UNIT 24 GENETICS IN AGRICULTURE, AND PLANT IMPROVEMENT

Structure **Page No.**

24.1 INTRODUCTION

You might have tried growing plants either in small pots or in your backyard at some time or the other. You probably tried your hand at vegetable gardening too. In this experience, did you encounter some common pests such as bugs and other insects that destroyed a large portion of your plants? If yes, then quite natually you might have either thought of writing off your gardening experience completely, or you might have tried some type of pest control. And, if you chose a toxic pesticide, you would worry about its possible toxic effects on you and your family as well as to the environment. Perhaps you chose an organic preparation, but it might have been more difficult and expensive to use than its synthetic counterpart. In such a situation, you probably wished that the vegetables or other plants had a built-in pest-repellent. This wish is something that the genetic engineers and biotechnologists have been thinking of for many years. They have tried to find solutions to such problems by applying the science of genetics. Besides building pest resistance, they have worked in many areas of plant improvement. Many important breakthroughs have been made. In the following sections you are going to study some important developments in the improvement of plants.

Objectives

After studying this unit, you should be able to:

- explain with examples, the various kinds of improvements made in plants by the application of the principles of genetics (Section 24.2).

24.2 GENETIC ENGINEERING OF PLANTS

Though plant breeding is an ancient art, it is based on sound scientific principles that aim at improving the economically important plants for the benefit of mankind. The traditional methods of plant impovement involve ***selective breeding*** in which parents with superior characteristics are used. The resultant progeny or hybrid is superior too, as it demonstrates the traits of both the parents. This phenomenon known as ***hybrid vigour*** or ***heterosis*** forms the basis of the improvement of many crop plants of which corn is a well-known example.

Ever since Mendel published the results of his famed breeding experiments in the garden pea, in 1866, the science of genetics developed at an unprecedented pace. And during the course it also developed several new tools and techniques. The application of modern methods such as the recombinant DNA technology has given a new impetus to plant improvement. Given below are some examples of plants, whose various qualities have been altered or modified to the desired levels by the application of the recombinant DNA technology.

24.2.1 Regneration of Plants from Callus Tissues and Protoplasts

Plants have a unique property, that is, *totipotency* which means that their cells are able to regenerate complete new plants. The differentiated plant cells are able to dedifferentiate to the embryonic state and subsequently to redifferentiate into new cell types. Thus, there is no separation of germ line cells from somatic cells as in higher animals.

When excised tissues from mature plants, known as *explants*, are placed in the appropriate sterile tissue culture conditions, notably in the presence of the plant hormone 2,4- dichlorophenoxyacetic acid (2,4-D), cells in these tissue explants often dedifferentiate and grow into highly unorganised cell masses (see Fig. 24.1)

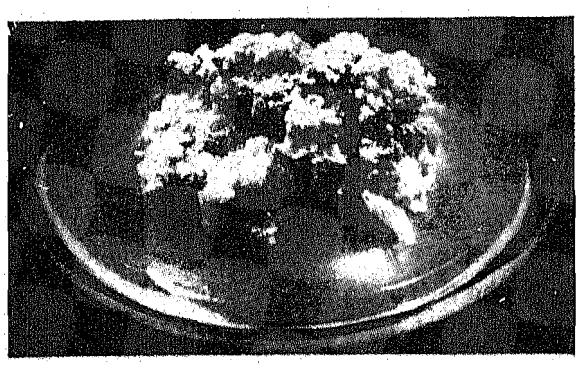


Fig.24.1: Undifferentiated callus growing ou synthetic culture medium.

called calli (or calluses, the singular form is callus) . If these undifferentiated callus cell-clumps are transferred to a growth medium lacking 2,4-D, but containing growth hormones such as kinetin, in many species plantlets will regenerate (see Fig. 24.2).

Fig.24.2: Plantlets regenerated from callus tissues growing ou sterile culture medium.

Moreover, with some plant species such as petunias, tobacco, tomato and potato, one can regenerate plants from even isolated protoplasts. These are single cells from which the walls have beeen removed by using appropriate enzymes, Protoplasts can be used in two different ways. Firstly, protoplasts of two different plants can be used to produce a hybrid cell from which a hybrid plant can be produced. This hybrid plant may be-subjected to-conventional plant breeding techniques if desired. Secondly, the protoplasts can be used as recipient cells for the introduction of specific genes being carried on vectors, that is, they can be transformed just as the *E.coli* cells.-In this manner a range of genetically engineered plants, including ones with increased yield, pest resistance and herbicide resistance can be generated.

The totipotency of plant cells is thus a major advantage for genetic engineering since it permits one to regenerate entire plants from individual modified somatic cells.

24.2.2 *The Ti Plasmid* of *Agrobacterium turnefaciens*

Till date, the most important tool in the genetic engineering of plants has been the Ti *plasmid* of the soil bacterium Agrobacterium tumefaciens. This bacterium causes, crown gall disease of dicotyledonous plants (see Fig.24.3). This disease is characterised by development of tumours or galls at the wounding sites which are often at the crown (hence the name crown gall). Crown is the junction between the root and the stem. Since the crown of the plant is usually located at the soil surface, this is where a plant is most likely to be wounded due to abrasion by soil particles or from the plant blowing in strong wind, and infected by the bacterium. However, A. turnefaciens can infect any part of the plant and induce formation of tumour.

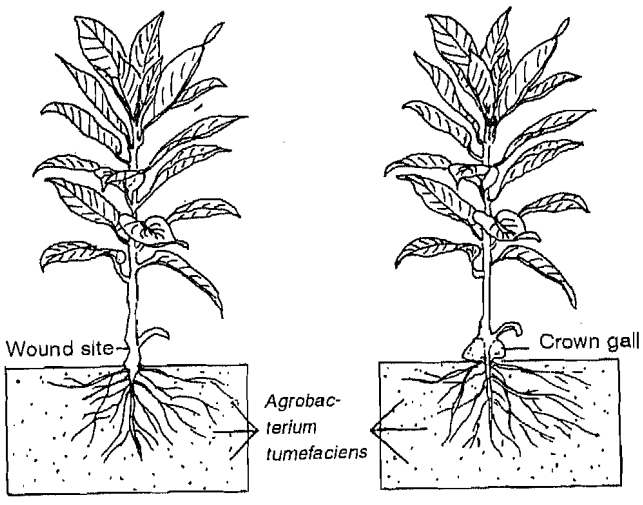
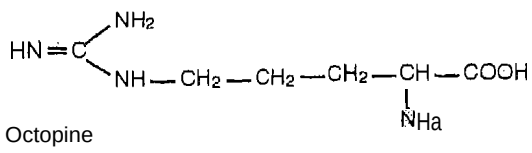


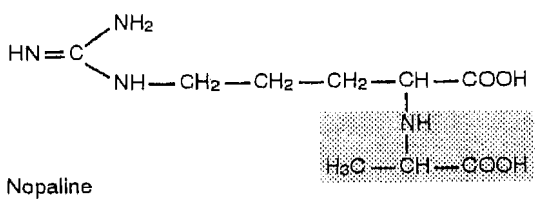
Fig.24.3: Cown gall formation on dicolyledonous plants after infection with the bacterium *Agrobacterium tumefaciens*.

