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Genetic Transformation of Sweet Potato for Improved Tolerance to Stress: A Review

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Abstract

The sweet potato (*Ipomoea batatas Lam*) is a major staple food in many parts of the world. Sweet potato leaves and tubers are consumed as food and livestock feed. Biotic and abiotic stresses affect yield leading to a reduction in production. This review analyzes factors limiting sweet potato production and the progress made towards stress tolerance using genetic transformation. Genetic transformation could enhance yield, nutritional value and tolerance to stress. Transgenic sweet potatoes tolerant to biotic and abiotic stress, improved nutritional value and higher yields have been developed. Sweet potato expressing the endotoxin *cry8Db*, *cry7A1* and *cry3Ca* genes showed lower sweet potato weevil infestation than non-transformed lines. Transgenic cultivar 'Xushu18' expressing the oryzacystatin-1 (*OC1*) gene showed enhanced resistance to sweet potato stem nematodes. Sweet potato line 'Chikei 682-11' expressing the coat protein (*CP*) exhibited resistance to the sweet potato feathery mottle virus (SPFMV). Transgenics expressing the rice cysteine inhibitor gene oryzacystatin-1 (*OC1*) also exhibited resistance to the SPFMV. Transgenic cultivar 'Kokei' expressing the spermidine synthetase gene *FSPD1* had higher levels of spermine in the leaves and roots, and displayed enhanced tolerance to drought and salt stress. 'Shangshu' variety expressing the *IbMas* has shown enhanced tolerance to salt stress. Transgenic 'Lixixiang' expressing *IbMIPSI* showed an up-regulation of metabolites involved in stress response to drought, salinity and nematode infestation. Transgenic 'Yulmi' sweet potato transformed with copper/zinc superoxide dismutase (*CuZnSOD*) gene showed an enhanced tolerance to methyl viologen induced oxidative and chilling stress. Similarly, transformation of cultivar 'Sushu-2' with betaine aldehyde dehydrogenase (*BADH*) gene resulted in transgenics tolerant to salt, chilling and oxidative stress. Sweet potato varieties 'Kokei14' and 'Yulmi' transformed with the *bar* gene were shown to be tolerant to application of the herbicide Basta. The development of stress tolerant varieties will immensely increase the area under sweet potato production and eventually promote the adoption of sweet potato as a commercial crop. Sweet potato research and breeding for stress tolerance still faces technical and socio-political hurdles. Despite these challenges, genetic transformation remains a viable method with immense potential for the improvement of sweet potato.

Key words: Sweet Potato (*Ipomoea batatas Lam*), Stress, Genetic Transformation, Transgenic

1.0 Introduction

Sweet potato (*Ipomoea batatas Lam*) is a gametapetalous, creeping herbaceous dicot in the order Polemoniales and family Convolvulaceae (Agili *et al*., 2012). It is a perennial that is normally grown as an annual and is mostly cultivated for its tuberous roots that are rich in starch and other nutrients (González *et al*., 2008). Sweet potato is grown in a wide range of agro-ecological zones ranging from the tropics to the temperate regions both in the highlands and lowlands. Conventional propagation of sweet potato is done using cuttings. Due to its tolerance to a wide range of agro-ecological conditions, potential high yields, ease of cultivation, effective vegetative propagation and high nutritive value, sweet potato is suitable for cultivation in marginal lands which are often poor, making it an important food security crop (Agili *et al*., 2012; Ogero *et al*., 2012). In countries like Ethiopia where population growth is high and there is a constant threat of starvation, sweet potato forms an important component of the diet (Abdissa *et al*., 1991).

Sweet potato ranks seventh globally as an important crop after rice, wheat, maize, cassava, white potato and barley. In Africa, sweet potato ranks as the second tuber crop after cassava. Annual sweet potato production stands at 115 million tonnes with an approximate plantation area of 10 million hectares (Kumar 2007). The world's largest producer is China, followed by sub-Saharan Africa (Liu 2011; Luo *et al*., 2006). About 7 million tonnes of sweet potato are produced in sub-Saharan Africa accounting for 5% of global production. However, yields in Africa stand at less than 5 tonnes/ha compared to the world's average of 15 tonnes /ha. Africa's top

producers are Uganda, Rwanda and Kenya at 1.7 million tonnes, 960,000 tonnes and 725,000 tonnes respectively. In the East Africa region, cultivation is majorly done in areas around Lake Victoria (Mukherjee *et al.,* 2012; Shekhar *et al*., 2013).

2.0 Sweet Potato Uses

The tubers and leafy tops of sweet potato are mainly used as food, making it a major staple food in many countries of the world. The yellow and orange pigmented varieties contain carotenoids making it a rich source of vitamin A and C (Agili *et al*., 2012; Motsa *et al*., 2015). The yellow fleshed varieties have beta carotene which the human body can easily convert to Vitamin A. Consumption of these sweet potatoes contributes to a reduction in Vitamin A deficiency. Other important nutrients in sweet potato include vitamin B6, Vitamin E, manganese, iron, calcium and copper. It has been sown that only 125g of orange fleshed sweet potato is enough to provide the dose of beta carotene required by pre-school children (CIP). The sweet potato tuber mainly contains starch, little protein and almost no fat. The sweetness of the tuber is due to the presence of glucose. Sweet potato flour can be fermented to make wine. The flour can also be used to replace wheat to make cakes and buns (Islam, 2014).

