Effect of Different Auxins Alone and Mixture of Benzene Aminopurine (BAP) and Kinetin (Kn) Along with Auxin 2,4-D on *In Vitro* Growth and Multiplication of Callus in B5 Medium Derived from Embryonic Cotyledon Excised Explants of Ashwagandha (Cultivated)

[1]*Naveen Gaurav,* [2]*AP Singh,* [3] *Abhishekh Srivastava,* [4] *Arun Kumar,* [5]*Deepak Kumar,* [6]*Komal,* [7]*Hira Singh Gariya*

1. *Assistant Professor Department of Biotechnology, S G R R P G College Dehradun, U.K.*
2. *Professor, Department of Botany Govt. P.G. Science College, Rewa, M.P.*
3. *Assistant Professor, Department of Botany Govt. S.V. College Teonthar, M.P.*
4. *Assistant Professor, Department of Biotechnology, S G R R P G College Dehradun, U.K.*
5. *M.Sc, Department of Biotechnology, S G R R P G College Dehradun, U.K.*
6. *M.Sc, Department of Biotechnology, S G R R P G College Dehradun, U.K.*
7. *B.Sc Biotechnology, Department of Biotechnology, SGRRPG,College Dehradun, U.K.*

1 ABSTRACT

Ashwagandha (*Withania somnifera*) belongs to the member of family Solanaceae, having enormous aromatic properties and medicinal properties, has been included in an ancient (early) text of Ayurveda. It is very useful as an, contraceptive, amoebocide, bactericide, abortifacient, anodyne, and diuretic. But the risks of fungal infections are very high in these plants. Due to its over use this plants is going towards extinction so *in vitro* microprogation is a best method to protect this plant as well as to produced value added compounds in a very short time without any external environmental hazards. Higher percentage of *in vitro* morphogenic response was exhibited by explants mature embryo followed by explants mature cotyledon leaves. Half B5 medium (gamborg medium) with NAA, IBA, 2,4- Di-chlorophenyl acetic acid (2,4-D), BAP, Kinetin (Kn) and Sucrose (5% w/v) was employed either separately or in mixture. Cotyledonary leaves are generally produces callus in callus induction media and after several sub culturing its produces shoots and roots in shoot and root induction media. Regenerated plantlets were obtained successfully in the field after hardening. Mostly for *in vitro* organogenesis MS medium has been employed bus it has been also done in B5 medium with cotyledonary leave's explants of *Withania somnifera*.
Key words: *Withania somnifera*, Solanaceae, aromatic properties, *in vitro* microprogation, B5 medium, Sucrose etc.

2. Introduction

Withania somnifera (L.) Dunal (Family: Solanaceae, commonly known as Ashwagandha, English name: Winter cherry) is an important perennial plant species with immense therapeutic uses in traditional as well as

modern system of medicine (Datta, *et.al.,* 2010). Due to restorative property of roots, the species is also known as 'Indian Ginseng'(Tripathi, *et.al.*, 1996; Andallu & Radhika, 2000*;* Winters, 2006; Kumar, *et.al.*, 2010). The Indian Himalayan region (BHR), one of the richest reservoirs of biological diversity in the world, is undergoing irrational extraction of wild, medicinal herbs, thus endangering many of its high value gene stock. *Withonia somnifera L.* (Dunal) is a member of solanaceae, also known for thousands of years by Ayurvedic practitioners. *Withania somnifera* root contains flavonoids, alkaloids, steroid and many active functional ingredients (Kumar, *et.al.*, 2007). *Withania somnifera* consists of very high concentration of secondary metabolites that can be also known as bioreactors like steroidal lactones, alkaloids and flavonoides, which have effective properties and they used in ninety commercially Ayurvedic formulations (Sreerekha, *et.al.*, 2004). *Withania sominfera* are propagated in northern western region of Madhaya Pradesh in India, on about 400 ha (Khare, 1996; Thapliyal & Thapliyal, 2001).

Withania sominifera having small white flowers mainly in rainy and winter seasons that can be develop into fruit during the winter seasons. Plants products can be obtained from the roots, leaves, and branches, by using many different biological techniques. *Withania* which is also known as Ashwagandha having effective property can also used in blends and supplements which are designed to show many multiple effects. It is described as an herbal tonic and health food in Vedas and considered as 'Indian Ginseng' in traditional Indian system of medicine (Singh , *et.al.*, 2001).

