Bioengineering: An Interface with Biology and Medicine
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Lecture - 14
DNA Tools & Biotechnology-VI

Welcome back. In the last few lectures, we have discussed how different DNA tools have revolutionized the field of biotechnology and genetic engineering. Many times, I have given you examples which are especially on the medical field. Today, we are going to really focus an area based on the plant biotechnology and how genetic engineering has contributed towards that.

If you go back and think about my very first lecture when I started emphasizing the need for various type of global challenges think about abiotic stresses.

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For example, salinity, heat, drought, all of these are abiotic stresses which are affecting our land, the cultivation area severely throughout the world. Almost 20% of agricultural land is getting affected because of the salt stresses and large volume of the overall earth is getting desertified because of different drought situation which is happening throughout the world. As you can see here in couple of your images various type of natural calamities like Tsunami, Caterina.

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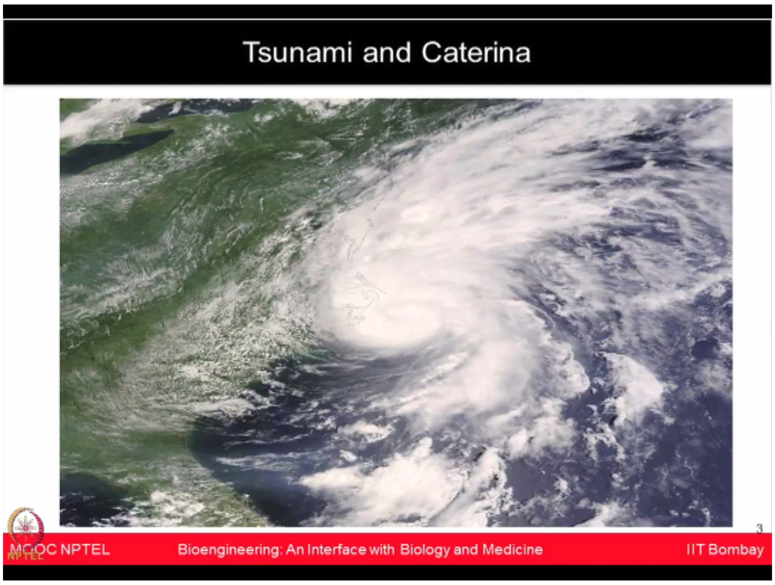

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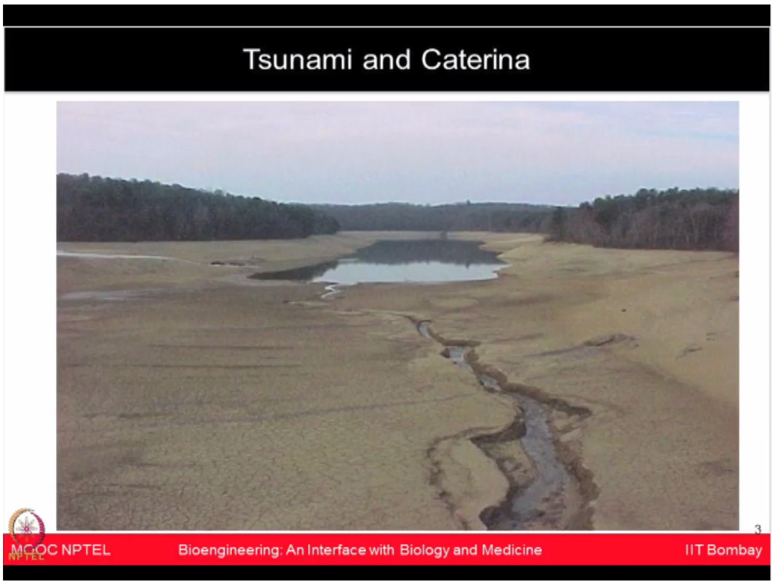

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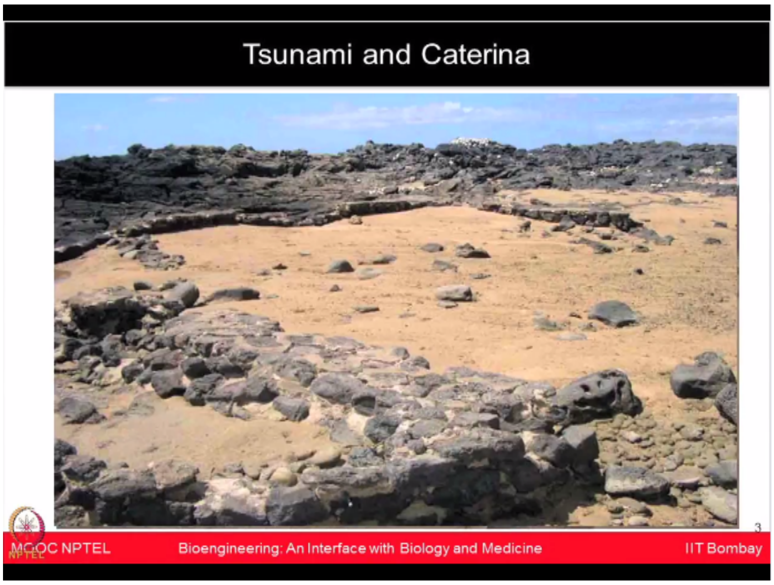

Many of these natural catastrophe, they leave huge impact not only on the climate that time but also for the years to come the whole land becomes barren and nothing could be grown on those kind of field. So these abiotic stresses they impose major limitations on food production in whole world.

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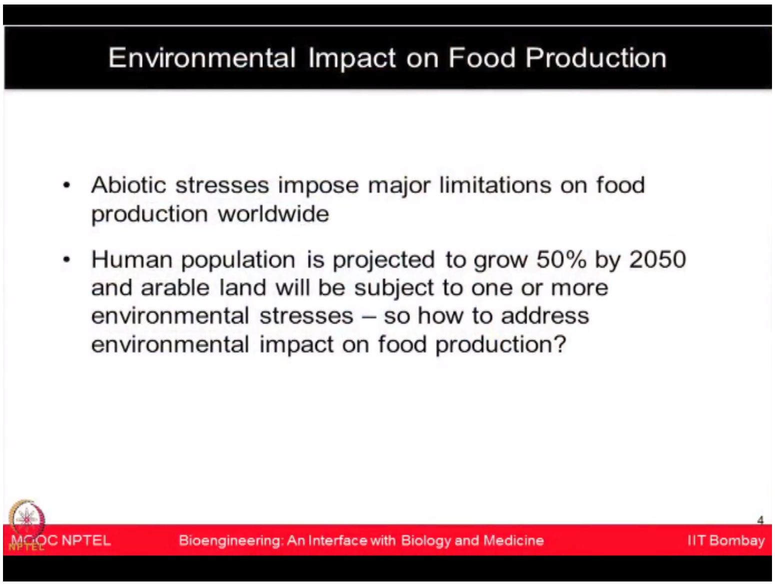

If you think about human population which is projected to grow almost 50% by 2050 and the land which is being used for the food crop cultivation is getting reduced over the period and it is also getting subjected to various type of environment to the stress conditions. So how are we going to address the environmental impact on food production?

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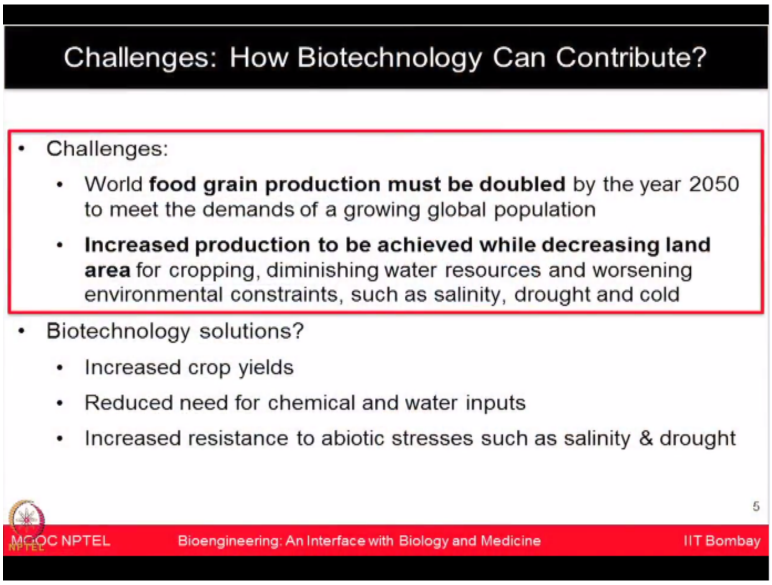

So we are facing many challenges globally. The world food grain production must be doubled by year 2050 because we have to meet the demand for the growing global world population and we have to increase the food production on the limited land area which is available to us and on one hand the water resources are getting diminished, lot of lands are getting affected because of the salinity, drought, cold and many stresses.

