Algerian People's Democratic Republic
Ministry of Higher Education and Scientific Research
Abdelhafid Boussouf University Center - Mila
Institute of Natural and Life Sciences
Department of Biotechnology

COURSE: BIODIVERSITY AND PLANT IMPROVEMENT

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For use by L3 « Biotechnology and Plant Improvement » students.

Objectives of Teaching

Methods of assessing and measuring biodiversity and strategies in plant improvement

Recommended Prerequisites:

- Plant Physiology and Propagation;
- Genetics;
- Genetic Basis of Biodiversity

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Abbreviations

1. **F2** : Second filial generation
2. **F3** : Third filial generation
3. **C(4, 2)** : Combination formula for calculating pairs
4. **FDis** : Functional Dispersion
5. **S.I** : Self-Incompatibility
6. **M.S** : Male Sterility
7. **SSD** : Single Seed Descent
8. **GMO** : Genetically Modified Organism
9. **NGS** : Next-Generation Sequencing
10. **SNP** : Single-Nucleotide Polymorphism
11. **PAVs** : Presence and Absence Variations
12. **CNVs** : Copy Number Variations
13. **CRISPR/Cas9** :Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR associated protein 9
14. *H'* : Shannon-Weaver index
15. S : total number of species (species richness)
16. i : a species in the study area
17. pi : proportion of species i relative to the total number of species S,
18. ni : number of individuals of species i
19. N = total number of individuals of all species (total abundance).

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CHAPTER I: BIODIVERSITY

INTRODUCTION

Biodiversity, a term derived from "biological diversity," encompasses all living beings and the ecosystems in which they evolve. It also includes the interactions within and between these systems. Essential for the maintenance of life on Earth, biodiversity provides numerous indispensable ecological services. This concept is fundamental for understanding and preserving the biological richness of our planet. It encompasses the diversity of living organisms, species, and ecosystems, and is crucial for ecosystem functioning and human well-being.

1. History of the Biodiversity Concept

The concept of biodiversity is relatively recent, having emerged in the 1980s. Before this period, scientists and ecologists primarily focused on the conservation of specific species and habitats. The term "biodiversity" was popularized to highlight the importance of biological diversity as a whole, in response to the rapid degradation of ecosystems caused by human activities such as deforestation, pollution, and urbanization.

A major turning point in the international recognition of biodiversity occurred at the Earth Summit in Rio de Janeiro in 1992, with the adoption of the Convention on Biological Diversity. This international treaty aimed to encourage nations to take measures to conserve biodiversity, use its components sustainably, and share the benefits arising from the use of genetic resources equitably.

2. Definition of Biodiversity

In 1984, Edward O. Wilson published "Biological Diversity," highlighting the concept of biological diversity for the first time. In 1992, biodiversity was defined as the "variability among living organisms from all sources including, inter alia, terrestrial, marine, and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species, and of ecosystems." Biodiversity includes all living species on Earth, their relationships with each other, as well as different genes, ecosystems, and species. Therefore, the concept of biodiversity encompasses all components and variations of the living world.

This broad definition, adopted by the Convention on Biological Diversity, encompasses three levels of diversity:

1. **Genetic diversity**: Variability of genes within a single species.
2. **Species diversity**: Variety of species in a given environment.

3. **Ecosystem diversity**: Variety of habitats and ecological processes.

3. The Three Levels of Biodiversity

3.1. Genetic Diversity (intra-species genetic diversity)

Genetic diversity refers to the variation of genes within a single species. This diversity is essential for the survival and adaptation of species, as it allows them to respond to environmental changes and resist diseases. For example, a plant population with high genetic diversity is more likely to contain individuals resistant to a new disease or extreme climatic conditions (figure 1).

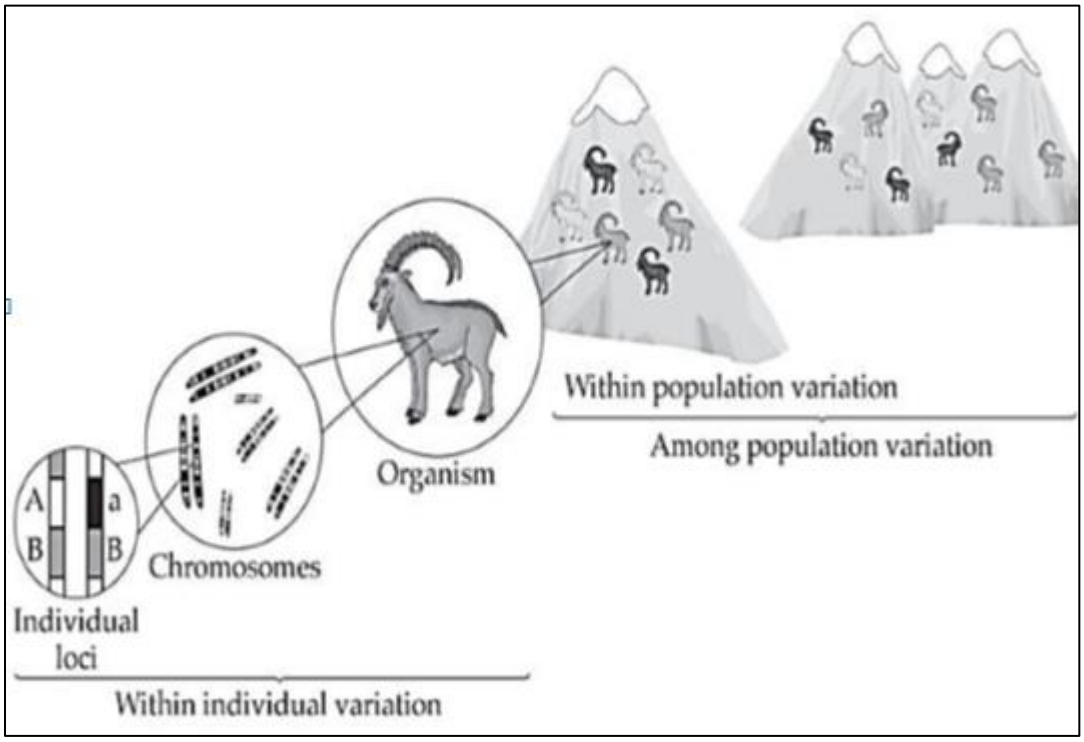

Figure 1 : Genetic diversity **(Gabriel Blouin-Demers, 2002)**

The importance of genetic diversity

- The raw material of evolution.
- Essential for species survival.
- The rate of evolutionary change within a group of organisms depends on the genetic variation present in the gene pool.
- To ensure the long-term persistence of species, genetic diversity is crucial.

3.2. Specific diversity (between species)

Specific diversity refers to the variety of species present in a given ecosystem. It is often measured by species richness, which corresponds to the number of different species in a habitat. High specific diversity indicates a healthy and resilient ecosystem capable of providing ecological services such as pollination, pest regulation, and water purification. For example, a tropical forest with a rich diversity of tree species, insects, and birds is more stable and productive than a pine monoculture (figure 2).

3.3. Ecosystem diversity

Ecosystem diversity concerns the variety of habitats, biological communities, and ecological processes within a landscape or region. Each ecosystem, whether it's a forest, desert, coral reef, or prairie, offers unique conditions that support different forms of life. This diversity ensures the maintenance of ecological functions such as nutrient cycling, climate regulation, and biodiversity conservation. For instance, wetlands filter water, forests store carbon, and coral reefs protect coastlines from erosion.

