The Journal of Animal & Plant Sciences, 23(5): 2013, Page: 1391-1397
ISSN: 1018-7081

STUDIES ON THE EFFECT OF SUCROSE, LIGHT AND HORMONES ON MICROPROPAGATION AND *IN VITRO* FLOWERING OF *WITHANIA SOMNIFERA VAR. JAWAHAR-20*

S. Nathiya, D. Pradeepa[*], T. Devasena and K. Senthil[**]

Centre for Nanoscience and Technology Anna University, Chennai- 600 025, India
[*]Department of Biochemistry, Biotechnology and Bioinformatics
[**]Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore- 641 043, India
Corresponding Author E-mail: aubio.ptc.kalai@gmail.com

ABSTRACT

Withania somnifera (solanaceae) popularly, known as "Ashwagandha" is one of the major herbal components of geriatric tonics mentioned in Indian system of medicine. In the present study, effect of various factors on *in vitro* germination such as sucrose concentration (1%, 2% and 3%) and light intensity (light and dark) in half strength Murashige Skoog medium (1/2 MS) has been studied. 1/2 MS supplemented with 2% sucrose incubated in dark at 25˚C was found to give maximum rate of germination compared to ½ MS supplemented with 1% and 3% sucrose. Different hormonal concentrations were used to test the efficiency of micro shoot formation at different time intervals after inoculation for a period of 90 days with regular subculturing. The maximum number of microshoots was developed in MS media supplemented with 0.5 BAP and 0.05 TDZ followed by 1.0 BAP and 0.05 TDZ. The well-propagated shoots were transferred to elongation media. MS medium supplemented with 1.0 KN and 0.05 BAP showed maximum elongation of shoots. The multiple shoots obtained from 0.5 BAP and 0.05 TDZ; 1 BAP and 0.05 TDZ were transferred to flower induction medium. Different hormonal combination were used to induce flowering, MS medium supplemented with combination of 1 KN and 0.05 BAP exhibits 66% of flowering followed by 0.1 BAP. The matured and immatured pollens in the flowers were observed by pollen staining which contribute to the mode of transfer of genes from one generation to another. Thus the number of matured pollens were more in the *in vitro* flowers similar to *in vivo* flowers. In this study an effective methodology for the *in vitro* seed germination, propagation and flowering was standardized.

Key words: *in vitro* flowering, micropropagation , pollen staining, *Withania somnifera.*

Abbreviations: BAP 6- Benzyl amino purine TDZ Thidiazuron MS Murashige and Skoog KN Kinetin DAI Days after Inoculation

INTRODUCTION

The genus *Withania* (Solanaceae), the Indian ginseng considered to be one of the best rejuvenating agents in Ayurvedha. The propagation of *W. somnifera* through seeds is restricted due to poor viability and death of young seedlings under natural environmental conditions. During the past few years, considerable efforts have been made for *in vitro* plant regeneration of this medicinally important plant species using various explants (Singh *et al*., 2006). The demonstration of hormonal regulation of growth, differentiation and organ formation in plants, the regeneration of plantlets from callus cultures and plant regeneration from cell suspension cultures paved the way for the real exploitation of this knowledge towards the benefit of mankind (Steward *et al*., 1958).

W. somnifera is a small, woody shrub that grows about two feet in height. It is an erect, evergreen, tomentose shrub, 30-150 cm high, found throughout the drier parts of India in waste places. Roots are stout fleshy, whitish brown; leaves simple ovate, glabrous, those in the floral region smaller and opposite; flowers inconspicuous, greenish or lubrid-yellow, in axillary, umbellate cymes; berries small, globose, orange-red when mature, enclosed in the persistent calyx; seeds yellow, reniform. The roots are the main portions of the plant used therapeutically. The bright red fruit is harvested in the late fall and seeds are dried for planting in the following spring (Gupta and Rana, 2007). Jawahar variety is released as a high yielding variety with high content of withanolides and is marked as a superior variety of *W. Somnifera* by Nehru Krishi Vishwa Vidhyalaya, Regional Agricultural Research station, Mandsaur (Purohit and Vyas, 2004).

