GENETIC DIVERSITY OF *Dimocarpus longan* Lour., IN SOUTHERN WESTERN GHATS

by

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THESIS

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DECLARATION

I, hereby declare that this thesis entitled, **"Genetic diversity of *Dimocarpus longan* Lour., in Southern Western Ghats"** is a bonafide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award to me any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

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1. INTRODUCTION

Longan (*Dimocarpus longan* Lour.), belonging to the family Sapindaceae, is an important commercially cultivated fruit tree in south east Asian countries like China, Taiwan, Thailand *etc.* Longan is reported as the third-highest produced minor tropical fruit by FAO during the year 2015-2017. Many countries have started the cultivation of longan as a commercially important fruit crop in recent years. Apart from the economic significance, longan has many nutraceutical values that helps increase the demand of longan fruit in markets. In India, longan fruit is one among the highly demanded imported minor tropical fruits, commonly imported from countries like Thailand, Taiwan, Florida *etc.*

From the past few decades, global demand for longan fruit has risen very rapidly due to its taste. It has an edible aril inside a leathery pericarp. It contains significant amount of bioactive polyphenols, vitamin C, volatile compounds, minerals, amino acids, flavanones, proteins, fat, carbohydrate, *etc*. The bioactive compounds from the fruit extract have shown pharmaceutical properties like antioxidance, anti-cancerous, anti- tyrosinase, immunomodulatory activity, antihypertensive, anti-glycated *etc.* Thus the importance of fruit can also be emphasized due to its richness in nutritional value. Sucrose, fructose and glucose are the major sugars present in the edible pulp whose content increases as it ripens. Large amount of polysaccharides are present in the fruit pulp and pericarp among which pulp contains the highest amount. Although, being naturally present in Western Ghats regions of Kerala, longan is not a cultivated species in the state.

D. longan is a huge shaded tree in the semi-evergreen and evergreen forests of Western Ghats regions at an altitude of 1700m. Longan is a subtropical fruit tree known to be originated in the southern regions of China also stretching to the Indo-Burma region.Gamble (1935) had reported its distribution from Konkan region to Tinnevelly region in Western Ghats. It also has its distribution along the Eastern Bengal and Western

Peninsula in India. Longan is a common tree species in the evergreen and semi-evergreen forests of Kerala.

Complete knowledge of the variability existing among the natural populations including both morphological and genetic variability assist in the better improvement of a forest tree. Morphological differences of a population may be influenced by the environmental conditions existing among different locations, so it is more ideal to study the genetic variability to understand the divergence existing among different populations for applying in the breeding programs. Knowing the existing morphological and molecular diversity can help to understand the potential of a species to accustom to the changing environmental conditions.

In spite of *D. longan* being a close relative of rambutan and litchi, which are gaining much popularity as a fruit crop in the state, the potential of longan as a fruit crop has not yet been investigated. The unavailability of good cultivar varieties also restrict large scale studies in longan fruit in India. This is the first study with the specific aims of documenting the variability of longan and identifying superior genotypes with promising fruit characters in southern Western Ghats for further improvement and domestication of the species. In this context, the present study was undertaken with the following objectives:

To analyze the morphological, biochemical and molecular diversity of *Dimocarpus longan* Lour. from Western Ghats of Kerala.

2. REVIEW OF LITERATURE

2.1 DESCRIPTION OF SPECIES

Dimocarpus longan Lour. belongs to the Sapindaceae family (soapberry family). It is commonly known as 'Dragon eye' (literally means eye of dragon) owing to the round and bright black seed of its fruit (Lin *et al.*, 2017). It is a popularly known commercial fruit tree in many regions of Southeast Asia *viz.* China, Thailand, Vietnam, India, *etc.* It is currently expanding its distribution in the tropical and subtropical regions all over the world (Menzel *et al.*, 1989). FAO (2018) reported the longan as the third-highest produced fruit over the three-year period 2015-2017 and was estimated to be nearly 3.4 million tones. Over the past decade, longan has shown a rapid rise in its global production, with a 4.6 percent average annual growth. IUCN has classified the species as near threatened. Recently, countries like China, Thailand, Australia, *etc*. have seen a boom in research in longan for exploring its commercial importance and developing improved varieties. Despite being a common indigenous species in the evergreen and semi-evergreen forests of India, its genetic potential still remains unexplored.

Longan is a highly shaded huge tree commonly found in semi-evergreen or evergreen forests occurring up to an altitude of 1700m (Tissot *et al.*, 1994). Longan grows and yields best in a climate with frost-free, short, cold winters and long, hot, and humid summers (Menzel *et al.*, 1989). Longan fruit is a drupe with white-colored succulent edible aril surrounding a relatively large black or dark brown colored shiny single seed. The edible aril is covered with a leathery, thin, indehiscent pericarp having brown color (Nakasone and Paull, 1998; Jiang *et al.*, 2002).

D. longan has a diploid genome (2n=30). *D. longan* is a close relative of litchi and rambutan which comes under the Sapindaceae family (Diczbalis, 2002). Longan and litchi are the most popular Sapindaceae members concerning their economically significant edible fruits with fleshy translucent juicy whitish aril with a sweet aroma.

Longan owes more resemblance with litchi in their origin, favorable environmental condition and fruit utility. But longan is smaller in size, the pericarp is yellowish-brown in color, aril having a milder flavor which is less acidic than that of litchi (Menzel, 2002; Wall, 2006). One-third of Asian people prefer longan over litchi due to its exorbitant taste (Menzel and Waite, 2005).

2.1.1 Sapindaceae Family

The Sapindaceae (in the order of Sapindales), is a family of flowering plants under the class Dicotyledoneae. It comprises approximately 20000 species from around 150 genera, primarily distributed in subtropical and tropical areas and to a lesser extent in northern temperate areas (Lora *et al.*, 2018). Sapindaceae family includes trees, shrubs, herbaceous plants, or sometimes woody vines. Family members are characterized by alternate, typically pinnately (sometimes palmately) compound, rarely simple leaves. Flowers are usually small and unisexual with appendaged petals, tricarpellary ovary, and unilateral extra staminal disk, fruits with arillate seeds. In India, the family is represented by nearly 20 genera (Xu and Deng, 2017). Sapindaceae family is well known for its important edible tropical fruit trees which have great economic significance both locally and globally. Among them, the most important fruit trees are rambutan (*Nephelium lappaceum*), longan (*Dimocarpus longan*), litchi (*Litchi sinensis*), and pulasan (*N. ramboutan-ake*) and huaya (*Melicoccus oliviformis* Kunth.). These trees which are currently categorized as minor tropical fruit trees are recently gaining commercial importance in the international markets owing to their sweet taste and nutraceutical values (Dong *et al.*, 2021).

2.2 ORIGIN AND DISTRIBUTION OF SPECIES

Dimocarpus longan is believed to have originated in South China or Southeast Asia (Jain *et al.*, 2013). Menzel *et al.* (1989) implied that longan was originated either in subtropical regions of China or in regions stretching between Burma and India. Longan is also native

to Southeast Asian countries including Sri Lanka and Bangladesh (Gunaratne *et al.*, 2011; Islam *et al.*, 2019). Longan has been cultivated in China for the past 200 years at an altitude of 150 m to 700 m (Lim, 2013; Lin *et al.*, 2017). As compared to other countries, China has the most extensive collection of longan germplasm (Paull and Duarte, 2011). Menzel and Waite (2005) had documented that the longan was emanated in the mountain belt that extends from Myanmar to South China and also likely in the Southwest regions of India and Sri Lanka. Longan was then introduced in some of the tropical countries like America, Africa, and South Europe *etc.* as a fruit tree or ornamental tree (Jain *et al.*, 2013). In recent years, the cultivation of longan has spread to countries such as India, Sri Lanka, and Myanmar as commercial fruit tree (Mishra *et al.*, 2018).

The number of cultivars in China exceeds 400, with early-maturing cultivars accounting for 14%, midseason cultivars 68%, and late-maturing cultivars 18%. The major cultivars are 'Wulongling', 'Fuyan' 'Chike' in Fujian, 'Chuliang' and 'Shixia' and in Guangdong, 'Guangyan' and 'Dawuyuan' in Guangxi, and 'Fenke' in Taiwan (Liu and Ma, 2000). Naturally distributed longan had recorded to produce thin-fleshed small fruits in early years giving high yield. Commercialization of longan began in late 1970s with the development of many commercial varieties from China, Thailand, Florida, Taiwan and Hong Kong with characters like greater flesh recovery and exceptional flavor (Menzel *et al.*, 1989).

Longan exist in thousands of varieties around the world, mostly landraces and farmer varieties. China alone maintains the germplasm of over 300 varieties in the National longan germplasm collection (Liu and Ma, 2000; Wu *et al.*, 2007). Longan is adapted to tropical or subtropical climates and occurs in both semi-evergreen and evergreen forest patches (Wall, 2006). *Dimocarpus longan* Lour. has two subspecies which consists of five varieties primarily distinguished by leaflet structure (Subhadrabandhu and Stern, 2005). The two subspecies are namely ssp. *longan* and ssp. *malesianus*. The *D. longan ssp. longan var. longepetiolulatus*, kindred to southern Vietnam; *D. longan ssp. longan var. obtusus* which is indigenous to Indo-China and *D. longan ssp. longan var. longan*

which is originally distributed from the region of Myanmar to South- China regions are under the *subspecies* longan. *D. longan ssp. malesianus var. echinatus* and *D. longan ssp. malesianus var. malesianus* which are native to southern Philippines and northern Borneo are under the *spp. malesianus* (Leenhouts, 1971). The third variety of *Dimocarpus longan ssp. longan var. longan* with large fruit, small seed and thick fleshy aril is generally used as a commercial fruit. The native species with evergreen and semi- evergreen forest as its natural habitat having small fruit and thin edible fleshy aril portion is classified as type first *Dimocarpus longan ssp. longan var. longan* (Mishra *et al.*, 2018). There are only fewer reports available on the distribution of *D. longan* in India. Among different varieties of *D. longan*, only a single variety has been reported in India so far, namely, *Dimocarpus longan subsp. longan var. longan* (Lim, 2013). In different parts of the country, longan is known by different names *viz.* ash-fol (Bengali), Kannakindeli (Kannada), Shempuvam (Tamil) and Chempoovam (Malayalam). In Kerala, *D. longan* is known by different names in different regions which include Chempoovana, Chempunna, Malampoovathi, Malampuvanna, Mulei, Poripunna, *etc.* (Lim, 2013).