Numerous studies have focused on biodiversity's response to climate constraints and adaptation mechanisms to climate change. To envision biodiversity's evolution in the face of environmental changes, an overview is essential. A key point is that the speed of adaptive mechanisms must be compatible with the timescale of climate disturbances. For some organisms, rates of genetic mutations are known (figure 2)

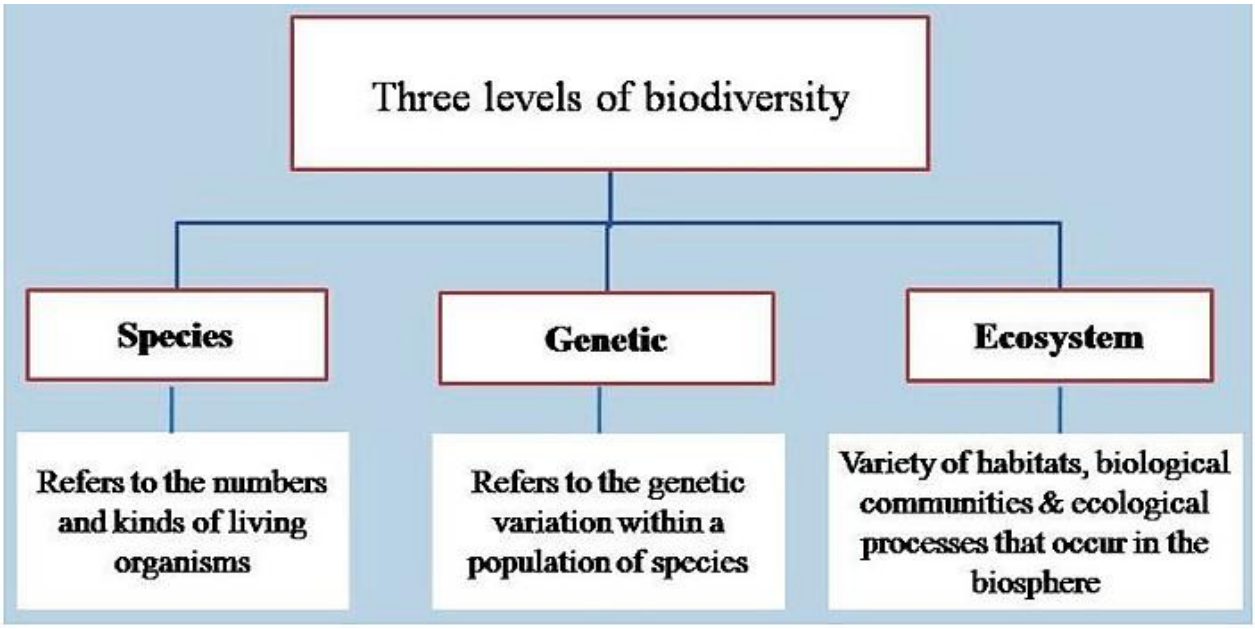

Figure 2 : The three levels of the biodiversity **(Chandrashekhar and *al*., 2022)**.

4. Economic (monetary) values of biodiversity

Biodiversity offers numerous direct and indirect economic values essential for human well-being and societal functioning.

✓ **Direct economic values**

1. **Food:** Biodiversity provides a variety of food resources. Plants, animals, and microorganisms form the basis of our diet. For example, fish caught in oceans and rivers, agricultural crops such as wheat, rice, fruits, and vegetables are all direct products of biodiversity.
2. **Medicines:** Many medicines are derived from biodiversity. Medicinal plants, fungi, and certain animals are used in modern pharmacology. For example, aspirin is derived from willow bark, and penicillin comes from a fungus.
3. **Materials:** Natural materials such as wood, fibers, and resins come directly from biodiversity. They are used in construction, textiles, and various industrial products.

✓ **Indirect economic values**

1. **Ecological services:** Ecosystems provide vital ecological services that support human activities. For example, forests purify air, wetlands filter water, and biodiverse soils enhance agricultural production.
2. **Crop pollination:** Insect pollinators, such as bees, play a crucial role in crop pollination. Without them, many crops would not reproduce effectively, leading to significant declines in agricultural yields.
3. **Climate regulation:** Ecosystems such as forests and oceans play an important role in climate regulation. For example, forests absorb carbon dioxide from the atmosphere, thereby helping mitigate the effects of climate change.

Figure 3 presents the different types of values recognized for biodiversity.

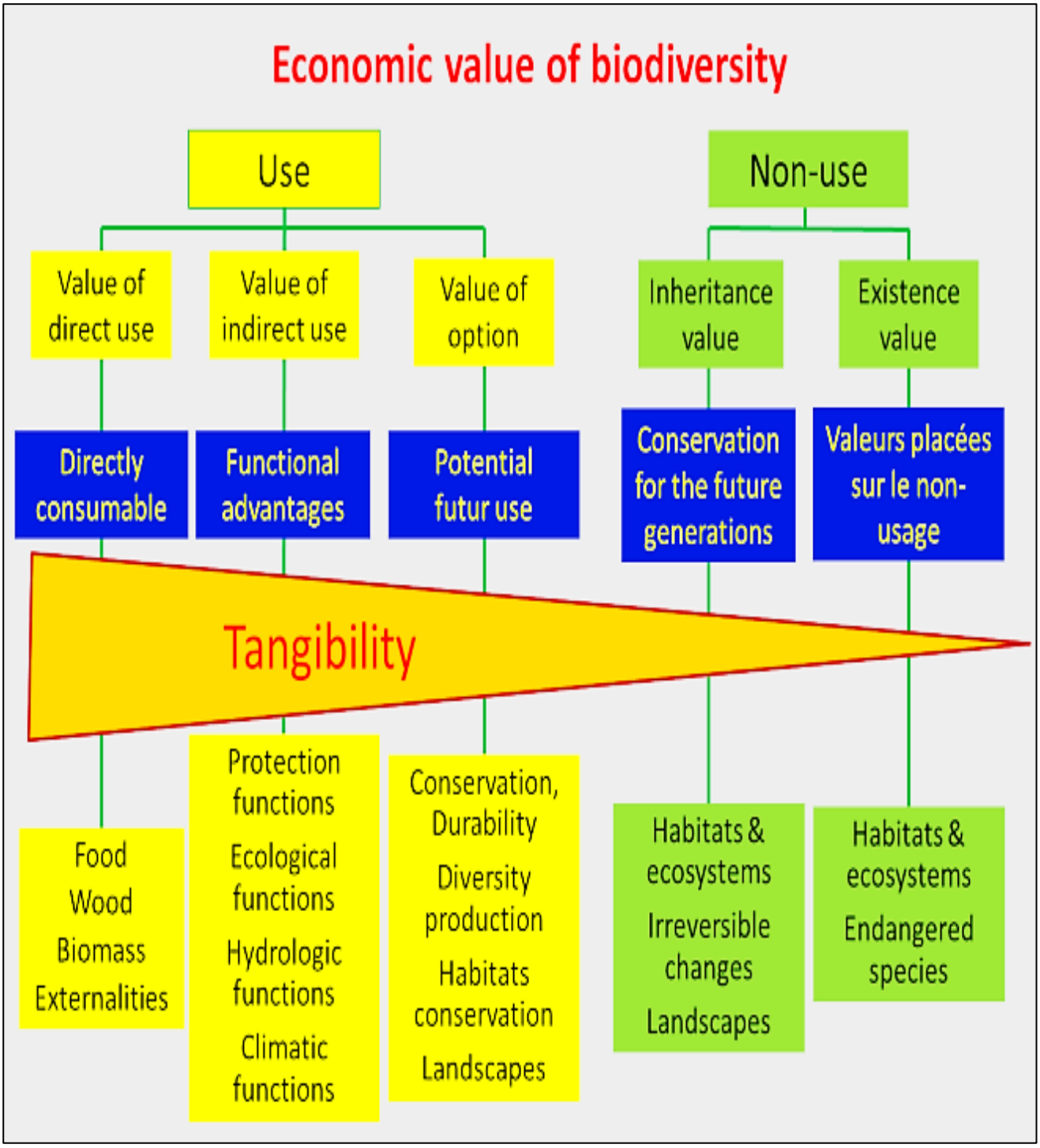

Figure 3 : The different types of values recognized for biodiversity **(Chevassus-au-Louis et *al.*, 2009).**

CHAPTER II: METHODS FOR EVALUATING AND MEASURING BIODIVERSITY

1. Biodiversity measures

1.1. Species richness (number of species)

Species richness is a straightforward indicator of biodiversity, corresponding to the total number of species present in a given ecosystem. This measure provides an initial estimate of the biological diversity of a habitat but does not take into account the relative abundance of species.

