

## EFFECTS OF GROWING MEDIA AND GROWTH HORMONES ON THE SPROUTING, ROOTING AND FIELD ESTABLISHMENT OF *WOODFORDIA FRUTICOSA* (L.) KURZ (DHAWAI) IN SUB HUMID FOOTHILLS OF EASTERN HIMALAYA

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**Abstract:** The common method of propagation of *Dhawai* (*Woodfordia fruticosa* (L.) Kurz, through seeds have low germination and seedling survival under normal conditions due to heavy rainfall immediately after the seed maturity and microbotic seed nature. Hence, an attempt on vegetative propagation through stem cuttings was made to observe the effect of different growth hormones, their concentrations and different growing media on its rooting and sprouting. Cuttings were treated with six different concentrations (IBA 250, 500, 750 and 1000 ppm), naphthalene acetic acid (NAA) + indole-3 butyric acid (IBA) (250 ppm each) by using quick dip method while, the untreated cuttings were used as control. The cuttings were planted in three different growing media: soil, sand, and soil + FYM in equal proportion. Results showed maximum survival (80.0 %) at 30 days in IBA- 750 ppm treated cuttings when planted in sand medium and minimum was with control in (soil + FYM). All the combinations of growth hormone and sand gave better results. In the second trial, sand media alone taken to evaluate the effect of different growth hormones, their concentrations in successive studies. Initiation of sprouting ranged from 12.00 to 15.33 days and completion of sprouting ranged from 18.00 to 25.67 days. Maximum survival (35.56 %) at 90 days was recorded in IBA 1000 ppm treated cuttings when planted in sand medium. Healthy and uniform *Dhawai* planting materials in Terai region of West Bengal can be produced by planting *Dhawai* stem cuttings treated with IBA 1000 ppm in sand medium, transplanting of the rooted cuttings to polybags containing soil and FYM in 3:1 ratio after two months with root ball and then transplanted to outfield with root ball.

**Keywords:** *Woodfordia fruticosa*, Vegetative propagation, Sprouting, Rooting, Survival

### INTRODUCTION

**W**oodfordia fruticosa (L.) Kurz, commonly known as Fire Flamed Bush or 'Dhawai' (Family: Lythraceae) is a traditional medicinal gregarious shrub widely present in India and South East Asia. This frost hardy, unpalatable species springs up naturally on landslips, abandoned cultivation and other open places, killing out grass, binding the soil together and acting as a useful soil-improver and an efficient nurse to tree species. All parts of the plant possess valuable medicinal properties but flowers are more prized in domestic and international markets for preparation of herbal medicines (Oudhia, 2003). Compounds like tannins, flavonoids, glycosides, sterols and polyphenols have been isolated from this species particularly in flowers and leaves (Khushalani *et al.*, 2006). The red dye extracted from the flowers of *Dhawai* is used extensively throughout India in perfume, leather and textile industry and this plant also yields gum similar to gum tragacanth (Grover and Patni, 2011). High demand of this species for its pharmaceutical value can be met with its mass cultivation which will also conserve the species in its natural habitat. Ensuring quality planting materials to the growers will not only popularize the species for its incorporation in the farming system but will also help in farm and income diversification of the growers. The species

can be propagated through seeds and vegetative means as well.

The plant flowers abundantly during summer and seeds are produced during rainy season which germinates within 15-20 days. Normally germination is very less with very little survival due to microbotic nature of the seeds (Shankar and Rawat, 2013; Mathew *et al.*, 2018). *In vitro* techniques have already been standardized for this species as a tool for germplasm conservation (Bulle *et al.*, 2012; Meena and Satish, 2015). Propagation through stem cuttings during monsoon was also recommended for better results (Joshi *et al.*, 1992; Shankar and Rawat, 2013). The increased genetic gains through mass propagation have been obtained in several medicinal plants (Swamy *et al.*, 2002). Root initiation in cuttings is affected by various factors like plant growth regulators, season, size and length of the cuttings (Hartmann *et al.*, 2002). Information on the propagation techniques of *Dhawai* is still meager. The mature *Dhawai* plant can be utilized for vegetative multiplication either collected from wild or from the maintained mother plants. Hence, the performance of vegetative propagation through stem cuttings as influenced by different growth hormones and growing media on sprouting, rooting and field establishment of *Dhawai* was attempted to standardize the propagation protocols through its

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stem cutting using growth hormone and growing media.

