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Minireview

Enhancing banana weevil (*Cosmopolites sordidus*) resistance by plant genetic modification: A perspective

Andrew Kiggundu[1,2]*, Michael Pillay[4], Altus Viljoen[3], Clifford Gold[4], Wilberforce Tushemereirwe[1], Karl Kunert[2]

[1]National Agricultural Research Organization (NARO), Kawanda Agricultural Research Institute (KARI) P. O. Box 7065 Kampala, Uganda.
[2]Department of Botany, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, 74 Lunnon Road, Hillcrest, Pretoria South Africa.
[3]Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, 74 Lunnon Road, Hillcrest, Pretoria South Africa.
[4]International Institute of Tropical Agriculture (IITA), East and Southern Africa Regional Centre (ESARC), P. O. Box 7878, Kampala, Uganda.

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Banana weevil is a serious pest of bananas and plantains in Africa. The development of resistant cultivars is seen as the long term and more sustainable control strategy. The difficulty in conventional breeding of bananas and plantains has prompted efforts towards the use of genetic transformation for banana and plantain improvement. In this review, the current status of banana weevil resistance, sources of resistance and resistance mechanisms is assessed. Further, current efforts and future prospects for identifying resistance genes outside the genus *Musa* with potential to control banana weevil in a transgenic approach are outlined and discussed.

Key words: Banana weevil, host plant resistance, pest resistance genes, transgenic plants.

BANANA WEEVIL AS AN AFRICAN PEST

The banana weevil is a pest of considerable importance in Africa and significantly affects banana and plantain production (Ostmark, 1974; Gold, 1998; Swennen and Vulysteke, 2001; Gold and Messiaen, 2000; Fogain et al., 2002). The weevil has been associated with rapid decline of banana plantations in east Africa (Gold et al., 1999b) and a phenomenon called "yield decline syndrome" in West Africa (Ortiz and Vulysteke, 1994). The adult weevils are free living, have a nocturnal habit, and rarely fly. Their eggs are deposited at the base of the pseudostem or on exposed corms. Upon hatching, the larvae tunnel through the corm to feed and develop. The tunnelling damages the corm and weakens the plant reduces, reduces water and mineral uptake, reducing bunch weight (yield) and causing plant toppling during windstorms. In severe weevil infestations, crop losses of up to 100% have been reported (Sengooba, 1986). The establishment of new plantings in infested fields may fail (Price, 1994) and yield loss appears to increase gradually reaching 44% in the fourth ratoon cycle (Rukazambuga et al., 1998).

CONVENTIONAL BANANA WEEVIL CONTROL

Banana weevil control is currently based on the application of cultural practices, such as the use of clean

*Corresponding author. Email: andrew.kiggundu@fabi.up.ac.za. Fax: +27-12-4203960.

Table I. Suggested sources of banana weevil resistance in *Musa* spp. taken from field trial cultivar screening experiments.

Cultivar	Genome group	Reference
Yangambi km-5	AAA	Fogain and Price, 1994; Lemaire, 1996; Kiggundu et al., 2003
Sannachenkadali	AA	Padmanaban et al., 2001
Sakkali	ABB	
Senkadali	AAA	
Elacazha	BB	
Njalipoovan	AB	
Pisang Awak	ABB	Kiggundu et al., 2003
FHIA03	AABB	
TMBx612-74	IITA hybrid*	
TMB2x6142-1	IITA hybrid	
TMB2x8075-7 TMB2x7197-2	IITA hybrid	
	IITA hybrid	
Long Tavoy	ABB	Ortiz et al., 1995
Njeru	AA	Musabyimana et al., 2000
Muraru	AA	
Calcutta-4	AA	Fogain and Price, 1994; Ortiz et al., 1995; Kiggundu et al., 2003
Bluggoe	ABB	Fogain and Price, 1994; Kiggundu et al., 2003
M. balbisiana	BB	Fogain and Price, 1994

* IITA – International Institute of Tropical Agriculture

planting material, systematic trapping of adult weevils, in an effort to control population build-up, and field sanitation whereby residues that may form breeding grounds for the weevil are removed (Gold, 2000; Gold and Messiaen, 2000). Although cultural control methods contribute to weevil management, both high labour input and material requirements are often limiting factors for adoption of these practices (Gold, 1998; Gold et al., 2001). The application of effective pesticides is economically not feasible for subsistence producers. In addition, the banana weevil has developed resistance to a range of commonly used pesticides (Collins et al., 1991; Gold et al., 1999a). Consequently, development of resistant plants has been suggested as a potential long-term intervention for weevil control, especially on small-scale farms, as the inclusion of such plants might be part of an integrated pest management (IPM) framework (Seshu-Reddy and Lubega, 1993; Gold et al., 2001).