1.2. Species diversity

Species diversity considers not only the number of species but also their relative abundance. Diversity indices such as the Shannon index and the Simpson index are commonly used to quantify this diversity.

- **Shannon index:** This index measures diversity by considering both the number of species and the evenness of their distribution.
- **Simpson index:** This index assesses the probability that two randomly chosen individuals in a sample belong to the same species.

Example of the Shannon-Weaver index:

This is the most commonly used index for measuring diversity, taking into account both species richness (number of species) in a given environment and species evenness (relative abundance of individuals within each species) **(Marcon, 2015)**.

It is expressed as a positive real number ranging from 0 (indicating a single species or a dominant species that overwhelmingly predominates) to 5 (when all species have the same abundance, being co-dominant). A high value of H' indicates high diversity. A value close to $H' = 0.5$ is already considered very low. This index is calculated by the following formula:

$$H' = -\sum_{i=1}^{S} p_i (\log_2 p_i)$$

Where:

H' = Shannon-Weaver index

S = total number of species (species richness)

i = a species in the study area

pi = proportion of species i relative to the total number of species S, calculated by the formula pi = ni / N, where ni = number of individuals of species i; N = total number of individuals of all species (total abundance).

$$\text{Log}_2\, pi = \ln(pi) / \ln(2)$$

Example of calculating the Shannon-Weaver index

Step 1: Data collection

Let's imagine we have a community with the following species and their respective abundances:

- Species A: 10 individuals
- Species B: 20 individuals
- Species C: 30 individuals
- Species D: 40 individuals

Step 2: Calculating the proportion of each species

The proportion (p_i) of each species is calculated by dividing the number of individuals of that species by the total number of individuals.

Total number of individuals = 10 + 20 + 30 + 40 = 100

- p_A = 10 / 100 = 0.10
- p_B = 20 / 100 = 0.20
- p_C = 30 / 100 = 0.30
- p_D = 40 / 100 = 0.40

Step 3: Calculating p_i * ln(p_i) for each species

Using the proportion values to calculate p_i * ln(p_i). Here, ln represents the natural logarithm.

- p_A * ln(p_A) = 0.10 * ln(0.10) ≈ 0.10 * (-2.3026) ≈ -0.2303
- p_B * ln(p_B) = 0.20 * ln(0.20) ≈ 0.20 * (-1.6094) ≈ -0.3219
- p_C * ln(p_C) = 0.30 * ln(0.30) ≈ 0.30 * (-1.2039) ≈ -0.3612
- p_D * ln(p_D) = 0.40 * ln(0.40) ≈ 0.40 * (-0.9163) ≈ -0.3665

Step 4: Summing the values of p_i * ln(p_i)

Adding the values obtained in the previous step:

H = - (Σ (p_i * ln(p_i))) = - (-0.2303 + -0.3219 + -0.3612 + -0.3665)

H ≈ 1.28

Result

The Shannon-Weaver index for this community is approximately 1.28. The higher this index, the greater the diversity.

This demonstrates how the Shannon-Weaver index can be used to quantify species diversity within a biological community.

1.3. Spatial Diversity

Spatial diversity assesses the variation of biodiversity across different geographic regions. It can reveal diversity gradients, such as those observed from poles to equator, and identify areas of high endemism where a large number of unique species are present.

Example of Spatial Diversity Calculation

Field Data

Let's imagine we have two plots (A and B) and we have recorded the following species in each plot:

- Plot A: Species 1 (10 individuals), Species 2 (5 individuals), Species 3 (2 individuals)
- Plot B: Species 1 (8 individuals), Species 2 (7 individuals), Species 4 (3 individuals)

Step 1: Calculation of Alpha Diversity

Alpha diversity measures the diversity of species within a single plot. We will use the Shannon index for each plot.

Plot A

Proportions:

p1 = 10 / 17 ≈ 0.588

p2 = 5 / 17 ≈ 0.294

p3 = 2 / 17 ≈ 0.118

Shannon Index:

H_A = - (0.588 * ln(0.588) + 0.294 * ln(0.294) + 0.118 * ln(0.118))

H_A ≈ - (0.588 * -0.531 + 0.294 * -1.227 + 0.118 * -2.136)

H_A ≈ 0.312 + 0.361 + 0.252 ≈ 0.925

Plot B

Proportions:

p1 = 8 / 18 ≈ 0.444

p2 = 7 / 18 ≈ 0.389

p4 = 3 / 18 ≈ 0.167

Shannon Index:

H_B = - (0.444 * ln(0.444) + 0.389 * ln(0.389) + 0.167 * ln(0.167))

H_B ≈ - (0.444 * -0.813 + 0.389 * -0.945 + 0.167 * -1.794)

H_B ≈ 0.361 + 0.368 + 0.300 ≈ 1.029

Step 2: Calculation of Beta Diversity

- Beta diversity measures the difference in species diversity between plots. We will use the Sørensen index to compare plots A and B.
- Number of species common to both plots: Species 1, Species 2 (2 species) Total number of species in both plots: Species 1, Species 2, Species 3, Species 4 (4 species)

Sørensen Index:

$$\beta = \frac{2\times(\text{nombre d'espèces communes})}{(\text{nombre total d'espèces dans les parcelles A et B})}$$
$$\beta = \frac{2\times2}{4+4} = \frac{4}{8} = 0.5$$

Step 3: Calculation of Gamma Diversity

Gamma diversity is the total diversity of species across all combined plots. In our case, gamma diversity is simply the total number of unique species in both plots combined.

- Total unique species: Species 1, Species 2, Species 3, Species 4 (4 species)

Results

- Alpha diversity (Plot A): $H_A \approx 0.925$
- Alpha diversity (Plot B): $H_B \approx 1.029$
- Beta diversity: $\beta = 0.5$
- Gamma diversity: 4 species

This example demonstrates how to calculate spatial diversity using alpha, beta, and gamma diversity indices. Alpha diversity measures diversity within each plot, beta diversity measures the difference in diversity between plots, and gamma diversity measures the total diversity of species across all combined plots.

1.4. Taxonomic Diversity

Taxonomic diversity examines diversity across different taxonomic levels (e.g., family, genus, species). This approach helps understand the evolutionary distribution of species and identify taxonomic groups that contribute most to biodiversity.

Taxonomic diversity is a measure of species diversity that considers not only the number of species (species richness) but also the evolutionary relationships among these species. A common method to calculate taxonomic diversity is using the Clarke and Warwick taxonomic diversity index.

Example of Taxonomic Diversity Calculation

Step 1: Data Collection

Let's imagine we have a community with the following species and their respective abundances, along with their taxonomic classification:

- Species A (Group 1)
- Species B (Group 1)
- Species C (Group 2)

- Species D (Group 3)

Step 2: Construction of a Taxonomic Matrix

We construct a taxonomic matrix where each cell indicates the taxonomic distance between two species. For simplicity, we consider the following distances:

- Distance between two species within the same group: 1
- Distance between two species from different groups: 2

	Species A	**Species B**	**Species C**	**Species D**
Species A	0	1	2	2
Species B	1	0	2	2
Species C	2	2	0	2
Species D	2	2	2	0

Step 3: Calculation of Average Taxonomic Distances

For each pair of species, we calculate the average taxonomic distance.