## MATERIALS AND METHODS

### Experimental details:

A greenhouse experiment was setup under natural environmental conditions at the field of the Department of Forestry, Uttar Banga Krishi Viswavidyalaya, Pundibari, in Terai region of West Bengal, India for two seasons during April-September 2018 and 2019. Terai region is a heavy rainfall area with an annual average of 2000-3500 mm and average minimum and maximum temperature of 18°C (January) and 33°C (August), respectively. The relative humidity of the region ranges from 55 % to 90 %. The experimental area is situated at an altitude of 43 m above mean sea level, intersected by 26°23'45.8" N latitude and 89°23'16.7" E longitude. The soil of the experimental plot was moist and well drained, rich in organic matter having sandy loam texture with pH ranging from strongly acidic to moderate. The fresh semi-hard wood branches were collected from Suntaley (Kalijhora Forest Beat), Kalimpong, West Bengal, situated at an altitude of 236 m above mean sea level, intersected by 26°56'1.8" N latitude and 88°27'4.4" E longitude. Leaves of the collected branches were removed and cut into 15 cm long segments each with 2-3 nodes. The first trial with 18 different treatment combinations, 20 cuttings each (replicated thrice) were quick dipped in five different hormonal concentrations *i.e.*, Indole-3 butyric acid (IBA, 250, 500, 750 and 1000 ppm), naphthalene acetic acid (NAA, 250 ppm) + IBA (250 ppm) with untreated cuttings as control in combination with three different growing media *viz.* soil, sand and equal proportion of soil and FYM. After dipping in hormones, the cuttings were planted directly in the polybag of size 20 cm × 20 cm × 35 cm filled with growing media as per treatment combinations. Heavy rains during the first trial after 30 days of planting had caused high mortality of rooted stem cuttings necessitating the repetition of the trial in the second year also but with some deletion and addition. Observation of first year trial clearly indicated sand as better growing media thus soil and soil with FYM was not repeated in the second-year trial. Sand media alone was used in combination of four different hormonal concentrations with (IBA 500, 1000 and 1500 ppm) and IBA + NAA (250 ppm each) along with untreated cuttings as control which was replicated thrice. Experiment of both the years was laid out in completely randomized design. Initiation and completion of sprouting and survival of the cuttings were recorded after 30 days of planting in the first-year trial while, in second year trial along with initiation and completion of sprouting, number of sprouts and roots, root length and survival of cuttings at 90 days after planting was recorded. A

cutting was considered to be rooted or survived if it had at least one primary root of about one millimeter long. For measurements, the cuttings were uprooted carefully to avoid damage to the roots. Selected healthy three-months-old rooted stem cuttings from second-year trial (with and without root ball) were transferred to polybag containing soil and FYM (3:1 ratio) media. These rooted stem cuttings were kept in the Soil + FYM media for two months after which randomly selected uniform healthy rooted stem cuttings were planted in the outfield with and without root balls (Mathew *et al.*, 2018). In each transplanting method 30 healthy well-established five months old rooted stem cuttings were used with three replications. Survival of the rooted stem cuttings at both the stages (*i.e.*, 1<sup>st</sup> stage at three months from sand media to Soil + FYM media and 2<sup>nd</sup> stage at five months from Soil+FYM media to outfield) was recorded. One and two-years-old *Dhawai* plant growth (height, collar diameter, number of branches, number of flowers and flower dry weight and economical yield *i.e.*, flower) parameters were also recorded.

### Data analysis:

A completely randomized design (CRD) was followed for the experiment. Data collected were analyzed using Analysis of Variance (ANOVA) and treatment means were compared using Least Square Difference (LSD) test at 5% level of significance. Duncan's test was performed to compare the significant difference between the mean values.

