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# *IN VITRO* SHOOT BUD INDUCTION FROM NODAL SEGMENT EXPLANTS IN INDIAN GINSENG- *WITHANIA SOMNIFERA* (L.) DUNAL

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Ashwagandha, A popular medicinal plant of family Solanaceae is well known for years as an important drug in ayurvedic literature. The active pharmacological components of Ashwagandha are steroid allactones of the withanolide type. Several chemotypes have been found differing in their withanilide content. The principal withanolide in Indian *Withania somnifera* are withaferin A and withanilide D. *In vitro* morphogenesis experiments were conducted using various auxins and cytokinins on MS medium. Direct shoot induction was obtained on 2, 4-D (2 mg/l); IAA (4 mg/l) and NAA (1 mg/l). Kn (1.5 mg/l) was found to be very effective for shoot buds proliferation. Various combinations of NAA and BAP; NAA and Kn, were tested for direct shoot bud induction. It was found that combination of NAA (1 mg/l) & BAP (0.5 mg/l) gave maximum response of shoot induction. Average no. of shoots induced was 7.8.

**Keywords**: Ethno-medicinal; *in vitro* culture; Micropropagation; Nodal segments; *Withania somnifera*.

## Introduction

The use of herbal medicines is fast growing in developed countries. About 40% of compounds used in pharmaceutical industry are directly or indirectly derived from plants because the chemical synthesis of such compounds is either not possible and/or economically not viable. Therefore, a large number plant species (especially medicinal) are under threat of extinction due to their over exploitation[1]. The micro-propagation technique has great potential for rapid and large-scale multiplication of true to type planting material**.**

*Withania somnifera* (L.) Dunal is popularly known as Ashwagandha or Winter Cherry[2] or Indian Ginseng, is a plant of Solanaceae or nightshade family. It is used as herb in Ayurvedic medicine. In Ayurveda, the roots of *W. somnifera* are used to prepare the herbal remedy ashwagandha, which has been traditionally used to treat various symptoms and conditions.

*Major Alkaloids-*

Indian ginseng's pharmacological activity has been attributed to two main withanolides, withaferin A and withanolide D[3]. Withaferin-A is therapeutically active withanolide reported to be present in leaves. In addition to alkaloids, the roots are reported to contain starch, reducing sugars, glycosides, dulcitol, with ancil, an acid and a neutral compound. The amino acids reported

from the roots include aspartic acid, glycine, tyrosine, alanine, glutamic acid and cysteine.

*Pharmacological activities of Ashwagandha-*

Centuries of Ayurvedic medical experience using *Withania somnifera* have revealed it to have pharmacological value as an antibiotic, aphrosidiac, astringent, anti-inflammatory, diuretic, narcotic, sedative, and tonic. Ashwagandha has been found to provide potent antioxidant protection. It stimulates the activation of immune system cells, such as lymphocytes and phagocytes.

*Ashwagandha as medicinal herb-*

Ashwagandha is considered to be one of the best rejuvenating agents in Ayurveda. Its roots, seeds and leaves are used in Ayurvedic and Unani medicines. Ashwagandha root drug finds an important place in treatment of rheumatic pain, inflammation of joints, nervous disorders and epilepsy. Dried roots are used as tonic for hiccup, cold, cough, female disorders, as a sedative, in care of senile debility, ulcers, etc. Leaves are applied for carbuncles, inflammation and swellings. Leaf juice is useful in conjunctivitis. Bark decoction is taken for asthma and applied locally to bed sores. Ashwagandha and its extracts are used in preparation of herbal tea, powders, tablets and syrups. It is also an exceptional nerve tonic and nourishes the nerves and improves nerve function to maintain calm during stressful conditions. It also nourishes crucial mind and body connection and psychological immune response.

Traditionally *W. somnifera* is propagated from seeds, but the mature and healthy seeds are not always available for germination. The viability period of seeds is very short and their germination is also poor[4]. Conventional propagation methods have proved to be inadequate to meet this challenge. Large scale production through plant *in vitro* regeneration will provide a means of putting the plant onto the market at lower prices[5]. Many earlier studies have reported *in vitro* propagation of Ashwagandha by using nodal areas[6] and shoot tips[7-10].

**Material and Methods**

*Collection of plant material and surface sterilization-*

Fresh apical twigs of *Withania* were collected from wildly growing plants in the institute premises. Nodal segments along with a few leaves were collected from healthy and disease free plants. Branches with 3-4 nodes (5 cm) were picked and carried in the laboratory carefully. Prior good care of stock plants may lessen the amount of contamination that is present on explants. Plants grown in the field are typically dirty and hence extensive sterilization is required. Plant material was thoroughly washed under running tap water for 2-3 minutes. A 5% solution of liquid detergent-Extran (Merck, India) was used to surface sterilize and remove dirt from plant material.