Total number of pairs: C(4, 2) = 6 pairs

(Species A, Species B) = 1

(Species A, Species C) = 2

(Species A, Species D) = 2

(Species B, Species C) = 2

(Species B, Species D) = 2

(Species C, Species D) = 2

Step 4: Calculation of Taxonomic Diversity

We calculate taxonomic diversity (Δ) using the following formula:

$$\Delta = \frac{\sum d_{ij}}{n(n-1)/2}$$

where *dij* is the taxonomic distance between species *i* and *j*, and n is the number of species.

$\Delta = 1 + 2 + 2 + 2 + 2 + 2 / 6 = 11 / 6 \approx 1.83$

Result

The taxonomic diversity of this community is approximately 2. A higher value indicates greater taxonomic diversity, reflecting greater diversity in the evolutionary relationships among the species present in the community.

This simplified example demonstrates how to calculate taxonomic diversity using distances between species based on their taxonomic classification.

1.5. Functional Diversity

Functional diversity refers to the variety of biological traits that influence ecosystem functioning. It is essential for understanding the role of species in ecological processes such as pollination, decomposition, and nutrient cycling.

Example of calculating functional diversity

Step 1: Data collection

Let's imagine we have a community with the following species and their functional traits (e.g., plant height, specific leaf area, growth rate):

Species	Height (cm)	Specific Leaf Area (cm^2/g)	Growth Rate (g/day)
Species A	10	5	0.2
Species B	20	4	0.3
Species C	15	6	0.25
Species D	25	3	0.4

Step 2: Calculation of the functional distance matrix

We calculate the distance between each pair of species using their functional traits. A common method for this is Gower's distance, which allows for the consideration of traits with different units of measurement.

Gower's distance for a pair of species (*i*, *j*) is given by:

$$D_{ij} = \frac{1}{T} \sum_{k=1}^{T} w_k d_{ijk}$$

where:

- *T* is the total number of traits.
- *wk* is the weight of trait k (often equal to 1 if all traits are equally important).
- *dijk* is the normalized difference for trait k between species *i* and *j*.

To simplify, we will use simple unnormalized differences without weighting.

Calculating distances:

	Species A	**Species B**	**Species C**	**Species D**
Species A	0	$\sqrt{((20-10)^2 + (4-5)^2 + (0.3-0.2)^2)} \approx 10.02$	$\sqrt{((15-10)^2 + (6-5)^2 + (0.25-0.2)^2)} \approx 5.02$	$\sqrt{((25-10)^2 + (3-5)^2 + (0.4-0.2)^2)} \approx 15.03$
Species B	10.02	0	$\sqrt{((15-20)^2 + (6-4)^2 + (0.25-0.3)^2)} \approx 5.04$	$\sqrt{((25-20)^2 + (3-4)^2 + (0.4-0.3)^2)} \approx 5.1$
Species C	5.02	5.04	0	$\sqrt{((25-15)^2 + (3-6)^2 + (0.4-0.25)^2)} \approx 10.04$
Species D	15.03	5.1	10.04	0

Step 3: Calculating Functional Dispersion (FDis)

Functional dispersion (FDis) is calculated as the weighted mean of species distances to the community mean trait value.

1. **Calculating the mean trait values of the community:**

Mean height = (10 + 20 + 15 + 25) / 4 = 17.5 cm

Mean specific leaf area = (5 + 4 + 6 + 3) / 4 = 4.5 cm^2/g

Mean growth rate = (0.2 + 0.3 + 0.25 + 0.4) / 4 = 0.2875 g/day

2. **Calculating species distances to the mean trait values:**

Distance of Species A = $\sqrt{((10-17.5)^2 + (5-4.5)^2 + (0.2-0.2875)^2)} \approx 7.53$

Distance of Species B = $\sqrt{((20-17.5)^2 + (4-4.5)^2 + (0.3-0.2875)^2)} \approx 2.53$

Distance of Species C = $\sqrt{((15-17.5)^2 + (6-4.5)^2 + (0.25-0.2875)^2)} \approx 2.06$

Distance of Species D = $\sqrt{((25-17.5)^2 + (3-4.5)^2 + (0.4-0.2875)^2)} \approx 8.02$

3. **Calculating the weighted mean of distances:**

FDis = (7.53 + 2.53 + 2.06 + 8.02) / 4 ≈ 5.03

Result

The functional dispersion (FDis) of this community is approximately 5.03. A higher FDis value indicates greater functional diversity in the community, meaning that the functional traits of species are more diverse.

This example demonstrates how to calculate functional diversity using functional dispersion (FDis), considering the functional traits of species and their differences.

2. Difficultés de mesure de la biodiversité

Measuring biodiversity poses significant challenges, primarily due to the immense variety of life forms and the spatial and temporal scales involved. The main difficulties include:

- Sampling techniques: Selecting appropriate sampling methods for different habitats and taxonomic groups is complex and can introduce biases.

- Identification errors: Species identification can be challenging, especially for poorly known or cryptic taxonomic groups.
- Seasonal variations: Biodiversity can fluctuate seasonally, complicating the acquisition of representative measurements.

3. Les bioindicateurs de biodiversité

3.1. Les bioindicateurs d'accumulation

Accumulation bioindicators are organisms that accumulate specific substances in measurable quantities, reflecting the presence of pollutants or other environmental factors. For example, certain mollusk species accumulate heavy metals, indicating water pollution.

3.2. Les bioindicateurs d'effet ou d'impact

Effect or impact bioindicators are species or groups of species whose changes in abundance, diversity, or behavior indicate alterations in the ecosystem. For example, a decrease in amphibian populations may signal deteriorating water quality or habitat.

CHAPTER III: PLANT BREEDING STRATEGIES

Introduction

Genetic improvement of plants is an essential discipline in agronomy and biology aimed at developing new plant varieties that are more productive, resistant, and adapted to human needs. This science combines traditional breeding techniques with modern biotechnology approaches.

1. Definition of Plant Genetic Improvement

Plant genetic improvement involves manipulating the genetic characteristics of plants to enhance their agricultural performance. This can include increasing yields, disease resistance, tolerance to adverse environmental conditions, and improving nutritional qualities.

2. Objectives of Plant Breeding

The main objectives of plant breeding are:

- Increase crop yields: Develop varieties that produce more grains, fruits, or biomass per unit area.
- Improve nutritional quality of products: Enrich crops with vitamins, minerals, and other essential nutrients.
- Develop resistance to diseases and pests: Create varieties resistant to specific pathogens to reduce crop losses.
- Enhance tolerance to abiotic stresses: Select plants capable of surviving and producing under conditions of drought, salinity, cold, or extreme heat.
- Reduce dependency on chemical inputs: Breed varieties that require less fertilizer and pesticides, promoting more sustainable agriculture.

3. Role of the Plant Breeder

The plant breeder plays a crucial role by:

- Identifying traits of interest: Observing and characterizing desired traits in plants.
- Selecting suitable parents for crossing: Choosing plants with complementary characteristics for breeding.
- Evaluating the performance of resulting lines: Testing and selecting the best lines for desired traits. Their work involves a balance of science, art, and practical experience.

4. Domestication

Domestication is the process by which wild plants have been transformed into agricultural crops through human selection. This process involved selecting favorable traits such as seed size, reduced seed dormancy, and disease resistance.

- Some examples of domestication:

✓ **Domestication of plants:**

- **Wheat**: One of the first cultivated plants, domesticated for food production.
- **Maize**: Native to Central America, domesticated for its edible grains.
- **Rice**: Mainly cultivated in Asia, domesticated for its grains.
- **Potato**: Native to the Andes, domesticated for its edible tubers.

