The Magic of Heterosis: New Tools and Complexities

Gulzar S. Sanghera[1], Shabir H Wani[2*], Wasim Hussain[1], Wajida Shafi[2], A. Haribhushan[3] and Naorem B.singh[4]
1. SKUAST –K, Rice Research and Regional Station, Khudwani, Anantnag, 192102, J&K, India;
2. Central Institute of Temperate Horticulture, Srinagar, J&K, India;
3. Krishi Vigyan Kendra, Senapati, Manipur, 795 129 India;
4. Department of Plant Breeding and Genetics, COA, CAU, Imphal, Manipur 795 004 India
shabirhussainwani@gmail.com

Abstarct: Heterosis, or hybrid vigor, an unsolved puzzle and a 'miraculous' agricultural phenomenon, refers to the phenomenon in which hybrid progeny of two inbred varieties exhibits enhanced growth or agronomic performance. Converse of hybrid vigour is 'inbreeding depression' caused by increased homozygosity of individuals, which reduces survival and fertility of offspring. Agricultural heterosis was observed nearly 100 years ago when hybrid plants out yielded their inbred parents and today this "hybrid vigor" is a major provider for global food production. One of the most promising approaches to unravel the genetic basis for heterosis at the molecular level emerged through the availability of molecular markers, as they have provided a powerful approach to map and subsequently identify genes involved in complex traits. Molecular marker technology was used to identify the genomic regions that contribute to heterosis for a trait of interest. The advancements in functional genomics have created a novel avenue to study the genetic basis of heterosis at the gene-expression level. The genetic basis of heterosis has been debated with respect to the relative importance of dominance, overdominance and epistasis; where one of the problems has been the use of whole genome segregating populations where interactions often mask the effects of individual quantitative trait loci. In this review the phenomenon of heterosis and the modern concept of its genetic and molecular basis will be discussed.
[Gulzar S. Sanghera, Shabir H Wani, Wasim Hussain[1], Wajida Shafi, A. Haribhushan and Naorem B.singh **The Magic of Heterosis: New Tools and Complexities.** Nature and Science 2011; 9(11):42-53]. (ISSN: 1545-0740). http://www.sciencepub.net.

Key words: Heterosis; genetic basis; molecular basis; quantitative trait loci; gene expression level

1. Introduction

Heterosis, or hybrid vigour, an unsolved puzzle and a 'miraculous' agricultural phenomenon, refers to the phenomenon in which hybrid progeny of two inbred varieties exhibits enhanced growth or agronomic performance. Converse of hybrid vigor is 'inbreeding depression' caused by increased homozygosity of individuals, which reduces survival and fertility of offspring (Charlesworth and Willis, 2009). Inbreeding depression and heterosis are considered two aspects of the same phenomenon (Falconer, 1981; Mather and Jinks, 1982). Heterosis a universal phenomenon in the biosphere though most evident for adult traits like plant biomass or yield but is also apparent during embryo (Meyer *et al.,*2004, 2007; Jhanke *et al.,*2010) and early seedling development (Hoecker *et al.,*2006). The concept was introduced nearly a century ago and such a long history, with paramount agricultural importance and exploitation has generated several hypotheses regarding the genetic basis of heterosis; however, the molecular basis and heterotic gene expression underlying heterosis remains elusive (Shull, 1908; Hochholdinger and Hoecker, 2007). Heterosis is often expressed as Mid-parent heterosis, that is the difference in phenotype value between the heterozygous offspring and the mean of the homozygous parents, and Best-parent heterosis that describes the situation where the hybrid exceeds the best parent and is the underlying rationale for the widespread use of hybrids in many agricultural species. Hybrid vigor was first described by Charles Darwin. (1876) and was independently rediscovered by Shull. (1908) and East. (1908) who highlighted the high potential of this phenomenon for agriculture for the first time. The term "heterosis" was recognized by Shull to facilitate the description of this phenomenon and as short form for the phrase "stimulation of heterozygosis". Despite a dramatic long history of successes, especially in maize (Duvick, 2001), there is still a striking discordance between an extensive agricultural practice of hybrid vigor utilization and our understanding of the basis of heterosis (Coors and Pandey, 1999; Reif *et al.,*2006), and this hampers an effective exploitation of the phenomenon. Still, the production of new hybrids basically relies on empirical and time consuming approaches (Duvick, 2001). Despite this lack of understanding and one of the most complex issues, breeders have quite successfully manipulated heterosis to increase the vigor of many domesticated species (Springer and Stupar, 2007). One of the more striking examples of the utilization of heterosis has occurred in maize breeding programs over the last

