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Standardization of various factors for production of adventitious roots in selected varieties of *Withania somnifera* and estimation of total withanolides by High Performance Liquid Chromatography

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ABSTRACT

Withania somnifera (Dunal) popularly known as Ashwagandha, "Winter Cherry" and "Indian Ginseng". Its roots and leaves are used in a number of preparations for their anti-inflammatory, anticonvulsive, antitumor properties besides promoting vigour and stamina. Ashwagandha contains very high concentration of metabolites like steroidal lactones (Withanolides), alkaloids and flavonoids, so it is used in more than 200 commercially ayurvedic formulations. The annual requirement of *Withania sominifera* in India is about 9127 MT where as the estimated production in India is only 5905 MT. This requirement can be met by mass cultivation of adventitious roots using bioreactors. Adventitious roots induced by this form are considered to be genetically uniform, true to its type that gives rise to mass production of desired pharmaceutical compound. Seeds of varieties like Jawahar Ashwagandh-20 (JA-20), Arka Ashwagandha (AA), IIHR WS-48 and IIHR WS-32 have been raised in *in-vitro* conditions. Adventitious roots were induced from *in-vitro* leaves by varying factors. Half strength MS medium yielded more roots than full strength MS medium, combination of IAA and IBA (ranging from 0.025-0.01mg/l) were found to be ideal for adventitious root induction for each variety. Sucrose concentration (3-4%) in half strength MS media yielded more adventitious roots, with a light intensity of 16 hours photoperiod than darkness.

KEY WORDS: *WITHANIA SOMNIFERA* (DUNAL), IAA, IBA, HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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INTRODUCTION

Withania somnifera (Dunal) popularly known as Ashwagandha, Indian ginseng and Winter cherry belongs to the family *Solanaceae*. The plant's Latin name literally means, "sweat of a horse" due to the scent of the roots. The plant is commonly found in Africa, Mediterranian, India and North America. It is an erect branched under shrub up to 1.25 m in height, minute, smooth and shiny hairs throughout the plant. Leaves are ovate, with hairs and soft to touch. Flowers are greenish, the roots are fleshy, papery and whitish brown in color. The stems are around 3 to 4 feet in height. One plant survives for up to 4 to 5 years. Its stem contains fiber like texture. The leaves are oval shaped, 2 to 4 inches long and contain fiber. The flowers are blooming at the base of the stems are small, somewhat long with chimney shape and yellowish green in color. The flowers bloom from the base of the leaves and become red when wipe. The seeds are small, heart shaped, smooth and flat. The roots are rough, white from within, strong, transparent, thick and one to one and half feet long, (Geetha et al., 2018).

In Ayurveda and Unani medications the herb is majorly used for its high rejuvenating power. It is called as "The natural stress buster" due to its ability in making the human body copes with different kinds of stress (Rao, 2012). The roots of Ashwagandha help in boosting immunity power of the body. It is commonly prescribed for hiccup, bronchitis, dropsy, rheumatism and female disorders, the roots of this plant also prescribed for general sexual weakness in human beings (Kattimani *et al.,* 2000). Its roots and leaves are used in a number of preparations for their anti-inflammatory, anticonvulsive, antitumor, immuno-suppressive and antioxidant properties besides promoting vigor and stamina. Ashwagandha is increasingly becoming a popular adaptogenic herb and is available throughout the western world as a dietary supplement. Ashwagandha contains very high concentration of metabolites like steroidal lactones (Withanolides), alkaloids and flavonoids, so it is used in more than 200 commercially ayurvedic formulations.

Adventitious roots are the roots that are induced at unusual sites such as roots forming on leaves, which grow and branch rapidly (Dubrovsky and Rost., 2003). The roots induced by this form are considered to be genetically uniform, true to its type, that gives rise to mass production of desired pharmaceutical compound (Goel *et al* ., 2009). Adventitious root cultures provide a preferred platform to produce commercially important secondary metabolites (Khan *et al* 2017). Adventitious roots can harbor medicinally important compounds through different strategies like elicitation, temperature stress etc., (Rani *et al*., 2017).

