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Seed Germination of *Withania somnifera* (L.) Dunal

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Authors' contributions

This work was carried out in collaboration between all authors. Author AN performed experiments, statistical analysis, wrote the first draft of manuscript. Author ENS designed the study, managed the analysis and corrected the draft .Both authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: *Withania somnifera* (L.) Dunal or Ashwagandha is a valuable medicinal plant having a consistent demand in pharmaceutical industries. Traditionally it is propagated from seeds but it's germination capacity is poor. So in the present study different methods were considered to sort out a suitable method that can be used for its cultivation in the field of Hazaribag, Jharkhand, India.
Study Design: The study was done in the laboratory and field of Hazaribag, Jharkhand, India.
Place and Duration of Study: Department of Botany, Vinoba Bhave University, Hazaribag, Jharkhand, India. The study was carried out from July 2012 to August 2012 and again from mid June 2013 to August 2013.
Methodology: Different pre germination treatments including 24 hours water soaking , 48 hours water soaking, mechanical scarification, heat treatment at 50 degree Celsius (5min, 10min, 15min) and Gibberellic acid [GA3] (250µg/l, 500µg/l, 1000µg/l) treatments were proposed. The mean germination percentage and the mean germination time were calculated for each of the treatments.
Results: The results revealed that GA3 500µg/l treated seeds showed increased germination percentage in laboratory (86±0.34%) and in soil (84.1±0.36%) as well as reduced mean germination time in laboratory (5.8±0.41 days) and in soil (10.6±0.17 days). Whereas heat treatment at 50 degree Celsius (5min, 10min, 15min) drastically

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reduced germination percentage in laboratory (32±0.47%, 16±0.36%, 12±0.31%) and in soil (51±0.49%, 49.6±0.49%, 32.2±0.46%), respectively.
Conclusion: Pre treating fresh seeds of Ashwagandha with GA3 500µg/l for 24 hours before sowing can be adopted to overcome dormancy of seeds with good germination percentage. Likewise mechanical scarification of seeds can also be taken into consideration as an alternative, cost-effective and eco-friendly way to break seed dormancy which has also given favourable results.

Keywords: Withania somnifera; ashwagandha; pretreatment; seed germination percentage; mean germination time.

1. INTRODUCTION

Withania somnifera (L.) Dunal known as Winter Cherry in English or Ashwagandha in Hindi language as it is commonly called in India is a plant of immense medicinal importance. It has a wide application in pharmaceutical industries. It is an ingredient in many formulations prescribed for a variety of musculoskeletal conditions (e.g. arthritis , rheumatism) and as general tonic to increase energy, improve overall health and longetivity and prevent disease in atheletes, the elderly and during pregnancy [1,2]. Numerous studies conducted on the plant indicated that they possesses antioxidant, antitumor, antistress, antiinflammatory, immunomodulatory, heamatopoetic, antiageing, anxiolytic, antidepressive, rejuvenating properties and was also found to influence various neurotransmitter receptors in the central nervous system [3]. Ashwagandha is found to be major ingredient of various adaptogenic and antistress tonic [4]. *Withania* contains the active ingredients such as the steroidal alkaloids and lactones which is known as 'withanolides'. Withaferin A and withanolide D are the two important withanolides that was found to contribute to most of the biological or pharmacological actions of *Withania* [5]. Ashwagandha grows primarily in Madhya Pradesh, Rajasthan, Gujrat, Karnataka, Uttar Pradesh and Punjab states of India but not found wild in Jharkhand. According to an estimate the annual requirement of the drug in India is about 9127.5 tons far exceeds the annual production of about 5905.1 tons under cultivation [6,7]. The herb has been identified by the National Medicinal Plant Board of India as one of the thirty two selected priority medicinal plants, which are in great demand in the domestic and international markets [8]. The study of seed germination is fundamental for understanding the growth and development of plants. The successful cultivation of plants depend on the quality and germination behaviour of the seeds. Seed germination is one of the most key processes in the plant survival and growth among the stages of the plant life cycles in the arid and semiarid regions of the world [9]. Traditionally *W. somnifera* is propagated from seeds but the germination is very low [10-12]. There may be possibility of dormancy in the seeds that prevent germination immediately. Different reports on this species suggest that germination percentage can be improved by the application of presowing chemical treatments [13,14,] or by providing congenial growth environment [15-17]. Earlier studies have reported an increase in the seed germination percentage in *W. somnifera* seeds when pretreated with GA_3. Invigoration studies in *W. somnifera* treated with 100µg/l GA_3 resulted in vigorous growth of seedling under laboratory condition [18]. According to a recent work, the most effective treatment is GA_3 150µg/l concentration at 25ºC. [19]. Hot water soaking (80ºC for 5min) resulted highest germination percentage in *Rauvolfia serpentina* [20]. Another study revealed hot water treatment resulted in 70% of germination [21]. Water is an indispensable factor in the external environment for the stimulation of germination in seeds. Soaking the seeds in water at room temperature helps in softening the seed coats, removal of inhibitors and reduces the time required for germination and increases the germination