When a wound site in a dicotyledonous plant is infected by *A.tumefaciens*, two key events occur. One, the plant cells begin to proliferate and form tumours. Two, they begin to synthesise an arginine derivative called an opine. The opine synthesised is usually either *nopaline* or octopine (Fig.24.4) depending on the *A. tumefaciens* strain involved. These opines are catabolised and used as energy sources by the infecting bacteria. The *A. tumefaciens* strains that induce the synthesis of nopaline can grow on nopaline, but not on octopine and vice versa. Thus the particular bacterial strain is able to divert the metabolic resources of the host plant to the synthesis of opines, which are of no known benfit to the host plant, but provides sustenance to the bacterium.

(a) Arginine



(b) Octopine



(c) Nopaline

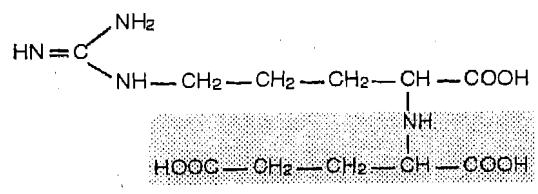


Fig.24.4: a) Chemical structure of the amino acid arginine, b) octopine, and c) nopaline

The genetic information for inducing the crown gall disease in plants is carried on a large, about 200,000 bp plasmid present in the *A.tumefaciens* cell (Fig.24.5). This plasmid is called the Tiplasmid, for its tumour-inducing capacity. Two components of the Tiplasmid are essential for the transformation of plant cells, and these are the T-DNA and the *vir region*. During the process of transformation, the T-DNA (refers

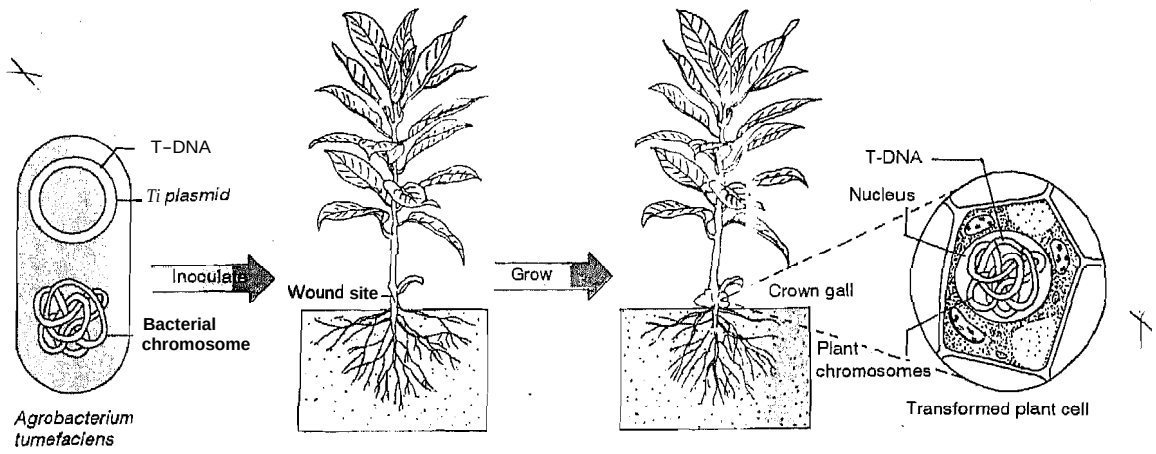


Fig.24.5: ***Agrobacterium tumefaciens,*** **bearing** the ***Ti plasmid.*** Cell of the **plant** tumour (contain (he ***T-DNA*** **segment of the** ***Ti plasmid*** **integrated** into ***(he*** DNA of the plant chromosome.

to transferred DNA) is excised from the Tiplasmid, and is transferred to a plant cell. Once in the plant cell, the T-DNA integrates into the DNA of the plant cell. The integration of T-DNA occurs at random chromosomal sites. The T-DNA in the ***nopaline-type Ti plasmid*** is about 23,000 bp having 13 genes. In *octopine* type ***Ti plasmids,*** there are two separate T-DNA segments. For convenience, we shall restrict to the nopaline-type Tiplasmids whose structure is shown in Figure 24.6.

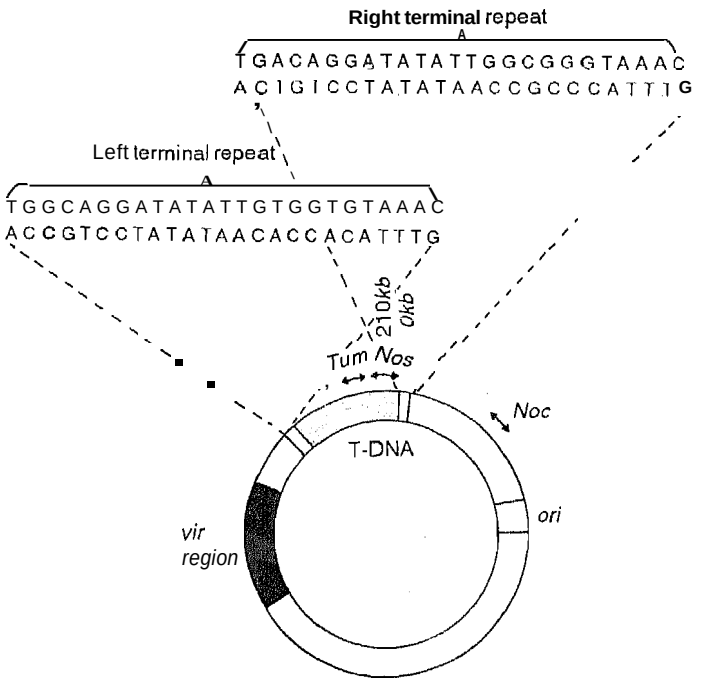


Fig. 24.6 : Diagrammatic representation **of the structure of** ***nopaline Ti plasmid.*** **The** description **of** symbols used in **the** figure are as **follows:** ***Ori,*** **plasmid orgin** of replication; ***Tum,*** region containing genes responsible for **tumour** formation or **the** genes coding for the **biosynthesis of phytohormones;** ***Nos*** **region contains** genes involved in **nopaline biosynthesis;** ***Noc*** **region** containing **genes** involved in catabolism ***of*** **nopaline;** viror **the virulence** region containing (he genes required for ***T-DNA*** **transfer** (From ***M.W.*** **Bevan** and M.D. **Chilton,** ***"T-DNA of the Agrobacterium Ti and Ri plasmids",*** **Annu,** Rev. Gent, **Vol.** 16, pp 357-384, 1982).

