**Modern problems of plant genetics.**

**Practical work 1**

**High-quality reference genome sequences.**

High-quality reference genome sequences are the prerequisite and basis for promoting fundamental and applied research in plants. Triggered by developments in computing power, sequencing technologies, and assembly methods, the genomes of more than 700 plants species, from non-vascular to flowering, have been released in the past 20 years (Sun et al., 2021). Third-generation sequencing technologies, such as those that use the PacBio and Oxford Nanopore platforms, can generate reads with significantly increased lengths and they have been widely applied along with well established assembly algorithms to construct large and complicated plant genomes at unprecedented high resolution (Koren et al., 2017; Cheng et al., 2021 ; Niu et al., 2022).

***Control questions:***

1. Main problems of plant genetics.

**Practical work 2**

**Major achievements in plant pan-genomics.**

Sequencing the genome of an organism is an important stage in its genetic research. Decoding the genomic sequence opens up wide opportunities for studying the structure of chromosomes, the distribution of repeated and coding sequences, and the identification and annotation of genes. When studying agricultural plants, this makes it possible to analyze the functions of genes and develop markers to search for associations with phenotypic traits. When solving these problems, the genome of a species is often represented by the sequence of one organism (the so-called reference genome). Recently, however, there is increasing evidence that large structural genomic variations, including gene copy number variations and gene presence/absence variations, are prevalent in crops, play a key role in the genetic determination of agronomically important traits, and lead to significant variation. functional set of genes and gene composition in representatives of the same species. Such structural variations cannot be represented on the basis of a reference sequence alone and are described based on the pangenome concept. *A pangenome is information about the complete set of genes of a taxon, among which one can distinguish a set of universal genes common to all representatives of the taxon and variable genes that are partially or completely specific to its representatives*. Analysis of pangenomes provides a more accurate understanding of the genetic diversity of the gene pool. Technologies for sequencing and analyzing pangenomes will enable large-scale studies of genomic variation, access to a wider range of genomic data in breeding programs, and will help speed up the selection of crop plants to create varieties with consistently high yields and stress resistance. The paper presents a brief overview of the study of pangenomes of agricultural plants, describes their structural features, methods and programs for bioinformatics analysis of pan-genomic data.

**Control questions:**

1. The plant pan-genomics
2. Sequencing the genome of plants

**Practical work 3**

**Creation of Haploid and Dihaploid plants.**

Pollen is a microspore produced by seed plants that appear in the form of dust. They are extensively used as an explant to produce haploid plants.

Doubled haploid (DH) is a genotype formed when haploid cells undergo chromosome doubling. Artificial production of doubled haploids is important in plant breeding.

Haploid cells are produced from pollen or egg cells or from other cells of the gametophyte, then by induced or spontaneous chromosome doubling, a doubled haploid cell is produced, which can be grown into a doubled haploid plant. If the original plant was diploid, the haploid cells are monoploid, and the term doubled monoploid may be used for the doubled haploids. Haploid organisms derived from tetraploids or hexaploids are sometimes called dihaploids (and the doubled dihaploids are, respectively, tetraploid or hexaploid).

*Genetics of DH population.* In DH method only two types of genotypes occur for a pair of alleles, A and a, with the frequency of ½ AA and ½ aa, while in diploid method three genotypes occur with the frequency of ¼ AA, ½ Aa, ¼ aa. Thus, if AA is desirable genotype, the probability of obtaining this genotype is higher in haploid method than in diploid method. If n loci are segregating, the probability of getting the desirable genotype is (1/2)n by the haploid method and (1/4)n by the diploid method. Hence the efficiency of the haploid method is high when the number of genes concerned is large.

*Applications of DHs plant breeding*

*Mapping quantitative trait loci*

Most of the economic traits are controlled by genes with small but cumulative effects. Although the potential of DH populations in quantitative genetics has been understood for some time, it was the advent of molecular marker maps that provided the impetus for their use in identifying loci controlling quantitative traits. As the quantitative trait loci (QTL) effects are small and highly influenced by environmental factors, accurate phenotyping with replicated trials is needed. This is possible with doubled haploidy organisms because of their true breeding nature and because they can conveniently be produced in large numbers. Using DH populations, 130 quantitative traits have been mapped in nine crop species. In total, 56 DH populations were used for QTL detection.

