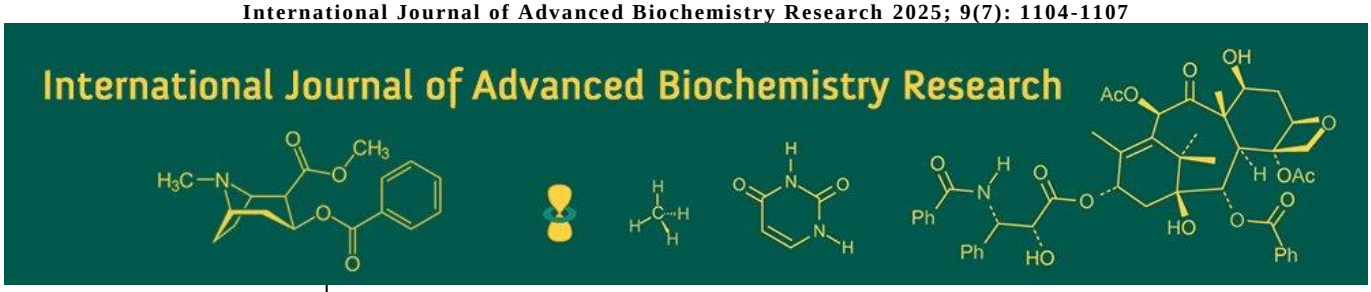

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Seed treatment induced enhancement of morpho-physiological, reproductive and seed quality traits in Ashwagandha

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Abstract
A field experimetn was undertaken to evaluate the influence of seed treatment on the morpho-physiological, reproductive and seed quality attributes of Ashwagandha. The experiment comprised eleven treatments *namely* Control (untreated), Water soaking for 24 hours and nine hormonal seed treatments including three concentrations each of Gibberellic Acid (1000, 1500, 2000 µM), Salicylic Acid (1000, 2000, 3000 µM) and Indole Acetic Acid (1000, 2000, 3000 µM), all applied as pre-sowing seed soaks for 24 hours. The trial was executed at the Research Farm of the AICRP on MAPB, NMPG, Dr. PDKV, Akola during the Rabi season of 2024 following a Randomized Block Design (RBD) with three replications. Among the treatments, seed treated with GA_3 @ 1000 µM for 24 hours resulted in the most significant enhancement of morpho-physiological and reproductive traits. This treatment recorded the maximum plant height (45.71 cm), number of primary branches plant^{-1} (8.84) and root length (35.66 cm) along with higher fresh root yield (993.23 g plot^{-1}), dry root yield (387.36 g plot^{-1}) and seed yield (166.61 g plot^{-1}). In terms of seed quality traits, the same treatment GA_3 @ 1000 µM exhibited superior germination percentage (69.41%), seedling length (7.48 cm) and seedling vigour index (519) reflecting improved seed metabolic activity and early seedling growth. These findings clearly establish that exogenous application of Gibberellic Acid (GA_3) @ 1000 µM as a seed treatment effectively enhances vegetative growth, root biomass production, reproductive success and seed quality in Ashwagandha under field conditions.

Keywords: Ashwagandha, seed treatment, gibberellic acid, root yield, germination

Introduction
Medicinal plants have long been vital to traditional health systems with Ashwagandha being a key species known for its adaptogenic and therapeutic properties (Singh *et al.*, 2011) [1]. Its roots contain important bioactive compounds like withanolides and withaferin-A (Mirjalili *et al.*, 2009) [2]. Native to India, Ashwagandha is mainly grown in Madhya Pradesh, Rajasthan, Maharashtra and Gujarat with rising global demand boosting its commercial cultivation (Verma *et al.*, 2019) [3]. However, it faces issues such as slow emergence and low germination rates, weak seedling vigor and poor field emergence (Rathore *et al.*, 2011; Ramesh and Sarang, 2015) [4, 5]. These are largely due to seed dormancy, hard seed coat and poor seed quality which affect plant uniformity, yield and phytochemical consistency (Yadav *et al.*, 2017) [6].

Ashwagandha faces inherent seed quality issues including low viability and poor vigour which hinder uniform and timely germination. Dormancy caused by physiological and physical factors such as hard seed coat and immature embryos restricts water absorption and gas exchange (Ramesh *et al.*, 2014) [7]. Endogenous inhibitors further delay germination leading to slow and uneven emergence often taking 10-15 days (Rathore *et al.*, 2011) [4]. This irregular seedling growth disrupts field operations and reduces plant stand, biomass and root yield. These challenges emphasize the need for effective seed enhancement methods to boost Ashwagandha's crop performance.

