



RESEARCH ARTICLE

Influence of bio-formulations and gibberellic acid on seed germination and seedling growth of *Dillenia indica* L.

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Abstract

The elephant apple (*Dillenia indica* L.) is an underutilised fruit crop of India, locally cultivated for food, medicinal and ornamental purposes. The fruits are rich in vitamin C and minerals and are a natural source of bioactive compounds. The present investigation was conducted under shade net conditions at the Hi-tech nursery unit of the Department of Horticulture, Post Graduate College of Agriculture, Dr Rajendra Prasad Central Agricultural University, Pusa, Samastipur (Bihar) during the year 2022–23. The experiment was laid out in a completely randomised design (CRD) and replicated 4 times. The research experiment was conducted on seeds of *D. indica* to evaluate the seed germination and seedling growth under the influence of different treatments viz., Control (plain seeds without soaking), soaking seeds in water, Jeevamruth solution, Panchagavya solution and gibberellic acid (GA₃) solutions for 24 hr. The results revealed that the seeds soaked for 24 hr with GA₃ at the rate of 600 ppm exhibited the shortest time to initiate germination (31.36 days) whereas GA₃ at the rate of 800 ppm showed the highest germination percentage (69.88 %) compared to other treatments. The seedling growth parameters, such as primary root length, secondary root count, plant height and leaf number varied significantly under the influence of different treatments.

Keywords: chalta tree; jeevamruth; panchagavya; plant growth regulator; seed germination

Introduction

Elephant apple (*Dillenia indica* L.), commonly known as the Chalta tree, is native to Southeast Asia, particularly in India, Sri Lanka, eastern southern China, Vietnam, Thailand, Malaysia and Indonesia. It is spread across India's Sub-Himalayan area, Assam, Meghalaya, Arunachal Pradesh, North Bengal, Bihar, Orissa, Madhya Pradesh and Gujarat. The genus *Dillenia* has approximately 60 species, out of which *D. indica* and *Dillenia pentagyna* Roxb. are native to India. Elephant apples are considered essential fruit crops because of their high nutritional value, which includes high levels of vitamin C, carbohydrates, fats, fibre, protein, minerals and fatty acids. In addition to nutritional components, many phytochemicals found in elephant apples have been identified as bioactive compounds with a broad range of biological activities (1, 2). The elephant apple is described as a medium-sized evergreen species, reaching up to 30 m in height. It features a straight trunk of moderate height, spreading branches that form a round and shaded crown. The bark is reddish-brown and peels off, while young branchlets are initially brown and covered in fine hair, which diminishes over time. Leaves grow in clusters at branch ends, with close veins that extend into serrated

edges. The upper leaf surface and lower veins are pubescent. The leaves have a narrow-winged petiole, around 2.5–5 cm long, which envelops the stem. The leaf blade is oblong or obovate-oblong, with parallel secondary veins and serrated margins. Flowers are solitary, large (12–20 cm in diameter) and the bud is over 5 cm wide. There are 5 rounded sepals, about 4–6 cm in diameter, thick and fleshy and 7–9 cm white obovate petals. The stamens are grouped distinctly, with anthers that release pollen through two pores. The fruit is edible, with an acidic flavour, containing 5 or more seeds in each carpel. Fruit growth follows a simple sigmoid growth curve, indicating that the fruit needed 140–160 days to reach maturity after fruit set. The seeds lack an aril, are enclosed in sticky pulp and have compressed shapes with hairy edges (1, 3).

The fruits are often found in local markets in Assam (Outenga in Assamese), Meghalaya (Soh-kyrbam in Khasi), Arunachal Pradesh and other Northeastern states of India. Indigenous communities highly value the fruits and they use them to prepare various culinary dishes, while immature fruits are used for pickling. The plants' leaves, bark and fruit have been used since time immemorial as medicine for the treatment of different ailments. It has been recognised as having notable antidiabetic,

antibacterial, antioxidant, analgesic, anti-inflammatory, dysentery, cytotoxic and hepatoprotective properties and has central nervous system (CNS) depressive effects (4–6). The trees have a great scope as well as an avenue tree for the development of beautiful landscaping, provided that the germination of seeds is improved (7). Apart from these, the fruits' mucilage has conditioning properties and is used naturally to wash hair. *Dillenia indica* is perpetuated naturally through seeds in the wild; however, the presence of mucilage on the seed coat acts as an adhesive and plays some role in obstructing seed germination. This gummy mucilage restricts the amount of water that seeds can absorb during the early stages of imbibition, but neither promotes nor inhibits germination. The seeds are morphologically dormant (8). Therefore, the present study was undertaken to evaluate methods for improving seed germination and seedling growth in *D. indica*.

