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Development of virus resistant sweetpotato using biotechnological approaches in Kenya

Gichuki S.T.[1], Nangayo F.[1], Machuka J. [2], Njagi I.[1], Macharia C.[1], Odhiambo B.[1], Irungu J [1] and Ndolo P.J.[3]
[1]KARI-Biotechnology Center, P.O. Box 57811, Nairobi, Kenya
[2]Kenyatta University, Nairobi, Kenya
[3]KARI- Regional Research Centre Kakamega, P. O. Box 169, Kakamega, Kenya

Abstract. The sweetpotato virus disease (SPVD) is one of the main constraints to sweetpotato production in Africa. It is caused by a number of sweetpotato viruses. The sweetpotato feathery mottle virus (SPFMV) is the most widespread of those viruses. Transformation of sweetpotato plants with viral coat protein genes may impart a coat protein-mediated resistance to SPVD. In 2001 twelve lines of sweetpotato variety CPT 560 transformed with the SPFMV coat protein gene were field tested under controlled conditions in four important sweetpotato growing agro-ecologies in Kenya. Based on virus resistance, yields of storage roots and vines, four transgenic lines were selected and evaluated further in 2002. Results from these field trials did not provide an adequate level of SPVD resistance as expected based on visual assessment of symptoms and tissue printing bioassays. Possible explanations for this unsatisfactory level of protection may be due to synergistic effects of other sweetpotato viruses in the environments where the lines were tested. Secondly it is possible that the first generation gene constructs were not efficient against the Kenyan strains of the virus since they were developed based on American strains of the virus. The second generations of transgenic plants are currently being developed at the Kenya Agricultural Research Institute (KARI) Biotechnology centre and are at various stages of development. These have improved gene constructs developed using Kenyan isolates of SPFMV. This project has been instrumental in development of the biotechnology framework in Kenya and capacity building in biotechnology.

Introduction

There is sufficient evidence that biotechnology has potential to boost global agricultural productivity in a sustainable way. The prospects are particularly bright for developing countries where conventional methods of breeding have not been able to cope with the myriad biotic and abiotic challenges constraining the agricultural sector. Being the economic mainstay for the majority of these countries, the sector's poor performance has contributed significantly to the escalating food insecurity, malnutrition, poverty and environmental degradation particularly in Africa. This has led many African governments and researchers to intensify the search for appropriate interventions including the integration of the cutting edge science of biotechnology into conventional approaches to agricultural commodity improvements. Problems however arise because the research capacity (human and infrastructural) in most of these countries is limited and the industrial world has dominated the biotechnological revolution, concentrating on crops perceived to be of global economic importance. African countries could import some of these technologies, but they are not usually tailored to the needs of small-scale subsistence farmers. In addition, "orphan crops" that are important to small-scale farmers but do not attract much research support internationally, would be neglected. One such crop is the sweetpotato [*Ipomoea batatas* (L.) Lam].

Sweetpotato is the world's seventh most important food crop after wheat, rice, maize, potato, barley, and cassava (CIP, 1996). It is one of the most important crops in densely populated East Africa (Low *et al*., 1997). The crop grows well on soils with limited fertility, is relatively drought tolerant, provides good ground cover, and is usually cultivated without fertilizer or pesticides (Ewell, 1990). Traditional cereal crops often fail in these same environments. Sweetpotato also thrives in fertile environments, producing remarkable amounts of energy quickly, often producing more edible energy than any other major food crop (CIP, 1996). Despite the crop's importance for human nutrition and environmental conservation, yields in Africa remain low – about 6 tons/ha compared to the global average yield of 14 tons/ha and a yield potential of more than 40 tons/ha (Ndolo *et al*., 1996).

The major constraints to sweetpotato production include continued use of low yielding and late maturing local cultivars, post-harvest yield losses, diseases and pests and inadequate planting material. The major pests includes insects such as the sweetpotato weevils, vertebrate pests such as moles, rats, porcupines and monkeys (Bashaasha *et al*., 1995;Kapinga *et al*., 1995). The most important disease in Kenya is the sweetpotato virus disease (SPVD) (Wambugu, 1991). In addition there is poor institutional support and recognition and inadequate research capacity in most African countries.

