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RESEARCH ARTICLE

Breaking morphophysiological dormancy in ashwagandha *Withania somnifera* (L.) Dunal seeds: A synergistic approach through scarification and GA_3 priming

R Muralidharan[1], D Thirusendura Selvi[1*], V Manonmani[1], A Ramalakshmi[2], L Nalina[3], P G Kavitha[4], V Vijayalakshmi[5] & B Venudevan[6]

[1]Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India
[2]Department of Food Process Engineering, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India
[3]Office of Controllerate of Examinations, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India
[4]Department of Nematology, Tamil Nadu Agricultural University, Coimbatore 641 003, Tamil Nadu, India
[5]Agricultural College and Research Institute, Tamil Nadu Agricultural University, Pudukkottai 622 104, Tamil Nadu, India
[6]Krishi Vigyan Kendra, Tamil Nadu Agricultural University, Virudhunagar 626 001, Tamil Nadu, India

*Correspondence email - thirusenduraselvi.d@tnau.ac.in

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Abstract

Ashwagandha [*Withania somnifera* (L.) Dunal], known as Indian winter cherry, has a rising demand worldwide with a market size of USD 837.5 million in the year 2025, but suffers significant cultivation difficulties. The major problem is that the freshly harvested seeds are notoriously difficult to germinate, creating a bottleneck in commercial production. This study investigates the dormancy-breaking treatments in Ashwagandha seeds using the Vallabh 1 variety. We found that 86 % of the freshly harvested seeds are viable and the remaining 14 % seeds are non-viable, but only 58 % naturally germinated. Physiological barriers in the seed coat cause the seed to imbibe slowly and our findings show that the embryo is undeveloped, resulting in morphophysiological dormancy. The seeds are subjected to scarification, GA_3 priming and combined treatment (scarification + GA_3 priming) in order to break this dormancy. The physiological barrier can be broken down by scarification and GA_3 boosts enzymatic activity to promote germination. The scarification for 3 min improved the germination to 68 %. However, the GA_3 primed seeds at 250-500 ppm achieved outstanding germination rates of 82 %-84 % and remarkable root development. Exceptional outcomes were obtained with the combination of moderate scarification and GA_3 priming at 500 ppm with 86 % germination, an average root length of 11.83 cm and enhanced seedling vigour. This simple combination provides a practical solution for commercial cultivation. The research provides a proven method to overcome the biggest cultivation challenge, supporting increased production of Ashwagandha for modern health needs.

Keywords: Ashwagandha; GA_3 priming; morphophysiological dormancy; scarification; seed dormancy

Introduction

Medicinal crops are becoming increasingly significant in today's rapidly evolving world, as people seek safe, sustainable and natural ways to enhance their health and well-being. Ashwagandha [*Withania somnifera* (L.) Dunal] is a medicinal plant of great importance, utilised in both traditional and modern healthcare systems. Ashwagandha belongs to the Solanaceae family, also known as the 'Indian winter cherry'. It has been highly exploited for its highly available bioactive compounds, such as alkaloids, steroids, flavones, glycosides, carbohydrates, tannins, coumarin, saponins, terpenoids and significant therapeutic values. It is a small to medium-sized shrub that ranges in height from 30 cm to 150 cm (1). The leaves of Ashwagandha are rich in withanolides, a steroidal lactone which is the most physiologically active component and used to make ointment, treat haemorrhoids and treat rheumatism; the berries are used to treat cuts, wounds, abscesses and inflammation (2). Ayurvedic and Unani remedies are made from the roots, which consist of withaferin A, the bioactive ingredient. It is used to cure heart problems, arthritis, bronchitis, leucoderma, tuberculosis and tumours. Stems are also used to treat diarrhoea and leg cramps. It's a 'rasayana' herb because of its adaptogenic and revitalising qualities. The phytochemicals present in these plants have various therapeutic properties such as anti-inflammatory, anti-microbial, anti-diabetic, cardioprotective, antihepatitic, anti-stress, immunomodulatory, anti-cancer and antioxidant (3).

