Course Name: Basics of Crop Breeding and Plant Biotechnology

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Lecture-58: Seed Sterilization and Transformation (Rice and Tobacco)

Well, so in this video we would like to discuss the sterilization process of rice seeds and tobacco seeds. So, those are two different types of seeds, rice seeds are relatively larger in size, while tobacco seeds are very tiny. So, you guys can see how the sterilization process is done for both the seeds, before initiating the plantlets, and before growing the plantlets in tissue culture conditions. And, after the rice seed sterilization, we will see how the plant transformation is done using the *Agrobacterium*-mediated gene transfer method. So, let us start the video. First, we need to dehusk the rice seeds ok, the husk part is removed.

Then in this way, approximately 100, 200 seeds are dehusked. Here, after those dehusked seeds are placed in an autoclaved conical flask. All these things will be conducted, the seed sterilization process will be conducted within the laminar airflow, under controlled conditions. So, first, we are washing the dehusked seeds using autoclaved double distilled water.

So, these things have to be done for a couple of times, 3 to 4 wash with double distilled autoclaved water. So, that if any dust particles are there, if any soil particles are there it could be removed. Thereafter, we have to add liquid soap to remove if any contaminants, soil debris particles, or any pollutants are available over there we need to clean it. So, in this way after adding the liquid soap we have to shake the flask containing the seeds for approximately 4 to 5 minutes. And after shaking we can see a lot of frothing has

occurred, then gradually we have to remove the water as well as the foam which has been created.

So, at least 3 to 4 times washing is needed to get rid of the soap particles. And, once we are trying to remove the water or the soap particles, then we need to burn the mouth part or neck part of the conical flask in the burner. So, that, no contamination should come from that part, from that opening part. So, here you can see most of the foam has gone the frothing has been removed mostly, we need to occasionally clean our hands by using 70% ethanol, and we need to sterilize our hands. So, this is another fresh autoclaved conical flask.

So, after initial washing by double distilled autoclaved water and washing with liquid soap, we need to use the main sterilizing material for rice seed sterilization, i.e., mercuric chloride ($HgCl_2$). So, now we are going to add mercuric chloride solution, and thereafter the thing will be transferred into the fresh autoclaved conical flask. After transferring this, we need to shake it vigorously for 3 to 4 minutes, shaking is done with $HgCl_2$ solution for proper sterilization of rice seeds. So, that if any fungal spore is available over there if any bacterial particles are available over there, it will be killed during this particular sterilization process. So, after 3 to 4 minutes we need to remove the mercuric chloride solution, then we have to rinse the seeds at least for 3 to 4 times using double distilled autoclaved water.

So, each time before pouring the water from the flask, we need to burn the mouth part or the neck part of the flask in the burner. So, in laminar air flow under controlled conditions, where a HEPA filter is available means, the high-efficiency particulate air filter is available over there within that container within that particular chamber we need to do all this process. So, here the initial process of sterilization of rice seed has been done, then as a number of times, we have used water and different solutions, we need to soak the seeds before putting them in the media. Some autoclaved petri plates have been used here with different forceps, and scalpels which are mostly used for plant tissue culture practices, those will be initially sterilized with 70% alcohol, and then it will be burnt in the burner. And, then we have to take some autoclaved blotting paper, in this

way, the blotting paper are kept in fresh sterilized petri plates and thereafter we have to put the seed over there for proper soaking.

So, the availability of water could be reduced, because if moisture is there the chances of contamination will be more. So, in this way, we have to soak the seeds using blotting paper, and autoclaved blotting paper, and eventually, it will be transferred into petri plates having suitable media. So, the plates are already made and now we have to put each seed, separately in this manner within the petri plates where callus-inducing media is available.

So, in 8 to 10 days once those plates are kept in the dark at around 27 to 28 °C we can see the callus formation. Here you can see the callus formation has been done and those calluses, have been broken out from the seed part, also and now we are showing the *Agrobacterium*-mediated transformation process.