The goal of clonal propagation or micropropagation is to obtain a large number of genetically identical, physiologically uniform and developmentally normal plantlets; preferably with high photosynthetic or photoautotrophic potential to survive the harsh *ex vitro* conditions, in a reduced time period and at a lowered cost (Jeong *et al*., 1995). The antioxidant activity and phytochemical analysis of both *in vitro* and *in vivo* grown leaves were studied *in W.*

somnifera it was identified that *in vitro* plants are able to produce and accumulate medicinally valuable secondary metabolites (Nathiya *et al*., 2012)

This study is aimed to establish an effective protocol for to find out the effect of sucrose and light intensity on seed germination, effect of BAP, TDZ and their combination on multiple shoot induction, influence of KN, BAP and their combination on elongation of shoots and rooting and finally to find out the efficacy of flowering in *in vitro* condition at different hormonal concentration.

MATERIALS AND METHODS

SOURCE OF MATERIAL

Plant Material: Seeds of a released variety of *W. somnifera*; Jawahar were collected from the University of Agricultural Sciences, Bangalore. Surface sterilized seeds were germinated *in vitro*; seedlings were maintained in medium for four weeks.

CULTURE MEDIA

Plant tissue culture media: Full strength and half strength of MS medium (Murashige and Skoog, 1962) were used for all the experiments with certain modifications.

METHODS

Media preparation and sterilization: The macro, micronutrients, vitamins and myo-inositol were taken from the stock solutions according to the requirement of plant tissue culture medium. Sucrose (30 g/l) was added and mixed well. The growth regulators at required concentrations were added. The pH of the media was adjusted to 5.6-5.8 using 0.1N NaOH or 0.1N HCl. 0.8% of the solidifying or gelling agent (agar) was added to the media and steamed to melt the agar. It was then dispensed in culture bottles (25 ml per bottle) and autoclaved at 15 lbs pressure at 121˚C for 20 mins.

IN VITRO GERMINATION OF SEEDS

Sterilization of seeds: *Withania* seeds (Jawahar Variety) were first washed in running tap water for 10 mins. These seeds were then soaked in water for 24 hours for imbibitions. The preliminary sterilization was done with 5% Teepol for 5 mins. Then the seeds were placed in 0.1% mercuric chloride for 12-13 mins. Finally the seeds were washed thrice with sterile distilled water.

Inoculation of sterilized seeds: The working area of the laminar airflow chamber was first surface sterilized with absolute alcohol. The Petri dishes and tools that were used during inoculation of the seeds (forceps, scalpel, sterile cotton, and paper towels) were sterilized in autoclave at 15 lbs 121˚C for 20 mins and kept in the laminar airflow chamber. The ultra violet light was switched on for 20 mins and switched off. The laminar airflow was put and hands were sterilized with 70% alcohol, forceps were dipped in alcohol, flamed, cooled and used for the inoculation. The surface sterilized seeds were carefully inoculated into various media for germination (Table. 1a).

The culture bottles, after inoculation of the seeds were incubated under two different conditions i.e., 16 hours photoperiod at room temperature and dark at 25˚C. The effectiveness of each treatment and different modes of incubation was assessed based on the germination percentage using the following formula.

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total no. of seeds inoculated}} \times 100$$

MICROPROPAGATION

Explant: The shoot tips from the *in vitro* germinated seedlings were used as the source for multiple shoot induction.

Initiation of multiple shoots: The shoot tips were carefully excised and inoculated on MS basal medium supplemented with various concentrations of 6-benzyl amino purine (BAP) and Thidiazuron (TDZ) (Table. 1b).

Multiplication of shoots: Fourty five days after inoculation, for multiplication, the initiated multiple buds were separated and sub cultured into respective fresh medium with the same concentrations of growth regulators as that of the induction medium.

Elongation of shoots: Fifteen days after sub culturing, the well-grown shoots of uniform height were transferred to the elongation medium (Table. 1c) and maintained for 30 days.

Root induction: The elongated plantlets were transferred to MS medium free from hormones for root induction medium **(**Table. 1d**)**.

Hardening: The well-organized plants were transferred from *in vitro* condition to *in vivo* condition for hardening. It was planted on a mixture of vermiculate and sand in the ratio 3:1. The plantlets were maintained carefully by gradually manipulating the temperature and humidity for acclimatization under greenhouse conditions.