D. longan has its distribution along the evergreen and semi-evergreen forests regions of Western Ghats. Longan had been reported from Soppina betta forests and Kodaku regions in Karnataka, parts of the Western Ghats of Maharashtra, tropical forests at Kodayar in Western Ghats parts of Tamil Nadu, tropical evergreen parts of Varagalaiar, and Anamalai parts of the Western Ghats (Ayyappan and Parthasarathy, 1999; Nayak *et al.*, 2000; Sundarapandian *et al.*, 2005; Kale *et al.*, 2009; Karun *et al.*, 2014). Mishra *et al.* (2018) have documented the nativity of longan in the southwestern region, Assam and southeastern parts of India. Occurrence of longan is also reported from Garo hills in Meghalaya in NE India, tropical wet evergreen forests in Kalakkad- Mundanthurai tiger reserve and southern Western Ghats parts of Kerala and Karnataka (Parthasarathy, 2011; Hazarika *et al.*, 2016). Kerala has a wide distribution of *D. longan* and can be regarded as a common species in the evergreen and semi-evergreen forests of Kerala including Thiruvanthapuram, Kollam, Pathanamthitta, Idukki, Palakkad, *etc.* (Murukesh and

Ashokan, 2018). Varghese and Balasubramanyam (1999) have described *D. longan* as a dominant species in Agasthyamala region of Kerala with a high percentage distribution frequency in Bonaccord (60%) and Karamana (80%). Prevailing vegetation of *Cullenia-Dimocarpus- Mesua* type in which *Dimocarpus longan* with mean IVI (Importance Value Index) of 14.99 dominating the lower altitude. There are reports on the natural distribution of *D. longan* in Silent Valley (Kunhikannan *et al.*, 2011), Nelliyampathy (Chandrashekara and Ramakrishnan, 1994) and Meppadi and Kalpetta at an altitude of 800 m- 1400 m (Narayanan *et al.*, 2010).

2.4 BOTANICAL DESCRIPTION

Longan is a huge tree with spreading branches growing up to a height of 20m and trunk diameter to nearly one meter (Tissot *et al.*, 1994; Lai *et al.*, 2000). Occasionally, trees in wild have large buttresses (Lai *et al.*, 2000). The trunk is brittle, rough and corky with a diameter up to one meter. Twigs are nearly 3-7mm in length, lenticellate, hairy and mostly dark brown.

Though different longan cultivars seem relatively uniform they differ in various morphological characters like in tree size, canopy density, and shape, leaf length and width, arrangement and leaf color, characteristics of bark, fruit yielding potential, fruit size and shape, fruit quality, flesh recovery and disease resistance (Menzel *et al.*, 1989).

2.4.1 Leaves

Leaves are alternately arranged paripinnately compound (hardly unifoliate) with 6-9 leaflets in a rachis. Leaves are spiral, without stipules with slightly swollen petiole. The leaflet is oblong-elliptic to oblong-lanceolate having dark glossy green on its upper surface and pale green color on the lower surface. Leaflets are stalked, alternatively (opposite) arranged, widest in the middle, glabrous or variably hairy (glabrous above and subglabrous beneath), papyraceous to thin-coriaceous, prominulous, oblique base and apex bluntly acuminate (Leenhouts, 1971). Young leaves are showy with a reddish-

brown tinge. Its color gradually changes from reddish-brown to light-dark green color at its mature stage. Leaves of longan have a noticeably asymmetrical base and acute apex. Leaves, flowers and fruits mostly occur on the terminal shoot (Menzel and Waite, 2005; Lim, 2013). Petiole length is approximately 2-10mm, while that of oppositely arranged leaflet length ranges from 1.15-10mm.

2.4.2 Flower

Longan is mostly monoecious with unisexual flowers. The inflorescence is a cymule with multiple branches with leafless panicles occurring either terminally or axillary. The inflorescence is compound dichasia, approximately 8-16 cm long, producing hundreds of flowers including staminate flowers (F1), functional male hermaphrodite flowers (F2), and functional females hermaphrodite flowers (F3) resembling litchi. Typically an inflorescence contains about 200 to 1000 functionally female flowers and nearly 1000 to 4000 functionally male flowers. Each F1 flower has eight hairy stamens (stamen filament longer than F2 an F3 flowers) arranged in a light brown disc in a single row and has underdeveloped ovary and stigma. The stamens of the F2 flowers consist of 8 sessile filaments with anthers producing viable pollens. F2 flowers show superior pollen germination over F1 male flowers. F3 flowers contain densely hairy and bicarpellate ovary and bilobed stigma with short and sterile anthers (Davenport and Stern, 2005; Pham *et al.*, 2015; Lora *et al.*, 2018). Generally, three to five flowers are present in cymules (small tertiary branches). Each inflorescence can have 5 to 80 alluring fruits at harvest. Only the central flower out of the five develops into a fruit. Longan has small inconspicuous flowers with glabrous cup-shaped petals which are 5 in number and have two calyx lobes. Usually, the petals do not exceed the length of the calyx, they are sub-glabrous on the outside and woolly on the inside. Flowers are yellowish-brown in color.

Longan flower is rich in nectar (Chang *et al.*, 2008). Flowering in longan is favored by low temperatures. Jia *et al.* (2014) had recorded flowering in China during the spring season (mid-March to mid-May).

2.4.3 Fruit

The mature fruit is small (1.5–2.0cm diameter), slightly conical, heart or spherical in shape. The fruits are edible with a single seed surrounded by white juicy aromatic fleshy aril. The edible aril is covered with a brown-colored indehiscent pericarp (Jiang *et al.*, 2002). The seed is round in shape with an attractive bright black color. Clusters of fruits grow outside the crown of the tree (Lora *et al.*, 2018). Fruit lobes are sub globular to nearly globular, occasionally aculeate or colliculate, nearly smooth (Leenhouts, 1971). The maturation period of fruits vary from 120 to 150 days, evidently longer than litchi. The pericarp is thin but leathery and tough. Its color changes from greenish-yellow to yellowish-brown upon maturing. Fruit diameter is about 1.5 to 3.5 cm with the highest quality fruits weighing normally 14g-18g (Menzel, 2002). The aril is white to off-white in color and total soluble solids amount to nearly 15%-25%. Aril accounts for 60%–75% of the fresh weight of the fruit. Each longan bunch has 50–100 fruits. Longan fruit has a sigmoidal growth curve (Huang, 1995). Different longan cultivars show the same pattern of fruit development, with the seed developing simultaneously with the pericarp. Depending on the cultivar, weight of mature fruit varies from 5g to 20 g. The top variety in China, 'Shixia', takes approximately 65 days to mature and attains an average weight of 15 g (Li and Li, 1999). According to Wara-Aswapati *et al.* (1994), the optimal harvest period ranges from 75-78 days from anthesis. Generally, a farmer assesses harvest maturity by observing the color and flavor of the fruit.

2.4.4 Seed

Seeds are glossy brown to black in color, having a round to ovoid shape which can be easily detached from the aril. Shape of seed apex can vary (flat, ovoid, or round).

2.3 UTILIZATION

2.3.1 Wood

Though wood is of good quality, longan woods are rarely used (Leenhouts, 1971). Fan *et al.* in 2019 have noted that the ultrastructure of longan wood have good aesthetic value including the special shape of coalescent openings on the inner surfaces of vessel elements and cavities of ray cells containing pearl-like granules of starch. Longan wood is thus used to manufacture various aesthetic products like handbags, decorative design works, and various other daily-use products.

2.3.2 Fruit

Longan fruit can be consumed as fresh, canned, frozen, dried, or processed (juice, wine, jelly, *etc.*) (Menzel and Waite, 2005; Lora *et al.*, 2018; Tang *et al.*, 2019). Juice of majority longan cultivars are fairly sweet and are also suitable for consumption without sugar (Menzel *et al.*, 1989). In China, longan is well known as a traditional medicinal tree attributed with the ability to enhance memory, promote metabolism and prevent insomnia and amnesia, various diseases including kidney disorders, leucorrhea, allergies, diabetes, cardiovascular disease, and even cancer as it contain several polyphenolic compounds like phenolic acids, flavonoids and polysaccharides (Zhong and Wang, 2010; Lim, 2013; Lin *et al.*, 2017). Traditional Chinese medicine has used the fruit as an agent to relieve pain by neural inflammation and swellings. In addition, longan pulp was being used as a stomachic, febrifuge, vermifuge, and even as an antidote against poisons. He *et al.* (2016) worked on the biosynthesis, antibacterial properties, and anticancer activity of silver nanoparticles against prostate cancer using *Dimocarpus longan* peel extract. Longan fruit contains a high concentration of inherent bioactive compounds. Longan fruit is prolific with an adequate amount of protein content, carbohydrates, fat, fiber, vitamins (including vitamin C, thiamine, riboflavin, carotene, *etc.*), minerals and amino acids. Longan fruit can be utilized as a readily available source of naturally derived antioxidants and/ or as an alternative in both food and pharmaceutical industries (Duan *et*

al., 2007; de Assis *et al.*, 2009; Yang *et al.*, 2011; Yi *et al.*, 2011; Issara *et al.*, 2014). Byproducts of longan including pericarp, seed, leaves, *etc.* can be made a candidate source for the extraction of natural polyphenols (He *et al.*, 2009; Jiang *et al.*, 2009; Prasad *et al.*, 2010). Longan extract is a potent food preservative to enhance the shelf life of vegetable oil, bakery items, and meat due to high phenolic content (Rojas and Brewer, 2008; Şahin *et al.*, 2017; Galanakis *et al.*, 2018).

