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IN VITRO CONSERVATION OF *WITHANIA SOMNIFERA* (L) DUNAL (ASHWAGANDHA) – A MULTIPURPOSE MEDICINAL PLANT

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ABSTRACT

Present work has been conducted on In Vitro conservation of a multipurpose medicinal plant, Withania somnifera commonly known as Ashwagandha through storage of germplasm derived from micropropagated progenies of the species. Direct regeneration of cotyledon explants and their multiplication have been optimized using cytokinins (BAP and KIN) at a concentrations of 0.5-3.0mg/l alone, BAP+KIN combined forms and combined with auxins (IAA and NAA) at 1.5mg/l and 2.0mg/l. MS medium supplemented with BAP(2.0mg/l) and IAA(1.5mg/l) produced maximum number of shoots per explant i.e., 57.4±0.59 and 50.0±0.40 were recorded from Withania somnifera WS1 and WS2 cultivars respectively. An improved In Vitro shoot bud elongation was achieved on MS medium fortified with 0.15mg/l GA_3. High frequency of rooting 64% and 60.1% and maximum number of 12.8±0.16 and 10.6±0.49 roots were produced on MS medium supplemented with IBA(5mg/l) in WS1 and WS2 respectively. After acclimatization, 85% of the micropropagated plantlets were successfully established in the field.

Keywords: Auxins, Cytokinins, Direct regeneration, Rhizogenesis.

1. INTRODUCTION

Application of biotechnology for conservation of important plant species has been given priority under circumstances, in particular when many valuable plant genetic resources are getting decimated rapidly from natural flora. *Withania somnifera* (L.) Dunal is a member of the family Solanaceae commonly known as Ashwagandha. 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 (Kiritikar and Basu, 1975). Besides roots, the other parts of this plant also useful for the treatment of inflammatory conditions and tuberculosis and exhibits excellent antitumor and anti-bacterial

activities (Devi and Sharada, 1992; Devi, 1996). The conventional method of propagating this species is through seeds, but seed viability is very poor and low germination limits its multiplication. There is no vegetative propagation of this plant. Tissue culture propagation could offer a valuable alternative and a reliable procedure for large scale propagation of it. Although earlier tissue culturists reported *In Vitro* micropropagation of *Withania* employing different explants, such as seed (Supe *et al.*, 2006), shoot apex (Sivanesan, 2007; De Silva and Senarath, 2009), axillary bud (Gita and Grover, 1999; Sabir *et al.*, 2007; Valizadeh and Valizadeh, 2011), hypocotyl (Kulkarni *et al.*, 2000), cotyledon (Gita Rani *et al.*, 2003; Jhankare *et al.*, 2011), leaf (Kulkarni *et al.*, 1996; Sivanesan and Murugesan, 2005; Joshi and Padhya, 2010) petiole (Ghimire *et al.*, 2010) and internodal (Govindaraju *et al.*, 2003; Valizadeh and Valizadeh, 2009). However, up to my knowledge there is no report of direct plant regeneration from cotyledon explants of *Withania somniefera*. Hence, the present study is an attempt to generate plantlets through direct regeneration of cotyledon explants of two genotypes of *Withania somnifera* (L.) Dunal.

2. MATERIALS AND METHODS

2.1. Explant Preparation

Seeds of *Withania somnifera* (L.) Dunal genotypes viz., WS1 and WS2 were obtained from Medicinal Garden of Botany Department, Andhra University, Visakhapatnam, India. The seeds were surface sterilized with 0.1% HgCl2 and repeatedly washed in sterile distilled water then they were then inoculated in glass containers with 50ml of half-strength MS medium (Murashige and Skoog, 1962) for germination. The cotyledon explants were derived from 15 days old seedlings grown *In Vitro* transferring on to culture media.

