Crop Improvement

Very Short Answer Questions

1. **List out the major achievements through Plant Introduction.**
   Ans: 1. Sonora 64 and Sonora 63 were introduced into our country from Mexico.
   2. IR-8 variety of Paddy was introduced from Philippines.

2. **What is clone? In which type of plants clonal selection is employed?**
   Ans: Progeny obtained from a single parent by vegetative propagation is called as clone. It is applicable to vegetatively propagated crops like potato and mango.

3. **What is emasculation? Mention its importance.**
   Ans: It is removal of anthers in female parent before opening of flowers. It prevents self-pollination.

4. **What is bagging? Mention the role of bagging in hybridization.**
   Ans: Covering the emasculated flowers of female parent with polythene or paper bags is called as Bagging. It prevents unwanted cross pollination.

5. **Who coined the word heterosis? State the important causes for heterosis.**
   Ans: The term heterosis was coined by Shull. The causes for heterosis are presence of more number of dominant genes in the offspring or heterozygosity.

6. **Who for the first time used the term Mutations? Who laid the foundation for mutational breeding?**
   Ans: Victor Hugo de Vriese used the term mutations. Stadler laid foundations for mutational breeding in plants and Morgan in animals.

7. **What are spontaneous mutations? Give examples.**
   Ans: Mutations that occur by themselves in nature are called as natural or spontaneous mutations. Oenothera nanella and Oenothera gigas are mutants of Oenothera lamarkiana.

8. **What are mutagens? Give two examples.**
   Ans: Any agent that causes mutations is called as Mutagen. Ex: Colchicine, Formaldehyde.

9. **How are artificial polyploids produced?**
   Ans: Artificial polyploids are produced by subjecting the zygote to cold treatment, treating the vegetative and floral buds with chemicals like Colchicine, Acenaphthene and Coumarins or X-rays.

10. **Name the alkaloid obtained from the seeds of Colchicum autumnale. What is its importance?**
    Ans: Colchicine. It is used to induce mutations as well as polyploidy in plants.

11. **Define hybrid vigour. Who first time identified hybrid vigour?**
    Ans: It superiority of hybrid over its parents in vigour, productivity and quality of produce. It was first identified by Koelreuter.
12. **Define pure lines. Who proposed pure line selection method?**

Ans: Progeny obtained from a single homozygous parent by self-pollination is called as pure line. It was proposed by Johannsen.

13. What are the achievements of mutational breeding?

Ans: The achievements are Disease resistance in IR 8 for blight, blast and leaf spot diseases, Sweedish variety of Barley with winter hardiness and Aruna variety of Castor.

Short answer Questions

1. Enumerate the main objectives of Plant breeding.

Ans: 1. Increasing the yield of the crop like grains, fodder, fibre, oil etc.

2. Improving the quality of produce like size, taste, colour, storability, shape, nutritional value etc.

3. Developing resistance.

4. Producing early maturing varieties.

5. Changing the agronomic and habit characters to make the crop suitable for harvesting and tolerant to various abiotic stresses.

6. Developing adaptability to various climatic conditions.

2. What is mutational breeding? Explain.

Ans: Production of superior varieties of plants by inducing mutations is called as mutational breeding. Induced mutations in plants were first caused by Stadler in Barley by using X-rays. The agents used to induce mutations are called as mutagens. These may be physical or chemical. Mutation breeding is quick method to bring about genetic variability in a crop. All mutations induced are not desirable. Only some are desirable. Desirable mutations are exploited for crop improvement.
Achievements: 1. Resistance to Leaf spot, Blast disease and Leaf blight in paddy variety IR-8 was achieved by mutational breeding.
2. Winter hardiness of grain in Sweedish variety of Barley
3. Early maturity in Aruna variety of Castor is developed by mutations.

3. Write briefly on polyploidy breeding.

Ans: Presence of more than two genomes in an individual is called as Polyploidy. In nature sexually reproducing organisms are in diploid condition. In them two sets of chromosomes are present. However in many plants, particularly in grasses the plants are either triploids or tetraploids or hexaploids or octaploids. The utilization of polyploidy for the improvement of crops is called as polyploidy breeding. It is usually useful in plant growing for their vegetative organs.

To bring about polyploidy, the zygote is treated with low temperature or the floral or vegetative buds are treated with chemicals like Colchicine, Coumarins, Acenaphthene or physical agents like X-rays.
The hexaploid variety of wheat called Bread wheat is developed by Polyplody breeding by using *Triticum monococcum, Aegilops speltoides* and *Aegilops squarrosa*.

4. **What is the botanical name of Bread wheat? Explain the origin of bread wheat schematically.**

Ans:

Triticum monococcum **(2n = 14) X** ***Aegilops speltoides*** **(*Triticum speltoides*) (2n=14)**

AA **BB**

↓

AB (2n = 14, sterile)

↓ doubling

AABB (4n = 28) X ***Aegilops squarrosa*** **(2n = 14) DD**

↓ **(*Triticum tauschi*)**

Triploid hybrid (3n = 21)

ABD (sterile)

↓ **Doubling**

Triticum aestivum **(6n = 42)**

Hexaploid wheat **AABBDD**

Long Answer Questions

1. **What is selection? Write briefly about the three types of artificial selection methods?**

Ans: Choosing the desirable plants for cultivation is called as selection. The three types of artificial selections are as follows.

A. Mass selection: It is the oldest method of selection and applied for cross pollinated crops. It has been practiced by farmers. The farmer chooses best appearing, vigorous plants from mixed population, collects seed from them and used the seed to raise the crop in the next season. It is continued for 8 years at the end of which a new variety is obtained. It is used to improve the yield and quality of the crop. Best results are obtained based on heterozygosity.

Advantages: 1. It is the easiest method and requires no scientific knowledge except some amount of skill. It is more an art than science.

2. This is the only method of selection applied for improving local cross pollinated varieties of crops.

Achievements: Many existing crops are result of mass selection.

1. Cotton: Most of the Indian commercial varieties of cotton like Dharwar American, Dodahatti local, Cambodias are developed by this method.

2. Bajra: Pusa Moti.

B. Pure line selection: Progeny obtained from a homozygous parent by self-pollination Is called as Pure line. Developing and choosing desired pure lines for cultivation is called as pure line selection. It was first developed by Johansen in *Phaseolus vulgaris*.

In this method, 150 to 1000 plants with desired characters are selected from the field and subjected to selfing after which the seed is collected. Seed of every plant is sown in separate row and the plants are raised. From every row the best plants are selected and subjected to selfing. The process is continued for 10 years. At the end desired pure lines are selected from the developed ones and released as new varieties to farmers for cultivation.

Advantages:

1. Local self-pollinated varieties are developed only by this method.
2. All the individuals of the line are phenotypically and genotypically same.

