Direct organogenesis of *Withania somnifera* L. from apical bud

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***Withania somnifera* (L.) Dunal, commonly known as (Ashwagandha) belongs to the family Solanaceae, is one of the important medicinal cash crop in many states of India. It is widely used in the treatment of inflammatory conditions, rheumatism, different types of tumor, tuberculosis, reproductive system, nervous system and is benificial to improve the vitality. In this work the seeds of *W.somnifera* were collected from Botanical garden of P.G. Department of Botany, Utkal University. The fresh seeds were inoculated in to ½ MS medium and the germination was initiated after 7 days. Germinated seedlings were allowed to grow for one month till the plantlet attain a height of 3-4 cm. Fresh plantlets were transferred to liquid MS medium for better devlopment of root and shoot system. Apical parts of the plant were excised and inoculated into fresh Revised Tobacco medium (RT) supplemented with 1mili gram/Litre of 2,4-D. The explant was allowed to grow for further differentiation. After 15 days it was observed that direct rhizogenesis was more than shoot development. Along with shoot/root differentiation there was also initiation of callus but frequency of callogenesis was very low. Further root and shoot development was maintained in the same medium for next two weeks, and the plants then transfered pots containing a sterilized mixture of garden soil, vermicompost and sand in (1:1:1) ratio. The pots were maintained at a temperature of 25±2^{0}C with required humidity and 16 hours light/day for 1 month. *In vitro* raised plantlets were successfully transferred to the field. About 90% of plantlets were survived in the garden soil. Overall a short term protocol was developed for propagation of *Withania somnifera*.**

Key words: Revised Tobacco medium, Rhizogenesis, Organogenesis, Seedlings, *Withania somnifera* L.

INTRODUCTION

Withania somnifera is known as one of the common medicinal plant. It is an herb producing wide range of withanolides having anti-tumor activity. Besides that the plant is also important for tropane alkaloids having antiarthritic, antirheumatic and anti-depression properties (Uma Devi et al*.,* 1995). The main aim of the research work includes: standardization of medium for *in vitro* germination of seeds of *Withania somnifera,* optimization of growth parameters for induction of callus, maintenance and regeneration of explant, Formulation of suitable media for rooting, shooting and hardening of the plant in order to achieve quality transplants, Development of a short term protocol for *in vitro* propagation of *Withania somnifera* L. Dunal. A medicinally important plant.

MATERIALS AND METHODS

Collection of seeds

Fresh seeds of *W.somnifera* were collected from the Botanical garden of P.G. Department of Botany, Utkal University, Bhubaneswar, Odisha.

Media for micropropagation

The media that are used in the present work for the micropropagation of explants were MS (Murashige and Skoog, 1962) and RT (Revised Tobacco) medium.

Table 1. Seed germination frequency in *Withania somnifera* on ½ strength MS medium without any growth regulator.

Medium	Weeks	Percentage of germination	Height of the explants in centimetre (mean±SEM)*
½ MS	1	0	-
½ MS	2	30	0.8±0.02
½ MS	3	50	1.9±0.03
½ MS	4	70	2.8±0.03
½ MS	5	95	4.1±0.01
½ MS	6	95	5.9±0.02

-: No growth
*: The difference in mean values among the treatments are statistically significant ($p<0.01$).
Data represents the mean of 5 replicates.

Table 2. RT medium containing different auxins for development of apical bud of *Withania somnifera.*

RT medium containing different conce ntrations of auxins (mg/L)		Status of shooting and rooting	Mean number of shoots per explants (Mean±SEM*)	Percentage (explants) with multiple shoots (Mean±SEM*)	Mean length of shoots (cm) (Mean±SEM*)
2,4-D Mg/L	NAA mg/L				
0.5	-	+	7.44±0.03	-	1.71±0.01
1.0	**-**	**+++**	**13.72±0.02**	**90.48±0.01**	**4.85±0.01**
1.5	-	++	10.20±0.03	68.34±0.03	2.51±0.01
-	0.5	+	4.16±0.05	-	1.10±0.05
-	1.0	+	5.76±0.02	-	1.54±0.02
-	1.5	++	8.36±0.02	62.40±0.06	2.21±0.01

Culture conditions

The cultured flasks or tubes were incubated in a culture room maintained under 16h/8h (light/dark), 45μmol m^{-2} s^{-1} irradiance level provided by cool-white fluorescent tubes (Philips, India), with 55-60% relative humidity and the temperature of the culture room was maintained about 25±2^{0}C.