So, over here the *Agrobacterium* suspension solution has been made, and from the Petri plates we are taking out different, properly developed callus, and it will be infected within the *Agrobacterium* solution, for approximately half an hour. So, in this way after putting the callus on the *Agrobacterium* suspension, we need to shake it gently not too much shaking is needed because the friable callus could be broken into small pieces. So, after approximately 30 minutes of incubation again, we need to soak the excess *Agrobacterium* solution available over there. Here also you can see that, for safety, scalpels which we are using frequently have to be sterilized, each and every time with 70% alcohol and by burning in the lamp. The autoclaved blotting papers are ready now here from, we have to take the callus which have been incubated with the *Agrobacterium* suspension.

On top of that again, we need to put at least one or two blotting papers to soak excess *Agrobacterium* solution because after this process, those calluses, and those infected calluses will be placed in co-cultivation media for 24 hours to 48 hours. Within that co-cultivation media, the *Agrobacterium* will do delivery of the Ti plasmid or the T-DNA region within the plant genome. Ok! If excess *Agrobacteria*, are there those bacteria can

grow subsequently also, but our target is not to grow *Agrobacteria*, right? Our target is to deliver the transgene, through this *Agrobacterium*, within the plant genome. So, we need to remove the excess *Agrobacterium* solution by soaking it, a number of times, using blotting paper. So, in this way callus is almost ready, now we have to place it on co-cultivation media, for 24 hours to 48 hours, then eventually it will be grown in selection media containing hygromycin, kanamycin, different antibiotics which are available in the construct, the binary vector. Ok! Based on that different antibiotic selections will be done subsequently.

So, now we are coming to, the tobacco seed sterilization process here you can see the tobacco seeds are very tiny, those are the *Nicotiana tabacum* seeds, and very small-sized seeds are there. So, first, we need to put the seeds in a small eppendorf tube, thereafter, again, we have to put the double distilled autoclaved water, and we need to imbibe the seeds for 3 to 4 hours. So, that the seeds will be swelled a little bit, and the sterilization process could be easier. So, after imbibition, we need to shake it several times and then we need to take out the water from this small eppendorf tube, for subsequent sterilization process. Here we have to use micropipettes, otherwise, those tiny seeds could be, means it can come out easily from the eppendorf tube.

So, in this way, first initial washing is done using double distilled autoclaved water, and if any dust particles are there, debris are there or the flower parts are there, or dry flower parts are there it will be removed during this washing process. So, that only seeds will be available within the eppendorf tube then we have to add 70% ethanol for 30 seconds. Ok! We cannot treat the ethanol treatment; means we cannot do the ethanol treatment for a longer period of time, because it can reduce the viability of the seeds. So, for 70% approximately 30 seconds, we need to shake those seeds and then we have to remove the 70% ethanol from the vial. The process is tricky because the seeds are very tiny, it can enter easily inside the micropipette, inside the tips of the micro pipette also.

So, once we remove the 70% ethanol, we need to wash it a couple of times, using double-distilled autoclaved water. We need to shake it vigorously, and here each time we need

to remove the water carefully, from the vial thereafter we have to use sodium hypochlorite which is the chemical, basically used for tobacco seed sterilization. In rice we use mercuric chloride, over here sodium hypochlorite is the main sterilizing agent. So, after adding sodium hypochlorite, we have to shake it for approximately 5 minutes and then we have to remove the sodium hypochlorite solution from the vial and then we need to wash it a couple of times using double distilled autoclaved water. So, that the trace of or traces of sodium hypochlorite should not be there at the final stage.

I think you guys are watching the video, and on the top of any petri plates or on the top of the water, which is being used for sterilization, our hand should not come above the open petri plates or above the water, which is being used we have to keep our hands aside. So, in this way, after 3 to 4 times rinsing, the seeds are almost ready, and then we need to transfer those sterilized tobacco seeds into the media. So, that the seedlings can grow from there, here you can see the sterilized seeds are being placed on media from each and every, seed the tobacco seedlings will come from, 3 to 5 days, we can see the seedling growth and within 2 weeks, we can get germination for most of the seeds, then we can transfer it separately and, we can put it in other media in the magenta box for subsequent analysis. Thank you.