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RESEARCH ARTICLE

Influence of Varying Concentrations of Indole-3-Acetic Acid (IAA) on Root Growth Characteristics of Blackgram (*Vigna mungo* L.) Varieties

K. Ashokkumar*, K.P. Krishnapriya, S.M. Dharshini, L. Dharani and G. Arun Prasath

School of Agriculture and Animal Sciences, The Gandhigram Rural Institute-Deemed to be University, Gandhigram, Dindigul, India.

Edited by:
Dr M. Prakash, PhD., Faculty of Agriculture, Annamalai University, Chidambaram, TN, India

Reviewed by:

Dr, Alagupalamuthirsolai, Sugarcane Breeding Institute, Coimbatore, India;
Dr V. J. Adavbiele, Ambrose Alli University, Edo State, Nigeria.

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*Corresponding author e-mail address:

biotech.ashok@gmail.com
(K. Ashokkumar)

ABSTRACT

The present study investigated the influence of indole-3-acetic acid (IAA) on seed germination and seedling vigor in three pulse varieties, VBN-4, VBN-8, and VBN-11. Seeds were treated with varying concentrations of IAA (0, 10, 20, 50, and 100 ppm), and parameters such as germination percentage, lateral root formation, root and shoot length, fresh and dry root weight, and vigor index were recorded. The results revealed a clear dose-dependent response, with low concentrations of IAA (10–20 ppm) significantly enhancing germination and seedling growth, while higher concentrations (50–100 ppm) exerted inhibitory effects. Among the varieties, VBN-8 exhibited the most pronounced positive response at 10 ppm, recording the highest vigor index (963), longest roots (10.7 cm), and shoots (11.5 cm). VBN-11 showed enhanced lateral root branching at 20 ppm, while VBN-4 displayed moderate improvements in vigor at 10 ppm. In contrast, all varieties demonstrated reduced germination percentage, shorter roots and shoots, and lower vigor indices at 50–100 ppm, confirming the inhibitory nature of excess auxin. The findings highlight that IAA acts as a growth regulator with a narrow optimal window, where low concentrations stimulate seedling establishment and vigor, but supra-optimal levels suppress growth. Seed priming with IAA at 10 ppm emerges as the most effective treatment for improving early seedling performance, particularly in VBN-8. These results provide valuable insights for optimizing auxin application in pulse crops and suggest future research on varietal-specific responses and field-scale validation to enhance sustainable crop production.

Keywords: IAA, seed germination, seedling vigor, *Vigna mungo,* root development, sustainable crop production

INTRODUCTION

Auxins, particularly Indole-3-acetic acid (IAA), are fundamental regulators of plant growth and development, influencing processes such as cell elongation, apical dominance, and root initiation. In legumes, auxin-mediated modulation of root system architecture is vital for nutrient acquisition, nodulation, and stress resilience. Root traits such as primary root length, lateral root formation, and root hair density directly contribute to improved nutrient uptake and yield stability, making auxin studies highly relevant for pulse crops like black gram (*Vigna mungo* L.) (Hu et al., 2021).

Previous research has demonstrated that exogenous application of IAA can either stimulate or inhibit root growth depending on concentration and genotype. Pandey et al. (2025) reported that moderate IAA levels enhanced germination and seed yield in green gram (*Vigna radiata*), while Shahzadi et al. (2022) showed that foliar IAA application alleviated lead stress in mung bean by improving macronutrient status and root development. In *Vigna mungo*, Chakrabarti et al. (2010) highlighted that symbiotic interactions with vesicular-arbuscular mycorrhiza (VAM) and *Rhizobium* enhanced endogenous IAA production, thereby improving nodulation and root growth. Broader studies in cereals also emphasize auxin's role in stress tolerance and yield potential, with Wang et al. (2018) linking auxin pathways to improved crop performance in rice.

Recent advances further underscore the complexity of auxin signaling. Jan et al. (2024) demonstrated the cross-talk between auxin and other phytohormones in shaping root system architecture, while Zhuang et al. (2025) discussed the role of Aux/IAA gene families in growth and stress responses. These findings suggest that auxin responsiveness is highly genotype-specific and concentration-dependent, reinforcing the need for targeted studies in economically important legumes.

