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Characterization of Genes and Promoters, Transformation and Transgenic Development in Sweet Potato

C. Mohan* • **Aswathy G. H. Nair**

Central Tuber Crops Research Institute, Thiruvananthapuram - 695 017, India

Corresponding author: * **cmsan99@gmail.com**

ABSTRACT

Sweet potato belongs to *Ipomoea* series batatas and is thought to have originated in Central America and Northern South America. World sweet potato production is around 106 million tons in an area of about 8.1 million ha. Asia is the world's largest sweet potato-producing region and China is the world's leading sweet potato-producing country accounting for about 76% of production. Sweet potato has not attracted the attention from modern geneticists that many other economically important crops have because of its high ploidy. The genes related to tuber storage protein, sporamin, sucrose metabolic genes, storage root-inducing genes, proteinase inhibitors, cystein proteniase, retrotranspogens, and senescence-related genes were studied in sweet potato; similarly, sporamin, wound regulated, peroxidase and GBSS promoters were used for developing transgenic plants. Transgenic plants sweet potato with biotic stress (weevil, virus), abiotic stress (drought, early frost, low temperature), and herbicide resistance as well as improved starch quality and fatty acid composition have been developed.

Keywords: sporamin, starch genes, storage root protein

Abbreviations: **AGPase**, ADP-glucose pyrophosphorylase; **APX**, ascorbate peroxidase; **CAT**, **c**hloramphenicol acetyltransferase; **CP**, coat protein; **CPR**, cysteine proteinase; **CuZnSOD**, CuZn-superoxide dismutase; **ER**, endoplasmic reticulum; **EST**, expression sequence tag; **GBSSI**, granule-bound starch synthase I; **GFP**, green-fluorescent protein; **GUS**, β-glucuronidase; **MT**, metallothionein; **MV**, methyl viologen; **NT**, non-transformed; **PAP**, purple acid phosphatase; **PAT**, phosphinothricin acetyltransferase; **PDI**, protein disulfide isomerase; **PI**, proteinase inhibitor; **POD**, peroxidase; **PPT**, phosphinothricin; **SAG**, senescence associated gene; **SPFMV**, *Sweet potato feathery mottle virus*; **SPLTI**, sweet potato leaf trypsin inhibitor; **TI**, trypsin inhibitor

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INTRODUCTION

Sweet potato (*Ipomoea batatas* (L.) Lam.) is the seventh most important food crop (both humans and animals) in the world with an estimated production of 106 million metric tonnes every year and currently grown in more than 100 countries. China is the largest producer (76% of the world's sweet potatoes), and half of the worldwide production is used either for animal feedstock or the production of starch-based products. Many genotypes can produce sizable quantities of anthocyanin and carotenoid plant pigments. Sweet potato with white- to cream-colored flesh is common in the Pacific, whereas US sweet potato normally has yellow to orange flesh (Bradbury and Holloway 1988). Some of these are currently considered as a source of natural dye because of their unique stability characteristics, while others (e.g. β-carotene, a vitamin A precursor) are known to be highly beneficial to human health and nutrition. In developing countries, sweet potato ranks third in value of production and fifth in caloric contribution to human diet.

The sweet potato has not attracted the attention from modern geneticists that many other economically important crops have. Sweet potato belongs to Ipomoea series Batatas and is thought to have originated in Central America and Northern South America (Austin 1988) or Mexico (McDonald and Austin 1990). Sweet potato is a hexaploid with 90 chromosomes that collectively hold a DNA content of 1050 Mbp. Cytological and genetic studies of sweet potato are difficult due to the sticky nature of small chromosomes and the self- and cross-incompatibility nature of reproductive behavior. Sweet potato is a complex polyploid that might have been derived from the ancient polyploidyzation of *I. triloba* L. (diploid, x=15) (A genome type) and two other B genome types, namely, *I. trifida* (diploid, x=15) and *I. tabascana* (tetraploid).

As an important material of industry and new energy resource crop as well as food, the increased production of sweet potato is desired, but this goal is often limited by diseases and pests. High male sterility, incompatibility, and the hexaploid nature of sweet potato have resulted in very lim-

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Invited Review

ited improvement of this plant by conventional hybridization. Gene engineering has been used for the successful introduction of foreign genes in the genome of many plant species. This technology offers great potential for the improvement of sweet potato.

GENE ISOLATION AND TRANSFORMATION

Isolation and characterization of sweet potato genes

1. Storage proteins

The pioneer works in molecular genetics on sweet potato were performed on the genes encoding two major proteins, sporamin and *β*-amylase, which specifically accumulate in the storage root (Nakamura 1992). They cloned and characterized their genes to study the molecular mechanisms regulating the formation of storage roots. Sporamin, which accounts for 80% of the total soluble proteins in sweet potato tuberous roots, consists of two polypeptide classes, A and B. The sporamin cDNA clones can also be classified into sporamin A and B subfamilies based on their sequence homologies, with intra-subfamily homologies being much higher than inter-subfamily homologies. The sequence of an essentially full-length cDNA for sporamin B was compared with that for sporamin A. The coding sequences of two cDNAs share 83% sequence homology. The sequences in the 5' and 3' non-coding regions show many deletions in addition to base substitutions. In the 5' and 3' non-coding region of sporamin B cDNA, there are 5 bp direct repeats with sequences complementary to each other and these repeats are absent in sporamin A cDNA (Murakami *et al*. 1986). The amount of sporamin present in other organs is very low, or even no detectable, in the normal field-grown plants. However, the stem of sweet potato plantlets grown on agar medium containing sucrose was found to accumulate large amounts of sporamin. Transgenic tobacco plants having a *CAT* (chloramphenicol acetyltransferase) fusion gene with the 5' upstream region of a sporamin A gene, *gSPO*-A1, show preferential expression of *CAT* activity in stems when the plants are maintained in axenic culture on sucrose medium as is the case for sporamin in sweet potato. Deletion analysis revealed that the DNA sequence of *gSPO*-A1 between -94 and -305, relative to the transcription start site, is important for its expression in tobacco. This region contains two of the previously postulated putative regulatory elements conserved between sporamin A and B genes (Hattori *et al*. 1990). The specific expression of a chimerical gene with a sporamin promoter in transgenic tobacco and potato has been observed and the promoters of sporamin and *β*-amylase genes are used to express the desired proteins in storage roots to improve their qualities and yield (Ohta *et al*. 1991).

2. Genes for sucrose metabolism in sweet potato storage root

Biochemical and molecular research on the starch biosynthesis has been started for developing the tuberous root of sweet potato. Genes that affect sucrose cleavage and related metabolisms may play a central role in the control of storage root development, storage sink strength and photosynthate partitioning. ADP-glucose pyrophosphorylase (AGPase) plays a central role in starch biosynthesis in both photosynthetic and non-photosynthetic plant tissues. It catalyzes the first step of starch biosynthesis: the production of ADP-glucose and inorganic phosphate from glucose 1-phosphate and ATP. Plant AGPase is characterized as a heterotetramer composed of two large subunits and two small subunits encoded by different genes. In sweet potato, cDNAs encoding both large and small subunit AGPase have been isolated and characterized (Bae and Liu 1997; Noh *et al*. 2004). They introduced cDNAs of the small subunit AGPase into tobacco plants. The transgenic tobacco over expressing the small subunit of AGPase were partially male sterile. Starch synthesis *via* AGPase and cell proliferation may work together in tuber formation in sweet potato root (Bae *et al*. 2001).

A nuclear *AmyB* gene from sweet potato encoding *β*-amylase (*βAmy*) that is abundant in tuberous roots and inducible in other organs by an exogenous supply of sucrose or polygalacturonic acid, was isolated and characterized. Genomic Southern blot hybridization, restriction maps of independently isolated phage 1 genomic clones, and the nucleotide sequence of *AmyB* compared with that of the cDNA, all suggested that *βAmy* of sweet potato is encoded by a gene that is present in a single copy per haploid genome. In the sequence of *AmyB*, the sequence that is identical to that of the cDNA was split into seven exons by six introns, and the transcription of this gene starts from multiple sites 26 to 30 bp downstream from a potential TATA-box sequence, 5′-TATATAA. In the 5'-upstream region of *AmyB* there are sequences homologous to those conserved in the 5'-upstream regions of genes encoding sporamin, which are regulated similarly to *AmyB* (Yoshida *et al*. 1992). Sweet potato cDNAs encoding granule-bound starch synthase I (*GBSSI*), *SBEII*, and ADP-glucose pyrophosphorylase small and large subunits (Bae and Liu 1997) have been cloned. The full-length cDNA and the gene for sweet potato *GBSSI* have been isolated and characterized (Kimura *et al*. 2000).

Lee *et al*. (2000) isolated cDNA clones encoding sweet potato AGPase large subunit (*iAGPLI*) from the cDNA library constructed from the tuberous root. Two clones were characterized and named *iAGPLI-a* and *iAGPLI-b*, they were 1,661 bp and 1,277 bp in length and contained partial open reading frames of 450 and 306 amino acids, respectively. Both nucleic acid and amino acid sequence identities between to genes were 83.8 and 97.3%, respectively. Based on the amino acid sequence analysis, both genes share the highest sequence identity (81%) with potato AGPase large subunit. The both genes were expressed predominantly in the stem and weakly in the tuberous root, and no transcript was expressed in other tissues. The sweet potato genome contains several copies of the *iAGPLI* gene. The cDNA of the starch-branching enzyme I gene (*IbSBEI*) in the sweet potato was cloned and sequenced by Hamada *et al*. (2006). The *IbSBEI* amino acid sequence was 81% identical to that of potato *StSBEI*. DNA gel blot analyses demonstrated that at least two copies of *IbSBEI* are present in the sweet potato genome. *IbSBEI* was strongly expressed in tuberous roots. Transcript levels in the roots of single leaf cuttings were extremely low during the first 15-40 days after planting and continuously increased up to 50 days, by which time the tuberous roots had almost completely developed. This indicates that *IbSBEI* may work in concert with the AGPase large subunit during the primary phase of starch granule formation.

