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The VIRCA Project
Virus resistant cassava for Africa

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Keywords: cassava, cassava mosaic disease, cassava brown streak disease, VIRCA, RNAi, virus resistance, confined field trial

Abbreviations: ABNE, African Biosafety Network of Expertise; ACMV, African cassava mosaic virus; BecA, biosciences eastern and central Africa; BRN, BioSafety Resource Network; CBSD, cassava brown streak disease; CBSV, cassava brown streak virus; CMD, cassava mosaic disease; CFT, confined field trial; CsVMV, cassava vein mosaic virus; DDPSC, Donald Danforth Plant Science Center; EACMV, East African cassava mosaic virus; IITA, International Institute of Tropical Agriculture; ILTAB, International Laboratory for Tropical Agricultural Biotechnology; IPR, intellectual property rights; KARI, Kenya Agricultural Research Institute; NaCRRI, National Crops Resources Research Institute; NANEC, National Network of Cassava Workers in Uganda; NARO, National Agricultural Research Organisation, Uganda; NBA, National Biosafety Authority, Kenya; NBC, National Biosafety Committee, Uganda; NPT, National Performance Trial; PBS, Program for Biosafety Systems; PIPRA, Public Intellectual Property Resources for Agriculture; SC, steering committee; UCBSV, Ugandan cassava brown streak virus; VIRCA, Virus Resistant Cassava for Africa project

The VIRCA (Virus Resistant Cassava for Africa) project is a collaborative program between the Donald Danforth Plant Science Center, USA the National Crops Resources Research Institute, Uganda and the Kenya Agricultural Research Institute, Kenya. VIRCA is structured to include all aspects of the intellectual property, technology, regulatory, biosafety, quality control, communication and distribution components required for a GM crop development and delivery process. VIRCA's goal is to improve cassava for resistance to the viral diseases cassava brown streak disease (CBSD) and cassava mosaic disease (CMD) using pathogen-derived RNAi technology, and to field test, obtain regulatory approval for and deliver these products to small landholder farmers. During Phase I of the project, proof of concept was achieved by production and testing of virus resistant plants under greenhouse and confined field trials in East Africa. In VIRCA Phase II, two farmer-preferred varieties will be modified for resistance to CBSD and CMD, and lead events identified after molecular and field screening. In addition to delivery of royalty-free improved planting materials for farmers, VIRCA capacity building activities are enhancing indigenous capability for crop biotechnology in East Africa.

Introduction

The VIRCA (Virus Resistant Cassava for Africa) project was conceived in 2005 as an initiative to bring effective solutions to the viral diseases that suppress cassava (*Manihot esculenta*) yields and reduce farmer incomes in East Africa. VIRCA is a collaborative research and development program established between the Donald Danforth Plant Science Center (DDPSC), St. Louis, MO, USA; the National Crops Resources Research Institute (NaCRRI) of the National Agricultural Research Organisation (NARO), Uganda; and the Kenya Agricultural Research Institute (KARI), Kenya. Based entirely within these public sector research organizations, the project's goal is to develop the intellectual and technical capacity to improve cassava varieties for resistance to cassava brown streak disease (CBSD) and cassava mosaic disease (CMD), and to field test, obtain regulatory approval and deliver these products to small landholder farmers. To this end, VIRCA is structured to include all aspects of the intellectual property, technology, regulatory, biosafety, quality control, distribution and stewardship components required for a GM product development and delivery process.

Adoption rates for transgenically modified crops have been dramatic. However, the vast majority of the area planted is dedicated to herbicide and insect tolerant commodities such as maize, soybean, cotton and canola,[1] most of which do not serve the food or income needs of small-scale farmers. The VIRCA project differs uniquely from these products in a number of important ways. These include: (1) the target is a major staple food crop essential to the wellbeing of resource-poor communities in sub-Saharan Africa, but one that has no importance within industrialized Northern agriculture; (2) the project is conceived and executed through North-South collaborations, solely within the public sector; (3) the need for virus-resistant cassava products is urgent, potentially critical in the case of CBSD; (4) the crop is vegetatively propagated and distributed through informal, non-structured processes; (5) no financial profit is intended for the technology developers, only the end-users; and (6) countries designated for release are Uganda and Kenya, where the regulatory

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systems for GM crops are still developing. All of the above bring significant challenges that shape VIRCA activities and all must be successfully addressed if its goals are to be achieved. This article describes VIRCA in the context of its goals, the crop and diseases addressed, the technologies being developed and how the project is structured to ensure timely delivery of relevant virus resistant planting materials to farmers in Uganda and Kenya.

VIRCA Targets: The Cassava Crop and Viral Pathogens in East Africa

In the East African countries of Uganda, Kenya, Tanzania, Mozambique, Rwanda, Burundi and Malawi, approximately 150 million people depend on the tropical staple cassava, with about 30 MT of roots produced annually.[2,3] Recent studies on cassava production and utilization in Eastern Uganda and Western Kenya reveal the central role the crop plays in food security and economic activity in this region. Cassava was determined to be the most important staple food in 67% of the poorer households surveyed with food security found directly related to the amount of cassava cultivated. Households that committed more than 0.6 ha to cassava were self sufficient in food.[4] Cassava also contributes more than any other single crop to household income, with 63% of households selling cassava products to generate average incomes of up to $90 per household per year.[4] In regions where up to 85% of the population exists on US $1 per day,[5] this makes cassava a significant component of the rural economy. With land use intensifying to supply the growing population (Ugandan population predicted to increase 18% by 2015[6]), cassava will continue to play an increasingly important role in the region's agriculture.[7,8] Any constraints that suppress cassava yields therefore have an immediate and severe impact on the wellbeing of resource-poor rural farmers and their families, and affect the urban poor through increased prices for cassava products.[9]