Materials and Methods

The present investigation was carried out under 50 % agro-shade net at the Hi-tech nursery unit of the Department of Horticulture, Post Graduate College of Agriculture, Dr Rajendra Prasad Central Agricultural University (RPCAU), Pusa, Samastipur (Bihar) during the year 2022–23. The experiment was laid out in a completely randomised design (CRD) with 3 replications and 6 treatments. Each replication consisted of polybags sown with 20 seeds, with a total of 360 seeds sown after application with different treatments viz., T₁: Control, T₂: Soaking in water (24 hr), T₃: gibberellic acid (GA₃) at the rate 600 ppm (24 hr), T₄: GA₃ at the rate 800 ppm (24 hr), T₅: Soaking in Jeevamruth solution (24 hr) and T₆: Soaking in Panchgavya solution (24 hr) (Fig. 1). The data obtained were subjected to Duncans' Multiple Range Test (DMRT) and significant differences among the treatments were determined at $p \leq 0.05$ level.

Preparations of Jeevamruth and Panchgavya solution

The preparation of Jeevamruth and Panchgavya was carried out using the locally available materials as given in Table 1 and 2. Various parameters were studied for improving the seed germination and seedling growth of elephant apple using bio-formulations and GA₃.

Days taken for initiation of germination

The number of days taken for initiation of germination was calculated from the date of sowing up to seed germination, where the average number of days was calculated.

Seed germination

The total number of seeds germinated for each treatment was

counted each day, from the first appearance till the period of completion of seed germination. It was calculated by using the following formula and expressed as a percentage.

$$\text{Seed germination} = \frac{\text{Total number of seeds germinated}}{\text{Total number of seeds sown}} \times 100 \quad (\text{Eq. 1})$$

Primary root length

Seedlings were carefully taken out from the poly bags and the roots were thoroughly washed with water. Plants were spread on the table and root length was recorded with a scale and units expressed in centimetres. The average length of the root was recorded at 120 days after sowing (DAS).

Number of secondary roots

Seedlings were spread on the table exposing the roots, where the total number of secondary roots was counted. The average number of secondary roots was observed at 120 DAS.

Seedling height

Height of seedlings was measured from the collar region (ground level) to the tip of seedlings at 120 DAS using a measuring tape and expressed in centimetres.

Number of leaves

Number of leaves was recorded by counting total functional leaves per seedling and the average was calculated and expressed as the total number of leaves per plant at 120 DAS.

Stem diameter

Diameter of the stem was measured using digital vernier callipers at 1 cm height from the base of the seedling and expressed in millimetres at 120 DAS.

Economic analysis

The benefit-cost ratio was determined by using the following formula.

$$\text{B : C ratio} = \frac{\text{Gross returns}}{\text{Cost of cultivation}} \quad (\text{Eq. 2})$$

Results and Discussion

Germination parameters

Number of days taken to initiate germination

Data recorded for the number of days taken to initiate germination were presented in Table 3. The minimum number of days taken to initiate germination was observed in T₃-treated seeds, i.e. GA₃ at

Table 1. Components of Jeevamruth for 100 L of water

Ingredients	Quantity
Cow dung	5 kg
Cow urine	5 L
Jaggery	1kg
Flour of gram (Tur, Moong and Cowpea)	1 kg
Live soil (Healthy soil)	500 g
Water	100 L

