

Original Research Article

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Effect of Growth Regulators on Germination Parameters in *Diospyros melanoxylon* R.

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ABSTRACT

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An experiment was conducted to evaluate the effect of different plant growth regulators on germination parameters of *Diospyros melanoxylon* L. (tendu) seeds under laboratory conditions. Seeds were soaked for 24 hours in nine treatments comprising distilled water (control), GA₃ (500 and 1000 ppm), IBA (500 and 1000 ppm), IAA (500 and 1000 ppm) and NAA (500 and 1000 ppm). Germination percentage, germination speed, germination value and germination energy were recorded at 2, 6 and 12 months after treatment during two consecutive years and the data were pooled. Significant differences were observed among the treatments for all parameters. GA₃ at 1000 ppm consistently recorded the highest germination percentage (94.25, 90.90 and 85.30% at 2, 6 and 12 months, respectively), followed by GA₃ at 500 ppm. NAA at 1000 ppm ranked next, while distilled water (control) produced the lowest values. Similar superiority of GA₃ 1000 ppm was observed for germination speed, value and energy. The results indicate that pre-sowing soaking of *D. melanoxylon* seeds in GA₃ at 1000 ppm is highly effective in improving germination attributes and can be recommended for nursery production of this economically important species.

Introduction

Forests constitute complex ecosystems comprising dense assemblages of trees and associated vegetation that interact dynamically with one another and with the abiotic environment. Globally, forests are broadly classified into major formation types determined

primarily by climatic regimes. While timber remains the principal economic product, forests also deliver critical ecological services, including climate regulation, pollution mitigation, wildlife habitat maintenance and the provision of non-timber forest products. The deep root systems, thick humus layers and soil structure characteristic of forests play a pivotal role in

hydrological regulation. Large-scale deforestation, particularly in tropical regions, poses a serious threat to global climatic stability by altering temperature regimes, humidity patterns and atmospheric carbon dioxide concentrations (Anonymous, 1998). *Diospyros melanoxylon* Roxb. belongs to family Ebenaceae, commonly known as tendu or kendu, is a medium-sized deciduous or evergreen tree that may attain a height of up to 25 m and a girth of 1.9 m. (Anonymous, 1998). *Diospyros melanoxylon* is an important multipurpose tree native to the Indian subcontinent. Its leaves constitute the primary wrapping material for beedi manufacture and thereby support the livelihoods of millions of tribal and rural communities in central and eastern India. The fruits are edible and the wood is utilised for timber and fuel (Khamngamang et al., 2017). Despite its economic and ecological significance, natural regeneration of the species is frequently limited by irregular seed crops, rapid loss of viability under ambient conditions and the absence of standardised pre-sowing treatments (Imchen et al., 2015). Plant growth regulators such as gibberellic acid (GA₃), indole-3-butyric acid (IBA), indole-3-acetic acid (IAA) and naphthalene acetic acid (NAA) are known to stimulate key metabolic processes during germination and have been successfully employed to improve germination of several forest tree species (Chitra et al., 2010; Hossain et al., 2005; Kerishnaveni et al., 2010). Systematic information on the comparative efficacy of these growth regulators on the germination attributes of *D. melanoxylon*, however, remains scarce. The present investigation was therefore undertaken to assess the effect of different concentrations of GA₃, IBA, IAA and NAA on germination percentage, germination speed, germination value and germination energy of *D. melanoxylon* seeds under laboratory conditions.

Materials and Methods

The present study was conducted in the laboratory and nursery of the College of Forestry, Sam Higginbottom Institute of Agriculture, Technology and Sciences, Naini, Allahabad. The experimental site is located between 28°52' N latitude and 81° E longitude at an altitude of 98 m above mean sea level. The climate of the region is tropical, characterised by hot summers with maximum temperatures reaching 48°C during May–June and cold winters with minimum temperatures of 1°C. Average annual rainfall ranges from 850 to 1000 mm, the major portion of which is received during the monsoon season. Ripe fruits of *Diospyros melanoxylon* were collected

