Course Name: Basics of Crop Breeding and Plant Biotechnology

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Lecture-55: Golden Rice, Bt Cotton, FLAVR SAVR

Hello everybody. Welcome to the SWAYAM NPTEL course on the Basics of Crop Breeding and Plant Biotechnology. Today we will discuss module 12, which is on genetic engineering in crop improvements. We will discuss some selected case studies. So, in this lecture, we will discuss mostly Golden Rice, Bt cotton, and FLAVR SAVR tomato; which means how transgenically those have been developed and what is their importance those things will be discussed. So, the concept that will be covered under this topic is Golden Rice, basically two types of Golden Rice, have been developed is Golden Rice 1, another one is Golden Rice 2.

So, some features there mean, different transgenes have been integrated in Golden Rice 1 and some modifications are there in Golden Rice2, which will be discussed. Then, we will discuss, Bt cotton because we know that cotton is an important cash crop. So, it is susceptible to a lot of lepidopteran insects, cotton bollworms, and once Bt cotton came into the market, then it was very popular, and very successful in India, as well as, across the world. Then, we will discuss, the FLAVR SAVR tomato, which is one of the first introduced transgenic food crops across the world.

So, here the transgenic fruits were consumed by different people, across different countries, and some issues arose later on we will be discussing those things also. So, let us start our discussion on Golden Rice. So, why there is a need for the development of Golden Rice, what is the need to develop Golden Rice? If we try to understand the scenario vitamin A deficiency, affects 250 million people worldwide, which means a

large number of people are affected by this malnutrition, caused by vitamin A deficiency, and which includes children below the age of 5, approximately 190 million children have been affected, and pregnant women approximately 19 million were affected, and it was reported by WHO, World Health Organization. Then, vitamin A deficiency is the major cause of preventive blindness in children, it increases the risk of disease, and due to severe infection or due to severe deficiency of vitamin A, we may see blindness and ultimately, we can see the death of the children. Then, approximately 5 lakh children go blind each year, due to vitamin A deficiency, and after losing their eyesight half of the disease children die within 6 months.

So, it is very tragic, but it is a real scenario. So, to get rid of this malnutrition, problem the Golden Rice initiation or initiative was taken. These are the importance of Golden Rice, first, it is an enhanced version of ordinary rice is Golden Rice, which is developed to combat the nutrient issue, as we are discussing vitamin A deficiency. So, its price is almost similar to the normal rice, available in the market and its taste is almost similar to the ordinary rice. You know in the market different types of rice are there, Miniket rice is there, Basmati rice is there, their price used to vary in different range, in different places, and the price of the Golden Rice which was expected or which is assumed that will not be too much higher.

Then, in the battle against vitamin A deficiency, the beta-carotene content of the Golden Rice plays a major role. So, the beta-carotene content has increased in Golden Rice, which will eventually produce vitamin A in our body, and vitamin A deficiency could be minimized due to the consumption of Golden Rice. So, vitamin A is one of the important micronutrients for growth and development, as well as, it maintains a healthy immune system in our body. So, this slide shows different developments in Golden Rice. Ok! In 1982, two famous scientists initiated the Golden Rice development program, under the Rockefeller initiative, the name of those two scientists was Professor Ingo Potrykus.

He was from the Swiss Federal Institute of Technology, and Professor Peter Beyer. So, these two names are very famous and they initiated the Golden Rice development in

1982. Thereafter, almost after 10 years of research, in 1992 many research groups joined this particular Golden Rice initiative, to pursue the Golden Rice project in New York. So, initially, it was started in another part of the world, but later on, some other research lab initiated that particular program in the US also. In 1999, using a genetic engineering strategy means, using transgenic technology, the Golden Rice development was initiated.

They integrated different genes, from one plant Daffodil, and a soil bacterium was used that is *Erwinia uredovora*, later on, we will discuss. And, by taking three different genes from, Daffodil, as well as soil bacteria, they eventually created Golden Rice, upon integration of those genes through transgenic technology within the rice genome, Golden Rice was found to be rich in beta-carotene content. Then, in 2004 further study was done on that and in collaboration with Syngenta, a rice variety, was created with approximately 20 times higher beta-carotene content compared to Golden Rice 1. So, here we are discussing mostly the Golden Rice 1. So, during this time, the Golden Rice 2 was developed, here beta-carotene content was much higher, and the gene was taken from a maize plant and the same soil bacteria *Erwinia uredovora*.

