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Molecular dissection of heterosis manifestation during early maize root development

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Abstract Heterosis is of paramount agronomic importance and has been successfully exploited in maize hybrid breeding for decades. Nevertheless, the molecular basis of heterosis remains elusive. Heterosis is not only observed in adult traits like yield or plant height, but is already detected during embryo and seedling development. Hence, the maize (*Zea mays* L.) primary root which is the first organ that emerges after germination is a suitable model to study heterosis manifestation. Various seedling root traits including primary root length and lateral root density display heterosis. Microarray studies suggest organ specific patterns of nonadditive gene expression in maize hybrids. Moreover, such experiments support the notion that global expression trends in maize primary roots are conserved between different hybrids. Furthermore, nonadditive expression patterns of specific genes such as a *SUPEROXIDE DISMUTASE 2* might contribute to the early manifestation of heterosis. Proteome profiling experiments of maize hybrid primary roots revealed nonadditive accumulation patterns that were distinct from the corresponding RNA profiles underscoring the importance of posttranscriptional processes such as protein modifications that might be related to heterosis. Finally, analysis of selected metabolites imply that a subtle regulation of particular biochemical pathways such as the phenylpropanoid pathway in hybrids might contribute to the manifestation of heterosis in maize primary roots. In the future, recently developed molecular tools will facilitate the analysis of the molecular principles underlying heterosis in maize roots.

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Introduction

Heterosis describes the superior performance of highly heterozygous F_1-hybrids compared to the average (midparent) performance of their genetically distinct homozygous parents. Repeated selfing of F_1-individuals over several generations leads to less vigorous plants by reducing the degree of heterozygosity of the progeny—a process called inbreeding depression. Heterosis is of outstanding agronomic importance and its extensive exploitation in maize breeding is based on the findings of East (1908) and Shull (1908). Collectively, three major genetic concepts are discussed to explain heterosis although little consensus has emerged concerning their contribution to the phenomenon. The "dominance" hypothesis attributes heterosis to the complementation of deleterious alleles in hybrids by the presence of superior alleles in one of the two parental inbred lines (Jones 1917). In contrast, the "overdominance" hypothesis accounts allelic interactions at one or multiple loci for the manifestation of heterotic traits (Shull 1908). Alternatively, interactions of non-allelic loci might explain the phenomenon of heterosis—a hypothesis called "epistasis" (Powers 1945). Recent studies tend to explain hybrid vigor by the contribution of each of the three genetic concepts to the manifestation of heterosis (Lippman and Zamir 2007). Although, it is generally assumed that heterosis is controlled by a considerable number of genes (Lamkey and Edwards 1998; Stuber 1999), linking the establishment of

heterotic traits with causal molecular mechanisms remains the major challenge in heterosis research. Heterosis can be categorized with regard to the deviation of phenotypic traits from the midparent value (MPV) in hybrid plants. The term MPV refers to the average value of a trait in the two parental inbred lines. Midparent heterosis (MPH) describes the deviation of a given trait from the MPV whereas best parent heterosis (BPH) refers to phenotypic values above the better performing parent (Hochholdinger and Hoecker 2007). Heterotic effects have been reported for various plant species, e.g. rice, wheat, sorghum, cotton, maize, sunflower, rapeseed, tomato (Duvick 1999), as well as for various traits, e.g. biomass, size, agronomic yield, pest resistance and tolerance to abiotic stress (Falconer and Mackay 1996). The highest degree of heterosis can be observed for adult traits (Falconer and Mackay 1996), although heterosis can already be detected during the early stages of embryo (Meyer et al. 2007) and seedling development (Hoecker et al. 2006). Today, the vast majority of maize (*Zea mays* L.) planted in North America and Europe are hybrids. Since the introduction of hybrids, maize yield increased from 1 to 8 tons per hectare in the US (Duvick 2001). According to the Food and Agriculture Organization (FAO) of the United Nations (http://www.fao.org) maize is one of the most important cereal crops and serves as staple food in many regions of the world with 785 million tons produced in 2007. Its agronomic importance and the intensive application of hybrid breeding in maize production, the availability of elaborate genetic tools (Candela and Hake 2008), and the recent release of a draft sequence of the maize genome (http://www.maizesequence.org) make this species an eminent object to study the molecular mechanisms of heterosis.

