**HETEROSIS AND INBREEDING DEPRESSION**

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**ABSTRACT**

Hybrids have played a great role in improving the productivity of various crops and has paved a way to cope up with the growing food demand. Heterosis is the mechanism of production of superior F1 hybrids compared to parents, while inbreeding depression is the opposite of this mechanism in terms of their manifest effects. Insights into the genetic, physiological, biochemical and molecular basis of these phenomena will help in better understanding and effective utilization in further breeding programs.

**Keywords –** Biochemical basis, Genetic basis, Heterosis, Inbreeding depression, Physiological basis.

1. **INTRODUCTION**

The dramatic increase in yield of different crops evident over the past 75 years was due to a ‘miraculous’ agricultural phenomenon named heterosis leading to yield advantage ranging from 15% to 50% depending on the crop. Hybrids are widely developed and used for field crops such as maize, sorghum and sunflower, for self-pollinated crop like rice the hybrids are grown extensively in China and are increasing in India. While, hybrids are widely used for many commercial flowers and vegetables due to suitability of hybrids for improved productivity.

1. **HETEROSIS**

The natural biological phenomenon of “Heterosis” was first observed by Koelreuter in tobacco and defined by Shull (1952) as the ‘Interpretation of increased yield, fruitfulness, size, resistance to biotic and abiotic stress, vigour or speed of development, manifested by cross-bred organisms as the specific results of unlikeness in the constituents of the uniting parental gametes as compared with corresponding inbreds’.

Heterosis works as a basic tool for improved production of crops in the form of F1 hybrids. The term heterosis was introduced by Shull in 1908, which refers to the phenomenon in which F1 hybrid obtained by crossing two genetically dissimilar individuals shows increased or decreased vigour over the parents. According to Miranda (1999), heterosis is the genetic expression of the superiority of a hybrid in relation to its parents. The manifestation of heterosis depends on genetic divergence of the two parental varieties.

Heterosis was earliest applied in maize after its scientific characterization followed by its use in other crops such as beet, sorghum, brinjal, onion, tomato, peppers, sunflower, rice, cotton. The term hybrid vigour is used as a synonym of heterosis, but it is generally accepted that the term heterosis describes both superior and inferior expression of the hybrids over their parents. While, hybrid vigour describes only the superior expression of the hybrids. This superior and inferior expression were designated as positive and negative heterosis respectively.

**CHARACTERISTICS OF HETEROSIS**

The degree of heterosis varies with genetic distance of the parents, more the heterosis with more genetic distance.

* Heterosis varies with the mode of reproduction.
* Heterosis varies with trait to trait and developmental stage of the plants.
* Heterosis is affected by environmental variations including both biotic and abiotic factors.

**CALCULATION OF HETEROSIS**

The three different terms used to describe the degree of phenotypic difference of a trait in a hybrid (F1) are:

1. **Mid-parent or Relative heterosis:** The increased performance of F1 hybrid over average performance of both parents (mid-parent) is termed as Relative or average or mid-parent heterosis.

* The formula for calculating relative heterosis was given by Turner (1953) as:

H (%) = x 100

Where, = Mean of F1­

**=** Mean ofmid parental value

1. **Heterobeltiosis:** The increased performance of F1 hybrid over better parent is called as heterobeltiosis.

* The formula for calculating heterobeltiosis was given by Fonesca and Patterson (1968) as:

