Cassava

Constraints to production and the transfer of biotechnology to African laboratories

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Author(s):
Bull, Simon E.; Ndunguru, Joseph; Gruissem, Wilhelm; Beeching, John R.; Vanderschuren, Hervé

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REVIEW

Cassava: constraints to production and the transfer of biotechnology to African laboratories

Simon E. Bull · Joseph Ndunguru · Wilhelm Gruissem · John R. Beeching · Hervé Vanderschuren

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Abstract Knowledge and technology transfer to African institutes is an important objective to help achieve the United Nations Millennium Development Goals. Plant biotechnology in particular enables innovative advances in agriculture and industry, offering new prospects to promote the integration and dissemination of improved crops and their derivatives from developing countries into local markets and the global economy. There is also the need to broaden our knowledge and understanding of cassava as a staple food crop. Cassava (*Manihot esculenta* Crantz) is a vital source of calories for approximately 500 million people living in developing countries. Unfortunately, it is subject to numerous biotic and abiotic stresses that impact on production, consumption, marketability and also local and country economics. To date, improvements to cassava have been led via conventional plant breeding programmes, but with advances in molecular-assisted breeding and plant biotechnology new tools are being developed to hasten the generation of improved farmer-preferred cultivars. In this review, we report on the current constraints to cassava production and knowledge acquisition in Africa, including a case study discussing the opportunities and challenges of a technology transfer programme established between the Mikocheni Agricultural Research Institute in Tanzania and Europe-based researchers. The establishment of cassava biotechnology platform(s) should promote research capabilities in African institutions and allow scientists autonomy to adapt cassava to suit local agro-ecosystems, ultimately serving to develop a sustainable biotechnology infrastructure in African countries.

Keywords Cassava · Transformation · Africa · Technology transfer · Biotechnology

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S. E. Bull · J. R. Beeching
Department of Biology & Biochemistry, University of Bath, Claverton Down, Bath, Avon BA2 7AY, UK

W. Gruissem · H. Vanderschuren (✉)
Department of Biology, Plant Biotechnology, ETH Zürich, Universitätstrasse 2, Zürich 8092, Switzerland
e-mail: hvanderschuren@ethz.ch

J. Ndunguru
Mikocheni Agricultural Research Institute (MARI), P.O. BOX 6226, Dar es Salaam, Tanzania

Cassava: a world crop

Cassava originated in South America where it was domesticated about 8,000 years ago and transported by Portuguese sailors to west Africa during the sixteenth century (Léotard et al. 2009; Olsen and Schaal 2007). Since then cassava production has spread across sub-Sahara Africa and to Asia and South East Asia. It is a staple food for approximately 500 million people in about 105 countries providing as much as a third of daily calorie intake (FAO 2008a, b). Thus, in the developing world cassava is amongst the top four most important crops (with rice, sugarcane and maize) with global production in 2009 estimated at 241 million tonnes. Africa, where cassava is grown primarily for food, is the largest producer with yields estimated to exceed 160 million tonnes per year (FAO 2008b). In Asia and South East Asia the crop is grown mainly for animal feed and industrial purposes (e.g. sweeteners, acids and alcohols), with increasing interest in

developing cassava for biofuel (Balat and Balat 2009; Schmitz and Kavallari 2009).

Cassava belongs to the genus *Manihot*, which comprises about 98 species ranging from small shrubs to tree-like relatives, including *M. glaziovii* that is used in some countries (e.g. Nigeria) for rubber production (Nassar 2008). However, it is the species *M. esculenta* Crantz (Fig. 1a), also known as manioc (French), muhogo (Swahili) and pondu (Lingala), which is grown predominantly in Africa by small-scale farmers for its starch-rich storage roots (Fig. 1b). Cassava is drought tolerant and can grow in a range of agro-ecologies including marginally fertile soils, ensuring that when other crops fail cassava roots can still be harvested. Furthermore, cassava is vegetatively propagated via stem cuttings that are used to multiply stocks and for planting; typically five to ten cuttings can be obtained from a single plant. This propagation technique means that in times of famine the farmer does not consume the "seed" of cassava, unlike other staple crops (e.g. maize). Despite these advantageous traits cassava production is generally mediocre with current yields barely averaging 20% of those obtained under optimal conditions, particularly in Africa (El-Sharkawy 2004, 2006; Fermont et al. 2009). The importance of cassava and the enormous potential for improvement therefore makes it a target crop for famine research to achieve the United Nations Millennium Development Goals (UN 2010).