✓ **Domestication of fruits:**

- **Apple**: Native to Central Asia, domesticated for its edible fruits.
- **Banana**: Native to the Indo-Malaysian region, domesticated for its nutrient-rich fruits.
- **Grapes**: Cultivated for fresh consumption, wine, and raisins.

✓ **Domestication of ornamental plants:**

- **Tulip**: Domesticated for its colorful flowers.
- **Rose**: Cultivated for its fragrant and aesthetic flowers.

These examples show how humans have selected and cultivated certain species to meet various food, utilitarian, and aesthetic needs.

5. Plant Reproduction Modes

Plant reproduction is the process by which plants produce new individuals or offspring. It is an inherent characteristic of all living organisms to continue or maintain their races by the mechanism of reproduction.

The following figure shows reproduction in plants.

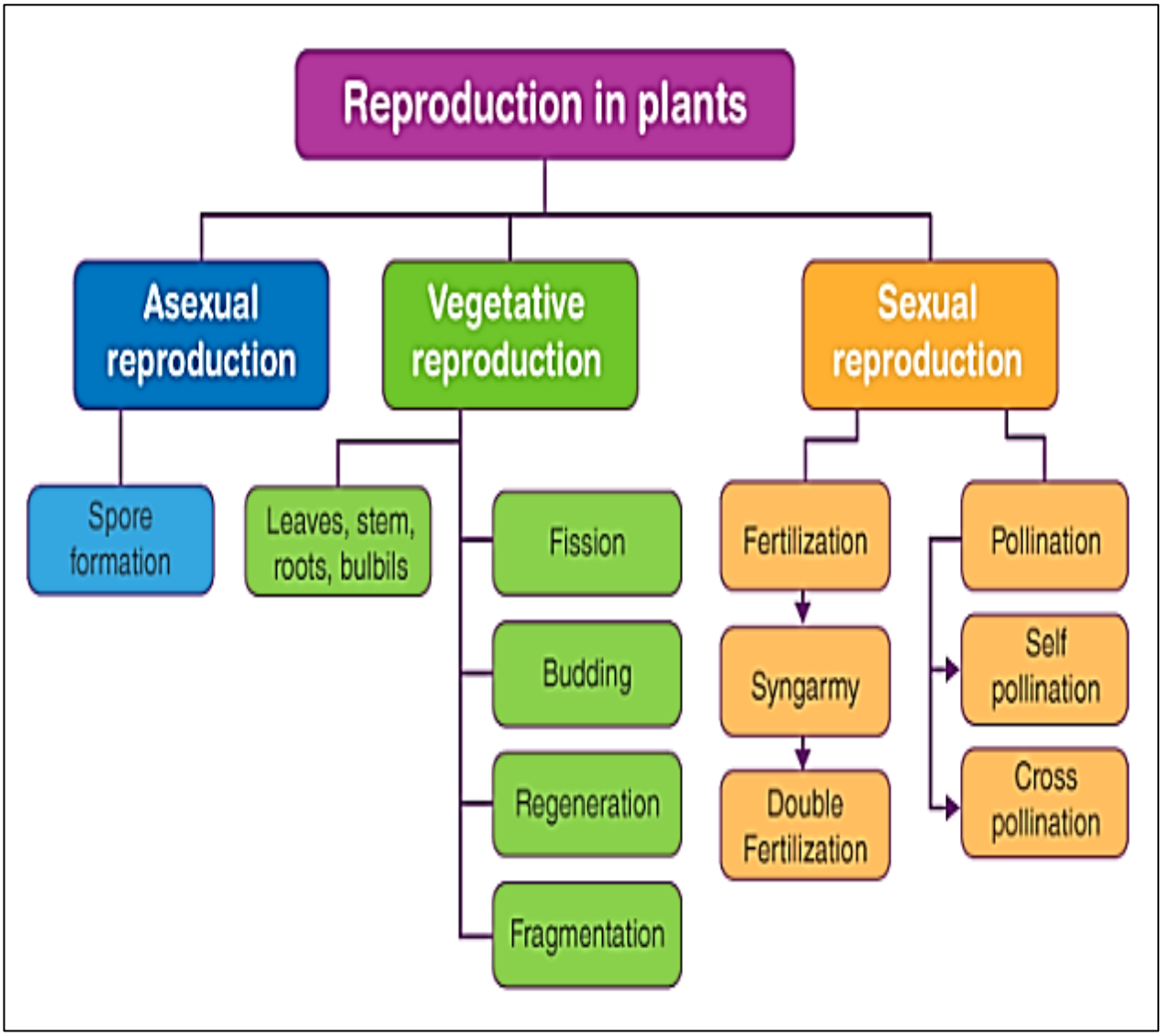

Figure 4 : Reproduction in plants **(Subramanian, 2023)**

5.1. Autogamy

Autogamy is self-pollination, where pollen from a flower fertilizes the ovule of the same flower, resulting in high genetic uniformity **(figure 5)**.

5.2. Allogamy

Allogamy is cross-pollination, where pollen from one plant fertilizes the ovule of another plant. This method promotes genetic diversity and variability within populations **(figure 5)**.

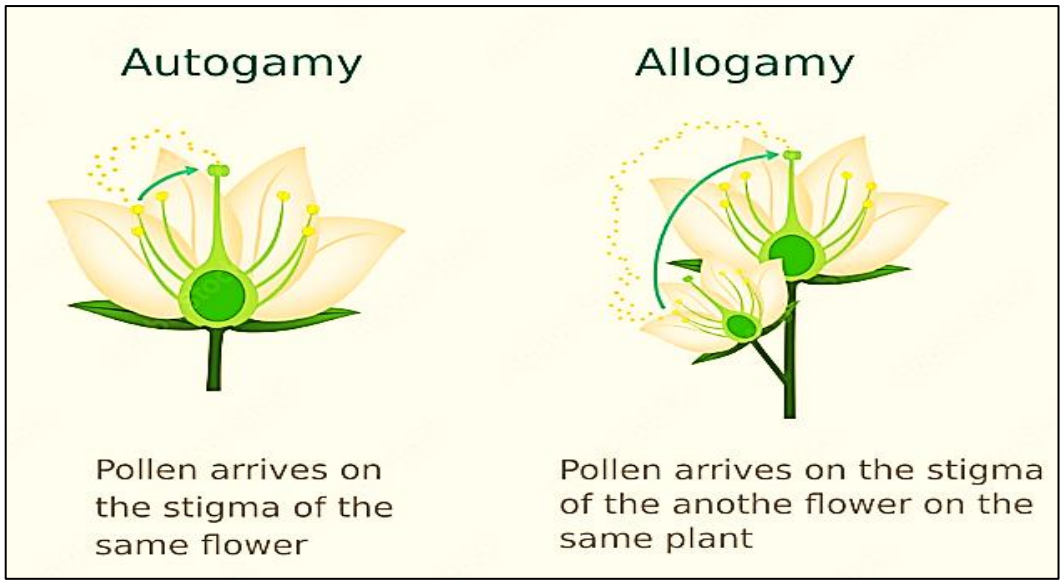

Figure 5 : Pollination of flower, allogamy and autogamy

(https://as2.ftcdn.net/v2/jpg/05/60/27/25/1000_F_560272500_Yx3TJeTBprxuFUs6flxfpRsXGLPTpUP5.jpg.)

5.3. Self-Incompatibility and Male Sterility

Self-incompatibility is a genetic mechanism that prevents self-pollination and promotes genetic diversity. First discussion on self- incompatibility by Darwin (1877), the term self-incompatibility was given by Stout (1917), Bateman (1952, 1954, 1955) gave explanation on incompatibility in three *Brassicas* plants namely, *Iberis amara* L., *Raphanus sativus* L. and *Brassica campestris* L.

Male sterility is a condition where plants do not produce viable pollen, used in hybrid seed production.

