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TISSUE CULTURE STUDIES IN *HEMIDESMUS INDICUS* (L.) R. BR.

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Abstract:- commonly known as known as Indian Sarsaparilla (Anantmool). In India, the plant recorded in almost throughout all parts. is highly valued in Indian system of medicine. It contains Aromatic, Anti-microbial, Anti-cancer, Anti-viral, Anti-inflammatory, Anti-pyretic, Anti-dysenteric actions (Satheesh et.al. 2008), Anti-oxidant, & Anti-hepatotoxic activities (Mohana et. al. 2005). Tissue culture studies of various replicates for in vitro growth. The important medicinal important phenolic compounds are present and so it is important make in vitro culture using different plant growth hormonal concentration on solid and liquid culture medium which produces multiple shooting and multiple rooting from single explants. Result shows sufficient increase in proliferations of shoot and roots by using proper concentration of plant growth hormones, nutrients and growth medium.

Keywords:*Hemidesmus indicus*, Tissue culture, Plant growth hormones, & Culture medium.

INTRODUCTION :

is a twining shrub of family Periploceae (formerly Asclepidaceae), commonly known as Indian Sarsaparilla (Anantmool). In India, the plant found within almost throughout all parts. It is found from the upper Gangetic plain eastwards to Assam and throughout central, western and southern India (*Gopiesh et al* 2007). The Moluccas and Sri Lanka are the other places of its distribution (*Globalherbal et al*, 2005). is highly valued in Indian system of medicine. Extracted plant materials are used in This plant useful in treatment of Inflammatory condition, Fever, Rheumatism, Leprosy,liver disorders (Nadanasaravanan and Namasivayam Nalini 2008). Root contains Aromatic, Anti-microbial, Anti-cancer, Anti-viral, Anti-inflammatory, Anti-pyretic, Anti-dysenteric actions (Satheesh et. al. 2008), Anti-oxidant, & Anti-hepatotoxic activities (Mohana *et. al.* 2005). Roots are used as addition in main treatment of snakebite and scorpion sting (Sircar *et. al*. 2007). As medicine "Anantmool' holds a reputed place in all systems of medicine in India (Neeta *et. al*. 2005). Due to multiple use of this plant has been indiscriminately collected from its natural habitat and becoming extremely rare by overexploitation (Sreekumar *et. al*. 2000). The natives use the roots internally in of premature graying of hairs, jaundice, eye related diseases.

Morphology of Root consisting of aligneous center, and with annular cracks. Stem and Branches: Elongate, narrow, twine anticlockwise are profusely laticiferous, narrow, woody and deep purple or purplish brown colo r with the surface slightly ridged at the node (Satheesh *et. al*. 2008). Leaves: simple, petiolate, exstipulate, opposite, entire, apiculate acute or obtuse, dark green above but paler and sometime pubescent below. Leaves of the basal par t of the shoot are linear to lanceolate (Warrier *et. al*. 2000). Flowers: Small, greenish yellow to greenish purple outside, dull yellow to light purplish inside, axillary, sessile racemes, imbricate with flowers, followed with scale-like bracts. Fruit: Two long slender spreading follicles. Seeds: many, flat, oblong, with a tuft of white silky hairs (Prasad et. al.1965, & Warrier *et. al.* 2000). Anatomically transverse sections of the fresh root are circular with a fairly regular outline. It is slightly porous strand of wood at center (Sharma *et. al*. 2000). Shows 3-15 layered cork (thick walled treatment xreddish brown), 2-3 rows of colorless phellogen, 1-2 rows of narrow thin-walled cells phelloderm, 2-3 layered thick walled polygonal parenchymatous cells with starch grains, prisms of calcium oxalate crystals (Warrier *et. al*. 2000). Cortex –

Wide, contains thin walled, large tangentially elongated cells contains simple and compound starch grains, prisms of calcium oxalate. Phloem – Narrow, with sieve tubes, phloem parenchyma, companion cells and uni-seriate medullary rays(Satheesh *et. al*. 2008). Cambium – Narrow, distinct. Xylem Vessels - Scattered with pitted walls, tracheids, thick walled fibers with uni seriate medullary rays (Shanthi *et. al*. 2010).