century (Hallauer and Miranda, 1981).In agriculture, the use of heterosis in different crop plants and animals has achieved great success and is considered essential to meet the world's food needs (Duvick, 1999). One common theme throughout the last century has been that no hypothesis of heterosis holds true for every experiment or every organism; magnitude of heterosis varies in different species and is the result of variation at multiple genomic locations and complex phenotypes that are often assessed for heterosis, such as yield, and are likely influenced by many (hundreds of) genes. Additionally, heterosis does not simply result from the overall genetic diversity within a hybrid, but is likely a reflection of diversity at specific important genes that contribute to a particular trait (Springer and Stupar, 2007). Though recent studies have determined the roles of non- additive gene expression, small RNAs, altered hormone levels and epigenetic regulation, including circadian-mediated metabolic pathways in hybrid vigor, which could lead to better use and exploitation of the hybrid vigor (Osborn *et al.,*2003; Okoh *et al*., 2007; Birchler *et al.,*2010; Chen, 2010). However, the knowledge on genetic mechanism of heterosis is limited due to biological complexity and limitations of research methodology and still a topic of research today.

***2.* Genetic models towards understanding of heterosis**

Dominance, real over dominance and/or pseudo over dominance and epistasis are the major genetic models invoked to explain hybrid vigor in the extensive scientific literature addressing heterosis in many crops (Lamkey and Edwards, 1999; Crow, 2000; Reif *et al.,* 2006). Although not always explicitly stated, these genetic hypotheses make the combination of a considerable number of genes and concurrently may play a role in hybrid vigor (Hochholdinger and Hoecker, 2007). However the basic question to be answered, what is the relative contribution of these gene actions in the manifestation of superior phenotype, is still revolving towards uncertainty, though dominance is considered more popular one (Charlesworth and Willis, 2009). These models have survived with various modifications and interpretations as the methods and specialties of biology have changed (Birchler *et al.,* 2010). Also, they were coined before the molecular concepts of genetics were formulated and are not directly connected with molecular principles. Although these classical hypotheses have provided guidance for experimentation, they are of limited utility to describe the molecular parameters that accompany heterosis. At molecular level, two models are considered to explain heterosis. One model considers that in hybrids having two different kinds of alleles an allelic expression in additive manner occur with the average of the parental expression levels. In the second model, the combination of different alleles causes gene expression changes in hybrids that deviate relative to mid parent (Birchler *et al.,* 2010).

The dominance hypothesis explains heterosis by the complementing action of superior dominant alleles from both parental inbred lines at multiple loci over the corresponding unfavorable alleles, leading to improved vigor of hybrid plants (Davenport, 1908; Bruce, 1910; Keeble and Pellow, 1910; Jones, 1917). An extension of the dominance hypothesis was recently suggested on the basis of DNA sequencing data (Fu and Dooner, 2002). Accordingly, functional genes are often absent in maize lines, and lines lacking different genes would complement one another in the F_1 hybrid, resulting in heterosis. However, in some instances the apparent loss of gene colinearity might be due to the movement of genes or gene fragments by helitron transposons to other genomic regions and thus the contribution of non-collinear regions of the genome to heterosis is unclear. Further it is unlikely that all species that display heterosis contain a degree of non-collinearity in their genome as high as that of maize.

Although complementation will certainly occur, as the major contributor to heterosis (Coors and Pandey, 1999; Crow, 1999), however for simple complementation to explain heterosis, the complementation across loci must be cumulative. There are several lines of evidence that suggest that mechanisms beyond simple complementation may be important in heterosis. The absence of a decline in the magnitude of heterosis (Duvick, 2001), the progressive heterosis in tetraploids, and the rapid rate of inbreeding depression in tetraploids have been cited as factors that suggest that dominance may be insufficient to explain completely about heterosis (Birchler *et al.,* 2010).Further some classical studies on hybrid vigor, however, point out the involvement of allelic dosage (rather than simple complementation) in the process. On the concept that heterosis results from the complementation of recessive detrimental mutations in the hybrid, one might expect that the magnitude of heterosis would decline with continuing accumulation of superior alleles in elite inbred lines. This however is not the result and it was observed that the magnitude of heterosis has not diminished but has increased slightly (East, 1936; Duvick, 1999). However, superior inbred lines have been difficult to identify, likely due to the large number of loci differing between two parents (Tsaftaris, 1995), an idea initially reported by East. (1936).