H (%) = x 100

Where, = Mean of F1

= Mean of better parental value

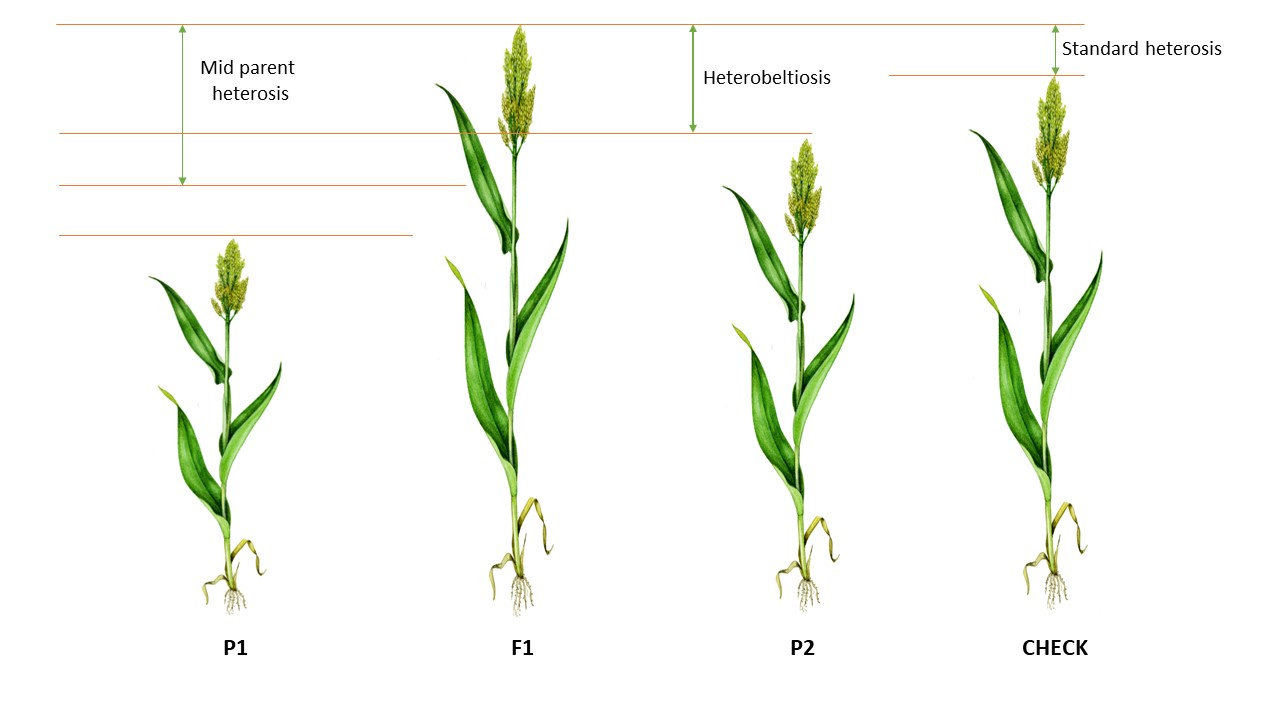
1. **Standard heterosis:** The increased performance of F1 hybrid over the best commercial variety available for that crop is called as standard or economic or useful heterosis.

* In practical application, heterosis over mid parent and better parent is of little importance since it does not offer any advantage. However, usage of standard heterosis helps in exploitation of hybrid depending on its performance in comparison to the best existing commercial hybrids/varieties (standard heterosis).
* It is calculated using the formula given by Virmani *et al*. (1982) as:

H (%) = x 100

Where, = Mean of F1

= Mean of standard check value



**Figure 1. Diagrammatic representation of Heterosis, Heterobeltiosis and Standard heterosis.**

1. **NATURAL CLASSIFICATION OF HETEROSIS:**

Heterosis was classified as Euheterosis and Luxuriance by Dobzhansky on the basis of fitness.

**EUHETEROSIS:** It is also referred as True heterosis as it confers higher fitness to individuals in sexually reproducing species and cross-pollinating species, where inter-crossing among inbred lines leads to increased fitness and vice versa in case of inbreeding depression.

Depending on the basis of arise of euheterosis, it has been classified as 1) Mutational euheterosis and 2) Balanced euheterosis.

1. **Mutational euheterosis:** It is a result of dominance gene action, due to masking of less adaptive deleterious recessive mutants by their more adaptive dominant counterpart in heterozygous state.
2. **Balanced euheterosis:** It is a result of over-dominance gene action, where heterozygotes confer superiority and high adaptive value over both the corresponding homozygote states.

**LUXURIANCE:** In contrast to euheterosis where the fertility is increased, here in luxuriance the hybrids exhibit poor adaptive value and fitness than the parents as the interspecific hybrids are generally sterile or poorly fertile. However, the hybrids may express considerable heterosis for vegetative characters. It is also referred to as pseudo-heterosis.

1. **CLASSIFICATION OF HETEROSIS BASED ON THE GENETIC DISTANCE OF PARENTAL LINES**

Heterosis was classified as intraspecific heterosis, intersubspecific heterosis and wide-hybridization heterosis on basis of genetic distance between parental lines.

1. **Intraspecific heterosis**

It is the heterosis resulting from crosses between two individuals belonging to same species. Due to ease in crossing, good seed set and low cost, it is a favored choice by most of the breeders.