The technique of micro propagation is applied with the objective of enhancing the rate of multiplication. Through the culture over a million of plants can be grown from a small piece of plant tissue within 12 months. Such proliferative rate of multiplication cannot be expected by any *in-vivo* methods. Large scale production through plant *in-vitro* regeneration will provide a means of putting the plant onto the market at lower prices. In addition, the technique is cost effective, relatively simple and can be performed by semi-skilled persons. A sustained supply of the source material often becomes difficult due to the factors like environmental changes, cultural practices, diverse geographical distribution, labour cost, selection of the superior plant stock, over exploitation by pharmaceutical industry (Kaur et al 2017)

Optimization of various tissue culture techniques become very important to explore *W. somnifera* at different aspects, as plants obtained from fields fileds are not enough for all in vitro studies. Therefore, effcient tissue culture techniques like, micropropaogation, regeneration, organogenesis, hairy root production, etc. have been established, (Vibha pandey *et al*. 2017).

The requirement of dried plant material for withanolides drug production in India is estimated to be 9127 tonnes against the annual production of 5905 tonnes (Sharda *et al*., 2007). Moreover, field cultivation is time consuming, laborious and not able to meet the Ashwagandha global market requirement (Sivanandan *et al*., 2012b; 2013a). To improve the commercial cultivation of Ashwagandha, biological advances must be made that should either increase yield or reduce time gap to assure quality (Banerjee *et al*., 1994).

The provision of alternative sources of *Withania somnifera* by encouraging its cultivation will go a long way in reducing their heavy dependence on the wild populations and also major diseases of plant like seed rot and blight can be overcome. The main objective of this research is to develop a reproducible protocol for adventitious root induction from in-vitro leaves, and comparative analysis of withanolides present in in vitro adventitious roots roots of different varieties and selection of best variety for mass propagation in bioreactors.

MATERIALS AND METHODS

Selection and establishment of plant material for micropropogation.

Seeds of four high yielding varieties of *Withania somnifera* like JA-20 (released variety from MPKVV, Madhya Pradesh) used as check in AICRP national trials, Arka Ashwagandha (released variety from IIHR, Bangalore), IIHR WS-32 and IIHR WS-48 were selected for the experiment.

The seeds were soaked in 300 ppm of gibberellic acid for 12 hours and washed with water. The seeds were

pre-treated using 100 mg Dithane M-45 fungicide for 15 minutes, washed with sterile water followed by 70%(v/v) ethanol for 1 minute, washed with sterile water and then with 0.1% (w/v) sodium hypochlorite(5% (w/v) available chlorine, NICE Kochi Solution) for 4 minutes. Then seeds were washed with sterile double distilled water 2-3 times to remove traces of sodium hypochlorite and dried. The seeds were then inoculated into half strength MS medium (Murashige and Skoog , 1962).

Procedure for adventitious root induction

Leaves from two month old explants were taken for production of adventitious roots, before inoculation, leaves were cut in middle with sterilized scalpel and placed on MS culture medium with the adaxial surface down. Different factors were varied–

1. Strength of medium: Half strength and full strength MS medium with selected combination of auxins were used to study adventitious root induction.
2. Sucrose concentration: Carbohydrate source plays an important role in maintaining osmoticum in plant tissue culture. Root initiation and development is a high energy process which requires the expense of available metabolic substrates such as sugars. Sucrose at different concentrations (2-7%) were tried.
3. Auxin treatment: In order to determine the optimal conditions for adventitious root induction, we tested various concentration of auxins (IAA and IBA) ranging from 0.025-0.01mg/L in 5 different combinations and compared it with control (without IAA and IBA).
4. Light intensity: The cultures in half strength MS medium with selected auxin combination were incubated at 25±2°C with 16 hours photoperiod under cool fluorescent light and for dark treatment the bottles were placed in shelves without light.

   The adventitious roots were observed after 15 days and parameters like number of roots per explant and percentage of explants response for root induction were studied.

