Rapid *in vitro* multiplication, regereration and rooting of Kalnesg (*Andrographis paniculata* Nees.)

Raajesh K. Yadav[1], Lakha Ram[2], Kumbha Ram Mahala[3], Raaz K. Maheshwari*[3]

1. **Department of Zoology, SS Jain Subodh PG College, Jaipur, Rajasthan**
2. **Department of Chemistry, JNMP Govt PG College, Phalodi, Jodhpur, Rajasthan**
3. **Department of Chemistry, SBRMGovt PG College, Nagaur, Rajasthan**

ABSTRACT

Andrographis paniculata is a promising new herb for the treatment of many diseases including HIV-AIDS and the myriad of symptoms associated with autoimmune disorders, and it has been used in bacterial dysentery, arresting diarrhea and in upper respiratory infections, tonsillitis, pharyngitis, laryngitis, pneumonia, tuberculosis, and pyelonephritis. Due to high medicinal property it's having high market demand in Homeopathy as well as in Ayurveda. The natural propagation of these plants is very poor and their populations are declining with an alarming rate Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used rural herbal remedies. Systematic approaches were made in order to achieve multiple shoot induction from seedling segment viz. cotyleuonary node, epicotyledonary node of *Andrographis paniculata*. In *Andrographis paniculata* BAP 13.31µM/lit with IAA. (20 shoots with 33.5mm length) from seedling segments. Amongst all the concentration of BAP with IAA tried, 13.31µM/lit BAP+5.70µM/lit IAA was found the best for further multiplication of *Andrographis paniculata* shoots. Incorporation of PVP (100Mg/lit) ascorbic acid (50Mg/lit) and citric acid (100Mg/lit), checked browning of culture.

K e y w o r d s*: Andrographis paniculata*; *Autoimmune disorders;* IAA; BAP; PVP

INTRODUCTION

Andrographis paniculata is one of the important medicinal plant species belonging to the family Acanthaceae. The plant is known by various vernacular names viz., Kalu kariyatu (Gujarathi), Birbhubat (Hindi), Banchimani (Marathi), Gopuramthangi (Malayalam and Tamil) and False water willow (English). The plant is an erect, annual herb, simple or slightly branched, growing up to a height of 20 to 60 cm. In the Indian Systems of Medicine, predominantly Andrographis *paniculata* is used against blood cancer. The leaf extract is recommended for oral consumption. Traditionally, the plant has been used as febrifuge, bitter tonic, astringent, anodyne and also for dysentery, cholera and diabetes. The ethanol extract of this plant used as diuretic and in sluggishness of liver and jaundice has been reported as the modern use of this plant. It is used to treat gastrointestinal tract and upper respiratory infections, herpes, sore throat, hepatitis and a variety of other chronic and infectious diseases. It exhibits antibacterial, antimalarial, antidiarrhoeal, cardiovascular activities and protection of liver and gallbladder.

Conventional vegetative propagation of this important medicinal plant is very difficult and too slow to meet the commercial quantities required. The heavy demand of andrographolide in Indian as well as international markets has motivated Indian farmers to start commercial cultivation of this medicinal plant. Considering the medicinal importance of A. neesiana, the present investigation undertaken to develop a rapid and efficient in vitro multiplication and regeneration system of this species, using in vitro nodal explants.

ACCUSTOMED PRACTICES

Andrographis paniculata has been reported as having antibacterial, antifungal, antiviral, choleretic, hypoglycemic, hypocholesterolemic, adaptogenic, anti-inflammatory, emollient, astringent, diuretic, carminative, anthelmintic, antipyretic, gastric and liver tonic. It has extremely beneficial activities antioxidant, antihelminthic, antipyretic, anticancer and antidiarrhoeal effects. [Sudhakaran MV 2012]. Because of its "blood purifying" activity, it is suggested in cases of raktapradoshaj vikara like leprosy, gonorrhoea, scabies, boils and skin eruptions, chronic and seasonal fevers. Swaras of leaves is given to infants for the relief in griping, irregular bowel habits, and loss of appetite. Its leaves and roots are also used in general weakness, febrile convalescence, gaseous distension related dyspepsia and in advanced stages of dysentery. Ayurvedic formulations used to treat liver disorders and can be widely used to treat neoplasm as mentioned in ancient Ayurvedic literature. [Saxena et.al.,1998] Andrographis paniculata is used for the treatment of pharyngolaryngitis, diarrhoea, dysentery and cough with thick sputum, carbuncle, sores and snake bites. Various preparations and compound formulas of the herb have been used with significant effect rates to treat infectious and noninfectious diseases, described for conditions such as epidemic encephalitis B, suppurative otitis media, neonatal subcutaneous annular ulcer, vaginitis, cervical erosion, pelvic inflammation, herpes zoster, chicken pox, mumps, neurodermatitis, eczema, and burns. A primary use of Andrographis paniculata is for the prevention and treatment of the common cold. It appears to have antithrombotic actions, so it is beneficial in cardiovascular disease. Pharmacological and clinical studies suggest that it has potential to treat in diseases like cancer and HIV infections Balachandran and Govindarajan ,2005].