Some of the genes on the T-DNA segment of the *Ti plasmid* encode enzymes that catalyse the synthesis of phytohormones such as the auxin indoleacetic acid and the cytokinin isopentenyl adenosine. These phytohormones result in the development of tumourous growth of cells forming crown galls. The T-DNA region is bordered by two repeated 25 bp sequences (see Fig.24.6), which are required for T-DNA 'excision and transfer. The deletion of either border sequence completely blocks the transfer of T-DNA to plant cells. The *vir* (virulence) region of the *Ti plasmid* contains the genes required for the T-DNA transfer process. These genes encode the DNA processing enzymes required for excision, transfer and integration of the T-DNA segment. Once the T-DNA region of the Ti-plasmid of *Agrobacterium tumefaciens* is transferred to plant cells it becomes integrated in the plant chromosomes. When this whole process of transformation became clear to the scientists, the potential use of *A.tumefaciens* in plant genetic engineering was obvious. Now, it was possible to introduce foreign genes into the T-DNA region and these genes would be transferred to the plant with the rest of the T-DNA segment.

In 1986, a research team led by Roger Beachy, at Washington University used *Agrobacterium* transformation to induce cross-protection against tobacco mosaic virus (TMV). The gene encoding the TMV coat protein was fused to the cauliflower mosaic virus promoter, and the fused gene was inserted into a Tiplasmid. Transgenic tobacco plants created by infection with the recombinant Tiplasrnid expressed TMV coat protein. The coat protein gene was stably integrated into the genome of the transformed plants and was inherited in a Mendelian fashion by their progeny. The transgenic offspring showed delayed onset of symptoms following inoculation with live TMV, and up to 60% of transgenic plants showed no symptoms at all. This research finding shows the tremendous potential of the use of *Ti plasmid* of *Agrobacterium tumefaciens*.

24.2.3 Gene Transfer in Monocotyledonous Plants

In the earlier section you have seen how the Tiplasrnid of *A.tumefaciens* can be employed as a vehicle for transferring foreign genes into the genome of a particular plant. This bacterium can work on in a limited range of plants, that is, the ones that are dicotyledonous. As so many of the economically important plants are monocotyledonous, there was a need to develop gene transfer technology that could be applicable to these plants. First, attempts were made to introduce DNA into plant protoplasts by transiently perturbing the cell membranes so as to make them permeable to macromolecules such as DNA. Based on this a successful technique called *direct gene* transfer was developed. It involved the addition of selected marker genes together with polyethylene glycol, which seems to stimulate membrane fusions. A second method with slight variation was also developed and it was known as *electroporation*. It employs a short pulse of high-intensity electrical current to disrupt cell membranes and render them temporarily permeable to DNA molecules. Although by using these procedures one could get transformed protoplasts of monocotyledonous plants but a poor percentage of these protoplasts could regenerate into plants.

Recently a novel procedure has been developed to carry on the transfer of DNA into intact plant cells by a microprojectile gun (Fig.24.7). This gun is used to shoot DNA-coated microprojectiles, the tungsten particles of 1 to 4 μm diameter, into plant cells. This method can be used both in culture cells as well as directly on the growing plants. This technique holds great promise for the future because of being technically simple, and also being applicable to all the plant species, including fungi and algae.

24.2.4 Herbicide Tolerant Plants

Herbicides are chemical agents that when applied to various types of plants, result in the death of the plant. These are used primarily to kill non-desirable plants like weeds, that compete with the desired plants say crops, for space, water and

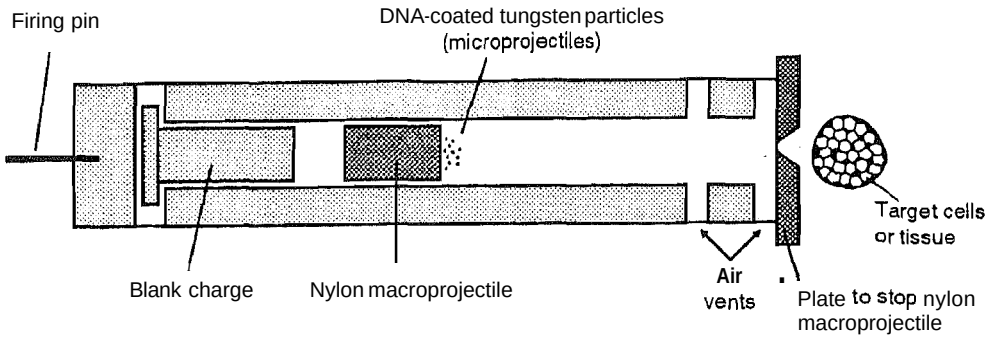


Fig.24.7: Dingrammatic representation of (he structure *of* a microprojectile gun. (After T.M. Klein, ED. Wolf, R.Wu, and J.G. Sanford. Nature Volume *327*, pp 70-73,1987).

nutrients. Unfortunately, the available herbicides seldom provide the degree of specificity that is desired, and most herbicides control only certain classes of weeds and not others. Broad-spectrum herbicides give good weed control, but, in so doing, usually have deleterious effects on the growth of the crop plant as well. As a result, scientists are now evaluating alternate approaches to weed control. The most promising approach, being considered presently is the development of herbicide-tolerant plant varieties for use with broad spectrum or totally nonspecific herbicides.

Though herbicides kill or inhibit the growth of the plants, but do not have any deleterious effects on animals. They act on plants by disabling the target enzymes in the metabolic pathways unique to plants such as those involved in photosynthesis or biosynthesis of amino acids. Glyphosate (see Fig. 24.8) is one of the most potent broad spectrum herbicides known, inhibits 5- enolpyruvylshikimate-3 phosphate synthase (EPSP synthase), an enzyme necessary in the biosynthesis of the aromatic amino acids tyrosine, phenylalanine and tryptophan in the chloroplast.