The green leafy parts have been used as a valuable source of livestock feed. The vines and foliage can be fed to livestock and they compare favorably with alfalfa hay (Sefasi *et al*., 2013). In the temperate regions, dried sweet potato tubers have replaced the traditional energy sources such as maize in cattle rearing. This has led to increased milk production and an increase in the vitamin A content in milk (Ozturk *et al*., 2012). Sweet potato has recently been exploited for medicinal purposes (Far *et al*., 2009; Islam 2014). The leaves have been used in the treatment of respiratory diseases and infections, gastro-intestinal relief as well as relief from bug bites and burns, reduction of fever, an immune booster as well as a powerful antioxidant (Sefasi *et al*., 2013). Flavonoid compounds from the tuber and the leaves have been shown to have therapeutic effects against stress and aging (Motsa *et al*., 2015). Purple colored sweet potato that was developed in the 1990s contains anthocyanins that have anti-oxidative or radical scavenging activity and health benefits to humans. Sweet potato is efficient in starch production and this has made it an attractive target for industrial production of biofuels, yeast and acetic acid (González *et al*., 2008; Chen *et al.*, 2013).

3.0 Effect of Biotic Stress on Sweet Potato

Sweet potato can grow well with minimal annual rainfall and on sandy fertile soils with good drainage. However, sweet potato has critical biotic delimiters including pests and diseases that affect production and reduce yields (Motsa *et al*., 2015). Damage by insects and diseases is usually manifested in the leaves, stems and tubers. Defoliation caused by damage to the leaves results in a significant reduction in yields since photosynthetic area of the plant is reduced (Ames *et al.*, 1997).

Viral diseases are the greatest threat to sweet potato production causing yield losses of up to 80% (Far *et al*., 2009). These diseases are usually systemic and are transmitted over generations leading to a constant reduction in yields (Liu 2011). Viral diseases in sweet potato cause chlorosis, deformed leaves, severe stunting and yield reduction (Mukherjee *et al*., 2012). Sweet potato feathery mottle virus has been frequently detected in sweet potatoes and is transmitted by aphids (Luo *et al*., 2006). Other viral diseases that affect sweet potato are sweet potato sunken vein virus (SPSVV), sweet potato virus disease (SPVD), sweet potato mild mottle virus (SPMMV), sweet potato latent virus (SPLV), sweet potato chlorotic fleck virus (SPCFV), sweet potato caulimo-like virus (SPCV), sweet potato ring spot virus (SPRSV), cucumber mosaic virus (CMV) and sweet potato chlorotic stunt virus (SPCSV). Prevalence of these viruses varies from region to region (Ames *et al*., 1997).

Several bacterial diseases are a threat to sweet potato production**.** Bacterial soft rot caused by *Erwinia crysanthemi* is characterized by black or brown soaked lesions occurring on the stems or petioles. Lesions enlarge rapidly on the stems and this may cause the stem to collapse resulting in wilting and ultimate plant death (Loebenstein, 2010). Sweet potato Pox is caused by a bacterium *Streptomyces ipomoea*. It is characterized by poor growth of plants, reduced yields and circular dark brown corky lesions on tubers which are V- shaped. Tubers crack and are distorted along with rotting feeder roots.

Apart from viral and bacterial diseases, fungal diseases are also known to affect sweet potato production. Fusarium rot and stem rot is caused by *Fusarium solani.* It is characterized by distortion of the base of the stems, deep dark rot extending deep into the tuber and the growth of white mold. Black rot caused by a fungus *Ceratocystis fimbriate* is characterized by stunting, wilting and yellowing of plants, drooping leaves, plant death and circular brown-black patches on the tubers. Altenaria blight is caused by *Alternaria bataticola*. Initial symptoms are small, brown to black oval lesions with a typical bull's eye appearance of concentric ring on leaves, stems and petioles (Mwanga *et al*., 2011). The leaf and stem scab disease is caused by a fungus *Elsinoe*

batatas. This disease is characterized by brown scabby lesions or spots occurring on the leaves. Little leaf disease, whose causative agent is unknown, is characterized by symptoms such as unusually small and round leaves which may also be yellowish in some cultivars. Stems become stunted, they do not creep and instead they grow erect. Internodes are normally very short resulting in a 'bushy' appearance. The roots as well are thin, short and highly branched with infected plants often producing none or very few tubers (Loebenstein, 2010).

Insects cause significant losses during outbreaks attacking sweet potato differently with the varying agro-ecological zones. They are a major problem especially during the dry period. The major pests are the Sweet potato weevil and the Giant termite. The three species of weevils in the genus Cylas, *Cylas formicarius, C. puncticollis* and *C. brunneus* are pests of sweet potato and are all found in Africa (Ames *et al*., 1997). The sweet potato weevil lays its eggs on the stems and roots, in the drier agro-ecological zones. The larvae then burrows into the tubers destroying them and significantly reducing their market value (Mukherjee *et al*., 2012). Giant termites are a major problem especially in freshly cleared land areas where established colonies have not previously been identified and cleared (Ngailo *et al*., 2013).

4.0 Effect of Abiotic Stress on Sweet Potato

Unlike the major staple food crops, sweet potato produces comparatively a high yield under relatively adverse conditions. Plant aspects such as growth, root development and productivity are heavily influenced by abiotic stresses such as drought, low temperature and salinity (Shao *et al*., 2014). Water deficit is a major abiotic stress limiting the production of many crop species worldwide. Drought is caused by lack of water in the surrounding plant environment. Water stress causes a change in the physiological and biochemical reactions in a plant, thereby affecting critical physiological functions such as water uptake, flowering, respiration and other growth parameters. Initial plant response to drought involves inhibition of shoot and root growth (Neumann, 2008). Drought is normally manifested in different forms in different plants. Dehydration occurs in three stages; - alarm, resistance and exhaustion. At the alarm stage, physiological processes such as transduction are similar to those of normal well-watered plants. The resistance stage is characterized by reduced photosynthetic capacity while in the exhaustion stage the plants try to adjust to the prolonged water stress to prevent death. Recovery of the plant is dependent on the provision of water through rain or irrigation (Amede *et al*., 2004).

