ATTED-II in 2016: A Plant Coexpression Database Towards Lineage-Specific Coexpression

Yuichi Aoki1,2,5, Yasunobu Okamura1,5, Shu Tadaka1, Kengo Kinoshita1,3,4 and Takeshi Obayashi1,2,*

1Graduate School of Information Sciences, Tohoku University, 6-3-09, Aramaki-Aza-Aoba, Aoba-ku, Sendai, 980-8679 Japan
2Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency, Kawaguchi, Saitama, Japan
3Institute of Development, Aging, and Cancer, Tohoku University, Sendai, 980-8575 Japan
4Tohoku Medical Megabank Organization, Tohoku University, Sendai, 980-8573 Japan
5These authors contributed equally to this work.

*Corresponding author. E-mail: obayashi@ecee.tohoku.ac.jp; Fax, +81-22-795-7179.
(Received September 4, 2015; Accepted October 20, 2015)

ATTED-II (http://atted.jp) is a coexpression database for plant species with parallel views of multiple coexpression data sets and network analysis tools. The user can efficiently find functional gene relationships and design experiments to identify gene functions by reverse genetics and general molecular biology techniques. Here, we report updates to ATTED-II (version 8.0), including new and updated coexpression data and analysis tools. ATTED-II now includes eight microarray- and six RNA sequencing-based coexpression data sets for seven dicot species (Arabidopsis, field mustard, soybean, barrel medic, poplar, tomato and grape) and two monocot species (rice and maize). Stand-alone coexpression analyses tend to have low reliability. Therefore, examining evolutionarily conserved coexpression is a more effective approach from the viewpoints of reliability and evolutionary importance. In contrast, the reliability of species-specific coexpression data remains poor. Our assessment scores for individual coexpression data sets indicated that the quality of the new coexpression data sets in ATTED-II is higher than for any previous coexpression data set. In addition, five species (Arabidopsis, soybean, tomato, rice and maize) in ATTED-II are now supported by both microarray- and RNA sequencing-based coexpression data, which has increased the reliability. Consequently, ATTED-II can now provide lineage-specific coexpression information. As an example of the use of ATTED-II to explore lineage-specific coexpression, we demonstrate monocot- and dicot-specific coexpression of cell wall genes. With the expanded coexpression data for multilevel evaluation, ATTED-II provides new opportunities to investigate lineage-specific evolution in plants.

Keywords: Arabidopsis • Comparative transcriptomics • Database • Evolution • Gene coexpression • Gene network.

Abbreviations: AUC, area under the curve; CS6, cellulose synthase 6; GH3, glycoside hydrolase 3; GH9, glycoside hydrolase 9; GO, Gene Ontology; MR, mutual rank; PCC, Pearson’s correlation coefficient; RNAseq, RNA sequencing.

Introduction

Identifying similarities in the expression profiles of different genes, or coexpression, can provide insight to elucidate gene function (Eisen et al. 1998, Walker et al. 1999). Backed up by the enlargement of public gene expression repositories, the usefulness of coexpression information has been expanding (Rung and Brazma 2013). Because the biological functions of paralogous genes are not clearly distinguished by their sequence similarities, a gene coexpression database is a prominent resource to estimate gene function, especially in plants, which generally have more paralogous genes than animals (Tang et al. 2008).

The quality of coexpression data is primarily based on the number of samples (Ballouz et al. 2015), and this characteristic limits application of coexpression analysis in non-model species. However, recent technical advancements in RNA sequencing (RNAseq) are overcoming this difficulty. During the last decade, gene coexpression databases have been constructed and used for a wide variety of species (Aoki et al. 2007, Usadel et al. 2009).

With the maturation of coexpression data, meta-analyses of coexpression are becoming increasingly important. Coexpression analysis based on a single platform will have technical biases associated with that platform. For example, the properties of cross-hybridization and the dynamic range of probes differ among microarray platforms, as does the signal-to-noise ratio. These technical biases can lead to false positives in the coexpression analysis. Therefore, multiple platform comparisons for a single species are an effective way to eliminate false positives. Examination of coexpression conservation between closely related species has similar benefits. In addition, comparisons between evolutionarily distant species have highlighted the evolutionary conservation of coexpression, which supports the functional relationship of the gene pairs, rather than the technical reliability (Stuart et al. 2003, Oti et al. 2008, Movahedi et al. 2011, Okamura et al. 2015). Some coexpression databases allow assessment of coexpression conservation (Obayashi and Kinoshita 2011, Obayashi et al. 2011).
Species-specific co-expression has also attracted researchers with an evolutionary viewpoint (Stuart et al. 2003, Oldham et al. 2006), but false species-specific relationships can also be generated by the technical bias of the platform or by different sample compositions. Therefore, species-specific coexpression analysis always requires evidence that the results are independent from both the platform characteristics and the sample composition.

