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Transcriptional profiling of ESTs responsive to *Rhizobium vitis* from 'Tamnara' grapevines (*Vitis* sp.)

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ABSTRACT

Genes related with defense responses were screened from the cDNA library constructed with *Rhizobium vitis*-inoculated or salicylic acid (SA)-treated 'Tamnara' grapevine (*Vitis* sp.) leaves. Among 13,728 expressed sequence tags (ESTs) from 'Tamnara' grapevine upon *R. vitis* inoculation and SA treatment, 6776 unigenes containing 1915 contigs and 4860 singletons were obtained. In gene ontology analysis, there were about 3200 clones related with biological process, 3555 with molecular function, and 3354 with cellular component genes. Proteins of secretory organ (35%), plasma membrane (30%), endoplasmic reticulum (20%), and vacuole (11%) were predicted. Photosynthesis-related genes and defense-related genes were most abundant. Among ESTs, 199 resistance-related ones were mapped to the genome of *Vitis vinifera* L. with three markers, GLP1–12, MHD98, and MHD145, which are known to be linked to resistance against powdery mildew. Approximately, 120 simple sequence repeats (SSRs) detected in cDNAs could be used as EST-derived SSR markers in disease resistant grape breeding.
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1. Introduction

Crown gall disease, caused by *Rhizobium vitis*, reduces the yield and vigor of grapevine, especially on *Vitis vinifera* L. and interspecific hybrids where climatic conditions favor freeze injury (Burr et al., 1998; Schroth et al., 1988). Incidence of crown gall disease was high on *V. vinifera* L. cultivars, but low on *V. labrasca* L. and hybrid cultivars (Burr et al., 1998; Park et al., 2000). 'Tamnara' grapevines, bred from the cross between 'Campbell Early' and 'Himrod Seedless', are moderately resistant to *R. vitis* as determined by controlled inoculations (Park et al., 2004).

The inheritance of complex characteristics can be elucidated by establishing their association with linked molecular markers, and identified candidate genes can be available for improving of traditional grapevine cultivars after further functional characterization. Although whole genome sequence of grapevines was completed (Jaillon et al., 2007), the information of infection and control of *R. vitis* remains to be elucidated.

Through the profiling expressed sequence tags (ESTs), at the transcriptional level with the marker selection, the efficiency of disease resistant grape breeding is expected to be improved (Doligez et al., 2002; Fischer et al., 2004). Levels of polymorphism and transferability tested by ten grape EST database-derived simple sequence repeats (SSRs) and their availabilities for breeding grapevines resistant to diseases were reported (Scott et al., 2000).

In this study, genes related with defense responses were screened and discovered from cDNA library constructed with *R. vitis*-inoculated or salicylic acid (SA)-treated 'Tamnara' grapevine leaves using Pipeline of the EST Analysis Service (PESTAS) system of Genome Research Center (GRC), Korea Research Institute of Bioscience and Biotechnology (KRIBB) (Nam et al., 2009), and transcriptional profiling of ESTs responsive to *R. vitis* from the grapevine leaves was constructed.

2. Materials and methods

2.1. Plant and pathogen materials

'Tamnara' grapevines grown in a greenhouse at 25–30 °C under natural light were sprayed with 0.5 mM salicylic acid (SA) on leaves,

Abbreviations: AOS, active oxygen species; CAT, catalase; CHS, chalcone synthase; ESTs, expressed sequence tags; GO, gene ontology; GST, glutathione S-transferase; LOX, lipoxygenase; PESTAS, Pipeline of the EST Analysis Service; PR, pathogenesis-related; PS, photosystem; LRR, leucine rich repeat; RuBP, ribulose-1,5-bisphosphate; SA, salicylic acid; TGICL, TIGR Gene Indices Clustering Tools.

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Table 1
Transcriptome features of 'Tamnara' grapevines.

Total reads		Total no. of assembled			Total known[a]		
Sequenced	High quality sequence[b]	Singleton	Contig	Total	BLASTX	InterPro Scan	Total
13,728	13,191	4860	1915 (883 bp)[c]	6776 (818 bp)[d]	6397 (1576)[e]	4835 (14)[f]	6411

[a] BLASTX cutoff *E*-value, $1e^{-5}$; InterPro Scan cutoff *E*-value, 0.01.
[b] Cutoff (Phred score 20 and sequence length 100 bp).
[c] Average size of contigs.
[d] Average size of total ESTs.
[e] Annotated BLASTX only.
[f] Annotated InterPro Scan only.

and inoculated with *Rhizobium vitis* Cheonan 493 (Yun et al., 2003). For the inoculation, the *R. vitis* strain was grown in LB medium and the bacterial cultures were diluted to OD_{600} approximately 1 (10^9 cfu/mL). The cultures were injected into the holes drilled on the internode of the grapevines and 0.5 mM SA was treated on both sides of the grapevine leaves. Leaves were harvested at 1, 3, 6, 12, 24, 48, and 72 h after SA treatment and *R. vitis* inoculation, and then immediately frozen in liquid N_2 and stored at −80 °C until used for RNA extraction. All harvested samples from each treatment were pooled and used for RNA extraction, screening differential expression of cDNA, and reverse transcription polymerase chain reaction analysis.

