

Effect of pre - soaking seed treatments on seed quality parameters of Ashwagandha (*Withania somnifera*)

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ABSTRACT The present investigation entitled, "Seed germination and growth studies in ashwagandha (*Withania somnifera*)" was carried involving different pre-soaking seed treatments with different chemicals viz., GA₃ @ soaking 100 ppm for 6 hr (T₂), GA₃ soaking @ 200 ppm for 12 hr (T₃), GA₃ soaking @ 400 ppm for 6 hr (T₄), KNO₃ soaking @ 1% for 6 hr (T₅), KH₂PO₄ soaking @ 1% for 6 hr (T₆), Thiourea soaking @ 0.5% for 6 hr (T₇), Cow urine soaking for 6 hr (T₈) and untreated seeds i.e. control (T₁). All the pre-soaking seed treatments had significant effect on root yield and growth contributing characters. The pre-soaking seed treatments significantly influenced the growth and root yield contributing characters of ashwagandha. The maximum root yield was observed in the GA₃ 400 ppm soaked for 6 hr, followed by GA₃ 200 ppm soaked for 12 hr and 1% KNO₃ soaked for 6 hr. The seeds pre-soaked with GA₃ @ 400 ppm took less number of days for field emergence, days to 50% flowering, higher shoot length, shoot diameter, shoot dry weight, root length, root diameter and root dry weight followed by GA₃ @ 200 ppm and 1% KNO₃. The root shoot ratio of seeds soaked with GA₃ @ 400 ppm recorded significantly superior root-shoot ratio than untreated seeds. Seed quality parameters viz., germination percentage, shoot length, root length, seedling dry weight and vigour index were improved by presoaking seed with GA₃ 400 ppm for 6 hr followed by GA₃ 200 ppm for 12 hr and 1% KNO₃ for 6 hr. The seed germination method was also standardised using different methods viz., between paper, top of paper and sand method at different temperatures viz., 20+2°C, 25+2°C and 30+2°C. Seed germination was significantly influenced by the germination method and temperature. The higher germination percentage (62.67 %) was recorded in top of paper method and 25+2°C temperature.

Key words: GA₃, KNO₃, germination, shoot length, root length, seedling dry weight, vigour index, *Withania somnifera*, ashwagandha

Ashwagandha (*Withania somnifera* L.) is an important medicinal plant that has been employed in Indian traditional medicinal systems viz., Ayurveda and Unani. It has wide range of applications in the treatment of various physiological disorders and diseases such as arthritis, asthma, cancer, inflammation, tuberculosis and typhoid.

Further, it has usefulness as bactericide, contraceptive, fungicide, sedative tonic [1]. Due to their adaptogenic and restorative properties, it is most effective on various systems viz., nervous system, digestive, urinary, circulatory and reproductive system [2]. Seed quality, especially the germinability and vigor is essential to establish adequate plant stand for crop production, which can be adversely affected by environmental factors.

The medicinal crop plants are multiplied through seed and are economically important worldwide. Although they are seed propagated, virtually no systematic work has been done either for their multiplication or to know the causes for their poor germination and keeping quality. As the germination of these medicinal crops is erratic, there is a need to study these problems especially on germination and seedling growth in order to enhance the quality for better and quick germination.

MATERIALS AND METHODS

The fresh seeds of ashwagandha (*Withania somnifera*) were collected from Medicinal and Aromatic Plant Project, Mahatma Phule Krishi Vidyaapeeth, Rahuri.

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The experiment was conducted in Seed Technology Research Unit (STRU), Laboratory, Mahatma Phule Krishi Vidyapeeth Rahuri during the period of 2011-2012. To standardize the suitable germination test method, seeds were kept for germination using different methods (Between paper method, Top of paper method and Sand method) at 20+2°C, 25+2°C and 30+2°C for 10 days and recorded the normal seedling percentage. Further, the seeds were subjected to seed treatments with (GA₃ 100ppm, GA₃ 200ppm, GA₃ 400ppm, KNO₃ 1%, KH₂PO₄ 1%, Thiourea 0.5%) and Cow urine for germination test, random samples of one hundred seeds in four replications. Ten normal seedlings were randomly selected from each replication and recording observation on germination percentage, root length (cm), shoot length (cm), dry weight of seedling (gm) and vigour index. The similar experiment was repeated under field conditions by superimposing the same type of treatments in randomized block design with three replications. The gross plot size was 2.70 x 3.00 m² and net plot size was 1.80 x 2.40 m². The observations were recorded on days to field emergence, days to 50 % flowering, shoot length (cm), shoot diameter (cm), shoot dry weight (g), root length (cm), root diameter (cm), root Dry weight (g) and root-shoot ratio. The data obtained from various observations were analyzed by using Factorial Completely Randomized Design (FCRD) and Completely Randomized Design (CRD) [3] and field data were analyzed by Randomized Block Design RBD method [4].