   The roots were subjected for HPLC analysis to estimate the total withanolide content.

5. Extraction of bioactive principles from *W.somnifera*

The adventitious roots extracted from *in-vitro* were washed twice with milli-Q water to remove the traces of agar, dried and powder dried using pestle and mortar. They were assessed for different components that contribute to total withanolides. The analysis was carried out by HPLC method (Agarwal and Murali, 2010). Two grams of dry root powder was extracted with 50 mL of methanol on boiling water bath for about 20 minutes and transfer the extract to a 250 mL beaker. Repeat the process 3-4 times till the extract was colorless. Then collected all the extracts and made up the volume to 100mL with methanol, mixed well and filtered through 0.45 micron membrane filter and these were subjected to analysis by HPLC with Photo Diode Array detector. Seven standards such as Withanoside IV, Withanoside V, Withaferine A, Withanolide A, Withanolide B, 12- deoxy Withanostramolide and Withanone were used to quantify the amount of various withanolides present in the root samples. Chromatogram was recorded at 227nm wavelength and later calculate the contents of individual withanolides by the using the formula and expressed as mg/100g dry weight basis.

$$\frac{\text{Area of the sample} \times \text{Standard Wt. (mg)} \times \text{Sample dilution} \times \text{Purity of standard}}{\text{Area of the standard} \times \text{Standard dilution} \times \text{sample weight (mg)}} \times 100$$

RESULTS AND DISCUSSION

The results from the present study demonstrated that standardization of different factors like strength of the medium, effect of photoperiod, sucrose concentration , combinations of auxins at different concentrations is essential for effective adventitious root induction in *Withania somnifera*.

In this study to determine the effects of media strength half strength MS medium with a combination of 0.25mg/l IAA and 0.75 mg/l IBA had higher number of induction response and also higher number of roots per explants (Table no 1 and 2). The results of the present study is similar to results of the previous studies on adventitious root induction in *Withania somnifera* by Wadegaonkar *et al* (2006) and Praveen and Murthy (2010), where half strength MS medium was chosen suitable for adventitious root induction. It contradicts with Yin *et al* (2013) induced adventitious roots in *Pseudostellaria heterophylla* in full strength MS medium with 3mg/L IBA using root explants.

Highest root induction response (98.2% in JA 20) and highest number of roots per explant (16 roots in WS 32) was observed in bottles placed under 16 hours photoperiod compared to darkness in all the varieties of *Withania somnifera* (Table 3 and 4). Explants incubated under darkness induced profuse callusing which subsequently turned brownish and hindered the induction of roots. Roots initiated were thick and longer in braches under 16 hours photoperiod compared to thin and brittle roots in darkness.

Table 1. Effect of strength of the medium on the induction response (%)

Treatment	Arka Ashwagandha	JA 20	IIHR WS 32	IIHR WS 48
Half MS	98.00±0.173a	98.00±0.520a	98.60±0.387a	97.40±0.316a
Full MS	95.4±0.173b	96.20±0.520b	96.40±0.387b	96.40±0.316b

Table 2. Effect of strength of the medium on number of roots per explant

Treatment	Arka Ashwagandha	JA 20	IIHR WS 32	IIHR WS 48
Half MS	13.80±0.070a	13.60±0.173a	14.20±0.173a	12.60±0.141a
Full MS	13.20±0.070b	13.20±0.173b	13.80±0.173b	12.40±0.141b

Table 3. Effect of photoperiod on adventitious root induction

Treatments	Induction Response (%)				No of roots per explant			
	AA	JA 20	WS 32	WS 48	AA	JA 20	WS 32	WS 48
16 hr photoperiod	97.8	98.2	97.6	97.6	13.5	15.25	16	16.45
Darkness	91.8	91.6	92	91.8	5.1	5.1	5.05	5.6
S.Em	0.2151				0.245			
CD 5%	0.619				1.75			
CD 1%	0.81				2.36			
CV	0.717%				4.69%			

