Micropropagation and in Vitro Flowering in Basella Spp.

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Abstract

*Basella* spp. a perennial vine of Basellaceae family used as a leafy vegetable. Phytonutrient of *Basella* spp. is being exploited in Indian medicinal system since antiquity for its antifungal, anticonvulsant, anti-inflammatory, antipyretic, antiulcer and analgesic properties. Propagation of *Basella* through seeds have limitations for germination and flowering, *in vitro* regeneration was studied on MS (Murashige & Skoog) media supplemented with various plant growth regulators, indole acetic acid (IAA), Indole butyric acid (IBA), 1-naphthalene acetic acid (NAA), N6-benzyladenine (BA), kinetin (KIN), zeatin (ZEA), gibberillic acid (GA3), and adenine sulphate (ADS), silver nitrate (AgNO3) as additives. The shoot bud initiation was observed in all the combinations studied showing a good response for direct regeneration. Shooting (84%) was observed in 12 days after inoculation in 1mg l⁻¹ BA + 0.1 mg l⁻¹ NAA. Liquid media containing 0.1mg l⁻¹ BA + 0.5 mg l⁻¹ KIN + 0.1 mg l⁻¹ IAA was preeminent in multiple shoots (22 ± 0.13) production with average shoot length (5.81 ± 0.19) in 5 weeks (wk). Supplementation of 40 mg l⁻¹ AgNO3 and 40 mg l⁻¹ ADS to media containing 1mg l⁻¹ BA + 0.1 mg l⁻¹ NAA resulted in enhanced number of elongated shoots with number of leaves. *In vitro* flowering was obtained on MS media containing 0.5mg l⁻¹ BA + 0.5 mg l⁻¹ GA3 concentrations. The survival rate of hardened plants was 90% after transferring to soil. This protocol can be efficiently used for mass production for regeneration of genetically transformed *Basella* spp. in studying its metabolite profile specially betalains and transformation of *Basella*.

Introduction

*Basella* spp. is an edible, annual or biennial succulent leafy climbing herb of family Basellaceae. *Basella* spp. is generally consumed as a leafy vegetable for its phytonutrient in tropical regions of Asia and Africa and is a native of Indian subcontinent. *Basella* spp. is commonly known with many vernacular names such as Indian spinach, Malabar spinach, climbing spinach, vine spinach, Ceylon spinach and creeping spinach (Roy et al. 2010; Kumar et al. 2016). The most widely used two common varieties are, *Basella alba* L. with green stem and *B. Rubra* L. with reddish-purple stem (Roy et al. 2010). *Basella* leaves are thick stalked ovate or heart-shaped with mucilaginous in nature. The flowers of *Basella* are bisexual, discreet on axillary spikes or on peduncles with white or pinkish or violet in colour (Kumar et al. 2016). The fruits are green in color initially, change to purple when matured, fleshy, sessile, ovoid or spherical in shape with single seed (Kumar et al. 2016). The phytonutrients content, pigment profile, bioactive phenolics and flavonoids of *Basella* spp foliage was reported (Kumar et al. 2016; Singh et al. 2016).

The most economically important part of *Basella* spp. is the mature fruits which are a rich source of red and yellow indole-derived pigments- Betalains with value-added potential for use as natural dye, in food colorants, in pharmaceutical, nutraceutical and cosmetic industries (Kumar et al. 2016). The plant has been reported for its antimicrobial, anticonvulsant, analgesic, anti-inflammatory, antimutagenic, antidiabetic, anticancerous, antiviral, depressant, hepatoprotective, wound healing, androgenic activities and for the treatment of anemia (Kumar et al. 2016).
As stem and leaves of *Basella* are mainly used in culinary practice, frequent harvesting delays flowering and stimulates growth of lateral shoots (Singh et al. 2016). The exploitation of economically important phytoconstituents of this ethanomedicinal plant resulted in an increased industrial demand and the cultivation through conventional methods using seeds or stem cutting is labor intensive. Therefore the *in vitro* micro propagation promises an efficient alternate method for mass propagation, production of disease free plants and conservation of germplasm. Auxin free Gamborg's medium was used to induce multiple shoots from nodal explants of *Basella* spp. (Norris et al. 1989). Similarly, Shekawat and Manokari (2016) studied in vitro shoot induction response for *B. alba*.