PHYTOCHEMICAL CONSTITUENTS

Therapeutically active constituent of Kalmegh found in aerial parts. Andrographis paniculata contains diterpenes, lactones and flavonoids and polyphenols. [Pandey et. al., 2011)]. Flavonoids mainly exist in the root, but have also been isolated from the leaves. Aerial parts contain alkanes, ketones, and aldehydes and the bitter principles in the leaves were due to presence of the lactone andrographolide named kalmeghin. Andrographis paniculata has various compounds in its aerial parts and roots and these are often used in extracting its active principles. Diverse factors such as geographical region, harvest time and processing method account for the variability in Sits chemical content. [Phosphane et. al., 2014; Li W.K. and Fitzloff J.F.,2004]. The primary active constituent of Andrographis paniculata is the Andrographolide. [Pandey et. al.,2011] It is colourless bitter in taste and crystalline and known as diterpenes lactone. There are four lactones in Andrographis paniculata viz., (1) 14- deoxyandrographolide and (2) andrographolide, (3) neoandrographolide (a non-bitter, C 3 O glucoside derivative of the major constituent andrographolide) and (4) 14-deoxy11,12-di-dehydro-andrographolide. Other compounds include 14-deoxy-11- oxoandrographolide, di-dehydro andrographolide/andrographolide D, 14deoxyandrographolide, non-bitter compound is neo andrographolide,

homoandrographolide, andrographosterin, andrograpanin, α sitosterol, stigma sterol. Apigenin-7, 4- dio-methyl ether, 5hydroxy 7, 8, 2, 3- tetramethoxy flavones, monohydroxy trimethyl flavones, andrographin, dihydroxy di-methoxy flavoue, panicolin, andrographoneo, andrographoside, andropani-culoside an andrograpanin, Isoandrographolide and skollcaflavone (912). Six ent-labdene diterpenoid i.e. 3-o-betaDglucopyranosyl-14, 19dideoxyandrographolide, 14-deox, 17hydroxyandrographolide, 19-o-[betaD-apiofuranosy 1-2betaDglucopyranoyl]-3, 14- dideoxyandiographolide, 3-obetaDglucopyranosyl-andro-grapholide, 12Shydroxy andrographolide and andrographatoside. These compounds showed inhibitor activity against several fungal and bacterial strains. Bharati et. al., 2011; Nyeem et. al., 2017;Chandran et.al.,2017].

MATERIALS AND METHODS

SEED GERMINATION

Plant material was obtained from in vitro seedling. Seeds were sterilized by sequential treatment (5% activated chlorine, 20 min) of sodium hypochlorite and 0.1% HCl solution for 5 min and washed with autoclaved distilled water 3–4 times and sprouted at half strength. Sucrose, seedlings were collected 5-6 weeks old.

SHOOT INDUCTION AND ELONGATION

Transplanting was used to reproduce several shoots. Inattentive to investigators on MS medium with different combinations and concentrations of plant growth connectors, ie BAP (2.21 toμM / lit to 22.19μM / lit), Kinetin (2.32μM / lit to 23.23μM / lit), IAA (0.5μM /lit) was disabled. 5.0 to 0.5μM / lit) and NAA (0.53 to 6.71μ M / lit.). Mean 50% Epicotiledonary explants with an auxin (IAA) in combination with two cytokinins (BAP and kinetin) showed shoot regeneration on BAP (2.21 μ M / lit), kinetin (2.32μM / lit) and IAA (0.57 μM / lit). A means of 3 shoots was generated on this medium, while achieving a length of 14 mm in 4–5 weeks. The concentration of BAP, kinetin and IAA increased. The percentage of shoot induction from explorers also increased with the number of shoots and their length in aseptic conditions. The maximum number of explorer regeneration (98.0%) found in combination with BAP (13.31μM / lit), kinetin (13.96μM / lit).) And IAA (7.13μM / lit) were produced on this medium with 45weeks with a mean 36.5 mm length. As a concentration of BAP, kinetin and IAA had reduced the percentage response of explant and shoot induction rates to 71.4–11.85%.

Epicotyledonary nodes from sterile shoots were cultured on MS salts, vitamins, and 3% sucrose with different concentrations of exosplastic (length 36.5 mm) 2, 4-D (4.52μM / lit to 22.62μM / lit), IAA + NAA (IAA 2.85μM. / Lit to17.12 μM / lit and NAA 2.68 μM / lit to 16.11 μM / lit) for 5 weeks. 2, 4-D (4.52μM / lit) NAA + IAA (IAA 2.85μM / lit and NAA 2.68 NAM / lit) were added to the medieval, producing 2 shoots with 5 mm length and callus induction. As concentrations of 2, 4-D increased 6.78 μM to 22.62 μM / lit, disrupting shoot formation. Small calluses of 2, 4-D (6.78μM / lit), medium calluses of 2, 4-D (9.04μM / lit) and huge calluses of 2, 4-D (13.57μM to 22.62μM / lit) by the explorers was inspired.