We constructed and developed ATTED-II (http://atted.jp), a coexpression database for plant species, which provides a parallel view of multiple coexpression data sets with network analysis tools (Obayashi et al. 2007, Obayashi et al. 2009, Obayashi et al. 2011, Obayashi et al. 2014). The user can effectively find functional gene relationships and design experiments to confirm the gene functions by reverse genetics and general molecular biological techniques (Obayashi and Kinoshita 2010). Here, we report updates to ATTED-II that include new and updated coexpression data and analysis tools. ATTED-II now includes eight microarray- and six RNAseq-based coexpression data sets for nine species (Arabidopsis, field mustard, soybean, barley, poplar, tomato, grape, rice and maize). Importantly, five species (Arabidopsis, soybean, tomato, rice and maize) are now supported by both microarray- and RNAseq-based coexpression data. Our assessment scores for the data indicate that the new coexpression data sets are of higher quality than any previous coexpression data sets in ATTED-II. These highly reliable coexpression data will enable us to detect lineage-specific coexpression. As an example, we demonstrate monocot- and dicot-specific coexpression with the updated ATTED-II. With the expanded coexpression data with multilevel evaluation, ATTED-II provides new opportunities to investigate lineage-specific evolution in plants.

Results and Discussion

The new co-expression data for nine species from 14 sources

We updated both the microarray- and RNAseq-based coexpression data (Table 1) in ATTED-II. For the microarray platform, tomato (Solanum lycopersicum microarray; Sly-m) was newly included, and additional microarray data for Arabidopsis (Arabidopsis thaliana; Ath-m), soybean (Glycine max; Gma-m), barley medick (Medicago truncatula; Mtr-m), rice (Oryza sativa; Osa-m) and grape (Vitis vinifera; Vvi-m) were downloaded from a public repository (Kolesnikov et al. 2015). For RNAseq-based coexpression data, field mustard (Brassica rapa; Br-), soybean (Gma-r), rice (Osa-r), tomato (Sly-r) and maize (Zea mays; Zma-r) were newly added, and the Arabidopsis coexpression data were updated (Ath-r). In total, ATTED-II provides information from 14 sources for the nine species. Among the nine species, five (Arabidopsis, soybean, tomato, rice and maize) are supported by data from both the microarray and RNAseq platforms, which enhances the reliability of the coexpression detection in these species described below.

Similarity among the 14 coexpression data sets

To provide an overview of the 14 coexpression data sets, we examined the similarities among them. We first quantified the similarity between pairs of coexpressed gene lists from different sources using the coexpression similarity (COXSIM) value, which is the weighted concordance rate of a gene list (Obayashi et al. 2013). Because the COXSIM values are calculated for every pair of corresponding guide genes, the median of the COXSIM values for all guide gene pairs was used to represent the similarity of the two coexpression data sets. Supplementary Fig. S1 shows the similarities among the 14 coexpression data sets. Among all data set pairs, the five pairs from the same species (Ath, Gma, Sly, Osa and Zma) showed the highest similarities, as expected. In contrast, the Mtr-m coexpression data showed similarity only to the Gma-r data (0.018), probably owing to the low quality of the barley data. The similarity table is also represented as a dendrogram in Fig. 1. In the dendrogram, the high similarities of the platform pairs (microarray and RNAseq) for the five species are represented as the longest branches, whereas barley medick, which did not show strong similarity to the other platforms, was placed near the root of the dendrogram. Importantly, this dendrogram reflects the phylogenetic branching of the brassica family (Ath and Bra) and the monocot–dicot branching, suggesting the potential to analyze the evolution of coexpression.

