ORIGINAL ARTICLE

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A database for poplar gene co-expression analysis for systematic understanding of biological processes, including stress responses

Abstract Reforestation in the humid tropics and arid zones, where trees are often subject to stresses, is an effective approach for mitigating global warming. Forestation with *Populus* species that are tolerant to the stresses in such regions has been conducted. The selection of poplar trees with higher stress tolerance leads to more efficient reforestation. The genome-wide bioinformatics approaches of gene function have been used for revealing the mechanisms of biological processes, including such stress tolerance. The decoding of the poplar genome has been followed by the genome-wide identification of genes and then the inference of gene function for systematic understanding of biological processes. To predict gene function in poplar, we analyzed poplar gene expression data using DNA microarray datasets obtained from the Gene Expression Omnibus database and developed a database for poplar gene co-expression analysis. Using the database, we illustrate the steps to retrieve two groups of co-expressed genes that are specifically expressed in experiments of hypoxic stress response in gray poplar, a flooding-tolerant tree species. Our database allows users to extract genes involved in biological processes, such as stress reaction, and then is useful for understanding such mechanisms in tree species.

Key words Co-expression · Database · Poplar · Biological processes · DNA microarray

Introduction

Reforestation in humid tropics and arid zones is an effective approach for mitigating global warming.[1] Study of the forestation of *Populus* species that are tolerant to stresses in such regions has been conducted; e.g., in the Chinese arid zone by the Japan Association for Greening Deserts project (http://www.sabakuryokka.org/). Poplar trees adapt to dry,[2–5] cold,[6,7] and flooding regions[8] with stress responses. Understanding of stress responses in poplar allows selecting poplar trees adequate for surviving in such regions, which then leads to better reforestation. Previous reports[2,8–16] have revealed the functions of individual genes involved in stress responses. A genome-wide analysis of poplar gene function is an approach to predict mechanisms of biological processes, including such stress tolerance.

In this decade, genomes of plants such as *Arabidopsis thaliana*[17] and rice[18] have been decoded, and their genomic information has been utilized to analyze the expression of genome-scale transcripts (mRNA) or transcriptomes using DNA microarray technology and the bioinformatics approach. Bioinformatics approaches have been developed along with the explosive accumulation of omics data, such as those of genome, transcriptome, and proteome, and the rapid progress of computer technology. Although the outputs from such approaches are indecisive, i.e., the identification of gene function requires evidence based on experimental approach, the approaches have been developed for and contribute to the prediction of gene function as a consequence of their genome-wide and high-throughput performance.

The genome draft of black cottonwood (*Populus trichocarpa*) was published in 2006 by Tuskan et al.,[19] and poplar DNA microarray techniques have been developed. Recent studies[8,16,20] have analyzed the poplar transcriptome using bioinformatics approaches, and the analyzed data have been deposited in public databases such as AspenDB (http://aspendb.uga.edu). Such transcriptome analyses in poplar have promoted the gene-level understanding of various types of biological processes, including stress responses. The tendency of genes involved in a common process to show a similar expression profile, or be "co-expressed," has been reported.[21,22] Therefore, co-expression analyses have been utilized to predict functional relatedness such as the commonality of metabolic pathways,[23] protein complex,[24] and stress responses.[25]

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Co-expression analysis in poplar has been performed since the decoding of the genome. Grönlund et al.[26] performed co-expression analysis to reconstruct the genome-wide co-expression network, in which genes are linked to other genes on the basis of gene-to-gene similarity in their expression profiles. An analysis using the co-expression network in plants has provided systematic information on gene-to-gene associations through microarray analyses performed in various experiments.[27,28] The information on experiments contributing to such associations promotes our understanding of functional relatedness between genes and biological processes such as the stress–response mechanism.

We constructed a poplar co-expression database for the genome-wide prediction of gene function based on poplar co-expression analysis. Ninety-five publicly available DNA microarray datasets were obtained from the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo/). We performed a co-expression network analysis using the datasets and extracted "co-expression modules," comprising co-expressed genes, which are tightly interconnected to each other. Information on the experiments contributing to gene-to-gene connections in co-expression modules was associated with the modules. As examples to extract co-expression modules, we demonstrate two modules that are composed of genes expressed in hypoxic stress–response experiments. Our database is available at http://webs2.kazusa.or.jp/kagiana/cop/.

