

TUBERCULOSIS

T Tuberculosis is caused by various strains of mycobacterium, usually *Mycobacterium tuberculosis*. It usually attacks the lungs but can also affect other parts of the body. It is spread through the air when people who have active MTB infection cough, sneeze, or spit. In most cases the disease is asymptomatic, latent infection, and about 10% latent infections eventually progresses to active disease. If untreated, it kills 50% of its victims. One third of the world's population is thought to be infected with *M. tuberculosis*, and every second a new infection occurs. About 80% of the population in many Asian and African countries test positive in tuberculin test.

DRUG RESISTANT TB

Until 50 years ago, there were no medicines to cure TB. Now, strains that are resistant to a single drug have been documented in every country surveyed. Drug-resistant TB is caused by inconsistent or partial treatment, when patients do not take all their medicines regularly for the required period because they start to feel better, because doctors and health workers prescribe the wrong treatment regimens, or because the drug supply is unreliable. A particularly dangerous form of drug-resistant TB is multidrug-resistant TB (MDR-TB), which is defined as the disease caused by TB bacilli resistant to at least isoniazid and rifampicin, the two most powerful anti-TB drugs. Rates of MDR-TB are high in some countries, especially the former Soviet Union, and threaten TB control.

MDR-TB

TB that is resistant at least to isoniazid and

rifampicin the two most powerful first-line anti-TB drugs are called the Multidrug-resistant tuberculosis (MDR-TB). It develops because the when the course of antibiotics is interrupted and the levels of drug in the body are insufficient to kill 100% of bacteria. This means that even if the patient forgets to take medicine, there are chances of developing MDR-TB. MDR-TB is treated with second line of anti-tuberculosis drugs such as a combination of several medicines called SHREZ (Streptomycin+isonicotinyl Hydrazine+ Rifampicin+ Ethambutol+ pyrazinamide) + MXF+cycloserine).

XDR-TB

When the rate of multidrug resistance in a particular area becomes very high, the control of tuberculosis becomes very difficult. This gives rise to a more serious problem of extensively drug-resistant tuberculosis (XDR-TB). XDR-TB is caused by strains of the disease resistant to both first- and second-line antibiotics. This confirms the urgent need to strengthen TB control. Thus, Extensively-drug resistant TB (XDR-TB) is a sub-set of MDR-TB which is further resistant to at least two more drugs which are second line drugs and is thus virtually incurable.

TDR-TB

Totally drug-resistant tuberculosis (TDR-TB) It is TB which is believed to be resistant to all the first and second line TB drugs. TDR-TB has resulted from further mutations within the bacterial genome to confer resistance, beyond those seen in XDR- and MDR-TB. Development of resistance is associated with poor management of cases. Drug resistance



testing occurs in only 5% of TB cases worldwide. Without testing to determine drug resistance profiles, MDR- or XDR-TB patients may develop resistance to additional drugs. TDR-TB is relatively poorly documented, as many countries do not test patient samples against a broad enough range of drugs to diagnose such a comprehensive array of resistance. The United Nation's Special Program for Research and Training in Tropical Diseases has set up a TDR Tuberculosis Specimen Bank to archive specimens of TDR-TB. India started its TB program with National TB Control Project in 1962 and used BCG as its main intervention. Few years later The Expanded Program on Immunization took over BCG vaccination (1978). This strategy and program didn't work for India and results were disastrous as BCG trials responded badly and showed no protection against infection by TB bacilli (1979). It was recognized that TB control project has been out of its reach and needs effective restructuring. India launched Revised National TB Control Program on the backdrop of WHO recommended DOTS strategy after piloting tests from 1993 to 1996. RNTCP is a fully Central Sponsored Scheme and works for free from diagnosis to treatment. It uses DOTS strategy of WHO and all component of STOP TB strategy of WHO. It had two phases. First phase was from 1998 to 2005 where focus was on ensuring expansion of quality DOTS services to the entire country. The second phase (2006-2011) concentrated on extensive services and set target to detect the rate of new smear positive cases (70%) and maintain a cure rate of at least 85%.

DOTS: Direct Observatory Treatment Short-course

It is a key component of the WHO campaign to Stop TB strategy. India's RNTCP is premised upon DOTS. It involves the

volunteer's (trained health professionals) based health services to patients, drugs and services are provided at the doorstep of patients and service provider keeps a track on the diseased. As a part of DOTS strategy health workers counsel and observe their patients swallowing each dose of powerful combination of medicines and keeping track on their complete drug usage. In 2012, WHO's Annual Report on TB reported that though DOTS saved lives from TB mortality

AIDS

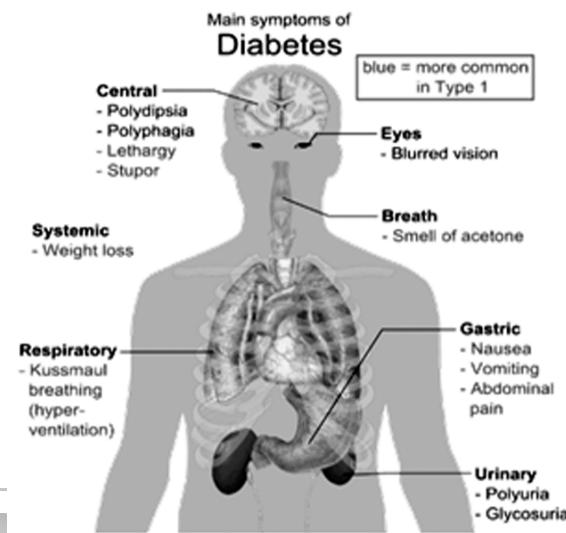
AIDS is an infectious disorder that suppresses the normal function of the immune system. It is caused by the human immunodeficiency virus (HIV), which destroys the body's ability to fight infections. Specific cells of the immune system that are responsible for the proper response to infections (T cells) are destroyed by this virus. Characteristically a person infected with HIV initially experiences no symptoms for a variable period of time. This may be followed by the development of persistent generalized swelling of the lymph nodes (AIDS-related lymphadenopathy). Eventually most patients infected with HIV experience a syndrome of symptoms that includes excessive fatigue, weight loss, and/or skin rashes. The later stages of HIV infection are characterized by the progressive depression of T cells and repeated infections that can even occur during a course of antibiotic therapy for another infection (superinfections). People with AIDS are particularly vulnerable to "opportunistic infections" from bacteria that other people normally fight off. *Pneumocystis carinii*, which causes severe inflammation of the lungs (pneumonia), is a common infection that affects people with AIDS. Cancers (malignant neoplasms), and a wide variety of neurological abnormalities, most notably the AIDS dementia complex, may also occur. These