The table 1 can clarify the difference between self-incompatibility and male sterility.

Table 1: Self-incompatibility and male sterility differences **(Padhan et Manas, 2014)**.

Self-incompatibility	Male sterility
▪ No seed set in self-pollination but possible in cross pollination.	▪ Un- functional pollen failure to set seed both in self and cross pollination.
▪ Self-Incompatibility utilized during hybrid seed production.	▪ Male sterility utilized during hybridization programme of breeding.
▪ S.I is less used because most of crop has no S.I.	▪ M.S can used more.

CHAPTER IV: POLYGENIC INHERITANCE

1. Genetic Variation

1.1. Nature of Genetic Variation

Genetic variation forms the basis of plant evolution and improvement. It can be caused by mutations, genetic recombinations, and gene flow between populations. Mutations are changes in DNA sequence that can introduce new traits. Genetic recombinations occur during sexual reproduction, mixing parental genes to create genetic diversity. Gene flow between populations, also known as genetic migration, can introduce new genes into a population.

2. Gene Action Modes

2.1. Intra-locus Interaction

Intra-locus interaction involves interactions between alleles of the same gene, including:

- **Dominance** : A dominant allele masks the expression of a recessive allele.
- **Co-dominance** : Both alleles are expressed equally.
- **Incomplete dominance** : The hybrid displays an intermediate phenotype between the two parents.

2.2. Inter-locus Interaction

Inter-locus interaction refers to interactions between genes located at different loci, including:

- **Epistasis :** One gene masks the expression of another gene.
- **Penetrance :** The proportion of individuals carrying a particular genotype that express the expected phenotype.
- **Expressivity :** The extent to which a gene is expressed in an individual's phenotype.

CHAPTER V: INBREEDING AND HETEROSIS

1. Inbreeding

Inbreeding refers to reproduction between closely related individuals within the same population. This phenomenon has significant effects on genetic diversity and population structure.

1.1. Effects of Inbreeding

Inbreeding leads to increased homozygosity within the population. This means individuals become more genetically similar as they share more identical traits through common ancestry, resulting in decreased genetic diversity.

1.2. Consequences of Inbreeding

Effects Consequences of inbreeding include several negative aspects for the population:

- Reduced vigor and fertility: Individuals from inbreeding often have fragile health and reduced fertility.
- Increased recessive genetic diseases: The likelihood of expressing recessive genetic diseases increases as homozygous individuals for these traits become more frequent.
- Decreased ability to adapt to environmental changes: With less genetic diversity, the population's ability to adapt to new environments or biotic stresses is reduced.

1.3. Effects of Inbreeding on Genotypic Frequencies

Inbreeding alters genotypic frequencies by promoting an increase in homozygous individuals and a decrease in heterozygous individuals within the population. This results in a less varied genetic distribution more concentrated in specific genotypes.

2. Heterosis (Hybrid Vigor)

Heterosis, also known as hybrid vigor, refers to the superiority of hybrids over their genetic parents in terms of several important characteristics.

2.1. Definitions

Heterosis occurs when purebred (homozygous) lines are crossed to produce heterozygous hybrids. These hybrids may exhibit improved growth, increased vigor, enhanced fertility, and often higher yields compared to their parents.

Heterosis is manifested by increased vigor in the offspring resulting from the crossbreeding of two genetically distant individuals. More specifically, it refers to the superiority of the hybrid compared to the better parent. Conversely, it leads to a decrease in vigor in the offspring resulting from crosses between related individuals.

This universal biological phenomenon in higher organisms reflects the advantage of genomic heterozygosity. Although the main hypotheses to explain this advantage, namely dominance and overdominance, were formulated at the beginning of the 20th century, it remains difficult today to precisely determine the relative importance of these two mechanisms. Heterosis is sometimes considered a mysterious phenomenon.

- **History of Heterosis concept :** Table 2 summarizes the history of Heterosis concept.

Table 2: History of Heterosis concept (**Chethana CK., 2024)**.

Pre- Mendelian		
1766	**Kolreuter**	- Hybrid vigour in Nicotiana
1799	**Knight A.**	- Principe of anti-inbreeding
1865	**Mendel**	- Hybrid vigour in peas
1877	**Darwin**	- Cross fertilization is beneficial
1880	**Beal**	- First published report of 51% increase in yied over parents
1891	**Johnson**	- Crossing gave better off-springs
1892	**Mc Cleur**	- Inbreeding imparted sterility Crossing imparted vigour

2.2. Explanation of Heterosis

Mechanisms of heterosis mainly include dominance, where one allele masks the expression of another, overdominance, where heterozygotes perform better than homozygotes for both alleles, and epistatic interactions, where the effect of one allele depends on the presence of other specific alleles.

3. Utilization of Heterosis

Heterosis is widely exploited in agriculture to develop plant and animal hybrids that combine the best traits of their parents. This includes the production of hybrid seeds aimed at increasing crop yields, disease resistance, and environmental robustness of crops.

Molecular Foundations of Heterosis

The observed greater vigor in the heterotic phenotype compared to its inbred parental lines results from the cumulative effect of genetic information processing through transcription, translation, and their regulatory mechanisms. With the advent of next-generation sequencing (NGS) technologies, genome-wide analyses of single-nucleotide polymorphisms (SNPs), insertions or deletions (indels), large structural variations such as presence and absence variations (PAVs), and copy number variations (CNVs) that contribute to phenotypic diversity have become more accessible.

To uncover the underlying mechanisms determining the degree of vigor differences between inbreds and hybrids, advanced molecular studies have been conducted to assess their transcriptomes, proteomes, epigenomes, and other regulatory mechanisms. Figure 6 provides an overview of the molecular foundations of heterosis.

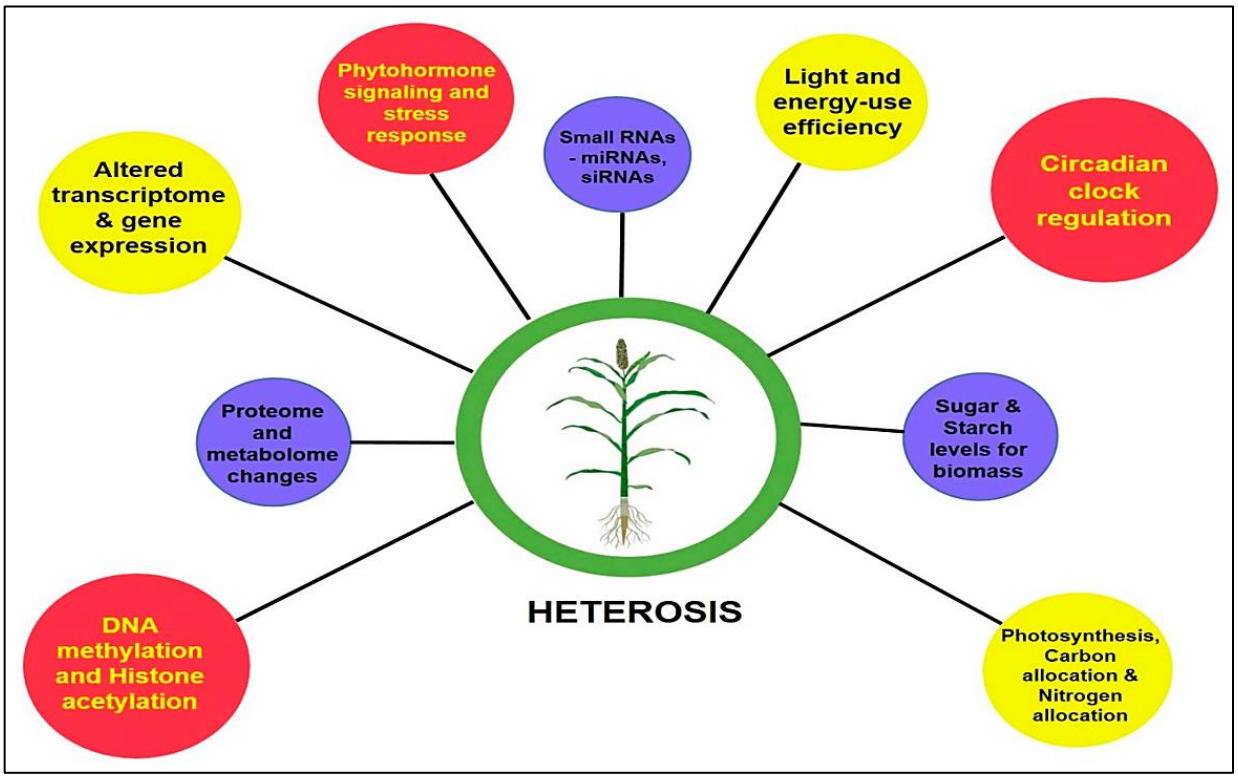

Figure 6 : Molecular changes involved in manifesting heterosis **(Rajendrakumar and *al*., 2015).**