Subculturing

In each and every step of *in vitro* propagation such as shooting and rooting, subculture was done within 3-4 weeks interval in the same medium.

Acclimatization

Well developed *in vitro* plantlets from rooting medium was taken out from the culture flasks and washed thoroughly to remove agar. The plants were then transferred to pots containing sterilised soil: sand: vermicompost (1:1:1) and kept in the culture room, subsequently to the greenhouse. Liquid MS medium was supplied to the pots at regular intervals for the survival of the small plants. The hardening plants after one month were transferred to normal field condition.

RESULTS

Seed germination and seedling development in MS medium

Freshly collected mature seeds used as inoculum exhibited maximum percentage of germination (90%). After 45 days of seed germination when the plantlets attained a height of about 6 centimetres (Table 1) the plants are transferred to liquid MS medium for better development of root and shoot system (Figure 1H).

Multiple shooting and development of apical bud in RT medium

The plantlets are maintained in the liquid MS medium for about six weeks for development of healthy root and shoot system, apical parts of the plant were excised and inoculated into fresh Revised Tobacco medium (RT) supplemented with two auxins i.e (NAA and 2,4-D) of different concentrations such as (0.5, 1.0, 1.5) mg/L. Initiation of shooting was observed in RT medium with all the auxin combination but profused rooting and frequent multiple shooting was only achived in RT medium containing 1.0 mg/L of 2,4-D (Figure 2 F), Maximum percentage multiple shoots are found (90.48±0.01) and average shoot length was highest (4.85±0.01) in RT medium containing 1.0 mg/L of 2,4-D (Table 2). RT medium with NAA of different concentration was not suitable for shooting and rooting except 1.5 mg/L NAA which exhibited rooting and mutiple shooting with very low frequency.

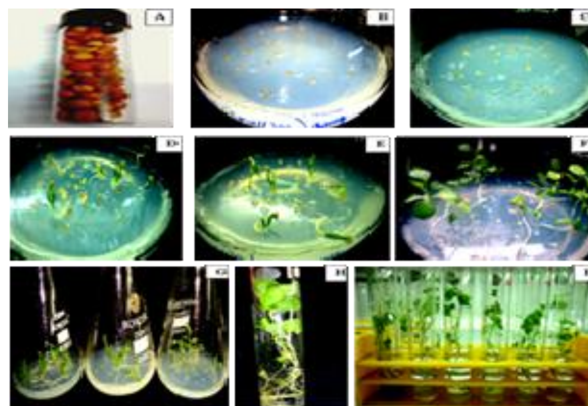

Figure- 1. **A**:- Seeds of *W. somnifera*, **B**:- Photo of inoculated seeds in 1st week, **C**:- Initiation of seed germination in 2nd week, **D**:- Germinated seeds in 3rd week, **E**:- Development of seedlings in 4th week, **F**:- Seedlings in 5th week, **G**:- Flasks containing seeds in 6th week, **H**:- Transfer of plant to liquid MS media, **I**:- plants with healthy root system in liquid media

