Food and Agriculture Organization of the United Nations

Manual on

Identification of Large Cardamom Variety in Bhutan

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INTRODUCTION

Large Cardamom is a perennial herb with subterranean rhizomes and 50-140 aerial leafy shoots. Each shoot has a height of 1.7 to 2.6 meters and possesses 9 to 13 leaves in each tiller. Leaves are glabrous on both sides with a prominent mid-rib. The inflorescence is a condensed spike with yellowish perianth. Each spike has 10-15 fruits. The Fruit is round or oval shape, capsule with reddish-brown color. Each capsule is tri-locular with many seeds (Spice Board of India).

Large cardamom is world's third expensive spice crop after saffron and vanilla. Saudi Arabia, India, Pakistan, Bangladesh, UAE, Europe, U.S.A, and Australia are its market. Major large cardamom producing countries are Indonesia, India, Nepal, China, and Bhutan but the species of the cardamoms differ geographically. India, Nepal, and Bhutan produce *Ammomum subulatum* based varieties whereas China produces varieties of the genus Ammomum other than subulatum species. Indonesian large cardamom varieties are based on *Ammomum compactum*. Java Island is a famous large cardamom producing area of Indonesia. Nearly 80 percent of large cardamom production of India is from Sikkim. Nepal's large cardamom production is concentrated in eastern Nepal i.e. Taplejung, Sankhuwasabha, Panchthar, Illam, Dhankuta, and Bhojpur. However, in recent years, its coverage is escalating towards western Nepal because of its lucrative price.

Large cardamom is an integral part of biryani in most of the Pakistani and Indian restaurants. It is also used as a kind of dessert after meal for fighting against bad breath. In Europe, it is used for bakery purposes. Large cardamom has several medicinal uses mostly in herb-based medicines. Its average productivity in Nepal is around 0.540 ton per hectare. In the year 2016, the total large cardamom production in Nepal was 6521 tons, 5623 ton in India, and 2245 tons in Bhutan.

ICIMOD's publication unveils that Nepal is the largest producer of large cardamom in the world. The price of large cardamom in Delhi market was INRs 1560 per kilogram in February 2019. But Siliguri based market sold cardamom at Rs. 1079/ kg in 2016.

There are several varieties of large cardamom. Some of the most popular varieties of large cardamom are Ramala, Seremna, Damberse, Zhangu-Golse, Golse, Bharlange, Jirmale, Madhuse and Ramse.

The India's Arunachal Pradesh originated varieties are Bebo dark red, Bebo light red, Boklok, Tali, Jaker, and Belak. Each variety exhibits different characteristics i.e. color of the pseudo-stem, height, leaf structure, bearing habit, the size of capsule, disease tolerance and drought tolerance.

Large cardamom is highly cross-pollinated crop. It is a challenge to maintain true to the type varieties of large cardamom unless maintained under separate facilities with proper management. This manual is developed as a guidebook for all professionals in research and extension system for identification of different varieties of large cardamom.

BOTANICAL CLASSIFICATIONS

Large cardamom is one of the popular spices found in the Zingiberaceae family. It is a tall, perennial, evergreen, herbaceous monocot plant (Kumar et al., 2012). The height of this plant is 1.5 to 3.0 m (Bisht et al., 2011) and on the upper part of the stem, there are leaves. The rhizomes are of dull red color and the flower buds protrude from the base of the rhizome. Spring is the flowering period of large cardamom. Short peduncle and buds covered with tight red bracts. The individual flowers remain open for three days or more. At the same time, new ones are opened. Flowering remains intact with the flowers for one month (Sharma et al., 2000). There are many classification systems but the botanical classification system is the one most frequently used.

Large cardamom has been botanically described as follows:

Kingdom	Plantae
Division	Spermaphyta
Sub-division	Angiospermae
Class	Monocotyledonae
Order	Scitaminea
Family	Zingiberaceae
Genus	*Amomum*
Species	*Subulatum*

OTHER CULTIVATED SPECIES

Other cultivated species in China is *Amomum sps*. It is bigger in size and is considered to be inferior to *Amomum subulatum* Roxb. Indonesian large cardamom variety is based on *Ammomum compactum*. (Emperor Akbar, 2016).

