Germination behaviour of seeds of *Withania somnifera* (L.) Dunal: a high value medicinal plant

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Abstract In order to evolve a quick method for smooth and optimum germination for *Withania somnifera*- a medicinally efficacious multipurpose plant, present investigation was carried to study the effect of physico-chemical treatments, storage, temperature, photoperiod and growth regulators (GA₃, IAA, IBA, 2–4 D and BA) on germinability. The most effective treatment is GA₃ at 150 µg/ml concentration at 25 °C. The optimal temperature for germination is 25 °C and continuous light favored germination showing that photoperiod has a significant role. The seedlings derived from seeds performed well when grown in a glasshouse. The data have implications for conservation and cultivation of the species studied.

Keywords Seed germination · Growth regulators · Temperature · *Withania somnifera*

Introduction

The demand for medicinal plants has increased globally due to the resurgence of interest and acceptance of herbal medicine. Most of the demand is being met through collection of large quantities of medicinal plants from wild populations. The methods of extraction employed are almost invariably

crude and unsystematic. As a consequence, the rates of exploitation may exceed those of local natural regeneration (Sharma et al. 2006; Pushpangadan and Nair 2001; Rao and Rajasekharan 2002). There is thus an urgent need to develop and implement regeneration and conservation strategies for the exploited medicinal plant species. The common means of regeneration and propagation of medicinal plants include seed-based, clonal and micro-propagation methods. Seed based multiplication is the most effective and convenient means for most species.

Withania somnifera (Solanaceae) also known as Winter cherry or Indian Ginseng is a herbal medicine that comes from the Ayurvedic tradition. Most of the scientific literature and herbal lore on the plant remedy therefore came from India. It is a predominantly sexually reproducing perennial herb (some types are annual) distributed in dry tropical regions of Asia and Africa. An erect branching undershrub reaching about 30–150 cm in height, usually clothed with minutely stellate tomentum; flowers are greenish or lurid yellow in axillary fascicles, bisexual, pedicel long, fruits globose, berries which are orange colored when mature, enclosed in a persistent calyx. This species flowers from March–May and berries start maturing in June–July under Indian conditions. Distinct berry colour red and orange are encountered in cultivated and wild population respectively as per Khanna et al. 2007. The two contrasting accessions have been collected and grown under uniform environmental conditions for the present seed germination studies. The number of seeds in red berries range from 18 to 24, whereas in orange berries it is 22–25. Moisture content of fully mature berries and seeds on an average is 37 % and 14 % respectively.

Some preliminary observations on seed germination have been reported in seedlots collected from different eco-

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geographical zones (Nigam 1987a, b). It is reported that the seed germination is poor and mortality rate of seedlings is high under field conditions (Karnick 1978; Kumar et al. 2001). However, detailed studies under controlled conditions are needed. This study is an effort in this direction.

Materials and methods

Seeds of two different accessions were collected from Manasa (Madhya Pradesh) in Central India coded as AGB-001 is dwarf type (40–50 cm) with orange berries and Jammu (Jammu and Kashmir) in North India coded as AGB-004 is tall type (90–110 cm) with red berries. As per Kumar et al. 2007; 2011 AGB-001 is a cultivated type while AGB-004 is wild one. These were grown under uniform environmental conditions and data recorded were of two complete seasons/populations. Fully mature berries of both the accessions were collected from cultivated populations, dried at room temperature for 15 days as the low moisture content is required before the germination can begin. If the seeds remain moist after dispersal, germination can be delayed for many months. The seeds were separated and stored in dry plastic containers. The moisture content tests of seedlots were conducted as per standards of International Seed Testing Association 1985. Seeds were treated with fungicide-0.3 % Dithane M45 solution for 15 min and washed before use. Assessment of seed viability was tested with 1 % solution of TTC (2, 3, 5-triphenyl tetrazolium chloride). The seeds were kept in TTC solution for 6 h in dark, thereafter the solution was drained off and seeds were washed with distilled water to check staining intensity. Hundred seeds were used in each case with four replications in Petri dishes (90 mm diameter) lined with Whatman No. 1 filter paper moistened with sterile distilled water. Germination was checked every 24 h under controlled conditions in germinators and seedlings removed. Physical treatments have generally been used to enhance germination and to increase seedling vigour. Seeds were mechanically scarified with sand paper and soaked in distilled water for 3 h before sowing. Running water treatment - Seeds were kept under running tap water for 3 h before sowing. For hormonal treatments, varying concentrations 150, 250, and 350 µg/ml of gibberellic acid (GA₃); indole acetic acid (IAA); indole butyric acid (IBA); 2–4 di-chlorophenoxyacetic acid (2–4-D) and benzyl adenine (BA) were used as pre-germination treatments. Seed germination of both the accessions was recorded at thermoperiods of 20° and 25 °C under continuous light (6–8 K Lux), diffused light (1–2 K Lux) and continuous darkness. For dark treatment, the Petri dishes were wrapped with black paper.