The enhanced shoot morphogenesis in presence of ethylene action inhibitors such as silver nitrate was reported in *Capsicum frutescens* (Sharma et al. 2008). The additives like silver ions in the form of silver nitrate or silver thiosulphate and adenine sulphate, incorporation in MS basal media has proved to enhance plant regeneration, multiplication, induction of somatic embryogenesis and also inducing *in vitro* flowers (Kumar et al. 2009; Jana and Shekhawat, 2011). Several reports are available on fortification of these ethylene inhibitors in MS media to check the morphological potential of the explants in *Coffea canephora* (coffee; Giridhar et al. 2004), *Capsicum frutescens* (Capsicum; Sharma et al. 2008), *Vanilla planifolia Andr.* (*vanilla*; Giridhar et al. 2001) and *Rivina humilis* (Harsha et al. 2012).

The flowering cycle through floral transition involves transformation of vegetative shoot apical meristem to floral meristem forming an important event in flowering plants. Flowering is a complex process regulated by different environmental and endogenous cues and its incidence in *in vitro* culture is not commonly observed (Sharma et al. 2008). Application of exogenous hormones to *in vitro* culture media can induce *in vitro* flowering in various plants (Yadav and Singh 2011).

Although meager studies on *in vitro* propagation have been performed previously (Norris et al. 1989; Shekawat and Manokari 2016), the achieved multiplication rate was inept, and GA₃, zeatin, adenine sulphate and silver nitrate in culture media were not tested. Apart from this, flowering of *in vitro* cultures in these species has not been described or reported. Moreover, a protocol for mass multiplication of Basella spp. is a prerequisite for genetic transformation of *Basella* spp.

In the current study the effect of different plant growth regulators on *in vitro* shoot regeneration and *in vitro* flowering in *Basella* spp. was studied.

**Materials And Methods**

**Plant source, explants surface sterilization and culturing**

*Basella alba* L. and *B. rubra* L. plants were collected from plant nursery of Bangalore University, have been maintained in RVCE college garden served as plant source of explant for this present study. The young tender leaves, nodes and shoot tips up to 5th node from the tip of branches of *Basella* were selected as explants for in vitro experiment. The explants were surface sterilized by initial washing with
Tween 20 under running tap water for 20 min. Then explants were treated with 1% Bavistin (w/v) for 1h with continuous shaking. After 1h, explants were washed 4–5 times with sterile distilled water, followed by 0.1% mercuric chloride (w/v) treatment for 3 min with thorough washing 3–4 times with sterile water to remove traces of mercuric chloride. The explants were later treated with 70% alcohol for 20 seconds and again washed thoroughly with sterile water. Finally blotted on sterile tissue paper towels and inoculated on MS media (Murashige & Skoog 1962) supplemented with different combinations and concentration of plant growth regulators such as indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), 1-naphthalene acetic acid (NAA), N6-benzyladenine (BA), kinetin(KIN), zeatin (ZEA), and gibberillic acid (GA$_3$). Also used adenine sulphate (ADS) and silver nitrate (AgNO$_3$) as additives. All the growth regulators and AgNO$_3$ were procured from Sigma-Aldrich, Bengaluru. All the chemicals and media components used were from HIMEDIA or otherwise mentioned.

**Axillary Bud Initiation And Shoot Multiplication**

MS basal media containing 3% sucrose and 0.7% of agar was augmented with different regimes of plant growth regulators were used for axillary bud initiation, the pH of the media was adjusted to 5.8 using 1 N NaOH/HCl. The additives like 40 mg l$^{-1}$ AgNO$_3$ and 40 mg l$^{-1}$ ADS were used in combinations with different concentration of BA and NAA. These concentrations were selected based on the preliminary studies and the best responses obtained only were chosen for further study. The media were sterilized at 121$^\circ$ C (15 psi) for 30 min. The explants were inoculated separately for *in vitro* regeneration on MS media containing 1–6 mg L$^{-1}$ BA, 1 & 2 mg l$^{-1}$ BA + 0.1 & 0.5 mg l$^{-1}$ NAA, 2 mg L$^{-1}$ BA + 0.5&1 mg L$^{-1}$ GA$_3$ and 0.25 mg L$^{-1}$ ZEA + 0.1 & 0.5 mg L$^{-1}$ GA$_3$ and the cultures were incubated in dark for a week (Table 1). After a week, the cultures were shifted to light with 16/8 h (dark/light) photoperiod at 26 ± 2$^\circ$ C, for 4-8weeks (wk). The microshoots were transferred to multiplication media after 4–8 wk (Table 2). The microshoots of the mother explants were subcultured repeatedly on fresh liquid MS media supplemented with 0.1 &0.5 mg l$^{-1}$ BA + 0.1 &0.5 mg l$^{-1}$ KIN with or without 0.1 mg l$^{-1}$ IAA and 40 mg l$^{-1}$ AgNO$_3$, incubated for 4–5 wks on orbital shaker at 60 rpm. After 5wk the shoots were excised and inoculated to shoot elongation media containing different combinations and concentrations of BA, GA$_3$, IBA, ADS and AgNO$_3$ (Table 3). The cultures were maintained under 16/8 h (dark/light) photoperiod at 26 ± 2$^\circ$ C, for 2 wk with regular subculturing. The shoots grown to a length of 2–3 cm were transferred to rooting media containing 0.5 mg l$^{-1}$ IBA in half strength MS media.
Table 1
Effect of plant growth regulators on *in vitro* shooting response of nodal explants in *Basella* spp. on MS media

