Gene Expression Variation Explains Maize Seed Germination Heterosis

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Abstract

Background

Heterosis has been extensively utilized in plant breeding, however, the underlying molecular mechanism remains largely elusive. Maize (*Zea mays*), which exhibits strong heterosis, is an ideal material for studying heterosis.

Results

In this study, there is a faster imbibition and development in reciprocal crossing Zhengdan958 hybrids than in their parent lines during seed germination. To investigate the mechanism of heterosis of maize germination, comparative transcriptomic analyses were conducted between reciprocal crossing hybrids and their parental lines. The gene expression patterns showed that 1324 (47.27%) and 1592 (66.44%) of the different expression genes between hybrids and either parental line display parental dominance up or higher levels in Zhengdan958 and Zhengdan958 reciprocal-cross, respectively. Notably, these genes were mainly enriched in metabolic pathways, including carbon metabolism, glycolysis/gluconeogenesis, protein processing in endoplasmic reticulum, etc.

Conclusion

Our results provide evidence for the higher expression level genes in hybrid involved in metabolic pathways acting as main contributors to maize seed germinating heterosis. These findings provide new insights into the gene expression variation of maize embryo and improve the understanding of maize seed germination heterosis.

Background

Plant heterosis, or hybrid vigor, refers to the phenomena that hybrid F1 generation over both of its parental homozygous lines in many traits such as biomass, grain yield, growth rate, resistance to biotic and abiotic stress, growth potential, and adaptability [1, 2]. While the utilization of heterosis has greatly increased the productivity of crop plants, the underlying molecular mechanisms remain enigmatic [3, 4]. To explain heterosis, three major theories, namely dominance, over-dominance and epistasis, respectively, were established. The dominance model believes that the better performance of hybrids was resulted by the aggregation of favourable alleles of both parents [5-7], among which the “good genes” pyramid in F1 were considered the key contributing to heterosis [8, 9]. The over-dominance hypothesis holds that the interaction between heterozygous alleles is greater than the homozygous alleles [10, 11]; while Epistasis refers to the interaction between non-alleles on chromosomes [12, 13].

In the last decade, the gene expression regulation network has been made to dissecting the genetic basis of heterosis with the rapid development of bioinformatics technology [14-16]. The additive and non-additive gene expression is a mixture of heterosis causal and effects [17]. The reason why these genes

expression as the given model is determined by up-stream divergences, including cis-elements variations, trans-acting factor divergence, and divergent epigenetic effects [18-20]. Cis-element variation is one reason of heterosis, since the hybrids gene expression was under the same trans action factor environment. It has been revealed that alleles from parental lines may controlled by the specific binding cis-elements, resulted as allelic specific expression [21]. In hybrid lines, the divergent trans action factors from one parental line may influence the cis-elements, existing in the promoter, of the allele from another parental line, resulting in gene different expression [22]. The transcription factors may also function by forming complexes, it added another layer of trans regulation divergence that combination of two or more transcription factors, or the competing binding of co-regulators, will change the DNA binding specificity [23]. It has reported that Epigenetics associated with distinct changes in the DNA methylation patterns may affect heterosis too [20, 24, 25].

Allele-specific expression (ASE, also called allelic expression or allelic imbalance), describing the expression variation between the two haplotypes in a diploid genome which showed heterozygous sites. Genome wide ASE were caused by cis-elements variants, genome imprinting, and nonsense-mediated decay [26, 27]. The interactions between trans and cis acting factors and their diversity further enhanced the complexity of ASE [28]. Allelic expression of multiple genes was considered to correlate to heterosis [29]. The extreme ASE, specific parental expression (SPE), was considered associated with dominant effect [30-32].

Seed germination is a fundamental process, which directly influences the development of maize plants and further affects grain yields. During the early stage of germination, seeds absorb water rapidly from the field. Secondly, the metabolic process, generating a large number of energies, is reinitiated with various enzymes and hormones. Finally, the radicle breaks through the seed coat and then activates seedlings establishment [33]. It is suggested that F1 hybrid seeds show a superior performance compared to its inbred parental lines in terms of seed germination [34]. It has also been proved that embryo expansion rate at early stages of maize seed germination is one of the heterosis traits [35]. It is good to adopt germinating seeds as the suitable material to dissect plant heterosis.