Materials and methods

We obtained gene expression datasets of 95 chips of Affymetrix Poplar Genome Array (Affymetrix), which are composed of 61 413 probes representing genes, from GEO. The data files were processed using Bioconductor 2.3.13 with R version 2.8.1 to obtain text-formatted data. Gene expression data in the files were standardized, and cosine correlation coefficients between all pairs of probes were calculated using our R program.

For functional characterization of a poplar co-expression module, information on functions of genes comprising the module may be insufficient. For better biological understanding of a poplar co-expression module, we used the information on functions of *Arabidopsis* genes that are best homologous to poplar co-expressed genes using BLAST. As an index of homology, a harmonic mean of mutual values of identities was calculated, referred to as the homology F-measure (HF).

Results

We constructed a database for poplar co-expression analysis using publicly available DNA microarray datasets, which is under the CoP database management system. It allows users to perform co-expression analyses of *Arabidopsis*, poplar, and soybean and to retrieve biological processes based on gene ontology.[29] By a query of a gene of interest, users can access to a page providing information on the co-expression module that is composed of genes co-expressed with the gene. In the portal page of the database (Fig. 1), steps to input required items are implemented as follows: (I) input a query word, e.g., a probe identifier, gene identifier, or *Arabidopsis* gene identifier (AGI code); (II) select a plant organism, e.g., select "Populus trichocarpa (poplar) Affymetrix"; (III) select an information type, e.g., select "Confeito (co-expression analysis)"; (IV) skip this step, which is not available for this purpose; and then (V) click the submit button.

Summarized descriptions of a co-expression module are composed of three parts (Fig. 2). Namely, the first part includes the information of a query gene identifier, tightness index of the module (referred to as the network F-measure; NF), and module size (the number of genes). The second part represents "Descriptions" of individual genes included in the module, composed of probe identifiers, representative public identifiers, and information on the homologous *Arabidopsis* genes; i.e., AGI code, HF, gene names, short descriptions of the function, and GO biological processes, respectively. The third part provides information on "Specific Experiments," in which the co-expressed genes are specifically expressed, composed of standardized scores of the genes, sample names, experimental identifiers of GEO, links to detailed description of experiments, and experiment titles. From the descriptions of the parts, users can obtain information on the module members that are specifically expressed under particular experiments.

To afford users the ability to retrieve co-expression modules, we illustrate the steps to retrieve a co-expression module by using the following example. In the portal site (http://webs2.kazusa.or.jp/kagiana/cop/):

I. Input "confeito," which is the word ready to retrieve a co-expression module with tight intramodular interconnections.
II. Select "Populus trichocarpa (poplar) Affymetrix."
III. Select "Confeito (co-expression analysis)."
IV. Make no selection.
V. Click the "Submit" button.

Next, in the page of a list of co-expression modules:

VI. Click "Ptp.4.1.A1_a_at" as an example probe identifier, whose *Arabidopsis* homologous gene is At5g15630, named "COBL4/IRX6 (COBRA-LIKE4)."

Then, a summarized description of the co-expression module that is composed of genes co-expressed with the query gene is displayed (see Fig. 2). The "Descriptions" part of this module is composed of functional descriptions of genes that may be involved in cell wall biosynthesis; i.e., "TUB2 (Tubulin beta-2)," "microtubule-associated protein (MAP65/ASE1) family," "COBL4/IRX6 (COBRA-LIKE4)," "FLA11 (fasciclin-like arabinogalactan-protein 11)," and "GAUT12/IRX8/LGT6 (GALACTURONOS-YLTRANSFERASE 12)." In the "Specific Experiments" part, the "Z-scores" of two samples for an experiment for root hypoxia (GSM328533 and GSM328532) are high

Fig. 1. The retrieval form of CoP database. The retrieval steps are as follows: (I) input a query word; e.g., an AGI code and a microarray probe name; (II) select a "Populus trichocarpa (poplar) Affymetrix"; (III) select "Confeito (co-expression analysis); (IV) skip this step; and (V) click the "Submit" button. When a single candidate gene is found, a page including information on a co-expression module that is composed of genes co-expressed with the gene is directly displayed. Otherwise, a page of a list of gene identifiers that are linked to pages including information on co-expression modules is displayed

Retrieval form

I. Input a query word

_____

For example

II. Select a plant organism

Arabidopsis thaliana (5k assays)   ▾

III. Select an information type

⦿ Confeito (co-expression analysis)
◯ Biological process

IV. Select an additional option

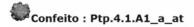When Biological process is selected in Step III, select <u>Evidence Code</u> Categories.