And on the same you know small area which is available for the food crop production; we have to increase the crop productivity. So what are the biotechnological solutions available to address this challenge? For example, we can increase the crop yield, we can reduce the need for the chemical and water inputs. We have to also increase the resistance for the plants towards the abiotic stresses such as salinity and drought. Also the plant should be more tolerant for the various type of insects and pests.

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So in order to improve the crop traits by using biotechnology, there are a couple of case studies I am going to discuss with you, the genetic modified organism or the GMO is one of the common and a very hot topic which I am sure you often read in the newspapers which you often hear about it. This is one of the GMO is one that has acquired one or more genes from another species.

And this can be done by using the genetic engineering artificial means right. So in some way that we can actually you know the across the barriers of different species and we can move genes from one to other organism for different type of traits and that is what is being utilized and exploited in the field of biotechnology. So let me take you the very first case study on developing these stress tolerant plants and especially the salt tolerant plant.

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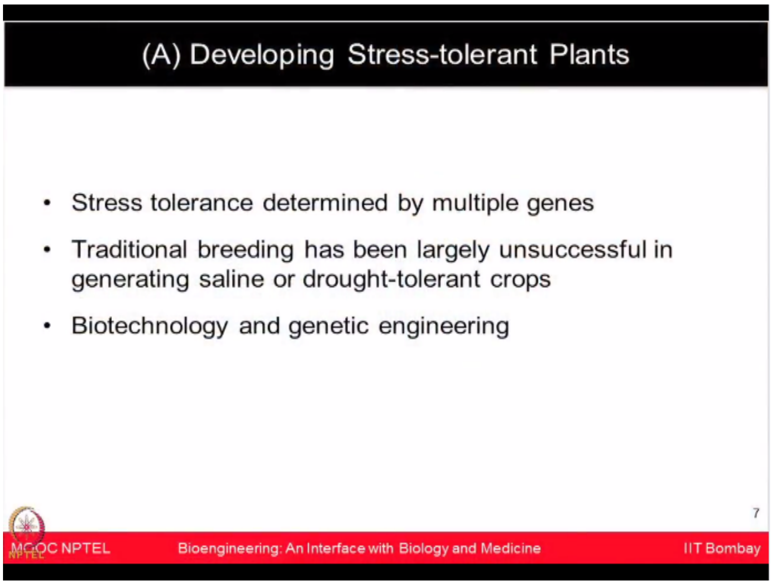

So as we discussed that you know there is a need to have the plants which could survive even if you have a high salt condition or drought condition still if those plants can live in this kind of you know environmental situation then probably we can have the more crop yield. So traditional breeding which is commonly employed has been unsuccessful majorly to generate the highly tolerant saline or the drought tolerant crops.

So again biotechnology and genetic engineering provides some solution to that.

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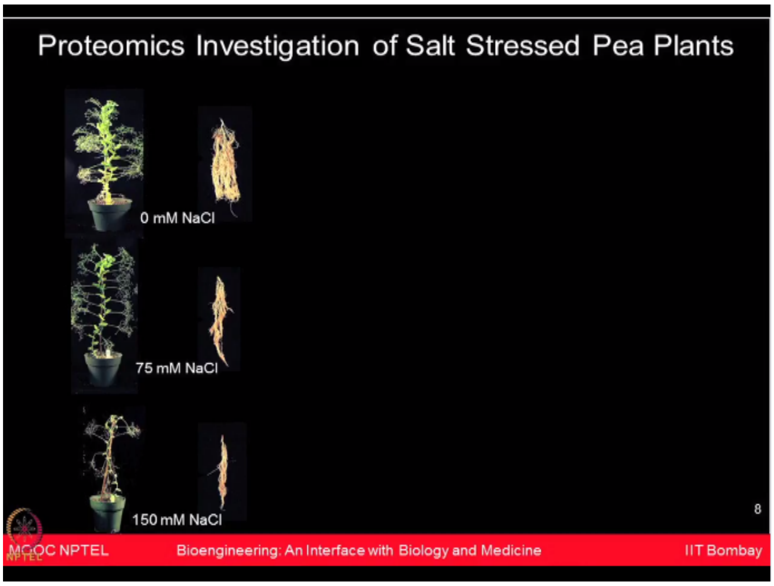

So in order to do that you know in this study the researchers looked into the pea plant and the pea plants were subjected to different type of stress conditions from 0 to 75 where 150 millimolar of the salt condition to look into the you know the medium and the high salt concentration that how that affects the pea plant and now the root and the shoots were excised and as you can see the roots are getting you know quite affected in these you know as the salt is increased concentration.

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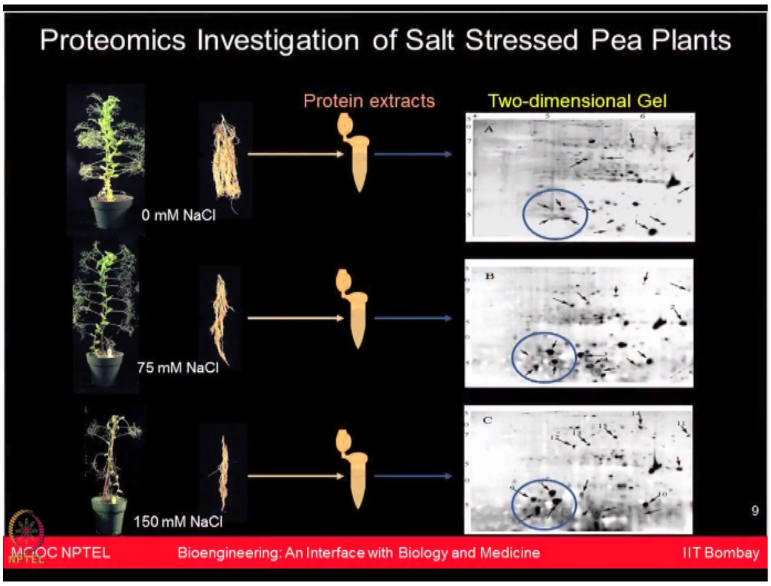

So proteins were extracted from these roots samples and now the proteins were separated using a gel for but which is 2-dimensional gel where proteins are separated based on the isolated point and their molecular weight. What are the spots which you can see on these gel images which are actually showing you that the proteins obtain from the roots which are affected from the salt conditions.

Now what kind of protein changes are being seen as the salt concentration is increasing? After doing this analysis as you can see in the highlighted circle part that you know a group of protein looks quite highly elevated and those proteins definitely showed that you know increasing response to this salt stress condition and of course one will be curious to know that what those proteins are right.

