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In vitro regeneration of withania somnifera L. dunal. A rare medicinal plant

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Abstract: Withania somnefera L. is a rare and endangered medicinal plant. It plays role in the prevention of chronic diseases, such as cardiovascular, carcinogenic, breast and prostate cancer. In addition to mental disorders, asthma, inflammation, arthritis, rheumatism, and tuberculosis, it has tremendous therapeutic value for a vast array of conditions. The therapeutic potential of W. somnifera is primarily attributable to the presence of tropane alkaloids, withanolides, and steroidal lactones. In vitro rapid propagation Withania somnefera was deliberated from shoot tip; node explants were cultured on (MS Murashige and Skoog) medium supplemented with B5 vitamins. In various concentrations of cytokinins and auxins ranging from 0.1 mg to 3.0 mg combinations of BAP and KN was good response from shoot tip and node. Highest number of callus induced in the concentrations of 2.5 mg BAP+2 mg NAA. Multiple shoot was noticed in 2.5 mg BAP and 1.5 mg NAA and 2 mg IBA. The present study enables the large scale production of W. somnifera L.using in vitro conditions and disease free plants.

Keywords: Withania somnifera, Micro propagation, Sterilization, growth regulators, Medicinal uses.

1. Introduction

Withania somnefera L. Dunal is erect evergreen perennial plant. It is commonly called Ashwagan that belongs to the family Solanaceae (Mishra, *et al.*, 2000,) It is a highly medicinal plant used to treat tuberculosis, rheumatism, and inflammatory conditions. Root and leaves also contains potent and preparations of anti-cancer properties, antiviral, antibacterial, antitumor, anticonvulsant, antibiotic, antistress, anti-inflammatory and antioxidant drugs present in several ayurvedic preparations (Kumar, *et al*., 2011,) *W. somnifera* proved 99.75% endangered and vulnerable medicinal plants (Kanungo and Sahoo, 2011).

Phytochemical constituents of Ashwaganda root include steroidalal kaloids and steroidal lactones, which belong to the withanolides class of constituents. This plant has produced twelve alkaloids,

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thirty-five withanolides, and several sitoindosides, which have all been isolated and studied. A withanolide with a glucose molecule at carbon 27 is called a sitoindoside. Two primary withanolides, withaniferin A and withanolide, account for the pharmacological activity of ashwagandha. The withanolides are essential hormone precursors that can be converted into human physiological hormones when required. They are the most significant bioactive constituents of Ashwagandha's roots. A Withaferin (George,.1984.) The present study ofmicro propagation technique to salve the problem has been large number of plant germination. The MS basal medium were supplemented with different concentration of auxin and cytokinins growth hormone IAA, IBA, NAA, 0.5 to 4.0 mg/L and cytokinins BAP, and KN, 0.5 to 4.0 mg/L are also used. *In vitro* propagation *W. somnifera* practiced and establishment of plants in the field conditions. (Thomas and Shankar, 2009).

2. Materials and Methods

2.1. Plant Collection and Sterilization of Explants

W. somnefera was collected from Thanjavur (Dt) during the month January 2021. The explants were then prewashed 10% percent (W/V) of bavistain methyl 1-3 benzimidizole carbonate solution and washed thoroughly in running tap water. The explants were subsequently and disinfection surface sterilized with 0.12% Hgcl2 (mercuric chloride) solution for 3-5 minutes and washed 2-3 times in sterile distilled water.

2.2. Culture Medium

Nutritional support must be required for optimal in vitro tissue growth. The nutrient media is comprised primarily of Effect of different BAP, IBA, and KN concentrations in shoot inorganic nutrients, carbon sources, vitamins, iron sources, amino acids, and natural supplements. All stock solutions were prepared and stored in amber – sterilised coloured bottles in a refrigerator at 40 degrees Celsius. In the current study, Ms Medium was supplemented with various concentrations of growth regulators. were added, such as Benzyl amino purine (BAP) Kinetin (KIN) 2, 4-Dichlorophenary acidic acid (2,4-D), Napthalic acetic acid (NAA), Indole – 3 butyric acid (IBA) were 0.5 to 3.5mg concentration was used. Multiple shoot was noticed BAP, NAA, IBA (0.1 to 3.5mg) were used. Required sucrose and other organic supplements were added. The final volume was composed of distillate water. To the aforementioned media, 0.8% (W/V) of sugar and Hi-media (agar) were added. Using a PH metre, the pH was adjusted to 5.8 - 5.9 with either 0.1 N NaOH or 0.1 N Hcl.