**Control questions:**

1. Applications of DHs plant breeding.

**Practical work 4**

**Aspects of Somatic Hybridization**

*Aspects of Somatic Hybridization*

***1. Fusion of Protoplast***

The protoplast is a cell without any cell wall. Since they are devoid of any cell wall, the fusion of protoplasts becomes easy without facing any incompatibility barriers, while fusing the two genomes. The fusion of protoplasts can be achieved by three methods:

*Mechanical fusion:* The protoplasts are allowed to fuse mechanically by putting them together on a depression slide. However, this technique can often destroy the protoplasts.

*Spontaneous fusion:* This is a natural process that happens during the enzymatic degradation of cell walls. The surrounding protoplasts often fuse together, without any physical interference, but these fusions cannot give rise to whole plants.

*Induced fusion:* Isolated protoplasts are fused together with the help of chemicals called fusogens, such as NaNO3, PEG, polyvinyl alcohol, lysozyme, dextran, fatty acids, electrofusion, etc. The mechanism of induced fusion is described below:

*Agglutination/adhesion*: The two protoplasts adhere together when brought in close contact by fusogens, such as polyethylene glycol(PEG) and NaNO3.

*Plasma membrane fusion:* The protoplasts membrane fuse together at the site of adhesion, which forms a cytoplasmic bridge between the two protoplasts. High pH and Ca2+ concentration can increase the rate of membrane fusion.

*Formation of heterokaryons:* The fused protoplasts round up to form a spherical homokaryon or heterokaryon.

***2.Selection of Hybrid Cells***

Not all the protoplasts fuse, only 20-25% fuse to form a heterokaryon. The whole mixture consists of homokaryons, heterokaryons and un-fused protoplasts. Therefore, methods are devised to select the hybrid cells from this heterogeneous mixture. There are three methods for the selection:

*Biochemical method:* In this method, biochemical compounds are used to select the fused cells from unfused cells. There are two methods:

Drug sensitivity: In this method, one of the protoplasts is resistant to antibiotics and the other protoplast will not be able to grow in its presence. For example: if protoplast 1 is resistant to actinomycin D, and protoplast 2 is not; after fusion, the fused protoplast will gain characteristics of both. When the cells are grown on a medium containing the antibiotic, protoplast 2 will not be able to grow, fused protoplasts will grow, and protoplast 1 forms small colonies that can be identified and separated.

*Auxotrophic mutants:* Auxotrophs are mutants that cannot grow in a minimal medium. The hybrids are able to grow in the minimal medium, whereas the parental cells do not grow, and thus the cells can be selected.

*Visual method:* This method is very tedious as it involves selecting the hybrid cells visually and mechanically. In this method, cells that grow on different media are fused to separate them visually after fusion. Another technique is to use a pipette called Drummond pipette to mechanically separate the hybrid cells.

*Cytometric method:* Modem techniques such as flow cytometry and fluorescent cells are applied in this method for easy selection of cells.

***3. Identification of Hybrid Plant***

The identification of hybrid plants after development from the hybrid cells requires molecular evidence. Here are some common approaches for the identification of hybrid plants.

*Morphology of hybrid plants:* The morphology of the hybrid plants is usually an intermediate of the two plants and can be identified easily. Hybrids such as pomatoes and topatoes are fusions of tomatoes and potatoes and can be easily distinguished from the mother plant.

*Isoenzyme analysis of hybrid plants:* Isoenzymes are forms of the same enzymes that catalyze different reactions. The hybrid plant consists of isoenzymes from one or both the parents. These isoenzymes can be analysed electrophoretically to verify hybridity.

*Symmetric and asymmetric hybrids:* When hybrid plants contain the chromosome numbers the same as their parents, they are called symmetric hybrids and are sterile in nature. Asymmetric hybrids are however abnormal and do not have a normal chromosome number or ploidy.

**Control questions:**

Somatic Hybridization: Aspects, Applications and Limitations

**Practical work 5**

**Chromosome Engineering**

Chromosome Engineering and Crop Improvement series summarizes landmark research and describes medicinal plants as nature’s pharmacy.

