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Standardization of Clonal Macropropagation Protocol of *Dillenia pentagyna* Roxb an Important and Endangered Medicinal Tree Species through Stem Branch Cuttings

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Abstract

Dillenia pentagyna belongs to family Dilleniaceae, is commonly known as Karkat. It is small deciduous tree reaching up to the height of 10-12 m with a straight bole. The species is distributed throughout in India including Andaman & Nicobar. It is highly important medicinal tree species. Its leaves, fruits and bark show antibacterial, anti-alpha glucosidase and antioxidant property. Due to its high medicinal value the natural occurrence of this species is declining day by day and gradually the species comes under the threat condition. The species is highly recalcitrant in nature, because it very difficult to propagate by conventional propagation methods.

Now there is an urgent need to develop an appropriate technology for its conservation and clonal propagation. An attempt has been made for standardizing the clonal propagation technique of this valuable medicinal species through stem branch cuttings under intermittent misting conditions in mist chamber. The optimum rooting response has been standardized by various parts per million (ppm) concentrations and treatment timings of root promoting hormones Indol-3 butyric acid-(IBA) and α-Naphthalene acetic acid (NAA). The optimum rooting response >60 percent was observed when the cutting were treated up to 20 minute with 500 ppm concentration of Indol-3 butyric acid. On an average 6 roots with10-15 cm length were induced from the cuttings after 30-35 days. A-Naphthalene acetic acid was failed to induce roots from the cuttings

Keywords: Propagation; IBA; NAA; Clonal; PPM; Recalcitrant

Introduction

The natural populations of some of the commercially important medicinal and aromatic plants species are gradually declining day by day from their natural habitats due to unsustainable harvesting practices, huge biotic pressure, recalcitrant nature of the species includes poor seeding and seed germination, non-availability of adequate amount of quality seeds/planting stocks and limitations in other propagation methods. Due to these factors most of the medicinal plant species comes under either rare, endangered, or threat category [1].

Therefore, today it is an urgent need to develop appropriate clonal propagation techniques through which the species can be multiplied easily and reintroduce in their natural habitats. Today there are several techniques and protocols are available for multiplying such taxa. Keeping under consideration of above situation of this valuable species, in this paper an attempt has been made for optimizing the induction of roots from stem branch cutting by standardizing the macro clonal propagation protocol. Different root promoting hormones (IBA, NAA) were tested in different concentrations ranging from 100 ppm to 1000 ppm. The different cutting size and seasonal rooting behavior were also highlighted in this paper.

About the species

Morphology: *Dillenia* is a genus of about 100 species of flowering plants of the family Dilleniaceae. The genus is named after the German botanist Johann Jacob Dillenius. In English, it is known as Dog Teak & in Hindi, it is known as Karmal. *Dillenia pentagyna Rox*. belongs to family Dilliniaceae (Karmal family). It is tall deciduous tree up to 15 m tall with short crooked and slender bole. The bark is greyish white. Leaves are simple, alternate, spiral, clustered at twig ends. Flowers are yellow in colour. Fruit and Seed are globose, seeds are 1 or 2 per carpel [1,2] (Figure 1).

Figure 1: View of *Dillenia pentagyna*.

Species distribution: Globally it is native to tropical and subtropical regions of southern Asia such as Bhutan, India, Indonesia, Malaysia, Myanmar, Nepal, Thailand, Vietnam and Australia (Figure 2). In India, it is mainly distributed in Himalayan terrain, also from Punjab to Assam, South India, Andamans, Gujarat, Madhya Pradesh, Mizoram and West Bengal (Figure 3).

Medicinal property and its uses: Recent studies have shown that

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Figure 2: Distribution at global level.

Figure 3: Distribution in India.

the extract of the bark of this plant had antibacterial and cytotoxic activity [1]. An ethnobotanical study among the various tribal and folk communities of Vindhya Region, Madhya Pradesh, reveal that they use the various parts of it for the treatment of their different ailments and diseases, *viz.*, delivery (bark), bone fracture (leaf), body pain (root), piles (leaf), diabetes (bark), diarrhea and dysentery (bark) [2]. Several other biological activities have been shown to be present in the secondary metabolites isolated from among those metabolites are terpenoids and flavonoids. The plant also shows antibacterial, antioxidant and anti-α-glucosidase activities and also used in the treatments of tuberculosis, fistula, sores, carbuncle, neuralgia, pleurisy and pneumonia. Barks and leaves are used for the treatment of diarrhea and dysentery.