neurological symptoms when of HIV, infects the nervous system. HIV is transmitted by three main routes: sexual contact, exposure to infected body fluids or tissues, and from mother to child during pregnancy, delivery, or breastfeeding (known as vertical transmission). There is no risk of acquiring HIV if exposed to faeces, nasal secretions, saliva, sputum, sweat, tears, urine, or vomit unless these are contaminated with blood. It is possible to be co-infected by more than one strain of HIV—a condition known as HIV superinfection. There is currently no cure or effective HIV vaccine. Treatment consists of highly active antiretroviral therapy (HAART) which slows progression of the disease. As of 2010 more than 6.6 million people were taking them in low and middle income countries. Treatment also includes preventive and active treatment of opportunistic infections. Current HAART options are combinations (or "cocktails") consisting of at least three medications belonging to at least two types, or "classes," of antiretroviral agents. Initially treatment is typically a non-nucleoside reverse transcriptase inhibitor (NNRTI) plus two nucleoside analogue reverse transcriptase inhibitors (NRTIs). Typical NRTIs include zidovudine (AZT) or tenofovir (TDF) and lamivudine (3TC) or emtricitabine (FTC). Combinations of agents which include a protease inhibitors (PI) are used if the above regimen loses effectiveness.

DIABETES

Diabetes, often referred to by doctors as diabetes mellitus, describes a group of metabolic diseases in which the person has high blood glucose (blood sugar), either because insulin production is inadequate, or because the body's cells do not respond properly to insulin, or both. Patients with high blood sugar will typically experience polyuria (frequent urination), they will become



increasingly thirsty (polydipsia) and hungry (polyphagia). Diabetes (diabetes mellitus) is classed as a metabolism disorder. Metabolism refers to the way our bodies use digested food for energy and growth. Most of what we eat is broken down into glucose. Glucose is a form of sugar in the blood - it is the principal source of fuel for our bodies. When our food is digested, the glucose makes its way into our bloodstream. Our cells use the glucose for energy and growth. However, glucose cannot enter our cells without insulin being present - insulin makes it possible for our cells to take in the glucose. Insulin is a hormone that is produced by the pancreas. After eating, the pancreas automatically releases an adequate quantity of insulin to move the glucose present in our blood into the cells, as soon as glucose enters the cells blood-glucose levels drop. A person with diabetes has a condition in which the quantity of glucose in the blood is too elevated (hyperglycemia). This is because the body either does not produce enough insulin, produces no insulin, or has cells that do not respond properly to the insulin the pancreas produces. This results in too much glucose building up in the blood. This excess blood glucose eventually passes out of the body in urine. So, even though the



blood has plenty of glucose, the cells are not getting it for their essential energy and growth requirements.

There are three types of diabetes:

(1) Type 1 Diabetes

The body does not produce insulin. Some people may refer to this type as insulin-dependent diabetes, juvenile diabetes, or early-onset diabetes. People usually develop type 1 diabetes before their 40th year, often in early adulthood or teenage years. Type 1 diabetes is nowhere near as common as type 2 diabetes. Approximately 10% of all diabetes cases are type 1. Patients with type 1 diabetes will need to take insulin injections for the rest of their life. They must also ensure proper blood-glucose levels by carrying out regular blood tests and following a special diet.

(2) Type 2 Diabetes

The body does not produce enough insulin for proper function, or the cells in the body do not react to insulin (insulin resistance). Approximately 90% of all cases of diabetes worldwide are of this type. Some people may be able to control their type 2 diabetes symptoms by losing weight, following a healthy diet, doing plenty of exercise, and monitoring their blood glucose levels. However, type 2 diabetes is typically a progressive disease - it gradually gets worse - and the patient will probably end up have to take insulin, usually in tablet form. Overweight and obese people have a much higher risk of developing type 2 diabetes compared to those with a healthy body weight. People with a lot of visceral fat, also known as central obesity, belly fat, or abdominal obesity, are especially at risk. Being overweight/obese causes the body to release chemicals that can destabilize the body's cardiovascular and metabolic systems. Being overweight, physically inactive and eating the

wrong foods all contribute to our risk of developing type 2 diabetes. The scientists believe that the impact of sugary soft drinks on diabetes risk may be a direct one, rather than simply an influence on body weight. The risk of developing type 2 diabetes is also greater as we get older. Experts are not completely sure why, but say that as we age we tend to put on weight and become less physically active. Those with a close relative who had/had type 2 diabetes, people of Middle Eastern, African or South Asian descent also have a higher risk of developing the disease. Men whose testosterone levels are low have been found to have a higher risk of developing type 2 diabetes.

(3) Gestational Diabetes

This type affects females during pregnancy. Some women have very high levels of glucose in their blood, and their bodies are unable to produce enough insulin to transport all of the glucose into their cells, resulting in progressively rising levels of glucose. Diagnosis of gestational diabetes is made during pregnancy. The majority of gestational diabetes patients can control their diabetes with exercise and diet. Between 10% to 20% of them will need to take some kind of blood-glucose-controlling medications. Undiagnosed or uncontrolled gestational diabetes can raise the risk of complications during childbirth. The baby may be bigger than he/she should be. Scientists from the National Institutes of Health and Harvard University found that women whose diets before becoming pregnant were high in animal fat and cholesterol had a higher risk for gestational diabetes, compared to their counterparts whose diets were low in cholesterol and animal fats.

Prediabetes

The vast majority of patients with type 2 diabetes initially had prediabetes. Their blood



glucose levels where higher than normal, but not high enough to merit a diabetes diagnosis. The cells in the body are becoming resistant to insulin. Studies have indicated that even at the prediabetes stage, some damage to the circulatory system and the heart may already have occurred.

Controlling Diabetes

All types of diabetes are treatable. Diabetes type 1 lasts a lifetime, there is no known cure. Type 2 usually lasts a lifetime, however, some people have managed to get rid of their symptoms without medication, through a combination of exercise, diet and body weight control. Researchers from the Mayo Clinic Arizona in Scottsdale showed that gastric bypass surgery can reverse type 2 diabetes in a high proportion of patients. They added that within three to five years the disease recurs in approximately 21% of them. Patients with type 1 are treated with regular insulin injections, as well as a special diet and exercise. Patients with Type 2 diabetes are usually treated with tablets, exercise and a special diet, but sometimes insulin injections are also required. If diabetes is not adequately controlled the patient has a significantly higher risk of developing complications.