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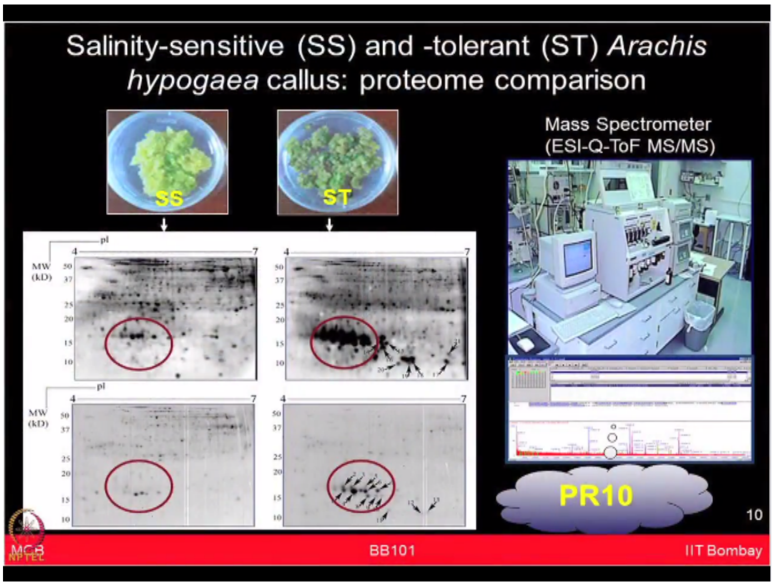

So another experiment was done where a salt sensitive and salt tolerant Arachis hypogaea another type of you know the plant was taken and again their proteins were compared using proteomic kind of investigation.

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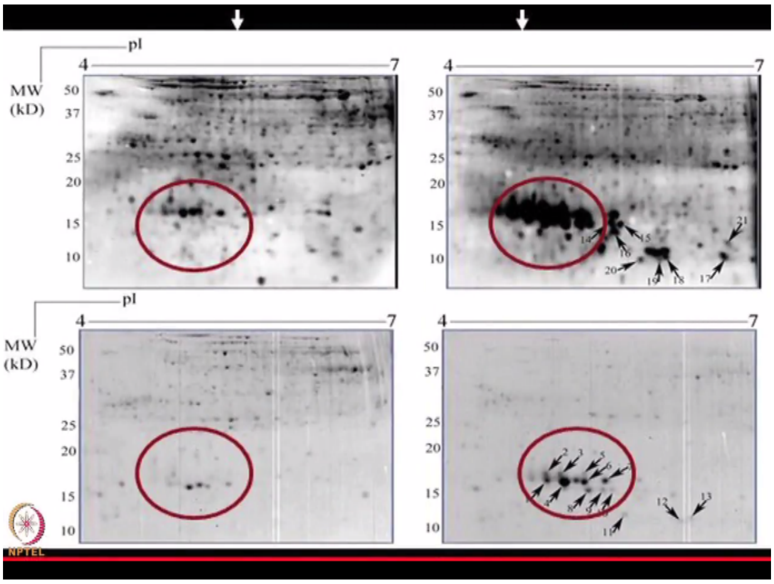

And as you can see on the left side, the protein obtained from these callus were separated on these gels two-dimensional gels and again on the lower side a group of protein a set of proteins are highly expressed which is shown in the circle red circle here which is we know very similar to the kind of pattern which you have seen in the last slide where I showed you the pea plants getting affected because of the high salt condition.

So looks like in the similar area of you know same molecular weight and same pI a set of proteins are getting highly overexpressed and what those proteins are. As we will go in the next module, I will talk to you about you know how protein technologies work and that time you will be also exposed to the mass spectrometry based proteomic investigation.

But for the time being to keep the context here you should know that you know the mass spectrometers are one of the powerful analytical tools which can be used to identify the proteins of interest where you can look at a peptide sequences and do the database search to identify what are the proteins.

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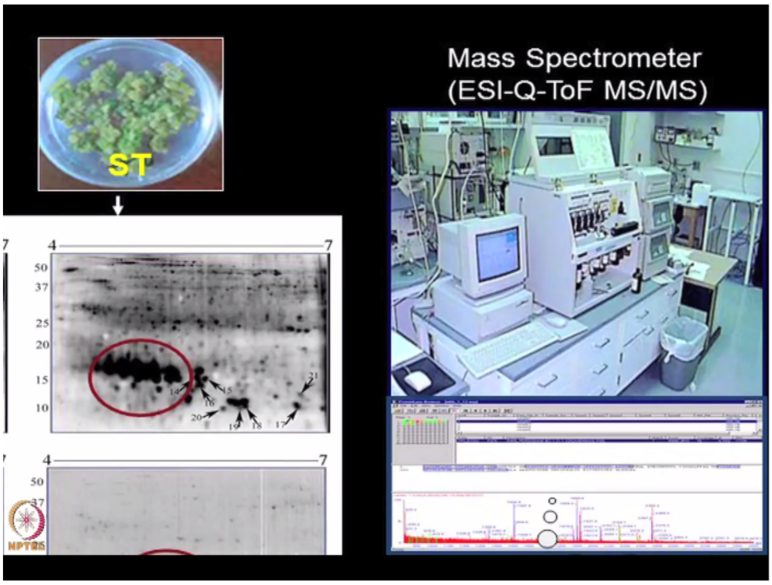

So in this case let say from this gel piece you have excised the protein sample, you have digested them to make peptides and now those peptides are subjected to the mass spectrometers to identify what is this protein of interest because it is what you want to identify that what this protein is.

So when researcher did this work, they identified this protein is a group of family of the protein which is pathogen resistant 10 protein, PR10 protein and there are couple of members of those proteins which were identified.

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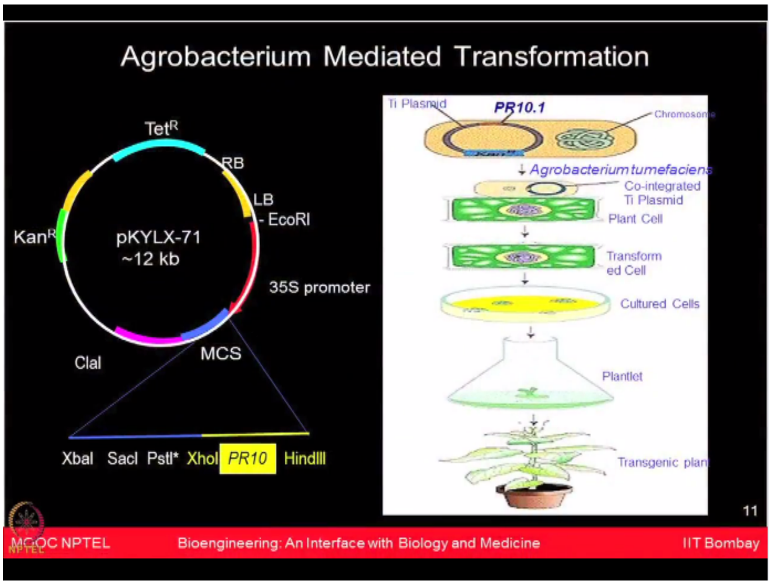

Now can we use this information and use it back in the genetic engineering approach is what is shown you in the next slide where an Agrobacterium mediated transformation system was used to do the genetic engineering approach. Now you know this protein PR10 which is coded by a gene PR10, can we introduce that in the plant system and whether this particular gene could boost up the plants tolerance to the environmental stress condition, for example salt stress condition.

And there are different you know the gene family for the PR10 is a large gene family. This specifically one member PR10.1 was used in this particular experiment. So you have been you know we have been discussing about the vector map, now you can see that you know this vector map different evolution sites are there, the promoter site is there and now we want to introduce this PR10 gene of our interest.

We are using here one of the Agrobacterium mediated transformation system in which the PR10 gene construct has been introduced now in the bacteria which is now getting you know co-integrated this plasmid inside the plant cell, how this exactly work I will you know elaborate in some more slides later on.

