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DNA Chip Gene Expression Analysis from *Panax ginseng* Adventitious Roots after Methyl Jasmonate Treatment

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Objectives

Methyl jasmonate (MeJA) treatment dramatically increase the ginsenocides contents of cultured *Panax ginseng* adventitious roots. To identify the genes related to the ginsenocide synthesis, we collected *P. ginseng* ESTs, analyzed the sequences, designed DNA chip, and performed gene expression analysis from MeJA treated *P. ginseng* adventitious roots.

Materials and Methods

○ Plant materials

Adventitious roots were induced from wild grown *P. ginseng* and cultured in Chungbuk National University.

○ DNA chip design & data analysis

A total of 9,020 *P. ginseng* EST clusters were collected and used for Combimatrix DNA chip probe design. The clusters were compared to non-redundant protein databases using BLASTX searches and were analyzed according to the Gene Ontology (GO) database (http://www. geneontology.org). DNA chip gene expression analysis was performed from the *in vitro* cultured adventitious roots after 0 h, 24 h, and 120 h of MeJA treatment.

○ Real-Time RT-PCR analysis

Total RNA was extracted from *in vitro* cultured *P. ginseng* adventitious roots after 0 h, 24 h, 72 h, and 120 h of MeJA treatment using Plant RNeasy Mini Kit (Qiagen, Germany) and subjected to real-time RT-PCR. SYBR Green master mix (Invitrogen, Carlsbad, CA) was used for quantification and *P. ginseng* rRNA was used as internal control.

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Results

○ HPLC analysis showed that ginsenoside contents was increased from 24 h after MeJA treatment and the contents were highest at 120 h.

○ The gene expression alteration of *P. ginseng* adventitious roots by MeJA treatment was analyzed by Combimatrix DNA chip analysis using 11,761 DNA chip probes.

○ A total of 51% of the probes showed increased expression level and 49% of the probes showed reduced expression level by MeJA treatment.

○ A total of 804 probes showed statistically significant changes of over 2 folds.

○ Jasmonate ZIM-domain (JAZ) gene expression was analyzed using real-time RT-PCR. JAZ expression level was significantly increased after 24 h of MeJA treatment.

Table 1. Annotation of the genes showed highest levels of gene expression level increment by MeJA treatment from DNA chip gene expression analysis.

Contig name	Gene name	Species
PGTT_cluster2103	(S)-reticuline oxidase-like protein	*Daucus carota*
PGTT_cluster400	Ribonuclease-like storage protein precursor, RNase-like major storage protein	*Panax ginseng*
PG07017E07	AP2-domain DNA-binding protein	*Datura metel*
PGTT_cluster2578	unnamed protein product	*Vitis vinifera*
PG07042E09	unnamed protein product	*Vitis vinifera*
PGTT_cluster2347	(S)-reticuline oxidase-like protein	*Daucus carota*
PGTT_cluster2488	Ribonuclease-like storage protein precursor, RNase-like major storage protein	*Panax ginseng*
PG07015G09	unknown	*Populus trichocarpa*
PGTT_cluster2382	LEC_PARPC Mannose/glucose-specific lectin	-
PG07031B08	RIP1_HORVU Protein synthesis inhibitor	-

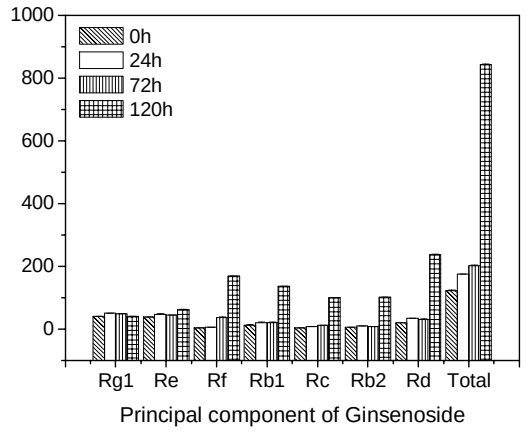

Figure 1. HPLC analysis of principal components of ginsenosides from the MeJA treated *P. ginseng* adventitious roots for 0h, 24h, 72h, and 120h.

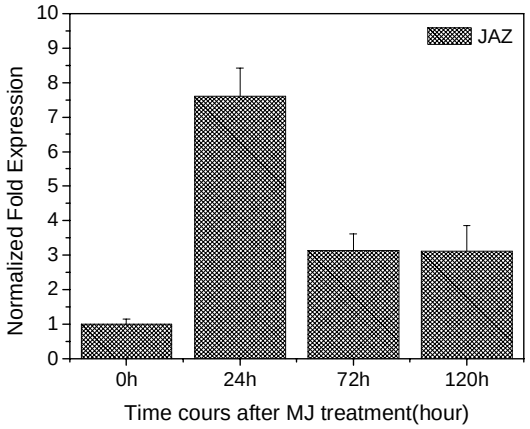

Figure 2. Relative expression level of Jasmonate ZIM-domain (JAZ) genes of *P. ginseng* adventitious roots elicited by MeJA. The fold differences in the level of expression in different time were presented as the means of three independent experiments with respective SE.