Drought often results in a reduction in root extension, stem extension and a reduction in the diameter of the internodes in sweet potato. Leaf growth is also affected eventually leading to a reduction in the overall leaf area (Farooq *et al*., 2009). Ultimately there is a reduction in the number of leaves, tubers roots and vines. With increase in water loss from the leaves, the total amount of amino acids, soluble sugars and carbohydrates decrease. Levels of potassium have also been shown to reduce significantly. Physiological changes resulting from drought in sweet potato include stomatal closure, resulting in reduced CO_2 intake and consequently a reduction in photosynthesis, plant growth and yield. Interference with plant growth results in reduced yields and eventual plant death under severe drought conditions (Motsa *et al*., 2015; Placide and Shimelis 2013).

Physiological stress is known to occur in plants during salt and cold conditions. Salinity is a major problem that affects crops in coastal regions. In sweet potato, root growth is more affected than the vine growth under high concentrations of NaCl thus lowering productivity. However, sweet potato is moderately salt tolerant and growth can be optimized as long as the Na^+/K^+ is maintained below 1 (Begum *et al*., 2015). Drought, salinity, cold, heat and chemical pollution often result in environmental conditions that lead to osmotic and oxidative stress (Wang *et al*., 2003).

5.0 Genetic Control of Stress Response

Adjustment to environmental stresses is one of the most required abilities of crop plants in times of global climatic changes especially in arid and semi-arid regions of Africa. Plant adaptation to environmental stresses is dependent upon the activation of cascades of molecular networks involved in stress perception, signal transduction as well as the expression of specific stress-related genes and metabolites.

Certain metabolites have been shown to play important roles in resistance to pests and a broad range of stresses in plants. One such metabolite has been identified to be the polyamine group. Polyamines are small poly-cations found in all eukaryotic organisms. Amines identified in plants include Spermidines (Spd), Putrescine (Put) and Spermine (Spm). These polyamines interact with different molecules such as phospholipids, nucleic acids and proteins thus activating and stabilizing them under stress. Transgenic sweet potato expressing the spermidine synthetase gene have been shown to be tolerant to paraquat induced oxidative stress, chilling and heat-mediated damages (Kasukabe *et al.*, 2006). In the sweet potato variety "Abees" polyamines were shown to accumulate in response to drought, salinity and chilling stresses (Far 2010). However, response is normally genotype dependent and varies with different plants (Chaves *et al*., 2002).

Sweet potato has phytochemicals like antioxidants, carotenoids and anthocyanins that are critical for plant response to oxidative stress. Antioxidants such as vitamin C, carotenoids and polyphenolic substances scavenge hydroxyl and peroxyl radicals during oxidative stress. They also control the oxidation of lipids and proteins of the cell membrane as well as help plants to resist attacks by insects. Proline, a molecular chaperone, is essential in the synthesis of proteins that help plants respond to drought and salt stress. In transgenic sweet potato enhanced for salt tolerance, the level of proline was shown to be higher as compared to the non-transformed salt stressed sweet potato plants. More proline accumulation in the transgenics helps in maintaining the osmotic balance between the intracellular and extracellular environment. Proline helps to maintain the protein integrity as well as enhancing the activity of different stress related enzymes (Liu *et al*., 2010).

The expression of genes encoding late embryogenesis proteins (LEA) generally increases in response to drought and has been shown to be induced by Abscisic acid (Pérez-clemente *et al.*, 2012). The late-embryogenesis-abundant (*LEA*) genes have been shown to be expressed in vegetative tissues during periods of water deficit. One such protein is dehydrin which stabilizes the proteins and membranes in plants that are stressed (Evers *et al.*, 2010). Sesquiterpenoid Abscisic acid (ABA) has been shown to accumulate in response to stress and therefore helps plants to acclimatize to environmental changes. ABA induces stomatal closure thus reducing transpiration and consequently environmental stress effects on sweet potato (Gao *et al*., 2011). Positive or negative regulation of ABA is controlled by genes such as the early response to dehydration *(ERD*) genes that code for the ERD proteins. One such gene is *ERD1* that prevents injury to the chloroplast membrane of *Arabidopsis*. *ERD15* in soy bean is a transcription factor that regulates transcription related to programmed cell death. Studies on roots of sweet potato treated with polyethylene glycol have shown that the *IbERD15* gene plays an important role in the defense response to drought (Shao *et al*., 2014).

Nudix hydrolases are a superfamily of proteins that require Mg^{+} and are spread amongst eukaryotes, bacteria, archaea and viruses (McLennan, 2006). The major function of cytosolic hydrolases is cleaning up of potentially hazardous ADP-ribose and regulation of the cellular NADH/NAD^{+} ratio in the plants. Accumulation of these substrates is often toxic to the cell and hence the intracellular levels need to be regulated. Nudix hydrolases are routinely termed as 'housekeeping' proteins and are associated with detoxification processes of plants under abiotic stress. They also induce signal transduction pathways when plants are attacked by pests and diseases (Ogawa *et al*., 2005).

6.0 Genetic Transformation of Sweet Potato

Transformation technologies for the genetic improvement of sweet potato have continued to be of interest over the years. Particle bombardment, electroporation, *Agrobacterium tumefaciens* and *Agrobacterium rhizogenes* mediated transformation have been attempted in the sweet potato (Luo *et al*., 2006; Chen *et al*., 2013). However, experiments using *Agrobacterium rhizogenes,* showed that there were morphological abnormalities in most of the regenerants and reproducibility of this method was low (Luo *et al*., 2006). Among the different methods that have been employed in gene transfer, *Agrobacterium tumefaciens* mediated transformation is the preferred alternative since it is easily achievable, uses rather simple equipment and it normally results in stable integration of single gene copies (Olive *et al*., 2014). Sweet potato transformation has been done in several cultivars in order to improve tolerance to both biotic and abiotic stresses.