Traditional farmers have used methods like seed soaking and bio-inoculants to improve Ashwagandha germination but these lack scientific validation. There is a major gap in standardized, cost-effective and eco-friendly seed treatment protocols for commercial

cultivation. Low seed viability and poor germination highlight the need for scalable enhancement strategies. It's crucial to assess not only germination and vigour but also traits like biomass, root yield and seed quality. This study aims to identify effective seed treatments to boost growth and yield in Ashwagandha

Materials and Methods

Ashwagandha was raised in the month of November, 2024 at the Research Farm of AICRP on MAPB, NMPG, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola using a Randomized Block Design with three replications having plot size of 3.0 m × 2.4 m. The study included eleven treatments comprised Water soaked (T_1) for 24 hours, Gibberellic Acid (GA_3) @ 1000 µM (T_2), 1500 µM (T_3) and 2000 µM (T_4) concentrations soaked for 24 hours; Salicylic Acid (SA) @ 1000 µM (T_5), 2000 µM (T_6) and 3000 µM (T_7) concentrations soaked for 24 hours and Indole Acetic Acid (IAA) @ 1000 µM (T_8), 2000 µM (T_9) and 3000 µM (T_{10}) concentrations soaked for 24 hours and Control (untreated) (T_0). At harvest, plant height was recorded by selecting five plants from the net plot and measuring from the base to the tip of the youngest fully opened leaf, and the mean was expressed in centimetres. The number of primary branches plant^{-1} was counted from the same plants, and the average was computed. For root studies, root was measured using a ruler to record root length. Fresh root yield was recorded plot^{-1} at harvest, followed by drying the same roots to obtain dry root yield in grams per plot. Seed yield was calculated by harvesting, drying, and threshing mature berries from the net plot, and drying the extracted seeds in a hot air oven at 70 °C to a constant weight. Seed quality was evaluated by conducting a germination test using the Top of Paper (TP) method on Whatman No.1 filter paper, with 400 seeds per treatment kept at 25 ± 2 °C and 80% relative humidity, germination was recorded on the 30th day and expressed as a percentage. Seedling length was measured from five randomly selected seedlings per replication. Seedling vigour index was calculated by multiplying germination percentage with seedling length.

Results and Discussion

The morpho-physiological and reproductive traits of Ashwagandha were noticeably influenced by the application of various seed treatments highlighting the effectiveness of pre-sowing interventions in enhancing seed quality. The seeds treated with Gibberellic Acid (GA_3) @ 1000 µM for 24 hours (T_2) outperformed significantly. It exhibited the maximum plant height (45.71 cm), number of branches plant^{-1} (8.84) and root length (35.66 cm) followed by GA_3 @ 1500 µM for 24 hours (T_3) and GA_3 @ 2000 µM for 24 hours (T_4), whereas the minimum values were exhibited in the Control (untreated) (T_0). The increase in plant height may be attributed to GA_3 mediated stem elongation through enhanced enzyme synthesis and cell expansion (Davies, 2013) [9], while greater branching could be due to stimulation of lateral bud development (Sahitya, 2013) [10] and longer roots may reflect better mobilization of food reserves (Taiz and Zeiger, 2010) [11].

The highest fresh root yield plot^{-1} (993.23 g), dry root yield plot^{-1} (387.36 g) and seed yield plot^{-1} (166.61 g) were recorded in the seeds treated with GA_3 @ 1000 µM (T_2), followed by GA_3 @ 2000 µM for 24 hours (T_4) and GA_3 @ 1500 µM for 24 hours (T_3), while the Control (untreated) (T_0) recorded the lower fresh root yield plot^{-1} (826.07 g), dry root yield plot^{-1} (322.17 g) and seed yield plot^{-1} (131.26 g). The improved root yield may be due to enhanced physiological activity and nutrient uptake leading to greater biomass accumulation (Shahzad *et al.*, 2021) [13]. GA_3 likely promoted cell elongation and division resulting in vigorous root growth. Similarly, seed yield enhancement may be attributed to improved vegetative growth, better flower retention and efficient assimilate partitioning (Taiz and Zeiger, 2010) [11].

The seeds treated with GA_3 @ 1000 µM (T_2) revealed the higher germination (69.41%), seedling length (7.48 cm) and seedling vigour index. The increased germination and vigour may be due to faster enzymatic activity and weakening of seed coat promoting radicle emergence GA_3 enhances hydrolytic enzyme synthesis like α-amylase and protease which help in mobilizing food reserves (Bewley *et al.*, 2013) [16]. These findings are in confirmation with Basra *et al.* (2003) [15] confirming GA_3's positive influence on seedling vigour and quality.

