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Micropropagation and tissue culture of the endangered medicinal plant (*Withania somnifera* L.) in Bangladesh

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Abstract

The present study was under taken to establish an efficient protocol considering various cultural aspects for plant regeneration, multiplication and rooting *in vitro* of *Withania somnifera* L. because of its medicinal value and to its survivability. Shoot tip and nodal segment were cultured aseptically on MS medium containing different concentrations and combinations of cytokinins (BAP and KIN) and auxins (NAA, IBA, and 2, 4-D) for direct and indirect regeneration. The explants were inoculated on MS medium supplemented with 8g/l Agar and 30g/l sucrose, different combination and concentration of plant growth regulators (auxin and cytokinins) was used for plant regeneration. The optimum pH was adjacent to 5.8 and autoclaved at 121±2°C for 20 minutes and incubated at 25±2°C under 16/8 hours photoperiod. In this study, the best shoot multiplication for direct regeneration was observed in 2.0mg/l BAP+0.2mg/l NAA, the longest shoot was observed 5.20cm and 5.00cm respectively. In case of indirect regeneration, highest response of callus was obtain by shoot tip (80.2%) and nodal segment (84.0%) culture on MS medium supplemented with 2mg/l 2, 4-D+0.2mg/l KIN. The best proliferation (6.5%) of adventitious bud was observed in BAP 2mg/l maximum rooting of the micro cuttings of regenerated shoots were successfully achieved with 2.0mg/l IBA and 1/2 MS+IBA2mg/l, the result was obtained 85% and 80% respectively. The plantlets were successfully transferred to soil and the percentage of survivability in such condition was obtains 87%. Micropropagation technique is the most effective way to rescue this medicinally endangers plant.

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Introduction

Ashwagandha is an important medicinal plant that belongs to the family of Solanaceae. It is also known as Winter Cherry (Andallu and Radhika, 2000). *W. Somnifera* proved to be 99.75% of the endangered medicinal plant (Siddique *et al.*, 2004). It is called as an herbal tonic and health food in Vedas and considered as 'Indian Ginseng' in traditional Indian system of medicine (Singh *et al.,* 2001). It is commonly used as anti-inflammatory, anticancer, anti-stress and immune-modulator, adaptogenic, central nervous system, endocrine and cardiovascular activities, respectively (Bhattacharya *et al.*, 1997; Mohanty *et al.,* 2004; Ahmad *et al.*, 2005).

It can also modulate the oxidative stress markers of the body. Its root part rich in alkaloids (withanine) (Majumdar, 1955), which are valuable constitutes in traditional Ayurvedic drug preparations against many diseases viz., hiccup, female disorders, cough, rheumatism and dropsy (Kirtikar and Basu, 1918). A number of pharmacological studies also indicate the multi-purpose medicinal potentiality of the plant which includes adaptogenic, antioxidant, anticancer and cardiovascular activities (Kulkarni and Dhir, 2008).

This plant are propagated because it has the potential therapeutic role in central nervous system related disorder, such as Parkinson's disease, Alzheimer's disease, epilepsy, etc (Tohda*et al.*,2005). The medicinal properties of this plant contain diverse active constituents such as withanolides, withaferin, withanosides, withanine, somniferine, sitoindosides, that's present in different parts of the plant (Kulkarni and Dhir, 2008).

Due to its medicinal value, multiple uses of this plant have necessitated its large-scale accumulation as raw material to the medicine industry (Antonisamy and Manickam, 1999). Conventional propagation of *W. somnifera* primarily via seeds (Kattimani*et al.* 1999) that's inadequate to meet the commercial demands because of the low viability of its stored seeds (Siddique *et al.,* 2004) and low seed germination rates (Vakeswaran and Krishnasamy, 2003). One of the problems for commercial cultivation, it takes long periods for seed germination strains productivity.

Tissue culture techniques can play an important role in the propagation and qualitative improvement of this medicinally important plant. Micropropagation of *W. somnifera* employs different explants such as shoot tips (Sen and Sharma, 1991; Furmanowa *et al.,* 2001; Ray and Jha, 2001), cotyledon (Rani*et al.*,2003), embryo (Kulkarni*et al.*, 2000), hypocotyl (Rani and Grover, 1999; Kulkarni *et al.,* 2000; Rani *et al.,* 2003), leaf disc (Rani *et al.,* 2003; Sharma *et al.*, 2010), root (Rani *et al.,* 2003), apical bud (Sivanesan, 2007), nodal segments (Kulkarni *et al.,* 2000; Sivanesan and Murugesan, 2008), auxiliary bud (Saritha and Naidu, 2007) has been demonstrated. Propagation used by seed, but seed viability is limited to additionally needed more than one year. Thus, an efficient*in vitro* propagation method may play an important role in rapid multiplication and germplasm conservation of this medicinally important herb. Abhyankar and Chinchanikar (1996) showed direct shoot regeneration from leaf discs grown on MS medium supplemented with indole-3-acetic acid (IAA), 6-benzyladenine (BA), and kinetin (KN) in various combinations. Kulkarni *et al.* (1996) resolutes direct shoot formation from leaf explants of *in vitro* grown seedlings using MS (Murashige and Skoog, 1962) medium containing IAA and IBA. No protocol has yet been standardized for micropropagation through callus using a variety of explants in *W. somnifera*.

