Comparison of Gene Expression Patterns in *Longissimus dorsi* of Pigs between the High-parent Heterosis Cross Combination Landrace×Large White and the Mid-parent Heterosis Cross Combination Large White×Meishan

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ABSTRACT : In order to detect the molecular mechanism of heterosis in pigs, the mRNA differential display technique was performed to investigate the differences in gene expression of pig's *Longissimus dorsi* between the high-parent heterosis cross combination Landrace×Large White and the mid-parent heterosis cross combination Large White×Meishan. Three pig purebreds, Large White, Meishan, and Landrace and four types of reciprocal F_1 hybrids were analyzed using nine 3'-end anchored primers in combination with ten 5'-end arbitrary primers and nearly 7,000 reproducible bands were examined. The patterns of gene expression of each cross combination were analyzed and eight common patterns (fifteen kinds) were found. When the results from the two cross combinations were put together and compared, eight different typical expression patterns were observed, these indicated that the patterns of gene expression of these two cross combinations had obvious differences. Gene expression correlation and cluster analyses of the two cross combinations indicated that the gene expression of the mid-parent heterosis cross combination was correlated with maternal effect, but in the high-parent heterosis cross combination, paternal effect acted in the gene expression of the hybrids or the gene expression of the hybrids was biased towards one parent. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 9 : 1192-1196)***

Key Words : Differential Display, Heterosis, Gene Expression Patterns, Correlation and Cluster Analyses, Maternal Effect, Paternal Effect

INTRODUCTION

mRNA differential display first described by Liang and Pardee (1992) is a fast, and efficient method for isolating and characterizing altered gene expression in different cell types. It was statistically shown that 80-120 primer combinations would be sufficient to cover all the transcript populations in the cell (Liang et al., 1993). This technique possesses the following advantages over other similar techniques: it is based on simple and established methods, more than two samples can be compared simultaneously and only a small amount of starting material is needed (Yamazaki and Saito, 2002). Our experiment used 90 primer combinations to perform differential display polymerase chain reaction (DD-PCR) and silver stain display to analyze gene expression differences of three pig purebreds, Large White (LW), Meishan (M), Landrace (LD) and four types of reciprocal F_1 hybrids: Large White (♂)× Meishan (♀) [LM], Meishan (♂)×Large White (♀) [ML], Large White (♂)×Landrace (♀) [LWLD], and Landrace (♂)×Large White (♀) [LDLW]. These animals belonged to two cross combinations: high-parent heterosis cross combination Landrace×Large White and mid-parent heterosis cross combination Large White×Meishan. These two cross combinations possessed one common purebred: Large White. This facilitated the comparison of results of the two cross combinations. This experiment dealt only with one carcass trait, the loin eye area, because this trait typically reflected the characters of two different kinds of heterosis (Figure 1). All the data came from the cross breeding populations slaughtered in 2002. From Figure 1, it can be seen that the loin eye areas of LM and ML are higher than the average value of their parental generation purebreds but lower than the top value of their parental generation purebreds, and the loin eye areas of LWLD and LDLW are both higher than the top value of their parental generation purebreds. So for the same trait, the two cross combinations exhibited different expression patterns. We therefore used the mRNA differential display technique to investigate the intrinsic differences of the molecular mechanisms of the two cross combinations.

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MATERIALS AND METHODS

Sample collection and total RNA extraction

Large White×Meishan and Landrace×Large White, two cross breeding populations were constructed in March, 2002. All the *Longissimus dorsi* muscle samples were collected from 180 days old pigs slaughtered in August, 2002. For each breed, total RNA was extracted from five male and five female pigs using the TRIzol Reagent Total RNA Extraction Kit (GIBCO, USA) and pooled. DNase I treatment of the total RNA was optional. Unless the total

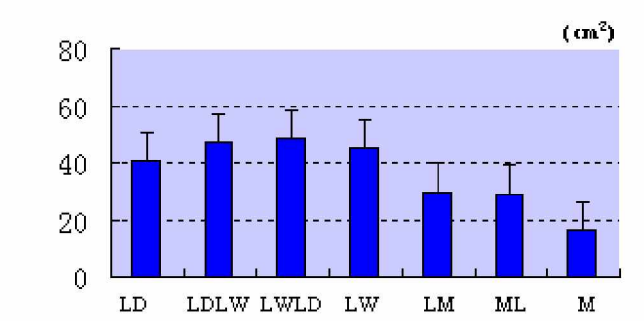

Figure 1. The bar graph of the average loin eye area values for seven types of pigs.

