Eukaryotic Gene Expression: Basics and Benefits

Prof. P N Rangarajan
Department of Biochemistry
Indian Institute of Science, Bangalore

Lecture No. # 36
Transgenic plants

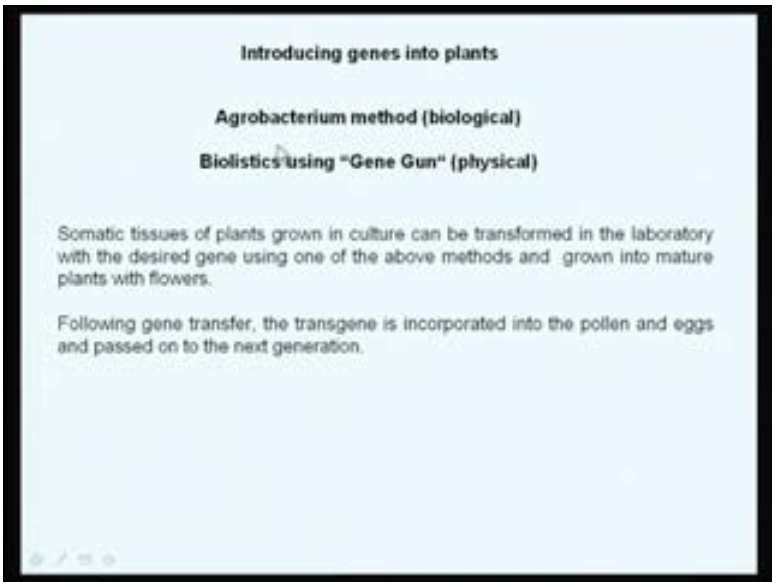
Welcome to this Eukaryotic Gene Expression, basics and benefits. In the last class, we discussed about expressing genes in transgenic animals. We started from transgenic mice, and how genes were being expressed in numbers of other mammals as well, including domestic animals. And, we also discussed at length, what are the strategies that are used for expressing genes in animal systems. And how, both for basic research as well as applied research.it has been very useful, not only in the... We have been able to understand; the function of genes, the function of promoters by generating transgenic mice, and transgenic animal models. We have also been able to express a number of useful genes for making recombinant proteins and recombinant pharmaceuticals.

So both, basic as well as benefits arising out of this transgenic technology has been discussed in the last lecture, in the animal systems. Today, we are going to spend some time to see, how people are trying to express genes in plants. How people are trying to generate transgenic plants, and what are the basic technology; that is involved in expressing genes in plants. And how the society got benefitted by the generation of transgenic plants. I have also would like to caution here, that this is the subject that is highly controversial. There has been a lot of controversy on the introduction of transgenic plants, into the environment.

There are a number of groups, which opposed to the introduction of transgenic plants into the fields, and the use of transgenic animals, both for the agriculture as well as for medical purposes. But what I am trying to focus here, mainly in this lecture is not to get into the controversies too much, but try to tell you the science of it, and also try to project some of the positive aspects, namely; how expressing some of the useful genes in plants, led to the development of very novel trades, and how society actually got benefitted by introducing these transgenic plants in agriculture. So, just as we have discussed about introducing the genes in transgenic plants how people use something like microware injection into the fertilized egg, or you can introduce genes to the

embryonic stem cells using viral vectors, and how you can carry out the germanaline transfer into animals to generate a transgenic mice. In the case of plants also, people use about two basic technologies for introducing genes into plants.

(Refer Slide Time: 02:45)

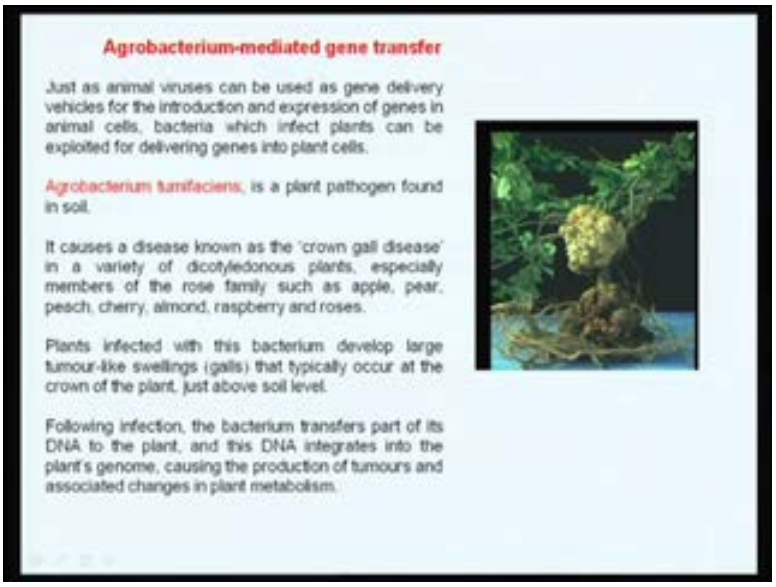


One is the agro bacterium mediated gene transfer, which can be called as the biological method of gene delivery. And in cases where, the agro bacterium mediated gene transfer is not very efficient, people use what is called as biolistics, just as we have seen in the case of gene delivery. In case of gene therapy, you can actually use gene gun or biolistics to pump or to forcefully deliver genes coated on gold particles into the plant cells. So, there are also several other minor techniques that people use for generating transgenic plants, but to the positive of time, we will confine ourselves to discussing these two major methods of generating transgenic plants. How transgenic plants can be generated by delivering genes, through the Agrobacterium meditating transfer or by using gene gun. So, basics strategy has been, somatic tissues of plants grown in culture, can be transformed in the laboratory with the desired gene, using one of the above methods and grown into matured plants with flowers.

So, following gene transfer, the transgene is incorporated into the pollen and eggs and therefore passed onto the next generation. So, the basically germline gene transfer, just as we have seen, because of transgenic animals, where we were introducing genes into the fertilized egg onto the E S cells, so that not all the animal which is generated, but the

successive generation also carries the transgene. The same way, by introducing genes through the Agrobacterium mediated gene-delivery or by biolistic methods. These transgenes also gets incorporated into the pollens and the eggs, and therefore, the transgenes gets passed on from generation to generation. So, what is this Agrobacterium mediated gene transfer.

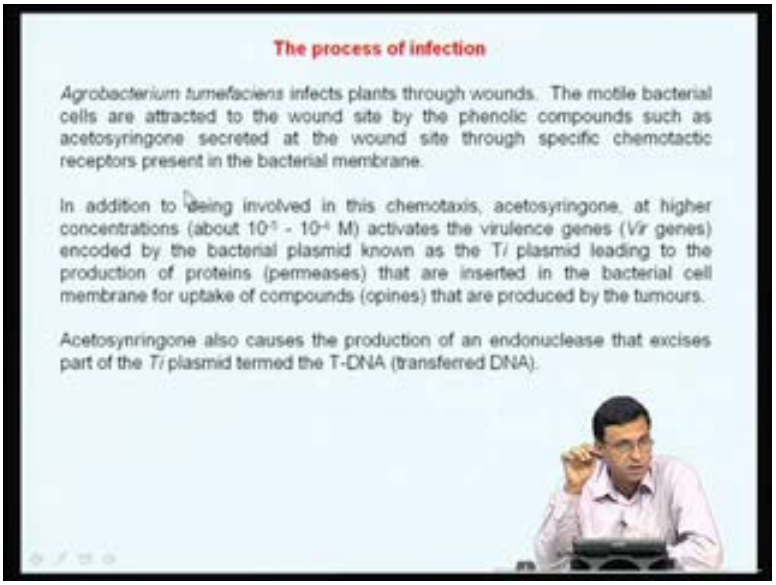
(Refer Slide Time: 04:23)



So, just as animal viruses are used for gene delivery vehicles, for the introduction and expression of genes in animal cells, bacteria which infect plants can be exploited for delivering genes into plant cells. And one of the most commonly used bacteria for the generation of transgenic plants, is a bacterium called Agrobacterium tumefaciens, which is a plant pathogen, normally found in soil, so it is soil microbe. Agrobacterium tumefaciens causes a disease called as crown gall disease. It is actually shown here, this is the crown gall, in a variety of dicotyledonous plants, especially members of the rose family; such as apple, pear, peach, cherry, almond, raspberry and roses and so on. So, it is basically plant pathogen. Plants infected with this bacterium develop large tumor like swellings called as galls, one of them is shown here. The typically occur in the crown of the plant just above the soil level. So, following infection, the bacterium transfers part of its D N A into the plant, and this D N A then integrates into the plants genome, causing the production of tumors and associated changes in plant metabolism.

So, here is a bacterium that actually naturally infects the plants, and during the infection process, it actually delivers its DNA into the plant. And therefore expresses its genes and also alters the plant metabolism. So, just as in the case of the, when we discuss gene therapy, how animal viruses do the same things; they infect the animal cells, push the genome into the animal cells, and then exploit the host machinery for its own survival. In the same way here is a plant a pathogen, a bacterium, which also infects the plants, pushes the D N A which goes and integrates the plant gnome, and then directs the plants to produce certain metabolites which is useful to you for the propagation of the bacterium. So, let us now see, how this basic phenomenon has been used, for generating a vector system for delivering genes into the plants.

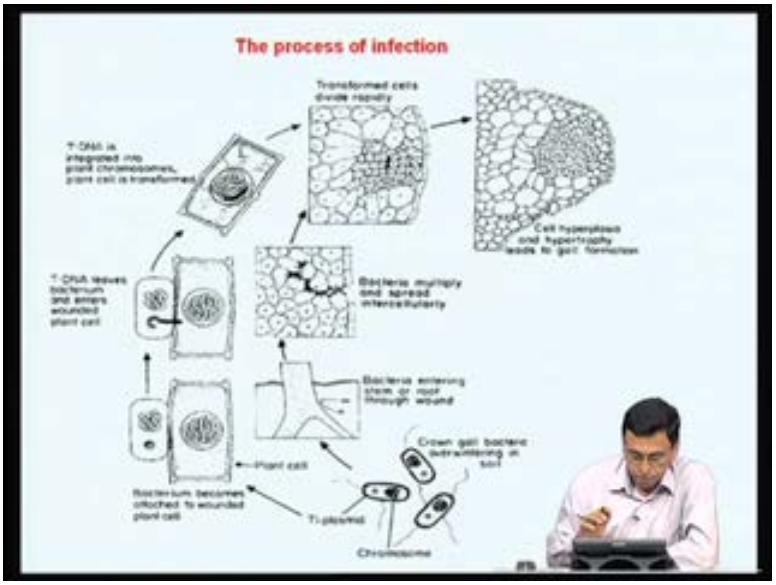
(Refer Slide Time: 06:24)



So, the process of infection as I just described, the Agrobacterium tumefaciens infects plants through wounds. The motile bacterial cells are attracted to the wound site by certain phenolic compounds, such as Acetosyringone, which is actually secreted by the plant at the wound site. And these compounds are sensed by specific chemotactic receptors, which are present on the bacterial membrane. So, the bacteria are attracted to the some of the chemo attractors, which are actually secreted by the wound. The wound may be happened naturally or during the propagation. So, when a plant gets wounded, it secretes certain chemical compounds; one of them is called the Acetosyringone, and the receptors present on the bacterial surface sense this, and the bacteria now gets attracted towards the wound. So this is basically called as Chemotaxis.

In addition, these compounds which are secreted by the plant wounds, such as Acetosyringone, at higher concentrations, activates certain genes known as virulence genes which is present in the bacteria. And these genes are encoded by a bacterial plasmid known as the T i plasmid, T i stands for tumors inducing plasmids. And as a result of the expression of this genes encoded by bacterial plasmid, certain proteins known as Permeases are actually inserted in the bacterial cell membrane, for the uptake of compounds that are actually produced by the tumors. And Acetosyringone also causes the production of an Endonuclease; that excises part of the T i plasmid termed as T D N A and transfers into the plant genome. So, let us now discuss in detail, how exactly this whole process of infection by the agro bacterium takes place, using the cartoon.

