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Emergence of Clones from the Nodal Explants of IUCN Red Listed Lindernia antipoda (L.) Alston

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ABSTRACT

An emerging tool for propagation and conservation of economically important crops enlisted as endangered, rare and threatened is in vitro studies. Tissue culture technique is potent and has opened extensive area of research for biodiversity conservation. The technique becomes successful with right choice of explants, medium composition and physical environment to support the growth and development. The technique aids in multiple field serving as the base and is a much essential field of study to enhance greenery. The current study aims in producing clones from nodal explants of the plant Lindernia antipoda which is least explored and has many therapeutic values. Beyond being a medicinal plant it is also an ornamental plant that can be used in aquarium. The nodal explants of linderniacean member Lindernia antipoda a least concern plant species of IUCN list was micropropogated in the MS basal medium with hormone BAP for shoot induction and multiplication and NAA for rooting. The well developed plantlets were hardened and acclimatized. The data's were interpreted using ANOVA and DMRT. The shootlets increased with increasing concentration of hormone and the maximum shoot of 100 with shoot length 4.59 was recorded in the10 micromolar BAP. Best concentration of rooting was found at 8 μ M NAA which had 14.8 \pm 0.83 number of roots having root length of 4.92 \pm 0.10 cm. The clones from the micropropagation process were healthy and similar to that of the in vivo plant in all aspects. The well developed plantlets were transferred to fields for further studies. KEYWORDS: Clones, Hormones, Node, Micropropagation, MS media

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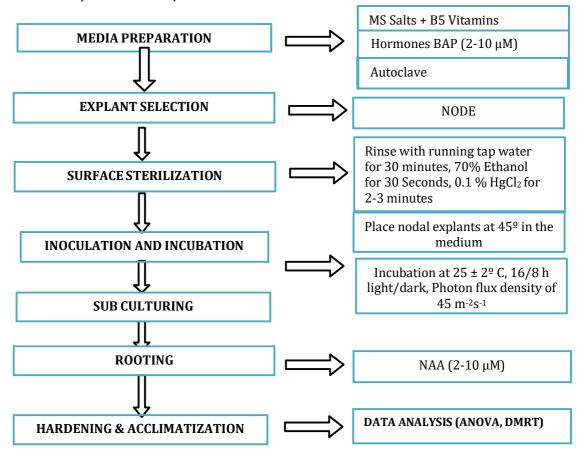
INTRODUCTION

Plants the worlds herbalism is a treasure of earth that evolved over millions of years. The plants grown under various physical and adapted to physiology, morphology and anatomy of various climatic conditions have contributed to the medication of mankind since prehistoric period. Numerous medicinal plants and their formulations are used by mankind in ethno-medical practice as well as traditional system of medicine in India [1]. Plant products play a beneficial role in the management of various disorders [2, 3]. Lindernia antipoda (L.) Alston is a weed in the paddy field belongs to the family linderniaceae is a least concerned plant as per the IUCN red list. The plant is a semi-wetland species found alongside the riverine and paddy fields. The genus is a resurrection genus while the plant *Lindernia antipoda* is an exception of that category. The plant is a small herb with purple colour flower bearing in the axillary position. Though the plant is a weed it has the therapeutic ability to cure emmenagogue, diarrhea, anthelmintic, vertigo, cough, and jaundice [4]. The plant has been exposed only for the ethnobotanical studies and it has to be explored for all other studies. The investigation focused on one of the unexplored field of the species namely plant tissue culture. There is only a single study on the micropropagation of the above plant that was published in 2016 by Jabir [5] the plant was subjected to the aseptic growth condition in the MS basal medium accorded with BAP and NAA to get clones of the plant. The research work has aimed to standardize a suitable protocol to get multiple clones of sparrow lindernia via aseptic cultivation. Thus conserving and economically supporting the humans in curing ailments.

MATERIAL AND METHODS

The nodal explant from the field was surface sterilized with tween, running tap water and distilled water for about 30 minutes. The sterilized explants were inoculated in the autoclaved MS medium augmented with MS salts, B5 vitamins and shooting hormone. The inoculated tubes were incubated at $25 \pm 2^{\circ}$ C under 45 m⁻²s⁻¹ photon density for a photoperiod of 16/8. The plantlets were transferred to rooting

medium and finally hardened and acclimatized. The data's were collected using ANOVA and DMRT and analyzed for the parameters like shoot number, shoot length, root number and length and percentage of responses of the explants in the aseptic medium.



RESULT AND DISCUSSION

The least explored plant species *Lindernia antipoda* node explants has been inoculated in the MS basal medium that was supplemented with BAP for shoot induction, multiplication and proliferation which was then transferred to rooting medium augmented with NAA. The initiation of the explant happened in the first 10 days of inoculation. The shoot induction frequency of the shootlets was 100 %. Some explants stopped growing after a month thus the frequency dropped because of physiological factors and other physical parameters. The concentration range of the shooting medium was 2- 10 micromolar. The shoots increased with increasing concentration depicting the demand for hormones. More the amount of hormonal concentration more the number of shoots and their length. The maximum concentration of 10 micromolar BAP had the highest number of shoots on an average of 100.2 with a standard deviation of 0.83 having shoot length of 4.54±0.19 cm. Though the lowest range of hormone had 65.8±0.83 shoots and with increasing micromolar of hormone elevated the shoot numbers by an average of 10 shoots we observed a fluctuation between 6 and 8 μ M. The shoot numbers increased only by 4 while it suddenly increased to 100. We observed a gradual increase in the shoot length.