Maize roots as a model for heterosis studies

Historically, most phenotypic analyses in heterosis research have focussed on aerial parts of model plants. Although the phenotypic manifestation of heterosis at the seedling stage has been recognized already in the early days of heterosis research (Kempton and McLane 1942; Murdoch 1940; Sprague 1936; Wang 1947) detailed analyses of early post-embryonic development in modern hybrid lines are rare (Hoecker et al. 2006; Wang et al. 2006). Studying the manifestation of heterosis during the early stages of root development in maize (Fig. 1) provides numerous benefits. The emergence of the primary root as first organ allows early morphological, histological and physiological analyses of the seedling (Hochholdinger et al. 2004b). This temporal pattern in maize seedling development also points to a key role of the early root system in heterosis manifestation as the primary and seminal roots are crucial for early seedling vigor. In addition, the very simple, well-defined

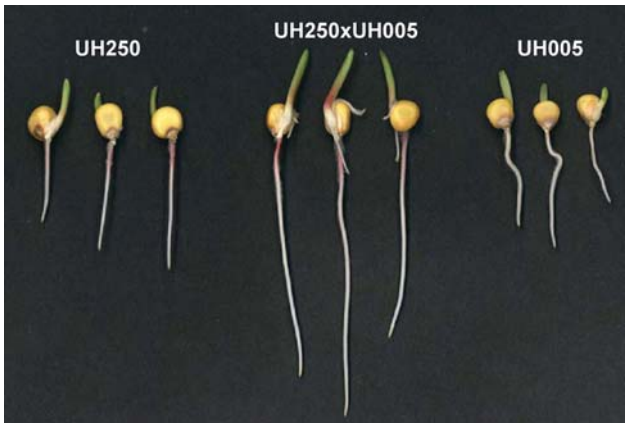

Fig. 1 Heterosis can already be observed during early seedling development as demonstrated for primary root length by 3.5 days seedlings of the maize hybrid UH250 × UH005, and its parental inbred lines UH250 and UH005

histological structure of the primary root with distinct longitudinal developmental zones allows detailed comparative phenotypic analyses. Moreover, root hairs can be used as phenotypic markers as their presence distinguishes the differentiation zone from the meristematic and elongation zones (Hochholdinger et al. 2004c). Finally, fast germination and growth of maize seedlings in laboratory environments allows high-throughput experiments under standardized conditions.

Manifestation of heterosis during early maize root system development

Only recently, a systematic survey of morphological and histological traits of young seedling roots in maize hybrids and their parental inbred lines has been performed (Hoecker et al. 2006). In this study, root traits of four German inbred lines of the flint and dent pools and their corresponding twelve reciprocal hybrids have been quantified (Hoecker et al. 2006). It was demonstrated that different root traits display distinct levels of MPH (Fig. 2). Lateral root density was determined by counting the lateral root primordia after Feulgen staining. This technique generates a purple precipitate in dividing cells and thus allows for the analysis of not yet emerged lateral root primordia. Lateral root density displayed on average 51% MPH over all twelve hybrids whereby individual hybrid lines displayed MPH levels up to 130%. Hence, lateral root density displayed on average the highest degree of heterosis of all surveyed traits (Hoecker et al. 2006). Primary root length (17–25% MPH between 3 and 7 days after germination) and cortical cell elongation (24% MPH at 5 days after germination) in the parenchyma of primary roots which is directly associated with root length displayed similar levels of average MPH.

