Theor Appl Genet (2010) 120:389–400
DOI 10.1007/s00122-009-1207-y

ORIGINAL PAPER

Heterosis in early seed development: a comparative study of F1 embryo and endosperm tissues 6 days after fertilization

Stephanie Jahnke · Barbara Sarholz · Alexander Thiemann · Vera Kühr · José F. Gutiérrez-Marcos · Hartwig H. Geiger · Hans-Peter Piepho · Stefan Scholten

Received: 1 May 2009 / Accepted: 22 October 2009 / Published online: 11 November 2009

Abstract Heterosis specifies the superior performance of heterozygous individuals and although used in plant breeding the underlying molecular mechanisms still remain largely elusive. In this study, we demonstrate the manifestation of heterosis in hybrid maize embryo and endosperm tissue 6 days after fertilization in crosses of several inbred lines. We provide a comparative analysis of heterosis-associated gene expression in these tissues by a combined approach of suppression subtractive hybridization and microarray hybridizations. Non-additive expression pattern indicated a *trans*-regulatory mechanism to act early after fertilization in hybrid embryo and endosperm although the majority of genes showed mid-parental expression levels in embryo and dosage dependent expression levels in endosperm. The consistent expression pattern within both tissues and both inbred line genotype combinations of genes coding for chromatin related proteins pointed to heterosis-related epigenetic processes. These and genes involved in other biological processes, identified in this study, might provide entry points for the investigation of regulatory networks associated with the specification of heterosis.

Introduction

Heterosis is the ability of hybrid progeny to outperform their parents with respect to various characteristics (Shull 1908). Since the phenomenon features agronomically important traits, like a high increase of biomass and faster development (Rood et al. 1988), investigations of heterosis in the last century typically employed full-grown plants (Shull 1952). Recently, developmental stages shortly after plant germination were additional targets of heterosis research (Swanson-Wagner 2006; Hoecker et al 2005, 2008). However, increased vigor is already present in early embryos (Wang 1947; Meyer et al. 2007) as well as whole

---

Communicated by T. Luebberstedt.

Contribution to the special issue “Heterosis in Plants”.

The microarray data of this study were deposited in Gene Expression Omnibus (GEO) at the National Centre for Biotechnology Information (NCBI) with the series entry GSE17754.

Electronic supplementary material The online version of this article (doi:10.1007/s00122-009-1207-y) contains supplementary material, which is available to authorized users.

S. Jahnke · A. Thiemann · S. Scholten (✉)
Biocenter Klein Flottbek, University of Hamburg,
Ohnhorststraße 18, 22609 Hamburg, Germany
e-mail: s.scholten@botanik.uni-hamburg.de

B. Sarholz
General Motors Powertrain Germany GmbH,
65423 Rüsselsheim, Germany

V. Kühr · H. H. Geiger
Institute of Plant Breeding, Seed Science,
and Population Genetics, University of Hohenheim,
70599 Stuttgart, Germany

J. F. Gutiérrez-Marcos
Warwick HRI, Wellesbourne, University of Warwick,
Warwickshire CV35 9EF, UK

H.-P. Piepho
Bioinformatics Department,
Institute for Crop Production and Grassland Research,
University of Hohenheim, 70593 Stuttgart, Germany

kernels (Römisch-Margl et al. 2010) shortly after hybridization, making early seed development suitable to study the specification of heterosis in plant life cycle.

In angiosperms the plant life cycle starts by double fertilization, which initiates seed development. By the fusion of an egg cell and a sperm cell a diploid zygote arises which develops into an embryo. The fusion of a second sperm cell with the central cell that contains two haploid cell nuclei, results in the formation of the triploid endosperm (Russel 1992; Goldberg et al. 1994; Drews et al. 1998). The triploid endosperm differs in principle from the embryo not only due to the genomic ratio, which is two to one for maternal and paternal genome, respectively, but also due to its development, function and fate (Olsen 2001, 2004). The endosperm functions as a sink tissue that acquires resources from the maternal tissue for use by the developing embryo and during germination (Brink and Cooper 1947; Lopes and Larkins 1993; Berger 1999). In addition, it maintains a high osmotic potential around the embryo, inherits mechanical support during the early embryonic growth (Lopes and Larkins 1993) and allocates hormones for the regulation of embryo growth and germination (Lopes and Larkins 1993; Hong et al. 1996; Olsen 1998). Because it archives these diverse tasks, the endosperm is of central importance for seed development (Laux and Jürgens 1997).

The integration of heterosis-associated changes in gene expression of diverse plants and tissue types might help to get an idea about the likely complex molecular basis of heterosis (Hochholdinger and Hoecker 2007). Two general modes of gene expression in hybrids can be distinguished (Birchler et al. 2003; Springer and Stupar 2007): on one hand, allelic additive expression with averaged parental expression levels, which is mainly attributable to physically linked control elements in *cis* and alternatively, non-additive expression which differs from the average of the parents can arise. This non-additive gene expression can arise when the combination of diverse alleles leads to new, *trans*-regulated interactions in a hybrid. Whereas a growing number of comprehensive analyses in plant as well as animal kingdom indicated that non-additive gene expression is prevalent in various hybrid situations (Adams et al 2003; Hämmerle and Ferrus 2003; Michalak and Noor 2003; Song and Messing 2003; Auger et al. 2005, Vuylsteke et al. 2005; Huang et al. 2006; Uzarowska et al. 2007), other investigations rarely identified non-additive expression pattern (Stupar and Springer 2006; Swanson-Wagner et al. 2006; Hoecker et al. 2008). Interestingly, the frequency of hybrid expression patterns outside the parental range highly varies between vegetative tissues, including embryos, and endosperm 13 and 19 dap (Stupar et al. 2007).

However, there is no information about the impact of heterosis on the seed shortly after fertilization at the proembryo stage and well before the mitotic peak and extensive reserve accumulation in endosperm. For a comparative study of both fertilization products at this stage we investigated heterotic traits in embryo and endosperm 6 days after fertilization in several modern maize hybrids and analyzed associated gene expression pattern. We applied a combined approach of suppression subtractive hybridization (SSH) and microarray hybridization to identify differentially expressed genes. A thematically focused cDNA microarray was generated and employed to gain functional expression information and to assess the proportion of non-additive gene expression in early hybrid tissues. Quantitative RT-PCR (qRT-PCR) analysis was used to validate the microarray data.

Material and methods

Plant material and tissue isolation by microdissection

The maize inbred line UH005 [National listing of plant varieties (NLPV), accession number (AC): M9379, European flint] from the flint pool, and the inbred lines UH250 (NLPV AC: M9005, Iowa Stiff Stalk Synthetic) and UH301 (NLPV AC: M8652) from the dent pool were grown in the glasshouse as described in Meyer et al. (2007). Six days after fertilization the isolation of embryo and endosperm tissues by microdissection were carried out as specified in Meyer et al. (2007). To analyze if hybrid vigor influence either fertilization products, embryo and endosperm, in an early stage of seed development three maize inbred lines were crossed in various combinations and both tissues of inbred and hybrid progenies were isolated.

A prerequisite for heterosis analysis is to warrant identical growth conditions for the hybrid and inbred genotypes. To ensure this, we did all pollinations by hand within the same hour and cultivated the plants next to each other in a greenhouse under standard conditions. The tissue isolation of each genotype was done in randomized order within 4 h.

