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Progress and Challenges for the Deployment of Transgenic Technologies in Cassava

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The production of genetically modified cassava (*Manihot esculenta* Crantz) plants is routine in the advanced laboratories that invested in this technology during the 1990s. The ultimate aim of those engaged in cassava biotechnology is to develop and deliver improved planting materials to farmers in the tropical regions. Transgenic plants are now being produced that express traits with potential agronomic importance. Although good science will remain an essential basis for this goal, if farmers are to benefit from this investment, it is essential that researchers and others involved in these programs adopt a mindset geared towards product development and delivery. The first field trials of cassava are now under way. This paper examines the major challenges facing public sector research organizations engaged in the transgenic improvement of cassava.

Key words: cassava, field trial, intellectual property, product development, transgenic.

Introduction

It is possibly among the vegetatively propagated "orphan crops" that transgenic technologies can have the biggest impact. Despite their importance as sources of both food and income for billions of people in the tropical and subtropical regions, yields in these crops, which include cassava (*Manihot esculenta*), plantains (*Musa* spp), sweetpotato (*Ipomea batatas*), taro (*Colocasia esculenta*), and yams (*Dioscorea* spp), have increased by less than 30% over the last 40 years. This contrasts with rice and wheat, which benefited greatly from investment during the Green Revolution and realized yield improvements of at least 80% and 100% respectively over the same period (Food and Agriculture Organization of the United Nations, 2004). The vegetatively propagated food crops therefore have a largely untapped potential for improvement in both quantity of production per unit area and for development as commodity products within developing economies. How can this potential be exploited, and how can the resulting benefits be passed to farmers and consumers in developing countries? In this article, we will use the root crop cassava as an example of the progress being made and the challenges faced in attempting to bring the potential of biotechnology to bear on vegetatively propagated crops. Great promises made by proponents of agricultural biotechnology—from both the private and public sectors—cite such potential as justification for continued investment into research and development in this field. It is hoped that this article will illustrate some of the realities involved in attempting to deliver on this rhetoric.

Production of enhanced germplasm through conventional breeding is problematic in the vegetatively propagated crops. Cassava, in common with the other species listed above, is characterized by strong heterozygosity and inbreeding depression. Breeding programs in cassava consist of crossing elite parents and screening thousands of first-generation progeny for desired characteristics (Ceballos, Iglesias, Perez, & Dixon, in press). Although important contributions to disease resistance and harvest index have been achieved in this manner (Jennings & Iglesias, 2002; Kawano, 2003), many believe that transgenic technologies offer the key to unlocking the full potential of cassava. Insertion of genetic material by direct gene transfer allows beneficial traits to be integrated rapidly into elite germplasm without changing the genetic background of the mother plant. In this way, desired traits can be introgressed into elite parent material prior to sexual crossing or into the products of a breeding program that lack a specific trait required by farmers. In other cases, existing farmer-preferred landraces and varieties can be modified to perform better in a given environment. An example of the latter approach is the introduction of transgenic resistance against cassava mosaic viruses into susceptible landraces grown by small farmers in Africa or into cultivars grown for commercial-scale starch production in southern India. Biotechnology also allows the introgression of beneficial traits not accessible through conventional means, such as herbicide tolerance and *Bt*-imparted resistance against stem borers and hornworms (Taylor, Chavarriaga, Raemakers, Siritunga, & Zhang, in press).

Present Status of Transgenic Cassava

The potential of transgenic technologies for improving cassava was first recognized in the late 1980s. At that time, a small group of scientists established the Cassava Biotechnology Network (CBN; Thro et al., 1999), to act as a mechanism to bring together researchers in developing and industrialized countries with a shared vision for using advanced molecular tools to improve the crop. It was not until 1996, however, that recovery of the first transgenic cassava plants was reported (Li, Sautter, Potrykus, & Pounti-Kaerlas, 1996; Raemakers et al., 1996; Schopke et al., 1996). This frustrating delay arose from an inability to generate the totipotent tissues required to produce transgenic plants. An interesting comparison can be made between rice and cassava, both of which were considered recalcitrant to in vitro manipulation in the mid-1980s. Within ten years, mostly as a result of initiatives by the Rockefeller Foundation, transgenic technologies for japonica rice became so routine that they were considered model systems for plant genetic transformation. Progress in cassava was distinctly slower, due to much lower levels of investment in the biotechnologies required for the improvement of the crop. With fewer than seven laboratories engaged in transgenic technologies for cassava worldwide, generating a critical mass of researchers proved to be difficult. The CBN, through its international meetings and small grant systems, was instrumental in encouraging collaboration between research groups in the United States, United Kingdom, The Netherlands, Switzerland, and the CGIAR center at Centro Internacional de Agricultura Tropical (CIAT) in Colombia. Breakthroughs in the ability to generate morphogenic culture systems (Li et al., 1996; Taylor et al., 1996) eventually resulted in the production of cassava plants transgenic for marker genes both via microparticle bombardment (Raemakers et al., 1996; Schopke et al., 1996) and *Agrobacterium*-mediated gene integration (Li et al., 1996).

