RESEARCH ARTICLE

Propagation Protocol of *Withania somnifera* (L.) Dunal and Phenotypic Adaptability of Transplanted Seedlings Raised at Various Altitudes

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Abstract

Plants in the temperate zone face extreme climate and resource constraints. The purpose of this study was to develop a propagation protocol for a medicinally important plant, Withania somnifera in a temperate climate. Climate change has an impact on plant morphology and reproduction. The phenotypic plasticity of developed seedlings transplanted at various altitudes was investigated. Three replicates of 25 seeds were sterilized with 1-ml L-1 tween 20, 1 g L-1 bavistin, and then treated with organic fertilizer (manure, organic compost, vermicompost, organic compost + vermicompost); inorganic fertilizer (urea, NPK, DAP, NPK+urea, DAP+urea), gibberellic acid (GA, plant growth regulator), and polyethylene glycol (PEG). Phenotypic plasticity was examined in three replicates of seven seedlings from each treatment at altitudes of 400, 900, and 1900 m. Germination parameters such as germination (100%), germination energy (86.67%), and vigour index (543.33) were maximum in seeds treated with gibberellic acid, whereas mean germination time (5.45 days) was minimum. Shoot length (58.77 cm), number of nodes per plant (77.67), and number of leaves per node (4.00) were also exhibited maximum during GA 500 mg L-1 treatment. Transplanted seedlings at high altitude showed a decrease in morphological traits such as shoot length, root length, and leaf area. Reproductive traits such as berries per plant and seeds per berry also decrease in seedlings. This study recommends 500 mg L-1 GA for seed propagation of *W. somnifera* for temperate regions. The study also concluded that when raising this species at different altitudes, it exhibits variation in morphological and reproductive traits. This ensures that Withania somnifera can grow in different altitudes, undergoing different morphological adaptations.

Highlights:

- A systematic protocol of Withania somnifera is developed for a temperate climate, which has not been previously documented.
- First comparative assessment of seedling performance and phenotypic plasticity at multiple altitudes (400–1900 m).
- GA at 500 mg L-1 was identified as the most effective for germination and vigor in the temperate region.
- The study fills a research gap in understanding the morphological and reproductive adaptability of *W. somnifera* under climate variations.

Keywords: Climate change, Germination, Gibberellic acid, Transplantation, Altitudes, Phenotypic plasticity.

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Introduction

Plants and associated biotic organisms benefit human communities in providing value-based products and economic ways (Gupta *et al.,* 2025; Gupta *et al.,* 2024). The Indian Himalayan region is home to over 1748 MPs, many of which are classified as rare, endangered, or threatened (RET) (Lodhiyal *et al.,* 2023; Gupta *et al.,* 2023). Threats like illegitimate harvesting, unreported trade, overexploitation, anthropogenic pressure, and climate change are some causes for the declining range of MPs worldwide (Mehta *et al.,* 2021; Dhar *et al.,* 2000). *Withania somnifera* (Solanaceae family), a valuable medicinal plant, is distributed in tropical and temperate parts of India but has a low population density (Obidoska 2004; Kaur *et al*., 2022). Climate change is having a serious impact on this species' population and survival (Kaur 2017; Saiyem *et al.,* 2022). As a result, plants alter germination efficiency, morphology and reproductive traits (phenotypic plasticity) (Caffarra and Donnelly, 2011). Considering its increasing demand and the impact of climate change on its growth, a reliable propagation protocol as well as a thorough understanding of phenotypic variations in this plant, are required. Seed propagation and plant phenotypic plasticity are crucial for analyzing the growth of plants (Nicotra *et al.,* 2010). This research focuses on the changes in seed propagation of *W. somnifera* by applying different growth stimulators, like organic or inorganic fertilizers or plant growth regulators (PGRs), along with the phenotypic adaptability of transplanted seedlings raised at various altitudes.

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Fig. 1: Propagation of *Withania somnifera* by seeds (a-c) Separation of seeds from berry (d) Sterilization of seeds using bavistin (e-g) Seed germination using PGR's in petridish on germinator and root trainers (h-i) Seed germination on soil using fertilizers (j) Early and late branching in seedlings treated with gibberellic acid and control (k) Root development using DAP+Urea and control (l) One year seedlings in open condition (m) Root development in seedlings (n) Transplantation in field condition (o-p) Flowering and berries formation in transplanted seedlings.