6.1 Transformation for Biotic Stress Tolerance

Transformation for weevil resistance is among the first genetic transformations that was attempted in sweet potato (Mwanga *et al*., 2011). This involved the use of proteins that reduced the digestibility of sweet potato for the insects. Sweet potato cultivar KB1 was transformed with a delta endotoxin gene *cry8Db* from *Bacillus thuringiensis* via *Agrobacterium* mediated transformation. The *cry8Db* gene codes for an insecticidal protein that has been shown to kill the larvae of many insects. Twenty one transgenic lines were regenerated from transformed calli. The transgenic plants were shown to have a lower sweet potato infestation level than the untransformed lines (Ngoc *et al*., 2015). Ugandan sweet potato landrace 'Kyebandula' has also been transformed with two genes for weevil resistance. *Agrobacterium* mediated transformation was used to introduce the two bacterial endotoxin genes *cry7A1* and *cry3Ca* into 'Kyebandula'. Although the transformation was successful as evidenced by positive PCR results, the transformed calli were not able to regenerate into whole plants (Sefasi *et al*., 2014).

Nematodes are a serious threat to sweet potato production. Transgenic sweet potato resistant to sweet potato nematodes was developed using the *oryzacystatin-1 (OC1)* gene from rice. The cultivar 'Xushu 18' was transformed via *Agrobacterium* mediated transformation. About 92.8% of the 2119 plants from 1710 cell aggregates were positive for the gene and exhibited an enhanced resistance to the stem nematodes. Among the

transgenics, only nine plants were fully resistant, 167 showed moderate resistance while 1943 were susceptible to the stem nematodes (Gao *et al*., 2011).

Sweet potato feathery mottle virus (SPFMV) causes Russet crack disease and is the most important sweet potato viral disease in Africa. To produce virus resistant sweet potato plants, a gene for the coat protein (CP) was introduced into the mesophyll protoplasts of the sweet potato line 'Chikei 682-11' via electroporation. The study reported that 19 plants were regenerated and among them, four were transgenics. All the transgenics expressing the *CP* gene showed high resistance to the SPFMV (Okada *et al*., 2001). Another attempt to introduce SPFMV resistance was done by interfering with the virus replication mechanism. This was done using a cysteine proteinase inhibitor that has been shown to inhibit proteolysis of polyproteins found in poly viruses. Based on this, a rice cysteine inhibitor gene *oryzacystatin 1(OC1)* was introduced into complete leaves of the sweet potato cultivar Jonathan, via *Agrobacterium* mediated transformation. Out of the twenty five transgenic lines obtained, eighteen of the transgenics were resistant to SPFMV. The non transgenics on the other hand were susceptible after inoculation with SPFMV infected grafts (Cipriani *et al*., 2001). Transgenic sweet potato CPT 560 resistant to SPMV was also developed in Kenya. However, the transgenic sweet potato developed was controversial since all lines tested were susceptible to the virus (Wambugu 2003; Ngailo *et al*., 2013).

6.2 Transformation for Abiotic Stress Tolerance

Salinity and drought are major abiotic stresses affecting production of sweet potato worldwide. Spermidine is a polyamine that has been shown to help plants adjust to environmental stresses. Genes coding for the protein spermidine synthetase have been used to improve environmental stress tolerance in sweet potato. A spermidine synthetase gene *FSPD1* was isolated from *Cucurbita ficifolia.* Embryogenic calli from the cultivar 'Kokei' was transformed with *Agrobacterium* EHA 101 harboring the binary vector plasmid PB1101-35S:*FSPD1*. Transgenic plants obtained were shown to have higher levels of spermidine in the leaves and roots and had an enhanced tolerance to drought and salt stress (Kasukabe *et al*., 2006). A novel gene *IbMas* isolated from a salt tolerant sweet potato variety was used to transform 'Shangshu', a widely cultivated sweet potato variety in China. Embryogenic suspension cultures were transformed and made to over-express the *IbMas* gene. Regenerated transgenics were then exposed to 86mM NaCl for assessment of salt tolerance. Overexpression of the *IbMas* was shown to regulate osmotic balance and increase reactive oxygen species (ROS) scavenging activity by increasing the proline levels. Photosynthesis and membrane integrity was also enhanced. The same study showed that 179 transgenics over-expressing the *IbMas* gene were shown to have a higher salt tolerance as compared to the wild type non transgenics (Liu *et al*., 2014).

Myo-inositol-1-phosphate synthase (*MIPS*) has been shown to improve tolerance to abiotic stresses in various plant species. *IbMIPS1* gene isolated from the sweet potato variety 'Nongda 603' was used to transform the variety 'Lizixiang'. Overexpression of the gene was then induced using NaCl, Abscisic acid (ABA) and Polyethylene glycol (PEG). Over-expression of *IbMIPS1* gene was shown to promote up- regulation of certain metabolites and genes involved in stress response. Transgenic sweet potato obtained were shown to have a significant enhanced tolerance to drought, salinity and stem nematode infestation (Zhai *et al.,* 2016). Embryogenic suspension cultures of 'Lizixiang' were also transformed with *LOW OSMOTIC STRESS 5 (LOS5)* gene. Twenty three transgenic plants expressing the *LOS5* gene were shown to have higher levels of Abscisic acid (ABA), superoxide dismutase (SOD) and proline that help plants to survive under different environmental stresses (Gao *et al*., 2011).

