ISBN: 978-81-968830-0-3

13. Heterosis Breeding

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Abstract

Heterosis breeding, also known as hybrid vigor, is a fundamental approach in plant and animal breeding to enhance agricultural productivity. It exploits the superior performance of hybrid offspring compared to their parents, resulting from increased genetic diversity. By crossing genetically distinct parents, desirable traits such as yield, disease resistance, and stress tolerance can be maximized. Heterosis breeding plays a crucial role in developing high-yielding varieties and improving agricultural sustainability. Understanding the genetic mechanisms underlying heterosis is essential for harnessing its full potential and ensuring food security in a changing climate.

Keywords

Hybrid Vigour, Heterozygosity, Cross Pollination, Dominance, Over Dominance.

13.1 Introduction:

In plants F_1 hybrids, resulting from crosses between genetically distinct parents can exhibit notable performance advantages over their individual parents, a phenomenon known as hybrid vigor or heterosis (Darwin, 1876; Shull, 1908; East & Jones, 1919; Shull, 1948). Joseph Koelreuter, in the 1700s, initially documented the earliest instance of heterosis, noting that F_1 hybrids of tobacco exhibited greater height than their parent plants.

In 1876, Charles Darwin described the heterosis phenotype in his work, "The effects of cross and self-fertilization in the vegetable kingdom." In 1914, George Harrison Shull introduced the term "heterosis" to replace "heterozygosis,", better expressing the superior performance of hybrids (Shull, 1948). Heterosis describes enhanced yield, increased resistance to pests and diseases and improved tolerance to environmental stresses compared with their parent plants. This advantage can be measured against the superior parent (best-parent heterosis) or the average of both parents (mid-parent heterosis).

The highest level of hybrid vigor is confined to the F_1 generation, requiring farmers to annually acquire F_1 seeds in order to maintain elevated crop performance. The commercial utilization of this phenomenon began with maize, where the first high-yielding hybrid cultivar, Funk 250 (a double-cross hybrid maize), was developed in 1922 (Troyer, 2009). In the 1970s, Yuan Longping made a significant breakthrough by creating hybrid rice varieties that demonstrated a substantial yield advantage, exceeding the output of inbred parental varieties by 10–20%. China widely adopted the cultivation of hybrid rice, allocating around half of the nation's total rice area to hybrids by the early 2000s (Cheng *et al*., 2007). In maize, a monoecious crop with distinct female (ear) and male (tassel) reproductive organs within the same plant, emasculation is easily accomplished by removing the tassels. This direct emasculation process, known as detasseling, has contributed to the cost-effective production of hybrid seeds in maize (Crow, 1998).

The production of maize has experienced a consistent six-fold or more increase since the adoption of hybrids began in the 1930s (Crow, 1998; Duvick, 2001). In India, maize production surged by 15.62 times from 1950 to 2017. Notably, the focus shifted significantly towards single cross hybrids (SCHs) at the onset of the 21st century, resulting in a remarkable genetic gain of 73 kg ha^{-1} yr^{-1} (Rakshit *et al.,* 2018). Conversely, self-pollinating crops like rice, wheat and barley, where the female (stigma) and male (anther) reproductive organs are located together in a floret and are predominantly cleistogamous, demand more difficult emasculation methods. These methods involve the utilization of male sterility systems such as chemical hybridizing agents (CHAs), cytoplasmic male sterility (CMS), and genic male sterility (GMS) (Whitford *et al*., 2013). Overcoming this significant hurdle is one of the reasons why only a few crops, such as maize, sunflower (Dimitrijevic & Horn, 2018), oilseed rape (Gils *et al*., 2008), rye (Gi *et al.,* 2003), tomato (Duvick, 1999), sugar beet (Bosemark, 2006), and rice (Chang *et al.,* 2016), have successfully commercialized hybrid varieties. Furthermore, attempts have been undertaken to establish hybrid seed production in wheat (Kempe *et al.,* 2014). The review details various models explaining heterosis, recent discoveries supporting genomic and proteomic changes in hybrids, molecular network studies, techniques for rapid exploitation of heterosis and its future prospects.