The present report deals with direct regeneration including callus induction and plantlet regeneration of *W. somnifera* from hypocotyl, root, and cotyledonary leaf segment explants of *in vitro*-raised seedlings.

Therefore, the present investigation was carried out to develop a high frequency of direct regeneration system of *W. somnifera* using shoot tip and nodal segment explants and to compare explants responses

to different hormone concentrations.

Material and methods

Collection of plants

In the present investigation, *W. somnifera* L. was used as experimental material. For *in vitro* culture nodal segments and shoot tips were used as explants. Explants were collected from the medicinal village of Natore.

Surface sterilization

Explants were excised from the field grown plants and washed thoroughly in running tap water for 30 min then immersed in savlon for 10 minutes and washed several times with distilled water. Explants immersed in 70% (v/v) ethanol for 1 minute. Subsequently they were sterilized on the surface with 0.1% $HgCl_2$ solution for 2 minutes and again washed well in distilled water for 4 times to remove the traces of $HgCl_2$. The explants were blotted in sterile filter paper to remove excess water and then remove the cutting ends.

Culture media and micropropagation

The all explants (1-2cm) were implanted on sterile medium consisting of salts and vitamins of MS medium (Murashige and Skoog 1962), supplemented with 8g/l Agar and 30g/l sucrose, different combination and concentration of plant growth regulators (auxin and cytokinins) for callus induction and shoot regeneration. The optimum pH was adjacent to 5.8 by using 0.1 N NaOH or 0.1 N HCl and autoclave at 121±2°C for 20 minutes. Cultures were incubated at 25±2°C under 16 hrs. photoperiod with 45µmol m^{-2} sec^{-1} irradiance provided by cool white fluorescent light (PHILIPS 40W tubes). The regenerated callus and the micro shoots were transfer into regeneration medium for callus to form adventitious bud and shoot elongation medium containing different concentrations of BAP and combination of BAP+NAA and KIN and BAP individually. Individual shoots, which were grown about 3-4cm long transferred to half strength MS medium containing IBA, IBA or NAA for rooting. The MS medium was supplemented with 2.0mg L^{-1} IBA and glucose, maltose, fructose, lactose or sucrose at 3% (w/v), to study the effect of carbon source on *in vitro* rooting. The rooted plantlets were transferred into plastic cups containing soil: sand: vermiculite (1:1:1). The plantlets were maintained under the same controlled environmental conditions for 3 weeks and watered once in 2 days with half strength MS basal salts, subsequently they were transferred to polythene cover and kept in greenhouse after four weeks the plantlets transfer to the field.

Data assortment and applied math analysis

Data were collected after 28 days of culture and 10 test tubes cultured for each concentration statistically analyzed by using Microsoft Excel and analysis of variance (Henselová*et al.*, 2012) followed by Duncan multiple range test at 5% probability level. Mean percentage and standard errors were carried out for each condition.

Results and discussion

Effect of different concentrations and combinations of BAP, KIN, BAP+KIN in MS media on direct proliferation of shoots from shoot tips and nodal segments of W. somnifera

In our study, best response was found when explants were cultured in MS media supplemented with 2mg/l KIN. Percentage of shoot initiation was 75% in shoot tips and 85% in nodal segments explants. Maximum shoot length was found in average 2.5cm and 3.15cm in shoot tips and nodal segments explants respectively. In case of BAP different concentration (0.5-5.0mg/l) were tested to observe their effect on shoot proliferation from shoot tips and nodal segments. Results of this study are presented in (Table 1 & Fig.1) the best response was found from explants cultured in MS media supplemented with 2.0mg/l BAP. Percentage of shoot initiation was 78% and 90% in shoot tips and nodal explants respectively. Highest shoot length was found 4.25cm and 4.90cm in shoot tips and nodal segments explants respectively. For combination of BAP and KIN it was observe that best response was found from explants cultured in MS media supplemented with 2.5mg/l BAP+0.1 KIN.

Table 1. Effect of different concentrations and combinations of BAP, KIN, BAP+KIN in MS media on direct proliferation of shoots from shoot tips and nodal segment.

Shoot tips				Nodal segments				
No. explants cultured	Average no. shoots/explants	% of explants responded	Mean shoot length(cm)	No. explants cultured	Average no. shoots/explants	% of explants responded	Mean shoot length(cm)	
BAP								
0.50	20	1.00	60	1.00	20	1.50	50	2.00
1.00	20	1.10	80	1.50	20	2.25	70	2.20
1.50	20	1.25	90	2.00	20	2.50	80	2.40
2.00	20	3.00	80	2.50	20	3.50	70	3.15
2.50	20	2.50	70	2.30	20	3.00	80	2.75
3.00	20	2.40	50	2.20	20	2.50	50	2.50
KIN								
0.50	20	2.50	50	3.00	20	3.10	70	3.20
1.00	20	3.25	60	3.30	20	4.25	80	3.40
1.50	20	4.70	70	4.10	20	4.75	85	4.20
2.00	20	5.00	78**	4.25	20	5.00	90**	4.90
2.50	20	3.20	75	3.75	20	5.00	70	4.00
3.00	20	3.00	60	3.10	20	4.10	60	3.20
3.50	20	2.80	30	2.50	20	3.60	40	2.40
4.00	20	2.70	20	2.00	20	3.20	20	2.10
4.50	20	2.10	12	1.70	20	2.25	20	1.80
5.00	20	1.50	10	1.50	20	1.70	10	1.50
BAP+KIN								
2.00+0.1	20	2.5	55	3.00	20	3.15	72	3.00
2.00+0.2	20	3.20	60	3.35	20	4.25	76	3.40
2.00+0.3	20	4.40	70	4.10	20	4.80	74	4.25
2.5+0.1	20	5.50	80**	4.50	20	5.00	92**	4.90
2.5+0.2	20	3.25	75	3.75	20	4.10	75	3.20
2.5+0.3	20	3.00	62	3.10	20	3.60	65	2.40
3.0+0.1	20	2.80	35	2.50	20	3.20	68	2.10
3.0+0.2	20	2.75	30	2.00	20	2.25	69	1.80
3.0+0.3	20	2.56	25	1.70	20	1.70	70	1.60

