

## Effect of seed pre-treatments on germination of ashwagandha (*Withania somnifera*)

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**ABSTRACT** Effect of seed pre-treatments on per cent germination of different seed lots of ashwagandha (*Withania somnifera* Dunal) among various physical and/or chemical treatments applied, pre-washing the seeds in running water under laboratory ambient temperature increased this germination values due to removal of certain inhibiting chemicals accumulated in seed coat. Thus, pre-washing seed treatment in running water for ashwagandha ranked as the best followed by application of gibberellic acid. Seed dormancy was due to certain chemicals present in seed coat, as pre-washing of seeds enhances seed germination. Pre-washing of ashwagandha seeds in running water, under laboratory ambient temperature ranked as the best, eco-friendly and economical.

**Key words:** *Withania somnifera*, seed, pre-washing, dormancy, GA<sub>3</sub>, germination

Ashwagandha, most important medicinal plant. It is a small or middle-sized shrub, growing 30 cm-1.5 m in height. Its commercial cultivation through seeds generally is late, erratic with poor germination [1] and substantial loss in seed viability. The major problem in its cultivation is poor germination percentage and establishment at field level. The quality of seed determines the potential for its field emergence, yield and its storability. The seed germination is first and foremost step in seed quality assurance, optimizing yields of a given seed lot. Seed testing protocols in some medicinal plants are available. Further, the International Seed Testing Association (ISTA) recommends and prescribes the seed testing protocols (Requirements of temperature and substratum regularly). The information on requirement of temperature and substrate for seed germination in ashwagandha was neither available in ISTA rules [2] nor any systematic study has been reported. Some fragmented information on seed germination in ashwagandha was available in literature [3] and that needs validation. In order to encourage its successful commercial cultivation

by seeds, standardization of germination testing procedure is essential as suggested for other medicinal crops [4]. In India, at least five different cultivars have been developed, adapted to different agroclimates. Presence of seed dormancy for a short period after maturity is beneficial in arresting seed germination within a fruit and also for adaptation in wild. However, long-period of seed dormancy poses problem in cultivating a species, when immediate sowing and/or seed testing is required. Since some pre-sowing treatments were reported to be effective in optimizing germination in seeds of various medicinal plant species [5, 6], therefore, application of pre-sowing treatments (Both physical and chemical) also needs to be investigated.

### MATERIALS AND METHODS

The experiment was conducted on procured seed lots belonging to different ecological regions, following ISTA rules [2] recommended for other Solanaceous crops (Tomato and brinjal) and previous work done on ashwagandha seeds [7] at IARI, New Delhi. From each, seed lot 400 seeds (100 seeds each in 4 replicates) of pure seed fraction

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was drawn at random and placed equidistantly on a double layer of moist filter paper discs in plastic Petri plates (11 cm diameter). Seed germination studies using different treatments *viz.* GA<sub>3</sub>, washing in running tap water, 2% thiourea, 0.2% KNO<sub>3</sub> for 24 hr, 1% H<sub>2</sub>SO<sub>4</sub> and pre-chilling at 4°C for 7 days in light at constant 25°C. Seeds were washed thoroughly with tap water. For this, seeds were kept in a muslin cloth bag, tied with running water tap for 24, 48, 72, and 96 hr, respectively, under laboratory ambient temperature. The washed seeds were taken at periodic intervals for seed germination using TP at 25°C in light. Seeds were treated with 100, 200 and 500 ppm aqueous solution of GA<sub>3</sub> (Laboratory grade) for 17 hr. Thereafter, the seeds were used for seed germination studies. The germination media were incubated in light at chosen constant temperature in walk-in-room germinator (Maintaining requisite temperature) and having 95±5% RH for 20 days. Only morphologically normal seedlings (Possessed all essential structures) were scored and the average percentage germination for four replicates was calculated.

