Research Article



Morphological and Propagation Studies in Some Chiuri types of Nepal

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Abstract

Diploknema butyracea (Chiuri) is a multi-use, under-utilized but economically and environmentally important native fruit tree species of Nepal. Earlier, four types of Chiuri were reported in Makwanpur district but recently about eight types has been reported from across the country. The main objective of the research was to study the morphology and propagation of six different types of Chiuri in Nepal. Leaves of six types of one year old grafted Chiuri was assessed, and vegetative propagation was tested in three types of Chiuri with side grafting and cleft grafting and during February, May, July, August and September 2021. Morphology of leaves showed differences in the pattern of leaves suggesting difference among them. Cleft grafting (47.7%) was found more effective than side grafting (41.7%) and early/mid-types were more responsive to grafting in contrast to late type. The best time for grafting early type of Chiuri was mid-July and not effective after August and before the month of May. Days to bud brust and first leaf opening was found earlier in cleft graft than side graft. Besides, the number of leaf was also higher in cleft graft in contrast to side graft suggesting better graft union in cleft graft than side graft. This research was focused on studying the morphology of the six types of Chiuri from four districts (Makwanpur, Chitwan, Rolpa and Dhading) and develop appropriate propagation technique for three different types of Chiuri collected from three districts (Makwanpur, Chitwan and Dhading).

Keywords : Chiuri, Cleft grafting, Diploknema butyracea, Morphology, Propagation

Introduction:

Chiuri (*Diploknema butyracea* (Roxb.) H. J. Lam), commonly known as the Butter tree in English and *Chiuri* in Nepali, is a multipurpose plant of the Sapotaceae family (Joshi, 2010; Uprety et al., 2013). Among different genera, *Diploknema is economically important* *and* native to Southeast Asia, the Himalayas, and southwestern China, with seven species in total in which *D. butyracea* is common in Nepal (Shrestha et al., 2022). Chiuri is a multi-use, under-utilized but economically and environmentally important native fruit tree species of Nepal (Zargar & Kumar, 2018) and spread in about

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58 districts (Bhattrai et al., 2024) in Nepal ranging from 400masl to 1400masl (Dahal et al., 2021).

It is also culturally very important to an indigenous seminomadic tribe of Nepal (Chepang) (Practical Action, 2010) and is perhaps only tree that is given to a daughter when they are married (Shakya, 2000) but this culture is not very commonly practiced these days (Poudel et al., 2024). Although, much has been researched and documented about the importance of Diploknema butyraceae tree to Chepang community yet in a recent study no significant economic gain has been found to have achieved by the community (Chikanbanjar et al., 2021a). Chiuri is mainly used economically for butter and bee keeping (Chikanbanjar et al., 2021a) but it can also be used for a host of other things such as, leaves as fodder, pulp as juice, seed kernels as biopesticides (Dahal et al., 2021). In recent times, the return from honey per household is the highest followed by butter and seeds (Poudel et al., 2024).

Recent studies have reported eight types of Chiuri from different parts of the country but is yet to be confirmed officially (Bhattrai et al., 2024). Four types of Chiuri were reported earlier from Makwanpur (Chikanbanjar et al., 2021b). They are Assare (Wayo), Sharawne (Lanyo), Bhaduree (Titya) and Mansiree (Tomyo); name in the parenthesis is chepang names. The recent studies further included four types; two types each from east Nepal (Aguwa and Pachhuwa) and west Nepal (Kalo and Jire) (Bhattrai et al., 2024), thus eight types were reported from different parts of the country. The morphological, chemical, and molecular studies of these types are yet to be undertaken and therefore lacks understanding on their cultivation technology and quality of butter or pulp.

