IN VITRO MICROPROPAGATION OF *Senna Alata* - AN ETHNOMEDICINAL PLANT THROUGH EMBRYOCULTURE

M.GURAVAIAH*, I. HATTI, I.RAMA KRISHNA, M.YANADI RAO & A.INDIRA PRIYADARSHINI


ABSTRACT

The use of medicinal herbs in the treatment and prevention of diseases is attracting attention by scientists worldwide. Micropropagation is the practice of rapidly multiplying stock plant material to produce a large number of progeny plants, using modern plant tissue culture methods. Micropropagation is used to multiply novel plants, such as those that have been genetically modified or bred through conventional plant breeding methods. Ayurveda and other traditional systems of medicines. Drugs obtained from plant are believed to be much safer and exhibit a remarkable efficacy in the treatment of various ailments. Vitamins are used as catalysts in various metabolic processes. It is necessary to supplement the medium with vitamins for best growth. Stages of micropropagation: 1 Selection and Establishment of Aseptic Culture, 2. Multiplication of propagules, 3. Plantlets Regeneration, 4. Preparation and Transfer to field. Micropropagation can be used to produce disease-free plants. Micropropagation produces rooted plantlets ready for growth, saving time for the grower when seeds or cutting are slow to establish or grow. It is the viable method of regenerating genetically modified cells or protoplast cells.

KEYWORDS

*Senna alata*, Embryoculture, Medicinal herbs, Micropropagation, plants, Drugs, Vitamins

INTRODUCTION

The use of medicinal herbs in the treatment and prevention of diseases is attracting attention by scientists worldwide. World Health Organization corroborated this in its quest to bring primary health care to the people. The plant kingdom has for long time served prolific source of helpful drugs, food, additives, flavoring agents, colorants binders and lubricants etc., As a matter of facts, it was estimated that about 25% of all prescribed medicines today are substances derived from plants (Bello *et al.*, 2005).

Countries in Africa, Asia and Latin American continents use traditional medicine to help meet some of their primary healthcare needs. In Africa today, up to 80% of the population uses traditional medicine in primary health care (WHO, 2006). Many African plants are used in traditional medicine as antimicrobial agents in which only few have been documented. However, in spite of vast improved health and longevity in the United State and Europe, millions of people are turning back to traditional herbal medicine in order to prevent or treat many illnesses (WHO, 2006) and to circumvent resistance of many human pathogens to conventional antibiotics, some of which have side effects like hypersensitivity and immunosuppression. In Nigeria, traditional medical practitioners use a variety of herbal preparation to treat different microbial diseases. For example, the Ebira tribes of Kogi State, Nigeria use the fruits of *Solanum melongena* for weight loss allowed as dietary delicacy (Bello *et al.*, 2005). The use of herbal medicine predates the introduction of antibiotics predates social, economic and religious barriers (Akinyemi *et al.*, 2000).

India is endowed with a rich wealth of medicinal plants. In India, medicinal plants are widely used by all sections of people either directly as folk remedies or indifferent indigenous systems of medicine or indirectly in the pharmaceutical preparations of modern medicines. India is one of the 12 mega diversity countries in the world and has 17,000 flowering plants. Of the designed 25 hotspots in the world the Eastern Himalaya and the Western Ghats are the 2 hot spots in India. 550 ethnic
tribes have rich traditional and indigenous knowledge in India. It is reported that these traditional healers use 2500 plant species and medicine. In recent years, there has been a tremendous range of interest in the medicinal plants especially those used in Ayurveda and other traditional systems of medicines. Drugs obtained from plant are believed to be much safer and exhibit a remarkable efficacy in the treatment of various ailments.

**Tissue Culture and Its Importance:**

**Micropropagation:**

Micropropagation is the practice of rapidly multiplying stock plant material to produce a large number of progeny plants, using modern plant tissue culture methods. Micropropagation is used to multiply novel plants, such as those that have been genetically modified or bred through conventional plant breeding methods. It is also used to provide a sufficient number of plantlets for planting from a stock plant which does not produce seeds, or does not respond well to vegetative propagation. Totipotency is the property, which is inherent in a plant cell that makes it possible for total development of cell or organ into a whole organism. It has been the basic for plant tissue culture as well as Micropropagation. The rate of plant regeneration *in vitro* varies greatly from one plant species to another. The *in vitro* growth and development of plant is determined by a number of complex factors include the genetic makeup of the plant, chemical factors (nutrient medium components: water, macro and micro elements, sugars and plant growth regulators) as well as gelling agent and all also physical growth factors (Pierik, 1988).

