Course Name: I Think Biology

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W6L33_Molecular Biology Techniques - BT Cotton - Part 2 (Case study)

Welcome to this lecture where we will be looking at BT cotton. In the first part of this lecture, I introduced you to the history of cotton in India and some aspects of the agro botany of cotton. In this lecture, we will be mainly looking at improvements to cotton and here we will be studying how hybrid cotton was produced in India for the first time. And we will also be looking at recombinant cotton, namely BT cotton and studying some of the science behind how BT cotton is produced and also trying to study how to assess the impacts of BT cotton in India. So first, let's look a little bit at some of the genetics of cotton, in particular its genome and how domestication changed some of the characteristics of cotton. So in this rather complicated figure, what you see is the development of domesticated cotton.

And so starting at the left, you have the ancestral species from a particular Gossypium species of cotton and this gave rise to different varieties I mean different diploid species of cotton and in particular we are interested in two species, Gossypium herbaceum, which is an old world species which was found in Africa and its genome is called AA and there was a new world species found in the Americas which is called Gossypium raimondii. And at some point, hundreds of thousands of years ago or perhaps even a million years ago, there was a hybridization event which happened between these two species which led to the development of a polyploid genome. So here you have a cotton which we now call Gossypium hirsutum which had a double duplicated genome so it had copies from both Gossypium herbaceum and Gossypium raimondii. So you have AA and DD.

So polyploidy is quite common in plants and generally it tends to produce plants which are more robust or showy. And the same thing seems to have happened in this species of cotton where the fiber length or the lint increased as compared to the herbaceum or the raimondii. And then this species Gossypium hirsutum was domesticated and farmers over perhaps a few thousand years selected and improved upon this original hirsutum species so that the fiber length was further increased. You can see actually the increase in

size if you look at the domesticated species which is shown on the right and compare it with the original progenitors of that species which are herbaceum and raimondii where the lint length is almost quadrupled. So this kind of improvement in cotton led to the modern species which are now used for producing fiber.

And we still practice kind of artificial selection. Only we do it in a very different manner than farmers were doing it in the past. We do it in test plots where basically to produce hybrids we choose plants which we want to cross. They might have certain characteristics which we are interested in improving upon. So we choose a female plant in the case of cotton and in that all the male parts of the plant are removed including the corolla and the anther sheath.

And then you have this emasculated flower bud over which you put a red bag just to signify that this process has been done. Then you choose a male plant and you again take out the corolla so you are just left with the male flower buds. And at a particular time of day you dust these anthers over the previously selected female flower. And typically one anther can be used to fertilize four to five female plants. And there is a particular time of day at which the stigma is most receptive in the morning.

So that's when this operation is done. And then after this fertilization has been done you cover these pollinated flowers with a white bag just to signify that this operation has been done. And so you know that which plants on your test plot have been fertilized by this manner. And this is how you produce a hybrid. Very similar to what Mendel did in his original experiments.

So a similar program was followed for cotton and actually the first successful cotton hybrid was produced in India. This was done by Prof. C.T. Patel at the Agricultural University in Gujarat.

And after many many trials he produced a hybrid which was particularly promising which was called Hybrid-4. And this was done by crossing two varieties which were called Gujarat 67 and an American Nectariless variety. And Prof. Patel found out that this Hybrid-4 had certain advantages. So because of its heterosis or hybrid vigor it had a very high yield.

And it had an improved bearing capacity which means the number of the amount of bowls which were produced. The size of the bowl was also larger and it underwent frequent flowering which again would have contributed to the increased yield. And as I said before the yield was found to be about 200% higher than a parent line. And so with the development of Hybrid-4 and then certain other hybrids which followed it in India

and other parts of the world cotton cultivation entered this new era. So going back to when I was talking about polyploidy in plants here is a question for you to think about. Natural hybridization occurs across many plant families. So how is it that plants can maintain species purity without too much mixing of their genomes? So what might be the some of the barriers to fertilization that you can think about.

Let's then move on to talk about recombinant technology. And one of the first experiments which was done was by a scientist called Paul Berg where he combined two viral DNA molecules. So Paul Berg had been studying in a lab which was looking at cancer states in animal cells during viruses.