3. Material and methods

Chemicals

All chemicals were mostly of Himedia, India and Sigma, USA and some of the chemical were also obtained from SRL, Qualigens and E. Merck, India.

Preparation of B5 medium:-

Added 23.23 grams weight of dehydrated medium in sterilized 600ml of double distilled water and to wash or clean the media vial by suitable and small quantity of double distilled water to remove out the traces of powder. Apply constant gentle animation to the solution in a proper way till the powder dissolves completely. Add heat stable supplements to obtain after autoclaving. Maintain the obtain pH of the medium by using 1N HCl/1N NaOH/1N KOH. Make up the final volume of media to one litre (1000ml) with continuous adding distilled water. Sterilize the medium or make the medium free from contamination by the process of autoclaving at 15 lbs or 121ºC for 15 minutes. Then cool the autoclaved medium to 45ºC prior addition of the filter sterilized heat sensitive supplements. Store the prepared medium at 2-8ºC away from direct light.

Commonly Used Planted Growth Regulators.

Substances (PGR's)	Solvent	Stock concentration	Sterilization	Storage conditions
1AA	1N Na OH	5 mg/1	F	O^0C
1BA	1N Na OH	0.5 mg/1	CA	O^0C
BAP	1N Na OH	20 mg/1	CA	O^0C
NAA	1N Na OH	2 mg/1	CA	4^0C

CA= Co-autoclavable with other media components
F= Filter sterilization with 0.22 micro Millipore filter

4. Results

Table 4.1: Effect of different auxins on *in vitro* growth and multiplication of callus in B5 medium derived from embryonic cotyledon excised explants of *Withania somnifera* or Ashwagandha (Cultivated):

S. No	Hormones (Auxins)	Conc. (mg/l)	Callus		Frequency of formation of callus (%)
			Fresh Weight (Gram)	Dry Weight (Gram)	
01	NAA	1.0	7.11±0.199	0.49±0.011	82±2.30
		2.0	5.86±0.129	0.38±0.006	75±1.43
		3.0	5.01±0.090	0.37±0.006	64±0.96
02	IBA	1.0	4.32±0.073	0.50±0.014	74±1.33
		2.0	3.45±0.045	0.39±0.007	62±0.81
		3.0	2.95±0.032	0.36±0.005	60±0.66
03	2, 4-D	1.0	5.01±0.095	0.39±0.007	71±1.21
		2.0	3.99±0.064	0.41±0.008	79±1.74
		3.0	3.87±0.058	0.33±0.004	66±1.06

(Mean [+ or -] represent Standard error).