Seed germination is influenced by various environmental factors, including the use of fertilizers or PGRs (Reed *et al.,* 2022). Low temperature reduces the seed germination in *W. somnifera* (Kambizi *et al.,* 2006). Therefore, for its better growth in the environment of high altitude requires a thorough understanding of the effects of fertilizers and PGRs. Morphological and reproductive traits of developed seedlings have a significant impact on population dynamics and evolutionary adaptations (Abdusalam and Li, 2018; Olejniczak *et al.,* 2018). Studies on many medicinal plants, such as *Hedychium coronarium* (de Castro *et al.,* 2016), *Berberis microphylla* (Radice and Arena, 2018), *Plantago lanceolata* (Hamann *et al.,* 2018), have been performed in this direction. According to these studies, parameters such as shoot length, root length, morphology of leaves, flowers, and number of seeds are malleable and change when being transplanted in different ecological variables (Radice and Arena, 2018; Hamann *et al.,* 2018). However, phenotypic plasticity may be influenced by genetic (Kumar *et al.,* 2007) or environmental factors (Yaqoob and Nawchoo, 2017). To gain a better understanding of the growth rate at different altitudes, a systematic study of *W. somnifera* was required.

Although some reports are available on the germination performance of *W. somnifera* through seeds (Kaur *et al.,* 2022; Namdeo *et al.,* 2021; Basak *et al.,* 2020), there is a paucity of literature on systematic nutrient dynamics for improving seed germination, seedling performance, and phenotypic plasticity of transplanted seedlings grown at different altitudes. This study aims (i) to assess the effects of different treatments (fertilizers or PGRs) on the germination and growth performance of *W. somnifera* (ii) to develop a reliable propagation protocol for seeds of *W. somnifera* (iii) to assess the morphological change and reproductive traits of transplanted seedlings in different altitudes. The uniqueness of this study lies in its integrated approach combining nutrient treatment effects with phenotypic plasticity analysis of *W. somnifera* seedlings across a broad altitudinal range under temperate climatic conditions. This fills a critical knowledge gap, as previous studies have not systematically evaluated nutrient-driven seedling performance and altitude-related morphological adaptations together. Such a comprehensive understanding is essential to optimize propagation protocols and support conservation and sustainable cultivation of *W. somnifera* in the face of climate change.

Table 1: Details of study sites

Sites	*Region*	*Latitude*	*Longitude*	*Altitude (m)*
Khurpatal	Temperate	79°40′35″ E	29°38′67″ N	1900
Bhujiyaghat	Sub-tropical	79°52′76″ E	29°31′21″ N	900
Haldwani	Tropical	79°51′78″ E	29°21′96″ N	400

Materials and Methods

Study area

The research was conducted at the Himalayan Botanical Garden in Khurpatal, Nainital district, Uttarakhand, India. The study area is situated at an elevation of 1600 to 1900 m, (29°23′09″ N) longitude, and (79°26′02″ E) latitude, situated in the temperate zone, with mean temperatures ranging from 10 to 27°C. Details of transplanted sites are represented in Table 1.

Soil collection and preparation

The soil was gathered from an oak forest. Soil was air-dried, fumigated, and treated with a systemic fungicide (1 g L^{-1} Bavistin) and insecticide (3 g L^{-1} Monocil) solution dissolved in distilled water. Physical and chemical properties of soil are presented in Table S1.

Table 2: Germination parameters of seeds treated with fertilizers and plant growth regulators

TT	*Treatment*	*Germination percent (%)*	*Germination energy (%)*	*Mean germination time (days)*	*Vigour index*
OF	Control	49.33 ± 0.27	17.00 ± 0.09	8.67 ± 0.02	189.33 ± 1.24
	Manure	51.33 ± 0.72	20.67 ± 0.09	7.43 ± 0.02	209.07 ± 0.19
	OC	57.33 ± 0.54	22.67 ± 0.0	7.51 ± 0.04	215.87 ± 0.50
	VC	66.67 ± 0.00	25.33 ± 0.22	7.43 ± 0.02	268.80 ± 0.54
	OC+VC	70.66 ± 0.47	42.67 ± 0.24	7.26 ± 0.02	289.47 ± 0.54
IF	Urea	69.33 ± 0.47	33.33 ± 0.00	6.16 ± 0.01	337.47 ± 1.02
	NPK	70.67 ± 0.27	54.66 ± 0.22	5.91 ± 0.01	343.73 ± 1.39
	DAP	74.67 ± 0.00	41.33 ± 0.09	6.12 ± 0.02	403.20 ± 2.01
	NPK+Urea	82.67 ± 0.27	50.66 ± 0.89	5.98 ± 0.03	473.73 ± 2.34
	DAP+Urea	90.67 ± 0.47	62.60 ± 0.88	5.90 ± 0.07	535.20 ± 1.22
PGRs	GA 250 mg L-1	77.33 ± 0.72	66.67 ± 0.88	5.70 ± 0.02	466.67 ± 0.45
	GA 500 mg L-1	100.00 ± 0.00	86.67 ± 0.00	5.45 ± 0.01	543.33 ± 0.56
	GA 1000 mg L-1	72.00 ± 0.47	46.60 ± 0.18	5.96 ± 0.03	450.27 ± 0.11
	PEG 250 mgL-1	100.00 ± 0.00	56.00 ± 0.00	6.16 ± 0.02	443.33 ± 0.11
	PEG 500 mg L-1	78.67 ± 0.27	29.33 ± 0.00	6.28 ± 0.01	430.80 ± 0.32
	PEG 1000 mg L-1	64.00 ± 0.47	20.00 ± 0.23	6.38 ± 0.03	345.60 ± 0.67
p- value		0.00	0.00	0.00	0.00
F value		0.00	85.33	65.75	104.11

*GA= Gibberellic acid, PEG= Polyethylene alcohol, OF= Organic fertilizer, IF= Inorganic fertilizer, PGRs= Plant growth regulators, TT= Type of treatment, OC= Organic compost, VC= Vermicompost. According to one-way ANOVA analysis, the treatments showed statistically significant effects on growth parameters at the 0.05 level of significance ($p \leq 0.05$). Data are represented as average values ± Standard Error.