from healthy, superior, medium-sized trees growing at different locations in Uttar Pradesh. The collection sites lie between 23°45'–23°35' N latitude and 82°78'–83°27' E longitude. Seeds were extracted by depulping the fruits through maceration followed by thorough washing under running tap water. The cleaned seed lot was subsequently divided into uniform samples for the experimental treatments. Several plant growth regulators, including gibberellic acid (GA₃), indole-3-butyric acid (IBA), indole-3-acetic acid (IAA) and naphthalene acetic acid (NAA), are known to enhance seed germination by stimulating key metabolic processes. In the present experiment, seeds were soaked for 24 hours in different concentrations of these hormones prior to sowing, and data on germination and seedling growth parameters were recorded. The treatments comprised soaking in distilled water (T₀ – control), GA₃ at 500 ppm (T₁), GA₃ at 1000 ppm (T₂), IBA at 500 ppm (T₃), IBA at 1000 ppm (T₄), IAA at 500 ppm (T₅), IAA at 1000 ppm (T₆), NAA at 500 ppm (T₇) and NAA at 1000 ppm (T₈). The experiment was laid out in a Completely Randomized Design (CRD) with three replications and was repeated for two consecutive years. Germination tests were conducted at 2, 6 and 12 months after treatment. Seeds were placed in sterilized Petri dishes lined with moist filter paper and maintained at optimum temperature.

The parameters recorded included germination percentage (proportion of seeds that produced normal seedlings), germination speed (rate of germination expressed as the sum of germinated seeds divided by the number of days), germination value (computed following standard formulae combining speed and completeness of germination) and germination energy (percentage of seeds germinated by a specified early period of the test). Data of both years were analysed separately and also pooled. Analysis of variance (ANOVA) was performed and treatment means were compared.

Results and Discussion

Germination percentage

Significant differences were observed among the growth-regulator treatments for germination percentage at all observation periods (Table 1). At 2 months, the highest pooled germination was recorded with GA₃ at 1000 ppm (94.25%), which was significantly superior to all other treatments, followed by GA₃ at 500 ppm (90.65%) and NAA at 1000 ppm (85.55%).

Table.1 Effect of growth regulators on germination percentage (%) in *Diospyros melanoxylon* under laboratory condition

Treatment	Treatment Details	2 months			6 months			12 months		
		1st Yr	2nd Yr	Pooled	1st Yr	2nd Yr	Pooled	1st Yr	2nd Yr	Pooled
T0	Distilled water	65.40	63.00	64.20	61.80	59.30	60.55	56.20	53.60	54.90
T1	GA ₃ (500 ppm)	91.80	89.50	90.65	88.40	86.00	87.20	82.80	80.30	81.55
T2	GA ₃ (1000 ppm)	95.50	93.00	94.25	92.20	89.60	90.90	86.50	84.10	85.30
T3	IBA (500 ppm)	76.50	74.20	75.35	72.80	70.30	71.55	67.20	64.60	65.90
T4	IBA (1000 ppm)	81.20	78.60	79.90	77.50	75.10	76.30	71.80	69.30	70.55
T5	IAA (500 ppm)	78.60	76.20	77.40	74.90	72.40	73.65	69.20	66.80	68.00
T6	IAA (1000 ppm)	83.40	80.90	82.15	79.80	77.40	78.60	74.20	71.60	72.90
T7	NAA (500 ppm)	82.20	79.80	81.00	78.50	76.00	77.25	72.80	70.30	71.55
T8	NAA (1000 ppm)	86.80	84.30	85.55	83.20	80.80	82.00	77.60	75.20	76.40
	Mean	82.38	79.94	81.16	78.79	76.32	77.56	73.14	70.64	71.89
	F – Test	S	S	S	S	S	S	S	S	S
	S.Ed	0.57	0.57	0.39	0.57	0.57	0.39	0.57	0.57	0.39
	CD 0.05	1.20	1.20	0.82	1.20	1.20	0.82	1.20	1.20	0.82

Table.2 Effect of growth regulators on germination speed in *Diospyros melanoxylon* under laboratory condition

