Towards the understanding of genetic and molecular basis of heterosis in wheat (*Triticum aestivum* L.)

Sun Qixin, Ni Zhongfu, Peng Huiru, Yao Yingyin, Du Jinkun, Liu Gang

Department of Plant Genetics and Breeding, China Agricultural University, Beijing, 100094, China

INTRODUCTION

The discovery of hybrid vigor or heterosis and its exploitation in modern breeding programs is one of the most important advances in plant improvement. Hybrid cultivars have been used commercially in many crop plants, and have made significant contributions to the world food supply [1]. Hybrid wheat was first commercialized in the United States in the 1970s [2]. Since then, it is cultivated in Australia, China, South Africa and India [2]. In India, the reported adoption of hybrid wheat was 60,000 acres in 2005. In China, more than 10 hybrid wheat cultivars have been registered by 2007, and hybrid wheat is planted in more than 10000 hectares annually, with yield advantage of 20%. Despite the extensive efforts in hybrid wheat breeding, however, mechanisms of wheat heterosis are largely unknown. Attempts have been made in our research group for last decade to understand genetic and molecular basis of heterosis in wheat.

GENETIC DISSECTION OF HETEROSIS

Up to date, based on QTL mapping, three types of QTL interaction responsible for the heterosis have been reported in different crops, i.e., overdominance in maize, dominance in rice and epistasis in rice. In this study, 188 recombinant inbred lines (RILs) derived from the cross between common wheat (*T. aestivum* L.) 3338 and spelt wheat (*T.spelta* L.) Altgold was used to construct an immortalized F_2 (IF_2) population consisting of 227 hybrids. The IF_2 population was used to investigate the genetic basis of heterosis. Firstly, a molecular marker linkage map comprising of 288 DNA markers was constructed, which covered 3905.9cM of total genome size, with an average distance of 13.6cM between the pair of markers**.** Secondly, heterotic loci were detected for six traits of the IF_2 population with modified composite interval mapping, including plant height, panicles per plant, grain number of major panicle, 1000-grain weight, spike length and yield per plant. A total of 39 heterotic loci (HL) were detected in one year for these six traits using their mid-parent heterosis as the input data. It was found that 23 out of the 39 HL showed negative dominance effect, which resulted in less net dominance effect of these traits. In addition, 27 out of the 39 HL showed over dominance effect, suggesting that over dominance effect at single locus is the main genetic basis of heterosis in the hybrid. Furthermore, QTL analysis was conducted for the RIL population using multiple years and sites phenotype data of the six traits. The results showed that these QTLs were distributed on 13 chromosomes, and they explained the variation from 5.04% to 43.53%. Comparison between the QTL and the HL of each trait revealed that most of them were not in the same chromosome region, which implied that heterosis and trait performance might be controlled by different sets of loci.

DIFFERENTIALLY GENE EXPRESSED PATTERNS IN HYBRID AS COMPARED TO THEIR PARENTS

Although all the genes in hybrid F_1 are derived from its parental inbreds, hybrid performance is quite different from its parental inbreds. Therefore, it is reasonable to speculate that differential gene expression between hybrids and their parents should contribute to the observed heterosis. In our earlier studies [3,4] we found that that both quantitative and qualitative differences could be observed. The quantitative differences include: (i) over-expression of parental genes in hybrids; (ii) under-expression of parental genes in hybrids; (iii) dominant expression of parental genes in hybrids. Qualitative differences are observed mainly as silencing of parental genes in hybrids, which includes: (i) silencing in hybrid of genes expressed in both parents; (ii) silencing in hybrid of genes expressed either in female or male parent. Expression in hybrid of genes only expressed either in male or female parent was also observed.

SOME OF THE DIFFERENTIALLY EXPRESSED PATTERNS ARE CORRELATED WITH HETEROSIS IN YIELD AND RELATED TRAITS

Next, we want to determine if these differentially expressed patterns are related to the observed heterosis. For this study, improved differential display was used in this study to analyse alterations in gene expression between hybrid and parents in leaves at jointing stage and heading stage in a wheat diallel cross involving 20 hybrids and nine parents, with the purpose of determining the relationship of differential expression patterns with heterosis in nine agronomic traits. About 2800 fragments were displayed from each hybrid and its parents in both developing stages. UPnF1 (fragments that occurred in either of the parents but not in F_1) pattern is negatively correlated with heterosis in seven traits, while F_1nBP (fragments observed only in F_1 but not in either of the parents) pattern is positively

correlated with heterosis in six traits, and UPF_1 (fragments present in one of the parents and F_1) pattern is positively correlated with heterosis in three traits. $BPnF_1$ (fragments observed in both parents but not in F_1) is not correlated with heterosis in any of the nine traits. We conclude that these differentially expressed genes, though functionally not known yet, play an important role for hybrids to demonstrate heterosis [5].