BANANA WEEVIL RESISTANCE AND RESISTANCE MECHANISMS

Considerable work has been done in screening diverse *Musa* germplasm for weevil resistance in Africa and Asia (Fogain and Price 1994; Pavis and Lemaire, 1997; Kiggundu et al., 1999; Kiggundu et al., 2003). Although plantains and east African highland bananas (EAHB) were found to be the most susceptible banana types, there were a few exceptions. For example, in India, Padmanaban et al. (2001) found that two plantains cultivars (Karumpoovan and Poozhachendu) were resistant to the banana weevil, while Fogain and Price (1994) found that the plantain cultivar Kedong kekang was resistant in Cameroon. Kiggundu et al. (2003) found that some highland banana cultivars including Tereza, Nalukira and Nsowe had intermediate resistance to weevils.

The large variability in weevil response observed in Musa germplasm and hybrid testing suggested that useful sources of weevil resistance are available in the Musa germplasm (Kiggundu et al., 2003). Candidates for use in conventional breeding for weevil resistance have been selected on the basis of very low levels of weevil damage in the field (Table 1). The AA genome progenitor *Musa accuminata* is more susceptible to weevils than the BB progenitor *Musa balbisiana* (Mesquita et al., 1984), and it is expected that AA sources of resistance might ultimately produce hybrids with better consumer acceptability. However, at this stage crosses with the more weevil-resistant BB type sources should not be completely excluded for obtaining a weevil-resistant hybrid (Mesquita et al., 1984; E DeLanghe, *personal communication*).

Classical resistance mechanisms (Painter, 1951) have been investigated in *Musa* germplasm, and antibiosis (factors affecting larval performance), rather than antixenosis (attraction), appears to be the most important resistance mechanism in banana (Mesquita et al., 1984; Ortiz et al., 1995; Abera et al., 1999; Kiggundu, 2000; Gold et al., 2001). Although some differences in attracting adult weevils to different cultivars have been identified no direct correlations between weevil resistance and plant damage were found (Buddenburg et al., 1993; Pavis and Minost, 1993; Abera et al., 1999; Kiggundu, 2000; Musabyimana et al., 2000). It was reported that differences in attraction have been due to environmental factors such as soil moisture around a cultivar with high sucker number (Ittyeipe, 1986).

Several banana plant phenological factors seem to contribute to weevil resistance. Corm hardness was the first biophysical factor associated with resistance. Whereas Pavis and Minost (1993) found a negative correlation (r = -0.47) between corm hardness and weevil damage, Ortiz et al. (1995) found no relationship between the two parameters in segregating plantain progenies. The latter suggested that other weevil resistance factors such as chemical toxins or anti-feedants might be playing role in weevil resistance. Kiggundu (2000) found that corm dry matter content, resin/sap production and suckering ability were negatively correlated with weevil damage. Whereas corm diameter and resin/sap production were important factors in EAHB accessions, corm dry matter content, corm hardness, resin/sap production and suckering ability (number of suckers) were significant parameters in the resistance response of recently introduced clones in east Africa. In large corms, the weevil larvae can complete their life cycle without borrowing too deep into the corm. This indicates that some form of tolerance exists in cultivars with large corms (Balachowsky, 1963).

High-performance liquid chromatography (HPLC) chromatograms from corm extracts of weevil-resistant AB (Kisubi) and ABB (Pisang awak) cultivars showed peaks that were absent when compared not only to susceptible clones, but also to some resistant clones of the AA (Calcutta 4) and AAA (Yangambi km5) genome group cultivars. This result possibly indicates a type of antibiotic mechanism that may be based on toxic compounds. These compounds are seemingly present in weevil-resistant cultivars with the BB genome suggesting that a different form of resistance may be present in weevil-resistant AA cultivars (Kiggundu, 2000). Furthermore, separation of a methanol extract from Pisang Awak (ABB) allowed the isolation of two highly polar sub-fractions with high anti-larval activity (Kiggundu, *unpublished data*). In these experiments, unique HPLC peaks were also found in susceptible cultivars, suggesting that the presence of compounds that may be related to weevil susceptibility.

