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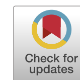
Original article

# Somatic embryogenesis and *in vitro* plant regeneration of Bacopa monnieri (Linn.) Wettst., a potential medicinal water hyssop plant

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*Bacopa monnieri* (Linn.) Wettst. commonly known as waterhyssop, Brahmi plant, traditionally used for memory enhancement, nerve tonic, epilepsy, central nervous system (CNS), antidepressant, anxiety, blood pressure and antioxidant activities. Due to pharmaceutical demands its lost natural habitat. At this juncture we describe a resourceful protocol for micropropagation of water hyssop plant. Surface sterilized leaf and nodal explants were inoculated on basal MS semi-solid medium added with PGRs; auxins, cytokinins. Highest calli formation from leaf explants was obtained on NAA (2.5 $mg^{-1}$) and showed (94.22%) accompanied via 2,4-D showed (2.5 $mg^{-1}$; 82.43%), maximum calli formation in nodal explants was obtained on 2,4-D showed (2.5 $mg^{-1}$; 71.14%) followed by NAA (2.5 $mg^{-1}$) showed (62.15%), in internodes explants uppermost calli formation was obtained from 2,4-D showed (2.5 $mg^{-1}$; 65.21%) followed by NAA (2.5 $mg^{-1}$) showed (52.14%). The maximum somatic embryogenic callus, calli induction and formation (84%) was observed on 2,4-D + KIN (2.0 + 1.5 $mg^{-1}$) amended solid medium. Uppermost shoot formation was observed in combination of IAA + BAP (1.0 + 1.0 $mg^{-1}$) showed (78.54%) shoot formation followed by IBA (2.0 $mg^{-1}$) alone showed (75.37%). The maximum shoot elongation was noticed from NAA + BAP (3.0 + 3.0 $mg^{-1}$) with 21.21 cm followed by NAA (2.0 $mg^{-1}$) showed (15.22 cm) although, chief root formation was obtained from IBA (2.0 $mg^{-1}$) with 83.75% root formation along higher number of roots (47.43%) per shoot. Followed by IAA (2.0 $mg^{-1}$) showed root induction (73.43%) and no of roots (38.54%) per shoot. In hardening under pot condition plants survivability (100%) was observed under glass house conditions, the present *in vitro* PTC techniques is extremely significant to gratifying its natural conservation.

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## 1. Introduction

The traditional source comes from plants, numerous chemicals used as fragrances, biochemicals, pharmaceuticals, foods, flavours and colours. Most expensive phytochemicals are products of plant secondary metabolism and acquire adequate structural or chemical complication, so that simulated synthesis is complicated (Leung, 1980). The plant tissue culture (PTC) process considered extremely important in expressions of profitable medicinal plant production in whereabouts the microbes free culture of cells, tissues and organs can be realize under aseptic conditions (Arjun, 2011). Moreover, this methods is functional for conserve important medicinal plants and attractive its secondary metabolites of engineering consequence throughout *in vitro* propagation techniques. Clonal propagation by PTC popularly called micropropagation; it also employed to defend luxurious germplasm and its implement for plant breeder (Murashige, 1974). Plant cells are distinctive capability to renew organogenesis (multicellular organs) when showing to an appropriate plant growth medium, nutrients and plant growth regulators (PGRs) (Hariprasath et al., 2015). An interchange conduit of plant regeneration is somatic embryogenesis which involves the formation of embryos from vegetative cells that are similar to seed embryos but extend lacking any sexual fusion of male and female gametes. A somatic embryogenesis method meaning for the clonally industrial unit in the generation of millions of disease free plants, comparatively tiny duration of time and at reduced price (Ishii et al., 2004; Nikam et al., 2009). It is a

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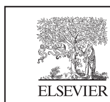


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significant implement in mutually applied and basic studies, as well as in marketable applications (Nagarajan et al., 2009). Although conservative breeding methods are significantly augmented the efficiency of modern crops, the biotechnology application might be rapidity up supplementary medicinal, agriculture crops development (Rao et al., 1996).