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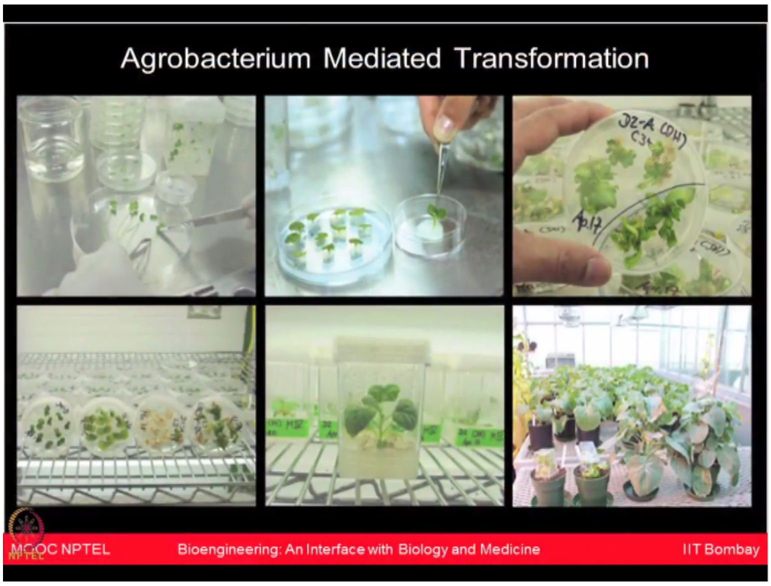

But ideally you are trying to introduce this particular gene of interest in the plant by using this transformation system which is Agrobacterium mediated transformation. Couple of images shown you here that how some of these things are experimentally done in the lab where you know when you want to introduce these genes from you know one system to the system then you have to do there are series of the experiments based on the tissue culture based methods in the lab inside the greenhouse condition.

And then slowly you can you know take these small implants where your gene of interest has been introduced. Now you are growing them further and you are trying to provide them you know the limited environmental conditions so that they can grow in the beginning and then slowly you want to test the effects of the gene which you wanted to test out.

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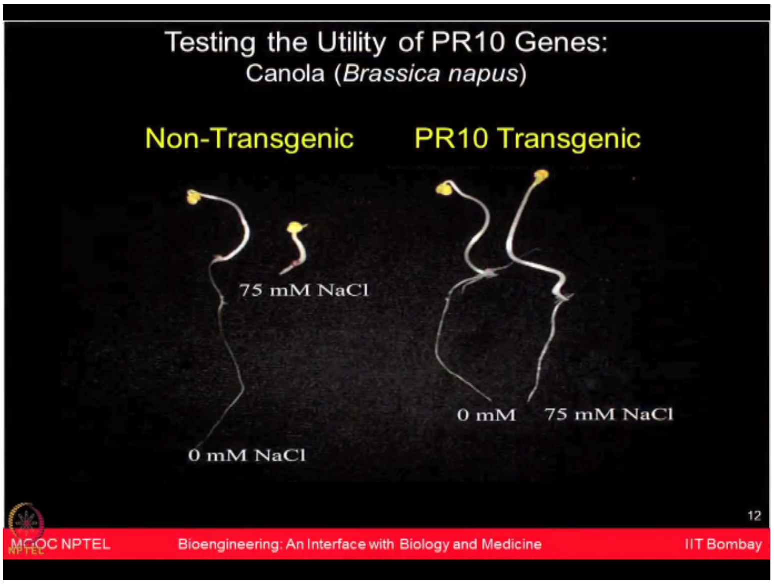

So in this case when researcher tested the utility of PR10 genes in a different crop because you know you want to you have identified the gene from P, what is the impact of this particular gene tolerance into another plant species which is Brassica napus or the Canola plant which is another plant of highly economical significance. So if you now look at the seedlings of the non-transgenic and the PR10 transgenic, they showed quite of a bit of difference that the no salt is of course you know both are surviving quite well.

But as the salt concentration is increased to 75 millimolar, in this case you can see that you know the transgenic plant which has incorporated the PR10 gene of interest is having much more tolerance.

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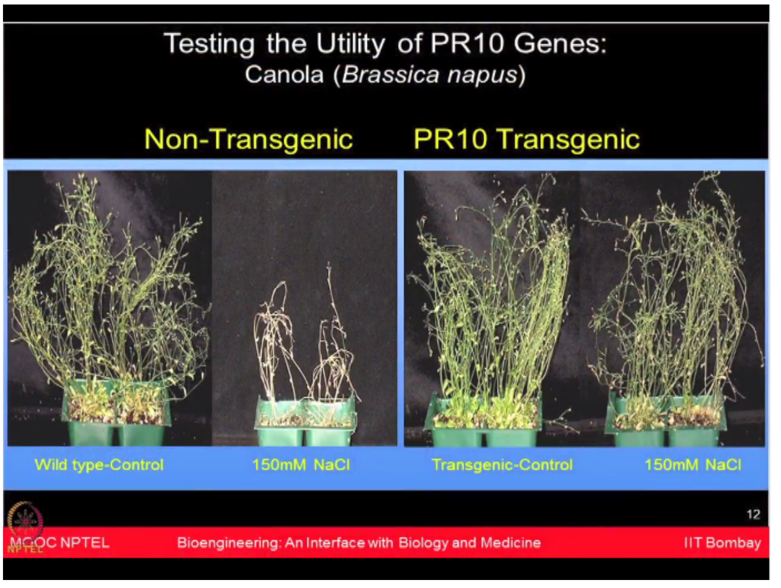

This is another image which is showing you the green house grown plants now. In the high salt condition even up to 150 millimolar, these plants were able to grow and survive as compared to the non-transgenic or the control plants and then similar utility was tested in the Arabidopsis plant.

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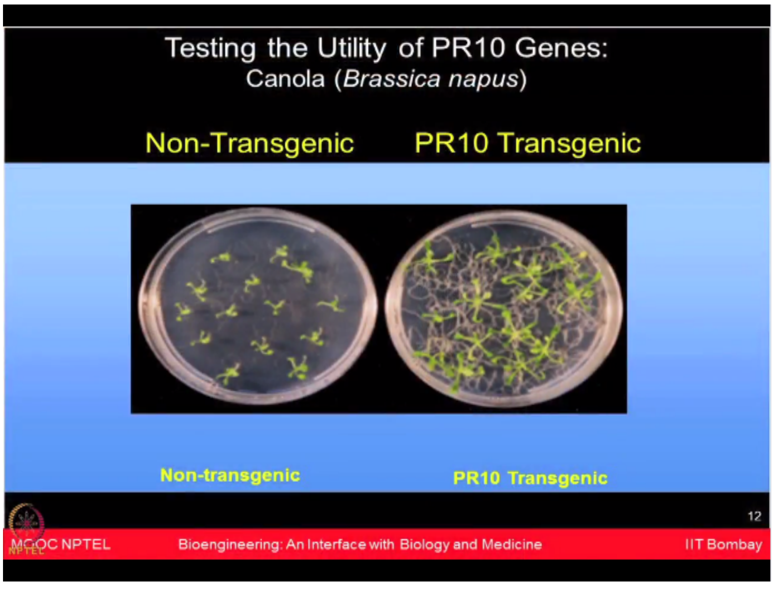

And now you can see that you know these are Arabidopsis seedlings are quite able to you know tolerate the high salt condition. So again this gene is doing something in the plant which is you know kind of increasing the plant tolerance towards the salt concentration and that was seen in the case of Canola, it is seen in the case of Arabidopsis.

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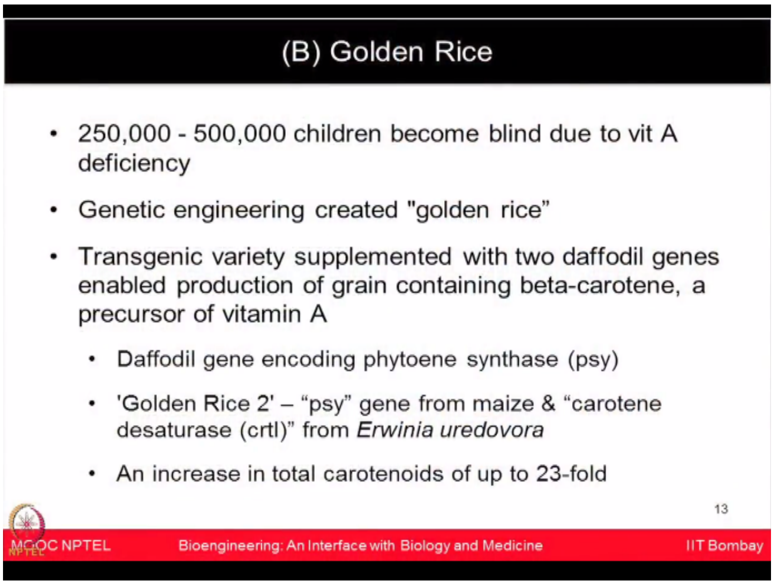

Let us come to another example of very successful example which is Golden Rice. As you know we are all aware that you know the large number of children become almost blind because of the vitamin A deficiency and came genetic engineering provides some solution to create the rice and one of that attempt was done which is known as Golden Rice.