In Kenya, the sweetpotato is an important food security and cash-generating crop with about 750,000 tons of fresh tubers being produced annually on an estimated 75,000 ha of land (FAO, 1999). The crop is also important for human nutrition especially the orange-fleshed varieties which contain high levels of b-carotene useful in combating vitamin A deficiency in children under the age of five and breast-feeding mothers (Low *et al.*, 1997). The Lake Victoria basin and the western Kenya highlands accounts for over 70% of national production followed by the Central Highlands and Eastern Kenya producing about 20%. Production is mainly by small-scale farmers.

The sweetpotato virus disease (SPVD) and the sweetpotato weevils are the main biotic constraints. In Kenya, the weevil is the single most important biotic production constraint and may account for losses of up to 80% on individual farms (Smit, 1997; Ngunjiri *et al.*, 1993). Among the viruses, the sweetpotato feathery mottle virus (SPFMV) is the most widespread in Kenya (Wambugu, 1991). The SPFMV belongs to the *Potyvirus* genus in the family *Potyviridae*. It occurs everywhere sweetpotatoes are grown, including Africa. SPFMV is transmitted non-persistently by aphids. It is not considered as a serious production constraint on its own. However, when it interacts with the sweetpotato chlorotic stunt virus (SPCSV), transmitted by the whitefly, it results into the sweetpotato virus disease complex (SPVD).

Biotechnological approach to virus resistance. Over the years researchers at the Kenya Agricultural Research Institute (KARI), in collaboration with the International Potato Center (CIP) have established that sweetpotato viruses have significant economic importance and that available virus control methods are ineffective or unaffordable by resource-poor farmers in Kenya. In 1991, KARI initiated a collaborative project having reached the conclusion that a biotechnological approach to virus resistance would add value to the search for a long-term solution for the control of SPFMV.

The project has been collaboratively carried out between KARI, Monsanto Company-USA, Danforth Plant Science Centre-USA, Agricultural Research Council (ARC)-Roodeplaat, Vegetable and Ornamental Plant Institute-South Africa, United States Agency for International Development

(USAID) and the International Potato Center (CIP). The International Service for the Acquisition of Agri-biotech Applications (ISAAA) brokered the project, which involved: negotiations for intellectual property (IP) rights donation, Material Transfer Agreements, fund-raising and identification of appropriate partners for the different implementation stages.

The broad objective of this partnership was to transfer virus resistance traits via genetic engineering to Kenyan sweetpotato varieties for use by subsistence farmers. Specific objectives werer to identify effective improved gene-construct/s, to build transformation capacity in Kenya, to transform local popular sweetpotato varieties, to test ago-ecological adaptation of transgenic sweetpotato through participatory methods, and to assess and identify impact of genetic engineering technology in smallholder farming systems.

Technology development and mock trials. Coat protein mediated resistance (CP-MR) for SPFMV control is a form of genetically engineered resistance, whereby the expression of viral coat protein genes in the transgenic plants induces a cross "protection-like'' phenomenon that confers resistance to the virus from which the gene was initially derived as well as to related viral strains (Beachy *et al*., 1990). It is still largely unclear what the molecular mechanisms of CP–MR are although some theories have been proposed. Monsanto donated the gene constructs and initial research on genetic transformation of six Kenyan sweetpotato varieties against SPFMV was done at Monsanto laboratories in the US. At the initial stages of the project, only one of the six sweetpotato varieties, CPT 560, was readily transformed with the donated constructs (Gibbons, 2000). The actual transfer of the transgenic sweetpotato technology from Monsanto to KARI took place in the year 2000.

Mock trials were carried out with conventional CPT 560 and other local varieties to familiarize KARI staff members with general trial procedures for the safe handling of transgenic crops. This was done prior to importation of the transgenic material. KARI and Monsanto scientists developed the transformation and regeneration protocol of sweetpotato variety CPT 560 at Monsanto Company, St. Louis (USA).

