Method of enhancing seed germination in *Chlorophytum* sp.

Mala Trivedi[1] & Rajesh K. Tiwari[2]

Amity Institute of Biotechnology, Amity University Uttar Pradesh, Lucknow campus, Uttar Pradesh, India

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Abstract

Chlorophytum sp. (Safed musli) is an important medicinal plant and used world wide in drug industry. Although Chlorophytum propagates through tubers in its natural state, but propagation rate is too slow to meet demand of high quality planting material for commercial cultivation. Seeds are difficult to germinate both under in-vitro as well as in field conditions. We have tried various concentrations of hormones (TDZ and IAA) together with cow & buffalo urine, electric field and found IAA (0.5 mg/l) is best for seed treatment for germination. Shoot cultures were initiated on MS medium (Murashige and Skoog, 1962) containing 290 mM sucrose and supplemented with IAA (0.5mg./l).

Keywords: ***Chlorophytum sp.,*** **hormones (TDZ and IAA), Murashige & Skoog, Electric field, Sucrose**

1. INTRODUCTION

Chlorophytum borivilianum, commonly known as Safed musli is a traditional medicinal plant which belongs to family Liliaceae. The genus includes about 300 species, which are distributed throughout the tropical and subtropical parts of the world. Tropical and subtropical Africa is the probable centre of origin of the genus, where about 85% of the species are found. In India *C. borivilianum* is mainly distributed in Southern Rajasthan, North Gujarat and Western Madhya Pradesh (Maiti and Geetha, 2005).

Thirteen species of *Chlorophytum* have been reported from India (Shariff and Chennaveeraiah, 1972). All these species differ in appearance; native species are sold as 'Safed musli' in the Indian drug market. Amongst these, *Chlorophytum borivilianum* produces the highest yield and highest saponin content (Shariff and Chennaveeraiah, 1972). Other important indigenous species are: *C. arundinaceum, C. tuberosum, C. laxum,* and *C. breviscapum* .

Chlorophytum borivilianum is a small perennial herb with a full crown of radical leaves appearing over the ground with the advent of summer rain. Its root tubers are fleshy, fascicled and directly originate from the stem disc devoid of any fibrous structure. They are cylindrical and 5 -20 in number. It has 6 -13 radical leaves spirally imbricate at the base, sessile in nature, linear or ovate with acute apex and slightly narrowed at the base. Then leaves spread horizontally, with smooth surfaces, wavy margins and parallel venation.

It is an annual herb with Tubers, Crown, Leaf and Flowers as different parts. Naturally occurs in forests of Gujarat, Madhya Pradesh and Maharastra States which are listed in the rare species of India. Safed Musli have annual demand around 35000 MT while only 15000 MT is produced. Safed Musli has natural oil, which is good manure for good and robust health and ideal for mother hood. It is also used in production of Chawanprash.

The root extract is primarily used as a tonic and rejuvenative for the reproductive system. For premature ejaculation, impotence, low sperm count in men. Used in chronic leucorrhoea, remedy for diabetes and arthritis. Basically this divine herb was available in abundance in our forests but due to unplanned and improper use of forest properties, this herb is in the verge of disappearance**.** Unfortunately the propagation of this highly valuable medicinal plant is not well managed scientifically and agriculturally which leads to several problems viz. misidentification of genuine plant species for alkaloid extraction, Year to year variability and degradation of active ingredient in process and storage of plant material and scarcity of planting material for domestication.

Purity of raw material for further processing is another problem which is also associated with raw roots as it is difficult to obtain ecologically unpolluted roots free from fungal and bacterial spores, insects and environmental contaminant. Low production of raw material is another hurdle for commercialization. Keeping in view the above associated problem, we tried to establish a foolproof *in vitro* regeneration system so that pure high yielding clones can be developed. The present study is a first step in this direction.

2. MATERIAL AND METHODS

Seeds of *Chlorophytum borivilianum* were procured form Central Institute of Medicinal and Aromatic Plants, Lucknow (CIMAP).

2.1 Plant Material and Culture Condition

Seeds were surface sterilized by 70% ethanol for 30 sec and 1% sodium hypochlorite solution for 15 min followed by three rinses with sterilized distilled water. All cultures were incubated at 26°C and 16-h photoperiod under 50 µmol/m^2/s irradiation provided by cool white fluorescent lamps in growth room. pH of the culture media were adjusted to 5.8 using NaOH (1N) or HCl (1N) before adding gelling agent (Agar-Agar, HIMEDIA). All culture media were sterilized by wet autoclave at 121°C for 15 min.

Total five following treatments were given to the seeds of *Chlorophytum* as per standard procedure and cultured *in vitro.* Equal number of seeds i.e., thirty seeds were soaked overnight in all the treatments–

a.) In TDZ (Thidiazuron) concentration 0.5 mg/l, 1.0 mg/l and 1.5 mg/l were used for overnight treatment.

b.) In IAA (Indol 3-Acetic Acid) conc.0.5 mg/l, 1.0 mg/l and 1.5 mg/l,

c.) Fresh Buffalo urine,

d.) Fresh Cow urine and

e.) Electric field 50 mA, 100 mA and 200mA just for 2 hrs.

The control seeds were soaked in distilled water.

