leaf canopy forms, little weeding is required. Such low input requirements mean that cassava is displacing crops that need more extensive weeding, irrigation, fertilizer and/or pesticide inputs. As a result, the total land committed to cassava cultivation in Africa has increased by 23% since 1990 (compared to 10% for maize over the same period) indicating that this crop remains preferred by farmers and will play an increasingly important role in the nutritional and economic well-being of Africans in the 21st century (FAO Database).

Nutritional Value of Cassava

Although possessing many desirable agronomic qualities, and an excellent source of energy, foods produced from cassava roots are not nutritionally balanced. Cassava is a high-energy food; consumption of 1 kg/day of cassava provides 130% of the daily energy requirement. However, cassava roots have the lowest protein:energy ratio (0.03) of all the major crops, (Scott et al., 2000) with a typical cassava-based diet providing <30% of the minimum daily requirement for protein. In addition, cassava-based meals provide only 10-20% of the required amounts of iron, zinc, vitamin A and vitamin E (Lyimo et al., 1991). Low levels of iron and zinc are compounded by the presence of the anti-nutrient phytate. Cassava is rich in phytate which chelates divalent minerals and inhibits their absorption from the human gastrointestinal tract. Foods with good iron and zinc bioavailability have a phytate/Zn molar ratio < 5:1 with inhibition occurring when the phytate/Zn is > 15:1 (Larsson et al., 1996). Many processed cassava products have a phytate/Zn molar ratio of 25, indicating that bioavailability of zinc, iron, calcium and copper from cassava-derived foods is low (Ferguson et al. 1989).

Cassava is also well known for the presence of toxic, cyanogenic glycosides within its leaves and roots. Cassava must be processed to remove cyanogens prior to human consumption, which usually consists of soaking and drying of mashed roots. When cyanogenic glycosides are ingested by humans they act as tissue toxins destroying neural and hepatic cells, and are metabolized to isothiocyanate which interferes with iodine metabolism and thyroxin production (Thilly et al., 1993). Properly processed cassava is a safe and invaluable source of food. However, in Africa, a number of cyanide-associated health disorders have been attributed to eating inadequately processed cassava, including goiter and tropical ataxic neuropathy from chronic cyanide consumption and permanent paralysis of the lower extremities and death from acute consumption of unprocessed cassava (Howlett et al., 1990; Tylleskar et al., 1992; Boivin, 1997; Banea-Mayambu et al., 1997). All these conditions are exacerbated in people suffering from under-nutrition.

Two of the most important constraints limiting full realization of cassava’s potential in Africa are the short storage life of harvested roots and the crop’s susceptibility to cassava mosaic disease (CMD). Within 72 hrs post-harvest (removal from plant) storage roots deteriorate such that they are inedible and unmarketable. It has been estimated that up to 26% of all cassava produced in Africa, some 18 million tons of fresh roots, is lost every year to post-harvest physiological deterioration (PPD) (Wenham, 1995). PPD has been demonstrated to be a polygenic trait, caused by a cascade of physiological events triggered by oxidative damage at the wound surface (Reilly et al., 2003).

CMD is endemic to all the cassava growing regions of Africa. Transmitted by at least six species of whitefly-transmitted geminiviruses, CMD is the single greatest constraint to cassava production in that continent. Incidence of CMD can be as high as 100% of all plants in all fields of a given region, with average yield reductions of 30-40%. Overall, it is estimated that more than 30 million tons of fresh cassava roots are lost each year to the impact of CMD.

Together, poor post-harvest durability and susceptibility of virus disease limit delivery of the full nutritional value of cassava to consumers in sub-Saharan Africa. Populations that rely on cassava as a major component of their diet are at risk from insufficient intake of zinc, iron, protein, vitamins A and E. In addition, cassava is rich in the anti-nutrients phytate and cyanogenic glycosides, which can also compromise human health. Enhancing the bioavailable levels of protein and micronutrients in commonly grown cassava cultivars, decreasing the anti-nutrient content and ameliorating losses from

CMD and PPD would improve human nutrition and health, food security and economic well-being of hundreds of millions of Africans.