Plant material was washed with distilled water 3-5 times and then sterilize with 70% alcohol for 30 sec. Further sterilization with 0.1% $HgCl_2$ was carried out under Laminar Airflow hood. Plant material was sterilized with freshly prepared aqueous solution of mercuric chloride for 3-5 minutes followed by rinsing with autoclaved distilled water several times. Explants were cultured on MS[11] medium containing 3% sucrose and solidified with 1% agar in Erlenmeyer flasks. Nutrient medium was sterilized by autoclaving at 15 psi for 20 minutes. Nutrient medium was fortified with various concentrations of auxins (2,4-D, IAA, NAA) and cytokinins (BAP and Kn). Nodal segments (0.5-1 cm long) were cut from sterilized plant material

and twenty five explants were cultured for each treatment. Cultures were incubated in growth chamber at 26±1°C provided with 16 h light and 8 hrs dark period. Initially, explants were cultured for 4-6 weak and then sub-cultured for shoot bud induction.

**Results and Discussion**

*Auxin Treatments* (Table-1)

**Table 1.** Response of direct shoot induction from nodal segments of Ashwagandha *(Withania somnifera)* cultured on MS medium supplemented with different Auxins (No. of explants/treatment =25)

| S. No. | Auxin | Conc. (mg/l) | No. of explants responded | Percentage response | Mean no. of shoots induced/explant |
|---|---|---|---|---|---|
| 1. | **MS only** | 0 (C) | 5 | 20 | 4.5 |
| 2. | **2,4-D** | 1 | 7 | 28 | 2.3 |
| 3. | | 2 | 12 | 48 | 3.0 |
| 4. | | 2.5 | 3 | 12 | 1.2 |
| 5. | | 4 | 5 | 20 | 2.6 |
| 6. | **IAA** | 1 | 3 | 12 | 1.0 |
| 7. | | 2 | 5 | 20 | 2.1 |
| 8. | | 2.5 | 3 | 12 | 1.8 |
| 9. | | 4 | 9 | 36 | 3.8 |
| 10. | **NAA** | 1 | 16 | 64 | 5.2 |
| 11. | | 2 | 11 | 44 | 1.6 |
| 12. | | 2.5 | 9 | 36 | 2.4 |
| 13. | | 4 | 3 | 12 | 3.2 |

2,4-D= 2,4-dichlorophenoxy acetic acid ; IAA= Indole-3-acetic acid
NAA= α- naphthalene acetic acid ; (C)= Control

Explants were cultured on MS medium supplemented with various Auxins (2, 4-D, IAA and NAA) in different concentrations. Plain MS (without any hormone) was set as control. Earlier, direct shoot induction from various explants. Like, node, internode, hypocotyl and embryos have been reported[12]. Direct regeneration of shoot buds was observed in MS basal medium supplemented with various concentrations of either benzyladenine (BA) or thidiazouron (TDZ) depending on the explant. Nodal explants formed multiple shoots on medium containing 0.1–5.0 mg/l BA. In the present investigation, good response of direct shoot induction was obtained on 2, 4-D (2 mg/l); IAA (4 mg/l) and NAA (1 mg/l). Maximum response was obtained on NAA (1 mg/l) supplemented medium upon which mean no. of shoots obtained was 5. 2. Shoots induced were morphologically normal and green. In a study[13], nodal segments were inoculated on MS medium supplemented with varying concentrations of NAA, showed 80-100% bud break. The maximum number of shoot per explant, average shoot length and nodes per shoot were recorded 1.3±0.1, 3.0±0.2 and 4.8±0.2 respectively on MS medium with 1.0 mg/l NAA.

*Cytokinin treatments* (Table-2)

To study the effect of cytokinins, nodal segments were cultured on MS medium supplemented with 6-benzylamino purine (BAP) and Kinetin (Kn). Both BAP and Kn proved to be very effective in inducing the shoot buds. Effect of Kn was more

**Table 2.** Response of direct shoot induction from nodal segments of Ashwagandha *(Withania somnifera)* cultured on MS medium supplemented with different Cytokinins (No. of explants/treatment =25)

| S. No. | PGRs | Conc. (mg/l) | No. of explants responded | % response | Mean no. of shoot buds induced/explant |
|---|---|---|---|---|---|
| 1. | **BAP** | 0.5 | 9 | 36 | 4.2 |
| 2. | | 1 | 13 | 52 | 4.8 |
| 3. | | 1.5 | 5 | 20 | 3.2 |
| 4. | | 2.5 | 7 | 28 | 1.8 |
| 5. | | 4 | 11 | 44 | 3.4 |
| 6. | **Kn** | 0.5 | 3 | 12 | 2.6 |
| 7. | | 1 | 7 | 28 | 1.8 |
| 8. | | 1.5 | 17 | 68 | 6.4 |
| 9. | | 2.5 | 11 | 44 | 4.2 |
| 10. | | 4 | 8 | 32 | 4.4 |

BAP- 6-benzylamino purine; Kn- Kinetin

promising as compared to BAP, wherein, a mean no. of shoot induced was 6.4. However, on BAP supplemented medium, maximum 4-8 shoot buds were obtained. The hormonal concentrations at which maximum response was obtained were BAP (1 mg/l) and Kn (1.5 mg/l). The shoot buds proliferated was normal, green and healthy. In an earlier study[14], obtained direct shoot bud induction from cultured nodal explants on medium supplemented with Kn (0.5 mg/l).