3. Results

Specific cell types differentiate from non-specialized meristematic cells during the normal growth of plant tissues, which results in an ordered fusion. The application of growth regulators to an in vitro culture medium containing suitable nutrients allows for the modification of plant developmental processes. The interactions of the major growth regulators can be utilised, but anxins can also cause cell growth and division on a basic level.

The explants shoot and nodal explants for micro propagation were selected from the same. The result showed that shoot formation was generated from direct organogenesis of explants, were detected in all treatment. After 15 days growth of callus was observed (Fig-1).

3.1. Callus Induction

The good development of callus types was observed in the MS media supplemented with BAP and NAA (2.5 mg/L) for best results for regeneration. The requirement of various plant growth regulators for induced callus and differentiation from nodal and leaves explants. After 14 days of culturing, the explants began to grow and callus formation occurred in the MS medium (Fig-1). The different concentration of growth hormone BAP, KN, NAA, are using in mass production of callus induction from MS media at 4 weeks culture (Table -1).

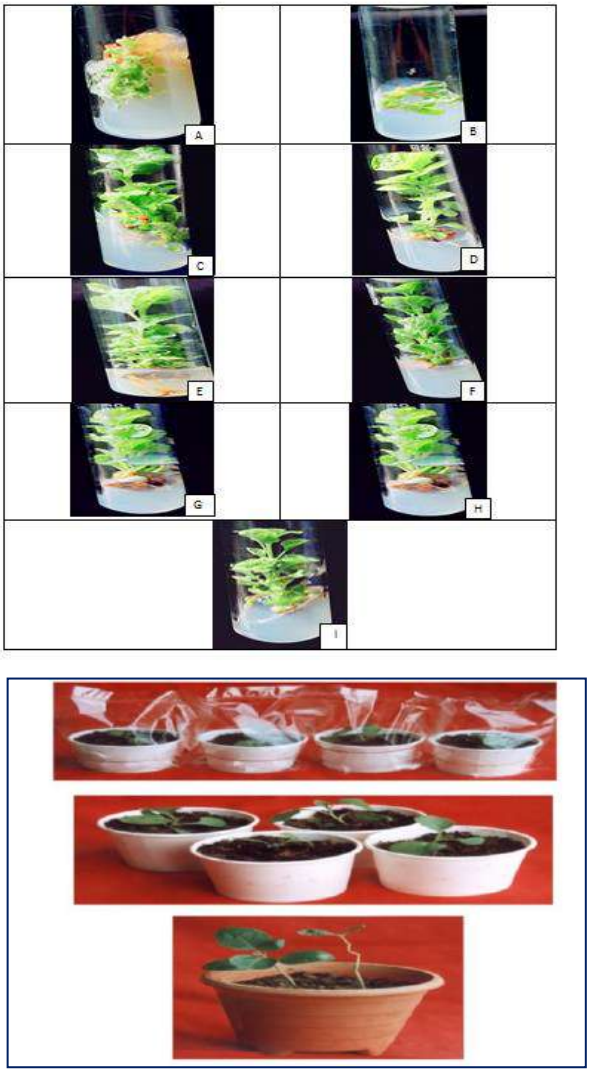

Figure 1. A &B: Callus induction, C&D Shoot induction, E Root induction, F to I Multiple Shoot production

