Plant Cell Bioprocessing
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Lecture – 02
History of plant cell and tissue culture

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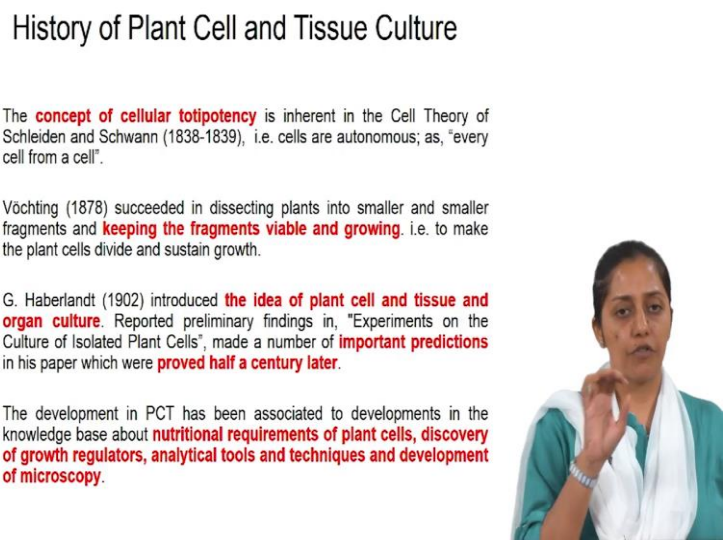

History of plant cell cultivation: When cell theory came into picture, parallel discoveries were going on in US and Europe during second world war. The cellular totipotent nature of plant cells was discovered around 1838-39. The idea was to see if the explants can remain viable in the medium for a longer time after taking it out from the plant.

So, the attempts were to keep the fragments which were cut from the parent plant growing and viable. It was necessary to identify what kind of media was needed to sustain that growth. IAA was then discovered. Went was able to find IAA, Indole acetic acid which is an auxin.

Initial discoveries involved the use of complex medium. When coconut milk was used, auxin was discovered. Further it was also discovered that there is a component in coconut milk which is unlike auxin, but is able to support the callus growth. Then in parallel it was also found how to induce callus from single cells. One of the techniques for inducing callus include the nurse tissue technique. A callus of some plant is taken and

a filter paper was placed on top of that callus, and the filter paper was allowed to get wet by the callus.

So, whatever came out of the callus was absorbed by the filter paper. And then on top of that filter paper single cell of a particular plant was placed and it was observed under sterile conditions. And they found that that the single cell started dividing and multiplying, and then callus was found to originate. So, these simple techniques of plant cell and tissue culture formed the basics of plant biotechnology. These were the initial discoveries which were very fast and quick and happened in parallel.

Then it lead on to refined media requirements depending on what kind of culture was required. The initial idea was to just maintain the explant and keep it viable. Then the callus induction was studied. Then somatic embryos also came into light by a simple experiment where carrot discs were put in the suspension. And, then because of agitation some of the single cells got dispersed and came in the medium. Because of the nutrient composition present in the liquid medium, these single cells, rapidly divided and formed embryoid like structures with both primordiums, shoot and the root primordiums later giving rise to the entire plant in the solid medium. This is how regeneration discoveries came into light.

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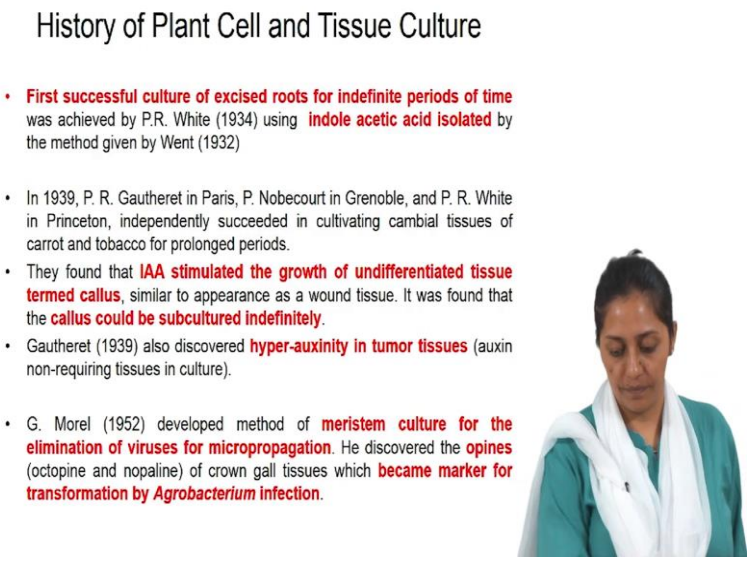