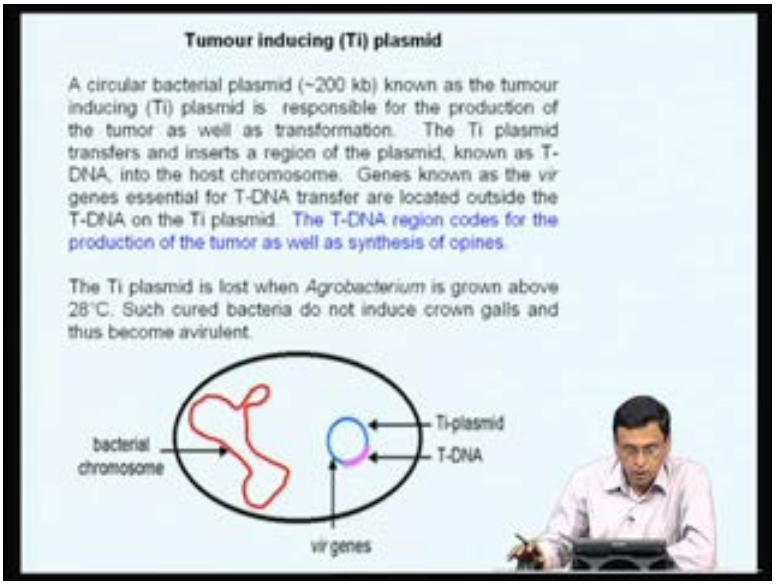
(Refer Slide Time: 08:02)



So, here are the soil bacteria. So when the plant gets wounded, this wound secretes certain compounds such as Acetosyringone, and by Chemotaxis, the receptrol present on the bacteria cell membrane. The bacteria sense these compounds, and get attracted towards the plant wound. And once it gets attracted towards the plant wound, the bacterium attaches to the plant cell and the bacterium. This is the base bacterial chromosome and here you have a bacterial plasmid, and a part of this bacterial D N A causes T D N A. Now, leaves the bacterium and enters the plant wound through specific bridges that are actually formed, between the plant cell and the bacterial cell. And then this D N A gets integrated into the main bacterial chromosome, and as a result of the expression of, some of these virulent genes, the plant is supposed to call gets

transformed, just as some of the animal virus when they go inside and cause transformation, leading to cancer. Here also, because of introduction of T D N A, the expression of the Plasmid coded genes. The plant cell starts producing, the wed genes of the bacteria mouse stars producing certain factor, which promote, which act as growth factors for the plants and promotes rapid cell division. And as a results the cells, plant cells surrounding the transform region develop very rapidly, and you get what is called as tumor or the crown gall. So, this is the basic physiology or the genetics by which, the bacterium induces a tumor formation in the plants, called as the crown gall.

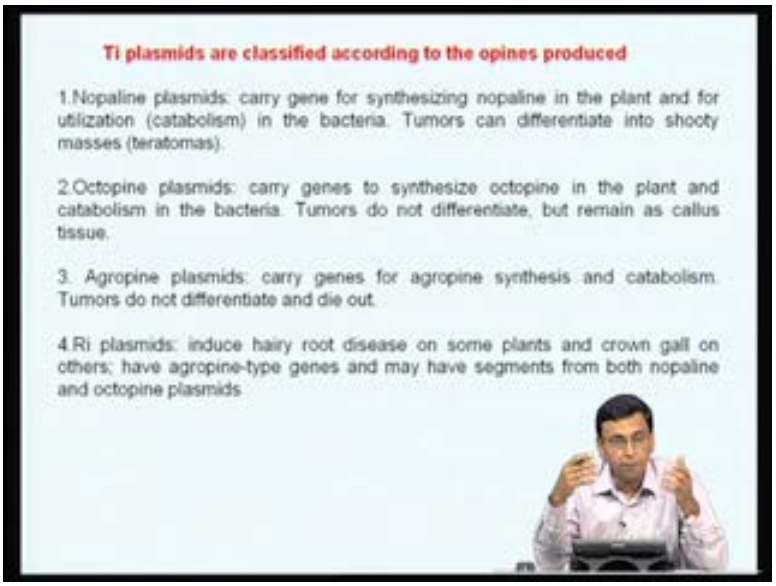
(Refer Slide Time: 09:37)



So, let us now focus on this plasmid, which is carried by the Agrobacterium Tumefaciens, known as the T A plasmid or tumor individual plasmid, because that is what is very essential for generating transgenic plants. Now, the T i plasmid is nothing, but a circular bacterial plasmid of about two hundred kilo bases in size, and this is known as the tumor inducing plasmid, is responsible for the production of the tumor as well as for transformation. Now, the T i plasmid transfers and inserts a region of the plasmid known as the T D N A into the host chromosome. So, here you have the agro bacterium tumefaciens, it has its main bacterial chromosome. It also has this T i plasmid of the tumor inducing plasmid, and within this plasmid, there is a small region denoted here in the pink color known as the T D N A, and this T D N A that gets enter to the plant genome.

The T D N A actually codes for production of tumor as well as synthesis of certain molecules known as opines. Now, these opines are the ones that promote growth of the plants, they act as plant hormones and promote the growth and multiplication of the plant cells, leading to the formation of the tumor. Now, this T i plasmid very very important, because if the T i plasmid is lost, as for example when you grow the bacterium above 28 degree centigrade, this plasmid is lost. And if I now take such bacteria which lack the plasmids, they cannot induce these crown galls and tumors, and therefore they become avirulent. Clearly indicating that, for induction of the tumor or a gall, further transformation, this T i plasmid is very essential.

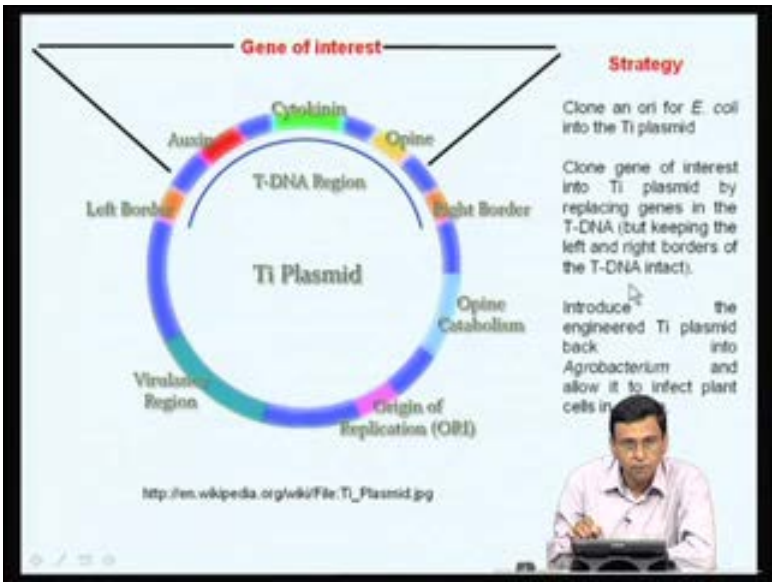
(Refer Slide Time: 11:11)



Now, there are number of T i plasmids depending upon what kind of opines they produce, there are water colors nopaline plasmids, which actually carry gene for synthesizing the compound called as nopaline in the plant, and for the utilization in the bacteria, and tumors that arise out of these nopalines, differentiate into shooty masses called as teratomas. There are other kinds of plasmid called as octopine plasmids. They carry genes, which synthesis compounds such as octopines in the plant, and catabolism in the bacteria. And in this, when bacteria carrying these octopine plasmids infect plants, the tumors do not differentiate, but remains as callus tissue. So, depending upon what kind of opines are produced, the morphology of the tumor varies.

There are water colors, certain agro bacterium tumefaciens carry water colors agropine plasmids. They carry genes for agropine synthesis and catabolism, and here also tumors do not differentiate and they die out. And finally the water colors are called as R i plasmids, they induce hairy root diseases on some plants and the crown gall on others. They have agropine type genes and may have segments from both nopoline and octopine plasmids. So, basically there are different types of T i plasmids; some of them produce nopolines, some of them produce octopines, agropines and so on and so forth, depending upon what kind of this opines are produced, the kind of crown gall, that is produced or the tumor that is produced varies.

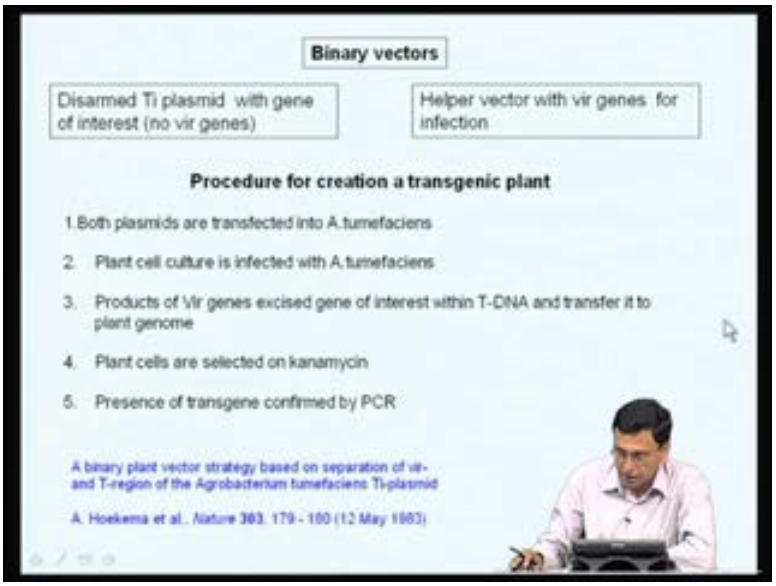
(Refer Slide Time: 12:36)



Now, so this is the cartoon of a T i plasmid, how does the T i plasmid look, basically the T i plasmid has what is called as left border and a right border, and the genes are actually codes for the auxins, opines and cytokines. It is these genes; that actually are responsible for promoting the growth and differential plant cells, leading to the formation of the tumor. For the insertion of this T region into the main bacterial chromosome, you required this right left border and right border. Any region that is put behind the left and right border, will now get inserted into the, leading to integration of this region into the plant gene. So, the strategy of making a transgenic plant has been, to take these genes that actually codes for some of these auxines cytokines and opines, and in their place put your gene of your interest. So, gene of your interest is inserted between the right border and the left border, so that these genes are replaced by your gene of your interest.

In addition, you also code a, include an origin of replication of equally origin of replication. So that once you make the recombinant construct, there can be made in large amounts in E coli, just as you have done for many of the other mammalian and east vector and so on and so forth. So, the strategy is, to introduce an origin of replication to the T i plasmid, so that this plasmid can be propagated in E coli cells. Then clone the gene of interest into a T i plasmid by replacing the genes in the T D N A, with the gene of your interest between the right and left border, and then introduce this (( )) T i plasmid, back into the Agrobacterium and allow it to infect the plant. So, what happens when this T D N A, T i plasmid gets inside the plant cell the region between the right and left border, now gets integrated into the plant genome. And therefore your gene of your interest with its promoter now gets expressed. So, this is the basic strategy by which Agrobacterium mediated to tumefaciens transgenseis carried out in plants.

(Refer Slide Time: 14:32)

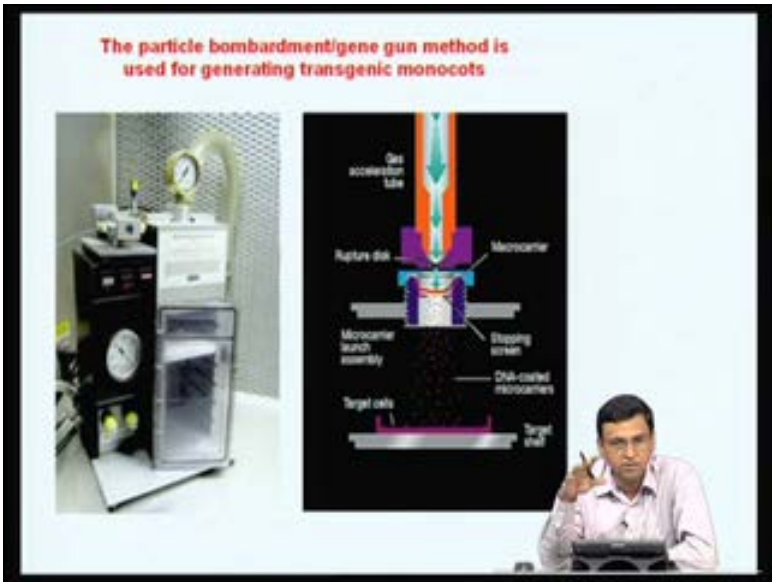


So, basically you construct what are called as binary vectors. First you generate a T i plasmid with your gene of interest; that is in the name of the place of the vir genes, you replace the vir genes or the virulene genes with your gene of your interest. And since you require the virulene genes for the transformation, you actually now express the vir genes on another plasmid called the helper vector. So, you have two plasmids you transform both these plasmids into the bacterium, and then make this bacterium carrying this recombinant plasmids, make it infect into a plant and you generate a transgenic plants. So, the basic procedure of creation of a transgenic plant using a Agrobacterium

tumefaciens is, the plasmids that is disarmed plasmid into the gene of interest as well as the helper vector containing the vir genes, are transected into agro bacterium tumefaciens.