2.3.3 Leaves

The leaves of *D. longan* leaves are extracted for natural dyes which can be used for coloring silk fabrics and are known to possess very good to excellent UV protection capacity (Mongkholrattanasit *et al.*, 2014). Longan leaves which are rich in wide range of phytochemicals are used to extract various natural polyphenols for medicinal purposes. The crude extract from *D. longan* leaves is known to have anti-hepatitis C properties (Apriyanto *et al.*, 2015).

2.5 PHENOLOGY OF THE SPECIES

The phenological behavior is an adaptation to the changes in both surrounding biotic and abiotic environment (Sundarapandian *et al.*, 2005). The phenology displayed by tropical forests differs from that of temperate forests due to lower level of seasonality in temperature and adaptations to photoperiod and thermoperiod (Reich, 1995). The tropical forests of the Western Ghats have limited data on phenological patterns of natural forest vegetation (Sundarapandian *et al.* 2005). Frankie *et al.* (1974) have recorded that the intense initiation of new leaf flushes occurs during the first dry season *i.e.* from late January to April in the wet forest regions of Central America where mean annual rainfall ranges between 2900mm to 5600mm. In wet forests, fruiting peaks during the second dry season, from August to October. According to Reich (1995), rain forests have a fairly flat pattern of annual leaf fall ranging from 5 to 13%.

Pham *et al.* (2016) characterized different phenological growth stages of longan according to the BBCH (Biologische Bundesanstalt, Bundessortenamt und Chemische industrie) scale. Longan has a poorly described phenology. Longan produces new leaves during pre-monsoon season reaching its peak in February and leaf expansion completes by March (Bhat, 1992). The juvenile period for longan is quite long, lasting nearly 7 to 8 years (Lora *et al.*, 2018). Peak period of commencement of flower buds often coincide with the leaf flushing and leaf shedding during the months of January to April in the Western Ghats (Sundarapandian *et al.*, 2005). New vegetative shoots flush during February- March (spring season) before inflorescence appears. Longan is a biennial- bearing fruit tree with flowering and fruiting once in every two years (Sundarapandian *et al.*, 2005; You *et al.*, 2012). Longan is a cross-pollinating species having duodichogamy, *ie.*, flower opening in each panicle occurs in three different progressive stages in which staminate flowers opening first followed by pistillate flower and finally the hermaphrodite flower (Crane, 2002). During the flowering phases of male and female flowers, there will be a period of overlap of 4 to 6 weeks. Sometimes, the flowering of different flower types overlaps even on individual trees, since inflorescences develop at the same time. According to Jia *et al.* (2014), longan has a single spring flowering period and the initiation of the floral bud occurs at low temperature period. Pham *et al.* (2016) reported that reproductive bud develops during spring season from April to early May. According to Leenhouts (1971) flowering occurs mainly during March-May and fruiting occurs mainly during July. Several climatic factors affect the amount, timing, intensity, and duration of flowering and fruiting in tropical ecosystems. These climatic factors include photoperiod, temperature and precipitation (Opler *et al.*, 1980; van Schaik *et al.*, 1993; Zee *et al.*, 1998; Krishnan, 2002). Flowering is a significant developmental stage in plants that is mediated by the regulatory networks that control shoot development in conjunction with networks that control responses to environmental cues. Flowering mainly depends on two major environmental factors *viz.* photoperiod and temperature (Song *et al.*, 2013; Jia *et al.*, 2014). Increased temperature and moisture during winter can induce flower reversion in *D. longan* (You *et al.*, 2012). Application of $KClO_3$

(potassium chlorate) can induce off-season flowering in cultivated longan trees (Manochai *et al.*, 2005).

Longan has its fruiting season between August and November (Ke *et al.*, 1992; Stern, 2005; Karun *et al.*, 2014; Mei *et al.*, 2014) and is in line with the study by Frankie *et al.* (1974) in the wet forest of Central America, fruiting peaks during August- October (second dry season). After initiation, longan fruit takes approximately four months, from June-July to October-November, for complete maturation (Pham *et al.*, 2013). Lin *et al.* (2001) reported that in China, long hot and humid periods during the months of August and September are most conducive period for fruit maturation.

Longan has a comparatively low amount of ethylene production after harvest and when stored at a lower temperature (1 ^{0}C to 4 ^{0} C), ethylene production remains more or less constant for about 30 days. Ethylene production increases upon decaying (Zhou *et al*, 1997).

Menzel *et al.* (1989) explored the phenological behavior of *D. longan* in different parts of China and Thailand where peak rainfall receives during summer-autumn, and reported that in Northern regions, the flowering season is from December to March (equivalent to June-September in Southern hemisphere) and fruit maturation occurs during July to September (equivalent to January to March in Southern hemisphere). As per the study conducted in Australia, there was difference in the flowering season in two different regions however, the fruit ripened more rapidly and was harvested from mid- January/February to mid-March/ April from both regions. In Taiwan, longan flowers from February to May, and fruits mature from July to September (Chang *et al.*, 2008).

It will take four to six months for the tree to bloom and harvest, depending on environmental conditions (Davenport and Stern, 2005).

2.6 MORPHOLOGICAL DIVERSITY

Morphological characterization is the most important step towards the identification and categorization of genetic resources (Kresovich and McFerson, 1992). Analyzing quantitative and qualitative traits are important for a better understanding of morphological diversity (Yao *et al.*, 2020). Rosado *et al.* (2018) have done a detailed morphometric analysis to study the intraspecific variability among three Acer species.

Kafkas *et al.* (2000) examined various qualitative features (including growth habit, tree vigor, branching pattern, leaf color, leaf persistency, leaflet and terminal leaflet shapes, shape of leaf apex, nut shape, nut texture, *etc.*) and quantitative characters (including leaf width and length, number of leaflet pairs, length of leaf petiole, leaflet width and length, nut weight, thickness, length and width of nut) to demonstrate morphological and phenotypical divergence of *Pistacia* species.

Sumathi and Balamurugan (2014) studied morphological traits to distinguish eleven varieties of oats (*Avena sativa* L.). They detailed the morphological traits of seed, *viz.* seed size and color, plant traits like growth habit, hairiness in stem, branching pattern, orientation, and leaf characters.

A variety of morphological characteristics, including the leaf shape, size of seed, size and shape of the fruits, as well as the texture and color of their fruits, have been traditionally used to distinguish cultivars of lychee and longan (Van Buren *et al.*, 2011).

Zerpa and Ming (2019) have done comparison studies in genomic and morphological diversity among most related species in Sapindaceae, namely, lychee (*Litchi chinensis*), longan (*Dimocarpus longan*), longli (*Dimocarpus longan subsp. malesianus* and *Dimocarpus confinis*), and rambutan (*Nephelium spp.*). Fruit of longli resemble that of longan and lychee, showing yellowish-brown pericarp, short sharp exocarp protrusions. *D. confilis* has a morphologically different trichome, whereas *D. longan subsp. malesianus* has a morphologically similar trichome as that of *D. longan*. Their flow

cytometry showed a significant variation in genomic size among *D. confilis*, longan and lychee.

VanBuren *et al.* (2011) explored the morphological variations (in fruit characters and trichome morphology) existing among the three closely related Sapindaceae members, longli (*D. longan subsp. malesianus* and *D. confinis*), longan (*D. longan*), and lychee (*L. chinensis*) cultivars.

For the proper management of germplasm and the establishment of breeding programs in litchi, molecular studies are essential alongside morphological studies (Lewandowski *et al.*, 2014).

Chaubey *et al.* (2001) studied the correlation existing between various tree morphological traits and fruit traits in *Litchi chinensis*. They concluded that there is a high genetic correlation between fruit weight and volume, fruit and aril weight, panicle length and breadth, plant height and leaf breadth, plant height and leaf volume, and chlorophyll *a* content and seed weight.

Lithanatudom *et al.* (2017) have detailed the differentiation between three varieties of *D. longan viz., D. longan spp. longan var. longan, D. longan spp. longan var. obtusus and D. longan spp. malesianus var. malesianus* using morphological (tree characters like habitat, twigs, petioles and rachis, leaflets, leaflet apex, fruit, leaf surface, and midrib) and molecular characterization.

2.7 LONGAN FRUIT

Longan has a non-climacteric fruit and will not ripen once get detached from the tree (Batten, 1986; Shi *et al.*, 2016). Fruit maturity can be evaluated by quantitative and qualitative analysis of various fruit characters including color of the pericarp, fruit weight, flesh sugar concentration and flavor, acid content, Soluble Solid Concentration (SSC) and sugar-acid ratio. Fruit weight, fruit size, aril size and TSS (Total Soluble Solid) are in parallel with consumption quality (Wara-Aswapati *et al.*, 1994). Flavor of the fruit is

best determined by SSC: TA ratio which falls slightly when stored at low temperature. As longan fruits mature, their solids content, sugar content, and ascorbic acid levels increase, while TA levels decrease. The pulp gets harden upon over maturation due to increased lignification and loss of water content. Major organic acids present in the longan pulp are malic acid, succinic and citric acids in the ratio 10:5:1 (Lin, 2002).