Achievements:

1. Groundnut: TMV – 3, RSB – 17
2. Paddy: CO 4, 6, 10, 14.

C. Clonal selection: Clone is a group of plants obtained from a single parent by vegetative propagation. The individuals of a clone are phenotypically and genotypically same but in heterozygous condition. It is applicable to vegetatively propagated crops like Banana, Ginger, Turmeric, Potato, Mango, Sugar cane etc. The type of propagules varies from plant to plant.

In this method healthy units form healthy plants are selected based on desirable phenotypic characters after enough field tests and clones are raised. Selection is made between the clones not within the clones unless mutations occur in the clone. The selected clones are subjected testing at various regional stations before they are multiplied and released to farmers for cultivation. It takes about 9 years to develop a variety which is based on the selected crop.

Advantages: Progeny of clone remain stable for any number of generations. If they have hybrid vigour, it is exploited for several generations.

Achievements: a. Potato: Kufri red and Kufri safed

b. Mango: Mundapa Pedda neelam

2. **What is hybridization and describe the procedure of hybridization.**

Ans: It is cross between genetically unlike parents with desirable characters. The method is as follows.

1. Selection of parents: It is the first step during this method. Homozygous plants with desirable characters are selected as parents. If a wild variety is selected as parent it is subjected to selfing to bring about homozygosity to desirable characters. The seeds of the both the parents is sown and the P generation is raised.
2. Emasculation: The flowers of female parent must be emasculated prior to opening of flowers or anthesis to avoid self-pollination. If the flowers are fairly larger it can be

done manually by using sterilized forceps and needle during which care should be taken to avoid injury to the ovary of the gynoecium. If the flowers are smaller and overcrowded, it can be done by dipping the inflorescence in hot water at temperatures between 45 to 50^0C for 1-10 minutes. The gynoecium withstands higher temperature than the anther.

Emasculation can be avoided in case of dioecious plants and male sterile plants considered as female parents.

3. Bagging: It is covering the emasculated flowers of female parent with polythene bags to avoid unwanted cross pollination.

4. Artificial Cross pollination: Pollen grains of male parent must be collected with help of brush or blotting paper and carefully kept on the stigmas of female parent and in this way artificial cross pollination is carried out. Soon after this, the flowers again rebagged and labels carrying the information about parental characters, date of pollination and expected date of seed setting to be attached to the female parent.

Seeds are collected from the female parent and preserved to rise the next generation. These seeds are sown and F_1 plants are obtained. These plants are subjected to selfing to get F_2 seed and later the generation. The desirable F_2 plants are selected and further improved by using different methods. Seeds are multiplied and finally released to farmers for cultivation.

Advantages:

New genetic recombinations can be created by hybridization. Many hybrids exhibit hybrid vigour. A large number of desirable characters can be incorporated into a single variety.

Quiz type Questions

1. **Which method/step in hybridization can be avoided when male sterile plants are used as a female parent during hybridization?**
   Ans: Emasculation.
2. State the reasons as to why emasculation and bagging are done respectively during the hybridization experiments.
   Ans: Emasculation is done to avoid self-pollination and emasculation is done to avoid unwanted cross pollination.
3. Which plant breeding method forms a basis for crop improvement?
   Ans: Selection
4. In pure line selection, there is no scope of incorporating new characters that do not exist in a population. Why?
   Ans: Because self-pollination is carried out this has no scope of inserting new characters not existing in population.
5. The characters which are in heterozygous condition remain the same throughout the period of clonal selection. Why?
   Ans: Because the progeny is obtained by vegetative reproduction which has no scope for genetical change unless mutations occur naturally.
6. State the reason for inbreeding depression in several crop plants.
   Ans: Continuous selfing.

7. How many types of genomes are present in bread wheat?
   Ans: Three types. One belonging to *Triticum monococcum*, second one to *Aegilops speltoides* and third one to *Aegilops squarrosa*.

Biotechnology- Introduction

Very Short Answer Questions

1. **Define Biotechnology?**
   Ans: It is the science of utilizing the properties and uses of microorganisms or to exploit cells and the cell constituents at industrial level for generating useful products essential to life and human welfare.

2. **Define interferon, Bioremediation, Bioinformatics, Proteomics and Genomics.**
   Ans: Interferon is host protein synthesized in response to viral infection and interferes with viral metabolism.
   Bioremediation is using living organisms to remove contaminants, pollutants and unwanted substances from soil and water.
   Bioinformatics is application of information sciences such as mathematics, statistics and computer science to increase our understanding of biology.
   Proteomics is the study of the array of proteins that an organism produces by using computer.
   Genomics is computer based study and designing of genome.

3. **Name any two biopesticides?**
   Ans: Bacillus thuringiensis, Baculoviruses like Nuclear Polyhedrosis Viruses and Cytoplasmic Polyhedrosis Virus

Short answer Questions

1. **Explain the role of biotechnology in medical field?**
   Ans:
   Biotechnology in Medicine: Cloned DNA produced by genetic engineering is used for commercial production of hormones like Insulin, Interferons and vaccines against rabies, polio, small pox, hepatitis B, foot and mouth disease. Microbes are commercially exploited for the production of vitamins like vitamin A, Riboflavin, Vitamin B_{12} etc., antibiotics like penicillin, erythromycin and other chemicals at low costs. Genetic engineering technique is also used in preventing, diagnosis and cure of many diseases by using DNA probes.

2. **Biotechnology has revolutionized the field of agriculture. Explain.**
   Ans: Biotechnology caused revolution in the field of Agriculture through tissue culture and organ culture. Intergeneric hybrid plants are produced through protoplast fusion and cell culture. Encapsulated seeds, disease and stress resistant plants are produced by using these methods.
   Ex: Bt. Cotton plant (It has Bacillus thuringiensis toxin which can kill various insect pests).
   Plants with improved nutritional quality, virus resistant, insect resistant, herbicide resistant plants are produced by using this technology.
   Ex: Transgenic Golden rice is produced by introducing three genes for Vitamin A synthesis in Taipei variety of Rice.

For better yield of crops, biofertilizers have emerged as alternative tool in place of chemical fertilizers due to discoveries in biotechnology. Similarly microbes with pesticidal abilities are discovered which minimized the usage of synthetic pesticides. Some of the biopesticides are Bacillus thuringiensis, Nuclear Polyhedrosis Virus and Cytoplasmic polyhedrosis virus.

3. **Discuss the application of biotechnology in industry.**
   Ans: Enzymes are commercially produced from plants, animals and microorganisms. Currently used animal enzymes are Lipase, Trypsin, Rennet etc. Most prevalent plant enzymes are Papain, Protease, Amylase, Soybean lipoxygenase etc. Glucose isomerase, α-amylase, protease etc. These enzymes are used in dairy, detergents, starch, brewing, wine and pharmaceutical industries.
   Ex: Papain is used as meat tenderizer, protease as leather softener. Rennet is used in preparation of cheese. Glucose isomerase is used in the preparation of artificial sweeteners, fructose syrup etc. Monosodium glutamate is used as food flavouring agent.