1. *Amomum delbatum*
2. *Amomum aromaticum*
3. *Amomum plauciforum*
4. *Amomum longiforme*
5. *Amomum corinostachyum*
6. *Amomum kingii*

There are other wild species

1. *A. spiceum*
2. *A. xanthophlebium*
3. *A. macranthunt*
4. *A. lllacrodells*
5. *A. hastilabiulll*
6. *A. eylindraceum*
7. *A. vivale*
8. *A. testaCellln*
9. *A. sqllarrosllnt*
10. *A. biflorum*
11. *A. maeroglossa*
12. *A. citrinll17l*
13. *A. uticolosum*
14. *A. ochrellm*
15. *A. uphalotes*
16. *A. leppaceum*
17. *A. aeilleatum*
18. *A. uligillosllm*

There are various wild species of cardamom found in the forest areas. The small cardamom is different from large cardamom. The Scientific name of small cardamom is *Elettaria cardamom* Maton. Guatemala produces the highest amount of small cardamom. Its genome 2n is 48. Large cardamom variability is also reported with 2n = 26, 34, 42 and 44 (Sharma and Bhattacharya, 1959) as mentioned by (Pradhan et al 2014).

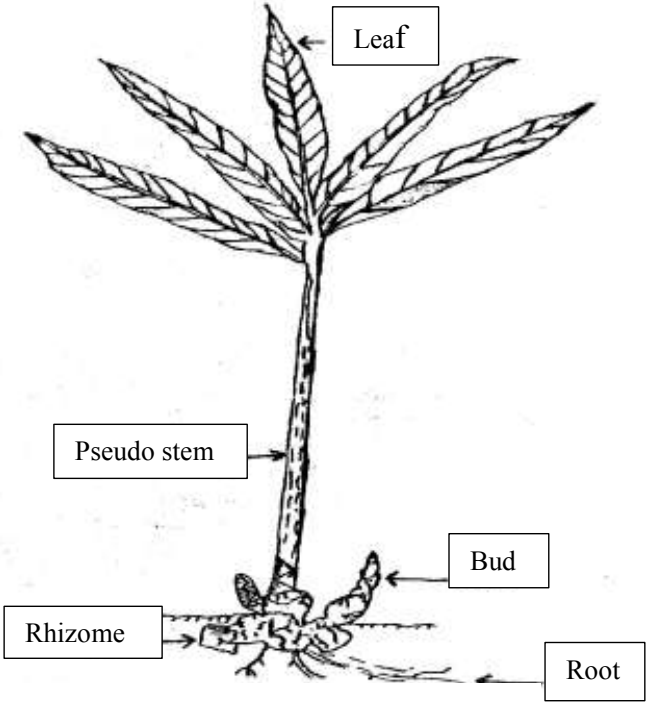

Figure 1: Various parts of Large Cardamom

Cardamom parts

It is a perennial evergreen monocotyledon plant, which has six parts.

1. **Rhizome:** It is a vital organ of cardamom, which has roots below and apical growing tips above. It has nice aroma. The figure below illustrates rhizome.

Figure 2 : Rhizome and roots

2. **Pseudo stem:** It is made of bundles of leaves wrapped around each other. The tips which do not bear flowers develop into pseudo stem and leaves emerge out of it. Pseudo stems are covered with leaves base or sheath. The color of pseudo stems depends on the varieties. Its height is around 1 to 2 meters; and one bush possesses 20 to 70 pseudo-stems. The number of pseudo stems depends on soil type and variety - Sawane, Ramse and Golse possess many tillers whereas Damberse and Chibse give lesser tillers.

Figure 3: Pseudo-stem & Spike

Figure 4: Emerging Buds of Pseudo-stem and spikes

a) **Golse:** Green colored and medium
b) **Ramse:** Monorish red and long
c) **Saune:** Green, long
d) **Jirmale:** Green and medium sized
e) **Madhuse:** Monorish red colored and dwarf
f) **Damberse:** Monorish red colored and short
g) **Bharlange:** Monorish red colored and long

3. **Leaf:** Leaves emerge from pseudo stems. Each pseudo stem has 5 to 13 leaves, depending upon the variety. Leaves are long, tapering towards tip, green and without hairs. Middle portions are wide and tapering towards tip. It has a long main vein in the center right from the base to the tip and secondary veins initiated from the main vein. Basal part of the leaf is covered by sheath. Leaves emerged from alternate directions. Cardamom leaves are droopy (Seremna) and vertical depending on the variety. Its green chlorophyll play role in photosynthesis and stomata beneath transpire and keep the plant healthy.

