Effect of Supplementing Mixture of Benzene Aminopurine (BAP) and Kinetin (KN) along with Auxin 2,4 D on Growth of Callus in Murashige and Skoog Medium Derived from Embryonic Cotyledon Explants of Withania Somnifera L

Naveen Gaurav
Assistant Professor
Department of Biotechnology
S G R R P G College Dehradun, U.K.

A. P. Singh
Professor
Department of Botany
Govt. P.G. Science College, Rewa, M.P.

Arun Kumar
Assistant Professor
Department of Biotechnology
S G R R P G College Dehradun, U.K.

Aditi Grover
Assistant Professor
Department of Biotechnology
S G R R P G College Dehradun, U.K.

Aviral Maithani
Student
Department of Biotechnology
S G R R P G College Dehradun, U.K.

Abstract
Withania somnifera (L.) Dunal (Family: Solanaceae, commonly known as Ashwagandha, English name: Winter cherry) is an important perennial plant species with immense therapeutic uses in traditional as well as modern system of medicine (Datta et al. 2010). Plants products can be obtained from the roots, leaves, and branches, by using many different biological techniques. One of the most important technique is plant tissue culture through which large number of plants can produced from a very short time and very small area with high quality. It can be easily possible by the use of plant growth regulators. Micropropagation by using cotyledonal leaves, meristem and the shoot culture is a best technique for the production of large numbers of identical or being same individuals. The formation of the right or exact copies of plants that mainly produce the good flowers, fruits, or have other desirable traits, to rapidly produce mature plants (Garg, et al., 2011). Ashwagandha having effective property can also used in blends and supplements which are designed to show many multiple effects. It is described as an herbal tonic and health food in Vedas and considered as ‘Indian Ginseng’ in traditional Indian system of medicine (Singh, et al. 2001).

Keywords: Winter cherry, perennial plant, Indian ginseng, medicine, therapeutic uses, herbal tonic, micropropagation etc

Introduction
Leaves as well as Cotyledonary excised small explants of Ashwagandha were responsible and introduced to evaluate the effect of various growth regulators upon the in vitro micro-propagation of direct shoot and root initiation processes. Explants were applied to generate callus, shoot and root regeneration. Ashwagandha is a straight, strong, erect, perennial shrub, evergreen and belong to a member of Solanaceae family, it is a widely used most important medicinal plant which is greatly useful in the treatment of inflammatory, anti-tumor agent (Devi, et al., 1993). Ashwagandha product is very well known for many years as an essential drug in Ayurvedic literature. Roots of the plant Withania somnifera generally exhibit antioxidant, immunomodulatory and haematopoietic properties (Mishra et al. 2000). Gita Rani and Avinash, (2003) observed or reported the callus induction or cultivation from the hypocotyls, root and cotyledonal leaf segments of Ashwagandha grown on MS medium provided with various different concentrations and combinations of the 2,4-di-chlorophenoxyacetic acid (2,4-D) and Kinetin plant growth hormone. Maximum callusing (near about 100%) was observed with root and cotyledonal leaf explants segments grown on Murashige and Skoog medium provided with a combination of 2,4-di-chlorophenoxyacetic acid (2,4-D) with BAP (Benzene Aminopurine) and Kinetin plant growth hormone. Roots of Ashwagandha mostly used in Unani and Ayurveda medicines.

Roots are very important and used as important medicines for hiccups, bronchitis, several female disorders, dropsy, rheumatism, lung inflammation, stomach and skin diseases. The ingredients in medicines are also used as for treating sexual
weakness in males and disability (Joshi et al. 2010). As per the record of red list of extinct species, forty four (44) species of plants are skillfully endangered, 113 endangered and 87 vulnerable. W. somnifera variety of Ashwagandha is proved and included to be 99.75% of the endangered and extinct medicinally important plant (Siddique et al., 2005). As over rate of harvesting of Ashwagandha that medicinal plant root is going and moving to be extinction condition in the region of Southern India (Manickam et al. 2000). Micro-propagation of Withania somnifera introduces various (excised pieces) such as shoot or stem tips (Sen and Sharma, 1991), auxiliary highly division phase meristems (Roja and Heble, 1991), auxiliary tip leaves, auxillary or apical shoot, hypocotyls, cotyledonary leaves (embryonic leaves) and root (small) segments (Rani and Grover, 1999) has been demonstrated. Propagation use by seed, but seed viability is limited to normally more than one year studied by Roja and Heble (1991) reported, callus formation from excised explants.