2.2. Micro Propagation

The basal nutrient medium containing MS salts and vitamins was used with IAA (Indole -3-acetic acid), NAA (Naphthalene acetic acid), BAP (6-Benzyl aminopurine) and KIN (Kinetin). The multiple shoots were induced in three independent experiments i) the effects of BAP and KIN were examined individually at the concentrations of 0.5-3.0mg/l ii) the combined effects of BAP and KIN was evaluated and iii) the effects of auxins and cytokinins inspected (IAA and NAA at the concentration of 1.5 and 2.0mg/l was combined with BAP and KIN). Subculture at every two weeks to the same medium the number of shoot buds were recorded after six weeks of culture, then the shoot buds were elongated on MS media with various levels of GA_3(0.05- 0.25mg/l) after two weeks of culture period. To test their rooting capacity, the *In Vitro* elongated shoots were excised and transferred on to MS media fortified with various concentrations of IBA (1.0-8.0mg/l). The rooting i.e., frequency of rooting (%), root length (cm) and number of roots per shoot were noted after two weeks of culture period.

2.3. *In Vitro* Conditions

All media were supplemented with 3% sucrose and 0.8% agar and the pH of the media was adjusted to 5.8 with 1N NaOH or 1N HCl prior to autoclaving. The cultures were maintained at 25

± 2^0C air temperatures in a culture room with a 16 hour photoperiod under an illumination of 20 m mol m-2s-1 photosynthetic photon flux density, provided by cool-white fluorescent light.

2.4. Acclimatization

Plants with roots were transferred after washing of the agar with distilled water and to pots with a mixture of soilrite (1:1). Potted plantlets were covered with transparent polythene membrane to ensure high humidity and watered every three days with half-strength MS salts solution for two weeks in order to acclimatize plants to field conditions. After two weeks the acclimatized plants were transferred to pots containing normal garden soil and maintained in greenhouse under natural day length conditions.

2.5. Statistical Analysis

The experiments were set up in Randomized Block Design (RBD) and each experiment was replicated thrice. Observations were recorded on the percentage of response, number of shoots per explant, number of roots per shoot and root length. Mean and standard errors were carried out for each treatment.

3. RESULTS

3.1. Effect of Cytokinins

Good *In Vitro* response was observed in WS1 and WS2 of *Withania somnifera* cotyledon explants were cultured on MS medium augmented with 0.5mg/l, 1.0mg/l, 2.0mg/l, 2.5mg/l and 3.0mg/l BAP and KIN alone. A progressive concentrations of BAP and KIN from 0.5-2.0mg/l resulted gradually increased *In Vitro* response while, further increase of BAP and KIN 2.5mg/l to 3.0mg/l resulted in development of poor *In Vitro* response. However, maximum response i.e., 80% (shooting) and 38.0±0.28(shoots per explant) in WS1 and 74.2%(shooting) and 32.6±0.64(shoots per explant) in WS2 was recorded at MS+2.0mg/l BAP (Table 1 and Fig. 1)

The combination of BAP and KIN did yield significant results in cotyledon explants. Noticeable response was seen on the medium augmented with 0.5-3.0mg/l BAP and KIN. A maximum of 95.1% in WS1 and 90.2% in WS2 cotyledons developed shoot proliferation when the concentration of 2.0mg/l BAP+2.0mg/l KIN. The shoots were subcultured on the same medium with same phytohormonal concentration, the average shoot number per explant was found to be 41.7±0.19 in WS1 and 40.7±0.19 in WS2 (Table 2)

3.2. Effect of Cytokinins and Auxins

Cotyledons of both genotypes (WS1 and WS2) showed very good response when the medium was augmented with BAP (0.5mg/l to 3.0mg/l) combined with IAA(1.5mg/l and 2.0mg/l) or NAA(1.5mg/l and 2.0mg/l). A maximum of 92.6% in WS1 and 87.6% in WS2 cotyledons developed shoot proliferation on MS+BAP(2.0mg/l)+IAA(1.5mg/l). The maximum average number of shoots produced per explant was found to be 57.4±0.59 in WS1 and 50.0±0.49 in WS2 (Table 3,4).

3.3. Elongation of Shoots

The multiple micro shoot clusters obtained through the culture of cotyledon explants from both WS1 and WS2 cultivars of *W. somnifera* were individually separated from the multiple shoot clusters and cultured on the MS medium containing gibberellic acid (GA_3) at different concentrations ranging from 0.05-0.25mg/l to enhance the length of the shoot (Table 5). The mean variation ranged from 4.8 to15cm in length. The maximum shoot length i.e., 15.0±0.20cm(WS1) and 11.3±0.52cm(WS2) was recorded on MS medium supplemented with 0.15mg/l GA_3.