In this work, gene expression and gene regulation of maize seed germination heterosis were obtained by RNA seq followed by bioinformatics analysis. The cis and trans regulation variations were experimental arranged to figure out the key up-stream factors influencing seed germination heterosis. Notably, 1324 (47.27%) in Zhengdan958 and 1592 (66.44%) Zhengdan958 reciprocal-cross of the different expression genes displayed above or equal to the level of higher parent pattern in hybrids. Further KEGG pathway analysis indicates that these genes are involved abundantly in metabolic pathways accounting for the carbon metabolism, glycolysis / gluconeogenesis, pyruvate metabolism. The ASE genes (116 in Z58*C72 and 579 in C72*Z58) were mainly enriched in protein processing in endoplasmic reticulum pathway. We propose that the high expression of metabolism process genes in hybrids promote metabolic rate and enable faster rates of energy acquisition, which may contribute to germination heterosis.

Results

Maize seed germination is a trait with heterosis.

Maize seed germination is a quite good point to study hybrid vigor, since during this stage the germinating seeds influenced less from the environment [34-36]. The first stage of seed germination is embryos imbibition, at this stage, embryos were germinated under the support of nutrition stored in endosperm. It has bare communication with outside environments before the radicle grow out of the tip. It was shown that at this stage, the reciprocal crossing hybrids were fully imbibition, with radicle protrusion initiated earlier than in the two parental lines (Fig.1A).

Introduction of RNA seq data

A total of 12 germinating embryo samples were collected for RNA-seq profiling, including four genotypes (Reciprocal-crosses and two parental lines), each with three biological replicates. Approximately 51–86 million clean reads were obtained for each sample. An overview of the RNA-seq data sets in this study is shown in Table 1. After low quality reads were filtered, more than 98% clean reads mapped to the maize B73 reference genome (B73_RefGen_v4).

Table 1. Overview of RNA-seq data.

Sample Name	Raw Reads	Clean Reads	Clean Reads Rate (%)	CleanQ30	Mapped Reads	Mapped Rate (%)
Z58-1	57,761,405	51,101,515	88.47	93.90;93.49	5026345015	98.36
Z58-2	93,888,829	86,546,723	92.18	90.63;88.68	8538699691	98.66
Z58-3	69,831,586	63,400,097	90.79	91.63;91.40	6270903594	98.91
C72-1	63,556,262	58,160,335	91.51	88.45;89.53	5722395361	98.39
C72-2	68,348,998	61,842,173	90.48	90.43;88.75	6084032980	98.38
C72-3	67,664,143	63,387,769	93.68	90.43;91.73	6246230757	98.54
Z58*C72-1	75,728,777	70,049,119	92.5	92.83;90.29	6915949519	98.73
Z58*C72-2	58,642,488	53,024,538	90.42	90.33;89.92	5238294109	98.79
Z58*C72-3	67,336,587	61,114,686	90.76	89.43;85.13	6016740837	98.45
C72*Z58-1	83,526,998	77,354,353	92.61	91.06;91.53	7607800618	98.35
C72*Z58-2	77,185,580	71,458,410	92.58	92.98;90.25	7024361703	98.3
C72*Z58-3	67,380,639	63,182,825	93.77	92.61;92.03	6220980950	98.46

Differentially expressed gene identification

To achieve a comprehensive overview of differential gene expression, we identified differentially expressed genes (DEGs) between all possible (N=6) pairwise comparisons of the four genotypes. When controlling FDR at 5%, 2870 genes were significantly differentially expressed between parental lines after setting a threshold ($\log_2$ fold change $> \pm 1$). In the comparison between the reciprocal hybrids, 501 specific genes were differentially expressed. The overall number of genes differentially expressed between the four hybrid-inbred line comparisons were estimated to be ranged from 1011 to 1932 genes (Fig.1B). Thus, the level of expression divergence between inbred-hybrid comparisons is higher than the comparison of the reciprocal hybrids and lower than the comparison of the two parental inbred lines. Interestingly, among the genes which differentially expressed between parental lines and reciprocal hybrids, the expression of the majority genes (67.81% to 91.49%) in reciprocal hybrids is higher than that in the parental lines. It has suggested that differential gene expression among hybrids and their parents play important roles in heterosis [8, 37]. Hence, these higher expressed genes may contribute more to heterosis during maize seed germination.