3. Storage root induction

Sweet potato develops two types of roots: fibrous roots and storage roots. There is not enough physiological and molecular information on the conditions that induce storage root formation in sweet potato. The expression sequence tag (EST) approach has been used in profiling the genes with enhanced expression in specific tissues. You *et al*. (2003) identified 22 differentially expressed genes in the early developmental stage of fibrous roots and storage roots. They suggested that those genes involved in gene regulation, signal transduction and development were possibly related to the storage root induction processes in sweet potato. Moreover, the development of sweet potato storage roots coincides with starch accumulation. Li and Zhan (2003) used ESTs to investigate the enzymes involved in sucrose metabolism and its immediate downstream biochemical reaction in the storage root and in fibrous roots. The *SuSy* (sucrose synthase) is the most active gene at the enlarging and starch-accumulating stages of sweet potato storage

roots.

A large fraction of DNA-binding proteins can be grouped into a small number of families defined by the presence of conserved DNA-binding domains such as the helix-turn-helix, homeo-domain, zinc finger and basic zipper motifs. MADS-box is a recognized type of DNA-binding proteins comprising of 180 nucleotides, encoding 60 amino acids that are highly conserved across developmental control genes from yeast, animals and plants. The MADS-box is a highly conserved sequence motif found in a family of transcription factors. The MADS-box genes are important developmental regulators found first in animals, then in yeast and subsequently in plants. Further, MADS-box genes have been cloned in many plant species and their role as homoeotic genes that control floral organ development are well established (Coen and Meyerowits 1991). It is very interesting that new MADS-box genes were isolated from pigmented and tuber-forming root tissue in sweet potato (Kim *et al*. 2002). These MADS-box genes from sweet potato were found to be expressed preferentially in root tissues; and these genes were suggested to play an important role in tuber initiation in sweet potato.

A new MADS-box gene designated as *Ib-MADS10* was cloned and its expression was characterized from sweet potato cv. 'Beniazuma' (Lalusin *et al*. 2006). The deduced amino acid sequence of the gene indicated high homology with members of the MADS-box family of transcription factors. This *IbMADS10* gene shares high amino acid sequence similarity with the *DEFH28* of *Antirrhinum majus* (64%). The *IbMADS10* is present in one or low copy number in the sweet potato genome. The gene is specifically expressed in the pigmented tissues such as in the flower bud, in the pink and in red roots, and hence, it was speculated that the *IbMADS10* gene might be correlated with anthocyanin biosynthesis in sweet potato. Another important aspect is the pigmented phenotypes of transgenic calli that ectopically express the *IbMADS10* gene, thereby supporting its involvement in the developmental regulation of pigment formation. Tissue printing result further strengthens the hypothesis that the *IbMADS10* gene is indeed involved in anthocyanin pigmentation in sweet potato. As the purpose of the *IbMADS10* gene is pigmentation, its function, therefore, resembles that of the transparent testa (tt) genes of Arabidopsis.

4. Enzyme genes isolated in sweet potato

Peroxidases (POD) are ubiquitous in the plant kingdom and form a diverse family of isoenzymes that reduce hydrogen peroxide in the presence of an electron donor. *PODs* are of great interest because they play a significant role in the response to environmental stresses in plants. Two cDNAs for anionic peroxidase *swpa2* and *swpa3*, were isolated from suspension cultures of sweet potato (Kim *et al*. 1999). *Swpa2* and *swpa3* encode polypeptides of 358 and 349 amino acids, respectively. The two genes responded differently to stresses such as wounding or chilling of leaves. *Swpa2* was strongly induced 48 h after wounding, but *swpa3* was not affected by this treatment. The two genes were also highly expressed upon chilling (4°C), but expression was reduced by prior acclimation at 15°C. In addition, both genes were strongly induced immediately after treatment with ozone, and expression had decreased to the basal level 12 h after treatment. The response of these two genes to stresses such as aging, wounding, and chilling are different from those of the *POD* genes (*swpa1* encoding an anionic product and *swpa1* a neutral *POD* that we described previously. The responses of the two genes were also different from each other. These results suggest that the two new *POD* genes are involved in overcoming oxidative environmental stress, and each *POD* gene may be regulated by cell growth and environmental stress in different ways.

Purple acid phosphatases (*PAPs*) contain a Fe(III)-Me(II) center in their active site and catalyze the hydrolysis of activated phosphoric acid esters and anhydrides in the pH range from 4 to 7. These metalloenzymes are resistant to inhibition by tartrate and show a Tyr-Fe(III) charge transfer band at about 560 nm responsible for the characteristic purple color of the enzyme. Physiological functions like triggering of seed dormancy and mobilizing organic phosphate have been suggested for *kbPAP*. The sequence of cDNA fragments of two isozymes of the PAP from sweet potato (*spPAP1* and *spPAP2*) has been determined by 5' and 3' rapid amplification of cDNA ends protocols using oligonucleotide primers based on amino acid information (Durmus *et al*. 1999). The encoded amino acid sequences of these two isozymes show an equidistance of 72-77% not only to each other, but also to the primary structure of the purple acid phosphatase from red kidney bean (*kbPAP*).

Protein disulfide isomerase (PDI) is a protein essential for formation, reduction or isomerization of protein disulfide bonds, depending on the reaction conditions in the lumen of the endoplasmic reticulum (ER). PDI is a member of the thioredoxin super family containing two thioredoxin like active sites, which act as catalytic sites for the isomerase activity. The PDI cDNA gene (*SPPDI1*) contains 1869 bp in length and encodes a protein of 503 amino acids. Primary structure analysis of the deduced protein revealed two thioredoxin-like active sites (CGHC), and an endoplasmic reticulum-retention signal at its C-terminus (KDEL), which is also found in PDIs of plants and animals. Although there is only about 65% amino acid identity between *SPPDI1* and other plant PDIs, the active site regions are almost identical. The mRNA level was found the highest in the storage roots, followed by sprouts and full-expanded green leaves; while was the lowest in sprouted roots and vein. In Western blot analysis, *SPPDI1* level was the highest in the storage roots, followed by sprouts, full-expanded green leaves and vein and no signal at all in sprouted root. These results suggested that the protein coded for by the sweet potato gene is a novel member of the PDI family in plants. *SPPDI* genes of sweet potato storage roots display differential gene expression patterns, which may be associated with the antioxidant functions they play in plant physiology (Huang *et al*. 2005).

The transit peptide (TP) sequence of sweet potato ADP-glucose pyrophosphorylase (ibAGP2- TP2) was found to be capable of targeting protein into the chloroplast in the Arabidopsis protoplasts. Kwak *et al*. (2008) fused the TP2 peptide to a β-glucuronidase (GUS) reporter gene and expressed in Arabidopsis under the control of the ibAGP2 promoter with the aim of dissecting the effect of the transit peptide in elevating foreign protein accumulation in the transgenic plant. TP2 dramatically elevated GUS protein accumulation regardless of developmental stage, but the level of the enhancing effect was developmental stage-dependent.

Cinnamyl alcohol dehydrogenase (*CAD*) is a key enzyme in the biosynthesis of lignin. Kim *et al*. (2010) isolated full length of a cDNA encoding CAD (*IbCAD1*) from suspension cells of sweet potato. The *IbCAD1* gene transcripts were highly induced by cold, wounding, and reactive oxygen species. Analyzed the transcriptional regulation of the *IbCAD1* gene in transgenic tobacco plants carrying the IbCAD1 promoter-GUS revealed that *IbCAD1* promoter expression was strong in the roots. *IbCAD1* promoter activity increased with increasing root age, and strong promoter expression was observed in the lateral root emergence sites and in root tips. The *IbCAD1* can be involved in JA- and SA-mediated wounding response and ABA-mediated cold response, respectively. So the *IbCAD1* gene may play a role in the resistance mechanism to biotic and abiotic stresses as well as in tissue-specific developmental lignification.

5. Proteinase inhibitor

Proteinase inhibitors (PIs) are classified into serine-, cysteine-, aspartic-, and metallo-PIs, according to the types of proteinase that they inhibit. In higher plants, PIs are shown to be particularly abundant in storage organs, such as seeds, tubers and endosperm. A large body of evidence indi-

cated that PIs functioned as storage proteins, regulate the endogenous proteinase activities, and suppress the exogenous proteinase activities from pathogens and pests. The defensive role of PIs was demonstrated by the observations that transgenic plants that over-expressing PIs are more resistant to insect attack than the control plants. Four types of PIs were found to accumulate rapidly in leaves in response to mechanical wounding or insect chewing, suggesting a direct role of PIs in plant protection.

A 7-kDa-proteinase inhibitor, designated *SPLTI* (Sweet Potato Leaf Trypsin Inhibitor) was partially purified from sweet leaves under water deficiency. The N-terminal amino acid sequence was determined and used to design two overlap degenerate primers for isolation of the *SPLTI* gene. Two full-length cDNA clones encoding proteinase inhibitor I (PI-I), designated *SPLTI*-a and *SPLTI*-b, were isolated. Both *SPLTI*-a and SPLTI-b are 98% identical to each other in both levels of nucleotide and amino acid sequence. Using SPLTI-a as a probe, Wang *et al*. (2003) found that SPLTI gene exhibited a leaf-specific expression pattern. Additionally, this was the first report that the *SPLTI* genes were up-regulated by water deficiency and chilling as well as osmoticant treatments in the PI-I family in plants. As other PIs, the *SPLTI* transcripts were induced by wounding and also by exogenous applications of abscisic acid and methyl jasmonate; however, accumulation of the wound-induced transcripts were restricted locally in the injured leaves, but not systemically. These distinct expression patterns provided a new insight to the regulation of PI-I gene family in response to environmental stresses. Wang *et al*. (2003) suggested that *SPLTI* could participate in defense systems against invasions of insects or bacteria as other PI-Is. Moreover, it may play a role against environmental stresses through regulation of endogenous proteolytic activities during leaf development.

6. Cysteine proteinase and Cysteine protease

The cysteine proteinase (CPR) is a family of enzymes that play an important role in protein degradation. These proteinases have been found in bacteria, eukaryotic microorganisms, animals and plants. CPR has been studied extensively because they appear to play a central role in a wide range of proteolytic functions in higher plants. In plants, CPR play a major role in intracellular and extracellular processes and are involved in the degradation of storage protein during seed germination, degradation of defective proteins, programmed cell death, leaf and flower senescence, development and ripening of fruits, zymogen activation and stress tolerance.