***BioCassava Plus* will develop cassava to improve the nutritional status of sub-Saharan Africans**

As a major crop of resource-poor farmers, cassava offers an ideal vehicle for delivering improved nutrition in a sustainable manner to rural areas where other nutritional and development programs are least effective. *BioCassava Plus* will develop novel cassava cultivars with increased levels of the nutrients presently lacking in the diet of hundreds of millions of Africans, and the increases will be such that consumers of *BioCassava Plus* cassava plants will receive a complete, balanced, and nutritious diet. Modern biotechnologies will be used to enhance the content and bioavailability of zinc, iron, protein, vitamin A, and vitamin E in cassava roots. In addition, cassava will be developed with improved post-harvest durability, reduced cyanogen levels, and elevated resistance to CMD; traits that are required to deliver the full benefit of the nutritionally improved cassava and to provide the necessary incentives for farmers to adopt and sustain the use of cassava germplasm developed within *BioCassava Plus*.

BioCassava Plus centers largely, but not exclusively, on the use of transgenic technologies to deliver enhanced products to farmers and consumers in Africa. The heterozygous nature of cassava and inherent inbreeding depression renders conventional breeding problematic, and dictates that sexual crossing alone is not capable of generating cassava germplasm that is nutritionally complete. Transgenic technologies provide a unique capability to integrate multiple traits for enhanced nutrition into farmer-preferred cassava varieties in a short period of time. Significant advances in producing transgenic cassava plants expressing agronomic traits, and their testing in East Africa, now make this, for the first time, a practical approach for delivering products to farmers.

The *BioCassava Plus* program consists of two approaches for improving the nutritional value of cassava roots. Existing technologies for which a proof of principle has been successfully demonstrated in other crops, such as maize, barley and potato will be modified and applied to cassava to enhance micronutrient, protein and vitamin content. Advantageous characteristics will be proven by a combination of plant composition studies, field testing in the tropics and human feeding trials, in order to assess stability of the trait and bioavailability of the enhanced nutritional content in cassava foodstuffs. Innovative research will also be applied to increase our understanding of the unique biochemistry and physiology of cassava’s nitrogen metabolism and post harvest characteristics.

Knowledge gained from the latter activities hold the key to developing future technologies to unlock the full potential of cassava.

Transgenic Technologies in Cassava: *BioCassava Plus* is reliant on the ability to integrate and express genes imparting enhanced nutritional qualities in cassava. The team assembled represents the world's most accomplished laboratories in this field, ensuring that the technical capacity to deliver improved cassava for field and feeding tests is in place. Since the first reported recovery of transgenic cassava plants by the researchers now based at the Danforth Center and the Institute for Plant Sciences ETH-Zentrum, team members have continued to both improve the technology and report production of transgenic cassava expressing agronomically important traits (Siritunga and Sayre, 2003, 2004; Zhang et al., 2003a, b).

Increasing Zinc and Iron Content and Bioavailability: The nutritional quality of cassava roots of 600 cassava cultivars has been extensively evaluated by Dr. Hernan Ceballos at CIAT. Investigators at CIAT have determined that none of cultivars screened can provide the daily allowance necessary for people having cassava as the most important food in their diet (CIAT, 1994). As part of the *BioCassava Plus* strategy, several transgenic approaches will be used to increase the essential trace metal content and its bioavailability in transgenic cassava. Genes encoding essential trace metal transporters will be co-expressed along with gene silencing constructs targeted to reduce endogenous phytate levels in transgenic cassava to both increase the trace metal content as well as make it more bioavailable.

Recently, Dr. Dan Schachtman's lab has demonstrated that the over-expression of a plant plasma membrane metal transporter (AtZIP) increases zinc content in specific plant organs (Ramesh et al., 2004). The ZIP zinc transporter is found in all plant species including monocots and as well as dicots and plays an important role in plant zinc and iron transport (Ramesh et al., 2003; Guerinot, 2000; Guerinot and Salt, 2001). Over-expression of the *Arabidopsis* ZIP1 in barley using a constitutive promoter increases short-term zinc uptake rates when plants are grown under zinc deficient conditions (Ramesh et al., 2004). In the long-term these transgenic barley lines had 2 to 3 fold higher zinc and iron content in seeds.

