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Research Article

Screening and Identification of Differentially Expressed Genes in Reproductive Stage of Rice (*Oryza sativa*) Using Digital Differential Display Tools

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Abstract

Growth and development of any plant tissue due to variation in genes expression level related to each tissue, indicates the specific pattern of genes expression. The whole of Expressed Sequence Tags (ESTs) of rice clustered in unigene sets in NCBI UniGene database that offer a platform for identifying differentially expressed genes in rice reproductive stage. This report illustrates a means to efficiency utilize this public database for gene expression (transcriptome) profiling reproductive stage. Using a data mining tool known as Digital Differential Display (DDD), comparisons were performed between 52 libraries of reproductive stage and 110 libraries of vegetative stage. DDD identified 812 specific unigene set in reproductive stage. Some of these genes encode for proteins such as prolamin, lipid transfer proteins, glutelin proteins and other unknown but novel gene sequences that's coded expression proteins without reveal functional. Also, part of genes screened was related to housekeeping genes. This study proves that the use of *in silico* analysis of the rice UniGene database led to the acceleration in create transcriptional profiles of known and novel genes in developing reproductive stage.

Keywords: Rice; Reproductive stage; Digital Differential Display (DDD)

Introduction

Rice (*Oryza sativa* L.) is one of the most important cereal crops worldwide, and the major source of human nutrition and livestock feed in many countries [1]. Considering the importance of reproductive phase of plants, identification of specific genes of a reproductive stage can be effective in improving the quality and quantity of cereals. In order to screen these genes, use of expression patterns is resultful [2]. Two analysis approaches, analog and digital used for the estimate of expression level. The analog approach based on oligonucleotide probe hybridizations such as microarray while the digital approach is based on the high-throughput generation of gene transcripts as in the case of Expressed Sequence Tags (ESTs) [2,3]. ESTs are a fast; inexpensive way to determine which genes are being actively transcribed in a tissue or organ at a given stage of development [4,5]. The one of high-throughput generation is Digital Differential Display (DDD) [2]. The goal of this study was to screen and identify genes specifically expressed in the reproductive stage of rice with DDD tool.

Methods and Materials

Extraction of rice cDNA library

In order to determine the level of genes expression was use a total of 623800 rice Expressed Sequence Tags (ESTs) (December 2015) that are available from the National Center for Biotechnology Information (NCBI) database. The ESTs sequences have been divided into162 library. The ESTs were computationally clustered into unigene set. Each unigene set is defined as hypothetical genes that based on sequence homology, originate from the same gene or expressed pseudogene in this study, the UniGene database was analyzed by an *in silico* tool known as Digital Differential Display (DDD).

Digital differential displays analysis

DDD is an online bioinformatics tool for identification of differentially expressed genes. The general framework of DDD is based on the comparison between the relative abundance of ESTs from various libraries [2,6]. To eliminate the difference caused by the unequal number of ESTs in each selected libraries, DDD uses the Fisher Exact Test. The output provided a numerical value in each pool denoting the fraction of sequences within the pool that mapped to the unigene cluster [2,7]. In the current work Comparisons between libraries related to reproductive phase and other libraries (Vegetative tissue) was used to identify unigene sets that were reproductive stage specific. Libraries chosen for DDD comparison are listed in Table 1. For those libraries that have sequences in UniGene, DDD lists the

Table 1: Summary of the libraries used for DDD screening.

Reproductive stage (Pool A)			Vegetative stage (Pool B)		
Tissue	library	ESTs	Tissue	library	ESTs
Panicle	25	135641	Callus	31	168798
Flower	19	134349	Leaf	39	177881
Seed	3	22829	Root	24	66590
Other	1	9271	Stem	10	135906
Mixed	1	12172	Vegetative meristem	3	4537
Unreported	2	21626	Mixed	3	36500
Total	52	335888	Total	110	590212

