

# IN VITRO DIRECT ORGANOGENESIS OF LINDERNIA DUBIA (L.) PENNELL

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### ABSTRACT

*Lindernia dubia* (L.) Pennell a least concerned emergent plant belong to the family linderniaceae was subjected to micropropagation technique for the standardization of *in vitro* direct organogenesis protocol. The experiment was carried out to explore, conserve and increase the distribution of the plant via aseptic condition and this serves as the first report of this plant species. Nodal segment from the elite mother plant was excised, surface sterilized and inoculated in Plant Growth Regular (PGR) Free MS basal medium for organogenesis following the standard plant tissue culture procedure. The strength of the MS salt was altered for obtaining the best regeneration capacity in both the medium. The result showed that half strength - Plant Growth Regulator free medium regenerated more plantlets from the selected explant than the other medium. In Hormone free MS media, only MS salt, Carbon source and B5 Vitamins were added. After a month of inoculation, the developed shootlets were sub cultured and raw data's for shoot & root number, length were calculated at 25<sup>th</sup> and 50<sup>th</sup> day from incubation. Statistical interpretations like ANOVA and Tukeys Multiple comparison Analysis were performed for significance. The regeneration percentage was 100 in all the sets. So, from the study it is concluded that the plant naturally possess essential growth regulators for its vegetative phase and under artificial conditions like *in vitro* conditions they doesn't require specific growth regulators.

### INTRODUCTION

Plants have been a significant source of medicine for centuries. The vast majority of earth relies on plant as they constitute the most significant source of life-saving medications, basic need (Abera, 2014) and a mutual or camouflage companion to millions

of living beings be it microscopic or macroscopic. Medications from these have uplifted the lifestyle and pharma potential of this current society. Drugs have been invented, produced for number of chronic and non-chronic, pathogenic diseases of olden times (Uttpal et al., 2020; Sharma et al., 2021). With increase in technology, and rapid change in climate and life style

conditions, emergence of new ailments has become inevitable. Cure to this are found in plants. Modern drugs use nearly one-quarter of medicinal plants or plant-derived compounds which has led to rapid decline of medicinal plants, along with habitat loss, natural calamities, and anthropogenic activities.

Every plant inhabits therapeutic abilities in them as they produce compounds called secondary metabolites which distinguishes from a normal plant (less or negligible production of secondary metabolites) used in traditional systems of medications like Ayurvedha, Siddha, Unani etc., plants based modern drugs (unique secondary metabolites - alkaloid, flavonoid, terpenoid, tannin, phenol, etc.,) that incorporate them with properties such as antioxidant drug, anti-inflammatory, anti-tumor, anti-diabetic drugs and lot more (Biswas et al., 2022; Mitra et al., 2022; Subhabrata et al., 2021). The geographical origin and distribution of the plant plays a vital role in the quality and therapeutic value of the plant (Kamboj, 2000).

As per, the International Union for Conservation of Nature (IUCN)'s latest updates (April 2025), a total of 47,000 species have been placed in the threatened with extinction category after assessing over 169,000 species. To protect both marine environment and land they emphasized the "need for double land and triple ocean protection" to safeguard the link of conservation and agricultural practices. In the IUCN category, we mainly focus on RET species but there is a category namely "Least Concerned" which requires more attention as 3,358 plant species of this category from 58,343 assessed plant species contributes to medicine worldwide as per IUCN 2023 records, similarly from the studies of Gowthami et al., 2021 it is evident that 366 least concerned (LC) species out of 2,143 species used for medicine in India. *Zingiber officinalis* (Royal Botanical Gardens, Kew, 1807; IUCN Red List Assessment (2021): DD), Turmeric, *Bacopa monnieri*, *Physalis angulata*, *Mecardonia procumbens*, *Lindernia dubia* (IUCN, 2019) are some of the LC that possess highly medicinal efficacy in antioxidant, anti-tumour, anti-inflammatory, anti-diabetes, anti-ageing, neuroprotective abilities and some are even commercialized (Fatima et al., 2022; Qasim et al., 2023; Anju Das and Geetha, 2024; Kausar et al., 2021; Pradeep K. Sharma and Sabiha Mansoori, 2019). The IUCN has reasoned that "abundant Populations, Stability, adaptability to environment and devoid of major threat" are strength of LC species while they are monitored closely to "focus on conservation effects, keeping in line with biodiversity and to detect early warning signs" of plant that fall into RET category and it further added a total of 40,000 trees are falling into least concern portion (IUCN, August 2025). Even though, most of the medicinal plants are under LC they are major source of essential drugs thus conserving and exploring the potential abilities of these plants plays a crucial role in biodiversity as well as pharmacology.