*Bacopa monniera* (L.) Wettst. belong to the Scrophulariaceae family; it grows in marshy or damp areas, bog garden, pond, glaburous, succulent, creeping herb. The entire plant a potential indigenous medicinal plant used in memory enhancement, nerve tonic, epilepsy, CNS, antidepressant, anxiety, blood pressure and antioxidant activity. It grows in Nepal, India, China, Sri Lanka, Vietnam, Taiwan, USA and Florida (Darpan et al., 2009). In India, the plant name commonly called as Brahmi, practiced by Ayurvedic fraternity, mainly used in polyherbal formulations, it's used for psoriasis, abscess and eczema, stimulate hair growth, skin, chronic rheumatism, skin diseases, asthma, leprosy, hoarseness and nervous system (Uma et al., 2010; Kumar et al., 2012). *Bacopa* or Brahmi secondary metabolies are bacosides A and B, facilitate refurbish damaged neurons, stigmasterol, sapogenins, flavonoids, triterpenoid saponins, *D*-mannitol, betulic acid, octacosane, β-sitosterol, amino acids nicotine, aspartic acid, α-alanine, glutamic acid, serine, herpestine, brahmines, hersaponin, stigmasterol, betulinic acid, β-sitosterol and stigmastenol (Jain and Kulshreshtha, 1993; Rastogi et al, 1994).

Based on the literature, medicinal property important and pharmaceutical industrial demands, the water hyssop plant was selected for tissue culture techniques. The current study was aimed to develop rapid, simple, competent protocol for somatic embryogenesis, organogenesis, *in vitro* and plant regeneration through different explants of *B. monniera*.

## 2. Materials and methods

### 2.1. Chemicals

Analytical grade chemicals supplied by E. Merck, Hi-Media, S.D. Fine Chemicals, Sigma and Qualigens Chemicals (U.S.A.) were used.

### 2.2. Resource of plant material

The entire new cleaned plant materials of *B. monniera* were purchased from local market; it's authenticated by **Dr. Raju Ramasubbu**, Taxonomist, Department of Biology, Gandhigram Rural Institute, Gandhigram, Tamil Nadu, India.

### 2.3. In vitro propagation of water hyssop

#### 2.3.1. Surface sterilization of explants

The healthy grown plant leaf, node and internodes were unruffled and wash carefully beneath running tap water, surface sterilization to produce contamination free plants were carried out under microbes free aseptic conditions. The explants were transferred to a beaker containing running tap water (10 min) without damage plant tissues, then explants washed with sodium hypochlorite (3%; 15 min) then explants were washed with $HgCl_2$ (0.2%) with dissimilar time duration, changing the solution at 5 min interval. The sterilized (surface) explants were washed through distilled (sterile) water for 3–5 times (5 min each). The explants were slashed into minute pieces (0.5–1.0 cm) slashed explants transfer to MS (Murashige and Skoog, 1962) semi-solid medium under *in vitro* conditions in a laminar flow chamber (Hariprasath et al., 2015).

#### 2.3.2. Plant tissue culture media preparation

The MS medium (1962) was used with 6 stock solutions individually; macro, minor, micro, iron and vitamins were equipped and stored. Auxins, cytokinins and *meso*-inositol, stock solutions were freshly prepared and used every month. Freshly prepared sucrose (30 $g^{-1}$; 3%), *meso*-inositol (100 $mg^{-1}$; 0.1%), solidifying agent agar (0.8%; 8 g/L), obligatory amount of plant growth hormones in additional to MS medium. Facing autoclaving (121 °C), 1.06 $kg/cm^2$ pressure (20 min), the MS medium was buffer with 1 N HCl and 1 N NaOH to regulate solutions pH (5.6–5.8). Subsequently, medium prepared was reserved beneath refrigerator situation awaiting additional use. The 25 ± 2 °C cultures were incubated with a 70–80% qualified humidity and (16/8 h Light/Dark) photoperiod under photon flux compactness (50 $\mu E\ mol/m^2/s^2$) (Vinothini et al., 2017).

