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Genome-wide comparative analysis of digital gene expression tag profiles during maize ear development

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ABSTRACT

The present study profiled and analyzed gene expression of the maize ear at four key developmental stages. Based on genome-wide profile analysis, we detected differential mRNA of maize genes. Some of the differentially expressed genes (DEGs) were predicted to be potential candidates of maize ear development. Several well-known genes were found with reported mutant analyses, such as, *compact plant2* (*ct2*), *zea AGAMOUS homolog1* (*zag1*), *bearded ear* (*bde*), and *silky1* (*si1*). MicroRNAs such as microRNA156 were predicted to target genes involved in maize ear development. Antisense transcripts were widespread throughout all the four stages, and are suspected to play important roles in maize ear development. Thus, identification and characterization of important genes and regulators at all the four developmental stages will contribute to an improved understanding of the molecular mechanisms responsible for maize ear development.

1. Introduction

Maize (*Zea mays*) is one of the most important crops and widely used model plant. Inflorescence and flower development are critically important for high yields in maize. Maize ears require a low concentration of nitrogen, thus making it more efficient and aiding in a sustainable production of crop without adding more fertilizer to the soil after harvest [1]. Various mutants have been discovered, providing insights into the molecular processes involved in the ear development [2–7]. However, understanding of the maize ear developmental dynamics at the transcriptome level is limited. Till now, only few studies have been conducted on the large-scale gene expression analyses of the maize ear, including, (i) evaluation of sequence-based expression profiles during reproductive organ development [8], (ii) study on the effect of water-deficiency on immature maize ear development [9], and (iii) discovery of novel microRNAs during maize ear development [10].

The B73 sequence assembly [11] enables analysis of maize ear development at a genome-wide transcriptome level. Owing to the dramatic decrease in the cost of sequencing and development of rapid and robust experimental procedures, it is now feasible to conduct a cost-efficient high-throughput profile analysis. For instance, by using digital gene expression (DGE) [12–15] and RNA-Seq [16–18] analyses, new genes have been discovered [19]. Furthermore, these technologies are useful for estimating the overall gene expression at different developmental stages or in different tissues [12,20], and in response to abiotic stresses [21,22]. Considering the significance of ears in maize production, it is of great importance to understand the molecular mechanisms involved in the maize ear development.

The objective of this study was to conduct a genome-wide comparative analysis of gene expression profiles to obtain an improved understanding of the molecular mechanisms of maize ear development during four developmental stages; the growth point elongation (I), spikelet differentiation (II), floret primordium differentiation (III), and floret organ differentiation phase (IV) [23] using a DGE approach. Ears of maize from all the four developmental stages were used to study the dynamics of mRNA expression. Quantitative real-time polymerase chain reaction (qRT-PCR) was performed using randomly selected

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DEGs, in order to validate their expressions across different developmental stages. The K-means clustering method was employed to further determine the co-expression of genes involved in the maize ear development.

2. Results

2.1. Sequence alignment and expression analysis

Library construction and sequence analysis were conducted [20]. Around 16.8 million high-quality raw reads were generated. After performing quality-control measures, 16.1 million clean tags were obtained for all the four stages (S1 and S2 Tables). Briefly, after removing low-quality and contaminating reads, clean tags were retained for further analysis. Subsequently, the 16.1 million clean tags were aligned against the maize genome (B73 RefGen_v2). The percentage of clean tags in the raw data for each developmental stage was 93.62%, 96.81%, 96.54%, and 96.56%, respectively. About 69.86% clean tags were mapped to the B73 reference genome with an average of 76.10% genes covered. Incompleteness of maize genome sequence data was probably one of the reasons for the occurrence of unmapped tags. Most tags were aligned to genic regions and the genic distribution of reads from mRNA reference sequences in all the four developmental stages (I–IV) showed that, a majority of tags (87.58%, 87.23%, 90.30%, and 90.95%, respectively) were mapped to exon regions and the remaining were distributed within introns, intergenic regions and repeat regions (S1 Fig.).

Majority of transcripts were expressed in all the four stages (Fig. 1A and S3 Table). The numbers of sense (Fig. 1A) and antisense (Fig. 1B) transcripts overlapping at all 4 stages were 11,970 and 4416, with a cut-off for gene expression at each stage of one tag per million (at least 4 reads). The number of genes that showed both sense and antisense expressions were 7230, 7052, 6918, and 6571 (Fig. 1C) for each developmental stage, respectively, and 10,456 in all stages in both sense and antisense expression. Of all the sense genes detected, only 74 genes were expressed uniquely in stage I, and were even lower than that of the other 3 developmental stages, suggesting the involvement of more genes in the maize ear development.