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>MS media composition (mg l(^{-1}))</th>
<th>Duration of bud breaking in days</th>
<th>Shooting response (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td>2</td>
<td>1 BA</td>
<td>14</td>
<td>76</td>
</tr>
<tr>
<td>3</td>
<td>2 BA</td>
<td>15</td>
<td>76</td>
</tr>
<tr>
<td>4</td>
<td>4 BA</td>
<td>17</td>
<td>73</td>
</tr>
<tr>
<td>5</td>
<td>6 BA</td>
<td>17</td>
<td>58</td>
</tr>
<tr>
<td>6</td>
<td>1 BA + 0.1 NAA</td>
<td>12</td>
<td>84</td>
</tr>
<tr>
<td>7</td>
<td>1 BA + 0.5 NAA</td>
<td>15</td>
<td>78</td>
</tr>
<tr>
<td>8</td>
<td>2 BA + 0.1 NAA</td>
<td>15</td>
<td>81</td>
</tr>
<tr>
<td>9</td>
<td>2 BA + 0.5 NAA</td>
<td>15</td>
<td>76</td>
</tr>
<tr>
<td>10</td>
<td>2 BA + 0.1 GA(_3)</td>
<td>17</td>
<td>80</td>
</tr>
<tr>
<td>11</td>
<td>2 BA + 0.5 GA(_3)</td>
<td>15</td>
<td>75</td>
</tr>
<tr>
<td>12</td>
<td>0.25 ZEA + 0.1 NAA</td>
<td>14</td>
<td>81</td>
</tr>
<tr>
<td>13</td>
<td>0.25 ZEA + 0.5 NAA</td>
<td>15</td>
<td>78</td>
</tr>
</tbody>
</table>

The shooting response in 1 mg l\(^{-1}\) BAP + 0.1 mg l\(^{-1}\) NAA combination was earlier with best response
Table 2
Effect of cytokinins, auxins and gibberellic acid on shoot multiplication in 5 wk after inoculation on MS media

<table>
<thead>
<tr>
<th>SL No.</th>
<th>Shooting media composition (mg l⁻¹)</th>
<th>No. of shoots</th>
<th>Shoot length (cm)</th>
<th>No. of leaves</th>
<th>Rooting response (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>00</td>
</tr>
<tr>
<td>2</td>
<td>0.5 BA + 0.5 KIN + 0.1 IAA</td>
<td>20.00 ± 0.49cb</td>
<td>5.5 ± 0.24a</td>
<td>11.2 ± 0.53b</td>
<td>77</td>
</tr>
<tr>
<td>3</td>
<td>0.5 BA + 0.1 KIN + 0.1 IAA</td>
<td>16.00 ± 0.64e</td>
<td>5.4 ± 0.17a</td>
<td>8.5 ± 0.47c</td>
<td>80</td>
</tr>
<tr>
<td>4</td>
<td>0.1 BA + 0.1 KIN + 0.1 IAA</td>
<td>20.10 ± 0.37cb</td>
<td>5.41 ± 0.22a</td>
<td>11.16 ± 0.58b</td>
<td>80</td>
</tr>
<tr>
<td>5</td>
<td>0.1 BA + 0.5 KIN + 0.1 IAA</td>
<td>22.13 ± 0.31a</td>
<td>5.81 ± 0.23a</td>
<td>14.25 ± 0.61a</td>
<td>81</td>
</tr>
<tr>
<td>6</td>
<td>0.5 BA + 0.5 KIN</td>
<td>21.00 ± 0.25ab</td>
<td>1.2 ± 0.21d</td>
<td>3.00 ± 0.25de</td>
<td>00</td>
</tr>
<tr>
<td>7</td>
<td>0.5 BA + 0.5 KIN + 0.1 IAA + 40 AgNO₃</td>
<td>5.10 ± 0.17d</td>
<td>4.07 ± 0.2ab</td>
<td>7.60 ± 0.41c</td>
<td>00</td>
</tr>
<tr>
<td>8</td>
<td>0.5 BA + 0.1 GA₃ + 0.1 IAA</td>
<td>8.43 ± 0.33g</td>
<td>5.43 ± 0.31a</td>
<td>8.14 ± 0.56c</td>
<td>61</td>
</tr>
<tr>
<td>9</td>
<td>0.5 BA + 0.5 GA₃ + 0.1 IAA</td>
<td>6.20 ± 0.24dh</td>
<td>4.84 ± 0.25ab</td>
<td>8.8 ± 0.62c</td>
<td>50</td>
</tr>
<tr>
<td>10</td>
<td>0.5 BA + 1.0 GA₃ + 0.1 IAA</td>
<td>10.17 ± 0.35f</td>
<td>2.67 ± 0.19c</td>
<td>5.13 ± 0.43d</td>
<td>37</td>
</tr>
</tbody>
</table>