Complications linked to badly controlled diabetes:

- Eye complications** - glaucoma, cataracts, diabetic retinopathy, and some others.
- Foot complications** - neuropathy, ulcers, and sometimes gangrene which may require that the foot be amputated
- Skin complications** - people with diabetes are more susceptible to skin infections and skin disorders
- Heart problems** - such as ischemic heart disease, when the blood supply to the heart muscle is diminished

- Hypertension** - common in people with diabetes, which can raise the risk of kidney disease, eye problems, heart attack and stroke
- Mental health** - uncontrolled diabetes raises the risk of suffering from depression, anxiety and some other mental disorders
- Hearing loss** - diabetes patients have a higher risk of developing hearing problems
- Gum disease** - there is a much higher prevalence of gum disease among diabetes patients
- Gastroparesis** - the muscles of the stomach stop working properly
- Ketoacidosis** - a combination of ketosis and acidosis; accumulation of ketone bodies and acidity in the blood.
- Neuropathy** - diabetic neuropathy is a type of nerve damage which can lead to several different problems.
- HHNS (Hyperosmolar Hyperglycemic Nonketotic Syndrome)** - blood glucose levels shoot up too high, and there are no ketones present in the blood or urine. It is an emergency condition.
- Nephropathy** - uncontrolled blood pressure can lead to kidney disease
- PAD (peripheral arterial disease)** - symptoms may include pain in the leg, tingling and sometimes problems walking properly
- Stroke** - if blood pressure, cholesterol levels, and blood glucose levels are not controlled, the risk of stroke increases significantly
- Erectile dysfunction** - male impotence.
- Infections** - people with badly controlled diabetes are much more susceptible to infections
- Healing of wounds** - cuts and lesions take much longer to heal



CHOLESTROL

Cholesterol is a form of fat found in the blood and all cells of the body. It is critically important in helping form cell membranes, steroid hormones and bile acid, but cholesterol can also build up in the inner walls of the arteries that supply blood to the heart. Those deposits contribute to the formation of plaque, which can cause the arteries to narrow, making them less efficient at transporting blood. Cholesterol is only slightly soluble in water; it dissolves into the (water-based) bloodstream only at exceedingly small concentrations. Instead, cholesterol is transported inside lipoproteins, complex discoidal particles with exterior amphiphilic proteins and lipids, whose outward-facing surfaces are water-soluble and inward-facing surfaces are lipid-soluble. Triglycerides and cholesterol esters are carried internally. Phospholipids and cholesterol, being amphipathic, are transported in the monolayer surface of the lipoprotein particle. There are several types of lipoproteins in the blood. In order of increasing density, they are very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL). Total cholesterol is defined as the sum of HDL, LDL, and VLDL. High levels of cholesterol contribute to build-up of plaque in the arteries, blood vessels that carry oxygen-rich blood to your organs and different parts of your body. Over time, plaque hardens and narrows your arteries. This condition is called atherosclerosis. Atherosclerosis limits the flow of oxygen-rich blood and can affect any artery in the body, including arteries in the heart, brain, arms, legs, pelvis, and kidneys. As a result, different diseases may develop based on which arteries are affected. Some of these diseases include:

- coronary heart disease – that affects the arteries the supply blood to the heart

- carotid artery disease - that affects the arteries located in the neck and deliver blood to the brain
- peripheral artery disease that affects arteries that carry blood to your head, organs, and limbs

Low-density Lipoproteins (LDL) This is the “bad” cholesterol, which increases your risk of heart disease. Too much LDL in the blood can lead to cholesterol build-up and artery blockages, a condition known as Hypercholesterolemia. Resistin, a protein secreted by fat tissue, has been shown to increase the production of LDL in human liver cells and also degrades LDL receptors in the liver. As a result, the liver is less able to clear cholesterol from the bloodstream.

Resistin accelerates the accumulation of LDL in arteries, increasing the risk of heart disease. Resistin also adversely impacts the effects of statins, the main cholesterol-reducing drug used in the treatment and prevention of cardiovascular disease. However, Abnormally low levels of cholesterol are termed hypocholesterolemia and it leads to depression, cancer, and cerebral hemorrhage.

High-density Lipoproteins (HDL) This is known as the “good” cholesterol because it works to slow the build-up of cholesterol by carrying it away from the arteries to be expelled from the body. High levels of HDL cholesterol seem to help protect against heart attack and other cardiovascular complications.

Fats contain long hydrocarbon chains, which can either be unsaturated, i.e. have double bonds, or saturated, i.e. have no double bonds. Partial hydrogenation of the unsaturated fat yields a trans fat. *Trans* fats, or trans-unsaturated fatty acids, trans fatty acids, are a type of unsaturated fats which became commonly produced industrially from vegetable fats for use in margarine, snack food, packaged baked goods and frying fast



food. Trans fat has been shown to consistently be associated, in an intake-dependent way, with risk of coronary heart disease in part by raising levels of the lipoprotein LDL (so-called "bad cholesterol"), lowering levels of the lipoprotein HDL ("good cholesterol"), increasing triglycerides in the bloodstream and promoting systemic inflammation.

VACCINATION

The word "vaccine" originates from the Latin Variolae vaccinae (cowpox), which Edward Jenner demonstrated in 1798 could prevent smallpox in humans. Today the term 'vaccine' applies to all biological preparations, produced from living organisms, that enhance immunity against disease and either prevent (prophylactic vaccines) or, in some cases, treat disease (therapeutic vaccines). Vaccines are administered in liquid form, either by injection, by oral, or by intranasal routes. T-cell memory is very important for long-lasting immunity, because T-cells control both humoral and cell mediated immunity. When the immune system recognizes a foreign antigen for the first time, an immune response is produced. When T cells are involved, immunological T-cell memory is produced. When the body encounters same

antigen subsequently, a stronger immune response is produced. This is because of existing immunological memory against that antigen. Further antigenic stimulus increases the immune response. First antigenic stimulus is "priming" whereas subsequent stimuli are "booster". This is the principle of active immunization. Vaccination involves deliberate exposure to antigen under conditions where disease should not result. Vaccination is aimed at inducing active immunity in an individual, so that subsequent contact with the microorganism following natural infection induces strong protective immune response. The protective immunity may involve secretion of neutralizing antibodies or production of memory CTL or Th1 cells. The use of vaccines is now being extended to immunize against tumors or to block fertilization (contraceptive vaccines). A vaccine is a suspension of whole (live or inactivated) or fractionated bacteria or viruses that have been rendered non-pathogenic, and is given to induce an immune response and prevent disease. Even though no vaccine is entirely safe or completely effective, their use is strongly supported by their benefit-to-risk ratio.

Type of vaccine	Examples
Live-attenuated	Measles, Mumps, Rubella, Varicella zoster
Inactivated	Hepatitis A, Influenza, Pneumococcal polysaccharide
Recombinant sub-unit	Hepatitis B
Toxoid	Tetanus, Diphtheria
Conjugate polysaccharide-protein	Pneumococcal, meningococcal, <i>Haemophilus influenzae</i> type b (Hib)



Properties of ideal vaccine:

- Provide long lasting immunity.
- Should induce both humoral and cellular immunity.
- Should not induce autoimmunity or hypersensitivity.
- Should be inexpensive to produce, easy to store and administer.
- Vaccines must also be perceived to be safe.