Table 4. Effect of photoperiod on number of days taken for adventitious root induction

Treatment	Arka Ashwagandha	JA 20	IIHR WS 32	IIHR WS 48
16 hours Photoperiod	24.00±0.316a	26.00±0.346a	26.60±0.141a	26.00±0.346a
Darkness	15.00±0.316b	14.80±0.346b	15.80±0.141b	14.80±0.346b

Praveen and Murthy (2010) also established adventitious roots from leaf segments of *Withania somnifera* on half strength MS medium(0.8%) agar with 0.5mg/L IBA, 30g/L sucrose incubated under 16 hours photoperiod with 100% of explants response for root induction.

Table 1- Values are mean ± standard error of five replications in three independent experiments, each with three explants per treatment. Means followed by the same letter are not significantly different at P<0.05 according to Duncan multiple range test in all tables Data were scored after15 days of culture

Table 2- Values are mean ± standard error of five replications in three independent experiments, each with three explants per treatment. Means followed by the same letter are not significantly different at P<0.05 according to Duncan Range Multiple Test. Data were scored after 15 days of culture

Table 5. Effect of Sucrose concentration on adventitious root induction response (%)

Treatments (Induction response %)	Arka Ashwagandha	JA 20	WS 32	WS 48
2% Sucrose	88.00±0.397b	82.40±0.465 b	80.40±0.389 b	88.20±0.499b
3% Sucrose	96.60±0.397a	86.80±0.465a	85.80 ±0.389a	98.40±0.499a
4% Sucrose	79.40±0.397c	78.60±0.465c	75.40±0.389c	77.00±0.499c
5% Sucrose	61.40±0.397d	61.60±0.465d	61.20±0.389 d	61.00 ±0.499 d
6% Sucrose	33.60±0.397e	35.60±0.465e	31.60±0.389 e	34.60±0.499 e
7% Sucrose	10.20±0.397f	11.00±0.465f	10.20 ±0.389f	10.60 ±0.499f

Table 6. Effect of Sucrose concentration on number of adventitious roots per explant

Treatments	Arka Ashwagandha	JA 20	WS 32	WS 48
2% Sucrose	11.40±0.319c	11.80±0.331c	10.60±0.294c	11.80±0.261c
3% Sucrose	17.60±0.319a	17.20±0.331a	15.00±0.294a	17.20±0.261a
4% Sucrose	12.80±0.319b	14.00±0.331b	12.40±0.294b	14.20±0.261b
5% Sucrose	8.80±0.319d	10.20±0.331d	9.8 ±0.294c	10.40±0.261d
6% Sucrose	7.40±0.319e	7.60±0.331e	8.60±0.294e	7.40±0.261e
7% Sucrose	5.40±0.319 f	5.60±0.331f	5.20±0.294e	5.40±0.261f

Table 7. Effect of auxins on adventitious root induction response (%)

Treatments	Arka Ashwagandha	JA 20	WS 32	WS 48
0 IAA+0 IBA	0.000±0.284f	0.000±0.330f	0.000±0.614f	0.000±0.703
0.25 IAA+0.75 IBA	96.60±0.284a	97.20±0.330a	85.80±0.614b	98.40±0.703
0.5 IAA+0.5 IBA	80.80±0.284c	80.60±0.330c	80.40±0.614c	82.20±0.703
0.75 IAA+0.25 IBA	76.80±0.284d	77.40±0.330d	75.60±0.614d	75.00±0.703
0 IAA+1 IBA	83.6±0.284 b	82.40±0.330b	97.6±0.614a	85.60±0.703
1 IAA+0 IBA	72.4±0.284e	68.00±0.330e	67.80±0.614e	65.00±0.703e