HARDENING PROCESSING

Once a reproducible process for large-scale plant regeneration is achieved *in vitro* and a sufficient number of plants are obtained, steps are taken in the next step - (1) hardening of plants i*n vitro* (2) Acceleration and finally transfer to (3) the area where plant performance is to be tested. These stages are important and important because the behavior of unequally grown plants is very different from that of gown plants *in vivo* and the plants are transferred from aseptic conditions to free living conditions, *in vitro* to the hard nutrients of the plants. A gradual decrease in levels is done with the promotion of root to moderate auxin and culture conditions. Acceleration *in vitro* involves exposure to plants to reduce relative humidity and the outdoor environment without disturbing or injuring delicate humidity and shoot systems. If a mixture of sterilized soil is used to move plants, it eliminates the transition changes but in some cases uncultivated soil can be used to move plants. Some plants produce a small callus *in vitro*, on which microorganisms begin to grow and move into the shoot due to infection, in which case sterilized soil should be used. The roots of agar and agar should follow and the plant should be washed gently before transfer. Once the roots are ready to move, uplift for autotrophic adaptation before transfer to the field position, and reposition. The plants under investigation are well adapted to the environment, but they differ in nature in the comfortable environment of culture vessels. These reflect a general decline of growth upon transfer to the region. The plants produced *in vitro* were hardened under aseptic conditions to harden. Rootlets developed a root system within 4-6 weeks on planting media. These plants were not transferred to fresh medium for hardening, but under low humidity (RH 50%) and slightly higher temperatures (30°C±2°C) and light (2000–2500 lux) of intensity under these cultural conditions a Only medium was allowed to grow. Plants were planted in 24 hours. In vitro regenerated plants in Andrographis *pancreatata* were transferred to pots containing dim soil with vermicite. Plants were uprooted after 48 hours on sand dune soil + vermiculite in a ratio of 1: 1. The soil mixture of natural habitat + vermiculite + gravel (stone fragments) in a ratio of 12: 1 was found suitable for the survival of *Andrographis paniculata* plants in pottery.

RESULTS AND DISCUSSION

Effect of Cytokinins and Auxins on shoot multiplication

Sprouted buds obtained from epicotyledonary nodes are cultured on MS medium containing BAP (13.31µM / lit), kinetin (13.93µM / lit) and IAA (7.13µM / lit) at the same concentrations as BAP, kinetin, and MS medium. There is a sub-culture. At low IAA (5.70µM / lit), shoots were sub-cultured at other combinations and concentration of PGR.

Plate.1 Shoot induction (BAP 1.5mg/L)

Plate.2 Shoot induction (BAP 2mg/L)

Effect of BAP + Kinetin + IAA

The obtained results are presented in Table-2. As concentrations of BAP (2.21µM / lit to 13.31 µM / lit), kinetin (2.32 µM / lit to 13.93µM / lit) and IAA (0.57µM / lit to 2.85µM / lit) increased shoot. The number of shoots increased. In 25 shoots containing Kinetin (13.93 µM / lit) and IAA (2.85µM / lit), containing BAP (13.31µM / lit) on MS, 29.5 mm length shoots regenerated from the same shoot. The maximum number (25 digits) was produced on this medium. Shoots number and length decreased with increasing concentrations of BAP (17.75 µM to 22.19µM / lit), kinetin (18.58µM / lit to 23.23µM / lit) and IAA (5.70µM / lit to 11.41µM / lit). Length (23.5 mm) and number of seedlings (18) elicited increased cytokinin and auxin with reduced calling at the shoot base.

Effect of Kinetin + NAA

The obtained results are presented in Table-2 which proves that kinetin in combination with NAA was not found to be as effective as BAP + kinetin + IAA for induction as well as enhancement of shoots from subculture shoot buds. Kinetin (2.32µM / lit to 23.2µM / lit) was included in NAA (1.34µ M / lit to 13.42µM / lit) medium. Only 3 shoots (length 9.5 mM) were produced at low concentrations of kinetin (2.32µM / lit) with NAA (1.34µM / lit). As the concentration of kinetin increased (13.93 µM / lit) with increased concentrations of NAA (8.05µM / lit), the number and length of shoots increased (21 shoots, with 26 mm in length). Number of shoots and number of shoots at high concentrations of kinetin (23.23µM / lit) + NAA (13.42µM / lit)) (16 shoots with 20.5 mm length).