*In vitro* plant regeneration by organogenesis usually involves induction of shoot buds that leads to development of shoots from the explants tissue, followed by transfer to a root induction medium for root formation and development. Organogenesis is of two types, direct organogenesis and indirect organogenesis. In direct organogenesis, either the shoot or root is induced directly from the pre-existing cells in the explants without undergoing an intervening callus phase (Brown and Thorpe, 1986; Christianson and warnick, 1988). Indirect organogenesis involves an intervening callus phase proliferation and growth, followed by shoots or roots induction. Organogenesis by direct rather than indirect pathway is preferred due to the problems with somaclonal variations confronted in culture (Richie and Hodges, 1993).

The pathway in which embryos have been induced from a somatic cell or group of somatic cells is referred as somatic embryogenesis. Somatic embryogenesis, which occurs directly from cells of the explants tissues without an intervening callus phase, is called direct embryogenesis (Conger et al., 1983 and Raghavan, 1986). Direct somatic embryogenesis was initially reported in soya bean (Lazzeri et al., 1985 and Finner, 1988), maize (Armstrong and Green, 1985 and Vain et al., 1989), and later in pea, chick pea (Murthy et al., 1996), Pigeon pea and groundnut. Indirect embryogenesis is a pathway in which somatic embryos get induced and developed from proliferated callus (Mc William et al., 1974: Williams and Maheswaran, 1986). The auxins, which were provided in the initiation phase of embryogenic culture, generally lead to induction of callus proliferation along with Embryogenesis pathway (Evans et al., 1981 Normaura and Kmanine, 1985).

One of the advantages of plant tissue cultures is that the greater availability of biomass provides researchers with an opportunity to assay the yield of secondary metabolites at specific stages of growth and differentiation (Srivastava et al., 1993, Datta and Srivastava, 1997, Pandi et al., 2000).

The media used for micropropagation of different explants must have Nitrogen, Phosphorus, Potassium, Calcium, Magnesium, Sulphur in major quantity, which are called as Macro elements while Boron, Manganese, Iron, Copper, Zink, Molybdenum, Chloride, Nickel, Iodine and Cobalt requiring in small quantity are called as Micro elements.

Macronutrients and most micronutrients are provided for plant culture media in the form of salts. In weak aqueous solution, such as plant media, each soluble salt
dissociates into two ions, one positively charged, the other negatively charged. Plants absorb the nutrients they require from nutrient media, almost entirely as ions. An ion is an atom or a group of atoms, which has gained either a positive (a cation) or a negative (anion). Calcium, and Magnesium and Potassium are absorbed by plant cells as the respective cations Ca$^{+2}$, Mg$^{+2}$ and K$^+$. Nitrogen is mainly absorbed in the form of ammonium (the cations, NH$_4^+$) or nitrate (the anion NO$_3^-$). Phosphorus as Phosphate ion (HPO$_4^{2-}$) and (HPO$_4^{3-}$) and Sulphur as Sulphate ion (SO$_4^{2-}$) (Shah et al., 1985). The most important step in deriving a plant tissue culture medium is the selection of macronutrient ions in the correct concentration and balance. The salts normally used to provide the macro elements in tissue culture media also provide ions of the elements sodium and chloride because plant cells tolerate high concentration of sodium and chloride without injury, these ions frequently given little importance when contemplating media changes. Salts containing them are considered as convenient sources of N, K, Ca, Mg, or Mg, because they effectively permit the addition of just one the major nutrients (Mc Cleonodon 1976; George, 1993). Calcium helps to balance anions within the plant, but unlike potassium and magnesium, it is not readily mobile. Because of its capacity to link biological molecules together, the element is involved in the structure and physiological properties of cell membranes and middle lamella of cell walls.