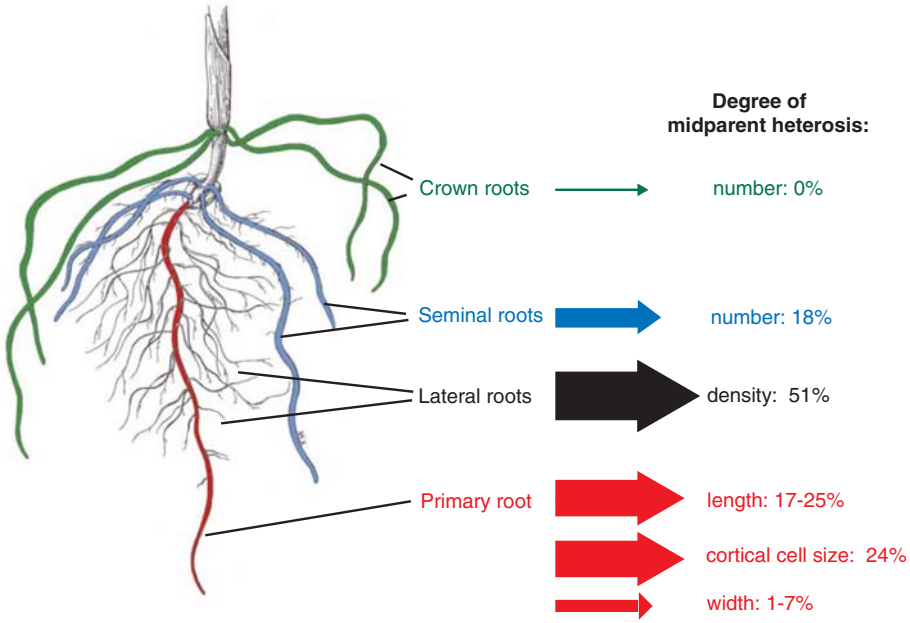

Fig. 2 Different traits of the maize seedling root system display different degrees of midparent heterosis

In contrast, primary root width displayed on average only 1–7% MPH between 3 and 7 days after germination. While maize always forms a single primary root the number of seminal and crown roots varies between different genetic backgrounds. The number of seminal roots displayed on average 18% MPH in hybrids. In contrast, the number of shootborne crown roots formed at the first shoot-node displayed less variability than the number of seminal roots. Therefore, on average no MPH was observed for this trait (Hoecker, unpublished observation). This data illustrates that heterosis in young seedling roots is manifested as early as 3 days after germination. An increased primary root length, lateral root density, and seminal root number might significantly contribute to early seedling vigor and thus provide hybrids an advantage over less vigorous homozygous inbred line seedlings. This study underscores the suitability of the very young maize seedling root system as a model to study the early events of heterosis manifestation before significant phenotypic differences between inbred lines and hybrids are developed.

Transcriptome profiling of early heterosis manifestation in maize roots

High-throughput profiling techniques such as microarray hybridization experiments allow for heterosis-related global transcriptome surveys in inbred lines and hybrids. Recently, global gene expression levels were analyzed during the very early stages of heterosis manifestation in 3.5-day-old maize primary roots (Hoecker et al. 2008a). Goal of this study was to identify genes that are expressed in a nonadditive manner between inbred lines and hybrids. Gene expression levels of four different inbred lines and the twelve corresponding reciprocal hybrids generated from these inbred lines were compared. The hybridization scheme (Keller et al. 2005) of this experiment compared 168 inbred and hybrid cDNA samples on 84 cDNA microarray chips that represented 10,649 unique maize transcripts. In order to increase the resolution of this microarray experiment each microarray chip was scanned at six different scanning intensities after hybridization. Subsequently, the data was combined using a non-linear latent regression model (Piepho et al. 2006). In total, 1941 of the 10,649 (18%) distinct microarray features displayed expression levels that were distinct from additivity in at least one hybrid (FDR < 5%). Nonadditive gene expression patterns in only a fraction of the surveyed genes is in line with the outcome of several other high-throughput gene expression studies that surveyed heterosis-related gene expression patterns in different organs of maize hybrids and demonstrated that additivity was the prevailing global expression pattern in these tissues (Meyer et al. 2007; Stupar and Springer 2006; Swanson-Wagner et al. 2006). However, other studies in maize also reported predominant nonadditive gene expression (Uzarowska et al. 2007) or a similar number of additively and nonadditively expressed genes (Guo et al. 2006). A simple comparison of these maize transcriptome studies is difficult because they significantly differ in the genotypes that were investigated, in the experimental design, the applied profiling techniques, and the data analysis tools (Hoecker et al. 2008a). A comparison of the maize primary root and maize shoot apical meristem transcriptomes that were surveyed in the same genotypes with the help of the same microarray platforms, experimental designs, and data analysis tools revealed less overlap in