Environmental stresses on plants often result in accumulation of reactive oxygen species (ROS) leading to oxidative stress. To help sweet potato adjust to oxidative stress, the cultivar 'Yulmi' was transformed with the genes Copper/Zinc (CuZn) superoxide dismutase (*CuZnSOD)* and ascorbate peroxidase *APX*. The two proteins are key detoxification enzymes found in the chloroplast for regulation of ROS levels in the cell. Embryogenic calli from shoot meristems was transformed using PDS-1000/Hg particle delivery system. To induce oxidative stress, the transgenic plants were exposed to 5µl methyl viologen and chilling at 4^{o}C. Transgenic sweet potato plants expressing the two genes in their chloroplasts had elevated levels of SOD and APX proteins and were shown to have an enhanced tolerance to methyl viologen induced oxidative stress and chilling stress (Placide and Shimelis 2013). Nucleoside diphosphate kinases (*NDKs*) are commonly occurring housekeeping genes which are known to control the intracellular levels of nucleoside triphosphates (NTP). Overexpression of the *NDPK2* gene in *Arabidopsis thaliana* showed that the transgenics had an enhanced tolerance to diverse environmental stresses. Shoot meristem derived calli of the sweet potato cultivar 'Yulmi' were transformed via particle bombardment using the PDS-1000/ Hg particle delivery system with the *NDPK2* gene. To induce stress, transgenics were subjected to Methyl Viologen (MV) treatment, cold treatment at 4^{o}C, salt treatment (200mM NaCl) and water withdrawal for 12 days. The transgenic sweet potato plants showed a marked increase in the level of stress

protection enzymes ascorbate peroxides (APX), catalase (CAT) and superoxide dismutase (SOD). These antioxidant enzymes confer an enhanced tolerance to multiple environmental stresses. Sweet potato cultivar 'Yulmi' was also transformed via *Agrobacterium* mediated transformation with the *SCOF-1* gene. *SCOF-1* expression reduces the accumulation of lipid peroxidation and damage to photosynthetic efficiency (Kim *et al*., 2011).

Sweet potato cultivar 'Sushu- 2' was transformed with the betaine aldehyde dehydrogenase *(BADH)* gene from spinach (*Spinacia oleracea*). Transgenic sweet potato over-expressing the *SoBADH* gene were shown to be tolerant to a multiple of stresses including salt, low temperatures and oxidative stress due to improved protection against cell damage and a marked reduction in the accumulation of reactive oxygen species (ROS) in the cells (Fan *et al.,* 2012). Transgenic sweet potato expressing the soy bean cold- inducible zinc finger protein (*SCOF-1*) showed enhanced tolerance to different low temperature stresses. A salt induced S- adenosyl methionine dependent Methyl transferase gene called *IbSIMT1* was used to transform sweet potato cultivar 'Shangshu 19' via *Agrobacterium* mediated transformation. Embryogenic suspension cultures were transformed and made to overexpress the *IbSIMT1* gene under salt stress and Abscisic acid treatment. SOD and proline content in the transgenics was shown to be elevated as compared to the non-transformed wild type sweet potato. The level of hydrogen peroxide was also reduced, thereby reducing oxidative stress in the plants. The transgenic plants showed an enhanced tolerance to salt and ABA induced stress.

Otani *et al*., 2003 introduced the herbicide resistance *bar* gene for improvement of new sweet potato cultivar 'Kokei 14'. Embryogenic calli obtained from the shoots meristems was transformed via *Agrobacterium* mediated transformation with the *GUS* and *bar* genes, Transgenic sweet potato expressing the *bar* gene were resistant when sprayed with the herbicide Bialaphos while the non-transformed plants showed severe necrosis on the leaves (Otani *et al*., 2003). Similarly, transgenic sweet potato resistant to the herbicide Basta has also been developed. Using *Agrobacterium* mediated transformation, embryogenic calli obtained from the shoot apical meristems of the elite Korean sweet potato cultivar 'Yulmi' was transformed with the *bar* and *GUS* genes and transformed calli regenerated into whole plants. When sprayed with the herbicide, the transgenic sweet potato showed more tolerance to the herbicide and did not show any damage, unlike the non transgenics which were highly susceptible and died within 14 days (Choi *et al*., 2007). The *bar* gene for herbicide resistance has also been introduced into sweet potato cultivar 'Yulmi' via particle gun bombardment. Embryogenic calli obtained from the shoot apical meristem was transformed with 3 different plasmids pCAMBIA3301, pBAR-PHY13 and pBAR-ABF13 each carrying the *GUS* and *bar* genes. The *bar* gene expression was then studied by application of the herbicide Basta at a higher concentration than that used by *Otani* et *al* (2003). Nine of the transgenic lines obtained were shown to be tolerant to the herbicide and remained green while the non transgenics turned brown and died within seven days (Yi *et al*., 2007).

7.0 Challenges Facing Genetic Transformation of Sweet Potato

Genetic transformation is currently viewed as the most promising alternative for improvement of major crops. However, most of the transformation attempts on sweet potato have not been easily applied as sweet potato is highly recalcitrant and most of the time these technologies are genotype dependent (Far *et al*., 2009; Shekhar *et al*., 2013). The recalcitrant characteristic of sweet potato can be attributed to the genetic makeup of the different varieties, production of phenolic compounds and anthocyanins by different sweet potato cultivars (Placide and Shimelis 2013). Low transformation efficiencies have been recorded for sweet potato, further limiting the use of this technology. Transgenic approaches to improving sweet potato are mostly useful for single traits. However, most traits of economic importance are polygenic and are quantitatively inherited, thus a major drawback for the technology (Ngailo *et al*., 2013).

Conventional breeding remains the most popular way of breeding for drought tolerance and tolerance to other stresses. This involves selection of the right parents for crossing. Parents with the desired traits are selected and the crossing done. Genotypes that thrive under drought and saline conditions are seen as good parents and are crossed with susceptible genotypes. Wild relatives are often seen as having a greater gene pool from which genes of interest can be obtained. This has been successful in plants such as peanuts and rice. However, due to the low heritability of drought tolerance and lack of efficient selection strategies, production of drought tolerant sweet potato cultivars has been difficult (Agili *et al*., 2012). In addition, effective and efficient transmission of genes of interest from selected parents to their progeny needs comprehensive knowledge on gene action (Mwije *et al*., 2014). Although conventional breeding has enabled improvement of qualities and traits in other crops, this has remained difficult in sweet potato. The inadequacy of conventional breeding in sweet potato improvement can be attributed to a high male sterility, the hexaploid nature, self and interspecific incompatibility of sweet potato (Chen *et al*., 2013; Shekhar *et al*., 2013). Consequently, these characteristics have resulted in difficulties in

producing varieties with novel germplasm for desirable traits.

