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Exploiting the use of biotechnology in sweetpotato for improved nutrition and food security: Progress and future outlook

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ABSTRACT

Sweetpotato (*Ipomoea batatas*) production is expanding faster than any other major food crop in sub-Saharan Africa (SSA), with about 13.4 million tons of roots from 3.2 million hectares in 2005. However, major constraints, including sweetpotato weevils (SPWs) and sweetpotato virus disease (SPVD) cause almost total crop loss on susceptible cultivars. This paper reviews examples where biotechnology in particular biofortification and genetic transformation could be used to improve sweetpotato for nutritional quality and food security. Expression of Cylas-active *Bacillus thuringiensis* (Bt) Cry proteins in sweetpotato could result in varieties potentially with field resistance against SPWs. Post transcriptional gene silencing (PTGS) mode of resistance to SPVD is promising. Quantitative trait loci (QTL) for dry-matter, starch, ß-carotene content, and yield have been identified in a hexaploid sweetpotato mapping population of 'Tanzania' (female, cream-colored flesh) x 'Beauregard' (female, orange-fleshed storage roots). Biotechnology approaches offer an attractive option of integrating some desired traits into farmer preferred sweetpotato cultivars in a more effective manner than conventional breeding.

Keywords: *Ipomoea batatas, Biofortification, Cry toxins, Quantitative trait loci*

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Importance of Sweetpotato in Sub-Saharan Africa (SSA)

Sweetpotato, *Ipomoea batatas* L. (Lam.) is an important food crop in more than 100 countries, and is ranked according to FAO data as the seventh most important food crop globally, grown on 9 million hectares, yielding about 124 million tons, with an average of about 13.7 ton/ha (FAOSTAT, 2007). In the developing world, sweetpotato is especially important because it is a highly adaptable crop that generates large amounts of food per unit area and unit time during relatively short rainy periods, giving it an advantage over major staples. Sweetpotato also has flexible planting and harvesting times, tolerates high temperatures and low fertility soils, is drought tolerant, is easy to propagate and maintain, and yields well in adverse conditions. Furthermore, compared to other crops, sweetpotato requires fewer inputs and labour, making it particularly suitable for households threatened by migration or diseases such as HIV/AIDS (Jayne *et al*., 2004).

These characteristics make sweetpotato ideal as a crop for poverty alleviation. They also make it more suitable for mitigating food shortages and famines that occur under the most severe socio-political circumstances such as in war-zones that are common in Africa. Although China accounts for about 85% of the world production, sweetpotatoes are also an important food staple in Asia, Africa, and the Caribbean, and South America, where they are an important source of carbohydrates, vitamins A and C, fibre, iron, potassium, and protein (Table 1) (Woolfe, 1992).

Table 1. Energy and Vitamin A yields of sweetpotato and other major starchy staples

Crop[a]	Average tropical yield (tons/ha)	Edible energy value (MJ/kg)	Edible energy per ha (10^3 MJ)	Available vitamin A value RAE[c] µg/100 gms	Vitamin A per hectare (RAE/ha)	Mean growth period (Days)	Edible energy (MJ/ha /day)
Sweeetpotato[d]	7	4.8	27.2	0-2500	0 to 175 million	140	194
Cassava[d]	9	6.3	45.6	0-75	0 to 6.5 million	330	138
Yam[d]	7	4.4	26.2	0-7	0 to 490,000	280	94
Banana	13	5.4	41.4	3	0 to 390,000	365	113
Rice[b]	2	14.8	20.8	0	0	140	149
Maize	1	15.2	18.8	0-30	0 to 300,000	130	145
Sorghum	<1	14.9	11.2	0	0	110	101
Millet	<1	15	8.2	0	0	100	82

Source: Low et al. (2009)

[a] Cereals, air-dry; roots/tubers/bananas fresh. [b] Paddy Rice. [c] RAE = Retinol Activity Equivalent

[d] Andrade et al. (2009); cassava varieties above 5 µg/100 gms still under development.

Sweetpotato is one of the most widely grown root crops in SSA, covering about 3.2 million hectares, with estimated annual production of 13.4 million tons of roots in 2005 (FAOSTAT, 2007). It is predominantly grown in small plots by poorer farmers, and is often referred to as the "poor man's food" (Woolfe, 1992). Its ability to produce relatively good yields under marginal conditions, its flexible planting and harvesting times provide roots and leaves during hunger seasons, and its good yield response to better management are factors driving its expansion in SSA, especially in land constrained countries with high population densities, justifying its reputation as the "classic" food security crop (Woolfe, 1992). FAOSTAT (2007) show that the area planted to maize in SSA is 9 times greater than to sweetpotato, but the latter is expanding faster than any other major food crop in SSA. All African countries recorded significant growth (3.1% annual growth) in sweetpotato area during 1991-2006. Currently 34.5% of global sweetpotato area is in Africa representing a significant increase from 4.6% crop area in 1961 (Srinivas, 2009).

