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Study on Sterilization Methods and Shoot Formation of Teak (*Tectona grandis* L.f.) Tissue Culture

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ကျွန်းတစ်သျှူးစတင်မွေးမြူခြင်းအဆင့်တွင် မျိုးပွားတစ်သျှူးများအား ပိုးသန့်ခြင်းနည်းစနစ်များကို လေ့လာခြင်း

ပြည့်ပြည့်ဝင်း၊ သုတေသနလက်ထောက်-၃
ဖြူဖြူနှင်း၊ သုတေသနလက်ထောက်-၂
ရွှေလုံး၊ သုတေသနလက်ထောက်-၃
နွယ်နွယ်ဝင်း၊ သုတေသနလက်ထောက်-၃
အုန်းလွင်၊ ပါမောက္ခ

စာတမ်းအကျဉ်း

တစ်သျှူးမျိုးပွားခြင်းသည် မိခင်အပင်နှင့် မျိုးရိုးဗီဇတစ်ထပ်တည်း တူညီသည့် အပင်များထုတ်လုပ်နိုင်သော၊ စီးပွားဖြစ်ထုတ်လုပ်နိုင်သော နည်းလမ်းတစ်ခုဖြစ်ပါသည်။ သင့်တော်သောပိုးသတ်ခြင်း နည်းစနစ် မရှိပါက တစ်သျှူးမျိုးပွားရာတွင် မှိုနှင့်ဘက်တီးရီးယား ပိုးမွှားများ ဝင်ရောက်ခြင်းတို့ ဖြစ်စေနိုင်ပါသည်။ ဤစာတမ်းတွင် ပိုးသတ်ခြင်းနည်းစနစ် (၃)မျိုးအား စမ်းသပ်ဆောင်ရွက်ထားပါသည်။ ၄င်းနည်းစနစ်များအနက် စမ်းသပ်မှုနံပါတ်(၃)သည် အကောင်းဆုံးနည်းလမ်းတစ်ခု ဖြစ်ကြောင်းတွေ့ရှိရပါသည်။

Study on Sterilization Methods and Shoot Formation of Teak (*Tectona grandis* L.f.) Tissue Culture

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Abstract

Tissue culture is an ideal method for production of genetically identical plants and proved to be commercially feasible method. But without proper sterilization method, tissue culture is susceptible to various fungal and bacterial attacks. In this paper, 3 different sterilization methods are trialed. The study suggests sterilization methods: 1) the tips were dipped in Homine , Kasumin for each 5 minutes and HgCl2 for 3min, and moved to laminar flow for cutting (culture). In the final step, the cut tips are dipped in Clorox and stir the solution containing the tips by hands for about 30 seconds, 2) The tips were washed with tap water for 15 minutes, Homine for 5 minutes, HgCl2 for 3 minutes and Clorox solution for 2 minutes and washed with DDW, 3) The tips were washed with tap water for 15 minutes , Homine , Kasumin and Ethanol 70% + Tween 20 for 5 mintues. Then they were washed with DDW and were moved to laminar flow for cutting (culture). The cut tips were dipped in HgCl2 for 1min. In all steps, after treating chemical solutions, the tips were washed with double distilled water (DDW). In trial 1, test tubes subjected to sterilization method S1 gives 7contamination free test tubes, S2 8 tubes and S3 8 tubes, respectively. In trial 2, method S1 gives 6 tubes, S2 7 tubes and S3 9 tubes. In trial 3, method S1 gives 5 tubes, S2 6 tubes and S3 gives 8 tubes. The result shows that sterilization method No.3 is the most appropriate for Teak tissue culture technique. After 30 - 45 days, the aseptic auxiliary shoots were transferred to a fresh medium supplemented with different concentrations of 6-benzyl amino purine (BAP) and Kinetin (KN) treatments.

Contents

Study on Sterilization Methods and Shoot Formation of Teak (*Tectona grandis* L.f.) Tissue Culture

1. Introduction

Teak (*Tectona grandis* L.f.) belongs to the family Verbenaceae, which is an economically important, large deciduous, reaching over 30 m in height in favorable conditions. It's uses included flooring and parquet, ship building, furniture-making and in building construction. Teak is grown naturally in India, Myanmar, Thailand and Laos.

Teak is traditionally reproduced though seeds, but in most cases, germination is difficult due to the hard seed coat, low seed quality and late seed production. Also, controlled pollination has turned out to be difficult in teak. Poor germination rate leading to a low production of seedlings further contributes to the paucity of planting material. To enhance teak plantation development, many efforts have been made in the research on improvement of the establishment of mass multiplication.

At present, tissue culture method is proved to be efficient and commercially feasible method for establishment of teak plantations. Tissue culture technique has become an adequate method for producing genetically identical plants on a large scale and in a short time for the plantation establishment. However, there are many varieties of methods in tissue culture and some are found to produce poor shoot proliferation and susceptible to fungal and bacterial attacks. Therefore, it is necessary to experiment which methods are capable of producing maximum number of vigorous plants for a successful plantation.