In 2002, Dr. Richard Sayre's lab identified a novel iron assimilatory protein, FEA1, from the unicellular algae, *Chlamydomonas reinhardtii*. FEA1 was able to complement yeast high-affinity iron transporter (*fet3* and *ftr1*) mutants (Rubinelli et al, 2002). This FEA1 protein has been transgenically transferred to *Arabidopsis* roots and its expression substantially enhances plant seedling emergence by 50% under conditions where iron is a limiting nutrient. Additional gene silencing strategies will be used to inhibit phytate production and increase the bioavailabilty of essential trace metals.

Enhancing protein content in cassava roots:

A variety of transgenic strategies will be used to increase the protein content in cassava. These strategies focus on increasing both the pool size of free amino acids available for protein synthesis in roots and the expression of nutritious root storage proteins.

Recently, the Sayre lab demonstrated a novel relationship between cyanogens and amino acid/protein synthesis in cassava. They selectively inhibited the expression of enzymes that catalyze linamarin synthesis in leaves and roots by antisense inhibition of *CYP79D1/D2* gene expression (Siritunga and Sayre, 2003). Transgenic plants in which the expression of these genes was selectively inhibited in leaves had a 60-94% reduction in leaf linamarin content. Surprisingly, these plants had more than a 99% reduction in root linamarin content. In contrast, transgenic plants in which the CYP79D1/D2 transcripts were reduced to non-detectable levels in roots had normal root linamarin levels. These results demonstrated that linamarin is synthesized in leaves and transported to roots. Transgenic plants having reduced leaf and root linamarin contents were unable to grow in the absence of NH_3. It was proposed that linamarin functions as a mobile form of reduced nitrogen for root amino acid synthesis (Siritunga and Sayre, 2004). The partitioning of linamarin between storage in root vacuoles and assimilation of linamarin-derived CN into amino acids represents a potential control point for regulating linamarin storage or amino acid synthesis. There is now a substantial body of work indicating that free-amino acid pool sizes in plants regulate the plant’s ability to produce proteins (Tabe et al., 2002; Hagan et al., 2003). The Sayre lab will engineer root linamarin metabolism and partitioning to increase root protein content while also reducing root cyanide toxicity.

An additional strategy for increasing the protein content of cassava is to over-express cassava root storage proteins and/or artificial storage proteins in transgenic cassava. Dr. Ceballos and Dr. Claude Fauquet’s lab will identify potential candidate cassava root storage proteins by proteomic analyses of high-protein cultivars of cassava. The identified root protein storage genes will then be over-expressed in transgenic cassava cultivars to increase protein content.

Dr. Peng Zhang has genetically transformed plants of the West African cassava cultivar 60444 with an artificial storage protein *ASP1* gene, designed to be rich in essential amino acids (Kim et al., 1992). Analysis of regenerated tissues confirmed expression of the transgene at both the RNA and protein levels. Total protein content of *in vitro* leaves was found not to differ from the non-transgenic plants, but levels of the amino acids proline and serine were significantly elevated and asparagine, alanine and methionine depressed compared to controls (Zhang et al., 2003b). These data demonstrate that it is possible to manipulate protein quality in cassava through a transgenic approach.

Enhancing Vitamin A and E Content: Both vitamin A and E are present in suboptimal concentrations in cassava roots. Since both vitamins share common biosynthetic precursors we will explore elevating the levels of both vitamins by increasing flux rates through common precursors as well as specifically targeting enzymes regulating the synthesis of individual vitamins.

Dr. Ed Cahoon's lab has successfully used molecular strategies for increasing vitamin E content in plants. The committed step in the biosynthesis of the tocotrienol class of vitamin E is catalyzed by the enzyme homogentisic acid geranylgeranyl transferase (Cahoon et al., 2003). The cDNAs encoding this enzyme were isolated from barley, rice and wheat. Transgenic expression of the barley homogentisic acid geranylgeranyl transferase in *Arabidopsis* resulted in a 10-15 times increase in vitamin E accumulation in leaf tissues. Similarly, over-expression of the same gene in maize seeds led to six-fold increase in total content of vitamin E antioxidants. Related research by other labs involving up-regulation of homogentisic acid phytyltransferase, the committed step in tocopherol synthesis, resulted in a four-fold and two-fold increase in the tocopherol class of vitamin E in *Arabidopsis* leaves and seeds, respectively (Collakova and DellaPenna, 2003; Savidge et al., 2002).