Despite these insights, systematic investigations of exogenous IAA effects on root traits in black gram varieties remain scarce. Most existing studies focus on *Vigna radiata* or model plants, leaving a gap in understanding varietal differences in *Vigna mungo*. Given the importance of root system architecture for nutrient uptake, stress resilience, and sustainable productivity, this study was undertaken to evaluate the impact of varying IAA concentrations on root growth dynamics in three black gram varieties (VBN 4, VBN 8, and VBN 11). The study aimed to identify optimal auxin levels for enhancing root traits and to document varietal responsiveness, thereby addressing a critical research gap and providing insights for crop improvement and sustainable management strategies.

MATERIALS AND METHODS

Experimental Site and Plant Material

The experiment was conducted under controlled environmental conditions at the Genetics and Plant Breeding laboratory, School of Agriculture and Animal Sciences, Gandhigram Rural Institute, Tamil Nadu, India. Observations were recorded for 21 days post-germination, corresponding to the seedling stage of black gram (*Vigna mungo* L.) (Hu et al., 2021). Certified seeds of three varieties, VBN-4, VBN-8, and VBN-11, were used. Seeds were surface sterilized with 0.1% mercuric chloride ($HgCl_2$) for 2 minutes and rinsed thoroughly with distilled water before germination (Chakrabarti et al., 2010).

Seed Germination and Establishment

Sterilized seeds were germinated on moist, sterilized germination paper under dark conditions for 48 hours. Uniform seedlings were transferred to plastic trays containing sterile vermiculite. To ensure standardized hormone exposure, seeds were also placed in germination papers rolled vertically and partially submerged in beakers containing respective IAA concentrations. Each roll contained 10 seeds per treatment, replicated three times, following the roll paper technique that provides uniform exposure and facilitates visualization of root system architecture.

IAA Treatments

Exogenous indole-3-acetic acid (IAA) was applied via irrigation at concentrations of 0 ppm (control), 10 ppm, 20 ppm, 50 ppm, and 100 ppm (Figure 1). Analytical grade IAA was dissolved in ethanol and diluted with distilled water to prepare treatment solutions (Pandey et al., 2025). Beakers were clearly labeled and maintained in a controlled environment chamber for 48 hours in darkness to facilitate germination, followed by placement under a 16/8-hour light/dark photoperiod.

Figure 1. Experimental setup with IAA Solutions (0, 10, 20, 50, & 100 ppm) using the roll towel method.

Growth Parameter Measurements

Root growth parameters were recorded 21 days after treatment. Primary root length was measured using a scale, while the number of lateral roots was counted manually under a stereo microscope. Root biomass was determined by recording fresh weight immediately after harvest and dry weight after oven drying at 70 °C for 48 hours. Root hair density was quantified from 1 cm above the root tip using microscopic imaging and a grid count method (Shahzadi et al., 2022; Jan et al., 2024).

Statistical Analysis

Data were analyzed using Analysis of Variance (ANOVA) under a Completely Randomized Design (CRD). Mean separation was performed using Duncan's Multiple Range Test (DMRT) at a 5% significance level (Wang et al., 2018; Zhuang et al., 2025)

RESULTS AND DISCUSSION

The effect of varying concentrations of indole-3-acetic acid (IAA) on seed germination and seedling growth of three varieties (VBN-4, VBN-8, and VBN-11) revealed clear differences in response patterns. At lower concentrations (10–20 ppm), all varieties showed enhanced germination percentage. Additionally, at lower concentrations, all three varieties exhibited increased vigour index, and shoot/root growth was compared to the control (Figures 2 & 3; Tables 1 & 2). However, higher concentrations (50–100 ppm) resulted in reduced germination, shorter roots and shoots, and lower vigor indices, indicating inhibitory effects of excess auxin.