Data are Mean ± SE (standard error), values followed by a same letters in the same column are not significantly different (Tukey’s test, $P<0.05$)
Table 3
Effect of auxins, cytokinin, gibberellic acid, ADS and AgNO₃ on elongation after 5 wk of inoculation on MS media

<table>
<thead>
<tr>
<th>Sl No.</th>
<th>Elongation media composition (mg l⁻¹)</th>
<th>No. of shoots</th>
<th>Shoot length (cm)</th>
<th>No. of leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control 0.0 mg l⁻¹ NAA + 0.1 mg l⁻¹ IBA + 0.1 mg l⁻¹ GA₃</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>2</td>
<td>1 mg l⁻¹ BA + 0.1 mg l⁻¹ NAA + 40 mg l⁻¹ AgNO₃</td>
<td>5.20 ± 0.15ᵇ</td>
<td>04 ± 0.42ᵇ</td>
<td>8.00 ± 0.61ᶜᵈ</td>
</tr>
<tr>
<td>3</td>
<td>1 mg l⁻¹ BA + 0.1 mg l⁻¹ NAA + 40 mg l⁻¹ ADS</td>
<td>3.24 ± 0.19ᶜ</td>
<td>3.2 ± 0.24ᵇᶜ</td>
<td>6.50 ± 0.58ᵈ</td>
</tr>
<tr>
<td>4</td>
<td>1 mg l⁻¹ BA + 0.1 mg l⁻¹ NAA + 40 mg l⁻¹ AgNO₃ + 40 mg l⁻¹ ADS</td>
<td>7.57 ± 0.19ᵃ</td>
<td>05 ± 0.53ᵃ</td>
<td>12.00 ± 0.51ᵃ</td>
</tr>
<tr>
<td>5</td>
<td>1 mg l⁻¹ BA + 0.1 mg l⁻¹ IBA + 0.1 mg l⁻¹ GA₃</td>
<td>7.95 ± 0.24ᵃ</td>
<td>5.3 ± 0.61ᵃ</td>
<td>10.20 ± 0.5ᵃᵇ</td>
</tr>
<tr>
<td>6</td>
<td>1 mg l⁻¹ BA + 0.5 mg l⁻¹ IBA + 0.1 mg l⁻¹ GA₃</td>
<td>4.45 ± 0.2ᵇ</td>
<td>4.2 ± 0.39ᵇᶜ</td>
<td>9.00 ± 0.53ᵇᶜ</td>
</tr>
</tbody>
</table>

1 mg l⁻¹ BA + 0.1 mg l⁻¹ NAA + 40 mg l⁻¹ AgNO₃ + 40 mg l⁻¹ ADS was significant (p < 0.05) in favoring elongation with multiple shoots. Data are Mean ± SE (standard error), values followed by a same letters in the same column are not significantly different (Tukey’s test, P< 0.05)

In vitro flower induction

For in vitro flowering, multiple shoots formed from shoot tip explants were selected after 8 wk of culturing, and subcultured on to MS media augmented with 0.25 mg l⁻¹ ZEA + 0.1 mg l⁻¹ NAA. The regenerated shoots were transferred to liquid media containing 0.1 mg l⁻¹ BA + 0.1 mg l⁻¹ KIN + 0.1 mg l⁻¹ IAA for shoot multiplication for 5wk, followed by their transfer to MS medium supplemented with 1 mg l⁻¹ BA + 0.5 mg l⁻¹ GA₃ + 0.1 mg l⁻¹ IBA for elongation up to 4 wk. The shoots elongated up to 2–3 cm were excised and transferred to half MS media with 2% sucrose supplemented with 2 mg l⁻¹ IBA + 0.5 mg l⁻¹ GA₃ for 3wk and later transferred to only 0.5 mg l⁻¹ IBA containing media for in vitro flowering and subsequent rooting. The cultures were maintained under 16/8 h (dark/light) photoperiod at 26 ± 2º C and parameters like number of days to flowering and average numbers of flowers produced were recorded after 2 wk of inoculation.

