World Journal of Agricultural Research, 2018, Vol. 6, No. 1, 15-19
Available online at http://pubs.sciepub.com/wjar/6/1/4

DOI:10.12691/wjar-6-1-4

Effect of Maturity Stage of Donor Plant on Propagation of *Diploknema butyracea* through Branch Cuttings

Aabid Rasool Zargar*, Dinesh Kumar

Silviculture Division, Forest Research Institute, Dehradun, Uttarakhand
*Corresponding author: writetoaabid@gmail.com

Abstract *Diploknema butyracea* an important multipurpose species owing to its great economic and medicinal value is facing threat as the exploitation levels have reached all time high. The species is failing to regenerate in spite of reasonable seed production. Therefore, in order to augment the natural regeneration, special attention needs to be given for its propagation. Thus, vegetative propagation is a better option, as it ensures purity of clonal or true-to-type propagation of elite tree. The present investigation was conducted to study the effect of maturity stage of donor plants on rooting of branch cuttings of *Diploknema butyracea*. Mature and juvenile branch cuttings of 2-3 cm diameter and 16-20 cm length classes were planted in the non-mist propagation chambers for sprouting and rooting of cutting. Maximum 92.20% sprouting was observed in juvenile cutting which got reduced to 37.20% in mature donor plants. The maturity stage of the donor plant had significant effect on the mean length and mean number of sprouts per cutting. Maximum (3.63) mean number of leaves was recorded in juvenile cuttings. Percent rooting decreased as age of the donor plants increased. Maximum (66.70%) rooting was observed in juvenile branch cuttings which decreased to (4.40%) in cutting collected from mature donor plants. Juvenile branch cuttings showed significant survival percentage (87.80%) and survival percentage during hardening (77.80%) than mature ones. Maturity stage had a significant effect on the mean number and mean length of roots per cutting after 64 days of planting.

Keywords: *Diploknema butyracea, vegetative propagation, maturity stage, branch cuttings*

Cite This Article: Aabid Rasool Zargar, and Dinesh Kumar, "Effect of Maturity Stage of Donor Plant on Propagation of *Diploknema butyracea* through Branch Cuttings." *World Journal of Agricultural Research*, vol. 6, no. 1 (2018): 15-19. doi: 10.12691/wjar-6-1-4.

1. Introduction

Diploknema butyracea (Roxburgh) H. J. Lam, (Syn. *Bassia butyracea* Roxburgh, Syn. *Madhuca butyracea* (Roxburgh) Macbride, Syn. *Aesandra butyracea* (Roxburgh) Baehmi, Syn *Illipe butyracea* (Roxburgh) Engler) belongs to family Sapotaceae, many of whose species produce edible fruits and other economic fruits, is a medium sized deciduous tree locally known as *Cheura* and popularly known as Butter tree. This fast growing multipurpose tree is a native of Nepal and is distributed from India through Nepal to Philippines and from Kumoan eastwards to Sikkim and Bhutan [1]. The population of this species is almost localized in Pithoragarh district particularly the areas bordering Nepal. It also occurs sporadically in tropical moist deciduous, semi deciduous and evergreen forests of Andaman Islands [2]. It is a fast growing tree borne oilseed found at an elevation of 400-1400 masl mainly along steep slopes, ravines, cliffs and in shady valleys [3]. In its natural habitat, the mean annual rainfall ranges from 1000-2000 mm. Temperature varies from 24^{o}C to 27^{o}C. Medium soils with deep granules are considered good for Cheura plantations.

It is a deciduous tree with a straight trunk. Flowers are yellow coloured with a fragrance. The fruit is berry, oval in shape with three seeds. Cheura starts flowering in the month of October-November and fruits start ripening in June-July. The seed contains considerable amount of fat, known as *Phulwara butter* [4], which has been classed along with commercial Morwa bassia fats [5]. The potential use of Cheura products is found in different fields such as confectionary, pharmaceuticals, vegetable ghee production, candle manufacturing and soap making. It has been found effective for rheumatism. The flowers are used as a source of alcohol. The defatted seed contains protein and saponins which are toxic. The cake produced after processing Cheura is used as manure [6].

Vegetative propagation is widely used to multiply elite plants selected from natural population by improving yield and quality, shortening rotation age and allowing some of the biological problems hindering reforestation to be circumvented [7,8]. The role of vegetative propagation is also important in respect of species which do not produce fertile seeds or species like *Diploknema butyracea* in which the seeds remain viable only for a short period. Reproduction of this nature is also important where seeds have high economic value as in Cheura.