The new version was donated to Bangladesh, Indonesia, Philippines to combat vitamin A deficiency, through the Golden Rice network. Now let us discuss the different genes, gradually which have been used in Golden Rice 1, and later on we will move into the Golden Rice 2 part. So, three genes have been used in Golden Rice 1 development, one is the *psy* gene i.e., phytoene synthase, the next one is the *lcy* gene i.e., lycopene beta cyclase, and another gene is *crtL* or carotene desaturase. So, these three genes were mostly used in Golden Rice 1 development, under different types of promoters. So, from the daffodil plant, the *psy* gene that is phytoene synthase and lycopene beta cyclase was taken while from another soil bacteria, *Erwinia uredovora*, *crtL* or carotene desaturase gene was taken.

Eventually, once those genes were PCR amplified, were cloned from the cDNA, thereafter it was used to develop the over-expression gene construct within the plasmid, eventually, those plasmids were transformed into the *Agrobacterium* strain, and with the

help of those *Agrobacterium* strain, ultimately within the plant genome, these three genes were transferred ok. In this way, the transgenic Golden Rice was developed, which produced more pro-vitamin A in rice embryos. So, let us see what is the pathway of beta-carotene production in a plant system. So, that's why those genes were used *phytoene synthase, lycopene beta cyclase, and carotene desaturase*, it will be clear. So, in the plant system from isopentenyl pyrophosphate that is IPP, the geranyl geranyl diphosphate or GGPP is produced.

Then, from here we have to pay attention with the help of the enzyme phytoene synthase, this GGPP or geranyl geranyl diphosphate, which is converted into phytoene. Then in the plant system, a number of enzymes are there, like the carotene desaturase, then phytoene desaturase with the help of those enzymes, that phytoene is eventually converted into lycopene. Then another enzyme, is their lycopene beta cyclase, that enzyme converts lycopene into beta-carotene. So, in this way from IPP, beta-carotene is produced, this is the basic pathway. So, if you think about the bacterial system, in the bacterial system a robust carotene desaturase enzyme is available i.e., *Erwinia uredovora*.

So, that enzyme was found to be effective in converting phytoene into lycopene. So, in Golden Rice 1, this phytoene synthase was used from Daffodil, the lycopene beta cyclase was used from Daffodil, and carotene desaturase was used from *Erwinia uredovora*, to produce beta-carotene content or to increase the beta-carotene content in Golden Rice 1. Now these are the different constructs that were used for Golden Rice 1 development. If you see about this particular construct, pZPsC over here *phytoene synthase* gene was available and *crtL* or carotene desaturase from *Erwinia uredovora* was available. The phytoene synthase was expressed under glutelin promoter.

So, that it will be expressed in the seed tissue, and a nos terminator, was there because you know for over-expression of every gene, we need to have a promoter, we need to have a terminator those things. So, glutelin promoter and nos terminator were used, for expressing the *crtL* or carotene desaturase gene, initially at the prior part of this gene, a transit peptide was attached from the pea rubisco small subunit. So, it would be targeted

to a specific part, and it was expressed under 35S promoter and nos terminator was there, and all this thing was available in between the left border and right border region, means within a plant transformation vector. While, in another construct i.e., pZLcyH over here you can see a *lcy* that is a *lycopene beta cyclase* gene was there under the control of glutelin 1 promoter, and 35S terminator was used while *aph* IV a selectable marker gene was used under 35S promoter, and 35S terminator. It was also available in between the left border and the right border.

So, these two constructs were used to generate Golden Rice1, and you guys can go through this particular literature, the engineering of the provitamin A i.e., beta-carotene, biosynthetic pathway into carotenoid-free rice endosperm, means initially the endosperm was carotenoid free. So, what are the limitations of Golden Rice1? Let us discuss that the production of beta-carotene or provitamin A is not enough for the children's dosage. It is only 1.6 microgram per gram of rice, the beta-carotene production was approximately 1.6 microgram per gram of rice.

Therefore, to fulfill the dosage of a particular child, he or she must intake 10 kg of rice per day, should if he supposed to take Golden Rice 1 to get rid of the vitamin A deficiency, but that is not possible for a kid to consume 10 kg of rice per day. So, then scientists started working to enhance the beta-carotene content in Golden Rice 1, and eventually, they developed Golden Rice 2. In Golden Rice 2, mostly this construct was used if you go through this particular literature. So, in this construct, they have used the *phytoene synthase* gene from *Zea mays*, which is from the maize plant, earlier it was used from Daffodil. So, they have used *phytoene synthase* from maize plant, under glutelin promoter and nos terminator, then the *crtL* that is carotene desaturase was used it was taken from *Erwinia uredovora* the same bacteria which was used in Golden Rice also. Here a rubisco chloroplast transit peptide was attached, with this for proper targeting of this particular protein and it was also expressed under glutelin promoter. So, eventually, they found that the production of beta-carotene became enormously high, at least 20-fold higher compared to Golden Rice 1. Here, the promoter they have changed, earlier if you recall the last slide, they used the 35S promoter, the bacterial promoter was used over

there. Another thing in Golden Rice 2, no *lcy* gene was used that is lycopene beta cyclase gene, because it was found that that gene is available in the rice system. So, that enzyme or that protein is available.