Viral diseases represent the single most important biotic limitation to cassava production in sub-Saharan Africa, and particularly in East Africa, where CBSD and CMD combine to impact the crop (**Fig. 1**).[10] The causal agents of both diseases are transmitted by whiteflies,[11-13] with further spread resulting from subsequent planting of infected stem cuttings. In East Africa, CMD is caused by single or dual infections with the DNA geminiviruses African cassava mosaic virus (ACMV) and East African cassava mosaic virus (EACMV).[14,15] CMD has been an important constraint to cassava production in Africa since the 1930s, with a devastating epidemic originating in Uganda in the late 1980s that subsequently progressed west and southwards through Central Africa.[16,17] CMD continues to suppress cassava production throughout the Lake Victoria region,[10,18] reducing root yields by as much as 80% in severely infected fields (**Fig. 1**).[19,20]

CBSD has recently emerged to become a serious and immediate threat to cassava productivity in East Africa.[10,21,22] The causal agents of CBSD are newly confirmed as members of two species belonging to the genus *Ipomovirus* in the family *Potyviridae*: *Cassava brown streak virus* (CBSV) and *Ugandan Cassava brown streak virus* (UCBSV).[23,24] First reported in Uganda in 2004, incidence of CBSD in cassava fields increased from 22% of surveyed districts in 2005 to 72% by 2008.[25,26] Although known to be endemic in Coastal Kenya since the 1990s,[27] CBSD was also recently confirmed impacting the crop in Western Kenya, with incidences as high as 93% reported within cassava plantings.[13] CBSD symptoms can be subtle and hard to detect on aerial tissues, but still result in severe necrosis within portions of the edible storage roots. Damage often remains unknown until harvest, but has a significant impact on usable yields and marketability of the crop (**Fig. 1**).[28] CBSD is considered a more immediate threat than CMD to cassava production in East Africa due to its recent and rapid increase in geographical distribution, and because levels of resistance to the latter disease exist in farmer-grown germplasm. An important feature of CBSD in East Africa is that the disease often appears highest, and severity greatest, in CMD-resistant varieties recently promoted and widely adopted for management of the CMD epidemic[29] (Hannington Obiero, personal communication, August 2011). Presently, no source of strong resistance to CBSD has been identified in cassava genotypes that possess characteristics acceptable to farmers and consumers.[10] Conventional breeding programs to tackle CBSD are ongoing but offer an uncertain solution to this threat.

Technical Approaches and Progress

VIRCA will develop two products. First will be the cultivar TME204 modified for resistance to CBSD and second, Ebwanateraka modified for resistance to both CBSD and CMD. Each will be tested over several years under repeated field trials using vegetatively propagated stakes to ensure the robust nature and durability of the resistance traits. Phase I of the VIRCA project ran from 2005 to 2010. During this period, activities focused on generating proof of concept in transgenic cassava for technologies capable of imparting resistance to CMD and CBSD.

VIRCA's strategies for generating resistance to CMD and CBSD are based on pathogen-derived RNAi technology.[30,31] The overarching concept is to genetically modify cassava plants, retain those with low (1 to 2) copies of the integrated T-DNA and high levels of transgenically derived siRNAs, and transition to the greenhouse for challenge with the viral pathogens. Those showing reproducible levels of resistance are then selected for evaluation under confined field trials in Uganda and Kenya. The goal has been to identify which gene constructs impart robust resistance to ACMV and EACMV and for these to be used in subsequent production of virus-resistant products, followed by field testing, regulatory assessment and eventual release to farmers.

Twenty-six inverted repeat constructs were produced for development of an RNAi approach to control CMD. These ranged from 1.4 to 3.0 kb in size designed to cover all coding regions of the A and B components of ACMV and EACMV. The constitutive Cassava vein mosaic virus (CsVMV) promoter[32] was used to drive expression of the hairpin constructs. A transient expression system in which the constructs were inoculated into intact tobacco leaves was utilized as a quality control step to