Long Answer Questions

1. **Give an account on the scope and applications of biotechnology.**
   Ans:
   Biotechnology in Medicine: Cloned DNA produced by genetic engineering is used for commercial production of hormones like Insulin, Interferons and vaccines against rabies, polio, small pox, hepatitis B, foot and mouth disease. Microbes are commercially exploited for the production of vitamins like vitamin A, Riboflavin, Vitamin B_{12} etc., antibiotics like penicillsin, erythromycin and other chemicals at low costs. Genetic engineering technique is also used in preventing, diagnosis and cure of many diseases by using DNA probes.
   Biotechnology in Agriculture: Biotechnology caused revolution in the field of Agriculture through tissue culture and organ culture. Intergeneric hybrid plants are produced through protoplast fusion and cell culture. Encapsulated seeds, disease and stress resistant plants are produced by using these methods.
   Ex: Bt. Cotton plant (It has Bacillus thuringiensis toxin which can kill various insect pests).
   Plants with improved nutritional quality, virus resistant, insect resistant, herbicide resistant plants are produced by using this technology.
   Ex: Transgenic Golden rice is produced by introducing three genes for Vitamin A synthesis in Taipei variety of Rice.
   For better yield of crops, biofertilizers have emerged as alternative tool in place of chemical fertilizers due to discoveries in biotechnology. Similarly microbes with pesticidal abilities are discovered which minimized the usage of synthetic pesticides. Some of the biopesticides are Bacillus thuringiensis, Nuclear Polyhedrosis Virus and Cytoplasmic polyhedrosis virus.
   Biotechnology in Industry: Enzymes are commercially produced from plants, animals and microorganisms. Currently used animal enzymes are Lipase, Trypsin, Rennet etc. Most prevalent plant enzymes are Papain, Protease, Amylase, Soybean lipoxygenase etc.

Glucose isomerase, α-amylase, protease etc. These enzymes are used in dairy, detergents, starch, brewing, wine and pharmaceutical industries.
Ex: Papain is used as meat tenderizer, protease as leather softener. Rennet is used in preparation of cheese. Glucose isomerase is used in the preparation of artificial sweeteners, fructose syrup etc. Monosodium glutamate is used as food flavouring agent.
Biotechnology in Food: Food biotechnology is a viable alternative to food problems and a solution to nutritionally influenced diseases such as diabetes, hypertension, and cancer. It helped to produce proteins from microbial source. Such proteins are called as Single Cell Proteins. Commercial cultivation of mushrooms has started at industrial level as these are rich source of proteins, vitamins, minerals and essential amino acids. More over SCP and mushrooms can be grown by using domestic, agricultural and industrial wastes as substrates.
Biotechnology and Environment: Environmental biotechnology mainly deals with the usage of microbes to reduce the burden of toxic substances on environment. Environmental quality can be improved by using genetically engineered microbes which can degrade the substances that cannot be naturally degradable. Microbes are used for bioremediation for the degradation of wastes which can be converted to compost, and used for the production of mushrooms, SCP and biogas.

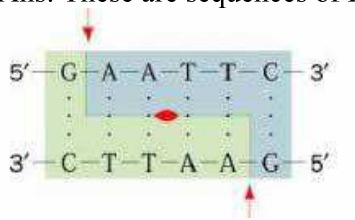
Quiz type Questions

1. **Name the enzyme that revolutionized the conventional breeding methods.**
   Ans: Restriction endonuclease.
2. **Name the toxin present in genetically engineered cotton variety which can kill effectively kill many arthropods.**
   Ans: Toxin of Bacillus thuringiensis or Bt. Toxin.
3. **Name the precursor rice variety responsible for the production of transgenic golden rice.**
   Ans: Taipei
4. If the meat has to be made tender for an easy cooking, which enzyme is popularly used?
   Ans: Papain
5. **In order to solve criminal cases and parental disputes with natural evidences which latest technology comes handy?**
   Ans: DNA finger printing.
6. **If a complex compound has to be rapidly degraded into simpler compounds using certain bacteria, which process is recommended?**
   Ans: Bioremediation.
7. **What is the term used to study the protein production of an organism using the computer technology.**
   Ans: Proteomics.

GENETIC ENGINEERING

Very Short Answer Questions

1. **What are molecular scissors? Where are they obtained from?**
   Ans: Restriction endonucleases are called as molecular scissors. They are obtained mainly form prokaryotes like bacteria.
2. **Name any two artificially restructured / modified plasmids.**
   Ans: pBR322, pUC 101
3. **What are genetically modified crops? Give an example.**
   Ans: Crops into which desired genes of altogether different organisms are inserted by genetic engineering are called as genetically modified crops. Ex: Flavr-Savr.
4. **What are Plasmids?**
   Ans: Extra chromosomal DNA rings seen in organisms like Bacteria are called as Plasmids.
5. **What are the transgenic plants?**
   Ans: Plants modified genetically by using recombinant DNA technology are called as Transgenic plants.
6. **What are the vectors used for Gene cloning in plants?**
   Ans: It is Ti Plasmid of *Agrobacterium tumifascience*.
7. Enumerate the major steps in Genetic Engineering.
   Ans: 1. Isolation of desired gene from the donor organism. 2. Insertion of gene into gene cloning vector, 3. Insertion of rDNA into suitable host organism, 4. Selection and screening of desired recombinants.
8. **What is *Eco*RI? How does it function?**
   Ans: EcoRI is a restriction enzyme obtained from E.coli R strain. It identifies the palindromic sequence GAATTC on the DNA strand and cuts it between G and A to form staggered ends in the DNA fragment.
9. **What are cloning vectors? Give an example.**
   Ans: The DNA molecules or organisms like bacteriophages used for cloning the desired genes are called as cloning vectors. Ex: Plasmids, Bacteriophages, Cosmids.
10. **What is rDNA?**
    Ans: It is DNA into which the desired gene of donor organism is inserted by using the enzymes restriction endonuclease and ligase.
11. **What is the mode of action of restriction enzymes? Give an example.**
    Ans: The restriction enzymes recognize the certain palindromic sequence in the donor genome and cut the genome into fragments. Ex: The enzyme Eco RI recognizes the sequence GAATTC on both the strands of DNA and cuts the DNA between G and A of both the strands and forms fragments of various sizes of donor genome.
12. **What are palindromic sequences?**
    Ans: These are sequences of DNA which read same in both strands in reverse direction.



13. **What are genetically modified organisms? Give any two examples.**

Ans: Organisms into which genes of altogether different organisms are inserted by rDNA technology are called as genetically modified organisms. Ex. Transgenic E.coli that produces human insulin called Humilin, Bt.cotton.