## RESULTS AND DISCUSSION

### Effect of growing media and growth hormone on cuttings of Dhawai

#### First year trial:

The media used for planting the cuttings significantly influenced the time required for initiation of sprouting, completion of sprouting and the survival of the cuttings in the 1<sup>st</sup> year trial (Table 1, 2). Survival of *Dhawai* stem cuttings at 30 days (48.89 %) was significantly higher with sand medium as compared to the other media used (*viz.* soil- 32.22 % and Soil + FYM in equal proportion- 15.56 %). Sprouting in cuttings planted in sand media initiated and completed at 10<sup>th</sup> and 21<sup>st</sup> day while, in soil media it was 14<sup>th</sup> and 24<sup>th</sup> day and in Soil + FYM it was 12<sup>th</sup> and 22<sup>nd</sup> day, respectively. Sprouting initiation and completion of cuttings planted in sand medium (Plate 1.a) was significantly earlier than the cuttings planted in other two media. Growth regulators also significantly influenced the survival of cuttings (observed at 30 days after planting), initiation and completion of sprouting over control (Table 1). The survival of the cuttings was highest (40.0 %) when dipped in 250 ppm IBA, while those treated (dipped) with other treatments had four per cent lower survival than the best though the difference was non-significant. All hormonal dip

treatments of cuttings significantly had about 6-7 days and 5-7 days earlier initiation and completion of sprouting than the control cuttings. The treated cuttings initiated sprouting on 11-12<sup>th</sup> day of planting while, completed sprouting after 21-22 days of planting. The initiation, completion and survival of the cuttings, however was statistically at par among the different hormonal dipping treatments.

A significant ( $p < 0.05$ ) interaction effect of growing media and growth hormones on initiation, completion and survival (30 days after planting) of *Dhawai* cuttings was observed (Table 2). The highest survival of 80.0 % was achieved with 750 ppm IBA dipped cuttings when planted in sand medium while, lowest survival was observed when cuttings were planted in the media without any hormonal dipping (control). There was no survival when untreated cuttings were planted in the soil and FYM media in equal proportion, while the survival of the untreated cuttings planted in sole soil and sand media was 6.68 % and 20.00 %, respectively. Delay in sprouting initiation and rotting of the untreated cuttings in the FYM based planting media resulted into complete mortality of the cuttings. Combined effect of growth hormone and growing media significantly improved initiation and completion of sprouting and survival of cuttings as compared to sole treatment of growth hormone or growing media. All the varying combinations of growth hormones performed significantly better in the sand medium (33.33-80.0 %) as compared to the soil (26.68-46.67 %) and soil + FYM medium (6.68-26.68 %).

Notable sprouting was observed in the cuttings but maximum got wilted within a few days due to poor root initiation causing mortality and poor survival (Plate 1.b). Early shoot formation might have created a competitive situation between roots and shoots for nutrient within the cuttings causing its mortality due to nutrient deficiency (Thai, 1979). Moreover, the climate of the study area is moist tropical (Anonymous, 2001) with high rainfall and humidity might also caused rotting and consequently mortality of the cuttings (Schmitz *et al.*, 2013).

#### **Second year trail:**

The effect of growth hormones in different combinations and concentrations on survival, number of sprouts, number of roots, and root length of stem cuttings of *Dhawai* when planted in sand media during 2<sup>nd</sup> year trial is given in table 3. Thus, the actual duration was reduced to two months in the following standardized protocol. Sprouting initiation, survival, number of sprouts and number of roots after 90 days of planting was non-significantly influenced by the various hormonal dip treatments as compared to untreated control but completion of sprouting and root length was significantly influenced by the hormonal treatments over control. Sprouting of the cuttings initiated on 12.00-15.33 days after planting while completed on 18.0-25.67 days after planting. Highest survival (35.56 %) was observed in cuttings