Major production and nutrition constraints

Although the area under sweetpotato is expanding rapidly in SSA farmers have to struggle to overcome several challenges. The major constraints to increased sweetpotato productivity include, declining soil fertility, drought, low yielding varieties, sweetpotato diseases (mainly sweetpotato virus disease (SPVD), and *Alternaria* blight), and insect pests, (mainly, sweetpotato weevils). Shortage of high quality planting materials, and limited range of processing and utilization options, leading to high post harvest losses, estimated between 30-35%, are also important production constraints. In addition, low nutritive value (low ß–carotene) of non-orange-fleshed sweetpotato and marketing problems limit availability of food with health attributes and processed products to consumers. Research to address these production and market constraints requires a sustained effort in the medium (2-5 years) to long term to produce adapted technologies such as pest resistant varieties. Biotechnology approaches offer an attractive option of integrating desired traits into farmer and consumer preferred sweetpotato cultivars. Examples where biotechnology could be used to improve sweetpotato significantly for nutrition and food security are highlighted below.

Target traits and strategies using biotechnology tools

Biotechnology tools are used to complement conventional approaches particularly for traits where conventional breeding efforts and integrated crop management efforts have till now not produced durable resistances particularly for pests and diseases. In sweetpotato, the target traits are mainly sweetpotato weevil and virus disease resistance.

Sweetpotato genetic transformation for weevil resistance. One of the most important causes of sweetpotato production losses worldwide is the sweetpotato weevil, *Cylas* spp. (Sutherland, 1986). Sweetpotato weevils are the most important biological threat to productivity, marketability, and sustainability in areas with significant dry periods.

The most ubiquitous *Cylas* species worldwide is *C. formicarius elegantulus* (Summers). However, in SSA the primary species are C. *puncticollis* (Boheman) and *C. brunneus* (Fabricius), which may cause losses of up to 60-100% depending on severity of attack (Smit, 1997; Stathers *et al.*, 2003). The primary damages caused by *Cylas* spp. larvae are partial consumption of the root, unacceptable microbial contamination, and production of toxicants by the sweetpotato root making it unfit for consumption (Uritani *et al.*, 1975; Sato and Uritani, 1981; Woolfe, 1992). The eggs are laid right underneath the skin and when hatched larvae tunnel into the sweetpotato root. This makes weevils extremely difficult to control because strategies such as use of insecticides, and integrated pest management (IPM) have primarily targeted adults. Pheromone mass-trapping of *Cylas* males has been successful in Cuba for the control of *C. formicarius* (Lagnaoui *et al.*, 2000). However, no differences in storage root infestation levels and female mating was observed when mass-trapping experiments were conducted

in Uganda using pheromones of the African species *C. puncticollis* and *C. brunneus* (Smit *et al*., 2001). The losses due to weevils limit the crop's potential contribution to reducing poverty and under-nutrition.

A recent survey on the socio-economic impact of weevils in Uganda, reports an average yield loss of over 28% between wet and dry seasons (Kiiza *et al.*, 2009). According to Qaim (2001), weevil resistance sweetpotato cultivars generated from biotechnology applications if deployed in Kenya would create welfare gains of US $ 9.9 million and an approximate internal rate of return on research investment of between 33 to 77%.

Breeders and biotechnologists seek to develop plants that are resistant to the weevils. Conventional breeding of sweetpotato is problematic due to factors such as hexaploid genomics, high heterozygosity, low seed set and self- and combining- incompatibilities. Conventional breeding alone is unlikely to provide all the solutions to sweetpotato improvement.

Weevils have the capacity to adapt and develop resistance to active proteins and compounds found or introduced into new varieties. Hence, long term control requires use of multiple strategies, to increase barriers to the rapidly evolving pests that pose a threat to the durability of available resistance.

Progress in breeding with resistance to weevils has been slow mainly due to scarcity of varieties with significant levels of resistance (Stevenson *et al.*, 2009). Nonetheless, weevil resistance was among the first traits for which genetic transformation was applied in the crop. Early work focused on transformation with proteins that decrease the digestibility of sweetpotato for insects. Sweetpotato was transformed with a cowpea (*Vigna unguiculata*) trypsin inhibitor (CTI) and the mannose binding snowdrop lectin (Newell *et al*., 1995); a soybean (*Glycine max*) Kunitz-type trypsin inhibitor (SKTI) and a rice (*Oryza sativa*) cystein proteinase inhibitor (OCI) (Cipriani *et al*., 1999, 2001).