13.2 Genetic Basis of Heterosis:

Heterosis or hybrid vigor, has been harnessed in various crops to enhance multiple traits, particularly focusing on improving yield (Duvick, 2001; Srivastava *et al.,* 2020). It is crucial to recognize that yield is not an isolated trait but rather the result of the complex interplay of multiple traits and their interactions. The predominant genetic explanation for heterosis in hybrid crosses lies in the genetic architecture of the parent organisms (William and Pollak, 1985). The genetic makeup of inbred or pure lines is largely homozygous, meaning that the genes or alleles influencing the growth and development of an organism are fixed in homozygous conditions. In hybrids, the alleles influencing growth and development complement each other, leading to the manifestation of heterosis or hybrid vigor.Some hypotheses explain the genetic basis of heterosis: (i) the dominance hypothesis by Davenport in 1908, (ii) the overdominance hypothesis independently proposed by East and Shull in 1908 (East, 1908; Shull, 1908), (iii) the epistasis hypothesis presented by Jinks and Jones in 1958 (iv) Progressive heterosis and dosage component and (v) Hemizygous complementation. A graphical representation of these hypotheses is shown in Figure 1.

13.2.1 Dominance Model:

The "Dominance" model suggests that heterosis arises from the complementary interaction of recessive alleles at different loci, with superior alleles from both parents in a hybrid cross (Bruce, 1910; Keeble and Pellew, 1910). Inbred parents carry homozygous alleles with adverse effects (inbreeding depression), which are masked in hybrid combinations as superior alleles complement the effects of inferior ones. These complementation's occur across multiple loci, preventing the expression of deleterious effects caused by recessive alleles and resulting in a more robust F_1 hybrid. As a result, natural selection works to diminish deleterious alleles or promote their close linkage with beneficial ones. In this model, it suggests that heterozygosity may not be the primary factor contributing to heterosis; instead, the increased number of superior loci plays a crucial role. However, there remains uncertainty about whether all complementation's of recessive alleles lead to an additive effect on the final phenotype. Initially favored, the dominance model faced objections as genetic knowledge developed. Issues included the lack of homozygous individuals with all dominant alleles and the absence of asymmetrical distribution in F_2 generations. Jones (1917) addressed the two objections, explaining the practical impossibility of recombining all dominant genes in one individual due to linkage. Consequently, obtaining superior homozygous individuals is practically impossible, explaining the symmetrical distributions in F_2. This model suggests that heterozygosity may not be the primary factor contributing to heterosis; instead, the increased number of superior loci plays a crucial role. However, uncertainty remains about whether all complementation's of recessive alleles lead to an additive effect on the final phenotype.

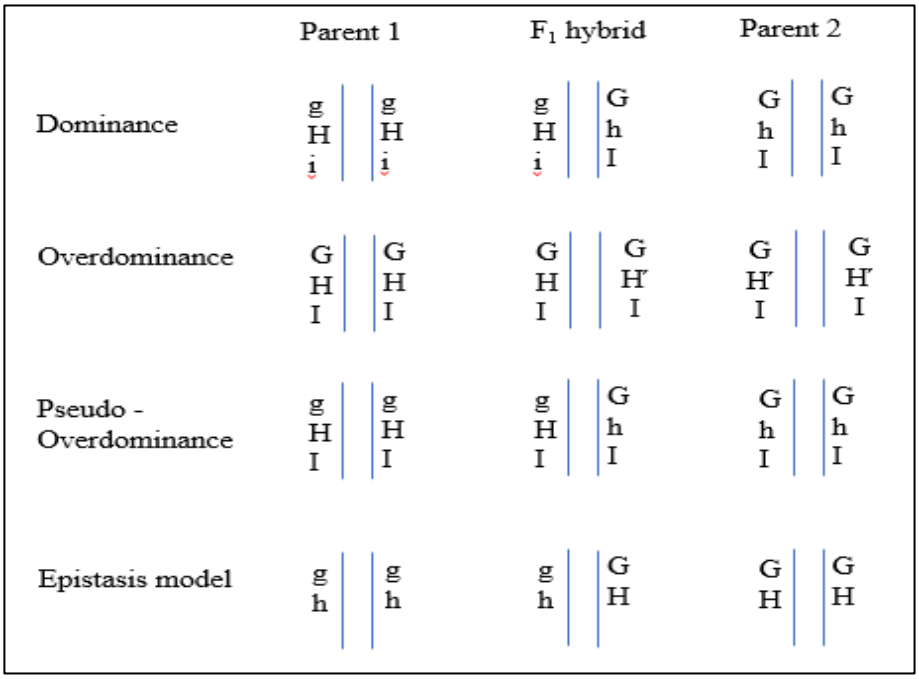

Figure 13.1: illustrates genetic models for heterosis. The diagrams depict the hypothetical phenotype or trait, showcasing the influence of multiple linked or unlinked loci (e.g., 'g', 'h', 'i').