Cysteine proteinase (CPR) cDNA clone (*SPCPRPP*) of sweet potato storage roots were isolated by differential display (Huang *et al*. 2005). The open reading frame in this cDNA encodes a pre-proprotein of 371 amino acids with conserved catalytic aminoacids of papain and specifically induced in the storage roots. Active recombinant *SPCPRPP* protein was able to digest the 22 kDa sweet potato trypsin inhibitor (TI) protein. A smaller peptide (14 kDa) was obtained as a digestion product. These results suggest that CPR is responsible for initiation of degradation and remobilization of stored 22 kDa TI during sprouting of SP storage roots.

Granulins are a family of evolutionarily ancient proteins that are involved in regulating cell growth and division in animals. Chen *et al*. (2006) isolated a full-length cDNA, *SPCP3*, from senescent leaves of sweet potato. *SPCP3* contains 1389 nucleotides (462 amino acids) in its open reading frame, and exhibits high amino acid sequence homologies (*ca*. 64-73.6%) with several plant granulin-containing cysteine proteases, including potato, tomato, soybean, kidney bean, pea, maize, rice, cabbage, and Arabidopsis. Gene structural analysis shows that *SPCP3* encodes a putative precursor protein, *via* cleavage of the N-terminal pro-peptide, it generates a protein with 324 amino acids (from the 139th to the 462nd amino acid residues), which contains two main domains: the conserved catalytic domain with the putative catalytic and the C-terminal granulin domain. The *SPCP3* gene expression was enhanced significantly in natural senescent leaves and in dark- and ethephon- induced senescent leaves, but was almost undetectable in mature green leaves, veins, and roots. Phylogenic analysis showed that *SPCP3* displayed close association with a group of plant granulin-containing cysteine proteases which have been implied to be involved in programmed cell death. In conclusion, sweet potato *SPCP3* is a functional, senescence-associated gene.

7. Other protein genes in sweet potato

Metallothionein (MT) is a group of proteins with low molecular masses and high cysteine contents, and is classified into different types, which in general contains two domains (domain 1 and domain 2) with typical amino acid sequences. Two full-length cDNAs (Y459 and G14) encoding MT-like proteins were isolated from leaves of sweet potato. Their open reading frames contained 249 and 195 nucleotides (82 and 64 amino acids) for Y459 and G14, respectively, and exhibited a relatively low amino acid sequence similarity. Gene structure studies showed that Y459 had the conserved domain 1 region of type 2 MT. The two MT-like protein genes of sweet potato display differential gene structures and gene expression patterns, which may be associated with the diverse roles and functions they play in plant physiology in order to cope with particular developmental and environmental cues (Chen *et al*. 2003). Thioredoxins, the ubiquitous small proteins with a redox active disulfide bridge, are important regulatory elements in a number of cellular processes. They all contain a distinct active site, WCGPC, which is able to reduce disulfide bridges of target proteins. Initially described as hydrogen carriers in ribonucleotide reduction in *E. coli*, they were found to serve as electron donors in a variety of cellular redox reaction. Three full-length cDNA clones, designated TRX1, TRX2, and TRX3 encoding different but similar thioredoxin h polypeptides, were isolated from sweet potato (Huang *et al*. 2004). These three thioredoxin h clones were similar to each other and contained the canonical WCGPC active site and the important structural and functional amino acids that were conserved in thioredoxin sequences. mRNAs corresponding to all three thioredoxin genes were found to have the highest level in the storage roots; those corresponding to TRX2 and TRX3 were detected at the next higher level in flowers. Three thioredoxin h genes of sweet potato storage roots display differential gene expression patterns, which may be associated with the diverse roles and functions they play in plant physiology.

8. Retrotransposons in sweet potato

Numerous plant retrotransposons (transposable genetic elements) have been characterized (Kumar and Bennetzen 1999). The LTR retrotransposons possess long terminal repeats and have been identified in many plant genomes. However, only three families (Tnt1, Tto1, Tos17) have been demonstrated to include elements that are capable of transposition. Genetic alterations (mutations) are believed to be likely results from the activity of retrotransposons, the majority of which can be activated by stresses, for example, from viruses and tissue culture. In the sweet potato genome, Tahara *et al*. (2004) isolated an active LTR retrotransposon that can be activated by tissue culture. Tahara used the LTR retrotransposon to identify sweet potato cultivars by insertion polymorphism (Ooe *et al*. 2004).

9. Senescence-related genes in sweet potato

Leaf senescence represents endogenously controlled degenerative processes which ultimately lead to organ death. It is a highly regulated, ordered series of events involving the decline of photosynthetic activity, disintegration of

chloroplasts, breakdown of biomolecules, loss of chlorophyll, and the recycling of valuable nutrients to other parts of the plant. Leaf senescence is regulated by the coordinated expression of specific genes, and many senescence-associated genes (SAGs) have been identified in senescing leaves. These gene transcripts are either elevated from a basal level, or are senescence-specific. The encoded gene products are principally involved in degradation or remobilization of biomolecules, but they are also involved in protecting cell viability for completion of the senescence process. A number of SAG cDNA have been cloned and characterized (Huang *et al*. 2001). Most of these clones show significant sequence homology to known proteins such as enzymes involved in protein degradation, enzymes involved in the glyoxylate cycle and gluconeogenesis, and defense proteins. A novel senescence-associated gene (*SPA15*) of sweet potato was characterized for its structure and expression in sweet potato (Yap *et al*. 2003). The protein coding region of the gene consists of 13 exons encoding 420 amino acids. The expression patterns of the *SPA15* gene in sweet potato reveals that the transcripts of *SPA15* are specifically induced in the senescing leaves, and the temporal profile of *SPA15* protein accumulation is correlated with that of *SPA15* transcripts and *SPA15* is specifically associated with the cell wall.

10. Transcription factors in sweet potato

MYB transcription factors play important roles in transcriptional regulation of many secondary metabolites including anthocyanins. Kim *et al*. (2010) cloned the R2R3- MYB type IbMYB1 complementary DNAs from the purple-fleshed sweet potato. The transcripts of IbMYB1 were predominantly expressed in the purple-fleshed storage roots and in the leaf tissues accumulating anthocyanin pigments. The IbMYB1a was transiently expressed in tobacco leaves under the control of a constitutive cauliflower mosaic virus 35S promoter, a root-specific and sucrose-inducible sporamin promoter, and an oxidative stress-inducible sweet potato anionic peroxidase2 promoter. The IbMYB1a induced massive anthocyanin pigmentation in tobacco leaves and up-regulated the transcript levels of the structural genes in anthocyanin biosynthetic pathway. Kim *et al*. (2010) suggested that the IbMYB1 gene can be applicable to a visible marker for sweet potato transformation with intragenic vectors, as well as the production of anthocyanin as important nutritive value in other plant species.

Promoters isolated from sweet potato

The promoter is an important factor for the expression of the introduced foreign genes. In sweet potato, CaMV35S promoter has been used commonly in transformation studies. However, the expression level of a fatty acid desaturase gene from tobacco (*NtFAD3*) driven by the CaMV35S promoter in transgenic sweet potato plants was lower than that driven by a modified CaMV35S promoter, El2*Ω* (Mitsuhara *et al*. 1996), on the basis of not only Northern blot analysis, but also fatty acid composition (Wakita *et al*. 2001). It is necessary to use an appropriate promoter for each gene in a transformation study of sweet potato. Moreover, promoters of organ-specific genes would be forceful, for example promoters of sporamin and *β*-amylase which are expressed specifically in the storage root of sweet potato (Ohta *et al*. 1991).

1. Sporamin promoters

Sweet potato has produced two well-characterized promoters. These promoters drive expression of two of the most abundant proteins in the tuberous storage roots of sweet potato, sporamin and β-amylase (Maeo *et al*. 2001). Sporamin makes up 60–80% of total soluble proteins in the sweet potato storage organ. It is composed of two multigene subfamilies, A and B, which contain approximately 10 total members. Analysis indicates that sporamin was expressed almost exclusively in the storage tuber, with a small amount of expression in stems (1-4.5% soluble protein) (Maeshima *et al*. 1985). When approximately 1000 bp of the gSPOA1 promoter was fused to the reporter gene, *cat*, and expressed in transgenic tobacco, it was found that various sugars caused induction of *cat* to high levels in the stem. Induction was retained when a deletion was made to 2305 bp, but abolished with a deletion to 294 bp (Hattori *et al*. 1990). The gSPO-A1 promoter shares two conserved sequence elements with another sporamin promoter that appears to act independently of each other. One directs expression to the phloem (2305 and 2237 bp), and the other to the pith parenchyma (2192 to 294 bp) (Ohta *et al*. 1991).

Similar deletion experiments were also performed with the sweet potato β-amylase promoter fused to the *GUS* reporter gene. α-Amylase is developmentally regulated and highly expressed in the storage root where it is involved in starch synthesis. A -1267 bp promoter fragment drove high *GUS* expression in the stems of tobacco when induced by sugar, but deletion to -786 bp completely abolished the induction. Continued deletion analysis found a carbohydrate metabolic signal-responsible element-1 (CMSRE-1) comparable in sequence to an element found in the sporamin promoter (Maeo *et al*. 2001). This 82 bp region contained the TGGACGG sequence that plays an essential role in the sugar-inducible expression of the truncated promoter of the sporamin gene. Deletion or base substitutions of this element in the truncated β-Amy promoter abolished the sugar inducible expression, the results suggesting that the TGG ACGG element plays an important role in the coordinate induction of expression of genes for β-amylase and sporamin by sugars.