Seed collection and preparation

The plant was authenticated by the Botanical Survey of India, Dehradun (Accession number: BSD 91789). Seeds were obtained from the Central Institute of Medicinal and Aromatic Plants (CIMAP), Pantnagar, Uttarakhand, India. Seed cover was manually removed with sterile forceps and the separated seeds were air dried (Fig. 1a-c). The moisture content of the seeds was determined by oven drying seeds (80 ± 2°C) up to a constant weight and calculated using equation 1. The tetrazolium test was performed eight times using 50 seeds per replicate (Lakon, 1942). The viability percentage of seeds was calculated using equation 2. Seeds were sterilized using 1 mL L^{-1} tween 20 (nonionic detergent) for 10 minutes, 1 g L^{-1} bavistin (fungicide) for 20 minutes, and rinsed three or four times in autoclaved distilled water (Gupta *et al*., 2025) (Fig. 1d).

$$\text{Equation 1: } \textit{Moisture content of seed} = \frac{\text{Fresh weight of seed} - \text{Dry weight of seed}}{\textit{Fresh weight of seed}}$$

$$\text{Equation 2: } \textit{Viability percentage of seed} = \frac{\textit{Stained seeds}}{\textit{Total seeds}} \times 100$$

Seed germination experiment

Fertilizers and plant growth regulators were applied to 75 seeds (triplicate of 25) for each treatment (Fig. 1). Fertilizer decomposition necessitates the presence of light, temperature, oxygen, moisture, aerobic and anaerobic organisms. Thus, organic or inorganic fertilizers were applied in plastic pots under greenhouse conditions (25–30°C). Organic fertilizers used in the study were included: manure (M), organic compost (OC), vermicompost (VC), and organic compost + vermicompost (OC+VC). Seeds were sown in soil enriched with organic fertilizers and sand (2:1:1, w:w:w). Inorganic fertilizers that were used in the experiment were urea (46 mg N kg^{-1}), NPK (12-32-16 mg kg^{-1}), DAP (18–46-0 mg kg^{-1}), NPK+urea, and DAP+urea. A solution of 2 g L^{-1} inorganic fertilizers was sprayed at the time of sowing (Tanwar *et al.,* 2014). Plant growth regulators were applied to a sterilized petri dish containing Whatman filter paper No. 1 and treated with different concentrations (250, 500, and 1000 mg L^{-1}) of gibberellic acid (GA) or polyethylene glycol (PEG), separately (Zhang, 2014; Salvi, 2015; Pandey *et al.,* 2018). Petridishes were kept at 28°C because previous trials showed that germination rate at 28°C was maximum as compared to 14, 21, and 35°C. Filter paper was moistened with autoclaved distilled water on alternate days. The seed was considered germinated when the radicle began to emerge (>2 mm).

Transplantation of seedlings

Plants treated with various stimulants (fertilizer or PGRs) were transferred from the greenhouse to field conditions after a year. Transplantation was performed in three different altitudinal variations of the Nainital district, Uttarakhand, India: Haldwani

(424–524 m), Bhujiyaghat (850–1000 m), and Khurpatal (1600–1900 m), which lie in tropical, subtropical, and temperate zones, respectively (Table 1). Three replicates of seven seedlings from each treatment were observed for one year to study phenotypic plasticity with respect to morphological and reproductive traits.

Data recording and analysis

The viability of a seed population was estimated using the germination percentage (GP), which was calculated using equation 3 (Scott *et al.,* 1984). Germination energy (GE) was calculated using equation 4. Mean germination time (MGT) was calculated using equation 5 (Orchard, 1997). Vigor index (VI) was estimated using equation 6 (Baki and Anderson, 1973). Seedling growth parameters (morphological and reproductive) were determined following standard procedures. Shoot length (from soil surface to green apical tip) and root length were measured using a vernier caliper (Krauss *et al.,* 1999) Sprouted nodes per shoot (nodes with leaves in a shoot from base to apical tip) and sprouted leaves per node (leaf in each node of a shoot from base to apical tip) were counted. Leaves with a minimum length of 5 mm or above were considered and counted (Oliveira *et al.,* 2021). The number of seeds per berry and the number of berries per plant were both counted. The germination and growth parameters were represented as mean values ± SE (standard error). SPSS software version 21.0 (SPSS Inc., Chicago, USA) was used to perform standard error, one-way ANOVA and MANOVA at a level of significance ($p < 0.05$).