The ability of branch cuttings to regenerate varies with the plant species. While some regenerate easily, others regenerate with difficulty and still others do not regenerate at all and are thus, obstinate. The regeneration through branch cuttings is affected by both internal and external

factors. Maturity stage of donor plants have been reported to have negative influence on the performance of rooted branch cuttings [9]. A general decline in rooting ability, root quality, reduction in survival has been associated with donor plants that have reached a state of reproductive or ontogenetic maturity [10,11]. Clark [12] identified stem anatomy, rooting co-factor levels, endogenous rooting inhibitors and presence of preformed root initials as four areas linking maturation state and rooting. The potential to initiate adventitious root formation in many woody plant species is one of the characteristics which has been observed to change with developmental age [13].

Propagators usually select healthy, vigorous, well-matured, shoots with viable buds as the source of cutting material. Cuttings made from the juvenile or young plants generally root best and produce roots of higher quality [14,15]. Gurumurti *et al.* [16] and Paton *et al.* [17] reported that the stem cuttings made from mature trees completely failed to root. Problems associated with maturation are more severe in some clones and cultivars than others [18]. Geneve *et al.* [19] stated the control of maturation is mainly a function of the development of the vegetative meristem, which is determined mainly by the number of cell divisions rather than its chronological age. Loss of rooting potential often is not marked by the arrival of the capacity for sexual reproduction and therefore can be related to other factors. Williams *et al.* [20] observed that poor-rooting ability in woody plant species was related to suberization of the cortex. Favre [21] observed that too much or too little lignification gave minimal rooting, while Dalet and Cornu [22] found lignification retarded the emergence of adventitious roots.

Another phenomenon associated with ageing is that tissues found at different locations on the same tree differ in juvenility [23]. In Douglas-fir, the rooting potential of the upper two-thirds of the tree was found to be significantly less than the lower one-third of the crown [24]. Branch order also plays a significant role in rooting potential. Secondary lateral branches tend to root better than do primaries [25]. Though, secondary's exhibit more plagioitropic growth. However, Miller [26] found that differences in rooting responses attributed to secondary and primary shoots were eliminated when exogenous auxins were applied.

Tewari and Dhar [27] studied the germination behaviour of *Diploknema butyracea* seeds and reported low germination. Seeds of *Diploknema butyracea* loose viability in 20 days and loss in viability is drastic when moisture content falls below 25 % [28]. Reports on successful vegetative propagation are almost rare. Tewari and Dhar [29] observed improved sprouting but no successful rooting by vegetative propagation. Similar result in stem cuttings was obtained by Sundriyal and Sundriyal [30]. Attempts have been made to regenerate the species through clonal propagation [31] as well as seed germination [32]. Having such a great economic and medicinal value *Diploknema butyracea* is facing extinction because of relentless anthropogenic pressure [33]. These species are failing to regenerate in spite of reasonable seed production [1]. Branch cuttings are classified based on their maturity as juvinile and mature [34]. These cuttings are important because of the ease by which plants grow from them although, some are more difficult to root than others hence this study was conducted to examine the type branch cuttings that is most suitable for propagating *Diploknema butyracea* in the nursery.

2. Materials and Methods

The experiment was conducted at the campus of Forest Research Institute (FRI), Dehradun located at 30°20'10.31''N latitude and 77°59'55.32"E longitude. Dehradun is in the influence of sub-tropical climate and experiences about 2200 mm rainfall annually. Major part of the rainfall is received from mid-June to mid-September.

Branch cuttings of 2-3 cm diameter and 16-20 cm length from the mature trees (obtained from Bambustem of FRI campus) and juvenile plants (obtained from nursery of FRI campus) were obtained from healthy plants free of apparent physiological disorders, diseases and insect infestations very early in the morning when the plant is fully turgid with a sharp thin-bladed pocket knife. Cuttings were severed from the primary axes to avoid the plagiotropic growth.

The experiment was laid out in a complete randomized block design (CRBD). There were 3 blocks with twenty cuttings per treatment per block for a total of 120 cuttings. Different non mist propagation chambers served as different blocks. The cuttings were firstly treated with Bavistin by dipping them in 1% solution of fungicide for 30 minutes. Thereafter 8000 ppm IBA hormone was applied in their powder formulations to the basal 1 cm portion of cuttings. The branch cuttings were inserted vertically. A gap of 6-7 cm was left between adjacent cuttings on both sides. Water was sprinkled over the cuttings. The top of the chamber was kept closed all the time except for operations or data collection.

After initiation of lenticels burst, the cuttings were observed at weekly interval to examine their sprouting. A branch cutting was recorded to have sprouted if leaf primordium was visible. The total number of sprouted cuttings under each treatment were counted and expressed as percentage of total branch cuttings. Length of sprouts produced on each cutting was measured and expressed in cm.

Removal of rooted cuttings was started after 64 days of planting. A cutting was considered to be rooted if a minimum of one root ≥ 1cm in length was present. The branch cuttings producing root was recorded. All the cuttings where callus formation was observed on the day of observation were removed. Unrooted cuttings were left undisturbed so as to form roots in subsequent weeks. The branch cuttings surviving for 9 weeks or 12 weeks and developing callus or roots were counted and expressed as percentage of total branch cuttings for survival percentage.

