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Economization of Datura Plant Using Planttissue Culture

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Abstract: *The subsequent research deals with a fundamental plant tissue culture technique where by my primary aim is to develop a callus of the Datura (innoxia spp.) plant. Following this, a comparative study of the extractive values of the callus cultured with the help of Murashige and Skoog media and its alterations with Datura plant was carried out. My other experiment with the newly developed callus included bringing about variations in the media to find out the finest possible combination of nutrients required by the Datura Extractions were performed by two different methods, both consisting of dissimilar organic solvents used for extraction and different soaking times. The extractive values following the callus growth were calculated and the essential aim of the experiment was to develop a callus with a higher extractive value than the original explants (seed) yet with a retained therapeutic potency. For this the crystals obtained from both the methods were weighed and there extractive values compared to the preparatory material were calculated. This helped us get an understanding about the variations that needed to be made in the media to get a constructive resultant callus growth. The extractive value obtained by the first method was the maximum. Hence the results were favorable for the supplementary study to be persistent.*
Keywords: *Economization, Media, DaturaInnoxia, Plant Tissue Culture, Callus, Extractive Values.*

I. Introducing

Plant tissue culture is a practice used to propagate plants under sterile conditions, often produce clones of a plant and have several advantages over traditional methods such as: a) The production of multiples of plants in the absence of seeds or necessary pollinators to produce seeds. b) The regeneration of whole plants from plant cells that have been genetically modified.

DaturaInoxia consists of the dried leaves and flowering tops of DaturaInoxia Linn.Belonging to family Solanaceae. Daturainoxia is an annual shrubby plant that typically reaches a height of 0.6 to 1.5 meters. The flowers are white, trumpet-shaped, 12–19 cm long. The fruit is an egg-shaped spiny capsule, about 5 cm in diameter. All parts of the plant are anodyne, antispasmodic, hallucinogenic, hypnotic and narcotic. It has been used in the past as a pain killer and also in the treatment of insanity, fevers with catarrh, diarrhea and skin diseases. The plant contains several alkaloids, the most active of which is scopolamine. This is a potent cholinergic-blocking hallucinogen, which has been used to calm schizoid patients. The leaves contain 0.52% scopolamine, the calices 1.08%, the stems 0.3%, the roots 0.39%, the fruits 0.77%, the capsules 0.33%, the seeds 0.44% and the whole plant 0.52 - 0.62% The tissue which is obtained from the plant to culture is called an explant. Explants are then usually placed on the surface of a Murashige and Skoog medium or (MSO or MS0(MS-zero)), Which is a plant growth medium used in the laboratories for cultivation of plant cell culture and composed of Macronutrients such as Ammonium nitrate ,Boric acid,Cobalt chloride,Magnesium sulfate,Cupric sulfate, Potassium phosphate, Ferrous sulfate,Potassium nitrate ,Manganese sulfate, Potassium iodide,Sodium molybdate ,Zinc sulfate and $Na_2EDTA \cdot 2H_2O$a.Common organic additives such as myo-Inositol,Nicotinic acid,Pyridoxine hydrochloride,Thiaminehydrochloride,Glycine (recrystallized), Sucrose.

II. Materials And Methods

Prepararion of Media

Take 2g agar and 3.5g Murashige and Skoog media in a conical flask. Add distilled water and dilute to 100ml.Cover the flask with aluminum foil. Autoclave for 15 minutes after the first whistle. After autoclaving is complete, keep the autoclave untouched for some time in order to ensure that all the pressure has been released. Transfer the media to an aseptic area. Here the media was transferred in the culture tubes under the laminar flow. Slants were prepared. The media was allowed to solidify before proceeding further.

Surface Sterilization

The next step involves sterilizing the parts of the plant that are to be used for callus formation.
The parts of the plant that were taken were- Shoot, Root tip, Leaf, Stem, Flower, Bud, Seed Sodium hypochlorite solution was used to sterilize them. These explants were kept in the solution for about 5-10 minutes and transferred to a Petri plate containing distilled water where they were washed and further cultured.

The seeds that were used for culturing were also of various types.

- From a freshly cut fruit.
- From a dried fruit that had burst open naturally.
- From a dried fruit that had not yet burst open to disperse the seeds.

Culturing

Separate culture tubes were prepared and labeled for each of the explants, i.e. leaf, stem, shoot, root tip, flower and bud and seeds. The explants are placed carefully on the media. The culture tubes are then sealed with parafilm in order to prevent any kind of contamination. Light and dark conditions are maintained for the explants so that normal growth can take place.

Sub Culturing

After some days of the initial culturing, the explants are then transferred to a fresh media. Sub culturing is done in order to provide a continuous supply of nutrients to the explants so that they can show the required growth. Other conditions same as the culturing.