The common selectable markers used during sweet potato transformation are neomycin phosphotransferase *II* (*nptII*) and hygromycin phosphorase (*hpt)* genes. However, the major obstacle has been that sweet potato has high levels of intrinsic resistance to antibiotic selection agents. In addition, the use of two selectable markers makes the development of gene constructs difficult. The use of antibiotics as selectable markers also increases the biosafety testing procedures required before the transgenics can be declared fit for release into the environment and for human consumption (Song *et al*., 2004; Luo *et al*., 2006). Regeneration protocols for sweet potato have been shown to be highly genotype dependent making it expensive and time consuming to come up with improved varieties. Most of the existing transformation and regeneration protocols for sweet potato are confined to particular cultivars which further limits the development of new varieties (Ngoc *et al*., 2015; Ogero *et al*., 2012).

In addition to the technical challenges, there are multiple concerns on the release of transgenics into the environment. Concerns arise due to the likelihood of cross pollination of transgenics with related species. A commonly used gene for herbicide resistance is the *bar* gene. Sexual transmission of this gene to related plants could result in the creation of 'super weeds' which are herbicide resistant. Adoption of genetically modified crops has been met with resistance especially from the religious groups, activists and the general population. This stems from the conviction that transgenics and their possible effects on human health and the environment have not been fully understood. The effects of horizontal gene transfer and antibiotic resistance are of major concern and have been highlighted repeatedly.

8.0 Conclusion

Genetic transformation is currently among the promising alternatives that can be used for breeding of improved plant varieties since it allows for transfer and introduction of foreign genes within and across species. Several transformation attempts have been made with different crops. There has been a remarkable increase in improved crop varieties and novel cultivars resulting from this technology. At the moment, there are transgenic sweet potatoes that have been engineered to be tolerant to salt, drought, extreme temperatures, pest and diseases. This will increase the yield potential and increase the area under sweet potato production. Consequently, this will promote the adoption of sweet as a commercial crop alongside its use as a source of food. With improved tolerance to the various abiotic and biotic stresses, the potential of sweet potato as a food security crop will increase. However, genetic engineering of sweet potato is greatly limited by genotype dependency and high research costs. Mitigating the bottlenecks in genetic transformation can provide a major breakthrough in improvement of sweet potato production.

References

Abdissa, T., Dechassa, N., & Alemayehu, Y. (1991). Sweet Potato Growth Parameters as Affected by Farmyard Manure and Phosphorus Application at Adami Tulu , Central Rift Valley of Ethiopia. *Agricultural Science Research Journal*, *2*, 1–12.

Agili, S., Nyende, B., Ngamau, K., & Masinde, P. (2012). Selection, Yield Evaluation, Drought Tolerance Indices of Orange-Flesh Sweet potato (*Ipomoea batatas Lam)* Hybrid Clone. *Journal of Nutrition and Food Sciences*, *2*, 2–9.

Amede, T., Schubert, S., & Stahr, K. (2004). Mechanisms of drought resistance in grain legumes I: Osmotic adjustment. *SINET: Ethiopian Journal of Science*, *26*, 37–46.

Ames, T., Smit, N. E. J. M., Braun, A. R., & Skoglund, L. G. (1997). *Sweet potato : Major Pests , Diseases, and Nutritional Disorders*. *International Potato Center*.

Begum, F., Haque, M. A., Alam, M. S., & Mohanta, H. C. (2015). Evaluation of sweet potato genotypes against salinity. *Bangladesh Journal of Agrilcultural Research*, *40*, 249–257.

Chaves, M. M., Pereira, J. S., Maroco, J., Rodrigues, M. L., Ricardo, C. P. P., Osório, M. L., & Pinheiro, C. (2002). How plants cope with water stress in the field. Photosynthesis and growth. *Annals of Botany*, *89*, 907–916.

Chen, L., Xu, C., Du, Z., & Hamaguchi, T. (2013). Establishment of *Agrobacterium* -Mediated Transformation System in Sweet Potato (*Ipomoea batatas*) by Culture of Leaf Segments for Functional Analysis of ASG -1 , an Apomixis- Specific Gene. *British Biotechnology Journal*, *3*, 458–470.

Choi, H. J., Chandrasekhar, T., Lee, H. Y., & Kim, K. M. (2007). Production of herbicide-resistant transgenic sweet potato plants through *Agrobacterium tumefaciens* method. *Plant Cell, Tissue and Organ Culture*, *91*, 235–242.

Cipriani, G., Fuentes, S., Bello, V., Salazar, L. F., Ghislain, M., & Zhang, D. P. (2001). Transgene expression of rice cysteine proteinase inhibitors for the development of resistance against sweet potato feathery mottle virus. *CIP Program Report*, *1*, 267–271.

El-Far, M. A., Koyro, H.-W., & Berberich, T. (2014). Polyamine Action in Sweet potato Plants in Response to Environmental Stresses. In *"Bridging the gap between increasing knowledge and decreasing resources"* 513–516).

Evers, D., Lefvre, I., Legay, S., Lamoureux, D., Hausman, J. F., Rosales, R. O. G., Schafleitner, R. (2010). Identification of drought-responsive compounds in potato through a combined transcriptomic and targeted metabolite approach. *Journal of Experimental Botany*, *61*, 2327–2343.

Fan, W., Zhang, M., Zhang, H., & Zhang, P. (2012). Improved Tolerance to Various Abiotic Stresses in Transgenic Sweet Potato (*Ipomoea batatas*) Expressing Spinach Betaine Aldehyde Dehydrogenase. *Plos One*, *7*, 44–73.