1. **Intersubspecific heterosis**

It is the heterosis resulting from crosses between two individuals belonging to different subspecies. The hybrids produced exhibit more heterosis as compared to the than intraspecific hybrids.

Eg: Hybrids obtained by crossing different subspecies of rice i.e., between indica and japonica rice exhibits higher yield and better agronomic performance than hybrids developed between intervarietal crosses.

However, intraspecific hybrids involving parents with more favourable genes and higher adaptability may show greater heterotic potential than intersubspecific hybrids. Apart from this, discordance between parental genomes leads to reproductive incompatibility and poor seed setting.

1. **Wide-hybridization heterosis:**

It is the heterosis resulting from crosses between two individuals belonging to different species or genus.

Eg: F1 obtained between - *Brassica oleracea* x *Brassica rapa* (interspecific hybrid)

F1 obtained between – *Triticum spp*. x *Secale spp*. (intergeneric hybrid)

Higher heterosis is observed in wide hybridization due to geographical and reproductive isolation, but due to huge genomic discordance between parental genomes leads in reproductive incompatibility and poor seed setting.

1. **INBREEDING DEPRESSION:**

Inbreeding refers to crossing of individuals that are closely related by descent. Selfing is the intense form of inbreeding, followed by sib-mating. It leads to increase in homozygosity.

Inbreeding depression is opposite to heterosis in terms of their manifest effects. It is defined as the loss or reduction in mean phenotypic value in traits associated with physiological efficiency and reproductive ability. Increased homozygosity can lower the fitness either due to increased homozygosity for partially recessive detrimental mutations or due to increased homozygosity for alleles at loci with heterozygote advantage (‘overdominance’).

**Outcomes of Inbreeding:**

* With each inbreeding, there is increase in homozygosity.
* It leads to appearance of deleterious homozygous recessive forms, which were otherwise concealed by their dominant counterparts.
* It leads to inbreeding depression and thereby reduction in fitness due to loss of heterozygosity.
* Leads to increase in variance as a consequence of separation of population into true breeding homozygous sub-populations.

**Degrees of Inbreeding depression:**

* The magnitude of inbreeding depression varies in different crop species, such as high, moderate, low and lack of inbreeding depression.
* Cross-pollinated species having large number of deleterious recessive alleles favour heterozygosity to mask the detrimental effects of the homozygous condition of deleterious recessive alleles. Thus, inbreeding depression is expressed in such species on occurrence of inbreeding.
* Crops such as Alfalfa and carrot exhibit high degree of inbreeding depression, with no survival of large population showing lethal characteristics and the lines which survive yield less than 25% as compared to open-pollinated varieties.
* Crops like Sorghum, maize and bajra exhibiting moderate inbreeding depression show reduced fertility and yield are reduced to 50% of the open-pollinated varieties.
* In crops such as most of the cucurbits, onion, sunflower and rye exhibit low inbreeding depression where there is no much loss of vigour and fertility.
* In self-pollinated species, unfavourable recessive genes in homozygous condition are eliminated and the favourable homozygous state are not injurious and they show lack of inbreeding depression.

1. **HYPOTHESES FOR HETEROSIS AND INBREEDING DEPRESSION**

Different hypotheses are given to understand the complex phenomena of heterosis. They are:

1. **GENETIC BASIS OF HETEROSIS**

The three theories explaining the genetic basis of heterosis as well of inbreeding depression are – Dominance hypothesis, overdominance hypothesis and epistasis hypothesis.

1. **Dominance hypothesis of heterosis**

This hypothesis was first proposed in 1908 by Davenport, which was further expanded by Bruce, Keeble and Pellew in 1910. It is amongst the oldest but still the most accepted explanations for heterosis. Here, favorable genes controlling growth and development are dominant and unfavorable genes are recessive. It is due to allelic interactions where heterosis is a result of masking of unfavourable and deleterious recessive alleles by the favourable dominant allele in the heterozygous condition.