Significance of the coexpressed gene list

When coexpression is supported by data from multiple platforms in the same species or closely related species, the coexpression can be regarded as reliable. Because selection of the best platform to assess the coexpressed gene list of interest depends on multiple factors, we used the maximum COXSIM value (maxCOXSIM) between the target gene list and each reference gene list as the measure of supportability of the target coexpressed gene list (Obayashi et al. 2013). The maxCOXSIM was then compared with the null distribution to calculate the statistical significance. We slightly refined the null distribution of the maxCOXSIM to estimate a more realistic P-value. The previous null distribution of maxCOXSIM was constructed by randomization of the individual gene list. However, the actual coexpressed gene list has two types of constraints that reduce the degrees of freedom. One constraint concerns the characteristic of correlation. When both of the two variable pairs A–B and B–C are correlated, the pair A–C is not independent. The other constraint concerns gene expression patterns. Compared with the conceptually possible variation in gene expression patterns, actual gene expression patterns in cellular systems are very limited. Therefore, independent randomization of the gene list allows too many degrees of freedom and consequently leads to overestimated P-values. To achieve more realistic P-values of maxCOXSIM, we used the distribution of the actual COXSIM values between any combination of guide genes of the Arabidopsis (Ath-r) and rice (Osa-r) data sets as the null distribution of the COXSIM values (Supplementary Fig. S2). Based on this COXSIM distribution, the thresholds of maxCOXSIM were defined, after Bonferroni correction, for 13
Fig. 1 Hierarchical clustering of coexpression data. Data sets were hierarchically clustered by the complete linkage method. The pairwise similarities among all coexpression data sets are shown in Supplementary Fig. S1. Because COXSIM values are not exactly symmetric, the median of the COXSIM for a pair is not exactly symmetric. Therefore, the average values of the median COXSIM between one target and one reference, and vice versa, were used to represent symmetric similarity between data sets, and 1 – similarity was used to represent the distance between data sets. The coexpression data set version is shown in parentheses under the data set ID. 'CodonS' is the codon score from Table 1.

**Table 1** Coexpression data in ATTED-II version 8.0

| Common name | Scientific name | Data set ID | Version | No. of genes | No. of samples | Codon score | Reproducibility score | Release date |
|-------------|-----------------|-------------|---------|--------------|---------------|-------------|-----------------------|-------------|
| Arabidopsis | *Arabidopsis thaliana* | Ath-m | c6.0 | 20,836 | 15,275 | 2.29 | 2.57 | August 31, 2015 |
| | | Ath-r | c2.0 | 25,296 | 1,401 | 1.67 | 2.20 | August 31, 2015 |
| Field mustard | *Brassica rapa* | Bra-r | c1.0 | 35,431 | 257 | 1.63 | 1.25 | August 31, 2015 |
| Soybean | *Glycine max* | Gma-m | c2.0 | 15,902 | 1,115 | 1.63 | 1.61 | August 31, 2015 |
| | | Gma-r | c1.0 | 42,787 | 410 | 1.73 | 1.48 | August 31, 2015 |
| Barrel medick | *Medicago truncatula* | Mtr-m | c2.0 | 6,226 | 909 | 1.08 | – | August 31, 2015 |
| Rice | *Oryza sativa* | Osa-m | c5.0 | 20,625 | 2,098 | 1.59 | 1.79 | August 31, 2015 |
| | | Osa-r | c1.0 | 17,548 | 222 | 1.49 | 1.64 | August 31, 2015 |
| Poplar | *Populus trichocarpa* | Ppo-m | c1.0 | 21,909 | 404 | 1.29 | 1.59 | August 31, 2015 |
| Tomato | *Solanum lycopersicum* | Sly-m | c1.0 | 5,786 | 401 | 1.07 | 1.51 | August 31, 2015 |
| | | Sly-r | c1.0 | 23,195 | 288 | 1.34 | 1.27 | August 31, 2015 |
| Grape | *Vitis vinifera* | Vvi-m | c2.0 | 9,564 | 245 | 1.18 | 1.32 | August 31, 2015 |
| Maize | *Zea mays* | Zma-m | c2.0 | 11,069 | 755 | 1.62 | 1.94 | August 31, 2015 |
| | | Zma-r | c1.0 | 22,592 | 1,571 | 2.55 | 1.85 | August 31, 2015 |

* -m, microarray-based coexpression data; -r, RNAseq-based coexpression data.

b Coincidence score between coexpressed gene lists and codon similarity gene lists. The agreement between the two types of gene lists is quantified using the COXSIM value for each guide gene. The median COXSIM value (1E-02) was used to assess overall performance. A higher score indicates better performance.