Phosphorus is a vital element in plant biochemistry. It occurs in compounds which are involved in energy transfer. Protein and nucleic acids synthesis and contributes to the structure of nucleic acids. Phosphorus is absorbed in to plants in the form of phosphate ions by an active process, which requires the expenditure of respiratory energy. It is used in plants as the fully oxidized orthophosphate PO$_4^{3-}$ form. High concentrations of dissolved phosphate can depress growth, possibly because calcium and some microelements are precipitated from solution and their uptake is reduced. The phosphate in culture medium is also inadequate for static cultures of some plants, or where a large amount of tissue or organs are supported on a small amount of medium. When phosphate levels are increased to obtain a more rapid rate of growth of a culture, it can be advisable to investigate the simultaneous enhancement of the level of Myo-inositol in the medium (Murashige, 1974; Lumsden et al., 1990; George,1993; Mohamed,1997).

Magnesium is an essential component of the chlorophyll molecule and is also required specifically for the activity of many enzymes, especially those involved in the transfer of phosphate. Magnesium sulphate is used as a source of magnesium in most of the media. The role of micro elements is as follows:

1. Boron: Helps in Ca$^{+2}$ ion uptake.
5. Molybdenum: It is important in enzymes involved in nitrogen metabolism.

Vitamins are used as catalysts in various metabolic processes. It is necessary to supplement the medium with vitamins for best growth. Thiamine is basic vitamin required by all cells and tissues. Other vitamins used are Nicotinic acid (B$_3$), Inositol, Biotin, Folic Acid, Ascorbic Acid, Riboflavin.

**STAGES OF MICROPROPAGATION:**

**Stage I. Selection and Establishment of Aseptic Culture**

This step is concerned with selection of typical, healthy, disease free mother plants. If necessary, test for virus presence is carried out and if found positive it is eliminated. Selection of plant is followed by preparation
of explants, surface sterilization and transfer to appropriate media. Sterilization is carried out through soaking in a calcium hypochlorite solution or in 0.1% mercuric chloride solution.

**Stage II. Multiplication of propagules:**
This stage is concerned with rapid multiplication of the regenerative system for obtaining large number of shoots. To achieve this, medium and tissue factors are optimized empirically. *In vitro* produced shoots are used as explants to produce more shoots. The shoots may be obtained by any of the approaches mentioned above (axillary, adventitious or embryogenesis). Orchids are multiplied by protocorm formation. Normally, medium for stage I and II is same, but minor changes may be made in the concentration of cytokinins or nitrogen to increase the shoot proliferation.

**Stage III. Plantlets Regeneration**
Plantlets are produced through isolated shoots or germination of somatic embryos. For this purpose, shoots of a length or age are required, which may be produced by the culture medium combinations. Shoots are separated manually from clusters and transferred on a rooting medium containing an auxin. A pulse treatment with high concentration of an auxin and transfer on hormone free medium is preferred over continuous growth of shoots on an auxin containing medium. The difficult to root species, particularly tree species, may require modifications in nutritional or environmental conditions such as activated charcoal, high temperature or liquid medium with filter paper bridge for high rooting. Cultures for rooting are placed in low light intensity (about 200-1000 lux, compared to stage II) or the lower portion of the culture tubes is covered with black paper to facilitate root induction. Low salt strength favors the rooting. In some herbaceous plants, *in vitro* produced shoots are pulse-treated with auxin and transferred directly to pot mixture, there they root.

**Stage IV. Preparation and Transfer to field:**
This stage is concerned with transfer of plantlets in pots, their hardening and establishment in soil. A successful tissue culture method of propagation must result in re-establishment in soil of a high frequency of the tissue culture derived plants. Stage IV is intended for the successful transfer of the propagules to soil. Hardening of plants imparts some tolerance to moisture stress and plants become autotrophic from heterotrophic condition. When plantlets are taken out from the culture tubes, adhering agar is removed by careful washing with running tap water and plantlets are transferred in a soil: vermiculate: (1:2) mixture and placed in mist-house under high humidity conditions. Plantlets are irrigated with a dilute nutrient solution or pure water. In laboratory, plantlets are covered with glass beakers. Plantlets are exposed to decreasing humidity by slowly exposing the plant (by removing glass covers) or reducing the mist period in the glass house. Plantlets develop cuticle and their stomata start functioning. During this period high light intensity may also be provided (3000-1000 Lux). Hardened plants are then transferred to glass or poly-house (tent like structure are erected by mounting polythene or polycarbonate sheets on metal frame support) with normal environmental conditions. Plants irrigated frequently and their growth and variation are monitored regularly. Plants may be kept up to flowering (flowering plants) or transferred to fields (plantation crops) after 4 to 6 weeks of acclimatization.

