

## Original Research Article

# Effects of light intensity and imbibition frequency of *in vivo* and *in vitro* propagated seeds of *Withania somnifera* (L.) Poshita on germination

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## ABSTRACT

### Keywords

*Withania somnifera*;  
Poshita variety;  
Germination

The present study investigates the effect of light intensity and imbibition frequency on seed germination and early seedling development of *Withania somnifera* (L.) Poshita variety. The plant is normally propagated through seeds. The number of plants obtained through *in vivo*, when the seeds are soaked in water for 24 and 48 hours are few in number. From the trials conducted, the seeds soaked for 72 hours, initially incubated in dark conditions were found to exhibit 100 per cent germination within 3 days. From the different concentrations of sucrose in half MS medium that were tested, the half MS medium with 3% sucrose was found to give 100% germination within 3 days of inoculation.

## Introduction

Pharmacological significance of *Withania somnifera* (L.) is due to the presence of secondary metabolites like withanolides (steroidal lactones) and alkaloids (Mirjalili *et al.*, 2009). The withanolides are steroidal compounds and bear resemblance, both in action and appearance to the active ginsenosides of Asian ginseng. Studies show that the plant has been used as an antioxidant, adaptogen, aphrodisiac, liver tonic, anti-inflammatory agent, antitumour, astringent and more recently, to treat ulcers, bacterial infection, venom toxins and senile dementia. The medicinal herb has always supported clinical trials and animal

research support the use for anxiety, cognitive and neurological disorders, inflammation, hyperlipidemia and Parkinson's disease (Gupta and Rana, 2007). Its increasing therapeutic benefits continuously attract the attention of pharmacologists for biomedical investigations on plant extracts and its isolated phytochemicals (Mulabagal *et al.*, 2009).

Sangwan *et al.* (2004) were of the opinion that high quality pharmaceuticals can be harvested from plant parts which are uniform both qualitatively and quantitatively. *There are reports that*

among 25 accessions of *Withania somnifera* (L.), the morphometric and root yield showed higher values in wild accessions as compared to the cultivated ones whereas, superiority in terms of Withaferin A content is exhibited by cultivated accessions (Kumar *et al.*, 2007). Such variations often lead to inconsistent therapeutic and health promoting properties of various commercial *Withania* preparations (Dhar *et al.*, 2006).

Another important point related to the cultivation is the high dormancy and low germination percentage of the seeds. Also, the seed viability is limited to one year (Rani and Grover, 1999), making the long term seed storage duration futile. The study is conducted to develop a method for high germination per cent of seeds.

## Materials and Methods

### *In vivo* germination of seeds

The Ashwagandha seeds (*Withania somnifera* (L.) Poshita variety) were collected from CIMAP (Central Institute of Medicinal and Aromatic Plants, CSIR), Lucknow. Hundred seeds of the Poshita variety were soaked in water for 24 hours, 48 hours and 72 hours and then sowed in soil. Germination percentage of the seeds were calculated.

### *In vitro* germination of seeds

Seeds were soaked for 24 hours / 48 hours / 72 hours and washed with 5% Teepol for 5 minutes followed by rinsing with sterilized distilled water. The seeds were sterilized with 0.1% (w/v) HgCl<sub>2</sub> for 5 minutes with continuous shaking followed by washing with sterile distilled water for three times, 5 minutes each. The seeds were inoculated on a series of jars

containing half MS basal medium with 1 to 6% of sucrose respectively. Later the cultures were kept in two laboratory conditions,

1. First set of bottles were incubated in dark condition at  $25 \pm 2^\circ\text{C}$  for a period of one week and then transferred to 16/8 hours photoperiodic condition with the light intensity of 3000 lux.

2. Second set of bottles were kept continuously under 16/8 hours photoperiodic conditions at  $25 \pm 2^\circ\text{C}$  with the light intensity of 3000 lux.

Both sets of seeds soaked for 24 hours, 48 hours and 72 hours were subjected to the trials under the two sets of environmental conditions and the data obtained were recorded. After germination, healthy and vigorously growing seedlings were selected and used as the source of explants.

### Statistical analysis

The experiments were carried out using a completely randomized design. Each treatment was repeated three times and each replicate consisted of twenty explants. Data were analyzed by analysis of variance (ANOVA) to evaluate significant difference between means. Data variability was expressed as the mean  $\pm$  SD. The results were analyzed by Sigma Stat version 3.1 of statistical package.

## Results and Discussion

Twenty five each of the Poshita variety soaked in water for 24 hours, 48 hours and 72 hours and sowed in pots containing soil gave the following results. The seeds soaked in water for 72 hours exhibited 100% germination within 10 days (Plate 1b). Thus, it is evident from the results that longer soaking time enhanced the

germination percentage. When seeds are non-dormant, imbibition of water is sufficient enough to trigger germination. Under normal conditions, the process can be completed within 48–72 hours after seed imbibition (Piskurewicz et al., 2008). Similar result was observed in the study as it showed showing 100% germination of the seeds soaked for 72 hours; before sowing.