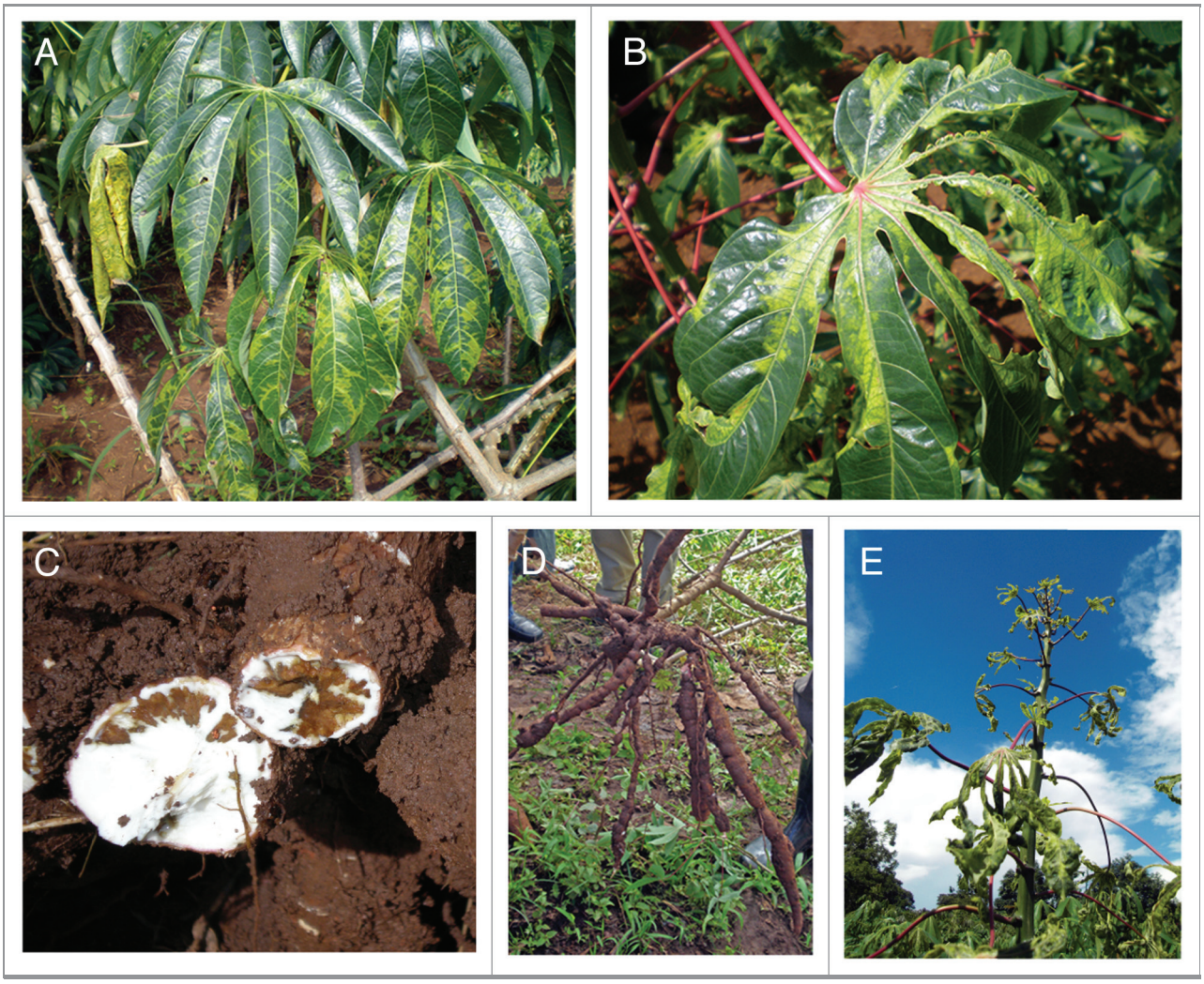

Figure 1. Cassava brown streak disease and cassava mosaic disease symptoms. (A) CBSD symptoms on infected leaves of the cassava cultivar TME204. (B) CBSD-induced necrosis in storage roots. (C) Deformation and reduced storage root development due to CBSD. (D) Typical mosaic leaf symptoms due to CMD. (E) Impact on shoot development in cultivar Ebwanateraka dual-infected with ACMV and EACMV.

confirm functionality of each construct and expression of the relevant virus sequence-specific siRNAs. In the case of ACMV, subsequent microparticle bombardment of the inoculated tobacco plants with an infectious clone of ACMV-Kenya[33] also allowed determination of a construct's capacity to suppress CMD development.[34] Those constructs capable of imparting resistance were used to generate transgenic cassava. As a result, five constructs targeted against ACMV and 10 against EACMV were retained for integration into cassava. When efforts commenced to address CBSD, the only known sequence for this RNA virus was the coat protein (CP) region of UCBSV.[25,35] Thus, inverted repeat constructs consisting of different lengths of this CP region driven by the CsVMV promoter were generated and utilized in attempts to control this disease.

A total of more than 1,250 independent transgenic plant lines were produced with the above constructs in the CBSD and CMD susceptible cassava cultivar 60444 over a period of three years.[36] Production and molecular analysis of this number of transgenic events necessitated a fundamental change in the manner in which activities at the International Laboratory for Tropical Agricultural Biotechnology (ILTAB), DDPSC were handled. Prior to commencing VIRCA, ILTAB operated like the vast majority of academic research laboratories, with each researcher making his/her constructs, and producing and analyzing their own transgenic events. Numbers of plant lines produced in this way are modest and testing is often elaborate. This approach was not suitable for generating the quality and quantity of information required to confidently determine which constructs within the VIRCA project were most effective for imparting the desired resistance traits. To solve this problem, a pipeline approach was developed in which all activities, including construct production, transgenic plant production, molecular screening, propagation, viral challenge and greenhouse care are handled by a team of specialist technical staff, with scientists dedicated to managing operations to ensure maximum flow of materials into and through the pipeline. This cassava pipeline is designed to regenerate transgenic plants

and screen for T-DNA copy number, vector backbone integration and siRNA accumulation at the in vitro plant stage. Events failing to meet required criteria are discarded and the remainder forwarded for micro-propagation, establishment in the greenhouse and challenge with respective viral pathogens. Researchers from all three VIRCA partner institutes have played, and continue to play, integral roles in establishing and running the pipeline. The processes and major components of the cassava pipeline operated at ILTAB are summarized in **Figure 2**.

As a vegetatively propagated species, cassava presents additional challenges because all transgenic events remain at the T_0 stage, must be multiplied clonally and maintained as whole plants in vitro or in the greenhouse. In order to handle the volume of plant material and data being generated, centralized data collection and analysis tools have been developed and quality control and transgenic event tracking systems put in place. While such working practices are common in the private sector and operate at scales greatly elevated from those reported here, they represented a major shift in organization and operational intensity for the VIRCA team. For example, in excess of 46,000 actions for plant propagation and sampling were recorded within a web-based tracking system (CassavaTracker) by one VIRCA technician in 2009 alone. In the same year, 12,000 transgenic plants were challenged with geminivirus pathogens to screen for efficacy of RNAi constructs to control CMD.

Testing cassava plants modified for resistance to CBSD was achieved by grafting stems of transgenic events to cassava carrying known infections with CBSV or UCBSV. Earlier results in tobacco[33] were confirmed showing that full length and N-terminal inverted repeat constructs from the CP region of UCBSV could impart strong resistance to the homologous virus, and in the case of a delta full-length CP construct, also some level of cross protection to CBSV.[37] Likewise, significant resistance to ACMV and EACMV has been recorded. In the case of ACMV, constructs generated from full length and N-terminal regions of the *AC1* and the *AC2* gene have generated results in which 75–100% of the challenged plants resisted development of disease symptoms. For EACMV, the most effective regions identified to date are derived from full-length and C-terminal regions of the *AC1* and *AC2* genes, which generate siRNAs capable of imparting 60 to 70% resistance against this pathogen.

Greenhouse screening for resistance to CMD and CBSD has been a valuable tool, facilitating elimination of constructs imparting insufficient capacity to control these diseases. However, growth conditions and disease transmission methods differ significantly from those in the field in East Africa, where both CMD and CBSD are transmitted by the insect vector.[12,13] Although whitefly transmission studies under confinement at the National Research Institute, UK have confirmed CMD greenhouse challenge data, only testing under field conditions with naturally vectored disease pressure can determine if the approaches being developed in VIRCA are valid for controlling one or both diseases. After approval by the Ugandan National Biosafety Committee, a confined field trial (CFT) of transgenic virus-resistant cassava was established in October of 2009. At the time of writing, three more CFTs, two for CMD and a third for CBSD, are underway at NaCRRI, with one for CMD ongoing in Kenya (**Fig. 3**). Another CMD resistance trial will be planted in late 2011.