2.2. RNA extraction and cDNA library construction

Total RNA was extracted separately from *R. vitis*-inoculated, SA-treated, and control leaves using the modified pine tree method of removing polysaccharides and phenolic compounds (Chang et al., 1993). Total RNAs of all time courses, 0.5, 1, 3, 6, 12, 24, 48, and 72 h, were collected in each tube for each series of the SA-treated and the *R. vitis*-inoculated grapevines. cDNA libraries were constructed using ZAP-cDNA Synthesis Kit (Stratagene, La Jolla, CA, USA) from total mRNA isolated from the *R. vitis*-inoculated leaves.

2.3. Expressed sequence tag (EST) sequencing and data analysis

ESTs were sequenced with 3730 Big Dye Terminator v3.1 Cycle Sequencing Kit and Sequencer 3730XL DNA Analyzer (Applied Biosystem, Foster City, CA, USA). PESTAS was used for analyzing the ESTs from the *R. vitis*-inoculated and the SA-treated leaves. PESTAS is available at http://pestas.kribb.re.kr/. Supplementary information is available at http://pestas.kribb.re.kr/pestas.jsp (Nam et al., 2009). EST sequences were pre-processed using Phred with 20 of cutoff value to extract high quality regions from raw sequence data. Cross_Match and SeqClean were used for vector and contaminant trimming, respectively. After repeat masking with the RepeatMasker, EST sequences were clustered and assembled into contigs and singletons to reduce the inherent redundancy and to build unigenes sets, using the TIGR Gene Indices Clustering Tools (TGICL). At each step, ESTs of less than 100 bp length were removed. To obtain more relevant functional annotation results, Blast2GO was used (Götz et al., 2008). The functional annotation was performed using the BLAST search in PESTAS system, InterPro Scan, SignalP, PSORT II, and TMHMM-based algorisms. The BLAST search and InterPro Scan were performed setting an *E*-value less than $1e^{-5}$ and 0.01, respectively. Simple sequence repeats (SSRs) in the cDNA of 'Tamnara' grapevines were detected using Tandem Repeat Finder program with basic parameters (Benson, 1999). A worldwide web server interface http://atc3.biomass.mssm.edu/trf.html has been established for automated use of the program.

2.4. RNA slot blot analysis

Total RNAs of 5 μg were used and the RNA mixtures were denatured at 65 °C for 10 min and then blotted membranes using the Bio-Dot SF (Bio-Rad, Hercules, CA, USA). RNA samples were transferred and immobilized to Hybond-N^+ nylon membrane with a UV-crosslinker. The digoxigenin (DIG) labelled cDNA probes were generated from total RNA with DIG 11-dUTP (Roche, Penzberg, Germany) and AMV reverse transcriptase. DIG Easy Hyb, DIG Wash and Block Buffer set, and CDP-Star, Ready-to-Use (Roche, Penzberg, Germany) were used for hybridization, washing, and detection. Detected blots were exposed to X-ray films and analysed with a BioRad GS-800 densitometer and Quanity one software (Bio-Rad, Munich, Germany).

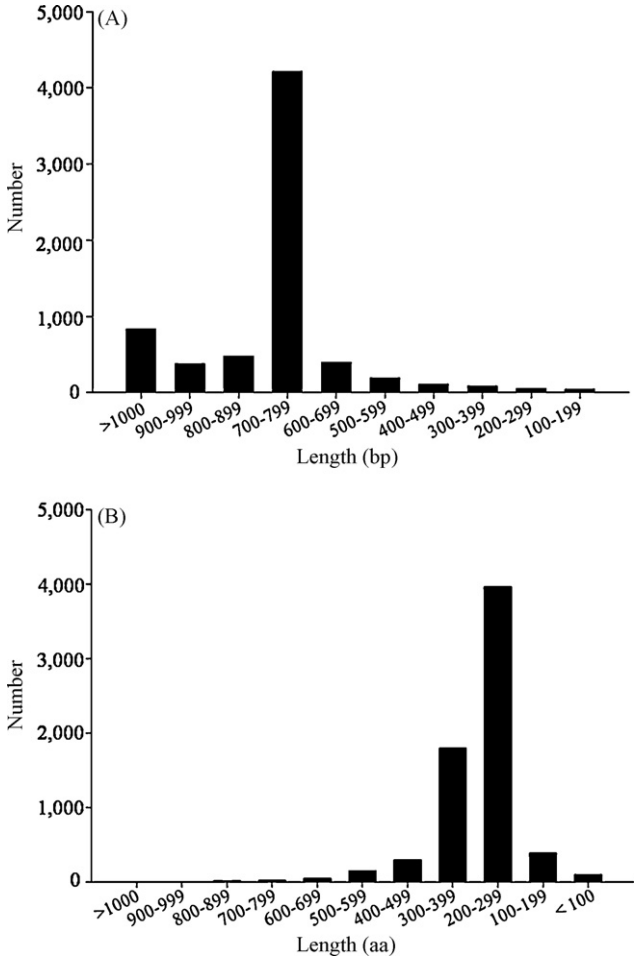

Fig. 1. Distribution of nucleotide (A) and amino acid (B) length for 6776 unigenes induced by *R. vitis*-inoculated and SA-treated 'Tamnara' grapevines.

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