Far, M. El, Mangoury, K. El, & Elazab, H. E. M. (2009). Novel Plant Regeneration for Egyptian Sweet potato (*Ipomoea Batatas Lam)* Abees Cultivar via Indirect Organogenesis Stimulated by Initiation Medium and Cytokinin Effects. *Australian Journal of Basic and Applied Sciences, 3*, 543–551.

Gao, S., Yu, B., Yuan, L., Zhai, H., He, S., & Liu, Q. (2011). Production of transgenic sweet potato plants resistant to stem nematodes using oryzacystatin -I gene. *Scientia Horticulturae*, *128*, 408–414.

Gao, S., Yuan, L., Zhai, H., Liu, C., He, S., & Liu, Q. (2011). Transgenic sweet potato plants expressing an *LOS5* gene are tolerant to salt stress. *Plant Cell, Tissue and Organ Culture*, *107*, 205–213.

González, R. G., Sánchez, D. S., & Guerra, Z. Z. (2008). Efficient regeneration and *Agrobacterium tumefaciens* mediated transformation of recalcitrant sweet potato (*Ipomoea batatas L*am) cultivars . *Asia Pacific Journal of Molecular Biology and Biotechnology*, *16*, 25–33.

H. G. Ashok Kumar, A. T. K. and K.-W. Y. (2007). An Efficient and Rapid Plant Regeneration System for Sweet Potato (*Ipomoea batatas Lam*). *European Journal of Horticultural Science*, *72*, 85–89.

Ingram, J., & Bartels, D. (1996). the Molecular Basis of Dehydration Tolerance in Plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, *47*, 377–403.

Islam, S. (2014). Nutritional and Medicinal Qualities of Sweet potato Tops and Leaves. *Plant Science*.

Kasukabe, Y., He, L., Watakabe, Y., Otani, M., Shimada, T., & Tachibana, S. (2006). Improvement of environmental stress tolerance of sweet potato by introduction of genes for spermidine synthase. *Plant Biotechnology*, *23*, 75–83.

Kim, Y. H., Kim, M. D., Park, S. C., Yang, K. S., Jeong, J. C., Lee, H. S., & Kwak, S. S. (2011). *SCOF-1*-expressing transgenic sweet potato plants show enhanced tolerance to low-temperature stress. *Plant Physiology and Biochemistry*, *49*, 1436–1441.

Liu, D., Wang, L., Zhai, H., Song, X., He, S., & Liu, Q. (2014). A novel α/β-hydrolase gene *IbMas* enhances salt tolerance in transgenic sweet potato. *Plos One*, *9*, 1–22.

Liu, Q. (2011). Sweet potato Omics and biotechnology in China. *Plant Omics*, *4*, 295–301.

Liu, X., Chen, M., Li, M., Yang, C., Fu, Y., Zhang, Q., Liao, Z. (2010). The anthocyanidin synthase gene from sweetpotato [*Ipomoea batatas Lam*]: Cloning , characterization and tissue expression analysis. *African Journal of Biotechnology*, *9*, 3748–3752.

Low, J. W., Arimond, M., Osman, N., Cunguara, B., Zano, F., & Tschirley, D. (2007). A Food-Based Approach

Introducing Orange-Fleshed Sweet Potatoes Increased Vitamin A Intake and Serum Retinol Concentrations in Young Children in Rural Mozambique. *The Journal of Nutrition*, *137*, 1320–1327.

Luo, H. R., Maria, M. S., Benavides, J., Zhang, D. P., & Zhang, Y. Z. (2006). Rapid genetic transformation of sweet potato (*Ipomoea batatas Lam*) via organogenesis. *African Journal of Biotechnology*, *5*, 1851–1857.

Loebenstein, G. (2010). *The Sweet Potato*. (D. G. Loebenstein & Dr. George Thottappilly, Eds.) (1st ed.). Springer.

M. Farooq, Wahid, A., D., N. K., & Basra, F. S. M. A. (2009). Plant drought stress : effects , mechanisms and management. *Agronomy for Sustainable Development, Springer Verlag (Germany)*, *29*, 185–212.

McLennan, A. G. (2006). The Nudix hydrolase superfamily. *Cellular and Molecular Life Sciences*, *63*, 123–143.

Motsa, N. M., Modi, A. T., & Mabhaudhi, T. (2015). Sweet potato (*Ipomoea batatas Lam)* as a drought tolerant and food security crop. *South African Journal of Science*, *111*, 1–8.

Mukherjee, A., Kanti, S., Rajasekhara, K., Ramesh, R., & Ray, C. (2012). Sweet Potato : Gains through Biotechnology. *Fruit, Vegetable and Cereal Science and Biotechnology*, *6*, 30–42.

Mvuria, J. M., & Ombori, O. (2014). Low Cost Macronutrients in the Micropropagation of Selected Sweet Potato [*Ipomoea batatas Lam*] varieties. *Journal of Agriculture and Environmental Sciences*, *3*, 89–101.

Mwanga, R. O. M., Ghislain, M., Kreuze, J., Ssemakula, G. N., & Yencho, C. (2011). Exploiting the use of biotechnology in sweet potato for improved nutrition and food security : Progress and future outlook. In *Agro-Biotechnology, Biosafety and Seed Systems in Developing Countries* (pp. 25–31).

Mwije, A., Mukasa, S. B., Gibson, P., & Kyamanywa, S. (2014). Heritability Analysis of Putative Drought Adaptation Traits in Sweet potato. *African Crop Science Journal*, *22*, 79–87.

Ndagijimana, V., Kahia, J., Asiimwe, T., Sallah, P. Y., Waweru, B., Mushimiyimana, I., Njenga, P. (2014). *In vitro* effects of gibberellic acid and sucrose concentration on micropropagation of two elite sweet potato cultivars in Rwanda. *International Journal for Biotechnology and Molecular Biology Research*, *5*, 1–6.