Equation 3: $$Germination\ percent = \frac{Total\ number\ of\ seeds\ germinated}{Total\ seeds\ tested} \times 100$$

Equation 4: $$Germination\ energy = \frac{Number\ of\ seeds\ germinated\ on\ day\ interval}{Total\ number\ of\ seeds\ tested} \times 100$$

Equation 5: $$Mean\ germination\ time = \frac{\Sigma n \times t}{\Sigma n}$$

Where, n is the number of newly germinated seeds at time t; t is the time since the start of the germination test

Equation 6: $$Vigor\ index = Germination\ percent \times Seedling\ height$$

Results and Discussion

Effects of fertilizers and plant growth regulators (PGRs) on seed germination

The Moisture content and viability percentage of the seeds were 9.67 ± 0.21 and 84.67 ± 0.67%, respectively. Seeds germinated in all fertilizer and PGR treatments. PGRs showed the maximum germination response, followed by inorganic (IF) and organic fertilizers (OF). Seeds treated with gibberellic acid (GA) 500 mg L^{-1} at 28°C in the laboratory induced the maximum germination percent (100%), germination energy (86.67%), and vigour index (543.33), while mean germination time (5.45 days) was the minimum (Table 2). Treatments had a significant and greater effect on germination parameters ($p \leq 0.05$) than the control (Table S1). Some reports are available on the germination

Table 3. Growth parameters of seedlings treated with fertilizers and plant growth regulators

TT	*Treatment*	*Shoot length (cm)*		*Root length (cm)*		*Sprouted node per plant*		*Leaves per node*	
		6 month	12 month	6 month	12 month	6 month	12 month	6 month	12 month
	Control	25.97 ± 0.59	37.93 ± 0.85	6.27 ± 0.48	9.21 ± 1.32	20.00 ± 0.94	38.00 ± 2.16	2.67 ± 0.00	2.78 ± 0.24
	Manure	27.05 ± 0.91	40.72 ± 1.47	9.00 ± 1.25	14.09 ± 0.92	26.67 ± 1.44	45.00 ± 2.62	2.00 ± 0.00	2.67 ± 0.31
	OC	28.73 ± 0.22	41.78 ± 1.09	10.47 ± 0.88	15.59 ± 0.48	24.00 ± 1.25	47.67 ± 1.91	2.47 ± 0.01	3.00 ± 0.16
	VC	29.97 ± 0.55	42.07 ± 0.83	9.64 ± 1.46	14.67 ± 1.25	26.00 ± 1.41	48.00 ± 1.89	3.00 ± 0.00	3.44 ± 0.36
OF	OC+VC	30.00 ± 0.94	43.05 ± 1.19	11.15 ± 0.80	16.23 ± 0.59	25.67 ± 1.52	50.33 ± 2.67	3.33 ± 0.01	3.64 ± 0.36
	Urea	28.07 ± 0.52	42.35 ± 0.95	10.82 ± 1.01	15.83 ± 1.65	29.67 ± 2.88	54.33 ± 2.42	2.28 ± 0.00	2.67 ± 0.57
	NPK	29.00 ± 0.36	43.92 ± 1.22	13.50 ± 0.47	18.57 ± 0.51	25.33 ± 1.44	58.67 ± 0.98	2.56 ± 0.24	2.78 ± 0.24
	DAP	29.80 ± 0.22	45.01 ± 0.81	14.02 ± 1.42	19.55 ± 1.97	26.00 ± 2.83	58.67 ± 1.78	2.67 ± 0.31	2.89 ± 0.24
	NPK+Urea	30.50 ± 0.41	48.10 ± 0.54	15.60 ± 0.57	21.64 ± 1.87	26.67 ± 2.37	60.33 ± 1.91	2.78 ± 0.22	3.00 ± 0.16
IF	DAP+Urea	30.85 ± 0.33	52.35 ± 1.18	16.60 ± 0.97	22.29 ± 1.12	27.00 ± 2.62	61.00 ± 1.70	3.33 ± 0.16	3.67 ± 0.33
	GA 250 mg L-1	30.03 ± 0.31	45.82 ± 1.22	12.18 ± 1.27	16.08 ± 1.12	27.33 ± 2.18	73.33 ± 2.60	3.67 ± 0.31	3.44 ± 0.09
	GA 500 mg L-1	33.57 ± 0.62	58.77 ± 1.21	13.47 ± 0.75	18.47 ± 1.66	33.67 ± 2.37	77.67 ± 3.31	4.00 ± 0.24	4.00 ± 0.00
	GA 1000 mg L-1	29.97 ± 0.91	43.30 ± 2.47	11.98 ± 1.53	16.03 ± 1.07	25.00 ± 2.05	69.00 ± 1.89	3.11 ± 0.76	3.00 ± 0.16
	PEG 250 mgL-1	28.80 ± 0.57	40.81 ± 1.00	13.38 ± 2.35	15.61 ± 1.40	22.67 ± 0.72	44.67 ± 1.19	3.16 ± 0.55	3.00 ± 0.00
	PEG 500 mg L-1	27.00 ± 1.69	37.47 ± 2.36	13.06 ± 1.24	12.90 ± 1.14	21.00 ± 1.25	41.00 ± 1.25	2.00 ± 0.32	3.00 ± 0.16
PGRs	PEG 1000 mg L-1	26.25 ± 0.93	35.03 ± 0.98	11.58 ± 1.65	12.07 ± 0.84	20.00 ± 1.89	37.67 ± 0.98	3.08 ± 0.06	2.78 ± 0.24
p- value		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F value		85.33	4.83	65.75	4.53	104.11	5.56	3.29	3.02