14. Write any two biosafety issues concerned with genetically modified crops?

Ans: 1. Through genetically modified crops allergens may be transferred to humans and animals which may show detrimental effect on health. 2. Due to molecular farming the fundamental nature of vegetable is changed.

15. What is molecular farming?

Ans: Usage of genetically modified crops as bioreactors for large scale production of useful products to mankind.

Short answer Questions

1. What are transgenic plants? Mention the beneficial aspects of transgenic plants

Ans: Plants whose genome is modified by inserting desired genes of distant donor organism are called as transgenic plants.

Benefits of GMP: 1. Transgenic plants are virus resistant (Papaya with ring spot resistance), Insect resistant (Bt cotton), Herbicide resistant (roundup ready soybean), fungal pathogen resistant (transgenic potato resistant to Phytophthora) and bacterial pathogen resistant (Tomato resistant to Pseudomonas) and show increase in productivity.

2. Transgenic plants like Flavr Savr tomato are bruise resistant and show delayed ripening which makes it suitable for food processing.

3. Transgenic Taipei variety of rice which is rich in Vitamin A can prevent childhood blindness.

4. Transgenic male sterile plants like Brassica napus can be used directly as female parents in hybridization without subjecting it to emasculation.

5. Transgenic plants like Basumati variety of rice is resistant to abiotic and biotic stress.

2. List out the applications of genetic engineering.

Ans: 1. rDNA can be used for production of Growth hormones, vaccines, and commercial chemicals.

2. rDNA technology is used to detect and cure genetical diseases. Genetical diseases in plants can be cured by gene therapy.

3. Abnormal gene expressions due to genetic disorders can be removed by gene transfer therapy which used rDNA technology.

4. DNA finger printing which is based on rDNA technology helps in identifying the criminals and solving parentage disputes.

5. Most of the transgenic plants and animals produced are beneficial to the society.

6. Pollution can be curtailed or reduced by using transgenic microbes.

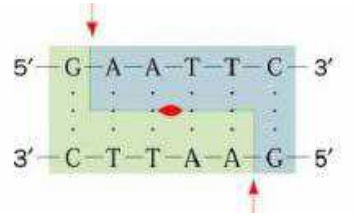
Long Answer Questions

1. **Explain the various steps in recombinant DNA technology.**

   Ans: The various steps in recombinant DNA technology are 1. Isolation of desired gene, 2. Insertion of the isolated gene into a suitable vector, 3. Introduction of recombinant vector into the host and 4. Selection of the transformed host cells.

   1. Isolation of desired gene: It has the following steps.

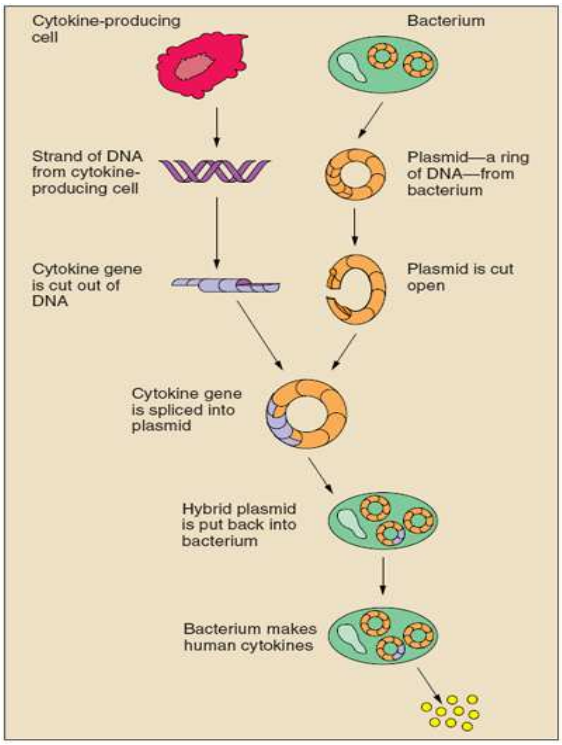
   a) Digestion of cell wall by cell wall degrading enzymes like cellulases, hemicellualases and Pectinases.
   b) Dissolving the unit membranes by detergent treatment.
   c) Treating the obtained protoplasmic contents with phenols, proteases, ribonucleases to dissolve all the contents except DNA and later separating them by gradient centrifugation.
   d) Precipitation of purified DNA by the addition of chilled ethanol.
   e) Cutting the DNA into fragments by treating with restriction endonucleases. Usually endonucleases that form sticky ends in DNA fragments are used.



   f) Separation of DNA fragments by Gel electrophoresis.
   g) Selection of desired DNA fragments by Southern blotting technique.

II. Insertion of isolated gene into suitable vector: The isolated gene is inserted into the suitable vector to get several copies of the gene which is known as Gene cloning. Cloning vector acts as a vehicle to carry the gene into the suitable host cell or organism. The vector may be a plasmid or bacteriophage or cosmid. Usually plasmids such as pBR322, pUC 19, 101 are used.

The plasmid DNA is opened with the restriction endonuclease which is used to isolate the gene. The two sticky ends of the plasmid are now joined to the ends of desired gene or DNA fragment by hydrogen bonding. Later the enzyme DNA ligase forms phosphor ester bonds between the vector DNA and the gene to form recombinant DNA or chimeric DNA which is circular hybrid DNA.



III. Introduction of recombinant vector into the host: For the expression of desired gene the rDNA is introduced into suitable bacterial host cell by transformation. The cells with rDNA are called as transformed cells. The transformation can be enhanced by using Calcium chloride. Inside the host cell the cDNA starts replicating. The transformed cells divide and form their clones on the medium.

IV. Selection of transformed host cells: It can be done by either without using probes or by using probes. In this the desired transformed cells are selected.

a) **Without using probes**: As an example, antibiotic resistance gene has to be cloned, the transformed cells are first incubated in a medium without antibiotic for about an hour to allow the antibiotic resistance gene to be expressed. Later, these cells are transferred to a medium containing antibiotic. The cells which have expressed the gene will survive and the others die.

b) **By using probes**: When transformed cells are cultured on medium several cells are formed. All the cells may not contain the desired gene. To select the cell with desired gene, colony hybridization method is employed. In this gene specific labeled probes are used which identify the desired gene in the transformed cells. These cells or colonies are separated from the non-transformed cells and cultured further.
Ex: Human gene for insulin production is isolated from human genome and inserted into the plasmid. The rDNA is incorporated into E.coli bacterial cell. This genetically modified E.coli started expressing the insulin gene in culture and producing insulin which is called as Humilin.