dipped with 1000 ppm IBA which was 15.56 % higher than cutting with control and 6.67-20.0 % higher than other hormonal dipped treatments. Cuttings dipped with 1000 ppm IBA also produced highest number of sprouts (2.67) and roots (3.00) along with longest roots (14.83 cm) which was 1.50-3.60 cm longer than the cuttings of other treatments. There was a significant difference in effect of different concentrations of rooting hormones and growing media (sand) on sprouting and rooting of stem cuttings of *Dhawai*. Application of growth hormones induces rooting on stem cuttings (Hartmann *et al.*, 2002). Auxins are widely used rooting hormones which are known to increase rooting (Ibrahim *et al.*, 2015). The exogenous application of IBA might have caused the vascular differentiation of cells to support root formation (Phuyal *et al.*, 2018). The efficiency of IBA in various concentrations, enhancing root proliferation as well as root numbers have been recognized by several earlier studies in different species including genus *Woodfordia* (Joshi *et al.*, 1992; Swamy *et al.*, 2002; Negash, 2003; Amri *et al.*, 2010; Mehraj *et al.*, 2013; Kalko Al-Zebari and Al-Brifkany, 2014). *Dhawai* stem cuttings treated with 1000 ppm IBA also gave best result in terms of both rooting and survival (Plate 1.c). Similarly, among the growing media, sand performed better as compared to soil or soil + FYM medium. Sand as growing media was successfully used for rooting and survival of stem cuttings of many plants earlier including *Dhawai* (Daoud *et al.*, 1995; Napagoda and Yakandawala, 2009).

#### **Standardization of seedling production through stem cutting and their field establishment**

*Dhawai* cuttings were transplanted with root ball in polybags containing Soil + FYM in 3:1 ratio and hardened in shade for one month to aid its field establishment (Plate 2.a). Survival per cent of *Dhawai* in two different transplanting stages (first stage was in poly bags and second stage was in outfield) with and without root ball were recorded (Figure 1). Maximum survival was recorded of the cuttings transplanted with root ball (70.33 % and 93.33 % in the 1<sup>st</sup> and 2<sup>nd</sup> stage of transplanting, respectively) which was 47.0 % and 66.66 % higher than the survival achieved in the cuttings without root ball, respectively. The entire stem cuttings failed to survive when they were transplanted directly to the outfield without hardening. Growth and yield traits of established cuttings of *Dhawai* in field (two years old) was recorded in Figure 2. More the age, more the number of flowers and its dry weight (Figure 2, Plate 2.b). *Dhawai* stem cuttings were well established (Plate 2.c) in the field of the department of forestry, U.B.K.V campus, Pundibari, West Bengal. These results further glimpse a successful introduction of *Dhawai* from higher elevation (Kalimpong hills) to lower elevation (Terai zone) for commercial cultivation and scientific conservation

(Plate 2.d & e). Two stage transplanting were also found effective for successful seedling production of *Dhawai* by Mathew *et al.* (2018) also. It was observed that *Dhawai* plants were most susceptible to transplanting shock which can be overcome by planting with root ball in early morning or late evening to avoid desiccation for maintaining the turgidity of the seedlings (Burdett, 1990).

## CONCLUSION

Vegetative propagation of *Dhawai* cuttings under varying levels of growth hormone concentrations in different growing media provides an insight into the positive link between hormone treated cuttings and a

growing medium. We conclude that *Dhawai* stem cuttings treated with 1000 ppm of IBA and planted in sand medium is best for survival, initial growth and propagation success. Many studies showed that *Dhawai* was difficult to root species nevertheless, 35.56 % survival of cuttings through vegetative propagation achieved in the present study is encouraging and can be used to propagate it in *Terai* region of West Bengal till further research generates more success. However, direct transplanting of the rooted cuttings in the outfield should be avoided unless the cuttings were hardened in the shade for one month in poly bags containing with growing medium of soil and FYM in 3:1 ratio and then transplanted with root balls only.