Rooting And Acclimatization

After elongation for 2wks the individual regenerated shoots from nodal cultures were separated carefully from the multiple shoots and transferred to rooting media containing half strength MS salts, 0.01% myo-
inositol, and 3% sucrose supplemented with (0.5-4 mg l⁻¹) IBA solidified with 0.7% agar. The well rooted plants were transferred to plastic cups containing sterile soilrite mixture. Plants were usually covered with polythene bags to maintain the humidity for 1–3 wk for acclimatization. Polythene covers were gradually opened before transferring plants to glasshouse.

**Statistical analysis**

All the experiments were repeated thrice for each combination of plant growth regulators and 10 explants were used per treatment. Number of shoots; shoot regeneration percentage; mean shoot length and number of leaves data were recorded after 5wk of fresh media transfer. The data recorded were subjected to one-way analysis of variance (ANOVA) followed by Tukey's test ($P<0.05$) using GraphPad Prism 8 software. Data was presented as Mean ± SE (Standard Error).

**Results**

The axillary bud breaking in the nodal and shoot tip explant was observed by 12 to 17 days after inoculation onto MS media supplemented with alone or in combination of plant growth regulators 1–6 mg l⁻¹ BA, 1 & 2 mg l⁻¹ BA + 0.1 & 0.5 mg l⁻¹ NAA, 2 mg l⁻¹ BA + 0.5&1 mg l⁻¹ GA₃ and 0.25 mg l⁻¹ ZEA + 0.1 & 0.5 mg l⁻¹ GA₃ (Table 1). The shoot bud initiation was observed in all the combinations exhibiting virtuous response to direct regeneration (Fig. 1a-e). The early bud breaking was observed within 12 days after inoculation in 1mg l⁻¹ BA + 0.1mg l⁻¹ NAA combination with higher response to shooting (84%). However, in 2 mg l⁻¹ BA + 0.1 mg l⁻¹ GA₃ combinations the bud breaking was comparatively delayed (17days) but a shooting response of 80% was achieved (Table 1).

The effect of different plant growth regulators on mass multiplication of induced shoots were studied for 5 wk on both liquid and solid MS basal media. The shoots induced/ regenerated were multiplied on media containing different combinations of IAA, BA, KIN and GA₃ (Table 2). The liquid media containing 0.1mg l⁻¹ BA + 0.5 mg l⁻¹ KIN + 0.1 mg l⁻¹ IAA combination was best in producing more number of multiple shoots (22.13 ± 0.31) with average shoot length (5.81 ± 0.23) and number of leaves (14.25 ± 0.61) respectively (Fig. 1f). However, the increase in concentration of IAA did not induce multiple shoots but enhanced the rooting. The media containing 0.5 mg l⁻¹ BA + 0.5 mg l⁻¹ KIN without IAA showed an increased number of lateral shoot buds (21.0 ± 0.25) but with decreased shoot length (1.2 ± 0.21) and number of leaves (3.0 ± 0.25) (Table 2). Effect of different concentration of (0.1, 0.5 and 1mg l⁻¹) GA₃ was studied to check the elongation of shoots. The MS media containing 0.1 mg l⁻¹ GA₃ with 0.5 mg l⁻¹ BA + 0.1mg l⁻¹ IAA combinations showed better results in terms of number of shoots (8.43 ± 0.33), shoot length (5.43 ± 0.31) and number of leaves (8.14 ± 0.56) compared to increased concentrations of GA₃. It was also observed that the presence of low concentration of 0.5 mg l⁻¹ IBA with 1 mg l⁻¹ BA + 0.1 mg l⁻¹ GA₃ was favourable in inducing and elongating the multiple shoots with increased shoot number (7.95 ± 0.24) and length (5.3 ± 0.61) in 5wk after inoculation (Table 3). The liquid and solid media were tried to
check for efficient multiplication from nodal and shoot tip explants. The liquid media proved effective in producing more number of multiple shoots with continuous sub culturing at regular intervals of 3 wk.