Effect of 2, 4-D and IAA + NAA on callus induction

Effect of MS + IBA and MS + IAA + NAA on rooting

The excised shoots produced on MS medium containing BAP (13.31µM/lit), kinetin (13.93µM/lit) and IAA (2.85µM/lit) alone were transferred on rooting medium. The medium on which the multiple shoots were produced showed marked influence on subsequent rooting behavior of such shoots originally grown on MS + Cytokinins and Auxins. The results obtained are presented in Table-4. The rooting of micro shoots was influenced by concentration of IBA. On lower concentration of IBA (1.23µM to 3.69µM/lit) these shoots did not root and could be rooted only on medium containing 4.92µM/lit to 24.60µM/lit IBA. However even on these concentrations more time was required (25-30 days) for rooting. As the concentration of IBA increased from 4.92µM/lit to 24.60µM/lit the

percentage of rooting in microshoots enhanced from 64.7 to 98.3% on 30 days. Maximum percentage (98.3%) of rooting on medium containing IBA 24.60µM/lit. On further increase in the concentration of IBA rooting could be achieved (up to 24.60µM/lit IBA) and (up to 8.05µM/lit NAA + 8.56µM/lit IAA) beyond this rooting was inhibited and excised shoots produced callus. Thus IBA 18.09µM/lit and NAA 8.05µM/lit + IAA 8.56µM/lit were found to be optimum for maximum rooting microshoots of *Andrographis paniculata*. The roots so produced attained 38.9mm length in 4-5 weeks.

Plate.3 Roots initiation in IAA (1.5 mg/L) after 15 days

Plate.4 Hardening

***In vitro* Propagation with varying concentration of Auxins and Cytokinins**

Shoot cultures created *in vitro* were the source of nodal explants (single node with 1cm of length) that were vaccinated on semacidolid MS medium with varying concentration of semicidal {benzyl aminopurine (BA), Kintin (Kn)} and cytokinins {indole butyric acid (IBA)} to find the best combination based on the maximum number of promotions. 16 combinations, including basal medium (control), were tested. Cultures were maintained under 16-h photoperiod at 25±2°C. Shoot cultures of convergence were established *in vitro* and in shoot number, shoot length and propagation number were recorded after 8 weeks of culture to identify elite accession. The triplet includes 10 cultures in each treatment.

***In vitro* regeneration through callus from selected accession**

Selected accession leaf, internode, and nodal sections obtained from the stream were cultured on MS media with different combinations and concentrations of BA and 2, 4-Dichlorophenoxy Acetic Acid (2, 4-D). Cultures were maintained at 25±2°C under a 16-h photoperiod. Callus diameter and morphological characteristics were recorded after 4 weeks of culture. Each treatment consisted of 10 cultures, in triplicate.

***In vitro* multiplication of selected accessions on different media**

Selected accessions were broadcast on five different media: MS, Nitsch, B5, SH, and White for rapid dissemination. All media were supplemented with 0.4 BA and growth was observed after 8 weeks of culture. Single node explorers were transferred into culture tubes (borosil; 25 × 150mm) 15ml of culture medium. The medium was autoclaved at 121°C for 15 min and pH adjusted 5.8 before autoclaving. Cultures were kept for 16-h photoperiod at 25±2°C. Growth on various mediums was compared to MS + 0.4 BA because MS medium is well established for *in vitro*

proliferation of *Andrographis peniculata*. More and more publicity for *in vitro* proliferation was identified based on the purpose of conservation. Each treatment includes 10 cultures in the triplet.