Figure 2. Hardening of Plant

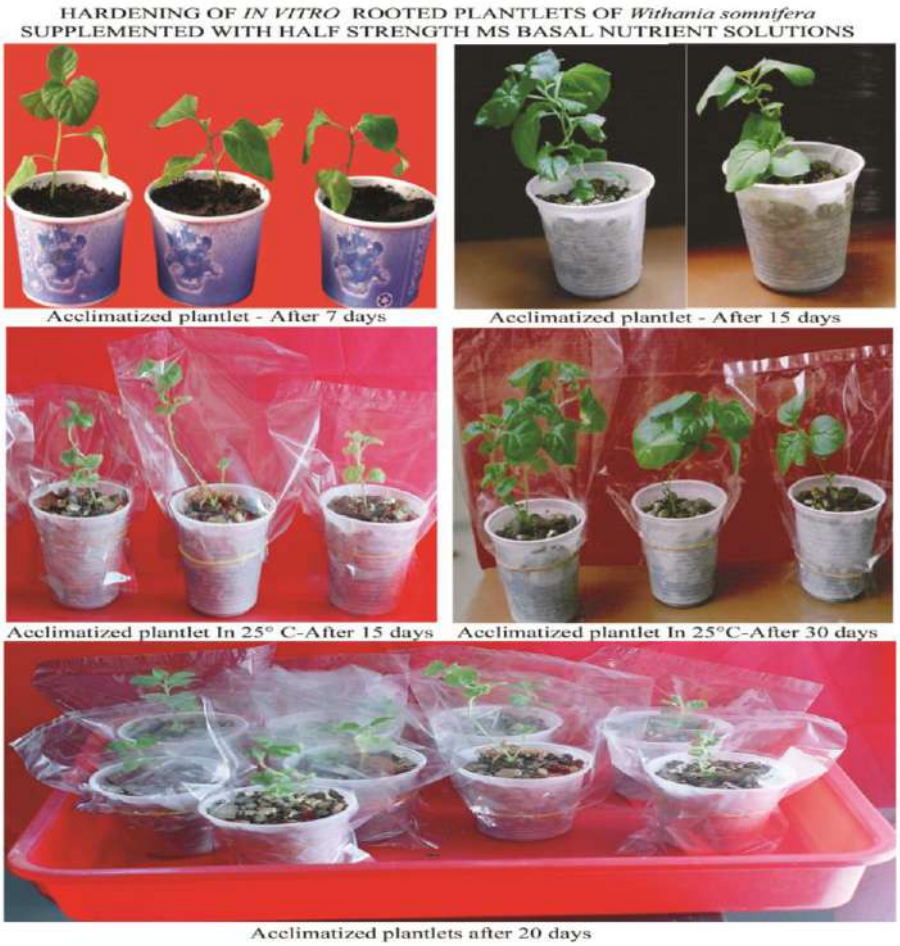

Table 1. Effect of various concentrations of BAP, 2-4D, and NAA in callus induction on MS media from leaves and shoot tip explants after 4 weeks of culture.

Growth regulators (mg/L)			Culture showing response	
BAP	2-4D	NAA	(%)	Basal callus
0.5	-	0.5	55	+
1.0	-	1.0	65	+
1.5	-	1.5	72	++
2.0	-	2.0	85	+++
2.5	-	2.5	95	++++
-	0.5	0.5	70	+
-	0.5	1.0	72	+++
-	0.5	1.5	80	+++
-	0.5	2.0	90	+++

-	0.5	2.5	92	+++
0.5	0.5	0.5	80	++
1.0	0.5	1.0	82	++
1.5	0.5	1.5	95	+++
2.0	0.5	2.0	81	+++
2.5	0.5	2.5	90	+++

Callus induction: + poor ++ moderate +++ high response.

Table 2. After 4 weeks of culture, the effect of various concentrations of BAP, IBA, and KN on MS medium from nodal and shoot tip explants

MS Medium Growth regulators (mg/L)			Culture Showing Response (%)	Mean shoot/ explants	Mean shoot length (cm)	Basal Callus
BAP	IBA	KN				
0.5	0.5	-	70	4	4.5	+++
1.0	1.0	-	75	5	4.8	+++
1.5	1.5	-	90	7	4.2	++
2.0	2.0	-	85	4	5.1	+++
2.5	2.5	-	95	6	5.0	+++
-	0.5	0.5	75	3	2.5	++
-	1.0	1.0	85	5	5.0	+++
-	1.5	1.5	90	7	7.2	+++
-	2.0	2.0	95	9	7.0	+++
-	2.5	2.5	85	4	5.1	+++
0.5	0.5	0.5	65	4	5.1	+++
1.0	1.0	1.0	85	6	5.2	+++
1.5	1.5	1.5	90	8	6.2	+++
2.0	2.0	2.0	87	6	5.2	++
2.5	2.5	2.5	90	5	5.0	++

Shoot induction have been indifferent grades viz.

- No response + poor ++ moderate +++ high

Table 3. Effect of various concentrations of BAP, NAA, and IBA in root formation on MS medium from nodal and shoot tip explants after 5 weeks of culture.