As the identification of resistance mechanisms and weevil resistance genes within *Musa* continues, it is useful to develop biotechnology tools and protocols for the effective transfer of genes into *Musa* cultivars.

WILL GENETIC MODIFICATION OF BANANA PLAY A ROLE IN WEEVIL RESISTANCE?

The continual need to increase food production necessitates the development and application of novel biotechnologies to enable the provision of improved crop varieties in a timely and cost effective way. Table 2 provides examples of genetically modified (GM) plants that have been produced to date worldwide with increased resistance to different pests using genes from various sources. Although remarkable achievements have already been made in banana transformation, the identification and introduction of useful genes into banana to reduce losses caused by the banana weevil is still a major challenge. A variety of genes are available for genetic engineering for pest resistance (Carozi and Koziel, 1997; Sharma et al., 2000). Among these are proteinase inhibitors. *Bacillus thuringiensis* (Bt) toxins, plant lectins, vegetative insecticidal proteins (VIPs), and alpha-amylase inhibitors (AI).

Potential of proteinase inhibitors

The two major proteinase classes in the digestive systems of phytophagus insects are the serine and cysteine proteinases. Serine proteinase activity is present in Lepidoptera, Dictyoptera and Hymenoptera, while the cysteine proteinase activity is characteristic for Odoptera and Hemiptera. Coleopteran insects, including the banana weevil, mainly use cysteine proteinases (Gatehouse et al., 1985; Murdock et al., 1987). More recent studies indicate a combination of both serine and cysteine proteinases in the Coleoptera (Gerald et al., 1997). The combination of both cysteine and serine proteinases is considered amore advanced digestive strategy. Proteinase inhibitors are expressed naturally as a plant defence against insect attack (Green and Ryan, 1972; Ryan, 1990; Pernas et al., 2000; Ashouri et al., 2001). They operate by disrupting protein digestion in the insect mid-gut via inhibition of proteinases.

The development of genetically modified (GM) banana with weevil resistance is currently the focus of the Uganda National Agricultural Research Organisation (NARO), the Forestry and Agricultural Biotechnology Institute of the University of Pretoria (FABI) and the

Table 2. Examples of transgenic plants with increased resistance to insects conferred by genes from various sources. Bt - Cry – *Bacillus thuringiensis* crystal proteins; BAAI - Bean alpha amylase inhibitor; GNA - snow drop lectin; OC-I Oryzacystatin-I cysteine protease inhibitor from rice (*Oryza sativa*); CC-I corn cystatin; CPTi - Cowpea trypsin inhibitor.

Gene	Insect-pest	Transgenic plant	Observed effect	References
*Bt - Cry*1Ab	Rice leaffolder *Cnaphalocrocis medinalis*	Rice	High stable resistance – no symptoms	Ye et al., 2003
*Bt - Cry*03Aa	Colorado potato beetle	Potato	Increased resistance	Willson et al., 1992
*Bt - Cry*03Aa	Cotton boll weevil	Cotton	Increased resistance	Willson et al., 1992
CpTi	Rice stem borers *Chilo suppressalis* and *Sesamia inferens*	Rice	Significant resistance	Xu et al., 1996
OC-I	Colorado potato beetle (*Leptinotarsa decemleata*)	Potato	Increase resistance	Lecardonnel et al., 1999
CC-I	Maize grain weevil *(Sitophilus zea mais)*	Maize	Increased resistance	Irie et al., 1996
BAAI	Bean bruchids	Adzuki beans	Increased resistance	Ishimoto et al., 1996
BAAI	*Collasobruchus* spp.	Pea	Increased resistance	Schroeder et al., 1995
GNA	Peach potato aphid	Potato	Increased resistance	Gatehouse et al., 1997
GNA	Sugarcane grub (*Antitrogus consanguineus*	Sugarcane	Increased resistance	Nutt et al., 1999