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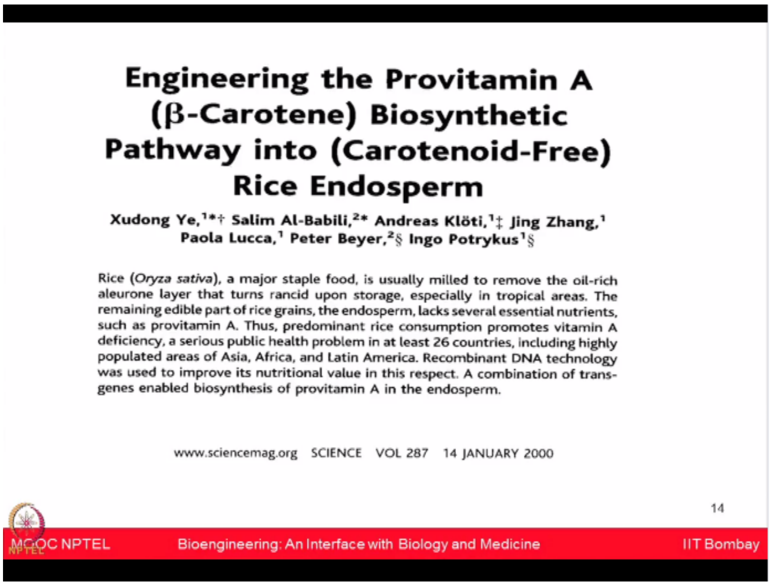

I have shown you here two highly cited publications of this area where they have engineered the provitamin A or the beta carotene biosynthetic pathway into the carotenoid-free rice endosperm and another study which was which has shown that you know you can improve the nutritional value of Golden Rice by increasing the provitamin A contents and they have expressed in this case a psy transgene which increase the carotenoid contents of the maize callus.

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So in these studies what they did they wanted to look at the transgenic variety production which could be supplemented with two Daffodil genes which can enable the production of the grain containing beta carotene which is a precursor of vitamin A.

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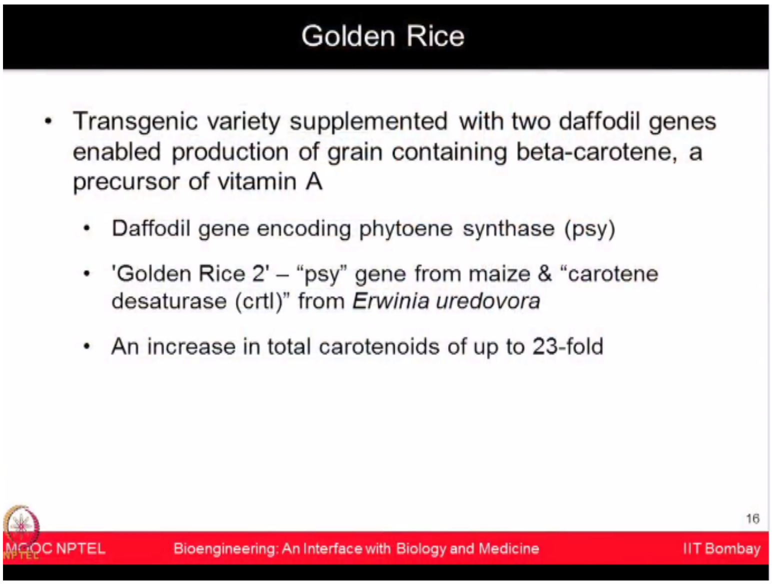

The Daffodil genes encode phytoene synthase or the psy and this Golden Rice 2 contains the psy from maize and carotene desaturase from Erwinia uredovora and overall the observed an increase in the total carotenoid of up to 23 folds. So after discussing the successful example of rice which is Golden Rice which is grown in the large areas and has really changed you know the nutritional quality of the rice, now let us come to the third most you know cited topic resistance to insect pest.

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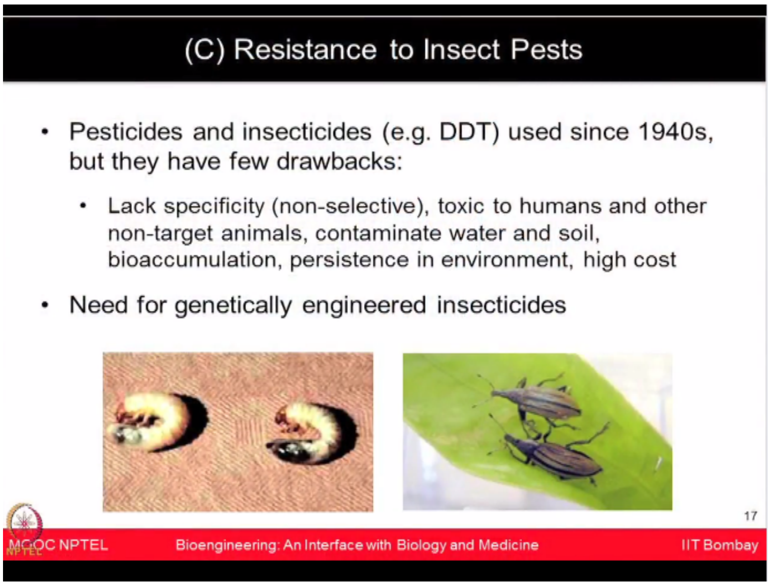

The large number of you know crops and the food crops in fact even get affected because of the pests and insects which affect when they are grown in the field. So the pesticides and insecticides are used to protect the plants from the impact of these pests and insects which are you know very harmful for the plants.

However, these insecticides and you know these chemicals are quite you know detrimental for even a human health. So you know since 1940s you know people have started reporting many drawbacks of the existing insecticides including DDT which lack its specificity, it is non-selective, it is actually toxic to the human health as well as many of the non-target animals.

Once you spray these type of you know chemicals on the plant then they come from water inside the soil and they also get bio-accumulated. You can see that they are getting persisted in the environment, it is very tough to remove them from the environmental conditions and of course there is a huge cost of spraying these chemicals on the plant to protect them from these pests and insects.

So can genetically engineered insecticide crops could be created which can you know boost the immune system of the plant for that matter so that the plants can now tolerate these kind of infections and still survive in the field. To do that a highly successful you know example comes where people have used the gene from Bacillus thuringiensis or the Bt gene.

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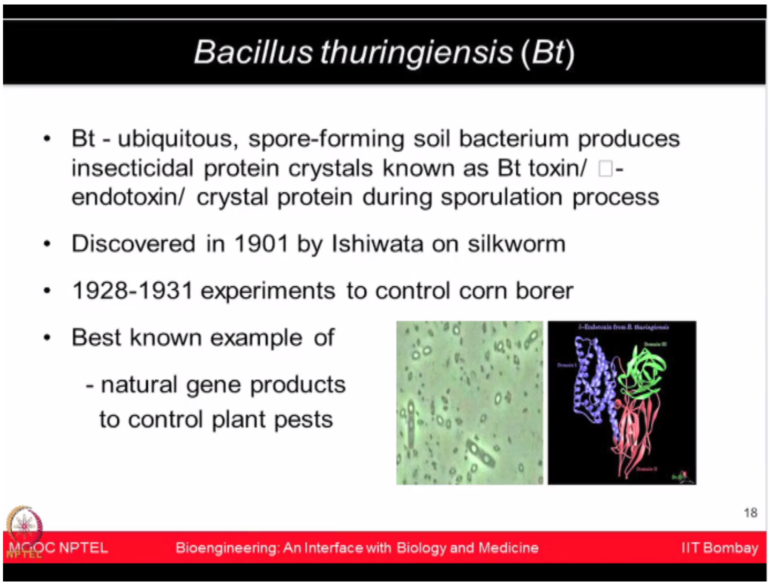

So Bt is the ubiquitous, spore-forming soil bacterium which produces insecticidal protein crystals which is known as Bt toxin. This is one of the endotoxin crystal protein which is produced during the sporulation process of this bacteria. It was discovered in 1901 by scientist Ishiwata silkworm. During 1928 and 1931, they performed some experiments to control the corn borer.