Table 1. Influence of varying IAA concentrations on the number of lateral roots and root biomass parameters of black gram varieties (21 DAS -days after sown)

Variety	IAA (ppm)	Number of Lateral Roots/plants	Fresh Root Weight /plant (g)	Dry Root Weight/plant (g)
VBN-4	0	11.0^{de}	0.062^{ef}	0.022^{ef}
VBN-4	10	14.0^{bc}	0.071^{ab}	0.027^{bc}
VBN-4	20	13.0^{cd}	0.068^{cd}	0.026^{cd}

VBN-4	50	8.0^{gh}	0.049^{hi}	0.018^{gh}
VBN-4	100	6.0^{hi}	0.041^{gh}	0.015^{hi}
VBN-8	0	10.0^{ef}	0.064^{de}	0.023^{de}
VBN-8	10	15.0^{b}	0.075^{a}	0.029^{a}
VBN-8	20	14.0^{bc}	0.072^{ab}	0.028^{ab}
VBN-8	50	9.0^{fg}	0.052^{fg}	0.019^{fg}
VBN-8	100	5.0^{i}	0.038^{hi}	0.014^{i}
VBN-11	0	9.0^{fg}	0.059^{ef}	0.021^{ef}
VBN-11	10	13.0^{cd}	0.069^{bc}	0.026^{cd}
VBN-11	20	18.0^{a}	0.067^{cd}	0.025^{cd}
VBN-11	50	8.0^{gh}	0.048^{gh}	0.017^{gh}
VBN-11	100	5.0^{i}	0.039^{i}	0.015^{hi}
CV (%)	-	5.1	4.5	4.2
CD (p=0.05)	-	1.4	0.005	0.003

Note: CV (%), Coefficient of variation; CD, Critical difference(p=0.05); Means followed by the same letter(s) within a column are not significantly different according to Duncan's Multiple Range Test (DMRT) at p = 0.05.

Table 2. Influence of varying IAA concentrations on growth parameters and vigor index of black gram varieties (21 DAS)

Variety	**IAA (ppm)**	**Average Root Length (cm)**	**Average Shoot Length (cm)**	**Vigor Index**	**Observation**
VBN-4	0	7.8^{ef}	9.4^{de}	624^{ef}	Normal
VBN-4	10	9.6^{bc}	10.2^{bc}	864^{bc}	Better
VBN-4	20	9.2^{cd}	10.1^{bc}	828^{cd}	Good
VBN-4	50	6.1^{gh}	7.5^{gh}	427^{fg}	Slightly stunted
VBN-4	100	5.3^{hi}	6.4^{hi}	318^{gh}	Inhibited
VBN-8	0	8.2^{de}	9.8^{cd}	656^{de}	Normal
VBN-8	10	10.7^{a}	11.5^{a}	963^{a}	Excellent
VBN-8	20	10.2^{ab}	11.0^{ab}	918^{ab}	Very Good
VBN-8	50	6.7^{fg}	8.2^{fg}	469^{fg}	Moderate
VBN-8	100	4.9^{i}	5.8	294^{h}	Poor
VBN-11	0	7.5^{ef}	8.7^{ef}	600^{ef}	Normal
VBN-11	10	9.4^{bc}	10.0^{bc}	846^{bc}	Good
VBN-11	20	9.0^{cd}	9.8^{cd}	810^{cd}	High branching
VBN-11	50	6.2^{gh}	7.4^{gh}	434^{fg}	Reduced growth
VBN-11	100	5.1^{hi}	6.1^{hi}	306^{h}	Poor
CV (%)	-	4.6	4.9	7.2	-
CD (p=0.05)	-	0.7	0.8	72	-

Note: CV (%), Coefficient of variation; CD, Critical difference(p=0.05); Means followed by the same letter(s) within a column are not significantly different according to Duncan's Multiple Range Test (DMRT) at p = 0.05