Two well-documented products exist in which accumulation of provitamin A (β-carotene) in plant tissues has been achieved through the expression of transgenes, resulting in some cultivars with vitamin A content increased 50-fold (Shewmaker et al., 1999; Ye et al., 2000). The first committed step in vitamin A synthesis is catalyzed by phytoene synthase, which uses the same substrate as the vitamin E precursor. Cahoon's success in using the transgene approach with vitamin E, the observation that there are natural cassava roots with high vitamin A content and the success using the transgene approach in other species with vitamin A indicate that this approach is likely to allow the *BioCassava Plus* team to develop cassava with increased amounts of vitamins A and E.

Control of cyanogenesis: Recently, it has been hypothesized that linamarin, synthesized in leaves and transported to roots, is both stored in root vacuoles as well as providing reduced nitrogen for amino acid and protein synthesis in roots (Siritunga and Sayre 2004).

The Sayre lab will explore transgenic strategies to divert linamarin from storage to protein synthesis in roots. This approach to enhancing root protein levels is expected to substantially reduce linamarin content, resulting in a less toxic cassava cultivar. An alternative strategy for reducing cyanogen toxicity in cassava foods that has been utilized by the Sayre lab is to accelerate cyanogenesis and cyanide volatilization during food processing. To achieve this objective, the Sayre lab over-expressed the leaf-specific enzyme, hydroxynitrile lyase in roots and leaves, which catalyzes the breakdown of acetone cyanohydrin to cyanide (Siritunga et al., 2004). Expression of hydroxynitrile lyase in roots accelerated cyanogenesis more than three-fold, preventing the accumulation of acetone cyanohydrin in poorly processed cassava roots.

Post-harvest physiological deterioration: The molecular basis and control of accelerated post-harvest deterioration in cassava roots remains one of the most challenging aspects for understanding the novel physiology of cassava roots.

Results from Dr. John Beeching's lab have shown an oxidative burst in cassava roots within three hours of harvesting and have demonstrated the involvement of enzymes, including catalase, peroxidases and superoxide dismutases, and compounds (antioxidants and others) that modulate reactive oxygen species during PPD. These data have led to a model for PPD in which reactive oxygen species (ROS) and the enzymes and compounds that modulate them play central roles (Reilly et al., 2003). Recent unpublished data from microarray and Northern analyses have identified several genes that are expressed early during PPD. Of particular interest is a peroxidase, MecPX3, that is expressed within 12 hours of harvesting and is not expressed in unwounded roots, leaves or wounded leaves. This PPD-specific clone promises to be an extremely useful tool for the production of transgenic cassava designed to modulate PPD though enhancing the activity and abundance of anti-oxidant enzymes and compounds. Increased levels of antioxidants using transgenic approaches have been shown to reduce ROS in other plants; over-expression of γ-glutamylcysteine synthetase resulted in 2 to 3-fold increases in glutathione in *Arabidopsis* and poplar (Noctor et al., 1996; Xiang et al., 2001) while over-expression of D-galacturonic acid reductase increased ascorbate levels in strawberry (Agius et al., 2003).

The Sayre lab has demonstrated that ROS production is associated with the presence of cyanogens in transgenic cassava plants. Presumably cyanide blocks the mitochondrial electron transfer chain leading to ROS generation by complex 1. Therefore, strategies to reduce root cyanogen content may lead to increased shelf life for harvested cassava roots.

Dr. John Felman will analyze the volatile compounds produced during early and late stages of PPD to facilitate identification of biochemical pathways contributing to PPD.

Recently, Dr. Martin Fregene's research group identified a *Manihot* species, *M. walkerae* (MWa1001) cross that displays remarkably delayed PPD, i.e., roots remain intact for 30 days after harvest. A single successful cross with cassava showed a dramatically delayed PPD. The molecular and biochemical characterization of these crosses will provide useful information on the control of PPD in cassava.