Problems associated with the production and consumption of cassava in Africa

Cassava production in Africa is greatly constrained by a number of biotic factors, including cassava green mite (Skovgård et al. 1993), cassava mealy bug, cassava bacterial blight (Boher and Verdier 1994), cassava brown streak disease (CBSD; Hillocks and Jennings 2003; Hillocks et al. 2001) and cassava mosaic disease (CMD; Patil and Fauquet 2009). CMD is caused by whitefly transmitted begomoviruses (family *Geminiviridae*) for which several species have been identified throughout cassava growing regions of Africa (Berrie et al. 2001; Bull et al. 2003, 2006; Hong et al. 1993; Stanley and Gay 1983). The disease—characterised by a yellow-green mosaic of the leaves, leaf distortion, stunted growth and decrease in the size of root—is probably the most significant biotic constraint to cassava production in Africa. Although the true incidence and severity of CMD is difficult to quantify (Sseruwagi et al. 2004), *African cassava mosaic virus* alone is estimated to cause 28–40% crop losses totalling 28–49 million tonnes per year (Thresh et al. 1994, 1997). CBSD is also the result of a viral infection caused by cassava brown streak viruses. CBSD is characterised by brown symptoms in the storage root and brown streaks on the stem. There is only scant information about CBSD compared to CMD, especially concerning virus transmission, but recent publications offer new insights into the molecular characteristics of the virus and disease etiology (Mbanzibwa et al. 2010; Winter et al. 2010), proffering the opportunity to evolve disease resistance programmes for cassava.

Cassava production is also hindered by numerous abiotic factors that include infertile soils, post-harvest root deterioration, planting of unimproved traditional varieties and inadequate farming practices. The planting of sub-optimal material, for example unimproved varieties or diseased cuttings, is exacerbated by the virtue that cassava is vegetatively propagated; without an organised and

Fig. 1 Cassava (*Manihot esculenta* Crantz) (**a**) and storage roots harvested in Kenya (**b**), photograph courtesy of Charles Orek

systematic dissemination of disease-free and improved cultivars, inferior material may be grown and distributed between farmers. This problem is often compounded by inefficient planting densities, as well as poor weed, pest and disease management. Unfortunately, even effective farming practices and good yields can be significantly impeded by post-harvest physiological deterioration (PPD). This is an endogenous process that results in the appearance of blue/black streaks in the roots, colloquially known as 'vascular streaking' (Averre 1967), and is due to the oxidation of phenolic compounds, in particular scopoletin (a hydroxycoumarin involved in plant defense; Buschmann et al. 2000), by reactive oxygen species (Huang et al. 2001; Reilly et al. 2004, 2007). PPD can occur within 48 h after harvest depending on the cultivar and climate, and renders the root unpalatable and unmarketable (Drummond 1953). Ergo, various approaches are being implemented to tackle PPD and improve the shelf-life of cassava roots, including breeding (Morante et al. 2010) and biotechnology (Blagbrough et al. 2010; S. E. Bull et al., unpublished data; E. Nyaboga et al., unpublished data). Additional complications associated with cassava consumption also include the poor nutritional content of storage roots (Montagnac et al. 2009a) and the potentially toxic quantities of cyanogen compounds (Barceloux 2009; Kamalu 1995; Montagnac et al. 2009b; Sundaresan et al. 1987). The aforementioned biotic and abiotic factors altogether significantly impact upon crop yields, root quality, economic costs, marketability, consumer availability and commercial processes. These obstacles are fundamental to cassava research projects and breeding programmes today.