To date, the approach has been to generate plants engineered for resistance against single CMD or CBSD pathogens and assess performance in the greenhouse and field. This was determined as prudent in order to generate conclusive information regarding the efficacious nature of each specific gene construct. However, to be acceptable to farmers, any VIRCA product will have to possess robust resistance to both diseases. To this end, gene constructs have been designed, and transgenic plants have been produced and confirmed to simultaneously accumulate siRNAs against ACMV and EACMV, and against CBSV and UCBSV. Information generated from ongoing greenhouse screens and from CFTs planned for 2012 will determine which constructs will be selected for incorporation into the final stacked products for development, further testing and delivery to farmers.

VIRCA Phase II

The second phase of VIRCA commenced in 2011 and includes the International Institute of Tropical Agriculture (IITA) at Biosciences eastern and central Africa (BecA) as a fourth partner. Phase II is structured to use data generated during Phase I to develop two virus-resistant products. The first addresses the immediate need for farmer-preferred germplasm resistant to CBSD[10,38] and will employ RNAi technology to incorporate stacked resistance against CBSV and UCBSV. Proven track records for existing products utilizing RNAi technology to control RNA viruses,[39,40] promising data from greenhouse and field testing[37] and the urgent need to address increasing impact of CBSD on cassava production in East Africa has stimulated an immediate start to production of transgenic plants

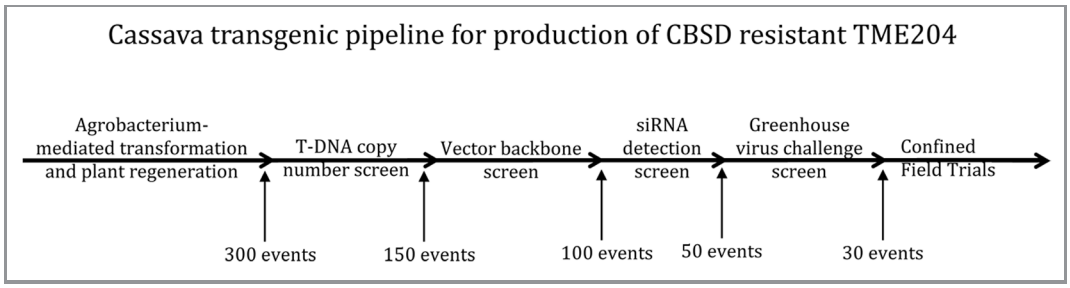

Figure 2. Schematic representation of major processes within the cassava pipeline underpinning VIRCA plant production and screening of virus resistant plants prior to confined field testing in East Africa.

