

(362) Identification of Pathogen of Powdery Mildew and Analyses of Ribosomal DNA-ITS Sequence on Melon

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DNA was extracted from the strain of pathogen of powdery mildew on melon using a modified CTAB method. ITS sequence (524bp) was initially amplified from the pathogen using the universal primers ITS1 and ITS4 (registered No. EU294368). Comparing to the nucleotide sequences acquired from GenBank database, the strain was clustered into the homogeneity with *Podosphaera fusca* (EF137855, EF137859 and EF137853), with a homology of 100%. The different phases in life cycle of *Sphaerotheca fuliginea* were observed through the method of coomassie brilliant blue staining clearly, included germinating conidia, primary germtube, hyphae, colidiophore, and colony. Morphologic characteristics of *S. fuliginea* were conidia ellipse, colorless, catenulate and its average length was 29.07 m, average width was 17.82 m. The identifying host showed infection. The results of morphological, molecular host identification showed that pathogen of powdery mildew on melon was *S. fuliginea*.

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(363) Genetic Diversity of *Swida wilsoniana* (Wanger.) Sojak Clones using ISSR Markers

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Swida wilsoniana (Wanger.) Sojak ('Wilson' dogwood) is an ideal biodiesel plant because its fruits have higher oil content. Based on plant vigor and fruit yields, we selected 15 clones from natural populations of Jiangxi, Guangxi, and Hunan provinces. A total of 96 leaf samples (six for each clone) were collected in Mar. 2006 for DNA extraction. Total DNA was quantified and 20 ng per sample were used for intersimple sequence repeat (ISSR) analysis. Preliminary screening from 100 primers yielded 10 primers [(TC)8A, (AG)8YA/(AG)8CTA, GCG(AC)6A, (CA)8RC/(CA)8AGC, (CTC)6, (GACA)4, (GATA)2(GACA)2, (TGCA)4, (GGAGA)3, (ACTG)4] for the ISSR analysis. A total of 106 discernible markers ranged from 100–2000 base pairs were generated. Among them, 84.9% of bands were polymorphic markers. Total number bands per primer produced ranged from 8 to 15. The average number of bands for each primer was 11. The observed number of alleles (no), effective number of alleles (ne), Nei's gene diversity (h), and Shannon's information index (I) was 1.8401, 1.403, 0.2878, and 0.4472, respectively. Those data showed a high level of genetic diversity among their provenance. The UPGMA dendrogram indicated that the distribution pattern of 15 clones was coherent with their geographical

origins. The results could be applied for future genetic improvement, identification, and conservation of 'Wilson' dogwood germplasm.

(364) Biosynthesis and Regulation of Steroidal Glycoalkaloids in Wild Potato, *Solanum chacoense* Bitter

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Steroidal glycoalkaloids (SGAs) are secondary metabolites produced by approximately 350 species in the Solanaceae family. SGAs are reported to be important for pest resistance and flavor enhancement at low concentrations but may be toxic to humans and other mammals at high concentrations. Studies on sterol/SGA biosynthesis have implicated squalene synthase as a key regulatory enzyme because it catalyzes an irreversible step from the mevalonic acid pathway. However, the regulatory mechanisms of squalene synthase are not yet known. A study was conducted to elucidate the distribution pattern of SGAs and to clone the squalene synthase gene in order to determine a relationship between SGAs and gene expression levels. *Solanum chacoense*, a wild potato species was used a model plant from which tissues were harvested at specified developmental stages and analyzed for SGA content. Additionally, a partial cDNA of squalene synthase gene was isolated from leaves of high and low SGA producing accessions of *S. chacoense*. The results so far show that the isolated squalene synthase partial cDNA has high sequence identity at the amino acid level to other annotated plant squalene synthase genes in the conserved domains. This suggests that the isolated cDNA is potentially functional. The results from the SGA analysis suggest a qualitative and quantitative tissue- and age-dependent biosynthesis of SGAs. Shoot meristems had the highest levels of total SGAs of 75.56 mole/g DW. Regenerative tissues such as, auxiliary shoots, flowers and belowground stolons had 36, 35, and 55 mole/g DW, respectively. The roots and stems showed the lowest amounts of SGAs of 6 and 7 mole/g DW, respectively. There was no clear relationship between leaf age/position and SGA content. These findings pave way for future work in the cloning and characterization of the full squalene synthase gene and determination of a relationship between different SGA contents and gene expression levels in plant tissues.

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(365) Marker Discovery in Tomato using Microarray-based Target Capture with Next-generation Sequencing

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Tomato has notoriously low levels of intraspecific sequence variation and it is often difficult to find molecular markers that are polymorphic between tomato cultivars. The objective of this project is to implement a method in tomato to efficiently screen large numbers of genomic loci to identify sequence variations that can be converted into useful molecular markers. The approach combines microarray-based genomic selection (MGS) for the enrichment of targeted genomic sequences with next generation sequencing of the selected targets. Custom microarrays were designed containing 50 mer oligonucleotides that are complimentary to tomato genomic sequences with known genetic map positions (e.g., BACs with hits to existing genetic markers, sequenced RFLP probes, and CosII markers). The sequences were screened to eliminate simple repeats and used in BLAST searches to exclude multi-copy sequences. Total genomic DNA was fragmented and ligated to linker sequences to facilitate PCR amplification and multiplex sequencing. The fragmented DNA was hybridized to the microarrays and the eluted captured DNA