Quiz type Questions

1. **How can the DNA fragments be separated when the complete length of DNA is treated with restriction endonucleases?**
   Ans: DNA fragments are separated by Gel electrophoresis method.
2. **Mention the logic of using the radioactive gene specific probes during colony hybridization**.
   Ans: The probe nucleic acid strand has the nitrogen base sequence complementary to the sequence of desired gene in the transformed cells. These probes hydrogen bond with the desired gene due to nitrogen base complementarity. Moreover they show radioactivity, hence can be easily identified among the cells.
3. **What shall be the sequence of nitrogen bases found in the sticky end 5′→3′ of a DNA strand, which is cut by *Eco* RI.**
   Ans: 5′ AATTC---------G 3′
   3′G---------CTTAA 5′

4. **Decide the ratio between the ester bonds and hydrogen bonds that are broken in each palindromic sequence of a DNA when treated with *Eco* RI during the formation of sticky ends.**
   Ans: 1:4
5. **Which natural process is comparable to the laboratory process of genetic engineering?**
   Ans: Crossing over.
6. **Which enzyme is recommended during recombinant DNA technology for sealing the nick in DNA?**
   Ans: DNA ligase.
7. **Given below is a sequence of nitrogen bases on piece of DNA. What specific name is given to such DNA?**

   5′ CGA TCG 3′
   3′ GCT AGC 5′

   Ans: Palindromic sequence.
8. **Calculate the number of covalent bonds which are broken when a plasmid vector is cut with *Eco* RI restriction enzyme**
   Ans: Two

Tissue culture

Very Short Answer Questions

1. **What is basal medium?**
   Ans: Nutrient medium consisting of all required inorganic nutrients and organic nutrients like sucrose, amino acids and vitamins but without growth regulators is called as Basal medium or minimal medium.

2. **Define explant.**
   Ans: Any living portion of the plant grown in tissue culture is called as Explant.
3. Define Rhizogenesis and Caulogenesis.
   Ans: Rhizogenesis is differentiation of root in callus in high auxin and low cytokinin concentrations. Caulogenesis is differentiation of stem in low auxin and high cytokinin concentrations.
4. **Define Somatic embryos.**
   Ans: These are asexual embryos formed during tissue culture either directly from explant or from callus.
5. **What is somatic embryogenesis?**
   Ans: It is formation of Embryo like structures from callus.
6. **What is micropropagation?**
   Ans: It is production of large number of plants from a cell or small piece of tissue in limited time and space.
7. **Differentiate between organogenesis and somatic embryogenesis.**
   Ans: Organogenesis is formation of roots and shoot from callus or directly from explant cultured on different concentrations of auxins and cytokinins. Somatic Embryogenesis is formation of embryo like structures from callus or explant.
8. **Differentiate the terms Callus and embryoids.**
   Ans: Callus is undifferentiated mass of tissue formed from explant in tissue culture. Embryoids are embryo like structures formed in tissue culture from callus or explant. These are also known as somatic embryos.
9. **What are synthetic seed?**
   Ans: Sodium alginate encapsulated somatic embryos or embryoids obtained through tissue culture are called as synthetic seed or artificial seed.

Short answer Questions

1. **Enumerate the applications of Plant Tissue culture technique?**
   Ans:1. Millions of plats can be produced without seed in relatively less time, space from small amount of plant material – Micropropagation.

   2. Variations shown by tissue cultured plants (somaclonal variations) are exploited for the improvement of crops.

   3. Virus free plants can be obtained from virus infected plants by shoot tip culture.

   4. In case of crops like Papaya, female plants are required more than male plants. By this method only those plants required can be obtained.

5. Artificial seed can be produced by using sodium alginate which can be stored for longer duration.

6. High value products of medicinal plants are easily collected by culturing medicinal plants.

7. Plant transgenics is mainly dependent on tissue culture.

Long Answer Questions

1. Explain briefly the steps involved in tissue culture.

Ans: The various steps involved in tissue culture are as follows.

1. Preparation of Nutrient Culture Medium: Conditions such as minerals, water, CO_2, O_2, and light energy are provided to the tissue. The nutrient culture medium consists of all essential micro and macro nutrients, amino acids, vitamins and carbohydrates dissolved in distilled water. The pH is adjusted to 5.6 to 6.0. To provide support to the explant the medium is solidified by addition of agar. Though several culture media are formulated by different scientists, the most commonly used one is Murashige and Skoog (MS) medium.

Medium not containing growth regulators is called as basal medium or minimal medium. It used for germinating seeds, callus cultures and developing aseptic seedlings to select as explants. To get complete plant or embryoids from the explant or callus, the medium is supplemented with auxins like IAA, NAA or 2,4-D and cytokinins like Kinetin and Benzyl amine and several other compounds. The medium is dispensed into the glass vials such as glass tubes, flasks or bottles and the opening is plugged with non-absorbent cotton. Cotton plugs allow the exchange of CO_2 and O_2 but prevent the entry of microorganisms.





2. Sterilization of Culture medium: It is making the culture medium free of living microorganisms. Since the medium rich in nutrients, the unwanted microbes which may seek entry into medium during preparation of the medium. Hence the medium should be made microbial free. The sterilization is carried out in an autoclave which works like domestic pressure cooker. The medium is autoclaved at 121^0C, 15lb pressure



for 15 minutes. The culture vials be checked in the next day morning for any microbial growth. Only non-contaminated one must be selected for inoculation of explant.

3. Preparation of Explant: Any living part of the plant which is introduced to culture medium to grow into full fledged plant is called as explant. Usually young and healthy parts of the plant are selected as explant. If an explant is selected from a potted plant, the selected part of the plant carries several microbes on it and it has to be surface sterilized by using either Sodium hypochlorite solution or Calcium hypochlorite solution and later rinsed in distilled water to remove the traces of sterilizer. The explant may develop from seeds and the aseptic seedlings are selected. For this purpose, the seeds must be surface sterilize by using 0.1% Mercuric chloride solution and the seeds be transferred to minimal medium where the seedlings are formed from the seeds and these can be used as explants.





4. Inoculation of explant: Placing the prepared explant on the culture medium under aseptic conditions is called as Inoculation. It is carried out in laminar air flow chamber which maintains aseptic conditions.



5. Incubation for Growth: The inoculated culture vials with explants are incubated in culture room where suitable light intensity, moisture, tempeature are provided. Incubation is carried out for 3 to 4 weeks during which the cells of explant absorb the nutrients, grow and undergo repeated divisions to produce a proliferating mass of undifferentiated cells called callus. It may also form directly roots and shoot. Formation of roots and shoot from callus or directly from explant is called organogensis which includes rhizogenesis and caulogenesis. Sometimes embryo like structures are developed from callus and the phenomenon is called as somatic embryogenesis and the embryos are called embryoids or somatic





embryos or asexual embryos. These somatic embryos are transferred to separate culture media to develop into complete plants. The embryoids may be encapsulated with sodium alginate to form synthetic seed or artificial seed.