nonadditively expressed genes than expected by chance (Hoecker et al. 2008a). This implies at least for the primary root and the shoot apical meristem organ specificity of nonadditive gene expression. This notion is also supported by the fact that in maize different organs and developmental stages display different degrees of heterosis (Springer and Stupar 2007b). Further investigation of the 1941 genes that were nonadditively expressed in young maize primary roots revealed that the expression levels of 89% of these genes fell between the parental expression levels whereas only 11% of the genes were above best or below the low parent values. Subsequent qRT-PCR analyses of a subset of 64 genes confirmed these results and demonstrated that global expression trends are conserved in different hybrids (Hoecker et al. 2008a). Reciprocal expression differences were only found for a small fraction (6%) of the nonadditively expressed genes. Among the 64 nonadditively expressed genes that were analyzed via qRT-PCR a *SUPEROXIDE DISMUTASE 2* (*SOD2*) showed expression levels consistently above the MPV in all twelve analyzed hybrids. The enzyme SOD2 is involved in the detoxification of reactive oxygen species and plays a protective role which could be relevant for the better performance of hybrids compared to their parental inbred lines. In summary, this microarray study provides evidence for organ specific patterns of nonadditive gene expression in hybrids. Moreover, the notion that global expression trends in particular organs at a given developmental stage are conserved between different hybrids is supported. Finally, it appears that nonadditive expression patterns of key genes contribute to the early manifestation of heterosis.

Proteome analysis of heterosis manifestation in maize primary roots

Proteomics technology combines the resolution of two-dimensional electrophoresis with the sensitivity of mass spectrometric protein identification (Rose et al. 2004). This technology has been successfully applied to analyze different aspects of maize root development (Chang et al. 2000; Dembinsky et al. 2007; Hochholdinger et al. 2004a; Hochholdinger et al. 2005; Liu et al. 2006; Sauer et al. 2006; Wen et al. 2005). Recently, nonadditive protein accumulation patterns in 3.5-day-old primary roots of a maize hybrid and its parental inbred lines were studied via a proteomic approach (Hoecker et al. 2008b). Two-dimensional gel electrophoresis revealed that 49% of all proteins detected in hybrid primary root protein extracts accumulated nonadditively compared to only 18% nonadditively expressed transcripts in maize primary roots at the same developmental stage. Moreover, while on the transcriptome level only 11% of the nonadditively expressed genes displayed above high or below low parent expression, 51% of the nonadditively accumulated proteins belonged to these categories. ESI MS/MS identification of these proteins related most of these proteins to metabolism or defense (Hoecker et al. 2008b). The difference in the overall additivity patterns and the extent of nonadditive gene expression and protein accumulation in maize primary roots might be due to differences in protein versus RNA stability or could be explained by protein modifications that cannot be detected on the RNA level. Identification of nonadditively accumulated protein also provides cues on biochemical pathways that display different activities in inbred lines and hybrids. In hybrid primary roots several isoforms of three enzymes of the phenylpropanoid pathway were accumulated below the midparent value (Hoecker et al. 2008b). A subsequent analysis of seven metabolites related to this pathway revealed that *trans*-coumaric and *trans*-cinnamic acid were also accumulated significantly below the midparent value in hybrids. Consistently with these results the levels of other metabolites of this pathway tended to be below the midparent value. In summary, the down-regulation of transcripts, proteins and metabolites related to the phenylpropanoid pathway which is involved in lignification, growth and defense (Dixon et al. 2002) might imply a role of these processes in the manifestation of heterosis.