Cassava breeding and transformation

Cassava research relies upon continuous advances in both knowledge and technology for researchers to effectively undertake and implement projects aimed at improving the crop. Conventional breeding programmes have long been key in encouraging these advances and with the establishment of the International Institute of Tropical Agriculture (IITA) in Nigeria and the International Centre of Tropical Agriculture (CIAT) in Colombia, in addition to other international research centres and national agricultural research systems (NARS), the last 30–40 years have seen improved knowledge of the crop, enhanced productivity and modernisation of cultural practises (Ceballos et al. 2004; Kawano 2003; Nassar and Ortiz 2010). Traditional breeding has resulted in the introgression of important traits into the cassava germplasm with major improvements recorded for bacterial blight resistance, virus resistance (Hahn et al. 1980; Okogbenin et al. 2007), protein content (Chávez et al. 2005) and starch quality (Ceballos et al. 2007). However, traditional breeding techniques face several limitations, notably the heterozygous nature of the crop renders it difficult to identify the true breeding value of parental lines, also there is only limited knowledge of inheritance traits that have agronomic importance (Ceballos et al. 2004; Nassar and Ortiz 2010). Thus, production of improved plant lines by conventional breeding can take approximately 10 years from the first parental crossing to distribution of the improved plants (Rudi et al. 2010). Moreover, introgression of the selected trait(s) into locally adapted and farmer-preferred cultivars without affecting their favoured characteristics remains difficult. Notwithstanding these complications, advances in molecular mapping (Akano et al. 2002; Okogbenin et al. 2007), sequencing of cDNA clones and expressed sequence tags (Anderson et al. 2004; Lokko et al. 2007; Sakurai et al. 2007), marker-assisted breeding (Rudi et al. 2010) and in particular the recent elucidation of the cassava genome sequence (Cassava Genome Project 2009) offer exciting new tools for both conventional breeding and biotechnology research.

The ability to use biotechnology to enhance cassava was proved possible in the mid 1990s with the production of transgenic material by two separate research groups. Researchers at ETH Zürich (Li et al. 1996) used *Agrobacterium*-mediated transformation of somatic cotyledons that were regenerated via organogenesis. At the same time researchers at the International Laboratory for Tropical Agricultural Biotechnology (ILTAB) and the University of Bath (United Kingdom) regenerated transgenic plantlets from totipotent tissue known as friable embryogenic callus (FEC) that was transformed by microparticle bombardment (Schöpke et al. 1996; Taylor et al. 1996). Subsequently a combination of the two techniques (*Agrobacterium*-mediated transformation of FEC; González et al. 1998; Zhang et al. 2000) became more widely adopted. However, despite the original techniques being published approximately 15 years ago, the uptake and success rate by laboratories has been particularly poor. In Africa, the production of transgenic material has been communicated (Hankoua et al. 2006) but maintaining the technique appears to be problematic, while publications from several western laboratories report generating only a few independent transgenic lines (e.g. Chellappan et al. 2004; Ihemere et al. 2006; Vanderschuren et al. 2007). The lack of uptake has been largely attributed to the technique(s) being complicated and labor-intensive, but also affected by low regeneration efficiency of plantlets from somatic embryos (Baba et al. 2008), intrinsic variation (including tissue quality) between transformation experiments (Schreuder et al. 2001), difficulty in using the protocol with farmer-preferred cultivars and the potential for chimeras and somaclonal variation (Raemakers et al. 1997; Raemakers et al. 2001; Zhang and Gruissem 2004).

Despite the potential complications associated with transformation, recent publications highlight the capacity of the crop to be improved. Welsch et al. (2010) increased vitamin A content in the roots using over-expression constructs containing a phytoene synthase gene. Zhang et al. (2003a) improved protein content via the expression of *asp1* (an artificial storage protein) and more recently developed plants with enhanced drought resistance (Zhang et al. 2010). Gene silencing techniques have brought about a reduction in cyanogen content (Jørgensen et al. 2005), improved starch for industrial applications (Raemakers et al. 2005), as well as developing resistance to *cassava mosaic virus* in transgenic cassava (Vanderschuren et al. 2007, 2009; Zhang et al. 2005). Of course, developing transgenic cassava is not always undertaken with the exclusive aim to improve the crop, but as with other species it provides a useful tool to improve our understanding of the plant. For example, Beltrán et al. (2010) and Zhang et al. (2003b) published research addressing promoter specificity in cassava. These various publications highlight the capacity for cassava to be improved to tackle a number of the constraints noted above, as well as reinforce the need to develop cassava transformation techniques to accelerate research.

Scientists from the University of Bath (UK) and ETH Zürich (Switzerland) experienced many of the complications with transformation systems first hand while developing transgenic cassava to study post-harvest root deterioration and virus resistance (Vanderschuren et al. 2007, 2009), as part of the BioCassava Plus initiative funded by the Bill & Melinda Gates Foundation. Despite good resources the consistent difficulty to reliably generate transgenic cassava stimulated a comprehensive review of the protocol(s), resulting in the recent publication by Bull et al. (2009). Numerous improvements simplified the procedure ensuring it is more robust, reliable and requires minimal expertise in tissue culture techniques. As such it has received interest from several groups internationally and appeared to be suitable for implementation in other laboratories, including those in developing countries.

