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Comparative study of differentiation of plants morphology of natural originated & *in vitro* originated *Withania somnifera* plants

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Abstract
Modern plant tissue culture is practiced under aseptic or favourable conditions under filtered air. Living plant materials which was taken from the environment or free space are being naturally contaminated or will show contamination on their external surfaces (and sometimes interiors) with pathogenic microorganisms, so surface sterilization of initial materials (explants) in chemical solutions (usually Sodium or calcium hypochlorite or mercuric chloride) is done. Mercuric chloride is mostly used as a plant sterilant now a days, unless other sterilizing agents which require for removal of contamination are dispose found to be least effective, as it is very dangerous and harmful to use, and is very difficult to dispose of. Explants are then generally placed or maintained on the upper surface of a solidified culture medium (normally MS) but during some cases it will be inoculate directly into a liquid nutrient medium, normally when cell suspension cultures are required. Solid nutrient and liquid nutrient media usually consists of hormones. Solidified media are prepared or produced from the liquid media with the use of a gelling or solidified agent, generally a purified agar. The constituents of the solid medium or the requirement of the plant growth hormones and the nitrogenous source (nitrate/ammonium salts and amino acids) have produces morphological effects of the grown tissues that from the initial explants. For example, high concentration of auxin responsible for the high multiplication of roots whereas an excess cytokinin may yield shoots. A balance concentration of auxin and cytokinin will often generate an unorganised and undifferentiated growth of cells, i.e. callus, but the morphology of the outgrowth depends on the different plant varieties as well as the suitable medium composition.

Keywords: Plant tissue culture, aseptic conditions, Solid nutrient, Explants, auxin, cytokinin, callus

1. Introduction

At recent days there are a large number of reports contained regarding in lab propagation of *Withania somnifera* L. Dunal by applying various pieces of tissue known as explants such as shoot tip explants (Sen and sharma, 1991) [11], nodal segments explants (Kulkarni, *et al.*, 2000) [5], axillary meristem explants (Roja, *et.al.*, 1991) [9], axillary shoot explants, and hypocotyls segments and root segment explants (Rani and Grover, 1999).

Soni, *et al.*, (2011) [13] 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).

Patel & Krishnamurthy, (2013) [7] did work on the micro-popagation of *Withania* explants with increased regeneration potential raised through *in vitro* viability and germination of seeds of two different genotypes of Ashwagandha. Regenerated plantlets were produced successfully or safely in the field region after the primary and secondary hardening.

In vitro micro-propagation of medicinally important plants was obtained for the generation or for the production of the multiple or various other different shoots and roots of Ashwagandha. Shoots were induced or executed from the mainly axillary buds of the Ashwagandha on Murashige and Skoog's (1962) [6] or MS media basal media provided with the variable or almost different concentrations of the BAP (0.10, 0.15, 0.20, 0.25 & 0.30mg/L) along with GA_3. Axillary bud explants which is an small pieces of the tissues processed initiation or increment of the shoots within five to six days of the transfer, with the favourable and optimum concentration of 0.25mg/L and it will be found to be highly efficient for the various or multiple shoot generation within the minimum time period or short time period. The generated or originated and formed shoots will be successfully rooted or planted on the MS basal medium added

with NAA (optimum concentration 0.5mg/L) along with GA3 and the plantlets acclimatized in soil (Sharma, *et al.*, 2009) [12]. Irshad, *et al.*, 2013 [3] did work on *Withania somnifera* and proved that it is an important medicinal plant and utilized by all over the world in pharmaceutical industry or in medicine production. Generally Ashwagandha propagates vegetatively with in its natural or exceptable state, but propagation rate is too slow or too late to meet demands of high quality planting material for the useful commercial cultivation. A rapid or regularly without any distruption and highly efficient method for the micro-propagation method for the selection of Ashwagandha by the auxiliary branching method and using the shoot tip as explants was standardized. Shoot cultures were initiated on MS medium containing BA (0.5 – 2.0 mg/L) with NAA (0.2-0.5 mg/L) containing 4.5 gms/l agar, and 3% commercial sugar. The regeneration nutrient medium that encourage the largest numbers or maximum numbers of shoots formation in the petiole explants and leaf explants when Murashige and Skoog (MS) medium was supplemented with 6 -benzyladenine (BA) alone or with naphthalene acetic acid (NAA) with suitable concentration. The frequency of shoot regeneration was highly affected by the different or various types of explants, the useful carbon and energy source, the orientation or genotype of the different explant, and the basal medium which will be used in the reorientation medium. The presence of 1 mg/l Indole 3-butyric acid to the nutrient medium was highly efficient in inducing or originating root formation. The regenerated or reformed (plantlets small plant) were acclimatized and grow in the greenhouse environment and successfully or efficiently transferred to the field, with a high survival rate. The acclimatized and survived plants possessed normal flowering and were not morphologically distinct from the seed-derived mother plants (Ghimire, *et al.*, 2010).