**Different techniques of micropropagation and their applications:**

**Seed culture**
- Increasing efficiency of germination and germling production in seeds, difficult to germinate *in vivo*.
- Precocious germination by application of plant growth regulators.
- Induction of multiple shoot formation and organogenesis by application of plant growth regulators.
- Elimination of viruses as seeds does not carry viruses.

**Embryo culture**
- Overcoming embryo abortion due to incompatibility barriers.
- Overcoming seed dormancy and self sterility of seeds.
- Embryo rescue in distant hybridization where endosperm development is poor.
- Production of monopolides.
- Shortening of breeding cycle.

**Ovary or Ovule culture**
- Production of haploid plants.
- Recovery of hybrid embryos overcoming embryo abortion at very early stage of development of zygote due to incompatibility barriers.
- Achievement of In vitro fertilization.

**Anther and Microspore culture**
- Production of haploid plants.
- Production of homozygous diploid lines through chromosome doubling, thus reducing the breeding cycle.
- Genetic transformation using microspores.
- Production of useful gametoclonal variants.
- Mutation investigations easier with single set of chromosomes.
- Fixation of certain genetic characters from heterozygous source materials.

**Organ culture**
- Mass production of plants of elite and rare germplasm.
- Production of calli, shoots and roots for production of secondary metabolites.
- Development of germplasm banks for rare and endangered plants.

**Shoot apical meristem culture**
- Production of virus free germplasm.
- Mass production of desirable genotypes.
- Facilitation of international exchange.
- Cryopreservation or In vitro conservation of germplasm.
- Phytosanitary transport.

**Somatic embryogenesis**
- Mass multiplication of elite of germplasm.
- Production of artificial seeds.
- As source material for embryogenic protoplasts.
- For genetic transformation.
- Production of primary metabolites specific to seeds such as lipids in oil seeds.

**Callus Culture**
- Production of plantlet through somatic embryogenesis or organogenesis.
- For obtaining virus –free plants.
- For generation of useful somaclonal and gametoclonal variants.
- As a source of protoplast and suspension cultures.
- Production of useful secondary metabolites.
- For biotransformation studies.
- Selection of cell lines with valuable properties such as resistance to disease, herbicides, overproduction of secondary metabolites etc.

**Genetic Transformation**
- Introduction of foreign DNA to generate novel genetic combinations.
- Transfer of desirable genes for disease and pest resistance from related or unrelated plant species into high yielding susceptible cultivars.
- Study of structure and function of gene.
- Induction of hairy roots or sooty teratomas for over-production of secondary metabolites, naturally present in mother plant.
- Production of novel secondary metabolites absent in parent plant.

**Micro grafting**
- Overcoming graft incompatibility.
- Rapid mass propagation of elite scions grafted on root stocks having desirable traits like resistance to soil-borne pathogens and disease.
- Multiplication and survival of difficult to root species as well as of transformants.

**Advantages of Micropropagation:**
Micropropagation has a number of advantages over traditional plant propagation techniques:
The main advantage of Micropropagation is the production of many plants that are clones of each other.

1. Micropropagation can be used to produce disease-free plants.
2. Micropropagation produces rooted plantlets ready for growth, saving time for the grower when seeds or cutting are slow to establish or grow.
3. It is the viable method of regenerating genetically modified cells or protoplast cells.
4. Some plants with very small seeds, including most orchids, are most reliably grown from seeds in sterile culture.
5. A greater number of plants can be produced per square meter and the propagation can be stored longer and in a small area.

Disadvantages of Micropropagation:

Micropropagation is not always the perfect means of multiplying plants. Conditions that limit its use include

1. It is very expensive, with a labor cost of more than 70%.
2. A mono culture produced after Micropropagation, leading to a lack of overall disease resistance, as all progeny plants may be vulnerable to same infection.
3. Some plants are very difficult to disinfect from fungal organisms.