13.2.2 Overdominance Model:

Heterosis cannot be solely explained by the complementation of deleterious alleles, leading to the emergence of another hypothesis in classical genetics known as the "Overdominance" model. This model asserts that heterosis results from the superiority of heterozygotes over each of the homozygotes. The interactions among diverse alleles in heterozygous genotypes, absent in either of the homozygous states (dominant/recessive), contribute to superior trait performance. Different alleles in heterozygous conditions can lead to the production of heterodimeric protein complexes with greater activity, contributing to heterosis (Shull, 1908). While the overdominance model is still debated, reports on "single-locus over-dominance" are significant (McKeown *et al*., 2013). Single locus heterosis has been observed in crops like rice, wheat and tomato exemplified by the Single Flower Truss (SFT) locus increasing tomato yields by up to 60% (Krieger *et al*., 2010). Identifying single-locus over-dominant loci has the potential to streamline breeding, allowing direct manipulation of relevant loci and improving predictions of heterotic hybrids. Genetic maps routinely map quantitative trait locus (QTLs) related to heterosis in various crop species (Wallace *et al*., 2014). Challenges may arise due to tightly linked superior alleles (pseudo-overdominance) or nonallelic/interallelic/epistatic interactions, leading to false positives. Fine mapping can help identify true over-dominant loci.

13.2.3 Pseudo Overdominance Model:

The discrepancy between the Dominance and Overdominance models led to the emergence of the "Pseudo-overdominance" model. This model is grounded in the idea that certain small genomic regions in hybrids may exhibit variations in repulsion at two or more different genes. These variations complement each other, resulting in superior phenotypes that mimic an Overdominance action (Birchler *et al.,* 2010).

The model suggests that homozygous dominant (favorable) alleles are linked with recessive (unfavorable) alleles in parental lines. However, after hybridization, they transition to a heterozygous state and function as an Over dominant locus.

13.2.4 Non-Allelic Interactions or Epistasis:

The term "epistasis," coined by William Bateson in 1907, denotes the deviation from expected Mendelian ratios in experimental crosses. Epistasis involves the interaction of genes from at least two loci, influencing the phenotypic expression of a trait.

The study by Powers (1944) suggested that intra-allelic and inter-allelic interactions, along with crosstalk between genes and the environment, contribute to the phenomenon of heterosis. Even in scenarios with no dominance or partial dominance in certain genes not receptive to improvement in quantitative characters, heterotic expression is observed.

The gene action at individual loci involved in epistasis can express additively or dominantly. Three major forms of epistatic interaction between genes are additive-additive, additive-dominant and dominant-dominant epistasis. For example, a study by Liang on the genetic basis of heterosis in Upland cotton (*Gossypium hirsutum* L.) revealed a significant boost in

hybrid productivity for boll number per plant, directly contributing to lint yield due to epistatic interaction. To understand heterosis at the metabolic level, metabolite profiling was conducted on two mapping populations of Arabidopsis by Lisec et al. In the first population, researchers identified 147 QTLs for metabolite absolute mid-parent heterosis (aMPH), as well as 153 and 83 QTLs for enhanced additive and dominant effects, respectively. In conclusion, epistasis was recognized as a significant contributor to metabolite heterosis in Arabidopsis.

13.2.5 Progressive Heterosis and Dosage Component:

Allelic diversity plays a crucial role in influencing the magnitude of heterosis in polyploid hybrids. The increased level of heterozygosity in polyploids, often termed progressive heterosis (Chen, 2010). East (1936) first reported progressive heterosis in the interspecific cross between two allotetraploids, *Nicotiana tabacum* and *Nicotiana rustica*, which brought together four different genomes, resulting in exceptional and higher than intraspecific crosses.

The phenomenon of progressive heterosis has been observed in various plant species, including alfalfa, potato, and maize. Despite not aligning with classical dominance and overdominance models, progressive heterosis has been briefly explained by proponents of the dominance model, linking allelic diversity and allele dose. However, dosage effects of alleles influencing heterosis expression appear distinct from progressive heterosis.

