Biotechnology

New Technical programme

1.	**Title**	**Micropropagation of Malabar neem (*Melia dubia)***
2.	**Background Information**	*Melia dubia* is a imperative multipurpose tree used in bio energy production, paper and pulp industry, plywood industry as well as having medicinal uses. *M. dubia* belongs to the Meliaceae family. It is significantly distributed in India, Iran, Pakistan, Argentina, Brazil, Bermuda, China, Australia, and Malaysia. All the parts of *M. dubia* were used as a medicine and various kinds of treatment such as for wound healing, treatment of skin, against insect pests, and gastro intestinal infections. It has a antibacterial, antifungal, antiviral, hypoglycaemic, antineoplastic, antileprosy and antihelmintic properties. Conventional propagation of *M. dubia* shows poor success due to hard stony seed coat. Therefore to cope up with the elevated demand of various industries, *in vitro* regeneration is the possible approach to prepare true to type plantlets. Therefore it is an immense requisite to develop an optimized protocol for micropropagation of *M. dubia.*
3.	**Objective**	1. To standardize establishment media for node 2. To standardize multiplication media 3. To standardize the media for rooting of *in vitro* regenerated shoots 4. To standardize pot mixture for acclimatization of *in vitro* regenerated plantlets
4.	**Principal Investigator & Associates**	1. Dr. Swati Patel (Assistant Professor) : PI 2. Dr. R. M. Patel (Principal & Dean) : Co-PI
5.	**Location and Agro-climatic zone:**	Aspee Shakilam Biotechnology Institute, NAU, Surat South Gujarat Heavy Rainfall Zone-II
6.	**Name of Res Scheme & B.H**.	12248
7.	**Year**	2021
8.	**Crop & variety**	Malabar neem (*Melia dubia)* Explant: Node
9.	**Experiment detail**	**Statistical design:** • CRD (Completely Randomized Design) with 10 bottles per treatment and 3 repetition • 5 repetitions for root induction and acclimatization

A) Treatments for shoot establishment

Treatment no.	Media
TE_1	MS
TE_2	MS + 0.1 mg/L BAP
TE_3	MS + 0.5 mg/L BAP
TE_4	MS + 1.0 mg/L BAP
TE_5	MS + 1.5 mg/L BAP
TE_6	MS + 2.0 mg/L BAP
TE_7	MS + 0.1 mg/L BAP + 0.1 mg/L NAA
TE_8	MS + 0.5 mg/L BAP + 0.1 mg/L NAA
TE_9	MS + 1.0 mg/L BAP + 0.2 mg/L NAA
TE_{10}	MS + 1.5 mg/L BAP + 0.2 mg/L NAA
TE_{11}	MS+ 2.0 mg/L BAP + 0.5mg/L NAA

B) Treatments for shoot multiplication

Treatment no.	Media
TM_1	MS + 0.25 mg/L BAP
TM_2	MS + 0.5 mg/L BAP
TM_3	MS + 1.0 mg/L BAP
TM_4	MS + 0.25 mg/L BAP + 2.5 mg/L GA_3
TM_5	MS + 0.5 mg/L BAP + 2.5 mg/L GA_3
TM_6	MS + 1.0 mg/L BAP + 2.5 mg/L GA_3
TM_7	MS + 0.25 mg/L BAP + 30 mg/L Adenine sulphate
TM_8	MS + 0.5 mg/L BAP + 30 mg/L Adenine sulphate
TM_9	MS + 1.0 mg/L BAP + 30 mg/L Adenine sulphate
TM_{10}	MS + 0.25 mg/L BAP + 2.5 mg/L GA_3 + 30 mg/L Adenine sulphate
TM_{11}	MS + 0.5 mg/L BAP + 2.5 mg/L GA_3 + 30 mg/L Adenine sulphate
TM_{12}	MS + 1.0 mg/L BAP + 2.5 mg/L GA_3 + 30 mg/L Adenine sulphate

C) Treatments for root induction of *in vitro* regenerated shoots

Treatment no.	Media
TR_1	½ MS
TR_2	½ MS + 0.1 mg/L IBA
TR_3	½ MS + 0.5 mg/L IBA

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<tr><td>TR_4</td><td>½ MS + 1.0 mg/L IBA</td></tr>
<tr><td>TR_5</td><td>MS + 0.1 mg/L IBA</td></tr>
<tr><td>TR_6</td><td>MS + 0.5 mg/L IBA</td></tr>
<tr><td>TR_7</td><td>MS + 1.0 mg/L IBA</td></tr>
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D) Treatments for acclimatization of in vitro regenerated plantlets
<table>
<tr><th>Treatment no.</th><th>Pot Mixture</th></tr>
<tr><td>TH_1</td><td>Soil: vermicompost (1:1)</td></tr>
<tr><td>TH_2</td><td>Soil: vermicompost: cocopeat (1:1:1)</td></tr>
<tr><td>TH_3</td><td>Soil: vermicompost(1:2)</td></tr>
<tr><td>TH_4</td><td>Soil: cocopeat: vermicompost (1:2:1)</td></tr>
<tr><td>TH_5</td><td>Sand: Soil: vermicompost (1:1:1)</td></tr>
<tr><td>TH_6</td><td>Cocopeat: vermicopost:sand (1:1:1)</td></tr>
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<td>10.</td>
<td>Observation to be recorded</td>
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A) Shoot establishment
1. Contamination (%)
2. Establishment of explants (%)
3. Frequency of explants showing bud break (%)
4. Days taken for shoot induction
B) Shoot multiplication
1. Number of shoots per culture
2. Shoot length (cm)
3. Number of internodes
C) Root induction
1. Frequency of shoots showing root induction (%)
2. Days to root induction
3. Root length (cm)
4. Number of root per shoot
D) Hardening of regenerated plantlets
1. Percent survival
2. Days taken for establishment
3. Shoot length (cm)
4. Number of leaves after 4 weeks
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<td>11.</td>
<td>Expected outcome</td>
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• A well optimized protocol for production of true to type plantlets of M. Dubia
• Exploited for commercial mass clonal propagation of economically important species
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