Conservation is one of the best strategies to get hold of biodiversity loss. Technoscience is widespread tree that rooted from science and technology (Latour, 2014). In this regard, the field of science and technology studies (STS), explain human-technology-plant interactions which is *in vitro* technology includes the conservation of plants i.e., plant tissue culture, germplasm storage (Holmes and Cavanagh, 2016; Berger-Tal and Lahoz-Monfort, 2018). Techniques for conserving and propagating numerous rare and endangered plant species have been published by Bhat et al., (1995). *In vitro* culture techniques, which provides a practical tool for mass multiplication, rapid regeneration, germplasm conservation of rare, endangered, threatened, and significant plants, and secondary metabolite production, have attracted more attention in recent years (Baskaran and Jayabalan, 2005). According to Maruyama et al., (2007), PTC provides innovative methods for plant production, replication, bioactive chemical extraction, genetic enhancement, and preservation. Additionally, due to straightforward extraction techniques and the absence of substantial pigment quantities, chemicals from tissue cultures may be easier to purify, thus lowering manufacturing and processing costs (Lozovaya et al., 2006).

The long back history of plant tissue culture starting from the concept of plant totipotency in 1902 by Haberlandt, cell theory

of Schleiden (1838) and Schwann (1839), to first success reports of tissue culture from mesophyll tissue has evolved constantly to imprint its hold in application of genetic biodiversity conservation, breeding and biopharmaceutical production (Schleiden, 1838; Schwann, 1839; Tang et al., 2020; Sussex, 2008; Haberlandt, 1902; Gautheret, 1983). By carefully adjusting plant growth hormones and nutrients, micropropagation of phytomedicinal species makes use of this trait and enables us to create identical offspring that express the same or higher levels of their beneficial metabolites. The proliferation and differentiation of somatic cells are redirected when phytohormones are added to the culture medium (Skoog & Miller, 1957).

The modification of hormones in the growth medium is one of the main sources to create tissue culture protocols. Using a variety of biosynthetic pathways, auxin stimulates cell division, select cell fate, and establishes environmental controls that affect overall root development (Overvoorde et al., 2010). In the absence of exogenous auxins, *in vitro* root induction depends on endogenous auxins produced at the shoot apex and transmitted downward (Grieneisen et al., 2007). Changes in the auxin gradient enable the plant to regulate growth, and the established auxin gradient enables cells to keep information about their growth and development after the initial impulses that led to cell differentiation (Grieneisen et al., 2007). By introducing exogenous auxins, these developmental processes can be artificially stimulated to encourage root cell differentiation. The *in vitro* environment is extremely stressful for cultured explants (Kevers et al., 2004). So micropropagation in plant tissue culture aids in the multiplication, commercialization, and all-time availability of medicinal, aromatic, and ornamental crops, orchids, halophytes, lithophytes, and xerophytes in an aseptic environment. One such least concerned plant species that needs exploration and conservation is *Lindernia dubia* (L.) Pennell from linderniaceae. *Lindernia dubia* is an annual, wetland species commonly called as Yellow-seeded false pimpernel found in Eurasian agricultural rice fields. The genus accommodates weeds (*L. dubia* & *L. procumbens*), medicinal (*L. crustacea*, *L. ciliata* & *L. ruellioides*), ornamental (entire *Lindernia* genus) and desiccation tolerant (*L. brevidens*), sensitive (*L. strabocmosa*) species. With taxonomical and morphological articles (Curt et al., 2024; Prasad and Sunojkumar, 2014; Prajapati et al., 2021; Vishnyakov, 2025; eflora of India) as the only published work, this study aids in understanding the need of conserving the LC species, to explore its potentiality in applied fields for human benefits. This investigation serves as the first report on *Lindernia dubia* other than morphology and taxonomy as far our knowledge.