RESULTS AND DISCUSSION

Effect of germination test methods and temperature on germination

The different germination test methods *viz.*, between paper method, top of paper method and sand method and different temperatures *viz.*, 20+2°C, 25 +2°C and 30+2°C were used for standardization of germination test. The higher germination percentage was recorded

in the top of paper (58.67%), followed by between paper method (52.11%) whereas, the lowest germination percentage (21.78%) was observed in sand method (Table 1). The top of paper method was ideal for conducting germination test in ashwagandha [5]. Significant differences were observed in germination percentage in response to different temperatures. The higher germination percentage was recorded at 25+2°C (47.00%), followed by 30+2°C (44.89%) temperature. However, the lowest germination percentage was recorded at 20+2°C temperature (40.67%). The interaction between the germination methods and temperature had non significant effect on germination percentage. However, the highest germination percentage was recorded in top paper method at 25+2°C (62.67%), followed by sand method at 30+2°C temperature (58.00%). The lowest germination percentage (20.67%) was observed in between paper at 20+2°C temperature (T₁M₁) Ellis *et al.* [6] reported the use of 'top of paper' or 'between paper' as suitable method for germination test in *Hibiscus sabdariffa* and *Catharanthus roseus* at 20+2°C temperature. Similar findings were reported by Vakeswaran [5] in *Withania somnifera* and Suryawanshi *et al.* [7] in *Solanum viarum*, *Artemisia pallens* and *Cassia angustifolia*. Jain *et al.* [8] reported that higher seed germination observed at temperature 20-30+2°C in ashwagandha.

Effect of pre-soaking treatments on seed quality parameters in laboratory

The effect of pre-soaking seed treatments (Table 2) were significant in respect of the quality parameters *viz.*, germination percentage, root length (cm), shoot length (cm), seedling dry weight (gm) and vigour index. The pre-soaking treatments, GA₃ @ 400 ppm recorded higher germination percentage (76%) followed by GA₃ 200 ppm (72%) and 1% KNO₃ (69.33%) over control (58%). The growth regulators stimulate and promote seed germination in a wide variety of tree crops. The seeds soaked in GA₃ improved the

Table 1. Effect of the methods and temperatures and their interaction on germination percentage

Temperatures	Germination methods			Mean
	Between paper	Top of paper	Sand method	
20 + 2 °C	46.00 (42.70)	55.33 (48.07)	20.67 (27.00)	40.67 (39.25)
25 + 2 °C	56.33 (48.67)	62.67 (52.37)	22.00 (27.96)	47.00 (43.00)
30 + 2 °C	54.00 (47.30)	58.00 (49.61)	22.67 (28.42)	44.89 (41.78)
Mean	52.11 (46.22)	58.67 (50.02)	21.78 (27.79)	44.18 (41.02)
	Temperature	Method	Interaction	
SEM±	0.73	0.73	1.27	
C.D at 5%	2.19	2.19	NS	

germination under laboratory than IAA and IBA [9].

There were significant differences in shoot length, root length, seedling dry weight and vigour indexes in response to different soaking treatments. The highest values of these parameters were recorded in GA₃ 400 ppm, followed by GA₃ 200 ppm and 1% KNO₃. Verma *et al.* [10] noticed improvement in vigorous growth of seedling under laboratory conditions resulted due to soaking in GA₃ @ 100 ppm. Similar findings were also reported [5, 11].

The improvements in shoot and root length of seedling due to GA₃ treatment which enhanced metabolic activity, earliness in germination and seedling growth [11]. These results are in conformity with findings in *Cassia tora*, *Withania coagulens*, *Argemone maxicana* and *Ocinum sanctum* [12].

There were statistically significant differences in seedling dry weight due to different pre-soaking seed treatments. The seeds pre-soaked with GA₃ and KNO₃ resulted in increased root shoot dry matter by

elongation of shoot length, root length. Similar trends were also reported by other workers [13-14].

The increased in vigour index was due to increased in germination percentage and root shoot length by pre-soaking seed treatments with plant growth regulators. Similar findings were reported by in tulsi, ashwagandha, periwinkle and kalmegh [15-16].

Effect of pre-soaking treatments on seed quality parameters in field

The seed quality parameters were significantly influenced by different pre-soaking seed treatments (Table 3). All the soaking treatments were performed better for seedling emergence in the field over control (9.67 days). This might be due to activation of cells, which resulted in mitochondrial activity enhancement leading to phase of germination and better plant growth due to supply of plant growth regulators. Similar findings were earlier reported [10-11, 13].

Amongst the treatments, GA₃ soaking @ 400 ppm for 6 hrs recorded faster field emergence (6.33 days), followed by GA₃ soaking @ 200

Table 2. Effect of pre-soaking seed treatments on quality parameter in Ashwagandha under laboratory conditions.