* Heterosis is not due to heterozygosity itself.
* The number of homozygous recessive alleles are more in the hybrids as compared to both parents.
* Heterosis is directly proportional to the number of beneficial dominant alleles.
* Heterosis is due to additive effect of favorable alleles exhibiting either partial or complete dominance.
* The number of dominant alleles is always more in the hybrids than their parents. Thus, hybrid possessing higher value of heterosis than the two parents.
* Inbreeding depression is due to homozygous condition of deleterious recessive alleles.
* **Objections:**
* Failure to isolate true breeding homozygous inbred lines with all dominant genes which are as vigorous as the F1 hybrid.
* Absence of skewed distribution towards dominant genes in F2 population.
* **Explanations for objections:**
* Jones proposed Dominance of linked gene hypothesis in 1917, stating that dominant and recessive genes would be linked together making it difficult to isolate inbred lines containing all dominant genes.
* Due to governance of the quantitative traits by polygenes and effects of environment the distribution curve would be symmetrical.

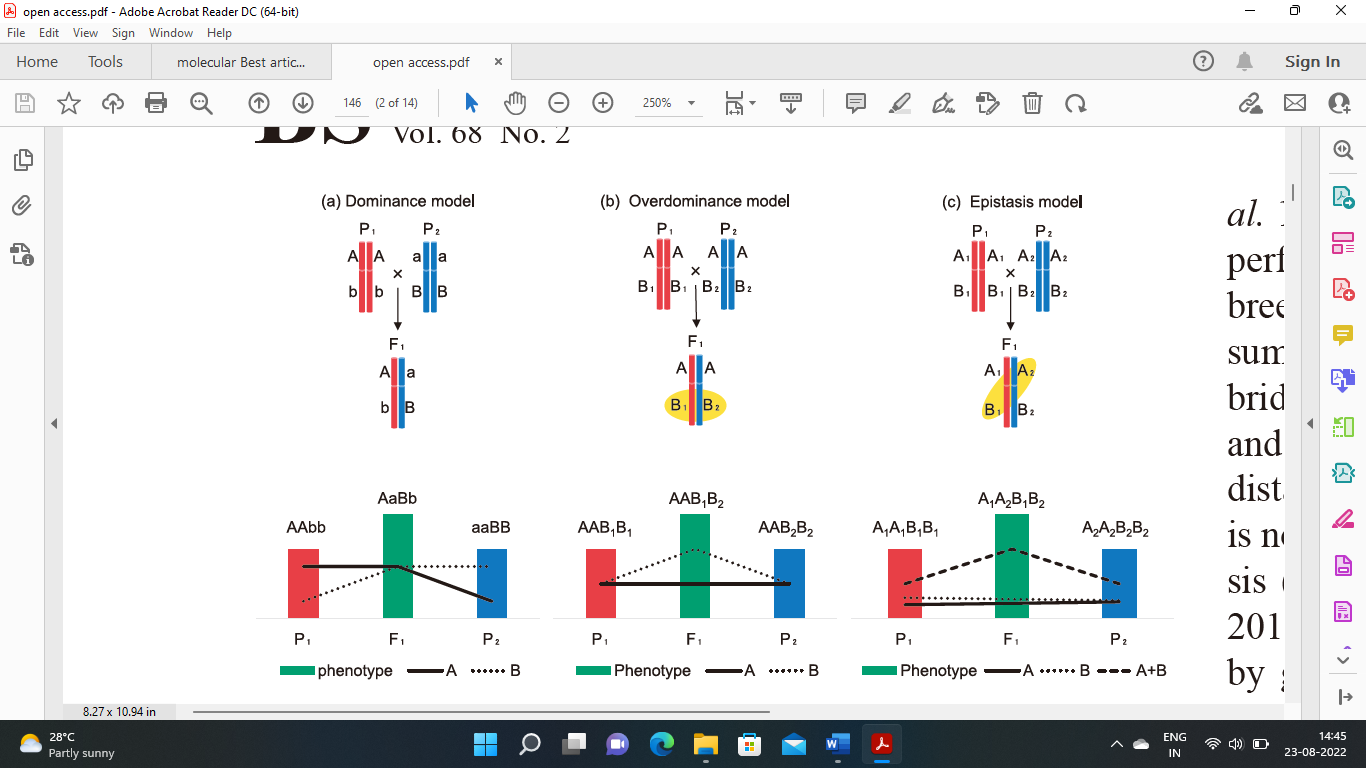
1. **OVER-DOMINANCE HYPOTHESIS**

This hypothesis was proposed in 1908 by Shull and East independently. Heterosis is a result of allelic interactions leading in heterozygote superiority i.e., the heterozygous state at atleast some of the loci leads to superior performance compared to both the homozygotes.