Table 4: Morphological and reproductive parameters of transplanted seedlings of Withania somnifera in Haldwani (400 m), Bhujiyaghat (900 m) and Khurpatal (1900 m)

TT	*Treatment*	*Altitude (m)*	*Shoot length (cm)*	*Root length (cm)*	*Leaf area (mm2)*	*Berry per plant*	*Seeds per berry*
Organic Fertilizer	Control	400	60.27 ± 8.18	19.00 ± 2.02	368.50 ± 66.65	123.67 ± 11.57	25.67 ± 1.91
		900	56.73 ± 6.73	17.55 ± 1.89	318.58 ± 49.46	112.00 ± 9.42	21.00 ± 2.05
		1900	49.73 ± 4.68	14.88 ± 2.13	274.22 ± 47.35	96.00 ± 11.12	16.67 ± 0.98
	Manure	400	64.09 ± 2.50	21.42 ± 1.05	402.83 ± 51.25	150.33 ± 7.85	30.33 ± 3.57
		900	59.42 ± 3.43	18.58 ± 2.28	354.53 ± 49.74	137.33 ± 16.83	24.33 ± 2.60
		1900	51.42 ± 2.85	15.20 ± 1.21	300.25 ± 46.45	105.67 ± 10.88	20.67 ± 2.60
	OC	400	67.84 ± 6.82	22.54 ± 1.10	447.25 ± 53.85	157.67 ± 11.36	31.33 ± 4.06
		900	63.51 ± 4.96	20.54 ± 1.98	384.82 ± 49.54	142.67 ± 10.08	25.33 ± 1.44
		1900	54.51 ± 1.86	17.67 ± 1.73	342.55 ± 61.64	112.00 ± 13.59	22.00 ± 2.83
	VC	400	68.40 ± 1.94	21.91 ± 3.28	475.65 ± 60.59	162.67 ± 18.39	33.33 ± 3.34
		900	65.07 ± 4.65	20.01 ± 2.61	427.48 ± 38.46	151.33 ± 6.62	30.67 ± 2.60
		1900	56.73 ± 3.91	16.90 ± 1.75	372.32 ± 36.90	117.33 ± 11.25	25.33 ± 2.42
	OC+VC	400	72.72 ± 3.19	24.56 ± 2.49	513.22 ± 90.15	170.33 ± 7.09	36.00 ± 4.98
		900	68.72 ± 3.75	22.03 ± 2.01	449.05 ± 70.68	164.67 ± 7.66	32.00 ± 2.94
		1900	60.39 ± 6.83	19.14 ± 1.69	398.22 ± 81.25	132.33 ± 9.27	27.00 ± 2.86
Inorganic Fertilizer	Urea	400	71.29 ± 3.35	22.49 ± 2.76	464.00 ± 60.06	150.00 ± 6.60	32.00 ± 2.94
		900	66.05 ± 4.06	20.49 ± 1.82	407.25 ± 54.35	138.67 ± 15.50	26.00 ± 1.89
		1900	59.42 ± 4.14	18.49 ± 1.88	358.25 ± 38.23	109.67 ± 10.34	24.33 ± 2.33
	NPK	400	74.25 ± 4.65	24.24 ± 2.28	490.92 ± 63.01	161.00 ± 11.86	33.00 ± 3.83
		900	69.58 ± 4.31	21.91 ± 1.81	412.75 ± 41.87	144.33 ± 14.65	27.33 ± 2.13
		1900	62.58 ± 5.96	19.70 ± 3.72	367.25 ± 36.61	120.33 ± 16.51	22.33 ± 2.23
	DAP	400	77.67 ± 1.91	27.24 ± 2.79	506.30 ± 47.43	165.33 ± 16.50	33.00 ± 4.97
		900	72.63 ± 2.06	23.90 ± 2.07	449.47 ± 58.33	153.33 ± 5.81	28.33 ± 2.60
		1900	63.63 ± 4.45	21.57 ± 1.90	393.47 ± 48.37	128.00 ± 14.76	24.00 ± 2.05
	NPK + Urea	400	83.10 ± 5.26	28.64 ± 2.30	523.05 ± 54.88	186.00 ± 12.76	35.67 ± 4.25
		900	76.77 ± 2.66	24.97 ± 2.14	482.97 ± 40.81	165.33 ± 7.25	30.00 ± 2.87
		1900	66.77 ± 6.05	22.31 ± 2.07	423.05 ± 53.17	135.67 ± 11.85	28.67 ± 3.14
	DAP + Urea	400	88.02 ± 6.52	30.09 ± 2.52	545.33 ± 54.99	200.67 ± 23.10	37.00 ± 2.87
		900	81.00 ± 9.10	27.42 ± 1.60	502.92 ± 99.94	174.00 ± 18.79	33.67 ± 2.13
		1900	71.02 ± 5.07	25.42 ± 1.60	442.33 ± 55.99	146.67 ± 20.63	30.67 ± 2.88
Plant growth Regulators	GA 250 mg L^{-1}	400	89.16 ± 3.73	23.41 ± 2.52	573.50 ± 83.71	195.67 ± 18.24	37.67 ± 3.81
		900	83.82 ± 4.18	20.74 ± 0.71	526.67 ± 65.07	164.00 ± 12.81	34.33 ± 2.42
		1900	71.82 ± 4.80	18.41 ± 1.89	460.00 ± 51.38	137.67 ± 15.35	28.00 ± 2.62
	GA 500 mg L^{-1}	400	93.44 ± 3.65	25.47 ± 1.66	613.83 ± 80.26	212.00 ± 15.56	40.00 ± 2.49
		900	89.10 ± 3.95	23.61 ± 1.35	538.75 ± 45.89	182.67 ± 15.54	35.33 ± 1.78
		1900	76.44 ± 6.72	21.37 ± 1.79	488.42 ± 35.33	152.33 ± 12.11	31.00 ± 1.70
	GA 1000 mg L^{-1}	400	86.63 ± 5.12	22.69 ± 1.75	535.08 ± 86.07	173.67 ± 19.73	35.33 ± 4.88
		900	81.97 ± 3.30	20.36 ± 0.55	483.92 ± 69.52	159.00 ± 7.76	32.00 ± 2.45
		1900	70.77 ± 2.87	17.92 ± 1.29	446.58 ± 59.81	125.67 ± 6.90	25.33 ± 2.72