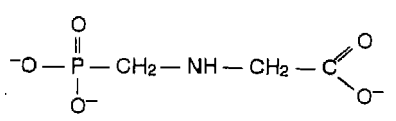


Fig. 24.8: The chemical structure *of* glyphosate, an active ingredient of many herbicides.

Presently, the major thrust is to identify and transfer herbicide resistance genes into major crop plants. The following three strategies are used for attaining herbicide resistance.

i) Stimulate overexpression of target protein of the herbicide, so that enough of it will escape disablement by the herbicide.

ii) Insert a genetically altered form of target protein that is less sensitive to the herbicide.

iii) Insert an enzyme that disables the herbicide.

The first and the second approaches have been used to produce plants that are resistant to glyphosate. A resistant petunia, in which the **EPSP gene** is highly amplified, was isolated by growing cultured cells in increasing levels of the herbicide. Petunias transformed with a fusion gene linking the viral promoter to a wild-type EPSP gene also show resistance. A mutant EPSP gene ***(aro*** A) isolated from ***Escherichia coli*** is 6,000 times less sensitive to glyphosate than to the wild-type enzyme, but it lacks the plant 'transit **peptide'** that is needed to transport EPSP into the chloroplast. To overcome this, a fusion gene was constructed, **linking** the ***aro A*** coding region with a dicot transit **peptide** sequence. Petunias and tobacco

plants transformed with this fusion gene express the *aro A* protein in the chloroplast, in addition to their own **EPSP.**

The third approach to glyphosate resistance has yet to be achieved. However, metabolic pathways have been defined in species of *Pseudomonas* and other bacteria that allow the use of glyphosate as a sole phosphatesource. Efforts are under way to clone the genes that encode these glyphosate-degrading enzymes.

24.2.5 Resistance to Pests

Pesticides and insecticides are chemical agents that kill various kinds of insects and other pests, while leaving plants and most animals relatively unharmed. Unfortunately, many pesticides are non-selective. They kill insects which are beneficial to plants and the environment along with those which are not. In addition, pesticides can be toxic to humans and other non-target animals and they contaminate soil and water supplies. This environmental contamination may then lead to a second level of adverse effects on both plant and animal life. Furthermore, prolonged use of insecticides can lead to the development of insect strains which are no longer susceptible to the toxic effects of those chemicals. Such a development has already resulted in the appearance of DDT resistant mosquitoes. You may recall that in the past few decades, **DDT** was widely used for the control of malaria-causing mosquitoes. Because of all these problems a major goal of genetic engineers has been to provide plants with natural endogenous resistance to a variety of pests through techniques of genelic engineering.

The best known examples of the use of natural gene products to control plant pests are the insect toxins of ***Bacillus thuringiensis***. Each of the ***B.thuringiensis*** toxin genes codes a large protein that aggregates to form protein crystals in spores, and those protein crystals are highly toxic to certain insects. To exploit this lethal characteristic, B. ***thuringiensis*** spores are combined with water to form a mixture which can be sprayed over an insect-infested area. When insect larvae ingest the bacterial spores, they get killed due to the toxic protein crystals. This treatment was effective but its insecticidal effects were transient due to limited field survival of spores, so long-term insecticidal activity required repeated applications of spores.

Genetic engineers tried to find solution to this problem by applying the techniques of recombinant DNA technology. In one line of research, scientists isolated genes which encode the B. ***thuringiensis*** toxin and inserted those genes into other organisms such as ***E.coli*** and the bacterium ***Pseudomans fluorescens*** which are better suited for survival in field. ***P.fluorescens*** lives on roots of many different types of plants, including corn. When these transformed bacteria (***P.fluorescens***) were sprayed on corn plants, these bacterial cells colonised the root area. As a result the B. ***thuringiensis*** toxin is synthesised at the site of plant itself, this helped the plant to ward off their pests.

A second line of research involved linking the ***B.thuringiensis*** toxin gene to a constitutively expressed promoter and introducing it via the Ti ***plasmid,*** directly into the cells of the plant which is to be protected. One such study which took place in Belgium involved the insertion of the toxin gene into cells of tobacco plant. Analysis of tissues of the mature transformed plants showed that the tobacco plant indeed synthesised ***B. thuringiensis*** toxin. When the treated plants were infected with tobacco hornworm larvae, very few of them survived, and the damage was negligible. In contrast, the control tobacco plants which were not transformed with the engineered ***Tiplasmid,*** all died within approximately two weeks. The inserted gene clearly provided protection to the tobacco plants.

Based on similar lines, work is underway to identify genes for salt and drought tolerance in plants.

SAQ 1

Match the items of coloumns I and II. Write the correct choice in the space provided.

I			II
i)	**Explants**	[ ]	a) affects target enzymes of plant's metabolic pathways
ii)	Crown gall	[ ]	b) **tissues culture**
iii)	Electroporation	[ ]	c) Bacillus *thuringiensis*
iv)	Herbicide	[ ]	d) direct transfer of DNA to protoplasm
v)	Natural pesticide	[ ]	e) *Ti plasmid*

24.2.6 Resistance to Frost Formation

If the temperature falls below 32°F(O°C) ice crystals form and water freezes. Ice crystals, forming in the interior of the cell often destroy the cell wall with the result that cell death soon follows. This phenomenon can be illustrated by freezing a firm tomato or even some peas. **As** the temperature drops, ice crystals form and disrupt the delicate cells of tomato. When the frozen tomato is later allowed to thaw, only a soggy, limp fruit remains which was once quite firm. A similar effect can be seen on growing plants which are subjected to freezing temperatures.

The presence of the common bacterium *Pseudomonas syringae* facilitates ice crystals or frost formation on a plant. P. *syringae*, found on many types of plants, contains a protein which acts as a site of nucleation for the formation of ice crystals at temperature of 32°F (0°C). In contrast, when *P.syringae* or other nucleating agents are absent, ice crystals do not form until the temperature drops to approximately 20°F(–7°C).

Scientists found that the nucleation ability of P. *syringae* results from a single gene in the bacterial genome. By removing this gene, scientists created a strain of bacteria which, when applied to growing plants does not provide the necessary site of nucleation for frost formation even when temperature drops briefly as low as 23°F(*–5°C).* This genetically engineered bacterial strain called ice minus was developed at University of California in Berkley. It has a great potential for future use in the prevention of frost damage to a variety of crops.