3.4. *In Vitro* Rooting

The elongated shoots of WS1 and WS2 cultivars of *W. somnifera* were subcultured individually on rooting medium containing IBA in different concentrations to induce rhizogenesis. *In Vitro* rooting started in the second week of culture and complete plantlets worth transplantation were obtained in four weeks of culture period. MS medium fortified with IBA 1.0mg/l-5.0mg/l accelerating the rhizogenesis further increase of IBA 6.0mg/l-8.0mg/l resulted decreased rhizogensis as depicted in table 6. A maximum of 64% WS1 and 60.1% WS2 shoots showed root induction with maximum root number per shoot (12.8±0.16 in WS1 and 10.6±0.49 in WS2) and root length (11.0±0.05cm in WS1 and 9.2±0.54cm in WS2)was recorded under the influence of MS medium supplemented with IBA(5.0mg/l).

Table-1. Effect of Cytokinins (BAP and KIN) on multiple shoot induction from cotyledon explants of two cultivars of *W. somnifera* (L.) Dunal

S. No.	Media	*W. s* cv. Jawahar(WS1)		*W. s* cv. Local(WS2)	
		Shooting (%)	Shoot no./ explant	Shooting (%)	Shoot no./ explant
1.	Control	-	-	-	-
2.	MS + BAP(0.5mg/l)	36.3±0.28	21.9±0.67	34.4±0.68	18.0±0.71
3.	MS + BAP(1.0mg/l)	42.0±0.38	26.7±0.19	41.1±0.33	22.0±0.33
4.	MS + BAP(1.5mg/l))	62.6±0.64	28.0±0.38	57.4±0.59	23.8±0.46
5.	MS + BAP(2.0mg/l)	80.0±0.52	38.0±0.28	74.2±0.35	32.6±0.64
6.	MS + BAP(2.5mg/l))	75.3±0.52	36.1±0.33	73.7±0.38	29.8±0.46
7.	MS + BAP(3.0mg/l)	73.7±0.38	34.9±0.35	71.1±0.23	28.0±0.72
8.	MS + KIN(0.5mg/l)	41.3±0.47	11.7±0.19	37.7±0.38	9.12±0.23
9.	MS + KIN(1.0mg/l)	43.9±0.35	14.0±0.13	39.7±0.50	10.7±0.38
10.	MS + KIN(1.5mg/l)	63.9±0.35	18.7±0.38	46.7±0.19	17.0±0.70
11.	MS + KIN(2.0mg/l)	78.0±0.38	28.0±0.33	68.6±0.49	26.0±0.28
12.	MS + KIN(2.5mg/l)	75.3±0.52	20.2±0.29	62.0±0.33	15.7±0.50
13.	MS + KIN(3.0mg/l)	68.5±0.96	18.2±0.77	60.1±0.33	13.9±0.35

Table-2. Combined effects of Cytokinins (BAP+KIN) on multiple shoot induction from cotyledon explants of two cultivars of *W. somnifera* (L.) Dunal

S. No.	Media	*W. s* cv. Jawahar(WS1)		*W. s* cv. Local(WS2)	
		Shooting (%)	Shoot no./ explant	Shooting (%)	Shoot no./ explant
1.	Control	-	-	-	-
2.	MS + BAP(0.5mg/l) + KIN(0.5mg/l)	63.6±0.49	28.9±0.73	56.0±0.28	24.3±0.52
3.	MS + BAP(1.0mg/l) + KIN(1.0mg/l)	70.0±0.40	35.5±0.21	58.1±0.23	33.7±0.50
4.	MS + BAP(1.5mg/l) + KIN(1.5mg/l)	84.0±0.38	38.4±0.59	75.3±0.52	34.0±0.72
5.	MS + BAP(2.0mg/l) + KIN(2.0mg/l)	95.1+0.75	41.7±0.19	90.2±0.78	40.7±0.19
6.	MS + BAP(2.5mg/l) + KIN(2.5mg/l)	62.0±0.33	37.4±0.50	43.9±0.35	22.7±0.19
7.	MS + BAP(3.0mg/l) + KIN(3.0mg/l)	58.0±0.28	25.0±0.40	40.7±0.38	14.0±0.70

Table-3. Combined effects of Cytokinin (BAP) and Auxins (IAA and NAA) on multiple shoot induction from cotyledon explants of two cultivars of *W. somnifera* (L.) Dunal.