For instance, crossing allotetraploid plants (AABB) back with diploid progenitors (AA or BB) results in inferior progenies (AAB or ABB) than the allotetraploid itself, attributed to reduced genome dosage in the progeny (Brichler *et al*., 2010). Studies on triploid hybrids suggest that heterosis is controlled by dosage-sensitive factors and involves allelic diversity across the genome.

Contradictory findings exist, such as in sugar beet triploid hybrids, where the genome dosage effect was not observed, and hybridity alone was responsible for heterosis manifestation (Hallahan *et al.,* 2018). This challenges the complementation theory of heterosis, emphasizing the significance of dosage effects beyond allelic complementation.

13.2.6 Hemizygous Complementation:

The genomes of individuals within a species are typically expected to exhibit genetic colinearity, representing a similar set of genes. However, deviations from genetic colinearity and genomic aberrations have been observed in maize inbred lines (Hochholdinger and Hoecker, 2007). For instance, in the bz region, a comparison between inbred lines McC and B73 revealed a deletion of four genes in B73, whereas McC had 10 genes (Fu and Dooner, 2002). In inbred line BSSS53, there were 22 gene copies of the a-zein storage protein subfamily z1C, compared to only 15 z1C genes in B73 (Song and Messing, 2003). Another study comparing 72 selected genes between B73 and Mo17 identified the absence of 27 genes in one of the inbred lines (Brunner *et al.*, 2005). In hybrids, compensatory effects, such as the presence of duplicate copies in the genome of other lines, may mitigate the impact of deletions in one line on trait expression. However,

the presence of at least a single copy of such genes contributed by either parent can contribute to heterosis in hybrids, a phenomenon termed hemizygous complementation. Inbreeding depression is explained as the loss of these hemizygous genes in subsequent selfing generations (Fu and Dooner, 2002). While a high level of heterosis in maize can be linked to significant genomic nonlinearity in inbred lines, other mechanisms might contribute to heterosis in species lacking such a degree of nonlinearity in the genome (Hochholdinger and Hoecker, 2007).

13.3 Epigenetics and Epigenomics Role in Heterosis:

Epigenetics refers to the study of heritable alterations in gene functions, without any changes in the DNA sequence, leading to varied phenotypes based on the epiallele profile. DNA methylation, expression of small RNA (sRNA) and histone modifications are fundamental mechanisms governing epigenetic regulations in both plants and animals (Calarco *et al*., 2012).

13.3.1 DNA Methylation:

DNA methylation involves the introduction of methyl groups to the bases of a DNA molecule through the action of the enzyme DNA methyltransferase (He *et al*., 2014). This process modulates gene expression by suppressing active transposons, pericentromeric repeat elements, and genetic promoters. In plants, DNA methylation predominantly targets cytosines, specifically at CG, CHG, and CHH sites (Dapp *et al*., 2015).

The stronger correlation of increased cytosine methylation levels and heterosis in hybrids compared to parents emphasizes the significant influence of methylation. The pace at which natural variation in DNA methylation patterns accumulates surpasses that of spontaneous genetic mutations, highlighting the capability of diverse independent epialleles to contribute to heterosis (Fujimoto *et al*., 2012).

There are two mechanisms governing the regulation of allelic methylation patterns (epialleles) in hybrids: Trans-Chromosomal Methylation (TCM) and Trans-Chromosomal Demethylation (TCDM). TCM entails the direct initiation of *de novo* methylation, while TCDM induces demethylation in hybrids (Greaves *et al.*, 2012).

These mechanism helps in maintaining a balanced methylation state at homologous alleles in hybrids, a crucial factor for heterotic expression. The inheritance patterns of Trans-Chromosomal Methylation (TCM) and Trans-Chromosomal Demethylation (TCDM) at specific genomic loci in the F_2 generation of an Arabidopsis cross (Ler-0 × C24) revealed a stable transmission of methylation patterns (Greaves *et al*., 2014). Methylation of DNA in pericentromeric heterochromatin regions is predominantly facilitated by the chromatin remodeler protein DDM1 (DECREASED DNA METHYLATION 1), a key regulator of heterosis. Impaired functioning of DDM1 results in modified methylation patterns and diminished phenotypic expression in hybrids (Kawanabe *et al*., 2016).

In addition to genetic factors, environmental elements like planting density can lead to a reduction in methylation in hybrids, impacting the extent of heterosis (Tsfartis *et al*., 1999).