percentage [22]. These previous works were adopted to check the germination percentage and analysed to find a better option that can be used for the cultivation of Ashwagandha in the ecological condition of Hazaribag.

2. MATERIALS AND METHODS

2.1 Sample Collection and Plantation

The experiments were conducted at Hazaribag, Jharkhand, India during June 2013 to August 2013. Hazaribag is located at 23.98ºN, 85.35ºE at an elevation of 604m above sea level. The soil type used was silt and 50% sand with pH6.8, organic carbon (0.2)% and 81% water holding capacity [23]. Seeds were collected from the healthy berries of the plant of Ashwagandha from a local nursery at Hazaribag. The seeds were removed from the berries, air dried for a week and kept in air tight plastic bags at room temperature for a maximum period of fortnight until used for experiment. Ashwagandha seeds were sown with the onset of monsoon i.e. in the mid June 2013 to August 2013. Different methods of germination were applied to compare germination percentage. The work was done both in laboratory condition and in the field.

2.2 Pre Germination Treatments

Different pre germination treatments were proposed before putting the seeds to germination test. These were mechanical scarification with sand paper; 24 hours water soaking ;48 hours water soaking; heat treatment (50 degree Celsius) for 5min, 10min & 15min; GA_3 (250µg/l, 500µg/l,1000µg/l) for 24 hours and control.

2.3 Seed Germination Methods in Laboratory

Twenty five seeds were placed on blotting paper backed with moist cotton wool in a petridish (15cm×15cm) for each of the different methods of germination undertaken. Three replicas were made for each set. Demineralised distilled water was used as germinating medium. The petridishes were moistened daily. Under constant temperature and moisture, the observations were done everyday regularly for 30 days. Radicle emergence was considered as a criterion of seed germination [24].

2.4 Seed Germination in Field

In order to study the germination behaviour of Ashwagandha in the ecological condition of Hazaribag, it was necessary to conduct germination treatments in the field. Local soil was filled in earthen pots. Thirty seeds were sown in each pot at a depth of 1cm after undergoing different pre germination treatments. There was no application of fungicides to the seeds. There were three replicas for each set and the pots were watered regularly. Seeds were observed daily for the emergence of radicle. The observations were done for 30 days and the germination percentage was calculated.

2.5 Germination Percentage

$$\text{Germination \%} = (\text{no. of seeds germinated} \div \text{total no. of seeds in each set}) \times 100$$

Using the daily counts, the mean germination time (MGT) was calculated for each set up using the formula given below [25].

$$\text{Mean Germination Time (MGT)} = \sum n\, D / \sum n$$

Where n = no. of seeds newly germinated at time D
D=days from the beginning of germination test
$\sum$ n= final germination