### 2.4. Statistical analysis

The statistical analysis were influenced by ANOVA (One-way Analysis of Variance) to calculate the noteworthy of dissimilarity of resources of an assortment of management groups, with the DMRT (Duncan's Multiple Range Test) by means of SPSS (Statistical Package for the Social Sciences) (Version 16.0) level (5%) package, values are presented as significance ($P < 0.05$).

## 3. Results and discussion

The *in vitro* plant rejuvenation is based on exploitation of organic, inorganic and ingredients in the selected MS medium, surroundings of plants, explants type and plant species. Biotechnological tackle are significant for genetic enhancement, reproduction of important medicinal plants throughout accept the procedures such as *in vitro* plant regeneration (Tripathi and Tripathi, 2003). The *in vitro* regeneration establishment consistent protocols are a precondition for function of genetic manipulation methods to augment production of medicinally significant secondary metabolites (Nikam et al., 2009).

### 3.1. Callus and calli formation from nodal and leaf explants

The undifferentiated biomass accumulation of cells of parenchymatous are recognized as callus. For callus induction, the surface sterilized leaf and stem (node, internode) explants slice into minute pieces (0.5–1.0 cm) and placed on MS basal semi-solid medium supplemented with PGRs; auxins and cytokinins (2,4-D, NAA, IAA, BAP (0.5–2.5 $mg^{-1}$), alone and in combination, different concentrations, incubated under dark condition (5 days), then its transferred L/D (16/8 h) conditions for callus and calli induction and formations. Early callus stimulation was perceived on the wounded water hyssop leaf and stem explants surfaces after seven days of inoculated on MS medium (Table 1). The callus, calli secured from leaf, stem explants were noticed that friable, pallid with soft surfaces, and then it's curved to compact and green after revelation to luminosity conditions. Subculture was performed on every 4th weeks subsequent to *in vitro* callus, calli maintain the culture, and with the medium has same and dissimilar MS medium composition. From leaf explants highest calli development was perceived on NAA (2.5 $mg^{-1}$) it showed (94.22%) followed via 2,4-D showed (2.5 $mg^{-1}$; 82.43%), lowest calli formation was obtained on IBA (2.5 $mg^{-1}$), respectively (Table 1; Fig. 1). From nodal explants maximum calli formation was obtained on 2,4-D showed (2.5 $mg^{-1}$; 71.14%) followed with NAA (2.5 $mg^{-1}$) showed (62.15%), fewest calli formation was noticed on IBA (2.5 $mg^{-1}$) showed (50.32%), respectively. from the internodes explant upper-