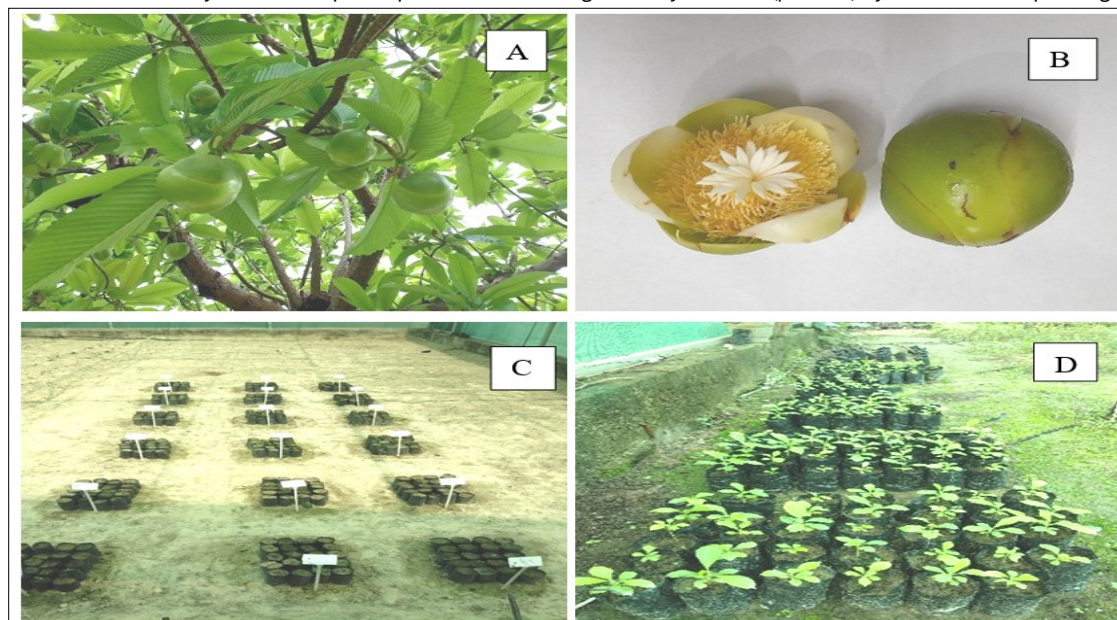
Table 2. Components of Panchgavya for 100 L of water

Ingredients	Quantity
Cow dung slurry	4 kg
Fresh cow dung	1 kg
Cow urine	3 L
Cow milk	2 L
Curd	2 L
Cow butter oil	1 kg
Water	100 L

Table 3. Effect of different bio-formulations and gibberellic acid (GA₃) on seed germination in elephant apple

Treatments	Number of days taken to initiate germination (days)	Germination percentage (%)	Primary root length (cm)	Number of secondary roots
T ₁ : Control	64.61 ^a	22.38 ^d	7.06 ^{dc}	15.17 ^d
T ₂ : Soaking in water	52.11 ^{ab}	31.13 ^{bcd}	8.61 ^{cd}	21.17 ^{cd}
T ₃ : GA ₃ at 600 ppm	31.36 ^d	62.38 ^a	10.74 ^{abc}	24.92 ^{bc}
T ₄ : GA ₃ at 800 ppm	31.61 ^d	69.88 ^a	11.51 ^{ab}	25.67 ^{bc}
T ₅ : Jeevamruth (24 hr)	44.61 ^{bc}	36.13 ^b	11.86 ^a	33.42 ^a
T ₆ : Panchgavya (24 hr)	42.36 ^c	34.88 ^{bc}	10.11 ^{abc}	28.42 ^{ab}
C.V. (%)	14.33	15.13	15.59	18.15
SE(m)	3.18	3.24	0.78	2.25
CD (0.05)	9.46	9.62	2.31	6.68

Values within column followed by the same superscript letter were not significantly different ($p < 0.05$) by Duncans' multiple range test.

**Fig. 1.** Planting material: (A) fruiting plant; (B) flower with immature fruit; (C) seed sowing and layout of different treatments; (D) healthy growth of seedlings.

the rate of 600 ppm (31.36 days), followed by T₄ (GA₃ at the rate of 800 ppm) (31.61 days), while the maximum number of days was reported in treatment T₁(Control) (64.61 days). Among the bio-formulations, treatment T₆(Panchgavya) showed minimum days taken to initiate germination (42.36 days) and was statistically at par with T₅(Jeevamruth) (44.61 days). The early germination of seeds treated with GA₃ was also reported in Rangpur Lime and papaya fruit crop (9–11).

Germination percentage

The germination percentage varied significantly among the treatments. The highest germination percentage was found in T₄ (GA₃ at the rate of 800 ppm) (69.88 %) and was statistically at par with T₃(GA₃ at the rate of 600 ppm) (62.38 %), whereas the lowest germination percentage was reported in T₁(Control) (22.38 %). Among the treatments of bio-formulations, the seeds treated with T₅(Jeevamruth) recorded 36.13 % germination, which is comparatively lower. The better germination with treatments under gibberellic acid may be due to proper activation of enzymes and utilisation of nutrients (12).