Examines the use of molecular technology for maintaining authenticity and quality of plant-based products

Details reports on individual medicinal plants including their history, origin, genetic resources, cytogenetics, and varietal improvement through conventional and modern methods, and their use in pharmaceutical, cosmeceutical, nutrition, and food industries

Explains how to protect plants with medicinal properties from deforestation, urbanization, overgrazing, pollution, overharvesting, and biopiracy

Brings together information on germplasm resources of medicinal plants, their history, taxonomy and biogeography, ecology and biodiversity, genetics and breeding, exploitation, and utilization in the medicine and food industries

Written by leading international experts and an innovative panel of scientists, Medicinal Plants offers the most comprehensive and up-to-date information on medicinal plant genetic resources and their increasing importance in pharmaceutical and cosmeceutical industries, medicine, and nutrition around the world. <https://www.ncbi.nlm.nih.gov/books/NBK216396/>

Crop Improvement Methods:

1. Traditional breeding
2. Marker-assisted breeding
3. Transgenic technology
4. Gene Editing

<https://learn.genetics.utah.edu/content/cotton/crop>

**Control questions:**

1. Plant Genetic Resources

**Practical work 6**

**The tools of recombinant DNA technology**

Recombinant DNA Technology. Recombinant DNA technology is an extremely important research tool in biology. It allows scientists to manipulate DNA fragments in order to study them in the lab. It involves using a variety of laboratory methods to put a piece of DNA into a bacterial or yeast cell. Once in, the bacteria or yeast will copy the DNA along with its own. Recombinant DNA technology has been successfully applied to make important proteins used in the treatment of human diseases, such as insulin and growth hormone.

A technique mainly used to change the phenotype of an organism (host) when a genetically altered vector is introduced and integrated into the genome of the organism. So, basically, this process involves the introduction of a foreign piece of DNA structure into the genome which contains our gene of interest. This gene which is introduced is the recombinant gene and the technique is called the recombinant DNA technology.

There are multiple steps, tools and other specific procedures followed in the recombinant DNA technology, which is used for producing artificial DNA to generate the desired product. Let’s understand each step more in detail.

<https://byjus.com/biology/recombinant-dna-technology/>

**Control questions:**

1. Creation of Recombinant DNA

**Practical work 7**

**Genetic transformation of plants.**

Plant genetic transformation (PGT) is a process where DNA is introduced into plant cells, tissues, or organs using molecular and cellular biology methods.

Through plant genetic transformation, we can discover a gene and its function, understand traits of interest and favor breeding programs by producing novel and genetically diverse plant materials.

In a plant transformation protocol, step-by-step wet lab activities are performed to introduce the foreign DNA (exogenous DNA) and evaluate its insertion. Thus, PGT comprises:

Delivery of the DNA into a single cell and

Regeneration into entire fertile plants.

Plant Transformation basic steps - first DNA is delivered into the plant cell. The plant is then regenerated with new genetic information

However, researchers are not always interested in developing a whole genetically transformed plant (stable transformation), instead, they may want to quickly test the expression of a foreign gene in the plant tissue (transient transformation). Furthermore, how you transfer foreign DNA into plant cells can be divided into: protoplast-mediated, biolistic-mediated, and Agrobacterium-mediated techniques.

Table 1.1. Comparison for stable and transient transformation.

|  |  |  |
| --- | --- | --- |
|  | ***Advantages*** | ***Disadvantages*** |
| **Stable transformation** | * A transgenic line can be produced * The target gene is inherited * A metabolite can be constantly expressed * Plants are used as biofactories | * Months to years in the transformation process |
| **Transient transformation** | * It is a fast method * It can easily be scaled up for commercial uses * It can be used to produce recombinant proteins * It can be used for the targeted silencing of genes | * It has a highly variable transformation efficiency |

*Plant genetic transformation strategies*

Stable transformation:

Depending on their goal, researchers may want to develop a stable genetically transformed plant variety to massively produce new plant materials with desirable traits for agriculture, like disease resistance.

With stable transformation, the foreign DNA is fully integrated into the host genome and expressed in later generations of the plant.

This type of plant transformation is used for longer-term research. For instance, a stable transformation project can take months, even years, to be thoroughly developed.

Stable transformation diagram - foreign DNA is integrated into the host genome and expressed in later generations of the plant.