**Table 1**
Calli formation from different explants of waterhyssop plant.

| Types of PGRs | Concentrations (mg$^{-1}$) | Calli formation (%) | | |
|---|---|---|---|---|
| | | Leaf | Node | Internodes |
| 2,4-D | 0.5 | 16.54 ± 0.57$^{l}$ | 24.24 ± 0.57$^{k}$ | 08.32 ± 0.88$^{k}$ |
| | 1.0 | 28.21 ± 0.11$^{i}$ | 39.66 ± 0.66$^{h}$ | 19.22 ± 0.33$^{i}$ |
| | 1.5 | 42.88 ± 0.88$^{g}$ | 49.35 ± 0.00$^{f}$ | 30.22 ± 0.33$^{g}$ |
| | 2.0 | 64.32 ± 0.00$^{d}$ | 60.43 ± 0.33$^{c}$ | 44.45 ± 0.57$^{d}$ |
| | 2.5 | 82.43 ± 0.33$^{b}$ | 71.14 ± 0.33$^{a}$ | 65.21 ± 0.33$^{a}$ |
| NAA | 0.5 | 21.33 ± 0.33$^{j}$ | 21.21 ± 0.88$^{l}$ | 17.21 ± 0.33i |
| | 1.0 | 42.76 ± 0.66$^{g}$ | 32.22 ± 0.33$^{i}$ | 26.34 ± 0.66$^{h}$ |
| | 1.5 | 64.51 ± 0.5$^{d}$ | 47.44 ± 0.57$^{g}$ | 37.32 ± 0.33$^{f}$ |
| | 2.0 | 82.43 ± 0.33$^{b}$ | 59.32 ± 0.00$^{d}$ | 44.75 ± 0.57$^{d}$ |
| | 2.5 | 94.22 ± 0.00$^{a}$ | 62.15 ± 0.33$^{b}$ | 52.14 ± 0.33$^{b}$ |
| IBA | 0.5 | 08.21 ± 0.0.33$^{o}$ | 07.43 ± 0.33$^{p}$ | 07.34 ± 0.33$^{k}$ |
| | 1.0 | 13.45 ± 0.33$^{m}$ | 14.11 ± 0.88$^{n}$ | 16.43 ± 0.88$^{j}$ |
| | 1.5 | 10.11 ± 0.57$^{n}$ | 28.87 ± 0.33$^{j}$ | 27.75 ± 0.88$^{g}$ |
| | 2.0 | 21.22 ± 0.66$^{j}$ | 39.21 ± 0.0.33$^{h}$ | 39.23 ± 0.33$^{e}$ |
| | 2.5 | 44.21 ± 0.33$^{f}$ | 50.32 ± 0.57$^{f}$ | 49.12 ± 0.33$^{c}$ |
| 2,4-D + BAP | 0.5 | 08.14 ± 0.00$^{o}$ | 09.22 ± 0.33$^{o}$ | 05.14 ± 0 66$^{l}$ |
| | 1.0 | 19.11 ± 0.00$^{k}$ | 19.33 ± 0.33$^{m}$ | 11.11 ± 0 00$^{j}$ |
| | 1.5 | 38.42 ± 0.33$^{h}$ | 34.21 ± 0.33$^{i}$ | 22.45 ± 0 33$^{h}$ |
| | 2.0 | 54.22 ± 0.66$^{e}$ | 43.21 ± 0.88$^{h}$ | 35.61 ± 0.66$^{f}$ |
| | 2.5 | 71.65 ± 0.88$^{c}$ | 52.65 ± 0.0.33$^{e}$ | 21.45 ± 0.66$^{h}$ |

a The values represent the means (±SE) of three independent experiments. At least 60 cultures were raised for each experiment. Mean values within a column followed by the same letter are not significantly different by Duncan's multiple range test ($P \geq 0.05$).

