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In vitro studies on *Rivina humilis* L. *(Phytolaccaceae)*-An important medicinal and dye yielding Plant

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Abstract

The present study *in vitro* culture of *Rivina humilis* was carried out in MS medium supplemented with varying combination of growth regulators. The study helped to find out most suitable combination of growth regulators for shoot and root regeneration of *Rivina humilis*.

1.Introduction

Medicinal plants have been the subject of man's curiosity since times immemorial. Almost every civilization has a history of medicinal plant use. Approximately 80% of the worlds developing countries rely on traditional medicines for their primary heath needs, and about 85% of the traditional medicine involves the use of plant extract. Plants have the ability to synthesize a wide variety of chemical compounds. Many of these phytochemicals have beneficial effects on long-term health when consumed by humans, and can be used to effectively treat human diseases. It is well known that plants are the main source of valuable products and some useful basic material such as cellulose, wood and rubber. In addition, secondary products like terpenoids, cardenolides, coumarins, anthraquinones, flavonoids, glucosinolates and alkaloids are also produced by plants and are used as drugs, flavors, pigments and agrochemicals. (Amiri *et al*., 2011).

In recent years there has been a large increase in the number of research laboratories using tissue culture techniques to investigate many fundamental and applied aspects of plants. However the use of these techniques is not confined to research alone. Tissue culture techniques are being exploited by many commercial laboratories. (Misra 2009).Chemical compounds in plants mediate their effects on the human body through processes identical to those already well understood for the chemical compounds in conventional drugs; thus herbal medicines do not differ greatly from conventional drugs in terms of how they work. This enables herbal medicines to be as effective as conventional medicines, but also gives them the same potential to cause harmful side effects (Street and Shillito., 1977).

In vitro cell and tissue culture methodology is envisaged as a mean for germplasm conservation to ensure the survival of endangered plant species, rapid mass propagation for large scale revegetation and for genetic manipulation studies. Plant tissue culture studies were carried out for the preservation of medicinal plant resources and efficient production of pharmaceutically important secondary metabolites (Evan *et.al*. 1986).

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Rivina humilis Linn. belonging to the family phytolacaceae, coming under order Caryophyllales was selected for the present study.The plant is commonly known as Pigeon berry, baby peppers and blood berry. *Rivina humilis* is cultivated as an ornamental in warm regions throughout the world and is valued as a shade-tolerant groundcover. It is also grown as a houseplant and in green houses. The red berries used for cosmetics. Antibacterial activity of *R. humilis* has been reported. The berries of *Rivina humilis* contain a pigment known as Rivianin or Rivinianin, which has molecular formula $C_{24}H_{26}N_2O_{16}S$. It is very similar to betanin, the pigment found in beets The fruit also contains the betaxanthin humilixanthin (Dieter *et al*; 1987).

2. Materials and Methods

2.1. About the Selected Plant

An important medicinal and dye yielding plant *Rivina humilis* Linn. was selected for the present study. *Rivina humilis* Linn (Phytolaccaceae), is an erect, vine-like herb, reaching a height of 0.4–2 m. Flowers are on racemes 4–15 cm long with a peduncle 1–5 cm in length and pedicels 2–8 mm long. Sepals are 1.5–3.5 mm in length and white or green to pink or purplish. The fruit is a glossy, bright red berry 2.5–5 mm in diameter. This plant is usually found in Forests, thickets, shell middens, hammocks, roadsides, and disturbed areas.

2.2. Instruments and Glass wares

Culture tubes were used for the culture of plant material. The liquid detergent like Teepol were used for cleaning culture vessel were thoroughly washed with double distilled water and dried at 100°c using hot air oven. The cleaned vessels were plugged with cotton plug made of non absorbent cotton. Other glass wares include beakers, conical flasks, glass rod, pipette, scalpels, forceps, sterilized cotton, weighting balance, Petri plates, razor, spirit lamp, non-absorbent cotton plugs, test tube racks etc.

2.3. Culture Media

Murashige and Skoog's (MS) medium was used for the culturing of the plant material. Agar was used for solidifying the nutrient medium.