Future directions: new tools to answer old questions

Understanding the principles of heterosis is a long-standing challenge in plant breeding and genetics. While early heterosis studies focused on morphological and physiological analyses of inbred lines and hybrids, the advent of molecular biology introduced novel molecular tools to dissect heterosis. Among those, allele-specific analysis of gene expression in hybrids is a promising approach to understand *cis* and *trans* influences on gene expression in hybrids (Guo et al. 2004; Guo et al. 2008; Springer and Stupar 2007a). Maize is in particular suited for such analyses as its genome is highly polymorphic (Haberer et al. 2005). This becomes apparent by the high content of repetitive sequences and tandem duplications in the maize genome, and the extensive degree of sequence polymorphisms between different maize inbred lines (Springer and Stupar 2007b). The analysis of 592 unigenes of the maize inbred lines B73 and Mo17 revealed that on average single nucleotide polymorphisms (SNPs) occur every 73 bp (Bi et al. 2006). The presence of SNPs has been successfully applied to study allele-specific expression in maize hybrids by WAVE dHPLC (Guo et al. 2003, 2004), or dideoxyterminator assays (Springer and Stupar 2007a; Woll et al. 2006). The major drawback of these approaches is their low-throughput. In contrast, tag-based sequencing techniques such as massively parallel signature sequencing (MPSS) allow high-throughput gene expression analyses (Brenner

et al. 2000; Harbers and Carninci 2005; Vega-Sanchez et al. 2007; Velculescu et al. 1995) and have already been applied to study genome-wide allele-specific gene expression in maize hybrids (Guo et al. 2008).The recent development of new high-throughput sequencing methods including pyrosequencing (Margulies et al. 2005), ligation- or polymerase-based sequencing by synthesis (Mardis 2008) will provide new insights into allele-specific gene expression patterns. In addition, these techniques allow for global analyses of DNA methylation patterns (Cokus et al. 2008), genome structure, (Brunner et al. 2005; Fu and Dooner 2002; Morgante et al. 2005; Song and Messing 2003; Wang and Dooner 2006), epigenetics (Hollick 2008), and only recently appreciated RNA populations such as non-coding RNAs, small RNAs or compartmentalized RNAs (Kapranov et al. 2007). Finally, metabolic fluxes (Ettenhuber et al. 2005; Spielbauer et al. 2006), and integrated networks (Andorf et al. 2009) will receive more attention and facilitate new insights into the molecular principles underlying hybrid vigor.

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References

Andorf S, Gartner T, Steinfath M, Witucka-Wall H, Altmann T, Repsilber D (2009) Towards systems biology of heterosis: a hypothesis about molecular network structure applied for the *Arabidopsis* metabolome. EURASIP J Bioinform Syst Biol:147157

Bi IV, McMullen MD, Sanchez-Villeda H, Schroeder S, Gardiner J, Polacco M, Soderlund C, Wing R, Fang Z, Coe EH (2006) Single nucleotide polymorphisms and insertion-deletions for genetic markers and anchoring the maize fingerprint contig physical map. Crop Sci 46:12–21

Brenner S, Johnson M, Bridgham J, Golda G, Lloyd DH, Johnson D, Luo SJ, McCurdy S, Foy M, Ewan M, Roth R, George D, Eletr S, Albrecht G, Vermaas E, Williams SR, Moon K, Burcham T, Pallas M, DuBridge RB, Kirchner J, Fearon K, Mao J, Corcoran K (2000) Gene expression analysis by massively parallel signature sequencing (MPSS) on microbead arrays. Nat Biotechnol 18:630–634

