IN *Vitro* Propagation of medicinal plants species of conservation concern of Lonar forest

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INTRODUCTION- Lonar forest is the biggest forest around salt water lonar lake. Around lonar lake more forest area is present .In the forest area many medicinally important plant species is present .But due to villegers activity the species of plant are going to be endangered. This leads to conservation of plant species of forest area. There are many species of plant which is in need of conservation as, *Tinospra sinesis(*Lour.), *Holestemma-ada-kodien* , *Terminalia arjuna* (Roxb.) *Limmonia accidissima Linn*. These spescies are medicinally important. The villegers activity of cutting plants in more numbers causes depletion of the species. conservation of plant species is need of time today. conservation is carried out by in *vitro* propagation so that more numbers of plant species are produced and then planting are carried out it will increase the plant species.

Key words- Lonar forest ,in vitro propagation ,conservation ,Lonar lake.

Material and Methodology-For the present work followings are the plant species of lonar forest are selected.

1) *Limmonia acdissimia Linn*.

2*) Terminalia arjuna (Roxb.)*

3) Tinnospora sinesis (Lour).

4) Holostemma –ada kodein schult.

Collection and Transportation of Samples

Plant parts were collected from above listed plant species available in Lonar forest. Parts were cut by using sharp object and kept in zeep lock bag. The zeeplock bags containing plant part were kept in thermostable container containing cooling pads.
Ample quantity of samples were collected at a single visit to avoid seasonal variation in physical, biochemical and morphological structure of plant parts, this may interfere with the study results. Sample were collected during late monsoon season (Sept 2015).
Samples were transported to working laboratory and kept immediately at refrigerated temperature. Samples were processed on the next day of arrival to laboratory

Processing of Sample:

Desired plant parts were cut out from the collected samples. Shoot tips from plant parts *Limonia acidissima,* and *Terminalia arjuna* were separated by sterile using dissecting blade. As well as nodes were cut out from the plant parts of , *Tinospora sinensis* and *Holostemma ada-kodien* by sterile using dissecting blade. These separated parts were used as explant.
The separated explants were sterilized.

Table : Disinfection protocol

Explant Type	First Rinse Double Distilled Water	Disinfectant	Second Rinse Double Distilled Water	Final Rinse Double Distilled Water
Shoot Tips	10 Min.	70% Ethanol 3 Min	5 Min.	5 Min’
Node	10 Min.	0.1% Mercuric Chloride 1 Min	5 Min .	5 Min.

Shoot tips were thoroughly rinse with continuous flow of double distilled water for 10 min followed by keeping it in 70% ethanol for 3 min. Shoot tips were rinse twice with sterilized double distilled water after ethanol treatment. Shoot tips thus processed were used for inoculation in MS (Murashige and Skoog) medium.

Nodes were thoroughly rinse with continuous flow of double distilled water for 10 min followed by keeping it in 0.1% mercuric chloride for 1 min. Nodes were rinse twice with sterilized double distilled water after mercuric chloride treatment. Nodes thus processed were used for inoculation in MS (Murashige and Skoog) medium. The care was taken that all explant of particular species used were approximately of same
dimension.

Preparation of Stock Solution for Auxins and Cytokinins

- L-Nephthalene Acetic Acid (NAA)- 1% solution of NAA was prepared aseptically with sterile double distilled water
- Indole-3 Acetic Acid (IAA)- 1% solution of IAA was prepared aseptically with sterile double distilled water
- Benzyl Amino Purine (BPA)- 1% solution of BAP was prepared aseptically with sterile double distilled water
- 6-Furfuryl Aminipurine (Kinetin)- 1% solution of Kinetin was prepared aseptically with sterile double distilled water All stock solutions were prepared under aseptic condition and kept in sterile screwcap containers at refrigeration temperature. The stock solutions were used in MS medium at different concentrations as and where required.

Preparation of Murashige and Skoog Medium

- Readymade dehydrated powder of Murashig and Skoog medium (PT100) prepare by HiMedia® was used
- Accurately weighed dehydrated MS medium and dissolved it in desired quantity of water (e.g. 4.14 g for 100 mL, 41.4 g for 1L) in glass beaker.
- The content was heat to boil for complete dissolution of media and liquefaction of powdered agar.
- Media was cooled to 55 to 60° and the pH of media was adjusted to 5.7±0.5 with the help of 1N NaOH and 1N HCl.
- Approximately 15 to 20 mL molted MS media was transferred to culture tube. Tubes were plugged wrapped and autoclaved at 121°C and 15lbs pressure for 15 min.
- After autoclaving culture tubes are adjusted in three sets of five tubes for each plant species
- Each set of culture tube was aseptically added with different concentrations of auxins NAA and IAA (0.2 to 1.0mg/L) after cooling them to 45 to 50°C
- Furthermore, culture tubes are also adjusted in two sets of five tubes for each plant species
- Each set of culture tube was aseptically added with different concentrations of cytokinins BAP and Kinetin (0.4 to 2mg/L) after cooling them to 45 to 50°C.
- Moreover, five sets of culture tube containing different concentration of auxins and cytokinin (KIN: IAA, KIN:NAA, BAP: IAA, BAP:NAA) was prepared in 2:1 ratio.
- Total 12 set of culture media tube were prepared for each plant species. The detailed information is shown in following Table 3.4

Auxin/Cytokinin					
IAA x 2	0.2	0.4	0.6	0.8	1.0
NAA x 2	0.2	0.4	0.6	0.8	1.0
KIN x 2	0.4	0.8	1.6	1.8	2.0
BAP x 2	0.4	0.8	1.6	1.8	2.0
KIN:IAA	0.4:0.2	0.8:0.4	1.2:0.6	1.6:0.8	2.0:1.0
KIN:NAA	0.4:0.2	0.8:0.4	1.2:0.6	1.6:0.8	2.0:1.0
BAP:IAA	0.4:0.2	0.8:0.4	1.2:0.6	1.6:0.8	2.0:1.0
BAP:NAA	0.4:0.2	0.8:0.4	1.2:0.6	1.6:0.8	2.0:1.0

Table : Sets of Culture tube with auxin and cytokinin

Inoculation and Incubation of Explant

- The finished and sterilized explants were picked up with sterile forceps and inoculated at centre position of media surface in culture tube.
- The inoculation was carried out under aseptic conditions in pre-sterilized laminar airflow.
- All sets of culture tube except only cytokinin culture tubes were inoculated with explant
- All inoculated tubes were kept for incubation at 25±2°C, 55 to 60% relative humidity and in light intensity of 2200 lux, with 16 hour day light.
- Inoculated tubes were incubated for 6 weeks.
- Tubes were daily observed for callus formation, shoot quantity and shoot length.

- After 6 weeks of incubation callus from set of auxin was transferred to culture tube set of cytokinin
- All culture tubes were further incubated at 2525±2°C, 58 to 62% relative humidity and 12 hr photoperiod of 2000 lux for 4 to 6 weeks.
- Tubes were daily observed for shoot quantity, shoot length, root formation and root length.

Result- Acclimatization and establishment in pots and field

- The plantlets with well-developed roots were brought out of culture medium.
- Roots were properly cleaned with continuous flow of sterilized double distilled water to remove portion of media stick to them
- The plantlets were transferred to pots containing Vermicompost and river soil (1: 1)
- Pots were kept in green house for hardening up to 3 to 4 weeks with regular watering
- Hardened plants were then transferred to specially developed (5x5M) bed for further development with regular watering
- Observation were made, regarding height or length, quality of leaf, vulnerability to diseases and environmental impact, on monthly basis for 12 (Climbers and shrubs) to 18 months (tree).

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