Plant biotechnology to help meet the millennium development goals in Africa

Emerging technologies offer new prospects to promote the integration of crops and their derivatives from developing countries into the global economy (Brink et al. 1998). Plant biotechnology is one such technique that enables innovative advances in agriculture and industry and has the potential to broaden knowledge and provide solutions to some of the most intractable challenges faced in African countries (Delmer 2005; Thomson 2007), in particular eradicating extreme poverty and hunger—goal 1 of the Millennium Development Goals (UN 2010).

The implementation of plant biotechnology in Africa requires an infrastructure of various disciplines, incorporating scientific expertise, policy, regulatory and institutional frameworks (Gopo and Kimeri-Mbote 2005). In Africa, only a few countries produce transgenic material and it remains problematic to "commercialise" the products for dispatch to farmers and growers. The lack of uptake of biotechnology is in part due to many African institutes simply not having the capacity or necessary expertise to undertake basic tissue culture techniques and to develop transgenic material (Wekundah 2003). As such, most transgenic crops grown in Africa are imported. In 2009, transgenic maize was grown in Egypt and South Africa, transgenic cotton in Burkina Faso and South Africa and transgenic soybean also in South Africa (James 2009). In addition, there are insufficient policies and regulatory frameworks, such as biosafety, education and long-term environmental assessments to govern the use of plant biotechnology downstream of the laboratory for agricultural improvement and industrial applications. These considerations are not exclusive to Africa but apply to many other countries where transgenic crops are developed and grown (Rommens 2010). Thus, despite the potential for plant science to contribute significantly to achieve the Millennium Development Goals, there is a pressing need to strengthen the biotechnology infrastructure in Africa.

Programmes to tackle the issues discussed above are being established in Africa with support from international research institutes and organisations. For example, the ten eastern and central African countries have received support from the Association for Strengthening Agricultural Research in Eastern and Central Africa (ASARECA) to build capacity in plant biotechnology. Additionally, the Biosciences for Eastern and Central Africa (BecA) in Kenya—a New Partnership for Africa's Development (NEPAD) initiative—have enabled the building of state-of-the-art facilities to support African countries in plant biotechnology research. Other laboratories in Tanzania, Rwanda, Uganda, Kenya, Malawi, Mozambique and Zambia have been incorporated in NARS programmes to strengthen their capacity in CMD diagnostics with support from the Bill & Melinda Gates Foundation and led by scientists at MARI, Tanzania. To be successful, these capacity building strategies must be integrated into international research to ensure a favourable and competitive environment for sustainable development in Africa.

Plant biotechnology transfer to Tanzania: a case study

As part of a supplementary grant received from the Bill & Melinda Gates Foundation BioCassava Plus programme, a knowledge and technology transfer partnership (KTTP) between ETH Zürich, the University of Bath and MARI in Dar es Salaam, Tanzania, was established. New facilities were built at MARI with funds from the Rockefeller Foundation but unfortunately local scientists lacked the expertise to successfully undertake transformation experiments. Therefore, following official approval for contained research and an initial training phase for staff at MARI, the KTTP commenced. MARI scientists working with the Europe-based researchers have since produced in vitro embryos of elite and farmer-preferred cultivars grown in Tanzania (i.e. Kibandameno, TME7, Mahando, Katakya, Sagalatu, Mzungu and Milundikachini). The early stages in the transformation procedure have been successfully implemented as attested by the production of transgenic FEC from cultivar TMS60444 and the regeneration of embryos on antibiotic selection media (Fig. 2a, b). The use of a pCAMBIA binary vector containing the GUS reporter gene (accession number AF234297.1) allows an easy evaluation of progress since transformed material (FEC, cotyledons, plantlets, etc.) develop a blue precipitate following a GUS assay (see Bull et al. 2009 for details; Fig. 2c, d). From this on-going initiative to establish a sustainable cassava transformation platform, we draw on our experiences and highlight below some criteria that we consider important for a successful KTTP in Africa.