Currently, the Chiuri trees comes to bearing only after 6-10 years because these trees are raised from seeds. The lack of appropriate planting material of elite mother stocks is limiting the commercial cultivation of Chiuri in Nepal despite increase in demand of Chiuri butter (Chikanbanjar et al., 2021b). Chiuri is mainly propagated by seeds although limited success was found in air layering (30%) stem cuttings (79%) (Tiwari & Dhar, 1979), and juvenile stem cuttings (77.8%) (Zargar & Kumar, 2018). In contrast, Zargar and Kumar (2018) reported rooting from matured cuttings failed (0%). While cuttings taken from juvenile stem success was good (Zargar & Kumar, 2018), the type of stem cuttings used is not clear (Tiwari & Dhar, 1979), thus it is assumed that those cuttings could be the juvenile ones as the success rate is similar (Tiwari & Dhar, 1979; Zargar & Kumar, 2018). Besides, the time to flowering and bearing fruit for a juvenile cutting may take much longer, and therefore it may not be very economical to use it as a planting material for commercial cultivation of Chiuri (Subedi et al., 2022). Thus, there is lack of an appropriate technique to develop good quality material for Chiuri (Zargar & Kumar, 2018). Recently, Chiuri

type Wayo or Asaree type could be grafted with limited success. The success percentage was 53% and the graft technique used was cleft (Subedi et al. 2022)

This research therefore focused on studying the morphology of the six types of Chiuri from four districts (Makwanpur, Chitwan, Dhading and Rolpa) and develop appropriate propagation technique for three different types of Chiuri collected from three districts (Makwanpur, Chitwan and Dhading).

Materials and methods:

Methodology for leaf morphology study of Chiuri

Leaf morphology is the study of the shape, size, and structure of leaves. It is an important tool for plant identification, classification, and ecological studies. Leaf morphological traits, such as leaf colour, shape, orientation and degree of marginal dissection, are often treated as diagnostic of species and have a long history of use for species identification (Cope et al., 2012; Foster & Gifford, 1989). The research site was Floriculture Development Center located at Godawari, Lalitpur, Nepal. The materials used for the morphological study of leaves of the *Chiuri* of different places were magnifying glass, ruler, camera, notebook, and laptop.

Location for propagation of Chiuri

This study was conducted from February 2021 to December 2021 in Floriculture Development Center, Godawari, Lalitpur district of Nepal. Godawari is located at 27.6^o North latitude and 85.37^o East longitude and at an altitude of 1550 masl. Godawari comes under warmtemperate humid agro-climatic condition. It receives an average annual rainfall of 354.03 mm (DHM, 2024).

Climatic conditions of the experiment site

The Meteorological data, viz., temperature, number of rainy days, relative humidity, and average sunny hours of the experimental site are presented in Table 1.

Plant materials

The scions used in this experiment represented 3 different flowering types viz early, mid, and late which were obtained from Makwanpur, Chitwan, and Dhading districts of Nepal. The rootstocks were prepared from the one-year old seedling plants. The rootstocks were obtained from Beekeeping Development Program, Bhandara, Chitwan which were prepared from the *Chiuri* seeds collected from Rolpa district. Healthy mother trees of bearing stage were selected for preparing scions. Scions from previous year's growth, approximately 6 inches in length, were carefully cut from the mother trees, wrapped in moist jute bags and transported to the experimental site. The scions were kept moist and under shade until they were grafted onto the rootstocks.

Table 1: Climate data of Godawari (2021)

Month	Temperature			Precipitation		Average Relative Humidity (%)	Average sunny hours (Hours)*
January	18.4	3.4	1	0	0	80.9	7.5
February	20.0	5.0	1	9.2	2	79.5	8.0
March	23.4	8.4	-	4.9	2	66.7	9.7
April	25.8	10.4	-	93.1	9	67.3	10.1
May	24.3	14.1	-	187.9	15	80.3	9.1
June	26.1	17.5	-	245.6	20	82.9	7.0
July	26.0	18.5	-	537.5	27	85.0	5.5
August	25.5	18.3	-	378.1	28	85.5	5.9
September	26.6	17.2	-	206.9	21	86.2	6.3
October	25.6	14.0	-	41.0	7	85.0	7.5
November	20.9	6.5	-	0	0	81.3	7.3
December	18.0	3.1	1	50.2	2	85.7	7.1

Source: Department of Hydrology and Meteorology, climate-data.org

Note: * = (Data: 1991 - 2021); Rainy days means rainfall >1mm rainfall.

Treatment details

The experiment was carried out in a three factor randomly complete block design (RCBD) but were not statistically analyzed due to unavailability of sufficient samples (samples number not uniform for different treatments) at different time.