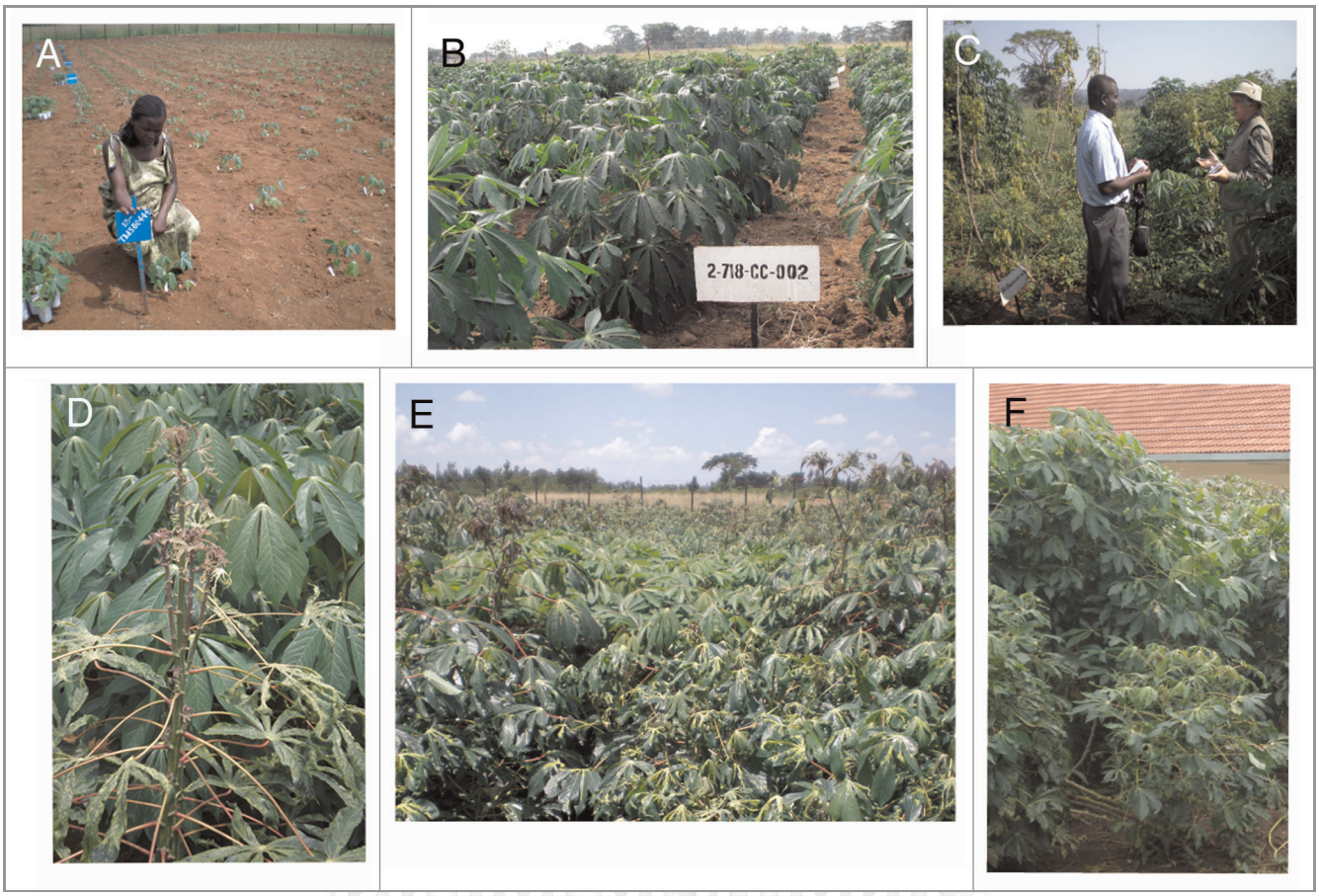

Figure 3. Confined field testing of plants modified for resistance to CMD and CBSD. (A) Planting mosaic resistance trial at KARI Alupe field station, Kenya. (B) Plants modified for resistance to Ugandan cassava brown streak virus at four months after planting at NaCRRI. (C) VIRCA managers discuss a cassava mosaic disease field trial. (D) siRNA expressing plants of 60444 showing significant resistance to cassava mosaic disease compared with the susceptible, non-modified cv Ebwanateraka (foreground). (E) Modified cv 60444 (middle ground) showing resistance to CMD. (F) siRNA-expressing plants of cv 60444 generated from clonal stake cuttings showing significant resistance to CMD (background) compared with non-modified cv 60444 (foreground).

effective against this disease. Choice of target germplasm is known to be a critical component of GM product uptake by farmers, especially in outcrossing, heterozygous crops such as cassava. After consultation with East African breeders, the cultivar TME204 was selected as the initial background for integration of CBSD resistance. A landrace of West African origin,[41] TME204, was introduced to Uganda by NARO in the early to mid 2000s and assessed through on-farm, participatory trials and demonstration plots. It was ranked highest by participating farmers, being scored excellent for plant establishment, resistance to CMD, early maturation, yield, mealiness, flavor, cooking qualities and market value.[42,43] As a result of its popularity, by 2009, TME204 was cultivated in 23 out of 26 districts in Uganda and found to be the predominant cassava variety in 17 of these districts.[26,29] TME204 is also a favored cultivar in Western Kenya and the Lake Victoria region of Tanzania (H. Obiero and Edward Kanju, personal communication, August 2011). However, as for all introduced materials, it has proven to be highly susceptible to CBSD.[29,44] It is considered therefore that a TME204 product modified for resistance to CBSD will be perceived favorably and adopted, if available, by small landholder farmers in Uganda and the Lake Victoria region.

After consultation with technical and regulatory experts, the vector plasmid pCAMBIA2300[45] was significantly modified to reach industry standards for regulatory compliance, and an expression cassette was inserted, designed to generate siRNAs against the CP sequences of both CBSD causal agents. Transgenic plant production has commenced and timelines for plant recovery and molecular screening place first CFTs for selection of CBSD-resistant TME204 events to commence late 2012.

The second VIRCA product will be more complex and thus have a longer development and delivery pathway. It will target the once popular, but highly CMD- and CBSD-susceptible cultivar Ebwanateraka (**Fig. 1**).[7,15] Data from ongoing CFTs will provide important information as to which genetic elements will best control ACMV and EACMV to be stacked within the same T-DNA with those imparting resistance to CBSD.

Conceptual and Organizational Structure of VIRCA

VIRCA's purpose is delivery of virus-resistant cassava with improved yields to farmers in Uganda and Kenya. Successful production and delivery of genetically modified crop products

requires not only strong technical capacity, but also coordinated efforts in intellectual property issues and regulatory requirements encompassing confined field testing and product approval processes associated with food and environmental safety assessment. The conventional model for crop biotechnology product development and commercialization is for the above components to be developed in-house by large agricultural companies. However, this leaves many staples, including cassava, orphaned by a technology approach that can deliver significant benefits; and many talented scientists in the developing world without experience in applying biotechnology for improvement of their crops. Cognizant of this reality, capacity for all components of the GM product development process have been built into the VIRCA project plan. These continue to be strengthened as the project progresses from proof of concept to production and testing of farmer-preferred products. Approaches taken to establish the required capacities in a local context are described below.

Since the earliest days of plant biotechnology, it has been presumed that the public sector would take the lead in delivering its benefits to resource-poor farmers in the world's developing regions.[46-48] Although research activities have been somewhat equal between the public and private sectors, deployment of products has been almost exclusively from the latter.[49] Most public sector organizations, especially academic institutions, are not structured to achieve these goals. Consequently, to date only virus-resistant papaya, and plum pox virus-resistant plum, both by the United States Department of Agriculture, and more recently, bean virus-resistant products by EMBRAPA, Brazil,[40] have been developed and deployed from the public sector.

The VIRCA project is organized to overcome these constraints. Sustained funding over Phase I of the project has allowed strong collaborative relationships to be built between the partner organizations and experienced teams of scientists and technicians retained at all locations. Institutional support for the project has been strong in all cases and significantly at DDPSC where VIRCA remains an important component of its mission statement. Access to high quality tissue culture and plant growth facilities has been critical to VIRCA's progress to date, as has been the extensive experience in cassava field testing and virology at NaCRRI and KARI. Recognizing the challenges involved in a complex product development project such as VIRCA, a management structure was established to coordinate and drive the scientific expertise and R&D workload at the three institutions. A Project Director leads a core team of researchers across all institutes consisting of experts with specific responsibilities for intellectual property, coordinating molecular and transgenic technologies, for performing in-region field testing, regulatory affairs, outreach strategies, training and capacity-building functions. A product development manager oversees day-to-day activities and communication between the partners, ensuring that actions meet previously defined timelines. Through organizations such as the BioSafety Resource Network (BRN) and Program for Biosafety Systems (PBS), external consultants are engaged as needed to provide guidance on project issues including data management, quality control tools, outreach and the East African regulatory environment. The management team reports to a steering committee (SC) consisting of experts in the fields of GM crop product development and the cassava crop in Africa. Access to such expertise underpins VIRCA activities and ensures its product focus. The SC formally assesses progress in all aspects of VIRCA activities every six months. It ensures that data accumulated is of the quality and quantity required for critical decisions to be made along the product development pathway, that resources are allocated effectively, and what additional actions are required for VIRCA to reach its goals within stipulated timelines and budget.

