



Transferring a cassava (*Manihot esculenta* Crantz) genetic engineering capability to the African environment: Progress and prospects

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Key words: biotechnology, cassava, capacity building, genetic transformation

Summary

The procedures required to produce genetically transformed cassava were developed and are now in place in three laboratories in the USA and Europe. Future implementation and sustainability of transgenic technologies for the agronomic improvement of cassava will depend, however, on transferring these capabilities to locations where cassava has an important socioeconomic niche. If successful, such countries can apply the technology towards their particular needs. Training scientists from the developing countries in the transgenic biotechnologies is of primary importance in this effort. There are, however, many other factors including the availability of laboratory supplies, equipment, suitably experienced support staff, sufficient funding levels and biosafety considerations, which must be addressed and put in place before a transgenic program can be fully implemented in a given country. A transgenic capability is being transferred from the International Laboratory for Tropical Agricultural Biotechnology (ILTAB), USA, to the University of Zimbabwe. Three southern African cassava varieties were induced to form embryogenic suspension cultures at ILTAB and have been transferred to Zimbabwe. These tissues are presently being used as the basis of genetic transformation programs in both laboratories. Problems encountered in the transfer process as well as possible solutions aimed at adapting the available protocols will be presented.

Abbreviations: ILTAB – International Laboratory for Tropical Agricultural Biotechnology; ACMD – African cassava mosaic disease; IPR – Intellectual Property Rights

Introduction

Cassava improvement programs are aimed at addressing the limiting factors in cassava production and utilization. These include increased resistance to pathogens and insect pests, reduced losses to post harvest deterioration and improved starch characteristics. Conventional breeding for the agronomic improvement of cassava is hindered by the crop's inherent heterozygous nature, inbreeding depression and the polygenic and recessive nature of many desirable traits (IITA, 1990). Genetic engineering has been identified as a powerful tool to overcome these limitations by allowing the introduction of desirable traits directly into farmer-preferred cultivars (Thro et al., 1999).

Successful application of transgenic technologies in cassava will depend not only on technical advances but also on transfer of knowledge, tools and expertise to the countries in which cassava has an important socioeconomic role. Protocols for the production of genetically transformed cassava plants were first reported in 1996 (Schöpke et al., 1996; Li et al., 1996; Raemakers et al., 1996), and have since been improved and extended to a total of six cultivars (Taylor et al., this issue; Arias-Garzon et al., 2000). Although a tropical crop, the technical expertise required to develop and apply genetic transformation technology in cassava presently exists only in laboratories in Europe, the USA and at the Centro Internacional de Agricultura Tropical (CIAT), Colombia. Different countries

face differing constraints to cassava agriculture, each of which must be addressed by improvements to the relevant cultivars. If cassava biotechnology is to impact the quality of life for the small farmer, these countries must be able to develop and direct the transgenic technologies to their own specific requirements. Establishing an indigenous capability for the production of transgenic cassava in the tropics also shortens the distance between the technologies and the intended end user and should help ensure that products fulfill real, rather than perceived requirements. Transferring technologies and building capacity for sustained implementation of agricultural biotechnology within developing countries poses significant challenges to the advanced laboratories, developing countries scientists and the funding bodies supporting cassava biotechnology.

This paper will outline ongoing collaboration between the University of Zimbabwe and the International Laboratory for Tropical Agricultural Biotechnology (ILTAB), St Louis, Missouri, USA, which is aimed at transferring a genetic transformation capability for resistance to African cassava mosaic disease (ACMD) to Zimbabwe. Africa is the largest cassava-producing region in the world. The ongoing collaboration between the University of Zimbabwe and ILTAB will be used as an example to highlight the challenges involved in transferring and sustaining a transgenic capability to the African environment. We will outline the progress being made and the perceived obstacles which must be overcome before this can be made a reality.

Requirements for transferring a cassava genetic transformation capability with special reference to Zimbabwe

Establishing an effective genetic transformation capability for a given crop requires two factors: (1) tissue culture facilities and expertise to generate target tissues for transgene insertion and to enable subsequent recovery of transgenic plants and (2) molecular biology techniques, expertise and equipment for constructing plasmid vectors for genetic transformation and for the analysis of the transgenic plants produced. Requirements for the transfer of tissue culture and molecular biology techniques to developing countries present different challenges.

Tissue culture requirements and constraints

Conditions and expertise necessary for cassava tissue culture already exist in many laboratories in Africa (Chishimba & Lingumbwanga, 1997; Jorge, 1997; Jorge et al., 2000; Danso & Ahiabu, 1997; Ng & Ng, 1997). While these techniques are capital intensive, requiring the construction and maintenance of growth room facilities and laminar flow hoods, this is counterbalanced by relatively low consumable and running costs. *In vitro* micropropagation is the most common activity presently taking place within African laboratories. Numerous institutes also have expertise in the induction and regeneration of somatic embryos (Danso & Ahiabu, 1997) which is the basis of all reported transformation protocols in cassava (Schöpke et al., 1996; Raemakers et al., 1996; Li et al., 1996).