Plant growth Regulators	PEG 250 mg L^{-1}	400	61.81 ± 4.85	18.61 ± 1.65	322.83 ± 38.41	85.67 ± 7.95	27.67 ± 1.44
		900	56.48 ± 4.19	17.34 ± 0.76	279.83 ± 23.08	78.00 ± 9.57	24.33 ± 1.19
		1900	51.48 ± 6.32	15.68 ± 1.48	247.83 ± 46.07	70.67 ± 8.85	22.33 ± 1.59
	PEG 500 mg L^{-1}	400	60.13 ± 3.98	17.23 ± 0.83	307.75 ± 26.60	78.00 ± 9.93	23.33 ± 0.72
		900	54.60 ± 4.06	16.70 ± 0.92	264.42 ± 25.38	71.33 ± 5.52	20.00 ± 1.25
		1900	49.60 ± 4.71	14.36 ± 1.06	223.50 ± 10.62	65.33 ± 6.50	17.67 ± 1.19
	PEG1000 mg L^{-1}	400	58.36 ± 3.33	17.00 ± 1.15	295.50 ± 51.94	74.00 ± 9.89	20.67 ± 1.66
		900	51.03 ± 4.02	16.00 ± 1.89	254.08 ± 25.94	67.33 ± 8.65	15.33 ± 1.91
		1900	48.36 ± 4.04	14.00 ± 1.15	203.08 ± 8.11	57.00 ± 8.81	14.67 ± 1.36
	Treatment	*p- value*	0.00	0.00	0.00	0.00	0.00
		F value	9.38	6.37	6.46	12.94	5.49
Altitude		*p- value*	0.00	0.00	0.00	0.00	0.00
		F value	11.99	11.73	7.43	10.98	19.66

performance of *W. somnifera* (Kaur *et al.,* 2018; Niyaz and Siddiqui, 2015; Khanna *et al.,* 2013). Vermicompost was found to be effective in seed germination and seedling growth (Kaur *et al.,* 2018). GA demonstrated the highest germination percentage in soil and laboratory conditions (Niyaz and Siddiqui, 2015). 150 µg L^{-1} of GA at 25 ºC exhibited the highest seed germination as compared to other PGRs (Khanna *et al.,* 2013). In the present study, GA proved to be the most effective in promoting seed germination compared to other fertilizers and plant growth regulators. This is likely due to GA's ability to break seed dormancy and stimulate the synthesis of hydrolytic enzymes like α-amylase, which mobilize stored reserves such as starch, proteins, and lipids. Additionally, GA promotes cell elongation and counteracts abscisic acid (ABA), collectively enhancing the physiological processes required for germination.