Management of intellectual property rights. Access to critical intellectual property rights (IPR) has often been cited as a limiting factor in the ability of the public sector and developing countries to develop and deploy crop biotechnology products.[49,50] The VIRCA project requires that all products emerging from its research and development work be unrestricted for royalty-free distribution to smallholder farmers in sub-Saharan Africa. This necessitates that every technology used in the development of, or incorporated into the final product, be free of IPR constraints that could impact product development costs and/or hinder freedom of distribution. The project employs a range of technologies including trait and selectable marker genes, regulatory elements, Agrobacterium-mediated transformation and hairpin-induced gene silencing methodologies, and other proprietary approaches. DDPSC developed some of the required technology, but substantial amounts have been acquired from commercial entities possessing the relevant IPR. Early in the project a thorough review was undertaken to insure that the pertinent IPR was identified. This was completed by a team of project scientists, patent lawyers, and licensing practitioners at DDPSC and also independently by PIPRA, an IPR review and management organization.[50] All relevant technology access issues were addressed by obtaining license agreements or non-assert agreements in which the IPR owner agrees not to assert their patent rights. In all cases, providers of the technologies have granted the necessary approvals. It is our experience, therefore, that by addressing these issues early and with due diligence, IPR need not be a critical or limiting factor in humanitarian-based projects such as VIRCA.

Confined field testing. Evaluating technology under local field conditions is an essential aspect of a product development project like VIRCA. Development of crop biotech products is similar to those of conventional breeding, where full evaluation of genotype by environment (G × E) interactions is critical to line selection and advancement. This is especially relevant for VIRCA with respect to virus strains and species that differ by and within regions.[19,51,52] In addition, cassava is a vegetatively propagated crop in which maintenance of resistance expression must be confirmed over successive generations cloned from the initial hemizygous T_0 events.

Prior to full risk assessment for food, feed and environmental safety, plant lines with new traits are tested under confinement conditions where the regulated plant material is controlled and restricted to a specific area of the environment. Such CFTs are subject to regulatory approval prior to implementation, and to regulatory oversight before, during and after their completion. VIRCA's approach is to evaluate efficacy of different siRNA-generating pathogen-derived constructs in the easily transformed

cultivar 60444 with performance in the field used as the final determinate of the genetic elements to be integrated into farmer-preferred cultivars.

While thousands of CFTs have been performed in North America, only two such trials (sweet potato and maize) had been completed in Kenya and none in Uganda when VIRCA commenced in 2005.[53] Critical activities during VIRCA Phase I therefore included constructing of CFT sites and infrastructure in both Uganda and Kenya and to obtain their certification by the national regulatory bodies. In consultation with regional regulators, the varying objectives and testing required at different stages of the product development process have been determined. Field staff have been trained in CFT operation, data collection and compliance record keeping. Applications for field testing have been compiled and timely approvals pursued and obtained from the Ugandan National Biosafety Committee (NBC) and Kenyan National Biosafety Authority (NBA) in order to perform CFTs and advance the project. As a result, a total of 26 independent transgenic events will have been tested under field conditions by the end of 2011, many over two subsequent generations, in these two countries.

The VIRCA CFT program began at NaCRRI, Namulonge, Uganda in October 2009, with establishment of the first transgenic field trial of virus-resistant cassava. This was designed to test genetic elements for RNAi-based control of EACMV in the cultivar 60444. Under the same approval, a repeat of this CFT was established in October 2010, and an evaluation of resistance stability initiated, using stakes of two promising events generated from plants grown through the first CFT. To date, these trials have confirmed greenhouse data and demonstrated siRNA-mediated resistance to EACMV and its stability across two full growing seasons. In order to extend the geographic scope of testing, a field testing site was developed at the KARI Alupe experiment station, in Western Kenya, and a CFT initiated in May 2011. A CFT to test efficacy of RNAi technology against CBSD was also established at Namulonge in November 2010, with additional plant lines scheduled for testing in 2012 at the KARI Mtwapa station. Situated in Coastal Kenya, Mtwapa provides a different environment from both Namulonge and Alupe, and as the presumed center of origin of CBSD in East Africa,[54] is expected to expose the transgenic events to a greater range of genetic variation across the UCBSV and CBSV causal agents.

It is the goal of VIRCA to work with appropriate regulatory officials to deliver CBSD- and CMD-resistant cassava cultivars to farmers as rapidly as possible. Failure to deliver in a timely manner has significant consequences regarding costs[55] and providing farmers with options to safeguard their crop from the impact of these diseases, most especially the emerging epidemic of CBSD. To this end, a carefully detailed CFT process, linked to regulatory-approved data collection, has been planned for the product development process. Transgenic events of TME204 generated through the cassava pipeline will be characterized in laboratory screens and those with acceptable genetic and phenotypic characteristics advanced to an initial CFT selection screen at NaCRRI, scheduled to commence in late 2012. Following this preliminary selection phase, an expected 20 events with high levels of field proven CBSD resistance will be taken forward for advanced event selection trials through three cycles of field testing. Advanced selection of CFTs will be conducted at multiple locations in Uganda and Kenya, representing the important agro-ecological cassava-growing zones of both countries. The sites required are being developed in collaboration with East African cassava breeders, virologists and regulators. Field data generated will be utilized to determine final event selection, to compile regulatory packages for use in safety evaluations, and to comply with National Performance Trials. This approach has been specifically designed to be fully compliant with all regulatory processes and to ensure that robust data are collected in a timely manner, thereby placing improved cassava varieties into the hands of farmers as soon as possible.