2.4.* Preparation of Culture media*: The components of most plant tissue culture media are inorganic nutrients (macronutrients and micronutrients), carbon source, organic supplements, growth regulators and a gelling agent. For the preparation of media, required quantity of MS medium and sucrose were dissolved in distlled water and final volume of media was made with double distilled water. The pH was adjusted by using 1 N HCl and 1 N NaOH. For the preparation of semisolid media 0.8% agar added. The media were boiled for proper mixing. The composition of MS media according to Dodd's and Robert method (1962) was follows

Table .1 **List of plant growth regulators used in the study**

COMPOUND	CHEMICAL FORMULA	MOLECULAR WEIGHT

AUXINS IBA NAA	 Indole-3 butyric acid ($C_{12}H_{13}NO_2$) Naphthalene acetic acid($C_{12}H_{10}O_2$)	 203.20 186.20
CYTOKININS BA KIN	 6-benzyl-adenine($C_{12}H_{11}N_5$) Kinetin(6-furfuryl amino purine) ($C_{10}H_9N_5O$)	 225.00 215.20

Table. 2 **Composition of MS media**

CONSTITUENTS	CHEMICAL FORMULA	AMOUNT (mg/l)
MACRONUTRIENTS		
Ammonium nitrate	NH_4NO_3	1650.00
Potassium nitrate	KNO□	1900.00
Calcium chloride	$CaCl_2.7H_2O$	440.00
Magnesium sulphate	$MgSO4.7H_2O$	370.00
Potassium dihydrogen phosphate	KH_2PO4	170.00
MICRONUTRIENTS		
Manganese sulphate	$MnSO_4.4H_2O$	22.30
Zinc sulphate	$ZnSO_4.7H_2O$	8.60
Boric acid	H3BO3	6.20
Potassium iodide	KI	0.83
Sodium molybdate	$Na2MO_4.2H_2O$	0.025
Copper sulphate	$CuSO_4.5H_2O$	0.25
Cobalt chloride	$CoCl_2.6H_2O$	0.25
IRON		
Disodium ethylene diamine tetracetate	Na EDTA	37.30
Ferrous sulphate	$FeSO_4.7H_2O$	27.80
SUGAR		
Sucrose		3000.00
ORGANIC COMPOUNDS		
Meso inositol		100.00
Nicotinic acid		0.50
Pyridoxine HCl		0.50
Thiamine HCl		0.10
Glycine		2.00
AGAR		0.8%
pH		5.7

The prepared medium was then poured into properly autoclaved dried tubes with 10 ml each and the tubes were plugged. It was then autoclaved for 20 minutes at 121°C. Then autoclaved tubes kept in a rack and kept to solidify. The prepared medium was kept for 24 hours before inoculation

2.5. Sterilization

2.5.1. Sterilization of equipments

The conical flask, petriplates, beakers, needles, forceps, blades and scalpels were thoroughly washed in tap water. They were then rinsed in distilled water and dried. Blotting papers were put in the Petri plates and they were covered with aluminium foil. The scalpels, surgical blades, needles, conical flask etc were also covered with aluminium foil. All of them were sterilized by autoclaving at a pressure of 151b/sq inch for 25 minutes.

2.5.2. Sterilization of nutrient media and double distilled water

Nutrient media were sterilized by autoclaving. For this, the media were covered with aluminium foil and subjected to autoclaving by using a pressure cooker of about 151b/sq inches for 20 minutes. As a result of which the microorganisms were killed. Higher pressure may cause the decomposition of carbohydrate and other composition of media. The media were allowed to cool after autoclaving. The tubes were stored in a clean sterile area before inoculation. The double distilled water was taken in conical flask and the mouth was covered with aluminium foil. It was then subjected to autoclaving.

2.5.3. Sterilizing culture rooms

The culture rooms were first cleaned by gently washing walls and floor with a detergent soap. This was followed by whipping them with sodium hypochlorite solution and 95% ethyl alcohol. Extreme care was needed to avoid the spreading of any contamination that may be settling on their surface. The process of sterilization of culture rooms was repeated at regular intervals. The laminar air flow cabinet was sterilized by switching on UV light for 30 minutes. It was then wiped with ethyl alcohol before initiating inoculation process.