Effects of fertilizers and plant growth regulators (PGRs) on seedling growth

All treatments (OF, IF and PGRs) showed a significant ($p \leq 0.05$) effect on shoot length (SL), root length (RL), number of nodes per plant (NP) and number of leaves per node (LN) of *W. somnifera* (Table S2). Treatments had shown a greater effect on the growth of seedlings after 6 and 12 months of duration as compared to the control. However, seeds treated with GA had higher SL (58.77 cm), NP (77.67), and LN (4.00) than other treatments (Table 3). *Withania coagulans* cultivated in Navsari, Gujarat, India (Mayur *et al.,* 2022) and Saravan, Iran (Ziaei and Jafari, 2022) yielded similar results. Other medicinal plants that have shown efficacy for seedling growth using GA included *Aegle marmelos* (Rout *et al.,* 2020), *Dracocephalum kotschyi* (Bidabadi and Mehralian, 2020), *Argimonia eupatoria* (Saffari *et al.,* 2021) and *Cuminum cyminum* (Rasooli *et al.,* 2021). Some studies on *W. somnifera* showed that integrated nutrient management using OF and IF promotes maximum seedling growth (Shrivastava *et al.,* 2018, Manohar *et al.,* 2012). Vermicompost and castor cake were also found effective in improving seedling growth of *W. somnifera* (Basak *et al.,* 2020). However, there has been no report regarding the effect of GA on the seedling growth of *W. somnifera*. The present study confirmed that GA plays a substantial role in seedling growth. This could be because GA activated amylase metabolism, which converts carbohydrate, stored in the seed, into sugar, which provides the required energy and nutrition to the seedling for its proper growth (Dilip *et al.,* 2017).

Effects of treatments and altitudes on morphological and reproductive traits of transplanted seedlings

Seedlings of *W. somnifera* transplanted at various altitudes have shown phenotypic plasticity. Both altitude and treatment of seedling with stimulants (fertilizers or PGR) showed a significant effect at ($p \leq 0.05$) on morphological and reproductive traits as compared to the control (Table S3). The current study found that morphological traits such as shoot length, root length, and leaf area showed variation along altitudes and observed a maximum at low altitude (400 m) and a minimum at high altitude (1900 m) (Table 4). Seedling growth of *Euptelea pleiospermum* (Wu *et al.,* 2021) and *Parthenium hysterophorus* (Rathee *et al.,* 2021) was also found to be favorable at low altitudes. In contrast, *Cardamine pratensis* and *Plantago major* showed better seedling growth at high altitude (Bakhtiari *et al.,* 2019). Reproductive traits of *W. somnifera,* such as the number of berries per plant and the number of seeds per berry, were maximum at low altitude and minimum at high altitude. However, different patterns of seed production were observed in *Pedicularis siphonantha*, which showed maximum seeds at low altitude (Dai *et al*., 2017), and *Parthenium hysterophorus*, which exhibited higher seed production at high altitude (Rathee *et al*., 2021). Phenotypic plasticity observed in *W. somnifera* across altitudes can be attributed to a combination of environmental factors and soil conditions. High altitude has low temperatures, reduced soil fertility, and shorter growing seasons, which impose stress on seedlings that limit their morphological and reproductive growth. Also, plants at high altitude may have reallocated their stored nutrient reserves toward survival rather than biomass or seed production (Abdusalam and Li, 2018; Wu *et al.,* 2021).

Conclusion

The current study concluded that gibberellic acid has a positive effect on seed germination and seedling growth of *W. somnifera*. Seedlings developed by propagation, transplanted at various altitudes, showed the best response at low altitude as compared to mid and high altitudes. Treatments and altitude impart a significant effect on morphological and reproductive plasticity. The study is useful because it provides a propagation protocol for populations who involved in the propagation of this plant, especially in temperate regions. The study also concludes phenotypic plasticity of *Withania somnifera* at various altitudes due to climatic or nutrient availability. Further research is needed to determine how different treatments and altitudes affect the various phytochemicals, including the bioactive molecules responsible for the medicinal value of *Withania somnifera*.

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Author Contribution Statement

RG designed, performed the experiment; data curation and analysis; statistical analysis, preparation of figures and tables, conceptualization and wrote the first original draft of the manuscript; NL, ST, LSL supervised and finalized the manuscript; HKC and NM edited the language.

Conflict of interest

The corresponding author declares that there is no conflict of interest on behalf of all authors.