The emergence index (Ei) as per Baskin 1969, speed of germination (SG) in accordance with Babeley and Kandya 1986 and coefficient of velocity of germination (CVG) as described by Kotowski 1926 were calculated by using following statistical formulae:

$$Ei = n1/dn1 + n2/dn2 + n3/dn3 + \dots nx/dnx$$

$$SG = n/t$$

$$CVG = En/E(nt) \times 100$$

Where n=No. of seeds germinated on the day 1

T or d=Time or days from soaking

The data was statistically analysed as per Panse and Sukhatme 1967. The values were mean±SD for eight samples and critical difference (CD) values were calculated at 5 % level of significance.

Results and discussion

Variability is exhibited in seed shape, size, weight and texture of Ashwagandha. Moisture levels of mature seeds also differ in different accessions. The average seed weight of 100 seeds of AGB-001 is 228±3.46 mg with 15.26 % moisture (30 days after harvesting). Seeds are more or less rounded with smooth texture. In AGB-004 the seeds are more or less oblong with oily texture having average seed weight of 148±3.96 mg (100 seeds) and moisture level of 13.87 %. Results depict that *Withania somnifera* seeds do not require any seed rest for longer time. Data presented in Table 2 show that physical treatments are significantly effective at 25 °C in both the accessions. In AGB-001 mechanical scarification and running water treatments gave more or less similar results (65 % and 67 % germination), whereas in AGB-004 the germination percentage was lower (52 % and 55 %), both significant at 5 % level. However at 20 °C, AGB-004 exhibited very poor germination percentage 8 % and 11 % against 37 % and 43 % in AGB-001, which in both cases was not significant against control.

Results indicate that out of six growth regulators, gibberellic acid (GA₃) treatment was effective in enhancing the germination. The optimum response was recorded at 150 µg/ml concentration in both types at 25 °C. In AGB-001 highest germination 98 % at 25 °C was recorded against 55 % in control, whereas it was 60 % in AGB-004 against 40 % in control. Only 4 days were required for onset of germination which was found to be significant at 5 % level. The response was relatively poor and statistically insignificant in all the treatments at 20 °C in both the accessions (Tables 1 and 2). The optimum temperature required for maximum germination is 25 °C which got significantly increased by application of gibberellins. Seeds treated with GA₃ start germination after 4–5 days of sowing in Petri

Table 1 Effect of physical and chemical treatments on germination of *Withania somnifera* (L.) Dunal at 20°C temperature (data from periodic observations compiled after 3 weeks)

Treatment	Days required for onset of germination	Germination %	
		AGB-001	AGB-004
1. Control	11	35 (36.7)	09 (17.4)
2. Physical			
i) Mechanical scarification	8	37 (37.4)	08 (16.4)
ii) Running water	8	43 (40.9)	11 (19.3)
3. Growth regulators			
A Gibberellic acid			
i) 150 µg/ml	7	48 (43.8)	13 (21.1)
ii) 250 µg/ml	7	35 (36.2)	10 (18.4)
iii) 350 µg/ml	7	34 (35.6)	10 (18.4)
B Indole acetic acid			
i) 150 µg/ml	9	23 (28.6)	08 (16.4)
ii) 250 µg/ml	8	23 (28.6)	10 (18.4)
iii) 350 µg/ml	7	21 (27.2)	09 (17.4)
C Indole butyric acid			
i) 150 µg/ml	10	29 (32.5)	08 (16.4)
ii) 250 µg/ml	8	32 (34.4)	13 (21.1)
iii) 350 µg/ml	9	26 (30.6)	08 (16.4)
D α-Naphthyl acetic acid			
i) 150 µg/ml	9	26 (30.6)	06 (14.1)
ii) 250 µg/ml	9	30 (33.2)	05 (12.9)
iii) 350 µg/ml	8	28 (31.9)	08 (16.4)
E 2-4-dichloro phenoxy acetic acid			
i) 150 µg/ml	10	20 (26.5)	08 (16.4)
ii) 250 µg/ml	10	20 (26.5)	06 (14.1)
iii) 350 µg/ml	9	22 (27.9)	06 (14.1)
F Benzyl adenine			
i) 150 µg/ml	9	15 (22.7)	05 (12.9)
ii) 250 µg/ml	10	19 (25.8)	06 (14.1)
iii) 350 µg/ml	10	18 (25.1)	06 (14.1)
CD at 5 %		NS	NS