The vaccine vial may contain relevant antigen, adjuvant (usually alum), preservatives and/or traces of protein derived from the cells in which the vaccine agent was cultured e.g. egg protein

TYPES OF VACCINES**A. KILLED VACCINES**

When it is unsafe to use live microorganisms to prepare vaccines, they are killed or inactivated. These are preparations of the normal (wild type) infectious, pathogenic microorganisms that have been rendered nonpathogenic, usually by treatment with using heat, formaldehyde or gamma irradiation so that they cannot replicate at all. Such killed vaccines vary greatly in their efficacy.

Advantages

- Safe to use and can be given to immunodeficient and pregnant individuals.
- Cheaper than live attenuated vaccine
- Storage not as critical as live vaccine

Disadvantages

- Since the microorganisms cannot multiply, a large number are required to stimulate immunity.
- Periodic boosters must be given to maintain immunity.
- Only humoral immunity can be induced.
- Most killed vaccines have to be injected.

- Presence of some un-inactivated microbes can lead to vaccine-associated disease.

B. LIVE ATTENUATED VACCINE

These vaccines are composed of live, attenuated microorganisms that cause a limited infection in their hosts sufficient to induce an immune response, but insufficient to cause disease. To make an attenuated vaccine, the pathogen is grown in foreign host such as animals, embryonated eggs or tissue culture, under conditions that make it less virulent. The strains are altered to a non-pathogenic form; for example, its tropism has been altered so that it no longer grows at a site that can cause disease. These tend to be less virulent for the original host. These vaccines may be given by injection or by the oral route. A major advantage of live virus vaccines is that because they cause infection, the vaccine very closely reproduces the natural stimulus to the immune system.

Advantages

- Infectious microbes can stimulate generation of memory cellular as well as humoral immune responses.
- Since these can multiply in the host, fewer quantities must be injected to induce protection.
- A single administration of vaccine often has a high efficacy in producing long-lived immunity. Multiple booster doses may not be required.
- Whole microbes stimulate response to antigens in their natural conformation. They raise immune response to all protective antigens.
- Some live vaccines can be given orally; such vaccines induce mucosal immunity and IgA synthesis, which gives more protection at the normal site of entry.



- Oral preparations are less expensive than giving injections.
- They can lead to elimination of wild type virus from the community

Disadvantages

- May very rarely revert to its virulent form and cause disease.
- Live vaccines cannot be given safely to immunosuppressed individuals. Administration of live attenuated vaccines to people with impaired immune function can cause serious illness or death in the vaccine recipient.
- Since they are live and because their activity depends on their viability, proper storage is critical.
- Spread to contacts of vaccinee who have not consented to be vaccinated. In some cases, it turns out to be an advantage.

C. SUBUNIT VACCINES

Subunit vaccines contain purified antigens instead of whole organisms. Such a preparation consists of only those antigens that elicit protective immunity. Subunit vaccines are composed of toxoids, subcellular fragments, or surface antigens. Administration of whole organism, as in case of pertussis was found unfavorable immune reactions resulting in severe side effects. The effectiveness of subunit vaccines is increased by giving them in adjuvants. Adjuvants slow antigen release for a more sustained immune stimulation.

Advantages

- They can safely be given to immunosuppressed people
- They are less likely to induce side effects.

Disadvantages

- Antigens may not retain their native conformation, so that antibodies produced

against the subunit may not recognize the same protein on the pathogen surface.

- Isolated protein does not stimulate the immune system as well as a whole organism vaccine.

Peptide vaccines

Peptide vaccine consists of those peptides from the microbial antigen that stimulates protective immunity. Synthetic peptides are produced by automated machines rather than by microorganisms. Peptide immunogenicity can be increased by giving them in ISCOMS, lipid micelles that transport the peptides directly into the cytoplasm of dendritic cells for presentation on Class I Major Histocompatibility Complex. Injected peptides, which are much smaller than the original virus protein, induce an IgG response.

Advantages

- If the peptide that induces protective immunity is identified, it can be synthesized easily on a large scale.
- It is safe and can be administered to immunodeficient and pregnant individuals.
- Disadvantage
- Poor antigenicity. Peptide fragments do not stimulate the immune system as well as a whole organism vaccine.
- Since peptides are closely associated with HLA alleles, some peptides may not be universally effective at inducing protective immunity.

D. CONJUGATE VACCINES

Conjugate vaccines are primarily developed against capsulated bacteria. While the purified capsular antigen can act as subunit vaccine, they stimulate only humoral immunity. Polysaccharide antigens are T independent, they generate short-lived immunity. Immunity to these organisms requires opsonizing



antibodies. Infants cannot mount good T-independent responses to polysaccharide antigens. By covalently linking the polysaccharides to protein carriers, they are converted into T-dependent antigens and protective immunity is induced.

E. RECOMBINANT VACCINES

The vaccines are produced using recombinant DNA technology or genetic engineering. Recombinant vaccines are those in which genes for desired antigens of a microbe are inserted into a vector.

Techniques

- Using the engineered vector (e.g., Vaccinia virus) that is expressing desired antigen as a vaccine
- The engineered vector (e.g., yeast) is made to express the antigen, such is vector is grown and the antigen is purified and injected as a subunit vaccine. Other expression vectors include the bacteria Escherichia coli, mutant Salmonella spp., and BCG.
- Introduction of a mutation by deleting a portion of DNA such that they are unlikely to revert can create an attenuated live vaccine.
- Live attenuated vaccines can also be produced by reassortment of genomes of virulent and avirulent strains.
- Genes coding for significant antigens are introduced into plants, such that the fruits produced bear foreign antigens. This is edible vaccine and is still in experimental stage.

Advantages

- Those vectors that are not only safe but also easy to grow and store can be chosen.
- Antigens which do not elicit protective immunity or which elicit damaging

responses can be eliminated from the vaccine. Example Cholera toxin A can be safely removed from cholera toxin.

Disadvantages

- Since the genes for the desired antigens must be located, cloned, and expressed efficiently in the new vector, the cost of production is high.
- When engineered vaccinia virus is used to vaccinate, care must be taken to spare immunodeficient individuals.

F. DNA VACCINES

These vaccines are still in experimental stage. Like recombinant vaccines, genes for the desired antigens are located and cloned. The DNA is injected into the muscle of the animal being vaccinated, usually with a "gene gun" that uses compressed gas to blow the DNA into the muscle cells. DNA can be introduced into tissues by bombarding the skin with DNA-coated gold particles. It is also possible to introduce DNA into nasal tissue in nose drops. Some muscle cells express the pathogen DNA to stimulate the immune system. DNA vaccines have induced both humoral and cellular immunity.

Advantages

- DNA is very stable, it resists extreme temperature and hence storage and transport are easy.
- A DNA sequence can be changed easily in the laboratory.
- The inserted DNA does not replicate and encodes only the proteins of interest.
- There is no protein component and so there will be no immune response against the vector itself.
- Because of the way the antigen is presented, there is a cell-mediated response that may be directed against any antigen in the pathogen.