Transient transformation:

In other cases, researchers are more interested in understanding a gene or protein function. In this case, developing a whole genetically transformed plant is not necessary. Instead, a tissue portion (e.g. detached leaf) would be enough to evaluate the expression of a foreign gene.

Here, transient transformation allows temporary introduction or silencing of genes to determine their expression. Therefore, the foreign DNA is not integrated into the host cell. A transient method can also be used for gene silencing by expressing small interfering RNAs (siRNAs) and microRNAs (miRNAs) in plant tissues. In this case, a transient transformation can take days or weeks.

**Control questions:**

1. Genetic engineering methods.

**Practical work 8**

**Agrobacterium-mediated plant transformation.**

The ability of Agrobacterium to transfer DNA to plant cells been harnessed for the purposes of plant genetic engineering. Since the initial reports in the early 1980s using Agrobacterium to generate transgenic plants, scientists have attempted to improve this “natural genetic engineer” for biotechnology purposes. Some of these modifications have resulted in extending the host range of the bacterium to economically important crop species. However, in most instances, major improvements involved alterations in plant tissue culture transformation and regeneration conditions rather than manipulation of bacterial or host genes. Agrobacterium-mediated plant transformation is a highly complex and evolved process involving genetic determinants of both the bacterium and the host plant cell. In this article, I review some of the basic biology concerned with Agrobacterium-mediated genetic transformation. Knowledge of fundamental biological principles embracing both the host and the pathogen have been and will continue to be key to extending the utility of Agrobacterium for genetic engineering purposes.

**Control questions:**

**Practical work 9**

**Particle bombardment method.**

Particle bombardment used method for genetic transformation of plants and other organisms. Millions of DNA-coated metal particles are shot at target cells or tissues using a biolistic device or gene gun. The DNA elutes off the particles that lodge inside the cells, and a portion may be stably incorporated in the host chromosomes. A protocol for the generation of transgenic grapevines via biolistic transformation of embryogenic cell suspension cultures is detailed in this chapter. In a typical experiment, transient gene expression averaged nearly 8000 "hits" per bombarded plate. Five months after bombardment, there were nearly five putative transgenic embryos per bombarded plate. About half of the embryos were regenerated into confirmed transgenic plants. The basic bombardment procedures described are applicable to a wide range of plant genotypes, especially those for which embryogenic cell cultures are available. All users of particle bombardment technology will find numerous useful tips to maximize the success of transformation.

**Control questions:**

1. Particle bombardment of plants.

**Practical work 10**

**Main problems of food safety.**

Food security from the viewpoint of any state is the ability of the state regardless of external and internal threats to meet the needs of the population in food in volumes, quality and assortment that correspond to accepted standards and safety regulations.

Key issues:

1. “Is our food safe in terms of application of new biotechnologies?”

2. “What measures should be taken in terms of convergence of global food market to standardize approaches, regulation and to provide consumer safety”?

Main problems of food safety:

1. Low-income citizens must choose cheap (often counterfeit) and low-quality food.

2. A trend of general decline in the quality of all food and increasing levels of anxiety towards food production with using genetic engineering.

3. Increase in the flow of genetically modified foods/organisms (GM foods/ GMOs) and products from them: soybeans, corn, canola, rice, potato, pumpkin,

papaya, etc.

4. Extension of intellectual property rights to genetically modified breeding and seed material by large trans-national corporations (TNCs).

5. Low level of awareness of both farmers and consumers about food safety standards.

6. A large number of international and domestic legal regulations (more than 300).

7. Complexity of harmonizing requirements to ensure competitiveness and high food safety standards.

Use of modern technologies in living systems (and agriculture is a set of living systems) raises questions of risks and security, as it is associated with dual-use sciences.

In modern scientific literature, this issue has long been given increased attention.