Initial results in no-choice feeding tests showed moderate increase of weevil resistance in two transgenic events produced by Newell *et al*. (1995) with the West Indian sweetpotato weevil (*Euscepes postfasciatus*) in a screenhouse bioassay (Zhang *et al*., 2000). However, the strategy of using proteinase inhibitors was later abandoned due to the relatively small increase of resistance observed and because there were concerns regarding nutritional impact of such proteins on the human diet. More recently, toxins from *Bacillus thuringiensis* (Bt) were tested against the two African weevil species, *C. puncticollis* and *C. brunneus*, and against the American and Asian species, *C. formicarius*.

A diet incorporation methodology provided reliable toxicity measures of seven Cry proteins from Bt strains, which were chosen based on prior evidence of toxicity (Maingi *et al*., 2002) or known anti-Coleopteran activity. All Cry proteins evaluated were toxic to both species at concentrations less than 1 µg/gram diet, and Cry7Aa1, ET33/34, and Cry3Ca1 had LC_{50} values below 1 µg/gram diet against both species (Ekobu *et al.*, 2010). These tests demonstrated the feasibility of transformation of sweetpotato varieties potentially conferring field resistance against these pests. Four of these Cry toxins were selected for plant expression because of their toxicity and low sequence identity, which is important for the potential of cross-resistance. Studies to help establish the potential for cross-resistance are currently ongoing. Several gene constructs have been developed using chemically synthesized sequences for the coding and polyA regions and optimized for sweetpotato, and sweetpotato promoters to express high level in the storage root. For weevil and virus resistance, transformation with *Agrobacterium* is underway for African varieties that are amenable to transformation and regeneration both in Kenya and Uganda (Gichuki *et al*. 2007; Kreuze *et al.*, 2009).

Sweetpotato genetic transformation for virus disease (SPVD) resistance. SPVD is a synergistic viral disease caused by co-infection of a crinivirus, *Sweet potato chlorotic stunt virus* (SPCSV) with a potyvirus, *Sweet potato Feathery Mottle Virus* (SPFMV). Whereas most sweetpotato cultivars are highly resistant to most viruses including SPFMV, infection with SPCSV renders them highly susceptible and the severe disease, SPVD, follows. Therefore SPCSV can be considered a major target for which resistance is required, although increasing resistance to SPFMV may also reduce damage caused by SPVD. The causal viruses of SPVD are transmitteed by whiteflies (*Bemisia tabaci;* SPCSV) and aphids (*Myzus persicae;* SPFMV), and during vegetative propagation of sweetpotato by humans. The disease causes stunting, mottling and deformation of sweetpotato leaves and up to 99% loss in yield (Njeru *et al.*, 2004; Mukasa *et al*., 2006).

Sources of good resistance, useful in breeding programs, are yet to be identified for SPCSV and SPVD. However, local varieties and improved varieties such as New Kawogo and NASPOT 1, respectively, exhibit reduced incidence of the SPVD in the field compared to other cultivars, a type of resistance for which the mechanism has not yet been elucidated (Mwanga *et al*., 2002; Kreuze *et al.*, 2009).

Viruses that cause SPVD belong to the family *Closteroviridae* and *Potyvidiridae* which are known to be highly variable and have a high rate of evolution leading to emergence and re-emergence of epidemics. Therefore, long term control requires use of multiple strategies, to increase barriers to the rapidly evolving pathogens that pose a threat to the durability of available resistance (Kreuze *et al*., 2009).

A transgenic approach is a good option for integrating SPVD resistance into farmer and consumer preferred cultivars. Indeed, for other crops, transgenic plants genetically transformed with similar genes have been produced, tested in greenhouse and field conditions, and in several cases developed for commercial release. Field trials of crops engineered for resistance to viral diseases have been approved in Canada, the United States, Mexico, China, Kenya and other countries (Fuchs and Gonsalves, 2007). Sweet potato transformed with the coat protein (CP) encoding sequence of SPFMV was resistant to SPFMV following experimental inoculation by grafting (Okada *et al.*, 2002). This type of pathogen-derived resistance (PDR) has been used against some viruses in many crop species (Latham and Wilson, 2008).