Disadvantages

- Potential integration of DNA into host genome leading to insertional mutagenesis.
- Induction of autoimmune responses: anti-DNA antibodies may be produced against introduced DNA.
- Induction of immunologic tolerance: The expression of the antigen in the host may lead to specific non-responsiveness to that antigen.

G. ANTI-IDIOTYPIC VACCINE

An antigen binding site in an antibody (paratope) is a reflection of the three-dimensional structure of part of the antigen (epitope). This unique amino acid structure in the antibody is known as the idiotype, which can be considered as a mirror of the epitope in the antigen. Antibodies can be raised against the idiotype by injecting the antibody into another animal. This anti-idiotype antibody mimics part of the three dimensional structure of the antigen. This can be used as a vaccine. When the anti-idiotype antibody is injected into a vaccinee, antibodies (anti-anti-idiotype antibodies) are formed that recognize a structure similar to part of the virus and might potentially neutralize the virus. Advantage: Antibodies against potentially significant antigen can be produced. Disadvantage: Only humoral immunity is produced. There is no cellular immunity and poor memory. Identification and preparation of idiotypes is labour intensive and difficult.

DIABETES

PLANT TISSUE CULTURE

Plant research often involves growing new plants in a controlled environment. These may be plants that we have genetically altered in some way or may be plants of which we need

many copies all exactly alike. These things can be accomplished through tissue culture of small tissue pieces from the plant of interest. These small pieces may come from a single mother plant or they may be the result of genetic transformation of single plant cells which are then encouraged to grow and to ultimately develop into a whole plant. Tissue culture techniques are often used for commercial production of plants as well as for plant research.

Plant tissue culture is the technique of maintaining and growing plant cells, tissues or organs especially on artificial medium in suitable containers under controlled environmental conditions. The part which is cultured is called explant, i.e., any part of a plant taken out and grown in a test tube, under sterile conditions in special nutrient media. This capacity to generate a whole plant from any cell/explant is called cellular toti-potency. In fact, the whole plant can be regenerated from any plant part (referred to as explant) or cells. Gottlieb Haberlandt first initiated tissue culture technique in 1902.

Hormones used in Plant Tissue Culture:

1. Auxins neoline (Indole-3-acetic acid, Indole-3-butyric acid, Potassium Salt—Naphthalene acetic acid 2, 4-Dichlorophenoxyacetic acid p-Chlorophenoxy acetic acid)
2. Cytokinins (6-Benzylaminopurine, 6-Dimethylallylaminopurine (2ip), Kinetin)
3. Gibberellins (Gibberellic Acid)
4. Abscisic Acid (ABA) (Abscisic Acid)
5. Polyamines (Putrescine, Spermidine)

Environmental Conditions:

There are three important aspects in vitro (outside the living organism and in an artificial environment) culture namely:



1. Nutrient Medium:

The composition of plant tissue culture medium can vary depending upon the type of plant tissues or cell that are used for culture. A typical (generalized) nutrient consists of inorganic salts (both micro and macro elements), a carbon source (usually sucrose), vitamins (e.g., nicotinic acid, thiamine, pyridoxine and myoinositol), amino acids (e.g., arginine) and growth regulators (e.g., auxins like 2,4-D or 2,4-dichlorophenoxyacetic acid and cytokinins such as BAP = benzylaminopurine and gibberellins). Other compounds like casein hydrolysate, coconut milk, malt extract, yeast extract, tomato juice, etc. may be added for specific purposes.

Plant hormones play important role in growth and differentiation of cultured cells and tissues. An optimum pH (usually 5.7) is also very important. The most extensively used nutrient medium is MS medium which was developed by Murashige and Skoog in 1962. Usually a gelling agent agar (a polysaccharide obtained from a red algae *Gelidium amansii*) is added to the liquid medium for its solidification.

2. Aseptic Conditions (Sterilization):

Nutrient medium contains ample sugar which increases growth of microorganisms such as bacteria and fungi. These microbes compete with growing tissue and finally kill it. It is essential to maintain aseptic conditions of tissue culture. Thus sterilization means complete destruction or killing of microorganisms so that complete aseptic conditions are created for in vitro culturing.

3. Aeration of the Tissue:

Proper aeration of the cultured tissue is also an important aspect of culture technique. It is achieved by occasionally stirring the medium by stirring or by automatic shaker.

Methods of Plant Tissue Culture:

Plant tissue culture includes two major methods:

- (A) Type of in vitro growth-callus and suspension cultures.
- (B) Type of explant— single cell culture, shoot and root cultures, somatic embryo culture, meristem culture, anther culture and haploid production, protoplast culture and somatic hybridisation, embryo culture, ovule culture, ovary culture, etc.

Types of Plant Tissue Culture

Callus and Suspension Cultures

In callus culture, cell division in explant forms a callus. Callus is irregular unorganised and undifferentiated mass of actively dividing cells. Darkness and solid medium gelled by agar stimulates callus formation. The medium ordinarily contains the auxin, 2,4-D, (2, 4-dichlorophenoxy acetic acid) and often a cytokinin like BAP (Benzyl aminopurine). Both are growth regulators. This stimulates cell division in explant. Callus is obtained within 2-3 weeks. A suspension culture consists of single cells and small groups of cells suspended in a liquid medium. Usually, the medium contains the auxin 2,4-D. Suspension cultures must be constantly agitated at 100-250 rpm (revolutions per minute). Suspension cultures grow much faster than callus culture.

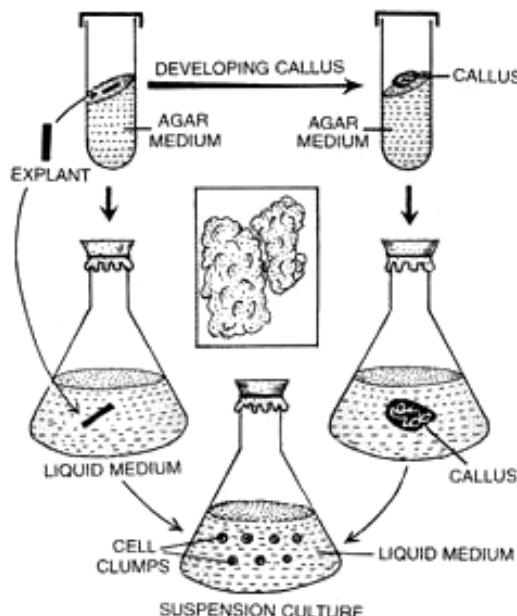
Sub culturing

If tissue cultures are kept in the same culture vessel, they die in due course of time. Therefore, cells/tissues are regularly transferred into new culture vessels containing fresh media. This process is called sub culturing. It is important to note that during subculture; only a part of the culture from a vessel is transferred into the new culture