*Auxin + Cytokinin treatments* (Table-3)

Nodal segments of Ashwagandha were also cultured on nutrient medium supplemented with Auxins and Cytokinins in combinations. Combination of NAA & BAP and NAA & Kn, at different concentrations were tested for direct shoot bud induction. It was found that combination of NAA (1 mg/l) & BAP (0.5 mg/l) gave maximum response of shoot induction. Average no. of shoots induced was 7.8 on these concentrations. A combination of NAA (2.5 mg/l) and Kn (1 mg/l) also gave good results. Combinations of higher concentrations of NAA+BAP and NAA+Kn were not suitable for shoot bud induction from nodal segments.

Earlier, it has been reported[15] that **a**mong the various combinations of media tested, the MS medium supplemented with BAP (1.5mg/l) and IAA (1.5mg/l) proved to be the best optimized medium with a maximum shooting (95.0%).

*Elongation of shoot buds induced from Nodal Segments*-

In the subsequent steps of *in vitro* plant regeneration, shoot buds induced from nodal segments were sub-cultured after 4-6 weeks on nutrient medium supplemented with Auxins and Cytokinins in various combinations. NAA and IAA were main Auxins used with Kn in combinations. Low concentration of NAA-1 (mg/l) along with low level of Kn (0.5 mg/l), had been very promising in elongation of shoots. The juvenile shoot buds were sub-cultured in culture tubes for elongation. On the above combination, 84% shoot buds elongated into shoots. The mean length of shoots was measured to be 8.6 cm. Whereas[16], achieved optimum shoot regeneration on MS medium was supplemented with 0.5-3.0 mg/l BA/Kn

**Table 3.** Response of direct shoot induction from nodal segments of Ashwagandha *(Withania somnifera)* cultured on MS medium supplemented with different NAA, BAP and Kinetin (No. of explants/treatment =25)

| S. No. | Hormone Concentration (mg/l) | | No. of explants responded | % response | Mean no. of shoots induced/explant |
|---|---|---|---|---|---|
| | NAA | BAP | | | |
| 1. | 1 | 0.5 | 21 | 84 | 7.8 |
| 2. | 1 | 1 | 17 | 68 | 2.3 |
| 3. | 1 | 2.5 | 12 | 48 | 5.2 |
| 4. | 2 | 0.5 | 8 | 32 | 3.6 |
| 5. | 2 | 1 | 11 | 44 | 1.8 |
| 6. | 2 | 2.5 | 5 | 20 | 2.9 |
| 7. | 2.5 | 0.5 | 3 | 12 | 0.5 |
| 8. | 2.5 | 1 | 9 | 36 | 2.1 |
| 9. | 2.5 | 2.5 | 12 | 48 | 3.2 |
| 10. | 4 | 0.5 | 13 | 52 | 3.4 |
| 11. | 4 | 1 | 6 | 24 | 5.2 |
| 12. | 4 | 2.5 | 8 | 32 | 2.8 |
| | NAA | Kn | | | |
| 13. | 1 | 0.5 | 5 | 20 | 1.6 |
| 14. | 1 | 1 | 11 | 44 | 3.4 |
| 15. | 1 | 2.5 | 9 | 36 | 2.8 |
| 16. | 2 | 0.5 | 6 | 24 | 1 |
| 17. | 2 | 1 | 8 | 32 | 0.8 |
| 18. | 2 | 2.5 | 3 | 12 | 2.2 |
| 19. | 2.5 | 0.5 | 15 | 60 | 3.6 |
| 20. | 2.5 | 1 | 19 | 76 | 2.4 |
| 21. | 2.5 | 2.5 | 11 | 44 | 1.8 |
| 22. | 4 | 0.5 | 3 | 12 | 3.4 |
| 23. | 4 | 1 | 5 | 20 | 2 |
| 24. | 4 | 2.5 | 13 | 52 | 1.8 |

alone or in combination with 0.5-2.0 mg/l NAA. The elongation response in terms of length of shoots was maximum on the medium supplemented with IAA (2 mg/l) and Kn (0.5 mg/l). Approx. 88% explants exhibited elongation of shoots. However, the mean length of elongated shoots was a little less (7.5 cm), as compared to the NAA+ Kn supplemented medium. On other concentrations, response was moderately good.

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