**MATERIAL AND METHODS**

**A. Chemicals:**
All chemicals were mostly of HiMedia, India and Sigma, USA and some of the chemical were also obtained from SRL, Qualigens and E. Merck, India.

**B. Nutrient Media:**
The in vitro morphogenic responses of the plant tissues which are cultivated are normally affected by the various constituents of the culture media or growing media. Both macro and micro-element of the media plays a major role in plant regenerations and morphogenesis (Murashige and Skoog, 1962). Most media additionally contains myo-inositol at a concentration of 100mg/l, B5 vitamins along with MS basal macro and micronutrients

**C. Sugar Concentration:**
A carbon source is essential for the cells, tissue, or organ cultures for in vitro regeneration. Sucrose is almost universally used for micro-propagation purposes, as it readily utilizable by cells. Sucrose concentration of 30g/l was found to be optimal for growth of Withania.

**D. Medium Used For Tissue Culture:**
Medium used for Tissue Culture for in vitro growth and regeneration of Ashwagandha was the standard MS medium (Murashige and Skoog, 1962) containing macronutrient salts, micronutrient salts, vitamins, Fe-EDTA, 0.01%(w/v) myo-inositol along with 3%(w/v) sucrose. The media composition is listed as follows: -

**E. For MS Media, Four Stock Solutions were Prepared as Follows:**
- Stock I macronutrients 10x
- Stock II micronutrients 100x
- Stock III Fe-EDTA 100x
- Stock IV Vit. And AA 100x

The stock solutions I, II and IV were prepared by dissolving appropriate amounts of salt in MQ water but stock solution III was prepared by weighing FeSO₄ 7H₂O and sodium salt of EDTA 2H₂O separately in the required quantities, dissolving them separately by slight warming together and stored in dark container, because I is light sensitive. The above stock solutions were kept at 4°C after autoclaving. During media preparation, the final concentration of each component was kept 1x and pH was adjusted to 5.8± 0.1.

**F. Medium and glassware sterilization:**
All the tissue culture media and vessels were steam sterilized by autoclaving at 15psi (1.04 kg/cm²) pressure at 121°C for 20 min. thermolabile substance were sterilized separately filtration (0.22um Millipore) then added to the autoclaved media when it was cooled at 40-45°C and mixed thoroughly. The media were then dispensed into autoclave culture tubes of radiations sterilized Petri dishes at allot to solidify. The glassware the solutions biodegradable detergent (labolene, India) and rinsed with double distilled water, over dried at 80°C for 2 hours, followed by most heat sterilization the instrument used for tissue culture, viz. forceps, needles, scalpels, spatula etc. which is make contamination less by washing or dipping in 70% ethanol followed by burner flaming and then cooling in sterilized water at regular intervals while using.

**RESULTS AND DISCUSSION**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Cytokinins</th>
<th>Concentration</th>
<th>Auxins (2,4-D)</th>
<th>Callus</th>
<th>Frequency of formation of callus</th>
</tr>
</thead>
</table>

Effect of supplementing mixture of Benzene aminopurine (BAP) and kinetin (Kn) along with auxins 2,4 D on growth of callus in Murashige and Skoog medium derived from embryonic cotyledon explants of Withania somnifera (Cultivated)

All rights reserved by www.journalforresearch.org
Effect of Supplementing Mixture of Benzene Aminopurine (BAP) and Kinetin (KN) along with Auxin 2,4 D on Growth of Callus In Murashige and Skoog Medium Derived from Embryonic Cotyledon Explants of Withania Somnifera L

(J4R/ Volume 01 / Issue 12 / 002)