MATERIAL AND METHODS

Plant materials: Plants collected from Sinhgrah Fort Pune, (18° 21' 49.12" N & 73° 45' 17.99" E)

Procedure for explant :- Collected plants were washed in tap running water with Tween20. stem nodal portions were cut off up to 2-3 cm each node and wash in tap water for 30 min. Cut explants were treated with 1 % of antifungal powder (w/v) for 30 min with continuous shaking followed by 3 time wash with sterilized autoclave distill water. Treatment with $HgCl_2$ was given for 5-6 min with shaking and surface sterilization with 70% alcohol for 15-20 seconds. Explants were washed 5 times with sterile distilled water. All procedure was done in under aseptic condition in laminar air flow cabinet. The explants were inoculated on medium.

Preparation of Stock solution for Murashige and Skoog's medium (MS)

3% Sucrose and 0.8% agar was used in each 1000 ml of solid MS full medium and half proportions used for MS1/2 medium. Macro stock 50 ml, Micro stock 5 ml, Organic 1 ml, Fe stock 5 ml, Sucrose (3%) 30 gm, and Agar (0.8%) 8gm were used. Final volume was 1000 ml for 1 liter MS full strength was made and pH was adjusted to 5.8. For preparations of $MS_{1/2}$ strength Macro stock 25 ml, Micro stock 2.5ml, Organic 0.5 ml, Fe stock 2.5 ml, Sucrose (3%) 15gm and Agar (0.8%) 4gm were used. 1000 ml of final volume were make for 1 liter $MS_{1/2}$ medium and pH was adjusted to 5.8

Different Hormonal concentrations with MS and $MS_{1/2}$ mediums

1) MS (CON)
2) MS + BAP (2 mg/lit) + NAA (0.1mg/lit)
3) MS + BAP (2mg/lit) + NAA (0.05 mg/lit)
4) MS + KN 1.15μM + 0.054 μM
5) MS ½ (CON)
6) MS ½ + IBA 1mg/lit
7) MS ½ + IBA 1.5mg/lit
8) MS ½ + IBA 2.0mg/lit
9) MS ½ + IBA 2.5mg/lit
10) MS ½ (CON)
11) MS ½ + IBA 1.5mg/lit
12) MS ½ + IBA 2.0mg/lit
13) MS ½ + IBA 2.5mg/lit

Solid Medium

Liquid Medium

Inoculation: In laminar air flow cabinet surface sterilized cut pieces of 2-3 cm explants are put into the Petri plates. Both ends of explants are cut with surgical bled and Slant of tubes which was autoclaved is used to inoculate the explants

Root culture: Well grown roots from subculture size up to 1-1.5 cm were cut, clear the solid medium present on, and washed with D W. 3-4 pieces of these root are transferred to flask (500 ml) containing 50 ml of liquid $MS_{1/2}$ medium (with different concentrations of PGR and control) and cultured with agitation (70 rpm) in dark.

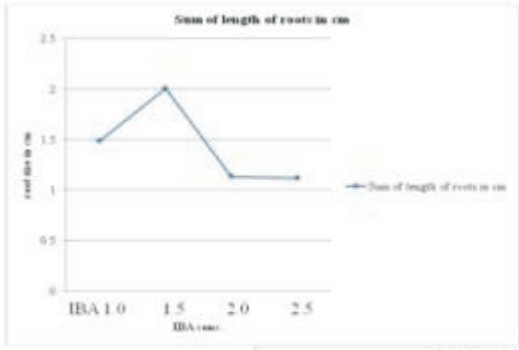
Anatomical study: The comparative anatomical study was carried out for in vivo and in vitro plants. were 2nd leaf, stem (of 2nd nodal sector), and newly grown root tips of *in vitro* and *in vivo* plants. Transverse sections of leaf, stem, and root were taken and stain with safranine and light green final sections were mount in 50% glycerin. The observations made for microscopic study of leaf trichomes stem trichomes and numbers per microscopic fields. Different 20 field of microscopic, observation of in vitro with 20 field of study of in vivo was taken.