**Control questions:**

1. Development of modern biotechnologies and risks of implementing in agriculture.

2. Main problems of food safety.

**Practical work 11**

**Plant microbiota and their interactions.**

Plant microbiota and their interactions are highly diverse and multiple factors shape community assembly and functioning. While recognized since the 19th century, the investigation of and interest in plant-associated microbiota only started to bloom since the 800s. Due to the high potential of microorganisms to improve plant growth, stress resilience and health, numerous microbial inoculants have been developed, but many of them show poor performance in the field. Several approaches may lead to improved field success such as designing smart microbial consortia, the selection of agricultural management practices favoring microbiota with beneficial functions or a new generation of plant breeding approaches. Last but not least the development of suitable formulations and delivery approaches is highly important for any field application. Our understanding of plant microbiota, its functionality and its exploitation has substantially increased in the last years. However, a better understanding is needed on how inoculants modulate the resident microbiome, how complex microbiota and the holobiont affect the activity of the applied strain or how microbial inoculants colonize the plant environment in the field.

Microbes in a community interact with each other and the host plant, so it is important to capture as much of the diversity of a microbiome as possible. To do so requires the use of global analyses such as metagenomics, metatranscriptomics and metaproteomics, which allow simultaneous assessment and comparison of microbial populations across all domains of life. Metagenomics can reveal the functional potential of a microbiome (the abundance of genes involved in particular metabolic processes), whereas metatranscriptomics and metaproteomics provide snapshots of community-wide gene expression and protein abundance, respectively.

Metatranscriptomics has revealed kingdom-level changes in the structure of crop-plant rhizosphere microbiomes. Th relative abundance of eukaryotes in pea and oat rhizospheres was fie-fold higher than in plant-free soil or the rhizosphere of modern hexaploidy wheat.

**Control questions:**

1. Plant microbiome.
2. Selection of plants for efficient interaction with plant microbiota

**Practical work 12**

**NGS is tightly bound to bioinformatics.**

NGS strategies allow a single template molecule to be directly used to generate millions of bases at low cost with a less cumbersome laboratory protocol. There are three NGS platforms widely used nowadays that are considered to be second-generation sequencing:

(1) the Genome Sequencer FLX+/454 from Roche which is capable of producing over a million reads of up to 800 bases per 10 hour run, yielding a total of 0.7±1 Gbp at a price of approximately $90 per megabase;

(2) the Genome Analizer from Illumina, of which the latest version, HiSeq2000, yields 100 Gbp of bases per day (26±150 bp read length) at a cost of $4 per megabase; and

(3) the Applied Biosystems SOLiD (Sequencing by Oligo Ligation and Detection) that produces 10±300 Gbp of short reads (up to 75 bp) per run at a similar cost.

The three platforms offer the paired-end sequencing technique. As a result, even large plant genomes can count on relatively inexpensive deep coverage with reads of 100 bp and paired-end libraries from 1 to 5 kbp (we will see that deep coverage does not allow for complete plant sequencing). A detailed description is beyond the scope of this article, and several reviews illustrate the rapid evolution of these and the newest NGS technologies (to cite a few, [25±31]). While 454 FLX+ and Sanger technologies are considered to produce long reads (600±800 pb in average), the other two produce short reads (<150 bp in average). Short-read technologies compensate the shortness of the sequences with a high coverage, so that bacteria can be successfully sequenced with a 40×±50× coverage, but as the genome increases in complexity, coverage of 100× may still be inefficient [32±34]. In contrast, long-read technologies do not need such deep coverage, with 20×±30×

being enough for a good compromise between costs and assembly quality. NGS is becoming the new sequencing standard for the following reasons:

(1) simplification of the sequencing process (DNA cloning is not required);

(2) miniaturization and parallelization (low cost); and

(3) good adaptation to a broad range of biological phenomena (genetic variation, RNA expression, protein-DNA interactions, gene capture, methylation, etc.).

But not everything about NGS is an advantage:

(i) the base calls are at least tenfold less accurate than Sanger sequencing base calls;

(ii) the sequence length is shorter than in Sanger technology and requires dedicated assembly algorithms; and

(iii) the quality of the NGS assemblies is also lower than Sanger assemblies.

**Control questions:**

1. NGS technology
2. NGS is tightly bound to bioinformatics.

**Practical work 13**

**Effect of abiotic and biotic stresses on food production.**

According Sustainable Development goals until 2030 we should have zero hunger and undernourished people in the world. But to achieve this goal plant breeders must improve plants in order to produce at least the double than is produced now. This is not a easy pathway because we have only few years, but considering that plant breeding programs normally take several years to produce improved genotypes, also the further improved plants should face with pest, disease and other abiotic factors that are increasing with the current climate changes. In this regards, we will discuss the situation of hunger in the world and the remaining available land to increase food production, point out effects of biotic and abiotic factors on the food production and present some ways that can be used to fastening plant breeding.