Table 1: Varieties and their leave characteristics

Variety	Leaves
Seremna	Droopy and vertical
Ramala	Broad and slightly elongated
Damberse	Leaver are short and erect the veins of leaves
Jirmale	Green leaves, length 24 cm width 5.05 cm
Chibese	Light green leaves, sword type leaves
Bharlange	Leaves having wavy margins, length 32.67 cm width 5.92 cm
Saune	Ovate and board leaves length 29.34 cm and width 6.66cm
Golse	Narrow and erect leaves 27 cm long and 5.38 cm wide
Ramse	Green colored narrow, 25.7 cm long and 6.47 cm wide
Madhuse	Length 25cm and width 6.12 cm

4. **Inflorescences:** Two types of buds emerge from the rhizomes; one that develops into pseudo stem and another develop into flowering buds. The flowering buds become flowers after attaining 1 to 1.5 cm in height; and the Inflorescence is 10 to 16 cm long. One pseudo stem contains one to four inflorescences. Flowers emerge from this inflorescence. One inflorescence contains 20 to 25 flowers. Flowers will be light yellow and vary in size, according to varieties. Cross-pollination is essential for fruits though there are both male and female parts in the flower. The pollination is mostly by bumble bees, bees, and other insects. The seed formation takes place after successful pollinations. The pictures below illustrate the Inflorescence.

Figure 5: Inflorescences, Flowers, Flowering buds and pseudo stems

5. **Capsules:** Each inflorescence possesses 15 to 12 capsules. The sizes of the capsules are 2.3 cm long and 1.5 cm wide. The color of the capsules is light red to brown. A dried capsule weighs 0.5 to 1.45 grams. The color and size of the capsule differ with the variety to variety.

Figure 6: Dried capsules

Table 2: Varieties and their capsule characteristics

Variety	Capsule character
Ramse	Long shaped, medium sized and medium quality. Dry weight of 20 capsules is 15.4 g
Golse	Big and round, plump and weighty Dry weight of 20 capsule 14.5 g
Jirmale	Big and round
Sawane	Big grey colored triangular in tip and round at base but smaller than Bharlange. Dry weight 20 capsules is 12.1 g
Chibese	Small capsule.
Zongu-Golse	Big and bold capsules Dry weight of 20 capsule is 17.2 g
Seremna	Medium sized weighty capsules
Ramala	Round medium sized, dark pinkish in color. Dry weight of 20 capsules is 16.1 g
Damberse	Big sized and tasty
Bharlange	Big sized long capsule
Madhuse	Dry weight of 20 capsule is 18.3 g

Table 3: Physical properties of cardamom capsule

Particulars	Minimum	Average	Maximum
Length in cm	2.25	2.2	2.3
Width in cm	1.35	1.1	1.35
Ratio of Length/Width	1.82	1.70	2.01
Bark %	36.14	32.5	38.8
Seed %	60.11	58.11	62.11
Pulp %	2-3	-	-

6. **Seed:** Seeds are always inside the capsules. One capsule possesses 25 to 80 seeds. The Immature capsule possesses white seeds. It turns red in color as the capsule matures and finally it will attain black color. The seed is covered with sticky pulp. Dried large cardamom possesses 61 % seed and 37 % bark and the remaining part will be pulp.

Figure 8: Fresh capsule

Figure 7: Dried capsule with seed

Table 4: Varieties and 1000 seed weight

Variety	**Seed weight 1000 seeds in gram**
Ramse	18.3
Golse	16.3
Zongu-Golse	10.9
Sawane	15.09
Madhuse	15.8
Ramala	14.5

Chemical properties of large cardamom are presented below.