c Coincidence score with reference data sets represented by the median of the normalized COXSIM value (1E-01). A higher score indicates better performance.
guide gene lists. The thresholds of maxCOXSIM for $p < 0.1$, $p < 0.01$ and $p < 0.001$ are 0.081, 0.189 and 0.377, respectively, and are represented as one, two and three stars on the ATTED-II coexpressed gene page. As an example, the supportabilities for the coexpressed gene list of CS6/At5g64740 are available in http://atted.jp/cgi-bin/coex_list.cgi?gene=836595. The proportions of genes of each significance level in each data set are shown in Fig. 2. This supportability analysis suggests that Arabidopsis, soybean, rice and maize are the best species for coexpression analysis.

Performance of overall gene coexpression data

We then assessed the 14 sets of coexpression data using three independent scores: (i) the Gene Ontology (GO) score; (ii) codon score; and (iii) reproducibility score. Because rich gene annotation resources are available for Arabidopsis, we first tested for enrichment of the GO biological process annotations (GO score; Obayashi et al. 2014). The GO scores showed improvement with each update of the Arabidopsis coexpression data (Table 2). Compared with the improvement seen with addition of RNAseq data (Ath-r), the improvement with the addition of microarray data (Ath-m) from c5-0 to c6-0 was small, implying saturation of sample variation for this platform. Although the GO score is informative for Arabidopsis, this score is not generally applicable for the numerous species that lack comprehensive GO annotations (Obayashi et al. 2014).

As the second assessment, we determined the codon score, which is a coincident score between a coexpressed gene table and a gene–gene codon usage similarity table (Obayashi et al. 2014). Because codon usage information is available for genes in any species, the codon score can be applied to any coexpression data. The codon scores (Table 2) also showed general improvement with addition of Arabidopsis coexpression data, in good agreement with the GO scores [Pearson’s correlation coefficient (PCC) = 0.91].

The third assessment score is based on the similarity of the coexpression data sets (Fig. 1; Supplementary Fig. S1). As discussed above, reproducible coexpression data can be assumed to be of high quality. However, to use supportability directly as a measure of overall quality of a data set, the quality of the reference data set should be considered. Generally, lower quality reference data do not affect maxCOXSIM, which is the highest COXSIM value among all reference data sets. In most cases, taking the maximum value means selecting the data sets of the closest or the same species if available. However, the data set of the closest species is not always the best choice in terms of similarity assessment. For example, microarray probes for a particular gene are not always available, or they may cross-hybridize, and thus be omitted from coexpression calculations. In such cases, the second or third best platform would be selected based on the maxCOXSIM value. Thus we can expect higher maxCOXSIM values if an ideal reference platform can be used. Therefore, we normalized the maxCOXSIM values by the
Table 2 Consistency of the three assessments for Arabidopsis coexpression data in ATTED-II

| Data set ID | Version | No. of genes | No. of samples | GO score | Codon score | Reproducibility score | Release date |
|------------|---------|--------------|----------------|----------|-------------|-----------------------|-------------|
| Ath-m      | c6.0    | 20,836       | 15,275         | 7.08     | 2.29        | 2.57                  | August 30, 2015 |
| Ath-m      | c5.0    | 20,836       | 11,171         | 7.02     | 2.24        | 2.55                  | May 17, 2013   |
| Ath-m      | c4.1    | 20,906       | 1,388          | 5.48     | 1.56        | 2.08                  | April 8, 2008  |
| Ath-m      | c4.0    | 20,906       | 1,388          | 5.06     | 1.59        | 1.82                  | March 18, 2008 |
| Ath-m      | c3.1    | 20,703       | 771            | 4.96     | 1.46        | 2.14                  | September 12, 2007 |
| Ath-m      | c3.0    | 22,263       | 771            | 4.96     | 1.46        | 2.10                  | May 25, 2006   |
| Ath-r      | c2.0    | 25,296       | 1,401          | 4.81     | 1.67        | 2.20                  | August 30, 2015 |
| Ath-r      | c1.0    | 25,838       | 328            | 4.27     | 1.59        | 1.67                  | August 17, 2013 |

Table 2. Consistency of the three assessments for Arabidopsis coexpression data in ATTED-II.