The plant cell culture is then infected with this agro bacterium tumefaciens carrying both this plasmids, and the products of vir genes excise the gene of interest with T D N A and transfer to the plant genome, and plant cells which carry these plasmids are now selected by incubating them in the presence of kanamycin, growing them in presence of kanamycin. So, all the cells which are not taken up the D N A, which have not been infected by the agro bacterium tumefaciens will die. So, only those which have got infected will now survive, and then you confirm the transgene by P C R, just as we are dealing the case of the transgenic mice. It is a detail protocol of how the first binary vector was actually constructed, and was published in nature 1983s is given in this particular paper. A binary plant vector strategy based on separation of vir and t region in agro bacterium tumefaciens plasmids. One can go read this paper in detail, to understand how exactly this binary vectors are constructed, and how they were used for generating transgenic plants.

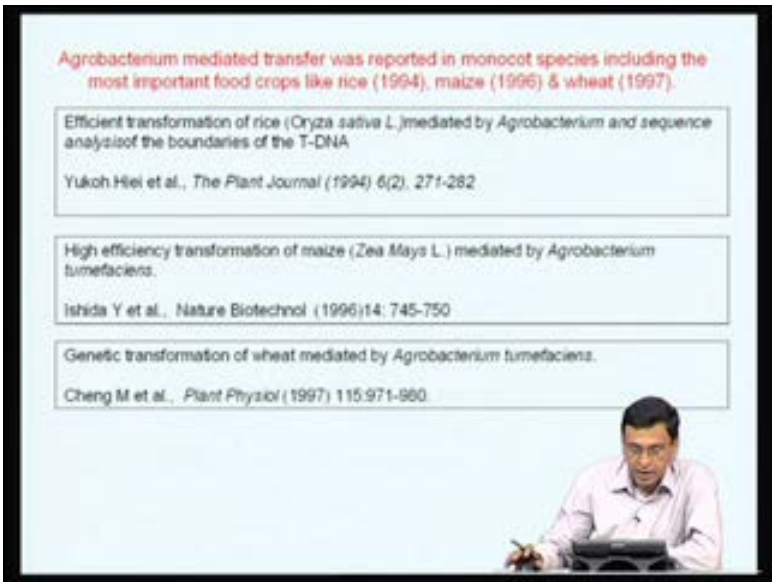
(Refer Slide Time: 16:18)



Now, the agro bacterium mediated tumefaciens is primarily infects the dicotyledon plants, it cannot really infect monocot plants. So during the early time of transgenesis of plants, the agro bacterium mediated gene transfer was primarily used for generating

transgenic dicot plants. But, since this bacterium cannot infect monocot plants, an alternate strategy was developed for introducing genes into monocotyledon plants, and that is the gene gun or the biolistic method or the particle bombardment method. Again this we have discussed in the case of gene therapy using wherein, actually again we use a gene gun, where you take your D N A of your interest, mix with gold particles and shoot them into the tissue using gene gun. In the same way, you use a gene gun based system for delivering gold coated D N A, and you just using a helium gas, when you pump or push this gold coated particles coated with D N A, with the high pressure using the helium gas, and the tissue culture cells or the plant callus cells now get transected by this gold particles carrying the D N A, and they get disease of the transformation is carried out. So, in the case of monocots, during the early years, the generation of transgenic plants was being carried out using the biolistic method of the gene gun method, using gold particles coated with D N A of your interest, because the agro bacterium tumefaciens did not infect monocots.

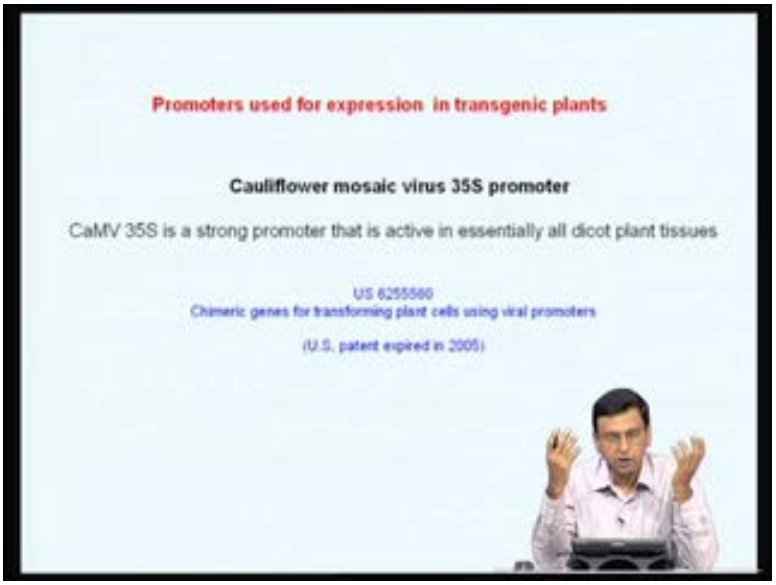
(Refer Slide Time: 17:56)



But, then later in 1994 to 1997, agro bacterium mediated transfer protocols were also developed for monocot species, especially for food crops; like rice, maize and wheat. Now a day's your monocot plants can be transformed using, agro bacterium mediated tumefaciens. I will not go into the details of it, I will just give the references here for the lack of time. So one can actually read some of these references to see, how these agro bacterium mediated gene transfer techniques were actually developed for monocots as

well. So, this is the paper efficient transformation of rice, mediated by agro bacterium and sequence analysis of boundaries of T D N A, published in plant journal 1994, gives how the agro bacterium mediated transfer was developed for rice. Similarly, in 1996 similar protocol was developed for introducing genes into maize by agro bacterium mediated tumefaciens, published in nature by technology. And again in 1997 a similar protocol was developed for introducing genes into wheat, published in plant physiology in 1997. So, a number of crop plants can now be, gene transfer can be carried out, using agro bacterium mediated gene transfer.

(Refer Slide Time: 19:08)

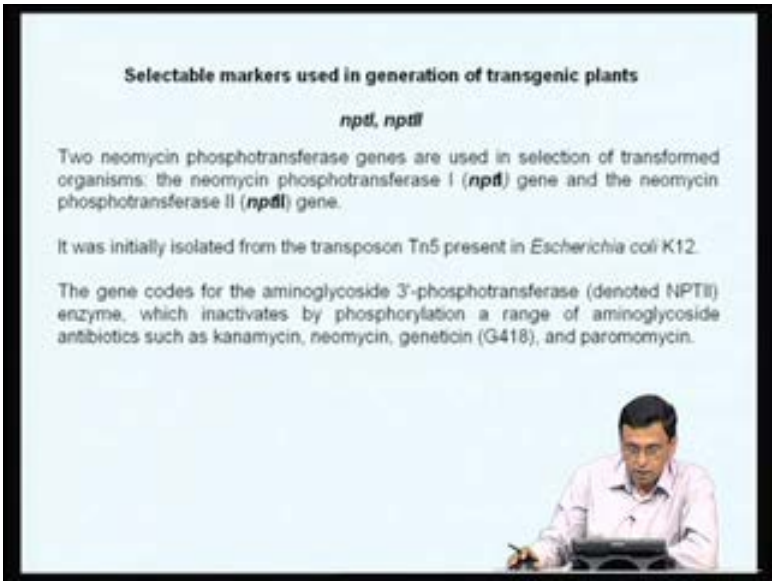


Now, what kind of promoters are used for expressing genes and transgenic plants. A wide just as we have seen in the case of the animal systems. A number of plant promoters are available, this could be virus or viral promoters which infect plants or it could be a natural plant promoter. They could be tissue specific or developmental stage specific and so on and so forth. But, one of the most widely used or most exploited promoters in generating transgenic plants has been derived from a virus called as cauliflower mosaic virus, and this promoter is made for 35 S gene.

So, this cauliflower mosaic virus 35 S promoter is one of most widely used promoters for expressing genes in generating transgenic plants. So, the 35 S promoter is a very strong promoter, that is active almost all dicot plants, and it is one of the most extensively used promoters for the transgenic plants. In fact this is this is a patent that is owned by

Monsanto, and we had a virtual monopoly for using this promoter for generating transgenic plants for long time. And patent only expired some time in 2005, and therefore anybody can actually use this 35 S promoter for generating transgenic plants, both for basic as well as for certain applications.

(Refer Slide Time: 20:22)

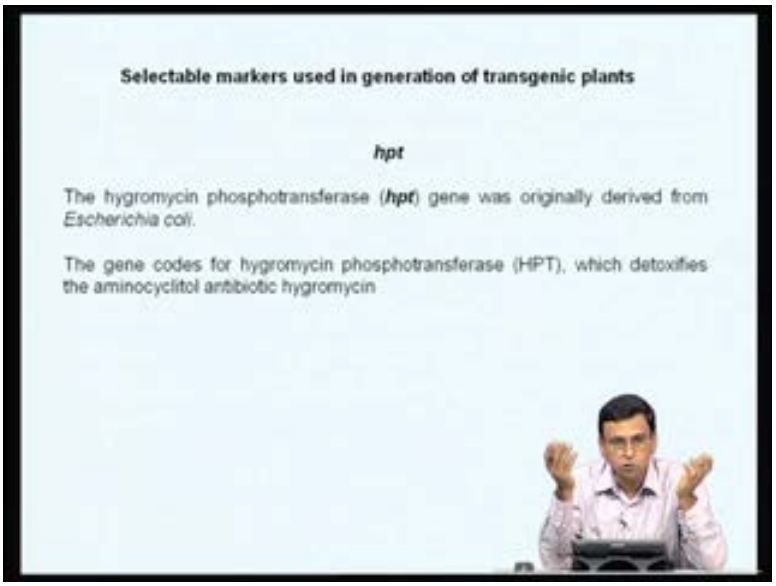


Now, what kind of selectable markers are used for generation in transgenic plants, because it is very important. When you do either the gene gun mediated gene transfer or use the acrobatic mediated gene transfer, not all cells will get transformed. So, you need to select those cells, which I have taken up, which got infected with the bacterium or which have taken up the D N A. and therefore usually the vector that where used, usually as to contain a selectable marker. Some of the common markers which are actually used, in the case of transgenic plants has been, what is called as a neomycin phosphotransferase gene. There are two such genes called as neomycin phosphotransferase is 1 and phosphotransferase 2, n p t 1, n p t 2 genes.

This was originally isolated from a transpozan, present in the escherichia bacterium, and the gene actually codes for the aminoglycoside three phosphotransferase, denoted as n p t 2. And this enzyme inactivates by phosphorylation a number of aminoglycoside antibiotics; such as kanamycin, neomycin, geneticin and paramomycin. So, cells which have not taken up this plasmid containing the selectable marker, they will get killed by this antibiotics. Whereas, cells which have taken up the antibiotic, because they express

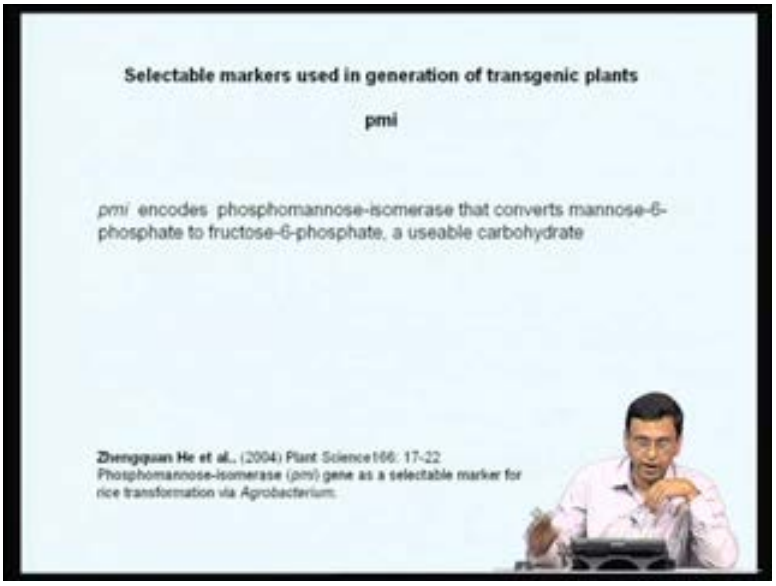
this phosphotransferase gene, it phosphorylize this antibiotic and makes it inactive, therefore they survive. So, we can select those cell which are taken up your gene of your interest or else those cells which are not taken up, will get killed by the antibiotic.