The phenomenon of progressive heterosis (Mok and Peloquin, 1975) suggests that increased

allelic diversity creates a more robust heterotic response. Progressive heterosis refers to the fact that double cross hybrid autotetraploids (ABCD) typically show greater vigor than single cross hybrids (AABB; CCDD and so on). If the explanation for progressive heterosis were solely complementation, increasingly superior alleles at any one locus must be added to the genotype with each different genome introduced in the tetraploid without contributing inferior alleles at other loci. The probability of this occurrence is low. This observation argues against simple complementation as the sole basis of heterosis; therefore, there must be an additional molecular explanation.

The inbreeding depression curves of autotetraploid plants and diploid plants are similar (Busbice and Wilsie, 1966; Rice and Dudley, 1974). If inbreeding depression is driven by homozygosity of recessive alleles, then it should proceed at a much slower pace in tetraploids. At any one heterozygous locus in diploids, selfing will result in 50% of the progeny being homozygous, whereas in tetraploids, selfing for a heterozygous locus (AAaa) will only produce homozygous alleles in F_1 1/18 of the progeny. The fact may be that allelic dosage impacts the magnitude of heterosis, and may be an additional argument why complementation of recessive detrimental alleles is an adequate model for heterosis (Birchler *et al.,* 2010). Evidence that such dosage component is consistent, that the first quantitative trait locus cloned, fruit weight 2.2 (fw2.2), shows a negative dosage effect on the size of tomato fruit (Frary *et al.,* 2000). The dosage of identical alleles, however, changes more rapidly than homozygosis and could potentially contribute to the otherwise unexpected more rapid progression of inbreeding depression.

Over-dominance hypothesis attributes heterosis to the superior fitness of heterozygous genotypes over homozygous genotypes at single locus (Shull, 1908; East, 1908; Crow, 1948; Stuber, 1994). It postulates that diverse alleles interact so as to create a superior function than that which could happen with homozygous alleles. Thus, a heterozygous individual may have an advantage due to the combination of both allozymes. For overdominance to produce superior phenotypes observations are repeatedly made that heterozygosity for a single gene or small genomic regions are needed to produce such response. Further a challenge for this model is to identify the best combination of a single genetic locus or a few loci that contribute to the overall heterosis, which seems to contradict the hybrid performance of many agronomic traits that are controlled by multiple genetic loci. Consequently, there is little support for single-gene over dominance (Lippman and Zamir, 2007).However a number of studies demonstrated the role of single genes in the manifestation of heterosis for various traits in Arabidopsis, Cereals and Tomato (Gustafson, 1946; Redei, 1962; Dollinger, 1985; Semel *et al.,* 2006; Krieger *et al.,* 2010). Though evident as examples of overdominance, it is possible that they involve dosage effects on regulatory networks that are not incompatible with the concept of multigenic control. If alterations to regulatory networks contribute to heterosis, then variation in single genes or multiple genes that are not necessarily the same in different varieties could also contribute (Birchler *et al.,* 2010). Further, by contrast to genome-wide heterozygosity, single loci with over dominant action contributes significantly to reproductive fitness as they have possibility to persist in the population because of no hybrid breakdown in subsequent generations due to recombination.

Jones (1917) first pointed out that linkage could cause considerable problems when attempting to identify overdominance, which gives rise to pseudo-overdominance. Pseudo-over dominance refers to a particular situation, in which tightly linked genes with favorable dominant alleles in repulsion phase in the parental lines result in an apparent over dominance when combined in the hybrid (Crow, 1952; Stuber *et al.,* 1992; Graham *et al.,*1997). For example, if beneficial dominant alleles were tightly linked to a deleterious recessive allele of another gene, one would have difficulty in producing the recombinant individual to identify such gene action. In that case, the pair of linked loci would mimic a single, over dominant locus, thus skewing a measure of true overdominance. The heterosis associated with pseudo-overdominance can dissipate in the selfing progeny because genetic recombination leads to the dissociation of the alleles from the repulsion state, which is exactly what is observed in a study with tomato hybrids (Semel *et al.,* 2006). This pseudo-overdominance can also arise from numerous alleles in recombination suppression regions where good and bad allele combinations are in repulsion (Gore *et al.,* 2009; McMullen *et al.,* 2009).