An example of lineage-specific coexpression supported by multiple platforms

Data from multiple platforms for a single species can confirm not only the existence, but also the absence, of coexpression. Among the five species with both microarray and RNAseq data, tomato had the lowest coexpression data quality (Table 1; Fig. 1). Therefore, to examine monocot- and dicot-specific coexpression, we compared two monocots (rice and maize) and two dicots (Arabidopsis and soybean). Based on the reciprocal best hit method, there were 7,882 orthologous groups composed of one gene from each of the four species. Among the 7,882 orthologous groups, 1,548 had coexpression data from all eight data sets (microarray and RNAseq data for the four species). Before investigating the differences between the monocot and dicot species, we first compared the average coexpression strength in the four microarray data sets (Ath-m, Gma-m, Osa-m and Zma-m) and the four RNAseq data sets (Ath-r, Gma-r, Osa-r and Zma-r) by the geometric average of the mutual rank (MR) values (Supplementary Fig. S3A). The coexpression averages within each platform were similar (PCC = 0.70), and platform-specific bias was not observed. Because most gene pairs are not coexpressed, the peak of the distribution of the average coexpression is around MR = 10,000, which is approximately half of the number of genes in each data set. Next, we compared the average coexpression strength of dicots (Ath-m, Ath-r, Gma-m and Gma-r) and of monocots (Osa-m, Osa-r, Zma-m and Zma-r) (Supplementary Fig. S3B). The general trend of the average coexpression was similar to that seen in the comparison of platforms (Supplementary Fig. S3A). Most of the gene pairs in dicots and monocots were not coexpressed (approximate MR = 10,000). However, some of the coexpressed gene pairs showed strong coexpression in both monocots and dicots, indicating evolutionarily conserved coexpression. The distribution of the average coexpression was expanded relative to the platform-specific coexpression distribution (Supplementary Fig. S3A), suggesting the existence of monocot-specific and dicot-specific coexpression.

Here, we focused on six gene pairs among four genes as examples of various coexpression patterns. Among the six gene pairs, one gene pair shows evolutionarily conserved coexpression, one gene pair is not coexpressed and the other four gene pairs show lineage-specific coexpression (Supplementary Fig. S3B). Interestingly, these examples revealed coexpression switching (Fig. 3). The gene encoding cellulose synthase 6 (CS6) was coexpressed with the gene for a protein of unknown function (Unk) in all eight data sets, and thus this unknown protein is very likely to be a cell wall rearrangement factor. In monocots, these two genes show coexpression with the gene for glycoside hydrolase 3 (GH3), which degrades the major hemicelluloses in monocots (xylan, arabian and arabinoxylan; Minic 2008). On the other hand, in dicots, these two genes are coexpressed with the gene for glycoside hydrolase 9 (GH9), which degrades glucan and cellulose (Minic 2008). Monocots and dicots use distinct sets of genes to construct the different types of cell wall (Yokoyama and Nishitani 2004, Minic 2008). By using gene coexpression, the difference in the individual gene modules
can be detected even in the common genes between the two lineages.

**Development of NetworkDrawer for subnetwork analyses**

Network representation is a suitable method to provide an overview of the module structure of multiple gene relationships, such as coexpression. ATTED-II provides NetworkDrawer, a coexpression network drawing tool. Coexpression network is generally scale free (Jordan et al. 2004, van Noort et al. 2004), meaning that the user has little control over the density of the network. NetworkDrawer draws edges for the three genes with the strongest coexpression with every gene. This constraint provides a medium-density network for a variety of query gene sets. However, even with this drawing constraint, large gene networks easily become too complicated to investigate manually. Therefore, an automatic network analysis tool is needed. To find biologically meaningful subnetworks in large coexpressed gene networks, we implemented an automatic subnetwork detection and analysis workflow, as previously introduced in a mammalian coexpression database (Obayashi et al. 2013). After the coexpressed gene network is drawn, a subnetwork detection algorithm automatically initiates. All of the detected subnetworks are then checked for enrichment of GO biological process annotations and cis-elements. Fig. 4 shows an example NetworkDrawer output page for the CS6, GH3 and GH9 genes discussed above. The gene network based on the Ath-m data set reflects stronger coexpression between the CS6 (TSD1 in the Fig. 4) and GH3 (PRC1 in the Fig. 4) genes than between either of these genes and the GH9 gene. In this case, three biologically meaningful subnetworks were detected, which generally correspond to each query gene.