Table 5: Chemical properties of large cardamom

Ingredients	**Amount in percentage**
Water	8.49
Volatile oil	2.0
Protein	6.0
Total ether extract	5.31
Nonvolatile ether extract	2.31
Volatile ether extract	3.00
Crude fiber	22.00
Carbohydrate	43.21

Ingredients	Amount in percentage
Alcohol extract	7.02
Ash	4.57
Water soluble ash	2.15
Water soluble alkaline ash	0.09
Acid soluble ash	0.90
Source: Cardamom cultivation, NSCDP, Lalitpur	

Large cardamom variety identification

Variety Identification in large cardamom can be done through two methods;

a. Morphological traits

Large cardamom varieties can be identified through careful observation of its morphological traits such as color, number and height of its pseudo-stem; size, shape and bearing habit of leaves; and size, shape of capsule, number of seeds per capsule and color of capsule.

b. Electrophoresis

Through electrophoresis, based on the protein bands computer can identify the variety. Its use is seen in case of small cardamom but there have been no reports available in case of large cardamom.

Morphological characteristics of large cardamom varieties

In order to distinguish large cardamom types depending upon its morphological characters, understanding the character of different varieties is very important. Different characters for different varieties are described in this section.

c. Damberse

Its capsules are bigger in size. It is less tillering, dwarf, reddish green stalk (pseudo stem), and big-sized capsules with more seed inside the capsule, tasty and fetch a better price in the market. It is cultivated at 700 to 1200 meters from sea level. Its leaves are upright and short. It ripens from September to October.

Variety Maintenance

Special farmers must be selected at least in four locations that can exert extra energy to save it from crosspollination with other unwanted varieties. Green net of fifty percent shade can be used in maintenance of the variety. It will save cardamom plant from pollination with other unwanted variety in the one hand while in the other hand it will save from the attack of aphid-like vectors, which transfer viral diseases.

Only selected personnel should be allowed to enter the plant areas in disinfected clothes and after dipping boots or legs inside lime water. No one should allow smoking in the vicinity of the area. Adequate manure applications, adequate health care must be practiced. Daily inspection of the mother plant must be practiced. The infected or diseased plant must be taken out and incinerated in the fire. The equipment used must undergo sterilization after use otherwise diseases will easily transfer upon the use of such equipment which is used in cutting or digging of the diseased plant. Fire sterilization is recommended for the equipment. Irrigation by sprinkler method can be installed for offering adequate moisture and humidity.

The area must be small for keeping the plants in ICU (Intensive Care Unit). The Government should offer a special grant for the purpose of variety maintenance. Interested people should collect seed and other planting materials from such stocks.

The periphery must not possess banana plants, peach plants and maize plants. These plants are alternate hosts of the Chirkey and Foorkey virus diseases. The aphid *Pentalonia nigronervosa*, *Myzuspersica* and *Ropalosiphum maidis* attack cardamom and transfer Foorkey and Chirkey viral diseases.

Fungal diseases mainly from *Collechtrochium geosporides* is becoming very serious which damage the crop very fast which put farmers at the risk of a higher rate of economic injuries. Periodic inspection of the cardamom field and regular sprays of the bordeux mixture in each 15 days interval can reduce chance of fungal disease incidence. Taking care of mother stock is very important because it will be the future of entire cardamom industry.

This variety maintenance principle will be applicable in other varieties too.

Figure 9: Damberse Variety of Large cardamom

Distinguishing Character

- Dwarf
- Less tillers
- Lower altitude (700-1200 masl)
- High quality bigger size capsules
- Tasty capsule

d. Jirmale

Its leaves and pseudo-stems are green in color. It produces a large number of tillers. It gives good production in low water availability conditions too. Its season of ripening is from August to September. In one spike, 22 to 37 capsules are available and each capsule possesses 56 seeds. It is somehow –tolerant to most diseases. This variety is also known as dryland cardamom. This variety is dwarf in size and is cultivated at 700 to 1,000 meters from sea level. Its capsule possesses 63 percent bark and 37 percent internal pulp and seed. It is also known as Salakpure. Heavy manure application is required each year.

Distinguishing characters

- Long green leaves and pseudo stem
- Disease tolerant
- Round & Big capsules

Figure 10: Jirmale variety of large cardamom

e. Seremna

Figure 12: Pseudo-stem of Seremna
Source: ICIMOD

Figure 11: Seremna showing drooping leaves
Source: Pakhribas

It has drooping leaves which are suitable for 1200 to 800 meters from mean sea levels. It is tolerant of Chirkey and Foorkey diseases. Regular application of water and manure will make it productive for 15 to 20 years. It is famous for its production. Plant height is 1.5 to 2 meters. It can be identified with its green colored leaves and pseudo stem. Capsules are big and long possess 65 to 70 grains. One plant gives 2 to 3 spikes. It is Indian variety from Sikkim area.