24.2.7 Enhanced Nitrogen Utilisation

As you know, plants can only utilise nitrogen that has been incorporated into chemical compounds such as ammonia, urea or nitrates. No green plants is capable of utilising diatomic nitrogen (N_2) directly from the atmosphere. In other words, a continuous supply of nitrogen in usable form or the *fixed* nitrogen must be available for the growth and development of plants.

For obtaining optimal yields of crops, farmers usually supplement the soil with fertilisers, nitrogen being its important constituent. Because the purchase of nitrogen fertilisers represents one of the major expenses incurred with current agricultural production methods, a major effort has been and continues to be devoted to the development of enhanced methods of biological nitrogen fixation.

Certain free-living soil bacteria such as *Azotobacter vinelandii* and Klebsiellapneumoniae directly convert atmospheric nitrogen to ammonia. These bacteria are valuable research material for conducting investigations on the mechanism of nitrogen fixation. In *Klebsiella,* there are 17 *nif* genes (nitrogen .

fixation genes) that are organised in seven operons. The situation in nitrogen fixation is very different from the examples discussed above, such as herbicide tolerance etc. You may recall in the instances discussed above, a single gene was constructed and transferred to plants. But in this case, it is quite difficult, if not impossible, to engineer 17 different genes and transfer all of them to the same recipient plant, and to coordinate their expression as well, so that all the components of the complex nitrogen fixing enzymatic machinery are synthesised in proper amounts and proper cells. Presently, we are still away by a few years from our goal.

Besides the free-living nitrogen fixers, the symbiotic nitrogen fixers too are very important sources of biologically fixed nitrogen. Can you guess we are talking about which organisms? It is, about the relationship between the genus *Rhizobium* and plants of family *Leguminosae*, i.e., peanuts, pea, soyabean, alfa-alfa etc. This kind of nitrogen fixation takes place in root nodules that develop due to the interaction of *Rhizobium* with roots of legumes. Thus nodule formation is dependent on the genetic information of both the plant and the bacteria. The nitrogenase that brings about reduction of nitrogen is encoded by the bacterial genome, but the fixed nitrogen is utilised for growth of both the bacteria and host legume plant. Once the mechanism responsible for establishing this symbiotic relationship, and for nodule formation are known and the genes that control these processes have been identified, it might be possible to use genetic engineering to modify nonlegume plants so that they too acquire the nitrogen-fixing capability. Presently, many research laboratories are working on this aspect to turn this dream to reality.

24.2.8 Improving Nutritional Value

Worldwide, the seeds of legumes and cereal grains are estimated to provide humans with 70 per cent of their dietry protein requirements. Protein molecules are composed of varying arrays of twenty different amino acids, of which human body can synthesise twelve. The remaining eight amino acids, called essential ***amino acids*** must be provided to the body by ingestion. This means that people must eat foods containing these eight amino acids to provide the complete proteins necessary for growth. All of these eight essential amino acids are present in a variety of animal products, including red meat, poultry and milk. In contrast, however, no source of plant food contains adequate supplies of all eight of the amino acids – they all lack at least one. Consider, for example, the beans. While the beans have more than enough of the essential amino acid lysine, they lack the amino acid methionine. Wheat and rice, on the other hand, contain suboptimal levels of lysine while containing a sufficient amount of methionine. When we consider that the majority of the world's population exists on a diet which rarely, if ever, contains meat, we realise how important it is for people to be able to get sufficient quantities of each amino acid from a plant-based diet. Therefore, it is desirable that plants contain complete proteins with all the amino acids required for human body.

The scientists are currently using the knowledge gained through genetic engineering in an attempt to alter the genes of a variety of plant proteins. For example, the genes of the beans could be altered so as to encode a protein which contains sufficient quantities of methionine. If the gene for phaseolin, the primary protein molecule of a beans could be altered to contain codons which specify the amino acid methionine without altering the overall structure or growth pattern of the plant, phaseolin could become a complete protein.

In addition to beans, the 'high lysine corn' is also worth mentioning. In cereals, the major seed storage proteins are called prolamines (zeins'in corn) and they have been found to have a lower lysine content. Therefore, the diets based on cereal grains are deficient in lysine. In the case of corn, the seed proteins are also deficient in tryptophan, and to a lesser extent, methionine. Because of the importance of cereals to man and animals, scientists have been working for several decades to develop varieties with increased lysine, tryptophan and methionine content. Considerable progress has been made, but the successes obtained had little

agricultural value. Some corn mutants such as ***sugary-1, floury-2*** and ***opaque-2*** have increased lysine and/or methionine content, but these strains have soft kernels and lower yields. These high lysine strains also have lower prolamine (zein) content.

Several corn genes encoding zeins have been cloned and sequenced. After this success, a blue-print for engineering 'high-lysine' corn by site-specific mutagenesis was prepared. Then these high-lysine coding sequences could be joined to strong promoters and reintroduced into corn plants by means of electroporation or a microprojectile gun. Recently, B.A. Larkins and colleagues introduced new lysine and tryptophan codons into a zein cDNA by site specific mutagenesis. And when RNA transcripts of these modified cDNAs were injected into Xenopus laevis oocytes, RNAs were translated efficiently and the 'high- lysine' zein products were found to self-aggregate into dense structures as seen in corn. These results give positive indication that 'high-lysine' corn might indeed be produced by means of genetic engineering. In fact, by using the same approach, one can tailor other plant storage proteins to the optimal amino acid compositions. Thus, by employing the genetic engineering techniques, the nutritional quality of seed storage proteins can be desirably improved.

24.2.9 Manipulating Gene Expression Using Antisense RNA

An unusual approach to the control of gene expression has scored preliminary success in changing an important commercial trait of tomatoes. Fresh tomatoes must be shipped or transported while still green, because ripe fruit is too soft and is bruised easily. The enzyme polygalacturonase, which breaks down plant cell walls, is primarily responsible for fruit softening. It has been found that transformation of an 'antisense' copy of the polygalacturonase gene in tomato plants decreases expression of the softening protein as much as by 90%.

The antisense gene was made by fusing a cDNA clone of the polygalacturonase in reverse orientation, to a constitutive promoter. This reverse or antisense gene was then linked to the T-DNA and transferred to the tomato plants. Each transformed plant thus carried an antisense gene, as well as a normal copy of the polygalacturonase gene. During gene transcription, antisense messenger RNA (mRNA) molecules are produced and these are complementary to normal mRNA molecules (Fig. 24.9). It is believed that antisense mRNAs bind to a proportion of normal RNAs, making them unavailable for translation into protein.