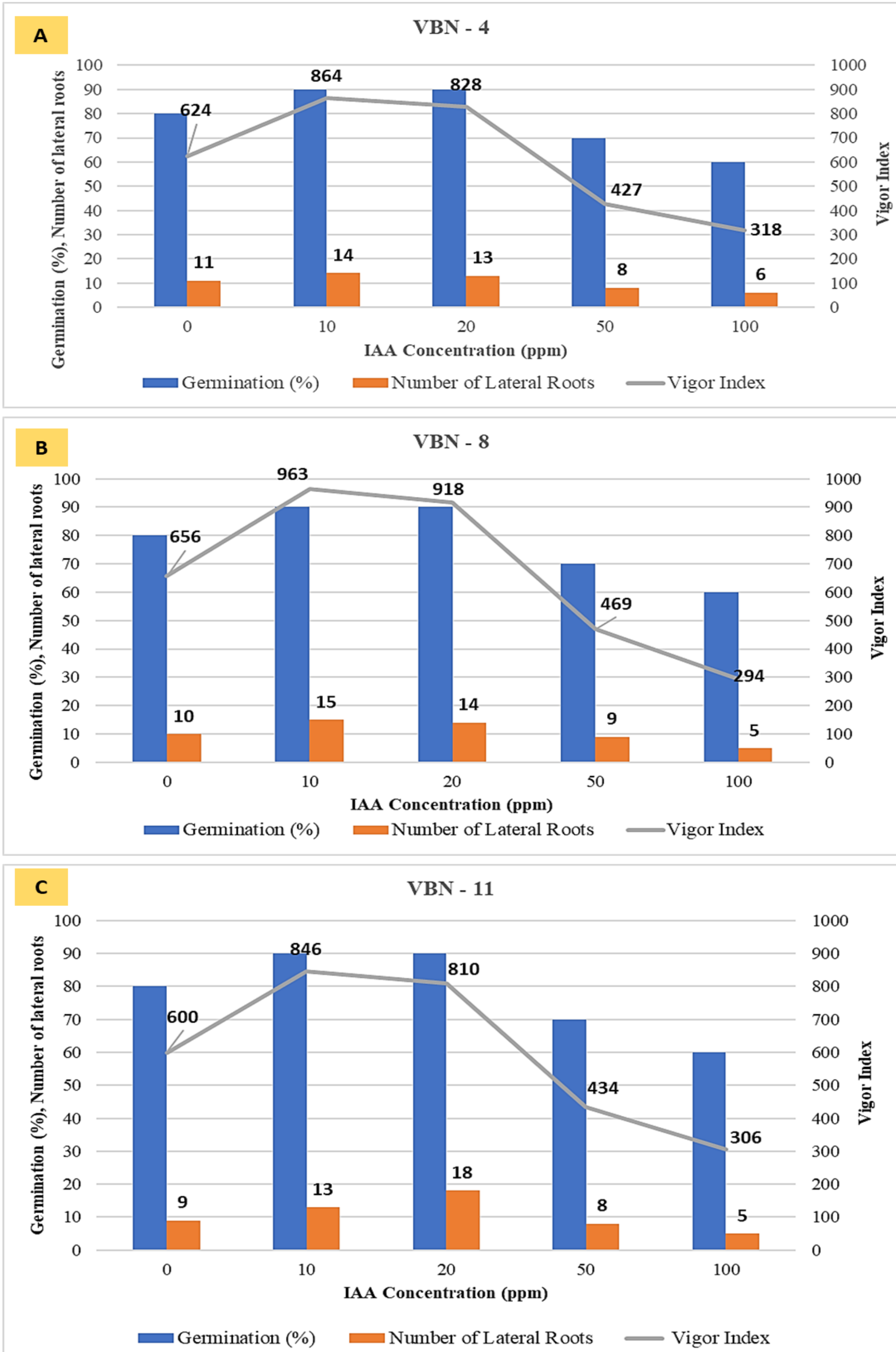

Figure 2. Effect of IAA concentration on germination percentage, number of lateral roots and root vigor index of three black gram varieties (A, B, & C represent VBN4, VBN8, & VBN11, respectively).