So viruses when they integrate themselves into the host genome cells they start producing more copies of the viral genome and we all know that. So Professor Berg thought whether this property could be used to produce copies of a DNA molecule which is of interest in a host cell. And so the first step would be to take a viral genome and splice that with a particular DNA molecule that you are interested in. And in this first experiment this was what was done where a simian virus molecule SV40 was spliced with another viral molecule called Lambda phage to produce a recombinant DNA molecule which was SV40 plus Lambda phage. And this first experiment was done in 1972 or at least was reported in 1972 I should say not done in 1972.

And this is a schematic of that original experiment. So first what you have to do is you have to linearize these viral DNA molecules. So this was done using an enzyme called a Nuclease which will cut up DNA. So Nucleases were used to cut both the SV40 genome and the Lambda phage genome. And then what the research team did was they added specific nucleotides to the ends of each of these viral DNA molecules.

So suppose you added adenine nucleotides to the SV40 then thymine nucleotides were added to the Lambda phage. And then these were mixed together in the presence of a DNA ligase and because of complementary base pairing between A and T the DNA ligase could then stitch up these two pieces of viral DNA molecules so that you had this recombinant molecule finally at the end. And like I said this experiment really hinged on previous discoveries. So these three enzymes had already been discovered and were used for the successful production of a recombinant molecule. This first experiment was then followed up by another one where two bacterial plasmids were spliced and then stitched together and then used to transform bacteria so that these bacteria would make more copies of this recombinant plasmid.

And again here this experiment benefited from two techniques. One was a heat shock technique which had been published which allowed for the uptake of DNA molecules into bacteria. And the other was the development of gel electrophoresis which allowed

you to separate DNA molecules by size and look at them on a gel. So this was really the start of the biotechnology revolution. Pretty soon after this experiment people showed that you could put a piece of eukaryotic DNA into a DNA plasmid molecule and allow bacterial species to express that eukaryotic DNA gene.

The recombinant technology was then applied to plants. So one publication which had a big impact came out in 1985 where they described, they showed a proof of concept for transferring antibiotic resistance genes into a plant. And they described a vector system which means a host and a plasmid system which could be used for transforming three different kinds of plants, petunia, tobacco and tomato. And this was how they did it. So they used a bacterium called Agrobacterium tumefaciens and it is known that this bacteria can infect plant cells and it has a plasmid which allow, which can go and integrate itself into the plant genome and then it induces more growth in the plant cell allowing the bacteria to multiply.

So this T i plasmid and this agrobacterium was used. So plant calluses were grown in a dish. So calluses, you can think of it like a plant embryo and then this was inoculated with a solution of agrobacterium which contained on the T i plasmid this antibiotic resistance genes. And then after the initial inoculum was done, plant shoots were grown. These were then transferred into media which would allow roots to grow and then finally these plants were grown and pots to the adult stage and then it was verified that they contained this antibiotic resistance gene in them.

If you look at the T i plasmid in more detail, it contains three main regions. One is this, it has an origin of replication like all plasmids. Then it has a virulence region. This contains genes which are responsible for snipping out and integrating a particular part of the plasmid in the host's genome and then that which is called the T-DNA region. This T-DNA region contains genes for plant hormones.

So this induces uncontrolled growth within the plant hormone and this also then will allow the bacterium to replicate itself. And so what is done normally is if you want to use a T i plasmid is that you take out this T-DNA region because you don't want that and then you put in there the DNA that you particularly want to have so that this part can be replicated in the host plant. So again here's a question. We are talking about snipping out pieces of DNA or adding pieces of DNA into a plasmid. How is it actually done and how do you confirm that this is what you have when you do this? So looking at the use of this recombinant technology in cotton in particular, in cotton a different bacterium was used which was Bacillus thuringiensis. This is a soil dwelling bacterium and it is also found on plant surfaces on leaves. And like many other species of bacteriums it undergoes a spore development phase when conditions are particularly unfavorable. And when it forms

these spores, this bacterium also synthesizes these crystal proteins which are part of the cell body. These crystal proteins contain a toxin which is called as a δ-endotoxin. And scientists have identified more than 500 different cry which stands for crystal genes that can make proteins.

So it is not clearly known why so many genes are used to produce such a variety of crystal proteins. Here is a schematic of the bacterium itself. So like I said in the vegetative phase it will produce a spore and then it also produces these crystals and the crystals can occupy a large part of the volume of the cell. So what will happen is that if the bacterium is found on a plant and then an insect ingests or eats up the plant then it will also take in the bacteria and these spores. And then these crystals, crystal proteins contain a toxin which will be detrimental to the health of the insect and it could also cause it to die.