(Refer Slide Time: 21:50)



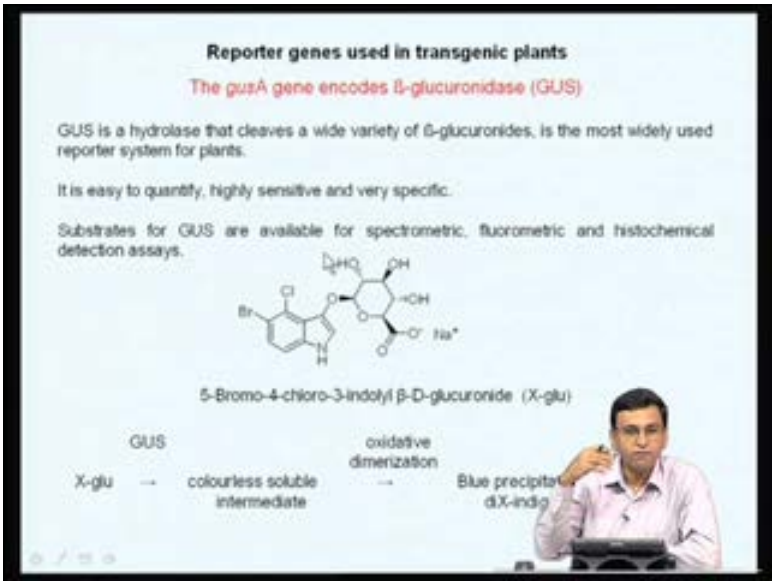
The other very commonly used selection marker in generating transgenic plants has been hygromycin phosphotransferase gene. Again derived from escherichia coli, this gene again codes for Hygromycin phosphotransferase. Again as in the case of the n p t, it actually detoxifies aminoglycoside antibiotic known as been hygromycin. So, basically the enzymes produced by this resident gene inactivate the antibiotic, and therefore cells carrying these genes become resistant to that antibiotic, whereas those cells which are not taken this antibiotic get killed.

(Refer Slide Time: 22:21)



There is also a non-antibiotic marker gene, which is now been used, actually this is came up with paper, this came up in 2004, where you can actually use an enzyme called phosphomannose-isomarase as a selectable marker gene, instead of an antibiotic resistant gene. And this gene which is called the p m i gene, actually converts mannose six phosphate to fructose six phosphate, which is a useable carbohydrate. So, if we now have cells which are expressing p m i, and which cells which are do not expressing p m i. If you now use mannose six phosphate as a carbon source, in the case of those cells we are expressing the p m i gene, mannose six phosphate get convert into fructose six phosphate, which can go into the glycolysis and the plant can use this carbohydrate. Whereas, those which do not express the enzyme, they cannot make use of the mannose six phosphate and therefore they die. So, instead of the antibiotic resistance marker, a metabolic enzyme can also be used as a selection marker, in the case of generating transgenic plants. So, we have the n p t 1 and n p t 2, h p t or the p m i genes, and these are the three genes which are routinely used for, generation of transgenic plants as selectable markers.

(Refer Slide Time: 23:36)

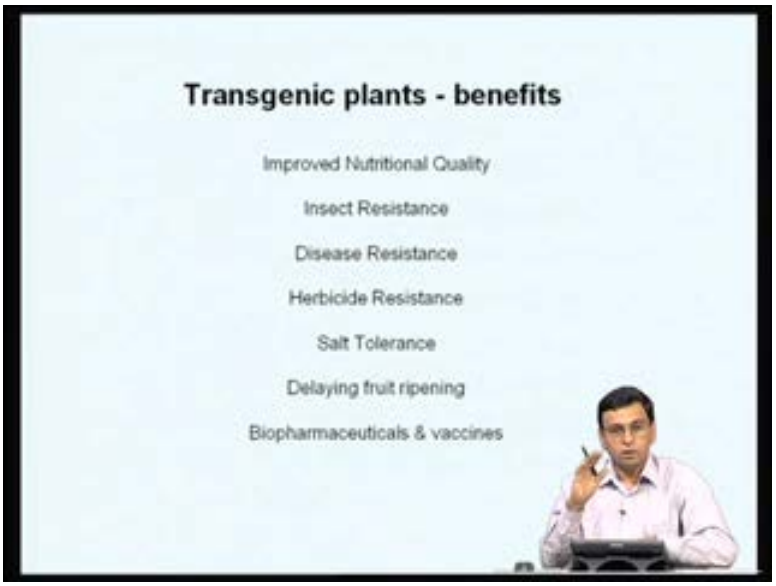


Now, what kinds of reporter genes are used for generation of transgenic plants, because suppose I want to study; what kind of promoter, what is the nature of the promoter, which cells, it is expressed, what are the cisacting elements of a natural promoter of a gene. If I have to identify just as promoters have been characterized in the case of transgenic mice and very valuable information was obtained, on the developmental regulation or tissue specific expression of number of promoters. People have also used this transgenic technology to understand the function or mapping this cis acting elements of various promoters, using the transgenic plants.

So, in such cases the strategy is, you take the promoter region of your interest, link it to a reporter gene, introduce them into the plants, either by biolistic method or using the agro bacterium mediated gene transferred. And then see, if the by activating the reporter gene and studying the reporter gene expression, you can understand what is the sis acting elements which are present in the promoter region. And one of the most commonly used reporter genes is, what is called as the GUS; that is beta glucuronidase. Remember in the case of the animal cells and in bacteria, we normally use what is called as beta glucuronidase. The equili beta glucuronidase is normally used for as a reporter in a number of microbial as well as the animal systems, because of plant systems, the b glucuronidise are abbreviated as gus, is the most popular reporter gene that is actually used.

Now, GUS is the hydrolase that gives a wide variety of beta glucuronidase, and is most widely used reporter system for plants, it is very easy to quantify highly sensitive and very specific, very important characteristics for a very successful reporter gene, and substance for GUS are available commercially. And you can assign the activity of the reporter gene or the GUS by spectrophotometric, fluorometric or histochemical methods. So, it is a very convenient and easy for detecting the reporter gene expression, and this is the compound, colorless compound that is actually used for the GUS activity measurement. And when this compound the bromo-chloro-indolyl-beta-d-glucuronide commonly known as the x-glu, it is normallycolorless, but when the GUS gene is expressed, it gets induces oxidize to dimerization, leading to the formation of blue precipitate. So, the appearance of blue color, indicates that the expression of the reporter gene.

(Refer Slide Time: 26:02)



So, what I have expressed so far, explained so far is, how one can generate transgenic plants, basically there are 2 major methodologies; one is using agro bacteria tumefaciens mediated gene transfer, using the tumor intubated plasmid, where introduce our gene of interest by inside right and left border of the T a plasmid, and then by expressing vir genes another plasmid using a binary vector system. You can actually generate a agro bacterium tumefaciens, expressing the transformed this plasmids, and then you make this bad bacterium infect the plants, and then select those cells are taken up or infected cells by using appropriate selection markers, and also discussed one of the most popular

promoter that are used for generating transgenic plants is the, thirty face promoter of the cauliflower virus.

There are also number of other promoters which are used, we will not get in the details, and if you want asses the activity of a promoter is a plant system, normally reporter genes such as glucuronidase, the GUS is normally used, but people also use luciferase or green fluorescent protein as select as the reporter gene, for saying plant promoters are generating transgenic mice, plus GUS has been the most popular reporter gene, that is used for a saying promoter activity in the main transgenic plants. So, these are the some of the basic aspects are discussed. Now, a number of variations, some of the features have discussed are possible, but we will not get into the details, or variations of these various protocols, but, I have given only basically general or basic idea, of how do you generate transgenic plant, and you want to express a gene of your interest.

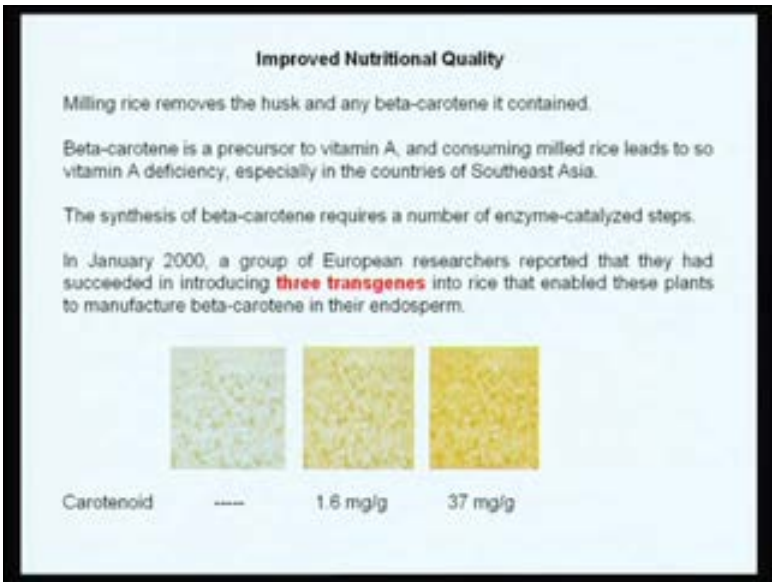
Normally, use a very important constitute promoter like a thirty phase promoter, or if you want to assign for a promoter activity of a unknown promoter, you can take the promoter, put it under a reporter gene such as GUS or g f p or luciferase, and then transform the plant, and then look for the reporter gene activity in various tissues of the plants. So, having explained the basic aspects of generating transgenic plants, let us now trying to spend some time and see, what are the benefits that came out of generation of transgenic plants. Now, one of the basic outcomes, on basic research has got enormously benefited, as I said people are using transgenic plants, for understanding the function of number of genes. People are also using transgenic plants for understanding the function of the number of promoters; this is the basic aspects of generating transgenic plants. So, by using transgenic plants, one has been able to map a number of tissue specific promoter, developmental stage specific promoters and so on and so forth. And also has been able to study, and understand a function of many normal genes. So, it has been extremely used, transgenic plants has been extremely useful in basic research for understanding gene functions as well as for understanding the promoter activity.

In addition, transgenic plants has been extremely useful, and a number of benefits come out of transgenic plants. So I am going to list some of the benefits that came out of generation of transgenic plants. It is not possible for me to explain all the benefits that came out of transgenic plants, so I have actually made a choice.As examples I am going to discuss, how by generating transgenic plants, you can improve nutritional quality of

plants, you can develop plants which are resistant to insecticides, you can develop plants which are resistant to certain diseases, you can also develop plants which are resistant to herbicides, you can develop plants which can tolerate salt, high concentration of salt. So, you can grow these plants in marshy areas, where normal plants cannot grow. You can actually by expressing certain genes especially those encoding antisense R N A, you can delay fruit ripening, again has lot of commercial applications. You can also use transgenic plants for the production of a number of bio pharmaceuticals, recombinant proteins as well as vaccines.

So, let us spend some time to see, how by using this basic approaches; that is by using this two basic approaches, that is either introducing genes, using biolistic method or agro bacterium mediated tumefaciens. What kind of genes have been introduced in the plants, transgenic plants, what kind of transgenic plants for generated, and how the society got benefitted for some of this transgenic plants. Now, one of the most exciting research that came out of the generation of transgenic plants, it is what has called, popularly known as the golden rice.

(Refer Slide Time: 30:27)



Now, we all use the rice, when you take the paddy, and then try to put them in rice mills. The husk is actually removed during this milling process along with the husk, the beta carotene, the aleurone layer which actually contains the beta carotene is also lost. Therefore, the rice, the polished rice that many of us actually eat, is actually depleted of

beta carotene. A beta carotene is very very important for vision and for the production of vitamin A, which is very essential, and without vitamin A, a number of physiological functions are affected, as we all know vitamin A gets convert retinoic acid and retinoic acid is a very important morph gene is required for development, and also if you consume excess amount of retinoic acid, it can lead to teratogenic.

Similarly, form the vitamin A you get a retinal, which is very important component for vision. So if you do not take sufficient amounts of vitamin A, it results in vitamin deficiency; causes blindness, and number of other health problems. So, especially people living in Africa and a certain developing countries, because of use of this continuous use of polished rice and their own supplement vitamin A by other, means such as vegetables and so on and so forth. They virtually develop blindness and number of other health hazard. So, people thought why cannot we develop rice, which actually now contains beta carotene. So, what actually was done is beta carotene is a precursor for vitamin A, and consuming milled rice leads to vitamin a deficiency, especially in country such as South East Asia.