Being the strongest adaptogen among herbal supplements, Ashwagandha has become a star in the global herbal medicine market (4). Ashwagandha exports from India have become the largest in the world (5). The ashwagandha market is expected to grow at a Compound Annual Growth Rate (CAGR) of 9.30 % from

2025 to 2034, from an estimated USD 837.50 million in 2025 to around USD 1864.51 million by 2034 (6). India is the primary producer and supplier of fine root grade ashwagandha globally (7). India leads the world in Ashwagandha export with 5344 shipments. In May 2025 alone, 241 shipments of ashwagandha were exported from India. This is a growth of 14 % sequentially from April 2025 and a 221 % year-over-year increase from May 2024 (8). The district of Neemuch in Madhya Pradesh, India, a key hub for cultivating high-quality ashwagandha, produced an estimated 1519 MT from 2170 ha in 2022-2023. An estimated 8000-10000 MT of ashwagandha dry roots is needed worldwide, while the average production from 10000 ha of land in India (Madhya Pradesh, Rajasthan, Uttar Pradesh, Andhra Pradesh and Karnataka) was 6720.7 MT (7, 9). Ashwagandha sales in the United States doubled from 2020 to 2021, reaching 92.4 US$M (10). The export market trend for India's ashwagandha plant parts, extracts and value-added products shows a sharp increase from 468 US$M to 877 US$M over the last ten years due to the demand for natural, generally recognised as safe (GRAS) certified nutraceutical supplements that boost immunity (5).

Medicinal plants are commonly propagated and regenerated using seed-based, clonal and micropropagation techniques. In the growing demand, the most efficient and practical method of growing medicinal plants is through seed. The purpose of this study is to develop and optimise effective pre-sowing treatment protocols that can significantly enhance the germination rate, thereby addressing the primary bottleneck in the commercial cultivation of this economically important medicinal plant. Given the increasing global demand for Ashwagandha in pharmaceutical and nutraceutical industries, coupled with the persistent challenges of low seed germination and dormancy that limit production scalability, there is a need to identify scientifically validated methods to ensure consistent crop establishment (11). This research aims to identify the type of dormancy and evaluate appropriate pre-sowing treatments to break seed dormancy mechanisms, ultimately providing farmers and commercial cultivators with reliable protocols that improve germination success rates, enhance production efficiency and support the sustainable supply of high-quality Ashwagandha raw material for traditional medicine and modern pharmaceutical applications.

Materials and Methods

This experiment was conducted at the laboratory of the Department of Seed Science and Technology, Tamil Nadu Agricultural University, Coimbatore 2025. It aimed to break the dormancy of freshly harvested Ashwagandha seeds by pre-sowing treatments to enhance germination. Freshly harvested seeds of the Ashwagandha variety Vallabh 1 (VA 1) were procured from V.P. Samy Organic Farm, Dindigul, Tamil Nadu. The imbibition pattern of the freshly harvested seeds was studied. Then the seeds were exposed to physical, hormonal and combined treatments and the various physiological parameters were studied using the roll towel method.

Imbibition

The imbibition pattern was studied in the freshly harvested Ashwagandha seeds. Initially, 2 g of seeds were taken and soaked in water and the weight of the seeds was noted at a 1 hr time interval. This experiment was continued until the weight of the soaked seeds remained static.

Viability test

The viability test was conducted using 0.1 % of 2,3,5-triphenyltetrazolium chloride by following the protocol described in previous studies for Solanaceae crops with eight replications of 50 seeds each (12).

Physical treatment

The freshly harvested Ashwagandha seeds were subjected to scarification using sandpaper. The seeds were rubbed between sandpaper for three different durations: 3 mins, 5 mins and 7 mins. Care should be taken while scarification to avoid injury to the seeds. The seeds that are not scarified are taken as control.

Treatment details

T_0 - Control

T_1 - Scarification (3 mins)

T_2 - Scarification (5 mins)

T_3 - Scarification (7 mins)

Hormonal treatment

The freshly harvested Ashwagandha seeds were exposed to priming with GA_3 solution for 24 hours. Different concentrations of GA_3 solutions were prepared by dissolving them in distilled water. The non-primed seeds are taken as absolute control and hydropriming (priming of seeds using water) is taken as positive control.