S. No.	Media	*W. s* cv. Jawahar(WS1)		*W. s* cv. Local(WS2)	
		Shooting (%)	Shoot no./ explant	Shooting (%)	Shoot no./ explant
1.	Control	-	-	-	-
2.	MS + BAP(0.5mg/l) + IAA(1.5mg/l)	75.0±0.33	30.2±0.38	72.7±0.38	23.8±0.46
3.	MS + BAP(1.0mg/l) + IAA(1.5mg/l)	91.1±0.33	36.0±0.67	78.9±0.35	34.0±0.72
4.	MS + BAP(1.5mg/l) + IAA(1.5mg/l)	87.8±0.23	42.7±0.19	84.2±0.59	38.4±0.66
5.	MS + BAP(2.0mg/l) + IAA(1.5mg/l)	92.6±0.18	57.4±0.59	87.6±0.49	50.0±0.40
6.	MS + BAP(2.5mg/l) + IAA(1.5mg/l)	88.8±0.46	47.7±0.50	85.7±0.50	42.7±0.19
7.	MS + BAP(3.0mg/l) + IAA(1.5mg/l)	74.1±0.44	40.0±0.70	82.0±0.38	32.6±0.64
8.	MS + BAP(0.5mg/l) + IAA(2.0mg/l)	65.4±0.43	32.0±0.70	62.0±0.33	28.2±0.78
9.	MS + BAP(1.0mg/l) + IAA(2.0mg/l)	76.0±0.40	34.2±0.78	65.8±0.46	30.0±0.70
10.	MS + BAP(1.5mg/l) + IAA(2.0mg/l)	80.1±0.33	42.8±0.65	74.4±0.92	40.0±0.72
11.	MS + BAP(2.0mg/l) + IAA(2.0mg/l)	50.0±0.28	26.6±0.49	51.7±0.38	24.0±0.40
12.	MS + BAP(2.5mg/l) + IAA(2.0mg/l)	36.2±0.58	20.0±0.38	44.9±0.35	18.7±0.38
13.	MS + BAP(3.0mg/l) + IAA(2.0mg/l)	26.0±0.70	11.7±0.19	29.3±0.52	10.2±0.69
13.	MS + BAP(0.5mg/l) + NAA(1.5mg/l)	41.3±0.47	22.1±0.68	36.2±0.86	21.7±0.50
15.	MS + BAP(1.0mg/l) + NAA(1.5mg/l)	46.6±0.49	32.5±0.18	42.7±0.19	30.0±0.38
16.	MS + BAP(1.5mg/l) + NAA(1.5mg/l)	79.6±0.92	34.7±0.23	63.9±0.35	33.7±0.50
17.	MS + BAP(2.0mg/l) + NAA(1.5mg/l)	96.4±0.70	25.0±0.40	79.6±0.92	18.7±0.38
18.	MS + BAP(2.5mg/l) + NAA(1.5mg/l)	73.8±0.46	24.0±0.28	60.4±0.92	14.7±0.50
19.	MS + BAP(3.0mg/l) + NAA(1.5mg/l)	62.5±0.19	23.3±0.52	53.4±0.59	11.7±0.19
20.	MS + BAP(0.5mg/l) + NAA(2.0mg/l)	46.0±0.28	22.0±0.33	35.0±0.70	20.0±0.40
21.	MS + BAP(1.0mg/l) + NAA(2.0mg/l)	53.9±0.61	26.1±0.33	47.4±0.92	22.0±0.33
22.	MS + BAP(1.5mg/l) + NAA(2.0mg/l)	56.2±0.86	31.1±0.30	48.6±0.49	29.4±0.59
23.	MS + BAP(2.0mg/l) + NAA(2.0mg/l)	61.1±0.33	14.7±0.50	55.0±0.38	13.0±0.70
24.	MS + BAP(2.5mg/l) + NAA(2.0mg/l)	58.2±0.86	10.7±0.38	46.6±0.49	8.12±0.23
25.	MS + BAP(3.0mg/l) + NAA(2.0mg/l)	24.6±0.49	8.6±0.49	37.0±0.70	4.60±0.49