Values in parentheses are arc sin√% of percent value

dishes, whereas it takes 7–8 days in control as well as other PGR treatments. Seeds of both the accessions showed that rate of germination is quite high during first 2 weeks and maximum germination is obtained from 13 to 15 days after sowing. Thereafter, there is not much increase in number of seeds germinated. Continuous light at 25 °C favored seed germination in both the accessions (55 %±2.56 in AGB-001 and 40 %±3.22 in AGB-004) although seeds showed relatively less germination under complete darkness (47 %±4.34 in AGB-001 and 35 %±1.73 in AGB-004). Data presented in Table 3 shows that the accession AGB-001 has greater value in terms of germination capacity (55 %), emergence index (4.58), speed of germination (10.05) and coefficient of velocity of germination (12.05) over the accession AGB-004 with these values as 50 %, 4.16, 8.33 and 9.21 respectively.

Effect of different physical and hormonal treatment on release of dormancy shows that compared to physical treatments, hormonal treatments were more effective at both the temperatures (20 °C and 25 °C). In recent years, targeted analysis of plant hormones has revealed novel aspects of seed physiology and complex metabolic balance between multiple plant hormones during development and germination (Nambara and Nonogaki 2012). Role of inorganic, organic nutrients, plant growth regulators and appropriating thermo and photoperiodic conditions have been demonstrated for breaking morpho-physiological dormancy and enhancing seed germinability (Ahmad et al. 1986; Bankar 1987; Sharma and Govil 1985; Vashisht et al. 1987; Verma and Tandon 1988). Since gibberellins are known to activate hydrolytic enzymes which increased osmotic content of seeds and hydrolyse the starch reserves in the endosperms,

Table 2 Effect of physical and chemical treatments on germination of *Withania somnifera* (L.) Dunal at 25°C temperature (data from periodic observations compiled after 3 weeks)

Treatment	Days required for onset of germination	Germination %	
		AGB-001	AGB-004
1. Control	8	55 (47.8)	40 (39.2)
2. Physical			
i) Mechanical scarification	7	65 (53.7)	52 (46.1)
ii) Running water	7	67 (54.9)	55 (47.8)
3. Growth regulators			
A Gibberellic acid			
i) 150 µg/ml	4	98 (81.8)	60 (50.7)
ii) 250 µg/ml	5	90 (71.5)	58 (49.6)
iii) 350 µg/ml	4	80 (63.4)	50 (45.0)
B Indole acetic acid			
i) 150 µg/ml	5	64 (53.1)	41 (39.8)
ii) 250 µg/ml	6	63 (52.5)	40 (39.2)
iii) 350 µg/ml	5	60 (50.7)	38 (38.6)
C Indole butyric acid			
i) 150 µg/ml	5	62 (51.9)	40 (39.2)
ii) 250 µg/ml	5	60 (50.7)	39 (38.6)
iii) 350 µg/ml	5	61 (51.3)	39 (38.6)
D α-Naphthyl acetic acid			
i) 150 µg/ml	6	50 (45.0)	33 (35.0)
ii) 250 µg/ml	7	52 (46.1)	36 (36.8)
iii) 350 µg/ml	7	53 (46.7)	36 (36.8)
E 2-4-dichloro phenoxy acetic acid			
i) 150 µg/ml	7	50 (45.0)	38 (38.0)
ii) 250 µg/ml	8	50 (45.0)	38 (38.0)
iii) 350 µg/ml	6	48 (43.8)	36 (36.8)
F Benzyl adenine			
i) 150 µg/ml	6	60 (50.7)	38 (38.0)
ii) 250 µg/ml	7	58 (49.6)	36 (36.8)
iii) 350 µg/ml	6	57 (49.0)	36 (36.8)
CD at 5 %		(15.02)	(07.31)

Values in parentheses are arc sin√% of percent value

stimulatory effect of GA₃ on germination of both dormant and non-dormant seeds has been widely reported (Paleg 1960). Exogenous application of 150 µg/ml GA₃ was found to be most effective in promoting germination. Washing of seeds under running water also favoured higher germination over control in both the accessions.