Since the first reports of transgenic plant recovery in cassava, efforts have focused on integrating genes with the potential to impart traits of agronomic interest. Genetically transformed plants have been recovered with reduced cyanogenic content (Siritunga & Sayre, 2003), resistance to infection by geminiviruses (Chellappan, Masona, Ramachandran, Taylor, & Fauquet, in press), expression of Bt proteins (Ladino et al., 2002), modified starch content (Raemakers et al., 2003; Uzoma, Arias-Garzon, & Sayre, 2003), and elevated protein content within the storage roots (Zhang, Jaynes, Potrykus, Gruissem, & Pounti-Kaerlas, 2003). In most cases, technologies successfully developed in other crops have been transferred to *Manihot esculenta*. Transgenic cassava plants produced to date represent proof of concept for the respective traits in this species, with efficacy of the transgenic strategy demonstrated at the laboratory and/or greenhouse level. For a more extensive review of the technologies used to generate these plants, the reader is referred to a recent review compiled in collaboration by the five laboratories with existing capacity to produce transgenic cassava (Taylor et al., in press).

The ultimate aim of transgenic programs in cassava is to develop and deliver improved planting materials to farmers in the tropical regions. Publication of technical achievements in scientific journals cannot, therefore, represent the end point of such endeavors, but are milestones in a product development and delivery process. As a first step in this direction, investment is being made to test existing transgenic plant lines under field conditions. Transgenic cassava plants have been field trialed in the US Virgin Islands and within screenhouse facilities in Western Kenya, and confined trials are in preparation at CIAT in Colombia.

Challenges to Deployment of Transgenic Cassava

As the initial technical hurdles have been overcome, new challenges are becoming apparent. Although not specific to cassava, these represent major hurdles that must be overcome if the technology is to move forward from proof of concept to product development, deregulation, and commercial release. The challenges being faced by the small group of researchers engaged in this effort are varied and include further technical questions, intellectual property rights, the underdeveloped regulatory environment and biosafety infrastructures within target countries, and of course accessing the funding needed to solve these issues. The inexperience of most public-sector research scientists in handling a product-delivery process, and the need to achieve these goals within the tropical regions, are also important factors that must be overcome in the technology transfer process.

Technical Issues

Desired traits within a transgenic plant must be expressed at the required level and in a predicable manner under conditions that the crop experiences during cultivation. Presently, little data is available regarding how transgene expression will be affected when genetically modified cassava plants are cultivated in the field in the tropics. As with other vegetatively propagated crops, obtaining homozygous transgenics in cassava is not practical, meaning that required levels of expression must be obtained and reliably sustained at the T_0 generation over many vegetative cycles. Such goals have been achieved for *Solanum* potato (Kaniewski & Thomas, 2004, this issue), but empirical studies on stability of transgene expression in cassava must be confirmed by carrying out field trials for specific traits in this crop. The challenges involved in establishing the required trials in the tropics are not trivial and are discussed below.

As with all crop species, significant variability exists in the response of different cassava cultivars to the available genetic transformation protocols. To date, most transgenic cassava plants ready for field trial are of model cultivars that have proved to be amenable to the genetic transformation protocols. In seed-propagated crops, it is possible to integrate a desired transgenic trait into one or a few easily transformable cultivars and then backcross these with local varieties to generate the desired products. In most cases, this is not an option for the vegetatively-propagated crops, because the aim is to enhance existing farmer-preferred germplasm or elite breeding lines without altering their existing desirable characteristics. As transgenic cassava programs move towards a product development mode, it is essential that capacity to produce genetically transformed plants is expanded into the agronomically most important varieties and breeding lines within the major cassava-growing regions.