6. Acclimatization of plantlets and transfer to pots: The seedlings formed in the above step must be gently acclimatized for their survival in natural conditions. The plants are gently washed to remove the traces of medium and transferred to plastic pots containing soil rite consisting of coconut shell powder and organic manure. The pots are covered with polythene bags and maintained in the laboratory at room temperature for 1 or 2 weeks. The polythene bag provides high humidity to help in acclimatization of plant. The bags are gently removed when the plant appears strong and healthy and it is transferred to a regular pot containing a mixture of soil and manure.



Quiz type Questions

1. **What happens if the glass vessels with culture medium are plugged with absorbent cotton?**
   Ans: The cotton absorbs atmospheric moisture and there won't be any exchange of gases.
2. **Specify the time, temperature and amount of pressure which is strictly required for sterilizing the nutrient medium.**
   Ans: Time is 15 minutes, temperature is 121^0C, and pressure is 15 pounds.
3. **Assume that a callus is subjected to high auxin and low cytokinin nutrient medium followed by high cytokinin and low auxin, what are the physiological phenomena which would result respectively. ?**
   Ans: At first roots are formed which is called as rhizogenesis and later shoot is formed which is called as caulogenesis.
4. **Name the materials involved in the preparation of soilrite.**
   Ans: Coconut shell and organic manure.

Single cell Protein

Very Short Answer Questions

1. **Define biomass.?**
   Ans: It is the mass living organism which is expressed in terms of fresh or dry weight.
2. **Why do fungal and bacterial SCP are not suitable for human consumption?**
   Ans: They have high nucleic acid content whose degradation forms uric acid harmful to health of human beings.
3. **Give the scientific names of torula yeast and bakers yeast.?**
   Ans: Torula yeast – *Candida utilis*, Bakers yeast – *Saccharomyces cerevisiae*
4. **Define single cell protein and give two examples.?**
   Ans: Protein rich microorganisms of a species used as a source of protein upon culturing, purification, drying and powdering is called as single cell protein. Ex: *Spirulina, Chlorella*

Short answer Questions

1. **What are the advantages of SCP?**
   Ans:

   1. Rich in high quality protein and poor in fat.

   2. Good source of B vitamin and minerals.

   3. Easy to manipulate the amino acid composition of the organisms.

   4. SCP organisms grow very fast and produce more protein quantity in less time and space.

   5. They can be grown cheap raw materials like agri and domestic waste and pollution due to this waste can be decreased.

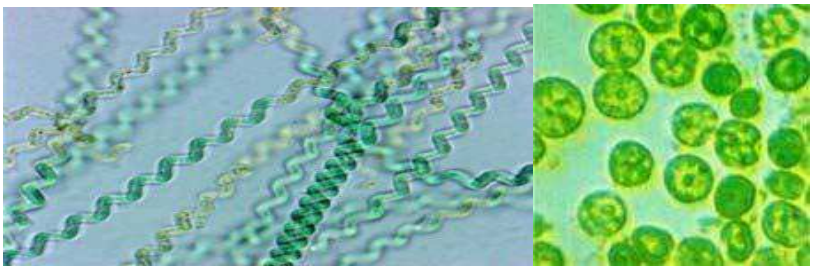
   6. Some SCP form valuable byproducts such as oils, fats and organic acids.

   7. These can be produced throughout the year.

   8. SCP can be produced in any locality and climatic conditions.

Long Answer Questions

1. **Give an account of algae, fungi and bacteria that are used for SCP production.?**
   Ans:
   1. Algae: Algae such as Spirulina maxima, Chlorella pyrenoidosa, Scenedesmusacutus and Dunaliellasalina are used as SCP. They are rich source of proteins (about 60%). They contain good amino acid composition except for the deficiency in sulphur containing amino acids.
   Disadvantages: 1. Have low cell density i.e. 1-2g dry weight/l of substrate.
   2. Severe risk of contamination during growth.

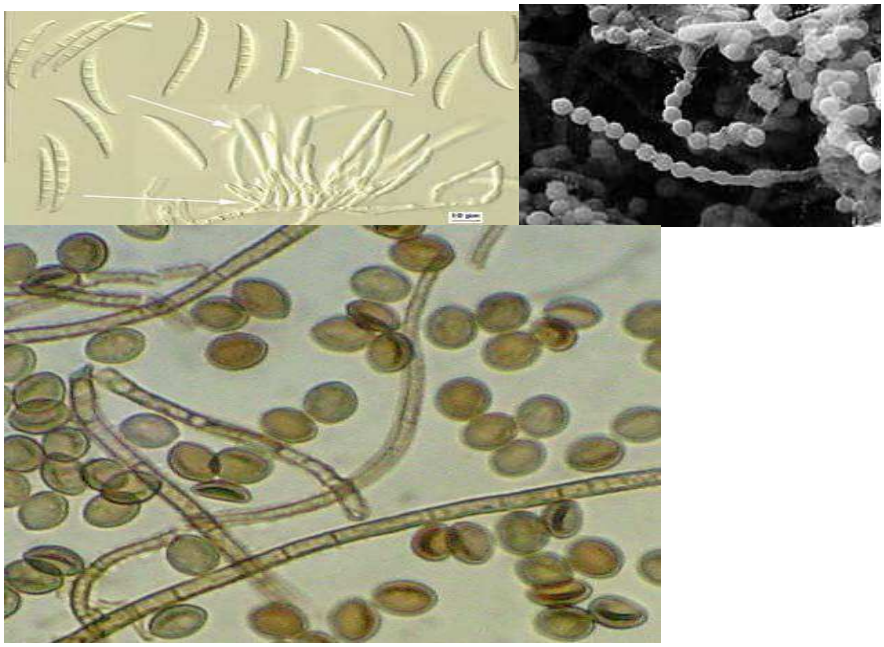


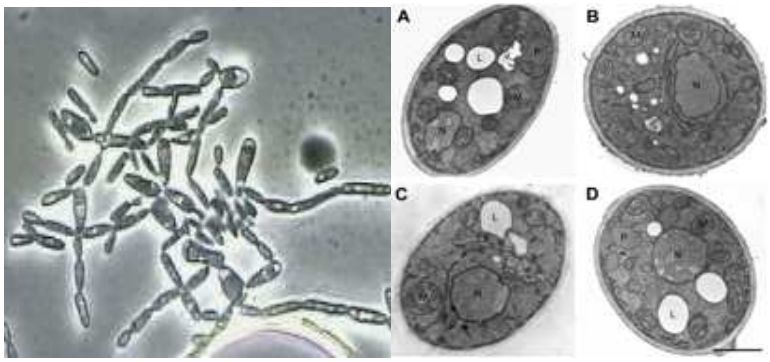


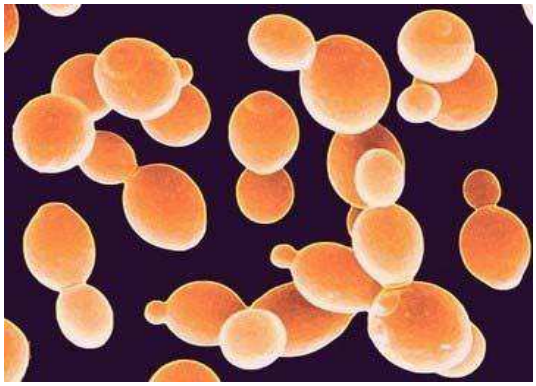
2. Fungi: The filamentous fungi like Chaetomiumcellulolyticum, Fusariumgraminearum, Paecilomycesvarioti and unicellular yeasts like Candida utilis, Candida lipolytica and Saccharomyces cerevisiae (Baker's yeast) are used for the production of SCP. They have high amount of nucleic acid RNA. Yeasts are rich in B-group of vitamins.