Beyond the nuclear genome, the organelle genome also plays a role in contributing to heterosis. The gene MutS HOMOLOG1 (MSH1) ensures stability in the organelle genome and disruption of MSH1 expression results in changes in various traits, such as dwarfism, leaf variegation, altered leaf structure, and delayed flowering in species like *A. thaliana*, sorghum, tomato, and soybean (Xu *et al.*, 2012). Other factors, including the expression of small interfering RNA (siRNA), influencing the heterotic gene expression by modifying the methylation pattern in hybrids (Chodavarapu *et al*., 2012).

13.3.2 Small RNAs (sRNAs):

Plant sRNAs falls into two main categories: microRNAs (miRNAs) and small interfering RNA or short interfering or silencing RNA (siRNAs), which were typically 21 and 24 nucleotides in length. Both miRNAs and siRNAs are noncoding RNAs with crucial roles in post-transcriptional and post-translational gene regulation. The miRNAs are generated from endogenous MIR (miRNA-encoding) genes through RNA polymerase II (RNA Pol II) and the DICER LIKE 1 (DCL1) protein. The RISC (RNA-Induced Silencing) complex and ARGONAUTE 1 (AGO1) endonuclease guides the mature miRNAs to cleave transcripts and thereby inhibit the translation of targeted mRNA (Bartel, 2004). siRNAs are generated through the collaborative action of RNA polymerase IV, RNA-dependent RNA polymerase 2 (RDR2), DCL2, DCL3 or DCL4. In combination with the RISC, these enzymes either facilitate mRNA cleavage or modulate the process of *de novo* DNA methylation and the formation of heterochromatin, a phenomenon referred to as RNA-dependent DNA methylation (RdDM) (Vaucheret, 2006). siRNA plays a crucial role in preserving genome stability by silencing transposons and providing protection against invasive viral RNA (Slotkin and Martienssen, 2007). Moreover, the involvement of sRNAs in heterosis has been documented in Arabidopsis (Shen *et al*., 2012), rice (Chodavarapu *et al*., 2012), wheat (Kenan-Eichler *et al*., 2011), and maize (Barber *et al*., 2012).

The role of sRNA to heterosis is predominantly substantiated indirectly through inference and correlation based on DNA methylation. For instance, HUA ENHANCER 1 (HEN1), an Arabidopsis methyltransferase, methylates mature sRNAs to enhance their stability. The crossing of a *hen1* mutant with *Ler-0* led to a diminished size and reduced plant vigor in F_1, suggesting a relationship between sRNAs and heterosis. Though, the heterotic expression remains unchanged in maize hybrids generated using the modifier of *paramutation 1* (*mop1*) mutant. *MOP1* is crucial for the synthesis of 24-nucleotide siRNAs in maize and is considered a homologue of *A. thaliana* RDR2 (Barber *et al*., 2012).

The divergent outcomes can be explained by HEN1's involvement in the stability of both siRNAs and miRNAs, while MOP1's role is limited to generating siRNAs. Therefore, in addition to sRNA production, the stability of the generated sRNAs plays a pivotal role in conferring heterosis.

The majority of current studies exploring potential networks between epigenetics and heterosis rely on statistical correlation models, lacking a clear elucidation of the underlying mechanism(s). The precise understanding of the contribution of epigenetics to heterosis is a complex journey that remains to be fully unravelled, presenting intriguing research for the field of plant breeding science.

13.3.3 Histone Modifications:

Histone modification impacts numerous genes and the adjacent regions on the associated DNA molecules. Consequently, studying the correlation between histone modification and heterosis is more intricate due to its inherent complexity. Significant efforts to explore the potential influence of histone modification on heterosis have primarily centered on the widely studied model genome of *Arabidopsis thaliana*. In 2009, Ni and collaborators, through the examination of the circadian clock and its associated genes in *Arabidopsis* F_1 hybrids, observed alterations in the transcription of these genes in conjunction with histone modifications (Ni *et al*., 2008). This discovery holds significance as the circadian clock plays a crucial role in various biological processes in plants, including starch biosynthesis and growth rate (Figure 13.2). Plants that synchronize their internal circadian rhythm with their living environments exhibit greater vigor compared to plants that do not maintain this synchronization (Kim *et al*., 2017). Hence, the transcriptional changes of genes related to circadian rhythms, mediated by histone epigenetics, could be linked to the performance of F_1 hybrids.