Phytohormones:

Hormones (gr.Hormaein=Provokes) are chemical substances, which are formed in smallest quantities in certain parts of an organism and from there they arrive to another part, where completely specific effects are cause.

Phytohormones widely used and their effects:

- Auxins: Auxins help in induction of cell division. Central the vascular system differentiation apical dominance, delay senescence, promote flowering, fruit setting, and ripening. They usually promote rooting. Commonly used auxins include, IAA (Indole-3-acetic acid), IBA (Indole-3 butyric acid), NAA(Naphthalene acetic acid), 2,4 D (2,4 dichlorophenoxy acetic acid).
- Cytokinins: Cytokinins promote shoot elongating, delay senescence and break dormancy. Cytokinins stimulate cell division, cell enlargement, chlorophyll synthesis and can reverse the effect of auxins in apical dominance, causes stomata opening and membrane decreased permeability. Commonly used cytokinins includes BAP (6- benzyle amino purine) and Kinetin.

AIM AND OBJECTIVES OF THE STUDY:

To establish standard reproducible protocol for rapid multiplication of S. alata: Seed germination is one of the major problems in S. alata to overcome this problem we aimed to establish are producible protocol. For the rapid multiplication of the species through zygotic embryo culture.

MATERIALS AND METHODS:

Plant material:

- Senna alata seeds

Laboratory requirements:

Chemicals and reagents:

- MS major elements, Minor elements, and Iron salts, Vitamins, Plant growth regulators and other supplements.
- Sucrose.
- Agar
- Other reagents such as HCl, NaOH, Buffer tablets Rectified spirit or Isopropyl alcohol

Glassware and other vessels

- Culture vessels preferably glass
- Glass-beakers or plastic (10 ml, 50 ml, 100 ml, 250 ml, 1 L)
- Measuring cylinder
- Micro pipettes
- Reagent bottle for storing stock solutions
- Conical flasks
- Glass rod for stirring medium
- Large vessels for mixing and heating medium
Equipments:
- Autoclave
- pH meter
- Water distillation unit
- Electronic balances
- Hot air oven
- Gas stove
- Laminar air flow chamber
- Glass bed sterilizer
- Air condition
- Tissue culture racks with fluorescent tubes and timer

Other apparatus:
- Spirit and distilled water
- Spirit lamp
- Forceps
- Surgical blades
- Blade holders
- Dropper bottles

Preparation of culture media:
The MS (Murashige and Skoog, 1962) medium required for the in vitro embryo culture of *S. alata* was prepared as mentioned in the steps below.

A) Preparation of stock solutions:
Macro nutrients and Micro nutrients, Vitamins Cacl2, Fecl2 etc., were prepared in their respective stocks of 1 mg/ml. The stock solutions were added to the medium as required.

Preparation of stock solution for MS medium:
1. **Stock- I (Micronutrients)**
   - a) NH4NO3 - 16.5 gm
   - b) KNO3 - 19.0 gm
   - c) MgSO4.7H2O - 3.7 gm
   - d) KH2PO4 - 1.7 gm
2. **Stock –II (Micronutrients)**
   - a) MNOSO4.7H2O - 2.230 gm
   - b) Znso4.7H2O - 860 mg
   - c) H3BO3 - 620 mg
   - d) NaMoO4 - 25 mg
   - e) Cuso4.5H2O - 25 mg
3. **Fe-EDTA (chelating agent) Stock- III**
   - a) Na2EDTA - 373mg

4. **Vitamins. Stock -IV**
   - a) Thymine Hcl - 25 mg
   - b) Hcl - 25 mg
   - c) Nicotinic acid - 25 mg
   - d) Glycine - 50 mg

5. **Stock –V CaCl2**
   - a) CaCl2.2H2O - 4.4 gm/100 ml

6. **Stock –VI**
   - a) KI - 41.5 mg/50 ml
   - b) Myo-Inositol - 100 mg/100 ml
   - c) 0.1 N NaOH - 300 mg in 50 ml distilled water
   - d) 0.1 N HCl - 3.6 ml/100 ml distilled water
   - e) Hgcl2 (0.1%) - 100 ml/100 ml

B) Preparation of BAP (PGR):
BAP or 6-Aminobenzyl Purina is the major PGR used in our present study for the preparation of 1mg/ml BAP, 20 mg of BAP was dissolved in 0.5 ml of 0.1N NaOH. The volume of stock was adjusted to 20 ml with distilled water.