Cassava mosaic disease: The control of viral diseases in cassava is a constantly moving target as new strains develop and areas of infection increase in magnitude. Recently, pathogen-derived resistance strategies have been developed for cassava by Fauquet and Dr. Nigel Taylor who integrated the *AC1* gene, a replication associated protein from African cassava mosaic virus (ACMV) into the genome of cv 60444. The resulting plants exhibited very high resistance to ACMV, and other geminivirus species from Africa and India (Chellappan et al., 2004). These plants are currently undergoing challenge with CMD pathogens in contained biosafety facilities in Western Kenya, in collaboration with KARI. Several decades of research into CMD, both in the field in Africa and laboratory, has generated a world-class level of expertise in the epidemiology and molecular biology of the cassava-infecting geminiviruses at this laboratory. This includes the recent development of a geminivirus-based gene silencing system for suppression of endogenous and transgene expression in cassava. Fauquet and Taylor have also developed an alternative system for imparting broad spectrum resistance to geminiviruses via a single stranded DNA binding protein (*g5p*) which interferes with geminivirus infection when expressed as a transgene in tomato and tobacco plants (Padidam et al., 1999; Yadav et al., 2004). Codon optimization and the use of leader sequences has been shown to increase g5 RNA expression 10-100 fold in transgenic cassava callus tissues, providing strong indications that improved versions of the *g5* gene have significant potential to impart resistance to all geminiviruses infecting cassava.

The Zhang lab has developed a strategy for improved RNA-mediated protection by expression of viral antisense RNAs as part of the 3'-untranslated region of a selectable marker gene. The targets of the antisense genes include the viral *AC1*, and *AC2* genes (required for replication of the viral genome). Antisense RNA genes containing coding sequences of these genes separately in antisense orientation in the 3'UTR of the hygromycin resistance gene were constructed. Linkage to the selectable marker facilitates selection for high-level expression of the antisense RNAs. Results demonstrated that antisense RNA expression interferes with viral DNA replication. Promising transgenic cassava lines were tested at the whole plant level using biolistic-mediated ACMV infection and found to be resistant to infection. Recently, the Zhang lab also pioneered RNAi mediated ACMV resistance in transgenic cassava. Several different RNAi constructs, designed to target the promoter and coding regions of ACMV, have been constructed. Initial results showed that ACMV replication was reduced by 90% in transgenic cassava expressing promoter hairpin regions of ACMV. The application of transgenic strategies to control viral pathogen will allow for rapid response to new viral variants having enhanced pathogenicity as well as facilitate rapid transfer of resistance traits to many cultivars.

Field trials in Africa: The *BioCassava Plus* research team is committed to moving nutritionally superior cassava from the laboratory to the field. Field trials will: (1) test the performance of new traits under field conditions, (2) involve African researchers in the project, providing an opportunity for capacity building and the incorporation of African social and cultural concerns, and (3) begin product development with African partners, thereby building local interest and ownership in the project. Investigators at IITA, including, Drs. Ingelbrecht, Dixon, Maziya-Dixon, and Herron, as well as investigators at the Kenyan Agricultural Research Institute, and Nigerian National Root Crops Research Institute, will be instrumental in conducting field performance trials and in disseminating the *BioCassava Plus* cultivars.

However, securing national approvals for field trials of transgenic plants in Africa is particularly challenging, because few African countries have workable biotechnology regulatory systems. The *BioCassava Plus* intellectual property and biosafety team (Lawrence Kent and Elizabeth Vancil) is uniquely qualified to address this challenge, because of their experience in collaborating with national partners in Africa to secure an approval for a transgenic trial in Kenya and applications to conduct transgenic cassava experimental field trials in Nigeria.