Morikami *et al*. (2005) generated transgenic tobacco plants that express the *GUS* gene under the control of the 305-bp 5'upstream promoter region of a gene coding for sporamin A of sweet potato. Expression of *GUS* in excised tobacco leaves was induced by sucrose, mimicking the sugar-inducible expression of the endogenous sporamin genes in sweet potato. Deletion of the sequences extending from position -305 (relative to the transcription start site) to -283 and from -146 to -87 resulted in an approximately 40-fold enhancement in *GUS* reporter expression. Furthermore, the sequence from -282 to -165 conferred sucrose-inducibility on the -89 core promoter of the *Cauliflower mosaic virus* (CaMV) 35S RNA gene. Analysis of internal deletions, linker scanning and the introduction of base substitutions in the sequence between positions -282 and -165 indicated that sucrose-responsiveness conferred by this region was dependent on the presence of two *cis*-acting elements, termed CMSREs (carbohydrate metabolite signal responsive elements) 1 and 2, which are separated by a spacer. A sequence similar or identical to the core of CMSRE-1 (TGG ACGG) is also present in the promoters of several other sugar-inducible genes, and sequences encompassing the TGGACGG-related motifs from two of these could functionally replace the CMSRE-1 in the truncated sporamin A promoter. These results suggest that the TGGACGG element plays an important role in the sucrose-inducible expression of a group of plant genes.

The sweet potato sporamin promoter was used to control the expression in transgenic potato of the *E. coli appA* gene, which encodes a bifunctional enzyme exhibiting both acid phosphatase and phytase activities. The sporamin promoter was highly active in leaves, stems and different size tubers of transgenic potato, with levels of phytase expression ranging from 3.8 to 7.4% of total soluble proteins. Phytase expression levels in transgenic potato tubers were stable over several cycles of propagation. Field tests showed that tuber size, number and yield increased in transgenic potato. Improved phosphorus (P) acquisition when phytate was provided as a sole P source and enhanced microtuber formation in cultured transgenic potato seedlings when phytate was provided as an additional P source were observed, which may account for the increase in leaf chloroplast

accumulation (important for photosynthesis) and tuber yield of field-grown transgenic potato supplemented with organic fertilizers. Animal feeding tests indicated that the potato-produced phytase supplement was as effective as a commercially available microbial phytase in increasing the availability of phytate-P to weanling pigs. This study demonstrates that the sporamin promoter can effectively direct high-level recombinant protein expression in potato tubers. Moreover, over-expression of phytase in transgenic potato not only offers an ideal feed additive for improving phytate-P digestibility in monogastric animals but also improves tuber yield, enhances P acquisition from organic fertilizers, and has a potential for phytoremediation (Hong *et al*. 2008).

2. Wound-regulated sporamin promoter

Sporamin, a tuberous storage protein of sweet potato, was systemically expressed in leaves and stems by wound stimulation. In an effort to demonstrate the regulatory mechanism of wound response on the sporamin gene, a 1.25 kb sporamin promoter was isolated for studying the wound-induced signal transduction. Two wound response like elements, a G box-like element and a GCC core-like sequence were found in this promoter. A construct containing the sporamin promoter fused to a GUS gene was transferred into tobacco plants by *Agrobacterium*-mediated transformation. The wound-induced high level of *GUS* activity was observed in stems and leaves of transgenic tobacco, but not in roots. This expression pattern was similar to that of the sporamin gene in sweet potatoes. Exogenous application of methyl jasmonate (MeJA) activated the sporamin promoter in leaves and stems of sweet potato and transgenic tobacco plants. In contrast, salicylic acid, an inhibitor of the octadecanoid pathway, strongly suppressed the sporamin promoter function that was stimulated by wound and MeJA treatments. The wound-response expression of the sporamin gene in aerial parts of plants is regulated by the octadecanoid signal pathway (Wang *et al*. 2002).

3. Peroxidase promoter

A strong oxidative stress-inducible POD promoter was cloned from sweet potato and characterized in transgenic tobacco plants and cultured cells in terms of environmental stress. A POD genomic clone (referred to as *SWPA2*) consisted of 1824 bp of sequence upstream of the translation start site, two introns (743 bp and 97 bp), and a 1073 bp coding region. *SWPA2* had previously been found to encode an anionic POD which was highly expressed in response to oxidative stress. Employing a transient expression assay in tobacco protoplasts, with five different 5'- deletion mutants of the *SWPA2* promoter fused to the *GUS* reporter gene, the 1314 bp mutant deletion mutant showed about 30 times higher *GUS* expression than the CaMV 35S promoter. The expression of *GUS* activity in transgenic tobacco plants under the control of the -1314 *SWPA2* promoter was strongly induced in response to environmental stresses including hydrogen peroxide, wounding and UV treatment. Furthermore, *GUS* activity in suspension cultures of transgenic cells derived from transgenic tobacco leaves containing the -1314 bp *SWPA2* promoter-*GUS* fusion was strongly expressed after 15 days of subculture compared to other deletion mutants. Kim *et al*. (2003) reported that the -1314 bp *SWPA2* promoter will be biotechnologically useful for the development of transgenic plants with enhanced tolerance to environmental stress, and particularly transgenic cell lines engineered to produce key pharmaceutical proteins.

The peroxidase gene (*Swpa4*) has been shown to be inducible by a variety of abiotic stresses and pathogenic infections in sweet potato. Ryu *et al*. (2009) isolated and characterized the promoter region (2374 bp) of swpa4. A transient expression assay in tobacco protoplasts with deletions from the 5'-end of SWPA4 promoter fused to the *GUS* reporter gene. The -1408 and -374 bp deletions relative to the transcription start site (+1) showed 8 and 4.5 times higher *GUS* expression than the CaMV35S promoter, respectively. *In silico* analysis indicated that four kinds of *cis*-acting regulatory sequences, reactive oxygen species-related element activator protein 1 (AP1), CCAAT/enhancer-binding protein alpha element, ethylene-responsive element (ERE) and heat-shock element, are present in the -60 bp region (-178/-118), suggesting that the -60 bp region might be associated with stress inducibility of the SWPA4 promoter.

4. Starch pathway promoters

A starch granule-bound starch synthase I (*GBSSI*) gene is regulated by a circadian clock in sweet potato leaves. In order to examine whether the promoter region is responsible for controlling a circadian expression of the *GBSSI* gene, the sweet potato GBSSI promoter was isolated and deleted to different lengths for functional analysis with a GUS reporter gene in transgenic *Arabidopsis* plants. Nuclear run-on transcriptional assays showed that the circadian control was regulated at the transcriptional rate level, and *de novo* synthesized proteins were necessary for controlling the rhythm. Promoter assays showed that the GBSSI promoter fragments containing six I-boxes, two putative circadian regulation elements (CAANNNNATC) and four circadian clock-associated 1 protein-binding sites (AATCT) maintained the activity to induce the circadian expression of the *GUS* gene. Similar to the *GBSSI* in sweet potato, *GBSSI*, soluble starch synthase and ADP-glucose pyrophosphorylase genes in *Arabidopsis* leaves also exhibited a circadian rhythm. These results suggested that common signals may exist in dicotyledonous plants to coordinate the circadian expression of genes involved in the transitory starch synthetic pathway (Wang *et al*. 2004).

Kim *et al*. (2009) used the sweet potato ADP-glucose pyrophosphorylase gene (*ibAGP1*) promoter and its TP as an expression system for the mass production of foreign proteins in potato. The *ibAGP1* promoter and its TP sequence were transformed into potato along with GUS as a reporter gene, and GUS activity was subsequently analyzed in the transgenic potato plants. In tuber tissues, GUS activity in transgenic plants carrying only the ibAGP1 promoter (ibAGP1::GUS) increased up to 15.6-fold compared with that of transgenic plants carrying only the CaMV35S promoter (CaMV35S::GUS). GUS activity in transgenic plants was further enhanced by the addition of the sweetpotato TP to the recombinant vector (ibAGP1::TP::GUS), with tuber tissues showing a 26-fold increase in activity compared with that in the CaMV35S::GUS-transgenic lines. The sweet potato *ibAGP1* promoter and its TP are a potentially strong foreign gene expression system that can be used for molecular farming in potato plants.

5. SRD1 root-specific promoter

Molecular farming of recombinant proteins require strong promoter to accumulate the desired protein in the tubers of sweet potato. For that purpose Noh *et al*. (2012) isolated the promoter region (3.0 kb) of *SRD1* from sweet potato and characterized its activity in transgenic Arabidopsis, carrot, and potato using the *GUS* gene as a reporter gene. The *SRD1* promoter conferred root-specific expression in transgenic Arabidopsis, with *SRD1* promoter activity increasing in response to exogenous IAA. The *SRD1* promoter directs strong expression restricted to the underground storage organs, such as fleshy taproots and tubers, as well as fibrous root tissues.

GENETIC TRANSFORMATION OF SWEET POTATO

Gene engineering has been used for the successful introduction of foreign genes in the genome of many plant species.

Table 1 Summary of published studies on the transformation of sweet potato.