In vitro propagation with varying concentration of auxins and cytokinins

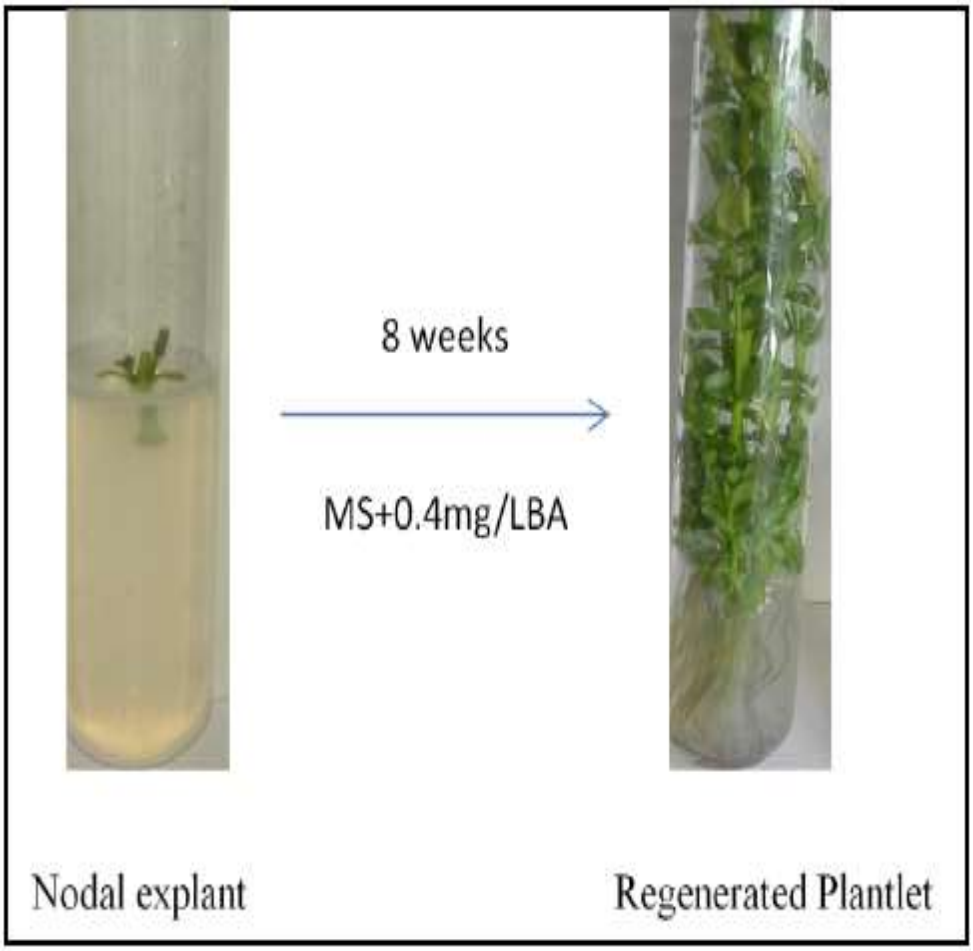
All combinations were viewed for the number of shoots, the length of the shoot and the number of promotions. Shoots appeared within 7 days of culture in all test combinations. The emergence of 2–4 shoots was observed in all treatments after 4 weeks. The difference in shoot prevalence in relation to different treatments became noticeable up to 8 weeks. Analysis of variance demonstrated a significant effect of treatment with respect to multiplication ($P < 0.01$). The MS basal medium showed an increase of 3.2 shoots from the nodal segment. BA addiction revealed a promotional effect on the number of shoots (20 - 30 shoots / explants) at concentrations of 0.2 (1.0-mg / liter BA). BA is greater than 1.0mg / lit was associated with a decrease in axial shoot proliferation. High amounts of BA (> 2mg / lit) result in simultaneous shoots with the basal callus (Sharma et al., 2007). Sharma and colleagues (2016) achieved the highest shooting prevalence in MS, fortified with a single node at 0.2mg / lit. Media supplementation with the knee showed an increase in the number of shoots (20–22 shoots / explants). There were no significant differences in many more shoots, which were recorded with further increase in the knee. Inclusion of IBA with BE and Ni eliminated the presence of calcus with the presence of multiple ends and decreased shoot length. The highest shoot growth was recorded with 1.0mg / lit, while the maximum shoot was seen with 0.4mg / lit, compared with MS (basal), MS + 0.2mg / lit BA and 0.4mg / lit BA. Shows highest shoot multiplication and MS + 1.0mg / lit. Single 3.2 in vitro regeneration of a single node for multiple shoots at 0.4mg / lit BA after 8 weeks of culture. Multiple shoot proliferation was recorded on average 30.4 shoots / 7.6 cm shoot lengths and 26.8 numbers of roots / investigators after 8 weeks of culture when 0.4mg / lit was used. Callus formation was not observed in this medium.

Effect of different growth hormones on shoot multiplication and number of roots after 8 weeks of culture

Treatments (mg/L)	Number of shoots/ explants	Shoot length (cm)	Root number
MS basal	3.25 ± 0.7^{l}	3.55 ± 0.8^{de}	7.40 ± 0.5^{i}
MS + 0.2BA	22.25 ± 0.5^{b}	8.97 ± 0.6^{ab}	23.2 ± 0.7^{b}
MS + 0.4BA	30.45 ± 0.6^{a}	7.96 ± 0.4^{bc}	26.85 ± 0.6^{a}
MS + 1.0BA	20.25 ± 0.5^{c}	9.56 ± 0.4^{a}	20.12 ± 0.6^{c}
MS + 0.2BA + 0.2IBA	11.2 ± 0.8^{g}	6.96 ± 0.4^{c}	12.10 ± 0.7^{f}
MS + 0.2BA + 0.5IBA	12.84 ± 0.5^{ef}	$3.21\pm0.4d^{ef}$	12.49 ± 0.6^{f}
MS + 0.4BA + 0.2IBA	11.80 ± 0.6^{fg}	3.75 ± 0.4^{de}	10.96 ± 0.5^{g}
MS + 0.4BA + 0.5IBA	16.20 ± 0.7^{d}	2.87 ± 0.4^{ef}	19.23 ± 0.6^{c}
MS + 1.0BA + 0.2IBA	13.70 ± 0.6^{e}	4.34 ± 0.4^{def}	15.62 ± 0.6^{d}
MS + 1.0BA + 0.5IBA	20.25 ± 0.6^{c}	3.79 ± 0.6^{de}	14.36 ± 0.5^{e}
MS + 0.5Kn	20.20 ± 0.5^{c}	6.86 ± 0.6^{c}	9.45 ± 0.7^{h}
MS + 1.0Kn	22.7 ± 0.8^{b}	6.87 ± 0.6^{c}	16.25 ± 0.7^{d}
MS + 0.5Kn + 0.2IBA	7.55 ± 0.5^{h}	3.89 ± 0.4^{de}	3.97 ± 0.7^{jk}
MS + 0.5Kn + 0.5IBA	3.74 ± 0.6^{jk}	1.97 ± 0.4^{fg}	3.53 ± 0.6^{kl}
MS + 1.0Kn + 0.2IBA	5.55 ± 0.6^{i}	2.86 ± 0.4^{ef}	2.46 ± 0.6^{l}
MS + 1.0Kn + 0.5IBA	4.75 ± 0.4^{ij}	1.01 ± 0.4^{g}	4.8 ± 0.5^{j}