Inheritance Classifications

According to comparing the expression level of hybrids and their parental lines, the genes divergently expressed between hybrids and parental lines were categorized into eight classes (Fig.2A). There are 2801 DEGs between Z58*C72 and either parental line. 222 (7.93%) were classified as additivity C72>Z58, 160 (5.71%) were additivity Z58>C72, 527 (18.8%) were C72 expression level dominance up, 67 (2.39%) were C72 expression level dominance down, 797 (28.5%) were Z58 expression level dominance up, 229 (8.18%) were Z58 expression level dominance down, 442 (15.8%) were transgressivity up, 357 (12.7%) were transgressivity down. Based on this classification scheme, 1324 genes (47.27%) of divergently expressed genes between hybrid and parental line were abundant in C72 or Z58 expression level dominance up category. 442 (15.78%) genes exhibited transgressivity up levels. Similar result was obtained in C72*Z58 (Figure 2). Furthermore, both in Z58*C72 or in C72*Z58, the most abundant type included Z58 expression level dominance up (approximately 50% of divergent expression genes) (Fig.2B). This suggest that the majority (63%) of the DEGs is expressed at parental dominance up or higher levels in the hybrid. These results indicated that the genes showing parental dominance up or higher levels pattern might correlate with the observed seed germinating heterosis.

Identification of Allele-specific expression genes

It has been suggested that ASE, or imbalance between the expression levels of two parental alleles in the hybrid, was associated with heterosis [38]. In order to further explore the potential relationship between ASE and heterosis, genome-wide analysis of ASE was performed by comparing the reads ratio of the parental alleles in transcriptome scale. A total of 116 and 579 genes showed ASE in Z58*C72 or C72*Z58, respectively. Out of the 116 ASE genes in Z58*C72, 41 of which overlap with the DEGs and the allelic ratio of these genes in hybrid is comparable to the parental proportions. Out of the 579 ASE genes in C72*Z58, 108 of which overlap with the DEGs and the allelic ratio of these genes in hybrid is similar to

the parental proportions. This means that the ASE genes divergently expressed between hybrids and parental lines are mainly due to cis-acting regulation.

Analysis of Functional Enrichment of Gene Ontology

Of the DEGs, almost half of the genes were in the parental dominance up level category. The distribution of genes in categories was similar to what has previously been observed for hybrids [18]. Consequently, these genes classified as parental dominance up and transgressivity up level may play important roles in heterosis. These genes were enriched to different classes and further analyzed using the AgriGOv2 database. The top 20 significantly enriched GO terms were shown in Table S2. In the biological process category, the most represented GO terms were response to temperature stimulus, response to heat, protein folding. Most of the molecular function-related genes were involved in unfolded protein binding, RNA binding, heat shock protein binding. For the cellular constituent category, the most terms were cytoplasm, cytosol, macromolecular complex (Fig.3A).

The KEGG analysis indicated that the identified genes were mainly enriched in protein processing in endoplasmic reticulum, proteasome, pyruvate metabolism, endocytosis, glycolysis / gluconeogenesis, carbon metabolism (Fig.3B). Notably, these pathways are significantly associated with metabolic regulation. the significant KEGG pathways were supplied in Table S3.

RT-qPCR confirmation

A subset of DEGs involving in metabolic pathways during maize seed germination were selected for RT-qPCR validation. These genes were mainly binned parental expression level dominance up category or above high parent expression level and involved in Carbon metabolism, Glycolysis/Gluconeogenesis and Pyruvate metabolism pathways. The RT-qPCR results suggested that the expression inheritance of selected genes was strong consistent with RNA-seq data (Fig.4), indicating that our result is reliable.

Discussion

Heterosis, or hybrid vigor, was broadly used in breeding programs to improve maize yield, however, its molecular bases is far from clearly described [39, 40]. It is believed that maize heterosis is traits specific, since heterosis of one trait cannot serve as an efficient predictor of another trait heterosis [41]. The germination results of our previous study have demonstrated that the seeds showed a superior germination ability in maize hybrid than their parental lines [36]. Similar to our previous results, Guo et al. reported that the significant alterations between hybrids and their parents in protein level might correlate with the superior performance in hybrids [42]. The first progress of seed germination is imbibition, during which the seeds influenced by no environmental factors other than water. Consequently, maize seed germination is a good trait to study heterosis.