2.7.1 Phytochemicals in Longan fruit

The longan fruit is a rich source of nutrients, energy, and other health benefits due to the presence of a large variety of useful metabolites (Wang *et al.*, 2020). The main functional metabolites of the longan fruit are polysaccharides, polyphenols (phenols and flavonoids), alkaloids and carotenoids. Metabolites like organic and amino acids, carbohydrates, hormones, vitamins, glucosinolates, flavonoids, and phenolics contribute to plant growth and development, stress adaptation, defense and stress adaptation. Primary metabolites play a crucial role in the survival of the species, including in photosynthesis and respiration (Raghuveer *et al.*, 2015). Functional metabolites in longan offer antihyperglycemic, anticancerous, anti-oxidative, anti-tyrosinase, immunomodulatory, antiosteoporotic, and anti-aging benefits to humans (Rangkadilok *et al.*, 2012; Shahrajabian *et al.*, 2019; Zhang *et al.*, 2020). Pulp is the most important part of longan fruit for consumption. In addition to the pulp, other parts of the longan fruit, such as the peel and the seed, may contain valuable bioactive components that are yet to be explored. Its chemical and biological composition is diverse and consists of a variety of compounds including phytochemicals, volatiles and anti-oxidants (Rakariyatham *et al.* 2021). There are variations in the concentration of metabolites present in different parts of the fruit based on the variety, environment and management practices. Compared to dried flesh, longan seed extract contains more amount of phenolic

compounds but slightly less than that is present in the pericarp. Longan seed contains highest amount of alkaloid content compared to other parts (Liu, 2012; Tang *et al.*, 2019).

2.7.1.1 Primary metabolites

Vijayvergia and Kumar (2007) in their study, extracted soluble sugar using distilled water and further by Loomis and Shull (1937) method and starch in order to quantify the total carbohydrate content from leaves, flowers, root and stem of *Nerium indicum*. In pomegranate juice, the level of sugars is strongly correlated with the amount of total soluble solids (TSS). TSS level in pomegranate juice ranges from 4.2- 8.5 g/100 g depending on cultivars, management and prevailing climatic conditions (Bar-Ya'akov *et al.*, 2019). TSS can be measured using a refractometer.

Sucrose, glucose and fructose are the main sugars in the pulp that contribute to its rich sweetness. Seeds are a good source of carbohydrate (37.1 – 38.9%), protein (7.2 – 9.3%), and fiber (3.6 – 7.9%) (Rakariyatham *et al.*, 2020). The fiber content in longan peel is higher than that of seeds, ranging from 33.4%- 83.9% (Issara *et al.*, 2014). SSC along with single fruit weight and edible rate make up the most valuable agronomic traits for breeding improved varieties (Jue *et al.*, 2021). Quantity of TSS and sucrose in longan pulp vary with number of days after flowering. According to Luo *et al.* (2021), TSS decreased by 13.49% and 40.61% in 111 DAF and 127 DAF respectively. The longan aril contained more free sugars and organic acids than the seed. A study by Yang *et al.* (2011) shows that longan has a higher content of crude protein (7.38%), carbohydrate (83.63%), and crude lipid (4.91%).

2.7.1.2 Secondary metabolites

All parts of longan fruit which include pericarp, pulp and seed are rich in secondary metabolites like polyphenolic compounds, phytate, amino acids, antioxidants and other phytochemicals (Dogan and Salman, 2007; Yang *et al.*, 2008; Zhang *et al.*, 2009). The concentration of these metabolites varies with varieties, environmental and management conditions. Total phenolic content varies among various cultivars. Longan seed and pericarp overheads pulp in various metabolite concentrations and longan seed holds the highest concentration of phenolic compound (Rangkadilok *et al.*, 2005; Wang *et al.*,

2020). Soong and Barlow (2004) studied the antioxidant activity and total phenolic content in the edible portion and seed of longan to compare with that of other important tropical fruits and found that longan has an appreciable antioxidant activity and phenolic content both in edible portion and in seed.

Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) vary among different longan cultivars in accordance with the difference in the environmental condition and location. In a study conducted by Tang *et al.* (2019) for comparing the TPC and TFC among 18 longan cultivars, it was found that cultivar Hualu Guangyan had the highest phenolic content (0.75 mg GAE/g), and cultivar Jiluan showed the least phenolic content (0.013 mg GAE/g). Hsieh *et al.* (2008) studied the anti-oxidative property of the active compounds extracted from longan flowers.

Phenolic compounds are bioactive compounds with one or more aromatic rings and hydroxyl groups in its backbone. It contributes to plant growth, reproduction, and resistance against pathogens. More than half of the naturally occurring phenolic compounds are flavonoids, making up the largest group of plant phenols (Balasundram *et al.*, 2006). During fruit growth, flavonoids concentration falls, while they rise during fruit maturity and after full maturity (Yen *et al.*, 2001; Zheng *et al.*, 2009).

Longan fruit has a short shelf life when stored at ambient temperature. Browning of the pericarp is the biggest problem that reduces the market demand for longan fruit as a commercial fruit (Campbell and Campbell, 2000). Longans have more shelf life at cold temperatures since they are more tolerant to lower temperatures than other tropical and subtropical fruits and are more likely to retain their flavor at cooler temperatures (Chattopadhyay and Ghosh, 1991; Jiang, 1999; Jiang *et al.*, 2002).

2.7.1 Nutritional value

According to Wall (2006), the nutritive value of longan fruit varies with the climatic condition, soil type, and fertility. Longan fruit is rich in nutrients including vitamin C,

provitamin A and a sufficient amount of other mineral nutrients that are important in the human diet like K, P, Mg, Cu, Fe, Mn and Zn (Hue *et al.*, 2000). Amongst the three important commercial fruits in the Sapindaceae family (longan, litchi and rambutan), the longan contains the greatest amount of vitamin C (Tongdee, 1997). Detailed data on the minerals in longan fruit is scanty. Minerals like potassium (2053.50 mg/100 g), calcium (122.60 mg/100 g), sodium (25.24 mg/100 g), and phosphorus (191.21 mg/100 g) contents are more abundant in longan aril, while iron (2.97 mg/100 g) and magnesium (69.83 mg/100 g) contents were more in the seed (Yang and Sim, 2021).

2.7.3 Medicinal properties

Polyphenol-rich food has recently drawn global attention owing to its cancer-preventive and cancer-therapeutic properties (Xiang *et al.*, 2008). Longan is known for its antioxidant and antiproliferative properties in addition to its anti-hepatitis and anti-tumor properties (Pan *et al.*, 2008; Puspita *et al.*, 2019). Shahrajabian *et al.* (2019) had listed out various health benefits of longan fruits to human health including protecting the brain, reducing inflammatory problems, blood pressure, boosting the immune system, boosting energy levels, helping in diabetics, *etc.*

Pan *et al.* (2008) investigated the efficiency of different methods for the extraction of phenol and reported that microwave-assisted extraction (96.78 mg/g) and soxhlet extraction method (90.35 mg/g) are superior among different methods. Yang *et al.* (2011) compared the efficiency of different extraction methods like conventional method using soxhlet extraction, ultrasonic-extraction and high pressure-assisted extraction method for the extraction of phenolic compounds and polysaccharides from the pericarp of longan and learned that use of high pressure-assisted extraction method or ultrasonic-assisted extraction method results in greater yields. Guo *et al.* (2003) evaluated the antioxidant properties of peel, pulp and seed using FRAP assay.

The by-products of longan including seed, pericarp, bark and leaves are highly rich in bioactive compounds that have potential utility as antioxidants and nutraceuticals. Some

of the important components are ellagic acid, gallic acid, geranin and corilagin (Sriwattana *et al.*, 2015; Sruamsiri and Silman, 2015; Rakariyatham *et al.*, 2020). Lampila *et al.* (2009) noted that flavonoids found in fruits have been studied extensively because of their numerous health benefits.

2.8 GENETIC DIVERSITY

Morphological traits are highly influenced by the environmental characters and they cannot be utilized for recognizing diversity among the accessions (Mariana *et al.*, 2011; Lewandowski *et al.*, 2014; Ho and Ngo, 2017). The ability of populations to adapt to the changing environments depends on the level of genetic diversity. Frankham *et al.* (2002) have described genetic diversity as the difference in the alleles and genotypes that are exist in a group *viz.* species, groups of species, or populations under study. Genetic diversity is a heritable variation that can be characterized using the measure of polymorphism, diversity in allelic frequency, and average heterozygosity (Frankham *et al.*, 2002; Rao and Hodgkin, 2002). The level of genetic diversity varies from population to population. Well-distributed species show a high degree of genetic diversity as compared to smaller populations, endangered and island species (Prout, 1969; Booy *et al.*, 2000; Ouborg *et al.*, 2006). Differences within and between species in biochemical properties (e.g. phytochemicals, isoenzymes), physiological characteristics, (e.g. growth rate, stress resistance), or morphological features including flower characters can be due to the variation in DNA sequence (Rao and Hodgkin, 2002). Understanding genetic diversity is crucial for the conservation of threatened species (Ouborg *et al.*, 2006).