The percentage of shoot initiation was 80% and 92% in shoot tips and nodal explants respectively. Highest shoot length was found 4.50cm and 4.90cm in shoot tips and nodal segments explants respectively.

So it is observed that when combination of BAP+KIN was used it gives better result from the previous experiments (Table 1 & Fig.1). Similar results were found in *Horlarrhena antidysentrica* (Datta and Datta, 1984), and *Asclepidas curassivica* (Pramanik and Datta, 1986).

Effect of different concentrations and combinations of BAP, BAP+NAA, BAP+KIN on shoot multiplication from shoot tips and nodal segments of W. somnifera

Among all these treatments best shoot multiplication was observed in 2.0mg/l BAP+0.2mg/l NAA supplemented in MS medium. Percentage of shoot initiation was 85% for shoot tips and 90% for nodal segments (Table 2 & Fig. 2).

In this combination, the number of shoot per explants was 6.00 in shoot tips and 7.00 in nodal segments. In this combination the number of shoot per explants were 3.25 in shoot tips and 4.10 in nodal segments. Different hormonal treatments were employed to regenerate shoots BAP (0.5-3.5mg/l) and KIN (0.1-0.2mg/l). Among all these treatment best shoot multiplication was observed in 2.0mg/l BAP+0.2mg/l KIN supplemented MS medium (Table 2 & Fig. 2).

Table 2. Effect of different concentrations and combinations of BAP, BAP+NAA, BAP+KIN on shoot multiplication from shoot tips and nodal segment.

Hormonal concentrations and combinations(mg/l)	Source of explants							
	Shoot tip				Nodal segment			
	No. explants inoculated	% of shoot regeneration	Mean no. shoot/explants	Average Shoot length(cm)	No. explants inoculated	% of shoot regeneration	Mean no. shoot/explants	Average Shoot length(cm)
BAP + NAA								
0.5 + 0.1	20	40	4.10	4.70	20	60	4.0	4.40
1.00+0.2	20	45	5.10	4.90	20	70	5.50	4.60
1.50+0.1	20	65	5.50	5.00	20	80	6.10	4.70
2.00+0.2	20	85*	6.00	5.20	20	90*	7.00	5.00
2.50+0.1	20	60	5.10	4.80	20	70	6.50	4.50
3.00+0.2	20	30	4.25	4.60	20	50	5.10	4.20
3.50+0.1	20	20	3.25	4.35	20	40	4.10	4.30
BAP+KIN								
0.5+0.1	20	25	4.10	4.60	20	35	4.0	4.35
1.0+0.2	20	35	4.70	4.80	20	40	5.45	4.55
1.5+0.1	20	55	5.20	4.90	20	60	6.00	4.65
2.0+0.2	20	75*	5.50	5.00	20	78*	6.80	5.00
2.5+0.1	20	65	4.80	4.70	20	68	6.30	4.45
3.0+0.2	20	45	3.75	4.50	20	47	5.00	4.25
3.5+0.1	20	35	3.50	4.20	20	38	4.00	4.10
BAP								
0.5	20	40	4.10	4.50	20	58	3.90	4.35
1.0	20	43	5.0	4.80	20	68	5.48	4.55
1.5	20	62	5.20	4.90	20	75	6.00	4.65
2.0	20	78*	5.80	5.10	20	83*	6.90	4.90
2.5	20	58	5.00	4.65	20	68	6.40	4.48
3.0	20	32	4.00	4.50	20	48	5.00	4.18
3.5	20	18	3.10	4.20	20	38	4.00	4.25

Table 3. Effect of different concentration and combination of 2, 4-D, 2, 4-D+KIN and NAA for callus induction.