2. Material and Methods

The seeds of the cultivated variety of *Withania somnifera* L. (Dunal) were obtained from the local nurseries. The Agriculture Research Station (ARS) Gulbarg produces the large stocks of Ashwagandha. The seeds taken is washed carefully and properly with the running tap water for 1-2 min and surface will be sterilized with the 70 % ethanol initially followed solution of mercuric chloride (0.1 % w/v) for 3-5 min and thoroughly rinsed 3-5 times in sterile double distilled water to remove the excess or traces of mercuric chloride and absorbed in double distilled water for four to six hours. The *in vitro* morphogenic responses of the plant tissues which are cultivated are normally affected by the various constituents of the culture media or growing media. Both macro and micro-element of the media plays a major role in plant regenerations and morphogenesis (Murashige and Skoog, 1962) [6]. Most media additionally contains myo-inositol at a concentration of 100mg/1, B_5 vitamins along with 3% sucrose and MS basal macro and micronutrients.

For hardening-off, 7 to 8 weeks old rooted shoots were withdrawed from the culture flacks. After giving the washing treatment to agar with water the rooted plantlets obtained from shoots were transferred to bags of polythene or small pots that contains vermi compost fertilizer, red soil and sand in the mixture ratio of 1:2:2 and transferred in a mist house for acclimatization. After successful acclimatization in the mist house for 2-months successfully grown plants are transferred to greenhouse. The plantlets were prosperously planted in the field. After transferred into the field maximum percent of plants approximately 90% of plantlets were generally survived in the garden soil.

3. Results

Table 1: Differentiation of plants morphology of natural originated & *in vitro* originated *W. somnifera* plants

S. No.	Days	CULTIVATED VARIETY						WILD VARIETY					
		Growth of shoots		Growth of Root		Growth of Leaves		Growth of shoots		Growth of Root		Growth of Leaves	
		Natural Cond.	*In Vitro* cond.	Natural Cond.	*In Vitro* cond.	Natural Cond.	*In Vitro* cond.	Natural Cond.	*In Vitro* cond.	Natural Cond.	*In Vitro* cond.	Natural Cond.	*In Vitro* cond.
1.	15	5.2±0.06	8.2±0.09	2.6±0.029	3.1±0.03	0.5±0.006	2.2±0.02	6.4±0.09	9.3±0.13	3.1±0.043	5.4±0.08	1.1±0.015	2.5±0.04
2.	25	8.3±0.13	10.1±0.16	4.2±0.067	6.3±0.10	1.4±0.022	3.5±0.06	10.6±0.19	13.2±0.24	5.4±0.097	8.7±0.18	2.2±0.040	4.3±0.09
3.	35	16.2±0.34	21.3±0.45	6.1±0.128	7.1±0.13	2.7±0.057	4.3±0.08	20.8±0.48	24.1±0.55	7.7±0.177	10.5±0.26	3.4±0.078	5.5±0.14
4.	45	44.1±1.10	49.4±1.24	8.3±0.208	10.5±0.24	3.9±0.098	5.4±0.12	50.5±1.41	58.6±1.64	10.9±0.305	15.4±0.43	4.8±0.134	6.5±0.18

(Mean [+ or -] Standard error).

In vitro plants having higher morphology than natural habitat plants. But in wild plants after successful *in vitro* operation the size of leafs, stem and roots is higher than cultivated variety plants (Table 1).