2.8.1 Genetic diversity assessment

Diversity analysis is usually carried out using different methods like cytological, morphological, molecular, and biochemical characterization. Cytological marker is related to cytological characters like size and structure of chromosome, arm ratio, position of centromere, banding patterns, genomic constitution, length of total genome, *etc.*

2.8.1.1 Biochemical marker

Biochemical marker involves banding patterns associated with proteins and several variants like isozymes. Molecular markers are used for studying variation between genotypes at DNA or RNA level (Bhanu, 2017). Molecular data can also be used to support the existing taxonomic identity of species under the genus *Dimocarpus* which is currently classified based on morphological characteristics. Morphological markers are visually discernible characters like phenotypic characters.

2.8.1.2 Morphological marker

Traditionally, morphological markers are the most widely used marker to identify, distinguish and study the variation among species for the purpose of plant breeding and crop improvement. As far as morphological and biochemical markers are concerned, there are only a restricted number of markers available and are highly influenced by the environmental conditions and plant development stage. Even with these shortcomings, plant breeders often employ these two markers (Kordrostami and Rahimi, 2015). Another limitation in using morpho-agronomic characters in studying genetic diversity is that, since they are phenotypic indicators, genetically distinct germplasms can have similar morphology (Tripathi *et al.*, 2013). Morphological markers are easy-to-use techniques that do not demand any technical skills and are a universally approved method for assessing diversity and genotype (Sumathi and Balamurugan, 2014; Kumari *et al.*, 2018).

2.8.1.3 Molecular markers

One of the most significant developments in molecular genetics has been the development and use of molecular markers to detect genetic diversity at DNA level.

Genetic markers are being used to substantiate phylogeny and taxonomic identity (Harlan, 1971; Frankham *et al.*, 1995). In comparison with enzyme, metabolite, and protein markers, DNA markers have the advantage of unlimited loci, high variability, and lack of environmental effects (Nguyen and Nguyen, 2009). Over the past decade,

DNA markers have been utilized in population studies in a way that has never been possible before because of the development of PCR for direct amplification of DNA fragments (Booy *et al.*, 2000). Molecular markers are widely used in Marker Assisted Selection (MAS) (Kordrostami and Rahimi, 2015). Genetic diversity will be reflected differently in different markers based on their properties (Rao and Hodgkin, 2002). Environmental conditions have a relatively less impact on molecular markers (Tsumura *et al.*, 1996; Hodgkin *et al.*, 2001). It can identify the sites of variation in the genome. Different markers examine different characteristics or scopes of variation (Bretting and Widrlechner, 1995). With the advent of PCR technologies, many markers are used for genetic studies including Random Amplified Polymorphic DNA (RAPDs), Simple Sequence Repeats (SSRs), Amplified Fragment Length Polymorphism (AFLPs), Single Nucleotide Polymorphism (SNPs), and microsatellites (Williams *et al.*, 1990; Hodgkin *et al.*, 2001). The genetic qualities possessed by different markers are different, some are dominant and some are co-dominant. It amplify expressed or non-expressed sequences. Variation in genetic makeup within-population can be assessed by evaluating the percentage of polymorphic genes, allele number in each polymorphic gene and proportion of heterozygous loci (Xiao *et al.*, 2005).

There are broadly two groups of molecular markers; hybridization-based marker (*e.g.* RFLP) and PCR-based molecular marker (*e.g.* RAPD, AFLP, ISSR, SSR, STP, *etc.*).

Molecular characterization of lychee has been carried out using isozymes, ISSRs, RAPD, SNP, and SSR markers (Aradhya *et al.*, 1995; Degani *et al.*, 1995; Anuntalabhochai *et al.*, 2000; Sim *et al.*, 2005; Peng *et al.*, 2006; Liu *et al.*, 2015).

2.8.3 Inter-Simple Sequence Repeat markers

ISSR amplifies the DNA fragments present between the identical repeating sequences oriented in opposite directions in the genome. ISSR technique uses microsatellite repeats to amplify the inter simple sequence repeats present in the genome. ISSR primers used can be either di, tri, tetra, or pentanucleotide repeats. ISSR targets the simple-sequence

repeats (microsatellites) present abundantly in the genome and measures the frequency within the genomes. The primers can be unanchored or more commonly 3' anchored or 5` anchored with one to four degenerate nucleotides. Amplification using unanchored primers does not require the complete genome sequence information and results in multilocus and strongly polymorphous patterns (Zietkiewicz *et al.*, 1994; Bornet and Branchard, 2001; Semagn *et al.*, 2006; Tarinejad *et al.*, 2015). ISSR is used for interspecific genomic fingerprinting (Fang and Roose, 1997). ISSR markers are being used to characterize many plants, including chrysanthemum, oilseed rape, *Eleusine* coracana, citrus, and apple (Fang *et al.*, 1998; Palai *et al.*, Goulão *et al.*, 2001; 2011; Havlíčková *et al.*, 2014; Venkatesan *et al.*, 2021).

RAPD markers are easy and fast to develop but one of the limitations of RAPD is its low reproducibility. Homozygous and heterozygous individuals will produce the same patterns (Nguyen and Nguyen, 2009). ISSR markers have emerged as novel DNA markers with great potential for crop improvement due to their high reproducibility (Tarinejad *et al.*, 2015).

Mahar *et al.* (2012) identified the genetic diversity in *Sapindus mukorossi* (soap nut) in the western Himalayas using 20 ISSR marker primers. They produced 258 bands (size ranging from 100 to 3000 base pairs), 152 out of which were clearly polymorphic, indicating 58.91% polymorphism. Sun *et al.* (2019) explored the genetic diversity and identified the loci associated with the fruit traits in *S. mukorossi* and S. *delavayi* using dominant ISSR markers. Out of the total 247 loci screened using ISSR primers, 18 loci corresponded to fruit traits.
Wang *et al.* (2013) used ISSR markers to assess the genetic variation existing in *Ricinus communis* population from North East China. Total 179 DNA bands were produced from 39 genotypes and showed an average polymorphism of 96.4%. The number of bands produced by different ISSR primers varied from a maximum of 23 bands (UBC856) to a minimum of 13 bands (UBC 823).

Clyde *et al.* (2003) assessed the genetic divergence in *Nephelium ramboutan-ake* using ISSR and RAPD markers and RAPD markers showed higher polymorphism (97.33%) compared to ISSR primers (87.10%).

2.8.4 Genetic studies in Sapindaceae family members

Zhou *et al.* (2020) identified and described NBS (Nucleotide-binding site) - encoding genes among the members of the Sapindaceae family such as *D. longan*, *Acer yangbiense* and *Xanthoceras sorbifolium*. Ekue *et al.* (2009) tried the cross-species transferability of SSR molecular markers generated for *Litchi chinensis* in other Sapindaceae members, *Blighia sapida* and *Nephelium rambutan-ake*. Shen *et al.* (2017) studied the genetic diversity among the germplasm of *Xanthoceras sorbifolium* (Sapindaceae) using morphological characters and SSR molecular markers.

Qiu *et al.* (2007) identified the genetic divergence among the scattered population of a rare endemic Sapindacea species *Dipteronia dyerana* using ISSR molecular markers. ISSR marker was used to study the genetic variation existing among population of tropical fruit crops under Sapindaceae like *Melicoccus bijugatus* Jacq. and *Melicoccus oliviformis* Kunth. (Martinez-Castillo *et al.*, 2019; Jiménez-Rojas *et al.*, 2021).

2.8.5 Genetic diversity in *Dimocarpus longan*

A number of biotechnological studies were performed on *D. longan* tissues and organs during the 1980s. Scientists began focusing on molecular-level studies during the 1990s.
Following this, genetic improvement in the longan field saw great progress.
PCR preferentially amplifies DNA fragments bound between sequences that are complementary to oligonucleotide primers employed. Amplification results can be influenced by many factors, including amplification conditions, DNA quality and quantity, primer suitability. The PCR can be standardized by adjusting the annealing temperature or increasing the number of total cycles, improving DNA quantity and quality using a more appropriate genomic DNA extraction method for *D. longan* and

altering various electrophoresis conditions (Mariana *et al.*, 2016). Xiao *et al.*, (2005) studied various genomic extraction methods in *D. longan* and concluded that the modified CTAB method can yield high-quality genomic DNA than conventional CTAB and SDS methods and $OD_{260/280}$ ratios of genomic DNA extracted using modified CTAB and SDS methods were 1.82 and 1.73 respectively.

Longan is a highly heterozygous species. The degree of heterozygosity is not completely explored. Genetic diversity and characterization among different longan cultivars were first done using isozymes during the 1980s but were not so effective. RAPD marker was the first PCR-based marker to be developed which allowed the differentiation of various cultivars and the identification of breeds. Lai and Lin (2013) generated a large extend of transcriptome profile of a longan cultivar 'Honghezi' from cultured embryos at various developmental stages and developed different unigenes for embryogenesis. Wang *et al.* (2015) used these transcriptome profiles and transcriptomes data of litchi to develop SNP markers for identifying and differentiating the cultivated and wild varieties of *D. longan* from China. Lin *et al.* (2017) through their study have testified that the overall polymorphism density of longan is between 0.05–0.12 SNPs and 0.004–0.007 indels per 10 kb of genomic sequence. Low level of genetic divergence can be attributed to the bottleneck effect suffered by longan during domestication. Arias *et al.* (2012) used RAPD, SRAP, AFLP and ISSR markers to generate linkage map of different cultivars of longan.
Weiping *et al.* (2003) studied genetic divergence and the relationship among 46 cultivars of *D. longan* using AFLP and amplified 103 polymorphic DNA bands with two pairs of primers.