Our study reported 10 μ M BAP to be the best hormone range for shootlet production of sparrow lindernia and the length increase with concentration similarly Molla *et al.*, [6] reported an increasing trend of shoot length in BAP which declined after 3 mg l⁻¹. The longest length of 4.54±0.19 cm was recorded in our plant while it was 3.79 cm in their study. Ma *et al.*, [7] reported 59.5 shoots from 5.0 μ M BAP in the *in vitro* cultivation of leaf explants of *Primulina tabacum*. Many researchers have report best results with BAP and there are studies that ingest BAP a standard and most suitable cytokinin for shootlet production.

The healthy and well grown shootlets of above 3 cm were transferred to the rooting medium that was prepared using NAA. The rooting frequency had 100 % in 2, 6 and 8 μ M while it reduced by 5 % in other concentration. The number of roots produced by the shoots were between 12- 15 and the length 1-5 cm. The roots increased till 8 micromolar and dropped back to original at higher concentration. The lowest concentration had 12.6±0.54 roots with 1.88±0.14 cm which is the lowest among the other concentrations. The consecutive concentrations had same number of roots 13.6 but the length of the roots varied 2.18±0.19 and 2.74±0.15 cm. though the highest concentration had the lowest number of shoots

(12.4) which is 0.2 or 1 root variation with 2 micromolar it possess the second maximum root length. NAA has proven to be a suitable hormone for the root induction and multiplication of many dicots, monocots, orchids and also horticultural plants. In direct organogenesis experiments NAA produces roots while some works report them as best hormones for calli induction also. In our study the best rooting status was found at 8 micromolar concentration which can be made as the optimal concentration to get good rooting thus helps in field transfer and acclimatization of the plant.

Table 1: Effect of BAP and NAA on shoot and root induction, multiplication of Lindernia antipoda (T) Alatan

			(L.) Aiston			
BAP (µM)	NAA (µM)	PERCENTAGE OF	NUMBER OF	SHOOT	NUMBER OF	ROOT
		RESPONSE (%)	SHOOTS	LENGTH	ROOTS	LENGTH
				(cm)		(cm)
2	-	95	65.8±0.83	2.46±0.11	-	-
4	-	95	72.6±0.54	2.78±0.13	-	-
6	-	100	82.4±0.89	2.92±0.14	-	-
8	-	95	86.8±0.83	3.74±0.28	-	-
10	-	100	100.2±0.83	4.54±0.19	-	-
-	2	100	-	-	12.6±0.54	1.88±0.14
-	4	95	-	-	13.6±0.89	2.18±0.19
-	6	100	-	-	13.6±0.54	2.74±0.15
-	8	100	-	-	14.8±0.83	4.92±0.10
-	10	95	-	-	12.4±0.54	4.24±0.13

Mean±Standard deviation of five replicates of three experiments

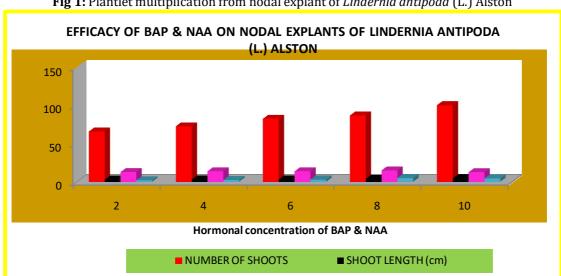
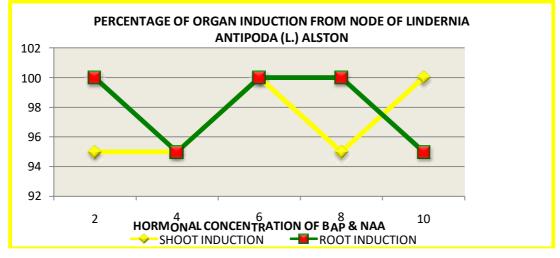


Fig 1: Plantlet multiplication from nodal explant of Lindernia antipoda (L.) Alston

Fig 2: Plantlet induction frequency from nodal explant of Lindernia antipoda (L.) Alston





CONCLUSION

The recent advancements in science and technology have taken every field ahead of our imagination. Some species have been uplifted from a lower category to next category thus they are conserved by various actions of government, non-government and local's effort. The study also aims to explore the ornamental and medicinally valuable plant *Lindernia antipoda* by microporopogation. The study provided a standard protocol for the aseptic culture of the plant.

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Conflict of Interest: The authors have no conflict of interest

Author Contributions: Rajkumar P- Contributed in conducting experiment, collecting and analysing data, paper preparation; **Dr. Jahirhussain G-** Research supervisor; **Karuniya Raja Viella G-** Data analysis and interpretation.

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