2.5.3. **Surface Sterilization of explants**

The nodes were used as explants .The explants were washed thoroughly in running tap water. It was then washed in detergent solution (Teepol) three times. The material was again washed 3 or 4 times in double distilled water to remove all soap remains.Inoculation was done under laminar air flow cabinet under aseptic conditions . Before transferring the materials into the laminar air flow cabinet, hands, beaker, and surface of conical flask were swabbed with 70% alcohol. The materials were then transferred to laminar air flow. There it was cut into appropriate size. The material was then treated with 0.1% $HgCl_2$ for 5 minutes. Conical flask was continuously whirled. This would help to reduce the penetration of the chemical into explants. The explants then washed thoroughly with double distilled water to remove all traces of $HgCl_2$.

2.6. Inoculation

Inoculation was done aseptic conditions in a laminar air flow cabinet. Laminar air flow cabinet sterilized by wiping with 70% alcohol and switch on the UV before 30 minutes of inoculation. After 30 minutes put the blower and light 10 minutes. During inoculation, the cotton plug of the culture tubes was removed in front of a spirit lamp. The spirit lamp kept there throughout the procedure. The explants were inoculated on the solidified medium

with help of a sterile pair of forceps. The explants were placed vertically into the medium. Before closing the tubes with cotton plugs, flame the rim of the culture tube to avoid possible microbial contamination. The tubes were then plunged tightly and were then transferred to the inoculation room. The tubes were incubated at 25±2°C under 16 hours photoperiod provided by white fluorescent tubes.

3. Results and Discussion

Nodal explants of *Rivina humilis* were successfully inoculated and kept under controlled environmental conditions. *In vitro* responses of the explants were recorded at frequent intervals. For regeneration of shoot from the node, MS medium supplemented with different concentrations and combinations of growth regulators were used. And for regeneration of root, MS medium supplemented with different concentrations and combinations of auxins were used. Culturing of nodal explants on MS media supplemented with various combinations of growth regulators showed responses and result presented as follows.

3.1. *In vitro* response of nodal explants to different growth regulators in shooting media:

1. **MS medium supplemented with 1 mg/l BA + 0.2 mg/l IBA :** In this medium single shoot formation /node was observed from nodal explants within 20 days.
2. **MS medium supplemented with 1 mg/l BA + 0.5 mg/l IBA:** In this medium single shoot formation /node was observed from nodal explants within 15 days .
3. **MS medium supplemented with 1 mg/l BA + 1 mg/l IBA:** In this medium single shoot formation /node was observed from nodal explants . The shoot elongation was observed in the same medium within 20 days.
4. **MS medium supplemented with 1.5 mg/l BA + 0.2 mg/l IBA:** The explants showed slight bulging after 5 days. Multiple shoot were formed after 15 days in 30% of the culture tubes. After 20 days, explants were turned to brown in colour, growth was very slow.
5. **MS medium supplemented with 3 mg/l BA + 3 mg/l IBA:** In this concentration and combination of growth regulators, the explants show maximum *in vitro* response. The nodes were bulged after 5 days of inoculation and after 13 days multiple shoots (2-5/node) were observed in 80% of the culture tubes. Shoots attain 1-4 cm in length. The shoots attain maximum size after 25 days.
6. **MS medium supplemented with 4 mg/l BA + 3 mg/l IBA:** After 12-15 days of inoculation callus was formed in 50% of the culture tubes. Shoots was initiated after 18-20 days. Shoot growth continued for 4-6 days. Shoots were sub cultured on the medium having same growth regulator concentration but no further growth was observed.
7. **MS medium supplemented with 5 mg/l BA + 5 mg/l IBA:** Slight bulging observed from the 13th day after inoculation but the growth rate was very slow. Only 20% culture tubes were responded. After 22 days growth was stunted and it become weak and did not show shoot initiation
8. **MS medium supplemented with 0. 5 mg/l KN:** Callus formation was observed in this media. But no shoots and roots were formed.
9. **MS medium supplemented with1 mg/l KN + 0.5 mg/l IBA:** Callus formation was observed in this

media. But no shoots and roots were formed.