Cultivar/genotype	Target tissue	Method used	Genes	Observation/ remarks	Reference
Not stated	*In vitro* whole plant	*A. rhizogenes*	Synthetic sequence	Transgenic plants	Dodds *et al*. 1991
'Jewel', 'TIS-70357'	Leaves and petioles	Particle bombardment	nptII, gusA	Transformed calli	Prakash and Varadarajan 1992
Five cultivars: 'Chugoku', 'Jewel', 'Beauregard', 'Kokei 14', 'Yulmi'	Leaves	*A. rhizogenes* 15834	nptII, gusA	Transgenic plants	Otani *et al*. 1993
Jewel'	Storage roots	*A. tumefaciens* LBA4404	nptII, gusA, cowpea trysin inhibitor, snow-drop lectin	Transgenic plants	Newell *et al*. 1995
White Star'	Embryogenic callus	*A. tumefaciens* EHA101	nptII, gus	Transgenic plants	Gama *et al*. 1996
'Kokei 14'	Embryogenic callus	*A. tumefaciens* EHA101	hpt, gusA	Transgenic plants	Otani *et al*. 1998
'Jewel'	Embryogenic callus	Electroporation	nptII, gusA	Transformed calli	Mitchell *et al*. 1998
'Jewel'	Leaves	*A. tumefaciens* C58C1	nptII, cryIIIA	Transgenic plants	Moran *et al*. 1998
'Beauregard'	Leaves and petioles	Particle bombardment, Electroporation	GFP	Transformed calli	Lawton *et al*. 2000
Kokei 14', 'Beniazuma'	Embryogenic callus	*A. tumefaciens* EHA101	hpt, NtFAD3	Transgenic plants	Wakita *et al*. 2001
'Beauregard'	Embryogenic callus	Electroporation, Particle bombardment	GFP	Transformed calli	Winfield *et al*. 2001
Tobacco cv. Petit Havan	Leaves	*A. tumefaciens* LBA4404	gusA, β-amy	Transgenic plants	Maeo *et al*. 2001
'Kokei 14'	Embryogenic callus	*A. tumefaciens* EHA101	hpt, GBSSI (sense)	Transgenic plants	Kimura *et al*. 2001
'Chikei 682-11'	Mesophyll protoplasts	Electroporation	gusA, hpt, SPFMV-S CP	Transgenic plants	Okada *et al*. 2001
'Jewel'	Embryogenic callus	*A. tumefaciens* A208SE	nptII, gusA	Transgenic plants	Liu *et al*. 2001
Nanging 51-93'	Embryogenic cell suspension	Particle bombardment	hpt, SPFMV-S CP	Transgenic plants	Okada *et al*. 2002
'Mary Anne'	Cell culture	*A. tumefaciens* LBA4404 EHA105, particle bombardment	gusA	Transient gene expression	Deroles *et al*. 2002
'Lizixiang'	Embryogenic callus	*A. tumefaciens*	nptII, gusA	Transgenic plants	Zhai and Liu 2003
Kokei 14'	Embryogenic callus	*A. tumefaciens* EHA101	hpt, bar	Transgenic plants	Otani *et al*. 2003
Beniazuma'	Stem	*A. tumefaciens* EHA105	nptII, hpt, gusA	Transgenic plants	Song *et al*. 2004
'Yulmi'	Embryogenic callus	Particle bombardment	nptII, SOD, APX	Transgenic plants	Lim *et al*. 2004
Lizixiang'	Embryogenic cell	*A. tumefaciens* LBA4404	nptII, OCI	Transgenic plants	Jiang *et al*. 2004
Kokei 14'	Embryogenic callus	*A. tumefaciens* EHA101	hpt, mouse adiponectin cDNA	Transgenic plants	Berberich *et al*. 2005
'Kokei 14'	Embryogenic callus	*A. tumefaciens* EHA101	hpt, FSPD1	Transgenic plants	Kasukabe *et al*. 2006
'Kokei 14'	Embryogenic callus	*A. tumefaciens* EHA101	hpt, SBEII RNAi	Transgenic plants	Shimada *et al*. 2006
'Lizixiang'	Embryogenic callus	*A. tumefaciens* EHA105	hptII, gusA	Transgenic plants	Yu *et al*. 2007
'Yulmi' – Korean elite	Embryogenic callus	Particle bombardment	gusA, bar	Transgenic plants	Yi *et al*. 2007
'Yulmi' – Korean elite	Embryogenic callus	*A. tumefaciens* EHA105	gusA, bar/pat	Transgenic plants	Choi *et al*. 2007
' Kokei 14'	Embryogenic callus	*A. tumefaciens* EHA101	hpt, GBSSI RNAi	Transgenic plants	Otani *et al*. 2007
'Yulmi' – Korean elite	Embryogenic callus	Particle bombardment	gusA, CuZuSOD, APX	Transgenic plants	Lim *et al*. 2007
'Xanthi' - tobacco	Embryogenic callus	*A. tumefaciens* EHA105	npt, swpa4	Transgenic plants	Kim *et al*. 2008
Huachano	Leaves	*A. tumefaciens*	Replicase of SPCSV	Transgenic plants	Kreuze *et al.* 2008
116 cultivars	Plant	*Electroporation*	CP of SPFMV	Transgenic plant	Okada and Saito 2008
' Kokei 14'	Leaves	*A. tumefaciens*	Dof zinc finger Transcription Factor	Transgenic plants	Tanaka *et al.* 2009
Tobacco	Leaves, tubers	*A. tumefaciens*	Cinnamyl alcohol dehydrogenase (ibCAD1)	Transgenic plants	Kim *et al*. 2010
'Yulmi' – Korean elite	Leaves, tuber	*A. tumefaciens*	Cu-Zn SOD, APX	Transgenic plants	Lu *et al.* 2010
'Jinhongmi'	Tuber	*A. tumefaciens*	MADS-box protein cDNA (SRD1)	Transgenic plants	Noh *et al.* 2010
---	Tuber	*A. tumefaciens*	Starch synthase II gene (SSII)	Transgenic plants	Takahata *et al.* 2010
'Yulmi' – Korean elite	Leaves	*A. tumefaciens*	Soybean cold-inducible zinc finger protein (SCOF1)	Transgenic plants	Kim *et al.* 2011
---	Embryogenic calli	*A. tumefaciens*	Late embryogenesis abundant 14 (LEA14)	Trangenic plants	Park *et al.* 2011
---	Tuber	*A. tumefaciens*	Hyperthermophilic α-amylase	Trangenic plants	Santa-Maria *et al.* 2011
Lizixiang	Plant	*A. tumefaciens*	Oryzacystain (OCI)	Trangenic plants	Gao *et al.* 2011
Sushu2,9,11Ayamurasaki, Wanslu1, Xushu 8,22	Embryogenic callus	*A. tumefaciens*	NptII GUS	Trangenic plants	Yang *et al.* 2011
Shinhwangmi, Yulmi	Tuber, calli	*A. tumefaciens*	β-cartoene hydroxylase	Trangenic plants	Kim *et al.* 2012
Kokei 14	Leaves, tuber	*A. tumefaciens*	α-Hordothionin	Trangenic plants	Muramoto *et al.* 2012
Yulmi	Leaves	*A. tumefaciens*	Cu-Zn SOD, APX	Trangenic plants	Wang *et al.* 2012

β-amy - amylase gene; APX-ascorbate peroxidase; cryIIIA – *Bacillus thuringiensis* endotoxin gene; CuZuSOD- CuZu superoxide dismutase; FSPD1 - Spermidine synthase; GBSS- Granule-bound starch synthase; gus - β-glucuronidase; hpt - hygromycin phosphor nptI1 - neomycin phosphotransferase; OCI - oryzacystain-I, NtFAD3 - fatty acid desaturase gene [gfp – green fluorescent protein gene; pat/ bar-phosphinothricin N-acetyltransferase; php-, luc – firefly luciferase gene; SBEII – starch branching enzyme; SPFMV-S CP – *Sweet potato feathery mottle virus* coat protein gene; swpa4-peroxidase

This technology offers great potential for the improvement of sweet potato. The successful application of this technology is greatly dependent upon the development of an efficient and reproducible transformation system. A great deal of effort has been made to develop an efficient transformation system in sweet potato. Particle bombardment and electroporation were attempted in sweet potato, only transient gene expression or transformed calli were observed (Prakash and Varadarajan 1992; Lawton *et al*. 2000), except for the report of Okada *et al*. (2001) who obtained a few transgenic sweet potato plants expressing the coat protein gene of sweet potato feathery mottle virus using electroporation. Using *Agrobacterium rhizogenes*-mediated method, Otani *et al*. (1993) obtained morphologically aberrant shoots from hairy roots induced on leaf explants of five sweet potato cultivars among 14 tested, but only one transgenic line was confirmed to be stable by Southern analysis. Transient expression of the *GUS* gene has been reported in leaf-derived embryogenic callus of sweet potato by electroporation (Mitchell *et al*. 1998). The green-fluorescent protein (*GFP*) gene from *Aequorea victoria* (Jellyfish) was expressed stable in sweet potato tissues by electroporation and particle bombardment (Winfield *et al*. 2001) using leaf and petiole segments from young *in vitro*-raised plantlets.

Agrobacterium tumefaciens-mediated transformation system has been widely used in many plant species because of its efficiency, simplicity, and stability of the introduced gene. Different explants such as leaves, petioles, stems, storage roots, and embryogenic calli have been used for *A. tumefaciens*-mediated transformation of sweet potato and stable transgenic sweet potato plants have also been reported, but in most cases only a low transformation efficiency was obtained (Newell *et al*. 1995; Gama *et al*. 1996; Moran *et al*. 1998; Otani *et al*. 1998, 2001, 2003; Wakita *et al*. 2001; Kimura *et al*. 2001; Song *et al*. 2004; Shimada *et al*. 2006). In comparison, apical meristem-derived embryogenic calli can give improved transformation efficiency in sweet potato (Gama *et al*. 1996; Otani *et al*. 1998), but they are not readily available target tissues for most of cultivars due to low frequencies of embryogenic callus formation in apical meristem cultures (Al-Mazrooei *et al*. 1997). Thus, considerable effort is still needed to develop an efficient transformation system in sweet potato. For that, Yu *et al*. (2007) developed efficient *A. tumefaciens*-mediated transformation was achieved using embryogenic suspension cultures. Cell aggregates from embryogenic suspension cultures were cocultivated with the *A. tumefaciens* strain EHA105 harboring a binary vector pCAMBIA1301 with *gusA* and hygromycin phosphotransferase II gene (*hpt II*) genes. Selection culture was conducted using 25 mg l^{-1} hygromycin. A total of 2218 plants were regenerated from the inoculated 1776 cell aggregates *via* somatic embryogenesis. *GUS* assay and PCR, dot blot and Southern blot analyses of the regenerated plants randomly sampled showed that 90.37% of the regenerated plants were transgenic plants. The number of integrated T-DNA copies varied from 1 to 4. Transgenic plants, when transferred to soil in a greenhouse and a field, showed 100% survival. No morphological variations were observed in the *ex vitro* transgenic plants.