Distinguishing character

- Drooping leaves
- Tolerant to Chirkey and Foorkey disease
- Capsules are long and bigger in size

f. Madhuse

This variety is not commercially cultivated in Nepal but cultivated in Kalimpong, India. Flowerings of this variety resemble that of Turmeric flower that grows from the soil surface. Local scientists and farmers of India found this variety as little bit resistance to Chirke-Furke diseases. NCARP, Pakhribas, Dhankuta started research in Madhusai for its suitability in Nepal and for disease resistance capacity. Stem color is red. Each Capsule size is 72.5mm. This variety performs well in lower altitudes (700-1000msl).

Distinguishing characters

- Dwarf variety
- More information about this variety is presented in table 7.

g. Ramala

This variety resembles Ramse and is cultivated above 1500 meters from sea level. The average capsules per spike is 65 to 70 and per capsule seed rate is 65 to 70 grams. It gives 2 to 3 spikes per plant. Per spike capsule rate is 10.

Figure 13: Ramala variety

Distinguishing characters

- Capsules are dark pink in color with 30-40 seeds
- Maroonish tillers
- Ready to harvest in October
- Leaves are broad and slightly elongated.

h. Bharlange

- This cultivar grows in low, medium and high altitude areas in South Regu (East Sikkim) and at high altitudes at Gotak (Kalimpong subdivision in Darjeeling district of West Bengal
- Its yield performance is exception ally high at higher altitude areas i.e. 1500 m and above.
- It is a robust type and total tillers may range from 60 to 150.
- Color of tillers is maroon-ish green to maroon towards collar zone; girth of tillers is more than that of *Ramse*.
- Each productive tiller on an average produces almost three spikes with an average of 20 capsules/spike

- Size of capsules is bigger and bold with 50-65 seeds. Harvesting begins in the last week of October
- This cultivar is also susceptible to *Foorkey* and *Chirkeys* disease (*NCARP, 2018)*

Figure 14: Bharlange Large Cardamom Variety

Distinguishing characters

- Big and bold capsules hold 50-70 seeds
- Slightly elongated capsules
- Flowering is from June to July depending upon altitude Fruit bearing tillers and spikes ratio are relatively high Plant height is around 2.4 m.
- ICIMOD recommends it in higher altitude above 1500 m to 2200m

i. Ramse

Figure 15: Left Leaves of Ramse middle Pseudo-stem of Ramse variety, right spikes of Ramse variety

Distinguishing characters

- Short spike length and difficult to harvest
- Grows in steep slopes
- Performs well in higher altitudes above 1500 m
- Harvesting in September-October
- Smaller capsules, each bearing 25-40 seeds per capsule Leaves are confined in the apical areas only.
- Red pseudo-stem

j. Golse

It has short & thick pseudo-stem and short leaves. Leaves emerged from basal part to apical part. Capsules of this variety are big, round and yellowish in color. It performs well in the elevation 1200 to 1600 meters. In Sikkim, it is cultivated in 600 to 1,000 meters in altitude. The pseudo stems are smaller than that of Ramse. The leaves initiation starts right from the basal part of the plant. The leaves are erect. Its leaf base beaks easily compared to the other variety. Its inflorescence is longer compared to Ramse. Its inner pulp and seeds are tasty and longevity is comparatively high. Harvesting starts from last September. This variety is seen to be infected with the Chirkey and Foorkey viruses.

Figure 16: Golse Large cardamom plant.

Distinguishing characters

- Bigger size capsules
- Spike length is high and easy to harvest
- Pseudo stem is smaller
- Leaf initiation is right from the base to the top

- It can be cultivated in 1200 -1600 mm altitudes
- Susceptible to viral diseases
- It has round and big sized capsules

k. Chibese

Farmers from the altitude 1,300 to 1800 meters can choose Chibese. A single fruit bearing tiller bears 2 to 4 spikes, each bearing around 10 to 20 capsules. This is a productive variety with good market potential. The figure below shows Chibese.