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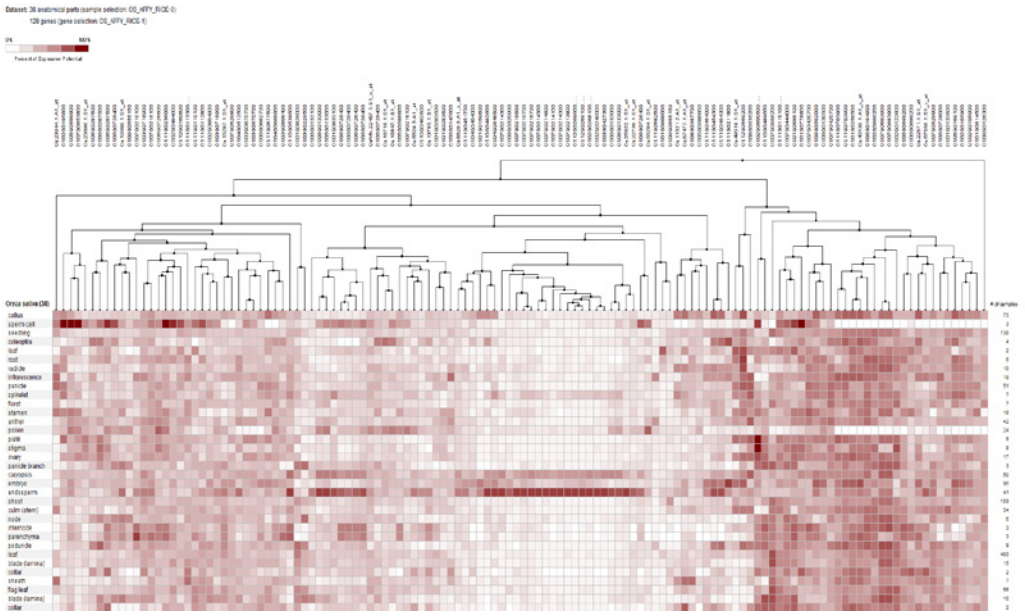

Figure 1A: Heat map and hierarchical clustering of 812 reproductive stage -specific genes based on their expression profile in different tissue. **(A)** High expression group.

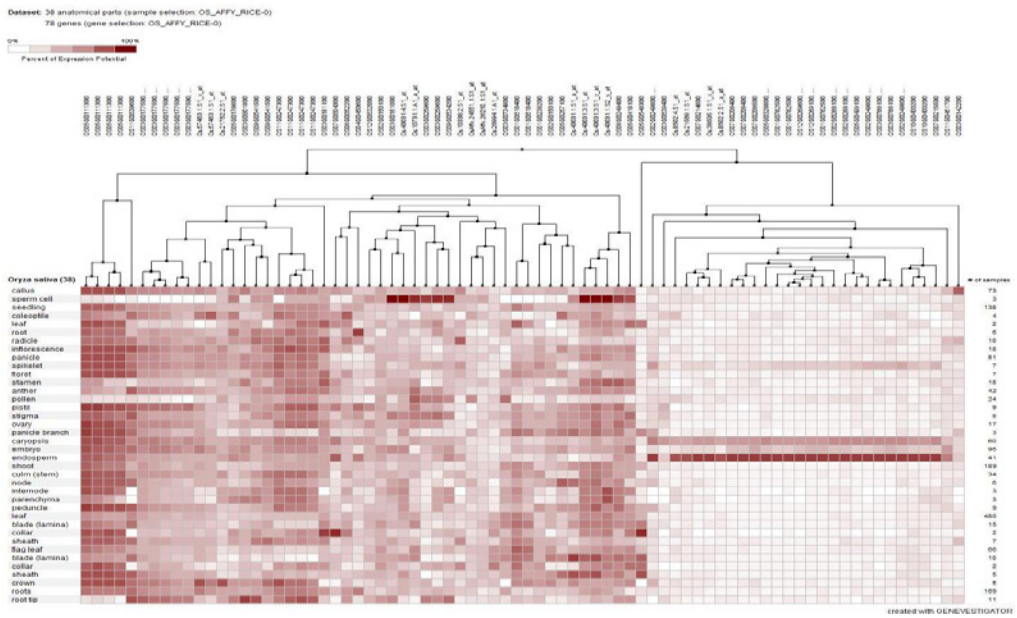

Figure 1B: Heat map and hierarchical clustering of 812 reproductive stage -specific genes based on their expression profile in different tissue. **(B)** Moderate expression group.

title and tissue source and provides a general description of each UniGene.