3. RESULTS

All experiment was repeated thrice with 15 replicates per treatment. Results obtained were compiled in Table-1. Close analysis of results depict that seed germination rate in all treatments was found significantly higher in comparison to Control in term of days to germination and percentage of germinated seeds. As compare to control (53% germination in 35 days) the seed germination enhanced to 86% in average 16 days which could be considered to be encouraging. The best response was obtained in seeds soaked in 0.5 mg/l concentration of IAA, it took only 12.4 days to germinate and percentage of seed germination was also high (80%). Response of other concentration of IAA (1.0 and 1.5 mg/l) was also found suitable in comparison to control (seeds soaked in distilled water).

We used weak electrical current and found that 50mA current was insignificantly enhancing the growth. However 100mA current was found equally effective as IAA which was 77% germination in 13.2 days. Above 100mA the current treatment was found adversely affecting the process. However, the other growth regulator Thidiazuron, used in the present study showed moderate response in all the concentration used. Cow and buffalo urine, which has been frequently used by farmers for the pretreatment of seeds were not found suitable as percent and days to germination were low and more respectively.

Table-1: Percentage of seed germination after giving various treatments

Treatments		Days to Germinate		% seed germination	
		In Dark	In light	In Dark	In light
IAA	(0.5 mg/l)	12.7±0.5	16±1	85±1	72.6±0.5
	(1.0 mg/l	18.7± 0.5	22.3±0.5	77±1	72±1
	(1.5 mg/l)	19.7± 0.5	21±1.7	81.3±1.5	61±1
TDZ	(0.5 mg/l)	25.6± 1.5	27 ±1	55.6±1.1	53.3 ±0..5
	(1.0 mg/l	26 ± 2	28 ±1	47±1	61±1
	(1.5 mg/l)	26.6±1.5	24.6 ±1.1	54±1	46± 1
Buffalo Urine		28±1	27.3± 0.57	49.3±0.5	42±1.7
Cow Urine		31.6±1.5	32.6±1.2	54±1.7	46.3 ±1.3
Electric field	50V	18±1	20.6±1.5	74±1.7	65±1.5
	100V	13.6±0.57	18.6±1.1	76.3±1.5	71±1.5
	200v	No germination	No germination	No germination	No germination
Control (Distilled water)		39 ±1	42±1.7	41±1.0	41±1

4. DISCUSSION

Results showed that the all treatments are effective and significantly enhancing the seed germination rate in less possible days. Auxin was found most effective in present study The reason being the treatment of auxin possibly increased the permeability of the membrane for the diffusing water (Hopkins 1995).

Electrical currents were also found every promising on breaking seed dormancy and enhancing the growth of plant in vitro (Killi, 2004). In present study we tried the week current to explore the possibility of using electrical current in inducing germination and subsequent growth. In present study 50mA current was found insignificantly enhancing the growth. However 100mA current was found equally effective as IAA. Exposure of seeds to electric current of 100 mA may lead to change the auxin and cytokinin ratio in the seeds leading to the germination of otherwise recalcitrant seeds. It may further be the matter of extensive investigation to study the weak electrical signaling on initial growth of plant system. Role of expression of some protein of activation of corresponding gene which may help in breaking seed dormancy should be worked out. We used fresh cow and buffalo urine for treatment for seed. The idea was based on the common rural practice in which farmers in Indian villages treat seed with fresh urines. However, results shown no significant effect on either parameter studied.

It is an established fact that phytohormones have an inherent role in derepressing specific genes to activate protein synthesis (Key 1969). The inherent character of auxin in improving water content, protein synthesis and promotion of cell division and elongation favored the process of seed germination (Hopkins 1995).

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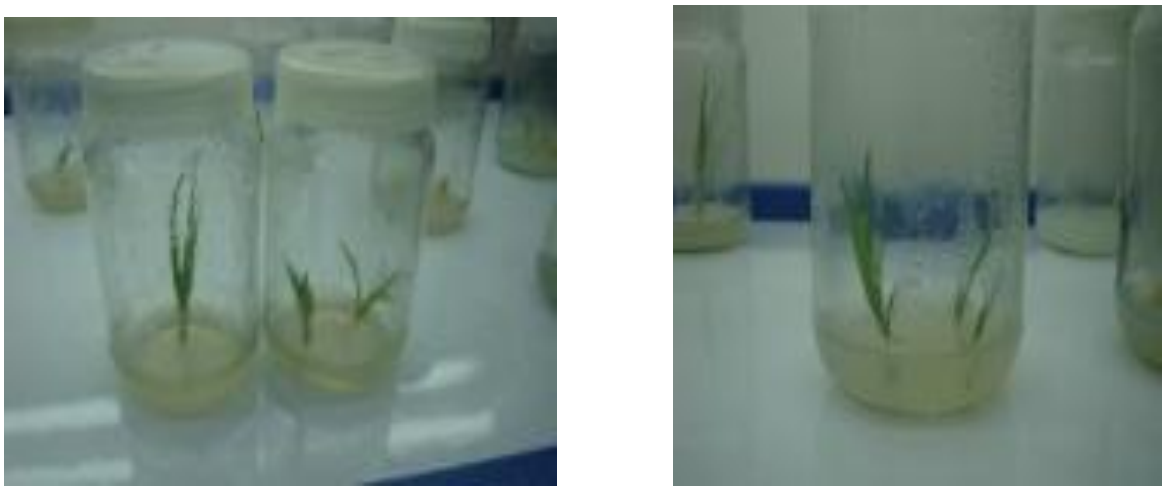

Fig.- 1 Plants under in-vitro conditions after germination of seeds with IAA treatment