Genetic introgression also can be made by crossing wild relatives with elite plants and applying recurrent selection in order to increase the elite genome in resistant plants. If the resistance gene is found in the cytoplasm of the wild species it should be used as mother plant in the crossing and recurrent selection in order to conserve the resistance gene, otherwise it is independent. This process can be accelerated by using assisted selection with molecular markers. On the other hand, the resistance gene can be transferred by genetic engineering to the desired crop.

The integration of in vitro techniques, like gametic embryogenesis, plant cell and tissue culture, somatic embryogenesis, protoplast fusion and mutagenesis to the modern biotechnology techniques, like: transgenic plants, synthetic biology, gene editing, interference RNAs and other OMICS technologies can be used to speed the resistance in improved plants to biotic and abiotic factors and to modulate plant root depth or change plant architecture. On the other hand, once susceptibility genes are identified, it is possible to use gene editing technologies in order to build resistant plants against different pathogens, and considering that QTLs are not race specific and that we can combine them with R race-specific resistant genes to promote an effective and durable resistance in different environments, both strategies are highly recommended to deal with climate changes. Thus, according to Cobb, et al., the public sectors should integrate new technologies to the Mendelian genetics and the principles of quantitative genetics in order to make vigorous change in crop productivity.

Another way to increase resistance is by using microbial biotechnology to enhance plant nutrition and/or promote biocontrol against pathogens, applying diethyl aminoethyl hexanoate to achieve resistance to abiotic stress, and use the proline to increase salt tolerance and low water condition.

**Control questions:**

1. Effect of abiotic and biotic stresses on food production.
2. In vitro techniques and modern biotechnologies in improved of plants.

**Practical work 14**

**Biosafety and regulation of genetically modified plants.**

Products of genetic engineering are a reality in our daily lives—whether as industrial and medicinal applications or for animal and human consumption. During the twenty-second year of commercialization of genetically modified (GM) crops in 2017, they were grown in 24 countries on 189.8 million hectares—19 developing and 5 industrial countries. Developing countries grew 53% of the global biotech crop area compared with 47% for industrial countries.

An additional 43 countries formally imported biotech crops for f ood, feed, and processing. The increased precision now possible in plant breeding using genome-editing techniques represents a big change from conventional breeding approaches, which, in large part, rely on random, uncontrolled chemical- or radiation-induced mutagenesis, and from genetic engineering, which relies on unpredictable insertions of isolated genes into the plant genome. I f conducive regulatory and social conditions are in place, genome editing could substantially increase the positive impacts of plant breeding on human welfare and sustainability.

The development and use of modern biotechniques are regulated by different countries and communities of states according t o t heir national laws and governance structures. Generally, the legal frameworks require submission of comprehensive scientifi evidence regarding the biology of the organism, its safety to human and animal health, and its effect upon the environment in which it will be released.

**Control questions:**

1. Biosafety and regulation of genetically modified plants.
2. Risk and safety assessment of genetically modified plants.

**Practical work 15**

**Plant Genome Editing via CRISPR/Cas DNA.**

***Crispr/cas systems for plant genome editing***

*CRISPR/Cas Systems.* The CRISPR/Cas system, comprising CRISPR repeat-spacer arrays and Cas proteins, is an RNAmediated adaptive immune system in bacteria and archaea that provides defense against phages and other invasive genetic elements by cleaving the invader’s nucleic acid genome. On the basis of their Cas genes and the nature of the interference complex, CRISPR/Cas systems have been divided into two classes that have been further subdivided into six types based on their signature Cas genes. Class 1 CRISPR/Cas systems (types I, III, and IV) employ multi-Cas protein complexes for interference, whereas class 2 systems (types II, V, and VI) accomplish interference with single effector proteins in complex with CRISPR RNAs (crRNAs). The CRISPR system that has been developed for genome editing is based on RNA-guided interference with DNA.

**Control questions:**

1. CRISPR/Cas Systems.
2. Plant Genome Editing via CRISPR/Cas DNA

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