Propagation of the plant: *Dillenia pentagyna* is commonly propagated through seeds but 10 to15 percent seeds germination percent is reported [3]. Due to lamination of seed propagation it is very essential to evolve its alternative propagation methods.

Due to over exploitation the species is gradually disappearing from the natural forest areas hence its conservation and propagation using alternative propagation techniques is very essential. This paper highlights the clonal propagation protocol of this valuable species through stem branch cuttings.

Material and Methods

Collection of propagation material

The propagation materials (stem branch cuttings) were collected from naturally grown plants. The cuttings were properly packed in gunny bags so as to maintain the moisture around them.

Preparation of cuttings for macropropagation

The cuttings were carefully brought in the mist nursery for further propagation. 8 to 9 mm thickness and 9 to 15 cm lengths of cutting were prepared (Figure 4). The cuttings were carefully washed with tap water and then treated with diluted solution of broad spectrum fungicide Bavistin to remove the microbial load from the cuttings.

Treatment of cuttings with root promoting hormones

Different concentrations of root promoting hormones (make Accumix) were prepared for optimizing the maximum root induction from the cuttings as mentioned in Table 1.

Placement of cuttings under polypropagators for induction of roots

The treated cuttings were placed in polypropagators on medium

Figure 4: Showing the types of cuttings.

Name of root promoting hormones	Ranges	Duration of Time for treatment
Indol-3 butyric acid-(IBA)	100 ppm- 1000ppm	10 to 40 min
α-Naphthalene acetic acid (NAA)	100 ppm- 1000ppm	

Table 1: Hormonal concentration.

grade pure sand with following congenial physical conditions:

a) Temperature: 35 to 45°C.

b) Humidity: 80 to 90% with intermittent spraying of water.

c) Spraying frequency: 3 to 4 times in summer and 2 to 3 times in other seasons.

Observations

The rooting responses from the cuttings were recorded at a weekly Interval as presented in the result and discussion part.

Transfer and shifting of cuttings/Stacklings

After the successful rooting from the cuttings, the rooted cuttings were sifted in 1:1:1 mixture of soil, sand and FYM and were maintained initially in partial shade and then transfer in open area.

Results and Discussion

The experiments were conducted from January 2014 to January 2015. The rooting response from the cuttings was recorded at weekly intervals (Tables 2-6). It was observed that, the rooting responses were directly influenced by various hormonal concentrations as well as their treatment durations. It was interesting to note that the medium sized cuttings (10 to 12 cm) which were semi hard wood in nature showed better rooting response as compared to long and hardwood cuttings.

Hormonal treatments		Concentrations in ppm	Sub treatments in min			
			10	20	30	40
Control	T0	0	NIL			
IBA	T1	100	T1a	T1b	T1c	T1d
	T2	200	T2a	T2b	T2c	T2d
	T3	300	T3a	T3b	T3c	T3d
	T4	400	T4a	T4b	T4c	T4d
	T5	500	T5a	T5b	T5c	T5d
	T6	600	T6a	T6b	T6c	T6d
	T7	700	T7a	T7b	T7c	T7d
	T8	800	T8a	T8b	T8c	T8d
	T9	900	T9a	T9b	T9c	T9d
	T10	1000	T10a	T10b	T10c	T10d
NAA	T11	100	T11a	T11b	T11c	T11d
	T12	200	T12a	T12b	124c	T12d
	T13	300	T13a	T13b	T13c	T13d
	T13	400	T14a	T14b	T14c	T14d
	T15	500	T15a	T15b	T15c	T15d
	T16	600	T16a	T16b	T16c	T16d
	T17	700	T17a	T17b	T17c	T17d
	T18	800	T18a	T18b	T16c	T16d
	T19	900	T16a	T16b	T16c	T16d
	T20	1000	T16a	T16b	T16c	T16d

No of hormonal groups-2
No of treatments-20
No of sub treatments-80
No of replications-3
No of cuttings per sub-treatment-25
Total no of cuttings/treatment-300 (R1+R2+R3)

Table 2: Experimental layout of cutting through hormonal treatments.