Yang *et al*. (2011) developed an efficient *A. tumefaciens*-mediated transformation using embryogenic suspension cell cultures of elite sweet potato cultivars, including 'Ayamurasaki', 'Sushu2', 'Sushu9', 'Sushu11', 'Wanshu1', 'Xushu18' and 'Xushu22'. Embryogenic suspension cultures were established in LCP medium using embryogenic calli induced from apical or axillary buds on an induction medium containing 2 mg l^{-1} 2,4-D. Suspension cultures were co-cultivated with *A. tumefaciens* strain with the hpt gene as a selectable marker and *uidA* gene as a visible marker. A total of 485 putative transgenic plant lines were produced from the transformed calli *via* somatic embryogenesis and germination to plants under 10 mg l^{-1} hygromycin and 200 mg l^{-1} cefotaxime. Plants showed 100% survival when 308 transgenics were transferred to soil in the greenhouse and then to the field. The development of such a robust transformation method suitable to a range of sweet potato genotypes not only provides a routine tool for genetic improvement *via* transgenesis but also allows us to conduct a functional verification of endogenous genes in sweet potato. Summary of studies on the transformation of sweet potato was given in **Table 1**.

Experimental protocol for *Agrobacterium tumefaciens*-mediated transformation

This protocol is based on that devised by Shimada and Otani (2007)

1. Embryogenic callus induction from shoot meristem tissues

Step 1. Culture sweet potato *in vitro* plants on LS medium (Linsmaier and Skoog 1965) supplemented with 3% sucrose and 0.25% Gelrite at 26°C under a 16 h photoperiod at 38 $\mu molm^{-2} s^{-1}$ from day light fluorescent tubes.

Step 2. Place shoot meristems (0.5 mm diameter) of *in vitro* plants onto LS medium for embryogenic callus induction, which is LS medium supplemented with 1 mg l^{-1} 4-fluorophenoxyacetic acid (4FA; Aldrich; or picloram), 3% sucrose and 0.32% Gelrite. Keep cultures at 26°C in the dark for 45 days.

Step 3. Maintain embryogenic calli by subculture on the same fresh medium every month. Use calli subcultured at least three times for the transformation.

2. Transformation

Step 1. Culture *A. tumefaciens* strain LBA4404 for 2–3 days at 26°C on Luria broth (LB; Sambrook *et al*. 1989) supplemented with 50 mg l^{-1} kanamycin, 50 mg l^{-1} hygromycin B and 1.5% (w/v) agar. Then, transfer the colony of bacteria to liquid LS medium and shake at 100 rpm for 30 min in the dark at 26°C.

Step 2. Soak the embryogenic calli in a bacterial suspension for 2 min and blot dry with sterile filter paper to remove excess bacteria.

Step 3. Then, transfer the calli onto co-culture medium, which is LS medium supplemented with 1 mg l^{-1} 4FA (or picloram), 10 mg l^{-1} acetosyringone (Aldrich), 1% glucose, 3% sucrose and 0.32% Gelrite, and culture for 3 days in the dark at 23°C.

Step 4. Wash the infected calli four times with sterile distilled water supplemented with 500 mg l^{-1} carbenicillin and then transfer onto selection medium, which is LS medium supplemented with 1 mg l^{-1} 4FA (or picloram), 25 mg l^{-1} hygromycin B, 500 mg l^{-1} carbenicillin, 3% sucrose and 0.32% Gelrite. Cultures were carried out in the dark at 26°C.

3. Selection and plant regeneration

Step 1. After 2 weeks of culture on the selection medium, wash the calli again as described above and then transfer to fresh selection medium. Sub-culture calli on fresh medium every 2 weeks.

Step 2. After 60 days of culture on selection medium, transfer the calli onto the somatic embryo formation medium, which is LS medium supplemented with 4 mg l^{-1} Abscic acid (ABA), 1 mg l^{-1} gibberellic acid (GA_3), 3% sucrose and 0.32% Gerlite, and culture at 26°C under a 16 h photoperiod at 38 $\mu mol\ m^{-2} s^{-1}$ from day light fluorescent tubes.

Step 3. After 21 days of culture on the somatic embryo formation medium, transfer the somatic embryos formed from hygromycin-resistant calli onto the plant formation medium,

which is LS medium supplemented with 0.05 mg l^{-1} ABA, 3% sucrose and 0.32% Gerlite for germination.

Transgenic sweet potato with agronomic importance

1. Biotic stresses

Some transgenic plants have been developed to withstand biotic stresses, such as weeds (Otani *et al*. 2003), insects (Newell *et al*. 1995; Moran *et al*. 1998), fungus, virus and nematode resistance (Okada *et al*. 2001). Herbicides are effective for weed control in the field production of sweet potato. Otani *et al*. (2003) introduced the phosphinothricin acetyltransferase (*pat*, *bar*) gene for herbicide resistance. Transgenic sweet potato plants exhibit bialaphos resistance and also show resistance to the commercial herbicide. Genetic engineering of herbicide tolerance in sweet potato may make weed control weeds more convenient and more economical during cultivation. The sweet potato weevil is the major sweet potato insect pest worldwide, especially in developing countries. To date, two reports have been published on transformed plants with insect-resistant genes. Newell *et al*. (1995) introduced cowpea TI and snowdrop lectin genes into sweet potato genome, but they did not confirm the insect tolerance of regenerated transgenic plants. Moran *et al*. (1998) regenerated transgenic plants 'Jewel' possessing the *cryIIIA* gene and observed the transgenic sweet potato plants to be less affected by weevils than the untransformed control plants in field tests.

Viruses are another main constraint to sweet potato production and a *Sweet potato feathery mosaic virus* (SPFMV) is the most widespread in the world. One of the most-serious diseases of sweet is russet crack disease caused by SPFMV. Okada *et al*. (2001) constructed an expression vector carrying the coat protein (CP) and *hpt* genes driven by cauliflower mosaic virus 35 S promoters. Introduced the expression vector into sweet potato variety 'Chikei 682-1'1 by the electroporation method. Among 449 calli obtained after antibiotic selection, 19 plants from seven independent calli grew to form adventitious shoots. Three transgenic lines were obtained from independent calli, based on analysis of the CP and *hpt* genes. To assay the virus resistance of the transgenic lines, each line was vegetatively propagated and then grafted with morning glory (*Ipomoea nil*) that had been infected with SPFMV-S (severe). A PAS-ELISA assay with polyclonal antiserum of the CP demonstrated that virus accumulation 3 months after grafting with the infected morning glory was suppressed in the transgenic lines as compared with non-transgenic ones. These transgenic lines were shown to be highly resistant not only to primary but also to secondary infection by SPFMV-S. The three transgenic lines with the CP gene of SPFMV-S can be used for coat protein-mediated resistance to the virus. Recently, in Kenya transgenic lines of sweet potato variety CPT560 transformed with SPFMV CP gene were tested in the field under controlled conditions to evaluate virus resistance, yield of storage roots and other characters (Gichuki *et al*. 2003).

Based on the previous report, the graft transmission of target specificity for RNA silencing using transgenic *Nicotiana benthamiana* plants expressing the coat protein gene (CP, including the 3' non-translated region) of SPFMV. Transgenic plants carrying the 5' 200 and 400 bp regions of CP were newly produced. From these plants, two silenced and two non-silenced lines were selected to investigate the manifestation of transitive RNA silencing by graft experiments. Non-silenced scions carrying the entire transgene were grafted onto either 5' or 3' silencing inducer rootstocks. When non-silenced scions were grafted onto 5' silencing inducer rootstocks, RNA silencing was induced in the non-silenced scions and spread toward the 3' region of the transgene mRNA. Similarly, when non-silenced scions were grafted onto 3' silencing inducer rootstocks, RNA silencing was induced in the non-silenced scions, but was restricted to the 3' region of the transgene and did not spread to the 5' region. In addition, results from crossing experiments, involving non-silenced and 3' silencing inducer plants, confirmed the above finding. This indicates that RNA silencing spreads in the 5'–3' direction, not in the 3'–5' direction, along the transgene mRNA (Haque *et al*. 2007).

Okada and Saito (2008) developed transgenic sweet potato lines using SPFMV CP genes, which had shown significant resistance to SPFMV. These plants were challenged by graft inoculation with field-infection SPFMVs and transgenic plants were highly protected than control plants. *Sweet potato chlorotic stunt virus* (SPCSV) is one of the most important pathogens of sweet potato. It can reduce yields by 50% by itself and cause various synergistic disease complexes when co-infecting with other viruses, including SPFMV. Because no sources of true resistance to SPCSV are available in sweet potato germplasm, a pathogen-derived transgenic resistance strategy was tested as an alternative solution by Kreuze et al. 2008. A Peruvian sweet potato landrace 'Huachano' was transformed with the replicase encoding sequences of SPCSV and SPFMV. Twenty-eight independent transgenic plants obtained and accumulation of transgene-specific siRNA was detected in most of them. None of the transgenic events was immune to SPCSV, but ten of the 20 tested transgenic plants exhibited mild or no symptoms following infection, and accumulation of SPCSV was significantly reduced (Kreuze *et al*. 2008).

Black rot of sweet potato caused by pathogenic fungus *Ceratocystis fimbriata* severely deteriorates both growth of plants and post-harvest storage. Antimicrobial peptides from various organisms have broad range activities of killing bacteria, mycobacteria, and fungi. Plant thionin peptide exhibited anti-fungal activity against *C. fimbriata*. A gene for barley α-hordothionin (αHT) was placed downstream of strong constitutive promoters of E12Ω and the promoter of a sweet potato gene for β-amylase of storage roots, and introduced into sweet potato commercial cultivar 'Kokei'. Transgenic E12Ω:αHT plants showed high-level expression of αHT mRNA in both leaves and storage roots. Transgenic β-Amy:αHT plants showed sucrose-inducible expression of αHT mRNA in leaves, in addition to expression in storage roots. Muramoto *et al*. (2012) reported the usefulness of transgenic sweet potato expressing antimicrobial peptide to reduce damages of sweet potato from the black rot disease and to reduce the use of agricultural chemicals.

Enhanced stem nematode resistance of transgenic sweet potato was achieved using Oryzacystatin-I (*OCI*) gene with *Agrobacterium tumefaciens*-mediated transformation by Gao *et al*. (2011). *A. tumefaciens* with *OCI* gene, *gusA* gene and *hptII* genes were used for transformation. Trans-genic plants exhibited significantly enhanced resistance to stem nematodes compared to the untransformed control plants by the field evaluation with stem nematodes. Stable integration of the *OCI* gene into the genome of resistant transgenic plants was confirmed and the copy number of integrated *OCI* gene ranged from 1 to 4.