Treatment details

T_0 - Control

T_1 - Hydropriming

T_2 - GA_3 (50 ppm)

T_3 - GA_3 (100 ppm)

T_4 - GA_3 (250 ppm)

T_5 - GA_3 (500 ppm)

Combined treatment

The best physical and hormonal treatments were combined and various physiological parameters were studied. The untreated seeds were taken as the control and hydropriming was the positive control. The combinational treatments were also compared with the best individual treatment.

Germination studies

The germination test was conducted using the roll towel method, with the towel placed in a plastic container. The germination setup was maintained in the germination room at a temperature of 25 ± 2 °C and a relative humidity (RH) of 95 ± 2 %. After counting the number of hard seeds, normal seedlings and abnormal seedlings on the 17th day, the percentage of germination was determined and the mean value was expressed as a percentage.

Root length

From the germination test, ten healthy seedlings were chosen and the primary root length was measured from the collar region to the root tip region. The calculated mean value was expressed in centimetres.

Shoot length

The seedlings used to measure root length were also used to measure shoot length. The length of the shoot was measured from the developing tip to the collar region. The calculated mean value was expressed in centimetres.

Dry matter production

Ten seedlings were collected in a brown paper bag, shade-dried for 24 hours and then dried in a hot air oven for an additional 24 hr at 80 °C. They were subsequently cooled in a desiccator containing silica gel for an additional 30 min. The dry weight of the seedlings was measured using an electronic weighing balance and the mean value was calculated and expressed as grams per 10 seedlings.

Allometric index

The allometric index was calculated based on the shoot length and root length of the seedlings, using the following formula

Allometric index = Root length/Shoot length

Vigour index

The vigour index was calculated using the formula outlined in earlier research and the mean was calculated and expressed as a whole number (13).

Statistical analysis

The data obtained from this experiment were statistically analysed using AGRES software and standard methods (14). The value in the percentage data was transformed into an arcsine value and a 5 % level critical difference was computed.

Results and Discussion

Imbibition and viability test

The seed propagation of Ashwagandha shows low germination potential. To address this issue, we have conducted imbibition and viability tests. Imbibition is a crucial factor that influences seed germination, exhibiting varying patterns among different species. The results showed that these seeds required 21 hr for imbibition (Fig. 1a). This slow trend of imbibition was associated with the presence of physiological barriers, which influence the seeds' ability to absorb water. The viability of freshly harvested seeds of Ashwagandha, as determined by the tetrazolium test, was 86 %. It is also revealed that most of the seeds have an underdeveloped embryo (Fig. 1b). A similar pattern of slow imbibition and an underdeveloped embryo was observed in some montane forest species, which is associated with morphophysiological dormancy (15). These results indicated that the freshly harvested seeds of Ashwagandha consist of morphophysiological dormancy. Hence, to enhance germination, seeds were exposed to different kinds of pre-sowing treatments.

Physical treatment

The results demonstrated that the seeds exposed to moderate scarification had a positive influence on germination. The seeds sacrificed for 3 min had a significant impact on seedling performance, with the highest germination percentage (68 %), representing a 17 % increase over the control. This improvement is attributed to seed coat rupture, which enhances the water imbibition and gas exchange during germination. The root length was also substantially improved by 7.51 cm compared to the control, 3.91 cm (Table 1). Similarly, in *Searsia pentaphylla*, the mechanical scarification enhanced the germination with a final germination percentage of 36 % higher than control seeds and supported root growth (16).

There was no significant improvement in shoot length found. The allometric index representing the root-shoot ratio and dry matter production was also notably higher in moderate scarification, 2.61 and 0.015g/10 seedlings, than control, 1.39 and 0.012 g/10 seedlings, respectively. Similarly, this treatment enhanced the vigour indices 706 vigour index I, 0.884 vigour index II, compared to the control 390 vigour index I and 0.754 vigour index II. Interestingly, the prolonged scarification for 5 min and 7 min resulted in reduced benefits compared to moderate scarification. The scarification for 5 min had a slight improvement when compared to the control, with 58 % germination, 6.11 cm root length, 2.62 allometric index, 0.014 g/10 seedlings dry matter production, 523 vigour index I and 0.868 vigour index II. No difference in germination was found in scarification for 7 minutes and the control seeds, but there was a difference in the root length (5.64 cm), shoot length (2.99 cm), 0.013 g/10 seedlings dry matter production, 501 vigour index I and 0.870 vigour index II. These comprehensive results of physical treatments showed that moderate scarification enhanced the overall performance of the seedlings. Even though scarification improved germination and produced vigorous seedlings, it increased the mortality rate of the seeds when exposed to prolonged scarification, which is evident from our results and similar results were also observed in *Mauritia flexuosa* (17).

