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Resurgence Of Multiple Shoots From Photosynthetic Explant Of Red Listed Lindernia Antipoda (L.) Alston

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ABSTRACT: Lindernia antipoda (L.) Alston a valuable ornamental wetland plant of Scrophulariaceae family. The in vitro studies on shoot induction and flowering in half-strength MS basal medium free of PGRs supported superior plantlets from photosynthetic explants (leaf). A series of modifications before complete shootlets were observed and the data were analyzed, interpreted, and recorded at an interval of 15 days. The explants 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. The plantlets gave in vitro flowers and fruit that were identical to the in vivo plant. The well-developed plantlets were hardened and acclimatized. Plants devoid of required amount of nutrients and growth regulators can encounter difficulties but in the present investigation the selected species marked a profound growth imparting the essentialities are available remarkably in plants by nature itself. In future a modified growth medium can be attained with no doubts. The current study aims in PGR free plantlet regeneration in half strength MS basal medium.

KEYWORDS: ***half-strength, hormone-free, in vitro flowering, in vitro fruiting, leaf, Lindernia antipoda***

ABBREVIATIONS: PGRs- Plant Growth Regulators, **MS Media- Murashige and Skoog Media**

1. INTRODUCTION

The correspondence of man and plants from ages civilized humans to extract and use plants as therapeutics beyond food and fodder. Plants vital for healthy living are rich in dietary sources of biomolecules, vitamins, and minerals. Traditional knowledge on medicinal plants sprung from expanded aboriginal cultures. Human beings have become so driven towards

technology over the years that they have forgotten its repercussions. Plant tissue culture serves as the key to retrieve genetically modified plants in crop biotechnology. The skill has significance in micropropagation, plant breeding, bioproduction, and conservation [1] and also as a tool of research [2]. The potent technique has unfolded an extensive area of research for biodiversity conservation. Micropropagation a user-friendly process for the rapid multiplication of commercial, and conservation plants. The non-monophyletic group [3] linderniaceae consists of 13 genera and 195 species many confined to neotropics. The genus *Lindernia* has curative ability in emmenagogue, diarrhea, anthelmintic, vertigo and cough. *Lindernia antipoda* (L.) Alston a familiar weed in the rice field of India, Japan, China, and wetlands is the least concerned species as per the IUCN 2009 list (RLTS.T168632A6524753) is a valuable aquatic ornamental plant in the aquarium trade [4]. These plants are potent enough to intensify, renew and maintain native habitats and add values ornamental [5]. The *in vitro* studies of aquatic and wetland plants *Myriophyllum spicatum* and *Potamogeton crispus* [6], *Ludwigia palustris* [7] have been delineated with a single study report for micropropagation and it is same for *Lindernia antipoda.* This is the first report on hormone free culture investigation on *L. antipoda* that intends to produce a new clone from photosynthetic explant (Leaf) via direct organogenesis.

2. MATERIALS AND METHODS

The leaf segments from field grown *Lindernia antipoda* (L.) Alston selected as the explants source for direct organogenesis. The nutrient medium for clonal propagation was half strength MS basal medium (without hormones). The molten mediums in glasswares were sterilized after scrutiny of p^H and inclusion of 0.8% solidifying agent (agar). The leaf explants were sterilized and inoculated after wounding; the cultures were maintained at standard incubation conditions (25±2°C; 16/8 hr photoperiod; 30-40 µM m^{-2} s^{-1} illumination). The developed shoots were subcultured in basal medium for shoot regeneration and multiplication in wider glassware to enhance the needed supplements. The full fledged plants were micropropagated till *in vitro* flowering and fruiting. The stacked and rooted plantlets were taken off and then transferred to cups containing sterilized hardening mixture. Each plantlet irrigated periodically and maintained under culture room conditions for about a month and later to normal laboratory conditions for 15 days. After a month the plantlets were transplanted to the field under shade for 3 weeks and then transplanted to the soil for acclimatization.

3. RESULT AND DISCUSSION

The inoculated leaf explants of sparrow *Lindernia* had swellings in the first week followed by numerous minute hairy roots from shoot primordial that arose from the wounds created by pricking. The explants of the plant showed a cotyledonary stage possessing trilobed leaf-like structures that differentiated to develop into an entire outlet of a leaf. The leaves were indistinguishable at the very early stage forming a funnel that opens up to a leaf. The development of shoots was active from the petiole, midvein, and serrations of leaf. The prior initiation of morphogenesis into leaf was a rachis like structure characterized by green, succulent and many active traits. Serrations of the leaf developed shoots while the lamina degenerates producing the reproduction pattern of *Bryophyllum.* (Fig. 1)

The culture showed multiple modifications in the third week of monoculture. The tri-lobed complexes differentiated into serration making the margin entire, distinguishable and original as that of *in vivo* leaf. The petiole, leaf width, length and size enlarged as days, and weeks passed to give off shootlets. The elongation of the funnel-like rachis at few points by active differentiation of cells created a surge forming nodal and intermodal segments. These formed shootlets that proliferated and multiplied to give multiple shoots. (Fig. 2).

Micropropagation is achieved through various explants including leaf *Saussurea involucrate* [8], nodal segment, intact seedlings, and cotyledonary node [9]. Current research utilized young green leaves for shoot induction. Atak and Celik [10] reported leaves as the most used explant source for *in vitro* culture in *Anthurium* while Martin et al., [11] observed a higher number of shoots in the discoloured lamina explants than green lamina.