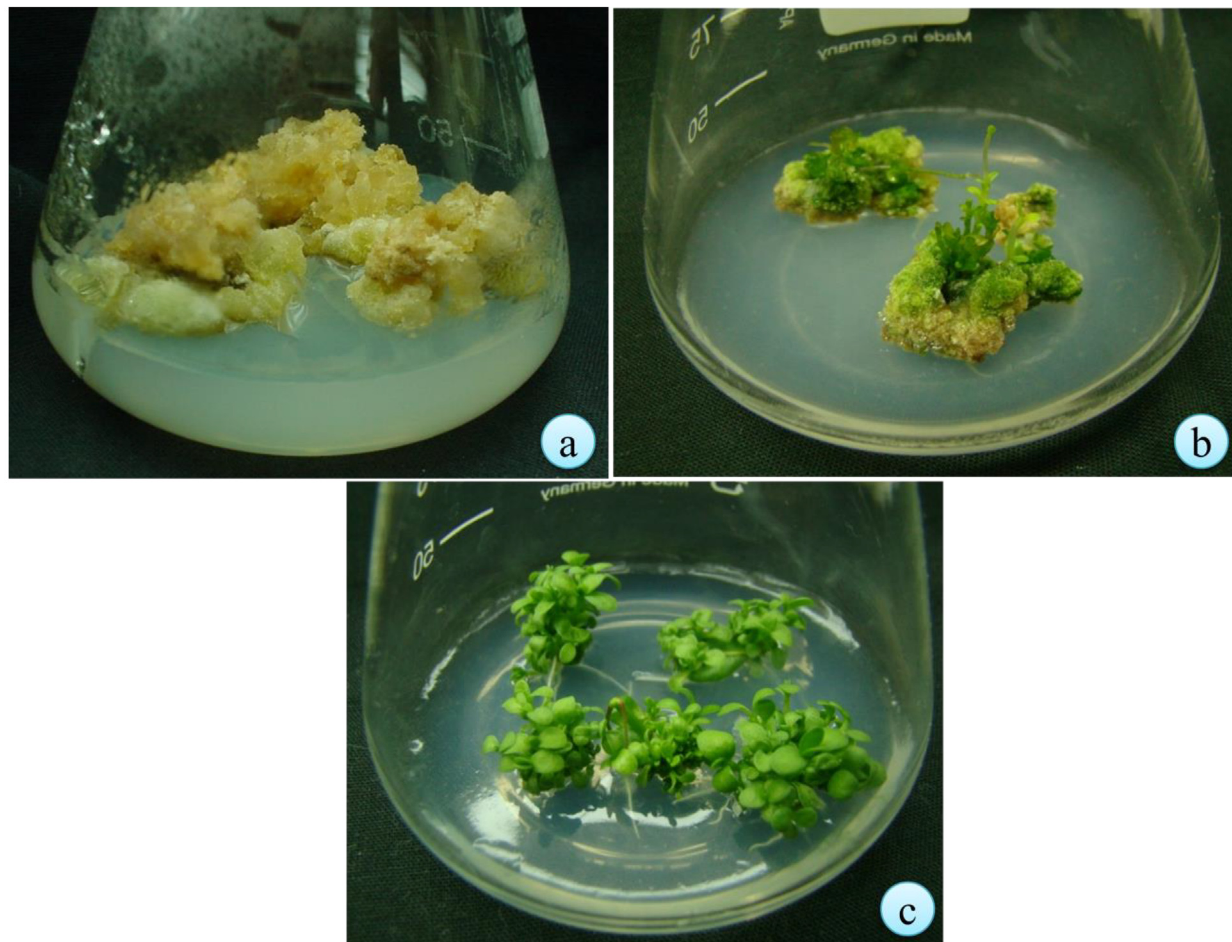


**Fig. 1.** Calli and multiple shoot formation. a. Calli formation, b. Shoot induction from organogenic calli, c. Multiple shoot initiation.

most calli formation was obtained from 2,4-D showed (2.5 $mg^{-1}$; 65.21%) followed in NAA showed (2.5 $mg^{-1}$; 52.14%), lowest calli formation was secured in amalgamation of 2,4-D + BAP (2.5 $mg^{-1}$) showed (21.45%), respectively. finally overall comparison of callus, calli induction, formation noticed from NAA (Table 1; Fig. 1).

The proliferation plant cells at wounded component of the explants might be auxins accumulation at the injury points, which encouraged the proliferation plant cells in the existence of PGRs (Ahmad et al., 2010). According to Ghosh and Sen (1994) recognized *Asparagus cooper* plant rejuvenation through *in vitro* callus culture from dissimilar explants. The 2,4-D + BA (2.5 μM + 5.0 μ M) combination of fashioned somewhat extra callus than mergers of BA/KIN (5.0 μM) among NAA (2.5 μM) observed good callus enlargement in BA/KIN (5.0 μM) with IAA (2.5 μM) has been noticed through Nikam et al. (2009). Callus formation from *Leptadenia reticulata* shoot-tip and nodal explants of selected medium possessions with BA/KIN is in connected with the account on *Holostemma ada-kodien* and *Gymnema sylvestre* (Martin, 2002; Komalavalli and Rao, 2000).

### 3.2. Somatic embryogenic callus and calli initiation, formation from leaf explants

The yellow-white embryogenic calli observed from water hyssop leaves explants were cultivated on the MS supplemented medium through NAA + KIN. The greatest somatic embryogenic callus, calli induction, development (84%) was obtained on 2,4-D + KIN (2.0 + 1.5 $mg^{-1}$) amended solid medium, 84% somatic embryo formation was observed (Fig. 2). After 30 d of background, the somatic embryos were separated and transferred to the regeneration medium. In MS medium augmented with KIN + 2,4-D (1.0 + 2.0 $mg^{-1}$) showed (71%) (Fig. 3).