## MATERIALS AND METHODS

**Selection of elite plant and explant:** Microbial-free cultures demand pathogen free explants that are selected from a healthy environment. Newly grown, mature *Lindernia dubia* plant parts were selected and collected with sterile scissors and a container from rice fields of Krishnarayapuram, Karur district, TN, India. The plants were digitally photographed in their natural habitat and uprooted for aseptic culture. From the elite plant, nodal regions were excised and used as explant for further culture.

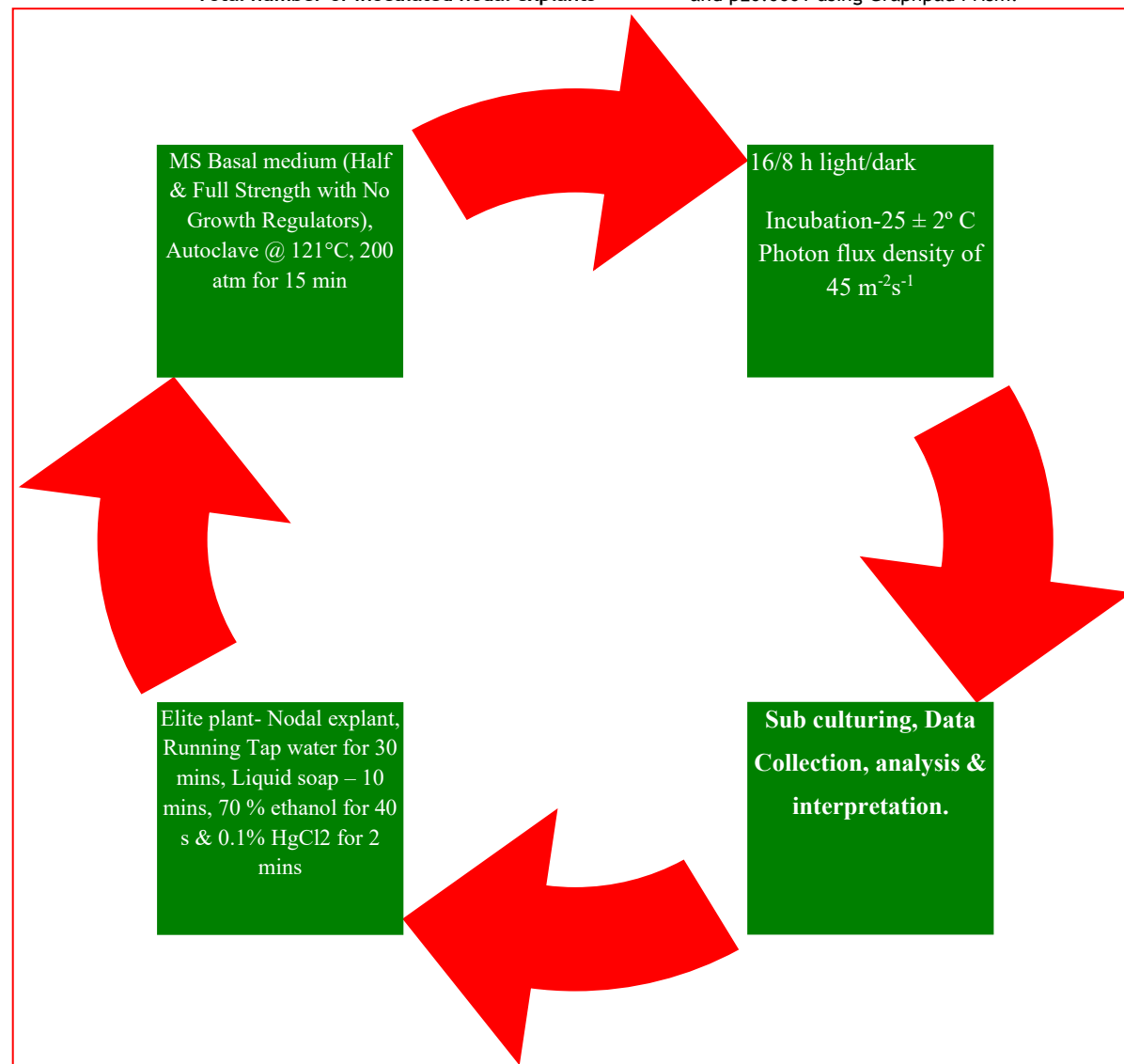
**Sterilization, inoculation and incubation:** To make the explant devoid of dirt and microbes, they were thoroughly washed with running tap water for 30 minutes and a drop of liquid soap is added and sterilized for 10 more minutes before completely washing with double distilled water. After this, sterilized nodal explants were taken into Laminar airflow chamber for aseptic sterilization for inoculation in the autoclaved MS Basal medium with no plant growth regulator (PGR). The explants were sterilized with mercuric chloride and ethanol to make it suitable for regeneration (to produce disease free plantlets). The MS basal medium was supplemented with micro, macronutrients, vitamins, carbon source, solidifier but no PGR's were added similarly, the strength of MS salts were altered into half - Strength (half the quantity of MS salt is mixed in 1L of distilled water) and full - strength (1L stalk). The pH was adjusted to 5.8 and autoclaved at standard pressure and time of micropropagation. The nodal explants were inoculated in the