Therefore, people asked the question, can you now express the genes, that are essential for the synthesis of beta carotene, so that now has rice which actually contains lots of beta carotene. So even when you polish this rice is still has lot of beta carotene. Now, to make beta carotene in the rice, you have to express the genes which are involved in the biosynthesis of beta carotene. And unfortunately this synthesis of beta carotene is a multi-step process. So, people realize that you need to express more than one gene, if you have to make beta carotene in plants. So, in January 2000, a group European researcher reported that, they have succeeded in introducing three transgenes into rice, that enable this plants to manufacture beta carotene in their endosperm. So, even if you loose the aleurone layer and lost the beta carotene, endosperm still has lots of beta carotene, and therefore such vitamin A deficiency can be avoided by eating such rice.

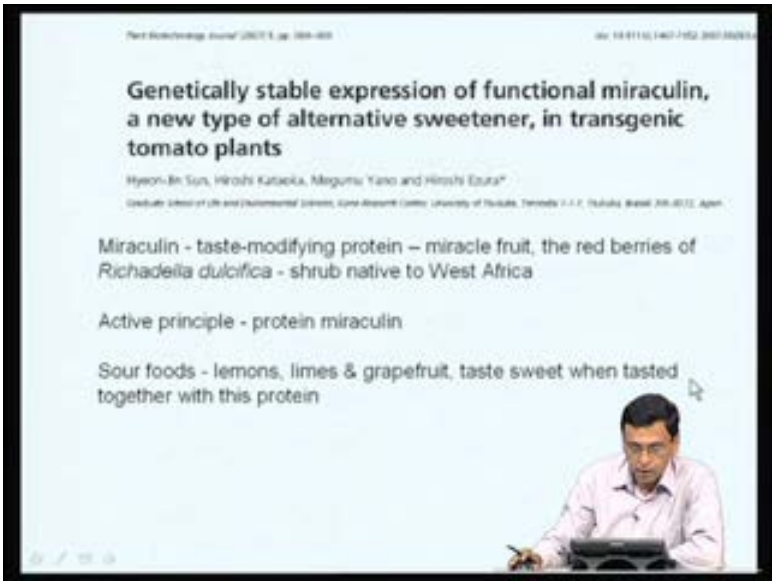
Here, I have just shown you the normal rice, which you can see is white, not colorless, because it does not contain any carotenoid, here is a transgenic rice which expresses now about hundred and one point six milligram per gram of beta carotene. And you can see it is now light yellow, and if you express higher amounts of carotenoids in this rice you can see its dark yellow. So, this is one of the golden moments of transgenic research, where people develop transgenic rice, containing high amounts of beta carotene, and thus you

can enhance the nutritive value of food crops by expressing certain trangenes, and this is one such example. So, this generation of golden rice created huge amount of excitement among researchers and as well as public.

(Refer Slide Time: 33:36)

Similarly, here are tomatoes which are purple why, two genes where expressed snap dragon, which induce the production of compounds called anthocynins. And when you express these two genes leading to synthesis of anthocynins, now the tomatoes turn purple. Now, it is not just the color that matters, these anthocynins are offer protection against cancers, cardio vascular diseases, age related degenerated diseases, and therefore there is evidence that anthocynins also have anti-inflammatory activity, promote visual activity, hinder obesity and diabetes. So, by consuming these kinds of transgenic tomatoes which are expressing these anthocyanins it has, it can actually promote your health, so again enhancing the nutritive value.

(Refer Slide Time: 34:21)



It is another example, genetically stable expression of functional miraculin, a new type of alternative stable sweetener in transgenic tomato plants. Now, what is this, now miraculin is a taste modifying protein, actually present in miracle fruit which are the actually red berries of richadella dulcifica, which is a shrub native to West Africa. So, they again took the gene encoding this miraculin protein, and then introduced in tomato plants or other plants. And what happens when you consume this fruits are expressing this miraculin, the taste changes, so sour fruits; such as lemons, limes and grape fruits taste sweet, when tasted together with this protein. So, somehow when you express this protein in some of this fruits, the sour taste is converted into sweet taste. So, you can see again enhancing the quality of the food by expressing certain transgenes in plants.

(Refer Slide Time: 35:16)

Here, is another example of benefits of generating transgenic plants, by expressing the appropriate genes and plants, tearless onion, we all know that when you cut the onions tears roll down your, why is that?; as onion are sliced the cells are broken, allinases brake down, I mean sulphoxides are generated, they are converted sulfonic acids, which are highly unstable, and they rearrange into a volatile gas, which defused by air, and then reaches the eye, and these acids now react with water to form a dilute solution of sulfuric acid, and tear glands produce tears to dilute and flush the irritant. This is how whenever you cut nions, you get tears. So, what happens, you know generate a transgenic onion by expressing a gene, that actually down regulates or reprocess the synthesis of lachrymatory factors synthase.

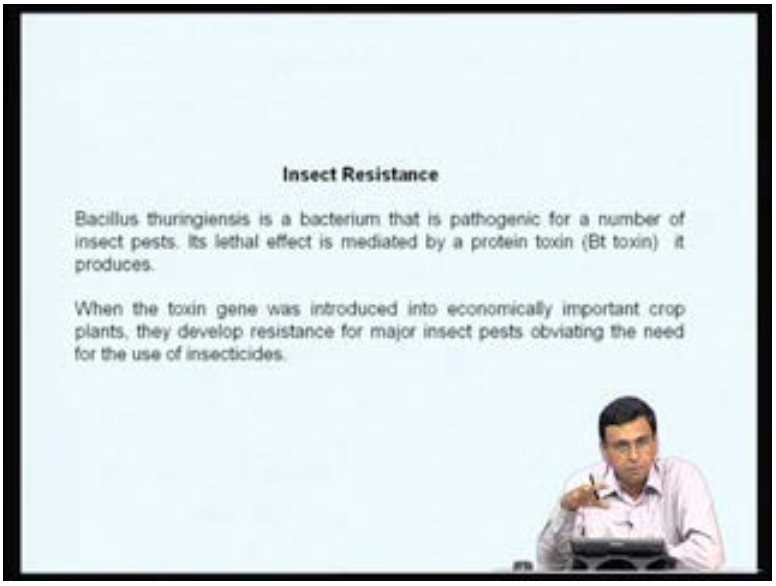
Now, this is the protein enzyme that is responsible for the production of this irritant, and if you use what is called as R N A I technology. I have still not discussed this R N A I technology in this course, we are going to discuss it may be in the next couple of classes. So, if you now repress the expression by using R N A I or R N A interference technology of a synthesis of a enzyme called lachrymatory factors synthase. The conversion of this valuable sulphur compounds in to the tearing agent is inhabited. So, now you have onions which does not induce tears. So you can see by expressing appropriate genes, either by activating certain enzymes or by inhibiting certain enzymes. Expression of certain enzymes, you can have very beneficial effects by generating such kind of transgenic plants.

(Refer Slide Time: 36:56)

Now, this is an example I want to give, who should not be under impression that, all these useful trades can be generated only by transgenic plants. Here is another very interesting example or called as rainbow cauliflowers, where you have purple, yellow cauliflowers. These were actually produced by traditional breeding methods by a company called syngenta. Actually it even has subsidiary in India, right in Pune, but these were developed by non-transgenic methods, and what is the significance of these, the orange cauliflower has higher the normal levels of beta carotene, that encourages healthy skin, just has you have the golden rice with high levels of beta carotene, you have a cauliflower containing high levels of beta carotene.

In the case of the purple, the purple color comes from the anthocyanin, again which helps prevents heart disease by slowing blood clotting. But, the point I want to make here is that, these kind of rainbow cauliflowers expressing again having useful nutrients well actual generated by non-transgenic means. So, let us not be under the impression that interesting trades can be incorporate into some of these agriculture plants, not only by transgenic technology, but even by conventional plant breeding, very useful trades can be introduced into the plants.

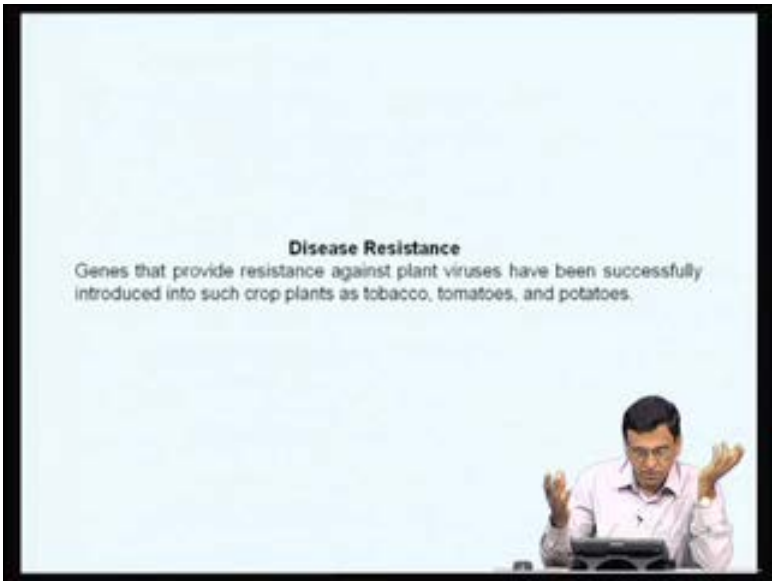
(Refer Slide Time: 38:09)



So, I have given a number of examples to tell you, how the nutritive value of food products can be enhance by generating various transgenic plants, by introducing appropriate genes, either by expressing certain genes or by inhibiting the expression of certain genes. Now, the other important example I would like to give is that, how you can generate transgenic plants which are resistant to insect pest. Bacillus thuringiensis for example, is a bacterium, that is pathogenic for a number of insect pests, why is that. Because this bacterium contains a protein called as B t toxin, bacillus thuringiensis toxin. So, when insect consume this bacteria, this bacteria produce this toxin and this toxin kills the insects. So, what do you do now, you now take this B t gene or the B t toxin gene, and generate a transgenic plant expressing the B t toxin.

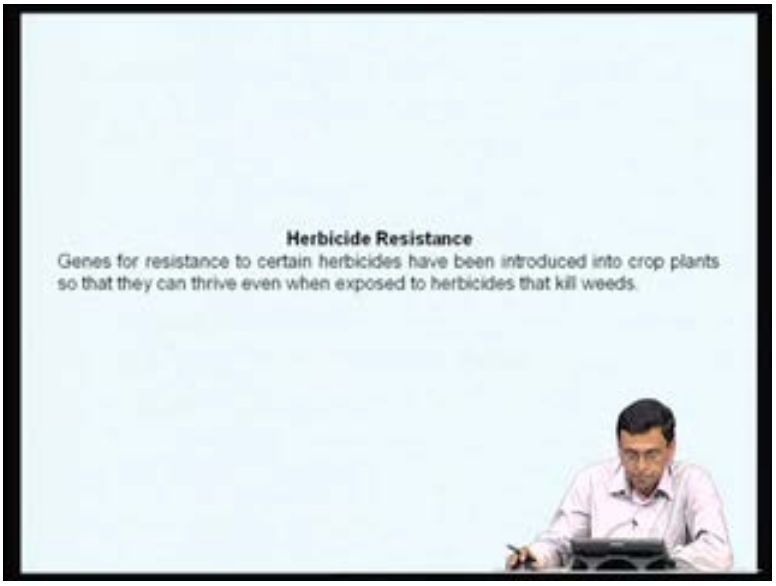
So, when the insects eat the plant leaves or the plant tissue, the B t toxin now enters the insect gut and it kills the insects, so you do not have to spray insecticides. So, the insect, use of insecticides can be completely avoided by generating transgenic plants, or important economical crop plants express a toxin gene. They can now develop resistance to major insect pairs, that thereby obviating the need for using insecticides. So, you can see by introducing think genes like B t toxin gene into important crop plants, and generating these transgenic verities, they can be made insect resistance. So, you do not have to spray insecticides to kill, to prevent insect from destroying this crop plants.