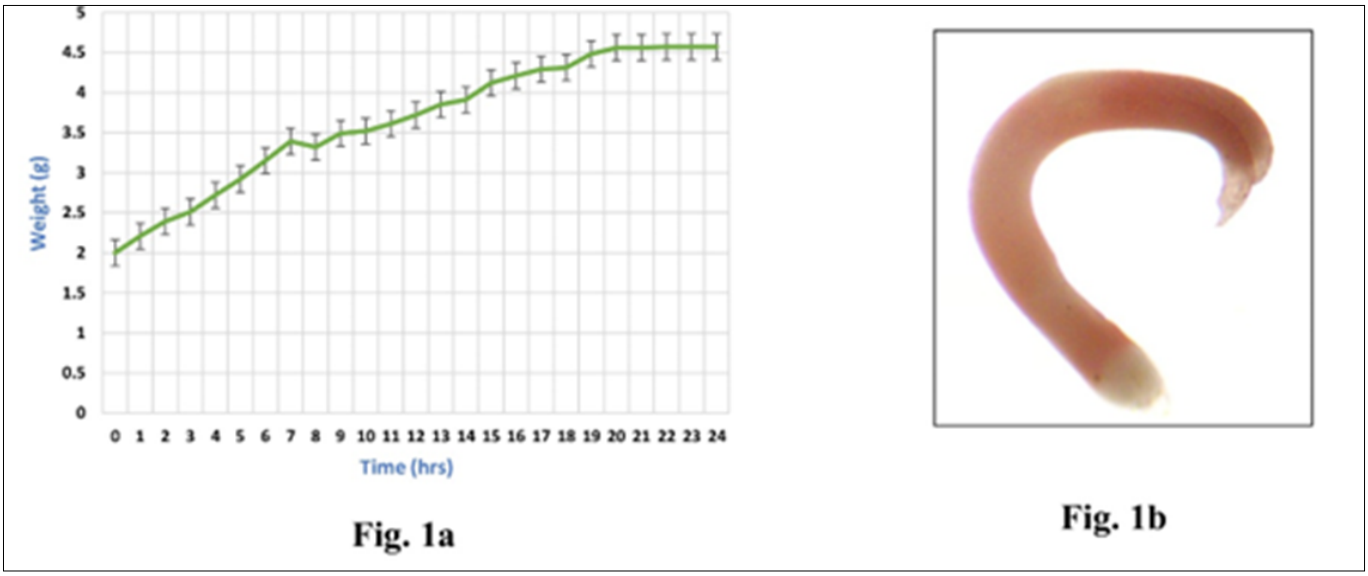

Fig. 1. a. Imbibition pattern and **b.** Underdeveloped embryo of freshly harvested seeds.

Table 1. Effect of scarification at different durations on germination, root length, shoot length, allometric index, dry matter production, vigour index I and vigour index II

Treatment	Germination (%)	Root length (cm)	Shoot length (cm)	Allometric index	Dry matter production (g/10 seedlings)	Vigour index I	Vigour index II
T_0 - Control	58 (49.60)	3.91	2.81	1.39	0.012	390	0.754
T_1 - Scarification (3 mins)	68 (55.55)	7.51	2.87	2.61	0.015	706	0.884
T_2 - Scarification (5 mins)	62 (51.94)	6.11	2.33	2.62	0.014	523	0.868
T_3 - Scarification (7 mins)	58 (49.60)	5.64	2.99	1.88	0.013	501	0.870
Mean	61 (51.36)	5.79	2.75	2.12	0.013	530	0.844
S. Ed	0.89	0.05	0.02	0.04	0.0002	7.44	0.012
CD (P=0.05)	1.949	0.120	0.060	0.090	0.0004	16.217	0.026

(Figure in the parenthesis indicates arcsine values)