autoclaved medium and incubated at the optimized parameters for regeneration (Fig 1).

**Data collection, analysis and Interpretations:** The incubated nodal explants were observed for initiation percentage after 7 days of inoculation. The initiation percentage was calculated using by

$$\text{Initiation percentage} = \frac{\text{Total number of nodes initiated}}{\text{Total number of inoculated nodal explants}} \times 100$$

Morphological observations were noted daily, after a 15 days the regenerated nodes were subcultured and data's on parameters like shoot and root length, number were recorded at 25<sup>th</sup> and 50<sup>th</sup> day of inoculation. The experiment was repeated 5 times for a full-fledged data analysis. Collected data's were arranged in randomized blocks two way ANOVA with Tukey's multiple comparison test was performed to obtain significance at  $p \leq 0.05$  and  $p \leq 0.0001$  using Graphpad Prism.



**Methodology for direct organogenesis of *Lindernia dubia* (L.) Pennell from nodal explant**

## RESULT AND DISCUSSION

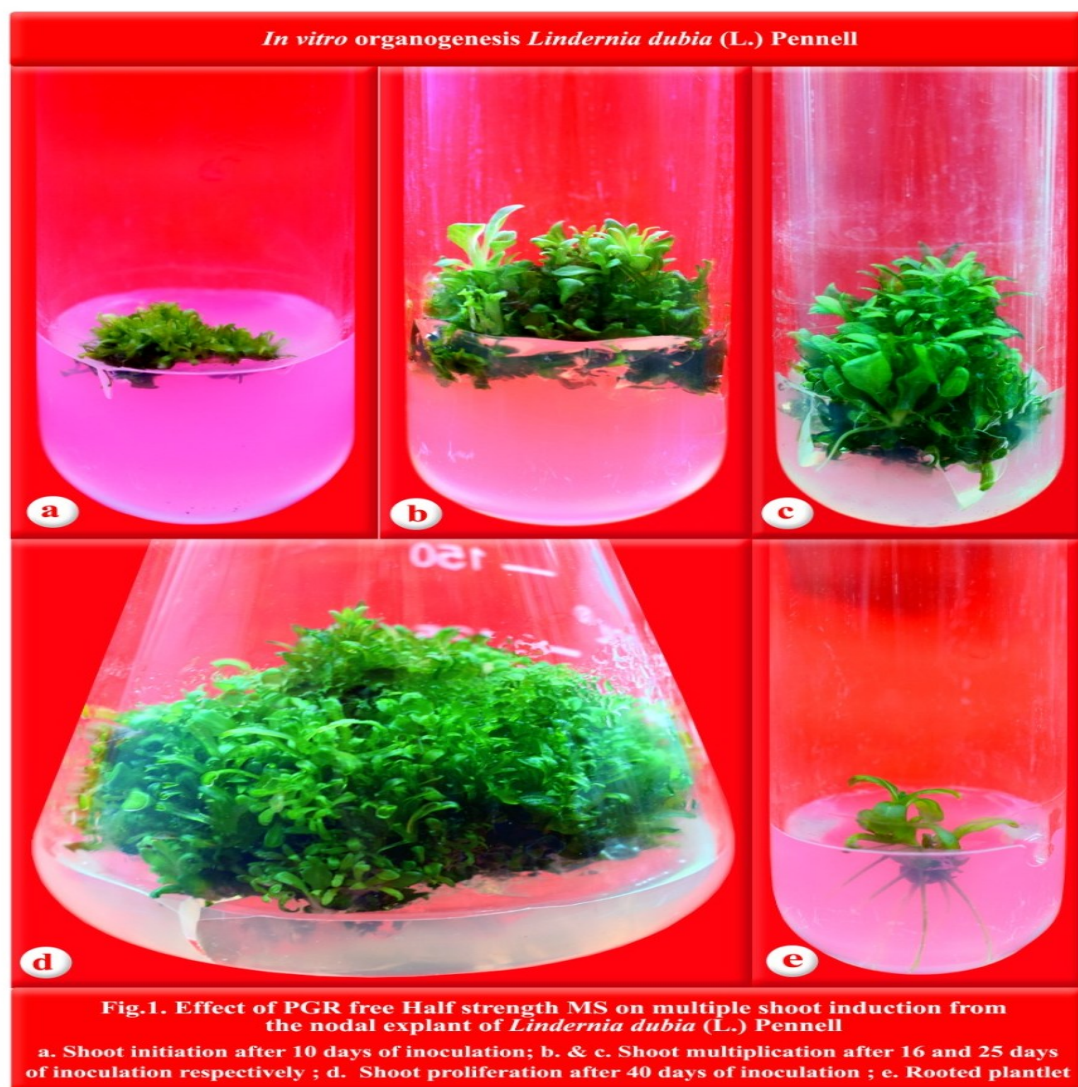
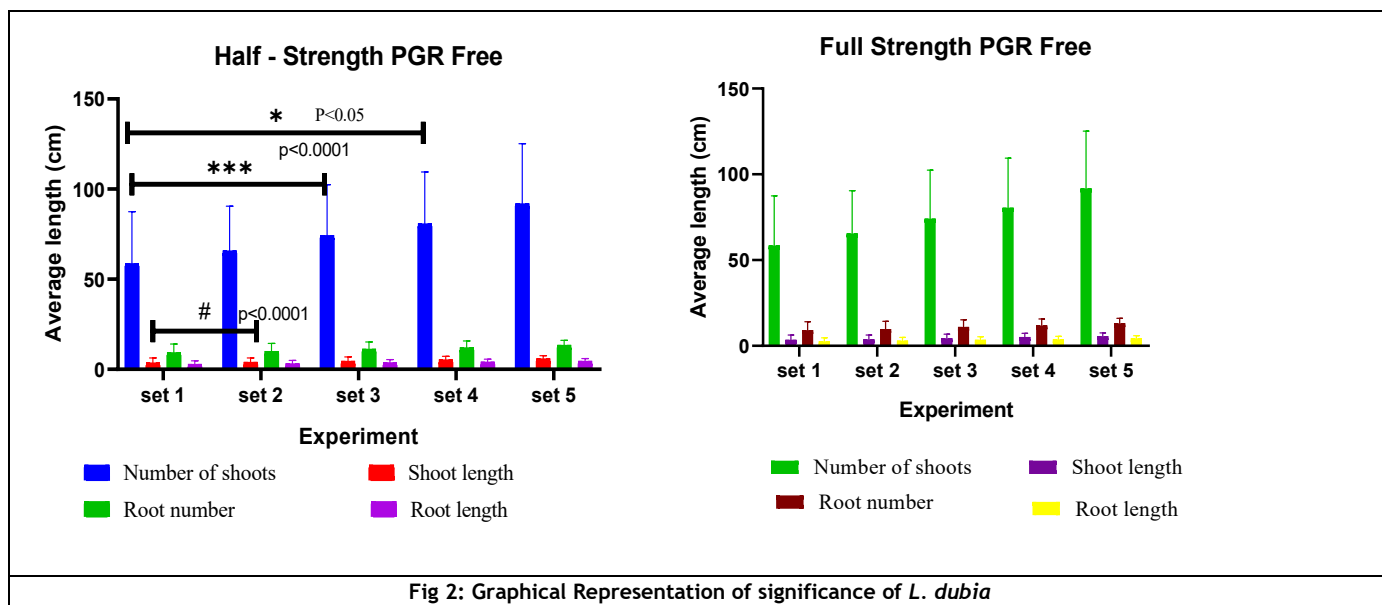
For plantlet initiation and multiplication, the nodal explants were grown on MS basal medium with no plant growth regulators in varied strength of MS salts (Half and Full strength). The shoots initiated after a week of inoculation. The initiation percentage