Table 8. Effect of auxins on number of adventitious roots per explant

Treatments	Arka Ashwagandha	JA 20	WS 32	WS 48
0 IAA+0 IBA	0.000±0.257d	0.000±0.245e	0.000±0.238f	0.000±0.371e
0.25 IAA+0.75 IBA	17.60±0.257a	17.20±0.245a	15.00±0.238b	17.20±0.371a
0.5 IAA+0.5 IBA	13.80±0.257b	13.80±0.245b	14.20±0.238c	12.20±0.371c
0.75 IAA+0.25 IBA	11.60±0.257c	11.60±0.245c	11.60±0.238d	13.00±0.371bc
0 IAA+1 IBA	13.60±0.257b	14.40±0.245b	17.40±0.238a	13.60±0.371b
1 IAA+0 IBA	11.40±0.257c	9.20±0.245d	8.80±0.238e	9.80±0.371d

Table 3- Values are mean ± standard error of five replications in three independent experiments, each with three explants per treatment. Data were scored after 15 days of culture. Growth Conditions- Media- Half MS supplement with selected auxin combination, photoperiod-16 hours, culture period-3 weeks at 25±2°C

Table 4- Values are mean ± standard error of five replications in three independent experiments, each with three explants per treatment. Means followed by the same letter are not significantly different at $P<0.05$ according to Duncan Range Multiple Test. Data were scored after 15 days of culture. Growth Conditions- Media- Half MS supplement with selected auxin combination, photoperiod-16 hours and complete darkness, culture period-3 weeks at 25±2°C

Carbohydrate plays an important role in maintaining osmoticum in plant tissue culture. Sucrose is considered as an unquestionably important carbon and energy source which is found in abundance in phloem sac involved in developmental process. From the observations of the above study, the optimal condition for adventitious root induction in *Withania somnifera* was half strength MS medium with 3% sucrose concentration, (Table 5 and 6).

It has been documented earlier that a sucrose concentration (3 %) was suitable for hairy root growth, whereas a too low or too higher a concentration of sucrose was adverse to adventitious root growth in *W. somnifera* (Sivanandhan *et al.* 2012 a). A lower concentration cannot provide enough energy and therefore may not be able to act as building blocks. However, higher sucrose concentration exhibited negative effect in growing cells. Nagella and Murthy (2010) recorded that 3 % sucrose was suitable for biomass accumulation and withanolide A production in cell suspension culture of *W.somnifera*. Sucrose at higher concentrations in the nutrient medium normally reduces cell biomass due to the increase of osmotic potential which subsequently reduces the uptake of nutrients. A similar result was obtained by Zhang *et al.* (2012) in *Periploca sepium* adventitious root culture and by Sivanandhan *et al.* (2012 a) in *W. somnifera* adventitious root and hairy root cultures.

Table 9. Analysis of bioactive principles from *W. sominifera* using HPLC.

Variety names	Withanoside IV	Withaferin A	Withanolide A	Withanolide B	Total Withanolide
Arka Ashwagandha	0.061±0.001 a	0.023±0.000a	0.000±0.000b	0.000±0.000b	0.084±0.001a
JA 20	0.018±0.001d	0.018±0.000c	0.002±0.000a	0.005±0.000a	0.043±0.001d
IIHR WS 32	0.0034±0.001b	0.022±0.000b	0.000±0.000b	0.000±0.000b	0.057±0.001b
IIHR WS 48	0.030±0.001c	0.017±0.000b	0.000±0.000b	0.000±0.000b	0.047±0.000c

The lowest induction response was observed in 7% sucrose concentration, from this result it was noticed that the number of roots per explant started decreasing at a higher root concentration of 5% and above. Higher amount of sucrose can retard the development of cultured cells (Wu et al., 2006) by causing cessation of the cell cycle when other nutrients are limited (Gould et al., 1981).

Table 5- Values are mean ± standard error of five replications in three independent experiments, each with three explants per treatment. Means followed by the same letter are not significantly different at P<0.05 according to Duncan Range Multiple Test. Data were scored after15 days of culture. Growth Conditions- Media- Half MS supplement with selected auxin combination, photoperiod-16 hours, culture period-3 weeks at 25±2°C

Table 6- Values are mean ± standard error of five replications in three independent experiments, each with three explants per treatment. Means followed by the same letter are not significantly different at P<0.05 according to Duncan Range Multiple Test. Data were scored after15 days of culture. Growth Conditions- Media- Half MS supplement with selected auxin combination, photoperiod-16 hours, culture period-3 weeks at 25±2°C.