The effect of additives AgNO$_3$ and ADS were studied on the induction and elongation of multiple shoots from nodal explants. The 1 mg l$^{-1}$ BA + 0.1 mg l$^{-1}$ NAA combination augmented with both 40 mg l$^{-1}$ AgNO$_3$ and 40 mg l$^{-1}$ ADS was best in producing more number of shoots with increased length and number of leaves compared to their individual combinations (Table 3). The effect of 40 mg l$^{-1}$ AgNO$_3$ was meager in terms of elongation or growth of explants in both liquid and solid media. However, the supplementation of silver nitrate in MS media enhanced the lateral branching in the shoots thereby increasing the number of multiple shoots. The shoots were successfully rooted and hardened (Fig. 1g-i). However, it was observed that response time taken for in vitro shooting was early in B. alba than to B. rubra.

In vitro flowering was observed in shoot tip cultures inoculated on to MS basal media containing a combination of ZEA with GA$_3$. Eight weeks initial culture of explants on this medium, the obtained shoot tip cultures further elongated in presence of 1 mg l$^{-1}$ BA + 0.5 mg l$^{-1}$ GA$_3$ + 0.1 mg l$^{-1}$ IAA, in 4 wk culturing (Fig. 2j). The shoots that attained length of 2–3 cm were excised and inoculated to rooting media containing 0.5 mg l$^{-1}$ IBA + 0.5 mg l$^{-1}$ GA$_3$ (Fig. 2k-l). The MS media combinations augmented with 0.5 mg l$^{-1}$ BA + 0.5 mg l$^{-1}$ GA$_3$ in elongation media favoured the induction of in vitro flowers. The incorporation of GA$_3$ in rooting media along with IBA and half strength MS basal media with reduced concentration of sucrose (2%) and nutrient stress for 4wk enhanced in vitro flowering. The in vitro flowers (80%) were formed from both apical and axillary buds (Table 4; Fig. 2j-p).

### Table 4

<table>
<thead>
<tr>
<th>Media compositions (mg l$^{-1}$)</th>
<th>In vitro response of media compositions</th>
<th>Duration of response</th>
<th>In vitro flowering response</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25 ZEA + 0.1 NAA</td>
<td>Shoot induction</td>
<td>4 wk</td>
<td>80%</td>
</tr>
<tr>
<td>0.1 BA + 0.5 KIN + 0.1 IAA</td>
<td>Multiple shoots</td>
<td>5 wk</td>
<td></td>
</tr>
<tr>
<td>0.5 BA + 0.5 GA$_3$ + 0.1 IAA</td>
<td>Elongation &amp; in vitro flowering</td>
<td>4 wk</td>
<td></td>
</tr>
<tr>
<td>1 BA + 0.1 IBA + 0.5 GA$_3$</td>
<td>Elongation</td>
<td>3 wk</td>
<td></td>
</tr>
</tbody>
</table>

The regenerated shoots were successfully rooted in IBA supplemented half strength MS media. In the current study, concentrations of 0.5, 1 and 2 mg l$^{-1}$ IBA were tried for in vitro rooting. The media containing 0.5 mg l$^{-1}$ IBA in half strength MS basal composition was sufficient in inducing root within 10 days after inoculation (Fig. 2j-p). However, in presence of higher concentration of IBA root formation
was delayed. Survival rate of 90% was achieved on transferring rooted plants to pots containing soilrite mixture (Fig. 1g-i and Fig. 2o-q).

**Discussion**

The *in vitro* plant regeneration approach is a pre-requisite for extracting key bioactive metabolite and also for studying the molecular mechanisms and understanding biosynthetic pathways. Also, *in vitro* plant regeneration is a successful technique involved in conservation of ethanomedicinal plants. In the present study, direct regeneration of *Basella* spp. was achieved by nodal and shoot tip explants. Initial bud breaking was observed by 12–17 days after inoculation on to MS media containing different combinations of auxins and cytokinins (Table 1). Good regeneration response was observed in all the combinations studied. Intermittent callus formation from explants during shoot bud proliferation stage is a setback for efficient proliferation and regeneration of shoots in *B. alba* when nodal explants were cultured on Gamboorg’s media devoid of exogenous auxins (Norris et al. 1989). In the present study, 1 mg l\(^{-1}\) BAP + 0.1 mg l\(^{-1}\) NAA in medium triggered higher response for shooting (84%) with early bud breaking by 12 days after inoculation under one week of initial dark incubation. Optimum concentration of specific cytokinin in culture medium for shoot bud initiation is vital as demonstrated in *Mimosa, Sida* and *Decalepis hamiltonii* (Sadheeshna et al. 2009; Sivanesan and Jeong 2007, Manjula et al. 2020). Many reports are have shown successful *in vitro* shoot regeneration using a combination of BA and NAA in different plants viz. with 8.8 µM BA and 0.5 µM NAA, in *Withania somnifera* (Saritha and Naidu 2007), 1.0 mg l\(^{-1}\) BA and 0.5 or 1.0 mg l\(^{-1}\) NAA in *Celosia argentea* (Bakar et al. 2014).