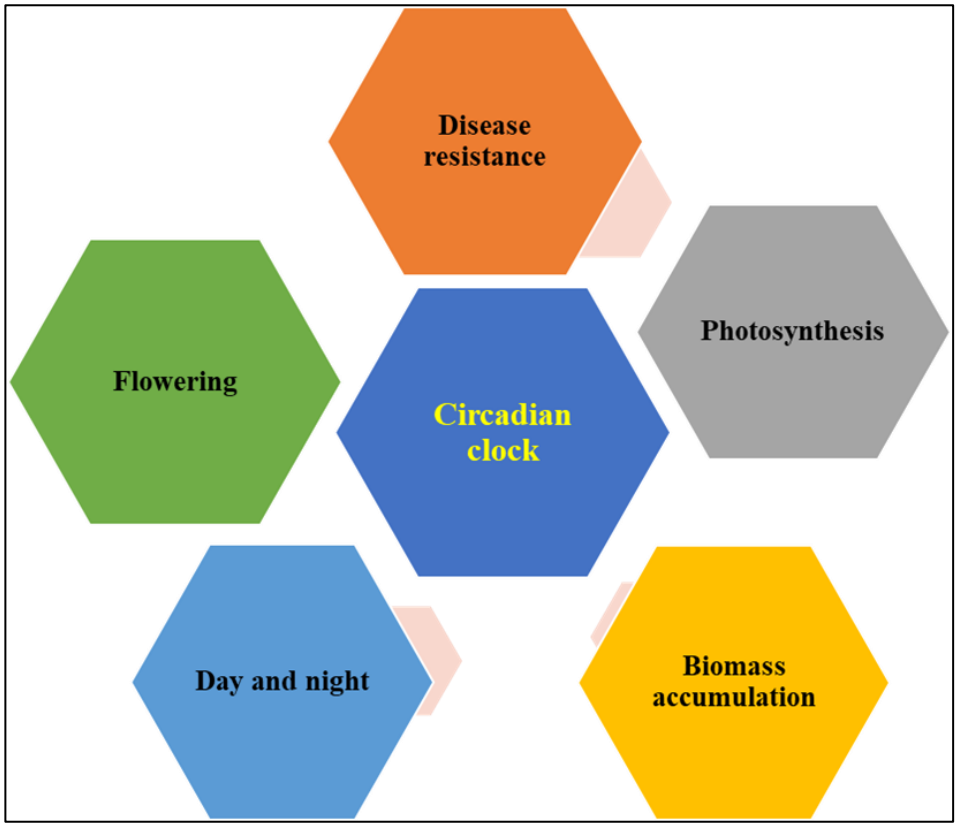

Figure 13.2: Various plant processes affected by Circadian rhythm

With supportive evidence from *Arabidopsis* research, additional investigations into crop plants have been undertaken to explore the connection between histone modification and heterosis. Notably, maize F_1 hybrids exhibited substantial expression variations in the key

histone HTA112 in comparison to their parental inbred lines, particularly in endosperm transcriptomes (Jahnke *et al*., 2010). The study served as an initial exploration into investigating specific histone modifications that regulate the performance of crop hybrids. In the case of rice, three global histone mark patterns (H3K4me3, H3K9ac, and H3K27me3) were examined across two rice subspecies, 'japonica' and 'indica', as well as their F_1 hybrids using high-throughput ChIP-Seq (He *et al*., 2010).

As a result, H3K4me3 (a mark associated with transcriptional activation) and H3K27me3 (a mark linked to transcriptional repression) exhibited distinct expression patterns between hybrids and their parental lines. These findings contribute to showcasing potential connections between alterations in epigenetic histone modification and heterosis.

13.4 Omics Approach:

Heterosis, commonly known as hybrid vigour, is a phenomenon in which the offspring of genetically diverse parents exhibit enhanced traits compared to their inbred parents. This phenomenon has been widely exploited in crop plants to improve yield, resilience, and overall performance. To unravel the molecular underpinnings of heterosis, researchers employ a suite of high-throughput technologies collectively known as "omics." These include genomics, transcriptomics, proteomics, and metabolomics, each providing a unique perspective on the genetic, transcriptional, protein, and metabolic changes associated with hybrid vigour.