Hormonal treatment

The results revealed that seeds primed with gibberellic acid (GA_3) had a profound influence on seed germination and seedling growth. It was found that the germination percentage increased progressively with increasing concentration, reaching a maximum of 84 % at 250 ppm and 82 % at 500 ppm, compared to the control (Table 2). This substantial improvement in germination demonstrates the hormone's potent capacity to break dormancy. Similar studies in *Leymus chinensis* showed that GA_3 treatment increased the seed germination rate by 14 % to 27 % and a study conducted in Ashwagandha showed that GA_3 at 150 µg/mL effectively enhanced the germination (18, 19). Application of GA_3 shifts the critical ABA/GA balance that controls dormancy and germination. The ABA/GA ratio is vital as the altered ratios directly correlated with dormancy-breaking potential (20). This improvement in germination may be due to the activation of gibberellin genes that stimulate the synthesis of hydrolases, particularly α-amylase present in the aleurone layer (21). Research indicates that the aleurone layer isolated from the dormant seeds has a higher response to the GA_3 and measures higher α-amylase secretion, which correlates with the sensitivity of dormancy depth to gibberellic acid treatment (22). Activation of these enzymes facilitates the mobilisation of stored starch food reserves by converting them into soluble sugars, enabling rapid embryo development and radicle emergence.

The results from our study showed that GA_3 priming performed superiorly to the hydroprimed seeds (66 %), which underscores the hormonal requirement for dormancy breaking. A dose-dependent response was found regarding root length, with the highest value recorded at 11.60 cm in 500 ppm, representing a remarkable increase compared to the control (3.92 cm) and hydropriming (8.48 cm). This significant increase in root length represents one of the striking findings of our study. Gibberellic acid supports cell division and elongation in meristematic tissues due to the enzyme induction process, explaining the pronounced root elongation observed in this study (23). Through several interconnected pathways, GA_3 enhances the root length by stimulating cell elongation. The cell elongation lowers the water potential of the cells and increases the water uptake and cell volume (24). Similarly, in lettuce, the hypocotyl cells are elongated on application of GA_3 (10 µM) (25). The gibberellins are accumulated in the endodermal region of the cells, which serves as the critical zone of GA_3 transporters (26). These mechanisms might be the reason for the enhancement of root length in our study. Shoot length also progressively increased with increasing concentration, showing a 3.16 cm increase compared to the control (2.65 cm) and hydropriming (2.70 cm).

Seeds primed with GA_3 exhibited a significant improvement in the allometric index, ranging from 3.07 to 3.89, in contrast to the control, which had an index of 1.47. Dry matter production and vigour indices (I & II) peaked at 500 ppm with 0.019 g/10 seedlings, 1210 and 1.64, respectively. Similarly, seeds primed at 250 ppm also positively impacted seedling enhancement, with a root length of 11.20 cm, a shoot length of 3.05 cm, a vigour index I of 1197, a vigour index II of 1.40, an allometric index and dry matter production comparable to seeds primed at 500 ppm. The improved allometric index indicates that GA_3-primed seeds promote balanced seedling growth and enhanced dry matter production. Recent research shows that seed priming with GA_3 induces morphophysiological and biochemical responses in plants, which supports our findings. From these substantial results, it is clear that seeds primed with GA_3 at 250 - 500 ppm enhanced overall seedling vigour and establishment potential (27).

Table 2. Effect of gibberellic acid seed priming on germination, root length, shoot length, allometric index, dry matter production, vigour index I and vigour index II

Treatment	Germination (%)	Root length (cm)	Shoot length (cm)	Allometric index	Dry matter production (g/10 seedlings)	Vigour index I	Vigour index II
T_0 - Control	58 (49.60)	3.92	2.65	1.47	0.013	382	0.754
T_1 - Hydropriming	66 (54.33)	8.48	2.70	3.14	0.016	738	1.056
T_2 - GA_3 (50 ppm)	65 (53.73)	8.37	2.72	3.07	0.014	721	0.728
T_3 - GA_3 (100 ppm)	74 (59.34)	10.91	2.80	3.89	0.017	1014	1.406
T_4 - GA_3 (250 ppm)	84 (66.42)	11.20	3.05	3.67	0.019	1197	1.462
T_5 - GA_3 (500 ppm)	82 (64.90)	11.60	3.16	3.67	0.019	1210	1.64
Mean	72 (58.05)	9.07	2.84	3.15	0.016	877	1.17
S. Ed	1.412	0.209	0.069	0.079	0.00	25.17	0.02
CD (P=0.05)	3.077	0.456	0.150	0.174	0.00	54.86	0.04