The length of inbred and hybrid embryo and endosperm tissues of two different interpool crosses using the genotype combinations UH301/UH005 and UH250/UH005 and one intrapool cross of the inbred lines UH250 and UH301 were compared several times in a period of two years. The comparisons were based on a total of 9, 3 and 5 investigation dates, respectively. Microscopic size measurements were done using at least 20 separate tissues per genotype and survey. Subsequently, sets of 20–25 of embryo and endosperm tissues, respectively, separated from each other were transferred into tubes and snapped frozen in liquid nitrogen. The tissues were stored at −80°C until use for molecular analyses.

Information about genetic distances between the different hybrid genotypes are given in Hoecker et al. (2005).

Synthesis of cDNA and suppression subtractive hybridization

After homogenization of the frozen embryo and endosperm tissue mRNA isolation and cDNA synthesis as well as SSH was essentially carried out as described in Le et al. (2005). Four subtractions were performed to generate cDNA pools enriched for genes higher expressed in embryo and endosperm tissue of the hybrid UH301 × UH005 (female parent is listed first) compared to both parental inbred lines. For this purpose the cDNA of the hybrid tissue was used as tester and cDNAs of one or the other parental inbred line as driver in forward subtractions. Cloning and transformation of subtracted cDNA populations as well as cultivation and storage of transformed bacteria were carried out as described in Meyer et al. (2007).

Microarray production and hybridisation

To generate probes for microarray production a PCR screening [3 μl bacteria suspension, 77.9 μl H_2O, 10 μl *Taq* buffer with $(NH_4)_2SO_4$ (Fermentas, St. Leon-Rot, Germany), 6 μl 25 mM MgCl, 0.3 μl 100 mM Primer NP1 and NP2R (BD Bioscience Clontech, Erembodegem, Belgium), 2 μl 10 mM dNTP, 0.5 μl *Taq* polymerase (Fermentas, St. Leon-Rot, Germany); 96°C: 2 min, (94°C: 30 s, 68°C: 4 min) × 35] using material of the cultured bacteria colonies as templates were carried out to check the inserts of each vector. 946 successfully amplified PCR fragments, which originated from one or the other subtraction conducted with embryo tissues, and 938 PCR fragments emanated from the two subtractions performed with endosperm tissues, were used as probes. All cDNA probes, control genes and four microarray spiking controls [*uidA* gene (462 bp, *Escherichia coli*), human estrogen receptor gene (464 bp), *green fluorescent protein* gene (378 bp, Pang et al. 1996), *DsRed-1* gene (680 bp, BD Bioscience Clontech, Erembodegem, Belgium)] were printed onto glass slides and hybridized as described in Le et al. (2005). The microarray hybridizations were carried out with unsubtracted target cDNA populations of 6 dap embryo and endosperm tissues generated by SMART technology (BD Bioscience Clontech, Erembodegem, Belgium). A total of 56 microarray hybridizations using cDNA of the genotypes generated by crosses of UH301/UH005 and UH250/UH301 were carried out. Our investigations of differential gene expression are based on three biological samples of each intrapool genotype and four biological replicates of each interpool genotype and were done by using a hybridization pattern employing the loop design showing in Fig. 2. For scanning an ArrayWorx-Microarray-Reader (Applied Precision, Issaquah, WA, USA) was used. The image files of the hybridized microarrays were analyzed with the software "GENEPIX 4.0" (Axon Union City, CA, USA) for data acquisition. All spots with higher background than foreground signal were excluded.

Statistical analysis

For normalization an intensity dependent loess regression and a scaling were carried out. In the process of scaling the median absolute deviation (MAD) of the slides were normalized to account for differences between arrays. The normalized data of each tissue type were analyzed by a linear mixed model regarding the signal intensity on the log2 scale as response variable. Genotype, dye, genotype × dye interaction and spotting/spot effect were taken as fixed effects, slide, slide × spotting/spot interaction and slide × dye interaction were considered as random effects for each spot. For microarray spiking controls also the random effects slide × block × dye and slide × block were included in the linear mixed model, since these gene fragments were printed in several blocks of the array. Linear contrasts between hybrid and parental mean as well as expression data of each genotype were calculated. The *P* values of the contrasts based on Wald tests were adjusted for multiple testing by controlling the false discovery rate (FDR) at 5% using the procedure of Benjamini and Hochberg (1995). The analyses for this study were generated using SAS software for Windows (version 9.1). Linear model analyses were performed by PROC MIXED, while the FDR adjustment was done by PROC MULTTEST.

Non-additive gene expression was investigated by using the formula $H - (P_M + P_P)/2$ for embryo and $H - (2 \times P_M + P_P)/3$ for endosperm tissue (H: hybrid expression level; P_M: expression level of the maternal inbred line; P_P: expression level of the paternal inbred line). *P* values were adjusted as described above. The allocations to six expression classes were done by comparing the expression level of hybrids and parental inbred lines. Above high parent (AHP): $H > \mathrm{HP}$; high parent: $H = \mathrm{HP} \pm 10\%$; higher than additive but not like high parent: $H < \mathrm{HP} \pm 10\%$; lower than additive but not like low parent: $H > \mathrm{LP} \pm 10\%$; low parent: $H = \mathrm{LP} \pm 10\%$; below low parent (BLP): $H < \mathrm{LP}$ (H: expression level of the hybrid, HP: expression level of the high expressing parental inbred line, LP: expression level of the low expressing inbred line).

Phenotypic data of the hybrid tissues were analyzed for heterosis by a linear mixed model: length = genotype + day of survey + error, in which genotype was taken as fixed effect, day of survey was considered as random effect by using the software PROC MIXED. The *P* values based on Wald tests were FDR adjusted as described above by using PROC MULTTEST. The mid-parent heterosis (MPH) values were calculated using the formula $\mathrm{MPH} = (H - (P_1 + P_2)/$

2)/(($P_1 + P_2$)/2) × 100 (where H is the value of the hybrid and P_1 and P_2 are the values of one or the other parental inbred line). Best-parent heterosis (BPH) were determined using the formula BPH = (($H - P_B$)/P_B) × 100 (where P_B represent the parent with the higher value). Hybrid increase (HI) was calculated using the formula HI = (($H - P_M$)/P_M) × 100 (where P_M is the value of the maternal inbred line).

Statistical analyses of the qRT-PCR data were performed as described in Meyer et al. (2007).

Sequence analysis

Inserts of plasmids of selected clones were sequenced. For BlastN searches against NCBI's non-redundant (nr) database and EST database, and for BlastX searches against NCBI's nr database and SWISSPROT (March 2009) the significance threshold of $E < 10^{-10}$ were used.

Real time PCR

For qRT-PCR three sets of cDNA of 6 dap embryos of all genotypes of the interpool cross UH301/UH005, isolated at three independent dates, were produced essentially as described in Le et al. (2005). Since the material was very limited we used a fraction of the first strand cDNA for SMART cDNA amplification as specified in Meyer et al. (2007). The amplified cDNA of each cDNA set was used to quantify differences in expression levels between the four genotypes. Gene-specific oligonucleotides (Sup. Tab. 1 in Electronic Supplementary Material) used in qRT-PCR were designed with primerselect software (Lasergene, GATC Biotech AG, Konstanz, Germany). For qRT-PCR experiments the iCycler iQ® detection system (BIORAD, München, Germany) and the qPCR MasterMix Plus for SYBR Green I (Eurogenetec, Seraing, Belgium) were employed. The used cycling parameter and qRT-PCR proceeding are described in Meyer et al. (2007) in detail. For statistical analysis the efficiencies of oligonucleotide pairs were estimated from cDNA dilution series and ranged between 84.8 and 97.6%.