Transgenic programs are multiyear, multimillion-dollar efforts and must generate products readily acceptable to the intended end users. Failure to genetically modify the relevant germplasm will result in a significant waste of resources, regardless of the effectiveness of the transgenic trait. To prevent this, one must identify which cassava varieties and landraces are dominant within a given region, what traits they are lacking, and what role they play within the local cropping system. For example, investing in the genetic enhancement of a cultivar primarily grown for sale to the local starch-processing factory may have a more significant impact on the well-being of farmers than the same enhancement applied to a variety primarily cultivated for on-farm consumption. It can be appreciated that this knowledge is not part of the average biotechnologist's repertoire but requires the skills and input of agricultural economists. In some situations, such as in Asia, India, and parts of Latin America, where cassava is increasingly grown on a commercial scale, identification of priority cultivars is relatively straightforward. In such cases, a few cultivars may be grown over tens of thousands of hectares in a situation not unlike maize in the United States. Africa, the world's largest cassava-producing region, presents a different scenario, where cassava remains very much a small-farmer crop grown in mixed stands as a source of food for the family. As a result, scores of landraces and varieties may be grown in any given region, and decisions regarding which landraces, varieties, and breeding lines to target for investment through transgenic improvement must be made with care and with significant input of local knowledge.

There are also technical challenges in culturing the prioritized germplasm and adjusting the transformation systems to allow efficient production of transgenic plants. The capacity to generate the 15–30 independent transgenic events required at the proof-of-concept stage must be increased tenfold (or more) in order to reliably generate plants of the quality required for release to farmers. Most public-sector research facilities are not experienced in, or geared up for, this level of output. To date, data is available for transgenic plant production in six cultivars of cassava. With the appropriate investment many more could be brought into the existing systems. For example, production of embryogenic tissues—the central component of transgenic protocols in cassava—has been reported in more than 60 varieties (Taylor et al., in press), so there can be optimism that farmer-preferred varieties from all the tropical regions can be genetically transformed with the appropriate investment. However, adapting the tissue culture systems to suit priority germplasm requires time and resources that the leading laboratories based in North America, Europe, and the CGIAR Centers would rather invest in trait development and transgene expression studies. It is considered important, therefore, that in the near future, development of the culture systems required for transformation of specific cassava varieties be carried out within the respective cassava-growing regions. To this end, training of scientists and technicians from the national agricultural research systems (NARS) within the tropical regions must be a priority within transgenic cassava programs (Taylor, Schöpke, Masona, & Fauquet, 1999).

Intellectual Property

Some claim intellectual property (IP) issues to be a major factor limiting the deployment of transgenic technologies in developing countries, while others consider this to be a trivial problem if the correct procedures are followed early in the product delivery program. The latter tend to be those knowledgeable in IP issues and with access to legal advice on such matters. The experience of the authors and other cassava biotechnologists falls somewhere between these views, but it is certain that IP presents a potential minefield which must be negotiated carefully. Ensuring compliance with IP consumes scarce resources and in some cases has prevented the development of potentially beneficial products in cassava.

Of the five laboratories engaged in production of transgenic cassava, two are partially funded by commercial starch companies interested in the use of biotechnology to modify the starch content of cassava storage roots. In these cases, the commercial backers have the experience and resources available to ensure that all IP issues are resolved prior to commencing product development. Within the public sector, naivety in the importance of such issues can lead to problems, although this is a situation that is improving as researchers gain hard-won experience in this area. Recent sublicensing of enabling technologies from Monsanto Company to the Donald Danforth Plant Science Center (see Horsch & Montgomery, 2004, this issue) for use by the cassava community provides an example of an increasing range of proprietary tools becoming available to those engaged in these efforts.

Acquiring freedom to operate with transgenes imparting valuable traits can also be problematic. When the intended aim is field deployment, lengthy and sometimes costly negotiations must be initiated with the owners of such technologies. In some cases—for example, the EPSP-synthase (*epsps*) gene, which imparts resistance to the herbicide glyphosate—significant efforts driven by farmer-identified needs in Colombia have failed to gain access for use of this gene in cassava. This is certainly disappointing for those involved, most especially as a subsequent ex ante study of the potential impact of glyphosate-resistant cassava indicates that this technology could be worth as much as $300 million to the Colombian economy over a 15-year period (Pachico & Rivas, 2003).