Data given as Mean ± SE. different letters in superscripted are significantly different at $p<0.05$ level using DMRT

***In vitro* shoot multiplication on MS (basal), MS + 0.2 mg/lit BA, MS + 0.4mg/lit BA and MS + 1.0mg/lit BA (L-R) after 8 weeks of culture**



Plantlet regeneration from nodal explants on MS + 0.4mg/lit BA after 8 weeks of culture

***In vitro* propagation is essential for germplasm conservation, which was achieved in *A. paniculata* by enhanced multiplication. Multiplication response on optimal medium (MS + 0.4mg/lit BA) was observed on 13 accessions in Table 3.2.**

Shoot multiplication in 13 accessions of *Andrographis paniculata* on selected media (MS+0.4 BA) after 8 weeks of culture

Accession No.	Number of Shoots/ Explant	Shoot Length (cm)	Number of Propagules
IC439118	24.65 ± 0.7^{c}	7.29 ± 0.8^{bc}	71.5 ± 0.6^{b}
IC426442	19.10 ± 0.7^{d}	7.02 ± 0.5^{bc}	54.6 ± 0.5^{f}
IC426447	19.20 ± 0.7^{d}	6.79 ± 0.5^{bcd}	58.2 ± 0.8^{e}
IC468878	19.25 ± 0.5^{d}	7.82 ± 0.3^{b}	$56.70\pm.9^{e}$
IC373640	24.99 ± 0.5^{c}	6.08 ± 0.4^{cd}	64.6 ± 0.6^{c}
IC344312	15.90 ± 0.6^{e}	5.59 ± 0.6^{d}	38.7 ± 0.4^{h}
IC531621	18.87 ± 0.6^{d}	6.27 ± 0.4^{cd}	41.5 ± 0.5^{g}
IC375976	16.90 ± 0.7^{e}	5.96 ± 0.4^{cd}	57.0 ± 0.7^{e}
IC353203	23.55 ± 0.5^{c}	6.80 ± 0.6^{bc}	70.1 ± 0.6^{b}
IC554588	30.45 ± 0.6^{a}	9.65 ± 0.4^{a}	78.4 ± 0.5^{a}
IC554586	27.20 ± 0.7^{b}	7.19 ± 0.4^{bc}	64.7 ± 0.7^{c}
IC554587	29.35 ± 0.6^{a}	7.15 ± 0.3^{bc}	61.6 ± 0.4^{d}
IC554585	24.20 ± 0.7^{c}	6.55 ± 0.4^{bcd}	70.4 ± 0.5^{b}

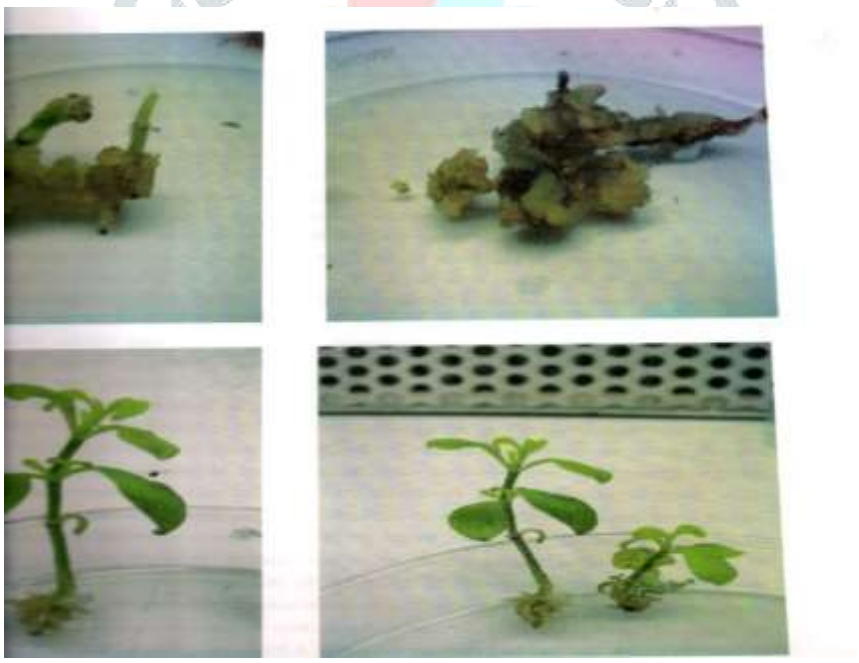
Data given as Mean ± SE. different letters in superscripted are significantly different at $p<0.05$ level using DMRT