2. Abiotic stresses

Abiotic stresses such as salt, low temperature, drought and early frost are also a serious problem for sweet potato production. By introducing a fatty-acid desaturase gene to increase the content of unsaturated fatty acid for low temperature tolerance and succeeded in modifying the fatty acid composition in transgenic sweet potato plants (Wakita *et al*. 2001). The low-temperature tolerance of these transgenic sweet potato plants were under testing. Oxidative stress is one of the major damaging factors in plants exposed to environmental stresses. Damage from multiple environmental stresses would be alleviated by manipulation of anti-oxidatives in chloroplasts.

POD reduces hydrogen peroxide (H_2O_2) in the presence of an electron donor. Extracellular POD can also induce H_2O_2 production and may perform a significant function in responses to environmental stresses *via* the regulation of

H_2O_2 in plants. Ten POD cDNA clones isolated from cell cultures of sweet potato. Among them, the expression of the *swpa4* gene was profoundly induced by a variety of abiotic stresses and pathogenic infections. Kim *et al*. (2008) reported transgenic tobacco plants over expressing the *swpa4* gene under the control of the CaMV 35S promoter were generated in order to assess the function of *swpa4* in plants. The transgenic plants exhibited an approximately 50-fold higher POD specific activity than was observed in control plants. Both transient expression analysis with the *swpa4-GFP* fusion protein and POD activity assays in the apoplastic washing fluid revealed that the swpa4 protein is secreted into the apoplastic space. In addition, a significantly enhanced tolerance to a variety of abiotic and biotic stresses occurred in the transgenic plants. These plants harbored increased lignin and phenolic content, and H_2O_2 was also generated under normal conditions. Furthermore, they showed an increased expression level of a variety of apoplastic acidic pathogenesis-related genes following enhanced H_2O_2 production. Kim *et al*. (2008), suggest that the expression of *swpa4* in the apoplastic space may function as a positive defense signal in the H_2O_2-regulated stress response signaling pathway.

Oxidative stress is one of the major factors causing injury to plants exposed to environmental stress. Transgenic sweet plants with an enhanced tolerance to multiple environmental stresses were developed by expressing the genes of both CuZn-superoxide dismutase (*CuZnSOD*) and ascorbate peroxidase (*APX*) under the control of an oxidative stress-inducible SWPA2 promoter in the chloroplasts of sweet potato plants (referred to as SSA plants). SSA plants were successfully generated by the particle bombardment method (Lim *et al*. 2007). When leaf discs of SSA plants were subjected to 5 μM methyl viologen (MV), they showed approximately 45% less damage than non-transformed (NT) plants. When 200 μM MV was sprayed onto the whole plants, SSA plants showed a significant reduction in visible damage compared to leaves of NT plants, which were almost destroyed. The expression of the introduced *CuZnSOD* and *APX* genes in leaves of SSA plants following MV treatment was significantly induced, thereby reflecting increased levels of SOD and APX in the chloroplasts. APX activity in chloroplast fractions isolated from SSA plants was approximately 15-fold higher than that in their counterparts from NT plants. SSA plants treated with a chilling stress consisting of 4°C for 24 h exhibited an attenuated decrease in photosynthetic activity (Fv/Fm) relative to NT plants; furthermore, after 12 h of recovery following chilling, the Fv/Fm of SSA plants almost fully recovered to the initial levels, whereas NT plants remained at a lower level of Fv/Fm activity. These results suggest that SSA plants would be a useful plant crop for commercial cultivation under unfavorable growth conditions. In addition, the manipulation of the antioxidative mechanism in chloroplasts can be applied to the development of various other transgenic crops with an increased tolerance to multiple environmental stresses.

Park *et al*. (2011) isolated the late embryogenesis abundant 14 (LEA14) cDNA from an EST library prepared from dehydration-treated fibrous roots of sweet potato. *IbLEA14* expression was strongly induced by dehydration, NaCl and abscisic acid treatments in sweet potato plants. Transgenic sweet potato non-embryogenic calli harboring Ib*LEA14* over-expression or RNAi vectors under the control of CaMV 35S promoter were generated. Transgenic calli over-expressing *IbLEA14* showed enhanced tolerance to drought and salt stress, whereas RNAi calli exhibited increased stress sensitivity. Under normal culture conditions, lignin contents increased in *IbLEA14*-overexpressing calli because of the increased expression of a variety of monolignol biosynthesis-related genes. Stress treatments elicited higher expression levels of the gene encoding cinnamyl alcohol dehydrogenase in *IbLEA14*-overexpressing lines than in control or RNAi lines. Based on the result, *IbLEA14* might positively regulate the response to various stresses by enhancing lignifications (Park *et al*. 2011).

β-Carotene hydroxylase (CHY-β) is a key regulatory enzyme in the beta-beta-branch of carotenoid biosynthesis and it catalyzes hydroxylation into both β-carotene to β-cryptoxanthin and β-cryptoxanthin to zeaxanthin in sweet potato. To increase the β-carotene content of sweet potato through the inhibition of further hydroxylation of β-carotene, the effects of silencing CHY-β in the carotenoid biosynthetic pathway were evaluated by Kim *et al*. (2012). A partial cDNA encoding CHY-β was cloned from the storage roots of orange-fleshed sweet potato to generate an RNA interference-*IbCHY*-β construct and introduced into cultured cells of white-fleshed cv. 'Yulmi'. Reverse transcription-polymerase chain reaction analysis confirmed the successful suppression of *IbCHY-β* gene expression in transgenic cultured cells. Down-regulation of *IbCHY-β* gene expression changed the composition and levels of carotenoids between non-transgenic (NT) and transgenic cells. In transgenic line #7, the total carotenoid content reached a maximum of 117 μg/g dry weight, of which β-carotene measured 34.43 μg/g dry weight. RNA-IbCHY-β calli increased abscisic acid (ABA) content, which was accompanied by enhanced tolerance to salt stress. Kim *et al*. (2012) reported, the down-regulation of IbCHY-β increased β-carotene contents and total carotenoids in transgenic plant cells and enhanced their antioxidant capacity.

Wang *et al*. (2012) studied the physiological indexes of leaves in transgenic sweet potato, which harbours two genes *CuZnSOD* and *APX* genes, with the stress-inducible SWPA2 promoter were evaluated under different concentrations of NaCl treatment. The activity of SOD, APX, POD and CAT in leaves of transgenic (TS) was always higher than non-transgenic (NS) under the same NaCl stress, respectively. And the decline range of chlorophyll and malonaldehyde (MDA) content in leaves of TS was lower than that of NS. All these results indicated that transgenic sweet potato had the resistance to salt tolerance. Therefore, there would be a great significance in efficiently utilizing saline land and alleviating the energy crisis by developing and planting transgenic sweet potato plants with salt tolerance.

Lu *et al*. (2010) studied the antioxidant activity of transgenic sweet potato containing elevated Cu/Zn SOD and APX in chloroplasts under water stress. The expression of antioxidant enzymes (SOD, APX and CAT) in transgenic (SOD, APX) plants cv. Yulmi was profoundly increased under drought stress and re-watering periods, but tuberization was poor. The expression of Cu/Zn SOD and APX in chloroplasts of sweet potato enhanced drought resistance and capacity for recovery from drought stress.

Low-temperature stress represents one of the principal limitations affecting the distribution and productivity of sweet potato. Transgenic sweet potato plants expressing the soybean cold-inducible zinc finger protein (SCOF-1) under control of an oxidative stress-inducible peroxidase (SWPA2) promoter. Increased SCOF-1 expression also correlated with enhanced tolerance to different low-temperature treatments at the whole plant level. TS plants treated with low-temperature stress (4 or 10°C for 30 h) exhibited less of a reduction in photosynthetic activity and lipid peroxidation levels than NT plants. The low-temperature stress in sweet potato can be efficiently modulated by over-expression of SCOF-1 (Kim *et al*. 2011).

3. Starch quality

Starch is the main product of sweet potato and provides important food processing and industrial materials. Amylose and amylopectin are the main polysaccharide components of most natural starches and the amylose:amylopectin ratio is the important factor in the textural properties of starch. A new sweet potato variety that contains starch with amylose-free or low amylose content would develop new industrial applications. The full-length sense cDNA of granule-bound starch synthase I (*GBSSI*) was introduced, which is one of the key enzymes to catalyze the formation of amy-

lose, a linear α(1,4)d-glucan polymer, from ADP-glucose, for the modification of starch structure. Of the 26 transgenic plants independently regenerated, one lacked amylose in the storage roots (Kimura *et al*. 2001). The amylose-free transgenic sweet potato plants were also successfully obtained more efficiently by inhibition of sweet potato *GBSSI* gene expression through RNA interference (Otani *et al*. 2003). Recently, double-stranded RNA-mediated gene silencing has been found to be an effective technology in the genetic improvement of crops as well as in functional genomic studies. Shimada *et al*. (2006) successfully obtained transgenic sweet potato plants, which had a starch with higher amylose content than non-transgenic plants (up to 25% compared to 10% in the control) by RNAi of starch branching enzyme II (SBEII). RNAi should offer a reliable technique for trait modification in agricultural crop improvement.

Amylose-free transgenic sweet potato plants were produced by inhibiting sweet potato *GBSSI* gene expression through RNA interference. The gene construct consisting of an inverted repeat of the first exon separated by intron 1 of *GBSSI* driven by the CaMV 35S promoter was integrated into the sweet potato genome by *A. tumefaciens*-mediated transformation. In over 70% of the regenerated transgenic plants, the expression of *GBSSI* was inactivated giving rise to storage roots containing amylopectin but not amylose. Electrophoresis analysis failed to detect the GBSSI protein, suggesting that gene silencing of the *GBSSI* gene had occurred. This shows that amylose synthesis is completely inhibited in storage roots of sweet potato plants by the constitutive production of the double-stranded RNA of *GBSSI* fragments. The RNA interference is an effective method for inhibiting gene expression in the starch metabolic pathway (Otani *et al*. 2007).