Lin *et al.* (2005) assessed the genetic variation in different longan accessions and its closely related species *Litchi chinensis* and *D. confinis* using AFLP and *rbc*L gene sequencing techniques.

Viruel and Hormaza (2004) used SSR markers to identify the genetic relationship and polymorphism among 21 lychee and four longan cultivars. Eleven out of 12 SSR markers were amplified DNA fragments of longan, eight of which were polymorphic. UPGMA cluster analysis method was employed for grouping of cultivars.

Ho and Ngo (2017) used RAPD markers to explore the genetic diversity of the *D. longan* population in Vietnam. Mei *et al.* (2014) have examined the geographic origin and genetic diversity among 5 accessions of *D. longan* from distinct geographical regions of China using RAPD and concluded that genetic relations among them do not correlate with their geographical locations. Chen *et al.* (2010) examined the genetic variance and genetic relation between 24 longan accessions from China using SCoT markers. 24 primers were screened from 80 ScoT primers were screened and 24 primers were used.

Chiang *et al.* (2010) explored the genetic relation among germplasm of 25 longan and litchi cultivars with ISSR markers. The study reported a polymorphism rate of 92.5% using 15 primers. They prepared a dendrogram using UPGMA cluster analysis and classified 24 cultivars into four groups.

VanBuren *et al.* (2011) compared of genomic size and genetic diversity existing among the three closely related species of Sapindaceae, longan, lychee, and longli, using ISSR and RFLP markers. The study inferred that Malesianus (longli) is not a hybrid of lychee and longan since its genome size is smaller than that of longan and lychee by 9% and 27% respectively.
Chen *et al.* (2012) compared and analyzed the efficiency of ISSR and SCoT (Start Codon Targeted) for assessing the genetic diversity of *D. longan* and concluded that the average number of DNA bands amplified by ISSR and SCoT were 7.8 and 10.58 respectively. They proved that both markers were effective in examining the genetic variation of longan germplasm.

Zhu *et al.* (2013) analyzed genetic divergence and relationships among different ecotypes of *D. longan* germplasm using ISSR markers. Polymorphism percentage observed was 76.97% with 12 primers. Genetic similarity coefficients of different ecotypes ranged from 0.57 to 0.92. The cluster analysis was performed using the UPGMA method.

Wen-shun *et al.* (2015) analyzed the genetic diversity among offspring of reciprocal hybrids of Shixia and Xiangcui cultivars of *D. longan*, using SSR markers. Lin *et al.* (2017) analyzed the whole-genome sequences of 13 cultivars of *D. longan* accessions to analyze the extent of genomic diversity. They compiled a draft genome sequence of a Chinese longan cultivar 'Honghezi' and identified approximately 31007 genes and 261.88 Mb of repetitive sequences.

Jue *et al.* (2021) worked out QTL (Quantitative Trait Loci) mapping of longan utilizing SNP marker and identified the candidate genes contributing to fruit quality and yield, characters that are important for the breeders to screen the best populations.

3. MATERIALS AND METHODS

The research project entitled "Genetic diversity of *Dimocarpus longan* Lour., in Southern Western Ghats" is carried out with particular objective of investigating the morphological and genetic diversity existing among the population of *Dimocarpus longan* along the southern Western Ghats and analyzing the biochemical variability persisting in the longan fruits from different locations. The study was undertaken at department of Forest Biology and Tree Improvement at College of Forestry, Kerala Agricultural University, Vellanikkara, Thrissur, during the period of 2019-2021.

3.1 MATERIALS

3.1.1 Selection of location

Natural populations of *D. longan* were identified through extensive literature survey of different published flora like Fisher (1917). Reconnaissance survey was conducted in different locations to locate the populations of *D. longan* all along the Western Ghats regions in Kerala. Six ranges across Kerala (parts of southern Western Ghats) were selected. Three ranges were selected from North of Palghat gap and three ranges were selected from South of Palghat gap. Population distribution of *D. longan* showed a slight difference among the selected locales varying from scanty to plenty from south of Palghat gap towards the north. The populations were identified through field visits.

Selected ranges in North of Palghat gap were Peruvannamuzhi range under Kozhikode Forest Division and Meppadi range and Kalpetta range under South Wayanad Division. Selected ranges in South of Palghat gap were Mankulam range under Mankulam Division in Munnar, Lakkom (Eravikulam National Park) under Munnar Wildlife Division, and Chimmony under Peechi Wildlife Division in Thrissur (Figure 1). The details on latitude, longitude, temperature and rainfall of selected locations is given in table 1.

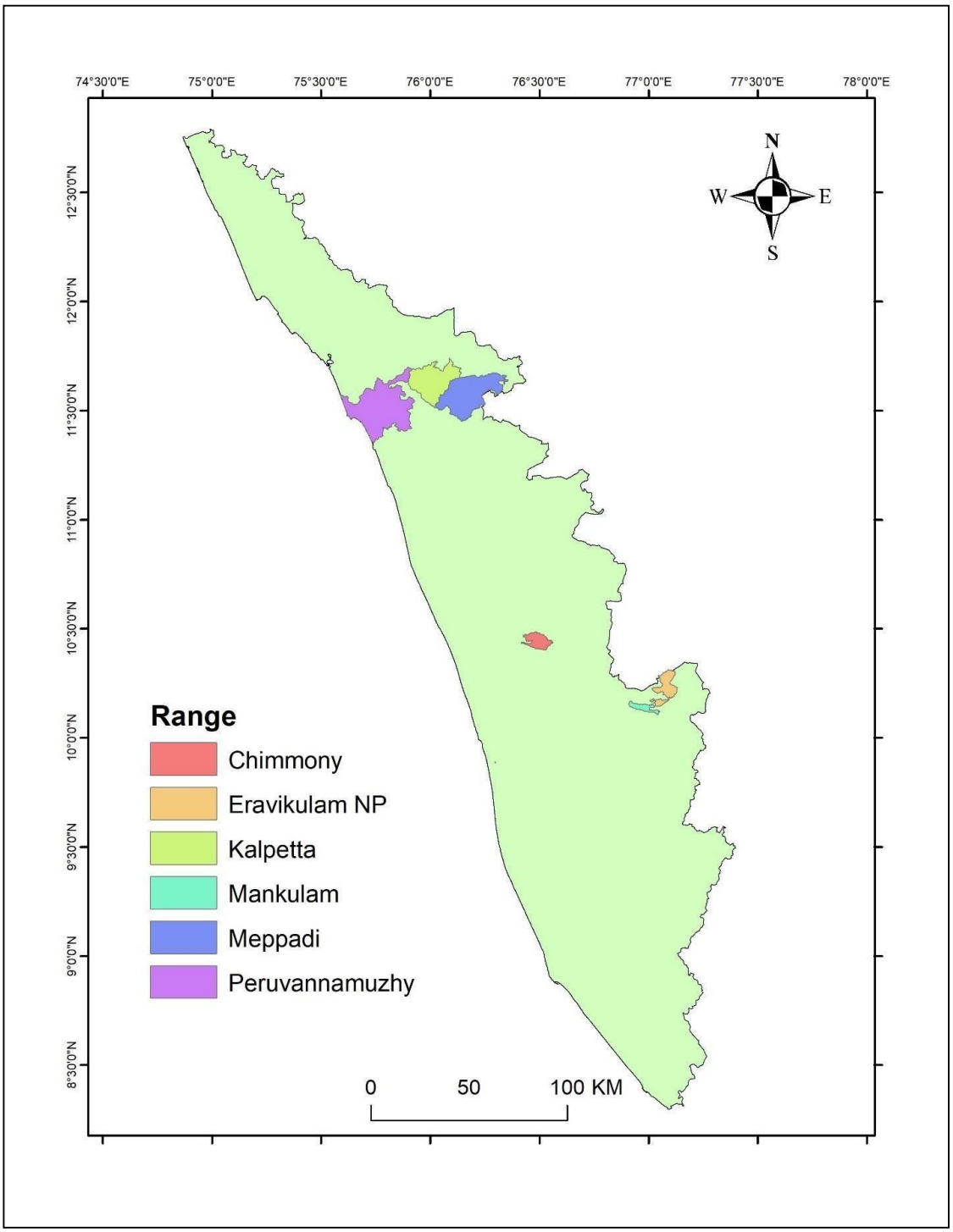


Figure 1. Selected ranges for the research study

3.1.2 Laboratory chemicals, glasswares and plasticwares

The chemicals that were used in the research study were of good quality Analytical Reagent (AR) grade procured from HIMEDIA, Merck India Ltd. and SISCO Research Laboratories. All plastic wares used were obtained from Tarson India Ltd. ISSR (Inter- Simple Sequence Repeats) primers and the Takara Mastermix.

3.1.3 Equipments

Molecular biology lab facilities and equipments available in Department of Forest Biology and Tree Improvement, College of Forestry and Cocoa Research station, KAU was utilized for carrying out the research study. Centrifugation was performed using High speed Refrigerated micro centrifuge (Eppendorf Centrifuge 5418R). The quality and quantity of extracted DNA was estimated using Nanodrop spectrophotometer Jenway- Genova Nano and Eppendorf Bio Spectrophotometer. DNA PCR amplification was done using Nexus gradient Thermal cycler (Eppendorf Mastercycler) and Horizontal gel electrophoresis unit BIO-RAD (USA) was used for agarose gel electrophoresis. Documentation of gel was done in Molecular Imager Gel Doc^{TM} XR+ imaging system (BIO-RAD).

3.2 GENERAL DESCRIPTION AND CLIMATE OF STUDY AREA

Evergreen and semi-evergreen forest patches in six ranges (three ranges from north of Palghat gap and three from south of Palghat gap) were selected for the study. *Dimocarpus longan* was found to be widely distributed species in both evergreen and semi-evergreen forests (Plate 1). Climatic data were collected from the working plan/management plan of each forest division.