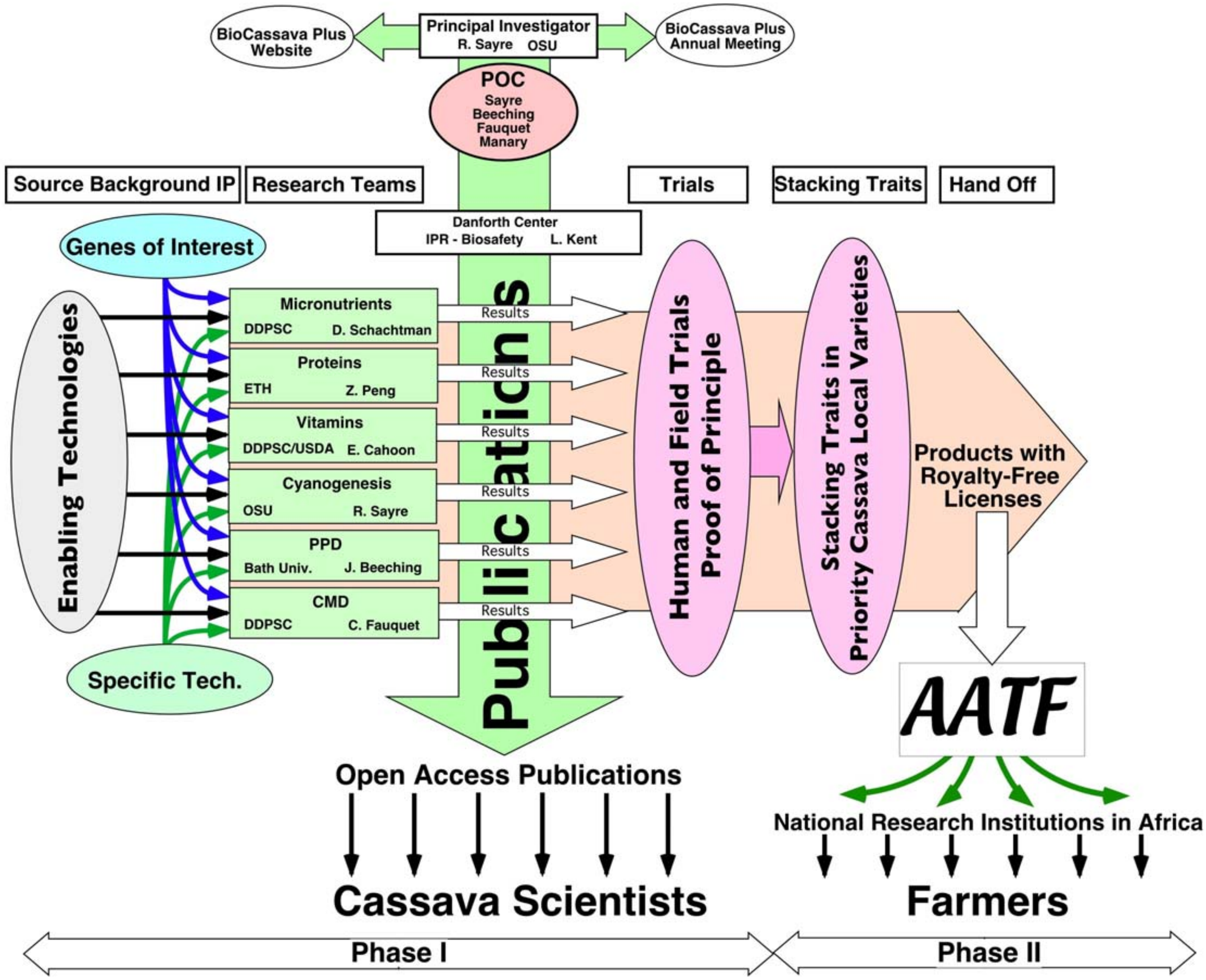
Although the team plans to prioritize Africa for its field trials, it also has access to a field trial site in Puerto Rico to be used for initial field screening of multiple lines, allowing the best to be selected for further trials in Africa. Dr. Dimuth Siritunga, from the University of Puerto Rico, brings with him considerable expertise in cassava biotechnology to facilitate this process. Regulatory approvals can be secured relatively quickly and easily for trials at this tropical US Commonwealth and appropriate facilities are already in place. In addition, the team will have access to cassava field trial sites at CIAT. The team's access to multiple potential field trial sites in Africa and the Americas maximizes flexibility and reduces vulnerability to regulatory bottlenecks.