For VBN-4, germination improved from 80% in control to 90% at 10–20 ppm, with notable increases in lateral roots (14 at 10 ppm) and average root length (9.6 cm). The root vigor index peaked at 864 under 10 ppm, suggesting optimal stimulation at this concentration. However, at 50 ppm, germination dropped to 70%, root length decreased to 6.1 cm, and vigor index fell sharply to 427. At 100 ppm, inhibition was evident, with only 60% germination and the lowest vigor index (318), confirming that auxin overdose suppresses growth (Table 2).

In VBN-8, the stimulatory effect of IAA was most pronounced. At 10 ppm, seedlings recorded the highest vigor index (963), longest roots (10.7 cm), and shoots (11.5 cm), with 15 lateral roots. Even at 20 ppm, performance remained high (918 vigor index), though slightly reduced compared to 10 ppm. Beyond 50 ppm, growth declined significantly, with vigor index dropping to 469 at 50 ppm and 294 at 100 ppm (Figure 2). This indicates that VBN-8 is highly responsive to low IAA concentrations but sensitive to higher doses.

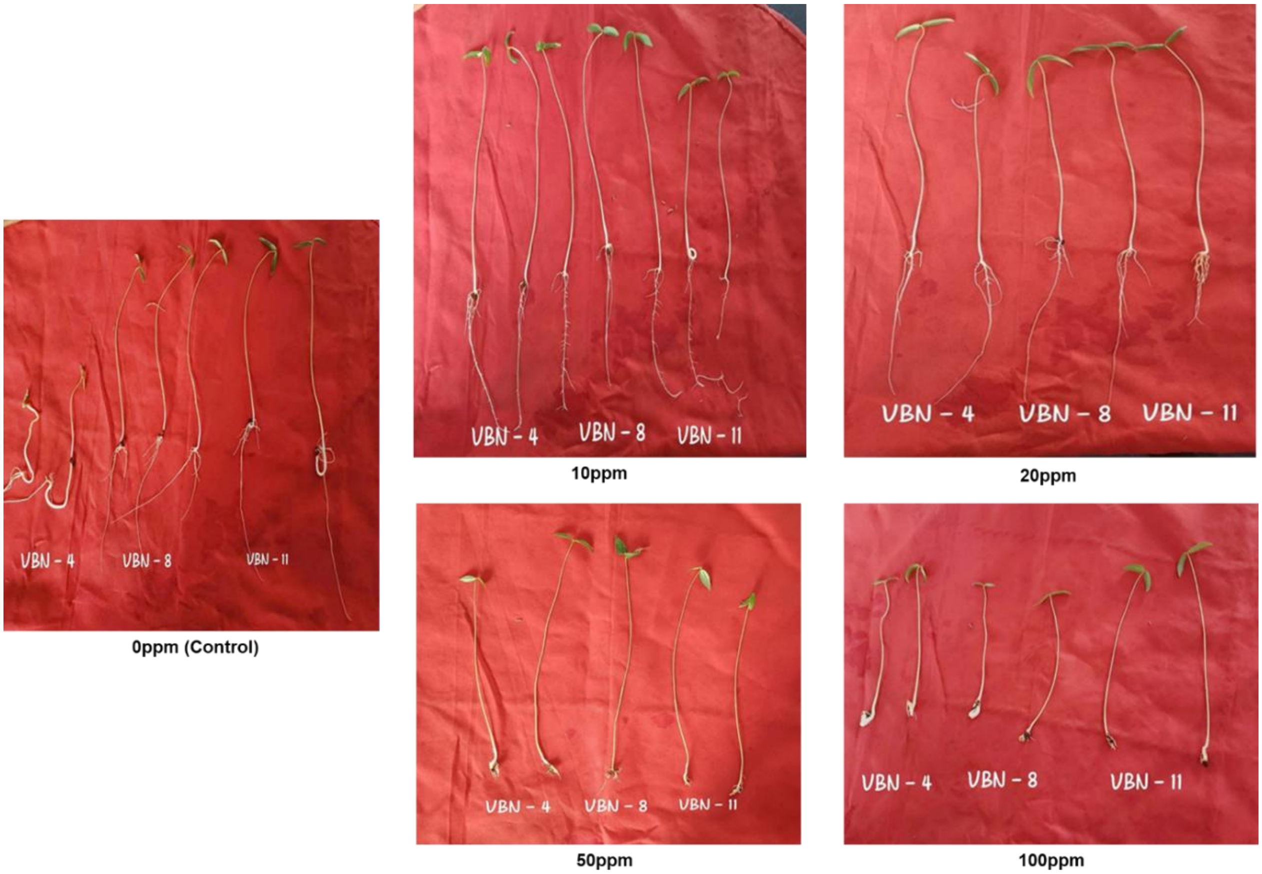

Figure 3. Influence of different IAA concentrations on seedling growth in black gram varieties.

For VBN-11, germination improved to 90% at 10–20 ppm, with remarkable branching (18 lateral roots at 20 ppm). The root vigour index was highest at 846 under 10 ppm, while 20 ppm maintained good performance (810). However, at 50 ppm, growth was reduced (434 vigor index), and at 100 ppm, poor development was observed (306 vigor index). This suggests that VBN-11 responds positively to moderate IAA levels, particularly in root branching, but suffers growth inhibition at higher concentrations.

DISCUSSION