Initiating a cassava transformation program in such laboratories should not be problematic but will require the relevant training and additional equipment, such as shakers and incubators for the culture of *Agrobacterium* and embryogenic suspensions, which are often not available in a clonal propagation facility. If direct gene transfer by particle bombardment is the preferred transformation method, a microparticle gun, such as the BioRad Biolistic[®] He1000 system must also be acquired along with the required support equipment including a vacuum pump and a reliable source of high grade helium. Such equipment has been acquired by the DGIS sponsored Cassava and Biotechnology project at the Crop Science Department of the University of Zimbabwe.

One of the major limiting factors for successful implementation of transgenic programs in developing countries is most often a lack of appropriately trained personnel (Taylor et al., 1998; Toenniessen, 1995). Although most of the transformation protocols are available as published papers it is often very difficult to reproduce a technique in a remote laboratory. This is especially the case with plant tissue culture systems which often require a substantial 'hands-on' experience before they can be fully appreciated. The most effective manner to ensure transfer of tissue culture technologies is for scientists from developing countries to spend a period of time training in the advanced laboratories. The induction of friable embryogenic callus in a new cultivar and the exact timing and procedures for *Agrobacterium* infection are most effectively transferred in this manner. Hands-on experience ensures that the workers learn directly from experienced workers in how to handle the best quality

tissues and are exposed to the latest developments in the transformation protocols.

Progress in the collaboration between ILTAB and the Crop Science department, UZ

In Zimbabwe we have began the process of transferring cassava transformation technology to the Crop Science Department at the University of Zimbabwe through the training of personnel at advanced labs such as ILTAB and Wageningen University, The Netherlands. Four cassava cultivars grown in Zimbabwe, MAus 7, L2, L11 and TMS 30337 were chosen for inclusion in the programme because of their known high embryogenic potential. They were transported to ILTAB by M.V. Masona, a postdoctoral scientist at the University of Zimbabwe. Organized embryogenic structures were induced at frequencies greater than 90% for Maus 7, L2 and L11 and 75% for TMS 30337. To achieve this immature leaf lobe explants were cultured on Murashige and Skoog basal medium (Murashige & Skoog, 1962) supplemented with 50 μ m picloram (Taylor et al., 1996). Friable embryogenic callus (FEC), which is utilised as the target for transgene insertion by particle bombardment (Schöpke et al., 1996) and *Agrobacterium*-mediated transformation (Gonzalez et al., 1998) of Maus 7, L2 and L11, was induced from these structures as described by Taylor et al. (1996) after 4 to 6 months of culture on Gresshoff and Doy basal medium (Gresshoff & Doy, 1974) containing 50 μ m picloram. Embryogenic suspension cultures were subsequently established from these tissues.

Successful induction of the tissues required to initiate genetic transformation in three out of the four Zimbabwean cultivars demonstrates both the applicability of this culture system to farmer-preferred African cassava cultivars and the value in training at laboratories such as ILTAB, where such expertise has been initially developed. Friable embryogenic callus from MAus 7, L2 and L11 has been returned to the Crop Science Department where they have been successfully maintained for more than 12 months.

During the training period at ILTAB/DDPSC, M.V. Masona has gained experience in the production of transgenic cassava plants from friable embryogenic tissues using both particle bombardment and *Agrobacterium*. Transgenic plants of the model cultivar TMS 60444 and L2, expressing the Rep gene of ACMV have been produced (Taylor et al., this issue; Ma-

sona et al., 2000). These transgenic cassava plants are currently being tested for resistance to ACMV.

Molecular biology requirements and constraints

Molecular biology techniques differ from those of tissue culture in being relatively sophisticated and requiring a high level of expertise. They also require reliable and continuous access to expensive equipment, including PCR machines and centrifuges and to perishable reagents such as restriction enzymes and polymerases and antibiotics. At this time well-equipped molecular biology laboratories are not widely established in Africa. Acquiring and sustaining the necessary capacity for such activities necessitates significant funding and on-going support from external sources, both for training scientific staff and providing them with the required resources in a continuous and reliable manner.

Establishing and maintaining capabilities for molecular biology in Africa remains a challenge. Basic molecular techniques are universal in nature and can therefore be obtained through a period of training at innumerable institutions in the North. However, specialized knowledge such as the development of pathogen-derived resistance strategies, or the construction of BAC libraries must be acquired at specific laboratories with established capabilities in this field. In general there is a shortage of personnel specifically trained in cassava transformation technology (Toenniessen, 1995). Although this staple food crop is the most important source of calories throughout much of Central Africa (Thro et al., 1999), there are less than twenty scientists in the world with hands-on experience in the production of genetically transformed cassava plants. Expanding the knowledge base is therefore an essential activity within cassava biotechnology at this time.

Genetic transformed plants must be confirmed and analysed using molecular methods, including Southern, Northern and Western techniques. Several institutes in Africa are equipped and capable of supporting such activities, even if all the required equipment may not be available under one roof. Locally based collaboration between university departments and institutions should therefore be considered vital in maximising the available equipment and expertise within a given location.