(Figure in the parenthesis indicates arcsine values)

Synergistic effect of physical and hormonal treatment

The combination of sacrification and GA_3 priming demonstrated a remarkable synergistic effect on seed germination parameters. The best treatments from physical and hormonal treatments were selected to study the combined impact. They yielded the highest values for all parameters, including germination percentage (86 %), root length (11.83 cm), shoot length (3.16 cm), allometric index (3.74), dry matter production (0.02 g/10 seedlings), vigour indices (1289 & 1.97 for vigour index I and II, respectively) (Fig. 2). This effect can be explained as a complementary mechanism of scarification and GA_3 priming. The scarification removes the inhibitors present in the seed coat, facilitating water uptake and gas exchange and GA_3 priming stimulates biochemical mechanisms for embryo growth and enzyme activity (28). The recent research has validated the synergistic approach across various plant species. The exogenous application of GA_3 following scarification in *Rheum khorasanicum* seeds improved the seed germination (29). Similarly, in *Koelreuteria paniculata* combination of chemical scarification and exogenous GA_3 application alleviated seed dormancy (30), supporting the efficiency of the combined approach. The significant improvement in germination percentage, root length and allometric index can improve the ability of the seedlings to uptake soil water and nutrients. This combination treatment represents a viable strategy for breaking seed dormancy in freshly harvested seeds and enhancing uniform germination.

Conclusion

The present study revealed that the freshly harvested Ashwagandha seeds consist of morphophysiological dormancy and effective strategies for breaking dormancy through pre-sowing treatments. The research demonstrated that the seeds exhibited 86 % viability, but showed poor germination (58 %) due to morphophysiological dormancy characterised by slow imbibition (21 hr) and underdeveloped embryos. The physical treatments showed that the moderate scarification for 3 minutes significantly enhanced the germination to 68 %, while also improving root length (7.51 cm). It