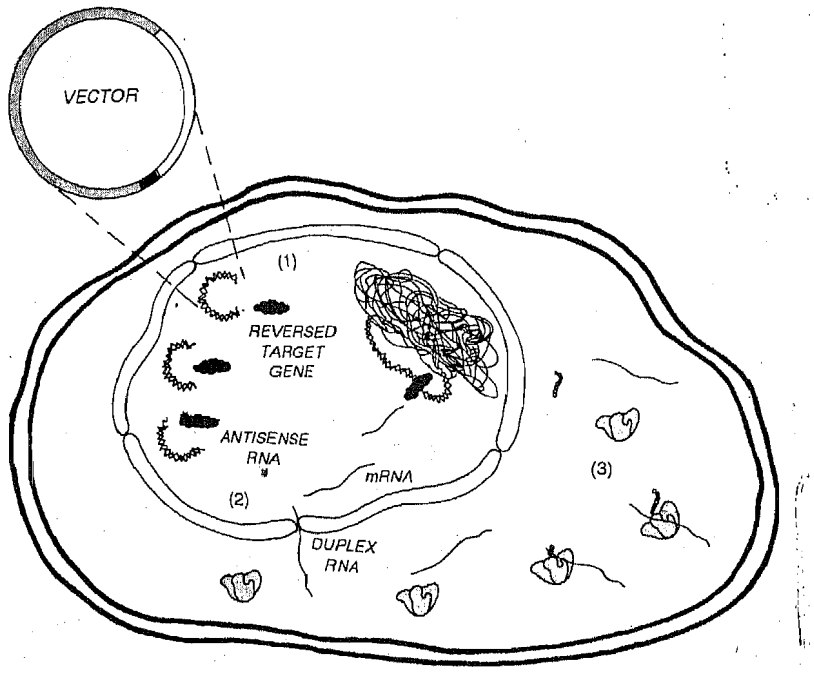


Fig. 24.9: Antisense RNA. a) The target gene in the plant chromosome expresses normal mRNA chromosome, and plasmid vector expresses complementary antisense RNA. b) Antisense RNA hybridises with the normal mRNA. c) When the ribosomes encounter the duplex RNA, translation is halted.

SAQ 2

Fill in the blanks with appropriate words:

i) Resistance to frost formation was developed by ..... a gene with nucleation ability in the *Pseudomonas* syringae.

ii) Klebsiella, a free-living bacterium having 17 ..... genes, organised into 7 operons, is a valuable material for research on enhanced ..... utilisation in plants.

iii) Beans have sufficient amount of amino acid ..... , but usually they are deficient in .....

iv) In cereals, that constitute the staple diet of a large section of people, the major seed storage proteins are .....

v) The result of transformation of an antisense copy of the ..... gene in tomatoes was better keeping quality of the fruit.

24.3 SUMMARY

This unit provides you an overview with the help of some representative examples, of some of the ongoing and anticipated applications of genetics in plant improvement. With the development of recombinant DNA and gene cloning techniques, biologists are now able to isolate and dissect almost any gene and any chromosomal segment of an organism. Transformation of plants with vectors based on *Ti plasmid* have opened the way for the genetic engineering of plants using recombinant DNA technology. It is expected that many types of desirably improved plants will result from the application of this new technology.

24.4 TERMINAL QUESTIONS

1) Write short notes on the following:
   i) Significance of regeneration of plantlets from protoplasts,
   ii) Microprojectile gun,
   iii) Herbicide-tolerant plants,
   iv) Ice-minus bacterium,
   v) Antisense RNA

2) Highlight the potential use of tissue culture in crop improvement.

3) Starting from the infection of a wound in a dicotyledonous plant, by *Agrobacterium* tumefaciens, outline the molecular events involved in the development of crown gall.

4) Comment on the following statement. 'The *Ti plasmid* of *Agrobacterium tumefaciens* – one of the most valuable tools in genetic engineering, has tremendous potential in plant improvement.' Support your argument with suitable examples.

5) Discuss the strategies adopted for imparting herbicide resistance to plants.

6) Explain with the help of a suitable example, the use of ***Tiplasmid*** in developing pest resistance in plants.

24.5 ANSWERS

Self Assessment Questions

1) i) b
   ii) e
   iii) d
   iv) a
   v) c

2) i) removing
   ii) *nif*, nitrogen
   iii) lysine, methionine
   iv) prolamines
   v) polygalacturonase

Terminal Questions

1 i) See Subsection **24.2.1**
   ii), See Subsection **24.2.3**
   iii) See Subsection 24.2.4
   iv) See Subsection 24.2.6
   v) See Subsection 24.2.9

2) See Subsection 24.2.1

3) See Subsection **24.2.2**

4) See Subsection **24.2.2**

5) See Subsection **24.2.4**

6) See Subsection **24.2.5**

GLOSSARY

adaptive **value**: refers to relative reproductive success of a genotype measured in terms of fecundity or the number of offspring left behind. Adaptive value is synonymous with Darwanian fitness and is symbolised as ω.

agglutinin: antibodies produced by agglutinogen that specifically react with the same agglutinogen (antigen). A test for agglutination makes it possible to classify people into four groups.

agglutinogen/Isoagglutinogen are the antigens that agglutinate or clump the red blood cells of the same species. Agglutination is caused by the interaction of the antigen on the surface of the cell with an antibody present in the serum.

allele : one of the forms of the gene at a particular locus that are members of the same gene pair, each kind of allele affecting a particular character somewhat differently than the others.

allelic frequency : the proportion of alleles in a population that belong to a given type.

allergy : originally defined as altered reactivity on second contact with antigen now usually refers to a type of hypersensitivity reaction.

alloantigen : antigen that differ between individuals of the same species.

allogenic : refers to intraspecies genetic variations.

amorph : a gene which has no demonstrable product like O gene.

antibody : a defense protein synthesised by mature B-cells (plasma cells) of a higher organism; it binds specifically to the foreign molecule that induced its synthesis.

antigen :a molecule which induces the formation of antibody.

antisense RNA.: RNA that is made from the DNA strand that is complementary to the sense strand of the DNA.

bactericidal : chemotherapeutic agent, i.e., compounds that have an irreversible lethal action.

basque : a member of a group of people of unknown origin inhabiting in France and Spain.

bilirubin : a degradation product of haeme.

bottleneck effect : random changes in the gene frequencies observed in a population where the population size is drastically reduced to one generation.