Analysis of multiplication demonstrated significant genotypic differences ($p<0.01$) in terms of multiplication response. The average number of shoots ranged from 15 to 30 while the shoot length and propagation numbers were 5.59 - 9.65 cm and 38 - 78 cm + 0.4 BA, respectively. Maximum number of shoots (30), shoot lengths (9.65 cm) and number of spreads (78) were recorded in IC 554588 in MS + 0.4 BA. Tiwari et al (2001) observed the shoots of the most bouncy buds on a 2.2µM BA medium with leaf explorers. Sharma et al. (2007), achieved similar results with 75 promotions at 22.2 seedlings / investigators and 0.2mg / lit after 8 weeks of culture. High concentrations of BA have been associated with the production of a massively packed mass of shoots, which was not readily distinguishable, whereas it was not applicable to low amounts of BA. Various researchers have also used different concentration of BA for shoot propagation in Bacopa (Tejavati and Shailaja, 1999).

***In vitro* regeneration through callus**

Callus induction was visualized using 3 explants, node, internode, and leaf explants. Compact callus was observed in leaf extracts by supplementing varying concentrations of BA and 2,4-D on MS medium. Leaf experiments were vaccinated in 5 different media combinations, including MA basal. No callus was observed

on MS basal medium. The average time for callus initiation varied from 14 to 20 days. Callus was first observed in leaf explants (14 days) followed by internodes and nodal segments. Dark green compact call with maximum diameter (3.6 cm) was observed from leaf discovery after 4 weeks when MS + 0.4 mg / liter BA was cultured at 0.2 mg / liter 2,4-D (Table-4) it was done. Calcus was transferred to plantlet regeneration medium (MS + 0.4mg / lit BA) after 2 subcultures. Rapid proliferation (2 weeks) from the callus of leaf exfoliation (fig. 3.3) compared to the node and internode was observed as the callus induced by node and internode explos showed proliferation after 3 weeks and 3.4 weeks of culture, respectively. Rapid growth of callus with 0.5mg / litre 0.5mg / lit NAA, 0.5mg / lit 2,2-D and 0.25 thyodizurone was observed on MS medium individually (Vijayakumar et al. 2010). Mehta and coworkers (2012) observed, 3.6 and 3.2 cm leaf 0.25mg / lit 2, 4-D + 0.5mg / lit Kn and 0.25mg / lit 2, 4-D + 0.1mg / liter BAP leaf. In the present study, leaf experts showed 100% calc formation at MS + 0.4mg / lit NAA + 0.2mg / lit 2,4-D. 76% and 84% of callus inductions were observed on the same medium with node and internode explorer, respectively. Varma et al. (2012) Callus induction on MS supplemented with 3mg / lit BA and 1.0mg / lit IAA after 2 weeks of culture. Mehta (2017) reported that MS showed only 50% response to callus induction, with varying concentrations of 2, 4-D. However, they considered MS + 1.5mg / lit 2, 4-D to be optimal for callus induction with all three implants, internodes, leafs and nodes. The data from the present study revealed the dominance of leaf explosives over internodes and nodal exoplanes in terms of callus feature, diameter, and propagation.



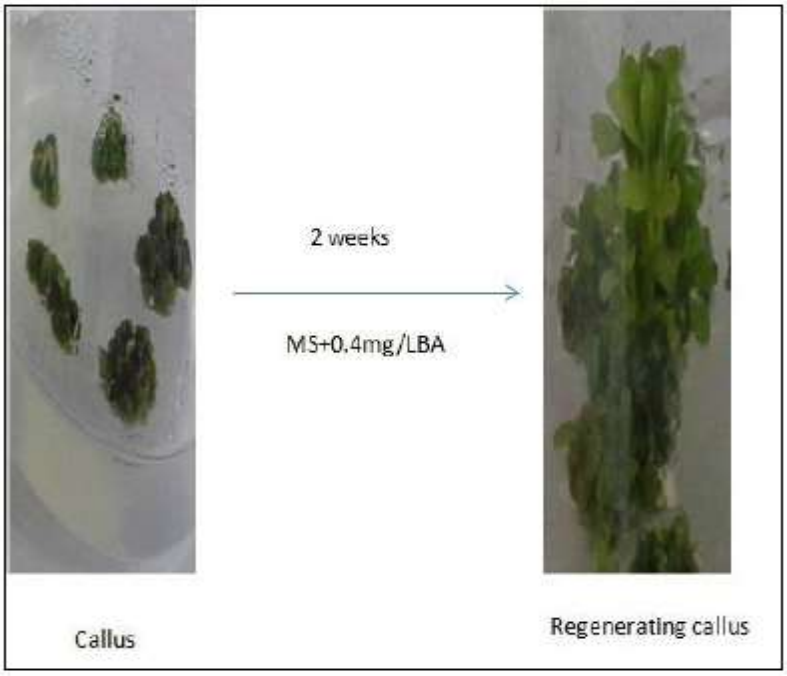
Callus formation and root induction of *Andrographis paniculata*