Plate 1. Distribution of *Dimocarpus longan* near Kakkayam Dam in Peruvannamuzhi forest range

Table 1. Latitude, longitude, altitude, temperature range and precipitation of selected location

Sl. No.	**Location**	**Latitude**	**Longitude**	**Altitude range(m)**	**Temperature range(^{0}C)**	**Precipitation range (mm)**
1	Chimmony, Thrissur	10º 22'N-10º 26'N	76º 31'E-76º 37'E	45 -1100	15 - 38	2500-3000
2	Mankulam, Idukki	10º 0'N-10º 10'N	76º 50'E-77º 0'E	340-1740	5 - 30	2500-3000
3	Lakkom, Munnar	10º 05'N-10º 20' N	77º0'E-77º10' E	900 -1500	5 - 30	2500-3000
4	Kakkayam, Kozhikode	11° 33'N-11° 39'N	75° 49'E-75° 55'E	600 -1500	16 -38	2500-3000
5	900Kandi, Meppadi, Wayanad	11° 30'N	76° 07'E	700 -2300	5 -30	3000-3500
6	Pookode, Wayanad	11° 32'N	76° 01'E	700 -2300	13 -35	3000-3500

3.2.1 Chimmony Wildlife Sanctuary

Chimmony Wildlife Sanctuary (CWS) is located in the Thrissur district of Kerala under Peechi-Vazhachal Wildlife Division (Figure 2). Sanctuary has a total area of 85.067km^2.
It is situated towards the western slope of Nelliyampathy hills, part of southern Western Ghats. The altitude varies between 45m to 1100m above mean sea level (MSL). The annual average rainfall of the region is 3000mm. Temperature prevailing in the area ranges from 15ºC to 38ºC. The hottest months are March-April and the coolest months are December- January. Major forest types include tropical evergreen forests, tropical semi-evergreen forests, moist deciduous forests, *etc.*

3.2.2 Mankulam Forest Range

Mankulam range is located in the Munnar district of Kerala under Mankulam Forest Division (Figure 3). Topography of the region comprises hilly and undulating terrain. Altitude ranges from 340m to 1740m above MSL. Temperature of the location varies between 5 ^{0}C and 30 ^{0}C. Annual average rainfall of the range varies from 2500mm to 3000mm. Humidity is very high during monsoon period. Major forest types in the region include west coast tropical evergreen forest (1A/C4) and west coast semi-evergreen forest (2A/C2).

3.2.3 Eravikulam wildlife sanctuary

Eravikulam is located in the Munnar district of Kerala under Munnar Forest Division (Figure 4). It is situated in the high ranges of the southern Western Ghats towards its western slope. Altitude of the division varies from 33m to 2695m. The region receives rainfall in the month of April- May in addition to the monsoon period from June- August and the heaviest precipitation is reported during June- July period. Mean annual temperature varies from 17.5 ^{0}C and 19.5 ^{0}C. Annual average rainfall of the range varies from 2500mm to 3000mm. The types of forests of this division are wet evergreen forest,

Figure 2. Chimmony range under Peechi Wildlife Division Thrissur district

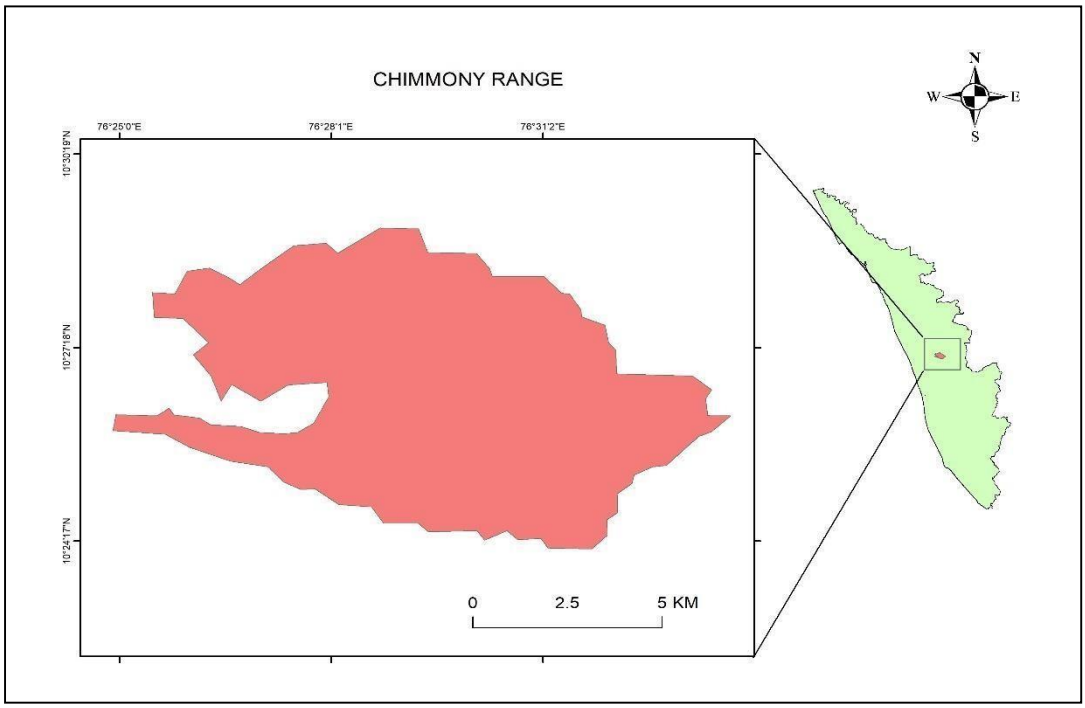
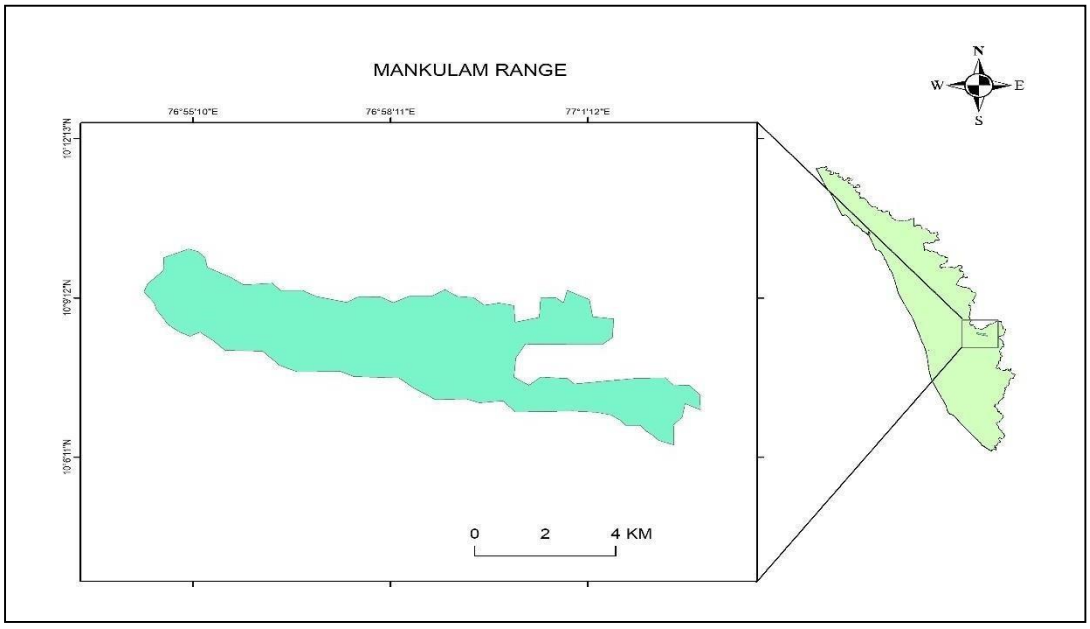


Figure 3. Mankulam range under Mankulam Division in Munnar district



semi-evergreen forest, moist deciduous forest, dry deciduous, broad-leaved hill forest, montane wet temperate forest, and grassland.

3.2.4 Kakkayam Forest Range

Kakkayam range is located in the Kozhikode district of Kerala under Kozhikode forest division (Figure 5). Altitude of the region varies between 600m and 1500m. It is located at a height 750m above MSL and it receives a pre-monsoon shower during April-May months and the regular monsoon during June- August. Temperature of the region varies from 16^0C to 38^0C, diurnal and seasonal variation being moderate, typical to tropical region. The hottest months are April-May and the coolest months are December- February. The forest types include west-coast tropical evergreen, west-coast semi- evergreen, southern moist mixed deciduous, southern hill-top evergreen forests, grasslands, and marshy grasslands.

3.2.5 Kalpetta Forest Range

Kalpetta range is located in Wayanad district of Kerala under the southern Wayanad division (Figure 6). The division has 325.34 km^2 of forests. Altitude of the division varies from 700m to 2300m above MSL. The tract lies to the East of Wayanad plateau, sloping gently towards East and North East. Temperature of this range varies between 13 ^{0}C and 35 ^{0}C. Average annual rainfall ranges from 3000mm to 3500mm. The region receives premonsoon showers during April- May and monsoon shower during the afternoon in the months October- November apart from the monsoon shower during June- August. Major forest types in the region are west coast tropical evergreen forest (1A/C4), west coast semievergreen forest (2A/C2), and southern tropical moist deciduous forest (3B/C1).