The sweet potato cultivar 'Quick Sweet' (QS) with a lower pasting temperature of starch is a unique breeding material. Takahata *et al*. (2010) studied the physiological impact of reducing the activity of starch synthase (SSII) on the starch properties in storage root, transgenic plants with reduced expressions of the SSII gene were evaluated. The pasting temperatures in transgenic plants were approximately 10-15°C lower than in wild-type plants. Distribution of the amylopectin chain length of the transgenic lines showed marked differences compared to that in wild-type plants. Based on the study, the expression of SSII in the storage roots of the sweet potato cultivar (QS) with low pasting temperature starch was notably lower than in cultivars with normal starch. The activity of SSII in sweet potato storage roots, like those in other plants, affects the pasting properties of starch through alteration of the amylopectin structure (Takahata *et al*. 2010).

Tanaka *et al*. (2009) characterized the functions of the sweet potato *SRF1* gene, which encodes a Dof zinc finger transcriptional factor preferentially expressed in the storage roots. Tanaka *et al*. (2009) isolated its full length cDNA and produced transgenic sweet potato plants with altered *SRF1* expression levels. Transgenic plants over-expressing SRF1 showed significantly higher storage root dry matter content compared to the original cultivar 'Kokei' or control transgenic plants. Among the enzymes involved in the sugar metabolism, soluble acid invertase showed a decreased activity in the transgenic plants. Gene expression level of *Ibbetafruct2*, which encodes an isoform of vacuolar invertase, was suppressed in the transgenic plants over-expressing the *SRF1* gene. These data suggest that *SRF1* modulates the carbohydrate metabolism in the storage roots through negative regulation of a vacuolar invertase gene.

4. Herbicide-resistant transgenics

Recent molecular breeding offers potentials for the production of sweet potato varieties tolerant to diseases and herbicides. Since herbicide resistant germplasm have not been identified in sweet potato or its related species, conventional breeding for herbicide-resistant varieties is not possible. Therefore, the use of *in vitro* genetic manipulation to produce sweet potato varieties with herbicide resistance is an obvious alternative.

The introduction of important agronomic traits into sweet potato has been achieved *via* plant transformation, such as insect resistance (Newell *et al*. 1995; Moran *et al*. 1998), virus resistance (Okada *et al*. 2001), fatty acid and starch (Kimura *et al*. 2001; Wakita *et al*. 2001) modifications. Most of the transgenic sweet potato plants have been produced using either neomycin phosphotransferase II (*nptII*) gene or hygromycin phosphotransferase (*hph*) gene as selectable markers. Recently, Otani *et al*. (2003) reported herbicide resistant sweet potato with the *bar* gene using a hpt/hygromycin selection system.

Herbicide-resistant sweet potato plants were produced through biolistics of embryogenic calli derived from shoot apical meristems. Plant materials were bombarded with the vectors containing the β-glucuronidase gene (*gusA*) and the herbicide-resistant gene (*bar*). Selection was carried out using phosphinothricin (PPT). Transformants were screened by the histochemical GUS and Chlorophenol Red assays. PCR and Southern-blot analyses indicated the presence of introduced *bar* gene in the genomic DNA of the transgenic plants. When sprayed with Basta, the transgenic sweet potato plants were tolerant to the herbicide. Yi *et al*. (2007), reported successful transformation of the *bar* gene confer herbicide resistance.

Transgenic herbicide-resistant sweet potato were produced through an *Agrobacterium*-mediated transformation system. Embryogenic calli derived from shoot apical meristems were infected with *A. tumefaciens* strain EHA105 harboring the pCAMBIA3301 vector containing the *bar* gene encoding phosphinothricin N-acetyltransferase (PAT) and the *gusA* gene encoding GUS. The PPT-resistant calli and plants were selected with 5 and 2.5 mg l^{-1} PPT, respectively. Soil-grown plants were obtained 28–36 weeks after *Agrobacterium*-mediated transformation. Genetic transformation of the regenerated plants growing under selection was demonstrated by PCR, and Southern blot analysis revealed that one to three copies of the transgene were integrated into the plant genome of each transgenic plant. Expression of the *bar* gene in transgenic plants was confirmed by RT-PCR and application of herbicide. Transgenic plants sprayed with Basta containing 900 mg l^{-1} of glufosinate ammonium remained green and healthy. The transformation frequency was 2.8% determined by herbicide application which was high when compared to biolistic method (Choi *et al*. 2007).

5. Fatty acid

The genes that have been previously introduced into sweet potato are marker and/or selectable marker genes and genes for resistance to insect and virus diseases. On the other hand, modification of plant metabolism by the transformation technique is an attractive way of generating new cultivars that would have novel traits such as an improved production of metabolites and the production of novel compounds. By introducing foreign DNA related to fatty acid metabolism in order to modify the fatty acid composition of the lipids, expecting thereby a functional or nutritional improvement. The ω-3 fatty acid desaturases are membrane-bound enzymes catalyzing the conversion of dienoic fatty acids (16:2 and 18:2) to trienoic fatty acids (16:3 and 18:3) in lipids, and they are found in microsome and plasmid membranes.

A tobacco microsomal ω-3 fatty acid desaturase gene (*NtFAD3*) under the control of the CaMV 35S promoter or an improved CaMV 35S promoter (El2Ω) was introduced into sweet potato. Transformed sweet potato plants were obtained from embryogenic calli following *A. tumefaciens*-mediated transformation. The transgenic plants grew normally to form storage roots and showed properties similar to those of the non-transgenic plants. The fatty acid composition in the transgenic line with a *NtFAD3* gene driven by the CaMV 35S promoter was similar to that in the non-

transformant. However, in the transgenic line that had a *NtFAD3* gene driven by the El2Ω promoter, linoleic acid (18:2) and linolenic acid (18:3) contents were 47.7 mol% and 24.8 mol%, respectively, which were significantly different from the 53.6 mol% and 11.3 mol%, respectively, in the non-transformant. The *NtFAD3* gene driven by the El2Ω promoter was expressed more strongly than that driven by the CaMV 35S promoter, thereby increasing the linolenic acid content in the transgenic sweet potato plants (Wakita *et al*. 2001).

6. Transgenic enzymes and proteins

Sweet potato accumulates large quantities of starch in the storage roots and has been shown to give comparable or superior ethanol yields to corn per cultivated acre. Starch conversion to fermentable sugars (i.e., for ethanol production) is carried out at high temperatures and requires the action of thermostable and thermoactive amylolytic enzymes. These enzymes are added to the starch mixture impacting overall process economics. Santa-Maria *et al*. (2011) isolated a gene encoding a hyperthermophilic α-amylase from *Thermotoga maritima* was cloned and expressed in transgenic sweet potato plant with the ability to self-process starch. No significant enzyme activity could be detected below 40°C, but starch in the transgenic sweet potato storage roots was readily hydrolyzed at 80°C. They also demonstrated that engineering plants with hyperthermophilic glycoside hydrolases can facilitate cost effective starch conversion to fermentable sugars. Furthermore, the use of sweet potato as an alternative near-term energy crop should be considered.

Adiponectin is a 30 kDa protein exclusively produced and secreted from adipocytes and as a cytokine has been found to link obesity, insulin resistance, and type-2 diabetes. Production of biologically active adiponectin in large scale is desirable for pharmaceutical applications. Mouse adiponectin cDNA was used for developing transgenic sweet potato plants *via Agrobacterium*-mediated transformation by Berberich *et al*. (2005). In the transgenic lines, all of which expressed high levels of adiponectin mRNA and a mouse adiponectin antiserum revealed that, in addition to a 29 kDa-protein which co-migrates with the adiponectin protein produced in *Escherichia coli* cells, a 31 kDa-protein was produced, indicative of a post-translational modification of the protein.

7. Tuber formation

A sweet potato MADS-box protein cDNA (*SRD1*) has been isolated from an early stage storage root cDNA library. The role of the *SRD1* gene in the formation of the storage root in sweetpotato was investigated by an expression pattern analysis and characterization of *SRD1*-overexpressing (ox) transgenic sweet potato plants Noh *et al*. (2010). *SRD1* mRNA was mainly found in the actively dividing cells, including the vascular and cambium cells of the young storage root. During the early stage of storage root development, the endogenous IAA content and *SRD1* transcript level increased concomitantly, suggesting an involvement of *SRD1* during the early stage of the auxin-dependent development of the storage root. SRD1-ox sweet potato plants cultured *in vitro* produced thicker and shorter fibrous roots than wild-type plants. The metaxylem and cambium cells of the fibrous roots of *SRD1*-ox plants showed markedly enhanced proliferation, resulting in the fibrous roots of these plants showing an earlier thickening growth than those of wild-type plants. The *SRD1* plays a role in the formation of storage roots by activating the proliferation of cambium and metaxylem cells to induce the initial thickening growth of storage roots in an auxin-dependent manner.

FUTURE PERSPECTIVES

As mentioned above, the biotechnology of sweet potato has developed very quickly during the past decade. Although transformation of sweet potato remains cumbersome, labor-intensive and genotype-dependent, some transgenic plants of sweet potato with agronomic importance have been produced in several countries. Transgenic sweet potato resistant to viruses and weevils should contribute to increased yield and food security in developing countries in the near future. Genetic engineering of starch can be used to improve the quality of sweet potato starch for the development of new dietary and industrial products. We have succeeded in producing transgenic sweet potato plants having amylase free starch. By manipulating the genes controlling the synthesis of starch, such as granule-bound starch synthase I (*GBSSI*), branching enzyme and debranching enzyme genes, we can obtain novel starches with modified amylase:amylopectin ratios for various uses. Further, because of the high production yield of its biomass, sweet potato would be a cost-effective alternative to microbial and animal systems as a target of "molecular farming" for the production of various biomolecules, such as high-value pharmaceutical polypeptides, industrial enzymes and biodegradable plastics (Shimada and Otani 2007). For the further improvement of multi-gene-controlled traits, such as yield, the accumulation of starch in storage roots and post-harvest properties, considerable progress in biotechnology is expected in the next 10 years.

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