The present study demonstrated that low concentrations of IAA (10–20 ppm) enhanced seed germination, root vigor index, and seedling growth across all three varieties (VBN-4, VBN-8, and VBN-11), while higher concentrations (50–100 ppm) inhibited growth. This biphasic response is consistent with the classical understanding of auxin physiology, where optimal levels stimulate cell division and elongation, but supra-optimal levels suppress root elongation and overall seedling vigor (Edelmann, 2022). In our study, VBN-8 exhibited the strongest positive response at 10 ppm, with the highest vigor index (963), aligning with reports that soybean seedlings treated with IAA-producing bacteria showed improved germination and nutrient uptake (Kangsopa, Singsopa, & Thawong, 2025).

The increase in lateral root formation at moderate IAA levels (e.g., 18 lateral roots in VBN-11 at 20 ppm) corroborates earlier findings that auxin plays a central role in root branching and lateral root initiation (Lewis et al., 2011). However, excessive auxin concentrations disrupt polar transport and inhibit lateral root development, explaining the reduced branching observed at 50–100 ppm. Similar inhibitory effects of high auxin levels on root elongation were reported in maize kernels treated with exogenous IAA, where germination and seedling vigor declined beyond optimal concentrations (Thanh et al., 2024).

The root vigor index trends in our study also align with seed vigor testing principles, which emphasize that vigor is a composite measure of germination percentage and seedling growth (Gupta, 1984). Our findings confirm that vigor indices peak at moderate auxin levels and decline sharply under inhibitory concentrations. Comparable results were observed in onion seeds, where moderate auxin application improved germination, but higher doses reduced seedling performance (Pokhrel et al., 2019).