☑ **X** (experimental): EP, IDA, IPI, IMP, IGI, IEP
☑ **S** (statement): TAS, IC
☑ **C** (computational): ISS, ISO, ISA, ISM, IGC, RCA
☑ **L** (electronic): IEA
☑ **N** (not available): NAS, ND

Submit

(6.8 and 6.7, respectively), indicating that these module members are specifically expressed through root hypoxic stress response.

When performing a query of "PtpAffx.14026.1.S1_s_at," a co-expression module for the query gene also included genes involved in cell wall biosynthesis; i.e. in the descriptions of *Arabidopsis* genes orthologous to the poplar genes in the module, "LAC10 (laccase 10)," "AGP10 (arabinogalactan protein 10)," "CESA8 (CELLULOSE SYNTHASE 8)," "TUA6 (tubulin alpha-6 chain)," "caffeoyl-CoA 3-*O*-methyltransferase, putative," "IRX9 (IRREGULAR XYLEM 9)," and "AGP2 "ARABINOGALACTAN-PROTEIN 2)." In the "Specific Experiments," three samples for an experiment for root hypoxia (GSM328484, GSM328533, and GSM328532) showed high Z-scores (6.1, 5.3, and 5.2, respectively). Two samples of them are common with those for the co-expression module in the previous paragraph, indicating that genes in both co-expression modules are partly involved in a particular biological process that is influenced by hypoxic treatment.

Confeito : Ptp.4.1.A1_a_at

_NF_: 0.952, 12 kernel genes

Descriptions

Probe name	Repr. public ID	GF	Corr.	Ave.G.X.	Max.G.X.	HF	AGI code	Short description	Biological process
Ptp.4.1.A1_a_at	CV238596	1.00	1.00	1536.7	38920.7	0.134	At5g15630	IRX6	plant-type secondary cell wall biogenesis
Ptp.2007.1.S1_s_at	CF236359	1.00	0.99	322.2	9481.1	0.159	At5g23750	remorin family protein	biological_process_unknown
PtpAffx.12420.1.A1_at	CV236172	1.00	0.97	896.9	25542.8	0.030	At3g01970	-	regulation of transcription, DNA-dependent
PtpAffx.1704.5.A1_a_at	CV248047	1.00	0.97	1265.3	26763.6	0.552	At5g62690	TUB2	response to cold
PtpAffx.29241.1.A1_at	BP936699	1.00	0.99	178.1	5159.1	0.161	At5g54690	GAUT12 (GALACTURONOSYLTRANSFERASE 12)	glucuronoxylan metabolic process
PtpAffx.102386.1.A1_at	CV229138	1.00	0.98	113.7	2751.2	0.051	At4g21216	unknown protein	-
PtpAffx.200222.1.S1_at	pmma418	0.95	0.99	78.7	2040.8	0.197	At1g27920	MAP65-8 (MICROTUBULE-ASSOCIATED PROTEIN 65-8)	biological_process_unknown
Ptp.3083.1.S1_s_at	AY607762.1	0.95	0.98	897.6	37923.4	0.023	At5g55750	hydroxyproline-rich glycoprotein family protein	biological_process_unknown
PtpAffx.49655.1.A1_at	DN496344	0.95	0.99	502.2	18220.1	0.025	At4g24290	unknown protein	biological_process_unknown
Ptp.1780.1.S1_at	AY935506.1	0.95	0.99	2143.2	54047.7	0.641	At1g27440	GUT2	biological_process_unknown
PtpAffx.25139.1.A1_s_at	CV225336	0.86	0.99	2052.5	41410.4	0.059	At5g03170	FLA11	-
PtpAffx.203942.1.S1_at	pmma7779	0.57	0.97	63.9	1767.2	0.027	At3g51290	proline-rich family protein	N-terminal protein myristoylation

More genes

Specific Experiments

The specific experiments, where these genes are specifically expressed on the basis of their z-scores, are listed in the below table. Each experiment is directly hyperlinked to the page in the GEO database.