**Table 1. Details on ashwagandha seed lots used for germination studies**

Cultivar	Strain/variety	Institution
JA-20	Variety	JNKVV, Jabalpur
JA-134	Variety	JNKVV, Jabalpur
Strain-1	Strain	IARI, New Delhi
Strain-2	Strain	IARI, New Delhi
Poshita	Variety	CIMAP, Lucknow

## RESULTS AND DISCUSSIONS

ANOVA showed that all the treatments studied were superior to the untreated (Control) seed germination value. Treatment T<sub>3</sub> (Pre-washing seeds for 24 hr in running tap water) registered highest germination value (66.2), followed by GA<sub>3</sub> treatment over the control (48.8). Results on seed germination using different concentration of GA<sub>3</sub> are given in table 2. ANOVA showed that all the treatments studied were superior to the untreated (Control)

seed germination values. Treatment T<sub>1</sub> (Treating ashwagandha seeds with 100 ppm GA<sub>3</sub> for 17 hr) registered maximum germination value (68.55%) over the control (52.10%). The next best treatments were use of GA<sub>3</sub> 200 for 17 hr and GA<sub>3</sub> (400 ppm) for 17 hr registered a minimum germination value (61.80%) among different concentrations of GA<sub>3</sub> used. Among various physical and/or chemical treatments applied, pre-washing the seeds in running water under laboratory ambient temperature increased the germination values due to removal of certain inhibiting chemicals accumulated in seed coat. Thus, pre-washing seed treatment in running water for ashwagandha (Medicinal plant) ranked as the best followed by application of gibberellic acid.

The results on seed germination percentages of ashwagandha seeds using TP method under 25°C in light with various durations of pre-washing the seeds in running tap water (MCD water supply) are presented in table 3. ANOVA showed that all the pre-washing seed treatments were superior (75.15-88.55%) than the control (72.85%). Statistically, pre-washing of seed for 48 hr registered maximum seed germination values (88.55%), followed by seed treatment for 72 hr pre-washing registered germination value (83.95%). Results indicative of 48 hr pre-washing of ashwagandha seed in running water was best for optimizing the seed germination values (86-90%). It was also reported that pre-washing of seeds increased seed germination in *Podophyllum* [8]. It is suggestive of presence of certain naturally occurring substances (Chemical) on seed coat which act as inhibitor for seed germination, were removed during washing of seeds in running water under laboratory ambient temperatures. It was also demonstrated that induction of secondary dormancy by a naturally occurring chemical [Abscisic acid (ABA) and four other terpenes] in leachate of *Nicotiana attenuate* seeds [9].

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Table 2. Germination (%) of ashwagandha seeds under different treatments

Cultivar	Treatment								
	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>	T <sub>6</sub>	T <sub>7</sub>	T <sub>8</sub>	T <sub>9</sub>
JA-20	48 (43.85)	57 (49.02)	65 (53.73)	67 (54.94)	54 (47.29)	49 (44.43)	58 (49.60)	50 (45.00)	57 (49.02)
JA-134	50 (45.00)	58 (49.60)	69 (56.17)	64 (53.13)	56 (48.45)	54 (47.29)	66 (54.33)	52 (46.15)	58 (49.60)
Strain-1	46 (42.71)	56 (48.45)	63 (52.53)	63 (52.53)	51 (45.57)	55 (47.87)	64 (53.13)	47 (43.28)	56 (48.45)
Strain-2	52 (46.15)	56 (48.45)	67 (54.94)	64 (53.13)	58 (49.60)	52 (46.15)	67 (54.94)	51 (45.57)	56 (48.45)
Poshita	48 (43.85)	57 (49.02)	67 (54.94)	63 (53.13)	58 (49.60)	50 (45.00)	66 (54.33)	49 (44.43)	57 (49.02)
Mean	48.8 (44.51)	56.8 (48.91)	66.2 (54.45)	64.8 (53.61)	55.4 (48.10)	52 (46.15)	64.2 (53.25)	49.8 (44.89)	56.8 (48.91)
CD (5%)	2.559								