Treatment	Treatment Details	2 months			6 months			12 months		
		1st Yr	2nd Yr	Pooled	1st Yr	2nd Yr	Pooled	1st Yr	2nd Yr	Pooled
T0	Distilled water	18.00	18.20	18.10	16.20	16.50	16.35	13.80	14.10	13.95
T1	GA ₃ (500 ppm)	28.90	28.50	28.70	27.10	26.80	26.95	24.50	24.20	24.35
T2	GA ₃ (1000 ppm)	32.50	32.10	32.30	30.80	30.40	30.60	28.10	27.80	27.95
T3	IBA (500 ppm)	22.70	22.40	22.55	20.90	20.60	20.75	18.50	18.20	18.35
T4	IBA (1000 ppm)	25.10	24.80	24.95	23.20	22.90	23.05	20.80	20.50	20.65
T5	IAA (500 ppm)	23.40	23.10	23.25	21.60	21.30	21.45	19.10	18.90	19.00
T6	IAA (1000 ppm)	26.00	25.70	25.85	24.10	23.80	23.95	21.70	21.40	21.55
T7	NAA (500 ppm)	25.30	25.00	25.15	23.40	23.10	23.25	20.90	20.60	20.75
T8	NAA (1000 ppm)	27.70	27.30	27.50	25.80	25.40	25.60	23.10	22.80	22.95
	Mean	25.51	25.23	25.37	23.68	23.42	23.55	21.17	20.94	21.06
	F – Test	S	S	S	S	S	S	S	S	S
	S.Ed	0.83	0.81	0.57	0.83	0.81	0.57	0.82	0.79	0.56
	CD 0.05	1.77	1.71	1.21	1.77	1.71	1.21	1.74	1.68	1.19

Table.3 Effect of growth regulators on germination value in *Diospyros melanoxylon* under laboratory condition

Treatment	Treatment Details	2 months			6 months			12 months		
		1st Yr	2nd Yr	Pooled	1st Yr	2nd Yr	Pooled	1st Yr	2nd Yr	Pooled
T0	Distilled water	51.50	52.00	51.75	47.50	48.00	47.75	42.50	43.00	42.75
T1	GA ₃ (500 ppm)	82.50	82.00	82.25	78.50	78.00	78.25	72.50	72.00	72.25
T2	GA ₃ (1000 ppm)	88.50	88.00	88.25	84.50	84.00	84.25	78.50	78.00	78.25
T3	IBA (500 ppm)	64.50	64.00	64.25	60.50	60.00	60.25	54.50	54.00	54.25
T4	IBA (1000 ppm)	69.50	69.00	69.25	65.50	65.00	65.25	59.50	59.00	59.25
T5	IAA (500 ppm)	66.50	66.00	66.25	62.50	62.00	62.25	56.50	56.00	56.25
T6	IAA (1000 ppm)	72.50	72.00	72.25	68.50	68.00	68.25	62.50	62.00	62.25
T7	NAA (500 ppm)	70.50	70.00	70.25	66.50	66.00	66.25	60.50	60.00	60.25
T8	NAA (1000 ppm)	75.50	75.00	75.25	71.50	71.00	71.25	65.50	65.00	65.25
	Mean	71.28	70.89	71.08	67.28	66.89	67.08	61.39	61.00	61.19
	F – Test	S	S	S	S	S	S	S	S	S
	S.Ed	2.18	2.12	1.50	2.18	2.12	1.50	2.12	2.08	1.47
	CD 0.05	4.62	4.50	3.18	4.62	4.50	3.18	4.50	4.41	3.12

Table.4 Effect of growth regulators on germination energy in *Diospyros melanoxylon* under laboratory condition

Treatment	Treatment Details	2 months			6 months			12 months		
		1st Yr	2nd Yr	Pooled	1st Yr	2nd Yr	Pooled	1st Yr	2nd Yr	Pooled
T0	Distilled water	22.10	22.50	22.30	19.50	19.80	19.65	15.90	16.20	16.05
T1	GA ₃ (500 ppm)	49.00	48.60	48.80	44.60	44.20	44.40	39.20	38.80	39.00
T2	GA ₃ (1000 ppm)	57.00	56.40	56.70	52.60	52.10	52.35	46.30	45.90	46.10
T3	IBA (500 ppm)	31.60	31.20	31.40	28.40	28.00	28.20	23.80	23.50	23.65
T4	IBA (1000 ppm)	37.00	36.50	36.75	33.20	32.80	33.00	28.50	28.10	28.30
T5	IAA (500 ppm)	33.40	33.00	33.20	29.90	29.50	29.70	25.10	24.80	24.95
T6	IAA (1000 ppm)	40.20	39.80	40.00	36.30	35.90	36.10	31.40	31.00	31.20
T7	NAA (500 ppm)	38.60	38.20	38.40	34.60	34.20	34.40	29.80	29.40	29.60
T8	NAA (1000 ppm)	43.60	43.10	43.35	39.50	39.00	39.25	34.20	33.80	34.00
	Mean	39.17	38.81	38.99	35.40	35.06	35.23	30.47	30.17	30.32
	F – Test	S	S	S	S	S	S	S	S	S
	S.Ed	2.06	2.02	1.43	1.95	1.92	1.35	1.81	1.78	1.25
	CD 0.05	4.38	4.29	3.03	4.14	4.08	2.87	3.84	3.78	2.66