Development of roots or shoots from explants involved in organogenesis depends on morphogenetic potentiality of the cells. Dedifferentiation, induction of organogenesis pathway and development of organ are the three distinct stages during organogenesis (de Kler *et al*., 1997). Supplementation of exogenous auxin is essential for adventitious root development (Pop *et al*., 2011). IAA and IBA induced adventitious roots from leaf explants after 12 days of culture. Protuberances developed in leaf explants within a week from the cut ends and adventitious roots directly developed from these protuberances in another week. The percentage of explants response for root induction and number of roots initiated per explants were recorded after 3 weeks of culture.

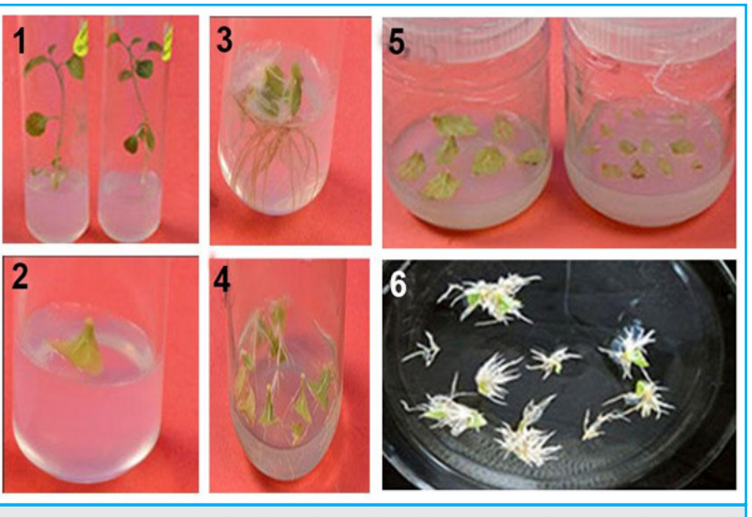

FIGURE 1A. 1) 2 month old explant of *Withania somnifera* , 2) culturing of leaves for adventitious root induction 3) best hormone treatment for adventitious root induction 4) adventitious root induction observed after 7 days of culture 5) mass production of adventitious roots in bottles 6) adventitious roots are washed and measured. Growth Conditions- Media- Half MS supplement with selected auxin combination, photoperiod-16 hours, culture period-3 weeks at 25±2°C

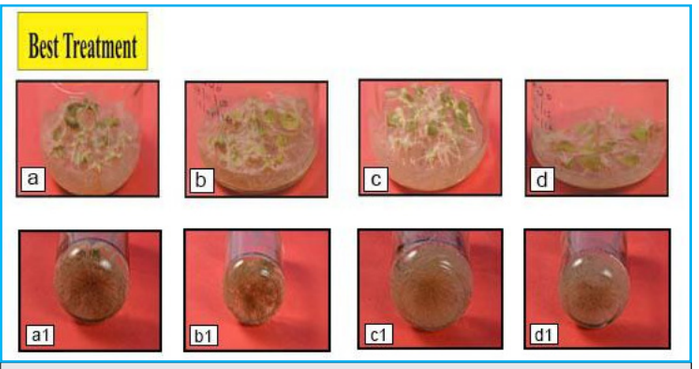

FIGURE 1B. Best culture conditions for mass production of adventitious roots in bottles. a, a1- Arka Ashwagandha variety with an auxin concentration of 0.25mg/L IAA and 0.75mg/L IBA, b,b1- JA 20 variety with an auxin concentration of 0.25mg/L IAA and 0.75mg/L IBA, c,c1 – IIHR WS 32 variety with an auxin concentration of 0.50mg/L IAA and 0.50mg/L IBA,d,d1 – IIHR WS 48 variety with an auxin concentration of 0.25mg/L IAA and 0.75mg/L IBA. Growth Conditions- Media- Half MS supplement with 3% sucrose concentration, photoperiod-16 hours, culture period-3 weeks at 25±2°C.