**Table 1.** Main effect of growing media and growth hormone on *Dhawai* stem cuttings

Growing Media			
	Initiation of sprouting days	Completion of sprouting days	Survival (%) at 30 days
Soil	13.78 <sup>a</sup>	23.94 <sup>a</sup>	32.22 <sup>b</sup>
Sand	10.39 <sup>c</sup>	20.50 <sup>b</sup>	48.89 <sup>a</sup>
Soil: FYM (1:1)	12.44 <sup>b</sup>	22.33 <sup>ab</sup>	15.56 <sup>c</sup>
Mean	12.20±0.15	22.26±0.39	32.22±0.79
Growth hormone			
IBA 250 ppm	11.89 <sup>b</sup>	22.00 <sup>b</sup>	40.00 <sup>a</sup>
IBA 500 ppm	10.89 <sup>bc</sup>	21.22 <sup>b</sup>	35.56 <sup>a</sup>
IBA 750 ppm	10.66 <sup>c</sup>	20.78 <sup>b</sup>	37.78 <sup>a</sup>
IBA 1000 ppm	11.77 <sup>b</sup>	21.78 <sup>b</sup>	35.56 <sup>a</sup>
NAA+IBA (250 ppm each)	10.56 <sup>c</sup>	20.56 <sup>b</sup>	35.56 <sup>a</sup>
Control	17.44 <sup>a</sup>	27.22 <sup>a</sup>	08.89 <sup>b</sup>
Mean	12.20±0.52	22.26±1.36	32.23±2.73

Means with the same alphabet are not significantly different from each other.

**Table 2.** Effect of growing media and growth hormone on cuttings of *Dhawai* during first trial

Growing Media	Growth hormone	Initiation of sprouting days	Completion of sprouting days	Survival (%) at 30 days
Soil	IBA 250 ppm	11.67	22.33	40.00
	IBA 500 ppm	11.67	22.00	40.00
	IBA 750 ppm	11.00	21.67	26.68
	IBA 1000 ppm	11.67	22.67	33.33
	NAA+IBA (250 ppm each)	12.00	22.33	46.67
	Control	24.67	34.67	06.68
Sand	IBA 250 ppm	10.67	21.00	60.00
	IBA 500 ppm	10.67	21.33	46.68
	IBA 750 ppm	09.00	19.33	80.00
	IBA 1000 ppm	10.33	20.33	53.33
	NAA+IBA (250 ppm each)	09.33	19.33	33.33
	Control	12.33	21.67	20.00

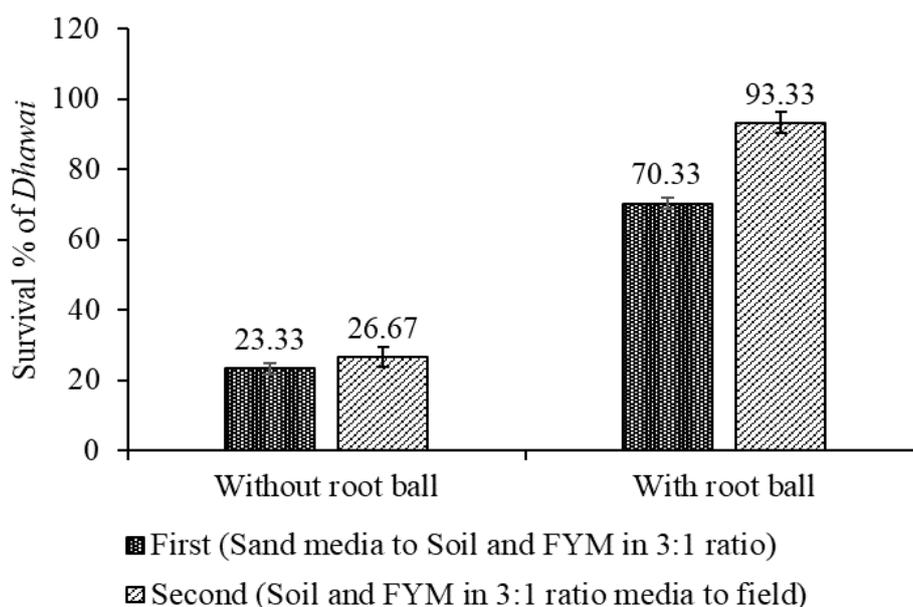
Soil: FYM (1:1)	IBA 250 ppm	13.33	22.67	20.00
	IBA 500 ppm	10.00	20.33	20.00
	IBA 750 ppm	12.00	22.33	06.68
	IBA 1000 ppm	13.33	23.33	20.00
	NAA+IBA (250 ppm each)	10.33	20.00	26.68
	Control	15.33	25.33	00.00
SEd		0.90	2.35	4.72
CD 5%		1.82	4.76	9.58
<i>p value</i>				
Growing Media		0.000*	0.004*	0.000*
Growth hormone		0.000*	0.000*	0.000*
Growing Media * Growth hormone		0.000*	0.018*	0.000*