Incubation of explants in dark play an important role in plant tissue culture technique as it induces the callusing in explants. In congruence with our studies, many reports have illustrated the effect of dark incubation on plant regeneration; in roses (Ibrahim and Debergh 2001), *Gerbera jamesonii Bolusex Hooker F.* (Talla et al. 2018). A dark incubation period for 10 days at the early stage of culture was found to be critical for higher regeneration of stored cheery cotyledon explants (Canli and Tian 2007).

*In vitro* shoot multiplication with supplementation of two cytokinins in MS basal media has been studied in number of plants and found effective over single cytokinin. In the present findings, MS liquid media containing two cytokines along with auxin (0.1 mg l\(^{-1}\) BA + 0.1 mg l\(^{-1}\) KIN + 0.1 mg l\(^{-1}\) IAA) produced more number of multiple shoots (22.0 ± 0.13) with average shoot length (5.81 ± 0.33), which was further substantiated by similar reports by Premkumar et al. (2011) in *Scopari adulcis* L. wherein, 4.44 µM BA + 2.32 µM KIN alone or along with auxins promoted *in vitro* flowering in the same. Sangeetha and Venkatachalam (2014) obtained highest frequency of multiple shoots in cucumber on MS media fortified with 1.0 mg l\(^{-1}\) BA and 0.51 mg l\(^{-1}\) KIN. The use of liquid media has many advantages over the solid media in better availability of plant growth hormones & nutrients, uniform dispersal of media, dilution of exudates like phenolic compounds and fast multiplication rates (Shekhawat et al. 2018). Liquid culture media for shoot regeneration studies were reported in many plants such as *Nothapodytes mimmonia* (Dandin and Murthy 2012). In our study, low concentration of IAA (0.1 mg l\(^{-1}\)) along with BA + KIN or BA
GA3 found effective in inducing the multiple shoots but increased concentration (0.5 mg l⁻¹) of IAA produced rooting response with multiplication in nodal explants (Table 1). Shekhawat et al. (2018) have optimized highly efficient stable micro propagation protocol for the medicinal plant *Turnera ulmifolia* L. on liquid MS media supplemented with 0.5 mg l⁻¹ BA and KIN with 0.1 mg l⁻¹ IAA. In the current work media containing cytokinin viz., 0.5 mg l⁻¹ BA + 0.5 mg l⁻¹ KIN without IAA showed an increased number of lateral shoot buds (21 ± 0.25) but with decreased shoot length (1.2 ± 0.21) and numbers of leaves (3 ± 0.25) (Table 2). Presence of low auxin concentration with cytokinin was found necessary for good morphogenesis of the shoots. Dalal and Rai (2004) observed that inclusion of indole-3-acetic acid (2.85 µM) into 6-benzyladenine- (8.87 µM) supplemented MS media triggered a high frequency of shoot proliferation in cotyledonal node explants of *Oroxylum indicum* Vent. In the present study, the MS media containing 0.1 mg l⁻¹ GA3 with BA and IAA combinations showed better response in terms of number of shoots (8.43 ± 0.33), shoot length (5.43 ± 0.31) and number of leaves (8.14 ± 0.56) compared to increased concentrations of GA3 (Table 2).

*In vitro* flowering techniques involves and also facilitate the understanding of the physiology of flowering and fruiting through controlled environmental cues, including plant growth regulators (PGRs) such as cytokinins, gibberellins, auxins, sugars, minerals, photoperiod, pH and temperature (Haque et al. 2016). *In vitro* flowering also serves as an essential tool in shortening the life cycle of plants in breeding cycle to generate better quality of plant traits to meet market demand (Mohammed et al. 2016). Ethylene, a potential inducer of flower senescence is synthesized by plants during certain development cues and in response to abiotic and biotic stresses (Kumar et al. 2009). The use of cytokinins as a common requirement for *in vitro* flowering has been observed plants such as *Rosa hybrida* (Kanchanapoom et al. 2009), *Spilanthes acmella* Murr (Yadav and Singh 2011).