A) Scion types

1. Chitwan	Early
2. Chitwan	Mid
3. Chitwan	Late
4. Makwanpur	Early
5. Dhading	Early

B) Grafting dates

- 1. February 10, 2020
- 2. February 27, 2020
- 3. May 28, 2021
- 4. July 11, 2021
- 5. August 8, 2021
- 6. August 28, 2021
- 7. September 14, 2021

C) Grafting method

- 1. Side grafting
- 2. Cleft grafting

Preparation of rootstocks

The plants were raised in polybags of 8"x10" size and kept under greenhouse condition (plastic house). The seeds for raising rootstocks were collected from various parts of Rolpa district. Selected seedlings were healthy, vigorous, pest and disease free, and uniform in size.

Grafting techniques

Two grafting techniques were adopted to propagate Chiuri vegetatively. These techniques where Side grafting was reported successful in Sapodilla (*Manilkara achras* (Mill.) Fosberg) (Kaur et al., 2022) which shares same family with *Chiuri* (Sapotaceae) and cleft grafting was reported successful earlier in *Chiuri* (Subedi et al., 2022).

Side grafting

Rootstock of about 1 year old and 6-12 mm diameter were trimmed at 15-20 cm from the soil level. A 3-4 cm long cut was made through the bark just into the wood and a short second cut was made at the base of the first cut, forming a notch. A scion with 2-3 buds, about 10cm and of equal or slightly smaller in diameter than rootstock was selected, and a slanting cut was made on one side equal in length to that made on the rootstock. A small cut was made at the base of the scion on the opposite side so that the scion will fit into the notch on the rootstock. The scion was placed into position on the rootstock with care to line up the junction between bark and wood on at-least one side.

Cleft grafting

The rootstock seedling was transversely cut at a height of 15- 20 cm above the soil level. The stub of the stock was then vertically split down for about 5 cm with a sharp knife. The scion containing 3-4 buds, and about 10cm was prepared like a wedge by a giving two slanting cut about 3 to 4 cm. long opposite to each other at the basal end. The scion was than inserted in the split of the rootstock. In both types of grafting methods, the cambial layer of rootstock and scion was brought into close contact and the graft unions were tightly wrapped with polyethene strip.

Care after grafting

The grafted plants in the polybags were watered after grafting and placed under low plastic tunnel covered with 50% shade net to ensure higher humidity and to protect from direct sunlight. The grafted plants were irrigated at 1-2 days interval depending upon the weather condition. The polythene sheet of the low tunnel and shade net were removed after one month of grafting. Polythene strip used for wrapping the graft joint, were removed after 9 months of grafting. De-suckering and weeding was done as per requirement.

Observation taken

Leaf morphology

Leaf samples were collected from 18 individuals of *Chiuri* from different places (Makwanpur, Dhading, Chitwan and Rolpa) in Nepal. Firstly, leaves of grafted *Chiuri* were closely observed. Then at least three representative samples of leaves from the plant were collected. We collected leaves from different parts of the plant, as leaf morphology can vary depending on the location of the leaf on the stem. The leaves of different plants of one year of age whose scion were of different locations of Nepal were taken for study. The leaves were then examined through magnifying glass and measured with the help of ruler. The measurement was recorded in the notebook and then to a laptop for further analysis. Photographs were also taken for reference and proof. The following features were observed and recorded.

Size: the maximum length of elongated part and the maximum width of narrow part (right angle to the elongated part) was measured in centimeters. (Ackerly et. al, 2002)

- Type of leaf: the type of leaf based on shape of the leaf can be described using a variety of terms, such as ovate, lanceolate, or cordate. (Foster & Gifford, 1989)
- Apex: the apex is the tip of the leaf. It can be described as acute, obtuse, or acuminate. (Foster & Gifford, 1989)
- Base: the base is the bottom of the leaf. It can be described as cuneate, cordate, or truncate.(Fatima & Javeed, 2018)
- Margin: the margin is the edge of the leaf. It can be described as entire, dentate, serrate, or crenate. (Viscosi & Cardini, 2011)
- 5. Venation: the venation is the pattern of veins in the leaf. It can be described as pinnate, palmate, or parallel. (Viscosi & Cardini, 2011)
- 6. Texture: the texture of the leaf can be described as smooth, rough, or hairy. (Viscosi & Cardini, 2011)

Then the observations were compared and analyzed with

other published sources. When studying leaf morphology, it is important to consider the variation that can occur within a species. Leaf morphology can vary depending on the environment in which the plant is growing. It is also important to be aware of the limitations of leaf morphology as a tool for plant identification. Some species of plants have very similar leaf morphology, and it can be difficult to distinguish them based on leaf morphology alone.