Gene expression divergence of mRNAs, miRNAs, and tRFs were shown to explain the superior vigor in hybrids compared to their parental lines [43, 44]. It is interesting to find that most differently expressed

mRNAs were higher expressed in hybrids, wherever miRNAs were largely repressed [36]. In the present study, the imbibition ability of hybrids was shown to be higher than both parental lines. Similar results were also corroborated by Meyer et al. [45], which stated that hybrids performed better than their parental lines owing to higher gene expression and greater metabolic efficiency during the early stage of germination. In the study researched by Li et al. [34], exogenous abscisic acid (ABA) sensitivity in hybrid B73/Mo17 was lower than in parental inbred lines and the content of abscisic acid in hybrid embryos declined more quickly. The authors stated that the reason of non-ABA sensitivity in hybrid B73/Mo17 is due to the declining ABA gene displaying an above high inbred parent pattern. Consistent with our study, the germination results suggest that the genes with high parent dominance pattern or above high parent pattern play important roles in maize seed germination process.

Previous studies have suggested that ASE is also considered as a mechanism of heterosis. According to the results for a genome-wide analysis of ASE obtained by Shao et al. [24], about 11.5% of the genes showed ASE which may cause dominance or overdominance effects in hybrid. Using RNA-sequencing technology, Hu et al. [29] discovered that 23.8% of expressed genes were identified as being significantly allelic expression and reported a cis-regulatory mechanism of ASE in F1 hybrids. In an elite maize hybrid Zhengdan808, 56.4% and 52.4% of analyzed transcripts showed significant allelic expression difference at the spikelet and floret stages, respectively [29]. However, in our study, A total of 116 and 579 ASE genes were obtained in Z58*C72 or C72*Z58, respectively. Notably, a subset of the ASE genes, 41 in Z58*C72 and 108 in C72*Z58, overlap with the DEGs between the hybrid and parental line. These ASE genes are mainly driven by cis-regulation. This phenomenon might be related to the mechanism of heterosis during maize seed germination.

Seed germination is a complicated process, which affects crop growth and development and ultimately affects grain yield. Because of the lack of photosynthesis, the initial nutrients are largely dependent on the reserve substances in seed at the seed germination and post-germinative seedling establishment stage [46]. With absorbing water, the metabolic process of seeds was activated rapidly and provided essential nutrition and energy for seed germination and development [47]. Our transcriptome results suggested that the majority of the DEGs between hybrid and parental line were involved in metabolic pathways, most of which were mainly enriched in Carbon metabolism, Glycolysis / Gluconeogenesis and Pyruvate metabolism. Notably, a large number of DEGs involving in metabolic pathways displayed parental dominance up or higher levels expression inheritance. Of them, the gene Zm00001d003247 encodes malate synthase, involved in pyruvate metabolism, carbon metabolism pathways, which is an important enzyme of the germination process [48]. The gene Zm00001d032187 encodes malate dehydrogenase, and loss-of-function mutants of its homologue in Arabidopsis affect maturation process and post-germination growth that leads to slow germination rates, shorter root length, and decreased root biomass [49]. Additionally, several pyruvate kinase genes were classified in parental dominance up or transgressivity up inheritance, such as Zm00001d026619, Zm00001d052494 and Zm00001d023379, whose expression level are correlate with cell proliferation [50]. In Arabidopsis, loss of pyruvate kinase activity alters the accumulation of storage oil delaying germination and seedling establishment [51]. Consistent with our present results, Lisec et al. stated that metabolite levels are significant correlate with

the biomass heterosis in *Arabidopsis thaliana* [52]. Similar study was also found in soya bean, in which the metabolic genes contribute to heterosis or hybrid vigor [53]. In rubber tree, carbohydrate metabolism are highly associated with growth heterosis, which was further validated by Yang et al [54]. Using transcriptome and metabolite analysis, Yi et al. revealed that the expression of genes involved in primary and secondary metabolism play important roles in biomass heterosis in rapeseed [55]. In conclusion, we propose that the expression levels of genes related to metabolic in hybrids are higher than those in parental lines and this promote faster metabolic rate in hybrids. Further, faster metabolic rate promotes energy acquisition, cell growth and reproduction, leading to heterosis.