There is also evidence for the role of epistasis in heterosis, i.e. the interaction of favorable alleles at different loci contributed by the two parents, which themselves may show additive, dominant, or overdominant action (Yu *et al.,*1997; Monforte and Tanksley, 2000; Li *et al.,*2001; Luo *et al.,*2001). Therefore, the genetic background and allelic interactions therein can have an effect on the heterotic contributions of individual loci. The dominance/overdominance debate becomes even more nuanced when contributions of epistasis are considered.

3. QTL and heterosis

One of the most promising approaches to unravel the genetic basis for heterosis at the molecular level emerged through the availability of molecular markers, as they have provide a powerful approach to map and subsequently identify genes involved in complex traits. Molecular marker technology was used to identify the genomic regions that contribute to heterosis for a trait of interest. Specific genes/QTL for individual traits contributing to heterosis for desirable traits can be used to enhance the performance of hybrids by transferring them into parental inbred lines through MAS but may be very challenging. The complex trait 'heterosis' is expected to be reflected by many genes, their wide genomic distribution, the combination and interaction of which may depend on the organism and trait under study (Korn *et al.,* 2008; Li *et al.,* 2008). To exploit heterosis by its best it is much needed to understand the nature of dominance, epistatic properties of these genes and how they interact with the environment (Coors and Pandey, 1999). Also marker-based QTL studies are inherently inefficient at detecting epistasis and one cannot exclude the possibility that some level of epistasis is occurring. Mapping and cloning QTL with heterotic effects will require more rigorous approaches, particularly with regard to the global phenotyping that is much expensive and time consuming. An alternative phenomic platform for each crop was proposed which would include a database of unbiased measurement of multiple traits (e.g., components to total yield are treated as individual traits and are recorded in well-characterized environmental conditions in term of seasons, locations, and years (Lippman and Zamir, 2007). Difficulties in defining specific heterotic phenotypes and individual loci that control them result predominantly from epistatic interactions among many segregating loci throughout the genome when F_2, backcross, or recombinant inbred line (RIL) populations are used (Li *et al.,* 2001; Luo *et al.,* 2001). Furthermore, when these populations segregating for the entire genetic background are used, the complex interactions often mask the effects of individual loci (Semel *et al.,*2006). However ILs, those are now widely available, helps in identifying and isolating QTL more effectively and feasibly, because any phenotypic difference between an IL and the recurrent parent is attributed to the introgressed chromosomal segment, thereby cleaning up most of the whole-genome epistatic interactions and eliminating the need for complicated statistical analyses (Lippman and Zamir, 2007). Recently phenomics study on tomato (*Solanum lycopersicum*) by Semel *et al.*(2006) has shown that ILs are greatly effective for identifying QTL contributing to heterosis, particularly those showing over dominance effect. Meanwhile, compared with the F_2 or the F_3 population, RILs as parents for producing testcross progenies offer few advantages as the effects of linkage is reduced, maximizes the genetic variance in testcross progenies and finally they are immortal. The main difference between RILs and ILs is the absence of variation for 'background' epistasis in the ILs, thereby increasing the power to detect QTL (Keurentjes *et al.,* 2007; Reif *et al.,* 2009). But with ILs epistatic interactions that are important in heterosis cannot be directly estimated (Li *et al.,* 2001; Luo *et al.,* 2001). Further based on single gene effects QTLs displaying over dominant gene action are not know, it is now imperative then to distinguish between true over dominance and pseudo- over dominance, that will require fine mapping and eventual cloning of over dominant QTLs, and make now possible through the availability of high-density molecular linkage maps.