Figure 17 : Chibese Variety

Distinguishing characters

- Smaller size capsules
- High production potential
- Single tiller bears 2-4 spikes
- Each spike bears 10-12 capsules

l. Sawane

This variety is grown at lower elevations (900 to 1,500m) in all cardamom growing areas of Sikkim and Kalimpong of India and Taplejung of Nepal. Plants are 1.5 to 2m in height and robust in nature. Leaves are ovate and broad with maroonish tillers. Capsules are relatively smaller than Bharlange and contain only 30 to 50 seeds. Flowering starts with on onset of rain, in the late March to May, and harvesting is done in September to October.

This variety is highly susceptible to disease. Bhutan should omit this variety. Sikkim 1 and Sikkim 2 are other varieties released in India which are selected from this variety.

Figure 18: Sawane variety

Distinguishing characters

Capsules are smaller than Bharlange containing 35-50 seeds

- Susceptible to diseases and pests
- Flowering starts at late March to May, harvesting follows during September to October.
- Its plants are longer like Ramse and leaves are smaller and wider like that of Golse
- Leaves color are dark green
- Pseudo stems are light green
- It is harvested in August

Long pseudo-stem, Short leaves

m. Zongu-Golse

This variety does well in the altitude range of 1,000 meters from mean sea level. It starts flowering from April, and capsule formation takes place from October. It is from the place Zhangu of Northern Province Sikkim. The pseudo stems are similar to that of Ramse. It is tolerant to diseases like local varieties. Leaves are narrow and tapering. Pseudo stems are green in color. Studies were conducted to assess the performance of eight varieties of large cardamom at mid-altitudes (660 meters MSL) in Arunachal Pradesh. Out of the eight varieties tested, Ramla, Sawane, Madhuse and Ramsey performed better and could be introduced for commercial cultivation at mid-altitude region of Arunachal Pradesh.

Distinguishing characters

- Capsules are big and bold with 50-70 seeds
- Disease tolerant cultivar
- Tillers are similar to that of Ramse Adaptive to lower elevations.

Figure 19: Zongu-Golse variety

The following tables will elaborate latest varietal descriptions based on the latest research at Arunachal Pradesh and Sikkim in India, and Pakhribas, Nepal.

Careful study of the table will further help to understand the varieties.

Table 6: Agro-morphological evaluation of large cardamom germplasm at Pakhribas, Nepal (NCARP, 2018) and ICIMOD 2017

SN	Traits	Ramse	Golse	Saune	Madhusai	Damberse	Bharlange	Jirmale
1	Plant height (cm)	65.8	52	51.4	45	62.8	51.4	44.8
2	Plant vigour	Good	Good	Good	Good	Good	Good	Good
3	Stem color	Red	Green	Green	Red	Red	Red	Green
4	Leaf length (cm)	25.7	27	29.34	25	32.67	32.67	24
5	Leaf breadth (cm)	6.47	5.38	6.66	6.12	6.68	5.92	5.05
6	Leaf color	Green	Green	Green	Green	Green	Green	Green
7	Number of adults tillers per bush	2	2	3	2	2	3	3
8	Number of new tillers per bush	7	7	5	9	9	6	7
9	Susceptibility to Diseases	Lower Elevation Yes	Leaf streak Viral	Fungal disease	Medium	Medium	Viral disease	Low
10	Number of Leaves	7	5	7	5	7	8	7
11	Altitude of cultivation	High	Low to Middle	Middle	Low to Middle	Low to Middle	High	Low

Based on such data we have to differentiate the varieties of large cardamom. Frequent cross-pollination and seed originated saplings can deteriorate varietal characters. Thus, variety maintenance practices must be carried out to find true to the type variety. Altitude based recommendation is presented in the table.

Table 7: Arunachal Pradesh based study on large cardamom

Variety	Mean Plant Height		Number of sucker/Plant		
	Four year	Cm	Four year	Mean	
Ramsey	100.1			17.52	
ZonguGolse	86.44			12.08	
Golse	107.2			13.68	
Madhuse	126.8			16.53	
Sawane	123.6			19.08	
Ramala	130.9			21.21	
Bebo Red	196.6			9.7	
Bebo light red	166.5			9.3	