### 3.3. Shoot induction, formation, multiple shoots from calli

Healthy developed calli transferred to basal MS medium combined with PGRs in dissimilar combinations, maximum calli induction was obtained from BAP (2.0 $mg^{-1}$) showed (97.75%) followed by NAA (2.0 $mg^{-1}$) showed (77.55%) (Table 2; Fig. 2), lowest shoot induction was observed on IAA (2.0 $mg^{-1}$) showed (74.32%), over all observation of shoot induction from BAP was noticed. For shoot formation auxin and cytokine alone and combined different concentrations used for shoot formations, uppermost shoot formation was observed in combination of IAA + BAP (1.0 + 1.0 $mg^{-1}$) showed (78.54%) shoot formation followed by IBA (2.0 $mg^{-1}$) alone showed (75.37%) (Table 2), lowest shoot formation was observed from NAA + BAP (2.0 + 2.0 $mg^{-1}$) showed (59.32%). Multiple shoot formation and shoot elongation (cm) was observed from PGRs alone and in combinations, different concentrations; highest multiple

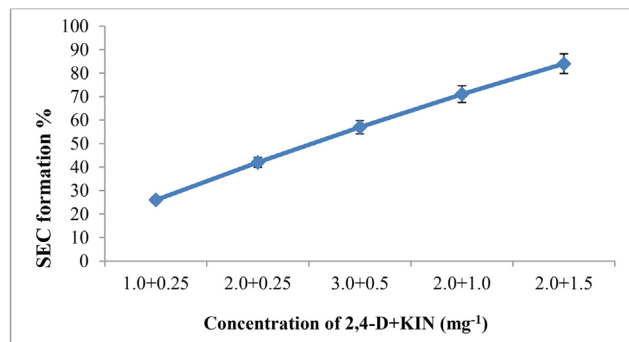


**Fig. 2.** Somatic embryogenic calli (SEC) formation from leaf explant.

shoot formation was observed from the combination of auxin and cytokinin (Table 2; Fig. 4). The elongated shoots per *in vitro* plants were transferred to fresh MS basal medium and incubated in light for another 4 weeks. The maximum shoot elongation was noticed from NAA + BAP (3.0 + 3.0 $mg^{-1}$) showed (21.21 cm) followed by NAA (2.0 $mg^{-1}$) showed (15.22 cm), lowest shoot elongation was observed from BPA (2.0 $mg^{-1}$) showed (7.50 cm).

Tejavathi and Shailaja (1999) suggested that the Brahmi plant shoot regeneration potential from IAA, similar results has also reported from *Cephaelis ipecacuanha* leaf callus on the MS medium augmented with KIN + NAA (4.5 $mg^{-1}$ + 0.1 $mg^{-1}$) (Rout et al., 1995). MS medium with BAP (2 $mg^{-1}$) shows uppermost shoot rejuvenation occurrence and number of shoots regenerated (6.0 ± 0.14 cm). The proliferation of shoot frequency was comparatively stumpy and smaller capacity shoots for every explant when MS medium was augmented via KIN (4.06 ± 0.44 cm; 60%) or TDZ (3.30 ± 0.48 cm; 45%) as a replacement for of BAP (Singh and Tiwari, 2011), supremacy of BAP greater than KIN for regeneration of shoots has been suggested in numerous systems counting *Pterocarpus marsupium* and *Andrographis paniculata* (Chand and Singh, 2004; Purkayastha et al., 2008). TDZ is extremely used for towering frequency regeneration of shoots commencing dissimilar explants in numerous species (Lata et al., 2009; Tiwari et al., 2001), excluding it is not effectual in container of *Clitoria ternatea*. MS basal augmented amid TDZ (1 $mg^{-1}$) unsuccessful to encourage regeneration of shoots from DEAs. Correspondingly, developed shoot buds on medium with TDZ were undersized and repeatedly spellbound and consequently unsuccessful to lengthen in numerous plant species such as *Balanites aegyptiaca* (Anis et al., 2010), *Rhododendron* spp. and *Dalbergia sisso* (Pradhan et al., 1998; Preece and Imel, 1991). Thus, BAP whilst supplementary unaccompanied in medium was mainly successful PGRs representative cytokinin particularly DEAs of *C. ternatea* for regeneration of multiple shoots (Singh and Tiwari, 2011).