* Heterosis is due to heterozygosity itself.
* More the number of divergent alleles in heterozygote, more will be the heterosis.
* It is also referred to as stimulation of divergent alleles, single gene heterosis or super dominance.
* Inbreeding depression is due to homozygosity itself.
* **Objections:**
* The concept of single locus heterosis may not hold good in case of quantitative traits.

1. **EPISTASIS HYPOTHESIS**

This hypothesis was suggested in 1952 by Gowen. It suggests that heterosis is attributed due to favourable epistatic interactions between non-allelic genes i.e., influence of one locus on expression of another locus.



Picture source: Fujimoto et al., 2018

**Figure 2. Hypotheses to explain genetic basis of heterosis.**

Phenotype is the sum of gene effects (A, B, A + B). (a) Dominance hypothesis- favourable dominant alleles (A and B) suppress or complement the deleterious recessive alleles (a and b). (b) Overdominance hypothesis – heterosis is due to heterozygosity (B1/B2) at the key locus. (c) Epistasis hypothesis- interaction of non-allelic genes (A2 and B1) from the parental lines leads to heterosis.

* Apart from the above classical hypothesis, Zhong has proposed active gene effect hypothesis by comparing the relationship between genomic imprinting and heterosis.

**Active gene effect hypothesis:**

* According to the active gene effect hypothesis, in homozygous condition only one allele is active due to genomic imprinting.
* While all genes are active in heterozygous condition due to non-occurrence of genomic imprinting. The additive interaction between the active genes causes expression of heterosis by increased overall effects of gene expression.
* DNA methylation and histone modification are affects caused by Genomic imprinting leading in differential expression of genes, as any alteration even in a single gene may cause changes in the entire network of gene expression. Through intensive studies on epigenetics, epigenetic factors such as DNA methylation, small RNAs, and histone modifications have been found to be involved in the development of heterosis in plants.

1. **PHYSIOLOGICAL BASIS OF HETEROSIS**

It forms a basis to understand the metabolic superiority of hybrids in terms of growth rate, size and other developmental characteristics as compared to inbreds.

* Ashyb’s hypothesis of greater initial capital was the first effort to explain physiology of heterosis, where it was stated that the greater initial embryo size and weight contributed to heterosis in hybrids.
* There are three stages of physiological manifestation of heterosis. They are:

1. **DEVELOPMENT OF EMBRYO AND SEED**

Hybrid vigour exhibited high positive correlation with embryo weight which was more in large seeds. It is mentioned in earlier studies that hybrid advantage was obtained in early stages of the embryo development.

1. **EARLY SEEDLING GROWTH:**

Ashby emphasized that heterosis was in the early post-germination growth but not in the later phases. Experimental findings in maize have revealed higher growth rate after germination in hybrids as compared to inbreds.

1. **LATER GROWTH STAGES:**

Ashby stated the maintenance of initial embryo seed and weight was responsible for later stage heterosis in hybrids. Hybrids were observed to absorb and utilize more amount of nutrients than the inbred lines. Root growth was more in hybrids.

1. **BIOCHEMICAL BASIS OF HETEROSIS**

It expresses the importance of biochemical reactions and role of major metabolic enzymes in manifestation of heterosis. It is related to gene-protein synthesis.

1. **Bottleneck concept of heterosis**

The concept of heterosis being depended on weakest link where inferiority of parents is presumed to be represented by inefficient alleles and not on the strongest link was termed as bottleneck concept by Mangelsdorf.

Suppose A1 and B1 are two dominant genes required to complete a biosynthetic pathway. The homozygotes A1A1B0B0 and A0A0B1B1 consisting of A0 and B0 deficient genes will fail to complete the biosynthetic pathway. Whereas, the heterozygous condition A1A0B1B0 consisting of both the dominant genes will be able to complete the biosynthetic pathway as a result of complementary non-allelic interaction of dominant genes, leading to expression of heterosis.

1. **Model of gene-gene product interactions**

Considering single locus dominance action, four models of gene-gene product interactions was suggested for superiority of heterozygotes.

1. **Supplementary action**

It considers that the two alleles of a gene produce different product and thereby perform different functions.

Eg: Gene A has two alleles – A0 and A1

A0 in homozygous state A0A0 produces ‘x’ product and A1 in homozygous state A1A1 produces ‘y’ product.

While the heterozygous state A1A0 produces both the products ‘x and y’ thus leading to hybrid superiority.