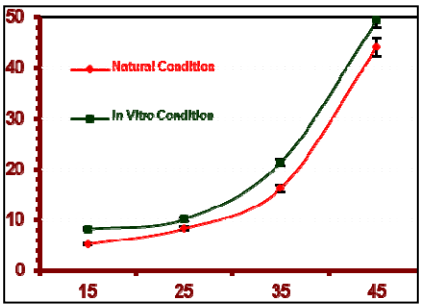

Table 4a: Line diagram showing growth of shoot (cms) of *Withania somnifera* (Cultivated)

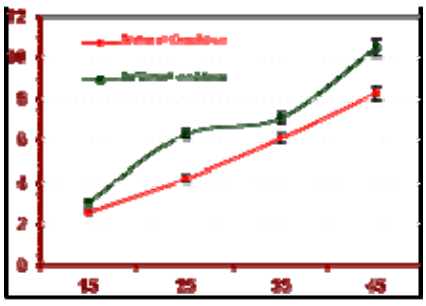

Table 4b: Line diagram showing growth of root (cms) of *Withania somnifera* (Cultivated)

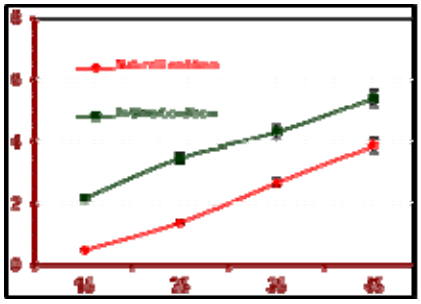

Table 4c: Line diagram showing growth of Leaves (cms) of *Withania somnifera* (Cultivated)

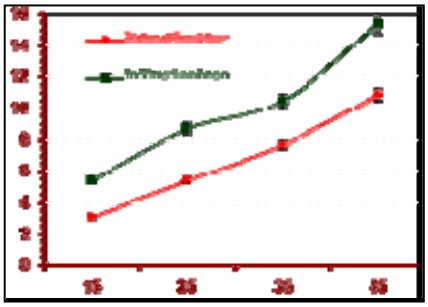

Table 4e: Line diagram showing growth of root (cms) of *Withania somnifera* (Wild)

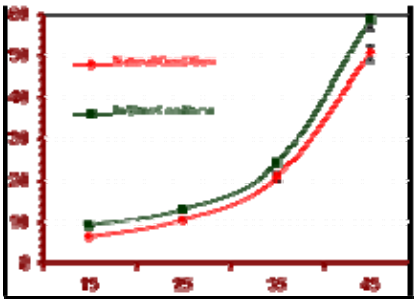

Table 4d: Line diagram showing growth of shoot (cms) of *Withania somnifera* (Wild)

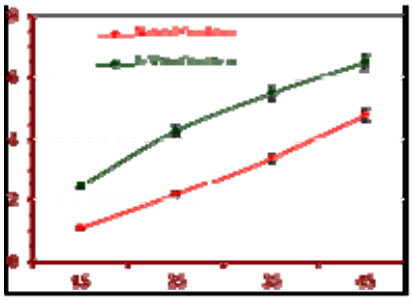

Table-4f: Line diagram showing growth of Leaves (cms) of *Withania somnifera* (Wild)

4. Discussion

Modern plant tissue culture is practiced under aseptic or favourable conditions under filtered air. Living plant materials which was taken from the environment or free space are being naturally contaminated or will show contamination on their external surfaces (and sometimes interiors) with pathogenic microorganisms, so surface sterilization of initial materials (explants) in chemical solutions (usually Sodium or calcium hypochlorite or mercuric chloride) is done. Mercuric chloride is mostly used as a plant sterilant now a days, unless other sterilizing agents which require for removal of contamination are dispose found to be least effective, as it is very dangerous and harmful to use, and is very difficult to dispose of (Aniel, *et al.*, 2011) [1]. Explants are then generally placed or maintained on the upper surface of a solidified culture medium (normally MS) (Pawar & Maheshwari, 2004) [8], but during some cases it will be inoculate directly into a liquid nutrient medium, normally when cell suspension cultures are required. Solid nutrient and liquid nutrient media usually consists of hormones. Solidified media are prepared or produced from the liquid media with the use of a gelling or solidified agent, generally a purified agar. The constituents of the solid medium or the requirement of the plant growth hormones and the nitrogenous source (nitrate/ammonium salts and amino acids) have produces morphological effects of the grown tissues that from the initial explants. For example, high concentration of auxin responsible for the high multiplication of roots whereas an excess cytokinin may yield shoots. A balance concentration of auxin and cytokinin will often generate an unorganised and undifferentiated growth of cells, i.e. callus (Sabir, *et al.*, 2008) [10], but the morphology of the outgrowth depends on the different plant varieties as well as the suitable medium composition (Joshi & Padhya, 2010) [4]

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