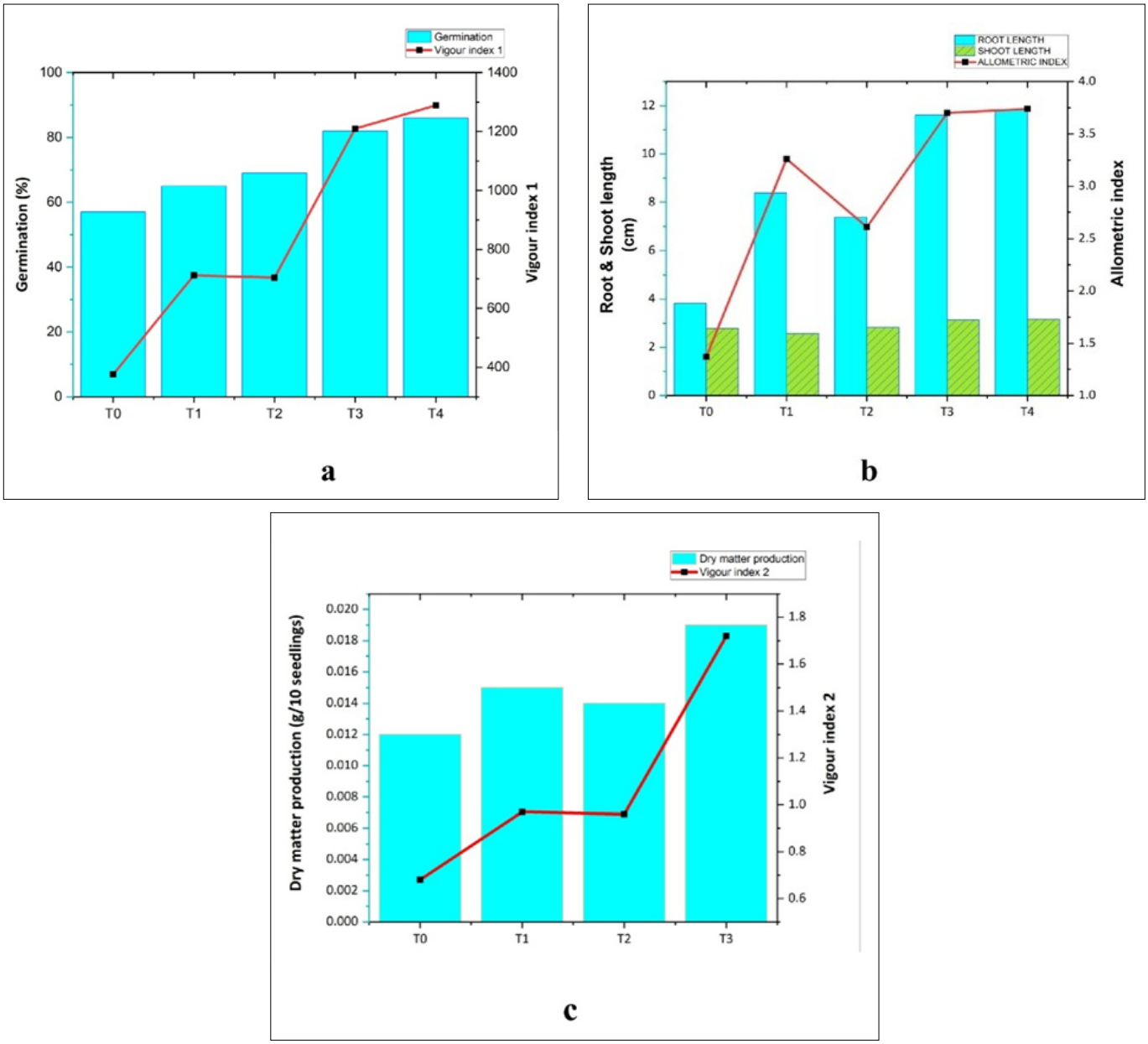

Fig. 2. Synergistic effect of physical and hormonal treatment on a. Germination and vigour index I, b. Root length, shoot length and allometric index, c. Dry matter production and vigour index II.
T_0 - Control T_1 - Hydropriming T_2 - Scarification (3 mins) T_3 - GA_3 (500 ppm) T_4 - Scarification (3 mins) + GA_3 (500 ppm).

also revealed that prolonged scarification (5-7 min) resulted in diminishing benefits. The hormonal treatment using gibberellic acid (GA_3) demonstrated superior dormancy-breaking potential, with concentrations ranging from 250 to 500 ppm, resulting in germination enhancement of 82 %-84 %. GA_3 priming at 500 ppm exceptionally enhanced the root length (11.60 cm), while significantly improving all vigour parameters. A balanced seedling development was seen as a dose-dependent response of GA_3 priming. The synergistic effect of moderate scarification (3 min) and GA_3 priming at 500 ppm yielded optimal results across all parameters, with 86 % germination, 11.83 cm root length, vigour indices (1289 and 1.97) and dry matter (0.02g/10 seedlings). This combined treatment effectively addressed the seed dormancy, providing a solution for commercial Ashwagandha cultivation, offering significant potential for meeting the growing global demand for this valuable medicinal crop. Future research should focus on validating the field performance of treated seedlings under diverse climatic conditions and exploring molecular mechanisms of dormancy for breeding improved cultivars. Additionally, cost-effective scaling of these protocols and their integration with precision agriculture technologies will be essential for widespread commercial adoption. Long-term studies evaluating the impact of seed treatments on final withanolide content and crop yield will further strengthen the practical application of these findings.

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Authors' contributions

RM conceptualised the study, experimented and prepared the original draft. DT assisted in the experimental work and contributed to drafting the manuscript. VM performed statistical analysis and contributed to reviewing and editing. AR supported statistical analysis and manuscript revision. LN contributed to data analysis and editing of the manuscript. PGK supervised the research and provided critical revisions. VV offered guidance during the study and assisted in manuscript refinement. BV contributed to supervision and provided final corrections to the manuscript. All authors read and approved the final version of the manuscript.

Compliance with ethical standards

Conflict of interest: The Authors do not have any conflicts of interest to declare.

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author(s) used “Grammarly” for language and grammar editing. After using this tool/service, the authors reviewed and edited the content as needed and takes full responsibility for the content of the publication.

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