Invitro regeneration and multiplication of *Andrographis paniculata*

Effect of BA and 2, 4-D on callus induction from leaf explants after 4 weeks of culture

Concentration of growth hormone (mg/L)	Callus diameter (cm)	Callus proliferation	Callus feature	% of callus formation
MS	0	0	0	0
MS + 0.2 BA + 0.2 2,4-D	2.7±0.6	Good	Brownish green, friable	92
MS + 0.4 BA + 0.2 2,4-D	2.8±0.9	Moderate	Green, nodular	84
MS + 0.2 Kn + 0.2 2,4-D	2.8±0.4	Good	Green, friable	87
MS + 0.4Kn + 0.2 2,4-D	3.6±0.5	Excellent	Dark Green, compact	100

Data presented as Mean ± SE.



Proliferation of callus on MS + 0.4mg/L BA after 2 weeks of culture

***In vitro* propagation on different media (MS, Nitsch, B5, SH, White)**

The nodal investigator of IC554588 selected from the above study was cultured on 5 different media supplemented with 0.4 BA to examine the efficiency of *in vitro* proliferation. Shoot emergence was observed within 12 days of culture. Analysis of multiplication demonstrated a significant effect of treatment ($P<0.01$) in relation to shoot multiplication. MS medium exhibited the maximum number of shoots (23 shoots/explants) and shoot lengths (8cm) followed by Nits Medium with 16 shoots/explore and shoot lengths (6cm). Root induction was first recorded in MS medium with the maximum number of roots (19 roots/culture) as well as the highest average number of proliferations (65), followed by Nitsch medium (45 propagation/culture). Table 3.5- b shows the influence of different media on the development parameters of monnery. Sharma et al., 2017 observed that SH recorded a greater number of shoots and shooting lengths than B5 after 4 weeks of culture. In the present study, maximum number of promotions and maximum marks were obtained on MS medium as compared to other tested media.

Effect of different media on growth parameters of *Andrographis paniculata* after 8 weeks of culture

Media	Number of shoots/ explants	Shoot length (cm)	Root number	Number of propagules
MS + 0.4 BA	23.35±0.5	8.15±0.5	19.1±0.7	65.1±0.85
Nitsch + 0.4 BA	16.85±0.9	6.13±0.3	15.3±0.7	45.4±0.8
B5 + 0.4 BA	13.3±0.8	5.54±0.5	11.85±0.8	36.25±0.7
S&H + 0.4 BA	9.55±0.7	4.69±0.5	9.2±0.6	21.25±0.5
White + 0.4 BA	9.15±1.1	2.17±0.4	6.55±0.5	19.8±0.6

Data given as Mean ± SE. different letters in superscripted are significantly different at p<0.05 level using DMRT

CONCLUSION

The size of explant and the overall quality of from which explants are to be obtained. There is sufficient residual cytokinin in shoots, thus little or no cytokinin is need in rooting medium. Higher concentration of cytokinins was shown to be deleterious to the initiation and elongation of roots of both monocotyledonous and dicotyledonous plants. The other factors known to affect rooting are carbohydrates abscisic acid. For obtaining uniformity in the result the size range of the shoots for rooting should be constant . In *Andrographis paniculata* rooting was obtained in the shoots of 10.5mm to 33.5mm length on MS medium without growth hormones as well as in hormones containing medium. The strength of inorganic and organic salts of MS medium also play significant role in the rooting behavior of shoots *in vitro* of *Andrographis paniculata* species. Similarly root could be induced in the shoots of *Andrographis paniculata* on MS full strength salts. The high salt concerning of macro-elements of MS when lowered to 3/4, 72, and 14 were found to be more beneficial. Similarly in *Andrographis paniculata* the rooting of *in vitro* shoots was achieved on MS salt of 12 strength medium. Among the various explants, shoot tips responded positively for shoot induction. MS medium fortified with BAP (2.5 mgl-1) was found highly responsive for shoot induction. The multiple shoot induction was achieved in MS medium + BAP (3.0 mgl-1). For shoot elongation, BAP (2.0 mgl-1) + GA3 (1.0 mgl-1) was found better. Rooting was best (94.85%) in ½ MS + IAA 0.5 mgl-1 + IBA 1.0 mgl-1 . Pot mixture containing vermiculite + red earth + sand (1:1:1) was found optimum for hardening.

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