Regulatory Challenges for Establishing Field Trials

Genetically modified crop plants must be tested in the environments where they will be cultivated by farmers. Multiple trails in different locations within a target country are required for determining efficacy of the transgenic trait, eliminating off-types generated during the tissue culture/transformation process, and selecting the optimal insertion events. Although field trials of transgenic crop plants are common in North America (more than ten thousand have been safely carried out in the United States since 1989), equivalent levels of experience do not exist in the countries where tropical crops such as cassava are central components of the staple diet. Although many developing countries have assembled the regulatory structures required to carry out field trials, the existing legislation is often outdated, and the process itself has never been put into practice. The result is that the human skills and biosafety infrastructure required to carry out field trials are lacking. This is most especially the case in sub-Saharan Africa, where to date outside of South Africa, only Kenya, Zimbabwe, and Burkina Faso have carried out field trials of transgenic plants, with the combined number in all three countries totaling fewer than ten.

This situation obviously presents significant obstacles for those wishing to develop a product delivery process within such regions. For example, an application to field trial cassava in Nigeria presently requires detailed answers to 150 separate questions. When this application was completed by the Donald Danforth Plant Science Center (DDPSC) in collaboration with its partners in that country, it reached almost 60 pages in length. Establishing a screenhouse trial of cassava in East Africa required the DDPSC to commit approximately $150,000 worth of resources, over a period of a year, to cover training of African staff, upgrading containment infrastructure, processing the application through the biosafety committees, and numerous exchange visits between Africa and the United States. Such levels of investment are a very significant undertaking for public research organizations. Decisions about whether to spend scarce resources on such endeavors have to be balanced against other demands, such as the need to publish in high quality peer-reviewed journals and thereby secure future funding for the research. It is hoped that programs such as Plant Biosafety Systems (PBS, funded by the United States Agency of International Development) will be successful in their efforts to modernize the regulatory environment in developing countries, and that as a record of safe field trials is established, the process will become progressively simplified and routine. Indeed, it is essential that this is achieved if transgenic crops are to be developed and released to farmers in these regions.

Funding for Transgenic Modification of Cassava

Sustaining required levels of funding remains a major concern for all research organizations engaged in genetic modification of cassava. Despite the importance of the crop—after rice and maize, cassava is the most important source of dietary calories in the tropics and is cultivated on a total area only 7% less than that committed to potato—resources available for the improvement of cassava are severely restricted. Cassava and the other orphan crops listed above are not cultivated in the industrialized nations and are therefore not mandated for the large investments spent in improving species such as maize and wheat. Indeed, cassava is most important in the world's least developed countries, where government support for agricultural research is insufficient and often nonexistent for development of the relevant biotechnologies. A review of the Agricola database illustrates this discrepancy in biotechnology investment. Agricola shows citations for 471 and 242 papers describing transgenic systems in maize and wheat respectively, but only 36 for cassava and two for plantain.

Most funding for transgenic improvement of the orphan crops is provided by aid agencies and charitable foundations. Although commendable, this support is most often piecemeal, and from the perspective of the researchers, unpredictable. As a result, at any given time, none of the laboratories engaged in transgenic cassava programs have guaranteed support for more than one to three years into the future. Building and maintaining research teams is thus difficult and means that expertise in the required tissue culture and transformation systems resides in less than a dozen individuals worldwide. There is no doubt that committing more resources to cassava in an intelligent and focused manner will increase the effectiveness of the technology and shorten delivery times to end users. The sources of this substantial long-term support for the development and application of transgenic systems in crops such as cassava and plantain are not easy to predict, but such support is necessary if the promises made for biotechnology in these crops are to be realized.

Conclusions

Significant advances in technical capacity of transgenic programs in cassava and other orphan crops mean that important and exciting challenges are being faced as the first steps are taken along a product development and delivery process. It is essential that these challenges are addressed and solved if the benefits of biotechnology are to reach end users in developing countries. The text above is not intended to convey pessimism, but to highlight the amount and type of work that needs to be done. Not all issues have been covered. For example, questions of how new transgenic crops such as cassava will be tested for food and environmental safety and deregulated for commercial release in the tropics, and who will pay for this process, have not been discussed. Instead, we have focused on the more immediate requirements as experienced by those involved in such research and development programs. These include the need to test prototype technologies in the field and to adapt transgenic capacity to include the most important farmer-preferred planting material. Although good science remains an essential base, on its own it will not deliver benefits to people in developing countries. Genuine commitment is required from research organizations and funding bodies to tackle the less glamorous tasks of improving regulatory infrastructure in developing countries and training scientists and regulators from these countries. If sustainable programs for transgenic improvement of tropical crops are to be achieved, it is also most important that capacity for development and deployment of genetically modified crops in developing countries is acquired by its own people and that they are actively engaged in driving all levels of the process—from priority setting to production and analysis of transgenic plants, field testing, and commercial deregulation.

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