Table-4. Combined effects of Cytokinin (KIN) and Auxin (IAA) on multiple shoot induction from cotyledon explants of two cultivars of *W. somnifera* (L.) Dunal

S. No.	Media	*W. s* cv. Jawahar(WS1)		*W. s* cv. Local(WS2)	
		Shooting (%)	Shoot no./ explant	Shooting (%)	Shoot no./ explant
1.	Control	-	-	-	-
2.	MS + KIN(0.5mg/l) + IAA(1.5mg/l)	65.7±0.19	28.2±0.67	61.4±0.72	24.1±0.33
3.	MS + KIN(1.0mg/l) + IAA(1.5mg/l)	67.0±0.38	36.8±0.34	65.7±0.19	36.0+0.67
4.	MS + KIN(1.5mg/l) + IAA(1.5mg/l)	75.3±0.52	42.8±0.65	72.7±0.50	38.6±0.91
5.	MS + KIN(2.0mg/l) + IAA(1.5mg/l)	84.0±0.70	50.2±0.59	78.0±0.38	46.8±0.40
6.	MS + KIN(2.5mg/l) + IAA(1.5mg/l)	81.3±0.52	45.0±0.40	75.2±0.92	42.2±0.62
7.	MS + KIN(3.0mg/l) + IAA(1.5mg/l)	78.9±0.35	38.0±0.70	73.0±0.28	36.2±0.58
8.	MS + KIN(0.5mg/l) +IAA(2.0mg/l)	63.6±0.49	41.4±0.59	56.0±0.28	40.0±0.70
9.	MS + KIN(1.0mg/l) + IAA(2.0mg/l)	70.0±0.40	44.0±0.40	58.1±0.23	41.0±0.40
10.	MS + KIN(1.5mg/l) + IAA(2.0mg/l)	84.0±0.38	48.0±0.70	75.3±0.52	44.7±0.19
11.	MS + KIN(2.0mg/l) + IAA(2.0mg/l)	72.6±0.64	36.3±0.52	63.6±0.49	34.2±0.78
12.	MS + KIN(2.5mg/l) + IAA(2.0mg/l)	62.0±0.33	26.1±0.33	43.9±0.35	24.0±0.40
13.	MS + KIN(3.0mg/l) + IAA(2.0mg/l)	48.0±0.28	20.4±0.59	30.7±0.38	18.7±0.38