complement : a group of serum proteins involved in the control of inflammation, activation of phagocytes and the lytic attack on cell membranes; the system can be activated by the interaction with the immune system.

electroporation: introduction of DNA fragments into a cell by means of an electric field.

epitopes : (antigenic determinants) small regions on the antigen that bind the antigen (paratope) to antibody.

erythroblast : one of the intermediate cells in the biosysthesis of erythrocytes.

estrogen : a female sex hormone.

exon : a protein coding region of a eukaryotic gene, the RNA transcribed Corn such a region.

founder effect : random changes in the gene frequencies observed in a small population founded as a non-representative of a larger population.

gall : a tumourous growth in plants.

gene flow : refers to movement of individuals from one population to another that results in either the formation of new alleles into the population or alteration in the frequencies of existing alleles.

genetic cloning: a process by which a large number of a DNA segment is produced after introducing the segment into a plasmid or other suitable vector. The replication of the cell or the phage vector results in clones.

genetic counselling : a communication process which explores the risks of producing a genetically defective child by a couple, especially when family history

of genetic disease exists.

genetic drift : random changes in allelic frequency that result from sampling of gametes from generation to generation; characterised of small population.

genetic engineering : linking two DNA molecules by in vitro manipulations for the purpose of creating a novel organism with desired characteristics.

genetic equilibrium : refers to a state where there is no change in the allelic frequenies of the population.

genotype : The genetic material inherited from parents, not all of it is necessarily expressed in the individual.

genotypic frequency : relative proportion of individuals in a population with a designated genotype.

heme : the iron-porphyrin prosthetic group of heme proteins.

hemolysis : disintegration of RBC membranes with subsequent release of haemoglobin.

heterozygote : a heterozygous individual with unlike members of any given pair or series of alleles that consequently produces unlike gametes.

HLA : the human leucocyte antigen also known as major histocompatibility complex (HMC).

homozygote : homozygote is an individual possessing a pair of identical alleles at corresponding loci on the homologous chromosome.

hybrid vigour (heterosis) : unusual growth, strength and health of heterozygous hybrids from two less vigourous homozygous parents.

hydrophobic: "water hating" non-polar molecules or groups that are insoluble in water.

immune response : the capacity of a vertebrate to generate antibodies to an antigen, a macromolecule foreign to it.

immunoglobulin: an antibody protein generated by a specific antigen.

locus : the site on a chromosome where a gene is located.

Mendelian population : a group of organisms of same species showing a common gene pool and capable of interbreeding.

mutation : a change in the nucleotide base pairs of a gene, or a rearrangement of genes within chromosomes so that their interactions produce different effects; a change in the chromosomes themselves.

natural selection : a process in nature whereby one genotype leaves behind more offspring than another genotype because of species adaption to the environment.

phenotype : the physical chemical expression of an organism's gene.

plasmid : an extrachromosomal genetic element consisting of double-stranded DNA that replicates autonomously from the host chromosome.

platelets : cell fragments also called thormbocytes found in the blood, synthesised in bones marrow that help in clotting.

pseudogene : a non-coding sequence of genomic DNA with homology to the given gene being probed.

randomly mating population : population consisting of individuals in which the probability of members mating with individuals of particular genotype is equal to their frequency in the population.

recombinant DNA technology: refers to techniques of gene cloning. The term recombinant DNA refers to the hybrid of foreign vector DNA.

restriction enzymes : These are endonucleases capable of recognising specific DNA sequences which they cleave. Useful enzymes in recombinant DNA technology.

restriction Fragment : a DNA segment excised from a larger DNA by restriction enzymes.

selection coefficient : the reduction in the relative fitness of a genotype. The genotype may be less well adapted to the environment and this may cause reduction in fecundity. Selection coefficient is related to adaptivevalue by theequation s (1-w).

totipotency : the capability of any cell of an organism to differentiate and develop into a complete organism.

FURTHER READING

1. Carlson, E. A. 1985. ***Human Genetics.*** Tata McGraw Hill Publishing Co. Limited, New Delhi.
2. Freifelder, David. 1993. ***Molecular Biology.*** Second Edition. Narosa Publishing House, New Delhi.
3. Gardner, E. J.; Simmons, M. J. & Snustad, D. P. 1991. Eight Edition. ***Principles of Genetics.*** John Wiley & Sons, Inc. New York.
4. Gupta, P.K. 1987. Second Edition. ***Genetics.*** Rastogi Publications, Meerut.
5. Klug, W.S. & Cummings, M.R. 1986. ***Concepts of Genetics.*** Second Edition. Scott Foresrnan & Co., Illinois.
6. Rao, K.R. 1986. Third Edition. ***Text Book of Biochemistry.*** Prentice Hall of. India (Pvt) Ltd., New Delhi.
7. Lloyd, J.R. 1986. ***Genes*** and ***Chromosomes.*** English Language Book Society, Macmillan, Hong Kong.

Dear Student,

While studying these units you may have found certain portions of the text difficult to comprehend. We wish to know your difficulties and suggestions in order to improve the course. Therefore, we request you to fill and send us the following questionnaire which pertains to this block.

QUESTIONNAIRE

LSE-03
Block-4

Enrolment No. | | | | | | | | | | |

1) How many hours did you need for studying the units?

Unit Number	19	**20**	21	22	**23**	**24**
No. of hours						

2) How Many hours (approximately) did you take to do the assignments **pertaining** to this block?

Assignment Number	
No. of hours	

3) In the following table we have listed **4** kinds of difficulties **that** we thought you might have come across. Kindly tick (√) the type of difficulty and give the relevant page number in the appropriate columns.

Page Number	Types of difficulties			
	Presentation is not clear	Language is difficult	Diagram is not clear	Terms are not explained

4) It is possible that you could not attempt some **SAQs and TQs.**

In the following table are listed the possible difficulties. Kindly tick (√) **the** type of difficulty and the relevant unit and question numbers in the appropriate columns.

Unit No.	**SAQ** No.	TQ No.	Types of difficulties			
			Not clearly **posed**	Cannot answer on basis of **information** given	Answer given (**at** end **of** Unit) **not** clear	Answer given is not sufficient

5) Were all the difficult terms included in the glossary. If not, please **list in** the space given below.

6) *Any* Other Suggestion(s)

To

The Course Coordinator (LSE-03; Genetics)
School of Sciences
Indira Gandhi National Open University
Maidan Garhi
New Dehli-110 068.