Z score	Sample name	Experiment ID	Description	Experiment title
6.8	GSM328533:Populus x canescens root hypoxia_rep_03	GSE13109	Description	Effect of hypoxia on gene expression in Grey poplar
6.7	GSM328532:Populus x canescens root hypoxia_rep_02	GSE13109	Description	Effect of hypoxia on gene expression in Grey poplar
0.1	GSM328484:Populus x canescens root hypoxia_rep_01	GSE13109	Description	Effect of hypoxia on gene expression in Grey poplar
0.0	GSM328567:Populus x canescens root hypoxia_rep_09	GSE13109	Description	Effect of hypoxia on gene expression in Grey poplar
0.0	GSM328292:Populus x canescens root control_rep_05	GSE13109	Description	Effect of hypoxia on gene expression in Grey poplar
-0.0	GSM327408:Populus balsamifera_1006_root midday_2	GSE13990	Description	
-0.0	GSM327402:Populus balsamifera_20071015_Etiolated_seedling_6d_2	GSE13990	Description	
-0.0	GSM327400:Populus balsamifera_20071015_Etiolated_seedling_6d_1	GSE13990	Description	
-0.0	GSM372098:Clone3200_LPI5_N-_8w_rep2	GSE14893	Description	Comparative transcriptomics analysis of Populus leaves under nitrogen limitation: clone 3200
-0.0	GSM372097:Clone3200_LPI5_N-_8w_rep1	GSE14893	Description	Comparative transcriptomics analysis of Populus leaves under nitrogen limitation: clone 3200
-0.0	GSM372043:Clone1979_LPI5_N-_8w_rep2	GSE14515	Description	Comparative transcriptomics analysis of Populus leaves under nitrogen limitation: clone 1979

Fig. 2. Functional descriptions of a co-expression module in which genes are specifically expressed in experiments of hypoxic stress response. The part of "Descriptions" includes genes involved in cell wall biosynthesis. The "Short description" column, which shows functional description of homologous _Arabidopsis_ genes, represents "TUB2 (tubulin beta-2)," "microtubule association protein (MAP65/ASE1) family," "COBL4/IRX6 (COBRA-LIKE4)," "FLA11 (fasciclin-like arabinogalactan-protein 11)," and "GAUT12/IRX8/ LGT6 (GALACTURONOSYLTRANSFERASE 12)." In the "Specific Experiments" part, the "Z score" of two samples for an experiment for root hypoxia are specifically high (6.8 and 6.7, respectively), indicating that these module members may be specifically expressed through root hypoxic stress response

Discussion

The database constructed in the present study provides information on poplar co-expression modules for predicting gene function and then contributes to understanding mechanisms of biological processes, including that of stress response. Using an identifier of a gene of interest or query words related to a gene of interest, users can retrieve a co-expression module that is composed of genes co-expressed with the gene. The database provides an approach to retrieve co-expression modules with tight intramodular connections using the term "confeito" as a query word. By using the term, a list of co-expression modules in which genes are tightly interconnected becomes available. To display a page including information on a co-expression module of interest, users can select the module from among the list without knowledge of gene function or biological processes.

Our database also provides information from experiments in which genes in a co-expression module are specifically expressed. Public databases of plant co-expression analyses such as ATTED-II (http://atted.jp/),[27] CSB.DB (http://csbdb.mpimp-golm.mpg.de/),[30] ACT (http://www.arabidopsis.leeds.ac.uk/act/),[31] and AspenDB are designed to extract co-expressed genes using gene-to-gene correlation data based on publicly available DNA microarray datasets. The correlation data between co-expressed genes are strongly influenced by gene expression data in experiments in which the genes are specifically expressed. Information on such experiments is useful for characterizing genes whose functions are unknown, particularly, in co-expression analysis of poplar, for which little information on gene function is available. Therefore, we provide such information on experiments to associate them with co-expressed genes that are specifically expressed in the experiments.

The co-expression modules described in the Results section indicate that co-expression analysis using poplar gene expression datasets is useful for extracting genes that are upregulated by stress treatments, such as that of hypoxic stress. The datasets for the present research, composed of 95 chips, include 30 chips of DNA microarrays for hypoxic stress experiments, leading to the extraction of co-expression modules involved in stress response. For predicting genes involved in various biological processes and understanding their mechanisms, gene expression datasets for a

wide variety of experiments are required. Although bioinformatics approaches such as co-expression analysis provide no evidence to identify gene function, the approaches have the capability to provide genome-wide information contributing to the identification.

The combination of transcriptome data with different omics data such as genome, proteome, and interactome may lead to better understanding of gene co-expression. Genomic data of plants are available at the TIGR Plant Transcript Assemblies database (http://plantta.jcvi.org/)[31] and the GeneChip Oncology Database (http://compbio.dfci.harvard.edu/tgi/plant.html).[32] *Arabidopsis* omics data including that of the genome, transcriptome, and proteome have been accumulated and are available at the TAIR database (http://arabidopsis.org/).[33] For combined analysis between omics data, omics data of plants such as poplar are further required. This combination of omics information allows plant biologists to understand the functionality of co-expression modules on the basis of further knowledge of molecular biology.

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