***In Vitro* Flowering:** The well elongated multiple shoots were transferred into the MS basal medium supplemented with various concentrations of kinetin and BAP (F0 to F11) for *in vitro* flowering (Table. 1e). Three-sub culturing was done with 30 days interval. A set of plants was also transferred to MS basal medium.

Staining of pollen: Flowers were collected during anthesis and stained with 1% acetocarmine and observed under microscope.

RESULTS AND DISCUSSION

***In Vitro* germination of seeds:** The commercial cultivation of *Withania* spp is associated with two major problems namely, the plant to plant variation in alkaloid and withanolide yield and quality as well as the long gestation period (4-6 years) between planting and harvesting (Jeong *et al*., 1995). Agronomically the plants are propagated through seeds since the plants do not have the natural ability for vegetative propagation and the variability generated by sexual recombination is very high. To overcome these constraints, biotechnological tools like plant tissue culture can be gainfully employed where cultures can be started from *in vitro* germination of seeds.

Effect of sucrose on seed germination: The effect of sucrose concentration on seed germination was studied by supplementing 1%, 2% and 3% sucrose in ½ MS basal media. Statistically significant difference was observed in germination percentage of seeds at varying sucrose concentration. From the (Table. 2) it was clear that seed germination on ½ MS supplemented with 2% sucrose on prolonged incubation (upto 40 days) was significantly higher (36.5±2.12) compared to ½ MS supplemented with 1% and 3% sucrose (17.5±0.70 and 10.5±0.70 respectively). The germination percentage for 40 days was calculated from the date of inoculation of seeds.

Effect of light intensity on seed germination: The influence of light intensity on germination of seeds was studied by maintaining one set of culture under dark at 25˚C and another set under a 16 hours photoperiod at 25˚C. Forty seeds were inoculated in ½ MS basal medium supplemented with 2% sucrose and incubated in dark at 25˚C showed significantly higher germination compared to the seeds germinated on 16 hours photoperiod at 25˚C (Table. 2).

Germination percentage was found to be higher in MS supplemented with 2% sucrose compared to MS media supplemented with 1% sucrose. The results indicated that MS supplemented with 2% sucrose incubated in dark at 25˚C gave significantly higher germination. MS with 2% sucrose incubated in 16 hours photoperiod at 25˚C gave better germination of 3.00±0.00 at 40 days (Fig. 1)**.**

Our study supported by (Kambizi *et al.,* 2006) they reported that temperature and photoperiod affected the germination of *W. somnifera* seeds. They found that the optimum condition for germination of the seeds was 18–25˚C under 16/8 hours' photoperiod and germination under light and dark was significantly higher than under continuous light or continuous darkness. But in our study the maximum germination was observed under continuous dark.

MICROPROPAGATION

Effect of BAP, TDZ and their combination on multiple shoot induction: The establishment of multiple shoot cultures of *W. somnifera* and their responses to different concentrations of several plant regulators are reported in this study. The shoot tips of one-month-old *in vitro* germinated seedlings were excised carefully and were inoculated into MS medium supplemented with various concentrations of BAP, TDZ and in combinations of both.

For all the experiments, MS was choosen as the nutrient medium because earlier reports described its usefulness in *W. somnifera* for *in vitro* experiments (Roja *et al*., 1991). Kulkarni *et al*., 1996 reported direct regeneration of *Withania somnifera* with sixteen shoots on an average from leaf explants.

Various concentrations of BAP were used to induce the multiple shoots. The results showed that among the different treatment groups, MS supplemented with 1.0 mg/L BAP (B3) gave the maximum number of microshoots per explant (11.5±0.70) in 45 days of incubation followed by B2 and B1 (Table. 3)

In B3 medium, the primordia were high compared to B1 and B2 treatment groups whereas the callusing is less compared to B1 and B2 treatment groups (Fig. 3). In the B3 treatment group, there are a number of smaller, continuously proliferating shoot buds present.

Our study related to (Shrivastava and Dubey, 2007) they indicated that best shoot induction (72%) was observed with MS medium supplemented with BAP (1 mg/l) in case of callus obtained from shoot tip explants. After two weeks, thin and long shoots with maximum length were recorded; similar result was observed in the present study.