Sub-treatments	No. of root/ cutting Avg.	Root length (in cm) Avg.	Avg. of rooting %
T1a	0	0	0
T1b	0	0	0
T1c	0	0	0
T1d	0	0	0
T2a	2	4.33	22.33
T2b	2.33	6.33	24.33
T2c	2	4.33	20.33
T2d	0.66	5.33	10.33
T3a	2.66	8.33	38
T3b	3.66	11.33	42.33
T3c	2.66	9.33	36.33
T3d	0	0	0
T4a	3.33	8	40.33
T4b	2.66	9.33	43.33
T4c	3.33	9	39
T4d	0	5	8
T5a	4.33	11	45.33
T5b	6.33	15.33	61.66
T5c	4.33	13.33	55.33
T5d	1	2.66	9.33
T6a	3.33	10.33	39.33
T6b	4.33	12.33	49.66
T6c	2.33	10.33	39.33
T6d	0	0	0
T7a	2.33	9.33	37.66
T7b	3.33	11.33	45.33
T7c	2	10.33	35.66
T7d	0	0	0
T8a	2.33	8.33	35.33
T8b	3.33	10.33	41.33
T8c	2	9.66	35.33
T8d	0	0	0
T9a	3.66	8.66	32.33
T9b	3.33	10.66	41.33
T9c	2.33	9.33	34.66
T9d	0	0	0
T10a	3.33	8.33	31.66
T10b	3.33	10.33	41
T10c	2.66	9.66	34.66
T10d	0	0	0

Table 3: Effect of different ppm concentration of IBA and time on the rooting percentage in Sami hard wood cuttings (Data of 3 replications).

In the present study two root promoting hormones (Table 1) were used and from the present findings, it was noticed that Indol-3 butyric acid (IBA) showed maximum rooting response as compared to α-Naphthalene acetic acid (NAA) (Tables 3-6).The maximum rooting response was observed from the semi hard wood cuttings when treated with 500 ppm concentration for 20 minutes of Indol-3 butyric acid (IBA) and on an average >60 percent rooting were induced within 30 to 35 days (Table 3) (Figures 6 and 7). It was also noticed that the hardwood cuttings showed 57 percent rooting response (Table 4). Seasonal variations were also recorded for root induction and it was observed that March to July was the suitable months for maximum root induction (Figure 5). Other concentrations of IBA showed moderate to poor rooting response. Comparatively α-Naphthalene acetic acid (NAA) was found less effective hormone as far as root induction is

Sub-treatments	No. of root/ cutting Avg.	Root length (in cm) Avg.	% of rooting Avg.
T1a	0	0	0
T1b	0	0	0
T1c	0	0	0
T1d	0	0	0
T2a	1	3.33	18.33
T2b	2	5.66	20.66
T2c	2.33	5	16.66
T2d	0	0	0
T3a	1	4.66	20.33
T3b	2.33	6.66	24.66
T3c	2.33	5.33	21.66
T3d	0	0	0
T4a	1	4.66	34.66
T4b	2.33	6.66	36.66
T4c	2.33	5.33	33.66
T4d	1	2.66	5.66
T5a	3.66	11	38.66
T5b	5.33	12.33	57
T5c	4.33	11.33	45.66
T5d	0.66	2.66	9.33
T6a	2.66	9.33	15.33
T6b	2.66	10.33	33.33
T6c	1.66	9.66	35.66
T6d	0	0	0
T7a	1.66	7.66	9.33
T7b	2.33	8	32.66
T7c	3	8.66	35.33
T7d	0	0	0
T8a	1.66	7.66	9.33
T8b	2.33	8	30.33
T8c	3	9.66	34.66
T8d	0	0	0
T9a	1.66	8.33	10.33
T9b	2.66	8.66	30.33
T9c	2.33	9.66	34.33
T9d	0	0	0
T10a	1.66	7.66	9.33
T10b	3.33	8	28.66
T10c	3	9.66	34.66
T10d	0	0	0

Table 4: Effect of different ppm concentration of IBA and time on the rooting percentage in hard wood cuttings (Data of 3 replications).

