Heterosis: emerging ideas about hybrid vigour

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Abstract

Perceived by Charles Darwin in many vegetable plants and rediscovered by George H Shull and Edward M East in maize, heterosis or hybrid vigour is one of the most widely utilized phenomena, not only in agriculture but also in animal breeding. Although, numerous studies have been carried out to understand its genetic and/or molecular basis in the past 100 years, our knowledge of the underlying molecular processes that results in hybrid vigour can best be defined as superficial. Even after century long deliberations, there is no consensus on the relative/individual contribution of the genetic/epigenetic factors in the manifestation of heterosis. However, with the recent advancements in functional genomics, transcriptomics, proteomics, and metabolomics-related technologies, the riddle of heterosis is being reinvigorated by adopting systems-level approaches to understand the underlying molecular mechanisms. A number of intriguing hypotheses are converging towards the idea of a cumulative positive effect of the differential expression of a variety of genes, on one or several yield-affecting metabolic pathways or overall energy-use efficiency, as the underlying mechanism for the manifestation of heterosis. Presented here is a brief account of clues gathered from various investigative approaches targeted towards better scientific understanding of this process.

Key words: Circadian clock, dominance, energy use efficiency, growth and development, over-dominance, yield.

Introduction

Heterosis or hybrid vigour is a phenomenon that describes the survival and performance superiority of a hybrid offspring over the average of both its genetically distinct parents. In plants, heterosis is known to be a multigenic complex trait and can be extrapolated as the sum total of many physiological and phenotypic traits including magnitude and rate of vegetative growth, flowering time, yield (in terms of inflorescence number, flowers per inflorescence, fruit or grain set and weight), and resistance to biotic and abiotic environmental rigours; each of them contributing to heterosis to a certain extent (Lippman and Zanim, 2007).

Clues from genetic studies

Conventionally, the manifestation of heterosis has been attributed to ‘Dominance’ as well ‘Over-dominance’ as the major underlying genetic factors. Later ‘Epistatic interactions’ and ‘Epigenetics factors’ were also found to contribute to heterosis. Emerging views now suggest that the combination of the parental pair has a major bearing on the extent of heterosis manifested by the hybrid for different traits and thereby affecting the overall performance of the hybrid (Chen, 2010). Hybrid formation could also be defined as an event where two adequately distinct genomes come together and undergo a phase of genomic turbulence before entering a state of homeostasis. This phase of genomic turbulence or ‘Genomic Shock’, as proposed by Barbara McClintock (McClintock, 1984), may cause the genome-wide relaxation of gene expression including transposon activation, ultimately leading to differential/novel expression patterns (Ha et al., 2009).

Clues from transcriptOMIC studies

A number of whole-genome transcriptome analyses have been carried out in successful inbred parental lines and the hybrid combinations to classify differential gene expression patterns...
into modes of gene action in a hybrid vis-à-vis its inbred parental lines and to correlate those changes to gains in biomass and yield (Kollippara et al., 2002; Guo et al., 2003, 2004, 2006; Auger et al., 2005; Bao et al., 2005; Huang et al., 2006a, b; Swanson-Wagner et al., 2006; Hochholdinger and Hoecker, 2007; Meyer et al., 2007; Song et al., 2007; Springer and Stupar, 2007; Uzarowska et al., 2007; Hoecker et al., 2008a, b; Stupar et al., 2008; Zhang et al., 2008; Gang Wei et al., 2009; Birchler et al., 2010; Frisch et al., 2010; Jahnke et al., 2010; Riddle et al., 2010). In another strategy, transcriptomes of large parental populations have been analysed to establish correlations between differential expression patterns in a number of parental inbreds to yield-related parameters in the hybrids resulting from their factorial crosses (Hoecker et al., 2008a; Stupar et al., 2008). As a universal code for heterosis related transcriptomic studies, the modes of gene action (for differentially expressed genes) have mainly been classified into additive, dominance, and over-dominance categories. The additive mode of gene action represents mid-parental expression values in the hybrid, while the dominance mode signifies both low and high parent-like expression. In the case of over-dominance or under-dominance, the expression value of a particular differentially expressed gene in the hybrid is either higher than the high parent or lower than the low parent value, respectively. The initial transcriptome based studies (Comings and MacMurray, 2000) explained heterosis as the establishment of more favourable gene expression levels in the hybrids compared with the parents. Several studies in maize and rice reported the prevalence of non-additive gene expression (Auger et al., 2005; Uzarowska et al., 2007; Hoecker et al., 2008a; Zhang et al., 2008; Fujimoto et al., 2012; Meyer et al., 2012), while others detected mainly additive gene expression levels (Swanson-Wagner et al., 2006; Meyer et al., 2007; Zhang et al., 2008). In contrast to the observation in maize (Swanson-Wagner et al., 2006), work on growth heterosis in larval pacific oysters revealed non-additive gene action to be the predominant mode of gene action during the active growth phase in the hybrids (Hedgecock et al., 2007). Of the two heterotic hybrids resulting from PA64s X93-11 and the Nipponbare X93-11 crosses, the first predominantly exhibited additive (Zhang et al., 2008), while non-additivity was the major mode of gene action in the second hybrid, at the younger stages of development (Zhang et al., 2008; He et al., 2010). In two recent reports, the manifestation of biomass heterosis in Arabidopsis Col-0 and C24 hybrids during the early stages of vegetative development has been attributed to the predominance of either dominant [high parent-like; intermediate; (Meyer et al., 2012) or over-dominant; higher than the high parent; (Fujimoto et al., 2012)] non-additive gene expression modes. The moderate non-additivity was suggested to lead to higher metabolic activity due to a better resource efficiency, and therefore enhanced performance in the hybrids (Meyer et al., 2012). Fujimoto and coworkers, however, have correlated extreme non-additivity, exhibited by a major proportion of differentially expressed genes, to increased photosynthesis and determination of cell size and number, which could play a central role in the manifestation of biomass heterosis (Fujimoto et al., 2012).