Neumann, P. M. (2008). Coping mechanisms for crop plants in drought-prone environments. *Annals of Botany*, *101*, 901–907.

Ngailo, S., Shimelis, H., Sibiya, J., & Mtunda, K. (2013). Sweet potato breeding for resistance to sweet potato virus disease and improved yield : Progress and challenges. *African Journal of Agricultural Research*, *8*, 3202–3215.

Ogawa, T., Ueda, Y., Yoshimura, K., & Shigeoka, S. (2005). Comprehensive analysis of cytosolic Nudix hydrolases in *Arabidopsis thaliana*. *The Journal of Biological Chemistry*, *280*, 25277–25283.

Ogero, K. O., Mburugu, G. N., Mwangi, M., Ngugi, M. M., & Ombori, O. (2012). Low Cost Tissue Culture Technology in the Regeneration of Sweet Potato (*Ipomoea batatas Lam*). *Research Journal of Biology*, *2*, 51–58.

Okada, Y., Saito, A., Nishiguchi, M., Kimura, T., Mori, M., Hanada, K., & Murata, T. (2001). Virus resistance in transgenic sweet potato [*Ipomoea batatas Lam*)] expressing the coat protein gene of sweet potato feathery mottle virus. *Theoretical and Applied Genetics*, *103*, 743–751.

Olive, S. F., Njoka, F. M., Mgutu, A. J., & Anami, S. E. (2014). Screening for Water Deficit Tolerance , Relative Growth Analysis and *Agrobacterium* -Infectivity in Tropical Maize [*Zea Mays L* .] Inbred Lines in Nairobi , Kenya, *2*, 218–224.

Otani, M., Wakita, Y., & Shimada, T. (2003). Production of herbicide-resistant sweet potato (*Ipomoea batatas Lam*.) plants by *Agrobacterium tumefaciens*-mediated transformation. *Breeding Science*, *53*, 145–148.

Ozturk, G., Nil, F., & Zihin, A. (2012). Field Performance of *in vitro* Sweet Potato [*Ipomoea batatas Lam*]

Plantlets Derived From Seedstocks. *Turkish Journal of Field Crops*, *17*, 1–4.

Pérez-clemente, M, R., & Gómez-Cadenas, A. (2012). *In vitro* Tissue Culture , a Tool for the Study and Breeding of Plants Subjected to Abiotic Stress Conditions. *Recent Advances in Plant in vitro Culture*, 1, 91–108).

Pham Bich Ngoc, Vu Thi Lan, Tran Thu Trang, Nguyen Hoai Thuong, Le Thu Ngoc, Chu Hoang Ha, & Le Tran Binh. (2015). *Agrobacterium*-Mediated Transformation of *Cry8db* Gene in Vietnam Sweet Potato Cultivar. *Journal of Life Sciences*, *10*, 262–271.

Placide, R., & Shimelis, H. (2013). Physiological mechanisms and conventional breeding of sweet potato (*Ipomoea batatas Lam.*) to drought-tolerance. *African Journal of Agricultural Research*, *8*, 1837–1846.

Sefasi, A., Ssemakula, G., Ghislain, M., Prentice, K., Kiggundu, A., & Mwanga, R. (2014). Transient Expression of β -Glucoronidase In Recalcitrant Ugandan Sweet potato and Putative Transformation With Two Cry Genes. *African Crop Science Journal*, *22*, 215–227.

Shao, H. H., Chen, S. D., Zhang, K., Cao, Q. H., Zhou, H., Ma, Q. Q., & Yong, B. (2014). Isolation and expression studies of the *ERD15* gene involved in drought-stressed responses. *Genetics and Molecular Research*, *13*, 10852–10862.

Shekhar, S., Agrawal, L., & Buragohain, A. K. (2013). Genotype independent regeneration and *Agrobacterium* mediated genetic transformation of sweet potato (*Ipomoea batatas Lam*). *Plant Tissue Culture and Biotech*, *23*, 87–100.

Song, G. Q., Honda, H., & Yamaguchi, K. I. (2004). Efficient *Agrobacterium tumefaciens*-mediated transformation of sweet potato (*Ipomoea batatas Lam.*) from stem explants using a two-step kanamycin-hygromycin selection method. *In Vitro Cellular and Developmental Biology Plant*, *40*, 359–365.

Wamalwa, N. L., Díaz, D., Tovar, J. C., Kreuze, J., Machuka, J., & Ghislain, M. Screening for regeneration and transformation efficiencies of African sweet potato cultivars, International Society for Tropical Root Crops (ISTRC), 37–40 (2006).

Wambugu, F. M. (2003). Development and transfer of genetically modified virus-resistant sweet potato for subsistence farmers in Kenya. *Nutrition Reviews*, *61*, 110–113.

Wang, W., Vinocur, B., & Altman, A. (2003). Plant responses to drought, salinity and extreme temperatures: Towards genetic engineering for stress tolerance. *Planta*, *218*, 1–14.

Wilson, J. E. (1988). *Sweet Potato (Ipomoea batatas) Planting Material* (Vol. 2).

Yang, X. (2010). Rapid Production of Virus-Free Plantlets By Shoot Tip Culture *in vitro* of Purple-Coloured Sweet Potato (*Ipomoea Batatas Lam* .). *Pakistan Journal Of Botany*, *42*, 2069–2075.

Yi, G., Shin, Y. M., Choe, G., Shin, B., Kim, Y. S., & Kim, K. M. (2007). Production of herbicide-resistant sweet potato plants transformed with the *bar* gene. *Biotechnology Letters*, *29*, 669–675.

Zhai, H., Wang, F., Si, Z., Huo, J., Xing, L., An, Y., & Liu, Q. (2016). A myo -inositol-1-phosphate synthase gene , *IbMIPS1* , enhances salt and drought tolerance and stem nematode resistance in transgenic sweet potato. *Plant Biotechnology Journal*, *14*, 592–602