Sub-treatments	No. of root/ cutting Avg.	Root length (in cm) Avg.	% of rooting Avg.
T11a	0	0	0
T11b	0	0	0
T11c	0	0	0
T11d	0	0	0
T12a	2	10.66	19.66
T12b	2.33	10.66	20.33
T12c	2	10.33	17.33
T12d	0	0	0
T13a	2	10.66	23.66
T13b	2.33	11.33	26.66
T13c	2	11.33	20.33
T13d	0	0	0
T14a	2.33	10.66	23.66
T14b	3.33	11.33	26.66
T14c	2	10.66	20.33
T14d	0	0	0
T15a	4	11.33	40.66
T15b	5	12.66	46
T15c	4	11	39.66
T15d	0.66	5.66	9
T16a	3	10.33	35.33
T16b	4	11	41.33
T16c	3	10.33	35.66
T16d	0	0	0
T17a	2	10.33	33.66
T17b	4	11	40.66
T17c	3	9.66	35.33
T17d	0	0	0
T18a	2	9.66	31.33
T18b	4	10.66	39.33
T18c	3	10.33	34
T18d	0	0	0
T19a	2	9.33	31.33
T19b	3	11	38
T19c	3	10.33	33
T19d	0	0	0
T20a	2.66	8.66	30.33
T20b	3	10	36.66
T20c	3	10.33	30.33
T20d	0	0	0

Table 5: Effect of different ppm concentration of NAA and time on the rooting percentage in Semi hard wood cuttings (Data of 3 replications).

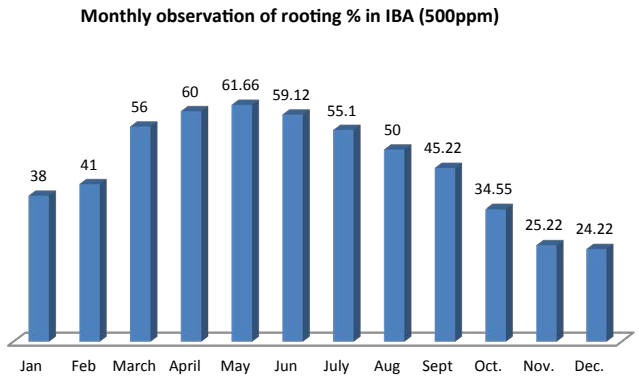

Figure 5: Monthly observation.

Figure 6: Induction of roots from the cuttings.

Sub-treatments	No. of root/ cutting Avg.	Root length (in cm) Avg.	% of rooting Avg.
T11a	0	0	0
T11b	0	0	0
T11c	0	0	0
T11d	0	0	0
T12a	1.33	4.33	15.66
T12b	2	9.66	18.66
T12c	2	9	15.33
T12d	0	0	0
T13a	1.33	8.33	16.66
T13b	3	9.66	19.33
T13c	2	8.66	16.33
T13d	0	0	0
T14a	2	9.33	24.66
T14b	2.33	10.33	28.33
T14c	3.33	10.33	25.33
T14d	0	0	0
T15a	4	9.33	34.33
T15b	4.66	11.33	46
T15c	4	11	39.66
T15d	0.66	5.33	9.66
T16a	3	8.33	31.66
T16b	3.33	9.66	40.33
T16c	3	10.33	35.33
T16d	0	0	0
T17a	3	8.33	26.33
T17b	3.33	9.66	39.66
T17c	2	8.66	25.33
T17d	0	0	0
T18a	2	9.33	25.33
T18b	3	10.33	39.33
T18c	2	8.66	23.33
T18d	0	0	0
T19a	3	9.33	24.66
T19b	3	9.66	39.33
T19c	2	8.66	22.66
T19d	0	0	0
T20a	2.33	8.33	24.33
T20b	3	10	39.66
T20c	2.66	9	20
T20d	0	0	0

Table 6: Effect of different ppm concentration of NAA and time on the rooting percentage in hard wood cuttings (Data of 3 replications).

Figure 7: Induction of roots from the cuttings.

concern and on an average 46 percent rooting were induced as well as induction of roots were also delayed (Tables 5 and 6).

Figure 8: Hardened plants of *Dillenia pentagyna*

The rooted plants were successfully shifted in potting mixture containing sand +soil+ FYM (1:1:1) (Figure 8). The similar kind of clonal propagation techniques through stem branch cuttings are also reported by several workers for other recalcitrant tree species [4-10].

Conclusion

From the above study, it is concluded that IBA was found to be the best root promoting hormone when the semi hardwood cuttings were treated at 500 PPM concentration for 20 minutes. The medium grad pure send was the suitable culture medium for induction of root because it helps the cuttings for proper air circulation and prevent cuttings from decaying. The optimum temperature for maximum rooting was found in between 35 to 45°C with 80 to 90% humidity with intermittent misting.

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