Regulatory pathway. Cultivation and consumption of cassava expressing the genetic sequences proposed in VIRCA will require approvals from regulatory bodies in Uganda and Kenya. Obtaining regulatory approvals is a significant component of any transgenic crop product development[55] and as such, a large proportion of the VIRCA Phase II effort will be committed to these activities. The regulatory plan uses existing international standards as a basis for determining the appropriate regulatory studies required for commercial approvals. Discussions are ongoing with regulatory authorities in East Africa to establish precise requirements for the regulatory pathway, study planning and execution of multi-location regulatory field trials. This includes conducting safety assessments, and creation and implementation of a plan that accurately describes the product, addresses questions or concerns and communicates progress to key stakeholders.

An important component of the regulatory plan is to continue providing adequate science-based information to regulatory authorities in Uganda, Kenya and the East African region. The VIRCA regulatory team is working with PBS and the African Biosafety Network of Expertise (ABNE) to support a science-based regulatory process in Uganda, Kenya and other African countries. Most regulatory activities will be performed in Africa, complemented by established international regulatory experts. On-site visits and evaluations will ensure laboratory practices are robust and consistent with accepted international standards for sample handling, data collection, experimental and analytical procedures, and compilation of results. Regulatory authorities place emphasis on such factors as (1) the origin (source) of the gene, (2) the function of the gene product and (3) any history of previous human dietary exposure and safe use. Safety assessments of each siRNA-generating sequence used to confer resistance traits expressed in VIRCA products will be completed. Significant literature and history of safe use of such transgenic approaches will aid this process. It is expected that a full safety assessment will also be required for each transgenic event intended for commercial release. This assessment will be performed on specific properties of the final event including molecular characterization of the integrated sequences, and compositional and agronomic characteristics of the transformed plant.

Assessment of transgenic crops includes consideration of unintended pleiotropic effects and is addressed by performing

extensive nutrient composition and agronomic performance analysis. Plant tissues from the lead event, selected through the CFT process described above, will be used to generate the required data. The field phase of the regulatory trial program, including trials for agronomic assessments and to generate samples for compositional analysis, will be conducted at multiple locations, representative of the agro-ecological zones where cassava is grown. Planning is underway to initiate regulatory consultations on requirements for subsequent commercialization of the TME204 and Ebwanateraka products with the dossier developed according to international standards.

Communications and outreach. In sub-Saharan Africa, only the Republic of South Africa has approved a food product developed using transgenic technology. Although biotechnology-derived products have been part of the food supply for two decades in other countries, questions remain on the African continent. The goal of the VIRCA communication strategy is to provide timely, credible and relevant information to various stakeholders including scientists, regulators, policy makers, media, farmers, and eventually distribution partners and processors, in the target countries. It is important that implementation of the communications plan follows the product development process. This takes into account laboratory work, field characterization, regulatory approval and product launch. The main VIRCA communication objectives are: (1) to enhance awareness of the VIRCA project among key stakeholders, (2) share appropriate technical information and evaluation techniques and (3) enhance capacity to generate and disseminate evidence-based information relevant to stakeholders. Project materials will be developed and effective delivery methods identified so that progress can be shared with key stakeholders and decision makers.

Capacity building. VIRCA is not designed as a capacity building project per se but has committed significant resources toward training personnel and enhancing the infrastructure required to achieve improved cassava varieties for farmers. As a result, the VIRCA project is contributing to increased capacity for crop biotechnology in Uganda and Kenya. In-country expertise, in consultation with international experts, has constructed CFT facilities, Biosafety level 2 screenhouses and plant tissue culture and transformation facilities at NaCRRI and KARI. Starting with no capacity in 2006, a single 0.28 hectare CFT site was established at NaCRRI in 2009, and subsequently expanded to a 3.6 ha location (**Fig. 4**), capable of handling five simultaneous field trials. With associated multiple screenhouses for performing smaller scale experiments and hardening transgenic plants prior to field testing, this represents one of the most comprehensively equipped GM crop testing facilities in sub-Saharan Africa. A fully functional plant molecular biology lab on the same station and a plant transformation facility due online by the early 2012 (**Fig. 4**) represent a full platform for production, analysis and testing of transgenic cassava and other crops.

Participation of knowledgeable personnel at NaCRRI and KARI has been critical to VIRCA's progress to date. All aspects of CFT planning and compliance, and most aspects of molecular plant analysis from ongoing and completed CFTs, are performed on-site by NaCRRI and KARI scientists. Production and multiplication of transgenic events in local varieties will commence at NaCRRI in 2012. These activities will continue to intensify as VIRCA transitions fully to the product development phase, with resulting increases in plant production and the CFT program described above. To perform these tasks, five scientists have been trained from NaCRRI in molecular virology, plant tissue culture and transformation and the regulatory requirements for conducting CFTs. Four of these researchers will complete graduate degrees through this work. In collaboration with the BioCassava Plus project[56] three KARI scientists are studying for PhDs in cassava biotechnology. These scientists, in addition to more than a dozen field staff trained in Kenya and Uganda for performing CFTs, comprise a cadre of researchers essential to the success of VIRCA, and represent potential future leaders for crop improvement programs in East Africa.[53]

VIRCA activities have contributed significantly to increased capacity in the regulatory environment in East Africa. Through applications to field test cassava transgenic for RNAi technology, Institutional Biosafety Committees at both NaCRRI and KARI have gained additional experience in handling submissions for CFTs. The Ugandan NBCs and Kenyan NBA have become familiarized with assessing applications for field evaluation of a vegetatively propagated, small holder crop developed by the public sector with full participation of their own national agricultural research organizations.

Varietal release and deployment. For VIRCA to benefit small landholder farmers, the approved planting materials must be multiplied and made available in relevant amounts to farmers in those areas where cassava is an important food security crop and where VIRCA products can have the most benefit. Virus resistant materials will also be made available to breeders in East Africa on completion of the required regulatory approvals.

Large-scale, rapid propagation of cassava is challenging since the crop is vegetatively propagated with a low multiplication factor—one cassava plant yielding between 10 and 15 stem cuttings. VIRCA will follow the deployment model established for virus-resistant cassava planting materials in response to the severe CMD epidemic for the 1990s and explore private sector participation in the multiplication and dissemination process. Uganda has developed the National Network of Cassava Workers in Uganda (NANEC), a template for integration of parties and actions at different levels for generation, deployment and adoption of improved planting materials.