Results

Heterosis exerts influence on early embryo and endosperm development

Morphologically 6 dap embryos of all genotypes could be assigned to the transition stage which preceded the formation of the cotyledon. Two distinct regions of the embryo, the embryo proper having small cytoplasmic cells and the suspensor, exhibiting lager vacuolated cells, were visible and no meristem was observable (Fig. 1a). The simultaneously isolated endosperm tissues featured the developmental stage where processes of cell proliferation and differentiation are prevalent (Fig. 1b).

Embryo and endosperm tissues of the individual inbred genotypes were clearly distinguishable with respect to size. The flint line UH005 had the smallest embryos with a maximum of 288 μm length. The inbred line UH250 exhibited the largest embryos with a maximum size of 572 μm. The UH301 embryos reached a maximum length of 450 μm. Also the endosperm of UH005 was notably small sized (2,120 μm). The endosperm of the dent lines UH250 and UH301 reached a maximum length of 2,915 and 3,180 μm, respectively.

All analyzed reciprocal hybrid embryos possessed a constantly measurable size increase with respect to the maternal inbred line (Fig. 1a). Five of the six hybrid genotypes had a size significantly above the mid-parent one and displayed mean MPH values of up to 50.8%. Only the mean size of the genotype UH005 × UH250 was significantly below the mid-parent value. Nevertheless, these embryos exhibited the highest increase in size with respect to the maternal inbred line resulting in a mean HI value of 35.3%. For the UH005 × UH301 embryos, exhibiting also a high mean HI value of 34.1%, a comparatively moderate mean MPH value of 7.3% was determined. The MPH values of both reciprocal hybrids of each interpool cross highly differ, since the hybrid embryo size increase adds to the profoundly variable female parent size. This fact leads to significant mean BPH values for the UH301 × UH005 and UH250 × UH005 embryos of 14.4 and 12.4%, respectively, possessing the larger female parent of the reciprocal hybrids. Both reciprocal hybrids of the intrapool cross featured a significant size increase with respect to the mean length of both more similar sized parental lines.

For endosperm of the UH301/UH005 interpool genotype combination heterosis was detectable for the UH301 × UH005 hybrid with a mean MPH value of 13.9% (Fig. 1b). The endosperm of UH005 × UH301 hybrids exhibited on average a significant smaller size compared to the mean of both parental inbred lines. BPH was detectable for the UH301 × UH005 hybrid tissue with a mean value of 3.5%. At diverse isolation dates a maximum size increase with respect to the maternal inbred line of up to 28.3% and up to 20.4% was detected for the endosperm of the UH301 × UH005 and the UH005 × UH301 hybrids, respectively. For endosperm of the UH250/UH005 interpool genotype combination heterosis was detectable for the UH250 × UH005 hybrid with a mean MPH value of 15.6%. The same hybrid showed no significant size increase in respect to the maternal inbred line. The reciprocal UH005 × UH250 hybrid exhibited an increase in size resulting in a mean HI value of 19.9%, notably at individual surveys an increase of up to 43% was traceable. For endosperm of the UH250/UH301 intrapool genotype combination heterosis was detectable for the UH301 × UH250

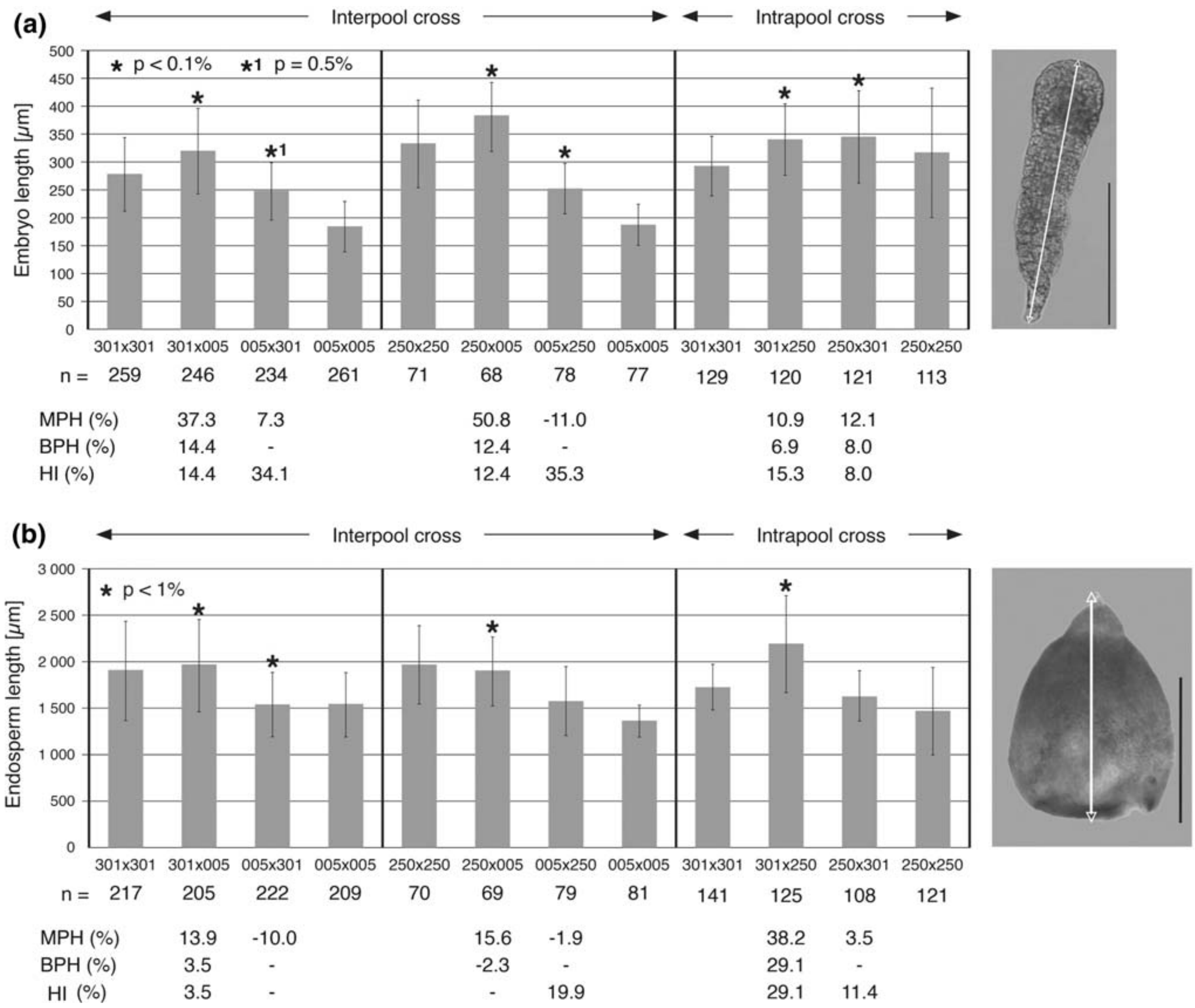

Fig. 1 Heterosis in 6 dap embryo and endosperm. Mean length (µm) of hybrid and inbred embryo (**a**) and endosperm tissues (**b**) of the three genotype combinations UH301/UH005, UH250/UH005 and UH250/UH301 is displayed. The measurements of the microdissected tissue types were done under the microscope as depicted on the *right* side of **a** and **b**. The *bar* given in the image of **a** and **b** represent a length of 200 and 1,000 µm, respectively. *Bars* in the diagrams show standard errors. Below the columns the genotypes, investigated numbers of tissues and mean values (%) of mid-parent heterosis [*MPH*: $(H - (P_1 + P_2)/2)/((P_1 + P_2)/2) \times 100$], best-parent heterosis [*BPH*: $((H - P_B)/P_B) \times 100$] and hybrid increase [HI: $((H - P_M)/P_M) \times 100$] are given. The *asterisk* (*, *[1]) exhibit the *P* value of statistic analysis testing the significance of each mean MPH value. *H* hybrid, P_1, P_2 parental inbred line 1 and 2, P_B best parental inbred line, P_M maternal inbred line

hybrid with a mean MPH value of 38.2%. Both reciprocal hybrid tissues showed significantly increased size with respect to the maternal inbred line resulting in mean HI values of 29.1 and 11.4% and MPH values of 38.3 and 3.5% for the hybrids UH301 × UH250 and UH250 × UH301, respectively. The endosperm of UH301 × UH250 displayed a mean BPH value of 29.1%, which reached up to 56.9%.