1. **Alternative pathway**

In this type of gene-gene product interaction, environment plays a critical role.

Eg: Gene A has two alleles – A0 and A1

A0 in homozygous state A0A0 produces ‘x’ product in environment 1 (E1) and A1 in homozygous state A1A1 produces ‘y’ product in environment 2 (E2). While the heterozygous state A1A0 produces both the products ‘x’ in E1 and ‘y’ in E2 leading to hybrid superiority.

1. **Optimal amount**

This type of gene-gene interaction differs in the quantity of gene product produced by different alleles.

Eg: Gene A has two alleles – A0 and A1

A0 in homozygous state A0A0 having no active allele produces too little of a gene product in ‘0.1x’ amount.

A1 in homozygous state A1A1 having two active alleles produces too much of a gene product in ‘2x’ amount. While the heterozygous state A1A0 having one active allele produces optimal amount ‘1x’ of the product, leading to hybrid superiority.

1. **Hybrid substances**

This type of gene-gene interaction produces different hybrid substance in heterozygous state which is not present in both homozygous states.

Eg: Gene A has two alleles – A0 and A1

A0 in homozygous state A0A0 ‘x’ gene product.

A1 in homozygous state A1A1 produces ‘y’ gene product. While the heterozygous state A1A0 produces ‘x + y + z’ gene products, having an additional hybrid substance ‘z’ owing to hybrid superiority.

1. **MOLECULAR BASIS OF HETEROSIS**

With development of novel molecular breeding tools, there are ongoing efforts to decipher the molecular basis of heterosis so that its powers can be harnessed and used more efficiently. Several laboratories have recently started to analyze different molecular aspects of heterosis.

The first step towards understanding the molecular basis of heterosis in the past was by quantitative trait loci (QTL) analyses. Recent studies have started to understand heterosis at levels of gene expression and genome organization.

Analysis of gene expression of molecular basis of heterosis can be done in three categories, as:

1. **Analysis at the level of genome organization**

Genetic collinearity is a basic notion which describes that individuals in a given species when observed at genome level contain the same gene content. Recent studies on maize inbred lines at genome level have observed presence of significant aberrations from colinearity on the microcolinearity level. High degree of non-colinearity in the genomes of maize inbred lines may be the responsible factor for high degree of heterosis.

In species where many genes belong to small gene families when undergo gene deletions, the inbred lines may only have minor quantitative effects on plant performance because these genes are frequently functionally compensated by duplicate copies elsewhere in the genome.

While, hemizygous complementation of many genes with minor quantitative effects in hybrids may result in significantly improved hybrid plant performance, which is consistent with the dominance hypothesis. This theory would also explain inbreeding depression caused by the loss of functional genes in subsequent generations when hemizygous genes are lost after several rounds of hybrid self-pollination.

1. **Analysis at the level of transcriptome**

Differential expression of genes in inbreds and hybrids at different developmental stages and tissues were observed to be associated with heterosis. These stidies were performed by high-throughput gene expression analyses via microarray profiling or GeneCalling. The non-uniformity in gene expression patterns may be attributed to the up-regulation and down-regulation of genes in hybrids relative to their parental inbred lines.

1. **Analysis of allele-specific contribution to gene expression**

Gene expression can differ based on allele-specific cis- and trans- regulation. The two alleles of a gene provide equal contribution to gene expression in the hybrid in trans-regulation, whereas two alleles contribute unequally to gene expression in case of cis-regulation in the hybrid. It was also observed that environment plays a greater role in allele-specific gene expression and thus heterosis, as compared to parent-of-origin effects.

**FIXATION OF HETEROSIS:**

In plants, hybrid vigour can be fixed in subsequent generations by the following methods:

1. **Vegetative propagation:**

Heterotic gene combinations can be maintained in further generations by following vegetative propagation of F1, where the progeny is passed with the exact genetic constitution as their parents due to occurrence of mitosis during the propagation.

1. **Apomixis:**

Apomixis leads to fixation of heterosis in crops as it does not change the genetic constitution and thereby heterotic gene combination as the seeds formed in this type of asexual reproduction are formed without fertilization leading to production of identical progeny as that of F1.

1. **Balanced lethal system:**

It is an approach of preferring permanent hybrids, where the dominant alleles of two traits each associated with a recessive lethal effect present in repulsion phase do not survive and only heterozygotes will survive.

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