EFFECT OF PLANT GROWTH REGULATORS ON INDUCTION OF ADVENTITIOUS ROOTS FROM DIFFERENT EXPLANTS OF *RIVINA HUMILIS* L.

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ABSTRACT

An efficient reproducible protocol for induction of adventitious roots from different explants of *Rivina humilis* L. has been developed. The explants cultured on MS and B5 media with different concentrations of auxins with 2.0 mg/L gibberellin as constant. The explants of *Rivina humilis* L. were responded uniquely with specific concentrations of auxins. However high frequency of adventitious root induction was found on B5 media than MS media. Petiole explants on B5 media supplemented with GA_3 (2.0 mg/L)+ IBA (2.5 mg/L), leaf and nodal explants on B5 with GA_3 (2.0 mg/L)+ IAA (2.0 mg/L), root explants on B5 with GA_3 (2.0 mg/L)+ NAA (5.0 mg/L) and stem callus on B5 with GA_3 (2.0 mg/L)+ 2,4-D (2.0 mg/L) showed high frequency of adventitious root formation. NAA (5.0 mg/L) with GA_3 (2.0 mg/L) found suitable to induce high frequency of adventitious roots from all explants used in the present study.

Key words: Adventitious roots, Growth regulators, *In vitro*, Nodal expalnt and *Rivina humilis* L

Abbreviations

IAA Indole-3-acetic acid, IBA Indole-3-butyric acid, GA_3 Gibberlic acid, NAA Naphthalene acetic acid
PVP Polyvinyl pyrolidine, 2, 4-D 2, 4 dichlorophenoxy acetic acid, MS Murashige and Skoog (1962) media
B5 Gamborg *et al.* (1968)

INTRODUCTION

Plant tissue culture techniques have been proved as a potential tool for the production of useful secondary metabolites. Thus, using this technology, secondary metabolites can be produced under controlled conditions, independent of geographical and climatic factors (Latiporn Udomsuk *et al*, 2009). Roots of numerous plant families are the site for biosynthesis and accumulation of major secondary metabolites including alkaloids, polyacetaline, sesquiterpenes and napthoquenones (Iyyakkannus and Byoung 2009). *In vitro* root culture has become an alternative method for the production of valuable secondary metabolites on commercial scale. Adventitious roots induced by *in vitro* methods showed higher rate of proliferation and active secondary metabolism (Hahn *et al.* 2003; Yu *et al.* 2005). Adventitious roots are natural, grown vigorously in phytohormone supplemented medium and have shown tremendous potentialities of accumulation of valuable secondary metabolites (Murthy *et al.* 2008). Adventitious roots have been reported in both technical and non-technical terms. Esau (1977) stated that adventitious roots are the root that arises from the site other than usual sites such as roots originating from stems or leaves. On the other hand, Barlow (1986) has defined adventitious roots as roots arise on parts of the plants not originating from the embryonic root; that is, the roots arise from parts of the shoot.

The medicinal property of a plant is due to the secondary metabolites produced in it. One such group of chemicals is flavonoids which are distributed in all parts of the plant (Buschmann 2002).

Production of isoflavonoids through adventitious root cultures of *Iris germanica* has been reported by Tomoyoshi *et al.* (2005) they noticed that the adventitious root cultures accumulated a high concentration of isoflavones that are capable to be utilized in medicine production . However, adventitious root formation is a complex process that is affected by multiple endogenous factors, including phytohormones and other environmental factors. The molecular mechanisms by which adventitious root formation is regulated are still poorly understood. Auxin plays a central role and may interact with other endogenous factors or environmental stimuli, such as light (Blakesley 1994, Celine *et al.* 2005) in induction of adventitious roots.

Available literature on *Rivina humilis* L. indicates that no *in vitro* studies has been undertaken, hence the present study is under taken to evaluate the effect of plant growth regulators, and manipulation of media

composition for the induction of adventitious roots from different explants of *R. humilis* L.

MATERIALS AND METHODS

Rivina humilis L. is an exotic economically important ornamental herb. The plant belongs to the family Phytolaccaceae and is native to tropical America (Ramaswamy and Razi 1973). A branched herb has simple alternative leaves with purple coloured flowers producing an attractive red coloured fruits, which has given a common name to the plant as "Blood berry". The berries are source of red dye and plant has claimed for higher values to treat cold, diarrheoa, marasmus, febrifuge and wounds.