**Materials and Methods**

**Construction of gene coexpression data**

To calculate coexpression from the microarray-based data, we downloaded the GeneChip CEL files from ArrayExpress (Kolesnikov et al. 2015). Mapping from probe to gene was based on National Center for Biotechnology Information (NCBI) Gene Expression Omnibus platform files (Barrett et al. 2013). Probes...
COXSIMg\( \leq \frac{1}{\max\{\text{coexpression measure} \}} \) is the number of genes in the top \( i \) genes in \( \text{list}_g \) with corresponding genes in the top \( i \) genes in \( \text{list}_r \). We previously used 100 for \( k \), meaning that we checked the gene correspondence of the top 100 coexpressed genes. However, the total number of genes in the gene list is different among the data sets, and thus the random inclusion rate of unrelated genes in the top 100 are different. Therefore, we have modified the number of genes in \( k \) to be the top 1% of all genes in \( \text{list}_r \). Typically, the values are approximately 100 for different species comparisons and approximately 200 for same species comparisons.

Because the best reference guide gene is initially unknown, we checked all possible reference guide genes. The reference guide gene set \( R \) is composed of the Blastp best hit genes from one target species in every other species. The selection of the genes with the highest similarity is independent of the data set composition, and thus the best-hit gene is sometimes not available in the reference data set, and, in that case, we did not use the data set as the reference. When multiple data sets are available for the species including the guide gene \( g \), the same gene in the other data set is also included in the reference guide gene set \( R \). The COXSIM values are calculated between the target guide gene \( g \) and every reference gene \( r \) in \( R \). The reference gene \( r \) that gives the maxCOXSIM value is regarded as the best reference guide gene.

\[
\text{maxCOXSIM}_g = \max_r \{\text{COXSIM}_{gr} \} = \max_r \left[ \frac{\text{COXSIM}_{gr}}{\text{COXSIM}_{nr}} \right]
\]

To assess the statistical significance, the maxCOXSIM value is then compared with the null distribution. To reflect the characteristics of the coexpression data, the actual COXSIM values between any combination of Arabidopsis gene \( g \) and rice gene \( r \) were used as the null distribution of the COXSIM values because almost all gene pairs are functionally independent (Supplementary Fig. S2). Based on this COXSIM distribution with Bonferroni correction for the 13 guide gene lists, the \( p \)-values of...
maxCOXSIM were determined. The thresholds of maxCOXSIM for \( p < 0.1,\) \( p < 0.01 \) and \( p < 0.001 \) were \( 0.081, 0.189 \) and \( 0.377, \) respectively.

Reproducibility score based on similarity of coexpression data

As suggested from Fig. 2, the maxCOXSIM\(_g\) value is one representation of the quality of a guide gene, and thus the median maxCOXSIM\(_g\) value for every guide gene in a data set reflects the total quality of the data set. However, the maxCOXSIM\(_g\) value should be considered from the viewpoint of the adequateness of the selected reference guide gene \( r. \) We designed the reproducibility score as a more accurate measure of data set quality:

\[
\text{Reproducibility} = \text{median} \left( \frac{\text{COXSIM}_{g} / \text{Reference Adequateness}_r}{\text{maxCOXSIM}_g} \right)
\]

Because different data sets from the same species are the best reference to check technical reproducibility (Supplementary Figs. S1, S2), the adequateness of the reference guide gene in the same species should be the highest, whereas that of the reference guide gene in the evolutionarily most distant species should be the lowest. We hypothesized that the conservation of gene coexpression could be approximated by using the conservation of the guide gene sequences. Based on this idea, we used the conservation ratio between protein sequences of the target guide gene \( g \) and the selected reference guide gene \( r \) to measure the adequateness of the selected guide gene \( r \) as the reference.

\[
\text{Reference Adequateness}_r = \frac{\text{Blastp bitscore from } g \text{ to } r}{\text{Blastp bitscore from } g \text{ to } g}
\]

The reproducibility score, which is the median of the normalized maxCOXSIM for all of the guide genes in a data set in Equation 3, is used as the coexpression quality value of the data set (Tables 1, 2).