Graft success percent

Graft success (%) = (Bhandari et al., 2021)

Analysis

The data entry and analysis were carried out by using Microsoft Excel 2016. Data were subjected to descriptive analysis and results were presented on average value.

Results:

Leaf Morphology of Chiuri

The length and width of leaves varied depending upon the species. The highest length and width were reported on Rolpa Early while the lowest length and width was reported on Raksirang Early. There was no difference on venation, phyllotaxy, and margins among the different species. However, variation was observed on leaf apex, base, and size of the leaves (Table 2; Figures 1-6).

Effect of graft time and technique on grafting success in early type of Chiuri

Time of grafting influenced the success rate of grafting in which no success of grafting during the months of February (0%), some success in May (30%), and best success rate in July (79%) (Table 3). Type of grafting technique didn't give positive results in February irrespective of graft technique (0%). However, in May and July, the graft technique influenced the success rate of grafting and Cleft (30% and 79%) was marginally better than Side graft (20% and 70%).

Effect of technique on grafting success of different types of Chiuri

Cleft grafting (47.7%) was more effective than side grafting (41.7%) but the success rate was higher in early and mid-types of Chiuri (47.5% and 48% respectively) than the late types (38.5%) (Table 4).

Effect of time of grafting on success of cleft grafting in Early type of Chiuri

Time of grafting significantly influenced the success rate of cleft grafting in early type of Chiuri. While there was no success in February, the success rate slowly increased in the month of May (30%), peaking in July (79%), slowly decreasing in August (10%) and no success in September (0%) (Table 5).

Effect on vegetative growth of plants grafted by

S. No.	Morphology	Raksirang Early	Raksirang Mid	Raksirang Late	Chitwan Early	Dhading Early	Rolpa Early
1	Maximum length (cm)	17.70	17.77	22.53	23.50	24.07	27.80
2	Maximum width (cm)	6.20	8.57	7.90	9.13	9.43	10.37
3	Type of leaf	Simple	Simple	Simple	Simple	Simple	Simple
4	Venation	Reticulate	Reticulate	Reticulate	Reticulate	Reticulate	Reticulate
5	Phyllotaxy	alternate	alternate	alternate	alternate	alternate	alternate
6	Tips/apex	acute	obtuse	acute	acute	acute	acute
7	Bases	cuneate	cuneate	cuneate	acute	cuneate	acute
8	Margins	undulate	undulate	undulate	undulate	undulate	undulate
9	Shape of leaf	elliptical	elliptical	elliptical	elliptical	elliptical	elliptical
10	Remarks	narrow at the base wide at the mid and narrow at the tip	narrow at the base, wider at the mid and tip	narrow at the base wide at the mid and narrow at the tip	narrow at the base wide at the mid and narrow at the tip	narrow at the base wide at the mid and narrow at the tip	leaf elongated, narrow at the base and tip, mid region relatively

 Table 2: Leaf morphology of Chiuri of different locations



Fig 1. Raksirang Early



Fig 2. Raksirang Mid



Fig 3. Raksirang Late



Fig 4. Dhading Early



Fig 5. Chitwan Early



Fig 6. Rolpa Early

different techniques

Days to first bud brust was earlier in cleft grafting (29.57days) in contrast to side grafting (37.03days). Similarly, bud brust was earlier in cleft graft in early (27.3days) or late type of chiuri (27.6days) or side graft in mid type of chiuri (29.7days). In contrast, bud brust was intermediate in cleft graft of mid types (33.8days) and side graft of late types (33days) whereas the latest was side graft of early types (53.8days). This trend was very similar to time to first opening of leaf (Table 6). The average length of rootstock after six months reduced (7.93cm) but the girth of rootstock increased (16.1mm).