There are some studies on the micropropagation techniques especially different media for teak tissue culture. In spite of describing the procedure of the sterilization on the explants, there is little or few study on the effect of sterilization methods on the tissue culture propagation of. That is why this research is to show on the effects of different sterilizations on teak tissue culture propagation. This study will intend to support some techniques in the propagation of teak.

In this research paper, we emphasized in finding the most suitable methods of sterilization and culture establishment.

2. Objective

1. To study the effect of different sterilization methods
2. To point out suitable sterilization methods in tissue culture

3. Materials and Methods

3.1 Explant source and sterilization

90 shoot tips and single nodes of young twigs from sexually mature teak plus trees are taken from Forest Research Institute Hedge Garden. Those teak plus tress or clones are taken from Pyi, Paukkaung and Moe Swe and planted in Forest Research Institute Hedge Garden.

The collected sample was washed thrice with tap water to remove the adherent dust particles and other contaminants. As a standard procedure, those shoot tips are sterilized using various methods. In this research paper, we used three different sterilization methods, 10 shoot tips for each method. The experiment was performed three times for each method. The sterilized tips are monitored for infection data and data is recorded every week. (Without hormone media was used as base standard media for all three methods).Fig 1.

3.2. Sterilization Method (1) (S1)

Firstly, the tips are dipped in Homine and stir it using a magnetic stirrer for 5 minutes. Then the tips are washed with DDW(double distilled water). After that, they are dipped again in Kasumin and stir it using a magnetic stirrer for 5 minutes. Then they are washed with DDW. In another step, they are dipped in HgCl2 and stir it using a magnetic stirrer for 3min and washed again with DDW. After finishing the wash, the tips are moved to laminar flow for cutting (culture). In the final step, the cut tips are dipped in Clorox and stir the solution containing the tips by hands for about 30 seconds. The tips are washed with DDW after wards.Fig 3. Fig 4.

3.3. Sterilization Method (2) (S2)

The tips are washed with tap water for 15 minutes. After that, they are dipped in Homine and stirred using a magnetic stirrer for 5 minutes. Then the tips are washed with double distilled water DDW. In the next step, the tips are sterilized in HgCl2 solution and stir it using a magnetic stirrer for 3 minutes. Then the tips are washed with DDW. Afterwards, they are dipped in Clorox solution and stirred using a magnetic stirrer for 2 minutes. Then the tips are washed with DDW. Finally, they are sent to laminar flow for cutting (culture) (Fig 3 and Fig 4.).

3.4. Sterilization Method (3) (S3)

In the beginning, the tips are washed with tap water for 15 minutes. Then, the tipsare dipped in Homine and stirred the solution using a magnetic stirrer for 5 minutes. Afterwards, the tips are removed from the solution and washed with DDW. For the next step, the tips are dipped in Kasumin and stirredwith magnetic stirrer for 5 minutes. Then they are washed with DDW. Next, the tips are dipped in Ethanol 70% + Tween 20 and stirred again using a magnetic stirrer for 5 minutes. After the stirring, the tips are washed with DDW. When above procedures are done, the tips are sent to lamina flow for cutting (culture). There, finally, they are dipped in HgCl2 and stir it using hand for 1 min. Then wash with double distilled water DDW.Fig 3. Fig 4

3.5. Media and Culture Conditions

The explants were cultured on Murashing and Skoog (MS) medium containing 3% sucrose was used in all the experiments. The pH of the medium was adjusted to 5.8 prior to the addiction of 0.6% agar (HiMedia Laboratories Private Limited Mumba, India). Medium was dispensed in 15 ml aliquots into culture tubes (25×150mm) and closed with non-absorbent cotton plugs. The medium was autoclaved at 1.1 kg cm pressure and 121˚ C temperature for about 15 min (Fig5).

3.6. Culture Establishment

The nodal explants were then singly placed in test tubes (25×150mm) with without hormone media sucrose (3%) and solidified with 0.6% agar was added to the medium to reduce browning. Medium pH was adjusted to 5.8 before autoclaving. After 30 - 45 days, the aseptic auxiliary shoots were transferred to a fresh medium supplemented with different concentrations of 6-benzyl amino purine (BAP) and Kinetin (KN) treatments.

Fig 1

Fig 2

Fig 3

Fig 4

Fig 5

Fig 6

Fig7

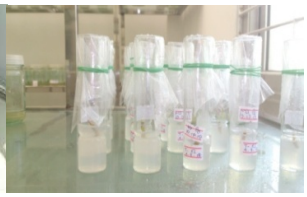


Fig 8

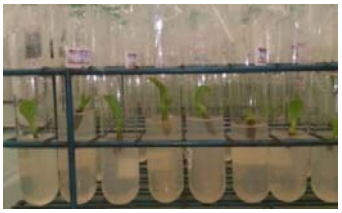


Fig 9

Fig 10

Fig 11

Fig 1. FRI Hedge Garden

Fig 2.Colleting explants from hedge garden

Fig 3. First stage of sterilization; washing with tap water

Fig 4. Sterilization with different sterilization methods using magnetic stirrer

Fig 5. Culture stage in laminar flow

Fig 6. Aseptic shoot tips after cutting

Fig 7. Placing shoot tips in test tubes with media

Fig 8.Placing shoot tips in test tubes with media

Fig 9. Shoot development after 30-35 days

Fig 11. After 45 days, transferred to subculture (multiplication stage)

4. Results and Discussions

4.1 Plant Response on sterilization

Three different sterilization methods (S1, S2, and S3) were performed, using 10 shoots for each method in one time. The shoot tips were put into different test tubes and kept in a separate shelf for each method. The objective of this test is to find the most effective method of sterilization for producing teak shoot tips free from any kind of contamination. The statuses of the sterilized shoots are kept in check and data recorded for once a week for 30 to 45 days. The shoot tips which are found to be attacked by fungi, bacteria or other forms of contamination are recorded as failure.