3. RESULTS AND DISCUSSION

Seed germination results of *W. somnifera* in laboratory and soil conditions are given in Table1. When it is compared with control, GA_3 application, mechanical scarification and 24 hours water soaking indeed increased the germination percentage having maximum value in laboratory i.e. 86±0.34 with GA_3 500µg/l. This is in conformity with the previous work [26]. Next to GA_3 application , the mechanical scarification was also effective in enhancing the mean germination percentage which varied from 68.8±0.46 to 78.6±0.41.This may be attributed to the fact that scarification of seeds have made imbibition of water through seed coat easier and hence improved germination percentage. This observation is further confirmed by the present study of 24 hours water soaking without scarification which has very little impact in enhancing the mean germination percentage .This is in conformity with the previous work [27] in which mechanical scarification with sand for 6 min. followed by soaking in GA_3 500µg/l for 5 hours significantly improved germination by 25% over control. Seeds treated with hot water i.e. 50 degree Celsius proved deteriorative for seed germination. This might be so, as hot water treatment might have proved injurious to embryo leading to germination inhibition.

Table 1. Seed germination % in laboratory and soil condition

Treatments	Mean germination %±SE in laboratory	Mean germination %±SE in soil
Control	61.3±0.48	53±0.49
24 hours water soaking	68±0.46	64.4 ±0.48
48 hours water soaking	42.6±0.49	62.1±0.49
GA_3 250µg/l	74.6±0.43	73±0.44
GA_3 500µg/l	86±0.34	84.1±0.36
$GA_3$1000µg/l	80±0.40	70±0.45
Mechanical scarification	78.6±0.41	68.8±0.46
Heat treatment 5min	32±0.47	51±0.49
Heat treatment 10min	16±0.36	49.6±0.49
Heat treatment 15min	12±0.31	32.2±0.46

The study of mean germination time (MGT) are given in Table 2 which reveals that in each of the set up the higher the MGT, the slower and more prolonged is the germination period. The MGT was found to be minimum in case of GA_3 500µg/l treated seeds in both laboratory and soil conditions. This means that it took less time for seed treated with GA_3 500µg/l to germinate. A good result was also shown in the case of mechanically scarified seeds which took less time to germinate than the control seeds.

Seed germination is affected by the ecological condition prevailing in the habitat and it depends on several environmental conditions including pH and type of soil. *W. somnifera* is rarely reported to grow well in sandy soil with pH 7.5 to 8 [28] from Eastern India including West Bengal plains [29]. Hazaribag also falls in the same zone with sandy soil and pH 6.8.Therefore, the germination percentage of seeds in control soil condition was very poor i.e. 53±0.49 Table 1.

Table 2. Mean germination time (MGT) in laboratory and soil condition

Treatments	Mean germination time (days) in laboratory±SE	Mean germination time (days) in soil±SE
Control	9.1±0.01	11.6±0.34
24 hours water soaking	7.8±0.71	12.1±1.90
48 hours water soaking	10±0.36	10.1±0.26
GA_3 250µg/l	6.5±0.38	10.9±1.42
GA_3 500µg/l	5.8±0.41	10.6±0.17
$GA_3$1000µg/l	5.4±0.69	11.9±0.87
Mechanical scarification	5.5±1.11	11.7±2.01
Heat treatment 5min	14.8±0.43	15.6±0.86
Heat treatment 10min	13.1±0.58	14.9±1.41
Heat treatment 15min	13.6±0.94	13.6±0.24

W. somnifera is commercially propagated by means of seeds because of lack of natural ability for vegetative propagation [30]. Stored seeds showed very poor germination percentage [31]. So, in the present study, seed germination percentage was studied both in laboratory condition and soil condition of Hazaribag using fresh seeds collected from ripe fruits. The germination percentage in the control soil condition was very poor. The different methods checked to improve germination percentage of seeds of Ashwagandha have to be adopted which will help in the cultivation of Ashwagandha in Hazaribag.

4. CONCLUSION

The seed germination of *W. somnifera* can be enhanced by pretreating seeds with GA_3 500µg/l for 24 hours before sowing. This increased germination percentage as well as reduced the mean germination time .The mechanically scarified seeds also showed a much favourable result. This suggest that mechanical scarification of seeds can be applied as a very suitable, cost–effective and eco-friendly method which is easy enough to be used by local, unskilled farmers to combat seed dormancy of Ashwagandha seeds and hence improve seed germination.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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