Brunner S, Fengler K, Morgante M, Tingey S, Rafalski A (2005) Evolution of DNA sequence nonhomologies among maize inbreds. Plant Cell 17:343–360

Candela H, Hake S (2008) The art and design of genetic screens: maize. Nat Rev Genet 9:192–203

Chang WWP, Huang L, Shen M, Webster C, Burlingame AL, Roberts JKM (2000) Patterns of protein synthesis and tolerance of anoxia in root tips of maize seedlings acclimated to a low-oxygen environment, and identification of proteins by mass spectrometry. Plant Physiol 122:295–317

Cokus SJ, Feng SH, Zhang XY, Chen ZG, Merriman B, Haudenschild CD, Pradhan S, Nelson SF, Pellegrini M, Jacobsen SE (2008) Shotgun bisulphite sequencing of the *Arabidopsis* genome reveals DNA methylation patterning. Nature 452:215–219

Dembinsky D, Woll K, Saleem M, Liu Y, Fu Y, Borsuk LA, Lamkemeyer T, Fladerer C, Madlung J, Barbazuk B, Nordheim A, Nettleton D, Schnable PS, Hochholdinger F (2007) Transcriptomic and proteomic analyses of pericycle cells of the maize primary root. Plant Physiol 145:575–588

Dixon RA, Achnine L, Kota P, Liu CJ, Reddy MSS, Wang LJ (2002) The phenylpropanoid pathway and plant defence—a genomics perspective. Mol Plant Pathol 3:371–390

Duvick DN (1999) Heterosis: feeding people and protecting natural resources. In: Coors JG, Pandey S (eds) The genetics and exploitation of heterosis in crops. American Society of Agronomy, Crop Science Society of America and Soil Science Society of America, pp 19–29

Duvick DN (2001) Biotechnology in the 1930s: the development of hybrid maize. Nat Rev Genet 2:69–74

East EM (1908) Inbreeding in corn. Conn Agric Exp Stn 1907:419–428

Ettenhuber C, Spielbauer G, Margl L, Hannah LC, Gierl A, Bacher A, Genschel U, Eisenreich W (2005) Changes in flux pattern of the central carbohydrate metabolism during kernel development in maize. Phytochemistry 66:2632–2642

Falconer DS, Mackay TFC (1996) Introduction to quantitative genetics, 4th edn. Longman, Harlow

Fu HH, Dooner HK (2002) Intraspecific violation of genetic colinearity and its implications in maize. Proc Natl Acad Sci USA 99:9573–9578

Guo M, Rupe MA, Danilevskaya ON, Yang XF, Hut ZH (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, Zinselmeier C, Habben J, Bowen BA, Smith OS (2004) Allelic variation of gene expression in maize hybrids. Plant Cell 16:1707–1716

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

Guo M, Yang S, Rupe M, Hu B, Bickel DR, Arthur L, Smith O (2008) Genome-wide allele-specific expression analysis using massively parallel signature sequencing (MPSS) reveals *cis*- and *trans*-effects on gene expression in maize hybrid meristem tissue. Plant Mol Biol 66:551–563

Haberer G, Young S, Bharti AK, Gundlach H, Raymond C, Fuks G, Butler E, Wing RA, Rounsley S, Birren B, Nusbaum C, Mayer KFX, Messing J (2005) Structure and architecture of the maize genome. Plant Physiol 139:1612–1624

Harbers M, Carninci P (2005) Tag-based approaches for transcriptome research and genome annotation. Nat Methods 2:495–502

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

Hochholdinger F, Guo L, Schnable PS (2004a) Lateral roots affect the proteome of the primary root of maize (*Zea mays* L.). Plant Mol Biol 56:397–412

Hochholdinger F, Park WJ, Sauer M, Woll K (2004b) From weeds to crops: genetic analysis of root development in cereals. Trends Plant Sci 9:42–48