The NANEC distribution framework is as follows:

(1) Initial multiplication to be performed at Institutional Multiplication Sites under the control or direction of NARO scientists, each site providing planting materials for 5 to 10 districts.

(2) Planting material from the Institutional Multiplication Sites go to intermediate sites established with farmer groups organized by the National Agricultural Advisory Services, women's groups, church entities or other NGOs.

(3) Intermediate multiplication sites distribute planting material to individual farmers, or to farmer groups, which then are informally distributed to family and neighboring farmers in each locale.

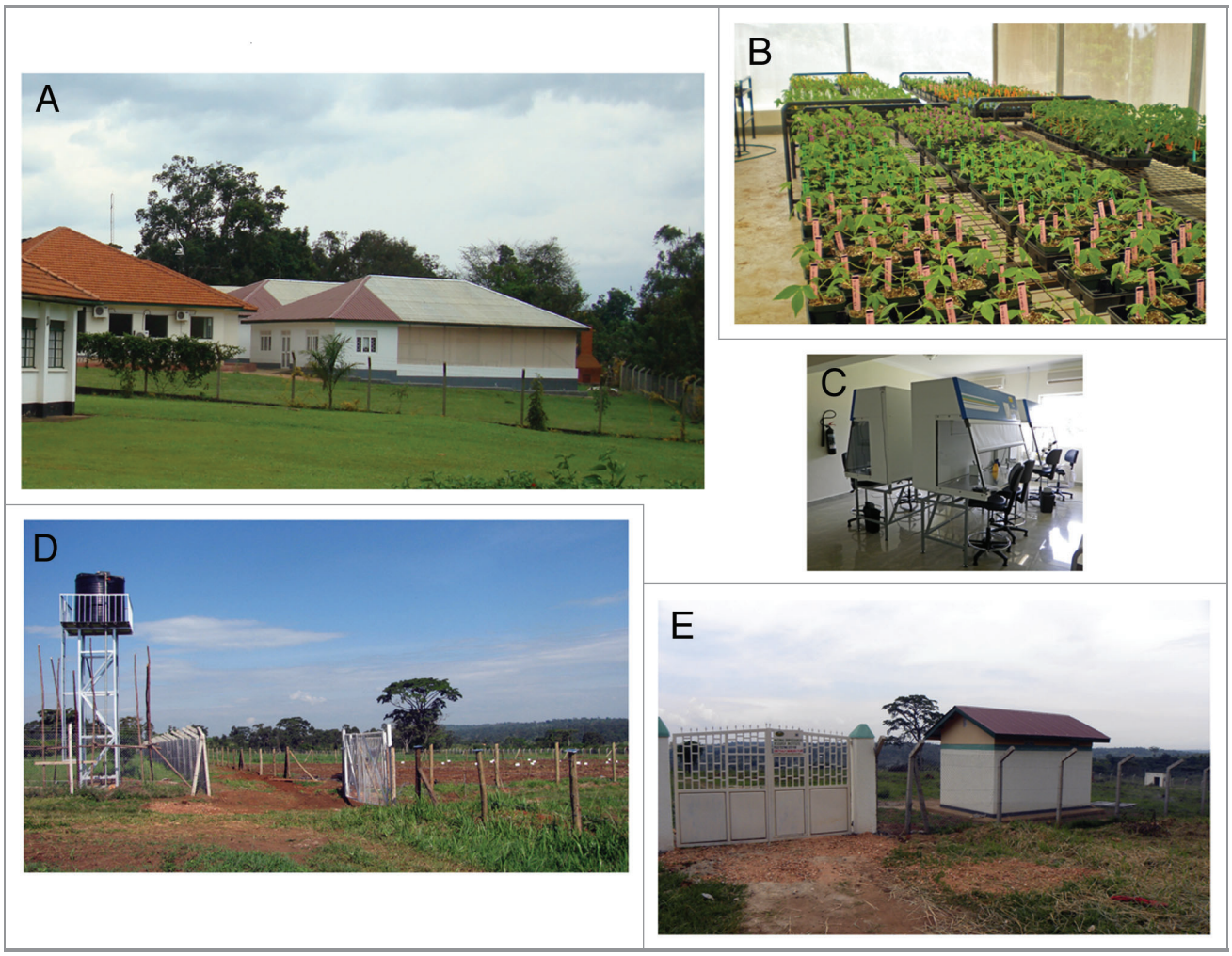

Figure 4. Recent infrastructure enhancements at NaCRRI, Namulonge. (A) Screenhouse and tissue culture facilities. (B) Cassava plantlets undergoing hardening from tissue culture in biosafety screenhouse. (C) Laminar flow hoods within tissue culture facility at Namulonge. (D) and (E) multi-hectare confined field trial site.

Using this approach, facilities in Uganda and Kenya best-suited to the efficient production and distribution of high value cassava lines will be engaged. Early stages of multiplication involving fewer participants and facilities will be launched prior to regulatory approval, and will be subject to confinement measures. Later stages of development with many participants are planned for initiation following regulatory approval for general release.

National performance trials (NPTs) are required in Uganda and Kenya prior to release. Such trials are used to evaluate new germplasm in comparison with existing varieties and to determine their uniformity and stability across relevant agro-ecological zones. NPTs take place over two or three years. VIRCA will work with regulators to determine how these can be integrated into the above field trial and multiplication stages in order to accelerate release to farmers while remaining fully compliant with regulations.

Conclusions

The VIRCA project is focused on utilizing crop biotechnology to improve the cassava planting material of small landholder farmers in the Lake Victoria region of East Africa. Through a comprehensive program of field testing and regulatory assessments, the goal is to complete all required analysis and have the first product, TME204 modified for resistance to CBSD, ready for distribution by 2015–2016. This will be followed by Ebwanateraka enhanced for resistance to both CBSD and CMD two years later. Many challenges remain, but VIRCA collaborators are committed to these goals and the potential it brings to benefit the livelihoods of farmers in East Africa, and to building indigenous capability for future crop improvement programs in the region.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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