In this study, we investigated genome-wide gene expression variation of maize embryo in germination stage of reciprocal hybrids zhengdan958 and their parental lines. Our research suggests that the majority of different expression genes were higher in hybrids than in their parental lines. The different expression genes were mainly enriched in metabolic pathways, such as carbon metabolism, glycolysis/gluconeogenesis, which plays important role in germination heterosis. Our work provides a comprehensive insight about the molecular mechanisms of heterosis during maize seed germination development.

Materials And Methods

Maize plants and planting field

Maize hybrid Zhengdan958 is the most widely cultivated hybrid in China, which exhibits high heterosis for grain yield [35]. Together with its parental inbred lines Zheng58 (maternal line, referred to as Z58 hereafter) and Chang7-2 (paternal line, referred to as C72 hereafter) were utilized for this study. The dent inbred line Z58 was selected from a synthetic population of Chinese domestic germplasm with Reid lineage, and the flint inbred line C72 was selected from the Chinese heterotic group Sipingtou [56]. In summer of 2018, the two parental lines were planted in farm of Henan agricultural university (Zhengzhou, China). Self-cross and Reciprocal-crosses were made for these two lines.

Seed germinating condition and sample phenotyping

After harvesting, eighty seeds of the reciprocal hybrids and each parental line were surface-sterilized in 70% (v/v) ethanol for 15 min, followed with rinsed three times with sterile distilled water. For each genotype, three biological replicates were prepared. The germinating embryos with 24 hours imbibition at a temperature of 28 °C in an incubator were immediately sampled and stored at -80 °C for further use.

RNA extracting and RNA seq (data analysis)

Embryo tissues of each line were separated from the seeds after 24 hours of dark incubating at 28°C submerged in sterile distilled water. The embryo tissues were peeled from the seeds, and each sample was mixed for immediate RNA extraction. Total RNAs were extracted with TRIzol reagent (Invitrogen) following the manufacturer's protocol. The total RNAs were processed to reverse transcription reactions

to obtain cDNAs following by constructing of RNA-seq libraries. The same cDNAs were supplied as RT-qPCR templates. To obtain the heterosis related gene expression, next-generation deep-sequencing was performed. For RNA-seq, 12 libraries were established and accessed by Agilent 2100 Bioanalyzer and ABI StepOnePlus RT-qPCR System for paired-end sequencing with Illumina HiSeq 2500 (Beijing Beirui Biotechnology, Beijing, China).

RNA-seq data analysis (including QC and DEG)

The raw data obtained by sequencing was filtered using Trimmomatic to remove adapters and low-quality reads [57]. The filtered reads were mapped to the B73 maize reference genome (B73 AGPv4, http://ensembl.gramene.org/Zea_mays/Info/Index) using Hisat2 [58]. Gene expression was then estimated using StringTie [59], and differential expression analysis of genes was performed using DESeq2 based on gene expression amounts with a fold change >2 and a false discovery rate (FDR) < 0.05 [60]. The gene ontology (GO) enrichment analysis of these DEGs was performed using agriGO v2.0 (<http://systemsbiology.cau.edu.cn/agriGOv2/index.php>) with singular enrichment analysis [61]. The Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were conducted using the web-based tool KOBAS (<http://kobas.cbi.pku.edu.cn/>) [62].