Discussion:

Leaf Morphology of Chiuri

The leaves of *D. butyracea* were found to be obovate in shape, with a length of 17.7-27.8 cm and a width of 6.2-10.37 cm. The apex of the leaves was obtuse or acute, and the base was cuneate. (Joshi, 2010) also stated the similar types of leaves shape and size of Chiuri. The margins of the leaves were entire, and the venation was reticulate.

Table 3: Effect of grafting time and technique on grafting suc	ccess of early type of Chiuri
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Grafting date	Chiuri type	Scion source	Grafting method	Success percentage (%)
10/2	Early	Chitwan	Side	0
10/2	Early	Chitwan	Cleft	0
27/2	Early	Chitwan	Side	0
27/2	Early	Chitwan	Cleft	0
28/5	Rootstock*	Chitwan	Side	20
28/5	Rootstock*	Chitwan	Cleft	30
11/7	Early	Chitwan	Side	70
11/7	Early	Chitwan	Cleft	79

*Grafting of rootstock on rootstock

Table 4: Effect of technique on grafting success of different types of Chiuri

Grafting date	Chiuri type	Grafting method	Success percentage (%)	Overall percentage of grafting method (%)
26/7	Early	Side	42	
26/7	Mid	Side	52	41.7
26/7	Late	Side	31	
26/7	Early	Cleft	53	
26/7	Mid	Cleft	44	47.7
26/7	Late	Cleft	46	

Date of grafting	Source of rootstock	Source of scion	Success percentage (%)
10/2	Chitwan	Chitwan	0
27/2	Chitwan	Chitwan	0
28/5*	Chitwan	Chitwan	30
11/7	Chitwan	Chitwan	79
26/7	Chitwan	Makwanpur	53
8/8	Chitwan	Dhading	17
28/8	Chitwan	Dhading	10
14/9	Chitwan	Dhading	0

Table 5: Effect of date of cleft grafting on graft success of early type of Chiuri

*Rootstock on rootstock grafted

Table 6: Vegetative growth of plants grafted by different techniques

Grafting technique	Type of Chiuri	Days to bud burst (days)	Days to first leaf open (days)	Length of rootstock after 6 months (cm)	Girth of rootstock after 6 months (mm)	No. of leaves/ scion after 6 months of grafting	Plant height after 7 months of grafting (cm)
Cleft	Early	27.3	40.2	9.2	18.2 (2.9)	11	27.1
Cleft	Mid	33.8	56	7	16.9 (2.0)	7	26.2
Cleft	Late	27.6	45	8	16.8 (2.8)	8	35.6
Side	Early	53.8	95.7	8.5	14.8 (2.4)	5.7	27.7
Side	Mid	29.7	54	7	15.1 (0.9)	6	16.7
Side	Late	33	53.8	7.9	14.6 (2.0)	10.2	30.6
Average		34.2	57.45	7.93	16.1	7.99	27.31

Number of leaves per scion was also higher in cleft graft (8.66) than side graft (7.33). The plant height was also higher in cleft graft (29.63cm) than side graft (25cm). Late types were taller in both the grafting technique (cleft:35.6cm and side:30.6cm) than mid and early types (Table 6).

The leaves were thinly coriaceous in texture, and they were pubescent to subglabrous beneath. The phyllotaxy of the leaves were alternate, and margins were undulate. Other study shows that leaf blades of D. butyracea of elliptic-oblong, ovate, or ovate-oblong, 17-35 cm X 8-17 cm, leathery, yellowish-brown to brown pubescent, base cuneate, apex obtuse to obtuse-acuminate (Royen, 1958). Similarly, study shows that leaves are thinly coriaceous, obovate, 18-35 x 9-16cm, obtuse or acute, base cuneate, pubescent to subglabrous beneath; petiole 2-3.5cm, pubescent; stipules triangular, c 2mm, early caduceus (Grierson & Long, 1999). The leaves are alternate, petioled, obovate-cuneate, obtuse-pointed, entire; the veins simple; and parallel; obtaining lengths of 15-30 cm with breadths 7-15 cm and petioles are 2.5-5 cm long (Oli et. al, 2024).