TDZ is also cytokinin (1-Phenyl 3- 1, 2, and 3-thiadiazol-5), which promotes cell division and shoots differentiation. In the present study, between the two different treatment groups with varying concentrations of TDZ used, MS supplemented with 0.05 mg/L TDZ (B4) gave the maximum number of microshoots per explant (9.5±0.70) in 45 days of incubation followed by B5 (Table. 3).

The proliferation of white friable basal callus declined at higher concentrations of TDZ and primordia gets increased, whereas multiple shoots were found to increase at low concentration of TDZ (Fig. 3).

The shoots obtained in BAP media were well-differentiated length and has less primordia compared to TDZ. However, the shoots in TDZ media were very short and stout. The basal callusing was found to be present in higher concentration in TDZ media compared to BAP. When comparing the multiple shoot induction in BAP and in TDZ, BAP was found to be a better multiple shoot forming phytohormone. While in TDZ, micro shoots are initiated within a week but with progress in time it

decreased due to the formation of primordial and no further differentiation was observed. Combinations of BAP and TDZ were used to induce multiple shoots. The results showed that among the different treatment groups, B10 showed maximum multiplication of shoots (18.5±0.70) at 45 days followed by B11 (14.5±0.70) and the multiple shoots was sub cultured in the same hormonal concentration to increase the number of shoots. The number of shoots was increased by prolonged incubation (Fig. 2)**.**

Basal callusing was observed in almost all the treatment groups but it was decreased by repeated sub culturing in the same media for two times at time interval of about 15 days to decrease the formation of basal callus. Primordia was increased if the hormone concentration of both BAP and TDZ increased and vice-versa. The brownish basal callusing was observed in the treatment groups B7, B10 and B11. The white basal callusing was observed in the treatment groups B6 and B8. The maximum shoot induction was observed in hormonal combination of BAP and TDZ than the multiple shoot induction in BAP and in TDZ alone.

Basal callusing was more in hormonal combinations than when hormones were used individually. Shoot elongation was observed in hormonal combinations. From the results obtained, it was identified that there is no statistically significant difference in multiple shoot induction and also maximum shoot induction was observed in hormonal combinations.

Elongation: Among the cytokinins, KN and BAP are frequently used in culture media for shoot elongation. Cytokines are the most important growth regulators for the elongation of plantlets. Various combinations of KN and BAP were used for elongation of shoots.

Effect of KN, BAP and their combination on elongation of shoots: The significantly higher multiple shoots compared to other hormonal concentrations obtained from MS medium supplemented with 0.5 BAP + 0.05 TDZ and 1 BAP + 0.05 TDZ were transferred to elongation media (Table. 4). From the shoots transferred from 0.5 BAP+0.05 TDZ and 1 BAP+0.05 TDZ to the MS medium supplemented with 1 KN +0.05 BAP (E6) showed maximum elongation of shoots (5.0±0.498 and 4.1±1.47 respectively) after 30 days of inoculation followed by MS medium supplemented with 1.5 KN+0.05 BAP (E8) (Fig. 5). Basal callusing was observed in all the hormonal combinations. Internodal space was increased in both E1 and E3 medium. No good elongation was observed in MS medium supplemented with KN alone.

Rosette formation was observed in E5 medium. Distinct internodal space was observed in MS medium supplemented with 0.1 BAP (E5). The combination of KN and BAP gave maximum elongation of shoots and the internodal space was increased than the hormones used individually.

Our results were supported by (Girija *et al*., 2006) reported that MS medium supplemented with KN and BAP combination is good for elongation compared to MS medium containing KN and BAP individually in *Ocimum sanctum*.

Root induction and hardening: Shoots from the elongation medium were excised and inoculated in hormone-free MS medium. The plants were rooted readily on growth regulator-free MS medium within 20 days (Fig. 6)**.**

The well-organized plants were transferred from *in vitro* condition to *in vivo* condition (Fig. 7**)**. The plants were maintained carefully by gradually manipulating the temperature and humidity until it acquires its threshold capacity to survive in the external environment. Only 50% of the transferred plants survived. The rest 50% dried due to less humidity and increased transpiration and was planted in green house. The green house and field have substantially lower relative humidity, higher light level and septic environment that are stressful to micropropagated plants compared to *in vitro* conditions. Approximately it takes two months for successful transfer on to the soil.