\*= Significant ( $\alpha= 0.05$ )

**Table 3.** Effect of sand growing media and growth hormone on cuttings of *Dhawai*

Treatments	Initiation of sprouting days	Completion of sprouting days	Survival (%)	90 Days After Planting		
				Number of Sprouts	Number of Roots	Root length (cm)
Control	12.67	18.00	20.00	2.00	1.67	12.07
IBA 500 ppm	15.33	21.67	15.56	2.33	1.67	13.17
IBA 1000 ppm	13.67	25.67	35.56	2.67	3.00	14.83
IBA 1500 ppm	12.00	24.00	28.89	2.33	1.67	12.10
IBA+NAA (250 ppm each)	15.00	23.33	20.00	1.67	1.33	12.57
SE(d)	1.15	1.21	7.30	0.42	0.56	0.52
CD 5%	2.57 <sup>Ns</sup>	2.70 <sup>**</sup>	16.27 <sup>Ns</sup>	0.94 <sup>Ns</sup>	1.24 <sup>Ns</sup>	1.15 <sup>**</sup>

\*= Significant ( $\alpha= 0.05$ ), Ns= Non significant ( $\alpha= 0.05$ )



**Figure 1.** Survival per cent of *Dhawai* in different transplanting stage with and without root ball

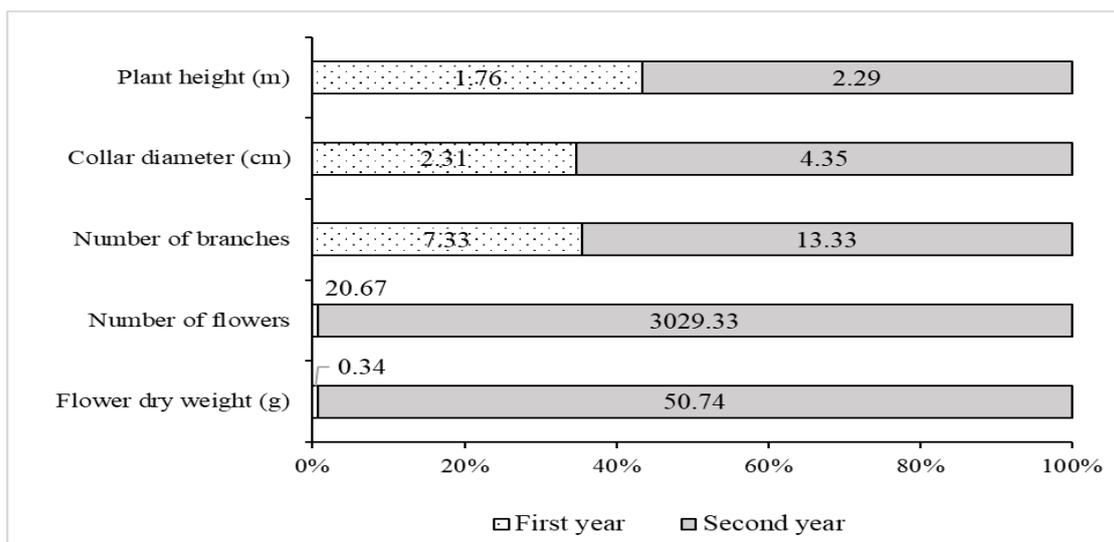


Figure 2. Growth and yield traits of established cuttings of *Dhawai* in field

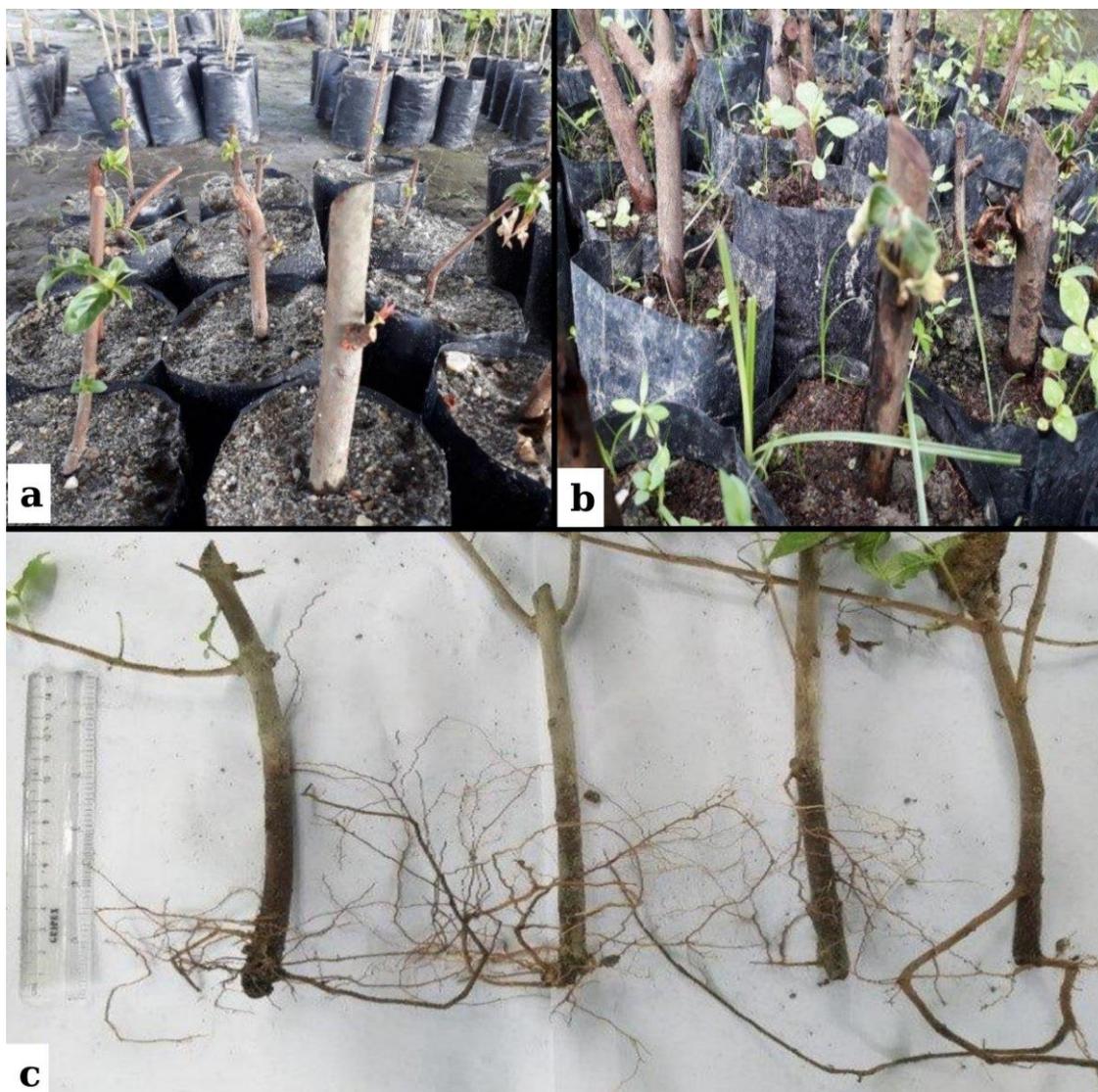


Plate 1. *Dhawai* sprouting, mortality and survival under nursery; a, Sprouting at 30 days; b, Mortality of cuttings during first year; c, Rooting and survival of IBA 1000 ppm treated cuttings in sand media at 90 days.



**Plate 2.** *Dhawai* transplanting and field establishment; a, Seedlings after first transplanting; b, One-year-old plant flowering in outfield; c, Two-year-old well-established *Dhawai* in outfield; d, *Dhawai* in natural habitat (Kalimpong hills); e, Vegetative Multiplication Garden (VMG) of *Dhawai*.

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