International Institute of Tropical Agriculture (IITA) (NARO/FABI/IITA). The objective of this research is to express exogenous cysteine proteinase inhibitors in bananas with the potential to control banana weevil. These inhibitors have already been used for insect control in GM plants (Leple et al., 1995). Cysteine proteinases are not part of the human digestive system, and therefore humans can consume cysteine proteinase inhibitors safely. The NARO/FABI/IITA group by interacting with the University of Laval (D. Michaud) is currently investigating the potential of plant derived cysteine proteinase inhibitors (phytocystatins) from rice and papaya in GM banana. Presently, cysteine proteinase activity has been identified in the mid-gut of the banana weevil and *in-vitro* studies have shown that these cysteine proteinases are strongly inhibited by both a purified recombinant rice (oryzacystatin-I (OC-I)) and papaya cystatin (Kiggundu et al., unpublished data). By using a newly developed bioassay system based on vacuum infiltration of banana stems, it was shown that recombinant papaya cystatin significantly reduced the early growth and development of weevil larvae (Kiggundu et al., unpublished data). In addition, the design of optimised inhibitors using site-directed mutagenesis is currently being carried out to improve the inhibition and stability of the inhibitors for weevil control. GM bananas expressing a modified OC-I gene targeting nematodes have been produced by the John Innes Centre (JIC, Norwich, UK) together with the University of Leeds, UK (P. Vain, John Innes Centre, UK. *personal communication*).

Although the phytocystatin strategy is interesting there are certain stumbling blocks to overcome. Some insects when exposed to a particular proteinase inhibitor selectively secrete other proteinases that are insensitive to that particular inhibitor. In other cases variation in the gut environment, especially pH, leads to inactivity of the inhibitor thus making the strategy a little more complicated. Strategies for overcoming these and other stumbling blocks in the use of proteinase inhibitors in a transgenic approach have been reviewed by Winterer (2002).

Other genes with potential

The expression and biological activity of the *Bacillus thuringiensis* (Bt) toxins has been investigated in GM plants for insect control. *Bt* gene technology is currently the most widely used system for Lepidopteran control in commercial GM crops (Krettiger, 1997). *Bt* genes are a group of more than fifty insecticidal crystal proteins. When ingested by an insect feeding on Bt expressing Gm plant, the proteins are solubilized in the alkaline environment of the insect's midgut and become toxic by binding to apical border brush membranes of the columnar cells. This causes lysis of the cells and eventual death of the insect. NARO in collaboration with the Centre de Coopération Internationale en Recherché Agronomique pour le Dévelopement (CIRAD) in France, is screening a range of newly isolated Bt toxins for banana weevil toxicity. This work is, however, currently being hampered by the absence of an artificial diet for the banana weevil affecting efficient screening under controlled conditions. The expression of a selected *Bt* gene for weevil resistance may be a rather long-term strategy since no potential *Bt* gene with high toxic effects against the banana weevil has been identified as yet. In earlier screening experiments, 80 Bt strains were tested against the banana weevil at the Katholieke Universiteit Leuven (KUL) without much success (Traore et al., unpublished data). It has been suggested that the acidic environment of the banana weevil gut may be influencing the solubility of the Bt toxin. (J-M.Vassal, CIRAD, personal communication).

Plant lectins confer a protective role against a range of organisms (Sharma et al., 2000). Lectins have been isolated from a wide range of plants including snowdrop, pea, wheat, rice and soybean. Their carbohydrate-binding capability renders them toxic to insects. A lectin from snowdrop, *Galanthus nivalis* agglutinin (GNA), is toxic to several insect pests in the orders Homotera, Coleoptera and Lepidoptera (Tinjuangjun, 2002). In collaboration with NARO, the effect of GNA and the *Aegopodium podagraria* lectin (APA) on the mortality and reproduction of three nematode species pathogenic to banana is being tested at KUL (Carlens, 2002). Similar work can be extended to banana weevil using *in vivo* assays. A major concern about the use of lectins, however, is that some of them, such as the wheat germ agglutinin (WGA), are toxic to mammals (Jouanin et al., 1998). However, the snowdrop and garlic lectins are toxic only to insects (Boulter, 1993), and are potential candidates for weevil control.