Table 8: Comparative analysis of cardamom varieties

Variety ↓	Fresh Weight of 20 capsules in gram		Dry Weight of 20 capsules in gram		Seed capsule		Weight of 1000 gram seed	
Year →	1999	2000	1999	2000	1999	2000	1999	2000
Ramse	67.6	67	15.5	15.4	41.6	42.3	18.3	18.3
ZonguGolse	65.66,	65.9	17.3	17.2	43’.6’0	39.7	10.8	10.9
Golse	56.34	55.9	14.3	14.5	29	33.4	16.6	16.3
Madhuse	70.24	72.5	18.7	18.3	84.8	87.4	15.5	15.8
Sawane	54.92	54.9	11.8	12.1	27.2	28.7	14.9	15.09
Ramala	74.58	81	16.5	16.1	59.2’0	58.3	14.9	14.5
Bebo Red	166.2’0	165	17.9	17.8	54.2	57.1	13.7	13.2
Bebo light red	198.1	201.2’0	19.7	19.9	52.2’0	55	20.2	20.6
CD at 5%	20.68	20.5	3.19	2.98	10.4	9.88	1.07	1.04

(Yadav & Dubey, 2001)

Table 9: Large cardamom cultivar diversity in Sikkim Himalaya

S.N	Variety	Morphological Traits	Capsule/Seed Characteristics	Altitude
1	Ramsey (cultivated on steep Slopes)	Tall & robust with large number of tillers. Plant height 1.5 to 2.0 meters, Tiller color Maroonish, narrow leaves.	Size Small 25-40 seeds	Above 1500
2	Sawane, Widely adapted cultivar	Tall & robust, Plant height: 1.5 to 2.0 meters, Leaves: Ovate and broad	Size: Bigger and bold, 35 - 50 seeds	(975-1500m masl) and high (> 1500 m
3	Bharlange	Plant height 1.5 to 2.0 meters Tiller color: Maroonish Leaves: Narrow leaves with wavy margins	Bold size Seeds 50-70	The productive tiller and spike ratio is high
4	Ramla	Plant height: 1.5 to 2.0 meters Tiller color: Maroonish Leaves: Broad and long	Color dark pinkish, 30-40 seeds	1000-1500m
5	Seremna	Plant height: 1.5 to 2.0 meters Tiller color: Green Leaves: Mostly drooping type	65-70 seeds	1000-1500 m, on an average 2-3 spike in each productive tiller with an average of 10 capsules in each spike
6	Golse	Leaves narrow and erect, Green tiller color, 1-1.5 m plant height, not robust.	50-70 seeds Bigger &Bold	1000 -1500 m

Electrophoresis

In this method, proteins are extracted from the grain, and the protein composition is used to identify the variety. In India, electrophoresis methods were in use at ICAR based facilities; we can use the facility and calibrate them. In small cardamom (*Elettaria cardamomum)*, there are reports of its use but are not seen in large cardamom. This device can be useful in other cases too. Its use can detect varietal purity effectively and accurately because of the computer added technology associated with it.

Molecular Methods

Molecular tools like PCR, RFLP, DNA Bar coding are recently used tools in the variety identification process.

Plant variety and cultivar identification are some of the most important aspects in the agricultural system. The large number of varieties or landraces among crop plants have made it difficult to identify and characterize varieties solely on the basis of morphological characters because they are non-stable and originate due to environmental and climatic conditions, and therefore phenotypic plasticity is an outcome of adaptation. To mitigate this, scientists have developed and employed molecular markers, statistical tests, and software to identify and characterize the required plant cultivars or varieties for cultivation, breeding programs as well as for cultivar right protection. (Nicholas et al 2012).

a) DNA bar coding

DNA barcodes allow non-experts to objectively identify species – even from small, damaged, or industrially processed material. Just as the unique pattern of bars in a universal product code (UPC) identifies each consumer product, a "DNA barcode" is a unique pattern of DNA sequence that identifies each living thing. Short DNA barcodes, about 700 nucleotides in length, can be quickly processed from thousands of specimens and unambiguously analyzed by computer programs.

With DNA barcodes, students can help discover and catalog biodiversity on our planet using tools developed at the DNA Learning Center.

b) Restriction Fragment Length Polymorphism (RFLP)

RFLP is a difference in homologous DNA sequences that can be detected by the presence of fragments of different lengths after digestion of the DNA samples in question with specific restriction endonucleases. RFLP, as a molecular marker, is specific to a single clone/restriction enzyme combination.

c) Complete Polymerase Chain Reaction (PCR)

PCR fragment analysis requires a method that supplies sufficient separation resolution. Despite this requirement, PCR fragments are commonly analyzed by slab gel electrophoresis. Though readily available in many laboratories slab gel electrophoresis is incapable of providing researchers with high-resolution separations of PCR fragments. Furthermore, agarose gel electrophoresis does not scale to effectively meet the needs of high-throughput environments, leading researchers to feel frustrated and out of time.