#### ***In vitro* germination studies of *Withania somnifera* (L.) *Poshita* variety**

The explants collected from field can have higher percentage of contamination than the explants taken from in vitro germinated seedlings. Since the seeds can be germinated under in vitro conditions at all seasons of the year, explants for tissue culture can be obtained throughout the year. It is to be noted that the seeds will germinate in the field only during the months of August-September.

#### **Effect of light and sucrose concentration on seed germination**

The number of seeds that germinated at different concentrations of sucrose, light intensity and various hours of soaking are depicted in figures 3 to 5.

Significant ( $P>0.05$ ) difference was observed in the germination percentage of seeds at varying sucrose concentration. The seeds soaked in water for 24 hours and 48 hours inoculated on half strength MS medium containing various sucrose concentrations showed that, the medium containing 2% sucrose gave the highest germination in dark-light cycle (65%) after 30 days of inoculation. (Fig.3,6 &7). The seeds soaked for 72 hours and inoculated in 3% sucrose gave 100% germination within 3 days under dark condition (Plate

3f).

According to Rao and Ravishankar (2002) sucrose has been used as a common carbon source for plant tissue and cell cultures, serving as the principal energy source and a component for secondary metabolite biosynthesis. The rate of biomass growth is usually directly correlated with sugar consumption. Lian et al. (2002) reported that 3% sucrose in cell suspension culture showed maximum ginsenoside production in *Panax ginseng*. Kambizi et al. (2006) have reported that temperature and light have significant effect on the seed germination of certain species of *Withania somnifera* (L.) on MS medium supplemented with 3% sucrose. The cultures supplemented with 3% sucrose showed optimum accumulation of withanolide A ( $2.51 \text{ mg g}^{-1} \text{ DW}$ ) (Nagella and Murthy, 2010).

Seeds generally germinate easily upon imbibition of water if they are harvested at the right time, dried and stored properly. In this study, an increase in percentage of seed germination to the fullest extent was observed when the seeds were soaked for three days, which may be due to the increased absorption of water into the seeds, consequently triggering the physiological processes in the seeds, leading to germination. From the germination studies of seed varieties of *Psophocarpus tetragonolobus*, Rudrapal et al. (1992) have reported that the genotype of the plant played a vital role in determining the germinability of the seeds. Seeds usually germinate at low light intensities, Hartmann and Kester (1975), Sacande and Some (1992). Bahuguna and Pyare (1992) and Ahmed (2000) indicated the importance of light intensity during early growth, since it enhances germination. In the case of *Withania somnifera* (L.) *Poshita* variety low

**Figure.1** Effect of different soaking times on the germination of seeds of *Withania somnifera* (L.) Poshita Variety - *in vivo*

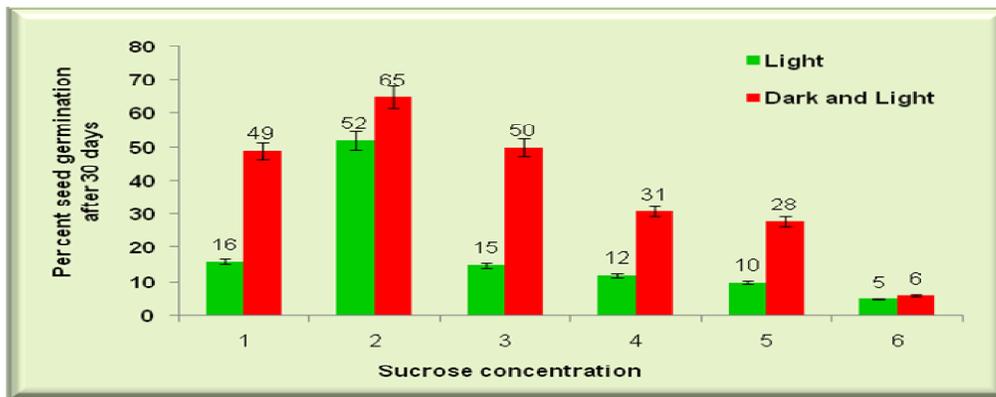
**Fig.1 (a)** Germination on 30<sup>th</sup> day after 24 hours of soaking in water