So, they did not express it, and over here *pmi* means a positive selectable marker was used phosphomannose isomerase, under the control of ubiquitin promoter and nos terminator. So, this was the construct of Golden Rice 2 and you guys can go through this particular literature, improving the nutritional value of Golden Rice through increased pro-vitamin A content. And, if you think about the importance of Golden Rice 2, here *phytoene synthase* gene, from maize was used with *carotene desaturase*, from *Erwinia uredovora* like Golden Rice 1. Both genes were expressed under endosperm-specific promoter means glutelin promoter endosperm-specific promoter was used. Golden Rice 2, produced 23 times more than 20 times carotenoids than Golden Rice 1. Ok!

In Golden Rice 2, 37 micrograms per gram of beta-carotene was supposed, to be produced. Therefore, to receive the recommended dietary allowance or RDA, a person should eat 75 grams of Golden Rice 2 per day, i.e., very important. It means it is manageable meaning a person can take, 75 grams of rice in a day. So, this was found to be highly manageable and beneficial and eventually, it was distributed to Bangladesh, the Philippines, and different countries for its popularity. Now coming to another exciting topic and the success stories of transgenic technology which is Bt cotton.

First, cotton is known as white gold, because it is the major cash crop in India and plays a crucial role in the Indian economy. It contributes 360 billion in export income and supports a large mass of people through its cultivation processing and trade. So, different cotton-related industries are there and cotton cultivation area ranges between 8 to 9 million hectares in India mostly in Maharashtra, and Gujarat in those regions, the cotton is highly cultivated. India, is the largest cotton-producing country, after China, and is the earliest country to cultivate and produce fibers using cotton. Now, these different types of cotton bollworms have been mentioned over here.

Bollworms are caterpillar types of insects, that mostly belong to the Lepidoptera family, and in their larval stage, they chew the leaves or different plant parts the bollworm, there in cotton the boll is formed, and the fiber is developed in the cotton boll. So, the bollworm is the kind of worm that can attack the cotton boll, but it will attack the cotton leaves also. Ok! So, different types are there like the American bollworm, *Helicoverpa armigira*, the pink bollworm that is *Pectinophora gossypiella*, the spotted bollworm, and the spiny bollworm. So, these 4 types of bollworms, are mostly found and out of these, the *Helicoverpa armigera*, which is an American bollworm, is the most prevalent and difficult to control. First of all, broad-spectrum insecticide resistance is there to kill those insects that attack the crop plants, we have to use insecticides.

But this, American bollworm, or *Helicoverpa armigira*, is resistant to a large number of insecticides. So, it cannot be controlled easily, then it is multiple times and has high polyphagy, which means it can attack different crops and different plants, and its feeding pattern is prolific also. So, if you see the annual expenditure on insecticides, in Indian agriculture. So, approximately 12 billion is spent on the control of bollworms in cotton, while only 4 billion is used for controlling other insects in cotton. For controlling bollworms in cotton, approximately 12 billion is invested, while 4 billion is utilized for controlling other pests in cotton, and 14 billion is spent by remaining agricultural use for other crops.

So, you can imagine, that how much cost is involved in cotton bollworm, control. Now, let us see the development of Bt cotton. In 1911, *Bacillus thuringiensis*, was discovered as a pathogen in flour moth. Ok! In flour moth, there this pathogen was initially detected in 1911. In 1938, *Bacillus thuringiensis*, was utilized as a biopesticide in France at that time.

In 1950, the biopesticide containing the soil bacterium was commercialized. The commercialization started at that time. In 1992, the gene responsible for the toxin was produced and introduced in cotton in the USA, which means this *Bacillus thuringiensis*,

was found to produce some toxin, that gene was initially isolated, and it was introduced in cotton in 1992. In 1996, cotton was grown in the USA, in 73000 hectares. So, gradually transgenic cotton was being famous.