MS medium Supplemented With 2,4-D(mg/l)	NO. explants Inoculated	Source of explants					
		Shoot tip culture			Nodal segment culture		
	20	% of explants Induced callus	Callus type	Color of callus	% of explants Induced callus	Callus type	Color of callus
0.1	20	--	--	--	--	--	--
0.5	20	--	--	--	--	--	--
1.0	20	42.2	Hard	White	43.3	Hard	White
1.5	20	56.6	Hard	White	62.23	Hard	White
2.0	20	76.32	Semi friable	Green	66.0	Semi friable	Whitish green
2.5	20	75.5*	friable	Green	78.42*	friable	greenish
3.0	20	53.20	Hard	Light Green	64.33	Semi friable	White
3.5	20	48.52	Hard	White	49.33	Hard	White
4.0	20	34.66	Hard	White	35.22	Hard	White
4.5	20	28.32	Hard	White	28.73	Hard	White
5.0	20	26.32	Hard	White	26.35	Hard	White
2,4-D+KIN							
1+0.1	20	--	--	--	--	--	--
1.5+0.1	20	56.2	Semi friable	Green	65.02	Semi friable	Green
2+0.2	20	80.2*	friable	Green	84.00*	friable	Green
2.5+0.2	20	75.2	friable	Green	76.2	friable	Green
3+0.3	20	55	Hard	Light Green	52.22	Hard	Light Green
3.5+0.3	20	40	Hard	Light Green	51.12	Hard	LightGreen
4+0.4	20	28.5	Hard	Greenish yellow	23.21	Hard	Light Green
4.5+0.4	20	27.4	Hard	White	24.12	Hard	White
5+.5	20	24.2	Hard	White	12.13	Hard	White
NAA							
1.0	20	--	--	--	- -	--	--
1.5	20	52	Semi friable	White	55	Semi friable	White
2.0*	20	65*	Friable	Greenish yellow	66*	friable	Greenish yellow
2.5	20	58	Hard	White	59	Hard	White
3.0	20	35	Hard	White	36	Hard	White
3.5	20	25	Hard	White	24	Hard	White
4.0	20	32	Hard	White	32	Hard	White
4.5	20	42	Hard	White	34	Hard	White
5.0	20	--	--	--	--	--	--

Percentage of shoot initiation was 75% for shoot tips and 78% for nodal segments. In this combination the number of shoot per explants were 5.50 in shoot tips and 6.80 in nodal segments. In this combination the average number of shoot per explants were 4.10 in shoot tips and 4.0 in nodal segments. All these treatments the best shoot multiplication was observed in 2.0mg/l BAP supplemented MS medium. Percentage of shoot initiation was 78% for shoot tips and 83% for nodal segments. In this combination the number of shoot per explants were 5.80 in shoot tips and 6.90 in nodal segments.

Table 4. Regeneration of adventitious bud from callus.

Callus initiation medium	Nature of callus	Regeneration medium	% of callus produced adventitious buds		Shoot elongation on the same medium
			Shoot tip	Nodal explants	
MS+2,4-D+KIN(2+0.2mg/l)	Light green to green in color and friable in nature	MS+BAP 2mg/l	6.2	6.5	*
		MS+0	5.2	5.5	***
MS+2,4-D 2.5mg/l	Greenish in color and friable in nature	MS+BAP 2mg/l	5.20	2.56	*
		MS+0	1.8	2.0	***
MS+NAA 2mg/l	Greenish yellow & friable in nature.	MS+BAP 2mg/l	--	--	--
		MS+0	--	--	--

Table 5. Effect of different concentrations of IBA and NAA for root induction.

Growth regulators (mg/l)	Sources of explants		
	In vitro grown shoots		
	% of root induction	Mean no. roots/explant	Mean length of the longest root(cm)
IBA			
0.5	70	2.00	3.00
1.00	75	4.00	3.00
1.5	80	4.00	2.50
2.00	85**	5.00**	3.50**
2.5	82	2.80	2.20
3.00	75	2.50	2.00
NAA			
0.50	50	2.00	2.20
1.00	60	3.00	2.90
1.5	65	3.50	2.50
2.0	70**	4.00**	3.00**
2.5	50	3.80	1.80
3.0	40	3.50	1.90
IBA+1/2 MS			
1.00	60	2.00	2.20
1.5	65	2.50	2.90
2.0	80**	3.00**	3.00**
2.5	80	2.60	2.20
3.00	70	2.30	2.00
3.50	50	2.10	1.80

Lowest Percentage of culture responded to multiple shoots in 3.5mg/l BAP.

In this combination the number of shoot per explants were 3.10 in shoot tips and 4.00 in nodal segments respectively. The similar results were also reported by several workers in *Adhatoda vasica, Rauvolfia serpentine* (Roja *et al*., 1991) and *Piper nijrum* Plant (Rani and Grover, 1999).

The output of the present investigation exposed similarities from various aspects with the findings of (Fanizza and Ricciardi, 1988) in *Ponsirous trifaliata*. It was also testified in *Vicia faba* L. (Selva *et al*., 1989) and (Sharma and Thorpe, 1990) in *Morus alba* L.

Effect of different concentration and combination of 2, 4-D, 2, 4-D+KIN and NAA for callus induction

The highest frequency (75.5%) of callus induction was observed in shoot tip culture on MS+2.5mg/l 2, 4-D medium. When the explants were cultured in media with low concentration of 2, 4-D (0.1-0.5) did not show any response.