Genomics involves the comprehensive study of an organism's entire genome, including the identification of genetic variations, single nucleotide polymorphisms (SNPs), and structural variations. Understanding the genetic makeup of hybrids and their parents is crucial for uncovering the inheritance patterns that contribute to the observed heterotic effects.

Proteomics and transcriptomics are complementary approaches that play crucial roles in unraveling the molecular mechanisms underlying heterosis in crop plants. Both methodologies provide insights into gene expression at different levels, helping to bridge the gap between genotype and phenotype. Their relevance to heterosis to support the statements were given below.

Transcriptomics focuses on the study of RNA molecules, providing insights into gene expression patterns. By comparing the transcriptomes of hybrids and their parents, researchers can identify differentially expressed genes (DEGs) and regulatory pathways that play a role in heterosis. Transcriptomics involves the study of gene expression at the RNA level. Through techniques like RNA sequencing (RNA-seq), it allows for the identification of differentially expressed genes (DEGs) between hybrids and their parents.

Transcriptomic studies provide a foundation for understanding the initial steps in the flow of genetic information. Differentially expressed genes (degs) associated with temperature tolerance, revealing key pathways involved in the response to stress (Shao *et al.*, (2019). Transcriptomic analyses identify DEGs associated with heterosis, providing information on specific pathways and biological processes. It aids in unraveling the genetic basis of hybrid vigour (Wei *et al*., 2019).

Proteomics involves the study of the entire set of proteins expressed by an organism. Examining the proteome allows researchers to understand post-transcriptional and post-translational modifications, providing a direct measure of protein abundance and function associated with heterotic traits. Proteomics, on the other hand, focuses on the study of proteins expressed in a biological system. It provides a direct measurement of protein abundance, offering insights into post-transcriptional and post-translational regulation. This is crucial as gene expression does not always correlate with protein abundance. The proteomic study identified and quantified phosphoproteins, providing information on post-translational modifications (Hu *et al*., 2015). Proteomic studies complement transcriptomics by revealing post-transcriptional modifications and providing a direct measure of protein function. This is crucial for understanding how gene expression changes at the mRNA level translate into altered protein function Li *et al*., (2018).

Both transcriptomics and proteomics are often integrated to gain a more comprehensive understanding of heterosis. Integrative analyses help decipher the intricate relationships between gene expression, protein abundance, and phenotypic traits in hybrids Ma *et al*., (2018).

The combined use of transcriptomics and proteomics enhances our ability to decipher the molecular basis of heterosis in crop plants, providing a more comprehensive view of the complex regulatory networks involved in hybrid vigour. The references provided offer examples of studies applying these methodologies to understand gene expression and protein regulation in various plant species.

Metabolomics explores the complete set of small molecules (metabolites) within a biological system. By profiling the metabolome of hybrids and their parents, researchers can identify specific metabolic pathways that contribute to the enhanced performance observed in heterosis. In this context, omics approaches offer a comprehensive and systems-level understanding of the molecular and biochemical changes that underlie the superior traits of hybrids.

The integration of genomics, transcriptomics, proteomics, and metabolomics data provides a holistic view, enabling researchers to connect genetic information with the dynamic processes occurring at the RNA, protein, and metabolite levels. This integrative approach is pivotal for deciphering the complex molecular networks governing heterosis and has significant implications for crop improvement and sustainable agriculture.

Metabolomics is another powerful -omics approach that contributes to the understanding of heterosis in crop plants. Metabolomics focuses on the comprehensive analysis of small molecules, or metabolites, within a biological system. These metabolites are the end products of various cellular processes and can serve as direct indicators of the physiological status of an organism. Studying the metabolome provides insights into the final outcomes of gene expression and protein activity, offering a unique perspective on the biochemical pathways associated with heterosis. As we delve deeper into the specific omics studies related to heterosis in crop plants, it becomes evident that these high-throughput technologies collectively contribute to unraveling the mysteries of hybrid vigour and provide valuable insights for crop breeding and agricultural sustainability.

13.4.1 Metabolic Profiling of Heterosis:

Metabolomics allows for the identification and quantification of a wide range of metabolites, including sugars, organic acids, amino acids, and secondary metabolites. By comparing the metabolite profiles of hybrids with those of their parents, researchers can identify specific metabolic changes associated with hybrid vigour. Wei *et al.* (2019) conducted metabolic profiling during apple fruit development and ripening using widely targeted metabolomics. The study revealed dynamic changes in metabolite abundance, providing insights into the metabolic pathways associated with heterosis.