Preparation of culture medium (for 1 liter):
The stock solution was added to about 700 ml of distilled water. To this solution, 3% (w/v) Sucrose was added and dissolved completely. The solution was made to a final volume of 1 lit with distilled water. The stock solution of BAP was added to the medium in varying concentrations (0.5-4.0mg/L). The pH of the medium was adjusted to 5.6±0.2 using 0.1 N HCl or 0.1N NaOH. Agar Agar which acts as solidifying agent is added to the medium after adjusting the pH. The medium was boiled on a heater for 15-20 min to till the Agar is completely dissolved. The medium was then dispensed into culture vessels at a rate of 50 ml per bottle and tightly capped using poly propylene caps; the medium was autoclaved for 15-20 min .at 121˚Cat 15 lbs pressure.

Plant materials:
The dried pods of *S. alata* were collected from the botanical garden of Kakatiya University, Warangal. The pods were carefully opened and the seeds were carefully separated. The seeds of *S. alata* were thoroughly washed.
under running tap water with 2-3 drops of tween 20 (detergent) followed by 0.2 % (w/v) Bavistine and again washed thoroughly under running tap water for 10-15 min. The seeds were subjected to mechanical scarification using a scalpel and in the laminar air flow cabinet, under sterile conditions, the seeds were sterilized with 0.1% (w/v) HgCl₂. For 90-120 sec. Followed by 3-4 rinses with sterilized distilled water and the seeds were then soaked overnight in sterile distilled water.

After overnight soaking, before inoculation, the seeds were again rinsed with sterile distilled water for 2-3 times. The seeds were blot on a sterile tissue paper. The embryos were carefully dissected and plated onto MS medium containing 3% sucrose (w/v) supplemented with various concentrations of BAP, solidified with 0.8% agar. The pH of the medium was adjusted to 5.7±0.2 using 0.1 N HCl or 0.1 N NaOH. Before addition of agar and the medium was autoclaved at 121°C for 15-20 min. The embryos were transferred to the culture room where they were allowed to germinate at a temperature of 25±2°C under a 16 hr photoperiod with a light intensity of 40-50 µmol·m⁻²·s⁻¹ maintained using white fluorescent tubes. Data on the germination of zygotic embryos, percentage of germination, shoot and root formation was recorded periodically.

**Explant Inoculation:**

The embryos that were carefully dissected from the seeds were transferred to the medium containing different concentrations of BAP (0.5-4.0 mg/L) and were inoculated in a culture room with 16 h photoperiod and light intensity and temperature using cool white fluorescent tubes were maintained at 25±2°C.

**RESULT AND DISCUSSION**

*S.alata* is an ethno medicinal plant which is known to possess a wide range of medicinal properties. Seed germination is a major problem in *S.alata*, even though germination is initiated by mechanical scarification, the embryos fail to develop. So, embryo culture is undertaken to solve the problem of embryo getting aborted.

The embryos which are carefully separated from the seeds of *S.alata* were cultured on MS medium augmented with different concentrations of BAP. Germination of the embryos was found to be 50% on MS medium (without BAP) which later developed into Callus which the other medium with BAP, the response was 100% with well developed shoot and root systems. The average number of days for seed germination was found to be between 5-8 days on MS medium and on the medium with BAP it is reported as 1-4 days. Effective shoot development was observed on MS medium supplemented with 2.0 and 2.5 mg/L BAP with a shoot length 2.94±0.09 and 2.91±0.09 cm and maximum rooting was observed on the medium supplemented with 1.5 mg/L. For was shown in the table below (Table-III).