Culturing Conditions

The cultured tubes are placed in an aseptic area. The temperature provided is room temperature.
Day and night conditions are maintained. The cultures are subjected to 12 hours light and 12 hours dark.
The plant DaturaInoxia was cultured in media containing various alterations.

Alteration 1

The explants placed in media containing 2g agar, 3.5g Murashige and Skoog and salt. Observations were recorded with 7-10 day's time intervals.

Alteration2

The explants were placed on media containing sugar. This was of two types-

1. The sugar was placed in the along with agar in Murashige and Skoog in the autoclave and was autoclaved.
2. The sugar was added after the agar and Murashige and Skoog were autoclaved, i.e., the sugar was not autoclaved but directly added to the media after it was autoclaved and before it could solidify.

The observations were recorded with 7-10 day's time intervals.

Alteration 3

The explants were cultured in a medium containing 2g agar, 3.5g Murashige and Skoog media and reconstituted coconut milk and the observations were recorded with 7-10 day's time intervals.

Alteration 4

The explants were cultured in a medium containing 2g agar, 3.5g Murashige and Skoog media and a fertilizer containing amino acids, and sea weeds and the observations were recorded with 7-10 day's time intervals.

Alteration 5

The explants were cultured in a media containing 2g agar, 3.5g Murashige and Skoog media and vitamin B complex and the observations were recorded with 7-10 day's time intervals.

Alteration 6

The explants were subject to media containing 2g agar, 3.5g Murashige and Skoog media and lysine extract and the observations were recorded with 7-10 day's time intervals.

Alteration 7

The explants were cultured in a media containing 2g agar, 3.5g Murashige and Skoog media and yeast extract and the observations were recorded with 7-10 day's time intervals.

Alteration 8

The explants were cultured in a media containing 2g agar, 3.5g Murashige and Skoog media and activated charcoal and the observations were recorded with 7-10 day's time intervals.

Alteration 9

The explants were cultured in a media containing 2g agar, 3.5g Murashige and Skoog media and an organic extract containing amino acids and triterpenoids and the observations were recorded with 7-10 day's time intervals.

Alteration 10

The explants were cultured in a media containing 2g agar, 3.5g Murashige and Skoog media and glycine and the observations were recorded with 7-10 day's time intervals.

Alteration 11

The antifungal agent ketoconazole was added to some of the culture tubes that contained seed as the explant and the observations were recorded with 7-10 day's time intervals.

Once these alterations in the media were made the explants were cultured as before-

Callus initiation in Salt   Callus initiation in salt,
Vitamin b complex and lysine

Figure 1: Callus Initiation

Fertilizer, Yeast extract, Vitamin B Complex, Activated Charcoal   Lysine, Salt, Organic Extract, Fertilizer

Figure 2: Culture Tubes

III. Results And Discussion

The various factors that encourage or retard the duration required for callus formation of DaturaInoxiaseeds were studied and the observations obtained are mentioned in the following table.

SR.NO.	ALTERATIONS	RESULTS
1.	Murashige and Skoog+ Agar+ Salt	Initiation of callus growth was observed after 5 days.
2.	a)Murashige and Skoog+ Agar+ autoclave Sugar	No initiation of callus was observed.
	b)Murashige and Skoog+ Agar+ non-autoclaved sugar	No initiation of callus was observed
3.	Murashige and Skoog+ Agar+ reconstituted coconut milk	No initiation of callus was observed
4.	Murashige and Skoog+ Agar+ Fertilizer containing amino acids and sea weeds	No initiation of callus was observed
5.	Murashige and Skoog+ Agar+ Vitamin B complex	Initiation of callus was observed after 5 days
6.	Murashige and Skoog+ Agar+ Lysine	Slow initiation of callus was observed after 10 days
7.	Murashige and Skoog+ Agar+ Yeast extract	Slow initiation of callus was observed after 7 days
8.	Murashige and Skoog+ Agar+ Activated Charcoal	Slow initiation of callus was observed after 10 days
9.	Murashige and Skoog+ Agar+ Organic extract containing amino acids and tri-terpenoids	No initiation of callus was observed
10.	Murashige and Skoog+ Agar+ Glycine	No initiation of callus was observed
11.	Murashige and Skoog+ Agar+ Ketoconazole	No initiation of callus was observed

IV. Conclusion

With various combinations of the composition of media, we have observed that the variations-

1. Murashige and Skoog + Agar+ Salt
2. Murashige and Skoog+ Agar+ Vitamin B complex
3. Murashige and Skoog+ Agar+ Yeast Extract
4. Murashige and Skoog+ Agar+ Lysine
5. Murashige and Skoog+ Agar+ Activated Charcoal

The above mentioned combinations have been proved to be better as compared to the others. Further, the most favorable explant for callus formation was found to be the seeds taken from the dried fruit. A lot of scope is still there to exploit the rationality between various nutritional supplement and methods of sterilization and effective technique of explant incubation. These studies will lead to economization of plant tissue culture techniques and its application in agricultural utility.

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