Comparison of the leaf morphology of D. butyracea of

Raksirang Rural Municipality with other *Diploknema species* of Chitwan, Dhading, and Rolpa revealed that *D. butyracea* of Raksirang has the shorter length and width compared with leaves of plant from Chitwan, Dhading, and Rolpa. The leaf morphology of *D. butyracea* is also distinct from that of other Diploknema species in its obovate shape and its reticulate venation.

The leaf morphology of *D. butyracea* is likely an adaptation to its environment. The large leaves and long petioles may help to capture more sunlight, while the pubescence on the underside of the leaves may help to protect the leaves from damage by insects, and other herbivores. Leaf morphological traits, such as leaf colour, shape, orientation, and degree of marginal dissection also vary with climate (Nicotra et al., 2011; Schmerler et al., 2012). This study has provided new information about the leaf morphology of *D.butyracea*. This information can

be used to improve plant identification and classification, and it can also be used to investigate the evolutionary history of the *Diploknema* genus.

Effect of graft time and technique on grafting success in early type of Chiuri

Graft time significantly influenced the grafting success in early type of Chiuri. Success was highest in July, moderate in May and nil in February. The highest success in July could be attributed to favorable climatic conditions with high relative humidity and better temperature conditions (Singh et al., 2012). In May, the success percentage could be moderate because the minimum temperature was lower than the ideal while February was not conducive at all from climatic perspective. Good success in July could be due to conducive environment, and optimum sap flow of the cambium. Seasonal variability affects the graft success and higher relative humidity and favorable temperature are key environmental factors for the graft success (Bhandari, 2021). Cleft graft was found better than side grafting irrespective of the time when the grafts were successful. It could be influenced by developmental anatomy of healing process which is also important in the entire process of grafting (Hartman et al., 1997).

Effect of technique on grafting success of different types of Chiuri

Cleft grafting showed higher success rate than side grafting in all types of Chiuri which might be due to better intimate contact between scion and rootstock. Cleft grafting yielded higher graft success than other methods as reported by (Beshir et al., 2019). Earlier, cleft graft was found better than side grafting in early type of Chiuri irrespective of timing of grafting. Similarly, early, and mid-types of Chiuri showed higher success rate than the late type of Chiuri.

Effect of time of grafting on success of cleft grafting in early type of Chiuri

Time of grafting influenced success rate of cleft grafting in early type of Chiuri as observed earlier. Success rate was nil in earlier (February) and later in the season (September) with the highest success in Mid-July. Success of grafting is influenced by both the physiology of the rootstock and scion, and the environment (Karimi & Nowrozy, 2017). The environment during the mid-July and late-July is almost similar but the sap flow in the cambium layer may be better in mid-July than late-July. It is therefore clear that the best time to graft Chiuri is mid-July and not before March and after August. However, further study, in the months of March-April and October can be done to assess the graft success to totally rule out those months. The months November to January is too cold and may not be conducive conditions for grafting.

Effect on vegetative growth of plants grafted by different techniques

Days to bud brust and leaf opening was earlier in cleft graft than the side graft. It suggests that the graft union is better and stronger in cleft than side grafting (Talukder et al., 2015; Sedaghathoor & Noie, 2016). Interestingly, earliest bud brust, and first leaf opening was in cleft grafted early types and latest in side grafted early types. This strengthen the notion that the cleft graft is more effective than side graft. The girth of the rootstock was bigger in cleft graft than side graft, and early types had higher girth irrespective of graft technique. Number of leaves was higher in cleft graft than side graft, and consequently the trend was similar in the plant height also. Interestingly, the plant height was higher in late types than the early and mid-types irrespective of grafting technique.

Conclusion:

Chiuri is a multi-use, under-utilized but economically and environmentally important native fruit tree species of Nepal and recently eight types has been reported from across the country. Cleft grafting was found more effective than side grafting and early/mid-types were more responsive to grafting in contrast to late type. The best time for grafting early type of Chiuri was mid-July and not effective after August and before the month of May. It is important to do detail morphological and butter quality studies of various types of Chiuri and standardize cultivation technology. Quality planting material and standardize cultivation technology is imperative to commercial farming of Chiuri,

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Declaration of conflict of interest and ethical approval:

No potential conflict of interest was reported by the author(s).

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