IN VITRO FLOWERING

Influence of KN, BAP and their combination on flowering: The multiple shoots obtained from 0.5 BAP + 0.05 TDZ and 1 BAP + 0.05 TDZ were well differentiated and maximum shoot induction was observed, and the shoots obtained in these medium was transferred to KN and BAP medium to induce *in vitro* flowering.

In the present investigation when the influence of KN and BAP concentration on flowering was observed, there was statistically significant difference in flowering between the various KN and BAP concentrations.

No flowering was observed from the MS medium supplemented with 0.5 BAP + 0.05 TDZ to KN and BAP medium. The flower induction was observed after 30 days from MS medium supplemented with 1 BAP + 0.05 TDZ to KN and BAP medium (Table. 5). The percentage of flower induction (66%) was significantly higher in MS medium supplemented with 0.1 BAP (F5) followed by 1.0 KN+ 0.05 BAP (F6). The buds were initiated in the axillary region and no buds were initiated in internodal region. On an average the number of flowers induced per explant was found to be 2 in MS medium supplemented with 0.1 BAP (F5) (Fig. 8**)**.

Pollen studies: The flowers of *W. somnifera* are pentamerous, antinomorphic, hypogynous and hermaphroditic. Each flower bears 5 epipetalous stamens and a pistil. Synchronous stigma receptivity, anther

dehiscence, relative length and close proximity of anthers and stigma predispose makes this species suitable for autogamy (Kaul *et al.,* 2005).

Pollen output per flower was calculated for both *in vitro* and in *vivo* flowers. The total number of pollens per flower vaired between *in vitro* and *in vivo* flowers. The amount of pollen was high in *in vivo* flowers when compared to the *in vitro* flowers. But the flowers from the plantlets derived from 0.1 BAP and 1.0 KN + 0.05 BAP showed an increased number of viable pollen than the flowers from other combinations of kinetin and BAP (Fig. 9**)**. This may be a reason for fruit setting in these two combinations alone.

In future, the work can be extended for the influence of growth regulators along with environmental factors and physiological state of the plant can be analysed to understand the mechanism of flowering.

Table. 1 Media compositions used

S. No.	Treatments	Media composition
	a. Germination of seeds	
1.	G1	½ MS + 1% sucrose
2.	G2	½ MS + 2% sucrose
3.	G3	½ MS + 3% sucrose
	b. Multiplication of Shoots	
4.	B0	MS0
5.	B1	MS + 0.2 BAP mg/L
6.	B2	MS + 0 .5 BAP mg/L
7.	B3	MS + 1 BAP mg/L
8.	B4	MS + 0.05 TDZ mg/L
9.	B5	MS + 0.1 TDZ mg/L
10.	B6	MS + 0.2 BAP+0.1 TDZ mg/L
11.	B7	MS + 0.5 BAP+0.1 TDZ mg/L
12.	B8	MS + 1 BAP+0.1 TDZ mg/L
13.	B9	MS + 0.2 BAP+0.05 TDZ mg/L
14.	B10	MS + 0.5 BAP+0.05 TDZ mg/L
15.	B11	MS + 1 BAP+0.05 TDZ mg/L
	c. Elongation of Shoots	
16.	E0	MS0
17.	E1	MS + 1 KN mg/L
18.	E2	MS + 1.5 KN mg/L
19.	E3	MS + 2 KN mg/L
20.	E4	MS + 0. 05 BAP mg/L
21.	E5	MS + 0. 1 BAP mg/L
22.	E6	MS + 1 KIN+ 0.1 BAP mg/L
23.	E7	MS + 1 KN+ 0.05 BAP mg/L
24.	E8	MS + 1.5 KN+ 0.1 BAP mg/L
25.	E9	MS + 1.5 KN+ 0.05 BAP mg/L
26.	E10	MS + 2 KN+ 0.1 BAP mg/L
27.	E11	MS + 2 KN+ 0.05 BAP mg/L
	d. Rooting	
28.	R	MS
	e. *In vitro* flowering	
29.	F0	MS
30.	F1	MS + 1 KN mg/L
31.	F2	MS + 1.5 KN mg/L
32.	F3	MS + 2 KN mg/L
33.	F4	MS + 0. 05 BAP mg/L
34.	F5	MS + 0. 1 BAP mg/L
35.	F6	MS + 1 KN+ 0.1 BAP mg/L
36.	F7	MS + 1 KN+ 0.05 BAP mg/L
37.	F8	MS + 1.5 KN+ 0.1 BAP mg/L
38.	F9	MS + 1.5 KN+ 0.05 BAP mg/L
39.	F10	MS + 2 KN+ 0.1 BAP mg/L
40.	F11	MS + 2 KIN+ 0.05 BAP mg/L