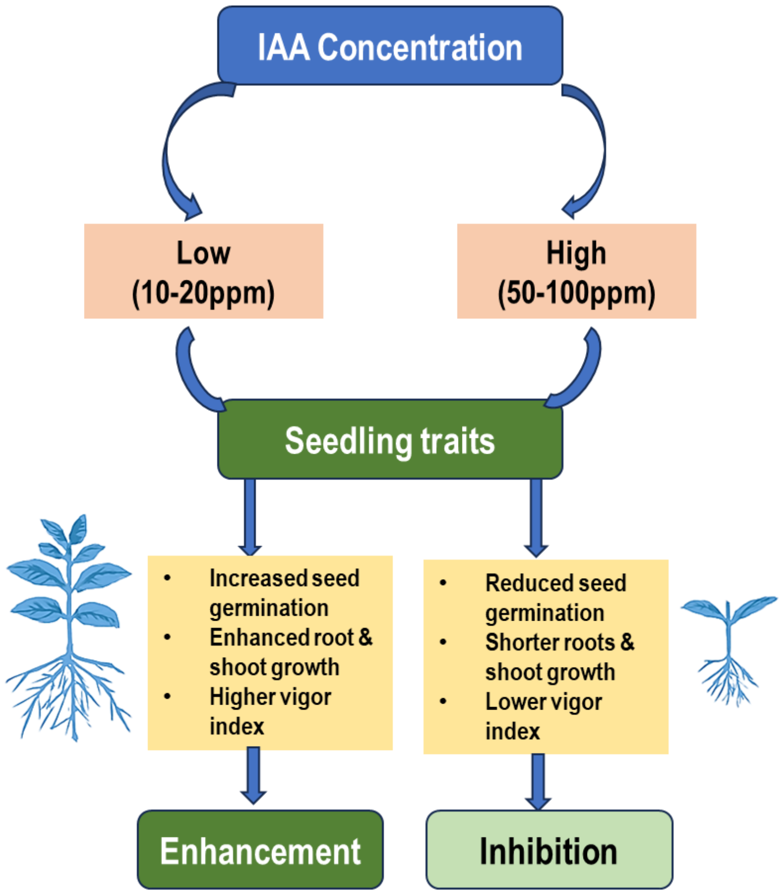

Figure 4. Conceptual diagram of the influence of IAA concentration on black varieties

The findings of this study clearly demonstrate that low concentrations of IAA (10–20 ppm) enhance seed germination, root and shoot growth development, and vigor index across all tested varieties, with VBN-8 showing the strongest positive response. In contrast, higher concentrations (50–100 ppm) were consistently detrimental, reducing germination percentage, root elongation, and vigor. The schema of the effect of IAA concentration on black varieties were displayed in Figure 4. These results highlight the importance of optimizing auxin levels for seedling establishment, as excessive application can inhibit rather than promote growth. Looking ahead, future research should focus on varietal-specific optimization of auxin treatments, integrating molecular approaches such as transcriptomics and metabolomics to uncover the mechanisms underlying enhanced root branching and vigor. Extending studies to later growth stages will help determine whether early vigor translates into improved yield and stress resilience. Moreover, exploring auxin interactions with other plant hormones, testing different application strategies (seed priming, foliar sprays, and soil drenching), and evaluating performance under abiotic stress conditions will provide a more holistic understanding of auxin's role in sustainable crop production. Aligning these insights with climate resilience and circular bioeconomy frameworks will ensure that auxin-based interventions contribute to environmentally friendly innovations in agriculture.

CONCLUSION

This study confirms that indole-3-acetic acid (IAA) exerts a concentration-dependent effect on seed germination and seedling vigor, with 10–20 ppm proving optimal for enhancing root branching, shoot growth, and vigor indices across VBN-4, VBN-8, and VBN-11. However, concentrations above 50 ppm were consistently inhibitory, reducing germination percentage and seedling performance. These findings emphasize the importance of carefully regulating auxin levels during seed priming to maximize early growth and establishment. Despite these promising results, several challenges remain. The varietal differences observed suggest that genetic variability influences auxin sensitivity, which complicates the development of a universal recommendation. Additionally, the long-term field performance of seedlings treated with IAA remains uncertain, as environmental factors such as soil type, moisture, and microbial interactions may alter auxin responses. Future research should therefore focus on field-scale validation of optimal IAA concentrations, exploring how environmental conditions and genetic backgrounds interact with auxin treatments. Studies integrating IAA priming with other growth-promoting practices, such as microbial inoculants or nutrient management, could provide more sustainable strategies for enhancing crop establishment. Moreover, molecular investigations into auxin signaling pathways in these varieties would help clarify the mechanisms underlying differential responses, paving the way for breeding programs that exploit auxin sensitivity for improved vigor and yield. In summary, while low-dose IAA priming shows clear potential for improving seedling establishment, addressing varietal variability, environmental interactions, and long-term performance will be critical to translating these findings into practical agricultural applications.

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AUTHORS CONTRIBUTION

All the authors contributed equally to this work. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL APPROVAL

Not applicable.

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AVAILABILITY OF DATA AND MATERIALS

All datasets analyzed and described during the present study are available from the corresponding author upon reasonable request.

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