However, resistance that works under controlled experimental conditions may not necessarily work under field conditions. This was the case for the first transgenic sweetpotato lines engineered for resistance to SPFMV using PDR. Their resistance broke down in East Africa (New Scientist, 7 February 2004, p. 7) possibly because the transgene was not from a locally prevalent SPFMV strain or because the plants became infected with SPCSV (Kreuze *et al.*, 2009). Resistance to potyviruses mediated by a rice cystein proteinase inhibitor (OCI) has been reported in tobacco (*Nicotiana tabacum*) (Gutierrez-Campos *et al.*, 1999). The OCI mediated resistance to potyviruses is thought to act through inhibiting the viral cystein proteinase NIa that processes the potyviral polyprotein. Closteroviruses also encode cystein proteinases to modify some of their proteins. Therefore, it was considered that expression of cystatins in transgenic plants might confer resistance to both viruses involved in SPVD. Cipriani *et al.*, (2001) reported increased resistance to SPFMV in sweetpotato plants of cv. 'Jonathan' transformed with the *OCI*. However, mixed infection with SPCSV still caused SPVD.

Post transcriptional gene silencing (PTGS) or RNA silencing, is a particular form of PDR. The PTGS strategy is based on the action of short interfering RNA (siRNA) molecules, which are formed in the plant in response to double stranded RNA (dsRNA) (Lindbo and Dougherty, 2005; MacDiarmid, 2005). The dsRNA molecules expressed in the transgenic sweetpotato events trigger naturally occurring defence mechanisms in the plants and serve as guides for enzymatic cleavage of complementary RNAs produced by SPFMV and SPCSV, thus destroying the corresponding viruses.

Recently, Kreuze *at al.* (2008) genetically engineered a Peruvian landrace sweetpotato variety 'Huachano' that is extremely resistant to SPFMV for resistance to SPCSV. In the case of Jonathan and Huachano as in many others (Karyeija *et al.*, 2000) the high levels of resistance to SPFMV breaks down following infection with SPCSV and the plants succumb to the severe SPVD. This shows the importance of the resistance to SPCSV in protecting sweetpotatoes against SPVD and other severe synergistic diseases induced by SPCSV with other viruses. The transgene was designed to express an SPCSV-homologous transcript that forms a double-stranded structure to efficiently prime virus-specific resistance through PTGS. Several transgenic lines accumulated only low concentrations of SPCSV after infection and no or only mild symptoms developed. These results showed that sweetpotato could be protected against the disease caused by SPCSV using PTGS. However, the low concentrations of SPCSV in the transgenic plants were still enough to break down the natural high levels of resistance to SPFMV. Hence, immunity to SPCSV seems to be required for prevention of the severe virus diseases in sweetpotato (Kreuze *et al.*, 2008).

PTGS may be lost following infection of the plants with a virus that is not targeted by PTGS (Latham and Wilson, 2008). This is explained by suppression of RNA silencing by the untargeted virus. RNA silencing is a fundamental antiviral defence system in plants and other cellular organisms. It becomes activated or primed by transgene-driven over-expression of viral RNA in cells of transgenic plants and by double-stranded structures of the viral RNA during infection of non-transgenic plants (Haasnoot *et al.*, 2007). Hence, PTGS actually activates the natural antiviral resistance to be ready for action when the target virus initially infects cells. However, PTGS is sequence-specific and therefore not able to target viruses that show less than ca. 87–90% sequence identity with the transgene sequence (Jones *et al.*, 1998). Consequently, the virus that circumvents PTGS will accumulate and produce proteins that suppress RNA silencing (Voinnet, 2005), which will convert the plant susceptible also to the virus that was the target of PTGS. For these reasons, the commonly encountered mixed virus infections in the field and the genetic variability of sweetpotato viruses pose an important challenge that needs to be addressed before sustainable virus resistance can be obtained (Tairo *et al.*, 2005).

Interference with silencing suppressor of SPCSV for controlling SPVD is another strategy of sweetpotato genetic transformation for virus disease resistance. SPCSV encodes an RNase3 protein which is a silencing suppressor (Kreuze *et al.*, 2005) and this protein by itself is able to provoke SPVD-like disease in RNase3 transgenic plants infected with SPFMV (Cuellar *et al.*, 2009). The RNase3 protein functions as a dimer and a mutation in the catalytic RNase3 signature motif (RNase3-Ala37,44) renders it dysfunctional, hence unable to suppress silencing (Kreuze *et al.*, 2005; Cuellar *et al.*, 2009). It is hypothesized that over- expression of the RNase3-Ala37,44 protein will interfere with the function of the wild-type protein expressed by SPCSV by binding to it, resulting in a dysfunctional RNase3-RNase3-Ala37,44 dimer, unable to suppress RNA silencing. Such transgenic plants might become resistant to SPVD.