So this feature makes it very advantageous to be used as insecticides. And if you look at it in more detail, the cry toxin needs to be ingested so it can't just be applied topically. So spraying it on a plant will not harm a farm worker. And in particular the cry toxin to work needs a high pH which is found in insect guts. It also has a very high level of specificity.

So particular crystal toxins act against particular insects. So this level of specificity is very very good so that you don't harm beneficial insects. It is also quite fast acting. So if an insect feeds upon it and ingests the cry protein then it stops feeding within a few hours and then death will follow within a few days. And death occurs because once the cry toxin is ingested it attaches itself to specific pores on the walls of the epithelium of the insect gut and then it starts to produce pores in this epithelium.

And then this allows the bacterial spore to enter and start multiplying inside the insect itself. And it is this bacterial replication which causes the insect to die. And then again like I said before they have a very high specificity and they have not been shown to have any kind of effect on vertebrates especially mammals. So all these qualities made them very very attractive to be used as insecticides. And so here is a timeline for the use of Bt in agriculture.

It has a very long history. After its initial discovery in 1901 and then again kind of been rediscovered in 1911. In 1915 was the first time people reported the presence of these crystals within Bt. And in the 1950s these three scientists showed that the insecticide action of Bt against Lepidopterans, moths in particular was due to the presence of these crystals. And so after that they started to be used as sprays and bioinsecticides in agriculture. And in fact after resistance to conventional pesticides grew in the 1980s the use of Bt sprays actually started to grow.

And so it was because of this long history that people thought of using them in recombinant technologies. So where you could insert the cry toxin gene into the plant host itself so that there is no need to spray the plant with this insecticide. And so some of the first Bt cotton plants were produced in 1990. Looking more at the timeline for transgenic cotton. So one of the first Bt genes which was successfully cloned and expressed was done in E.coli which is a standard kind of model organism in microbiology. And actually the expression of this gene in a plant was shown by 1990. And the first commercial cotton which was cultivated was in 1996 where one gene was being used, the Cry1Ac. And then since then there have been other varieties of cotton which have been put out commercially which contain two genes. There is also subsequent developments where there are varieties of cotton which contain three different genes which contain three different toxins which means they can be applicable against a larger variety of pests.

And here is an example of the effect of these Bt toxins on insects. This is from a trial done by the US Agricultural Service where on the left you see a control caterpillar from the cotton bollworm which is raised on a diet of conventional plants and then cotton bollworm which is raised on a diet containing these Bt toxins where the size of the caterpillar is much much smaller and it is less healthy. So after the production of and availability of commercial varieties of Bt cotton farmers took up its cultivation on a very large scale especially in the US and Australia and it was also introduced in India in 2002. So overall Bt cotton has been a success story worldwide. In India though there is some debate about the benefits accruing to farmers from the use of Bt cotton.

So we thought that this is a good case study to use to figure out how to assess the claims that a new technology makes. And so in this next part we will be talking about how to look at Bt cotton in India and figure out whether it has been a benefit or a detriment to Indian farmers. So if you look at the story of Bt cotton you have these kind of two opposing narratives. So if you ask like NGOs or environmental activists they will say that it has been a failure because there have been some instances of crop failure or they have linked Bt seeds and farmer suicides or there are questions about the sustainability of Bt cotton because of its high capital inputs. On the other hand if you look at certain other industry supported commentators or applied economists they will say that Bt has been a triumph because there has been a surge in cotton years after the introduction of Bt in 2002.

So then how do we actually look at this story and figure out what is happening. So here we will be using data which has been provided in the citation which is given on this slide. And in the graph there are shown two things. One is a timeline from 1950 to 2010

and then the left axis Y-axis shows the increase in cotton production in India. So you will see that it is increased very very slowly from 1950 to say 2000 but after 2000 there is a sudden jump in the amount of cotton grown in India.

And then the red line is the amount of Bt cotton area which is being cultivated. So right after its introduction in 2002 the area under Bt cotton cultivation jumped and by 2010 close to 90% of cotton being grown in India was Bt. So now the question is did this jump in the amount of cotton being grown in India or the yield could that be attributed to Bt cotton or not. So this is a classic case of is it correlation or is it causation.