Transcriptomic analyses have been further refined to reveal allele-specific expression in reciprocal hybrids but the significance of maternal or paternal effects on gene expression could not be established (Guo et al., 2004; Stupar and Springer, 2006). Recently, in maize and Arabidopsis, epigenetic modifications in circadian clock genes and altered gene expression resulting from differentially expressed small RNAs have been linked to increased biomass and, eventually, to heterosis (Ni et al., 2009; Groszmann et al., 2011). Surprisingly, over-dominant mode of gene action of a single flowering gene has been found to drive yield heterosis (Krieger et al., 2010). Data from transcriptomic studies has enhanced our understanding of the extent of genome-wide transcriptome fluctuations, while highlighting the importance of certain key biochemical pathways that may prove to be quintessential for the manifestation of heterosis. These studies have also revealed the common occurrence of genes from basic metabolisms like carbohydrate, protein, lipid, and hormone in many hybrids suggesting a possibility of higher-level regulatory mechanism(s), which is able to influence these pathways in most heterotic combinations.

**Clues from epigenomic studies**

When two genetically distant parental lines are combined to obtain hybrids, besides obvious interactions in cis and trans genetic factors, DNA methylation machinery and its components that maintain and regulate epigenomic status would also interact. To gain insights into the extent of the role played by the epigenetic machinery and its dynamics in the manifestation of heterosis, genome-wide methylation, miRNA expression, and siRNA distribution patterns have been analysed in the past three to four years (Ha et al., 2009; Groszmann et al., 2011; Shen et al., 2012). Rapid changes in siRNA population and non-additive expression of more than 50% miRNA was observed in Arabidopsis allo-haploids lines, suggesting the involvement of the small RNA machinery in growth, vigour, and adaptation (Ha et al., 2009). In a study involving the mapping of genomic regions contributing to 24-nt small RNA population in Arabidopsis thaliana C24 and Ler accessions and their intraspecific hybrids, positive correlation between genomic regions with reduced 24-nt small RNA levels, differential gene expression, and the respective changes in DNA methylation levels were revealed (Groszmann et al., 2011). Using genome-wide methylome profiling of the same parent–hybrid combination, Shen and co-workers found that both hybrids had increased cytosine methylation compared with the parents (Shen et al., 2012). The higher methylation levels were correlated with transcriptional down-regulation of more genes in the hybrids than the parental lines. These genes included central clock genes, CCA1 and LHY, which have previously been shown to have profound effects on biomass heterosis (Shen et al., 2012). This study further showed that increased DNA methylation in hybrids predominantly occurred in regions that were differentially methylated in the parents and covered by small RNAs, implying that RNA-dependent DNA methylation may be responsible for increased DNA methylation in reciprocal hybrids. Using the same parental combination Greaves et al. (2012) showed that methylome dynamics in the hybrid were frequently associated with regions where parental methylation levels were different. This means that the methylation levels of alleles from one parent were altered to resemble that of the other parent by a process that also involved changes in corresponding si-RNA levels.
A recent study involving small RNA profiling in a maize hybrid-parent combination revealed the non-additive differential accumulation of ~90% of miRNA species, along with the specific accumulation of three miRNA species in the hybrid compared with the parental lines (Ding et al., 2012). In this investigation, about two-thirds of the differentially expressed miRNA families exhibited down-regulation. These miRNA families included, miR168, miR164, miR169, miR156, and miR396 that are known to regulate ARGONAUTE 1 (AGO1), NAC Domain containing proteins (NAC1), Nuclear Factor Y (NFY), Spindle Pole Body (SBP domain proteins), and Zinc finger GRFs (GRFs) which are, in turn, known for their involvement in various development-associated pathways and those involved in energy and protein metabolism.