Taiz and Zeiger (2002) reported that roots may require a less concentration of auxin to grow, but root growth is strongly inhibited by its higher level because at this level, auxin induces the production of ethylene, a root growth inhibitor. The adventitious roots were also induced in leaf explants of *Withania somnifera* using a combination of IAA and IBA by Praveen and Murthy (2010). Combination of IBA and IAA performed better than individual treatment of auxin upon adventitious root induction in *Withania somnifera* (Sivanandan *et al* 2012a). Wadegoankar *et al* (2006) reported that a combination of IAA and IBA was effective in adventitious rot induction in leaves of *Withania somnifera* .

Hence the best auxin concentration for adventitious root induction with 96.6% root induction with 18.25 roots per explant was observed in Arka Ashwagandha variety with an auxin concentration of 0.25mg/L IAA and 0.75mg/L where as a combination 0.25mg/L IAA and 0.75mg/L IBA yielded in 97.2% root induction response and 15.12 roots per explant in JA 20. Induction response of 97.2% with 18.5 roots per explant was observed in an auxin concentration 0.25mg/L IAA and 0.75mg/L IBA of in IIHR WS 48 and induction response of 97.6% with 18.38 roots per explant was observed in explants inoculated into half strength MS medium with 0.50 mg/L IAA and 0.50 mg/L IBA for IIHR WS 32.

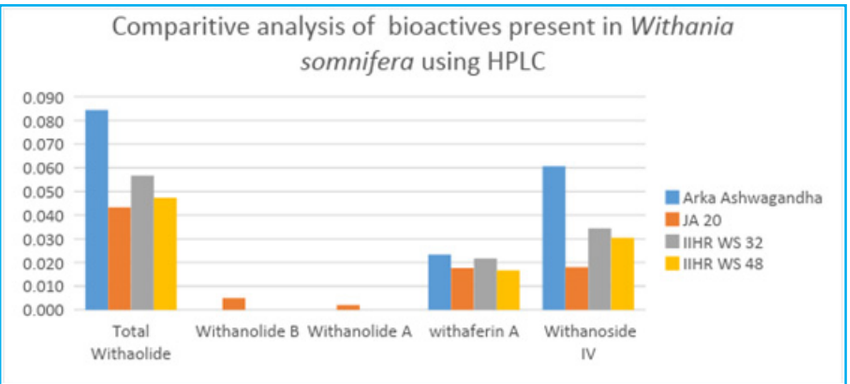

FIGURE 1C. Data represents mean ± standard error of three replicates; each experiment was repeated thrice.Comparision of total withanolide, withanolide B, withnolide A, withaferin A, and Withanoside IV among different varieties of Ashwagandha.