Human Studies: Dr. Mark Manary's research group has completed stable isotope studies quantifying zinc and iron absorption and status in children in Malawi with high phytate and reduced phytate diets, finding that 90% phytate reduction improves zinc bioavailability 2-fold (Manary et al., 2000; Manary et al., 2002). The Manary research group has also successfully introduced a novel, protein-rich, ready-to-use food for prevention and treatment of protein-energy malnutrition in Malawi through a series of 6 studies (Manary et al., 2004; Collakova and DellaPenna, 2003). This innovation is currently widely used through out Malawi by many thousands of children and promoted by a variety of governmental and non-governmental agencies. The Manary group will characterize the bioavailability of nutrients (trace metals, vitamins and protein) in the nutritionally enhanced cassava. This aspect of the *BioCassava Plus* research program will insure that the deliverable products have an impact on the nutrition of the consumer.

Global Access Strategy:

The *BioCassava Plus* Program is committed to providing unrestricted access to nutritionally enhanced cassava cultivars consistent with the principles outlined by the Public Intellectual Property Resource for Agriculture Program (http://www.pipra.org/). Member institutions of the *BioCassava Plus* program will sub-license relevant technologies and products to the African Agricultural Technology Foundation (AATF), which will in turn sub-license the set of technologies embodied in the enhanced planting materials to the National Agricultural Research Systems (NARS) (Figure 1). Before any materials can be distributed to farmers, regulatory approvals must be obtained at the national level. In the case of conventionally-bred cassava varieties, regulatory approvals usually require only a demonstration of good agronomic performance. In the case of genetically-engineered crops, however, the regulatory requirements will be more demanding, and it will be up to the NARS to take the lead in securing approvals based upon the submission and review of environmental and food safety data (Figure 1). The *BioCassava Plus* team will play an important role in generating this safety data working in cooperation with AATF. During Phase One, the *BioCassava Plus* team will conduct initial biosafety assessments of the major technologies to be employed by the team to enhance cassava. These assessments will give preliminary assessment information on the safety issues and allow us to focus our research on only those technologies that are safe and are likely to be considered so by regulators. During Phase Two, more detailed safety assessments will need to be conducted to develop full biosafety dossiers for the final products flowing from this project, namely cassava varieties with "a full range of optimal, bioavailable nutrients. Information generated through the *Biocassava Plus* program will be freely accessible through the web and peer-reviewed journals providing immediate electronic access. The BioCassava Plus Program is supported by the Grand Challenge in Global Health Program of the Bill and Melinda Gates Foundation.

BioCassava Plus - Global Access Strategy



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Non-technical Abstract: *BioCassava Plus* is a multidisciplinary team of scientists brought together to develop cassava cultivars that provide complete nutrition for sub-Saharan Africans. Two hundred and fifty million Africans rely on the starchy root crop cassava (*Manihot esculenta*) as their staple food. Cassava-based diets, however, are deficient in both macro and micronutrients. A typical cassava-based diet provides less than 30% of the minimum daily requirement for protein and only 10-20% of the required amounts of iron, zinc, vitamin A and vitamin E. *BioCassava Plus* will employ modern biotechnologies to improve the health of Africans through development and delivery of novel transgenic cassava germplasm with increased bioavailable levels of zinc, iron, protein and vitamins A and E. Consumers of the enhanced root will receive a complete compliment of macro and micronutrients from cassava alone. Effective delivery of the enhanced cassava will be achieved by linking optimal nutritional traits with improved post-harvest durability of the storage roots, reduced cyanogen content, and elevated resistance to viral disease; characteristics required to provide ample amounts of foodstuffs and the incentive for farmers to adopt and sustain the use of cassava cultivars developed within *BioCassava Plus*. The *BioCassava Plus* program is distinguished by an innovative combination of approaches that draw upon transgenic plant science, effective collaboration with African partners and field and human feeding tests to demonstrate efficacy of the improved cassava. Existing technologies, for which proof of principle has been demonstrated in other crops, will be modified and applied to cassava. In addition, novel strategies will be developed to engineer the unique biochemistry and physiology of cassava.

Specific Nutritional Objectives of *BioCassava Plus*:

- Increase bioavailable levels of iron and zinc levels in foods six-fold.
- Increase the protein content of cassava roots four-fold
- Increase levels of both vitamin A and vitamin E ten-fold

Specific "Delivery" Objectives of *BioCassava Plus*:

- Reduce cyanogen levels in cassava foodstuffs ten fold.
- Suppression of the rapid post-harvest physiological deterioration inherent to cassava roots
- Generation of robust geminivirus resistance in susceptible, farmer-preferred cultivars.