10. **MS medium supplemented with1. 5 mg/l KN +0. 5 mg/l IBA** Callus formation was observed in this media. But no shoots and roots were formed

3.2. Media for root regeneration: Explants were cultured in rooting media supplemented with auxin for development.

1. MS medium supplemented with 0.5mg/l IBA: In these medium root formation was observed within 15 days.

2. **MS medium supplemented with 1mg/l IBA:** Root initiation starts after 5 days of inoculation.

Table. 3 *In vitro* response of nodal explants of *Rivina humilis* on MS medium supplemented with varying combinations of growth regulators.

Sl. No:	Growth regulator (mg/l)	% of Response	No. of Shoots/node	No. of Roots/node
1	**1 mg/l BA + 0.2 mg/l IBA**	20	1.1±1.2	--
2	**1 mg/l BA + 0.5 mg/l IBA**	20	1.2±1.12	--
3	**1 mg/l BA + 1 mg/l IBA**	20	3.1±1.71	--
4	**1.5 mg/l BA + 0.2mg/l IBA**	30	5.3±1.89	--
5	**3 mg/l BA + 3 mg/l IBA**	80	6.1±2.7	--
6	**4 mg/l BA + 3 mg/l IBA**	50	1.8±1.35	--
7	**5 mg/l BA + 5 mg/l IBA**	20	1.8±1.17	--
8	**0.5mg/l KN**	70	--	--
9	**1 mg/l KN + 0.5 mg/l IBA**	80	--	--
10	**1.5mg/l KN+0.5mg/l IBA**	85	--	--
11	**0.5mg/l IBA**	40	--	9.3±2.12
12	**1mg/l IBA**	30	--	8.1±1.69

(MEAN ± SD)

Statistical analysis was done by Karl Pearson's standard deviation method.

Growth and organogenesis of explants in tissue culture is influenced by genotype of explants, environment and tissue development factors. In the present study the effects of cytokinin (BA) in combination with auxin (IBA) were studied. Effectiveness of 0.1% HgCl2 for the surface sterilization was reported earlier in plants like *Phytolacca esculent*

(Shi, 2003).In the present studies also 0.1% HgCl2 treatment was effective for the surface sterilization of explants of *Rivina humilis*.

(Juliani *et al*., 1999), reported shoot formation in medium containing low concentration of auxin and high concentration of cytokinin. Nodal explants of *Rivina humilis* also showed similar response. Results in the present experiments showed that BA was highly effective than KN for the multiple shoot induction of *Rivina humilis.* The simulative effect of BA over KN in direct shoot regeneration was reported earlier in many plants (Babu et al., 2003; Julani et a1., 1999; Sahoo and Chand 1998; Tiwari *et al*., 2001; Jagadishchandra *et al*., 1999; Komalavally & Rao, 1997; Naik *et al*., 1999; Vincent *et a1*., 1998;and Ulubelde *et al*., 1991).

Present studies also demonstrated efficacy of IBA for the rooting of *in vitro* raised shoots of *H. keralense.* Similar results showing efficacy of IBA in *in vitro* rooting was reported in many plant species (Andoh *et al*., 2005; Echeverrigaray *et al.,* 2000; Faisal & Anis, 2003 and Fracaro & Echeverrigaray, 2001). The protocol described here would be useful for conservation and rapid multiplication of *Rivina humilis*.

4. Conclusion

In the present study *in vitro* culture of *Rivina humilis* was carried out in MS medium supplemented with varying combination of growth regulators. The study helped to find out most suitable combination of growth regulators for shoot and root regeneration of *Rivina humilis*. From the observations noticed at regular intervals, it can be concluding that the equal amount of cytokinin and auxin will produce good result in shoot formation (BA + IBA). So a combination of BA and IBA was most effective for the direct shoot regeneration. The presence of auxin alone in the medium induces root formation. Maximum root regeneration was observed on MS medium supplemented with 0.5 mg/l IBA about 40% of the culture tubes were responded. Medium with 1 mg/l IBA showed about 30% of response.

5. References

Amiri, S., Kazemitabar, S,K., Ranjbar, G.A and Azadbakhat, M. (2011). *In vitro* propagation and whole plant regeneration from callus in Datura. *African J. Biotechnol.,* **10**:442-448.

Andoh,Y. Yahara, S. and Yoshizawa, N. (2005). *In vitro* regeneration of the medicinal plant *Phellodendron amurense* Rupr. Through excised leaves. Plant Cell Tiss. Org. Cult., **80:** 43-50.