Table. 2 Germination percentage of seeds in response to light intensity and sucrose concentration

Treatment	Media composition	Germination percentage	
		20 Days Interval	40 Days Interval
G1	½ MS supplemented with 1% sucrose incubated in 16 hours photoperiod at 25ºC	1.00±0.00	2.50±0.70
G2	½ MS supplemented with 1% sucrose incubated in dark at 25ºC	**12±1.41**	**17.5±0.70**
G3	½ MS supplemented with 2% sucrose incubated in 16 hours photoperiod at 25ºC	2.50±0.70	3.00±0.00
G4	½ MS supplemented with 2% sucrose incubated in dark at 25ºC	**16±1.41**	**36.5±2.12**
G5	½ MS supplemented with 3% sucrose incubated in 16 hours photoperiod at 25ºC	1.00±0.00	3.00±0.00
G6	½ MS supplemented with 3% sucrose incubated in dark at 25ºC	**4.5±0.70**	**10.5±0.70**
	SED	0.9129	1.0000
	CD (0.05)	2.2338	2.4470
	CD (0.01)	3.3845	3.7075

*Data represents mean ± SE of three replications with 40 seeds per replicate

Table. 3 Shoot induction percentage in response to varying hormone concentration

Treatment	Concentration of hormone (mg/l)	15 Days Interval	30 Days Interval	45 Days Interval
B0	MS medium without hormones	1.0±0.00	1.0±0.00	1.0±0.00
B1	0.2 BAP	3.5±0.70	4±0.00	4±0.00
B2	O.5 BAP	6.0±0.00	7.5±0.70	9.5±0.70
B3	1.0 BAP	**7.5±0.70**	**9.5±0.70**	**11.5±0.70**
B4	0.05 TDZ	**5.0±0.00**	**7.5±0.70**	**9.5±0.70**
B5	0.1 TDZ	3.0±0.00	3.5±0.70	4.0±0.00
B6	0.2 BAP + 0.1 TDZ	6.5±0.70	9.5±0.70	11.5±0.70
B7	0.5 BAP + 0.1 TDZ	5.5±0.70	6.5±0.70	12.5±0.70
B8	1 BAP + 0.1 TDZ	6.0±0.00	8.5±0.70	13.5±0.70
B9	0.2 BAP + 0.05 TDZ	7.5±0.70	10.5±0.70	13.5±0.70
B10	0.5 BAP + 0.05 TDZ	**9.0±0.00**	**12.5±0.70**	**18.5±0.70**
B11	1 BAP +0.05 TDZ	8.5±0.70	11.5±0.70	14.5±0.70
	SED	0.5000	0.6455	0.7071
	CD (0.5)	1.0894	1.4064	1.5407
	CD (0.1)	1.5273	1.9718	2.1600

*Data represents mean ± SE of three replications with 3 explants per replicate

Table. 4 Elongation of shoots in response to various concentrations of KN, BAP and their combination

Treatment	Conceentration (mg/l)		30 Days Interval 0.5 BAP + 0.05 TDZ to KN and BAP medium Elongation/explants (cm)	30 Days Interval 1 BAP + 0.05 TDZ to KN and BAP medium Elongation/explants (cm)
	KN	BAP		
E0	–	–	3.3±0.26	3.3±0.26
E1	1.0	–	0.90±0.28	1.0±0.28
E2	1.5	–	3.4±0.75	2.1±0.87
E3	2.0	–	2.9±0.58	2.5±0.60
E4	–	0.05	2.5±0.97	3.8±0.81
E5	–	0.1	4.0±1.3	3.9±0.86
E6	1.0	0.05	**5.0±0.49**	**4.1±1.47**
E7	1.0	0.1	2.9±1.02	3.6±1.47
E8	1.5	0.05	4.5±1.04	2.0±1.02
E9	1.5	0.1	3.3±0.75	3.2±1.40
E10	2.0	0.05	3.0±0.86	3.7±1.50
E11	2.0	0.1	3.4±0.73	3.2±1.08
		SED	1.5574	0.612
		CD (0.5)	3.1154	1.2247
		CD (0.1)	4.1432	1.6289

