BT-061

Enhancing Resistance Against African Weevils Through Development of Transgenic Sweetpotato Cultivars (Ipomoea batatas (L.)Lam.) Expressing cry7Aa1, cry3Ca1 and ET33-34 Genes

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Sweetpotato (*Ipomoea batatas*) is one of the most important food crops in tropical and subtropical countries. In Sub-Saharan Africa, sweetpotato is mainly produced for consumption and as a source of income by resource-poor farmers. However, their production is limited by severe damage caused by pests and diseases. The African weevils *Cylas puncticollis* and *C. brunneus* are the main biological constraints that may cause losses between 50 and 100%. Biotechnological approaches to control weevils include the introduction of genes encoding Cry proteins found to be active against these pests. To that end, several protocols for sweetpotato regeneration and transformation by organogenesis or somatic embryogenesis have been developed but their efficiency remains largely genotype-dependent and time-consuming. In this study, 31 African sweetpotato cultivars from CIP genebank were screened for regeneration and transformation efficiencies by organogenesis and somatic embryogenesis. Additionally, "Jewel" and "Jonathan" cultivars were used as organogenic and embryogenic controls, respectively. Regeneration by organogenesis was conducted using a two-step protocol including 2,4-D then thidiazuron, zeatin or kinetin while regeneration by embryogenesis was performed using a three-step protocol, each one using a different hormone (2,4,5-T, ABA and AG_3). Higher than 40% regeneration efficiencies were obtained for 8 cultivars (Jewel, Imby, Kawogo, Luapula, Mafutha, CIP440163, Zambezi and Ukerewe) with an organogenesis protocol and 8 cultivars (Jonathan, Imby, K51/3251, Bwanjule, CIP440163, SPK004, New Kawogo and KSP 11) with an embryogenesis protocol. Genetic transformation of sweetpotato with Jewel by organogenesis and Imby, CIP440163 and Jonathan by somatic embryogenesis has been achieved using chimerical genes coding for three of the most active proteins (Cry7Aa1, ET33-34, and Cry3Ca1) against African weevils. Transgenic events have been confirmed by kanamycin resistant calli test, PCR and Southern blot. Transcriptional activity and Cry protein accumulation are being tested in leaves and storage roots by Real time PCR and DAS-ELISA respectively.

BT-062

Accumulation of an Amylolitic Enzyme from *Microbacterium aurum* in Potato Plants Altered Starch Granule Morphology

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Starch is the major carbohydrate reserve in many crop plants. It consists of amylopectin and amylose biopolymers. Starch-binding domains (SBDs) from Carbohydrate Binding Module (CBM) family 20 have so far been used as a molecular tool to target different fusion proteins in starch granules. In an attempt to study, the possibility of producing porous starch granules in *planta*, a full length amylolytic enzyme consisting of two tandem CBM25 domains, three Fibronectin type III(FN3) domains and an α-amylase like domain of *Microbacterium aurum* were expressed in the tubers of a wild type potato cultivar (cv. Kardal) and an amylose-free (*amf*) potato mutant. Results showed that the full length protein accumulated in starch granules of both Kardal and *amf* transformants. The accumulation of the full length enzyme resulted to starch granule morphology alteration in both genetic backgrounds. The starches of the different transformants did not show significant differences in starch granule size distribution, apparent amylose content, and physico-chemical properties in comparison with that of untransformed control plants.

BT-063

The bHLH Transcription Factor DvIVS regulates Floral Anthocyanin Contents in Dahlia Cultivars

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Dahlia (Dahlia variabilis) is one of the most popular ornamental plants because of the huge variations in the color of its flowers; however, the molecular mechanism underlying the regulation of flower colors remains to be elucidated. We used MJOr and MJY, which are spontaneously occurring single-colored bud mutants from "Michael J" with orange variegation patterns in yellow petals. MJOr produced completely orange petals with anthocyanins, buteins, and flavones, whereas MJY produced completely yellow petals without anthocyanins. Gene expression analysis revealed that four structural genes in the anthocyanin synthesis pathway and a basic-helix-loop-helix (bHLH) transcription factor, DvIVS, were downregulated in MJY. A CACTA superfamily transposable element was found in the DvIVS genomic region of MJY and was named Tdv1. These results demonstrated that DvIVS is involved in the regulation of anthocyanin synthesis in dahlia flowers.

Next, 12 cultivars with different anthocyanin contents in their petals were analyzed to determine the relationship between DvIVS and anthocyanin contents. These cultivars were classified into four color groups depending on their anthocyanin content. Ivory white cultivars accumulated only flavones, whereas deep purple, purple, and pink cultivars accumulated different concentrations of flavones and anthocyanins. Among all these cultivars, positive correlations were identified between the anthocyanin contents and the expression of some structural genes in the anthocyanin synthesis pathway and between the expression of these structural genes and the expression of DvIVS. The DvIVS genome was classified into at least three types on the basis of polymorphisms in the promoter region. All high anthocyanin content cultivars (deep purple and purple cultivars) had Type 1 genome and expressed Type 1 transcripts. Thus, these results indicated that DvIVS contributes to the expansion of flower color diversity in dahlia by controlling floral anthocyanin contents.

BT-064

Functional Analysis Of Phytochelatin Synthase cDNA From *Eucheuma denticulatum*

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Phytochelatin synthase (PCS) catalyses the synthesis of phytochelatins (PCs) which are cysteine-rich proteins with important role in heavy metal detoxification found in various types of plants. PCS uses glutathione (GSH) as a substrate in the presence of metal ions like Cadmium, Cd^{2+} as the strongest inducer. The full-length clone for *Eucheuma denticulatum* PCS has been isolated using PCR and rapid amplification of cDNA ends (RACE) techniques with the size of 1.6kb which contains a single open reading frame encoding a protein containing 218 amino acids. Multiple sequence alignment of the *E. denticulatum* PCS protein sequence with other plants PCS protein sequences showed conserved region in the N-terminal sequence. The recombinant expression of PCS was performed in *Escherichia coli* using pET32b vector and SDS-PAGE analysis showed that a ~43 kDa insoluble protein was successfully expressed. To improve the recombinant expression, the PCS cDNA was subcloned into pQE2 expression vector. Subsequent SDS-PAGE and western blot analyses showed that the expected ~25 kDa soluble protein was successfully expressed. The soluble recombinant PCS protein was further purified using HisTrap™ HP column by AKTA-Prime system. The activity of the purified recombinant protein was then assayed to show that the PCS cDNA was functional.