Expression classification

To identify the classification of the different expression genes, DESeq-normalized expression values of each genotype for each gene were obtained. According to previously described by McManus et al.[63], the genes with more than 2-fold expression changes between hybrids and either parent were thought to have non-conserved inheritance. Based on the Log-transformed values of the ratio of hybrids and parental line, these genes were further classified into eight groups as displaying additive (with Z58 expression lower or higher than C72 expression), Z58 or C72 parental dominance (up and down), and transgressivity (lower or higher than both parent: down and up), respectively. Genes for which expression in the hybrid was higher than Z58 and lower than C72 was classified as additive C72>Z58 ($\log_2^{(\text{hyb}/\text{Z58})} > 1, \log_2^{(\text{hyb}/\text{C72})} < -1$). Genes for which expression in the hybrid was higher than C72 and lower than Z58 was classified as additive Z58>C72 ($\log_2^{(\text{hyb}/\text{Z58})} < -1, \log_2^{(\text{hyb}/\text{C72})} > 1$). Genes for which expression in the hybrid was higher than Z58 and equal to C72 were classified as C72 dominance up ($\log_2^{(\text{hyb}/\text{Z58})} > 1, \text{abs}(\log_2^{(\text{hyb}/\text{C72})}) < 1$). Genes for which expression in the hybrid was lower than Z58 and equal to C72 were classified as C72 dominance down ($\log_2^{(\text{hyb}/\text{Z58})} < -1, \text{abs}(\log_2^{(\text{hyb}/\text{C72})}) < 1$). Genes for which expression in the hybrid was higher than C72 and equal to Z58 were classified as Z58 dominance up ($\log_2^{(\text{hyb}/\text{C72})} > 1, \text{abs}(\log_2^{(\text{hyb}/\text{Z58})}) < 1$). Genes for which expression in the hybrid was lower than C72 and equal to Z58 were classified as Z58 dominance down ($\log_2^{(\text{hyb}/\text{C72})} < -1, \text{abs}(\log_2^{(\text{hyb}/\text{Z58})}) < 1$). Genes for which expression in the hybrid was higher than both C72 and Z58 were classified as transgressivity up ($\log_2^{(\text{hyb}/\text{C72})} > 1, \log_2^{(\text{hyb}/\text{Z58})} > 1$). Genes for which expression in the hybrid was lower than both C72 and Z58 were classified as transgressivity down ($\log_2^{(\text{hyb}/\text{C72})} < -1, \log_2^{(\text{hyb}/\text{Z58})} < -1$).

Allelic expression analysis based on re-sequencing SNPs

We used the SNPs between Z58 and C72 based on our previously re-sequencing data as the reference for ASE calling. For the results obtained from the Hisat2 comparison, GATK was used to perform mutation detection. Normalization of trimmed reads number was then performed using function Estimate Size Factors provided by DESeq2 package. For each gene, the ASE was identified by SNPs between two parental genomes. Three thresholds should be met to filter the ASE: (1) Every SNP had at least five reads to support it; (2) There was Significant deviation of the read counts from the two parental alleles; (3) the significant deviation of different SNPs in the same gene was not in different directions.

Gene expression verification

To verify the reliability and reproducibility of the results obtained in the RNA-Seq, the expression of some key genes involving in metabolic pathways were selected and further verified by RT-qPCR. The RT-qPCR primers are listed in Table S1. RNA samples were treated with DNase I and further reverse transcribed to cDNA using SYBR Premix Ex Taq II (Tli RNaseH Plus) kit. The RT-qPCR were run on a CFX96 RT-qPCR Detection System. The housekeeping gene *ZmActin1* was set as the endogenous control [64]. The relative expression of each gene was calculated with the $2^{-\Delta\Delta C_t}$ method [65].

Abbreviations

DEGs: Differentially expressed genes

ASE: Allele specific expression

GO: Gene ontology

RT-qPCR: Real-time quantitative PCR

SPE: Specific parental expression

Declarations

Ethics approval, guidelines and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The sequence datasets in fastq format of the current study are available in the NCBI Sequence Read Archive (SRA) database under Bioproject PRJNA788131 (<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA788131>).

Competing interests

The authors declare that they have no competing interests.

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Author' Contributions

DD and JHT designed the research. JW and QYW performed the experiments. JW wrote the manuscript, JWZ, KTD, ZYG, DSH, SJM, YL, XQQ, LQM revised the manuscript. All authors approved the final manuscript.

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Figures

Figure 1

Phenotypic observations of maize embryo imbibition and overview of differential gene expression. A The transcriptomes of maize embryo of 24 hours imbibition of zhengdan958 and their parental lines (C72 and Z58) were compared. B The differently expressed genes of six pairwise comparisons of the four genotypes are shown.

Figure 2

Inheritance classification of gene expression in reciprocal zhengdan958 A The scatterplots show the gene expression classification between reciprocal hybrids and their parental lines (C72 on the x axis and Z58 on the y axis). B The bar graphs show the number of genes in each expression inheritance category for Z58*C72 and C72*Z58 hybrids.

Figure 3

GO and KEGG enrichment analysis of parental dominance up or higher levels genes. A The top 20 GO term analysis of the shared genes. B The significant KEGG pathways of the shared genes.

Figure 4

The relative expression levels of twelve genes involved in metabolic pathways as assessed by RT-qPCR.

Supplementary Files

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