Disadvantages of Fungal SCP:

1. These are not suitable for human consumption because they have high amount of RNA which produce damaging levels of uric acid which can not be excreted out by human beings.
2. They show slow rate of growth compared to other SCP organisms.
3. There is risk of contamination during the production process.
4. Some strains produce mycotoxins, hence the strains should be screened.







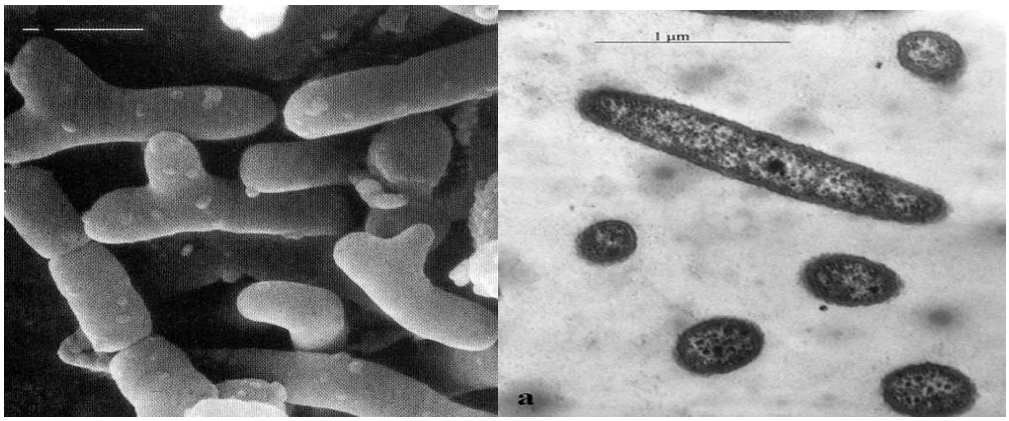
3. Bacteria: Brevibacteriumketoglutamicum, Methylophilusmethylotrophus etc. are bacterial SCP. These contain about 0.3 – 4.0% carbohydrates, 45-80 % proteins, 16 % lipids, 0.5 % β-carotene and 20% nucleic acid RNA. The protein is rich in amino acids such as lysine and methionine.

Disadvantages: 1. These are not suitable for human consumption because they have high nucleic acid content.

2. There is risk of contamination during production process.

3. There is problem in recovering the cells.

4. These should be carefully evaluated for endotoxin production particularly when gram negative bacteria are used.



Quiz type Questions

1. **Candida and Brevibacterium which are used as SCP are not suitable for human consumption. Why?**
   Ans: They have high RNA content which is harmful to human beings and cause health hazards.
2. **What is the importance of Methylophilousmethylotrophus?**
   Ans: It is bacterial SCP and rich in proteins with high content of essential amino acids such as Lysine and methionine.
3. **AUG is the initiating codon on m-RNA. It codes for an amino acid. Which group of SCP organisms is rich in that amino acid?**
   Ans: Bacterial SCP
4. **For which of the following SCP organisms careful evaluation for endotoxin production is necessary?**
   A. Algae B. Filamentous fungi C. Yeasts D. Gram negative bacteria.
   Ans: D. Gram negative bacteria

Mushroom Cultivation

Very Short Answer Questions

1. **Name the mushroom that emits light in the dark. Write briefly about it.**
   Ans: It is Armillariella mellea. It is parasitic fungus causes root rot in Apple and other forest trees. It is used in mines for luminescence. Its emission of light is described as Fox-fire.

2. **Name the fruiting body of mushrooms. Mention its parts.**
   Ans: Basidiocarp. It has pileus, stipe, gills, annulus, trama, sub-hyemnium, Hymenium with basidia and basidiospores.
3. **Name the spore bearing structures of basidiomycotina fungi. What type of spores they produce?**
   Ans: Basidium, it produces basidiospores.
4. **Mention poisonous mushrooms.**
   Ans: Amanita phalloides commonly called as death cap, Amanita muscaria commonly called as Fly Agaric and Amanita virosa commonly called as destroying angel.
5. **What is spawn?**
   Ans: It is seeding material used for the cultivation of mushrooms. It is dikaryotic mycelium of mushrooms present in sterilized organic matter.
6. **Define Flush?**
   Ans: Each crop of mushrooms produced at the end of mushroom production is called as Flush.
7. **Write the common and scientific names of four important cultivated mushrooms?**
   Ans: 1. White button mushroom – Agaricus bisporous, 2. Paddy straw mushroom – Volvariella volvacea, 3. Oyster mushroom – Pleurotus sojar-caju, 4. Shiitake mushroom – Lentinus edodes.

Short answer Questions

1. **Write briefly about food value of mushrooms?**
   Ans: 1. They have low carbohydrate and fat content. Good as food for diabetics and people with high blood cholesterol.

   2. Have 19 – 35% protein content which is much higher than the protein content of conventional vegetarian proteins like pulses animal proteins like milk, fish meat etc.