	Germination (%)	Shoot length (cm)	Root length (cm)	Seedling dry wt. (g)	Vigour index (I)
T ₁ : Control	58.00	1.74	1.05	1.06	161.74
T ₂ : 100 ppm GA ₃ soaking for 6 hrs	68.67	2.47	1.75	1.60	289.87
T ₃ : 200 ppm GA ₃ soaking for 12 hrs	72.00	2.78	2.00	1.77	344.55
T ₄ : 400 ppm GA ₃ soaking for 6 hrs	76.00	3.20	2.43	1.96	428.79
T ₅ : 1% KNO ₃ soaking for 6 hrs	69.33	2.54	1.81	1.64	301.29
T ₆ : 1% KH ₂ PO ₄ soaking for 6 hrs	60.00	2.34	1.42	1.31	205.97
T ₇ : 0.5% Thiourea soaking for 6 hrs	62.33	2.13	1.47	1.43	224.62
T ₈ : Cow urine soaking for 6 hrs	61.33	1.93	1.24	1.13	194.61
Mean	65.96	1.65	2.39	1.49	260.93
SEM ±	1.87	0.16	0.03	0.03	12.60
CD at 5 %	5.61	0.35	0.09	0.08	37.77

ppm for 12 hrs (7.00) and GA₃ soaking @100 ppm for 6 hrs (7.33). The 50% flowering was earlier in GA₃ soaking @ 400 ppm (92.67 days), followed by GA₃ soaking @ 200 ppm (95.33 days), and KNO₃ soaking @ 1% (95.67 days). The induction of earliness in days to 50 percent flowering by GA₃ and KNO₃ might be due to active involvement in physiological and biochemical processes of plant, which in turn resulted in earlier flowering. Similar trends were also reported by earlier workers [11, 14].

The treatment GA₃ soaking @ 400 ppm for 6 hrs maintained the highest shoot length (82.13 cm), shoot diameter (4.99 cm) and shoot dry weight (230.20 g) followed by GA₃ soaking @ 200 ppm for 12 hrs and KNO₃ soaking @ 1% for 6 hrs. The lowest shoot length recorded in control. It enhanced metabolic activities and increased seedling growth [11]. These results are in conformity with the findings of in ashwagandha [13, 17]. The pre-soaking seeds increased shoot diameter and dry weight by elongation of shoot length and due to increased in vigour, which may help in absorption of moisture and nutrients from the deep root zone. Similar finding of dry matter

assimilation were reported in ashwagandha [11, 14, 17].

The pre-soaking treatment with GA₃ soaking @ 400 ppm for 6 hrs also maintained the highest root length (35.70 cm), root diameter (6.75 cm) and root dry weight (23.16 g). In addition to this, GA₃ soaking @ 200 ppm for 12 hrs (28.53 cm, 6.36 cm & 20.02 g) and KNO₃ soaking @ 1% for 6 hrs (32.43 cm, 6.38 cm & 19.77 g) were also promising for root length, root diameter and root dry meter respectively. The improvement in root length due to seeds treated with GA₃ and KNO₃ probably due to enhanced metabolic activity and increases in seedling growth by Kattimani *et al.* [11]. A similar finding beneficial effect on growth was reported by Velmurugan *et al.* [17] and Krishana *et al.* [13]. The pre-soaking seed treatments increased root dry weight by elongation of root length and due to increased in vigour which may help in absorption of moisture and nutrients from the deep root zone. The treatment GA₃ soaking @ 400 ppm for 6 hrs was recorded beneficial root shoot ratio (0.43) than other treatments.

Table 3. Effect of pre-soaking seed treatments on shoot length (cm), shoot diameter (cm) and shoot dry weight (g) in field conditions.

Treatments	Field emergence (days)	50% flowering days	Shoot length (cm)	Shoot diameter (cm)	Shoot dry (g)	Root length (cm)	Root diameter (cm)	Root dry weight (g)	Root: shoot ratio
T ₁ : Control	9.67	99.00	59.68	3.17	107.19	28.53	4.90	15.11	0.48
T ₂ : 100 ppm GA ₃ soaking for 6 hrs	7.33	97.33	69.01	3.84	156.18	31.20	6.01	19.11	0.45
T ₃ : 200 ppm GA ₃ soaking for 12 hrs	7.00	95.33	77.03	4.04	181.23	34.10	6.36	20.02	0.44
T ₄ : 400 ppm GA ₃ soaking for 6 hrs	6.33	92.67	82.13	4.99	230.20	35.70	6.75	23.16	0.43
T ₅ : 1% KNO ₃ soaking for 6 hrs	7.67	95.67	71.98	3.94	168.85	32.43	6.38	19.77	0.45
T ₆ : 1% KH ₂ PO ₄ soaking for 6 hrs	8.00	100.67	65.28	3.53	120.89	30.07	5.38	17.76	0.46
T ₇ : 0.5% Thiourea soaking for 6 hrs	8.33	100.33	65.90	3.70	122.85	30.27	5.66	18.43	0.46
T ₈ : Cow urine soaking for 6 hrs	7.67	99.67	62.53	3.45	119.75	30.37	5.44	17.05	0.49
Mean	7.75	97.58	69.19	3.83	15.02	31.58	5.86	18.80	0.46
SEM±	0.49	0.47	0.94	0.09	1.48	0.68	0.13	0.47	0.008
CD at 5 %	1.49	1.44	2.85	0.29	4.49	2.05	0.38	1.42	0.025

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