QTL mapping has been increasingly used in recent years that provide link between genotype and phenotype for a complex trait heterosis. Numerous QTLs with different levels of dominant, over dominant, and epistatic effects have been mapped for heterosis in Maize (Stuber *et al.,* 1992; Beavis 1994; Lu *et al.,* 2003; Frascaroli *et al.,* 2007, 2009 ;Garcia *et al.,* 2008; Schon *et al*., 2010), Rice (Li *et al.,* 2001;Luo *et al.,* 2001 and Hua *et al.,* 2003 Tomato (Semel *et al.,*2006), Rapeseed (*Brassica napus*) (Radoev *et al*., 2008;Dong *et al.,*2007; Radoev *et al.,* 2008; Basunanda *et al.,* 2010) and *A. thaliana* (Kusterer *et al.,* 2007; Melchinger *et al.,* 2007;Meyer *et al.,* 2010). Besides the involvement of various gene actions found in these studies, all the three gene actions may condition heterosis in crops (Li *et al.,* 2008; Swanson-Wagner *et al.,* 2006). These diverse results indicate that heterosis may be caused by combinations of these mutually nonexclusive mechanisms. Frequently, the comparison across studies is confounded by differences in experimental design, genetic material, or statistical methods used for data analysis. Despite numerous marker-aided studies on the genetic basis of heterosis in various crops, results have not been conclusive, further from QTL studies on the importance of epistasis have been rather ambiguous (Stuber *et al.,* 1992; Cockerham and Zeng, 1996; Frascaroli *et al.,*2007). Even using dense genetic maps, marker intervals can still cover several hundred genes (Young, 1999), i.e. their genetic resolution is low and their ability to account for complex interactions between several or many genes and their products is limited.

QTL may not always directly control an individual agronomic trait but may instead be regulatory in nature, mediated by multi subunit

complexes, are dosage dependent that would contribute to the multi genic control of the ultimate phenotype (Birchler and Veitia, 2010).Furthermore the variation observed in the level of expression of a gene as a result of genotypic differences is referred to as an expression level polymorphism (ELP), and the QTL responsible for this type of variation have been described as eQTL (Jansen and Nap, 2001; Doerge 2002; Gibson and Weir, 2005). Advances in QTL analysis and genetic genomics involving identification of expression QTL (eQTL), have led to significant progress in genetic dissection of complex traits likely heterosis. When the transcript abundance is treated as a continuous trait for the purpose of mapping, it is termed an expression trait (eTrait). More specifically in eQTL the transcript level measured in a mapping population can be treated as a quantitative trait like any other phenotypic trait and have mapped it to local-acting or distant-acting expression quantitative trait loci (eQTLs) (Brem *et al*., 2005). The eQTL analysis, when compared with classical quantitative trait analysis, may provide relatively more detailed information about a gene network controlling a trait, because in this analysis, data on thousands of expression traits are recorded simultaneously, also there is a one-to-one relationship between an eTrait and a gene with its expression profile assayed in the mapping population. Provided with these tools, expression quantitative trait locus (eQTL) analysis has been applied to study inheritance of thousands of similar traits in the hope to find general rules of genetic control of transcriptional regulation (Brem *et al.,* 2002; Schadt *et al.,* 2003;). Also, the gene expression traits exhibit a high level of heritability (Keurentjes *et al.,* 2007), making their detection and manipulation more reliable. It has been shown that large number of both cis- and trans-acting eQTL are responsible for non additive genetic variation, which involves transgressive segregation and epistatic genetic variation that may sometimes alter an entire transcriptional network (Kliebenstein *et al.,* 2006; Keurentjes *et al.,* 2007; Potokina *et al.,* 2008). In future, it is hoped that eQTL analysis will be increasingly used as a supplement to classical QTL analysis for genetic dissection and manipulation of multiple traits. Further with the availability of novel genetic and genomic tools that allow for the integrated study of the complex interactions between genome organization and expression might contribute to a better understanding of heterosis.