The rooted cuttings were planted in polythene bag of size 22 cm x 13 cm having sand 2: soil 1: FYM 1 (v/v). The polythene bags were placed in similar non-mist propagation chambers for three weeks for establishment and acclimatisation. After twenty one (21) days, they were placed in shade house. After staying in shade house for twenty one (21) days, they were removed in the evening and placed in the open. The plants were now considered as hardened. The hardened rooted cuttings were planted in nursery bed. The size of the pit was 30 cm x 30 cm x 30 cm. Planting was done at such a depth that collar region was at

the level of soil surface. The plants were irrigated twice a week during summer and once a week after the end of monsoon rains. The spray of insecticides/ fungicides, weeding, hoeing etc was done manually as and when required. The rooted branch cuttings surviving the hardening phase were counted and this number was expressed as percentage of the number of rooted cuttings planted in polythene bags in non-mist propagation chambers at the start of the hardening procedure.

The data collected was subjected to analysis of variance (ANOVA).

3. Results

Maturity stage of the donor plant exhibited significant effect (P<0.01 level) on the percent sprouting of cuttings after 36 days of planting in non-mist propagation chamber. Percent sprouting decreased as age of the donor plants increased. Maximum 92.20% sprouting was observed in juvenile cuttings which got reduced to 37.20% in mature cuttings (Figure 1).

Maturity stage of the donor plant had significant effect (P<0.01 level) on the mean length of sprouts for all the three sprouts. Maximum length of sprouts was 11.50 cm in juvenile cuttings which decreased to 0.71 cm in mature branch cuttings (Table 1).

Mean number of sprouts per cutting and mean number of leaves per cutting varied significantly with maturity stage of donor plant. Maximum (1.79) mean number of sprouts per cutting was obtained in juvenile branch cuttings, while it was (0.47) in mature branch cuttings. Maximum (3.63) mean number of leaves per cutting was recorded in juvenile cuttings while cuttings collected from mature donor plants produced (0.66) mean number of leaves per cutting (Table 1).

Percent rooting decreased significantly as age of the donor plants increased. Maximum (66.70%) rooting was observed in juvenile cuttings which decreased to (4.40%) in mature cuttings after 64 days of planting in non-mist propagation chamber. Survival percentage of branch cuttings after 64 days of planting varied significantly among different ages of donor plant ranging from 87.80% in juvenile cuttings to 5.60% in mature cuttings. Juvenile cuttings showed significant (77.80%) survival percentage after 43 days of hardening period than mature ones (Table 2).

Maturity stage of donor plant had a significant effect on the mean number and mean length of roots per cutting after 64 days of planting. Maximum (5.69) mean number of roots per cutting was obtained in juvenile branch cuttings, while it was (0.04) in mature branch cuttings. Similar results were obtained on the mean length of roots per cutting with maximum (2.96 cm) obtained in juvenile branch cuttings (Table 3).