The emerging model

If plants are biological engines, which take light energy as input to assimilate basic elements into biomass, the net energy converted to biomass could be expressed as:

\[
\text{Energy}_\text{Biomass} = \text{Energy}_\text{input} - \text{Energy}_\text{consumed}
\]

Hence, either an increase in the input energy or a decrease in the energy consumed in basic metabolic processes could result in increased growth and biomass. A recent model by Goff emphasizes the role of energy-use efficiency in the manifestation of multigenic heterosis (Goff, 2011). According to this model, hybrids have more efficient growth than the inbreds because of the significant reduction in the energy-consuming processes of protein metabolism. Because of this fine-tuning, hybrids are able to conserve energy from basic metabolism, and translate it into higher growth rates and biomass (Hauben et al., 2009). The importance of increased energy input, on the other hand, has been highlighted by Ni and coworkers (Ni et al., 2009), who proposed that resetting of the circadian clock to a higher amplitude during the day leads to increased photosynthetic efficiency and, thereby, to growth vigour and increased biomass.

Taken together it is becoming evident that, in a biological system, a positive modulation of energy can translate into vigour and biomass. In different heterotic combinations, this modulation might be achieved by different means and at different phases of growth and development. This might explain the observed differences in the modes of gene action at various stages of development. In different heterotic combinations, both additive and non-additive modes of differential gene actions have been shown to be involved in the manifestation of heterosis. Correlations between the positive modulation of the circadian clock and the non-additive mode of gene action have mainly been found during the early development stages of heterotic hybrids (Fujimoto et al., 2012) and there is mounting evidence of the involvement of the epigenetic machinery in regulating this multigenic trait (Groszmann et al., 2011; Greaves et al., 2012). Delineation and functional characterization of major yield-related QTLs have resulted in identification of genes associated with specific traits like panicle branching, grain number, grain size, grain weight etc, which might have been contributing to heterosis. Introgression of naturally occurring (or synthetic) superior alleles of these genes could be used for directed heterosis (Fig. 1: Ashikari et al., 2005; Tamaki et al., 2007; Krieger et al., 2010; Mao et al., 2010; Miura et al., 2010; Li et al., 2011).

The next set of questions would be: how is the modulation of energy achieved to yield positive heterosis; mechanistically,
what are its components and how does mixing of two genomes affect their equilibrium? Certainly, when two adequately different genomes come together in a hybrid, the genome dynamics (depending on cis, trans, and chromatin/epigenomic interactions) translates into the differential expression of a large number of genes. The changes in gene expression patterns, whether additive or non-additive, would affect both regulatory and metabolic pathways depending on their affiliations and in a manner that would have a net positive or negative effect on the cumulative output of any individual pathway. It seems logical that, if an individual or a combination of differential gene expression patterns translates into positive modulation of any major regulatory pathway, it could have a major influence on the manifestation of heterosis (Fig. 2). But could we identify key pathways whose net positive modulation is obligatory for the manifestation of heterosis? The circadian clock and down-regulation of protein metabolism certainly seem two of those, but are these two sufficient? In another scenario, heterosis could still manifest if, instead of a major regulatory pathway, a group of downstream biochemical pathways are individually affected by the transcriptome dynamics and are able to bring net positivity to the system in terms of growth and development. However, to gauge the net positivity or negativity in the system for growth and development, a manageable number of standard markers (either biochemical or mode of gene actions) will have to be identified or developed. These markers would help define the currency of energy in all its denominations, and the net balance of this currency in the system would define the state and extent of heterosis. The development of such a currency system and understanding of its dynamics would bring us closer to the ultimate goal of predicting the heterotic potential of any unknown parental combination.

**Fig. 2.** A model outlining the mechanistic aspects of manifestation of heterosis. Mixing of two adequately distant genomes brings about cis, trans, and chromatin level changes in the hybrid, which results in differential expression of a number of genes. These expression patterns, which might represent additive or non-additive modes of gene action, may primarily affect a few major regulatory pathways which, in turn, send out regulatory cues that either individually or cumulatively affect a number of downstream metabolic pathways (including photosynthesis, starch and protein metabolism, etc) in either a positive or a negative manner. These individual pathways, whether placed on the input side or the consumption side of the energy equation, affect various aspects of growth and development. The net positivity or negativity in the system, therefore, would define the state and extent of heterosis.

**References**