MS Medium Growth regulators (mg/L)			Culture showing response (%)	Mean root/ explants	Mean root length	Basal callus
BAP	NAA	IBA				
0.5	0.5	-	60	11	09.95	-

1.0	1.0	-	75	13	11.05	-
1.5	1.5	-	78	16	11.05	-
2.0	2.0	-	85	14	13.45	-
2.5	2.5	-	92	18	13.45	-
0.5	-	0.5	70	13	12.75	-
1.0	-	1.0	75	15	11.05	-
1.5	-	1.5	85	16	09.95	-
2.0	-	2.0	93	19	13.75	-
2.5	-	2.5	96	18	13.80	-
0.5	0.5	0.5	80	10	12.75	-
1.0	1.0	1.0	85	14	13.45	-
1.5	1.5	1.5	75	17	09.95	-
2.0	2.0	2.0	90	19	13.75	-
2.5	2.5	2.5	95	16	13.80	-

Shoot induction have been indifferent grades viz.

No response + poor ++ moderate +++ high

3.2. Shoot Regeneration:

The highest frequency of shoots from node and leaves explants were observed in MS media containing 0.5 mg to 2.5 mg BAP, NAA, IBA, KN were used (Table- 2). The maximum shoot regeneration frequency i.e. 97% on MS basal medium supplemented with 2.5mg/L BAP, NAA 2.5mg/L and IBA 2.0mg/L respectively.

3.3. Shoot Multiplication:

Shoot multiplication of *W. somnifera* the different concentration of BAP, NAA, IBA 0.5 to 2.5mg/L used, IBA at 1.5mg/L has given 95% growth of shoot with a mean shoot length of 5.2cm (Table- 3). The maximum number of multiple shoots was observed at BAP 2.5mg/L with 95% of response with mean shoot length 5.0cm (Fig-3& 4).

3.4. Root Formation:

Among the different concentration of BAP, NAA, IBA 0.5 to 3.5mg/L were used, IBA at 2.5mg/L has given 97% growth of roots. A large number of lateral roots were identified on thedifferent explants cultivated at media proper cytokinins and auxins combination on the formation of roots*in vitro* conditions. (Fig-2&3). The highest numbers of roots from different explants were observed by on 45 days it has given 20roots per explants (Table- 3).

4. Discussion

Sangwan,*et al*., 2007 found that withanolide, regeneration in shoot cultures of *W.somnifera*. Multiple shoot cultures of *W. somnifera* from single shoot tip explants in MS agar medium

containing 2.0 mg BA/L (Ray&Jha, 2001,) To meet production demands, plant tissue culture is desirable. This technique has been developed for medicinal plant mass propagation (Thomas and Philip, 2005: Thomas and Shankar, 2009). The hormonal combinations of benzyl amino purine and kinetin affected both morphogenetic response and withanolide biosynthesis (1991, Sen and Sharma).

Govindraju et al. (2003) reported direct regeneration of the same plant from leaf explants; however, adventitious buds were formed when MS medium was supplemented with BAP 1.0 mg-3.0 mg, IAA0.5 mg, and 10 percent coconut milk (Shukla,et al., 2010). MS plates were seeded with auxiliary leaves, auxiliary shoots, hypocotyls, and root segments from W. somnifera, 1962 medium supplemented with 2 mg/l 2, 4-D and 0.2 mg/ml kinetin (Rani and colleagues, 2003). For the present study, the results when sub cultured on 25th day 15 shoots per explants were achieved (Table-3). The mean shoot length was 16.80 measured. BAP growth regulator 97% more effective than KN, (Gwo, 2001). It was suggested that the use of BAP as a cytokinis and NAA asauxin in an appropriate ratio. They investigated that BAP induced more shoot produced. Many investigators examined that uses of auxin KN, IBA, BAP was the optimum concentration for shoot and root regeneration (Lazzeri, 1986, Ravanta, 2003) concentrations of KIN (0.5-3.0 mg/l) in combinations for the purpose of organogenesis. (Li, 2005).

After inoculating the rooting medium for thirty days, the plantlets were removed from the culture tube and washed with distilled water. Before planting, these in vitro-grown plantlets were transferred to plastic containers containing a 1:1 mixture of sand and FYM for 18 days. A mixture of 75 percent plantlets was successfully hardened. Plant regeneration from the shoot apex of W. somnifera through the callus phase provided a significant opportunity for gene transformation of this important organism, according to the findings of this study (Rani and partners, 2003).

5. Conclusion

Withania somnifera *(*Dunal) L. is very rare medicinal plant. The *invitro* regeneration protocol is an efficient means of ex-situ conservation of plant diversity. We have demonstrated in this study the effects of growth regulators on morphogenic response of *W. somnifera* cultivated *invitro*. Appropriate different combinations and concentration of plant hormones result in higher yield of plant biomass and mass propagation by tissue culture technique.

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