Identification of heterosis-associated genes in 6 dap embryo and endosperm of two genotype combinations

To compare the transcript composition of 6 dap embryo and endosperm tissues of the interpool cross UH301/UH005 and the intrapool cross UH250/UH301, a thematically focused cDNA microarray was generated (Table 1). Populations of UH301 × UH005 hybrid cDNA were used for SSH, because this hybrid revealed the greatest crossbreeding advantage in embryos (Fig. 1a). 946 cDNA probes of embryo tissue originated in equal parts from the two subtractions UH301 × UH005 – UH301 × UH301 and UH301 × UH005 – UH005 × UH005, described in detail by Meyer et al. (2007), were used. Further, 938 cDNAs probes of endosperm tissue were generated by two subtractions UH301 × UH005 – UH301 × UH301 and UH301 × UH005 – UH005 × UH005 under the same conditions as for embryo tissue whereby half of the probes

Table 1 Content of the generated microarray

Number of cDNAs	Origin and description
822	cDNAs of Central cells (DW474971–DW476067)
73	cDNAs Central cells (CC) and egg cells (EC) (SSH: CC-EC, EC-CC, AC: DW474971–DW476067, Le et al. 2005)
946	cDNAs of 6 dap embryos (SSH: UH301 × UH005 − UH005 × UH005, UH301 × UH005 − UH301 × UH301, Meyer et al. 2007)
938	cDNAs of 6 dap endosperm tissues (SSH: UH301 × UH005 − UH005 × UH005, UH301 × UH005 − UH301 × UH301)
177	Gene fragments of chromatin associated proteins (http://www.chromdb.org)
54	Microarray spiking controls (present in each block) and control genes
120	cDNAs of two-celled and multicellular, 36 haf embryos (Okamoto et al. 2005)
9	Gene fragments of starch and nitrogen metabolism
3,139	Total number of cDNAs and gene fragments
2,685	Approx. number of unique genes (redundancy adjusted) under investigation

The number of spotted cDNAs and gene fragments as well as information about the origin and description are given. Furthermore, in consideration of redundancy the approximately number of unique genes under investigation is pointed out

stems from one and the other subtracted cDNA population. Subtracted cDNA pools of embryo revealed a redundancy of 13.3% (Meyer et al 2007). Based on this value the 1,884 cDNA probes represent approximately 1,633 unique genes. The second largest group of probes were given by 895 egg and central cell cDNAs which display active genes or stored transcripts of the female gametophyte, whereas 73 cDNAs originated from subtracted pools (Le et al. 2005) which were enriched for transcripts higher or especially expressed in either central cells (40 cDNAs) or egg cells (33 cDNAs). 822 cDNAs (AC: DW474971–DW476067) belonged to a non-subtracted cDNA population of central cells with a redundancy of 19.4% (S. Scholten, unpublished data). In total about 735 unique sequences, expressed before fertilization in female gametophytes, were spotted on the array. Furthermore, 120 probes of maize cDNA populations of two celled or multicellular embryos generated 36 h after in vitro fertilization (haf) (Okamoto et al. 2005), 177 gene fragments of chromatin associated proteins of maize (http://www.Chromdb.org) and 9 gene fragments of maize proteins of the starch and nitrogen metabolism were included.

To identify significantly (FDR adjusted P value <0.1) differential expressed genes, we compared the gene expression level of hybrids and parental inbred lines (Fig. 2). Verification of the microarray results was done by qRT-PCR. A total of 31 genes (Supplemental Table 1) which display in embryo tissue expression differences between genotypes of the interpool cross UH301/UH005 were analyzed. Overall, 48 of 66 differences in gene expression between hybrids and inbred parents, detected by microarray hybridization (FDR adjusted P value ≤ 0.1), were verified by qRT-PCR ($P \leq 0.15$). Overall, the qRT-PCR results are consistent with 73% of the microarray data.

Compared to the interpool cross UH301/UH005 fewer difference in gene expression were found in embryo and endosperm tissues of the intrapool cross UH250/UH301. The number of differentially expressed genes per tissue and genotype are given in Fig. 3. While the total number of identified differential expressed genes varied between embryo and endosperm, the composition of the detected genes and the overlapping genes between tissues and genotypes followed essentially the array composition (Fig. 3; Table 2). Five cDNA clones were determined to be differential expressed between inbred and hybrid genotypes of all tissues (Fig. 3; Table 3). These cDNAs show homology to a putative plastidic ATP/ADP transporter, a putative pyrophosphate-fructose 6-phosphate 1-phosphotransferase alpha subunit, a putative abscisic acid (ABA)-induced protein and one gene code for the histone H2A.

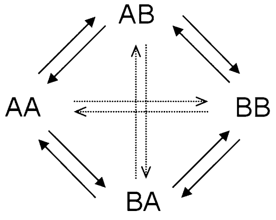

Fig. 2 Hybridization scheme. The *solid arrows* indicate conducted microarray comparisons between inbred lines and hybrids. *Dotted arrows* stand for in silico comparisons. *Double arrows* represent dye swap experiments. *AA* inbred line 1, *BB* inbred line 2, *AB*, *BA* reciprocal F_1-hybrids

Regarding the group of chromatin associated proteins we found in each tissue genes of diverse functional classes being differentially expressed between inbred and hybrid genotypes (Supplemental Table 2). Members of the four categories “histone” (HTA112), “histone modification” (HAC104, HAC111, SET115), “global transcriptional regulation” (GTB101, GTC101), and “interaction with methylated DNA” (MBD105) could be detected in either investigated tissues or both genotype combinations. Furthermore, differential transcription of the bromodomain-containing

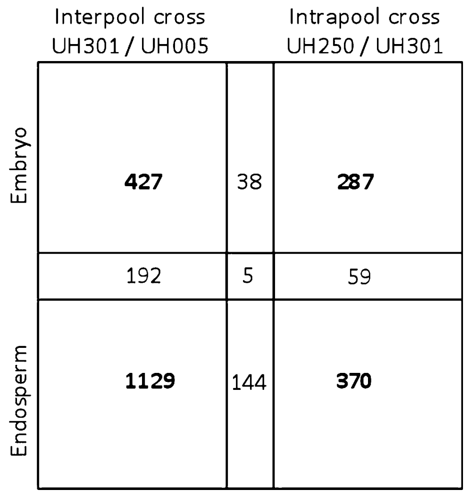

Fig. 3 Differential expressed genes. The Venn diagram gives an overview about the differential gene expression in embryo and endosperm of the interpool cross UH301/UH005 and the intrapool cross UH250/UH301, respectively. The number of genes which are significantly (FDR adjusted *P* value <0.1) differential expressed in at least one inbred/hybrid genotype comparison are given. The intersections display the number of genes, which were found in both interpool and intrapool cross or in both tissues types or in all tissues

protein BRD102 gene and the nucleosome assembly like protein NFD102 gene as well as the set protein SDG130, SDG135 and SET129 genes were identified in embryo and endosperm. Exclusively in embryos of both genotype combinations the SMH106 gene, belonging to the single myb histone protein group was differentially expressed. Differential expression specifically in endosperm tissue of both genotype combinations was found for the methyltransferase SET124, the global transcription regulator GTA101, the set protein SDG105, the “high mobility group family B” member NFD104, as well as for the nucleosome assembly like proteins NFA102, NFA103, NFA104, NFC101 and NFC102 genes.