Table 2

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Cytokinins</th>
<th>Concentration (mg/l)</th>
<th>Auxins (2,4-D) Concentration (mg/l)</th>
<th>Callus Growth (Fresh Weight) Gram</th>
<th>Dry Weight Gram</th>
<th>Frequency of formation of callus (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>BAP</td>
<td>0.5</td>
<td>2.0</td>
<td>4.8±0.06</td>
<td>0.39±0.005</td>
<td>70±1.47</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>2.0</td>
<td>6.2±0.13</td>
<td>0.48±0.010</td>
<td>84±2.27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5</td>
<td>2.0</td>
<td>5.4±0.10</td>
<td>0.43±0.008</td>
<td>68±1.22</td>
</tr>
<tr>
<td>02</td>
<td>Kinetin</td>
<td>0.5</td>
<td>2.0</td>
<td>5.2±0.08</td>
<td>0.41±0.006</td>
<td>65±0.78</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.0</td>
<td>2.0</td>
<td>7.8±0.21</td>
<td>0.65±0.018</td>
<td>72±1.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.5</td>
<td>2.0</td>
<td>6.5±0.16</td>
<td>0.50±0.013</td>
<td>67±1.01</td>
</tr>
</tbody>
</table>

(Mean ± Standard error).

Fig. 1a: Bar diagram showing effect of supplementing mixture of benzene aminopurine (BAP) and kinetin (Kn) along with auxins 2, 4 D on growth of callus (Fresh weight) in MS medium of withania somnifera (Cultivated)

Fig. 1b: Bar diagram showing effect of supplementing mixture of benzene aminopurine (BAP) and kinetin (Kn) along with auxins 2, 4 D on growth of callus (Dry weight) in MS medium of withania somnifera (Cultivated)

Cotyledon explants (size 1.5cm) were inoculated in full strength MS medium supplemented with 0.8% agar-agar and same concentration 2mg/l of 2, 4-D with 0.5mg/l to 1.5mg/l BAP, same concentration 2mg/l of 2, 4-D with 0.5mg/l to 1.5mg/l Kn. After two weeks of inoculation greenish colored callus was observed in different frequencies in different hormone concentration in MS medium. The results observed are depicted by the table 6.2.

Table – 2

Effect of supplementing mixture of Benzene aminopurine (BAP) and kinetin (Kn) along with auxins 2,4 D on growth of callus in Murashige and Skoog medium derived from embryonic cotyledon explants of Withania somnifera (Wild):-

All rights reserved by www.journalforresearch.org
Effect of Supplementing Mixture of Benzene Aminopurine (BAP) and Kinetin (KN) along with Auxin 2,4 D on Growth of Callus In Murashige and Skoog Medium Derived from Embryonic Cotyledon Explants of Withania Somnifera L (J4R/ Volume 01 / Issue 12 / 002)

<table>
<thead>
<tr>
<th></th>
<th>1.0</th>
<th>2.0</th>
<th>7.20±0.18</th>
<th>0.60±0.015</th>
<th>88±2.38</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.5</td>
<td>2.0</td>
<td>6.40±0.12</td>
<td>0.43±0.006</td>
<td>70±1.05</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>2.0</td>
<td>5.80±0.09</td>
<td>0.51±0.009</td>
<td>67±0.80</td>
</tr>
<tr>
<td>02 Kinetin</td>
<td>1.0</td>
<td>2.0</td>
<td>8.00±0.22</td>
<td>0.70±0.019</td>
<td>70±1.26</td>
</tr>
<tr>
<td></td>
<td>1.5</td>
<td>2.0</td>
<td>6.90±0.14</td>
<td>0.55±0.012</td>
<td>75±1.88</td>
</tr>
</tbody>
</table>

(Mean (+ or –) Standard error)

![Fig. 2a: Bar diagram showing effect of supplementing mixture of benzene aminopurine (BAP) and kinetin (Kn) along with auxins 2, 4 D on growth of callus (Fresh weight) in MS medium of withania somnifera (Cultivated)](image)

![Fig. 2b: Bar diagram showing effect of supplementing mixture of benzene aminopurine (BAP) and kinetin (Kn) along with auxins 2, 4 D on growth of callus (Dry weight) in MS medium of withania somnifera (Cultivated)](image)

MS medium having same concentration of 2, 4 D (2mg/l) with different concentration of cytokinins (0.5 to 1.5mg/l) also initiates the formation of callus in wild variety. Maximum formation of fresh and dry callus takes place in MS medium having 2.0mg/l 2, 4 D with 1.0mg/l BAP and 2.0mg/l 2,4 D with 1.5mg/l kinetin, in which maximum frequency of callus formation takes place with BAP (1.0mg/l BAP) but the frequency of formation callus in wild variety slightly higher than cultivated variety of Withania as recorded in table 6.10.

REFERENCES