4. Heterosis and gene expression

So far almost all of the documented studies on revealing the genetic basis of heterosis are limited to classical quantitative genetics and QTL mapping using molecular markers. The advancements in functional genomics have created a novel avenue to study the genetic basis of heterosis at the gene-expression level. The dynamic genome of an F1 hybrid is derived from its parents; hybrid performance is quite different from its parents due to extensive difference in gene expression in hybrids as compared to parents. The patterns of gene expression changes in hybrids results from unique regulatory interactions in hybrids, which give rise in quantitative variants, that may be responsible for the heterosis observed in the F_1 hybrid (Birchler *et al.,* 2010; Hochholdinger and Hoecker, 2007). Differences in gene expression thought to be an important source of phenotypic diversity, and complex trait that, in diploid organisms, results from transcription of both maternal and paternal allele (Knight, 2004; Fontanillas *et al.,* 2010). Genetic phenomenon like dominance, over dominance and epistasis are suggested to be generic features of gene regulatory networks and might be explained by mechanisms likely altered (Mrna) expression levels (Omholt *et al.,* 2000). The observed heterosis so produced due to allelic expression differences resulting from changes in a regulatory region is poorly understood because of its complexity and the lack of efficient methodology (Cowles *et al.,* 2002; Glazier *et al.,* 2002; Guo *et al.,* 2004; Xing *et al.,* 2010). Hybrid expression patterns can be additive as the average expression of parental lines or non additive as between high and low parent, above the high parent (over dominance), or below the low parent (under dominance) relative to the expression patterns observed in the inbred parents. These quantitative changes in gene expression may be the result of cis- or trans- variations in gene regulation (Wittkopp *et al.,* 2004). Cis- regulators are genetically tightly linked to a gene and influence transcription in an allele-specific manner. In contrast, trans-regulators are located elsewhere in the genome and modify gene expression by interacting with cis-regulators. Genes that are completely subject to cis-regulation reflect the relative expression levels of the parental inbred lines in the allelic ratio of gene expression in the hybrid. Genes that are exclusively regulated by trans-acting factors show equal expression of the two alleles in the hybrid. Genes that are subject to cis- and trans-regulation fall in between the two classes, that is, the relative allelic contribution to gene expression in hybrids for this class of genes neither displays neither the relative expression levels of the parental inbred lines nor an equal expression of both alleles. While pure cis-effects imply the preservation of parental regulatory function, differential expression between parents and hybrid due to trans-effects are caused by hybridization that brings two genomes together, allowing both alleles to be exposed to a common set of trans-elements.

Identification of genes associated with changes in expression patterns in hybrids is important for understanding heterosis. It had been shown that differential gene expression between hybrids and their parents that are involved in certain complicated regulatory networks may be underlying cause of heterosis. However responsible molecular mechanisms have not been determined and the function of specific genes associated with it is still unknown. Earlier several studies have reported non additive expression for number of genes in maize hybrids as compared to their parental inbred lines (Romagnoli *et al.,* 1990; Leonardi *et al.,* 1991; Song and Mesing *et al.,* 2003; Auger *et al.,* 2005).Based on few selected genes they were not sufficient to explain the relationship between different gene expression and molecular mechanism of heterosis at genomic level. Recently, with the advent of new genomic tools, non additivity was observed on genome wide scale, that have been analyzed in Maize (*Zea mays*), Rice (*Oryza sativa*) and Arabidopsis (*Arabidopsis thaliana*), that reveal complex transcriptional networks between parental inbred lines and hybrids to contribute to heterosis (Sun *et al.,* 2004; Swanson-Wagner *et al.,* 2006, 2009; Wang *et al.,* 2006; Meyer *et al.,* 2007; Uzarowska *et al.,* 2007; Zhuang and Adams, 2007; Chen *et al.,*2008; Guo *et al.,*2008; Hoecker *et al.,* 2008; Pea *et al.,* 2008; Stupar *et al.,* 2008; Zhang *et al.,* 2008; Li *et al.,* 2009; Tirosh *et al.,*2009; Wei *et al.,* 2009; Andorf *et al.,* 2010; He *et al.,*2010; Jahnke *et al.,* 2010; Paschold *et al.,* 2010; Riddle *et al.,* 2010;). Non additive gene expression, arise when the combination of diverse alleles leads to interaction in hybrids and novel patterns of gene action (Birchler *et al.,* 2010), is of common occurrence which suggests that altered trans-regulation in hybrids is quite prominent and plays important role in the manifestation of heterosis. Furthermore non-additive gene expression profiles have been documented in diploid and triploid maize hybrids and found that the non-additive effects in reciprocal diploid hybrids (AB versus BA) were similar to each other in contrast to the non-additive effects between the two types of triploid hybrids (Auger *et al.,* 2005), that may be indication that dosage of different genomes alters the nature of the non-additive expression, suggesting role of regulatory effects (Birchler *et al.,* 2005; Birchler and Veitia, 2007, 2010;).Additive gene expression was also prevalent in other studies for most of the genes (Guo *et al.,* 2003, 2006; Vuylsteke *et al.,* 2005; Stupar and Springer, 2006;). These provide evidence that in hybrids additive or nearly additive expression pattern caused by cis- regulation are prevailed that may lead to a potential mechanism of heterosis based on mid-parent levels of gene expression.