Reliable and efficient procedure

The optimized cassava transformation protocol (Bull et al. 2009) is the culmination of nearly 2 years research to troubleshoot each step of the procedure. A key improvement was the introduction of fine plastic mesh on which transformed tissue cultured material is spread for incubation on media. This approach (also used in transformation of *Brachypodium distachyon*; Alves et al. 2009) allows easy transfer of material to freshly prepared media, which not only potentially reduces fluctuations in nutrient

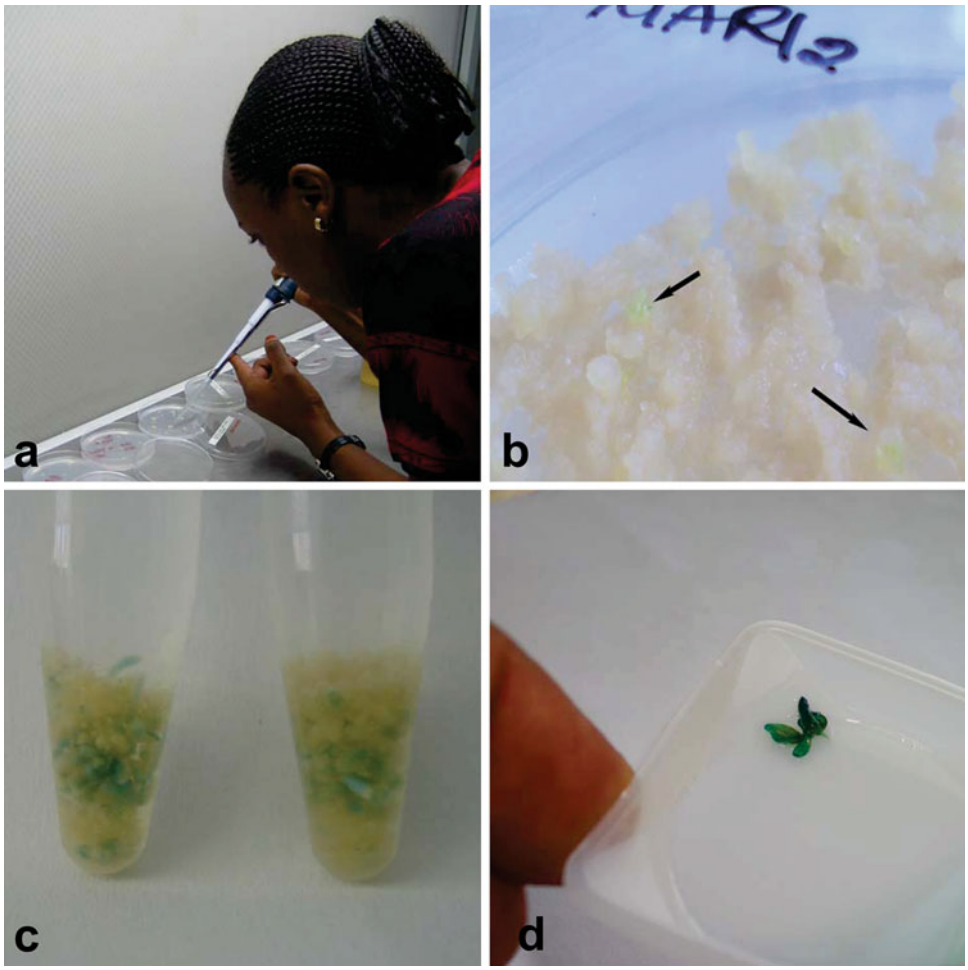

Fig. 2 *Agrobacterium* inoculation of FEC from cultivar TMS60444 (**a**), regenerating embryos (indicated by *arrows*) on hygromycin selection media (**b**), GUS assay using TMS60444 FEC (**c**) and regenerating embryo (**d**) transformed with pCAMBIA plasmid containing GUS reporter gene at MARI, Tanzania. Transformed material produces a blue precipitate

concentrations but also lessens the time required for handling tissue. This is especially important to prevent microbial contamination—a consideration for all research groups, but particularly relevant for laboratories in tropical climates where airborne pathogens/spores may be more prevalent. The concentration of antibiotic (hygromycin) for selection of transformed FEC was also optimised to improve regeneration capacity and efficiency. Collectively, the introduced modifications mean it is no longer necessary to perform time consuming high-throughput or repetitive screening of hundreds/thousands of plantlets to identify key lines with single T-DNA insertions. A reliable and robust system is vital for successful uptake by research groups that will invariably have different equipment, facilities, financial status and expertise.

Collaboration and communication between partners

All collaborative projects are based on effective communication and each partner having clearly defined goals. Since the generation of transgenic cassava is a lengthy process (approximately 6 months) it prevents continuous day-to-day guidance in MARI by the advisors, but short visits (several days) every few months coupled with email communication (weekly) proved sufficient to minimise the risk of any problems or issues arising. In addition, there is a broad level of management at MARI that requires several people to consent to approving project ideas and day-to-day decisions. As such it is important to maintain effective coordination and consultation between advisors and host institute staff to avoid delays in the research and without creating other complications.