The plant is well known for its red dye yield; even in the present study pigmentation and production of brown colouration on media is observed. This problem was overcome by treating explants with 0.8% (w/v) PVP prior to inoculation.

Explants

The seeds of *Rivina humilis* L. collected from the plants grown in the Botanical garden of Karnatak University Campus, Dharwad, India, were germinated. *In vitro* germinated plant parts like leaf, petiole, node and roots used as explants. The *in vivo* explants are obtained from the plants grown in the garden are also used in the present study.

Sterilization of seeds and *in vitro* explants.

Freshly harvested seeds of one year old plant were collected and subjected for surface sterilization. Seeds are washed with 1% (v/v) Tween 20 liquid detergent for 15 minutes and followed by three washes in sterile $D.H_2O$. Then it is treated for 5 minutes in Bavistin 1% (w/v) to kill fungal pathogens. After thorough washing, the seeds were rinsed with 70% (v/v) ethanol for 0.5 minutes in Laminar Air Flow Chamber. Further, it is treated with 1% (v/v) NaoCl for 1 minute, 0.15% (w/v) aqueous mercuric chloride ($HgCl_2$) solution for 1 minute and then it is rinsed three times in sterile $D.H_2O$. Finally the seed coat is ruptured with the help of sterile needle to break physical dormancy and again rinsed in sterile $D.H_2O$.The seeds thus sterilized were inoculated on to culture tubes containing basal MS media, later it is incubated in dark initially for a period of 72 hours and after transferred to light conditions. Thus grown 30 days old seedling organs are used as explants, i.e. petiole, leaf, node and root. Explants were inoculated on MS and B5 media formulated with GA_3 (2.0 mg/L) and different concentrations of auxins.

Sterilization of *in vivo* explants

Leaf, petiole, nodal and root explants collected from *in vivo* grown plants were surface sterilized. They were washed by 1% (v/v) Tween 20 for 15 minute followed by 5 minutes treatment with 1% (w/v) Bavistin and washed three times in sterile $D.H_2O$. These explants were taken into the Laminar Air Flow Chamber, where they were treated with 70% (v/v) ethanol for 0.5 min, 1% (v/v) Sodium hypochlorite for 1 min., 0.15% aqueous mercuric chloride and 3 times washed in sterile $D.H_2O$. Finally the explants were trimmed to appropriate sizes (5mm long nodal, petiole and root explants, 5X5mm leaf explants) and inoculated on to MS and B5 media.

RESULTS AND DISCUSSION

Adventitious rooting is a quantitative genetic trait regulated by both environmental and endogenous factors (Celene Sorin *et al.* 2005). Adventitious root culture provides reliable means and good prospects of eco-friendly synthesis of large number of secondary metabolites. The adventitious roots may be induced by manipulating culture media and or altering physical environment. An alternative method by transgenesis is also well employed and specially termed as, hairy root induction. There has been a large number of research papers published on transgenic root culture. However, in the present study induction of adventitious roots is attempted by manipulating nutrient media with different concentrations of auxins and constant use of GA_3 2.0 mg/ L. Ziauka and Kuusiene (2008) and Hansen (1988) have reported that the gibberellin is a root inhibitor; root formation was significantly decreased by addition of GA_3 on aspen explants, on the contrary, the present study it is observed that the positive effect of GA_3 on root formation in different explants of *R. humilis* L. However, the higher concentration of GA_3 above 3.0 mg/L inhibited root formation. Therefore it is optimized that 2.0 mg/L GA_3 for the better response of *in vitro* adventitious root induction with different concentrations of Auxins.

The explants are treated with 0.8% (w/v) PVP prior to inoculation to overcome browning, the similar type treatment was done by Gupta *et, al.* 1980) in *Tectona grandis* L. terminal and axillary buds were suspended in 2%sucrose solution and 0.7% soluble polyvinyl pyrolidine before planting on a semisolid medium.