The differences found in these expression studies might be the result of utilization of diverse species, differences in genotypes within species, distinct tissues and variety of microarray platforms applied in the various studies. However, it might also be an indication that in different tissues or developmental stages different global expression patterns might prevail, which might nevertheless be related to heterosis. This notion is supported by the observation that different tissues and organs within a hybrid plant display significant differences in their degree of heterosis (Melchinger, 1999).

These gene expression profiling studies represent a first step towards the definition of the complex gene expression networks that might be relevant in the context of heterosis. However, they cannot associate novel expression patterns in hybrids with any heterotic phenotypes, besides there is currently no direct link between the classical genetic hypothesis and these gene expression profiles. There has been no obvious consensus about genes that are differentially expressed in hybrids. It is tempting to relate such non-additivity of transcription to phenomena such as heterosis, but there is no evidence that this expression is responsible for phenotypic differentiation particularly in regard to economically important traits. Nevertheless, there does appear to be a correlation between the size of the fraction of genes that show non- additive expression and the magnitude of the heterotic response (Li *et al.,* 2009; Riddle *et al.,* 2010), but it is not clear if this effect is causative. Further it appears that the number of genes showing non-additive effects increases when increasingly divergent genomes are combined (Birchler et al., 2005; Birchler and Veitia, 2010).Up to date the studies of gene expression on the whole are ambiguous and as to whether any observed changes are correlative, causative, or predictive of heterosis (Birchler *et al.,* 2010). However, some attempts to correlate parental expression with hybrid performance show promising (Frisch *et al.,* 2010; Thiemann *et al.,* 2010).

5. Epigenetics as a cause of heterosis

"Epigenetics" refers to heritable (through mitosis or meiosis) alterations in gene expression that are independent of DNA sequence (Wolffe and Matzke, 1999); different epigenetically regulated forms of a gene are known as epialleles. Epigenetic regulation of gene expression is accomplished by DNA methylation, histone modifications, histone variants, chromatin remodeling, and may involve small RNAs. As allelic variation can include sequence differences (alterations in DNA sequence) or regulatory differences (altered expression levels and epigenetic changes) found in different parental genotypes, and as at some level, heterosis is the result

of variation between the parental lines, epigenetic variation, like genomic variation, could also combine to produce a heterotic phenotype. Thus, if epigenetic mechanisms are responsible for allelic- and locus-specific gene expression in hybrids and allopolyploids, they probably operate through cis- and trans-acting effects (Wittkopp *et al*., 2004; Wang *et al.,*2006), chromatin modifications, and/or small RNAs (Chen, 2007; Chen *et al.,*2008).Among the regulatory mechanisms, DNA methylation, is a major epigenetic regulatory phenomenon due to its important role in cellular activities and more importantly in transcriptional inactivation leading to gene silencing, and gene regulation (Dong *et al.,* 2006). Understanding the dynamics and inheritance patterns of DNA methylation is essential for elucidating epigenetic paradigms in plant development, evolution (Zhang *et al.,* 2007) and heterosis. The possible role of methylation in the expression of heterosis was first suggested by Tsaftaris *et al.,* (1997) in maize. Later, Tsaftaris and Polidoros, (2000) have suggested that DNA methylation could be considered as genome wide regulatory mechanism that affects the global expression of many genes involved in the manifestation of heterosis. DNA methylation is generally recognized to function to suppress gene expression as regulatory factors (Jacobsen and Meyerowitz, 1997; Jones and Takai, 2001). Basically heterosis is a result of ‘‘different alleles’’ being present at loci that contribute to the regulatory hierarchies that control quantitative traits (Birchler *et al.,* 2010). These ‘‘different alleles’’, however, can arise from differently methylated DNA. If so, homozygosity of methylated DNA in such regulatory factors suppresses gene expression, while its heterozygosity regulates depending on the gene actions, dominant, partial dominant or additive. Therefore, it can be suggested that inbreeding depression partly or primarily results from lower levels or fewer genes expressed simply due to homozygosity of methylated DNA in regulating factors, while heterosis is from higher levels or larger number of genes expressed simply due to heterozygous conditions between methylated and non-methylated DNA in the F_1 hybrid.