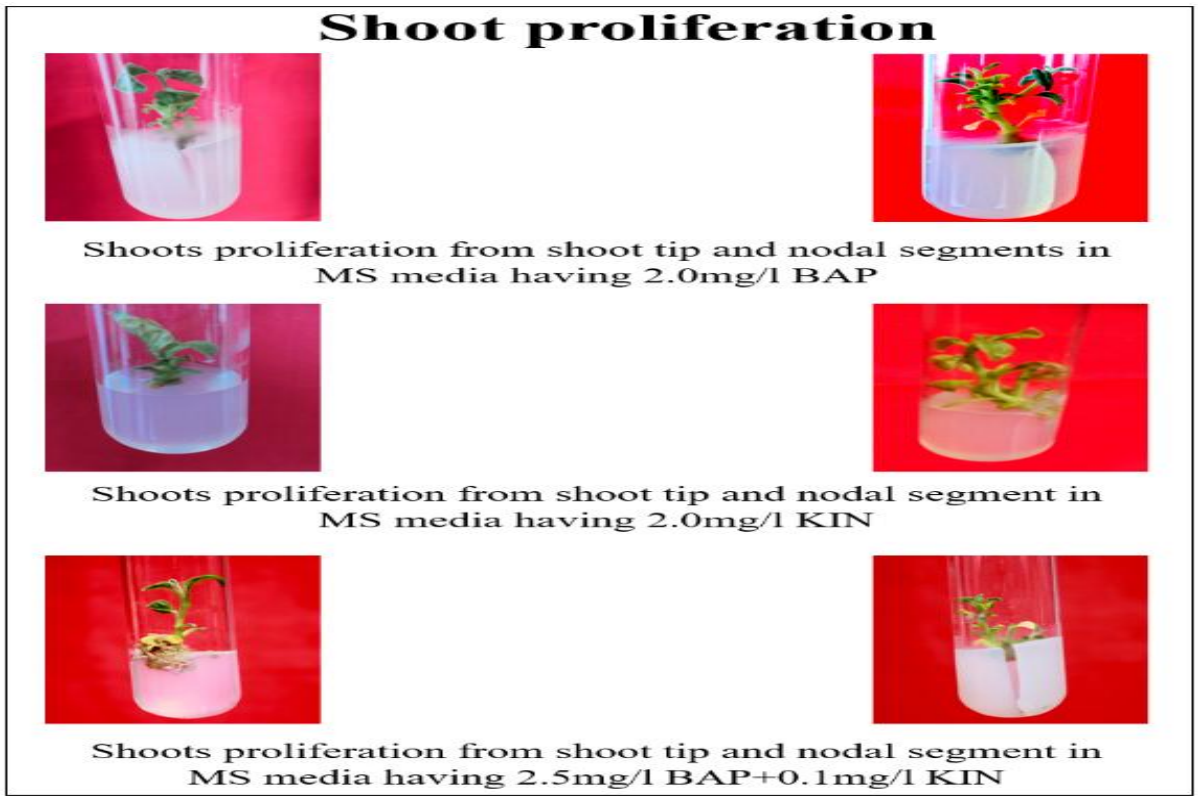

Fig. 1. Effect of different concentrations and combinations of BAP, KIN, BAP+KIN in MS media on direct proliferation of shoots from shoot tips and nodal segments.