Result and Discussion

This study explored the utility of DDD as a means of *in silico* digital transcriptome profiling to identify differentially expressed genes in reproductive stage of rice. The results of DDD comparison led to identification 812 unigene sets. Then, after the blast of sequences each of the unigene sets were assigned to a specific gene. Some of these genes encode for proteins such as prolamin, lipid transfer proteins, glutelin proteins and other unknown but novel gene sequences. Also, part of identified genes was related to housekeeping genes. Screened genes were divided into three groups, high expression (Figure 1A), moderate expression (Figure 1B), and weak expression based on expression level (Figure 1C). The heat map of investigated genes was provided by GENEVESTIGATOR (www.Genevestigator.com). The screened unigene sets by DDD should be converting to probeset that is necessary for heat map [7]. During the analysis, it is worthily noticed

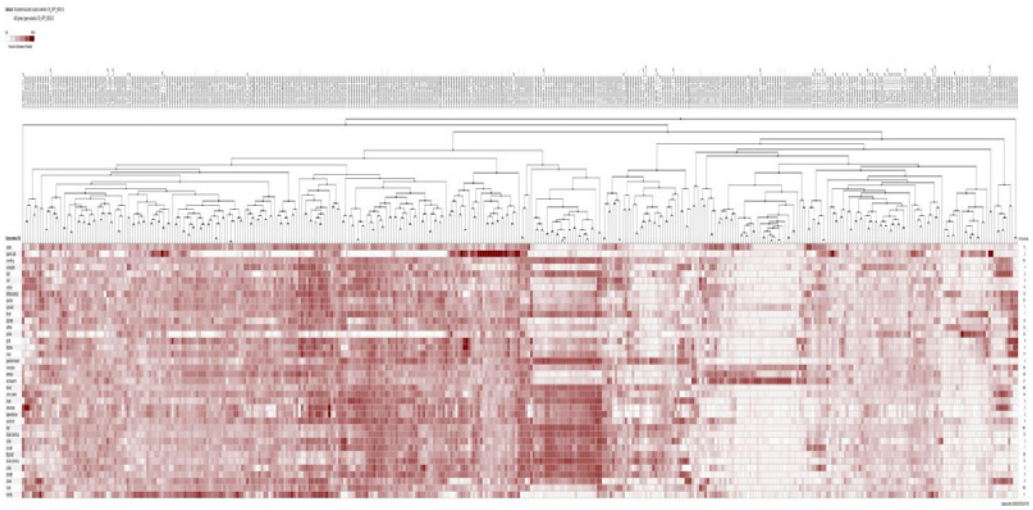

Figure 1C: Heat map and hierarchical clustering of 812 reproductive stage -specific genes based on their expression profile in different tissue. **(C)** Weak expression group. The accession number of each gene was listed on the above. The hierarchical clusters are color-coded: the largest values are displayed in red (hot), the smallest values are displayed in white, while the intermediate values are a lighter color of either white or red. Pearson correlation clustering was used to group the developmentally regulated genes.

that not all the unigenes screened have the corresponding probeset. Analysis of the heat map was confirming the specificity of screened genes in the reproductive stage. The results of developmental stage analysis demonstrated that screened gene expression in the flowering stage was more than other reproductive stages (results not shown). According to this analysis, the specificity of the genes identified in the reproductive stage will be confirmed experimentally using RT-PCR.

Conclusion

This study demonstrated the utility of Digital Differential Display (DDD) as a tool to analyze the rice transcriptome *via* the NCBI UniGene dbEST database. In this study, DDD enabled the identification of numerical differences in transcript frequency between individual or pooled cDNA libraries from various tissues of the reproductive stage [8]. These genes screened during this study will provide valuable information for further studies about the functions of these genes in rice. Bioinformatics methods can be very effective in acceleration to identify novel genes and improvement of costs. It should be noted that any DDD results should be validated experimentally using quantitative RT-PCR or other methods using a panel of genotypes and tissues.

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