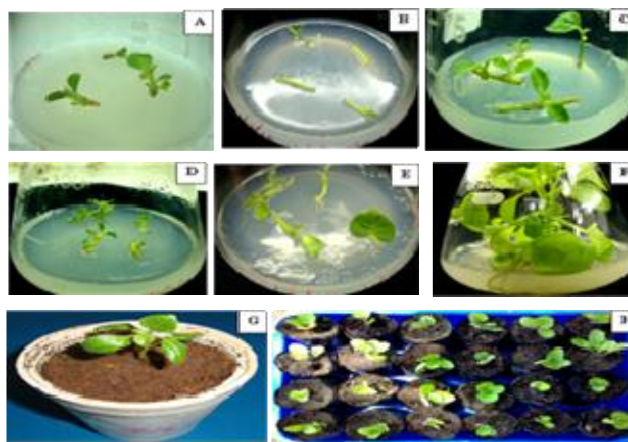

Figure 2. **A**:- Inoculation of *W. somnifera* stem and apical bud in RT medium containing NAA, **B**:- Inoculation of *W. somnifera* stem and apical bud in RT medium containing 2,4-D (1mg/L) **C**:- Initiation of shoot development in RT medium containing 2,4-D, **D**:- Development of multiple shoot **E**:- Initiation of root in RT medium containing 2,4-D, **F**:- Profuse rooting and plant development in RT medium, **G**:- Transfer of *in vitro* raised plant to sterilized soil supplemented with liquid MS media, **H**:- Plants finally transferred to poly bags containing garden soil, sand and vermicompost.

Transferred under the table: no explants are found in the highlighted boxes (Mean±SEM*)

+ : Shoot initiation
++ : Multiple shooting with less rooting
+++ : Multiple shooting with profuse rooting
- : Absent

*: The difference in mean values among the treatments are statistically significant ($p<0.01$). Data represents the mean of 5 replicates for each treatment. Data were recorded after 8 weeks of culture.

Acclimatization and field establishment

The newly developed plants are transfer to the sterile pots containing sterilized mixture of garden soil, vermicompost and sand in (1:1:1) ratio. The pots were maintained at a temperature of 25 ± 2^0C with required

humidity and 16 hours light/day for 1 month with required supply of liquid MS media. Subsequently healthy plantlets were transferred in to the glasshouse potted in natural garden soil for another two week. About 90% of plantlets were survived in the garden soil. All the potted plants placed at outdoor survived even after 8 weeks of transfer and showed good and healthy growth.

DISCUSSION

The natural population of *Withania* sps. are not easily amenable for vegetative propagation and seed raised plants need about 6-8 months to reach maturity after they are transferred to the garden soil, when they can be harvested for their natural, active components. To overcome these constraints, biotechnological tool like plant tissue culture can be gainfully employed where cultures can be started from chemically superior mother plants and harvesting of secondary metabolites can be done from such differentiated cultures. In this study an attempt has been made to use the *in vitro* germinated seedlings as the source of explants for rapid clonal propagation.

Sivanesan and Murugesan (2008). Reported that *Withania somnifera* is used in several indigenous drug preparations because of over exploitation. This plant had entered in the endangered plants' list. Tissue culture is an important technique where in numerous plant (clones) are could be produced from single explants. Therefore this plant was micropropaged using nodal explants which would lead to a large scale multiplication of this plant**.**

(Kulkarni et al, 1999, 2000) stated that nodal explants formed multiple shoots both from pre existing and *de novo* buds on MS medium containing 0.1 to 0.5 mg/L of BAP but in this present study the multiple shooting is achieved in RT medium containing auxins, hence auxins are also helpful in shoot development in *Withania somnifera*. About 90% of the plants are survived after they are transferred to the garden soil and this survival rate is higher in comparison to the result obtained by (kulkarni et al, 1999).

This report presents a reproducible and effective method of shoot regeneration directly from *in vitro* cultured shoot buds of *W. somnifera*. Rapid regeneration via adventitious bud formation, avoiding callus production is the system of choice because it minimizes the risk of somaclonal variation (Sivanesan, 2007).

Regenerated plants have transferred to the field with 90% survival rate which was an improvement in the survival rates obtained by (Rani et al, 1999) and (Manickam et al, 2000). In conclusion a simple efficient protocol was developed in the establishment of direct plant regeneration from apical bud explants of *W. Somnifera.* The protocol will have relevance in microcloning of the elite strain besides its implications in genetic engineering of this important medicinal herb in India.

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