The embryos were placed in there different positions (Viz, Half dipped, fully dipped, horizontal) to study the effect of the position on the germination of the embryo. The embryos that were placed horizontally produced callus while the embryos that were placed vertically into the media developed into healthy plantlets with an average germination percentage of 80%. Similar results were observed by Rambabu *et al.*, in *Givotia rottleriformis*. They also reported the formation of callus when the embryos were placed horizontal in the culture media and well developed plantlet formation was observed when the embryos were placed in the upright position.
Table –III- showing the % of embryo culture of germination shoot and root length in *S.*alata.*

<table>
<thead>
<tr>
<th>S.No</th>
<th>Concentration of BAP</th>
<th>% of response</th>
<th>Average of no of days for germination</th>
<th>Shoot length*</th>
<th>Root length*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>MS Basal</td>
<td>50</td>
<td>5-8 Days</td>
<td>CALLUS</td>
<td>---</td>
</tr>
<tr>
<td>2.</td>
<td>0.5</td>
<td>80</td>
<td>3-4 Days</td>
<td>1.96±0.04</td>
<td>1.59±0.05</td>
</tr>
<tr>
<td>3.</td>
<td>1</td>
<td>100</td>
<td>3-4Days</td>
<td>2.1±0.05</td>
<td>1.68±0.05</td>
</tr>
<tr>
<td>4.</td>
<td>1.5</td>
<td>100</td>
<td>1-2Days</td>
<td>2.14±0.03</td>
<td>2.23±0.06</td>
</tr>
<tr>
<td>5.</td>
<td>2</td>
<td>100</td>
<td>1-2Days</td>
<td>2.91±0.09</td>
<td>1.95±0.06</td>
</tr>
<tr>
<td>6.</td>
<td>2.5</td>
<td>100</td>
<td>1-2Days</td>
<td>2.94±0.09</td>
<td>1.66±0.05</td>
</tr>
<tr>
<td>8.</td>
<td>3</td>
<td>100</td>
<td>3-4Days</td>
<td>2.17±0.06</td>
<td>1.4±0.04</td>
</tr>
<tr>
<td>9.</td>
<td>4</td>
<td>100</td>
<td>3-4Days</td>
<td>1.8±0.03</td>
<td>1.2±0.04</td>
</tr>
</tbody>
</table>

*Mean ± SE
Ms basal medium with germination Callus formation with 5-8 days

A) 0.5 Cons of BAP with germination shoot formation

B) 1 Cons of BAP germination shoot and root formation

C) 1.5 Cons of BAP germination shoot and highly root formation

D) 2.0 and 2.5 Cons of BAP Good germination of shoot length

F & G) Shooting and Rooting plant was formation

H) The plant was transformed into vermiculate covered with polyethylene bags

I) The developed plants was transformed or soil
Table -IV: Showing the effect of position of embryos on germination shoot and root length of S.alata

<table>
<thead>
<tr>
<th>S NO</th>
<th>Embryo orientation</th>
<th>Days to shoot emergence</th>
<th>Germination (%)</th>
<th>Days to root emergence</th>
<th>No.of roots/plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Upright</td>
<td>2</td>
<td>100</td>
<td>6</td>
<td>8 ± 0.04</td>
</tr>
<tr>
<td>2</td>
<td>Embedded</td>
<td>4</td>
<td>100</td>
<td>8</td>
<td>10 ± 0.03</td>
</tr>
<tr>
<td>3</td>
<td>Embryo without cotyledons</td>
<td>3</td>
<td>100</td>
<td>10</td>
<td>8 ± 0.04</td>
</tr>
<tr>
<td>4</td>
<td>Horizontal</td>
<td>Callus</td>
<td>Callus</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

*Mean ± SE

CONCLUSION

*Senna alata* is an ethnomedicinal plant with a wide range of medicinal properties. Seed germination is a major problem in *S.alata*. The present study is undertaken to develop an efficient protocol for *in vitro* zygotic embryo culture in *S.alata*, which is an alternative to overcome problem of seed germination. *In vitro* culture of embryos was studied on MS basal medium and MS medium supplemented with various concentrations of BAP. Data on the development of embryos in all the culture media were reported periodically. From our results it can be concluded that the best suitable medium for embryo culture is MS medium supplemented with 2.0 mg/L and 2.5mg/L BAP when the embryos were placed vertically in the culture medium and the time recorded for embryo germination was only 1-3 days. Callus formation was reported in the MS basal medium and in all the culture media when the embryos were placed horizontally. The present study favors the *in vitro* culturing of zygotic embryos in *S.alata* where the seeds fail to germinate or takes longer time for germination.

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M.GURAVAIAH* IN VITRO MICROPROPAGATION OF Senna Alata - AN ETHNOMEDICINAL PLANT THROUGH EMBRYOCULTURE