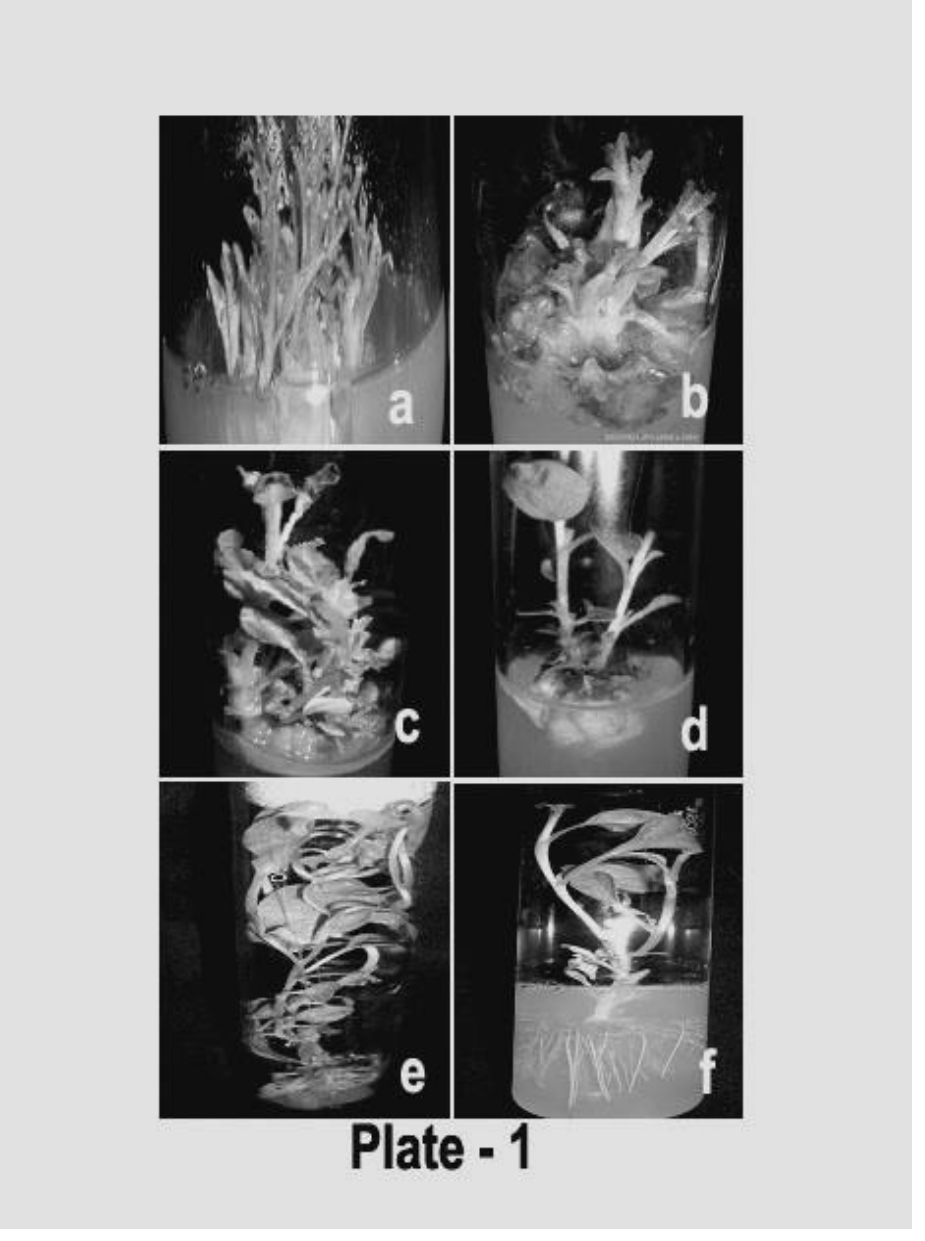
Table-5. Effect of GA_3 on enhancement of shoot length of *In Vitro* induced micro shoots of two cultivars of *W. somnifera* (L.) Dunal.

S. No.	Media Type	Shoot length(cm)	
		W. s cv. Jawahar(WS1)	*W. s* cv. Local(WS2)
1.	Control	-	-
2.	MS + GA_3(0.05mg/l)	8.0±0.08	6.4±0.59
3.	MS + GA_3(0.10mg/l)	11.4±0.12	10.6±0.49
4.	MS + GA_3(0.15mg/l)	15.0±0.20	11.3±0.52
5.	MS + GA_3(0.20mg/l)	12.4±0.24	9.1±0.33
6.	MS + GA_3(0.25mg/l)	10.2±0.18	4.8±0.46

Table-6. Rooting response of *In Vitro* elongated shoots of two cultivars of *W. somnifera* (L.) Dunal

S.No.	Media	*W. s* cv. Jawahar(WS1)			*W. s* cv. Local(WS2)		
		Root induction (%)	Root No. /explant	Root length (cm)	Root induction (%)	Root No. /explant	Root length (cm)
1	MS	-	-	-	-	-	-
2.	MS + IBA(1.0mg/l)	12.0±0.20	2.0±0.06	1.8±0.05	10.7±0.50	0.97±0.14	0.6±0.21
3.	MS + IBA(2.0mg/l)	14.0±0.08	2.2±0.02	2.6±0.04	12.6±0.49	1.14±0.24	1.2±0.26
4.	MS + IBA(3.0mg/l)	19.0±0.10	3.6±0.11	3.2±0.03	18.0±0.39	2.7±0.19	2.5±0.24
5.	MS + IBA(4.0mg/l)	25.0±0.19	6.4±0.08	5.8±0.03	21.9±0.31	4.1±0.34	4.7±0.58
6.	MS + IBA(5.0mg/l)	64.0±0.22	12.8±0.16	11.0±0.05	60.1±0.33	10.6±0.49	9.2±0.54
7.	MS + IBA(6.0mg/l)	40.0±0.09	8.0±0.04	7.2+0.09	38.0±0.38	6.7±0.19	6.3±0.27
8.	MS + IBA(7.0mg/l)	29.0±0.16	4.1±0.15	5.0±0.06	28.9±0.35	2.8±0.46	3.0±0.56
9.	MS + IBA(8.0mg/l)	16.0±0.24	3.0±0.05	2.8±0.10	15.1±0.23	1.01±0.32	0.4±0.26