(Refer Slide Time: 39:42)



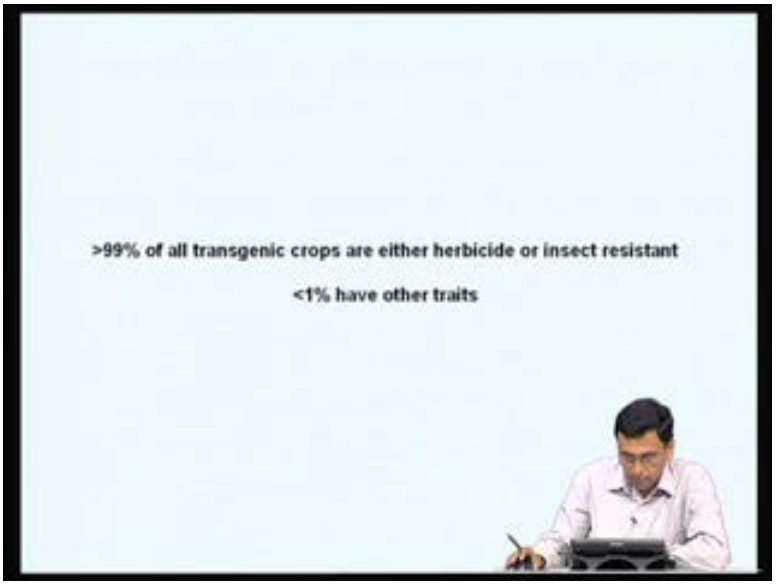
Again, disease resistance, this is the provide resistance again, viruses have been successfully introduce in the number crop plants; such as tobacco, tomatoes and potatoes. A, number of plant viruses cause a wide verities of diseases, especially many of the fruits like tobacco, tomatoes, potatoes and so on and so forth. Like in tobacco for example, have the tobacco mosaic virus and so and so forth. So, whenever the plants get infected with these viruses, they also express certain dieses resistant genes, and by engineering this gens which provide disease resistance. You can actually develop these plants which can now resist these viral viruses. There becomes a resistance many of this viral diseases.

(Refer Slide Time: 40:21)



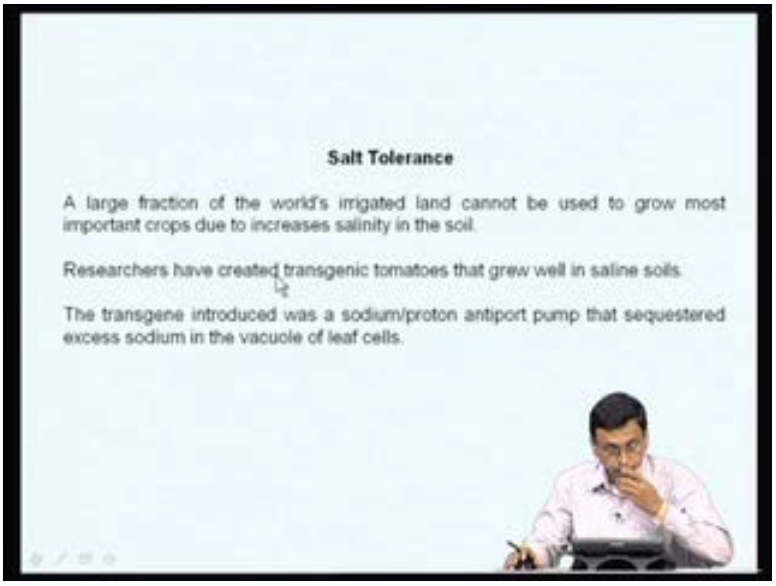
Herbicide resistance again, genes for resistances certain herbicides, have been introduced in the crop plants, so that they can thrive, even if when he exposed her besides that kill weeds. So, one of the major problems in agriculture is that, along with the crop plant or plant that you want to grow, a number of weeds also grow, along the side of the plants. So, how do we destroy these weeds, because the (( )) of the fertilize that you used, are consumed not only by your crop plant, by your plant of interest, but also weed decide, so which is a waste of money. Therefore, you want to actually kill the weed selectively, but not your crop plant. So by engineering herbicide resistance genes in to your crop plant of your interest. If you now spray this herbicide, your plant will not get affected, but the weeds which are actually growing along these plants, they get affected by these herbicides; therefore you can kill all the weeds, by using this kind of a herbicide resistant plant.

(Refer Slide Time: 41:16)



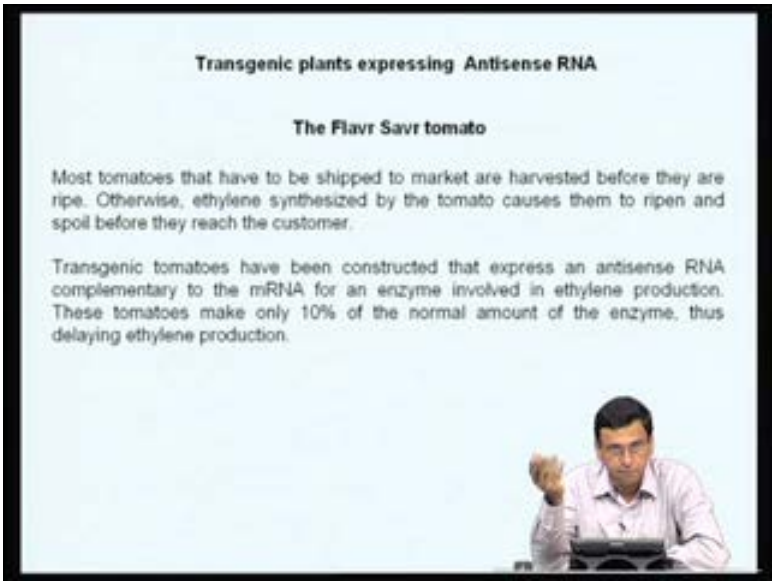
Now, the importance about, I have told you that can use, I have given a list of what kind of transgenic plants have been generated, you can use a nutrition value, herbicide resistance, insect resistance so and so forth. But almost more than 99% of the transgenic crops, which have commercial value, or either herbicide resistance or insect resistance crops. So, introducing genes that come for herbicide resistance or insect resistance has been one of the major aspects of generating transgenic plants, especially from the agriculture. So, the other trades, the various other trades that I have mention so far, contribute less than 1% of the total transgenic crops that we have today. So, majority of the transgenic crop that have been produced today, are primarily devoted for developing resistance to herbicides or developing resistance to insect pests.

(Refer Slide Time: 42:06)



So, some of this other ways that people are trying to developing in transgenic plants is, development of salt tolerance for example. A, large fraction of worlds' irrigated land cannot be used to grow most important crops, because of the salinity nature of the soil. The soil is highly saline, so normal plants cannot grow there. But many of the plants actually express what is called resistance genes, when you expose them to high, many plants which grow in marsh areas, they are tolerant to the salinity conditions, because they express genes which confirm resistance to salinity. Now, if he takes such genes which confirm resistance to high concentration, put them properly crop plants, you can grow crop plant marshy areas, which are otherwise not suitable for agriculture. So, research how created transgenic tomatoes that grew very well in saline soils. The transgene introduced was actually a sodium proton and antiport pump; that sequestered excess sodium in the vacuole of the leaf cells. So, by expressing a gene, that actually course a sodium proton antiproton pump. The salt that is injected in to the plant is actually sequestered vacuole, and therefore to the plant is able to survive in high salt conditions.

(Refer Slide Time: 43:20)

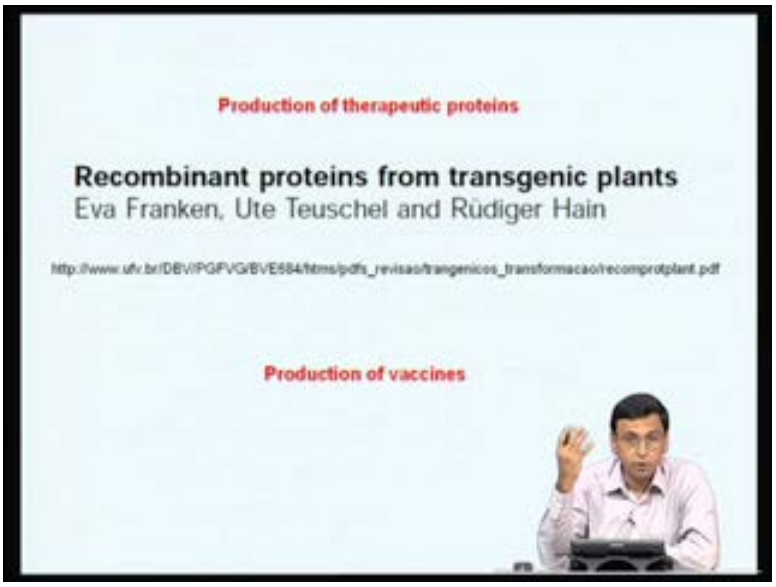


Again, transgenic plants expressing antisense R N A. Now when you express an antisense R N A. The antisense R N A will go and hybridize the sense R N A, which is normally present in the cells and as the result double stand R N A is formed, and translation is blocked. So, the R N A will not be translated and proton of your interest will not be produced. So, tomatoes can you now use this kind of antisense by technology to delay ripening of certain fruits, very famous example has been what is called the flavors sour tomato by produced by a company called Calgene. Now, most tomatoes that have to be shipped to the market, are harvested before they are ripe, otherwise the ethylene that is synthesized by tomato causes them to ripen and spoil before they reach the customer. So, from the place of production, that is from the farm to carry this genes towards to the actual marketing place, which is usually town or a city, you have to if you allow the fruits are ripen on the plant. And then transport most of the tomatoes will get damaged during the transport itself, so it is tomatoes loss. So, this is because, the tomatoes produce ethylene which induces fruit ripening.

Now, transgenic tomatoes have been constructed, that express an antisense R N A complementary to the m R N A for an enzyme involved in ethylene production, therefore production of ethylene is delayed. Now, these transgenic tomatoes make only ten present of to the normal amount of the enzyme, therefore it delays a ethylene production. So, you can plug them early, and then transport slowly, because it is producing much less ethylene. The fruit ripening is delayed, and as a result the damage due to fruit ripening

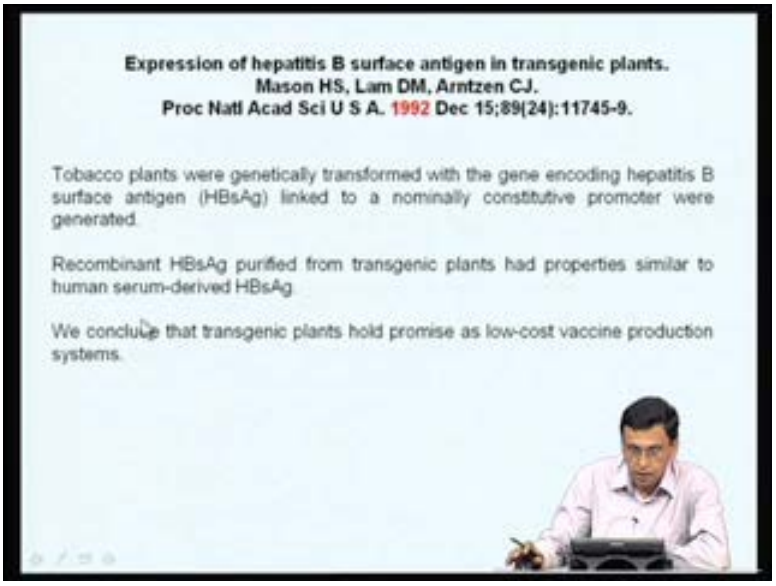
before diseases which is destination can be prevented. So, a company called Calgene actually developed this kind of a tomato, and call it has flavor sour tomatoes but, because of a number of oppositions for the introduction of some of this transgenic plants or transgenic fruits or edible crops, transgenic first there is lot of resistance for these things. These many of these transgenic fruits never saw the light of the day.

(Refer Slide Time: 45:26)



Production of therapeutic proteins; just as we have seen that farm animals are being used for production of a number of a therapeutic protein; such as a factor eight, factor nine and some growth hormones so on and so forth. You can also use plants, using again plants using transgenic technology, for a producing a number of recombinant protein which have medical or biologically importance. I will not go in to the details of the production therapeutic proteins, and number of such recombinant proteins has been produced. It is more or the same proteins which discussed in a last class using transgenic animals or using expression system, other expression systems. I have just given one review article here. One can go through review article and see, what kind of recombinant proteins have been produced in transgenic plants, and does it have any commercial value. But rather I would like to stick myself for the limited amount of time we have, to see what kind of vaccines can be produced by using transgenic plant and transgenic technology.