RNA samples were seriously contaminated with genomic DNA, DNaseI treatment was not necessary. If analysis of total RNA on a denaturing formaldehyde/agarose/EtBr gel revealed visible genomic DNA contamination then the total RNA sample was treated with DNase I before continuing with the first-strand cDNA synthesis.

RNA reverse transcription and first-strand cDNA synthesis

For each RNA sample, a single reverse transcription reaction was set up. This method was simpler than the multiple cDNA synthesis reactions required for similar methods. Every reverse transcription reaction only required 4 μg of total RNA per sample. In a sterile RNase-free microcentrifuge tube, 0.5 μg of the oligo (dT) 15 primer per microgram of the mRNA sample was added in a total volume of 15 μl with water. The tube was heated to 70°C for 5 minutes to melt the secondary structure within the template, and then cooled immediately on ice to prevent the secondary structure from reforming. After that, the tube was spun briefly to collect the solution at the bottom. The following components were added to the annealed primer/template in the order: 5 μl of M-MLV 5×Reaction Buffer, 1.25 μl of 10 mM dNTPs, 25 units of rRNasin® Ribonuclease Inhibitor (Promega, USA), 200 units of M-MLV RT (Promega, USA), Nuclease-Free Water to a final volume of 25 μl and mixed gently by flicking the tube. The tube was incubated for 60 minutes at 37°C, and then the efficiency of reverse transcription was checked on 1% agrose/EtBr gel.

Differential display PCR

PCR amplification of each reverse transcription product was carried out simultaneously with ten arbitrary primers and nine oligo (dT) primers. Thus 90 combinations of upstream and downstream primers were used. The 25 μl reaction system was: 2.0 μl cDNA, 2.5 μl 2 mM mixed dNTPs, 2.5 μl 10×Taq DNA polymerase buffer, 2.5 μl 25 mM $MgCl_2$, 2.0 μl 20 μM anchored "T" primer, 2.0 μl 20 μM arbitrary primer, 2.0 units of Taq DNA polymerase (1 U/1 μl) (JINMEI BIOTECH, China), and 9.5 μl sterile water. The PCR was done as follows: 94°C for 5 min, 40°C for 5 min, 72°C for 5 min, 3 cycles, followed by 30 cycles of 94°C for 1 min, 60°C for 1 min, 72°C for 2 min, then 72°C extension for 10 min, finally 4°C to terminate reaction.

Figure 2

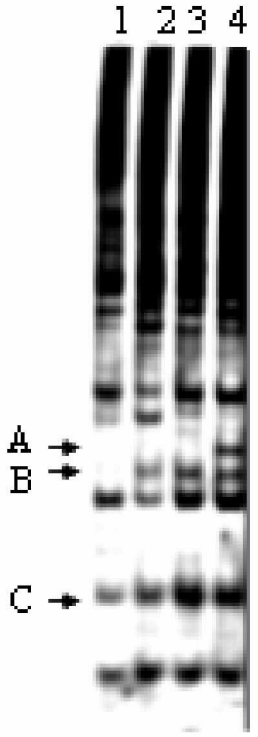

Figure 3

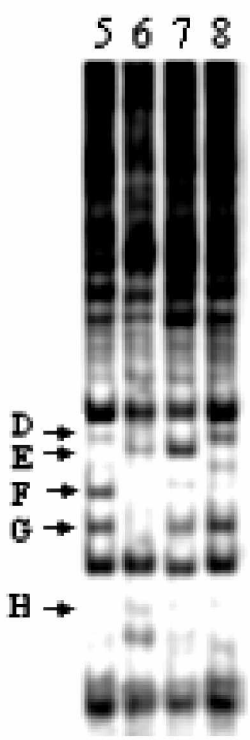

Figure 2 and 3. Eight kinds of typical gene expression patterns in each cross combination. 1, 2, 3, 4, 5, 6, 7 represent LD, LDLW, LWLD, LW, LM, ML, M, respectively. A, B, C, D, E, F, G, H with arrows indicate the bands of different expression patterns.

Non-denaturing polyacrylamide gel electrophoresis and silver stain

A 8% non-denaturing polyacrylamide gel was prepared by mixing 16 ml 30% acrylamide stock solution, 6 ml 10×TBE buffer, 37.5 ml sterile water, 400 μl 10% Ammonium Persulfate and 40 μl TEMED (BioRad, USA). The polyacrylamide gel was then pre-run for 30 min in 1×TBE buffer at 100 V. After that, 4 μl 6×gel loading solution was added to 20 μl PCR product, mixed well and loaded on the polyacrylamide gel; next, the gel was run in 1×TBE buffer at 100 V until the xylene cyanol reached the bottom of the gel; finally, silver stain was done as follows: the gel was fixed with 10% ethanol for 10 min, washed with 1% HNO_3 for 10 min, stained for 15 min using 0.2% $AgNO_3$, rinsed in distilled water three times, 3 min each time, developed in 3% NaCarbonate (with 0.004% formaldehyde), and reaction was terminated with 3% acetic

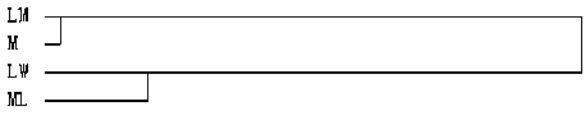

Figure 4. Cluster analysis result of gray values among mid-parent heterosis cross combination.