IDENTIFICATION AND CHARACTERIZATION OF DIFFERENTIALLY EXPRESSED GENES

Since genes are differentially expressed between hybrid and parents, and these differentially expressed patterns could be related to wheat heterosis, we want to know the categories of these differentially expressed genes. For this purpose, an interspecific hybrid between common wheat (*Triticum. aestivum.* L, 2n=6x=42, AABBDD) line 3338 and spelt (*Triticum. spelta* L. 2n=6x=42, AABBDD) line 2463 was used for expression assay. A modified suppression subtractive hybridization (SSH) was used to generate four subtracted cDNA libraries. A total of 748 non-redundant cDNAs were obtained, among which 526 had high sequence similarity to the GenBank entries and represent diverse of functional categories, such as metabolism (41.4%), cell growth and maintenance (18.2%), signal transduction (6.7%), photosynthesis (8.6), response to stress (3.3%), transcription regulation (1.9%) and others (9.8). The expression patterns of 68.2% SSH-derived cDNAs were confirmed by reverse northern blot, and semi-quantitative RT-PCR exhibited the similar results (72.2%) [6]. A genome-wide gene expression analysis in roots of the heterotic inter-specific hybrid 3338/2463 and its parental inbreds was also conducted by using Barley GeneChip. A total of 1187 genes displayed difference in gene expressions between hybrid 3338/2463 and its parents, and they can be clustered into eight differential expression patterns. Further analysis revealed that among these 1187 genes, 975 genes showed high sequence similarity to the GenBank entries, and represented diverse functional categories, such as metabolism, cell growth and maintenance, signal transduction, response to stress, transcription regulation and others. Fourteen genes were selected for RT-PCR analysis and expression patterns of 9 (64.29%) genes were confirmed. Remarkably, 380 differentially expressed genes could be mapped on the Chinese Spring deletion bins, and with the number of genes in seven homoeologous groups being 158, 148, 121, 140, 132, 94 and 127 respectively. It is concluded that a combination of systematic identification of differentially expressed genes with comparative mapping would provide further insight into understanding of molecular basis of heterosis [7].

We also found that some members of large gene families, such as Expansin, Ribosomal proteins, MYB, MADS-box, WRKY transcription factors were also differentially expressed between hybrids and parents, but with a tissues- or developmental stage-dependent manner, indicating the transcriptional regulation could participate in the differential gene expression [8-14].

ALLELIC EXPRESSION VARIATION IN WHEAT HYBRID

Allelic variation is common in the genomes of organism and provides raw materials for species evolution and breeding. Nucleotide sequence variation can potentially alter protein function or affect the level of gene expression. Recently, it has been reported that allelic variation in gene expression may contribute to human genetic disease and plant heterosis. The characterization of allelic variation is difficult in hexaploid wheat due to its large and complex genome. In this study, two EST-SSR markers were selected for allele-specific expression analysis in wheat. By using *CAU36* and CAU328, four and three allelic variations were identified in thirty-five wheat genotypes, respectively. The dHPLC analysis indicated that, for the two genes analyzed, unequal expression of the two alleles (biallelic) in uppermost internode of heading stage were detected in a group of cross combinations. Further investigation showed that the allelic expression variation detected by *CAU36* is positively correlated with plant height of some specific hybrids. It was also concluded that EST-SSR markers combined with dHPLC will be an efficient method for identification of allelic gene expression in wheat [15].

PROTEOME ANALYSIS BETWEEN HYBRID AND PARENTS

Although transcriptome analyses of gene expression have contributed to our understanding of the heterosis in rice, maize and wheat, changes on the level of mRNA do not necessarily indicate changes on the protein level. Therefore, differential protein expression between hybrid and its parental lines is still an area to be elucidated. We carried out a comparative proteomic analysis in seedling leaves and roots between wheat hybrid and parents. In roots, a total 45 differentially expressed protein spots were detected, and both quantitative and qualitative differences could be observed. Moreover, 25 of the 45 differentially expressed protein spots were identified, which were involved in diverse pathways. The expression patterns of the total proteins in seedling leaves were also compared between hybrid and its parent by using two-dimensional gel electrophoresis with two pH ranges for the first dimension separation. Moreover, 30 of the 49 differentially expressed protein spots were identified, which were involved in metabolism, signal transduction, energy, cell growth & division, disease & defense, secondary metabolism. These results indicated that hybridization between two parental lines can cause expression differences between wheat hybrid and its parents not only at mRNA levels but also at protein abundances, and the proteins differentially accumulated between hybrids and their parents were involved in diverse physiological process pathways, which might be responsible for the observed heterosis [16].