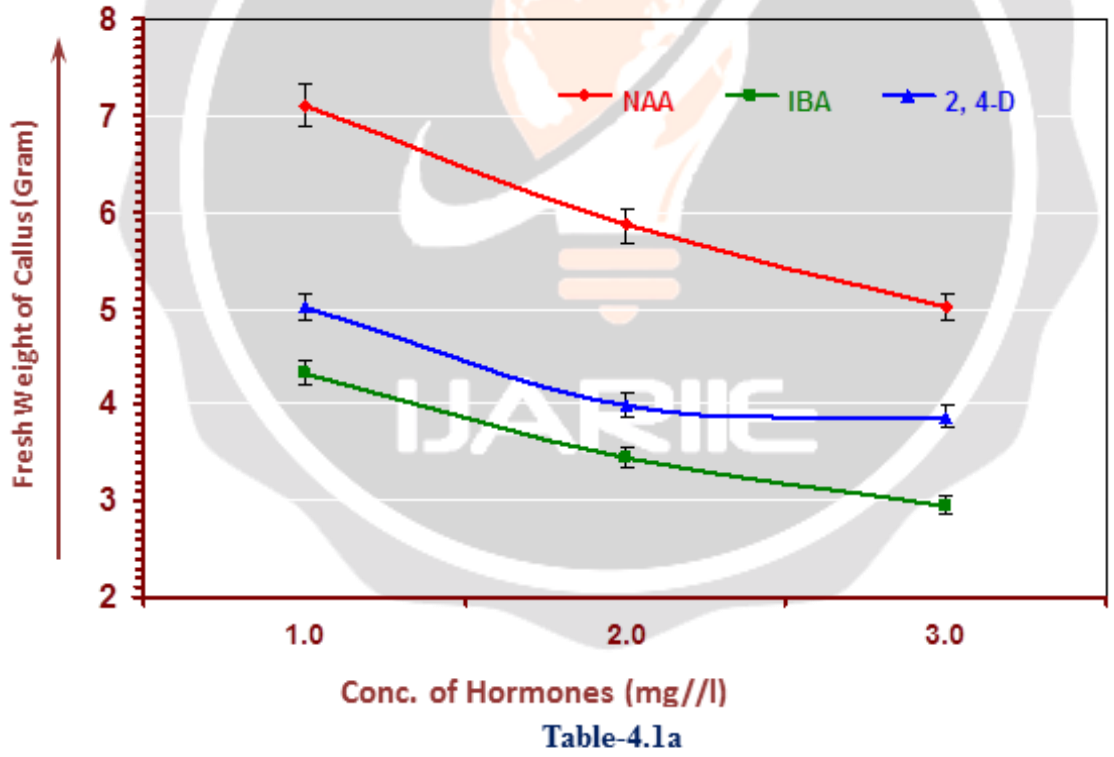

Table-4.1a

Bar diagram showing effect of different auxins on *in vitro* growth of excised cotyledons and formation of fresh weight of Callus in B5 medium of *Withania somnifera* (Cultivated)

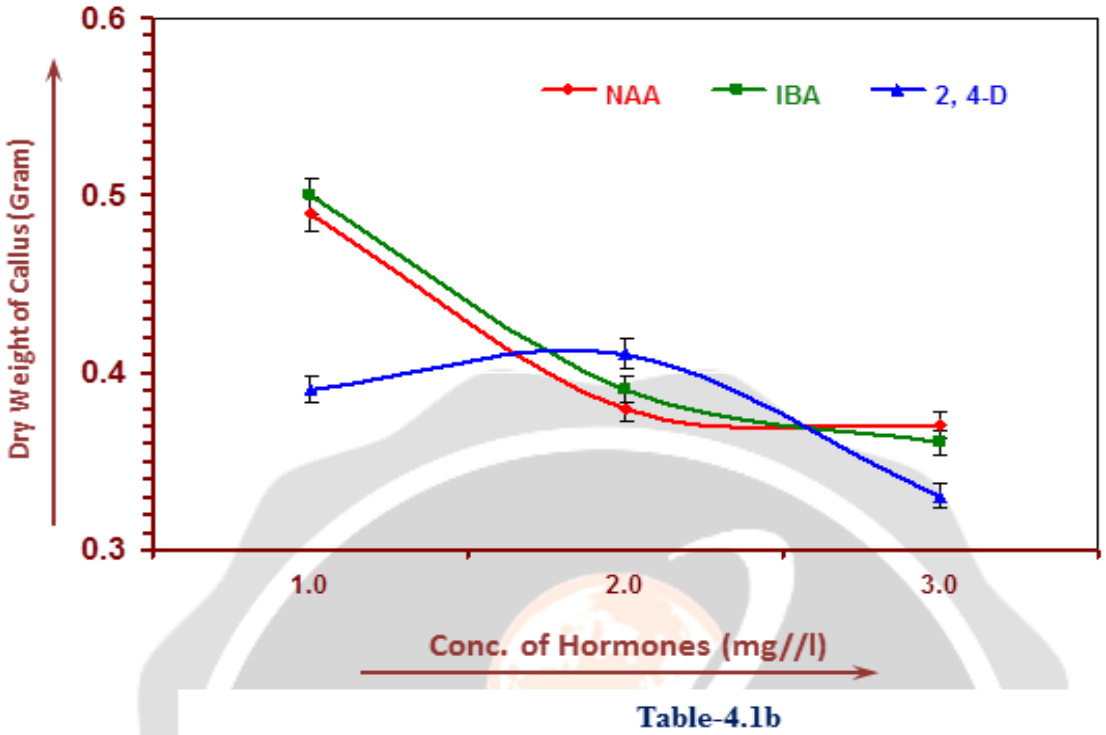

Table-4.1b

Bar diagram showing effect of different auxins on *in vitro* growth of excised cotyledons and formation of dry weight of Callus in B5 medium of *Withania somnifera* (Cultivated)

Cotyledon explants (size 1.5cm) were inoculated in full strength B5 medium supplemented with 0.8% agar-agar and same concentration (1mg/l - 3mg/l) of 2, 4-D, NAA and IBA . After two to three weeks of inoculation greenish colored callus was observed in different frequencies in different harmone concentration in B5 medium. The results observed are depicted by the table 4.1. B5 media with different concentration of different hormones (auxins) initiates callus formation. Maximum callus formation in cultivated explants occurs in B5 medium supplemented with 1mg/l IBA, 1mg/l NAA and 2mg/l 2,4 D. Maximum frequency of callus formation by *Withania* explants takes placed in B5 medium with NAA as observed are depicted by the table 4.1.