Then came *Agrobacterium* mediated transformations where people found that these natural vector systems can cause crown gall disease. When the crown galls were tested,

they found that they have hyper-auxinity. Hyper-auxinity means that they do not require any hormones in the medium to continue their growth. Hyper-auxinity was due to the presence of genes that inherently produced auxins in large amount. The analytical and microscopic techniques were discovered in parallel which helped in the development of plant biotechnology.

Opines were found to be present in these crown galls. Opines act as carbon and nitrogen sources for the bacteria. They are not expressed when present in the bacteria. But, once the bacteria infects the plant cell, the opines get expressed and they start getting produced, which is then utilized by the bacteria as food source. So, initially opine expression is transient.

Opines were used as markers for testing successful transformations. Later more fool proof confirmations such as PCR, was used to check whether T-DNA integration has happened or not.

Then meristem culture came into the picture. So, now, the objective was to see if virus free plants can be obtained which led to the development of meristem culture. Further in the course, the use of meristematic tissues for plant regeneration will be dealt in detail. The reason for the development of meristem culture is because they can lead to production of virus free plants. It is an interesting question to think upon as to why a meristematic region is free of virus and while the other matured parts of the plant are easily invaded by the virus.

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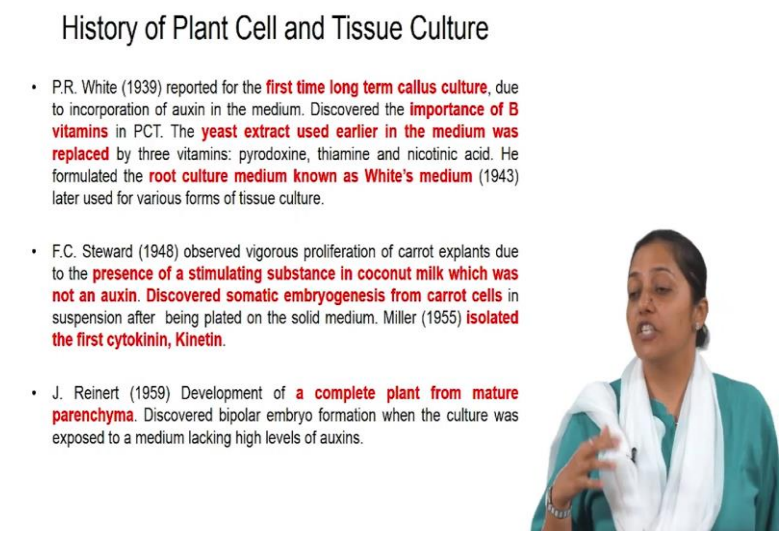

Initially complex media were used for *in vitro* plants such as the use of yeast extract which is also used now.. B vitamins were found to be very significant in induction of *in vitro* cultures plant cell and tissue cultures. So, yeast extract was replaced by three of these vitamins, pyridoxine, thiamine and nicotinic acid. So, gradually well-defined media compositions were developed.

Then White's medium came into the picture which was specific to root induction. Then MS media is used for callus induction. Half strength or quarter strength MS compositions also are being widely used which may impact the induction of *in vitro* cultures.