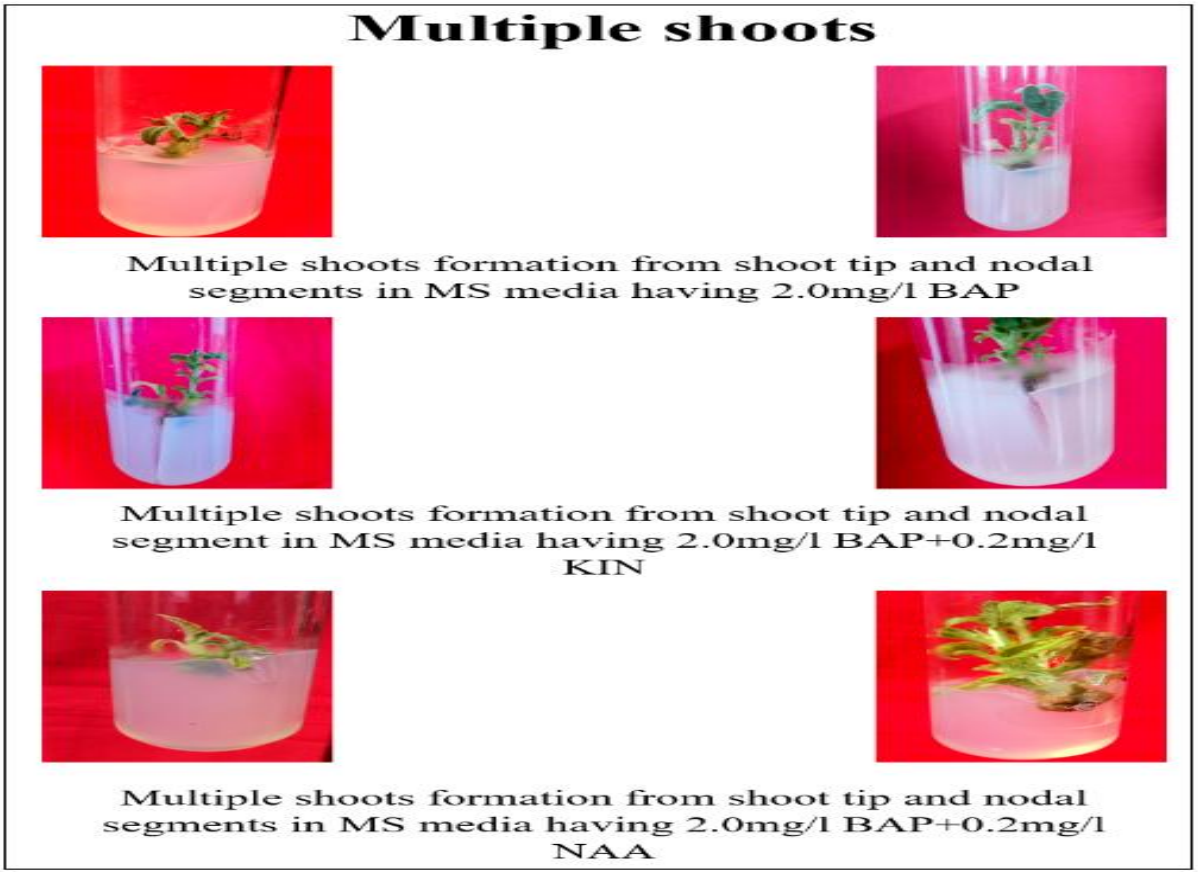

Fig. 2. Effect of different concentrations and combinations of BAP, BAP+NAA, BAP+KIN on shoot multiplication from shoot tips and nodal segments.

The lowest percentage of callus was observed on MS+5.0mg/l 2, 4-D which were mostly white and hard in nature. Deep green spot appeared in the light green to green and friable calli after two subcultures on the same medium (MS+2.5mg/l 2, 4-D). Similar to shoot tip explants, in nodal segment culture the highest percentage (78.42%) of callus induction was also observed on MS+2.5mg/l 2,4-D and the lowest (26.35%) on MS+5.0mg/l 2,4-D. Different concentration and combination of 2, 4-D+KIN were used. The highest percentages of callus were observed 80.2% for shoot tip and 84.00% for nodal segments when the media was supplemented with 2mg/l 2, 4-D+0.2mg/l KIN. The lowest (24.2%) on MS+5.0mg/l 2, 4-D+0.5 KIN in shoot tip culture. In case of nodal segment it was 12.13% in the same medium.

Deep green spot turn light green to green and friable calli after two subcultures on the same medium (MS+2mg/l 2, 4-D) +0.2 KIN.

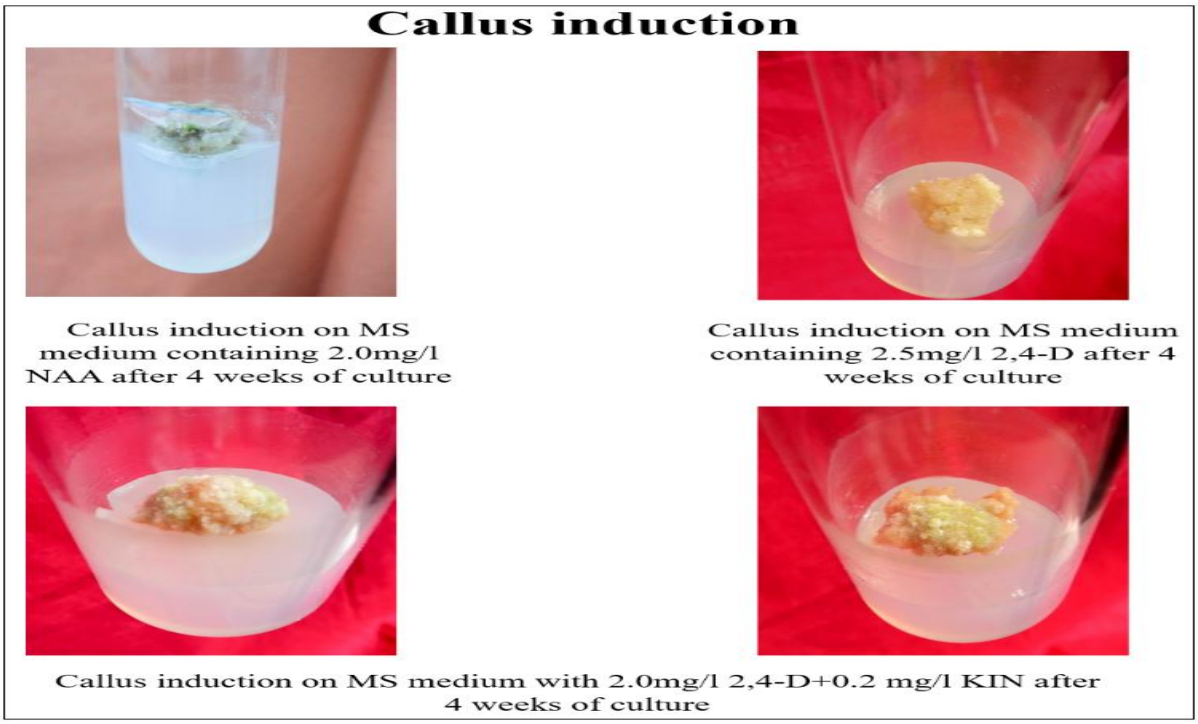

Fig. 3. Effect of different concentration and combination of 2, 4-D, 2, 4-D+KIN and NAA for callus induction.