The MS media supplemented with 1 mg l⁻¹ BA + 0.1 mg l⁻¹ NAA combination augmented with both AgNO3 and ADS was best in producing more number of *Basella* shoots with increased length and number of leaves compared to their individual combinations (Table 3). The effect of AgNO3 was meager in terms of elongation or growth of explants in both liquid and solid media. A synergistic effect of 4.4 µM BA + 2.85 µM IAA with, AgNO3 was observed in inducing multiple shoots of *Rivina humilus* L, and the elongation of micro shoots were achieved in 2.22 µM BA + 2.89 µM GA3 combination (Harsha et al. 2012). However, the supplementation of silver nitrate in MS media enhanced the lateral branching in the shoots thereby increasing the number of multiple shoots. Ibrahim and Debergh (2001) observed that addition of silver nitrate to shoot induction media significantly improved the regeneration percentage of three rose genotypes.

In the present study, *in vitro* flowering was observed in shoot tip cultures inoculated on MS basal media containing a combination of ZEA with GA3 that maintained on same media composition for 2 months. Anuar et al. (2017) obtained *in vitro* flowering of *Phlox paniculata* L. after 4 wk of culturing shoot tip segments on MS solid media supplemented with 11.2 µM zeatin (67.8 ± 3.2%) or 5.6 µM GA3 (48.8 ± 9.3%). Further regenerated shoot was multiplied on media containing 0.5 mg l⁻¹ BA + 0.5 mg l⁻¹ KIN + 0.1
mg l\(^{-1}\) IAA for 5 wk. After 5 wk subsequent subculturing to 1 mg l\(^{-1}\) BA + 0.5 mg l\(^{-1}\) GA\(_3\) + 0.1 mg l\(^{-1}\) IBA for 4 wk facilitated more elongation and induced *in vitro* flowering. Many studies have elucidated role of BA in, *in vitro* flower inductions in several plant studies such as *Momordica* and *Capsicum* (Wang et al. 2001; Haque et al. 2016). In the present investigations 1 mg l\(^{-1}\) BA in combination with GA\(_3\) and IBA helped in elongation and *in vitro* flowering in *Basella* spp (Table 4). Mohamed et al. (2018) developed an efficient protocol for *in vitro* flowering for *Impatiens balsamina* cv Dwarf Bush, on MS media supplemented with different concentrations of GA\(_3\), BA and KIN media.

Sharma et al. (2014) optimized effective protocol for *in vitro* flowering and micropropagation in *Swertia chirayita* using axillary bud explants using 1.0 mg L\(^{-1}\) BA and 70.0 mg L\(^{-1}\) adenine sulphate. In our study also, *in vitro* flower is induced in both with and without root response respectively. This is in line with induction of *in vitro* flower in absence of roots from shoot cultures of bamboo on media containing BA (Yuan et al. 2017). Our results revealed that the nutrient stress for 6–8 wk induced flowering and decreased carbon source has also played a vital role in flower development as reported in Arabidopsis cultures with reduction of mineral nutrition by JanKolář and JanaSeňková (2008).

**Conclusions**

In recent years the *Basella* spp. is being exploited for its nutritional properties with high economical value added bioactive compounds. This study optimized an effective regeneration method that signifies a quick and high multiplication rate protocol with induction of *in vitro* flowering. The study can augment in conservation of highly exploited *Basella* spp. for pharmaceutical, cosmetic and nutraceutical potentials.

**Declarations**

**Acknowledgement**

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**Conflicts of Interest and authors contributions**

Authors declare that they have no conflicts of interest in the publication.

**References**


**Figures**

![Figure 1](image-url)
In vitro regeneration in *Basella* spp. a-e shoot bud initiation from nodal segments on 1 mg l\(^{-1}\) BA + 0.1 mg l\(^{-1}\) NAA fortified MS medium f multiplication on MS liquid medium augmented with 0.1 mg l\(^{-1}\) BA + 0.5 mg l\(^{-1}\) KIN + 0.1 mg l\(^{-1}\) IAA g-i rooting on 0.5 mg l\(^{-1}\) IBA medium and hardening on soilrite and soil mixture

**Figure 2**

*In vitro* flowering in *Basella* spp. j induction of *in vitro* flowering on MS liquid medium containing 0.5 mg l\(^{-1}\) BA + 0.5 mg l\(^{-1}\) GA\(_3\) + 0.1 mg l\(^{-1}\) IAA k-l *in vitro* flower on rooting media containing 1 mg l\(^{-1}\) IBA + 0.5 mg l\(^{-1}\) GA\(_3\) m plantlet with root and *in vitro* flowers n *In vitro* flower with fruit intiation(F) o-q hardening and acclimatization of plant lets in soilrite and soil mixture