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Supplementary tables

Table S1: Physical and chemical properties of soil used for propagation

Parameters	*Soil depth*		
	0-10 cm	10-20 cm	20-30 cm
SMC (%)	4.26 ± 0.67	4.73 ± 0.71	5.26 ± 0.99
bD (g cm−3)	0.69 ± 0.01	0.77 ± 0.04	0.80 ± 0.04
Po (%)	73.46 ± 0.38	70.51 ± 0.64	69.23 ± 0.46
Sand (%)	59.14 ± 0.42	56.53 ± 0.20	49.46 ± 0.41
Silt (%)	16.43 ± 0.56	16.70 ± 0.70	19.77 ± 0.21
Clay (%)	24.43 ± 0.55	26.77 ± 0.87	30.77 ± 0.21
pH	6.90 ± 0.03	7.10 ± 0.08	7.30 ± 0.05
SOC (%)	3.94 ± 0.03	4.04 ± 0.07	3.51 ± 0.15
TKN (%)	0.16 ± 0.01	0.10 ± 0.01	0.06 ± 0.00
P (%)	0.0035 ± 0.00	0.0033 ± 0.00	0.0018 ± 0.00
K (%)	0.0229 ± 0.00	0.0161 ± 0.00	0.0097 ± 0.00

*SMC= Soil moisture content, bD= Bulk density, Po= Porosity, SOC= Soil organic carbon, TKN=Total Kjeldhal nitrogen, P= Phosphorous, K= Potassium

Table S2: Level of significance of germination and growth parameters using one-way ANOVA

Germination parameters		*Seed germination*					*Growth parameters*		*6 months*					*12 months*				
		SS	*df*	*Mean square*	*f*	*Sig.*			*SS*	*df*	*Mean square*	*f*	*Sig.*	*SS*	*df*	*Mean square*	*f*	*Sig.*
GP	BG	1020.00	15	68.00	0.00	.000	SL	BG	8959.66	15	597.31	85.33	.000	557.33	15	37.15	4.80	.000
	WG	.00	32	.00				WG	224.00	32	7.00			247.72	32	7.74		
	Total	1020.00	47					Total	9183.66	47				805.05	47			
GE	BG	8959.66	15	597.31	85.33	.000	RL	BG	18410.66	15	1227.37	65.75	.000	482.04	15	32.13	4.53	.000
	WG	224.00	32	7.00				WG	597.33	32	18.66			226.67	32	7.08		
	Total	9183.66	47					Total	19008.00	47				708.71	47			
GT	BG	18410.66	15	1227.37	65.75	.000	NP	BG	4.26	15	.28	104.11	.000	1577.81	15	105.18	5.56	.000
	WG	597.33	32	18.66				WG	.08	32	.00			604.66	32	18.89		
	Total	19008.00	47					Total	4.35	47				2182.47	47			
VI	BG	4.26	15	.28	104.11	.000	LN	BG	20.20	15	1.34	3.29	.002	13.95	15	.93	3.02	.004
	WG	.08	32	.00				WG	13.09	32	.40			9.84	32	.30		
	Total	4.35	47					Total	33.30	47				23.80	47			

Table S3: Level of significance of growth parameters by effect of replicates, treatments and altitudes using one-way ANOVA

Growth parameters		*Replicates*					*Treatments*					*Altitudes*				
		SS	*df*	*Mean square*	*f*	*Sig.*	*SS*	*df*	*Mean square*	*f*	*Sig.*	*SS*	*df*	*Mean square*	*f*	*Sig.*
SL	BG	20110.51	47	427.88	4.27	.000	15559.67	15	1037.31	9.38	.000	4320.04	2	2160.02	11.99	.000
	WG	9603.13	96	100.03			14153.97	128	110.57			25393.61	141	180.09		
	Total	29713.65	143				29713.65	143				29713.65	143			
RL	BG	2075.34	47	44.15	2.80	.000	1533.52	15	102.23	6.37	.000	511.67	2	255.89	11.73	.000
	WG	1509.65	96	15.72			2051.42	128	16.02			3073.31	141	21.79		
	Total	3584.99	143				3584.99	143				3584.99	143			
LA	BG	1406355.45	47	29922.45	2.29	.000	1145246.21	15	76349.74	6.46	.000	253479.14	2	126739.57	7.43	.001
	WG	1250500.44	96	13026.04			1511609.68	128	11809.45			2403376.75	141	17045.22		
	Total	2656855.89	143				2656855.89	143				2656855.89	143			
BP	BG	216059.88	47	4597.01	6.35	.000	172059.44	15	11470.63	12.94	.000	38491.01	2	19245.50	10.98	.000
	WG	69438.66	96	723.31			113439.11	128	886.24			247007.54	141	1751.82		
	Total	285498.55	143				285498.55	143				285498.55	143			
SB	BG	5132.22	47	109.19	3.39	.000	3223.33	15	214.88	5.49	.000	1794.09	2	897.04	19.66	.000
	WG	3932.66	96	32.21			5001.55	128	39.07			6430.79	141	45.60		
	Total	8224.88	143				8224.88	143				8224.88	143			

*This table signifies that the treatments, altitudes were significant or non-significant for growth parameter at level of Significance $p \leq 0.05$ where SS= Sum of squares, BG= Between groups, WG= Within groups; SL= Shoot length, RL= Root length, LA= Leaf area, BP= Berry per plant, SB= Seeds per berry.