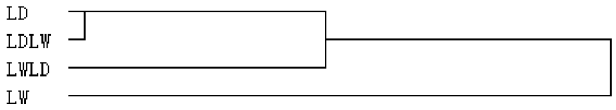

Figure 5. Cluster analysis result of gray values among high-parent heterosis cross combination.

acid, then dry the gel (Pan, 2002 and 2003).

Statistical method and program

Gel image scan was performed to find the bands and to measure the gray values of bands by using BandScan software version 4.50 (http://www.Glyco.com). The correlation and cluster analyses of the scan results were carried out using the CANCORR, CLUSTER and TREE procedures of SAS software version 8.1 (SAS Institute Inc., NC, USA).

RESULTS

The gel analysis indicated that the gene expression of the two cross combinations possessed eight common patterns (fifteen kinds), which included: i) Band only present in one purebred (two kinds) (Figure 2 (A)). ii) Bands only absent in one purebred (two kinds) (Figure 2 (B)). iii) Bands detected in all types of pigs (one kind) (Figure 2 (C)). iv) Bands only displayed in two purebreds but absent in two hybrids (one kind) (Figure 3 (D)). v) Bands only found in two hybrids (one kind) (Figure 3 (E)). vi) Bands only observed in one hybrid and one purebred (four kinds) (Figure 3 (F)). vii) Bands only not expressed in one hybrid (two kinds) (Figure 3 (G)); viii) Band only occurring in one hybrid (two kinds) (Figure 3 (H)).

For each cross combination, the gel images were scanned using software BandScan 4.50 and the gray values of the bands were imported into the computer, then cluster analyses were performed, results as Figure 4 and 5. It can be seen that the patterns of the two cross combinations had

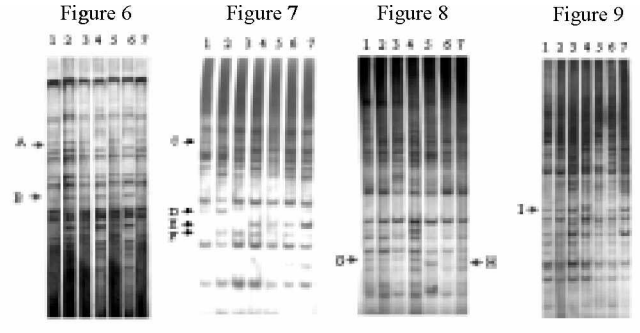

Figure 6, 7, 8 and 9. Typical gene differential display patterns between the two cross combinations. A, B, C, D, E, F, G, H, I with arrows indicate the differential display bands. 1, 2, 3, 4, 5, 6, 7 represent LD, LDLW, LWLD, LW, LM, ML, M, respectively.

obvious differences.

The gene expressions in the high-parent heterosis cross combination and the mid-parent heterosis cross combination not only possessed qualitative differences but also showed quantitative differences. When these two cross combinations were put together for comparison, we detected that some bands were down-expressed in the mid-heterosis cross combination hybrids while up-expressed in the high-parent cross combination hybrids (Figure 9 (I)) or vice versa. With regards to qualitative differences, nearly 7,000 reproducible bands were examined, and the results showed that the patterns of gene expression of the two cross combinations had obvious differences. Eight patterns of differential gene expression were observed: i) Bands only absent in one hybrid and one purebred of one cross combination but present in others of two cross combinations (Figure 6 (A)). ii) Bands displayed only in two hybrids belonged to different cross combinations and one purebred which were linked by the maternal effect but absent in others, such as Figure 6 B, only displayed in LW, LDLW, ML; iii) Band revealed only in one purebred (Figure 7 (C)); iv) Bands only observed in one hybrid and one purebred of one cross combination (Figure 7 (D)); v) Band detected only in mid-parent heterosis cross combination but absent in LD, LDLW, LWLD or vice versa (Figure 7 (E)); vi) Bands only absent in one purebred but occurring in other hybrids and other purebreds of the two cross combinations (Figure 7 (F)); vii) Bands only not expressed in two hybrids of one cross combination (Figure

Table 1. Correlation matrix of gray values of seven types of pigs.