Hochholdinger F, Woll K, Sauer M, Dembinsky D (2004c) Genetic dissection of root formation in maize (*Zea mays* L.) reveals root-type specific developmental programmes. Ann Bot 93:359–368

Hochholdinger F, Woll K, Guo L, Schnable PS (2005) The accumulation of abundant soluble proteins changes early in the development of the primary roots of maize (*Zea mays* L.). Proteomics 5:4885–4893

Hoecker N, Keller B, Piepho HP, Hochholdinger F (2006) Manifestation of heterosis during early maize (*Zea mays* L.) root development. Theor Appl Genet 112:421–429

Hoecker N, Keller B, Muthreich N, Chollet D, Descombes P, Piepho HP, Hochholdinger F (2008a) Comparison of maize (*Zea mays* L.) F_1-hybrid and parental inbred line primary root transcriptomes

suggests organ-specific patterns of nonadditive gene expression and conserved expression trends. Genetics 179:1275–1283

Hoecker N, Lamkemeyer T, Sarholz B, Paschold A, Fladerer C, Madlung J, Wurster K, Stahl M, Piepho HP, Nordheim A, Hochholdinger F (2008b) Analysis of nonadditive protein accumulation in young primary roots of a maize (*Zea mays* L.) F_1-hybrid compared to its parental inbred lines. Proteomics 8:3882–3894

Hollick JB (2008) Sensing the epigenome. Trends Plant Sci 13:398–404

Jones DF (1917) Dominance of linked factors as a means of accounting for heterosis. Genetics 2:466–479

Kapranov P, Willingham AT, Gingeras TR (2007) Genome-wide transcription and the implications for genomic organization. Nat Rev Genet 8:413–423

Keller B, Emrich K, Hoecker N, Sauer M, Hochholdinger F, Piepho HP (2005) Designing a microarray experiment to estimate dominance in maize (*Zea mays* L.). Theor Appl Genet 111:57–64

Kempton JH, McLane JW (1942) Hybrid vigor aid weight of germs in the seeds of maize. J Agric Res 64:0065–0080

Lamkey KR, Edwards JW (1998) Heterosis: theory and estimation.. Proceedings of the 34th Illinois Corn Breeders' School, Urbana, pp 62–72

Lippman ZB, Zamir D (2007) Heterosis: revisiting the magic. Trends Genet 23:60–66

Liu Y, Lamkemeyer T, Jakob A, Mi GH, Zhang FS, Nordheim A, Hochholdinger F (2006) Comparative proteome analyses of maize (*Zea mays* L.) primary roots prior to lateral root initiation reveal differential protein expression in the lateral root initiation mutant *rum1*. Proteomics 6:4300–4308

Mardis ER (2008) The impact of next-generation sequencing technology on genetics. Trends Genet 24:133–141

Margulies M, Egholm M, Altman WE, Attiya S, Bader JS, Bemben LA, Berka J, Braverman MS, Chen YJ, Chen ZT, Dewell SB, Du L, Fierro JM, Gomes XV, Godwin BC, He W, Helgesen S, Ho CH, Irzyk GP, Jando SC, Alenquer MLI, Jarvie TP, Jirage KB, Kim JB, Knight JR, Lanza JR, Leamon JH, Lefkowitz SM, Lei M, Li J, Lohman KL, Lu H, Makhijani VB, McDade KE, McKenna MP, Myers EW, Nickerson E, Nobile JR, Plant R, Puc BP, Ronan MT, Roth GT, Sarkis GJ, Simons JF, Simpson JW, Srinivasan M, Tartaro KR, Tomasz A, Vogt KA, Volkmer GA, Wang SH, Wang Y, Weiner MP, Yu PG, Begley RF, Rothberg JM (2005) Genome sequencing in microfabricated high-density picolitre reactors. Nature 437:376–380