*Data represents mean±SE of three replications with three explants per replicate

Table. 5 Flower induction in explants from 1.0 BAP + 0.05 TDZ to various concentration of BAP and KN

Treatment	Concentration (mg/l)		Percentage of flower induction	30 DAI Number of flower induced/ explant
	KN	BAP		
F0	–	–	0.00	0.00
F1	1.0	–	0.00	0.00
F2	1.5	–	16	0.30
F3	2.0	–	0.33	0.30
F4	–	0.05	16	1
F5	–	0.1	**66**	**2**
F6	1.0	0.05	**50**	**1.3**
F7	1.0	0.1	0.00	0.00
F8	1.5	0.05	50	1
F9	1.5	0.1	0.00	0.00
F10	2.0	0.05	33	0.3
F11	2.0	0.1	0.00	0.00

*Data represents mean of three replications with three explants per replicate

Acknowledgement: The author would like to acknowledge DBT, Ministry of Science and Technology, New Delhi- 3 for providing financial assistance. Proceeding No: DT/PR11845/GBD/27/168/2009, Dated- 4. 02.2009.

REFERENCES

Girija, S., S. Kavitha and S. Deepavathi (2006). Direct multiple shoot regeneration from shoot tips and nodal explants of *Ocimum sanctum* L. (Tulsi): A medicinal herb. Plant cell Biotechnology and Molecular biology. 7: 23-28

Gupta, G. L and A. C. Rana (2007). *Withania somnifera* (Ashwagandha): A Review. Pharmacognosy Reviews. 1: 129-136

Jeong, B. R., K. Fujiwara and T. Kozai (1995). Environmental control and photoautotrophic micropropagation. In: Horticultural Reviews (Ed. Janick J.) John Wiley and Sons Inc., New York. 17: 125-172

Kambizi, L., P. O. Adebol and A. J. Afolayan (2006). Effects of temperature, pre-chilling and light on seed germination of *Withania somnifera*; a high value medicinal plant. South African J. Botany. 72: 11–14

Kaul, M. K., A. Kumar and A. Sharma (2005). Reproductive biology of *Withania somnifera* (L.) Dunal. Curr. Sci. 88: 1375-1377

Kulkarni, A. A., S. R. Thengane and K. V. Krishnamurthy (1996). Direct *In vitro* Regeneration of Leaf Explants of *Withania somnifera* (L.) Dunal. Plant Science. 119: 163-168

Murashige, T and F. Skoog (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Planta. 15: 472-497

Nathiya, S., N. Santhi and Kalaiselvi Senthil (2012). A Comparative study on ontogenic expression of antioxidants and secondary metabolites in *Withania somnifera*. International Research J. Pharmacy. 3(1): 210-216

Purohit, S. S and S. P. Vyas (2004). Medicinal Plant cultivation: A scientific approach. Publisher Agrobios; Jodhpur (India). 547 P

Roja, G., M. R. Heble and A. T. Sipahimalini (1991). Tissue cultures of *Withania somnifera*: Morphogenesis and Withanolide synthesis. Phytotherapy Research. 5: 185-187

Shrivastava, S and P. K. Dubey (2007). *In vitro* Callus Induction and Shoot Regeneration in *Withania somnifera* (L.) Dunal. International J. Biotechnology and Biochemistry. 3: 1-12

Singh, A. K., R. Varshney, M. Sharma, S. S. Agarwal and K. C. Bansal (2006). Regeneration of Plants from Alginate-Encapsulated Shoot Tips of *Withania somnifera*(L.) Dunal, A Medicinally Important Plant Species. J. Plant Physiology. 163: 220-223

Steward, F. C., M. O. Mapes and K. Mears (1958). Growth and organized development of cultured cells. II Organization in cultures grown freely from suspended cells. Am. J. Bot. 45: 705-709.