Field trials in Kenya. This activity was aimed at evaluating agronomic performance, ecological adaptability and virus resistance ability of transformed (transgenic) CPT 560 under field conditions in Kenya. The first round of field trials was carried out during 2000/2001 at four centres of the Kenya Agricultural Research Institute namely the Regional Research Centre (RRC) Mtwapa located in the Coastal Lowlands, RRC Embu in Eastern Province, the National Agricultural Research Laboratory (NARL), Kabete on the outskirts of Nairobi and RRC Kakamega in Western Province. It involved 12 transgenic lines of sweetpotato variety CPT 560. During the trials, data were collected on crop establishment and crop vigour based on protocols developed by researchers at the International Potato Centre (CIP). Based on agronomic performance and virus resistance data using visual symptoms and NCM Elisa tissue print bioassays four transgenic lines were selected for the second round of field trials in 2002 across all the four agro-ecologies.

Prior to the introduction of the transgenic sweetpotato, Kenya had no operational biosafety system. The need to evaluate the genetically modified (GM) material stimulated the development and institutionalization of national biosafety guidelines and regulations. During the trials, measures were taken to minimize the chances of gene transfer between the trial crops and cultivated sweetpotatoes or their wild relatives. This was achieved through: Security enclosure using chain-link wire around all trial sites

- 24-hour security surveillance systems
- Restricted entry to the trial sites

- Regular monitoring and emasculation of flower buds from test plants
- Inclusion of guard rows and a barrier crop around the test site
- Destruction by burning of all material after harvest

Results from these field trials did not provide an adequate level of SPVD resistance as expected based on visual assessment of symptoms and tissue printing bioassays. A possible explanation for this is the synergistic effects of other Sweetpotato viruses in the in the environments where the plants were tested. Secondly the gene constructs used were developed from American strains of the virus whereas African strains are more virulent.

Current activities. A second generation of gene constructs has now been developed based on a Kenyan strain of SPFMV. The transformation and regeneration protocol for variety CPT 560 has been optimized at the KARI Biotechnology laboratory in Nairobi, Kenya. Concurrently, regeneration for other popular local varieties is being developed in the same laboratory. In addition capacity for molecular characterization and resistance testing is currently being developed in this laboratory. KARI has also initiated collaboration with Kenyatta university in training and research to enhance capacity building in gene technology in Kenya.

Achievements

The project serves as an example of collaborative linkages between the private and public sectors as well as technology transfer from the north to the south. This project was instrumental in the operationalization of the Biosafety system and opened up the approval system for the introduction of other genetically modified organisms in Kenya. A biosafety permit application for on-station field-testing has been granted to KARI by the Kenya Plant Health Inspectorate Service (KEPHIS) following approval by National Biosafety Committee.

The sweetpotato project has also helped to create awareness of biotechnology and related biosafety issues in Kenya. In addition, it has been instrumental to the increasing involvement of Kenyan biotechnology stakeholders in the global biotechnology debate.

Intellectual Property Rights (IPR) negotiations between Monsanto and KARI were successfully brokered by ISAAA and a royalty-free license agreement between the two institutions was signed in 1998. The agreement allows KARI to use and further develop the transgenic virus resistance technology in sweetpotatoes. KARI may also protect resulting transgenic varieties under the plant breeders' rights convention or similar regulations in Kenya and transfer the technology to any other country in Africa.

KARI has an operational biotechnology laboratory for further transformation of local African sweetpotato genotypes with technical support from Monsanto, CIP and Danforth Plant Science Centre. This laboratory is manned by fully qualified Kenyan scientists. In addition, KARI has developed full capacity for conducting laboratory, greenhouse and field experiments with transgenic plants.

KARI has also developed capacity to engage in genetic engineering for other traits in sweetpotatoes and other important crops. New improved gene constructs developed using a Kenyan SPFMV isolate have been used to transform a few popular sweetpotato lines. Some of these are undergoing multiplication, molecular characterization and virus challenge trials at the KARI Biotechnology centre. Institutions involved in biotechnology and biosafety development should establish linkages with centers of excellence to ensure access to the most up to date information.

Future activities

Resent studies indicate that the SPCSV is a key virus in the development of the SPVD. The sweetpotato weevil is also a major constraint to sweetpotato production. Pyramiding of virus and weevil resistance to ensure success in improvement of sweetpotato crop yields is an important objective for the future of this project. Future evaluations will also include food and environmental safety. Eventual commercialisation of the transgenic sweetpotato will follow assessment of its potential. The experiences gained in Kenya will be shared with partners in the ASARECA (Association for Strengthening Agricultural Research in East and Central Africa) region.

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