Meyer S, Pospisil H, Scholten S (2007) Heterosis associated gene expression in maize embryos 6 days after fertilization exhibits additive, dominant and overdominant pattern. Plant Mol Biol 63:381–391

Morgante M, Brunner S, Pea G, Fengler K, Zuccolo A, Rafalski A (2005) Gene duplication and exon shuffling by helitron-like transposons generate intraspecies diversity in maize. Nat Genet 37:997–1002

Murdoch HA (1940) Hybrid vigor in maize embryos. J Hered 31:361–363

Piepho HP, Keller B, Hoecker N, Hochholdinger F (2006) Combining signals from spotted cDNA microarrays obtained at different scanning intensities. Bioinformatics 22:802–807

Powers L (1945) Relative yields of inbred lines and F_1-hybrids of tomato. Bot Gaz 106:247–268

Rose JKC, Bashir S, Giovannoni JJ, Jahn MM, Saravanan RS (2004) Tackling the plant proteome: practical approaches, hurdles and experimental tools. Plant J 39:715–733

Sauer M, Jakob A, Nordheim A, Hochholdinger F (2006) Proteomic analysis of shoot-borne root initiation in maize (*Zea mays* L.). Proteomics 6:2530–2541

Shull GF (1908) The composition of a field of maize. Am Breed Assoc Rep 4:296–301

Song RT, Messing J (2003) Gene expression of a gene family in maize based on noncollinear haplotypes. Proc Natl Acad Sci USA 100:9055–9060

Spielbauer G, Margl L, Hannah LC, Romisch W, Ettenhuber C, Bacher A, Gierl A, Eisenreich W, Genschel U (2006) Robustness of central carbohydrate metabolism in developing maize kernels. Phytochemistry 67:1460–1475

Sprague GF (1936) Hybrid vigor and growth rates in a maize cross and its reciprocal. J Agricl Res 53:0819–0830

Springer NM, Stupar RM (2007a) Allele-specific expression patterns reveal biases and embryo-specific parent-of-origin effects in hybrid maize. Plant Cell 19:2391–2402

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

Stuber CW (1999) Biochemistry, molecular biology and physiology of heterosis. In: Coors JG, Pandey S (eds) Genetics and exploitation of heterosis in crops. American Society of Agronomy, Crop Science Society of America, Madison, pp 173–184

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

Swanson-Wagner RA, Jia Y, DeCook R, Borsuk LA, Nettleton D, Schnable PS (2006) All possible modes of gene action are observed in a global comparison of gene expression in a maize F1 hybrid and its inbred parents. Proc Natl Acad Sci USA 103:6805–6810

Uzarowska A, Keller B, Piepho HP, Schwarz G, Ingvardsen C, Wenzel G, Lubberstedt 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

Vega-Sanchez ME, Gowda M, Wang GL (2007) Tag-based approaches for deep transcriptome analysis in plants. Plant Sci 173:371–380

Velculescu VE, Zhang L, Vogelstein B, Kinzler KW (1995) Serial analysis of gene expression. Science 270:484–487

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

Wang QH, Dooner HK (2006) Remarkable variation in maize genome structure inferred from haplotype diversity at the bz locus. Proc Natl Acad Sci USA 103:17644–17649

Wang ZK, Ni ZF, Wu HL, Nie XL, Sun QX (2006) Heterosis in root development and differential gene expression between hybrids and their parental inbreds in wheat (*Triticum aestivum* L.). Theor Appl Genet 113:1283–1294

Wen TJ, Hochholdinger F, Sauer M, Bruce W, Schnable PS (2005) The *roothairless1* gene of maize encodes a homolog of *sec3*, which is involved in polar exocytosis. Plant Physiol 138:1637–1643

Woll K, Dressel A, Sakai H, Piepho HP, Hochholdinger F (2006) *ZmGrp3*: identification of a novel marker for root initiation in maize and development of a robust assay to quantify allele-specific contribution to gene expression in hybrids. Theor Appl Genet 113:1305–1315