And you know this was one of the best natural gene product which was used to control the plant pests and that gives an idea that can this type of genes be used to protect the plant from you know the deleterious effects of the insects and pests.

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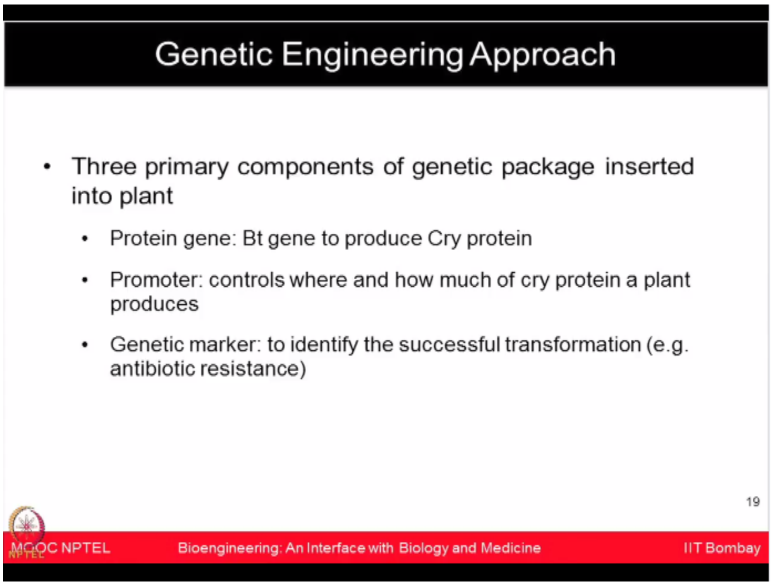

So how a genetic engineering approach could be used here? So there are 3 primary components of genetic packages which were inserted into the plant. The protein gene which is the Bt gene which is going to produce the Cry protein, a promoter sequence which controls where and how much of the Cry protein has to be produced in the plant and a genetic marker which can identify the successful transformation experiment for example the antibiotic resistance which can be used for this screening purpose.

So let us look at how the tumor inducing plasmid or Ti plasmid could be used to produce transgenic plants.

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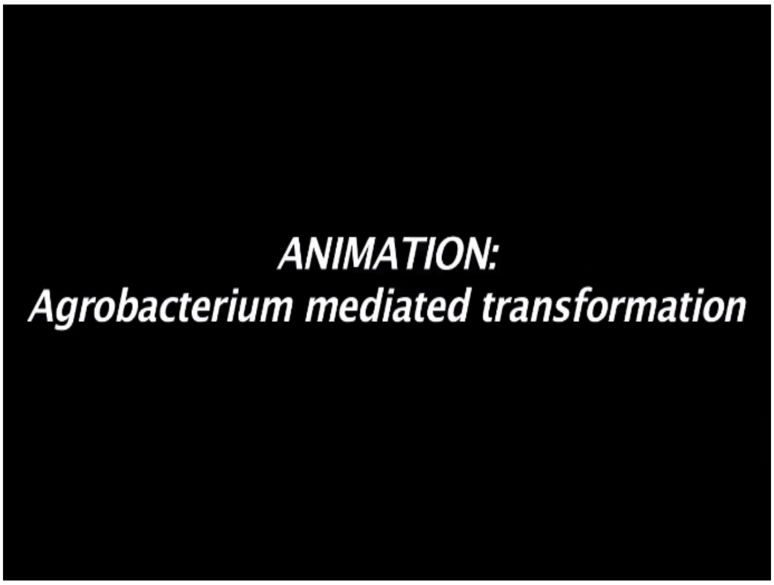

Let me explain you this in more detail in the following animation.

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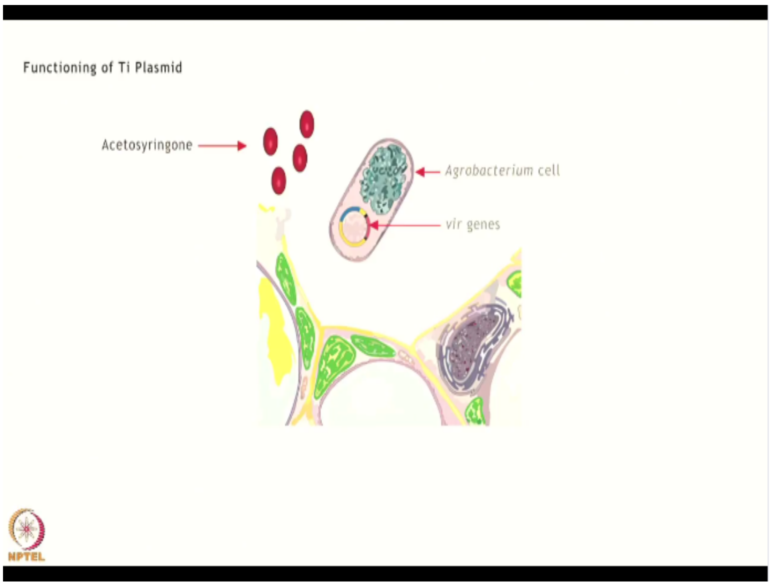

When a plant gets injured, there is release of the phenolic compound Acetosyringone which is detected by Agrobacterium tumefaciens.

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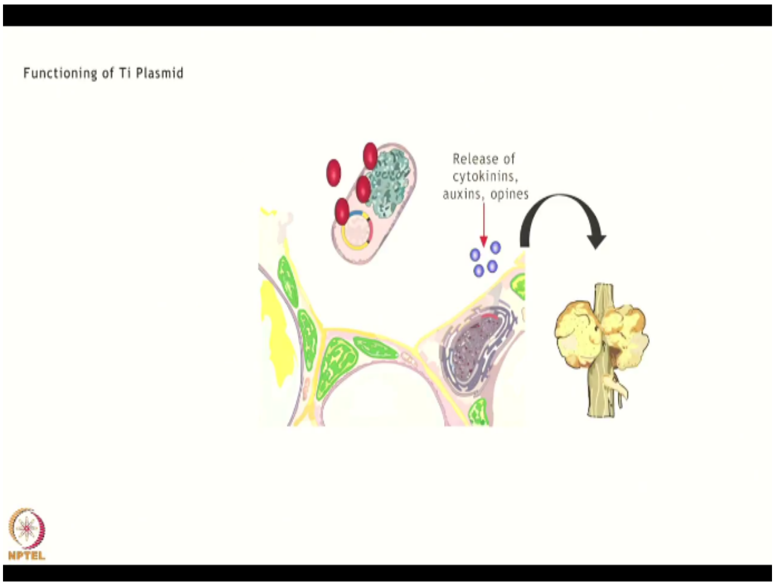

Upon detection the virulence genes of tumor inducing Ti plasmid get expressed which encode the enzymes that are essential for transfer of the T-DNA into the nucleus of the plant cell. Once the T-DNA gets integrated with a plant chromosome, there is release of cytokinins, auxins, etc which brings about tumor formation in the plant.

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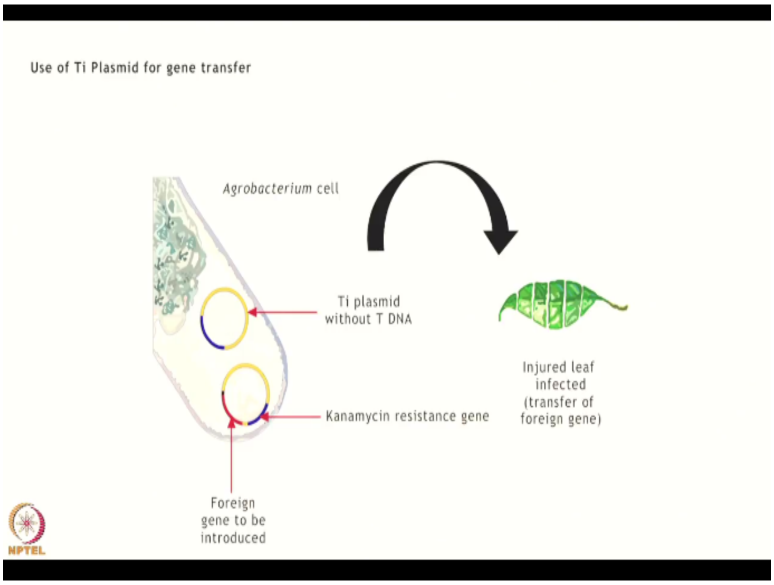

The useful property of infection by Agrobacterium has allowed several foreign genes of interest to be introduced into plant cells as per the requirement. One plasmid of the cell is a Ti plasmid without the T-DNA. The other plasmid contains the gene of interest along with antibiotic resistance genes placed in between two repeat units that are essential for gene transfer.