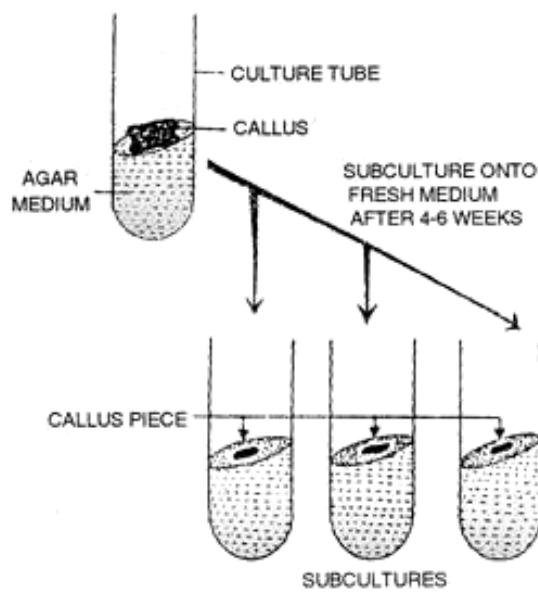


vessel. The callus and suspension cultures may be used to achieve cell biomass production,

regeneration of plantlets, production of transgenic plants and isolation of protoplasts.



Initiation of callus and suspension cultures.



Schematic representation of subculturing.

Single Cell Culture (Cell Cloning)

As stated earlier, cells derived from a single cell through mitosis constitute a clone and the process of obtaining clones is called cloning (asexual progeny of a single individual make up a clone).

Shoot and Root Cultures

Shoot culture is promoted by a cytokinin like BAR. However, root culture is promoted by an auxin like NAA (naphthalene acetic acid). The shoot and root cultures are generally controlled by auxin-cytokinin balance. Usually, an excess of auxin promotes root culture, whereas that of cytokinin promotes shoot culture. Roots culture from the lower end of these shoots to give complete plantlets.

Somatic Embryo Culture

A somatic embryo develops from a somatic cell. The pattern of development of a somatic

embryo is comparable to that of a zygotic embryo. Somatic embryo culture is induced by a high concentration of an auxin, such as 2,4-D. These embryos develop into mature embryos. Mature somatic embryos or embryoids germinate to give complete plantlets.

Establishment in the Field:

The plantlets are removed from culture vessels and established in the field. This transfer is done by specific procedures called hardening. During hardening, plantlets are kept under reduced light and high humidity. Hardening procedures make the plantlets capable of tolerating the relatively harsher environments outside the culture vessels.

Endosperm Culture

Tissue culture methods are also used for culturing endosperm. It is unique because it supplies nutrition to the developing embryo. It is also triploid in its chromosome

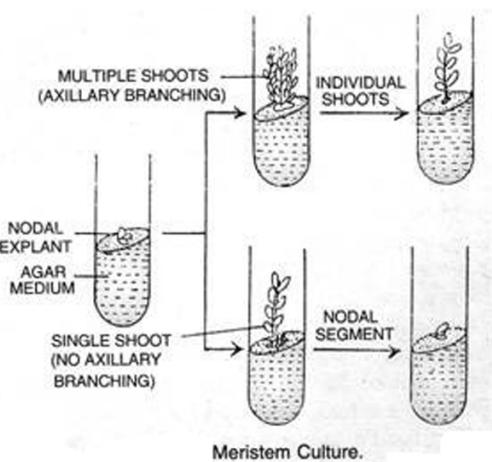


constitution. Triploid plants are used for the production of seedless fruits (e.g., apple, banana etc.). The technique of endosperm culture involves the following:

- (i) The immature seeds are dissected under aseptic condition. Endosperms along with embryos, are excised. Sometimes, mature seeds can also be used.
- (ii) The excised endosperms are cultured on a suitable medium and embryos are removed after initial growth.
- (iii) The initial callus phase is developed.
- (iv) The shoots and roots may develop and complete triploid plants are formed for further use.

Meristem Culture

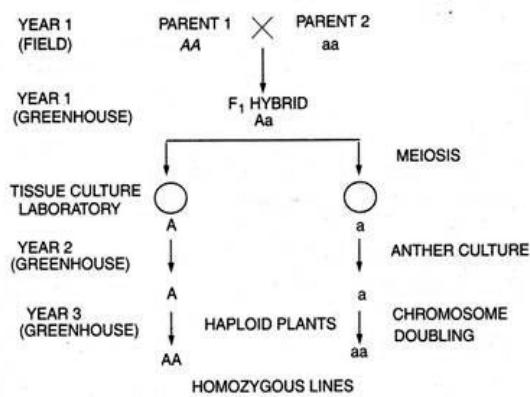
Meristem is a localized group of cells, which are actively dividing and undifferentiated but ultimately giving rise to permanent tissue. Although the plant is infected with a virus, yet the meristem is free of virus. Therefore, meristem can be removed and grown *in vitro* to obtain virus free plants. Cultivation of axillary or apical shoot meristems is called meristem culture. The apical or axillary meristems are generally free from virus. Meristem culture involves the development of an already existing shoot meristem and subsequently, the regeneration of adventitious roots from the developed shoots.



It usually does not involve the regeneration of a new shoot meristem. The explants commonly used in meristem culture are shoot tips and nodal segments. These explants are cultured on a medium containing a cyto-kinin (generally BAP). The plantlets thus obtained are subjected to hardening and, ultimately, established in the field. Meristem culture is carried out in Potato, Banana, Cardamom, Orchids (protocorm stage), Sugar-cane, Strawberry, Sweet Potato, etc. It is used in (i) Production of virus-free plants like potato, sugarcane, banana and apple, (ii) Germplasm conservation, (iii) Production of transgenic plants, (iv) Rapid clonal multiplication.

Anther Culture and Haploid Production:

An individual/cell having the chromosome number found in the gametes of the species is called haploid. Formation of haploid is called haploid production. Thus haploid individuals arise from the gametes. A haploid has only one copy of each chromosome. Haploids are sterile and of no direct value.



Production of homozygous lines using anther culture.
The two parents are shown to differ for only one gene, i.e., AA and aa.

When the chromosome number of a haploid plant is doubled, the plants of normal chromosome number for particular species are obtained. These plants are homozygous and are produced in 2-3 years. The chromosome



number of these haploid plants is doubled by using colchicine to obtain homozygous plants.

In nature, haploid plants originate from unfertilized egg cells, but in laboratory, they can be produced from both male and female gametes. Anther is the part of the flower of Angiosperms producing pollen (microspores), borne at the end of the stamens and usually consisting of four sporangia. When anthers of some plants are cultured on a suitable medium to produce haploid plants, it is called anther culture. The technique was developed by Guha and Maheshwari (1964) who cultured mature anthers of *Datura innoxia*. It is highly useful for the improvement of many crop plants. It is also useful for immediate expression of mutations and quick formation of purelines. This technique was first used in India to produce haploids of *Datura*. In many plants, haploids are also produced by culturing unfertilized ovaries/ovules. Sometimes, pollen grains are separated from anthers and cultured on suitable medium.