The most commonly used histochemical analysis in plant genetic engineering programmes is the *uidA* (GUS) visual marker gene (Jefferson, 1987). This is

an invaluable tool for developing genetic engineering technologies in new crop plants and cultivars and as a tool for teaching and training in the basic aspects of this technology. Although simple and convenient to use, the GUS system is dependent on expensive substrates that must be imported into an African country like Zimbabwe. This is also the case for the majority of chemicals and reagents required to sustain even a basic transformation capability. Therefore, in addition to training of personnel and the availability of minimal facilities, a reliable infrastructure must be established which can guarantee supply of essential consumables to the African (e.g. Zimbabwe) or developing country laboratory. Although often not considered as a primary concern, lack of timely access to consumables within a working laboratory is a reality for many African laboratories. This can undermine the effectiveness of a whole research program by limiting experimental progress, preventing the development of useful products, and most importantly leading to disillusionment of the trained staff. Significant investments in human and capital resources account for little if these fail to operate due to the inaccessibility of chemicals and other consumables.

Establishing centers of excellence within Africa

Identifying centers of excellence in Africa is therefore considered to be an essential component in successful technology transfer and capacity building. It is important to identify African scientists and institutions such as the University of Zimbabwe and the Scientific Industrial Research and Development Center (SIRDC) in Zimbabwe where interest and commitment to crop biotechnology has been demonstrated. Such institutes have the infrastructure and political backing necessary to implement and maintain a transformation research and development effort. Although many such projects exist for rice biotechnology through the efforts of the Rockefeller Foundation Rice Biotechnology Program, no such structured programs have been established for cassava or the other staple African food crops. New ground must be broken in this area if these technologies are to be established on the African continent and utilised for the benefit of the small farmer.

High turnover of trained personnel due to poor incentives and lack of resources can seriously frustrate efforts to build and maintain research teams and to generate an effective research base within African laboratories through the training of local personnel. This however, requires adequate local funding since

laboratories in Africa must compete with other institutes throughout the world in attracting and retaining good candidates.

IPR and biosafety implications

Currently genes of interest are available only in advanced laboratories and biotechnology companies. Prior to successful capacity building, researchers in Africa must obtain the genetic constructs carrying desired genes of interest required for a genetic transformation program. Once again this is best achieved through collaborations with advanced labs in the North. Questions of intellectual property rights for the acquisition and use of cloned genes, promoters and techniques are then unavoidable. Most patented molecular tools and genetic sequences are available for research purposes only. Efforts at advanced laboratories aimed at providing promoters e.g. the pCsVMV promoter (cassava vein mosaic virus) from ILTAB and constructs free from proprietary rights protection associated with other gene promoters should be encouraged (Taylor et al., 1998). There is therefore a need to generate the technical capacity for African scientists to identify, develop and protect their own technologies and to work with scientists in the North to facilitate this process.

Biosafety considerations are now causing increasing public concern and must be taken seriously when introducing transgenic materials and research activities into the African environment. In many cases, release of transgenic materials and field trials are not possible at this time, due to a lack of relevant bio-safety legislation in all but a handful of African countries. This is already acting as a bottleneck for the development of agricultural biotechnology on the continent and must be addressed if African farmers are to benefit from the new technologies. Establishing a critical mass of trained scientists who fully understand the issues and can knowledgeably contribute to the debate on the production and introduction of genetically modified plants, is an important issue in this area.

We concur with Herrera-Estrella (1999) concerning the issue of directly introducing end products (i.e., transgenic plants) in the short term. It should be considered, however, that transgenic plants produced by African scientists working in collaboration with advanced laboratories would be received better by the African countries compared to an entirely foreign effort. Often there is fear that African countries are being used to try out a technology that is being seriously

questioned in other parts of the world. This view is promoted by a lack of information and debate at the grassroots level. Africans must make their own informed decisions concerning these matters, as the perceived risks and advantages and cultural perspective differ from those in Europe and North America. The debate on the use of biotechnology has already taken place in Zimbabwe and biosafety regulations have now been put in place. The Zimbabwean government is committed to biotechnology as judged by its funding of the SIRDC, which includes a biotechnology institute. This has largely been a result of the presence, in the country, of highly respected local scientists who understand the potential benefits of biotechnology to Zimbabwean agriculture.

The present lack of legislation governing the release of genetically modified organisms in Africa should not prevent the initiation of transgenic programs for cassava as capacity building must address all the concerns outlined above. A period of 3–5 years is necessary to generate and analyse transgenic plants expressing transgenes with putative agronomic benefits. During this time all plant tissues remain *in vitro* or can be contained within growth chambers.

Conclusions

It is considered that cassava transformation technology can be transferred to the African environment when suitable institutes and individuals have been identified in that continent. This process is underway through a collaborative project between ILTAB and the Crop Science Department at the University of Zimbabwe. There is, however, a much greater need for capacity building, training and overall support for the application of biotechnology to the African situation. Africa faces many challenges over the coming decades. Agricultural biotechnology offers important possibilities for crop improvement for food and economic security. To make this a reality a much greater proportion of the resources of the North must be dedicated to applying biotechnology where it can have its greatest impact on the well-being of the world's population.

Acknowledgements

M.V. Masona was supported by DGIS and the Rockefeller Foundation. This is manuscript DDPSC 00-11.

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