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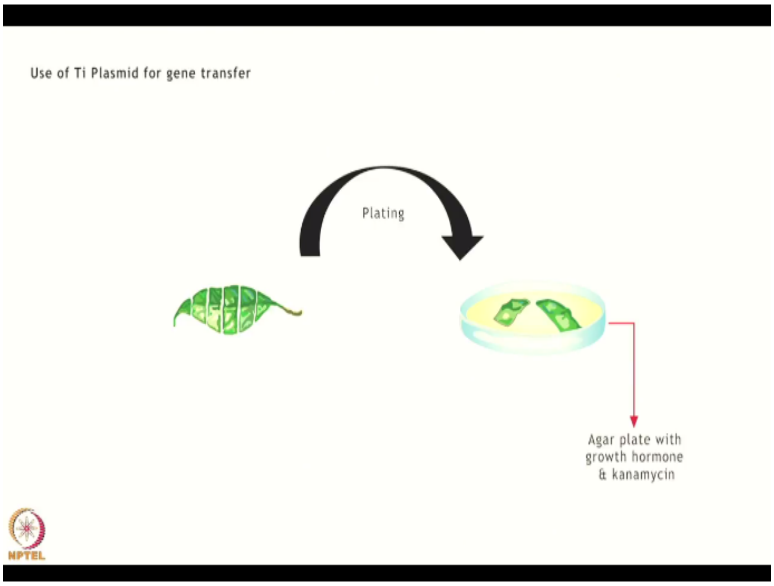

The gene of interest such as genes for pesticide resistance better yield etc invades the plant at the site of injury.

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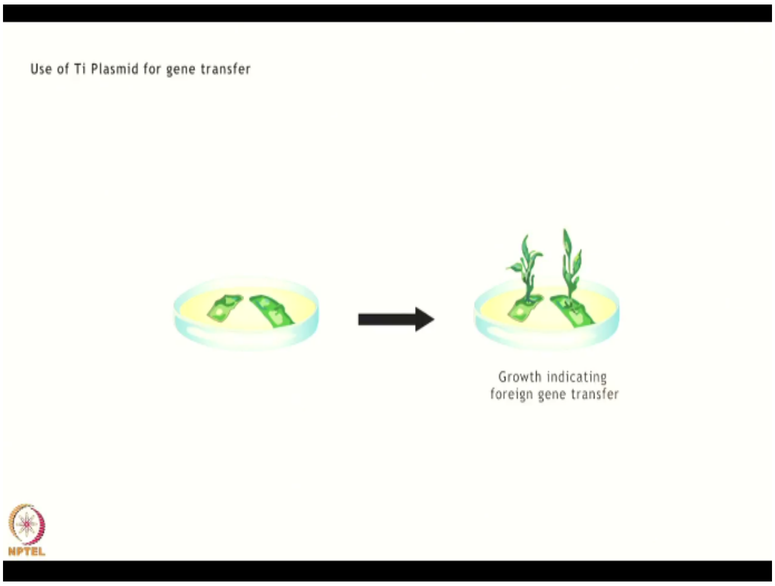

Once this happens the foreign gene gets inserted into the plant DNA which is confirmed by plating on to agar containing the suitable antibiotic. Only those which have taken up the gene will grow on such plates. Agrobacterium tumefaciens mediated transformation was used here.

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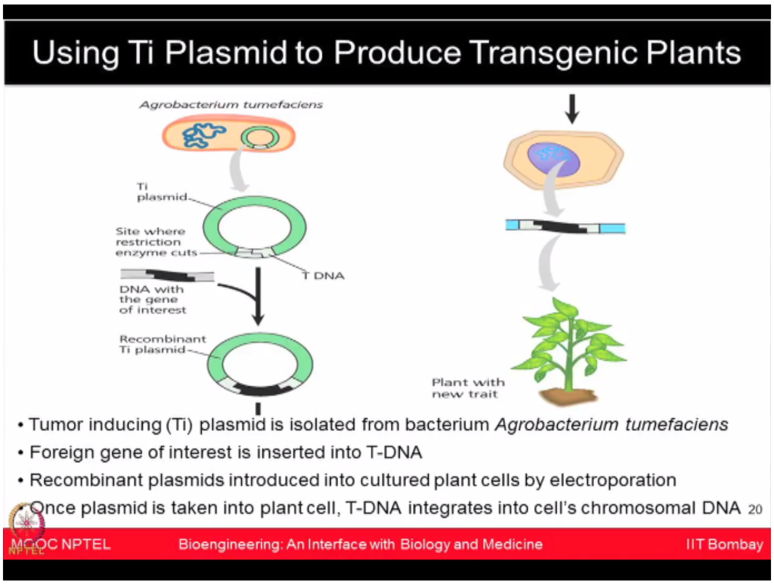

The Ti plasmid was isolated from the bacteria. Now the foreign gene of interest which is Bt gene in this case was inserted into the T-DNA. The recombinant plasmids were introduced into the cultured plant cells by the process of electroporation. Only those plant cells which took this plasmid where the T-DNA was integrated into the cells chromosomes, those were taken forward.

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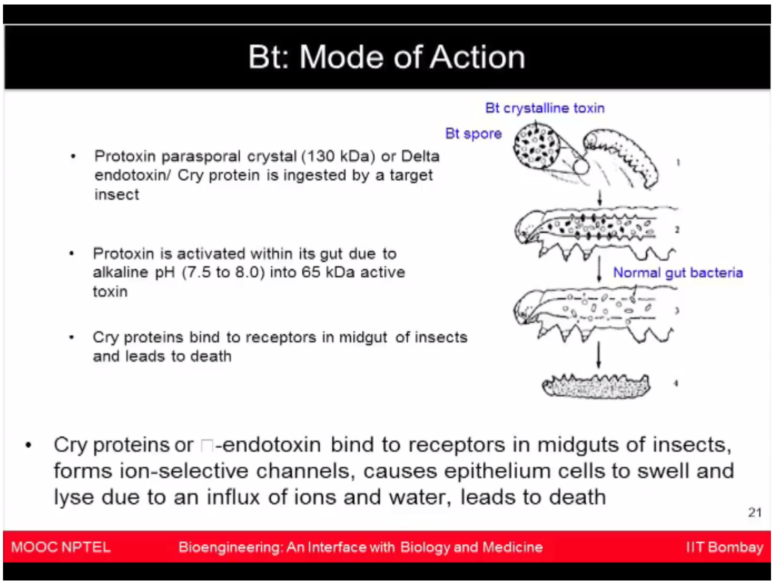

So what is a mode of action for this Bt gene? Initially, the protoxin parasporal crystals or Delta endotoxins they are ingested by the target insect. Now these protoxins are activated in their gut because of the alkaline pH which is there which activates this particular toxin. So you know after these crystals have been taken by the insect when it goes to their gut now that environment of alkaline pH is activating this toxin which was inactive earlier.

And now the Cry protein is going to bind to the receptors in the mid gut of insect which leads to the death of this particular insect. So the Cry proteins or endotoxin, they bind to the receptors in mid guts of insects. They form ion-selective channels causes epithelium cells to swell and they lyse because of the influx of ions and water and lead to the death of this insect. There have been large number of successful example for transgenic Bt crops which have been produced worldwide.