Non-additive gene expression in 6 dap embryo and endosperm

Both genotype combinations and tissues exhibited substantial differences concerning the numbers of non-additively expressed genes. In hybrid embryos of the intrapool and interpool crosses 12.6 and 4.5% non-additive gene expression was found, respectively. In hybrid endosperm tissue we found the opposite situation: intrapool and interpool crosses revealed 2.4 and 8.0% non-additively expressed genes, respectively. According to their expression levels the identified non-additively expressed genes were grouped in six expression classes (Fig. 4, Supplemental Table 4). Most genes being non-additively expressed in embryo as well as in endosperm resemble the expression level of one

Table 2 Composition of differential expressed genes

cDNA classes	Interpool cross		Intrapool cross		Overlapping genes			
					Interpool cross	Intrapool cross		
	EMB (%)	END (%)	EMB (%)	END (%)	EMB/END (%)	EMB/END (%)	EMB/EMB (%)	END/END (%)
Chromatin associated proteins	5.6	3.4	4.5	8.4	5.7	8.5	7.9	5.6
Gametes	21.6	35.9	40.8	39.7	20.8	50.8	31.6	46.6
6 dap tissues	68.1	56.2	49.8	45.9	68.8	35.6	50.0	42.4
Others	4.7	4.5	4.9	6.0	4.7	5.1	10.5	5.6

The percentages of differential expressed genes belonging to one of the main cDNA classes of the microarray are given per genotype and tissue. In addition, the percental composition of the groups of overlapping genes is listed

Table 3 Intersection of differentially expressed genes

Clone name	Accession number	Length (bp)	Blast X sequence similarity (accession number)	*E* value
KG9	GR421164	721	Pyrophosphate-fructose 6-phosphate 1-phosphotransferase alpha subunit (*Zea mays*) (ACG23899)	9.00*E*−125
HTA112	–	411	Histone H2A, core histone required for chromatin assembly and chromosome function	–
CC0447	DW475393	512	Plastidic ATP/ADP transporter (*Zea mays*) (ACG28895)	5.00*E*−56
CC53	DW475019	232	ABA-induced protein (*Zea mays*) (ACG47436)	3.00*E*−17
CCEC5D12	DN591093	400	No hit	–

Genes which were determined to be differentially expressed (FDR adjusted *P* value <0.1) between inbred and hybrid genotypes in embryo and endosperm of the interpool cross as well as the intrapool cross. The clone name, the accession number, the sequence length, Blast X sequence similarity and *E* value is given

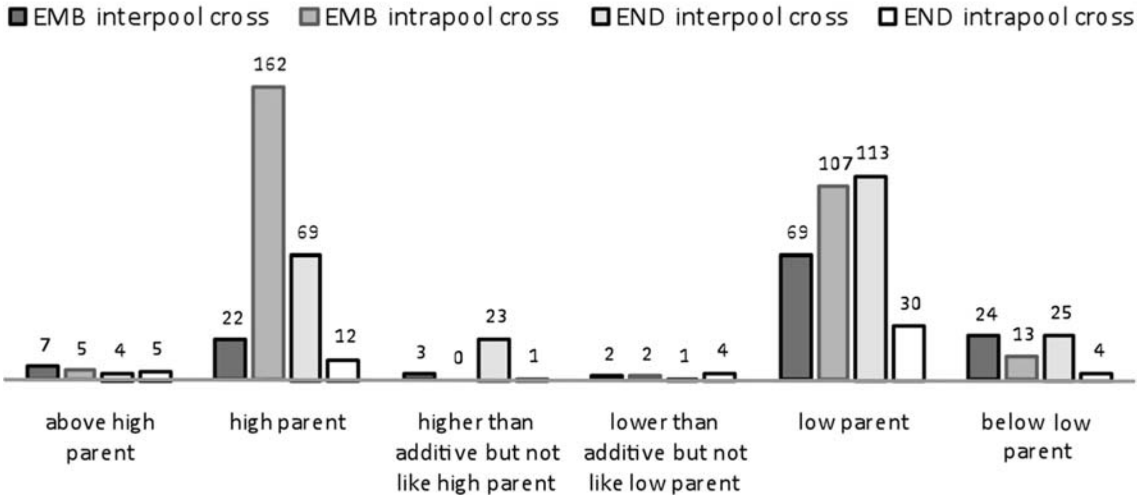

Fig. 4 Relative distribution of non-additively expressed genes detected in two different crosses revealed conserved expression trends among embryo and endosperm tissue. The *color* code used for the columns is specified in the legend. The numbers of genes *above the columns* indicate the absolute numbers of genes in each expression class. *EMB* embryo, *END* endosperm

or the other parent. Noticeably, the expression level of the low expressing parent (LP) was detected more frequently (45.1%) than that of the higher expressing parent (37.5%). This tendency was also found for genes with expression levels outside the parental range: 3.0 and 9.3% non-additively expressed genes showed expression AHP and expression BLP, respectively.

To reveal the extent of parental expression trends we analyzed the expression patterns of genes that displayed non-additive expression in both reciprocal hybrids. The classification according to their expression patterns is listed in Table 4. Due to a varying number of non-additively expressed genes of the reciprocal hybrid tissues only a small number of genes could be used for the classification. In both reciprocal hybrids, most genes (79.4%) exhibited HP or LP expression levels or an expression level higher or lower than the mid-parental value. We found fewer genes showing maternal like expression MLE in both tissue types (20.6%) and paternal like expression (PLE) could not be identified. In endosperm tissue the *dwarf8* (*d8*) gene exhibited striking overdominance (AHP) in both reciprocal hybrids of the interpool and intrapool cross.

Discussion

We showed that heterotic traits are established in both embryo and endosperm 6 dap. For three genotype combinations of modern European maize inbred lines the manifestation of heterosis in embryo and endosperm tissue was demonstrated by means of the trait “size”. These results are consistent with our previous results on 6 dap hybrid embryos of one European flint and dent genotype combination (Meyer et al. 2007). Importantly, heterosis was detectable independently of the date of survey and with similar values throughout, revealing of heterosis as a stable effect on embryogenesis. Compared to earlier studies on embryos during seed development 8 dap by Wang (1947) modern interpool hybrid embryos showed higher values of hybrid vigor earlier in development, most likely reflecting the enhancement of maize lines by extensive breeding efforts in the last decades. Similar to the findings of Wang (1947) the interpool MPH values of the reciprocal hybrids highly differ in that the hybrid embryo size increase adds to the highly varying female parent embryo size. The near equal parent embryo size of the intrapool combination is associated with similar MPH values for both reciprocal hybrid embryos, although these genetically more related genotypes results in relative moderate hybrid effects. Since genetic diversity is positively correlated to heterosis for yield (Smith et al. 1990; Guo et al. 2006), the different degrees of heterosis of the interpool and intrapool genotypes indicated that this correlation is already established in early embryos, well before germination.