Reasons of use of molecular markers in large cardamom

Molecular markers which detect variation at the DNA level overcome most of the limitations of morphological and biochemical markers hence, the potential application of RAPD markers has been demonstrated in many crops for a long time (Bowditch et al., 1993 and Zhang et al., 2011).A comparative analysis of the different methods of variety identification in terms of accuracy, cost and time will be essential to follow the techniques according to the need.

Molecular cardamom variety identification in Nepal (Methodology)

The following methodology was used to access the cardamom varieties in Nepal by molecular method.

The sixteen plant materials (*Amomum subulatum*) used in this study were collected from Taplejung, Panchthar and Ilam district of Eastern Nepal during 2012. Immature leaf samples were collected from various eco-geographical locations of different altitude irrespective of wild as well as cultivated genotype. The samples were transported to the laboratory as soon as possible and stored at -40^{0}C until DNA extractions. Geographical situations of collected samples were recorded with the help of GPS machine (GPS Garmin). Each sample was reduced to fine powder through mortar and pestle with the addition of liquid N2 and transferred to a 2.0 ml eppendorf tube. DNA extraction was performed using a modified CTAB method (Doyle and Doyle, 1990), where modification was done for CTAB buffer reaction time at 65^{0}C for1 hour. DNA concentration was estimated by Q5000 UV vis Spectrophotometer (Quawell).

RAPD amplification was performed with random decamers (Kit from Operon Technologies, Alameda, CA, USA). On preliminary experimentation sixty primers were tested but only twenty five arbitrary RAPD primers showed reproducible banding pattern and were chosen for the analysis based on highly readable and polymorphic bands as described by Pandiyan et al., 2010.

DNA amplifications were performed in 15 µl reaction volume containing 50ng template DNA, 2X Green Go Taq® Reaction Buffer (pH 8.5),400µM dATP,400µM dGTP, 400µM dCTP, 400µM dTTP and 3mM MgCl2. (Promega) and 0.6picomole of primer. The mixture was gently mixed and centrifuged in shaker for 60 seconds prior to adding two drops of mineral oil. After addition of mineral oil it was transformed to thermo-cycler, All the amplifications were performed in the same thermo-cycler (MULTIGENE OPTIMAX, Lab net International, Inc.) programmed for 1 cycle of 95° C for 5 min followed by 40 cycles of 95° C for 60 sec, 36°C for 1 min 30 sec, 72°C for 2 min, and final 1 cycle of 72°C for 10 min. Samples were kept at 2^0C until further analysis. 12 µl aliquots of amplification products were loaded in a 1% (w/v) agarose gel (Bioneer) for PCR product separation in 1X TAE buffer (Bioneer) in an electrical field (120 V) for 90 minutes. Gels was stained with Ethidium Bromide (0.5µg/ml for 30 min) and visualized under exposure of UV light under gel doc system (UVDI, Major Science). For scoring band and to confirm consistent amplifications during the whole experiment standard 1 kb Ladder (Bioneer) was used.

RAPD profiles of 16 different cultivars were scored on binary system as 1 for presence of the band and zero for absence of band. Only the bands that are clear and reproducible were scored. Polymorphic Information Content (PIC) for each primer were calculated as described by Roldan Ruiz et al., (2000) as PIC= 2Pi (1-Pi), Where Pi is the frequency of i^{th} null allele. Scored bands were subjected to study the Dice similarity coefficient with the help of NTSYS pc versions 2.01 and based on genetic distance UPGMA genetic maps were created. To confirm the clustering (Chaudhary, Raju et al, 2016)

For details, please consult the paper GENETIC DIVERSITY IN LARGE CARDAMOM (*Amomum subulatum* Roxb.) DISSECTED USING RAPD MARKERS. By Raju Chaudhary, Surya Kant Ghimire, Bal Krishna Joshi, Bisnu Raj Ojha) International Journal of Advanced Research 4(6):1443-1451 · June 2016

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