Babu, K.N., Sajna, A., Minoo, D., John, C.Z., Mini, P M., Tushar, K.V. and Ravindran, P.N. (2003). Micropropagation of Camphor tree *(Cinnamomum camphora). Pl. Cell Tiss. Org. Cult.,* 74: 179-183.

Dieter, S., Doris, S., Hans, R., Wilhelm, B., Lutz, G., and Victor, W. (1987). Humilixanthin a new betaxanthin from *Rivina humilis*. Phytochem., **26(**8): 2285–2287.

Dodd's, J.H and Robbert, L.W.(1962). *Experiments in plant tissue culture,* Cambridge University press, Cambridge, Lonndon, New York.

Echeverrigaray, S., Bias, S., Fracaro, F. and Sarafini, S. (2000). Clonal micropropagation of Roman Charnomile *(Anthemis nobilis* L.). Med. Plants, 7(2): 35-41.

Evan, J.S., Pattison, E. and Morris, P.(1986). Antimicrobial agents from plant tissue culture, in:

secondary metabolites in plant cell culture.

Faisal, M. and Anis, M. (2003). Rapid mass propagation of *Tylophora indica* Merill via leaf callus culture. *PIant Cell. Tiss. Org. Cult.,* 75: 125-129 .

Fracaro, F. and Echeverrigaray, S. (2001). Micropropagation *of Cunila galiodes,* a popular medicinal plant of South Brazil. *PIant Cell Ttiss. Org. Cult.,* 64: 1-4.

Jagadhishchandra, K.S. Rachappaji, S. Gowda. K. R. and Tarasaraswathi, K.J. (1999). *In vitro* propagation of *Pisonia alba* (L) Sapanogae (lettuce tree) a threatened species. *Phytomorphol.,* 49: 43-47.

Juliani H.R., Koroch, A.R. and Trippi, V.S. (1999). Micropropagation of *Lippia junelliana* (Mold.) Tronc. *Pl. Cell Tiss. Org.Cult.,* 59: 175-179.

Komalavalli, N. and Rao, M.N. (1997). *In vitro* micropropagation Gymnema *elegans* W&A, A rare medicinal plant. *Ind. J. Exp. Biol.,* 1088-1092.

Misra, S.P. (2009). The Plant Tissue Culture, *New central Publications, Kolkata*, pp: **29**-33.

Naik, S.K., Pattnaik, S.S. and Chand, P.K. (1999). In vitro propagation of pomegranate *(Punica granatum* L) through axillary shoot proliferation from nodal explants of mature tree. *Scient. Horticult.,* **79:** 175-183.

Sahoo, Y. and Chand, P.K. (1998). Micropropagation of *Vitex negundo* L. a woody aromatic medicinal shrub, through high frequency axillary shoot proliferation. *PIant Cell Rep.,* 18: 301-307.

Shi, H.P., Lang. P. and Quan, H. (2003). *Induction of hairy boots in Phytolacca esculenta and its saponin production.* South China University. Guangzhou, **1**:9-46.

Street, H.E and Shillito, R.D.(1977). Nutrient media for plant organ, tissue and cell culture In: Rechcigl M (Ed) CRC Hand Book in *Nutrition and Food*, Vol. **4**, *CRC Press* Boca Raton. New Delhi.

Tiwari. V., Tiwari, K.N. and Singh, B.D. (2001). Comparative studies of cytokinins on in vitro propagation of *Becopa monnieri.. PI. cell Tiss. Org. Cult.,* 66: 9-16.

Ulubelde, M., Ekim, M. and Tan, A. (1991). The aromatic medicinal plants in Turkey. In; Raychanduri S.P. (Eds.) *Recent Advances in Medicinal, Aromatic and spices Crops*. Today and Tomorrow's Printers and Publishers, New Delhi.

Vincent, K.A. Martin, K.P. and Hariharan, M. (1998). In vitro culture studies on *Kampferia galanga* L. In; Khan, I.A. and Khanum, A. (Eds.). *Role of Biotechnology in Medicinal and Aromatic Plants*. Ukaaz. Publ. Hyderabad, India.