was 100 in all the sets. Proliferation and multiplication followed thereafter, after 15 days the plantlets were sub-cultured and data's with respect to morphological parameters of the plant were recorded and tabulated in two times, 25 and 50 respectively for five sets of experiments (Table No 1; Fig 1 & 2)

**Table No. 1 : Clonal Propagation of *Lindernia dubia* (L.) Pennell in Plant Growth Regulator Free MS basal medium from nodal explant**

Strength	Number of shoots (Nos)		Shoot length (cm)		Root number (Nos)		Root length (cm)	
	25 days	50 days	25 days	50 days	25 days	50 days	25 days	50 days
Half - Strength	38.2±3.70	79±2.12	1.54±0.23	5.48±0.31	5.8±0.83	12.6±0.89	1.2±0.17	4.12±0.44
	48±2.23	83.2±2.38	2.1±0.24	5.56±0.27	6.6±0.54	13±0.70	1.68±0.16	4.4±0.38
	54.2±1.64*	94.2±1.64*	2.7±0.31#	6.14±0.26	8.2±0.83	14±0.70	2.26±0.55	4.82±0.19
	60.2±2.58***	101±2.54***	3.62±0.27	6.64±0.23	9.2±0.83	14.6±0.54	2.64±0.34	5.1±0.31
	68.2±4.32	115.4±4.15	4.42±0.31	7.02±0.51	11.2±0.83	15.2±0.83	3.28±0.29	5.48±0.16
Full - Strength	35.8±1.30	60.4±0.54	2.68±0.19	5.46±0.30	4±1	8.6±0.54	1.16±0.19	3.44±0.15
	45.4±1.94	66±1.41	2.86±0.63	5.76±0.49	4.6±0.54	9.8±0.44	1.7±0.23	3.74±0.16
	49.6±1.34	71.4±1.51	3.42±0.25	5.66±0.32	5±0.70	10.6±0.54	2.06±0.21	3.96±0.16
	53.2±0.83	73.8±0.83	4.22±0.44	5.74±0.55	5.8±0.44	11.2±0.83	2.44±0.11	4.3±0.12
	56±1.58	79.8±0.83	4.58±0.35	6.54±0.27	7.2±0.83	12.4±0.54	2.814±0.27	4.68±0.13