Table 4.2: Effect of supplementing mixture of Benzene aminopurine (BAP) and kinetin (Kn) along with auxins 2,4-D on growth of callus in B5 medium derived from embryonic cotyledon explants of *Withania somnifera* (Cultivated):-

S. No.	Cyto–Kinins	Conc. (mg/l)	Auxins (2,4-D) (mg/l)	Callus		Frequency of formation of callus (%)
				(Fresh Weight) Gram	Dry Weight Gram	
01	BAP	0.5	2.0	4.1±0.05	0.32±0.004	61±1.10
		1.0	2.0	5.2±0.11	0.41±0.009	74±2.00
		1.5	2.0	4.4±0.07	0.35±0.005	55±0.66
02	Kinetin	0.5	2.0	5.0±0.09	0.36±0.006	65±1.63
		1.0	2.0	6.9±0.19	0.56±0.015	63±1.32
		1.5	2.0	6.0±0.15	0.49±0.012	60±0.90

(Mean [+ or -] Standard error).

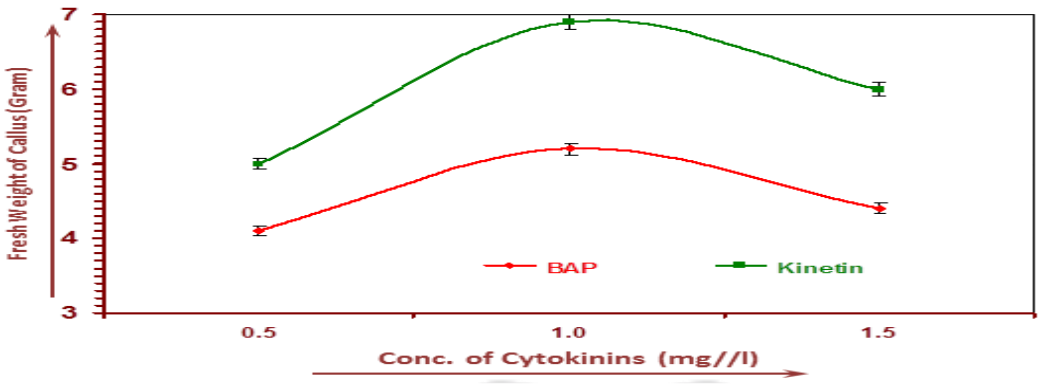

Table-4.2a

Bar diagram showing effect of supplementing mixture of Benzene aminopurine (BAP) and kinetin (Kn) along with auxins 2,4 D on growth of Callus (Fresh Weight) in B5 medium of *Withania somnifera* (Cultivated)

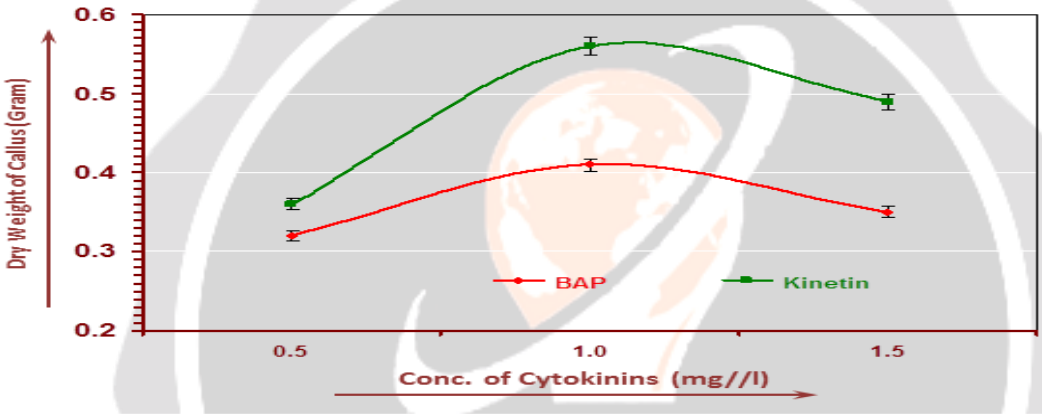

Table-4.2b

Bar diagram showing effect of supplementing mixture of Benzene aminopurine (BAP) and kinetin (Kn) along with auxins 2,4 D on growth of Callus (Dry Weight) in B5 medium of *Withania somnifera* (Cultivated)