2.2. Analysis of differentially expressed genes and validation by qRT-PCR

Based on a cutoff of at least 4 reads per gene, all reads that were mapped to genes were used for differential expression analysis combined with the DGE method for a genome-wide comparative analysis of data for all the 4 developmental stages. Comparative gene expression analyses were used for estimation of gene expression levels in all the four developmental stages (S4 Table). We calculated the number of tags corresponding to each gene in each library to estimate the gene expression levels and compare the difference in fold-change between the developmental stages [20]. Transcripts that showed differential expression levels are shown in S2 Fig. The up-regulated and down-regulated genes indicate the DEGs (Fig. 2, S2 Fig., and S5 Table). In total, the number of DEGs between two stages were as follows: 3325 between stages I and II (36% up- and 64% down-regulated in stage II), 4735 between stages I and III (57% up- and 43% down-regulated in stages III), 6398 between stages I and IV (46% up- and 54% down-regulated in stage IV), 3765 between stages II and III (71% up- and 29% down-regulated in stage III), 5178 between stages II and IV (60% up- and 40% down-regulated in stage IV), and 1698 between stages III and IV (35% up- and 65% down-regulated in stage IV).

To better understand the dynamic changes of gene expression in maize ear development during all the four developmental stages, further analyses of the DEGs were performed, especially of those genes in which up- or down-regulation gradually follow ear development (II *vs.* I, III *vs.* II, and IV *vs.* III; S5 Table). Among the DEGs identified, 1201, 2690, and 594 genes were up-regulated in stages II, III, and IV, respectively, compared with their own preceding stage. In contrast, the numbers of down-regulated genes were 2124, 1075, and 1104 in stages II, III, and IV, respectively (Fig. 2 and S5 Table). During the adjacent developmental stages, nearly two third of DEGs were up-regulated in developmental stage III (Fig. 2) *vs.* stage II or stage IV. This suggests that DEGs were more abundant in stage III, indicating an active ear development during stage III (floret primordium differentiation phase). Furthermore, the expression patterns of 9 DEGs are illustrated in S3 Fig. Interestingly, we found some well-known genes with reported mutants analyses during maize inflorescence development, such as, *compact plant2* (*ct2*), *zea AGAMOUS homolog1* (*zag1*), *bearded ear* (*bde*), and *silky1* (*si1*) [24].

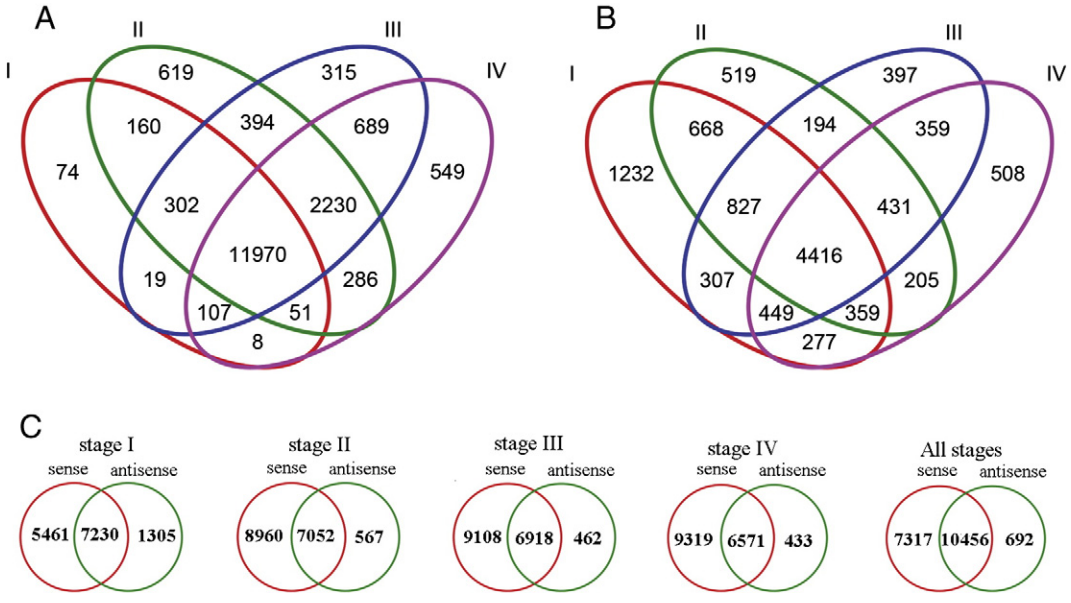

Fig. 1. Comparison of four development stages of maize ear. Comparison of genes expressed in sense (A) and antisense (B) directions in the four development stages. Overlaps show the number of genes shared between stages. (C) A Venn diagram shows the genes expressed in sense and antisense direction in each developmental stage, and all stages.