Predictive performance of GO terms by gene coexpression data

Using the GO biological process annotations downloaded from NCBI (August 10, 2015, gene2go), the Arabidopsis coexpression data were assessed (Table 2). Owing to the differences in the relevance of GO terms to our purposes along with their hierarchical topology, we selected particular GO terms for coexpression evaluation, as described previously (Kinosita and Obayashi 2009). We selected GO terms associated with 5–20 genes with comparable information content, resulting in 1,197 GO biological process terms for Arabidopsis. The genes associated with at least one selected GO term were then used in this assessment, resulting in 3,708 and 4,163 genes for the Ath-m and Ath-r data sets, respectively. All of the genes within each data set were divided into two groups, those sharing at least one GO term with another gene. The differences in the distribution of the degrees of coexpression were assessed using the partial receiver operating characteristic (ROC) area under the curve (AUC)\(_{501}\) (McClish 1989). Note that this GO score scheme is not applicable for most of the other species. For example, only 27 GO biological process terms have been identified for rice.

Coincidence score with codon similarity

Protein-coding sequences were retrieved from NCBI RefSeq (Brown et al. 2015). For each gene, the 61-dimensional vector was constructed from the number of codons in the protein-coding sequence. When multiple RefSeq sequences were available for a gene, the longest sequence was used for the calculation of codon usage. The PCCs between the vectors of any two genes were calculated and then converted to MRs, which were used as the codon usage similarity index. Codon similarity tables are also downloadable from the ATTED-II bulk-download page (http://atted.jp/top_download.shtml) in the same format as the coexpression tables.

Supplementary data

Supplementary data are available at PCP online.

Funding

This research was supported by the Japan Science and Technology Agency (CREST research project grant No. 11102558 to T.O.); Grants-in-Aid for Innovative Areas (grant No. 24114005 to T.O.), Scientific Research (grant Nos. 15K20863 to Y.A. and 15K18464 to T.O.) and Publication of Scientific Research Results (grant No. 15HP08044 to T.O.).

Acknowledgements

We thank Professors Kazuhiko Nishitani and Ryusuke Yokoyama (Tohoku University) for valuable discussions about cell wall rearrangement in monocots and dicots. We also thank Mr. Kota Jin for the web design of NetworkDrawer and the taxonomy icons. The super-computing resources were provided by the Human Genome Center, Institute of Medical Science, University of Tokyo.

Disclosures

The authors have no conflicts of interest to declare.

References

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., et al. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25: 3389–3402.

Aoki, K., Ogata, Y. and Shibata, D. (2007) Approaches for extracting practical information from gene co-expression networks in plant biology. Plant Cell Physiol. 48: 381–390.

Barrett, T., Wilhite, S.E., Ledoux, P., Evangelista, C., Kim, I.F., Tomashovskv, M., et al. (2013) NCBI GEO: archive for functional genomics data sets—update. Nucleic Acids Res. 41: D991–995.

Ballouz, S., Verleyen, W. and Gillis, J. (2015) Guidance for RNA-seq coexpression network construction and analysis: safety in numbers. Bioinformatics 31: 2123–2130.

Brown, G.R., Hem, V., Katz, K.S., Ovtetsky, M., Wallin, C., Ermolaeva, O., et al. (2015) Gene: a gene-centered information resource at NCBI. Nucleic Acids Res. 43: D36–D42.

Eisen, M.B., Spellman, P.T., Brown, P.O. and Botstein, D. (1998) Cluster analysis and display of genome-wide expression patterns. Proc. Natl Acad. Sci. USA 95: 14863–14868.

Jordan, I.K., Maríño-Ramírez, L., Wolf, Y.I. and Koonin, E.V. (2004) Conservation and coevolution in the scale-free human gene coexpression network. Mol. Biol. Evol. 21: 2058–2070.

Kinosita, K. and Obayashi, T. (2009) Multi-dimensional correlations for gene coexpression and application to the large-scale data of Arabidopsis. Bioinformatics 25: 2677–2684.

Kolesnikov, N., Hastings, E., Keays, M., Melnichuk, O., Tang, Y.A., Williams, E., et al. (2015) ArrayExpress update—simplifying data submissions. Nucleic Acids Res. 43: D1113–D1116.

Langmead, B., Trapnell, C., Pop, M. and Salzberg, S.L. (2009) Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biol. 10: R25.

McClish, D.K. (1989) Analyzing a portion of the ROC curve. Med. Decis. Making 9: 190–195.