13.4.2 Identification of Metabolic Pathways Contributing to Heterosis:

Metabolomics helps identify key metabolic pathways that are altered in hybrids, contributing to enhanced performance. Understanding how metabolites are regulated and interact provides information on the metabolic basis of heterosis. Riedelsheimer *et al.*, (2012) used metabolomics to study the metabolic basis of heterosis in maize. They identified metabolites associated with increased biomass and grain yield in hybrids. Integrating metabolomics with genomics, transcriptomics, and proteomics provides a comprehensive understanding of the molecular mechanisms underlying heterosis.

This integrative approach helps connect genetic information, gene expression, protein abundance, and metabolic outcomes. Yuan *et al.*, (2019) integrated metabolomics and transcriptomics to study heterosis in cotton. The combined analysis revealed coordinated changes in metabolite levels and gene expression, highlighting the interconnected nature of these molecular processes.

In summary, metabolomics plays a crucial role in unraveling the biochemical basis of heterosis in crop plants. By profiling the metabolome, researchers can identify specific metabolic pathways associated with superior traits in hybrids, providing valuable information for crop improvement and sustainable agriculture. Integration with other omics approaches enhance the depth of our understanding of the complex molecular networks governing heterosis.

13.5 Future Prospects of Heterosis:

The exploration of heterosis in crop plants holds great promise for the future of agriculture, with several exciting prospects on the horizon. These prospects encompass advancements in understanding the molecular mechanisms, improving breeding strategies, and harnessing heterosis for sustainable and resilient crop production. Here are some future prospects in the field of heterosis,

Precision Breeding and Genomic Selection Advances in genomics have paved the way for precision breeding approaches. The identification of key genes and genomic regions associated with heterosis allows for more targeted breeding strategies. Genomic selection, utilizing molecular markers and genomic information, can accelerate the breeding process by predicting hybrid performance based on genetic markers linked to heterotic effects.

Integrated Omics Approach The integration of genomics, transcriptomics, proteomics, and metabolomics will provide a more holistic understanding of the complex molecular networks underlying heterosis. Systems biology approaches will enable researchers to model and predict the interactions between genes, proteins, and metabolites, facilitating more informed breeding decisions.

Synthetic biology and genome editing Synthetic biology techniques, including genome editing technologies like CRISPR-Cas9, offer the potential to engineer specific genetic elements associated with heterotic traits. Precision genome editing allows for the targeted modification of genes to enhance desired traits, potentially unlocking new dimensions of heterosis.

Understanding Epigenetic Regulation Epigenetic modifications play a role in regulating gene expression and may contribute to heterosis. Future research will likely delve deeper into the epigenetic mechanisms underlying hybrid vigour. Targeting epigenetic modifications could provide additional avenues for manipulating and enhancing heterotic effects.

Expanding Heterosis to More Crops While heterosis is well-studied in major crops like maize and rice, future research will likely extend the understanding of hybrid vigour to a broader range of crops. Unlocking heterosis in diverse plant species could lead to improved yields, resilience, and nutritional content in a variety of agricultural products.

Climate-Resilient Hybrids Climate change poses significant challenges to agriculture. Future research in heterosis aims to develop hybrids that exhibit enhanced resilience to climate stress, such as drought, heat, and pests. Breeding for climate-resilient hybrids could contribute to sustainable agriculture by ensuring stable crop production under changing environmental conditions.

Quantitative Understanding of Heterosis Advancements in computational biology and quantitative genetics will enable a more detailed and predictive understanding of the genetic basis of heterosis. Mathematical models and simulations may aid in optimizing breeding strategies and predicting hybrid performance across different environments.

Incorporating Functional Genomics Functional genomics studies, including the characterization of gene function and regulatory networks, will provide deeper insights into how specific genes contribute to heterosis. Understanding the functional relevance of genes associated with hybrid vigour can guide targeted interventions for crop improvement.

In summary, the future of heterosis research holds exciting prospects, ranging from precision breeding and genomic technologies to the integration of omics approaches and the development of climate-resilient hybrids. These advancements have the potential to revolutionize agriculture by enhancing crop productivity, sustainability, and adaptability to changing environmental conditions. The interdisciplinary nature of heterosis research, combining genetics, genomics, and computational biology, positions it as a key area for innovation in the future of crop improvement.

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