In 1997, in China, it was grown in approximately 1 million hectares. Thereafter, in 1998 1.5 million hectares were in the USA, Mexico, Australia, Argentina, China, and South Africa. The transgenic cotton production has been increased in different areas. In 2002, Bt cotton was grown by 5 million farmers out of which 99 percent was from the developed countries.

By that time, most of the developed countries farmers were using this. In 2001, the commercialization of Bt cotton started by Monsanto, was approved. In 2003, increase in cultivation area up to 1 lakh hectare, in India. If you look at the cotton production curve in India, from 2003 up to 2010-11, there was a boom in cotton production in India. India became the cotton exporter, one of the big cotton exporters. Ok!

During this period, from 2003 to onwards till 2010 or 2012, by that time huge amount of cotton was being produced in India, and more than 90 percent of that cotton, was belonging to the Bt cotton category. So, how does it work, how the Bt cotton work? First, of all the gene was taken from, *Bacillus thuringiensis*. In *Bacillus thuringiensis*, the available gene is responsible for this toxic compound production, basically, those proteins are known as crystal proteins. Ok! Different genes are there like *cry*1Ab, *cry*1Ac, or *cry*1A, different crystals stand for *cry*. Ok! So, in this way, *cry*1Ab, *cry*1Ac *cry*1A different genes eventually, have been identified or discovered from different Bt strains, *Bacillus thuringiensis* strains.

So, initially, the first gene was cloned which was found to be suitable, it may be *Cry*1A or *Cry*1Ab whatever. So, it was placed in this way, a promoter was placed for expression, a terminator was placed for proper stopping the transcript, and the gene was

placed in between. Thereafter, it was initially inserted into a plasmid and then that plasmid was initially inserted into *Agrobacterium*, then using that *Agrobacterium tumefaciens* harboring this plasmid, the cotton callus or cotton explant was infected. Ok! So, thereafter in this way, the genetically modified Bt cotton was developed, there within the cotton genome this transgene has been integrated, which means our *cry* gene along with its promoter and terminator has been integrated. Now it has been found that in transgenic plants once it is fed by the insect larva, then the larva dies due to this toxin available on the Bt leaves, due to the availability of this toxin in the Bt leaves.

So, this is in a nutshell how does it work, later on, I will explain it once again. So, here you can know the mechanism, how this type of larva chews, the leaves of the transgenic cotton or Bt cotton, and how they will be killed. Ok! So, this is the mechanism of action of cry proteins, in the midgut of insect pests, first of all, this crystal protein or cry protein will be effective under alkaline pH. If we think about the human system in our gut, what is the pH its acidic in nature, in our stomach pH is acidic in nature, but in this type of lepidopteran insect, in their gut, the pH is alkaline in nature. So, once they ingest this type of leaf, the cotton leaves have the Bt toxin, once they chew and ingest it, within this leaf our protein will be available because within the cotton genome, our transgene has been delivered right the transgene having the *cry*1A or *cry*1Ac whatever has been delivered.

So, it is also producing protein, in the leaf, this protein will be available. So, initially, it is chewed by this particular larva then that protein will go into its gut, in its gut under alkaline pH conditions, this protein becomes active. Once it becomes active, then the activated toxin binds, to the epithelial receptors in the gut, lumen if you see the gut lumen structure like this in these types of receptors, basically this active protein can bind. Ok! Once, it binds over there, it can cause pore formation, ok, it can cause pore formation the cell wall leaks, and ultimately cell lysis takes place, eventually the larva dies due to lysis of different parts in their stomach, as well as, in their abdominal lumen. Ok! So, in this way, the Bt cotton was found to be highly successful, and as most of the money was being spent to control, this particular bollworm, once the Bt cotton was introduced, it was very famous.

So, few things we need to recall once again, first of all, it was not hazardous to human beings, because once such type of technology, comes to the market, before that thorough research has to be done, I think they have done it and it was effectively active under alkaline pH condition. So, in acidic conditions, which is available in human, these things will not work anyway. So, later on, different controversies came, and finally, Bt cotton has been, means Bt cotton production, has been minimized somehow, and again India became the cotton importer earlier, India was a cotton exporter. Ok! Now, another example of the success stories of transgenic technology is FLAVR SAVR tomatoes. So, why were, FLAVR SAVR tomatoes designed? Tomato is considered one of the most important fruit, usually, tomatoes are picked, before ripening from the vines as they soften during the ripening process and it is difficult to transport.