Root parameters

The data related to root parameters were carefully recorded at 120 DAS of seeds (Fig. 2, Table 3)

Primary root length

The maximum primary root length among the seedlings was reported in T₅(Soaking in Jeevamruth) solution (11.86 cm) and was found to be statistically at par with T₄(GA₃ at the rate of 800 ppm)

(11.51 cm), T₃(GA₃ at the rate of 600 ppm) (10.74 cm) and T₆ (Panchgavya) (10.11 cm). The minimum primary root length was recorded in T₁(Control) (7.06 cm). The better root growth may be due to proper supply of nutrients in both bio-formulations, which are required for root growth and development. The gibberellic acid also helps to promote root elongation by increasing indole-3-acetic acid (IAA), which may contribute towards root growth and development.

Number of secondary roots

The T₅ (Jeevamruth) treatment resulted in the highest number of secondary roots (33.42) and was significantly higher than all the treatments except T₆ (Panchgavya) (28.42). The lowest number of secondary roots was reported in T₁ (Control) (15.17), followed by T₂ (Water) (21.17). These observations are in close agreement with the findings regarding Rangpur lime and may be due to the rich nutrients of traditionally prepared bio-formulations; however, higher levels of GA₃ may show no significant impact on root number (9).

Seedling growth parameters

The data related to seedling height, number of leaves and stem diameter were also noted at 120 DAS of seeds (Table 4).

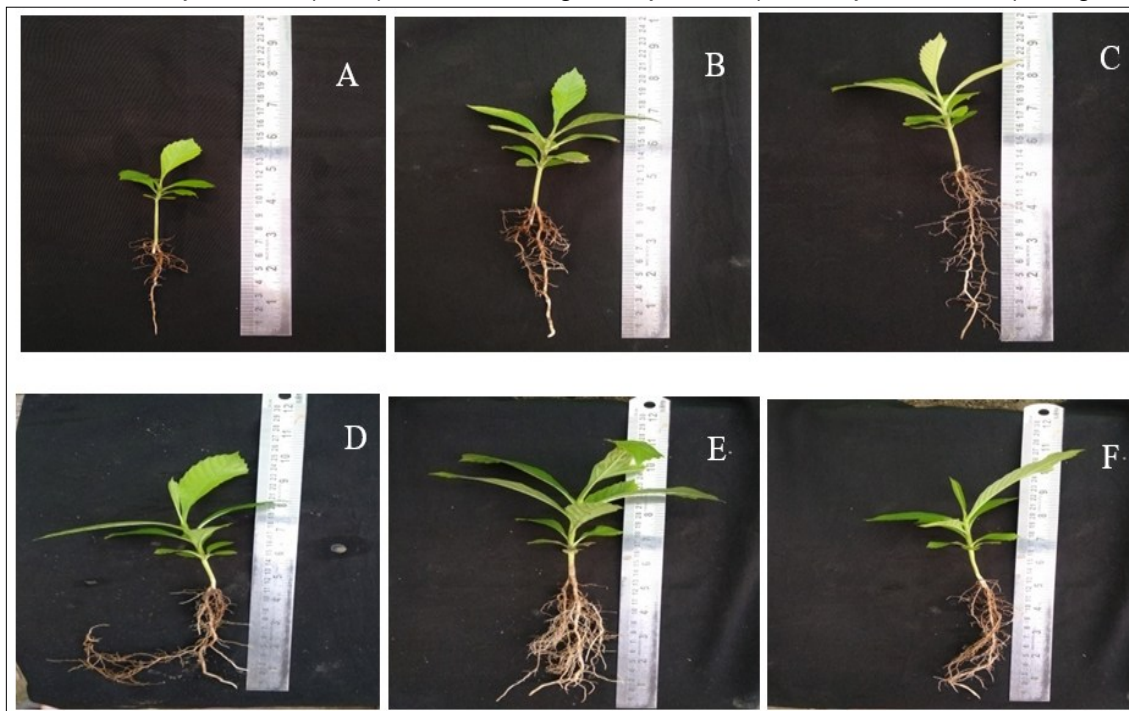
Seedling height (cm)

Observations related to seedling height varied significantly among the treatments. The highest seedling height was found in T₄- GA₃ at the rate of 800 ppm (12.66 cm) and was at par with T₃ (GA₃ at the rate of 600 ppm) (11.81 cm). While the lowest seedling height was