### 3.4. Root induction from in vitro shoot plants

The root induction, formation was obtained from different PGRs alone, which shoot developed from different concentrations of PGRs, the utmost root formation was obtained from IBA (2.0 $mg^{-1}$) showed (83.75%), it showed (47.43%) number of roots for every shoot. Followed by IAA (2.0 $mg^{-1}$) showed root induction (73.43%) and number of roots (38.54%) for every shoot. Lowest root induction was obtained from NAA (2.0 $mg^{-1}$) showed (53.21%) and no of roots (31.56%) per shoot was noticed. According to Vinothini et al. (2017) uppermost root induction quantity was obtained on IBA showed (2.5 $mg^{-1}$; 76.3%), whereas fewest induction of root was obtained with showed $GA_3$ (1 $mg^{-1}$; 40.2%). According to Gurel and Gurel (1998) in several plants species auxins are vigorous toward encourage root formation adventitiously. Silver nitrate (2.0 $mg^{-1}$) found that can successfully appearance of roots on the *Beta vulgaris* (sugar beet) leaf explants. BAP and NAA were found efficient *Echinacea pallid* and *Solanum laciniatum* for plant regeneration by *in vitro* (Sharma & Rajam, 1995). Moreover in *S. grandiflora*, *in vitro* regeneration in shoots was productively reached in the attendance of BAP (Pande et al., 2002).

### 3.5. Acclimatization

Well developed plantlet amid roots developed carefully detached from the *in vitro* culture medium, then detached plant roots cleaned throughout sterile distilled water under lab conditions, washed plantlets were carefully shifted to plastic cups contain uncontaminated soil and vermiculite in the ratio (2:1 v/v). The plants kept under (16/8 h L/D) photoperiods. The MS ½ strength salt solution as regularly poured under laboratory circumstances as

**Fig. 3.** Somatic embryogenic calli and single plantlet development. a. Somatic embryogenic calli formation from *in vitro calli*, b. Heart shaped somatic embryo formation, c. Single plantlet form somatic embryo.