(Refer Slide Time: 46:25)



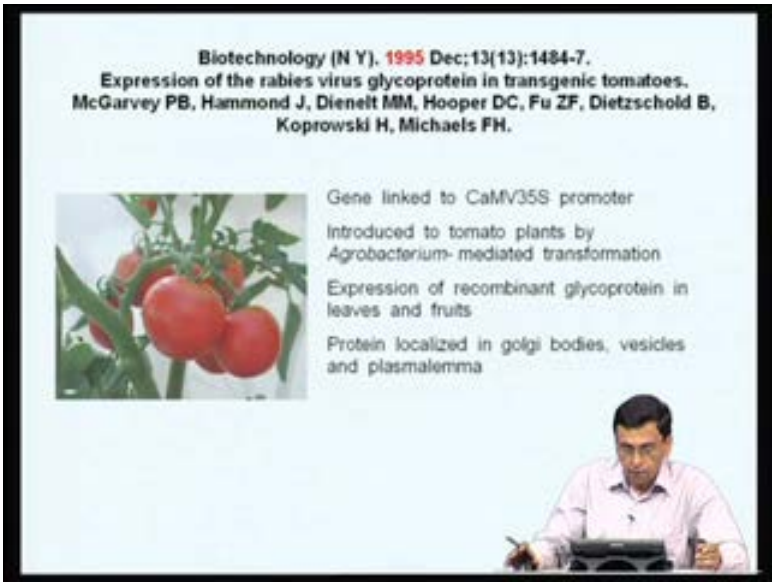
Now, as you all now vaccines are very important, in the two classes before we had actually discussed about D N A vaccines, how a failed gene therapy techniques was actually used for developing a D N A vaccine, by simply injecting D N A into in a encoded eukaryotic expression, mammalian expression vector. You can actually express the antigenic proteins, in the mammalian systems or inside our human or animal tissues, and therefore, it will get presented to our M L C class one or class two systems. And you get either a cell mediated immune response or a humoral immune response. So, here is the question. The question we are asking is, instead of expressing the gene coding of antigenic protein, either in yeast or E coli or in the mammalian cells, why do not we express a gene using a plant promoter, so that our plants actually produced now this viral antigen.

Now, this viral or the foreign antigen producing plants, can either purified or if you put in to the express the edible parts, by simply eating those edible parts of the plant, you can get an immune response, and therefore you can get protected against that particular pathogen. This idea of using plants as vaccine production factories, was proposed first by scientist called Charles Arntzen in the Arizona State University, and is the first paper that came out in 1992 in P N A's, the entitled expression of hepatitis b surface antigen in transgenic plants. So, in this paper they have actually shown the tobacco plants were generally genetically transformed, with a gene coding for hepatitis b surface antigene, linked to a nominally constitutive promoter. And recombinant hepatitis b surface antigen

which was purified from this transgenic tobacco plants, had properties very similar to that derived from the serum.

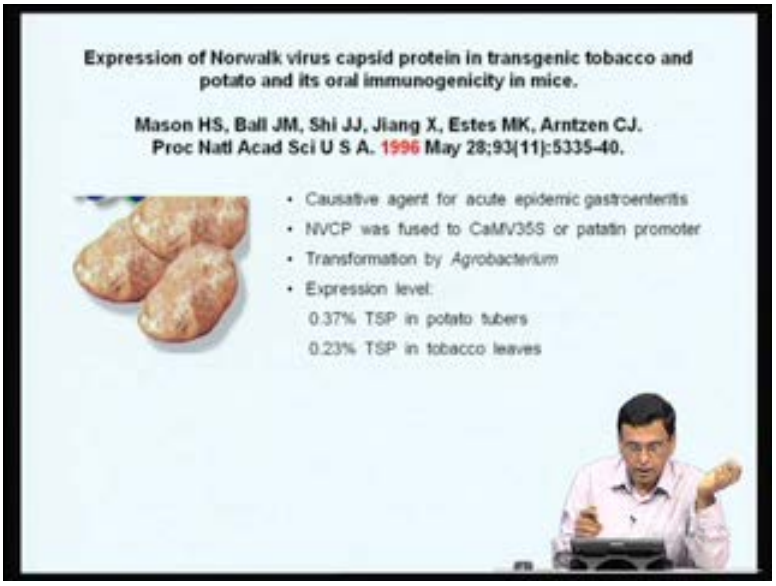
No, the traditional way of making hepatitis vaccine is to purify the virus like particles from the infected human patients. And they found that the hepatitis b surface antigen, which is expressed in this transgenic plants, is very similar to that produced from the human plasma. And therefore, they concluded that transgenic plants can hold tremendous promise as low cost vaccine production systems. So, instead of taking your gene, putting them in yeast or putting them equaline, and then making this recombinant protein, purifying this is and going them in a huge fomenters, and then purifying them and then giving them as a vaccine. Why cannot we express these genes right in the plants, and then see whether we can use plants as vaccine production platforms.

(Refer Slide Time: 48:52)



Now in 1995, this is the paper that came out, where they actually express the rabies virus glycoprotein in transgenic tomatoes, and actually shown, that this recombinant protein producing tomatoes can induce an immune response in animal models.

(Refer Slide Time: 49:11)



Now, in 1996 again Arntzen's group actually expressed what is called as norwalk virus capsid protein, in transgenic tobacco as well as potato. And then demonstrated if mice are fed with this potatoes, which consume this antigen, potato is expressing antigen they can develop an immune response.

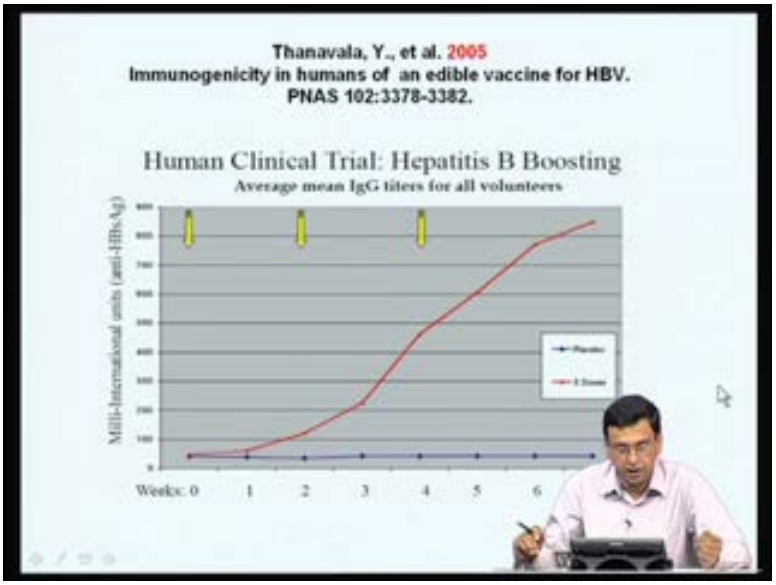
(Refer Slide Time: 49:32)

The first human clinical trial of a vaccine antigen produced in plants, was done in published in 1998. Immunogenicity in humans of a recombination bacterial antigen derived in a transgenic potato. So, in this paper they discussed about, how mice when

they feed these potatoes which express this hepatitis b surface antigen. They can actually develop, antibody against the hepatitis b surface antigen or humans which actually consume this raw potatoes, expressing the hepatitis b antigen. You can actually demonstrate the presence of antibodies against the viral antigen in their serum, indicating that the antigen which is consumed through the oral dot along potatoes, can actually stimulate our immune system to produce antibodies against the viral antigen.

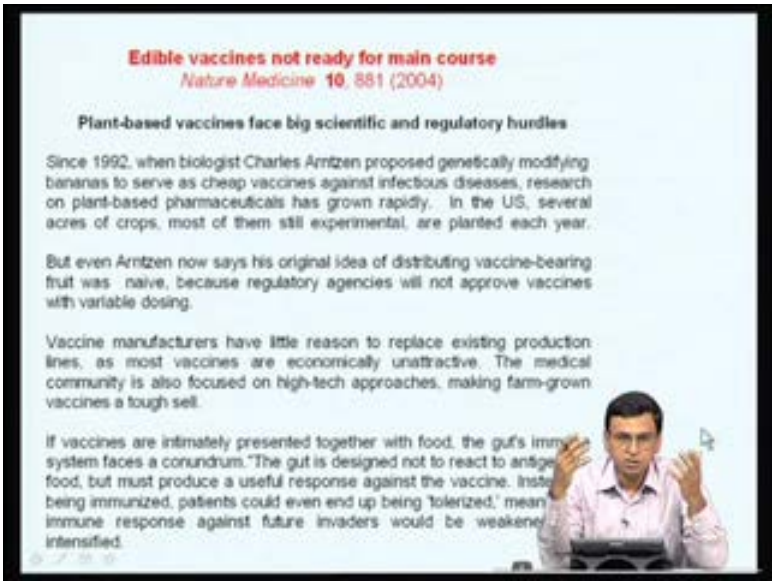
So, this was one of the landmark papers in the area of transgenic plants for used as edible vaccines. So, it generate a lot of excitements, as you do not have to produce antigens organisms like yeast or equaline, then use needles inject these antigens. But, simply consuming fruits or edible potions of a plant, expressing antigen, you can get immunized. So, over all vaccines or edible vaccines became a huge excitement, generate a lot of excitement among research as well as public.

(Refer Slide Time: 50:47)



So, here is for an example 2005, they actually demonstrated that the humans hepatitis b surface antigen, which is actually consumed through oral route, can elicited similar kind of response as generated by the normal hepatitis b antigen injected through the systemic root. So, the human volunteers who consumed these hepatitis b antigen expressing potatoes, can develop antibodies in their serum, whereas the control group did not show any antibiotic titles.

(Refer Slide Time: 51:21)



But, this excitement died down, because people soon realize, that this concept of edible vaccine also sounds very exciting, cannot really be a commercially success. A nice article actually appeared nature medicine, clearly saying that edible vaccines are not ready for the main course. Now, plants based vaccines face the biggest scientific and regulatory hurdles, because you just cannot express your vaccine antigen in a thing like, potato, tomatoes and then simply asked people eat this potato, tomato and get immunized, because these are all, the dose, the amount of antigen I have to take the quality, everything has to take properly regulated. Now, one can ask, for example should I use a big potato or a small potato, should I use a big banana or a small banana, how do you regulate the dose of the antigen you have to consume. Now, what is the amount of antigen that is produced from different batches of the plants. So, all these things have to be argued out.

And so the initial excitement that Charles Arntzen work actually developed slowly died down, because even Arntzen's now said, that his original idea of distributing vaccine bearing fruits was naïve, because regulatory agencies will not have approved vaccines with variable dosage, because you have no control over the expression antigens in these plants. It may vary from batch to batch, and you have no control on how much you ingest. So, vaccine manufactures have little reason to replace the existing production lines, as most vaccines are economically unattractive. So, if vaccines are ultimately presented together with food, the guts immune system also faces a conundrum. The gut

is designed not to react to antigens in the food, but must produce the useful response against the vaccine. So, instead of being immunized, patients could even end up being tolerance, developed tolerance meaning immune response against future invaders would be weaken and not intensified. So, this concept of using these edible vaccines slowly died down.

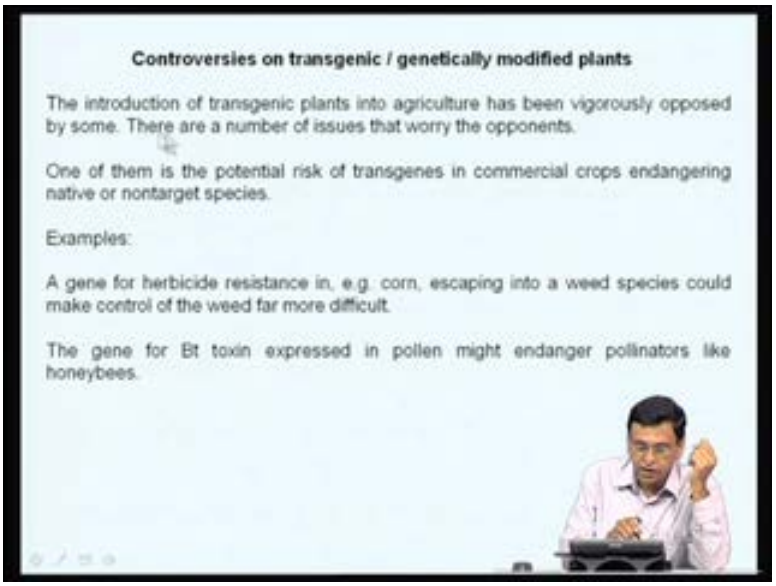
(Refer Slide Time: 53:11)

But what now Charles Arntzen, who is the pioneer in this technology, and believes in that; instead of original ideas consuming this fruits as edible vaccines. He now thinks that can actually take that is edible portion of these plants, freeze dry them, and then take these freeze dried material put it in the form of a capsules. And now it is, you can actually determine how much antigen is actually consumed. You can now regulate what dosage it is. So, the concept of edible vaccine has now become what is called as oral vaccine; that is you make antigens in plants, and then freeze dry them. Through here you can see freeze potato media or freeze dried tomatoes, and then push them into form of capsules, and if I now orally I take this capsules, you can develop the immune response. So, the concept of edible vaccine has now slowly changed to oral vaccine, but still as I speak today, still no company has really come forward to success fully commercialize this concept of plant based vaccines as yet, but it is likely to happen in the future.