The positive correlation of genetic distance and heterosis does not hold for early endosperm. The relative size increase of endosperm with respect to the maternal line and also the MPH values we observed, were generally lower for the interpool crosses and the intrapool crosses showed the far highest MPH values. This might be associated with the proposed role of endosperm as a ‘sensor’ for genomic imbalances (Costa et al. 2004). Interploidy crosses deviating from the normal 2:1 maternal:paternal genomic ratio show defective endosperm development (Rhoades and Dempsey 1966; Lin 1984; Scott et al. 1998; Alleman and Doctor 2000), suggesting that dosage effects play an important role in endosperm (Costa et al. 2004; Birchler 1993; Baroux et al. 2002). Indeed, the majority of genes in endosperm show expression values proportional to the parental

Table 4 Classification of non-additively expressed genes

	Embryo		Endosperm	
	UH301/UH005	UH250/UH301	UH301/UH005	UH250/UH301
No. genes non-additive in both hybrids	**5**	**6**	**18**	**5**
No. genes with MLE pattern	1	2	3	0
No. genes with PLE pattern	0	0	0	0
No. genes AHP or BLP in both hybrids[a]	0	0	1	1
No. genes AHP or BLP in one hybrid[b]	0	2	0	1
No. genes HP or LP in both hybrids	2	2	10	3
No. genes HP or LP in one hybrids[c]	2	0	2	0
No. genes below HP or above LP in both hybrids	0	0	2	0

The total number of non-additively expressed genes detected in embryo or endosperm tissue of both reciprocal hybrids in one or the other genotype combinations (UH301/UH005; UH250/UH301) is given in bold letters. The classification of these genes regarding specific types of non-additive expression patterns is reported below

Expression levels: of the high expressing inbred line (HP), of the low expressing inbred line (LP), above the high expressing inbred line (AHP), below the low expressing inbred line (BLP)

MLE maternal like expression, *PLE* paternal like expression

[a] No gene which exhibited AHP expression in one hybrid and BLP expression in the other hybrid was observed

[b] Only one gene was found in embryo of the intrapool cross UH250/UH301 which resemble a MLE but was expressed below the low expressing parent in one hybrid. The other listed genes displayed the same expression tendency in both reciprocal hybrids, but only in one hybrid an expression outside the range of the parents was found

[c] The listed genes exhibited the same expression tendency inside the range of the parents

dosage. In 10, 14 and 21 dap endosperm of four genotype combinations (Guo et al. 2003) and 13 and 19 dap endosperm of B73 and Mo17 (Stupar et al. 2007) this was demonstrated by comprehensive expression analysis. Also, in interpool and intrapool endosperm of the present study most gene expression patterns were not distinguishable from additivity. Therefore, a certain amplification of parental expression differences occurs for most genes in endosperm due to the parental genomic imbalance so that higher expression differences between more distant genotypes might already negatively affect its development. Supportive of this view, genetic distance affect transcriptional differences in endosperm much more than in embryos (Fig. 3). The evaluation of the number of differentially expressed genes between parental lines and hybrids showed that the combination of more distant genotypes results in more transcriptional differences. We found this tendency in embryo and more pronounced in endosperm. A positive relation of genetic distance and expression differences was also shown for a number of genotype combinations in above-ground seedling tissue 11 days after germination (Stupar et al. 2008) and immature ear tissues of a series of 16 maize hybrids that vary in their degree of heterosis (Guo et al. 2006).

Embryo and endosperm have diverse functions and fates and follow highly disparate modes of development. The embryo is formed from the zygote by asymmetric and symmetric cell divisions with progressive cell fate determination (Laux and Jürgens 1997) whereas the triploid nucleus of the primary endosperm initially divides without cytokinesis forming a syncytium during the first 3 dap before cellularization and differentiation take place (Sabelli and Larkins 2009). However, both tissues collectively showed heterotic traits in early development and exhibited a small number of intersecting genes that showed differential expression between the parental lines and hybrids in both genotype combinations. Two of these genes code for proteins of the central energy metabolism (ACG28895, ACG23899). Especially characteristics of the pyrophosphate-fructose 6-phosphate 1-phosphotransferase like energetic advantage mediation when glycolysis is the sole source of ATP (Mertens 1991) and a possible rate limiting quality in glycolytic carbon flow under conditions of high metabolic activity (Groenewald and Botha 2008) strikingly relate this gene to the higher growth rate of hybrid seed development. Homology of one clone to an ABA-induced protein (ACG47436) additionally involves this pivotal hormonal pathway, which regulate genetic and epigenetic processes (Chinnusamy et al. 2008). A possible relation of the hybrid state to epigenetic processes was further indicated by HTA112 among the intersecting genes, one of 15 known histone 2A variants in maize (http://www.chromdb.org). Variant histones are targeted to particular functional sites in the genome and have profound epigenetic consequences after incorporation into chromatin (Henikoff and Ahmad 2005). The common expression pattern and the function in central regulatory processes of each of these genes might indicate the association with heterosis to some extend. In addition, we identified members of three categories of genes coding for chromatin modifying proteins to be differentially expressed with a certain degree of commonness between either investigated tissues or the genotype combinations (Supplemental Table 2), which might indicate a contribution to epigenetic state regulation of hybrids. For hybrid embryos these indications are supported by the overrepresentation of the gene ontology terms nucleosome assembly and methylation-dependent chromatin silencing among the commonly differentially expressed genes in both genotype combinations (Fig. 3, Supplemental Table 3). It was suggested that an epigenetic perspective might be useful and complementary to conventional genetic explanations of the molecular basis of heterosis (Chandler and Stam 2004; Grant-Downton and Dickinson 2004). This might hold especially true for seed development shortly after fertilization, because the interplay of the diverse parental genomes has to be coordinated and adjusted for the onset of heterosis.

The extent of non-additive gene expression provides a measure for the interaction of the parental genomes, since deviations from the mid-parental expression level in hybrids require novel regulatory interactions in *trans*. A number of studies analyzing differential gene expression between inbred lines and hybrids in diverse tissues revealed expression trends from mainly mid-parental to mainly non-additive (Hochholdinger and Hoecker 2007). Our study revealed between 2.4 and 12.6% non-additively expressed genes in hybrid embryo and endosperm tissue. Compared to findings of other research groups this is a relative low number of genes outside the mid-parental or dosage dependent range. Based on microarray analyses of 14 days old seedlings Swanson-Wagner et al. (2006) determined 22% significantly non-additively expressed genes. Also by microarray analyses approximately 20% non-additive gene expression was detected in immature ears, 19 dap embryos and 11 days old seedlings (Stupar and Springer 2006). In primary roots 3.5 days after germination about 18% of 10,649 distinct microarray features displayed an expression level different from additivity in at least one of 12 inbred–hybrid combinations (Hoecker et al. 2008). In meristems non-additive gene expression prevailed (Uzarowska et al. 2007) and approximately half of the genes analyzed showed non-additive expression in immature ears (Guo et al. 2006). A high difference between the non-additively expressed gene fractions of the early endosperm, we analyzed, also exists to endosperm of later stages: at 13 and 19 dap 41.2 and 38.9% non-additively expressed genes were identified (Stupar et al. 2007).