Embryo Culture

Culturing young embryos on a nutrient medium is called embryo culture. Young embryos are obtained from the developing seeds. The embryos complete their development on the medium and grow into seedlings. In general, older embryos are more easily cultured *in vitro* than young embryos.

Embryo culture is useful as follows:

- (i) Orchid seeds do not have any form of stored food. Embryos of such seeds can be cultured to obtain seedlings and maximum seedling formation can be achieved. Embryo culture in orchids can be applied for rapid clonal propagation.
- (ii) In certain species, inhibitors present in the endosperm or seed coat make the seed dormant. Such embryos can escape

dormancy by culturing on a suitable medium.

- (iii) In certain hybrid seeds developed after interspecific crosses, the endosperm degenerates at an early stage and the young embryo is left with no food, consequently it also dies. Such young embryos can be excised from the seeds and cultured on the nutritive medium. Getting nutrition, they develop into seedlings which can be transplanted in the field.
- (iv) A popular example includes hybridization of barley and wheat with *Hordeum bulbosum* leading to the production of haploid barley and haploid wheat respectively. Haploid wheat plants have also been successfully obtained through culture of hybrid embryos from wheat x maize crosses.

Ovule Culture

Ovule culture technique is utilized for raising hybrids which normally fail to develop due to the abortion of the embryos at an early stage. Ovules can easily be excised from the ovary and cultured on the basal medium. The loss of a hybrid embryo due to premature abscission of fruits may be prevented by ovule culture. In some cases, addition of fruit/vegetable juice increase the initial growth.

Ovary culture

Ovary culture technique has also been successfully employed to raise interspecific hybrids between sexually incompatible species, *Brassica campestris* and *B. oleracea*. Ovaries are excised from the flowers and cultured at the zygote or two-celled proembryo stage for obtaining normal development on culture medium.

Sometimes coconut milk when used as a supplement to the medium promote formation



of fruits that are larger than those formed *in vivo* (within the living organism). In Anethum, addition of kinetin in the medium caused polyembryony which gave rise to multiple shoots.

Micro propagation

Micropropagation is the tissue culture technique used for rapid vegetative multiplication of ornamental plants and fruit trees by using small sized explants. Because of minute size of the propagules in the culture, the propagation technique is named as micropropagation. This method of tissue culture produces several plants. Each of these plants will be genetically identical to the original plant from where they were grown.

The genetically identical plants developed from any part of a plant by tissue culture/micropropagation are called somaclones. The members of a single somaclone have the same genotype. This micropropagation is also known as somaclonal propagation. It is the only process adopted by Indian plant biotechnologists in different industries mainly for the commercial production of ornamental plants like lily, orchids, Euca lyptus, Cinchona, Blueberry, etc. and fruit trees like tomato, apple, banana, grapes, potato, citrus oil palm, etc.

There are four defined steps in micro propagation method. These are:

- (i) Initiation of culture from an explant like shoot tip on a suitable nutrient medium.
- (ii) Shoot formation multiple shoots formation from the cultured explant.
- (iii) Rooting of shoots rooting of in vitro developed shoots.
- (iv) Transplantation the hardening of tissue culture raised plants and subsequent trans-plantation to the field.

Advantages of Micro propagation:

These are as follows:

1. It helps in rapid multiplication of plants.
2. A large number of plantlets are obtained within a short period and from a small space.
3. Plants are obtained throughout the year under controlled conditions, independent of seasons.
4. Sterile plants or plants which cannot maintain their characters by sexual reproduction are multiplied by this method.
5. It is an easy, safe and economical method for plant propagation.
6. In case of ornamentals, tissue culture plants give better growth, more flowers and less fall-out.
7. Genetically similar plants (somaclones) are formed by this method. Therefore, desirable characters (genotype) and desired sex of superior variety are kept constant for many generations.
8. The rare plant and endangered species are multiplied by this method and such plants are saved.

Regeneration of Plantlets:

1. Preparation of Suitable Nutrient Medium:

Suitable nutrient medium as per objective of culture is prepared and transferred into suitable containers.

2. Selection of Explants:

Selection of explants such as shoot tip should be done.

3. Sterilisation of Explants:

Surface sterilization of the explants by disinfectants and then washing the explants with sterile distilled water is essential.



4. Inoculation:

Inoculation (transfer) of the explants into the suitable nutrient medium (which is sterilized by filter-sterilized to avoid microbial contamination) in culture vessels under sterile conditions is done.

5. Incubation:

Growing the culture in the growth chamber or plant tissue culture room, having the appropriate physical condition (i.e., artificial light; 16 hours of photoperiod), temperature (-26°C) and relative humidity (50-60%) is required.

6. Regeneration:

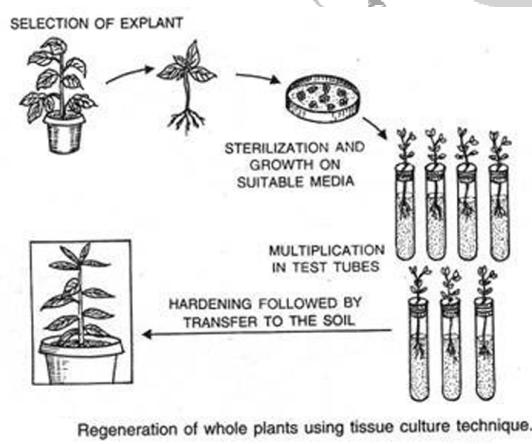
Regeneration of plants from cultured plant tissues is carried out.

7. Hardening:

Hardening is gradual exposure of plantlets to an environmental conditions.

8. Plantlet Transfer:

After hardening plantlets transferred to the green house or field conditions following acclimatization (hardening) of regenerated plants.

**Protoplast Culture and Somatic Hybridisation:**

When a hybrid is produced by fusion of somatic cells of two varieties or species, it is known as somatic hybrid. The process of producing somatic hybrids is called somatic