Analysis of Variance Table for survival rate

Source	DF	SS	MS	F	P
Rep	2	2.8889	1.44444		
Sterilization	2	8.2222	4.11111	9.25	0.0316
Error	4	1.7778	0.44444		
Total	8	12.8889			

Grand Mean 7.11111 CV 9.37

Table 1.LSD All-Pairwise Comparisons Test of Survival rate for Replication

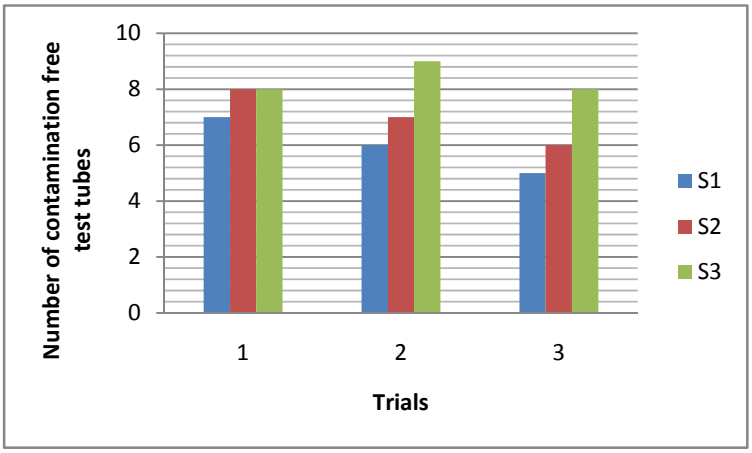
Rep	Mean	Homogeneous Groups
1	7.6667	A
2	7.3333	A
3	6.3333	A

Alpha 0.05 Standard Error for Comparison 0.5443
Critical T Value 2.776 Critical Value for Comparison 1.5113
Error term used: Rep*Sterilization methods**,** 4 DF There are no significant pairwise differences among the means.

Table 2. LSD All-Pairwise Comparisons Test of Survival rate for Sterilization Methods

Sterilization Methods	Mean	Homogeneous Groups
S3	8.3333	A
S2	7.0000	AB
S1	6.0000	B

Alpha 0.05 Standard Error for Comparison 0.5443
Critical T Value 2.776 Critical Value for Comparison 1.5113
Error term used: Rep*Sterilization methods, 4 DF There are 2 groups (A and B) in which the means are not significantly different from one another.



Status of three sterilization methods for three trials

As described above**,i**n trial 1, test tubes subjected to sterilization method S1 gives 7 contamination free test tubes, S2 8 tubes, respectively.In tril 2, method S1 6 tubes, S2 7 tubes and S3 9 tubes. In trial 3, method S1 gives 5 tubes, S2 6 tubes and S3 gives 8 tubes. Therefore, the sterilization method S3 is found to eliminate contamination other than S1 and S2.

5. Conclusion

Sterilization method is the first and foremost step in tissue culture of teak, not to mention it is a vital step. Contamination posts serious obstacles in tissue culture. This research successfully points out an effective sterilization method. No doubt this finding of effective sterilization method is an important finding for further research in tissue culture of teak.

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Analysis of Variance Table for survival rate

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Grand Mean7.11111 CV 9.37

Tukey's 1 Degree of Freedom Test for Nonadditivity

Source	DF	SS	MS	F	P
Nonadditivity	1	0.69878	0.69878	1.94	0.2577
Remainder	3	1.07900	0.35967		

APPENDIX 2

LSD All-Pairwise Comparisons Test of Survival rate for Replication

Rep	Mean	Homogeneous Groups
1	7.6667	A
2	7.3333	A
3	6.3333	A

Alpha 0.05 Standard Error for Comparison 0.5443
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Error term used: Rep*Sterilization methods, 4 DF There are no significant pairwise differences among the means.

LSD All-Pairwise Comparisons Test of Survival rate for Sterilization Methods

Sterilization Methods	Mean	Homogeneous Groups
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Error term used: Rep*Sterilization methods, 4 DF There are 2 groups (A and B) in which the meansare not significantly different from one another.

APPENDIX 3

Original Data of three sterilization methods chemical treatments by means of Replications

Sterilization Methods	Replications	Survival rate
S1	1	70%
	2	60%
	3	50%
S2	1	80%
	2	70%
	3	60%
S3	1	80%
	2	90%
	3	80%