For callus induction and subsequent plant regeneration, the explants were cultured on MS medium supplemented with different concentration of NAA such as; 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0mg/l. When the explants cultured on media with lower concentrations (1.00mg/l) as well as on higher concentrations (5mg/l) of NAA did not show any callus initiation. For both shoot tip and nodal segment culture, the highest percentage (respectively, 65% and 66%) of callus initiation was observed on medium having 2.0mg/l NAA and the calli were whitish green and semi friable in nature (Table 3 & Fig.3). It was reported by many workers in *W. somnifera* L. (Chopra *et al.* 1958) and *Vigna radiata* L. These findings of the present examination present derivatively resemble with the findings of (Ahmad *et al.,* 2005) and (Al-Khayri*et al.*, 1991). Shoot tips and nodal segment have been described as a good source of explant for callus induction by a number of workers in a number of plants (Bornman, 1983; Ohyama and Oka, 1980). Recently, many findings are accessible on callus initiation and subsequent plantlet regeneration from both herbaceous and vegetable crops (Furmanowa *et al.,* 2001).

Regeneration of adventitious bud from in vitro raised callus

For regeneration of adventitious bud the media was supplemented with MS+2mg/l 2, 4-D or NAA, which were green to light green in color and friable in

nature, shows the best morphogenesis. When they were sub-cultured on the regenerating medium, the calli began to develop adventitious buds after 6-8 weeks of culture on regenerating medium (MS+2mg/l BAP). The highest frequency of callus differentiated from adventitious bud was 6.2% on the medium containing MS+2mg/l BAP (Table 4 & Fig. 4). The complete plantlets of *Morus alba* were successfully establish 95% was reported by (M Sharma *et al.* 2010).

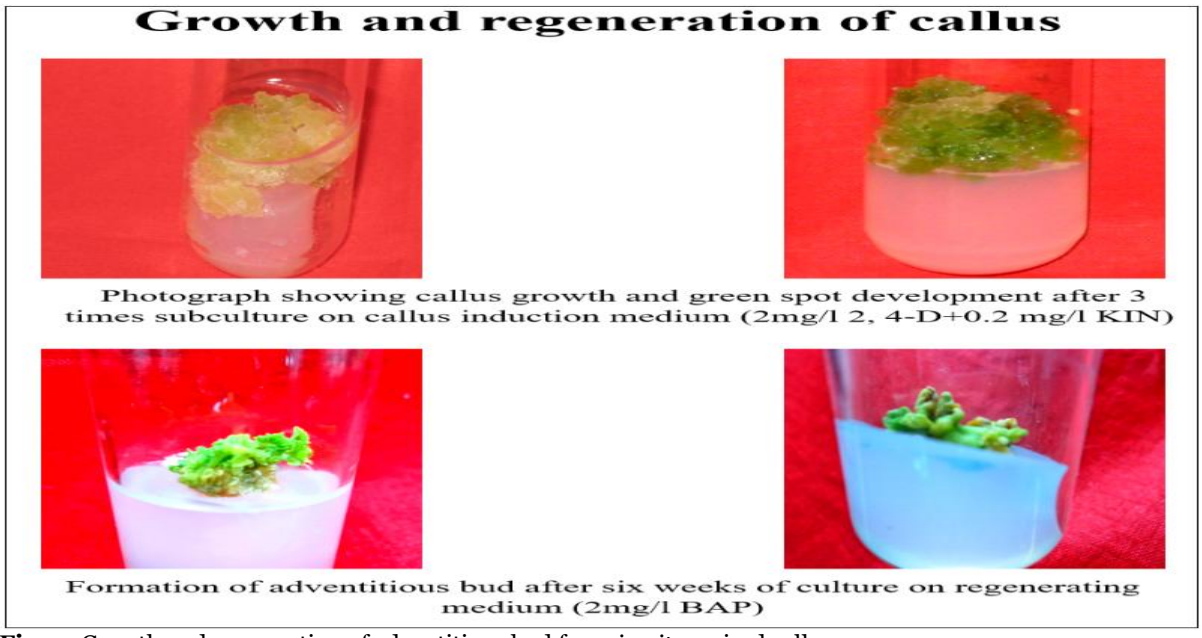

Fig. 4. Growth andregeneration of adventitious bud from *in vitro* raised callus.

Fig. 5. Effect of different concentrations of IBA and NAA for root induction.

Effect of different concentrations of IBA and NAA for root induction

In case of root induction *in vitro* grown micro shoots were transferred into MS-medium supplemented with different concentrations of IBA (0.5-3.0mg/l) and NAA (0.5-3.00mg/l). The highest percentage of root 85% formation was observed in 2.0mg/l IBA. The observation of 1/2MS+2.0mg/l IBA the percentage of root was observed approximately 80% and 70% in

2mg/l NAA. The lowest percentage of (40%) root induction was recorded in 3.0mg/l NAA. The highest average number of roots/shoot was recorded (5.0) in medium containing 2.0mg/l IBA, it was approximately 4.0 the media supplemented with 2.0mg/l NAA. The average highest length of roots (3.5cm) was recorded in 2mg/l IBA and 3.00cm in both 1/2MS+IBA 2mg/l and NAA 2mg/l (Table 5 & Fig.5). In most cases; morphology of roots was fragile, long and thick.