Correlations	LD	LDLW	LWLD	LW	LM	ML	M
LD	1.000	0.656**	0.523**	0.363**	0.124*	0.227**	0.132*
LDLW		1.000	0.582**	0.437**	0.123*	0.289**	0.188**
LWLD			1.000	0.498**	0.188**	0.287**	0.194**
LW				1.000	0.266**	0.560**	0.290**
LM					1.000	0.596**	0.614**
ML						1.000	0.313**
M							1.000

* Correlation is significant at the 0.05 level. ** Correlation is significant at the 0.01 level.

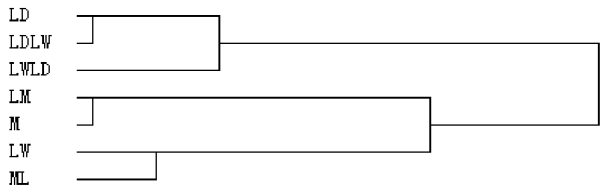

Figure 10. Cluster analysis result of gray values for seven types of pigs.

8 (G)); viii) Band only expressed in one hybrid (Figure 8 (H)).

The gel images were also scanned using the software Bandscan 4.50 and gray values of bands were imported into the computer when these two cross combinations were put together for comparison. Then correlation analyses were performed and results were presented in Table 1. It can be seen that in the high-heterosis cross combination Landrace×Large White, the correlation between LD and LDLW is higher than that between LD and LWLD (0.656>0.523), and the correlation between LW and LWLD is higher than that between LW and LDLW (0.498>0.437). Based on the results above, in the high-heterosis cross combination Landrace×Large White, we can infer that paternal effect may play an important role in the gene expression of the hybrids. Similarly, in the mid-parent heterosis cross combination Large White×Meishan, we can conclude that maternal effect influences the gene expression of the hybrids.

Cluster analysis for gray values was also done when these two cross combinations were put together for comparison and the results were presented in Figure 10. It can be seen that the proximity extent between M and LM is higher than that between M and ML, and the proximity extent between LW and ML is higher than that between LW and LM. Based on this, we can also infer that a maternal effect plays an important role in mid-parent heterosis cross combination Large White×Meishan. Similarly, we can conclude that a paternal effect influences the gene expression of the hybrids in the high-parent heterosis cross combination Landrace×Large White. These results agree with the cluster results obtained above (Figures 4 and 5) and correlation analyses (Table 1).

DISCUSSION

Heterosis is of utmost economic importance in animal breeding. The two earliest hypotheses regarding heterosis, the dominance hypothesis (Davenport, 1908) and the overdominance hypothesis (East, 1908; Shull, 1908) have competed for almost one century. However, its underlying molecular mechanism is still unknown. Our results show that both the high-parent heterosis cross combination and the mid-parent heterosis cross combination have eight common gene expression patterns (fifteen kinds). These agree with the results obtained by Wu et al. (2001) and Xie et al. (2003) from studying the heterosis in wheat using mRNA differential display. This shows that both animals and plants possess the common gene expression patterns of heterosis, but to our knowledge, until today there has been no report about using the mRNA differential display technique to study heterosis in pigs. We have also found that mid-parent heterosis cross combination is correlated with maternal effect, but for the high-parent heterosis cross combination it seems that paternal effect influences the gene expression of the hybrids or the gene expression of the hybrids is biased towards one parent. Are these universal phenomena of nature? If we can prove that, we can forecast the heterosis patterns of hybrids through analyzing gene expression patterns in the early life of a small number of individuals. Recently some investigators had also analyzed a lot of DNA molecular markers to elucidate the principles of heterosis (Xiao et al., 1995; Yu et al., 1997; Lu et al., 2003; Hua et al., 2003) but obtained different conclusions. A possible reason is that molecular markers only are correlated with traits but do not directly contribute to the heterotic traits. On the other hand, investigators can not find all markers correlated with traits which show heterosis. Compared with these, by using mRNA differential display with 80-120 primer combinations we can scan almost the differentially expressed genes at a time which directly contribute to the trait (Yamazaki and Saito, 2002), but mRNA differential display also has defects, such as false positives. In our experiment, in order to reduce false positives, we adopted the two-step method for PCR modified from the enhanced differential display protocol and used longer PCR primers (Linsken, 1995). Every PCR was repeated to validate the reproducibility of every band. Thus improved mRNA differential display is a suitable technique to study heterosis. In order to study the principles of heterosis in more details, we will continue to test the universality of our results obtained by using the mRNA differential display technique.

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