CHAPTER VI: METHODS OF GENERATING VARIABILITY

1. Creation of Varieties through Directed Intraspecific

Crosses directed intraspecific crosses involve crossing different varieties or lines within the same species to introduce new combinations of beneficial genetic traits for plant improvement.

- **Intraspecifique variation is caused by genetic and environment factors:**

Intraspecific variation (variation that accurs within a species) can be caused by a number of environmental and genetic factors.

- **Genetic:** all members of the same species have the same genes, however they have different alleles cause variations in an organism's phenotype (physical appearance). Exemple of variation caused by genetic factors include eye color and blood group. Genetic variation is inherited, as certain genetic factors can be passed on in families.
- **Environmental:** the phenotype of an organism can also be affected by environment factors. For example, plant growth is affected in the soil. Another example is Himalayan rabbits. These rabbits are affected by temperature. Their fur turns black when temperatures reach below 25 degrees.

1. Creation of Varieties through Directed Interspecific Crosses

Directed interspecific crosses involve crossing two different species to transfer specific resistance or tolerance traits from a wild species to a cultivated species.

The following table summarizes the differences between interspecific and intergeneric hybridization.

Table 3: Differences between interspecific and intergeneric hybridization **(Gauravrajsinh, 2019)**

Particular	Interspecific Hybridization	Intergeneric Hybridization
Parents involved	Involves two different species of the same gene.	Involves two different genera of the same family.
Fertility	Such hybrids vary from completely fertile to completely sterile	Hybrids are always sterile.
Seed Setting	More than intergeneric crosses.	Less than interspecific crosses.
Use in crop improvement	More than intergeneric crosses.	Less than interspecific crosses.
Release of Hybrid Variety	Possible in some crops.	Not Possible.
Evolution of new crops	Not possible, but evolution of new species is sometimes possible.	Sometime possible, e.g., Triticale.

2. Creation of Varieties through Mutagenesis

Mutagenesis is a technique that uses chemical or physical agents to induce random mutations in the plant genome, generating additional genetic diversity for selection purposes.

- They may directly damage DNA through their actions, often resulting in replication errors.
- Mutations occur randomly and are not directed by the organism's needs. Most mutations are caused by environmental factors, but they can also be induced in the lab using chemicals, radiation, or physical stimuli.
- These mutagens can be carcinogenic, as numerous mutations may lead to cancer in animals, although this is not always the case.
- All mutagens exhibit distinctive mutational signatures and some chemicals can become mutagenic through cellular processes.

Mutagens Types

Mutagens can be classified into three major types based on their origin. They are: Physical mutagen, Chemical mutagen and Biological mutagen (Figure 7).

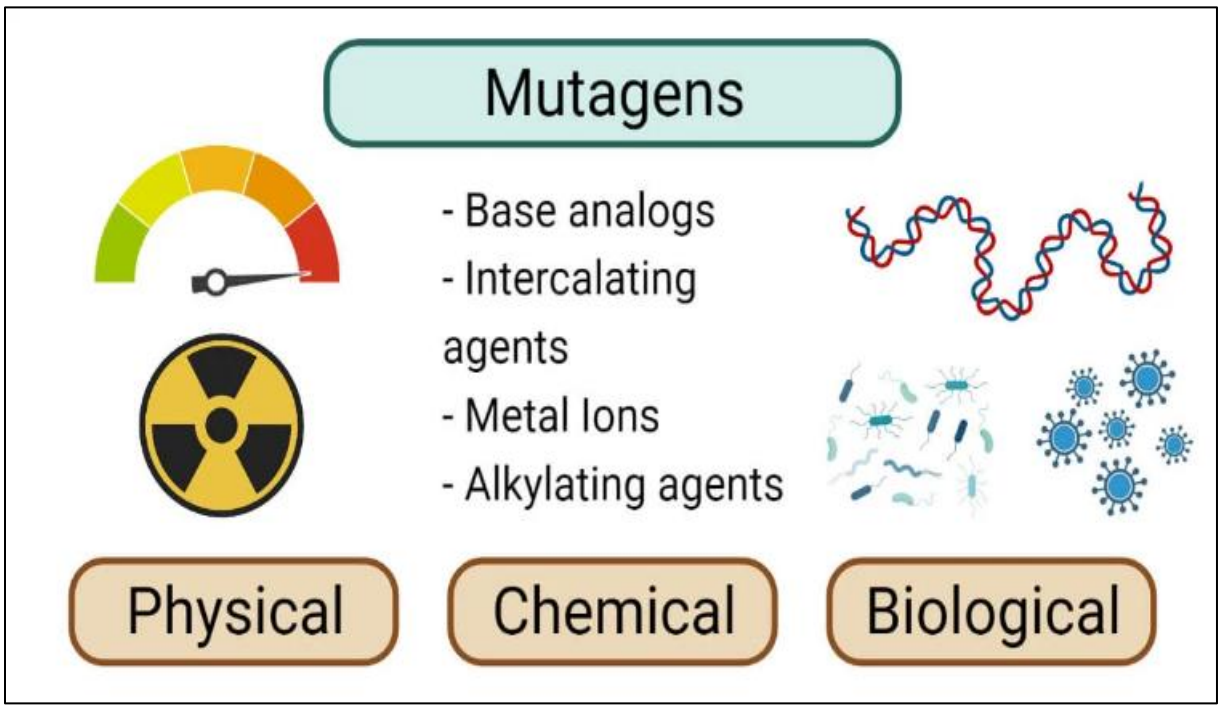

Figure 7 : Molecular changes involved in manifesting heterosis **(Sagar, 2023).**

a. Creation of Varieties through Protoplast Fusion (Somatic Hybridization)

Protoplast fusion is a method that combines genetic material from two distinct plant cells to create new genetic variations without relying on sexual reproduction.

• Two genetically different protoplast isolated from the somatic cells and areexperimentally fused to obtain parasexual hybrid protoplasts.

• The technique of fusion of isolated protoplasts from somatic cells andregeneration of hybrid plants from the fusion products, called somaticHybridization

• Somatic hybridization allows combining genomes of two desirable parents, irrespective of their taxonomic relationship.

• Somatic hybridization has a unique potential to combine both nuclear andcytoplasmic genes simultaneously unlike sexual hybridization or geneticengineering.

b. Creation of Varieties through Transgenesis

Transgenesis involves introducing foreign genes into a plant genome to confer specific traits such as herbicide or insect resistance.