Mean±SD of 5 sets of experiment; \* significant at  $p < 0.05$ ; \*\*\*, # significant at  $p < 0.0001$



From the tables, it is found that half - strength PGR (HS PGR) free medium is more efficient than the full - strength PGR medium. In the HS PGR free medium, the developed plantlets

were fleshy green, indistinguishable at the early weeks of inoculation. After 15 days, the plant parts were distinguishable, after first sub-culture the proliferation was faster when



compared to FS PGR free media. The parameter showed a constant increase which double after 50 days of inoculation. The maximum number of shoot and roots were found in the fifth set which is double the numbers found in 25<sup>th</sup> day. The highest shoot number was 115.4±4.15 with a length of 7.02±0.51 cm in HS PGR free media followed by 79.8±0.83 shoots of length 6.54±0.27 cm in FS PGR medium in the same set of experiment.

Even after the shootlet regeneration, the MS media was devoid of root promoting hormone as we observed simultaneous growth of roots which were white from beginning turning into green colour. They formed a network like structure and settled at the bottom of the container mainly conical flask. In test tube's they formed the same structure but settled at the walls of the tubes. The highest root number 15.2±0.83 and length 5.48±0.16 cm was observed in the same HS PGR free medium. The well-developed plantlets were hardened and the survivality was 90%.

In our current study, we have used either half strength or full strength for the complete regeneration of plantlet from the explant but researchers has altered between the strength for shoots and root proliferation. *Lessertia frutescens* from nodal explants has been subjected to Full strength MS medium for shoot while half strength for rooting (Shaik et al., 2010). Half strength MS medium with hormones has been successfully reported to be the most suitable media for *Strelitzia reginae* (Kumar et al., 2024), *Typhonium flagelliforme* (Rezali et al., 2017) while full strength for *Manihot esculenta* (Groll et al., 2002), *Turnera ulmifolia* (Manokari et al., 2018). Similar to our investigation, half - strength MS basal medium free of PGRs was used to regenerate *Lindernia antipoda* from leaf explant. That produced an average of 99.36±1.51 shoots with 5.07±0.29 cm length having 34.68±1.71 number of roots of length 4.07±0.16 cm (Jahirhussain et al., 2021; Jabir et al., 2016) while Watad et al., (1995) reported few shoots from hormone free culture in *Aconitum napellus*. Though there are only few studies on half strength PGR free micropropagation, still in plants that show lower production of plantlets for commercial purposes PGR free medium is effective as it implies the natural existence of growth supplements in the plant itself. Thus, the investigation shows half strength PGR free MS medium is the most suited media for large scale production of *Lindernia dubia* (L.) Pennell.

## CONCLUSION

*Lindernia dubia* an invasive weed of the family linderniaceae showed better efficacy for large scale production in half - strength PGR free MS basal media that the other tested media indication; this method, of culturing is efficient for conservation as well. Though, the plant is least concerned, still fewer exploration and minimal distribution in India has added curiosity in exploring this plant species deeper owing to existing medicinal benefits of this genus *Lindernia*. Thus, current investigation has shed a ray of hope in multiplication of *L. dubia* under aseptic condition to maintain the species population to aid in biodiversity balance. Future scope of this investigation avails in handy existence of enough material to carry out advanced studies like secondary metabolite production, analysis, quantification, and pharmacological studies to explore its medicinal potentiality.

## CONFLICT OF INTEREST

The authors declare that "They have no conflict of interest"

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