Vegetative insecticidal proteins (VIPs) are similar to Bt toxins. VIP1 and VIP2 are contained in supernatants of *Bacillus cereus* (Warren, 1997) while VIP3 can be found in the supernatants of *B. thuringiensis*. They function by causing gut paralysis followed by lysis of the gut epithelium cells, completely arresting gut function and leading to the death of the insect (Duck and Evola, 1997).

Alpha-amylase inhibitors (AI) and chitinase enzymes might also have a future potential for weevil control. Alpha-amylase inhibitors operate by inhibiting the enzyme alpha-amylase, which breaks down starch to glucose in the insect gut (Le Berre-Anton et al., 1997; Morton et al., 2000). They are divided into two types, AI-1 and AI-2, isolated from common and wild beans (*Phaseolus vulgaris*), respectively. Ishimoto et al. (1996) produced transgenic adzuki beans with enhanced resistance to bean bruchids, which are Coleopteran insects. Alpha-amylase inhibitors may be of interest for banana weevil control in GM banana. Chitinase enzymes are produced as a result of invasion either by fungal pathogens or insects. Transgenic expression of chitinase has shown improved resistance to *Lepidopteran* insect pests in tobacco (Ding et al., 1998). At KUL, a rice chitinase gene has been transferred into bananas with the objective of controlling fungal pathogens (Arinaitwe, 2002).

THE WAY FORWARD

Plant engineering has, without doubt, the potential to play a key role in the sustainable production of bananas and plantains in Africa. This has recently been realised by several African governments and international donors by providing, in general, better financial support for research into banana and plantain biotechnology. Such support includes the extensive training of young African scientists in plant biotechnology in both African and overseas research institutions, and the setting up of research networks in plant biotechnology on the African continent.

The current efforts at NARO, IITA and the African Centre for Banana and Plantain (CARBAP) in Cameroon, to develop weevil resistance by conventional means using carefully selected parents and especially improved diploids with resistance should continue to complement the biotechnology efforts. This will provide a two-pronged strategy that would increase the chances of developing resistance against the banana weevil. The development of rapid screening methods for testing weevil resistance in the field and laboratory is an essential step in developing resistant hybrids (Kéhé et al., 2000; Messiaen, 2002). A better understanding of resistance mechanisms and genes that control these mechanisms are among the priorities in developing successful resistance for the banana weevil.

Unfortunately, certain barriers exist in Africa that limit the opportunity to take advantage of the benefits plant biotechnology might offer. Affordability is one of the major barriers for the introduction of any costly biotechnology-derived product. Also, what might look exciting in the lab might not necessarily make a good and affordable product when considering the needs of small-scale farmers in Africa. In this respect, one should also consider whether the over-expression of a single selected native plant gene, as is done in many engineering approaches, is not too simplistic and ultimately ineffective in obtaining efficient pest resistance. Natural plant resistance is mostly polygenic. Recent evidence shows, however, that these sophisticated defence mechanisms have been lost during selection for domestication (Carlini and Grossi-de-Sa, 2001). Therefore, one approach would be to optimise a “resistance” gene by protein engineering, or a balanced interaction that involves the simultaneous expression of several protective proteins by using gene pyramiding or multiple resistance engineering (Winterer, 2002). In addition, when a triploid GM banana is produced, it will have to be propagated vegetatively for large-scale multiplication. This raises questions about the genomic integrity of the off-spring plant, such as the occurrence of mutations and epigenetic changes as a result of the plant transformation process (Karp, 1993; Phillips et al., 1994; Labra et al., 2001; Van der Vyver et al., 2003).

Without doubt, any GM banana expressing banana weevil resistance would have great value for the small-scale farmers. However, the current negative public perception of GM crops and concerns of bio-safety has to be addressed. Research on performance of GM bananas under African field conditions and the long-term response of weevils will be necessary to make a judgement of GM technology on weevil resistance. These concerns are important even in African countries, where crop losses due to stress are most severe and which would, therefore, benefit most from the successful development of GM crops.

The need to strengthen the whole transgenic approach by developing a wide range of technical expertise through training cannot be over emphasised. The benefits would include the development of integrated biotechnology teams with overlapping expertises required for success.

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