GENE REGULATORY PATHWAY IN HETEROSIS - GIBBERELLINS AND HETEROSIS OF PLANT HEIGHT IN WHEAT

Heterosis in internode elongation and plant height are commonly observed in hybrid plants, and higher GAs contents were found to be correlated with the heterosis in plant height. However, the molecular basis for the increased internode elongation in hybrids is unknown. For this study heterosis in plant height was determined in two wheat hybrids, and it was found that the increased elongation of the uppermost internode contributed mostly to the heterosis in plant height. Higher GA_4 level was also observed in a wheat hybrid. By using the uppermost internode tissues of wheat, we examined expression patterns of genes participating in both GA biosynthesis and GA response pathways between a hybrid and its parental inbreds. Our results indicated that among the 18 genes analysed, genes encoding enzymes that promote synthesis of bioactive GAs, and genes that act as positive components in the GA response pathways were up-regulated in hybrid, whereas genes encoding enzymes that deactivate bioactive GAs, and genes that act as negative components of GA response pathways were down-regulated in hybrid. Moreover, the putative wheat GA receptor gene *TaGID1*, and two GA responsive genes participating in internode elongation, *GIP* and *XET*, were also up-regulated in hybrid. A model for GA and heterosis in wheat plant height was proposed (Fig. 1). Our results provided molecular evidences not only for the higher GA levels and more active GA biosynthesis in hybrid, but also for the heterosis in plant height of wheat and possibly other cereal crops [17].

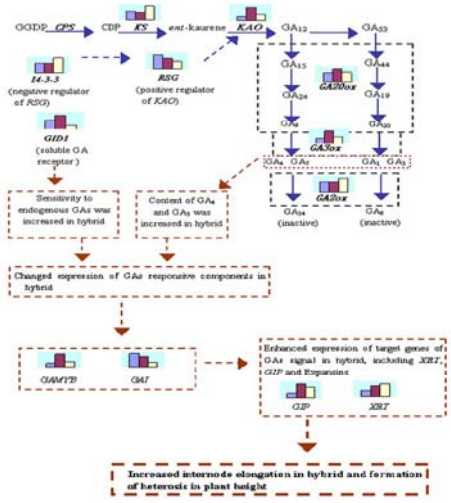

Fig.1 A proposed model for GA biosynthesis and response pathway in regulation of heterosis in plant height. Differential expressions of genes in GA metabolism and response pathways are listed in the box, with the bar heights representing the expression levels of female (left bar), hybrid (middle bar) and male (right bar) parent.

Acknowledgements

This work was financially supported by National Basic Research Program of China (2007CB109000), National Natural Science Foundation of China (30671297), Program for New Century Excellent Talents in University (No. NCET-05-0131) and 863 Project of China

REFERENCES

1. Duvick DN: 1997, In Genetics and Exploitation of Heterosis in Crops. Edited by Coors JG and Pandey S. American Society of Agronomy, Inc. Madison, Wisconsin, USA.pp19-29
2. Matuschke I, Mishra RR, Qaim M. 2007, World Development, 35: 1422–1435
3. Sun, Qixin, Zhongfu Ni & Zhiyong Liu ,1999, Euphytica , 106: 117-123
4. Ni, Z., Q. Sun, Z. Liu, L. Wu and X. Wang , 20 , Molecular and General Genetics, 263(6):934-938
5. Sun, Qixin, Limin Wu, Zhongfu Ni, Fanrong Meng, Zhangkui Wang and Zhan Lin, 2004, Plant Science, 166(3):651-657
6. Yao, Yingyin, Zhongfu Ni, Yinhong Zhang, Yan Chen, Yuhua Ding, Zongfu Han, Zhiyong Liu, Qixin Sun, 2005, Plant Molecular Biology, 58(3) :367-384
7. Zhang, Yinhong, NI Zhongfu, YAO Yingyin, ZHAO Jun and SUN Qixin, 2005, Progress in Natural Science 16：712-720
8. Wu, LM, Ni ZF, Meng FR, Lin Z, Sun QX, 2003, Molecular Genetics and Genomics, 270: 281-286
9. Chen, Rongmin, Zhongfu Ni, Xiuling Nie, Guoqing Dong, Yuxiang Qin, Qixin Sun, 2005, Plant Science 169:1146-1154
10. Lin, Zhan, Zhongfu Ni, Yi Zhang, Yingyin Yao, Haiyan Wu, Qixin Sun, 2005, Molecular Genetics and Genomics, 274:548-556
11. Yao, Yingyin, Zhongfu Ni, Jinkun Du, Xiuli Wang, Haiyan Wu, Qixin Sun, 2006, Plant Science, 170:579-586
12. Zhao, Tao, Zhongfu Ni, Yan Dai, Yingyin Yao, Xiuling Nie, Qixin Sun, 2006, Molecular Genetics and Genomics,276:334-350
13. Wang, Zhangkui, Zhongfu Ni, Hualing Wu, Xiuling Nie, Qixin Sun, 2006, Theor Appl Genet, 113:1283–1294
14. Wu, Hualing, Zhongfu Ni, Xiuling Nie, Ganggang Guo, Qixin Sun, 2008, Progress in Natural Science, 18:697-705
15. Han, Zongfu, Zhongfu Ni, Xiaona Wang, Lahu Lu, Yingyin Yao, Qixin Sun, 2008, Progress in Natural Science (in press)
16. Song, Xiao, Zhongfu Ni, YingyinYao, Zhenxing Li, Haiyan Wu, Yinhong Zhang, Qixin Sun, 2007, Proteomics，7, 3538–3557
17. Zhang, Yi, Zhongfu Ni, Yingyin Yao, Xiuling Nie, Qixin Sun, 2007, BMC Genetics 8：40