Effects of IAA, IBA, 2-4D and NAA on adventitious root induction

It is well established that the root development is controlled by hormonal signals, especially auxins (Celenza *et al.* 1995), and that environmental cues can act on the genetic programs for root development and their hormonal and metabolic control (Zhang *et al.* 1999; Martin *et al.* 2008). Roots of certain higher plants synthesize and secrete a remarkable diversity of secondary metabolites. In such cases, mass production of roots through either the induction of transformed roots (Hairy roots) using *Agrobacterium rhizogenes* or

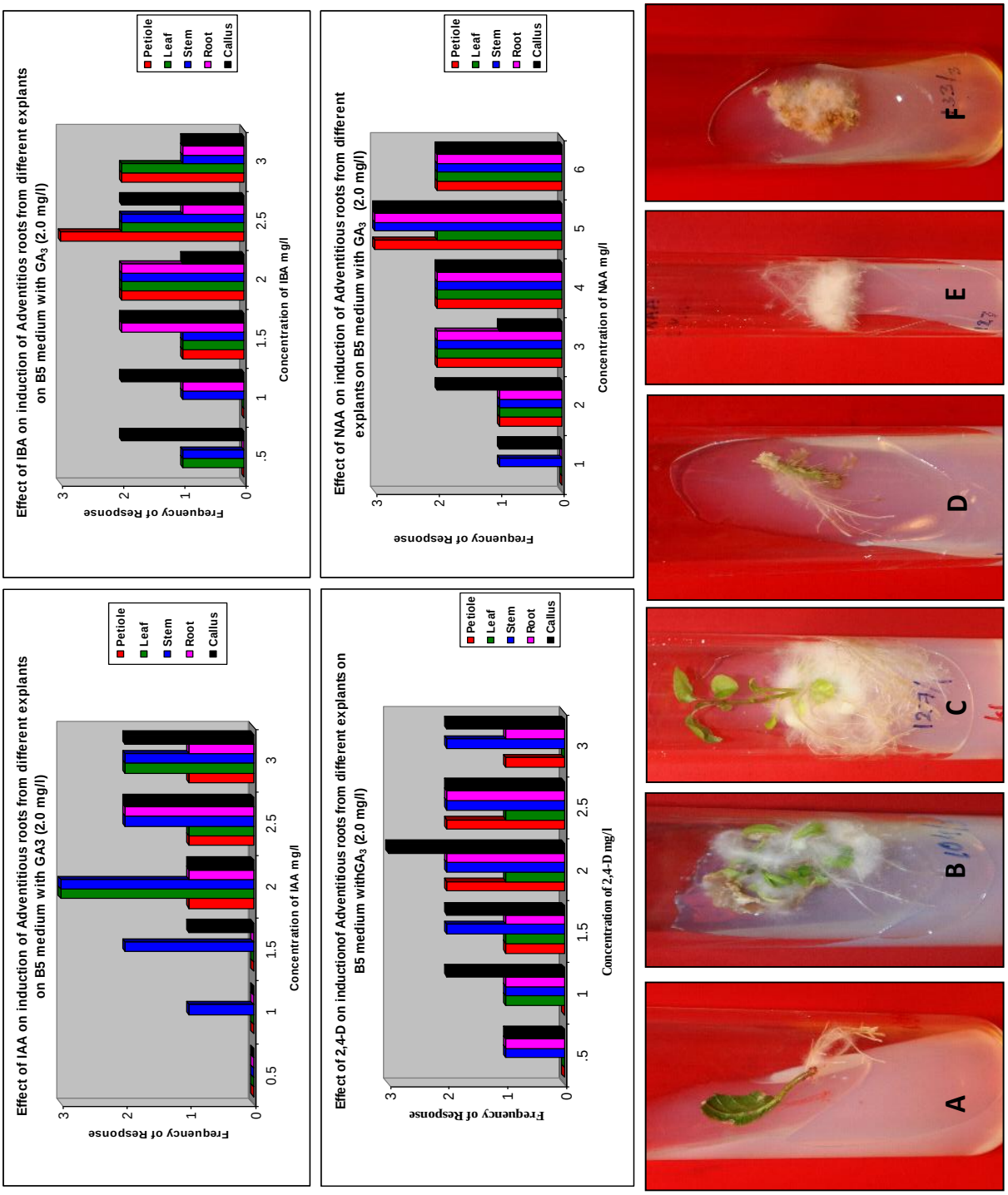

Fig: 2. Photographs showing adventitious roots from different explants of *Rivina humilis* L.

Frequency of Response: 0= No response, 1= Poor response, 2= Moderate response and 3= Good response.

A. Induction of Adventitious roots from petiole explants on B5+GA3 (2.0 mg/l) +IBA (2.5 mg/l).

B. Induction of Adventitious roots from leaf explant on B5+GA3 (2.0 mg/l) +IAA (2.0 mg/l).

C & D. Induction of Adventitious roots from nodal explants on B5+GA3 (2.0 mg/l) +IBA (2.5 mg/l).