Further non-additive gene expression is also controlled by posttranscriptional mechanisms via RNA-mediated pathways (Chen, 2007; He *et al.,* 2010). Small RNAs, including microRNAs (miRNAs) (Bartel, 2004), small interfering RNAs (siRNAs) (Baulcombe, 2004), and transacting siRNAs (tasiRNAs), mediate post-transcriptional regulation, RNA-directed DNA methylation, and chromatin remodeling. RNA interference (RNAi) is an evolutionarily conserved mechanism for modulating gene expression (Sanghera *et al.,* 2010). Evidence for the involvement of RNA-mediated gene regulation in heterosis came from characterization of five miRNA families in maize, and some miRNAs are differentially expressed between hybrid and its parental inbred lines (Mica *et al.,* 2006) proposed that if siRNAs from one inbred do not match genes from the other inbred, the resulting hybrid could exhibit novel patterns of gene expression, including over-dominance or under-dominance. As a result, short interfering RNAs (siRNAs) and microRNAs (miRNAs) are negative regulators of target transcript accumulation. Long non-protein coding RNAs (npcRNAs) are identified as precursors of miRNAs and siRNAs (Reinhart *et al.,* 2002; Hirsch *et al.,* 2006) and differentially expressed siRNAs and miRNAs between hybrid and its parental lines may be controlled by transcript levels of long npcRNAs. Recently in maize seedlings the differential expression of some siRNAs was controlled by transcript levels of a long npcRNAs named ZmHUR and was unregulated in hybrid (Xing *et al.,* 2010).

Recently gene expression profiling in Arabidopsis had suggested that genes involved in the circadian rhythm, such as LHY (LATE ENLONGATED HYPOCOTYL) and CCA1 (CIRCADIAN CLOCK ASSOCIATED 1), both MYB-like transcription factors, are associated with heterosis (Ni *et al.,* 2009). A circadian rhythm is an endogenously generated rhythm with a period of about 24 h, approximating the period of the rotation of the earth on its axis. Ni et al. (2009) reported a model related to circadian rhythms to explain heterosis, in which F_1 hybrid and allopolyploid of Arabidopsis gained advantages from the control of circadian-mediated physiological and metabolic pathways. In this model, two key factors, CCA1 and LHY (Alabadi *et al.,*2001), were epigenetically modified and repressed in the F_1 hybrid and allopolyploid during the day and further induced the expression of downstream genes involved in photosynthesis and carbohydrate metabolic pathways. The regulatory network involved in circadian clocks affect many physiological and developmental processes, including various metabolic pathways and fitness traits in animals and plants, and photosynthesis and starch metabolism in plants (Wijnen and Young, 2006). In addition, a regulatory network involving circadian-rhythms and light signaling pathways was also found in rice. The similarity of the regulatory network between rice and *Arabidopsis* may imply that the circadian rhythms regulatory network in hybrid might be one of the molecular mechanisms underlying heterosis in hybrid plants. Altering expression of a few genes in the circadian clock regulation to promote growth vigor is

reminiscent of single locus heterosis, which has been documented for the erecta and angustifolia loci in *A. thaliana* and SFT gene in tomato (Redei, 1962; Krieger *et al.,* 2010). However, the contribution of Epigenetics in producing superior phenotypes is still unknown. Though heterosis is of great use in crop improvement, the future of it lies in the unraveling of appropriate mechanisms at molecular as well as gene expression level. Otherwise, pre-mature conclusion of one mechanism will mislead our finding from the reality of heterosis mechanism in plants.

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Correspondence to
Shabir Hussain Wani (Ph.D.) (Plant Breeding & Genetics)
Research Associate (R.A.)
Biotechnology Laboratory
Central Institute of Temperate Horticlulture. (ICAR).
Near Old Airfiled. P.O SanatNagar, Rangreth,
Srinagar. 190007 Jammu and Kashmir, India. Tel: +91 194 2305044. Fax:+91 194 2305045.
shabirhussainwani@gmail.com

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