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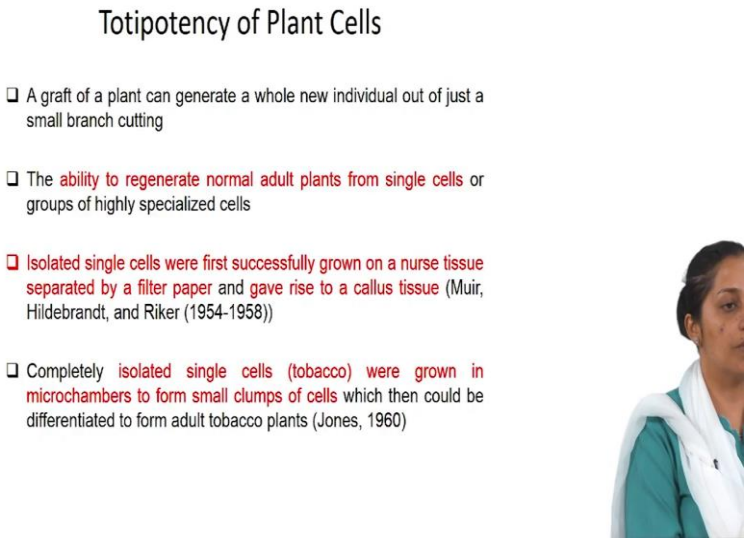

Totipotency is the potential of any plant cell to revert back and reprogram itself from a specialized function, and respecialize itself towards a different function.

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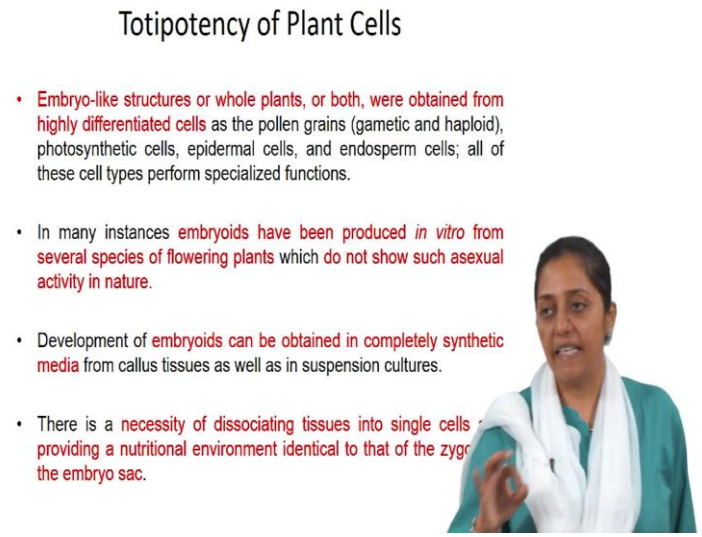

For a totipotent cell to regenerate into a plant, the cell should have embryogenic capacity so as to form primordiums for shoot and root generation. And, there has to be a nutrient environment created around the cell to allow it to rapidly divide, similar to a zygote. So, this will lead to regeneration of the cell into a whole plant.

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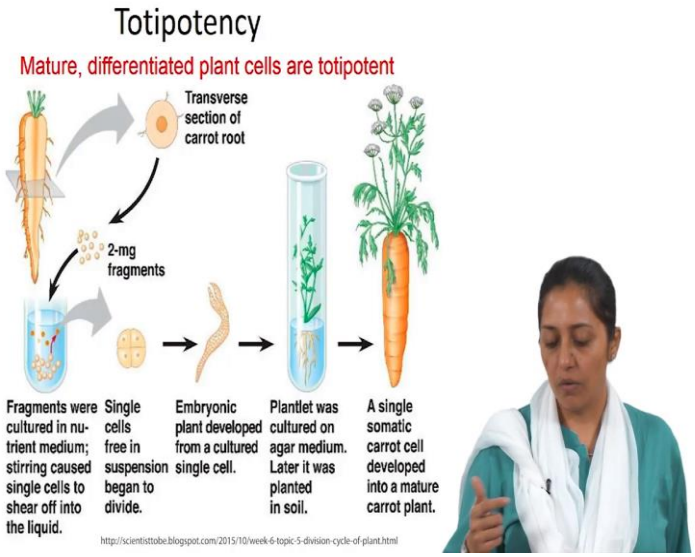

This slide shows how the entire plant was regenerated from an explant. A disc of carrot was suspended in the liquid. Some of the free cells, by agitation, get dispersed freely in suspension. Totipotent cells with embryogenic competence, start dividing and gets reprogrammed and behave like embryoids, leading to shoot and root primordium. And they are then transferred to the solid medium, giving rise to roots and the entire plant.