Other Target Traits for Genetic Modification. There are other traits that could be targeted for genetic modification, for example, drought tolerance (particularly survival of shoots during drought, and sprouting of storage roots at the beginning of the rainy season), starch (modification for quality and quantity), baking quality, protein content, and nematode resistance; some of these have been described by Kreuze *et al.* (2009).

Sweetpotato genetic mapping and genomics

Vitamin A deficiency (VAD) remains a serious threat to children under five years of age in SSA. In 2005, an estimated 43 million children in SSA under 5 years old were still at risk of VAD (Aguayo and Baker, 2005). The causal link between VAD and associated increased child mortality is well-established. Extreme mortality rates (60%) are linked with severe VAD, and even sub-clinical deficiency is associated with a 23% increase in pre-schooler mortality (Sommer and West, 1996). Development of orange-fleshed sweetpotatoes (OFSP) is essential for the improvement of the food supply and nutritional status of millions of people in developing countries, particularly in SSA. However, sweetpotato breeding is challenging due to its genetic complexity and marker-assisted breeding tools are needed to facilitate crop improvement.

Recently, the North Carolina State University, U.S.A. and the National Agricultural Research Organisation research team of Uganda identified quantitative trait loci (QTL) for nematode resistance, dry-matter, starch, ß-carotene content, and yield in a hexaploid sweetpotato mapping population derived from a cross between 'Tanzania', a cream-fleshed, high dry matter African landrace, and 'Beauregard', an orange-fleshed, low dry matter sweetpotato cultivar popular in U.S.A. Two parental maps were constructed using a population of 240 clones (Cervantes *et al.*, 2008a, b). In both parental maps, QTL analysis revealed the presence of 13 QTL for storage root dry-matter content, 12 QTL for starch content, 8 QTL for ß-carotene content, and 18 QTL for yield. Multiple QTL regression models developed for segregation of alleles in each parent explained 15-24% of the variation in dry matter content, 17-30% of the starch content, 17-35% of ß-carotene content, and 12-30% of the variation in yield (Cervantes, 2006; 2010). Molecular markers are an important genetic diversity analysis tool for increasing sweetpotato breeding efficiency (Yada *et al.*, 2010; Elameen *et al*., 2008). This work is a first step toward the long-term goal of developing marker-assisted breeding tools to aid sweetpotato breeding efforts. It also improves our understanding of the inheritance of these important traits in sweetpotato.

CIP and collaborating partners have also developed some genomic resources for sweetpotato breeding, supported by the Generation Challenge Program. They have 454-sequenced two normalized cDNA libraries and have established a gene index consisting of about 30,000 contigs and another 29,000 singletons. The gene index and database are available at the CIP website *(http://www.cipotato.org/sweetpotato_gene_index)*. CIP has produced a population from two heterozygous (2x) *Ipomoea trifida* accessions. This population serves for establishing a diploid reference map for sweetpotato. Currently, CIP has about 680 Diversity Arrays Technology (DArT) markers and 50 simple sequence repeat (SSR) markers to genotype this population; and the map based on *I. trifida* is expected to be ready by 2013. For rapid progress there is a strong need to make available more and better genomic resources for sweetpotato by increasing available sequence information. This could be done by increasing transcriptome sequencing of different tissues and clones for functional genomics. CIP, in collaboration with the University of Ghent is sequencing the genome of sweetpotato, cv Huachano (SOLiD4 complete genome shotgun sequencing with paired ends at 90x coverage of the haploid genome) which is expected to provide a wealth of new data for genomic studies and development of new molecular markers. To study QTL for some traits, it would be easier to do in the 2x population than in hexaploid sweetpotato, however, it might not be suitable to assess most traits of interest, although the parents do produce storage roots. The available DArT resource could be used to increase the number of markers for genotyping the mapping populations.

Conclusion

Africa faces major sweetpotato production constraints, including SPWs and SPVD which cause almost total crop loss on susceptible cultivars. Biotechnology could be used to improve sweetpotato significantly for nutrition and food security. Expression of *Cylas*-active *Bacillus thuringiensis* (Bt) Cry proteins in sweetpotato could result in varieties potentially with field resistance against SPWs. Post transcriptional gene silencing (PTGS) mode of resistance to SPVD is promising. Quantitative trait loci (QTL) for dry-matter, starch, ß-carotene content, and yield have been identified in a hexaploid sweetpotato mapping population of 'Tanzania' (female, cream-colored flesh) x 'Beauregard' (female, orange-fleshed storage roots). Biotechnology approaches offer an attractive option of integrating desired traits into farmer and consumer preferred sweetpotato cultivars to improve sweetpotato significantly for nutrition and food security.

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