   3. Rich in essential amino acids like Lysine and Tryptophan

   4. Rich in vitamins pantothenic acid, Vit.B_{12}, ascorbic acid etc.

   5. Agaricus bisporous is rich in P,K,Fe,and Cu but poor in Ca.

   6. They have high K to Na ratio and fibers. Hence, good for people with hypertension and hyperacidity.

2. Give an account of different types of mushrooms.

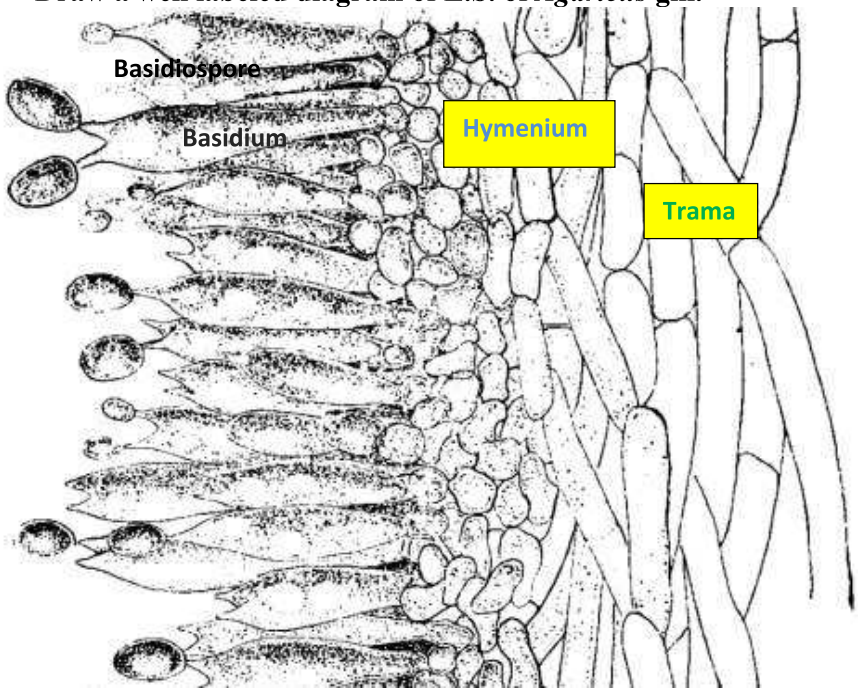
Ans: There are about 200 different species of edible mushrooms. Out of these, 40 species are found as economically useful. Only 8 species are commercially grown. Some of the important cultivated mushrooms grown in large scale are

Scientific name	Common name
1. Agaricus bisporus	White button mushroom (European or temperate mushroom)
2. Vovariella volvacea	Paddy straw mushroom
3. Pleurotus sojar-caju	Oyster mushroom
4. Lentinus edodes	Shiitake mushroom

Some mushrooms are mild to deadly poisonous. These are called as Toad stools. Amanita is poisonous genus among fungi. Some of its species are as follows.

Scientific name	Common name
1. *Amanita virosa*	Destroying angel
2. *Amanita phalloides*	Death cap
3. *Amanita muscaria*	Fly agaric

3. Draw a well labeled diagram of L.S. of *Agaricus* gill.



4. Give a brief account of Morphology of mushrooms?

Ans: Mushroom is the fruiting body of Agaricales. It is technically called as Basidiocarp.



It is developed from the knots of root like structure of mycelium called Rhizomorph. It has pin head stage, Button stage and Adult stage. It may be epigean or hypogean. It has a stalk called stipe and an expanded umbrella like structure known as Pileus. Lower surface of pileus has fertile membranous structures known as gills or lamellae. Stipe has a membranous circular structure around it known as Annulus. Gill has central region with vertical loosely arranged hyphae with elongated cells known as Trama, obliquely running short hyphae that form sub-hymenium and fertile outermost region Hymenium. Hymenium has fertile cells called basidia and sterile cells paraphyses. Basidium has four basidispores developed exogenously.

Long Answer Questions

1. **Explain briefly the cultivation process of white button mushroom.**

   Ans: There are two major steps involved in cultivation of white button mushroom. They are Spawn production and Mushroom production.





1. Spawn production: The seed material of mushroom fungi ix called as Spawn. It is produced in sterile organic matter. It is used as inoculum for mushroom production. It is produced in plastic bags or bottles containing straw or grains. Spawn production is done under aseptic conditions. For grain spawn production, the cereal grains are used either singly or in combination. The grains are boiled in water till all the water is absorbed by the grains. Then calcium carbonate is added to the grains at 20g/Kg of grains. In straw spawn making the straw is cut into 5cm long pieces and soaked in water for 5-10 minutes and mixed with 2% lime. After this, these are taken in containers or bottles and the mouth of the bottles is plugged with non-absorbent cotton. The bottles are sterilized in an autoclave at 15lbs pressure and 121^0C for 1½ hour and then cooled down. A small quantity of the fungus is inoculated into the bottles and later the bottles are incubated at 25^0C temperature for about two weeks. During incubation, the mycelium grows and infests the inoculated material to form a dense growth of mycelia called spawn. It is later used for mushroom production.

2. **Mushroom production:** It has four steps. These are compost preparation, Spawning, Casing and Cropping

A. Compost preparation: It is process of making compost from waste. It helps in rapid growth of mycelium. It is made by mixing paddy straw, urea, cotton seeds, rice bran and gypsum or by mixing wheat straw, chicken manure, urea and Gypsum. The Gypsum improves the texture of compost. After incubating the above raw materials for several days, good compost is formed which will be dark brown in color, lacks greasiness, free of ammonia smell, pH between 7 – 7.5, has 65 – 70% moisture and 2.2% nitrogen content. Once the compost is ready, it is filled into trays or boxes or shelves to make the beds and transferred into the room for pasteurization. The boxes then transferred to the production room at 24^0C and inoculated with spawn.



B. Spawning: A handful of spawn is spread over the compost and a small amount of compost is spread over the spawn. This step is called as Spawning. After this, the beds are sprinkled with water to maintain relative humidity between 70 to 80%. Within 15-20 days, at 25 $\pm 2^0$C the fungus grows luxuriantly.



C. Casing: After spawning, the bed is covered with thin layer of pasteurized soil (Casing) and incubation is continued for 8-10 days till the white button mushrooms start appearing. At this stage, the temperature is maintained at 16 $\pm 2^0$C and 95% relative humidity. If required fresh air is passed and beds are wetted with water as and when required.

D. Cropping: Mushrooms are harvested in button stage by gripping the cap and twisting gently when they are in size of 3-5 cm. An average yield of 10-15 Kg/100 Kg of compost is obtained by good growers within a period of 8-9 weeks. However, the yield depends on the quality of



the compost and the management practices.



Mushrooms must be consumed fresh because they have short shelf life**.** If stored at 5^0C, it can be extended to week days. At high temperatures they become brown colored and rotten. For transport to longer distances, they are specially processed and preserved in cans containing salt solution and cans are kept in boiling water for some time. The cans then sealed and dipped in cool water. Such canned mushrooms can be stored for about six months.

Quiz type Questions

1. **It is observed that from some forest trees, light is emitted during night times. What is that phenomenon called? Which organism is responsible for it?**
   Ans: It is called as Foxfire. The organism Armillariella mellea is responsible for it.
2. **It is not advisable to eat all types of mushrooms. Why?**
   Ans: Because some mushrooms like Amanita species are poisonous and its consumption may lead to death.
3. **Mushrooms are considered as a right protein food than fish. Why?**
   Ans: These have high protein content per unit volume of dry mushroom than fish.
4. **Indole-3-acetic acid (IAA) is synthesized from an amino acid. The same amino acid is present in mushroom. Name the amino acid.**
   Ans: Tryptophan
5. **Do you think mushrooms can also be used as ideal food for those suffering from hypertension and hyperacidity? If yes. Why?**
   Ans: Yes. Because they are poor in lipids and carbohydrates and rich in protein, vitamins, essential amino acids and minerals. Since they are rich in Potassium to Sodium ratio they are good food for persons suffering from hypertension.