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Expression analysis of several EST clones related to drought stress in sweetpotato (*Ipomoea batatas*L.)

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Drought stress impedes the growth and productivity of various crop species. Genome-wide expression analysis has identified a number of drought stress induced up- and/or down-regulated genes. Sweetpotato (*Ipomoea batatas* L.) is 7th important food crop in the world. To date, sweetpotato has not been exploited to understand the molecular basis of its drought stress tolerance, even though it is quite a tolerance to unfavorable growth conditions including drought condition. cDNA libraries were constructed from root tissues by subjecting to dehydration. The 983 clones was read in this experiment. The expressed sequence tags (ESTs) represent up to 773 independent genes, 124 clusters and 649 singletons. These 773 cDNAs could be classified into two groups based on the BLAST search results. The first group consists of 535 cDNAs which were assigned a function by aligning them with the translated sequences of the GenBank nucleotide sequence database using the BLASTX algorithm. The second group, comprising 238 cDNAs have no homologies with any genes in the database. The 538 cDNAs belong to different groups of genes involved in ion transport, cell division, metabolism, and protein synthesis, etc. Sequence comparison of these clones using BLASTX led to the identification of 14 putative abiotic stress-related genes. At different stages of development and stress conditions, the expression patterns of 14 genes were analyzed through RT-PCR in detail, and their direct and indirect relationship with drought tolerance mechanism is discussed. The putative ESTs will serve as useful resource to understand the molecular basis of abiotic stress tolerance in plants.

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Differential expression of *FLOWERING LOCUS C (FLC)* isolated from *B. rapa* L. *ssp. pekinensis* and effects on flowering time in Chinese cabbage.

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Chinese cabbage plants remain in the vegetative growth phase until they have experienced prolonged exposure to cold temperature, known as vernalization. This inhibition to flowering is due to high level of *FLOWERING LOCUS C (FLC)* expression. In order to increase product

value of Chinese cabbage by inhibiting floral transition, three genes (*BrFLC1*, *BrFLC2*, and *BrFLC3*) homologues to *AtFLC* genes, a floral repressor, were isolated from Chinese cabbage Chiifu. These genes showed high similarity to *AtFLC*, except that putative *BrFLC1* protein contained ten more residues than did *AtFLC*. These *BrFLC* genes were ubiquitously expressed, except that *BrFLC3* expression was not detected in roots. *BrFLC1* and *BrFLC2* showed stronger expression than did *BrFLC3* in unvernallized and vernalized Chinese cabbage plants. Expression levels of three *BrFLC* genes were lower in an early flowering of cultivar of Chinese cabbage plants, suggesting that BrFLC level is associated with flowering time of Chinese cabbage plants. In order to examine the expression of each *BrFLC* gene, upstream region (-2kb) was introduced into *Arabidopsis*, and GUS and gfp expression was observed in tissues. Constitutive expression of *BrFLC* genes significantly delayed flowering in *Arabidopsis*. Delay in flowering was also observed in transgenic Chinese cabbage plants overexpressing *BrFLC3*. These results suggested that *BrFLC* genes act similarly as does *AtFLC*.

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(O4-06)

Recombinant antibody confer complete resistance against DNA and RNA virus infection by selective genome degradation

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We present a novel strategy for creating virus-resistant transgenic plants, by ectopic expression of a catalytic single-chain variable fragment antibody (3D8 scFv) that exhibits nuclease activity. We successfully expressed 3D8 protein in tobacco with the *Agrobacterium tumefaciens* transient transformation assay (agroinfiltration). The M13mp18 plasmid DNA was digested in dose-dependent manner of purified scFv proteins in mixtures. 3D8 scFv protein expressed in plants had functionally active nuclease activity shown the catalytic activity of 3D8 protein expressed in *E. coli*. In order to investigate how 3D8 scFv protein could inhibit the virus multiplication at the early stage of virus infection on transgenic plants, the apical tips of leaf blade of wild type tobacco and Tx99-2 transgenic tobacco were inoculated with plant saps derived from the TMV infected tobacco. The upper and lower halves of each leaf blade were harvested to determine TMV multiplication at the different infection stages. RT-PCR analysis by using TMV coat protein primers showed that TMV accumulation started from 32 HPI on challenged upper leaf halves and moved onto lower leaf halves in wild type plants. However in case of scFv transgenic tobacco leaves, TMV was not detected. GFP fused 3D8 protein was observed green fluorescent signals in cytosol. Three endogenous RNAs of transgenic plants and wild type plants were not affected by exist of 3D8 protein in cytosol. Our results suggest that the transgenic expression of anti-nucleic acid antibodies is a highly effective approach for protecting plants from a broad spectrum of viruses, regardless of their genome types.

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