REGIONWISE LIST OF STUDY CENTRES FOR B.Sc. PROGRAMME

Sl.No.	Centre Code	Centre Address
1. HYDERABAD REGION (Andhra Pradesh)		
1	0102	V.R. College, Nellore-524001, **Andhra Pradesh**
2	0103	KBN College, Kothapet, Vijayawada-520001, **Andhra Pradesh**
3	0111	Aurora's Degree College, Hyderabad-500020. **Andhra Pradesh**
2. GUWAHATI REGION (Assam,Arunachal Pradesh & Sikkim)		
4	0401	Guwahati University, Guwahati-781014, **Assam**
5	0404	Bujhora Mahavidyala, Bongaigaon-783280, **Guwahati**
6	0408	Hendique Girls College, Guwahati-781100, **Assam**
7	0409(P)	Govt. Science College, Jorhat-785010, **Assam**
8	0411	Bajali College, Pathsala, Pathsala P.O. Barpeta District-781325, **Assam**
9	0414(D)	Debraj Roy College, Golaghat P.O. Golaghat-785621, **Assam**
10	0419	Lakhimpur Girls College, Khelman P.O. North Lakhimpur-787031, **Assam**
11	2401	Sikkim Govt. College, Tadong, Gangtok-737102, **Sikkim**
3. PATNA REGION (Bihar)		
12	0501	Vanijya Mahavidyalay, Patna University, Patna-800005, **Bihar** (Patna Science College, Patna, Bihar)
13	0504	B.R.S. Bihar University Library. Muzaffarpur-842001, **Bihar** (L.S College, Muzaffarpur, Bihar)
14	0505	Marwari College, (T.M. Bhagalpur University), Bhagalpur-812007, **Bihar**
15	0508	Purena College, Purnea-854301, **Bihar**
16	0509	Rajendra College, Chhapra-841301, **Bihar**
17	0515R	Balika Vidyapeeth, Lakhisarai-811311, **Bihar**
18	0521	Sindri College. P.O. Sindri-828122, Dhanbad, **Bihar**
19	0522	C.M. College, Kilaghat, Darbhanga, **Bihar**
20	0524	Bihar National College, Patna-800004, **Bihar**
21	0525	Mahila College, Chaibasa, P.O. Chaibasa-833201, Dist. West Singhbhum, **Bihar**
22	0528(D)	St. Columbas College, P.O. College More, Hazaribagh-825301
23	0529	Anugrah Narayan College, Boring Road, Patna-800013
4 DELHI REGION (1) (South and West Region, Gurgaon, Faridabad and Mathura)		
24	0707	MCRC, Jamia Millia Islamia, Jamia Nagar, New Delhi-110025
25	0711	Gargi College, Siri Fort Road, New Delhi-110049
26	0715	Acharya Narendra Dev College, Kalkaji, New Delhi-110019
5. DELHI REGION (2) (North and East Region including Meerut, Modinagar and Ghaziabad Districts of Uttar Pradesh)		
27	0728	Bhaskaracharya College of Applied Sciences, Veer Savarkar Complex, Pusa New Delhi-110012
28	0729	Kalindi College, East Patel Nagar New Delhi-110008
29	2743	Lajpat Rai (P.G.) College, Sahibabad-201005, **Uttar Pradesh**
6 AHMEDABAD REGION (Gujarat, Daman & Diu, Dadra & Nagar haveli)		
30	0901	L.D Arts College. Navrangpura, Ahmedabad-380009, **Gujarat**
31	0902	General Education Building, M.S University, Vadodara-390002, **Gujarat**
32	0906	JB Thacker Commerce College, Bhuj-370001, Gujarat (Lalan College, Bhuj, **Gujarat**)
33	0909	Nerv Progressive Education Trust, Mehsana-384002, **Gujarat**
34	0922(R)	Shree Gattu Vidyalaya, Plot No. 910, GIDC Estate, Ankleshwar, **Gujarat**
35	0928(It)	National Institute of Management and Information Technology (NIMIT) C/o Parag Ad., Jansatta press, **Rajkot-5**
36	2901	Govt. Arts College, Daman and Diu (U.T.)-396210
7. KARNAL REGION (Haryana and Punjab)		
37	1001	Mukanndlal National College, Yamuna Nagar-135001, **Haryana**
38	1005	Chhotu Ram College of Education, Rohtak-124001, **Haryana** (All India Jat Heroes Memorial College, Rohtak, **Haryana**)
39	1008	Govt. College, (Girls Wing), Sector-14, Railway Road, Kamnal-132001, **Haryana**
40	1009	Govt. P. G. College, Hissar-125001, **Haryana**
41	1012	Markanda National College, Shahabad, Kurukshetra, **Haryana**
42	1013	Government P G College, Jind-126102, **Haryana**
43	2201	D.A.V. College, Jalandhar-144008, **Punjab**
8 SHIMLA REGION (Himachal Pradesh and Chandigarh)		
44	1101	Government Boys College, Sanjauh, Shimla-171006, **Himachal Pradesh**
45	1105	Government College, Dharamshala-176215, **Himachal Pradesh**
46	1113	Govt. P.G. College, Bilaspur-174001, **Himachal Pradesh**
47	1115	Govt. Degree College, Recong Peio, Kinour Dist., **Himachal Pradesh**
9 JAMMU REGION (J&K)		
48	1201	University of Jammu, Department of Management Studies, Jammu Tawi-180001, **J&K** (Gandhi Memorial Science College, Jammu Tawi, J&K)
49	1206	Govt. Degree College, Kathua, **J&K**
50	1207	Govt. Degree College, Rajouri, **J&K**
51	1208	Govt. Degree College, Poonch, **J&K**
52	1223(P)	Gandhi Memorial College, Camp Raipur, Bantalab, Jammu-181123, **J&K**
10. BANGALORE REGION (Karnataka and Goa)		
53	0802	Dhempe College of Arts & Science. P.O. Box No. 222. Panjim, **Goa-403001**
54	1303	ISS College, Dharwad-580004, **Karnataka**
55	1320	Govt. Science College, Nirupathunga Road, Bangalore-560001, **Karnataka**
11. COCHIN REGION (Kerala and Lakshdweep)		
56	1401	Institute of Management in Govt. Vikas Bhavan, Thiruanatapuram-695011 **Kerala**, (University College, Thirumantapura, Kerala)
57	1403	JD T Islam, Calicut-673018, **Kerala**
58	1404	Catholicate College, Pathananthits-689645, **Kerala**
59	1405	Shri Narayan College, Kannur-670007
60	1412	St. Alberts College, Ernakulum-682018, **Kerala**