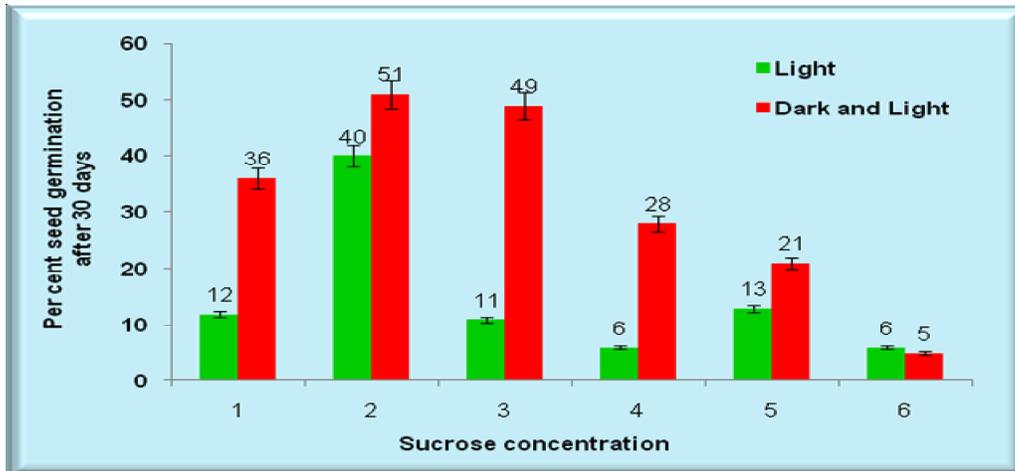
**Fig.2 (b)** Germination on 10<sup>th</sup> day after 72 hours of soaking in water



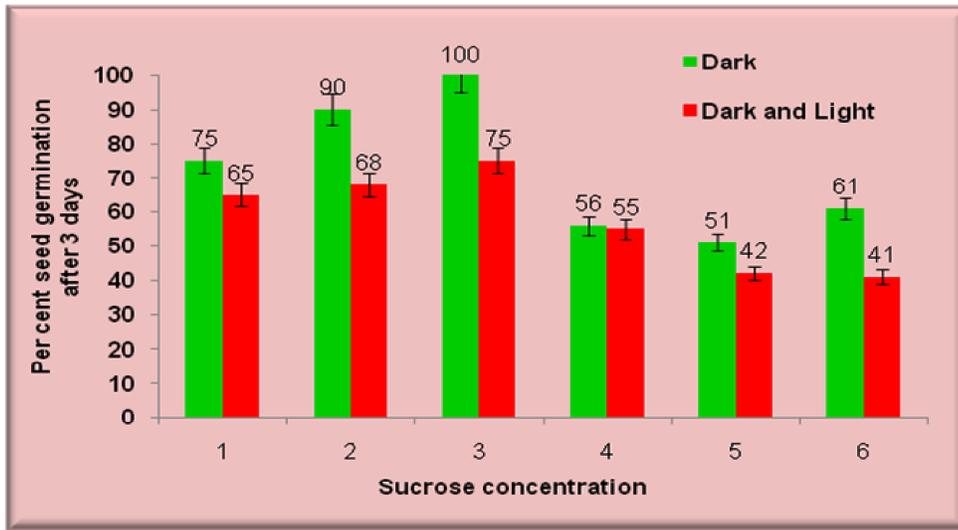
**Fig.3** Effect of ½ ms containing different sucrose concentrations and light intensity on *in vitro* cultured seeds after 24 hours soaking



**Fig. 4** Effect of ½ ms with different sucrose concentrations and light intensity *in vitro* after 48 hours soaking



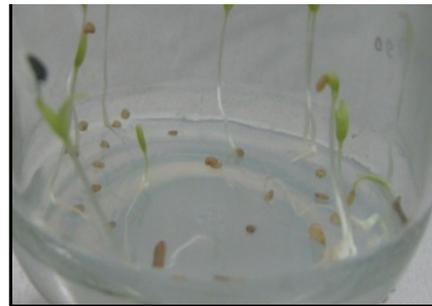
**Fig.5** Effect of different sucrose concentrations and light intensity *in vitro* after 72 hours soaking



**Fig.6** Germination of the seeds soaked for 24 hours observed on 30 days



**Fig.7** Germination of the seeds soaked for 48 hours observed on 30 days



**Fig.8** Germination of the seeds soaked for 72 hours observed on 15 days



intensity and high imbibition frequency induced high percentage germination seeds. These germination and acclimatization protocols which focus on propagating *Withania somnifera* (L.) Poshita seedlings by reintroduction can help in the conservation of the plant species which is at the risk of being endangered.

From these studies regarding the effect of ½ MS containing various concentrations of sucrose and light intensity on seed germination it is noted that dark condition in the initial days of incubation is more and the initial stage than it has to spend some energy containing compounds to defend against the effect of light. Conversely, in dark the entire energy coins can be spending for metabolic activities leading to germination. The germinated seeds showed flourishing growth when transferred to light because they start synthesizing food materials in presence of light.

## Conclusion

From these studies regarding the effect of ½ MS containing various concentrations of sucrose and light intensity on seed germination it is noted that dark condition in the initial days of incubation is more and the initial stage than it has to spend some energy containing compounds to defend against the effect of light. Conversely, in dark the entire energy coins can be spending for metabolic activities leading to germination. The germinated seeds showed flourishing growth when transferred to light because they start synthesizing food materials in presence of light.

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