So, if you harvest a ripe tomato, you cannot keep it at room temperature for a longer period, you have to keep it in freeze for a while, but the ripe one cannot be stored easily. Ok! The tomatoes are mostly picked before ripening for their easy transport. Ok! Then, picking the fruit before ripening hampers the taste and flavor, also that is true if we harvest green tomatoes, then its flavor will not be like the fully ripened tomato. The genetically engineered tomato was developed by a Californian company, Calgene in 21st May, in 1994. So, the FLAVR SAVR tomato was developed by Calgene, by using anti-sense RNA technology, to reduce the expression of enzyme PG i.e., polygalacturonase involved in the ripening process of the fruit. Ok!

So, it is an enzyme, that fasts the ripening process, that fastens the ripening process of the fruit. So, this enzyme's expression was reduced by anti-sense RNA technology. I will describe once again what is anti-sense RNA technology. Then polygalacturonase is present abundantly in ripe tomato fruit and is responsible for the softening of the ripe tomato. Ok! It can help in ripening, as well as, in softening of the tissue.

The FLAVR SAVR tomato is the first genetically engineered, whole food to be sold in the commercial market. So, let us describe the process, of how the FLAVR SAVR

tomato was developed. In normal tomatoes, the polygalacturonase, this enzyme is produced. So, let us assume, this is its mRNA for this particular enzyme, and suppose it is the protein of this particular enzyme. Let us take an example, of a sequence suppose its sequence is ATG and at last… sorry in mRNA ATG will not be there.

Suppose it was AUG, and over here the stop codon UAA was there. Suppose this one was the mRNA sequence. So, in the anti-sense RNA technology, if you think about this particular gene. So, it must need a double-stranded DNA, from where this mRNA will be transcribed. Let us assume its sequence was from 5' to 3', detection ATG …… at last TAA in its opposite strand the sequence will be TACATT.

So, transcription started from here. So, in anti-sense RNA technology, we know that here, 2 strands of DNAs are available one is the coding strand, another one is the template strand. Using the template strand, the mRNA is produced based on the complementarity. Ok! So, this mRNA is known as the sense mRNA, the sense strand. So, in this, FLAVR SAVR tomato, what did they do, they inverted the gene to generate an anti-sense strand. Ok! So, if we have to make the anti-sense strand, of this gene we have to start expression from here from this side. Ok!

So, we have to put the gene in this way. So, let us make the gene in anti-sense orientation. Suppose, I am starting from here this is the 5' end, we had TTA different bases were there, and over here it was CAT the 3' end. In another strand, it will be AAT, over here it will be GTA. So, this was the case for the normal tomato in this way the polygalacturonase gene was there, there from mRNA was being produced the protein was being produced and the natural rotting of the tomato, due to polygalacturonase was visible. What they have done in FLAVR SAVR tomato, in anti-sense mechanism using anti-sense mechanism we are discussing.

So, here they have inverted the gene in this way, and once the transcription will be taken place, this strand will be treated as a template strand. Ok! So, using that mRNA will be

formed. Let us see if the mRNA will be having UUA and CAU. This will be the mRNA, is not it because, it will be just similar to this strand except in, the place of T there will be uracil residue, because it will be complemented to this one, in this way the mRNA will be formed right. So, now in this transgenic tomato plant, this transgene they have integrated and they are from this anti-sense mRNA for polygalacturonase gene was being produced.

Now, in this plant this sense RNA was already available. So, let us see if these two come together. Ok! So, over here this one was the 5' to 3' end, right! of the mRNA. If we see from here AUG and at last UAA. So, these two mRNA can pair means A pairs with U, U pairs with A, and G pairs with C in this way, the sense mRNA of *polygalacturonase* and the anti-sense mRNA could pair, and that might reduce the protein production. So, as the protein production of polygalacturonase enzyme was reduced, no rotting was observed or detected for about 3 weeks over there.

So, why FLAVR SAVR have failed? First of all, the high production cost of genetically engineered tomatoes, along with the inexperience of the company to handle and grow tomatoes, which gradually led to the financial troubles of FLAVR SAVR. Then, the company was later bought by Monsanto, a multinational agricultural company, and then FLAVR SAVR disappeared from the market within 3 years after its introduction. Two major differences between genetically modified and normal tomatoes are that, the pectin wall took a longer time to degrade, and the new paste of tomato was higher in viscosity because the pectin wall was stronger, in the anti-sense RNA-mediated developed tomato. Ok! The paste was having higher viscosity, though it did not have any risk factor, but there was a change in taste, and it was not accepted by the consumers, and later on, it was obsolete. So, these are the differences related to Golden Rice and FLAVR SAVR and Bt cotton-related things.

I hope you have enjoyed the classes. So, do not forget to register for the examinations. Thank you.