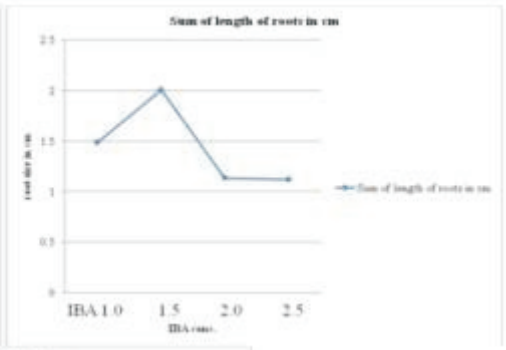
RESULT AND DISCUSSION

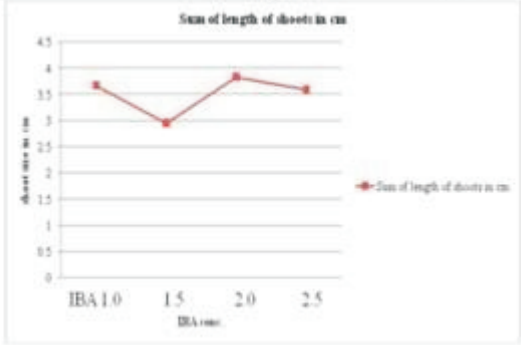
Statistically analysis of rooting and shooting observation

Sr. No	Medium +PGR mg/lit	Number of Replicates for observation	Sum of length of roots in cm	Number of Roots/replicate	Sum of length of shoots in cm	Tot no. of shoots/replicate
1	**MS CON**	20	0.912.9±0.21	0.970±1.21	2.514±2.80	1.025±0.254
2	MS +KN	60	1.10548±0.253	2.101±1.53	**5.575±3.1319**	1.75 ±0.8660
3	$MS_{1/2}$ CON	40	0.8±05.1072	1.5±2.010	1.9±2.45	1.3±2.234
4	$MS_{1/2}$+IBA 1.0	100	1.489±0.9222	2±1.1094	3.1875±2.7861	0.75 ±0.1670
5	$MS_{1/2}$+IBA 1.5	100	**2.009±1.4171**	2.0153±1.260	2.96153±1.5380	2.30 ±0.5201
6	$MS_{1/2}$+IBA 2.0	100	1.139±0.81135	**3.13636±1.5787**	3.8421±2.193	**3.01 ±2.3012**
7	$MS_{1/2}$+IBA 2.5	100	1.12246±0.8275	2.66667±1.4505	3.2021±1.6235	2.61±1.2540

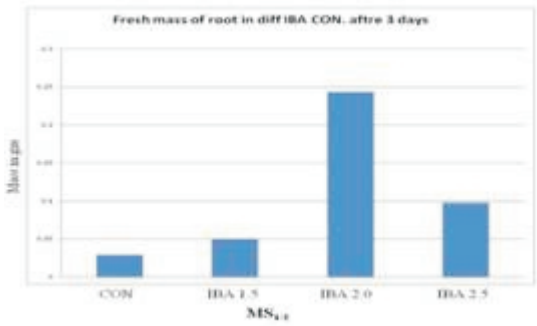
Graphical representation of rooting and shooting