Minic, Z. (2008) Physiological roles of plant glycoside hydrolases. Planta 227: 723–740.
Movahedi, S., Van de Peer, Y. and Vandepoele, K. (2011) Comparative network analysis reveals that tissue specificity and gene function are important factors influencing the mode of expression evolution in Arabidopsis and rice. Plant Physiol. 156: 1316–1330.

Obayashi, T., Hayashi, S., Saeki, M., Ohta, H. and Kinoshita, K. (2009) ATTED-II provides coexpressed gene networks for Arabidopsis. Nucleic Acids Res. 37: D987–D991.

Obayashi, T. and Kinoshita, K. (2009) Rank of correlation coefficient as a comparable measure for biological significance of gene coexpression. DNA Res. 16: 249–260.

Obayashi, T. and Kinoshita, K. (2010) Coexpression landscape in ATTED-II: usage of gene list and gene network for various types of pathways. J. Plant Res. 123: 311–319.

Obayashi, T. and Kinoshita, K. (2011) COXPRESdb: a database to compare gene coexpression in seven model animals. Nucleic Acids Res. 39: D1016–D1022.

Obayashi, T., Kinoshita, K., Nakai, K., Shibaoka, M., Hayashi, S., Saeki, M., et al. (2007) ATTED-II: a database of co-expressed genes and cis elements for identifying co-regulated gene groups in Arabidopsis. Nucleic Acids Res. 35: D863–D869.

Obayashi, T., Nishida, K., Kasahara, K. and Kinoshita, K. (2011) ATTED-II updates: condition-specific gene coexpression to extend coexpression analyses and applications to a broad range of flowering plants. Plant Cell Physiol. 52: 213–219.

Obayashi, T., Okamura, Y., Ito, S., Tadaka, S., Aoki, Y., Shirotta, M. and Kinoshita, K. (2014) ATTED-II in 2014: evaluation of gene coexpression in agriculturally important plants. Plant Cell Physiol. 55: e6.

Obayashi, T., Okamura, Y., Ito, S., Tadaka, S., Motoike, I.N. and Kinoshita, K. (2013) COXPRESdb: a database of comparative gene coexpression networks of eleven species for mammals. Nucleic Acids Res. 41: D1014–D1020.

Ogasawara, O., Mashima, J., Kodama, Y., Kaminuma, E., Nakamura, Y., Okubo, K., et al. (2013) DDBJ new system and service refactoring. Nucleic Acids Res. 41: D25–D29.

Okamura, Y., Obayashi, T. and Kinoshita, K. (2015) Comparison of gene coexpression profiles and construction of conserved gene networks to find functional modules. PLoS One 10: e0132039.

Oldham, M.C., Horvath, S. and Geschwind, D.H. (2006) Conservation and evolution of gene coexpression networks in human and chimpanzee brains. Proc. Natl. Acad. Sci. USA 103: 17973–17978.

Ori, M., van Reeuwijk, J., Huynen, M.A. and Brunner, H.G. (2008) Conserved co-expression for candidate disease gene prioritization. BMC Bioinformatics 9: 208.

Rung, J. and Brazma, A. (2013) Reuse of public genome-wide gene expression data. Nat. Rev. Genet. 14: 89–99.

Stuart, J.M., Segal, E., Koller, D. and Kim, S.K. (2003) A gene-coexpression network for global discovery of conserved genetic modules. Science 302: 249–255.

Tang, H., Bowers, J.E., Wang, X., Ming, R., Alam, M. and Paterson, A.H. (2008) Synteny and collinearity in plant genomes. Science 320: 486–488.

Usadel, B., Obayashi, T., Mutwil, M., Giorgi, F.M., Bassel, G.W., Tanimoto, M., et al. (2009) Co-expression tools for plant biology: opportunities for hypothesis generation and caveats. Plant Cell Environ. 32: 1633–1651.

van Noort, V., Snel, B. and Huynen, M.A. (2004) The yeast coexpression network has a small-world, scale-free architecture and can be explained by a simple model. EMBO Rep. 5: 280–284.

Yokoyama, R. and Nishitani, K. (2004) Genomic basis for cell-wall diversity in plants. A comparative approach to gene families in rice and Arabidopsis. Plant Cell Physiol. 45: 1111–1121.

Walker, M.G., Volkmut, W., Sprinzak, E., Hodgson, D. and Klingler, T. (1999) Prediction of gene function by genome-scale expression analysis: prostate cancer-associated genes. Genome Res. 9: 1198–1203.