Notably we identified a relatively low number of non-additively expressed genes in early embryo and endosperm despite the application of SSH to enrich for differentially expressed genes and an optimal hybridization design to assess expression level deviating from the mid-parental value in hybrids (Piepho 2005). For some instances this might be due to varying tissues analyzed, since different organs of a hybrid plant display significant differences in their degree of heterosis (Melchinger 1999). Alternatively or supplementary the developmental stage likely affects the amount of genomic interactions in hybrids since chromatin states specifying specific transcriptional patterns largely change during differentiation (Fransz and de Jong 2002). However, to answer if genetic interactions and non-additivity increase with developmental progression of the seed comparative studies of various stages of the same genotypes are required. Although we detected a relatively low number, the relative distribution of expression classes of non-additively expressed genes (Fig. 4) was consistent between the analyzed tissues and genotypes of our study and also very similar to the relative distribution found in primary roots of related European genotypes (Hoecker et al. 2008). The same classifications of gene expression pattern of immature ear, seedling, and embryo tissues from the maize inbred lines B73 and Mo17 also revealed consistency among the diverse tissues of the same genotypes (Stupar and Springer 2006) but large differences to the European genotypes. The number of genes with expression values between the parental levels was significantly lower in European genotype hybrids. These findings might indicate that the genotypes not only influence the number of non-additively expressed genes, as discussed above, but also the relative distribution of expression classes, i.e. the extent of gene expression deviation from the mid-parental level. The direction of expression variation seems not to be affected, since all genotypes and tissues showed a trend to expression levels of the low parent and below (Fig. 4; Hoecker et al. 2008; Stupar and Springer 2006).

Our gene expression analyses uncovered some genes displaying the same non-additive expression pattern in both reciprocal hybrids. Most of these genes showed expression levels of the low expressing parent in hybrids. Exceptionally the *d8* gene revealed overdominance in endosperm of all four hybrid genotypes analyzed. The maize *d8* gene is found to be an ortholog of the gibberellin (GA)-insensitive gene (*gai*) of *Arabidopsis thaliana* (Peng et al. 1997, 1999). GAI and D8 belong to the GRAS family of transcriptional regulators functioning as key components in GA signal transduction pathways to negatively regulate GA mediated responses in a mechanism of feedback regulation (Gao et al 2008). Recent studies revealed that they have broad interactions with other hormonal and environmental signaling and play important roles in many aspects of plant growth, development and adaption to environmental stresses (Fu and Harberd 2003; Achard et al. 2006, 2007). It is conceivable that D8 regulates processes that are connected to differentiation and proliferation of endosperm cells or to principal functions of early endosperm shortly after fertilization. Since our data refer to an overexpression of *d8* in early hybrid endosperm, this might prompt the further characterization of *d8* action in seed development and its possible role for heterosis.

In summary our data show distinct effects of hybridization on the fertilization products during early stages of seed development. Both tissues exhibited heterosis to different degrees associated with differential gene expression of similar pattern to varying extent. Outstanding genes with consistent expression pattern among tissues and genotypes provide entry points for further analysis of regulatory processes underlying hybrid vigor.

Acknowledgments For providing seeds of the inbred lines used in this study we thank Prof. Dr. A. Melchinger (University of Hohenheim) and his co-workers. We thank T. Okamoto for allocating cDNAs of zygotes and two-celled embryos and André Schützenmeister for data processing for GEO submission. Furthermore we are especially grateful to Petra von Wiegen, Marlis Nissen and Sabina Miaskowska for excellent technical help with embryo and endosperm isolation and microarray hybridization. For assisting the glass house work we are thankful to Bärbel Hagemann. We thank Prof. Dr. Erhard Kranz and Prof. Dr. Horst Lörz for providing working space and support. This work was financially supported by the Deutsche Forschungsgemeinschaft (DFG) grant SCHO746/2 within the framework program “heterosis in plants” and by the Eiselen-Foundation (Ulm).

References

Achard P, Cheng H, De Grauwe L, Decat J, Schoutteten H, Moritz T, Van Der Straeten D, Peng J, Harberd NP (2006) Integration of plant responses to environmentally activated phytohormonal signals. Science 311:91–94

Achard P, Liao L, Jiang C, Desnos T, Bartlett J, Fu X, Harberd NP (2007) DELLAs contribute to plant photomorphogenesis. Plant Physiol 143:1163–1172

Adams KL, Cronn R, Percifield R, Wendel JF (2003) Genes duplicated by polyploidy shows unequal contributions to the transcriptome and organ-specific reciprocal silencing. PNAS 100:4649–4654

Alleman M, Doctor J (2000) Genomic imprinting in plants: observations and evolutionary implications. Plant Mol Biol 43:147–161

Auger DL, Gray AD, Ream TS, Kato A, Coe EH Jr, Birchler JA (2005) Non-additive gene expression in diploid and triploid hybrids of maize. Genetics 169:389–397

Baroux C, Spillane C, Grossniklaus U (2002) Genomic imprinting during seed development. Adv Genet 46:165–214

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc 57:289–300

Berger F (1999) Endosperm development. Curr Opin Plant Biol 2:28–32

Birchler JA (1993) Dosage analysis of maize endosperm development. Annu Rev Genet 27:181–204

Birchler JA, Auger DL, Riddle NC (2003) In search of the molecular basis of heterosis. Plant Cell 15:2236–2239

Brink RA, Cooper DC (1947) The endosperm in seed development. Bot Rev 13:423–541

Chandler V, Stam M (2004) Chromatin conversations: mechanisms and implications of paramutation. Nat Rev Gen 5:532–544

Chinnusamy V, Gong Z, Zhu JK (2008) Abscisic acid-mediated epigenetic processes in plant development and stress responses. J Integr Plant Biol 50:1187–1195

Costa LM, Gutièrrez-Marcos JF, Dickinson HG (2004) More than a yolk: the short life and complex times of the plant endosperm. Trends Plant Sci 9:507–514

Drews GN, Lee D, Christensen CA (1998) Genetic analysis of female gametophyte development and function. Plant Cell 10:5–17

Fransz PF, de Jong FH (2002) Chromatin dynamics in plants. Curr Opin Plant Biol 5:560–567

Fu X, Harberd NP (2003) Auxin promotes Arabidopsis root growth by modulating gibberellin response. Nature 421:740–743

Gao XH, Huang XZ, Xiao SL, Fu XD (2008) Evolutionarily conserved DELLA-mediated gibberellin signaling in plants. J Integr Plant Biol 50:825–834

Goldberg RB, de Paiva G, Yadegari R (1994) Plant embryogenesis: zygote to seed. Science 266:605–614

Grant-Downton R, Dickinson H (2004) Plants, pairing and phenotypes: two’s company? Trends Genet 20:188–195

Groenewald JH, Botha FC (2008) Down-regulation of pyrophosphate: fructose 6-phosphate 1-phosphotransferase (PFP) activity in sugarcane enhances sucrose accumulation in immature internodes. Transgenic Res 17:85–92

Guo M, Rupe MA, Danilevskaya ON, Yang X, Hu Z (2003) Genome-wide mRNA profiling reveals heterochronic allelic variation and a new imprinted gene in hybrid maize endosperm. Plant J 36:30–44

Guo M, Rupe MA, Yang X, Crasta O, Zinselmeier C, Smith OS, Bowen B (2006) Genome-wide transcript analysis of maize hybrids: allelic additive gene expression and yield heterosis. TAG 113:831–845