E. Induction of Adventitious roots from root explant on B5+GA3 (2.0 mg/l) +NAA (5.0 mg/l).

F. Induction of Adventitious roots from callus explant on B5+GA3 (2.0 mg/l) +2, 4-D (2.0 mg/l).

Table: 1. Effect of auxins on induction of Hairy roots on B-5 medium supplemented with GA_3 (2.0 mg^{-1}) on different explants of *Rivina humilis* L.

IAA mg^{-1}	Petiole	Leaf	Node	Root	Callus
0.5	-	-	-	-	-
1.0	-	-	+	-	-
1.5	-	-	++	-	+
2.0	+	+++	+++	+	+
2.5	+	+	++	++	++
3.0	+	++	++	+	++
2,4-D mg^{-1}					
0.5	-	-	+	+	+
1.0	-	+	+	+	++
1.5	+	+	++	+	++
2.0	++	+	++	++	+++
2.5	++	+	++	++	++
3.0	+	-	++	+	++
IBA mg^{-1}					
0.5	-	+	+	-	++
1.0	-	-	+	+	++
1.5	+	+	+	++	++
2.0	++	++	++	++	+
2.5	+++	++	++	+	++
3.0	++	++	+	+	+
NAA mg^{-1}					
1.0	-	-	+	-	+
2.0	+	+	+	+	++
3.0	++	++	++	++	+
4.0	++	++	++	++	++
5.0	+++	++	+++	+++	+++
6.0	++	++	++	++	++

Abbreviations: '-' No response, '+' Poor response, '++' Moderate response, '+++' Good response.
Note: Based on density of roots in the culture tubes above observations are made.

the development of adventitious roots (nontransformed roots) by the manipulation of culture media is a reliable route for the production of secondary metabolites. The nontransformed root system necessitates a special mention because of easy of production of valuable pharmaceutical compounds without genetic modification of the plant genome (Martin *et al.* 2008). Additionally Muthy *et al* (2008) mentioned that in many transformation studies selectable marker genes are used to identify genetic transformation. Use of genetic markers has raised question of human health concern when the target material is a functional food. For these types of problems production of adventitious roots without transformation is better alternative method.

Nodal explants were found to be the best explants of *R. humilis* L. in adventitious root induction in the present study.

In the present investigation it showed that the IAA induced high efficiency of adventitious roots from leaf explants of *Rivina humilis* at 2.0 mg/L concentration (2.0 mg/L IAA) and even nodal explants are also responded better and induced adventitious roots. Similarly, Anna Pick Kiong Ling *et al.* (2009) considered IAA as the best auxin in induction of adventitious roots from leaf explants of *O. stamineus* L. They observed better rooting response on MS medium supplemented with IAA at 3.0 mg^{-1}.

The petiole and root explants have developed adventitious roots on B5 medium supplimented with IBA

(2.5 mg/L). Farzin M Parabia *et al.* (2007) reported adventitious root induction by micro shoots of *Leptadenia reticulata* L. on MS media supplemented with 2.5 mg/L IBA.

2,4-D is also induced adventitious roots from callus on B5 with 2.5 mg/L concentration. On the contrary Praveen et *al.* (2009); Goa *et al.* (2005) reported that 2, 4-D is responsible for callus induction.

In the present study it is observed that an efficient high frequency of adventitious root induction on B5 medium supplemented with NAA (5.0 mg/L). Among the explants used in present study, except leaf, all explants (petiole, node and roots) shown good efficiency of adventitious root induction on B5 supplemented with NAA 5.0 mg/L and GA_3 2.0 mg/L. Similarly Pandey *et al.* (2010) have reported that addition of 4.0 mg/L NAA promoted root growth from leaf explants of *Rauwolfia serpentine* L. Agnieszka Pietrosiuk *et al.* (2007) noticed B5/2 liquid medium supplemented with 0.5 mg/L NAA used to induce Hairy roots in nontransformed cultures of *Catharanthus roseus*. Altinkut *et al.* (1997) also reported that 1.0 mg/L NAA on MS increased rooting efficiency up to 78% in Red Chickpea.

Conclusion

Adventitious roots are the promisive structures for the large spectrum of accumulation of secondary metabolites. In the present study an attempt is made to induce adventitious roots by manipulating the hormone concentrations from the economically important ornamental herb *R. humilis* L. which is claimed to treat cold, diarrhea, marasmus, febrifuge and more diseases.

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