Cotyledon explants (size 1.5cm) were inoculated in full strength B5 medium supplemented with 0.8% agar-agar and same concentration 2mg/l of 2, 4-D with 0.5mg/l to 1.5mg/l BAP, same concentration 2mg/l of 2, 4-D with 0.5mg/l to 1.5mg/l Kn. After two weeks of inoculation greenish colored callus was observed in different frequencies in different harmone concentration in B5 medium. The results observed are depicted by the table 4.2. B5 medium having same concentration of 2,4 D (2mg/l) with different concentration of cytokinins (0.5 to 1.5mg/l) also initiates the formation of callus. Maximum formation of fresh and dry callus takes placed in B5 medium having 2.0mg/l 2,4 D with 1.0mg/l BAP and 2.0mg/l 2,4 D with 0.5mg/l kinetin, in which maximum frequency of callus formation takes placed with BAP (1.0mg/l BAP) as recorded in table 4.2.

5. Discussion

Chaudhry, *et.al.*, *(*2009) did work on Ashwagandha, the medicinal plant of India it will be used as an "adaptogen" and has been referred to as the 'ayurvedic ginseng due to their different effective property'. The recent methodology deals with the showing effect of different media explants, carbon sources, different concentration of agar and varying salt concentrations on in-lab propagation of Ashwagandha and its phytochemical effects. From taking different explants (node, internode, apical bud, petiole) and media (MS, B5) which is responsible for growth, the best results in terms of percent regeneration and growth index were present or obtained on MS media with cotyledonary leaves, nodal and apical bud explants. Callusing was obtained on MS+ NAA+ Kn with petiole and internodes as explants. Among the given different carbon sources like (sucrose, fructose, glucose), sucrose at three percent concentration was assume to be optimum for shoot induction. Among the certain decrease in agar concentration from 0.8% to 0.16%, there was increase in shoot regeneration and production and also the size of leaf.

Chitturi, *et.al*., (2010) established a protocol for *in vitro* production of Ashwagandha plants by which callus cultures of Ashwagandha from leaves were established or reported on growing nutritional MS (Murashige and Skoog) media having Kin and sugar sucrose (3% w/v). Liquid suspension cultures were established or placed and the amount of growth and production kinetics was studied and also be noted for further use. For growth kinetics MS media placed with Kinetin (0.1 mg/l) and sugar sucrose (3% w/v) without the use of the agar which was found to be very effective with good success and responsible for the maintenance and initiation of the suspension cultures from the calli without any contamination. Half B5 medium (gamborg medium) with 2,4- Di-chlorophenyl acetic acid (2,4-D) (1 mg/l), Kinetin (Kn) (0.1 mg/l) and Sucrose (5% w/v) was employed. Several different useful or less useful effects of the various precursors and elicitors which are used on the suspension cultures were studied.

Soni, *et.al*., (2011) developed an very useful and prospective protocol for the rapid and further *in vitro* propagation of Ashwagandha through the shoot bud culture. The *in vitro* multiplication of the auxiliary shoot will be isolated from the nodal explants or from small piece of tissues of field grown Ashwagandha propagated in MS medium and Gamborg B5 medium supplemented with the concentrations of 2,4- Dichlorophenoxy acetic acid (2, 4-D), Benzyl amino purine (BAP), Kinetin(Kn) and Naphthalene acetic acid (NAA).

Arumugam, & Gopinath (2011) worked on the Leaf and Cotyledon explants of Ashwagandha were used to evaluate or recognize the effect of the different growth regulators require in the process of the *in vitro* direct shooting and rooting initiation experiment. This new procedure was standardized for easy mass propagation or multiplication of the Ashwagandha medicinal plant (Kannan, *et.al*., 2014). Callus (Sabir, *et.al*., 2008) initiation was observed best in MS media (Murashige & Skoog.,1962) with 2,4- D (1.0-5.0mg/L) after 16-20 days (93%).

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