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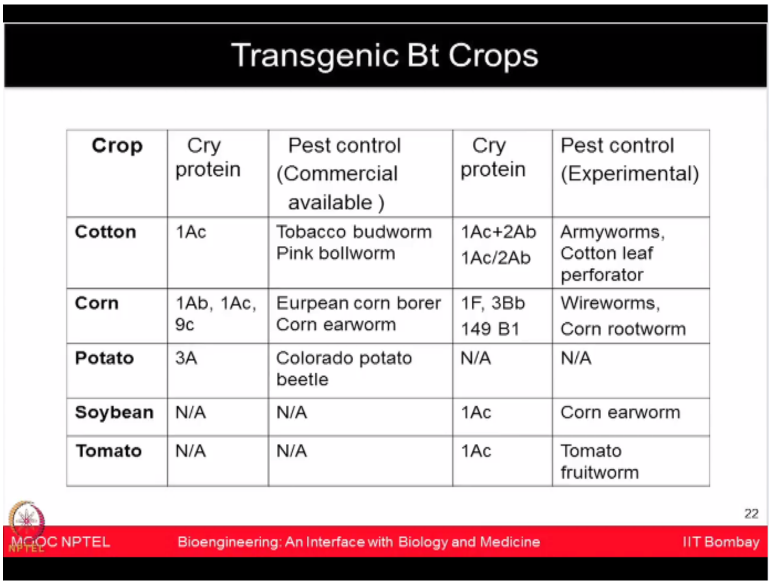

Crop	Cry protein	Pest control (Commercial available)	Cry protein	Pest control (Experimental)
Cotton	1Ac	Tobacco budworm Pink bollworm	1Ac+2Ab 1Ac/2Ab	Armyworms, Cotton leaf perforator
Corn	1Ab, 1Ac, 9c	Eurpean corn borer Corn earworm	1F, 3Bb 149 B1	Wireworms, Corn rootworm
Potato	3A	Colorado potato beetle	N/A	N/A
Soybean	N/A	N/A	1Ac	Corn earworm
Tomato	N/A	N/A	1Ac	Tomato fruitworm

I have shown in this table here the transgenic Bt cotton for example, Bt corn, Bt potato, Bt soya bean, Bt tomato. All of these crops having different type of Cry proteins which were inserted in the plant and as a result now the plant have shown the pest control for different type of pest.

So this particular area has really kind of you know transform has been very revolutionary because now large acres of the you know field where these plants are grown could be protected from these kind of infections and therefore you know the yield of the food can be very high; however, people have expressed concern that you know what are the potential risk of using these transgenics in the field and especially transgenic insecticidal crops.

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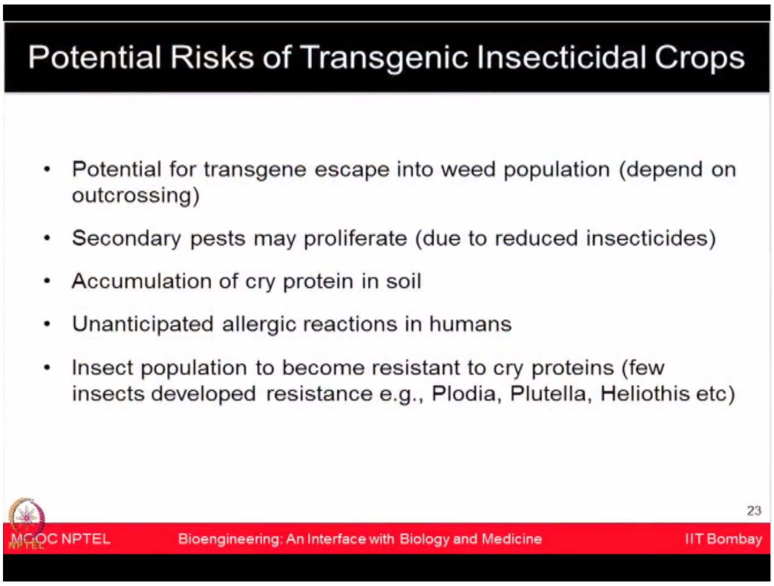

So you know one of the potential region could be that you know these transgene could escape into the weed population depending on the outcrossing from the other crops which are in the same you know neighboring regions. The secondary pests may also proliferate because if you are controlling one type of pest probably another type of you know the pest population might also increase.

You know the Cry proteins might also get accumulated in the soil over the period of time. There could be some sort of unanticipated reactions to the human like which could be allergic reactions and these insect population could also become you know resistant to these Cry proteins over the period of time. For example, you know a few cases have been reported where even plants have shown you know the insects have shown the resistance towards these Cry proteins.

So this must be you know I must admit this is one of the areas where people have both you know pros and cons for the technology. There has been large cases you know where you would have heard that you know because of the Bt crop lot of you know farmers committed suicide. Sometime the big multinational companies, they try to make lot of you know money out of this you know proprietary what they have from the licenses.

And as a result you know on one hand when we have seen that you know the huge benefits because of you know the increased yield which has obtained because of these transgenic crops. On other hand, there are you know certain regulatory issues which are still need to be handled. There are certain ethical issues, which still has to be addressed. So this area know I will leave that up to you to read more about it to you know get more educated in that particular direction.

And to find out you know kind of you know what you think is the best approach of you know this kind of transgenic crops but you know scientifically speaking these transgenic you know the GMO crops have really made a lot of you know increase in the nutritional value, increase in the you know certain components which can show a lot of you know increased productivity for the plants.

How you know we accept as a human being is something which remains you know a topic of you know discussion further. So in summary I think you know in the last several lectures, we have been talking about you know the gene cloning technology, the polymerase chain reaction, different ways of you know different lab techniques which we have given you demonstration to make the cDNA and do all kind of separation.

All of those knowledge moving you know as a part of the genetic engineering technologies is showing lot of application whether you talk about you know the human health or we talk about you know the plant biotechnology I am sure you can understand that you know by just manipulating the genes of interest, by knowing that you know what we are inserting, where we are inserting and what kind of trade they may have I think has made huge you know and the tremendous changes in overall applications.

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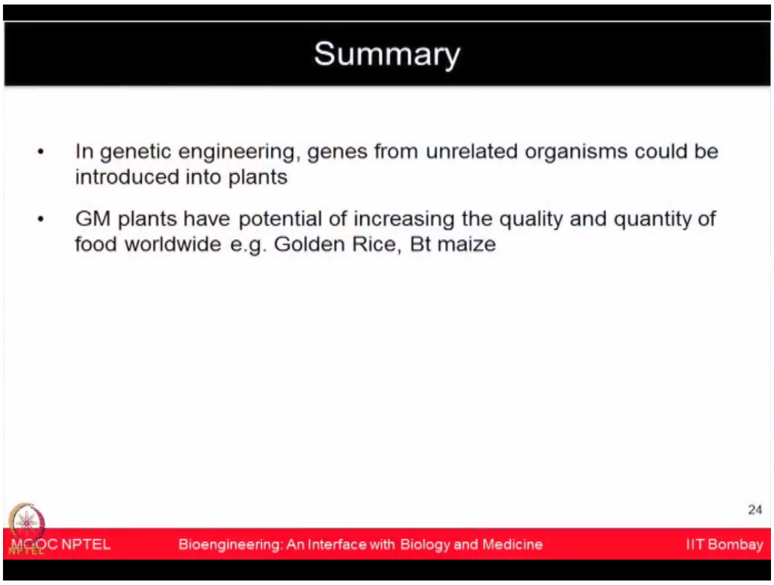

So in today's lecture in summary, we have talked about you know how genetic engineering technologies could be used to move the genes from one to other organisms and we have shown you a couple of examples of the salt tolerant plants to the Golden Rice plants and also looking at the you know the crops which are having highly insecticidal resistance properties.

The genetic modified plants have definitely lot of potential for increasing the quality and quantity of the food worldwide and there has been many example of you know the Bt crops but as I mentioned that you know how to take this kind of you know technologies forward and what are their you know ethical and economical consequences of that, I think this is an area of you know discussions and one need to you know be very open to look into the pros and cons of these technologies. Thank you very much.

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