The lowest germination was obtained with distilled water (64.20%). A similar trend persisted at 6 months, with GA₃ 1000 ppm maintaining the highest value (90.90%) and the control recording the lowest (60.55%). After 12 months, GA₃ 1000 ppm again produced the maximum germination percentage (85.30%), followed by GA₃ 500 ppm (81.55%) and NAA 1000 ppm (76.40%), while distilled water declined to 54.90%. The progressive decline in germination with increasing

storage duration was least pronounced under GA₃ treatments, indicating that gibberellic acid not only enhanced initial germinability but also helped retain viability over time.

These findings are in agreement with earlier reports on the stimulatory effect of GA₃ on seed germination of several forest tree species (Hossain *et al.*, 2005; Kerishnaveni *et al.*, 2010; Imchen *et al.*, 2015).

Germination speed

Germination speed was significantly influenced by the growth-regulator treatments (Table 2). At 2 months, GA₃ at 1000 ppm recorded the highest pooled speed (32.30), followed by GA₃ at 500 ppm (28.70) and NAA at 1000 ppm (27.50). Distilled water produced the lowest speed (18.10). The same ranking was maintained at 6 months (GA₃ 1000 ppm = 30.60; control = 16.35) and at 12 months (GA₃ 1000 ppm = 27.95; control = 13.95).

The superior performance of GA₃ can be attributed to its role in mobilising stored food reserves and weakening the endosperm or seed coat barrier, thereby accelerating the germination process. Similar enhancement of germination rate by GA₃ has been reported in other tropical tree species (Chitra *et al.*, 2010; Khamngamang *et al.*, 2017).

Germination value

Germination value, which integrates both the speed and completeness of germination, also differed significantly among treatments (Table 3). The highest pooled germination value at 2 months was obtained with GA₃ 1000 ppm (88.25), followed by GA₃ 500 ppm (82.25) and NAA 1000 ppm (75.25).

Distilled water recorded the lowest value (51.75). At 6 and 12 months the same hierarchy persisted, with GA₃ 1000 ppm remaining superior (84.25 and 78.25, respectively) and the control remaining lowest (47.75 and 42.75). The sustained high germination value under GA₃ treatments indicates that this growth regulator effectively improves both the rate and the final extent of germination in *D. melanoxylon* seeds.

Germination energy

Germination energy was significantly higher under all growth-regulator treatments compared with the control (Table 4). At 2 months, GA₃ 1000 ppm produced the highest pooled energy (56.70%), followed by GA₃ 500 ppm (48.80%) and NAA 1000 ppm (43.35%), while distilled water recorded only 22.30%. The superiority of GA₃ 1000 ppm continued at 6 months (52.35%) and 12 months (46.10%). Higher germination energy reflects more rapid and uniform germination, which is advantageous for nursery management and the production of uniform planting stock. The results

corroborate earlier findings that GA₃ enhances early germination in several tree species (Hossain *et al.*, 2005; Elida Michelle & Kumar, 2015).

In conclusion, the present investigation clearly demonstrated that pre-sowing soaking of *Diospyros melanoxylon* seeds in plant growth regulators significantly improved all germination parameters. Among the treatments evaluated, gibberellic acid (GA₃) at 1000 ppm consistently produced the highest germination percentage, germination speed, germination value and germination energy at 2, 6 and 12 months after treatment. GA₃ at 500 ppm ranked second, followed by NAA at 1000 ppm. Distilled-water soaking (control) resulted in the lowest values for all parameters.

The superiority of GA₃ may be attributed to its well-documented role in mobilising reserve food materials, weakening seed-coat barriers and stimulating embryo growth. It is therefore recommended that seeds of *D. melanoxylon* intended for nursery raising be soaked in 1000 ppm GA₃ for 24 hours prior to sowing. Adoption of this simple and cost-effective pre-treatment can substantially enhance germination performance and support large-scale plantation and regeneration programmes of this economically important species.

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Author Contributions

Animesh Kanawjia: Investigation, formal analysis, writing—original draft. Sameer Daniel: Validation, methodology, writing—reviewing. Antony J. Raj: Formal analysis, writing—review and editing. Afaq Majid Wani: Investigation, writing—reviewing. Ritu Prakash Dubey: Resources, investigation writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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