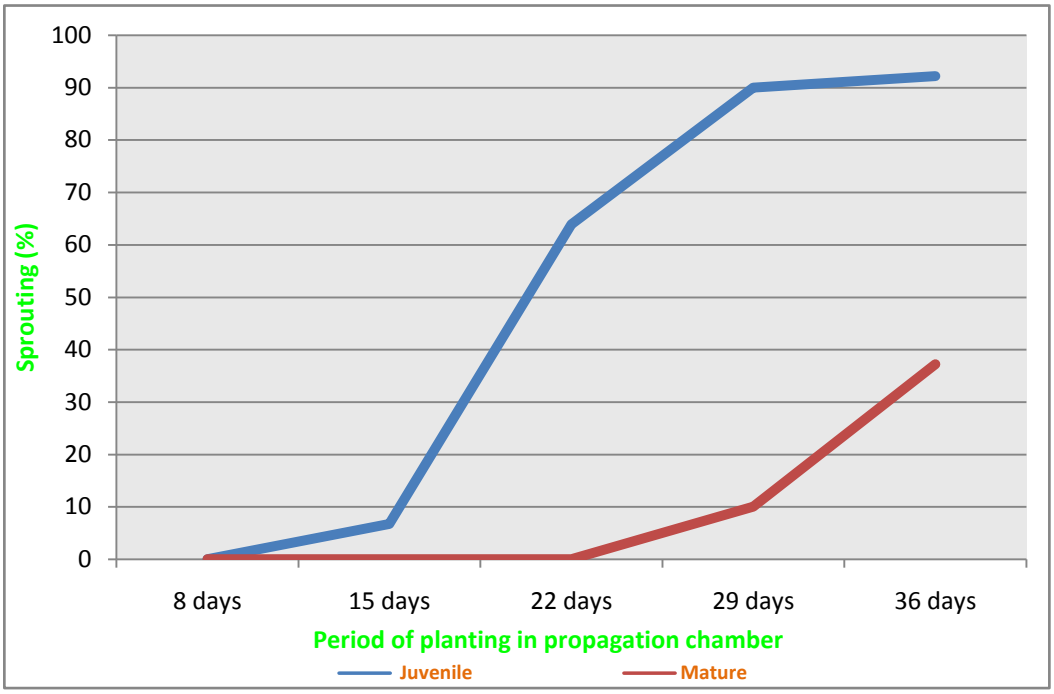

Figure 1. Sprouting percentage of branch cuttings of different maturity stages of donor plant at 8 to 36 days of planting in propagation chamber

Table 1. Effect of different maturity stages of branch cuttings on Max length of Sprouts, Mean no. of sprouts per cutting and mean no. of leaves per cutting

Maturity stage of donor plant	Max length of Sprouts	Mean number of Sprouts	Mean number of leaves
Juvenile	11.50	1.78 (1.34)	3.63 (1.91)
Mature	0.71	0.47 (0.68)	0.66 (0.81)
t-test **(α=0.05)**	Sig.	Sig.	Sig.

Values within parentheses are the transformed values (transformation applied: square root).

Table 2. Effect of different maturity stages of branch cuttings on rooting and survival till hardening

Maturity stage of donor plant	Rooting after 64 days of planting branch cuttings (%)	Survival after 64 days of planting branch cuttings (%)	Survival after hardening period of 43 days (%)
Juvenile	66.70 (54.78)	87.80 (69.59)	77.80 (61.92)
Mature	4.40 (12.11)	5.60 (13.70)	0.00 (0.00)
t-test (α=0.05)	Sig.	Sig.	Sig.

Values within parentheses are the transformed values (transformation applied: arc sine).

Table 3. Effect of different maturity stages of branch cuttings on mean number of roots per cutting and mean length of roots per cutting

Maturity stage of donor plant	Mean number of roots after 64 days of planting branch cuttings	Mean length of roots after 64 days of planting branch cuttings
Juvenile	5.69 (2.39)	2.96
Mature	0.04 (0.21)	0.00
t-test (α=0.05)	Sig.	Sig.

Values within parentheses are the transformed values (transformation applied: square root).

4. Discussions

Maturity stage of branch cutting greatly affected the rooting and sprouting of *Diploknema butyracea*, with juvenile branch cuttings rooting more readily than mature branch cuttings. Percent rooting and percent sprouting in branch cutting decreased as age of the donor plants increased from two years to 40 years (Table 2, Figure 1). Decrease in rooting potential of branch cuttings due to ageing and maturity of donor plants has been reported for a number of plant species by other investigators also [34,35,36]. It has been suggested that the reduction of root development may be caused by the presence of sclerenchyma tissue in the phloem of mature trees which obstruct the emergence of the root [37,38,39]. These studies found that the ease of rooting is directly related with the degree of sclerification of stem tissue. Increased sclerification as primary tissues mature explains the decrease in rooting ability observed in many species as juvenile tissue (softwood) matures into adult tissue (hardwood) [40].

The findings further show that the effect of IBA treatment on rooting and sprouting of *Diploknema butyracea* branch cuttings varied with maturity stage of the donor plant. The stimulatory effects of IBA on rooting and sprouting of branch cuttings of different physiological ages of several other woody plant species have been reported by many workers [34,41,42] and it has also been shown that an IBA treatment which promoted rooting more strongly suppressed shoot growth on cuttings of several plant species [43]. The differential effect of auxins on rooting and sprouting of cuttings have been ascribed to IBA caused differential rates of mobilization and translocation of nutrients to the growing root and shoot primordial [43,44].

In cuttings of 2 year (juvenile) plants externally applied IBA was generally ineffective or mildly promotive at lower concentration. Pal [41] has also reported similar results in juvenile cuttings of teak, which he explained on the basis of pre-existing optimal endogenous auxin content in the juvenile cuttings and that the externally applied IBA caused build up of supra-optimal concentration of auxin in the rooting zone causing thereby inhibitory effects. The higher auxin requirement for causing and promoting rooting in cutting of older trees may be due to a decrease in the content on endogenous auxins [45] or decreased sensitivity of ageing tissues to rooting promoters [46] and/or accumulation of inhibitory substances which inhibit rooting [34].

5. Conclusion

The results of the present study show that cuttings taken from juvenile donors not only show more profused rooting but also higher percent survival than the cuttings taken from mature donors. A decreased rooting ability due to maturity of donor plants leading to poor survival of rooted cuttings has already been reported as a common phenomenon for most woody plant species [47]. Thus, the findings of the present studies on *Diploknema butyracea* are in conformity with previous findings in other species.

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