So let's look at this in a little bit more detail. So what the authors of this study highlighted was that in order to tease out this relationship between the increase in cotton yield and the increase in the area under Bt cultivation we had to look at certain biases and they highlighted three different kinds of biases. There is a selection bias. So who are the farmers who can afford to grow Bt and so are these wealthy farmers and are we looking at the socio-economic strata of these farmers and on their land are farmers treating Bt cotton and non-Bt cotton in the same way or they are paying extra attention to the Bt plots. So there could be a selection bias, there could be a cultivation bias and there could also be a time term bias. So we showed that initial plot up to 2010 so how has cotton production changed in India after 2010 and could it be attributed to Bt or some other practices say the increased use of fertilizers or pesticides or the increased use of irrigation and so how do you tease out all these factors.

So on this graph we are showing you three different states and their cotton production. In particular we are showing cotton production from 2000 to 2018 and we are looking at cotton yields which means earlier we were just looking at the total amount of cotton but here when you look at yields you talk about the amount of cotton grown per unit area. So here we are looking at cotton kilograms of cotton grown per hectare of area. So then if you look at Gujarat you see that there is a sudden jump in the yield which is the black line and then it kind of stays flat after that. Say after 2002 there is a jump in the yield and then it starts to stay flat and there is even a dip at 2018.

And then if you look at the red line which is the state wise adoption of Bt, the percentage of land which is under Bt cultivation. Again there is a jump after 2006 and close to 90% of the land is under Bt cultivation by 2018. So what contributes to this increase in yield in Gujarat can it be attributed to Bt? We could say perhaps. Now if you look at Punjab the yield seems to flatline after 2006 and does not really grow even though most of the farmers are growing Bt cotton because after 2006 almost 90% of the land is under Bt cultivation. Similarly in Andhra Pradesh and Telangana yields do not seem to have grown, they have grown quite slowly even though most of the land is under

Bt cultivation.

So out of three states, two of the states do not really show a huge jump in yield even though most of the land is under Bt cultivation. One state Gujarat does show a jump in the yield. And the authors say that this could be due to the increased irrigation which was available due to the Sardar Sarovar dam becoming operational. So one conclusion that we could draw is that there could be other factors which are contributing to the overall increase in the amount of cotton being produced in India and we have to look at it state by state. And some of the factors that the authors highlight the use of fertilizers, the use of pesticides, just the amount of acreage could have increased, there could be other changes in technology.

The other thing that they highlight is that the amount of capital or money which is being spent on spraying for pests. So very interestingly they distinguish between two different kinds of pests. So you are spraying for lepidopterans or these bollworms or spraying for sucking insects. So if you follow the blue line across the years you see that there is a dip in this amount of money which is being used for sprays for lepidopterans and then after 2015 that also starts to show a slight increase. So what the authors say is that with the introduction of Bt indeed there was a drop in the amount of money which was needed to be used for spraying for lepidopterans but after a little while farmers started to see that some of these pink bollworm species started to show resistance to these Bt varieties being grown.

So again then you had to spray again for these pink bollworm pests. Along with that if you look at the spraying for sucking insects that started to show an increase because with the decrease in these bollworms you started to see other kinds of pests and we will know this very clearly as biology students that if there is a gap in the ecosystem and some other organism can come in to fill that niche. So with the drop in these primary pests, secondary pests started to be increased. And then finally to look at another factor which was the use of fertilizer. So again this is a graph which shows the yield which was similar to the earlier graph for Gujarat, Punjab and Andhra Pradesh and Telangana and you can also see the amount of fertilizer being used.

So again this is the fertilizer being used per unit area. So kilograms per hectares and that seems to follow the increase in yield in Gujarat. It seems to follow the increase in yield in Punjab and Telangana. So what the authors highlight here is that the Bt story is very complicated. Yes there was an increase in the amount of cotton being produced in India post the introduction of Bt but could this increase be attributed to Bt clearly? No it cannot because as you can see here in three different states yields either increased or stayed flat or increased very very slowly.

So you had different effects in different states. Again farmers started to use more fertilizers so that could also contribute to increase in yields. Third their capital costs for spraying initially dropped but then started to increase. So they dropped for the primary pest but increased for the secondary pests. So because of all these complicating factors the authors of that study are saying that you cannot attribute the increase in production of cotton to Bt in India. So while we are not saying that Bt in India is good or bad we are saying that we need to very very carefully consider any new technology that we are going to employ and the effects on Bt in India have not been as clear-cut as they have been in say the US or Australia where you have a more homogeneous agricultural system.

In India the agricultural system is very very diverse and in order to ensure that a technology is being used you need to have strict controls and measures to make sure that everybody is using it in the same way. So with that I will close this lecture and we can discuss more of these points in the subsequent tutorial class. Thank you.