Capacity building and appreciation for differences between Western and African laboratories

"The differing cultural, economic and environmental conditions between countries mean that there can be no one size fits all solution" (Beddington 2009). Facilities, expertise and laboratory management, for example, vary between all research groups and a fortiori between European and African laboratories. Therefore, for a KTTP to be genuinely successful it requires trained scientists to base the project at the host institute. As the initiative progresses then a visit to the supervising laboratories can help the African researchers hone their skills and knowledge. This approach maximises the input of the advisors, is directly beneficial to the host institute, optimises the financial support and learning process; a KTTP must encompass two or more dedicated scientists in the host institute for a programme of successive education to occur, thus avoiding the risk of an institute being left without expertise if people move elsewhere.

Foreseeing potential high risk problems and restrictions/limitations

As noted in the sections above, there may be fundamental differences between laboratories that need to be considered when planning a KTTP. For example, power failures, which are unprecedented in Western institutes, can be common place in laboratories in developing countries. In addition, the maintenance of equipment is potentially a high risk problem since the host institute may not readily have access to supply services and, in our experience, it may take several weeks or months for basic and essential equipment to be repaired or replaced. This is primarily due to companies not having adequate local stocks in Africa, usually requiring items to be ordered from abroad. A similar scenario occurs when obtaining chemical and general consumables, which can be exacerbated by serious delays at country border customs. Restrictions such as these are manageable but require foresight to predict and resolve in order to prevent significant losses to time, tissue culture stocks, materials and indubitably, motivation.

Sustained investment by funding organisations

The establishment of a new platform for technology requires a sustained investment for it to be successful in the long term. This is especially so with cassava transformation that requires nearly 6 months to undertake and relies on daily maintenance of stocks and propagation of in vitro material. Without continued funding there is the potential risk that transformation facilities, like those at MARI, will be abandoned and as a result remain located in just a few laboratories worldwide, most likely in developed countries. This will lead to groups outsourcing their research despite potentially being able to perform the work themselves. Whilst there are clear benefits to outsourcing some tasks, there is evidence to suggest that the production of transgenic crops in developing countries should be undertaken locally. It certainly ensures local intellectual and physical ownership of the products, thereby enhancing the probability of uptake by end users (Cohen 2005; Pray et al. 2002; Raney 2006).

Engaging government and local officials, scientists, farmers and the general public

Establishment of a transformation platform in Africa should allow the local scientists and officials to build confidence in the process and eventual products to coordinate more effectively the dissemination of information regarding transgenic cassava. Without this process, the undertaking of projects involving transgenic material is unlikely to progress beyond the laboratory phase. A recent

case study addressing farmers' knowledge of transgenic crops in Tanzania confirms the importance of improving awareness and education, highlighting that an infrastructure to link science, agriculture, health, development and communities is required (Lewis et al. 2010). The application to Tanzanian authorities for the approval of contained research activities at MARI was managed locally. The Tanzanian authorities were professional and cautious with the implementation of new research activities, but communication between local scientists and officials allowed steady progress and the opportunity to implement the KTTP. Clearly, the experience and advice that can be provided by some Western research groups, institutions, agencies and so forth is necessary to aid African researchers and government officials as the subject of transgenic crops is increasingly discussed. Ultimately, however, this is to provide Africans the autonomy to undertake their research and utilise new technologies as appropriate.

Outlook for cassava transformation in Africa

The case study above briefly outlines important considerations that we experienced while undertaking the KTTP in Tanzania. This venture will not produce a plethora of transgenic material with various desirable traits suitable for field tests in the coming months. Instead, however, we seek to begin the gradual expansion of a sustainable infrastructure to enable independence and allow African scientists to have greater ownership of their research activities. The programme in Tanzania is not unique, similar KTTP are underway in South Africa and also in Kenya with the support of researchers from ETH Zürich. Notwithstanding, establishing transgenic cassava is not the panacea to solving food shortages in Africa (Fermont et al. 2010) but it is an additional tool that along with traditional plant breeding and improvements in farming practises should better equip developing countries to tackle the many problems associated with cassava production and consumption. It is a necessary undertaking if the Millennium Development Goals are to be achieved. Complete success with the delivery of integrated and stable cassava transformation platforms in Africa is an important and realistic target that draws closer and with it we can ensure that the necessary knowledge, skills and responsibilities are transferred directly to the hands of those whose futures may rely upon it.

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