Temperature is an important environmental factor for germination and establishment of seedlings of a particular species. The optimal temperature for most of the agricultural

plants seems to be from 25 to 35 °C. The importance of temperature in seed germination has been emphasized by various workers (Bankar 1987; Young et al. 1981) and result presented in Tables 1 and 2 clearly show that at lower temperature i.e. 20 °C germination was poor irrespective of both the accessions, whereas at higher temperature i.e. 25 °C germination was good and significantly increased by the application of growth regulators. Critical examination of data shows that variation in germination in *Withania*

Table 3 Germination behavior in two accessions of *Withania somnifera* (L.) Dunal at 25°C

Accession	Dry wt. of 100 seeds (mg)	Seed viability (%)	Germination capacity (%)	Ei	SG	CVG
AGB-001	228±3.46	98±3.46	55±2.56	4.58	10.05	12.05
AGB-004	148±3.96	85±2.03	50±2.21	4.16	8.33	9.21

Ei Emergence index, SG Speed of germination, CVG Coefficient of velocity of germination

somnifera is mainly due to type differences. Barring a few types overall seed germination in *Withania somnifera* is low. Application of IAA, GA₃ or thiourea alone or in combination with mechanical scarification enhanced the germination percentage in *Withania somnifera* under field conditions (Hussain and Illahi 1988). Light is a critical regulator of seed germination in small seeded plants (Seo et al. 2009). Continuous light condition favoured early germination in both the accessions and it also enhanced germination as compared to continuous dark. This study revealed that seed germination is highly influenced by photoperiod in this plant. Seed size, weight, texture, moisture content and longevity are most important factors affecting germinability (Ruberts 1972; Zheng et al. 1998). Our results indicate that the seed moisture content (MC) is the most important factor affecting seed longevity. With the advancement of age, moisture content (MC) decreases considerably and germination increases upto optimum moisture level (6.89 and 6.49 %) in both the accessions. Thereafter, with the decrease in moisture content decrease in the germination percentage is recorded in both the types. The critical moisture content of *Withania somnifera* seeds ranges from 6.5 to 6.9 %. Harrington 1973 reported that seeds die faster at 4 % as compared to 6 % MC, which indicates that in majority of cases seeds can be stored at an approximate range of 6.0 to 6.5 % MC levels. Below this MC level longevity and viability gets considerably reduced. In *Withania somnifera* there are short-lived seeds, whose longevity is about 12 months. The short-lived seeds can be classified into two types by their physiological characteristics in shortage- one endures dry conditions and the other type- such as recalcitrant seeds loses viability during desiccation. Our results have shown that *Withania somnifera* fits in first type as evidenced by its natural preference to grow in dry conditions. Similar results have been reported by Huang et al. 2003 in *Haloxylon ammodendron*, a shrubby perennial of deserts in North- West China. Seeds remain viable upto 1 year, after which germination decreases considerably in all the accessions studied. This is due to seeds degenerate with age, although a certain percentage of various seeds regenerate after storage of many years. The reason for this loss of vitality is probably not the loss of stored food, for even aged seeds contain large amounts of reserve food material. However, the changes in the protein fraction of the seeds have been found to take place (Bhatia 1984). A decrease in the true protein content and an increase in smaller compounds such as amines, amides and amino-acids resulted in low germination. Longevity of seeds depends on the retention of the original structure of their proteins. The seeds of the *Withania somnifera* are light in weight. Seed size and weight are known to be related to germination and vigor as per Ruberts 1972. In the present study, the seed weight of two accessions of *Withania somnifera* showed some improvement in percentage of seed germination (Table 3). AGB-001

exhibited higher value (228 mg/100 seeds) over AGB-004 (148 mg/100 seeds). Also, variation in germination was mainly due to type differences rather than due to seed weight. The germination capacity (%) of AGB-001 and AGB-004 was practically 55 % and 50 % respectively but it showed higher value of emergence index (4.58), speed of germination (10.05) and coefficient of velocity of germination (12.05) as compared to AGB-004.

Conclusions

The results of the present study depicted synergistic activity of temperature, gibberellic acid and light indicated by improved seed germination, that can be exploited to obtain uniform seed germination under controlled conditions for raising large populations of desired accession for commercial cultivation.

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