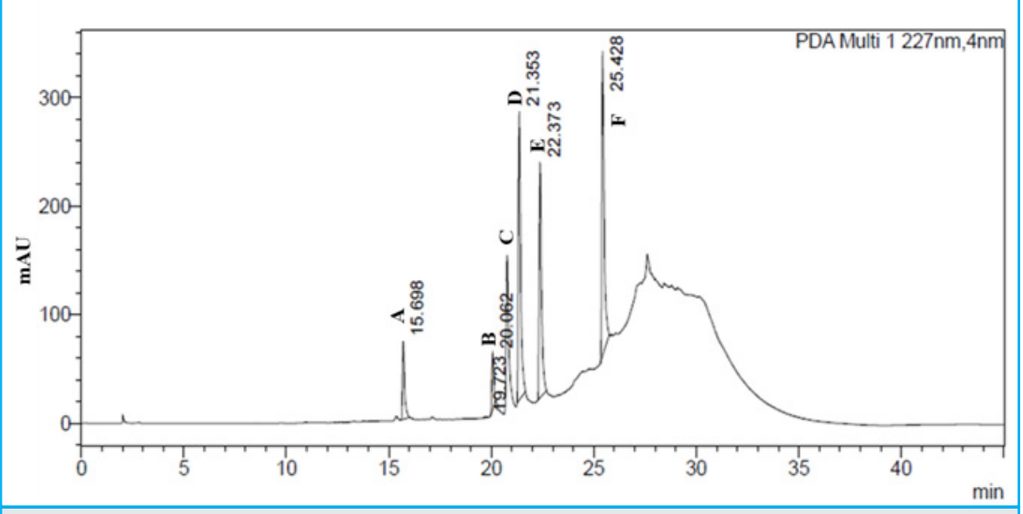

FIGURE 1D. Standard HPLC chromatogram of extracts of W. somnifera roots. A.Withanoside IV (Rt=15.69), B. Withanoside V (Rt=19.72), C. Withaferin A (Rt=20.06), D. 12-Deoxy Withastramonolide (Rt= 21.35), E. Withanolide A (Rt=22.37), F. Withanolide B (Rt=25.42)

(Table 7 and 8), At the end of 15 day after explant inoculation, the length of the root was between 0.5-1.0 cm and it was observed that the root length steadily increased with the increase in growth period with was approximately 4-5 cm after 30 days. These results indicate fast growing nature of adventitious roots.

Table 7-Values are mean ± standard error of five replications in three independent experiments, each with three explants per treatment. Means followed by the same letter are not significantly different at P<0.05 according to Duncan Range Multiple Test. Data were scored after15 days of culture. Growth Conditions- Media- Half MS supplement with selected auxin combination, photoperiod-16 hours, culture period-3 weeks at 25±2°C.

Table 8- Values are mean ± standard error of five replications in three independent experiments, each with three explants per treatment. Means followed by the same letter are not significantly different at P<0.05 according to Duncan Range Multiple Test. Data were scored after15 days of culture. Growth Conditions- Media- Half MS supplement with selected auxin combination, photoperiod-16 hours, culture period-3 weeks at 25±2°C.

Variation persist in accumulation of withanolides due to plant parts, developmental stages (Praveen and Murthy, 2010), plant part obtained from different types of cultures (Sharada *et al*., 2007; Singh *et al*., 2017) of *W. somnifera*. These studies establish relationship between morphology/condition of plant tissue and withanolide contents. Sivanandhan et al., 2012b, 2013b; Singh *et al*., 2017) used in vitro grown plants in different studies to develop adventitious roots, using different growth conditions. These developed roots were harvested to extract different combinations of withanolides.

The results for total withanolides analyzed in adventitious roots of four genotypes revealed significant difference among them (Table 9).

Among the genotypes, Arka Ashwagandha recorded high total withanolide content of 0.084% when compared to check JA-20 (0.043%). Among seven withanolides analyzed, withanoside V, 12- deoxy Withanostramolide and Withanone were not detected in all genotypes and withanoside IV constitutes highest in all the genotypes. Withanolide A and B were detected only in JA-20. Arka Ashwagandha and IIHR WS-32 contain high withaferin A and Withanoside IV when compared to Check JA-20.

Table 9-Values are mean ± standard error of three repeated experiments, each experiment was repeated thrice. Means followed by the same letter are not significantly different at P<0.05 according to Duncan Range Multiple Test.

CONCLUSION

From the present research a standard protocol has been developed for mass production of adventitious roots from *in-vitro* leaves in *Withania somnifera*. A variety with highest total withanolide content has been identi-

fied in comparision to JA 20. The requirement of dried plant material for withanolides drug production in India is high. Moreover, field cultivation is time consuming, laborious and not able to meet the Ashwagandha global market requirement. By transferring these roots in to suspension culture and mass propagating it in bioreactors reduce time gap compared to field grown roots and assure good quality *Withania somnifera* roots with high total withanolide content to cater the global demand. Large scale production through plant *in vitro* regeneration will provide a means of placing the plant onto the market at lower prices. In addition, the technique is cost effective, relatively simple and can be performed by semi-skilled persons.

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