Hämmerle B, Ferrus R (2003) Expression of enhancers is altered in *Drosophila melanogaster* hybrids. Evol Dev 5:221–230

Henikoff S, Ahmad K (2005) Assembly of variant histones into chromatin. Annu Rev Cell Dev Biol 21:133–153

Hochholdinger F, Hoecker N (2007) Towards the molecular basis of heterosis. Trends Plant Sci 12:427–432

Hoecker N, Keller B, Piepho H-P, Hochholdinger F (2005) Manifestation of heterosis during early maize (*Zea mays* L.) root development. TAG 12:421–429

Hoecker N, Keller B, Muthreich N, Chollet D, Descombes P, Piepho HP, Hochholdinger F (2008) Comparison of maize (*Zea mays* L.) F1-hybrid and parental inbred line primary root transcriptomes suggests organ-specific patterns of nonadditive gene expression and conserved expression trends. Genetics 179:1275–1283

Hong SK, Kitano H, Satoh H, Nagato Y (1996) How is the embryo size genetically regulated in rice? Development 122:2051–2058

Huang Y, Zhang L, Zhang J, Yuan D, Xu C, Li X, Zhou D, Wang S, Zhang Q (2006) Heterosis and polymorphisms of gene expression in an elite rice hybrid as revealed by microarray analysis of 9198 unique ESTs. Plant Mol Biol 62:579–591

Laux T, Jürgens G (1997) Embryogenesis: a new start in life. Plant Cell 9:989–1000

Le Q, Gutiérrez-Marcos JF, Costa LM, Meyer S, Dickinson HG, Lörz H, Kranz E, Scholten S (2005) Construction and screening of subtracted cDNA libraries from limited populations of plant cells: a comparative analysis of gene expression between maize egg cells and central cells. Plant J 44:167–178

Lin BY (1984) Ploidy barrier to endosperm development in maize. Genetics 107:103–115

Lopes MA, Larkins BA (1993) Endosperm origin, development, and function. Plant Cell 5:1383–1399

Melchinger AE (1999) Genetic diversity and heterosis. In: International symposium on genetics and exploitation of heterosis in crop plants, Mexico City, 17–22 August 1997, pp 99–118

Mertens E (1991) Pyrophosphate-dependent phosphofructokinase, an anaerobic glycolytic enzyme? FEBS Lett 285:1–5

Meyer S, Pospisil H, Scholten S (2007) Heterosis associated gene expression in maize embryo six days after fertilization exhibits additive, dominant and overdominant pattern. Plant Mol Biol 63:381–391

Michalak P, Noor MAF (2003) Genome-wide patterns of expression in Drosophila pure species and hybrid males. Mol Biol Evol 20:1070–1076

Okamoto T, Scholten S, Lörz H, Kranz E (2005) Identification of genes that are up- or down-regulated in the apical or basal cell of maize two-celled embryos and monitoring their expression during zygote development by a cell manipulation- and PCR-based approach. Plant Cell Physiol 46:332–833

Olsen OA (1998) Endosperm development. Plant Cell 10:485–488

Olsen OA (2001) Endosperm development: cellularization and cell fate specification. Annu Rev Plant Physiol Plant Mol Biol 52:233–267

Olsen OA (2004) Nuclear endosperm development in cereals and *Arabidopsis thaliana*. Plant Cell 16:214–227

Pang SZ, DeBoer DL, Wan Y, Ye G, Layton JG, Neher MK, Armstrong CL, Fry JE, Hinchee MA, Fromm ME (1996) An improved green fluorescent protein gene as a vital marker in plants. Plant Physiol 112:893–900

Peng J, Carol P, Richards DE, King KE, Cowling RJ, Murphy GP, Harberd NP (1997) The Arabidopsis GAI gene defines a signalling pathway that negatively regulates gibberellin response. Genes Dev 11:3194–3205

Peng J, Richards DE, Hartley NM, Murphy GP, Devos KM, Flintham JE, Beales J, Fish LJ, Worland AJ, Pelica F, Sudhakar D, Christou P, Snape JW, Gale MD, Harberd NP (1999) 'Green revolution' genes encode mutant gibberellin response modulators. Nature 400:256–261

Piepho H-P (2005) Optimal allocation in designs for assessing heterosis from cDNA gene expression data. Genetics 171:359–364

Rhoades MM, Dempsey E (1966) Induction of chromosome doubling at meiosis by the elongate gene in maize. Genetics 54:505–522

Römisch-Margl L, Spielbauer G, Schützenmeister A, Schwab W, Piepho H-P, Genschel U, Gierl A (2010) Heterotoc patterns of sugar and amino acid components in developing maize kernels. TAG (this issue)

Rood SB, Buzzell RI, Mander LN, Pearce D, Pharis RP (1988) Gibberellins: a phytohormonal basis for heterosis in maize. Science 241:1216–1218

Russel SD (1992) Double fertilization. Int Rev Cytol 140:357–390

Sabelli PA, Larkins BA (2009) The development of endosperm in grasses. Plant Physiol 149:14–26

Scott RJ, Spielman M, Bailey J et al (1998) Parent-of-origin effects on seed development in *Arabidopsis thaliana*. Development 125:3329–3341

Shull GH (1908) The composition of a field of maize. Proc Am Breeders Assoc 4:296–301

Shull GH (1952) Beginning of the heterosis concept. In: Gowen JW (ed) Heterosis. Iowa State College Press, Ames, pp 14–48

Smith OS, Smith JSC, Bowen SL, Tenborg RA, Wall SJ (1990) Similarities among a group of elite maize inbreds as measured by pedigree, F1 grain yield, heterosis, and RFLPs. TAG 80:833–840

Song R, Messing J (2003) Gene expression of a gene family in maize based on noncollinear haplotypes. PNAS 100:9055–9060

Springer NM, Stupar RM (2007) Allelic variation and heterosis in maize: how do two halves make more than a whole? Genome Res 17:264–275

Stupar RM, Springer NM (2006) Cis-transcriptional variation in maize inbred lines B73 and Mo17 leads to additive expression patterns in the F_1 hybrid. Genetics 173:2199–2210

Stupar RM, Hermanson PJ, Springer NM (2007) Nonadditive expression and parent-of-origin effects identified by microarray and allele-specific expression profiling of maize endosperm. Plant Physiol 145:411–425

Stupar RM, Gardiner JM, Oldre AG, Haun WJ, Chandler VL, Springer NM (2008) Gene expression analyses in maize inbreds and hybrids with varying levels of heterosis. BMC Plant Biol 8:33

Swanson-Wagner RA, Jia Y, DeCook R, Borsuk LA, Nettelton D, Schnable PS (2006) All possible modes of gene action are observed in a global comparison of gene expression in a maize F_1 hybrid and its inbred parents. PNAS 103:6805–6810

Uzarowska A, Keller B, Piepho H-P, Schwarz G, Ingvardsen C, Wenzel G, Lübberstedt T (2007) Comparative expression profiling in meristems of inbred-hybrid triplets of maize based on morphological investigations of heterosis for plant height. Plant Mol Biol 63:21–34

Vuylsteke M, van Eeuwijk F, Van Hummelen P, Kuiper M, Zabeau M (2005) Genetic analysis of variation in gene expression in *Arabidopsis thaliana*. Genetics 171:1267–1275

Wang FH (1947) Embryological development of inbred and hybrid *Zea mays* L. Am J Bot 34:113–125