Table 1: Effect of seed treatment on morpho-physiological traits in Ashwagandha

Treatments	Plant height (cm)	No. of branches	Root length (mm)
Control (untreated) (T_0)	32.96	7.56	28.79
Water soaking for 24 hrs. (T_1)	34.29	7.98	29.17
Gibberellic Acid (GA_3) @ 1000 µM for 24 hrs. (T_2)	45.71	8.84	35.66
Gibberellic Acid (GA_3) @ 1500 µM for 24 hrs. (T_3)	42.21	8.51	34.04
Gibberellic Acid (GA_3) @ 2000 µM for 24 hrs. (T_4)	41.54	8.74	35.60
Salicylic Acid (SA) @ 1000 µM for 24 hrs. (T_5)	36.02	8.03	29.70
Salicylic Acid (SA) @ 2000 µM for 24 hrs. (T_6)	36.67	8.09	30.28
Salicylic Acid (SA) @ 3000 µM for 24 hrs. (T_7)	36.59	8.26	30.90
Indole Acetic Acid (IAA) @ 1000 µM for 24 hrs. (T_8)	39.65	8.26	33.00
Indole Acetic Acid (IAA) @ 2000 µM for 24 hrs. (T_9)	36.72	8.27	33.87
Indole Acetic Acid (IAA) @ 3000 for 24 hrs. (T_{10})	40.98	8.43	34.27
F Test	Sig	Sig	Sig
SE(m)±	1.876	0.197	1.399
CD @ 5%	5.536	0.581	4.127
CV	8.44	4.13	7.50

Table 2: Effect of seed treatment on reproductive traits in Ashwagandha

Treatment	Fresh root yield (g plot^{-1})	Dry root yield (g plot^{-1})	Seed yield (g plot^{-1})
Control (untreated) (T_0)	826.07	322.17	131.26
Water soaking for 24 hrs. (T_1)	846.51	330.14	133.17
Gibberellic Acid (GA_3) @ 1000 µM for 24 hrs. (T_2)	993.23	387.36	166.61
Gibberellic Acid (GA_3) @ 1500 µM for 24 hrs. (T_3)	947.71	369.61	162.61
Gibberellic Acid (GA_3) @ 2000 µM for 24 hrs. (T_4)	954.07	372.09	158.55
Salicylic Acid (SA) @ 1000 µM for 24 hrs. (T_5)	851.70	348.07	135.17
Salicylic Acid (SA) @ 2000 µM for 24 hrs. (T_6)	892.49	332.16	143.18
Salicylic Acid (SA) @ 3000 µM for 24 hrs. (T_7)	929.92	348.39	139.48
Indole Acetic Acid (IAA) @ 1000 µM for 24 hrs. (T_8)	893.31	362.67	154.10
Indole Acetic Acid (IAA) @ 2000 µM for 24 hrs. (T_9)	941.21	367.07	146.09
Indole Acetic Acid (IAA) @ 3000 for 24 hrs. (T_{10})	941.59	367.22	157.56
F Test	Sig	Sig	Sig
SE(m)±	28.599	11.154	6.186
CD @ 5%	84.367	32.903	18.249
CV	5.44	5.44	7.24

Table 3: Effect of seed treatment on seed quality traits in Ashwagandha

Treatment	Seed germination (%)	Seedling length (cm)	Seedling vigour index
Control (untreated) (T_0)	49.75 (44.86)	4.64	231
Water soaking for 24 hrs. (T_1)	52.08 (46.02)	4.87	253
Gibberellic Acid (GA_3) @ 1000 µM for 24 hrs. (T_2)	69.41 (56.52)	7.48	519
Gibberellic Acid (GA_3) @ 1500 µM for 24 hrs. (T_3)	65.91 (54.23)	7.08	467
Gibberellic Acid (GA_3) @ 2000 µM for 24 hrs. (T_4)	61.08 (51.40)	7.24	442
Salicylic Acid (SA) @ 1000 µM for 24 hrs. (T_5)	55.75 (48.59)	5.08	283
Salicylic Acid (SA) @ 2000 µM for 24 hrs. (T_6)	59.25 (50.47)	5.32	315
Salicylic Acid (SA) @ 3000 µM for 24 hrs. (T_7)	59.08 (49.91)	5.76	340
Indole Acetic Acid (IAA) @ 1000 µM for 24 hrs. (T_8)	60.41 (50.89)	5.37	324
Indole Acetic Acid (IAA) @ 2000 µM for 24 hrs. (T_9)	59.58 (50.50)	5.88	350
Indole Acetic Acid (IAA) @ 3000 for 24 hrs. (T_{10})	60.75 (51.42)	6.68	405
F Test	Sig	Sig	Sig
SE(m)±	0.457	3.827	11.171
CD @ 1%	1.822	15.256	44.533
CV	1.42	5.68	5.78

(Figures in parenthesis are arcsine transformed values)

Conclusion

The study revealed that the pre-sowing seed treatment with Gibberellic Acid (GA_3) @ 1000 µM for 24 hours significantly enhanced the morpho-physiological growth root and seed yield and seed quality traits in Ashwagandha. This treatment recorded the highest plant height, branching, root length, fresh and dry root yield, seed yield, germination percentage, seedling length and vigour index. The improvements are attributed to Gibberellic Acid (GA_3) induced enzymatic activity enhanced nutrient mobilization and better assimilate partitioning. Thus, pre-sowing seed treatment with Gibberellic Acid (GA_3) @ 1000 µM for 24 hours emerges as the most effective approach to enhance vegetative growth, reproductive performance and seed quality in Ashwagandha under field conditions. These results offer a practical and cost-effective approach for improving crop performance and seedling establishment in medicinal plant cultivation.

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