**Table 2**
Shoots and roots developments of waterhyssop plant from *in vitro* calli with different PGRs.

| Types of PGRs | Concentrations ($mg^{-1}$) | Shoot induction | Shoot formation | Multiple shoot formation | Shoot elongation (cm) | Root induction | No of roots (%) |
|---|---|---|---|---|---|---|---|
| IAA | 0.5 | 49.63 ± 0.33$^{f}$ | – | – | 4.45 ± 0.33$^{f}$ | 48.55 ± 0.33$^{e}$ | 37.33 ± 0.33$^{c}$ |
| | 1.0 | 62.54 ± 0.57$^{e}$ | – | – | 7.21 ± 0.33$^{d}$ | 65.33 ± 0.33$^{c}$ | 45.31 ± 0.00$^{b}$ |
| | 2.0 | 74.32 ± 0.00$^{c}$ | – | – | 10.33 ± 0.33$^{c}$ | 73.43 ± 033$^{b}$ | 38.54 ± 0.57$^{c}$ |
| NAA | 0.5 | 47.31 ± 0.66 $^{g}$ | – | – | 7.85 ± 0.88$^{d}$ | 31.21 ± 0.00$^{f}$ | 18.45 ± 0.88$^{f}$ |
| | 1.0 | 62.32 ± 0.33$^{e}$ | – | – | 11.75 ± 0.33$^{c}$ | 45.55 ± 0.57$^{e}$ | 30.75 ± 0.57e |
| | 2.0 | 77.55 ± 0.57$^{b}$ | – | – | 15.22 ± 0.00$^{b}$ | 53.21 ± 0.33$^{d}$ | 31.56 ± 0.66$^{e}$ |
| IBA | 0.5 | – | 53.45 ± 0.66$^{e}$ | 26.21 ± 0.33$^{e}$ | – | 66.43 ± 0.33$^{c}$ | 33.32 ± 0.33$^{d}$ |
| | 1.0 | – | 64.56 ± 0.66$^{c}$ | 38.34 ± 0.66$^{c}$ | – | 72.35 ± 0.57$^{b}$ | 45.32 ± 0.33$^{b}$ |
| | 2.0 | – | 75.37 ± 0.57$^{b}$ | 49.75 ± 0.66$^{a}$ | – | 83.75 ± 0.33$^{a}$ | 47.43 ± 0.33$^{a}$ |
| BAP | 0.5 | 49.51 ± 0.33$^{f}$ | 34.21 ± 0.33 $^{g}$ | 19.54 ± 0.57$^{f}$ | 3.52 ± 0.33$^{f}$ | – | – |
| | 1.0 | 71.75 ± 0.66$^{d}$ | 47.22 ± 0.33$^{f}$ | 26.75 ± 0.00$^{e}$ | 5.21 ± 0.33$^{e}$ | – | – |
| | 2.0 | 97.75 ± 0.88$^{a}$ | 60.15 ± 0.57$^{d}$ | 38.85 ± 0.57$^{c}$ | 7.50 ± 0.00$^{d}$ | – | – |
| IAA + BAP | 1.0 + 1.0 | – | 54.55 ± 0.66$^{e}$ | 26.21 ± 0.33$^{e}$ | 4.33 ± 0.33$^{f}$ | – | – |
| | 2.0 + 2.0 | – | 65.43 ± 0.33$^{c}$ | 39.21 ± 0.33$^{b}$ | 7.21 ± 0.88$^{d}$ | – | – |
| | 3.0 + 3.0 | – | 78.54 ± 0.57$^{a}$ | 51.21 ± 0.33$^{a}$ | 10.25 ± 0.66$^{c}$ | – | – |
| NAA + BAP | 1.0 + 1.0 | – | 35.37 ± 0.00 $^{g}$ | 18.45 ± 0.88$^{f}$ | 11.21 ± 0.57$^{c}$ | – | – |
| | 2.0 + 2.0 | – | 47.45 ± 0.00$^{f}$ | 28.35 ± 0.66$^{d}$ | 16.55 ± 0.33$^{b}$ | – | – |
| | 3.0 + 3.0 | – | 59.32 ± 0.33$^{d}$ | 40.15 ± 0.33$^{b}$ | 21.21 ± 0.33$^{a}$ | – | – |

a The values represent the means (±SE) of three independent experiments. At least 15 cultures were raised for each experiment. Mean values within a column followed by the same letter are not significantly different by Duncan's multiple range test ($P \geq 0.05$).

primary hardening. Primarily the hard-edged plantlets were congested with polythene for maintain lofty virtual humidity 80%. After 2 weeks period, the polythene bags were removed and the potted plants were exposed to direct light and sterile distilled water was poured under pot condition plants survivability (100%) was observed under glass house conditions (Fig. 4).

## 4. Conclusion

In the current study, resourceful procedure for the Brahmi plant regeneration established through somatic embryogenesis. Plant tissue culture considered to be an appropriate coordination for great level production of medicinal plants. The present *in vitro*

**Fig. 4.** In vitro plant regeneration. a. Shoot and root formations from *in vitro* calli,b. Multiple shoot formation from *in vitro* calli, c. Hardening.

propagation method was found successful for the huge amount calluses production in water hyssop plant. Accordingly, the current micropropagation of PTC techniques are extremely significant for rewarding its marketplace requirements through micropropagation production, it increased without disturbing its natural environmental habitats.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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