Figure-1. *In Vitro* micropropagation of *Withania somnifera* L. Dunal. cotyledon explants: A,C&E: Multiple shoots proliferation, shoot elongation and rhizogenesis of *Withania somnifera* L. Dunal cultivar WS1., B,D&F: Multiple shoots proliferation, shoot elongation and rhizogenesis of *Withania somnifera* L. Dunal cultivar WS2.



4. DISCUSSION

Using *In Vitro* derived germplasm, it has been possible to conserve *Withania somnifera* under tissue culture conditions. To achieve the goal, an improved protocol for rapid *In Vitro* multiplication of the species has been developed as it is an essential prerequisite of the study. A high frequency regeneration protocol of *Withania somnifera*, standardized in this study is a simple, efficient and time saving. Combinations of cytokinins and auxins (IAA and NAA) in MS medium

was most suitable for shoot multiplication in its presence, shoot buds were differentiated directly from the cotyledon explants and with in six weeks of culture, innumerous propagules were developed in clusters showing high sprouting ability.

Cotyledon explants *In Vitro* response in cytokinins alone, combinations of BAP and Kinetin and combinations of cytokinins and auxins (IAA and NAA) induced shoot formation. In the present investigation reveals that auxins (IAA and NAA) combination with cytokinins (BAP and KIN) was more effective than cytokinin (BAP and KIN) alone in inducing shoot formation. Earlier work on isolated cotyledon explants of *Withania* genotypes has depicted that they also show a strong organogenetic potential when cultured in presences of BAP+2, iP, BAP+NAA, KIN+2,4-D and KIN+NAA (Gita Rani *et al.*, 2003; Jhankare *et al.*, 2011). These findings corroborate with our findings as multiple adventitious shoots were raised from isolated cotyledon explants of both genotypes *Withania* (WS1 and WS2) under the influence of combinations of cytokinins (BAP and KIN) with auxins (IAA and NAA). Low concentrations of BAP(0.5-2.0mg/l), KIN(0.5-2.0mg/l) alone or in combination with low concentrations of auxins IAA(1.5mg/l) and NAA(1.5mg/l) was registered to be the most effective for adventitious multiple shoot induction and shoot proliferation. On the other hand higher levels i.e., more than 2.5mg/l of BAP and KIN alone or combined with 2.0mg/l IAA and NAA was found less effective agreed with the earlier findings (Gita Rani *et al.*, 2003; Siddique *et al.*, 2004; Saritha and Naidu, 2007; Sivanesan and Murugesan, 2008; Valizadeh and Valizadeh, 2009; Ghimire *et al.*, 2010; Valizadeh and Valizadeh, 2011). The micro shoots were best elongated on MS medium supplemented with GA_3(0.15mg/l), the similar results were also reported earlier (Sivanesan, 2007; Logesh *et al.*, 2010; Jhankare *et al.*, 2011). Data from the results of the present studies show that the effect of IBA resulted significant rhizogenesis of *In Vitro* elongated shoots of WS1 and WS2 varieties of *Withania somnifera*, which corroborates with the earlier attempts, made in this line (De Silva and Senarath, 2009; Ghimire *et al.*, 2010; Joshi and Padhya, 2010; Jyoti Ranjan *et al.*, 2011) who also used IBA for such studies while, Kulkarni *et al.* (1996) and Shukla *et al.* (2010) reported rooting of micro shoot by IAA on the other hand and Sharma *et al.* (2010) and Satyajit and Santi (2011) reported rhizogenesis of *In Vitro* shoots by NAA.

In conclusion, the outlined procedure offers a potential system for conservation and mass propagation of *Withania somnifera* from cotyledon explants. In the present investigation BAP(2.0mg/l)+IAA(1.5mg/l) on MS media is more effective for shoot multiplication for both cultivars of *Withania* (WS1 and WS2). The MS medium supplemented with 5.0mg.l(IBA) is best for root induction.

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