Values in parentheses are arc sine transformed germination percentage

T<sub>1</sub>, control; T<sub>2</sub>, 500 ppm GA<sub>3</sub> for 6 hr; T<sub>3</sub>, 24 hr washing in running tap water; T<sub>4</sub>, 500 ppm GA<sub>3</sub> for 17 hr; T<sub>5</sub>, Presoaking seeds in 2% thiourea for 17 hr; T<sub>6</sub>, presoaking seeds in 0.2% KNO<sub>3</sub> for 24 hr; T<sub>7</sub>, washing (12 hr) + 500 ppm GA<sub>3</sub> for 17 hr; T<sub>8</sub>, 1% H<sub>2</sub>SO<sub>4</sub> for 5 min; T<sub>9</sub>, prechilling at 4°C for 7 days in light

Table 3. Germination (%) of ashwagandha seeds under different duration of washing

Cultivar	Treatment				
	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>	T <sub>5</sub>
JA-20	71.25 (57.61)	73.75 (59.21)	86.00 (68.03)	83.00 (65.65)	78.00 (62.03)
JA-134	73.50 (59.54)	74.25 (59.02)	90.00 (71.56)	85.75 (67.86)	80.25 (63.65)
Strain-1	72.00 (60.53)	75.75 (58.05)	<b>90.00</b> <b>(71.56)</b>	83.00 (65.65)	81.25 (64.38)
Strain-2	73.75 (60.00)	75.00 (59.21)	86.75 (68.70)	84.00 (66.42)	80.00 (63.44)
Poshita	73.75 (61.34)	77.00 (59.21)	<b>90.00</b> <b>(71.56)</b>	84.00 (66.42)	77.25 (61.55)
Mean	72.85 (60.13)	75.15 (58.63)	88.55 (70.27)	83.95 (66.34)	79.35 (62.94)
CD (5%)	2.714				

Values in parentheses are arc sine transformed germination percentages

T<sub>1</sub>, control, T<sub>2</sub>, 24 hr washing in running tap water; T<sub>3</sub>, 48 hr washing in running tap water; T<sub>4</sub>, 72 hr washing in running tap water; T<sub>5</sub>, 96 hr washing in running tap water

Table 4. Germination (%) of ashwagandha seeds at different concentration of GA<sub>3</sub> for 17 hr

Cultivar	Treatment			
	T <sub>1</sub>	T <sub>2</sub>	T <sub>3</sub>	T <sub>4</sub>
JA-20	67.00 (54.94)	<b>69.00</b> <b>(56.17)</b>	63.25 (52.71)	52.00 (46.15)
JA-134	68.50 (55.86)	65.25 (53.91)	60.75 (51.24)	55.00 (47.87)
Strain-1	68.75 (56.04)	68.75 (56.04)	63.75 (53.01)	<b>51.00</b> <b>(45.57)</b>
Strain-2	<b>69.25</b> <b>(56.35)</b>	70.25 (56.98)	61.00 (51.35)	51.25 (45.75)
Poshita	<b>69.25</b> <b>(56.35)</b>	68.75 (56.04)	60.25 (50.89)	51.25 (45.75)
Mean value	68.55 (55.92)	68.40 (55.80)	61.80 (51.80)	52.10 (46.20)
CD (5%)	1.994			

Values in parentheses are arc sine transformed germination percentages

T<sub>1</sub>, 100 ppm, T<sub>2</sub>, 200 ppm, T<sub>3</sub>, 500 ppm, T<sub>4</sub>, control

(61.80%) among different concentrations of GA<sub>3</sub> used. The results were in conformity with [10] and [11]. The GA<sub>3</sub> enhances the germination of seeds exhibiting physiological, morphological or morpho-physiological dormancy. The GA<sub>3</sub> treatment in breaking dormancy depends on concentration and length of incubation [12] also reported that GA<sub>3</sub> pre-treatment partially negated light induced inhibition of germination.

Seed dormancy was due to certain chemicals present in seed coat, as pre-washing of seeds enhances seed germination. Pre-washing of ashwagandha seeds in running water, under laboratory ambient temperature for 48 hr ranked as the best, eco-friendly and economical.

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