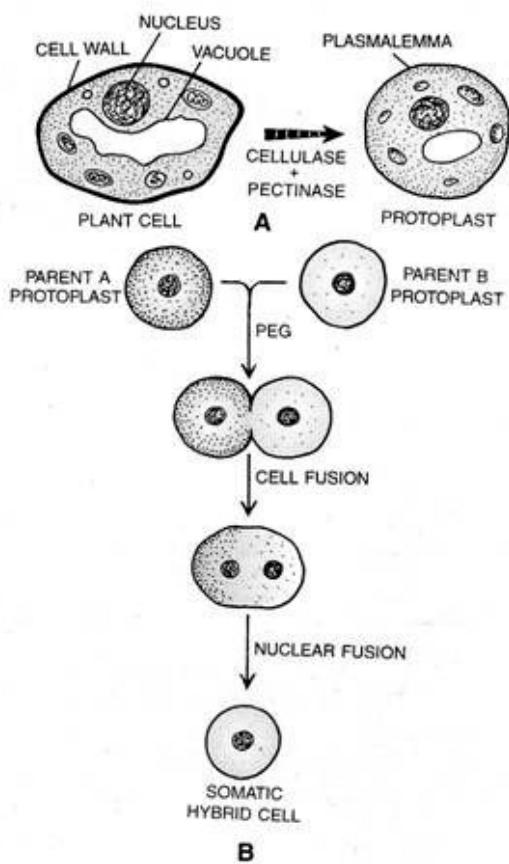
hybridisation. First, the cell wall of the plant cells is removed by digestion with a combination of pectinase and cellulase. The plant cells without cell wall are called protoplasts. The protoplasts of the two plants are brought together and made to fuse in a solution of polyethylene glycol (PEG) or sodium nitrate. The fusion of protoplasts with the help of chemicals is called chemo-fusion. Fusion of protoplasts with the help of high voltage pulse is known as electro-fusion. The fusion of protoplasts not only involves the fusion of their cytoplasm but also their nuclei. The fused protoplasts are allowed to grow on culture medium. Soon they develop their own walls when they are called somatic hybrid cells. The hybrid cells give rise to callus. Callus later differentiates into new plant which is somatic hybrid between two plants. Somatic hybrids in plants were first obtained between two species of Tobacco (*Nicotiana glauca* and *N. langsdorffii*) by Carlson et al in 1972. Successful somatic hybrids have also been got from different species of *Brassica*, *Petunia*, and *Solanum*.

Pomato is somatic hybrid between Potato and Tomato that belong to two different genera and **Bomato** is somatic hybrid between Brinjal and Tomato. Somatic hybrids are also produced between rice and carrot. The hybrid plant bears both fruits and tubers of the two parents.

- Protoplast technology has opened up avenues for development of hybrids of even asexually reproducing plants.
- There is a distinct possibility of development of new crop plants, e.g., Pomato.
- Somatic hybrids may be used for the production of useful allopolyploids (Individuals produced by interspecific polyploidy).



(d) Genetic manipulations can be carried out more rapidly when plant cells are in protoplast state. New genes can be introduced (e.g., male sterility, herbicide resistance). Mutations will be easier.



Somatic hybridisation. A, Production of protoplasts using a combination of pectinase and cellulase. B, Protoplast fusion induced by PEG ultimately yields somatic hybrid cells.

Artificial Seeds

There are many plants which neither have seeds nor produce a small quantity of seeds. To overcome this problem the concept of artificial seeds has become popular, where somatic embryos are en-capsulated in a suitable matrix composed of sodium alginate, along with substances like mycorrhizae, herbicides, fungicides and insecticides. The technique involved in the production of artificial seeds is based on cellular totipotency

and somatic embryogenesis. An artificial seed is a bead of gel containing a somatic embryo (or shoot bud) and the nutrients, growth regulators, antibiotic, etc. needed for the development of a complete plantlet. Artificial seeds may be produced using one of the following two ways: desiccated systems and hydrated systems. In the desiccated systems the somatic embryos (SEs) are first hardened to withstand desiccation and then are encapsulated. In the hydrated systems, the beads become hardened as calcium alginate is formed, after about 20-30 minutes the artificial seeds are removed, washed with water and used for planting. Hydrated artificial seeds become dry rapidly in the open air. Therefore, hydrated artificial seeds have to be planted soon after they are produced. In India, this technique of synthetic seeds is being done for sandalwood and mulberry at BARC (Bhabha Atomic Research Centre), Mumbai.

Advantages

- (i) They can be directly sown in the soil like natural seeds
- (ii) They can be stored upto a year without loss of viability
- (iii) They are easy to handle, and useful as units of delivery.

The only disadvantage of artificial seeds is the high cost of their production.

Practical Applications of Plant Tissue Culture:

The use of plant cells to generate useful products and/or services constitutes plant biotechnology. In plant biotechnology, the useful product is a plantlet. The plantlets are used for the following purposes:

1. Rapid Clonal Propagation

A clone is a group of individuals or cells derived from a single parent individual or cell through asexual reproduction. All the cells in callus or suspension culture are derived from



a single explant by mitotic division. Therefore, all plantlets regenerated from a callus/suspension culture generally have the same genotype and constitute a clone. These plantlets are used for rapid clonal propagation. This is done in oil palm.

2. Somaclonal Variation

Genetic variation present among plant cells of a culture is called somaclonal variation. The term somaclonal variation is also used for the genetic variation present in plants regenerated from a single culture. This variation has been used to develop several useful varieties.

3. Transgenic Plants

A gene that is transferred into an organism by genetic engineering is known as transgene. An organism that contains and expresses a transgene is called transgenic organism. The transgenes can be introduced into individual plant cells. The plantlets can be regenerated from these cells. These plantlets give rise to the highly valuable transgenic plants.

4. Induction and Selection of Mutations:

Mutagens are added to single cell liquid cultures for induction of mutations. The cells are washed and transferred to solid culture for raising mutant plants. Useful mutants are selected for further breeding. Tolerance to stress like pollutants, toxins, salts, drought, flooding, etc. can also be obtained by providing them in culture medium in increasing dosage. The surviving healthy cells are taken to solid medium for raising resistant plants.

5. Resistance to Weedicides

It is similar to induction of mutations. Weedicides are added to culture initially in very small concentrations. Dosage is increased in subsequent cultures till the desired level of resistance is obtained. The resistant cells are then regenerated to form plantlets and plants.

ANIMAL TISSUE CULTURE

The foundation of animal cell and tissue culture was laid by Jolly (1903) when he showed that animal cells could not only survive but could divide in culture medium. The actual beginning of animal cell culture and tissue culture was made by Harrison (1907) and later by Carrel (1912) who used frog's tissue in tissue culture. They successfully showed that animal cells can be grown indefinitely in culture medium just like microorganisms. Later tissues from warm blooded animals like chick and mammals were used as material for tissue culture purpose. Prior to the year 1950, parts of animal tissues were used as material for tissue culture, but later on dispersed cells were utilized for the culture purpose. With the advancement of techniques, cells and tissues from mammals are mainly used for tissue culture purpose.

Applications of Tissue Culture:

1. Animal cell culture was primarily aimed to study infection of animal viruses.
2. Later on it was used to produce a wide range of biological products of commercial importance such as antibodies, enzymes, hormones, immuno-regulators.
3. Recently tissue culture technique has been used in the manufacture of viral vaccines, tissue plasminogen activator, interferon-a, monoclonal antibodies and tumor specific antigens.
4. Production of Foot and Mouth disease vaccines (FMD vaccines) is the most important example of the use of large scale cell culture. There are several other vaccines including polio vaccine, bovine leukaemia virus (BLV) vaccines, rabies vaccines etc. which are produced on commercial basis using cell cultures.
5. Impact of new drugs can be evaluated using cell and tissue culture techniques.