Types

- **Transgenic plants** have genes inserted into them from other species. The inserted genes can come from species within the same kingdom (plant to plant) or between kingdoms (bacteria to plant). Often, the inserted DNA must be slightly modified to express correctly and efficiently in the host organism. Transgenic plants are used to express proteins, such as the cry toxins from *Bacillus thuringiensis*, herbicide resistance genes, and antigens for vaccinations.
- **Cisgenic plants** are created using genes from the same species or a closely related one, where conventional plant breeding can occur. Some breeders and scientists argue that cisgenic modification is useful for plants that are difficult to crossbreed by conventional means (such as potatoes). They also contend that cisgenic plants should not require the same level of legal regulation as other genetically modified organisms.

Advantages of Transgenic Plants

GM technology has been used to produce a variety of crop plants. As the global population continues to expand, food remains a scarce resource. Genetically engineered foods offer significant benefits by improving production yields, lowering transportation costs, and enhancing nutritional content. Developments in countries such as the USA and Canada have focused on conferring resistance to insects, pests, or viruses and producing tolerance to specific herbicides. While these traits benefit farmers, consumers often struggle to see any direct benefits other than reduced prices due to lower production costs. Several GM crops aimed at combating malnutrition are expected to be introduced for cultivation in the next five to ten years.

c. Creation of Varieties through Somatic modifications

Somatic modifications include tissue culture techniques and the use of genetic editing (CRISPR/Cas9) to introduce targeted changes into the plant genome, thereby creating new varieties.

CHAPTER VII: SELECTION METHODS

1. Selection

Selection in plant improvement involves identifying and breeding individuals with desirable traits to enhance crop performance.

Selection objectives

The objectives of selection are numerous. Generally, the primary criterion mentioned is productivity, which depends on many factors. This can result from reducing yield-limiting factors. However, productivity potential can also be enhanced by optimizing plant physiology: increasing photosynthetic activity, improving growth, enhancing the migration and distribution of assimilates, and/or mobilizing the plant's reserves (grains, roots, etc.).

Plant species also exhibit varying degrees of plasticity. Some plants, such as wheat, require significant varietal adaptation to soil and climate conditions. For farmers, one of the most important factors is resistance to diseases and pests. This not only affects yield but also the farmer's income. Indeed, other solutions, such as the use of treatment products, can increase the operational costs of the crop.

For a long time, selection has taken into account the qualitative needs and industrial constraints of processors. Important selection factors include the intrinsic quality of the harvest, its sanitary condition, the homogeneity of batches, their suitability for storage, as well as the technological qualities for processing and uses (bakery, biscuit making, crushing, etc.). These criteria are diverse and codified in specifications.

Expectations for agriculture are increasing and diversifying: greater respect for the environment, combating climate change, improved nutritional quality, production of molecules, etc. Research is already incorporating these new criteria to enable, for example, the production of energy via biomass, the creation of non-food substances such as specific oils for industry, and even the manufacture of vaccines or medications.

1.1. Selection in Heterogeneous Population

This selection method aims to choose the best individuals from a genetically diverse population, thus exploiting natural variation to improve plant characteristics.

a) Definition and Context

- **Genetic Diversity**: Heterogeneous populations encompass individuals with diverse genetic backgrounds and phenotypic traits.

- **Selection Objective**: The aim is to identify and breed individuals with desirable traits or combinations thereof to enhance population fitness and productivity.

b) Challenges and Considerations

- **Trait Variation**: Managing variability in traits across a heterogeneous population poses challenges in trait identification and selection.
- **Genetic Drift**: Small subpopulations within a heterogeneous group may experience genetic drift, impacting allele frequencies over time.
- **Adaptation**: Selection strategies often focus on enhancing adaptation to specific environmental conditions or improving overall resilience.

c) Methods and Techniques

- **Phenotypic Selection**: Traditional methods rely on observable traits related to yield, disease resistance, and other agronomic traits.
- **Genomic Selection**: Modern approaches integrate genomic data to identify markers associated with desired traits, improving breeding efficiency.
- **Hybridization and Introgression**: Utilizing crossbreeding to harness hybrid vigor (heterosis) and introduce novel genetic variation for targeted improvement.

d) Applications

- **Crop Improvement**: Crucial for developing cultivars with enhanced yield, quality, and resilience to biotic and abiotic stresses.
- **Livestock Breeding**: Applies to breeding programs aimed at improving productivity, disease resistance, and other economically important traits in livestock.
- **Conservation Genetics**: Important for maintaining genetic diversity and adaptive potential in endangered species and wild populations.

e) Future Directions

- **Precision Breeding**: Advances in genomic technologies and bioinformatics are expected to revolutionize selection strategies, enhancing precision and accelerating genetic gain.

- **Climate Adaptation**: Given climate change challenges, there is growing emphasis on selecting for traits that confer resilience and adaptation to changing environmental conditions.

2. Selection after Hybridization

After crossing two genetically distinct parents to create a new hybrid population, the most promising individuals are selected to continue the genetic improvement program.

1. **Definition and Context**
    - **Hybridization**: The crossing of genetically distinct individuals or species to combine desirable traits.
    - **Selection Objective**: Refers to the process of selecting and breeding hybrid offspring that exhibit superior traits for further cultivation or breeding programs.
2. **Challenges and Considerations**
    - **Heterosis**: The phenomenon where hybrids exhibit enhanced vigor or other desirable traits compared to their parents.
    - **Trait Expression**: Ensuring stable and consistent expression of desired traits across hybrid generations.
    - **Genetic Stability**: Maintaining genetic integrity and stability over successive generations of hybrids.
3. **Methods and Techniques**
    - **Phenotypic Selection**: Traditional method involving observation and selection based on phenotypic traits such as yield, disease resistance, and quality characteristics.
    - **Molecular Selection**: Utilization of molecular markers to facilitate more precise and efficient selection of desired traits.
    - **Genomic Selection**: Application of genomic technologies to predict breeding values and select individuals based on their genetic potential.

4. **Applications**
    - **Crop Improvement**: Critical for developing hybrid varieties with improved yield, stress tolerance, and quality traits in major crops.
    - **Livestock Breeding**: Important for enhancing productivity, disease resistance, and other economically valuable traits in livestock species.
    - **Conservation and Restoration**: Used to reintroduce and improve genetic diversity in endangered or threatened species through hybridization and selective breeding.
5. **Future Directions**
    - **Precision Breeding**: Continued advancements in genomics and biotechnology are expected to enhance the precision and speed of selection after hybridization.
    - **Environmental Adaptation**: Focus on selecting hybrids with traits that confer resilience and adaptation to changing environmental conditions, including climate change.

3. Selection Methods

3.1. Pedigree Selection

Pedigree selection involves tracking plant pedigrees over multiple generations to identify and use the best genetic combinations in breeding programs.

In the pedigree method, selection begins in F2. This allows for the rapid isolation of desirable traits in the case of qualitative inherited characteristics such as disease resistance, seed color, etc. Quantitative traits, especially yield, are more challenging to evaluate during the early generations (F2 and F3) based on individual plants.

3.2. Selection by Single-Seed-Descent (SSD) Method

This method involves growing a single seed from each selected plant over multiple successive generations, effectively fixing desired traits.

This approach is also known as single seed descent (SSD). Each F2 plant contributes with a single seed to the F3 generation, each F3 plant contributes with a single seed to the F4 generation, and so on. This method is simple and cost-effective. It only requires harvesting

one seed per plant and sowing them collectively in the next generation. Selection using this method is particularly convenient when multiple generations can be obtained per year. The use of off-season greenhouses helps expedite the generation development process.

3.3. Backcross

Backcrossing is used to introduce specific traits from one parent into an elite line while retaining the desired genetic background, particularly useful for improving traits while maintaining overall plant performance.

The Backcross is typically used when a variety with desirable traits has a weakness, such as susceptibility to a specific disease, which can be corrected by introducing one or a few specific genes. Its principle involves gradually eliminating all genes from a donor parent, except for the one that confers resistance to a specific disease, in order to integrate it into the desired variety.

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