This is the first report on *L. antipoda* in half strength basal medium without hormones for shoot multiplication and *in vitro* flowering. Jabir et al., [4] reported shoot multiplication of *L. antipoda* where hormone free medium was just a control. The present investigation produced more shoots in basal medium that was in harmony with the single existing report on *Lindernia*. (Table 1). The shoot and root induction frequency was 100% in *Lindernia antipoda.* The number of shoots regenerated after 2 weeks had an average of 41.76±0.82 from 5 trails. Of that first and fourth trial showed elevation in number (42.8±0.83, 42.4±1.14) respectively than other experiments. The shoot number doubled in the next 15 days to 99.36±1.51 numbers. The second trial gave a best result 101.2±9.25 while the lowest was seen in the first trial. Watad et al., [12] reported fewer numbers of shoots in hormone-free MS basal medium.

The shoot length of the produced shoots ranged between 3- 4 cm. the mean of the five experiments on shoot length delineated 3.63±0.37 cm from 3.24±0.80cm, 3.96±0.64 cm, 3.22±0.94 cm, 3.82±0.37cm, and 3.94±0.20 cm respectively from repeated experiments. The shoot length hiked up with prolonging days. The average of first experiment 5.38±0.69 cm was prior in shoot length.

As the shoot multiplied and proliferated so as the roots. The shootlets were denied rooting media for root initiation. The roots initiated simultaneously after a few days of shoot primordial development. They gave white minute hairy roots that modified into rootlets. The number of roots was 1/3rd of shoot numbers. The mean numbers after 30 days were 34.68±1.71 with 4.07±0.16 cm length. The root length oscillated between ranges 2 and 3 in the first half of incubation period that grew in the next half. The length 4.36±0.18 cm was the highest recorded in 30 days which was reported in the 5th trail. The even-numbered trials had roots of 4.04±0.19cm and 4.06±0.20 cm while the odd numbers had 3.94±0.42 cm and 3.96±0.13 cm.

The subcultures infer multiplication in 2nd week which gave 245 shoots from a single leaf with simultaneous development of roots. The 3rd and 4th-week shoots had profuse roots with branched adventitious roots from nodes that vary numerically from 2 to 5 which formed nestle like appearance. The variations have aroused due to *in vitro* stress. A month-old shoots produced flowers in half-strength MS medium that were identical to *in vivo* plant in eidonomy. A 65 days old plantlet produced mature fruit having seeds that dehisce horizontally with vivipary mode of germination in the 10th week. *In vitro* studies on flowering and fruiting have been put down with hormonal medium on many studies including *Andrographis lineata* [13]. Mature and well established *in vitro* plantlets of *Lindernia antipoda* were hardened in the vermiculite soil, farmyard manure mixture in the

ratio 2:1:1 with survival of 85–90 %. In the 11th week the hardened plantlets were taken for gardening with establishment and acclimatization rate of 80 %.

4. CONCLUSION

A simple and standardized protocol for the development of clones of *Lindernia antipoda* (L.) Alston has been successful with much efficiency in the half strength MS basal medium. Several studies have reported negative or zero growth in the control medium (medium devoid of hormones) but in the present study hormone-less medium suited best for multiple shoot regeneration. Thus proving that plants have a sufficient amount of natural PGRs even to make them spring into a complete plant from single tissue under controlled aseptic conditions. This can be used to conserve the red-listed plants.

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1. TABLE 1: Effect of PGR's free Half strength MS medium on multiple shoot induction in *Lindernia antipoda* (L.) Altson.

No of Days	**Experiment**	**No. Of Shoots**	**Average**	**Shoot Length (cm)**	**Average**	**No. Of Roots**	**Average**	**Root Length (cm)**	**Average**
15 days	1	42.8±0.83	41.76±0.82	3.24±0.80	3.63±0.37	18.4±2.30	18.84±1.42	3.04±0.36	3.02±0.07
	2	41.2±1.30		3.96±0.64		16.6±1.14		2.98±0.16	
	3	40.8±0.83		3.22±0.94		19.8±1.30		3.14±0.18	
	4	42.4±1.14		3.82±0.37		20.2±1.30		2.98±0.31	
	5	41.6±1.14		3.94±0.20		19.2±0.83		2.96±0.18	
30 Days	1	97.8±9.60	99.36±1.51	5.38±0.69	5.07±0.29	32.2±0.83	34.68±1.71	3.94±0.42	4.07±0.16
	2	101.2±9.25		4.94±0.15		34.6±1.67		4.04±0.19	
	3	99.2±8.40		4.64±0.29		34.2±0.83		3.96±0.13	
	4	98±8.21		5.14±0.34		36.8±1.30		4.06±0.20	
	5	100.6±7.46		5.28±0.16		35.6±1.14		4.36±0.18	

Fig. 1. Effect of Half strenth MS salt in 15 days culture on *Lindernia antipoda* (L.) Alston
a. Shoot initiation from leaf explant ; b. Shoot multiplication ;
c. Rooting ; d. & e. Shoot proliferation.

Values are mean±SD replicates of 5 plantlets in 5 consecutive experiments

Fig. 2. Effect of Half strenth MS salt in 30 days culture on *Lindernia antipoda* (L.) Alston
a. & b. Shoot multiplication and proliferation from leaf explant ;
c. Boottom view of roots ; d. Hardening .