(Refer Slide Time: 54:09)

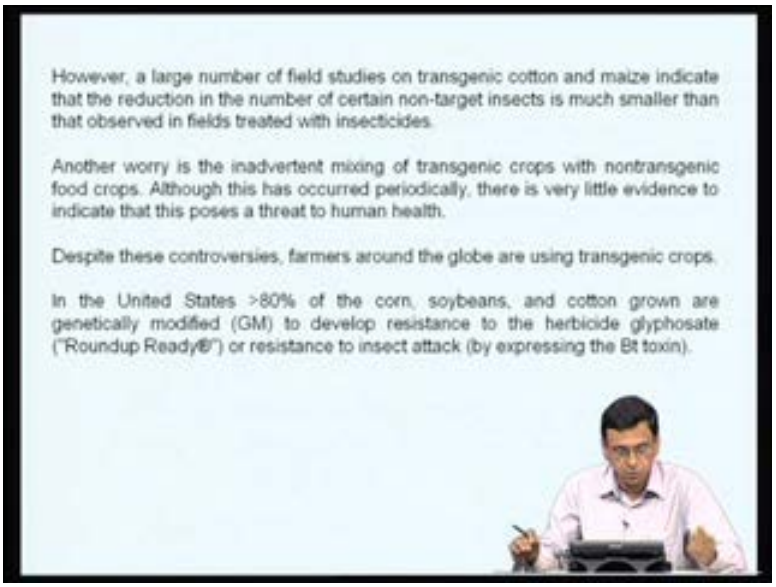
I think there is very nice article written by Charles Arntzen, one can download this p d f file from the website called upstream and downstream manufacturing of vaccines in tobacco. This is a facility that is present in called the bio design institute in the Arizona State University. Primarily and there is also a nice article which appeared just recently this year on transgenic plant based oral vaccines. One can read this to get exited more about very useful information of transgenic plants for many vaccines manufactures.

(Refer Slide Time: 54:40)



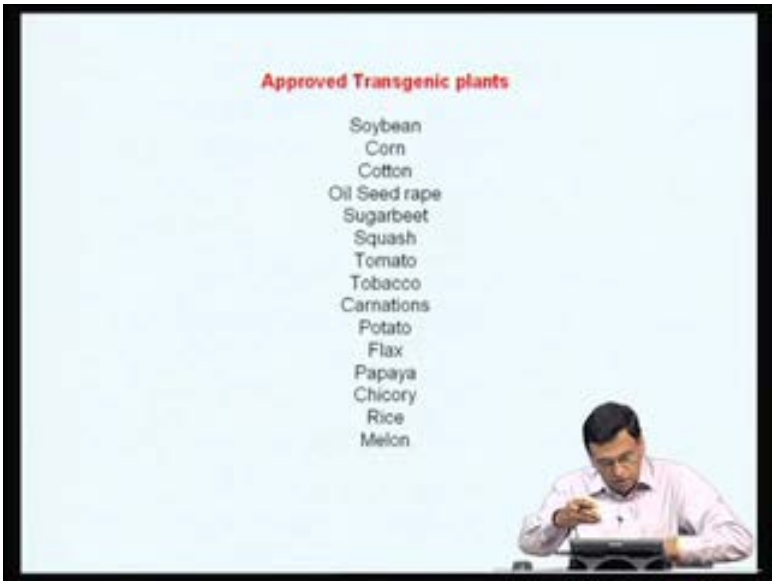
It is lot if controversies on transgenic plants, as I said introduction of transgenic plants into agriculture has been vigorously opposed by many groups. There, are number of issues that worry the opponents. One of them is the potential risk of transgenes in commercial crops, endangering native or non-target species. For example a gene for herbicide resistance; that is in corn may escape in to a weed species, and now weed may now express there is herbicide resistance gene, and therefore it may become much more difficult to control the weed. Similarly, the gene coding for a B t toxin expressed in pollen, might endanger pollinators like honey bees. Now you do not want to kill the honey bees, because they are naturally fun materials, but there are all insects then they also die, because they consume this B t toxin producing, they get contaminated by the pollen containing B t toxin. So, number of such opinions expressed.

(Refer Slide Time: 55:32)



But, a large number of field studies on transgenic cotton and maze indicated that, reduction in the number of certain non-target insects is much smaller than that observed in fields created the insecticides, because insecticides also pose the same problem. So, there are number of other things, but the take home message is that, despite all this concerned, in United States for example, for more than eighty percent of the corn, soya beans and cotton grown are all using genetically modified plants, which all have resistance to herbicides or resistance to insecticides.

(Refer Slide Time: 56:01)

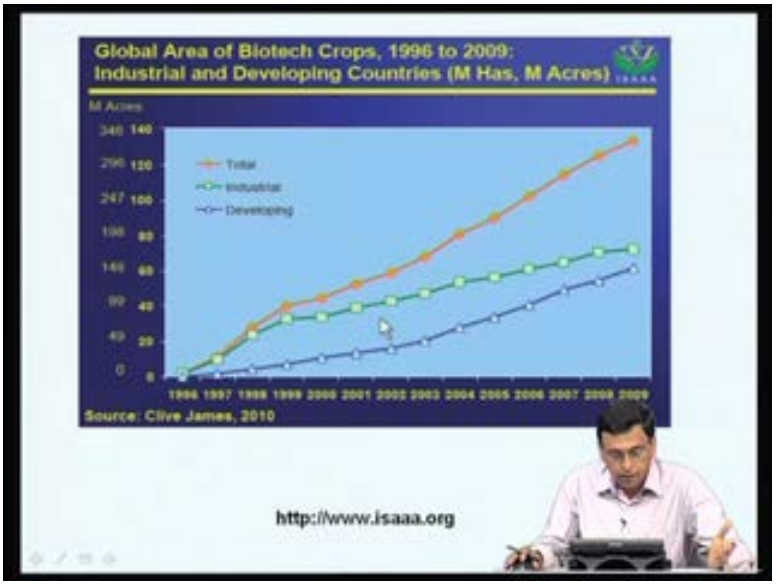


So, a number of transgenic plants have been approved for by regulatory agencies, both for edible as well as non edible purposes.

(Refer Slide Time: 56:09)

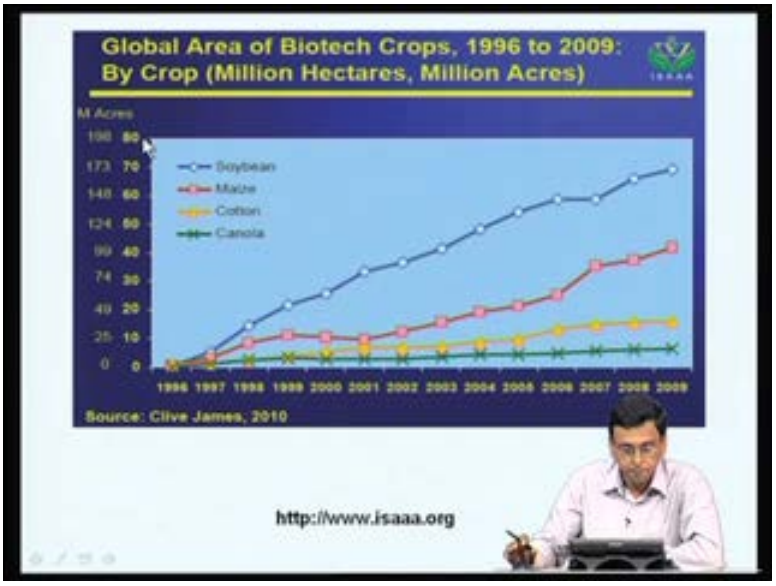
The acreage of transgenic crops has gone from which will be 0 in the year 1995 to all most 345 million acres in 20009.

(Refer Slide Time: 56:18)



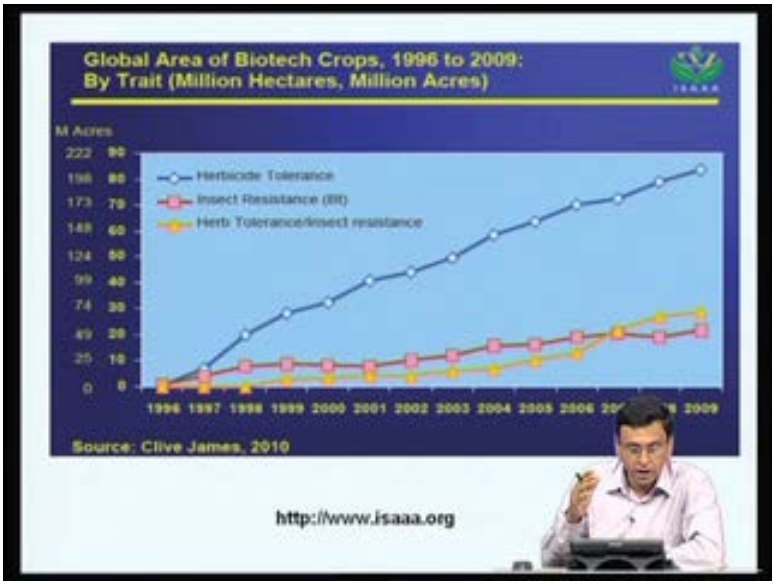
And here the some of the statistics here, if you can go to his website, you can get the things you can see here from 1996, when the first transgenic crop was introduced till last year. You can see, if you look at the number, total global area of biotechcrops has been continually increasing, both are industrial countries and developed countries, as well as the almost 345 million acre, is now under cultivation of transgenic crops.

(Refer Slide Time: 56:44)



Similarly, a number of crops you can see soybeans, maize, cotton, canella, all there is a study increase in the growing transgenic crops all over the globe.

(Refer Slide Time: 56:59)



Similarly, the global area of, if you go by the trades, the herbicide residence is the top most transgenic crops expressing, which are resistance herbicides are occupy the top of the chat, this is followed by those which are insecticide resistant or herbicide resistance. So, these of the majority of the transgenic crops there are being produced now, either on a herbicides resistance genes, insecticide resistant genes and so on and so forth.

(Refer Slide Time: 57:22)

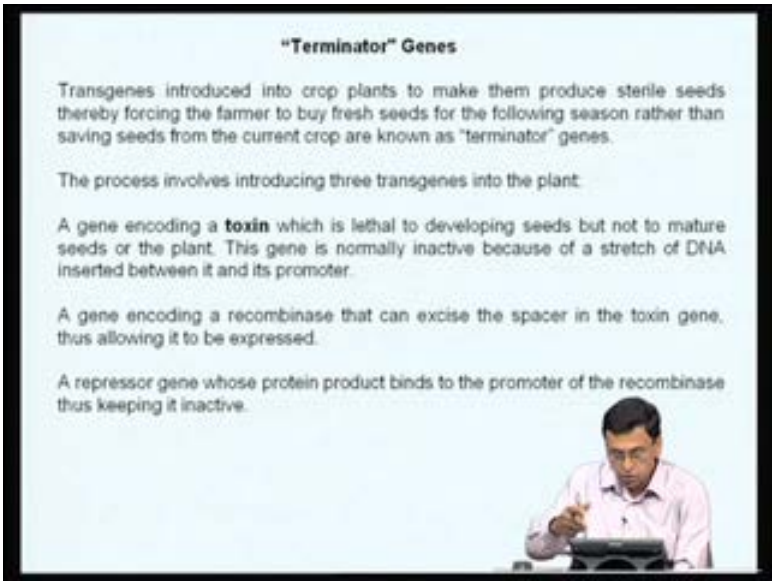
And you can see the global area of biotech crops again in nineteen nineties 2006, a record of 14 millions farmers in 25 countries, planted 135 million hectors in 2009. A

sustain increase of seven percent or 9 million hector 2008. What, I am trying to say is that every year the transgenic crop growth is increasing.

(Refer Slide Time: 57:42)

I think one can just go through these statistics to show that the global status of commercialize in 2009.

(Refer Slide Time: 57:49)



I will not go into the details; that are what is called as terminator genes, again there are some concerns. One can go through this and try to understand what these concerns are.

(Refer Slide Time: 57:58)

I have just list out some of the references here, this references actually summarizes all the eukaryotic expression system that we have discussed so far, yeast insect and plant expression system. One can go through and understand more.

(Refer Slide Time: 58:09)

(Refer Slide Time: 58:13)

This website actually gives the global status of commercialized biotech or G M crops, and these are some of the biotech companies Monsanto, B A S F, Dow, Bayer, DuPont, Syngenta. These are some of the leading bio companies which are actually expressing a number of genes, and developing transgenic plants for a number of commercial applications. Thank you.