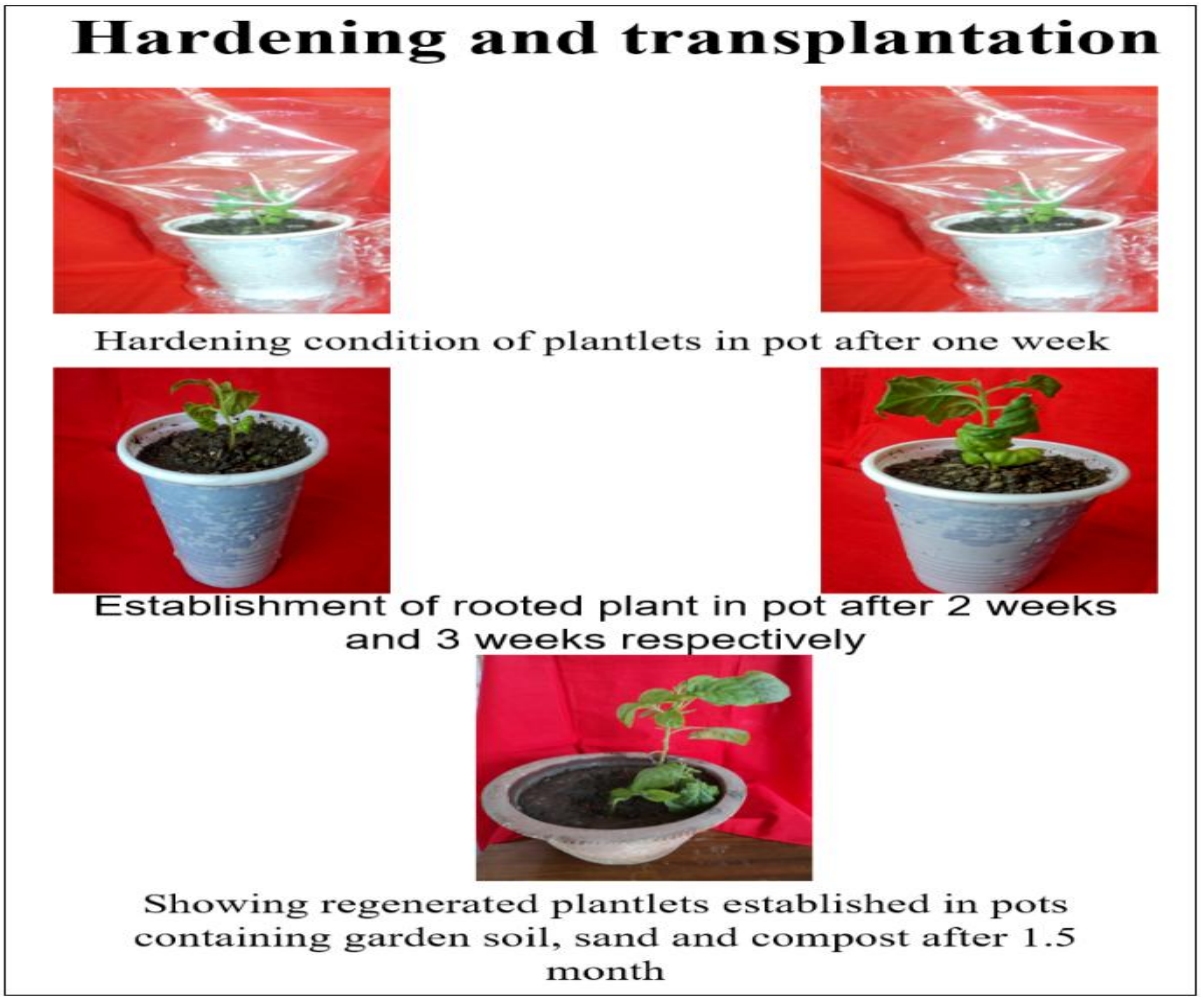

Fig. 6. Hardening and transplantation of the plantlets in different week intervals.

Hardening and transplantation of the plantlets

The plantlets of *W. somnifera L.* were subsequently subcultured and finally transferred aseptically to the greenhouse after 3 week of inoculation covered with polythene bag after four week the microplants were transferred to the soil. The percentage of such microplants survivality was observed 87% (Fig. 6).

Conclusion

In this study, the best performance for shoot proliferation was found in MS media supplemented with 2.0mg/l BAP. The best shoot multiplication was observed by using shoot tip and nodal segment in 2.0mg/l BAP+0.2mg/l NAA supplemented with MS medium. In case of indirect regeneration, highest frequency of callus induction was obtained by shoot tip (80.2%) and nodal segment (84.0%) culture on MS medium supplemented with 2mg/l 2, 4-D+0.2mg/l KIN. The best proliferation (6.5%) of adventitious bud was observed in BAP 2mg/l. Maximum rooting of the micro cuttings of regenerated shoots were successfully achieved with 2.0mg/l IBA and 1/2 MS+IBA2mg/l.

The plantlets were successfully transferred to soil and the percentage of survivability in such condition was

87%. So, the development of direct regeneration of Ashwagandha plantlet through tissue culture method by using nodal segment and shoot tip is the most effective way.

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