Table 4. Effect of different bio-formulations and GA₃ on seed germination in elephant apple

Treatments	Seedling height (cm)	Number of leaves	Stem diameter (mm)
T ₁ : Control	9.49 ^{cd}	7.25 ^d	1.87 ^d
T ₂ : Soaking in water	9.61 ^{bcd}	9.00 ^{abcd}	2.35 ^{bcd}
T ₃ : GA ₃ at 600 ppm	11.81 ^{ab}	10.25 ^{abc}	3.07 ^a
T ₄ : GA ₃ at 800 ppm	12.66 ^a	10.75 ^a	2.87 ^{ab}
T ₅ : Jeevamruth (24 hr)	11.09 ^{bc}	10.00 ^{abc}	2.50 ^{abc}
T ₆ : Panchgavya (24 hr)	9.96 ^{bcd}	10.50 ^{ab}	2.60 ^{abc}
C.V. (%)	14.25	14.54	16.07
SE(m)	0.77	0.70	0.20
CD (0.05)	2.28	2.08	0.61

Values within column followed by the same superscript letter were not significantly different ($p < 0.05$) by Duncans' multiple range test.


Fig. 2. Effect of different bio-formulations and gibberellic acid (GA₃) treatments on seedling and root growth: (A) T₁: control; (B) T₂: water; (C) T₃: GA₃ at 600 ppm; (D) T₄: GA₃ at 800 ppm; (E) T₅: Jeevamruth; and (F) T₆: Panchgavya.

reported in T₁ (Control) (9.49 cm), followed by T₂ -Water (9.61 cm). The results obtained with GA₃ treatments are in accordance with earlier findings in papaya and Kagzi lime (13, 14).

Number of leaves

The observations related to the number of leaves show not much variation with other treatments but are significantly higher than the control. The maximum number of leaves was found in T₄ (GA₃ at the rate of 800 ppm) (10.75) and minimum in T₁ (Control) (7.25).

Stem diameter (mm)

There was a substantial variation in the stem diameter observation with the control treatment. T₃ (GA₃ at the rate of 600 ppm) had the largest stem diameter (3.07 mm), which was comparable to T₄ (GA₃ at the rate of 800 ppm) (2.87 mm), T₆ (Panchgavya) (2.60 mm) and T₅ (Jeevamruth) (2.50 mm). On the other hand, T₁ (Control) had the lowest stem diameter (1.87 mm). These findings closely align with those of earlier findings reported in papaya and Rangpur lime (9–11).

Economic analysis

Benefit : cost ratio

The highest benefit-cost ratio was reported in seeds treated with T₃ (GA₃ at the rate of 600 ppm) (2.69), while the minimum was in T₁ (Control) (1.08). Nevertheless, among the traditionally prepared bio-formulations, the treatment T₅ (Jeevamruth) recorded a B : C ratio of 1.58, which was comparatively higher than the control (Table 5).

Conclusion

It can be concluded that among the treatments, GA₃ at the rate of 800 ppm required fewer days to initiate seed germination with maximum germination percentage in elephant apple, followed by GA₃ at the rate of 600 ppm. The treatments having traditionally prepared bio-formulations viz., Jeevamruth and Panchgavya also reported better germination percentage when compared to control, but their effect has provided substantial improvement in

Table 5. Cost of cultivation for sowing of 360 seeds in each treatment

Treatments	Cost of cultivation (₹)	Gross income (₹)	Net income (₹)	B : C ratio
T ₁ : Control	333.32	360	26.68	1.08
T ₂ : Soaking in water	333.32	500	166.68	1.50
T ₃ : GA ₃ at 600 ppm	383.32	1000	616.68	2.61
T ₄ : GA ₃ at 800 ppm	416.64	1120	703.36	2.69
T ₅ : Jeevamruth (24 hr)	366.64	580	213.36	1.58
T ₆ : Panchgavya (24 hr)	378.64	560	181.36	1.48

root growth parameters. This reflects their role in the development of strong and healthy seedlings.

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Authors' contributions

DAN conducted the field trials and wrote the manuscript. CM contributed to conceptualisation, validation and visualisation of research. TM prepared the treatments. NK, RA, PKN and RS reviewed and edited the manuscript. AV and PM contributed to data curation and analysis. All authors have read and approved the final manuscript.

Compliance with ethical standards

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

Ethical issues: None

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