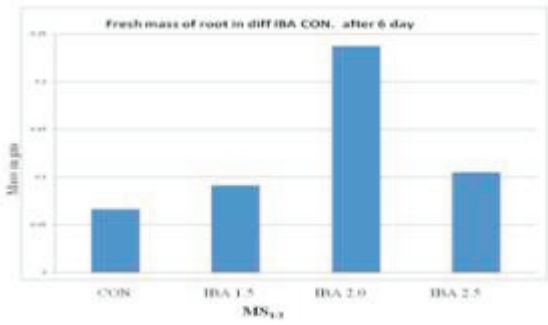






Fresh mass of root in different IBA concentration

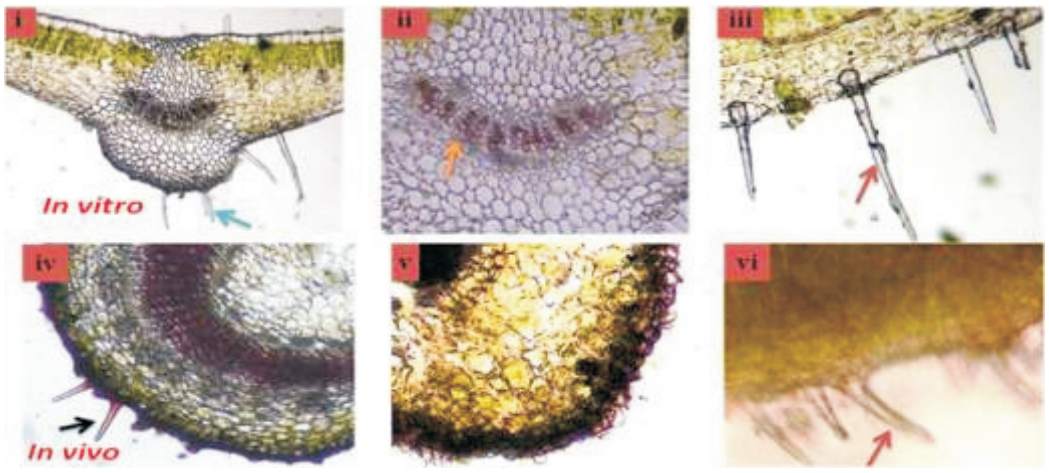




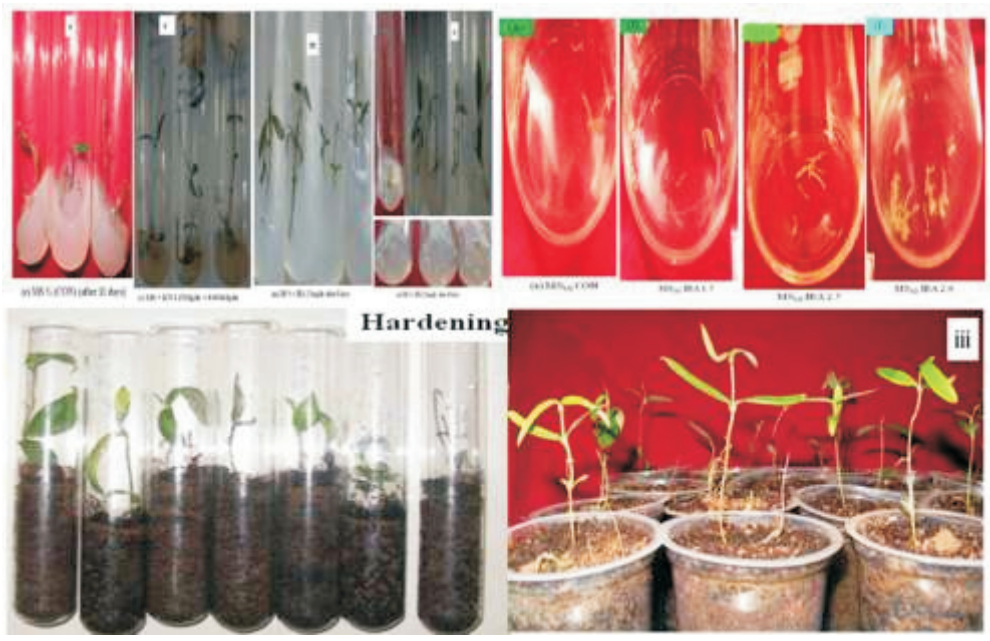
Comparison of number of trichomes *in vivo* and *in vitro*:-

No. of Field	Number of *In vivo* trichomes	No. of Field	Number of *In vivo* trichomes	No. of Field	Number of *In vivo* trichomes	No. of Field	Number of *In vivo* trichomes
1	11	1	7	11	8	11	5
2	12	2	8	12	10	12	6
3	12	3	9	13	9	13	5
4	14	4	6	14	10	14	6
5	9	5	4	15	10	15	5
6	9	6	7	16	8	16	7
7	10	7	4	17	7	17	6
8	9	8	5	18	10	18	5
9	6	9	9	19	12	19	6
10	12	10	5	20	8	20	6
				Tot.	196		121
				Mean	9.8		6.05

***In vivo* and *in vitro* anatomical observation :-**



Shoot, Root culture observation and Hardening of plants:-



CONCLUSION

In vitro multiplication of shoot on MS supplemented with Kinetin and NAA were showing maximum response and the mean number of shoots per explants is more. Were mean number of shoot length was more in MS ½ supplemented with IBA 2.0 mg/lit. This finding was similar to the findings of Sreekumar and Seeni (1998). In vitro multiplication of roots on MS ½ supplemented with IBA 1.5 mg/lit were showing maximum response and more number of rooting in liquid culture is obtained by MS ½ supplemented with IBA 2.0 mg/lit, which showing significance increase fresh mass of root. Comparative anatomical study of in vivo and in vitro plant material shows more number of trichomes in in vitro culture as compared to in vivo.

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