3.2.6 Meppadi Forest Range

Meppadi range is located in Wayanad district of Kerala under the southern Wayanad division (Figure 7). The division has 325.34 km^2 of forests. Meppadi has 112.61 km^2

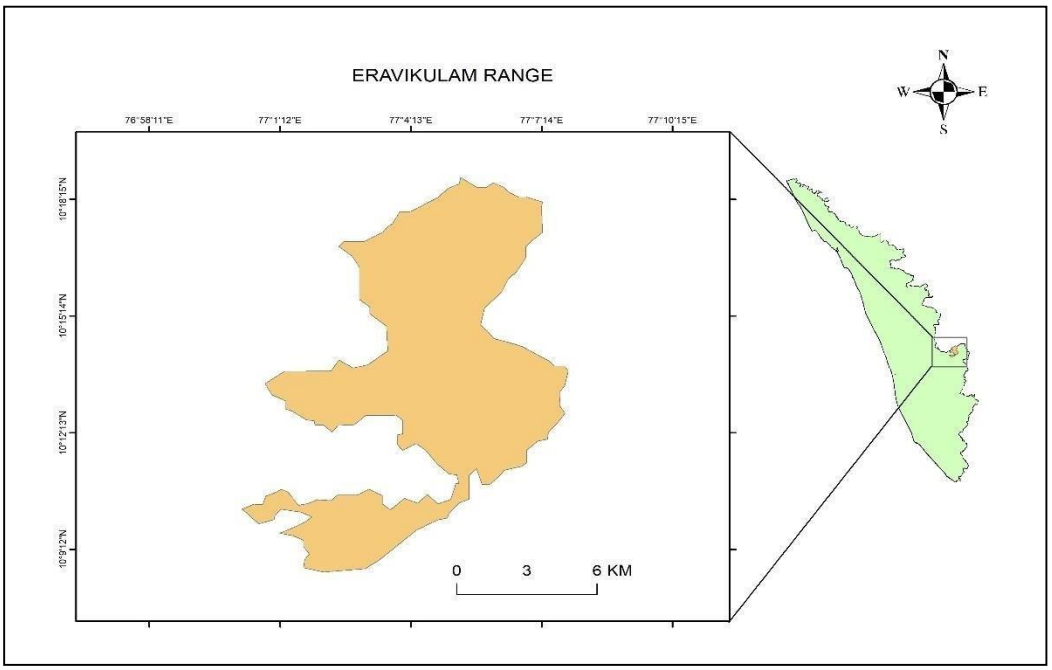


Figure 4. Eravikulam range under Munnar Wildlife Division in Munnar district

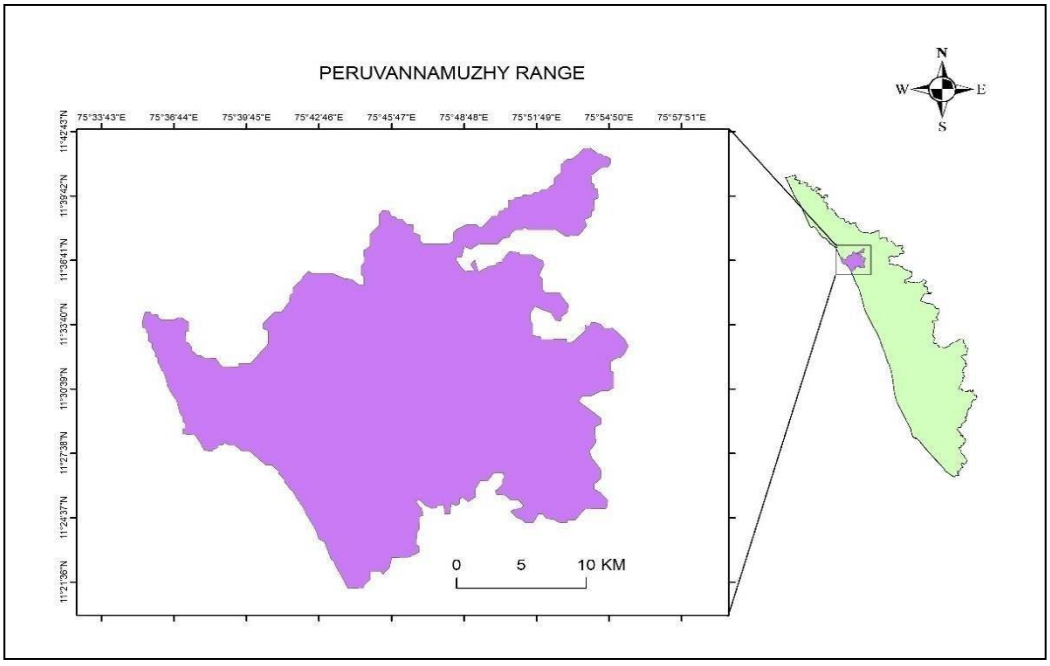


Figure 5. Peruvannamuzhy range under Kozhikode Forest Division in Kozhikode district

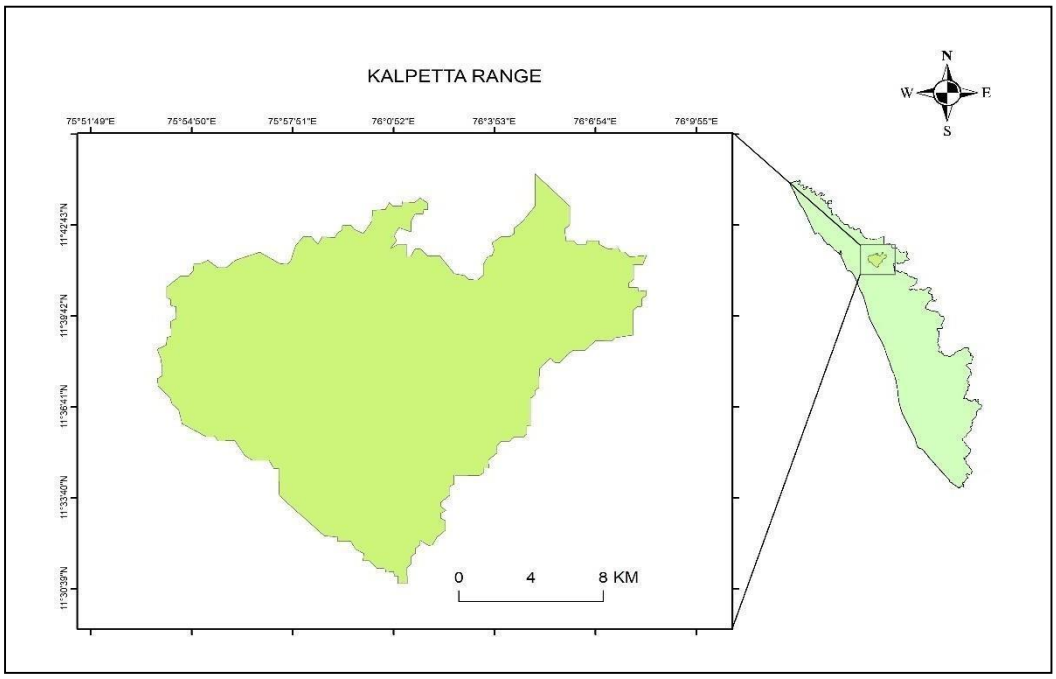


Figure 6. Kalpetta range under South Wayanad Forest Division in Wayanad district

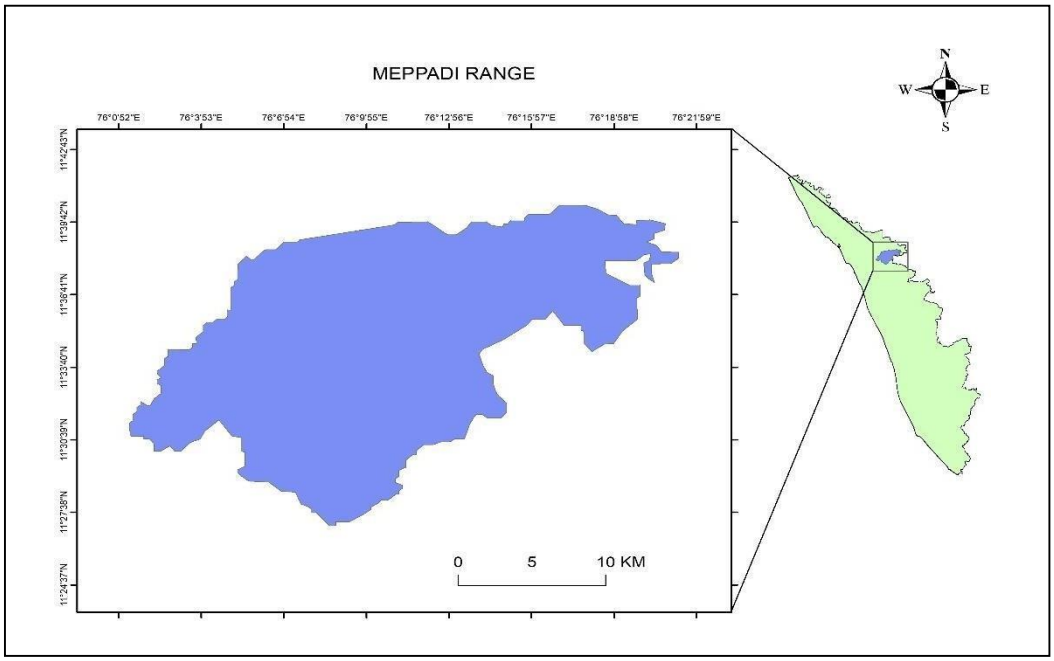


Figure 7. Meppadi range under South Wayanad Forest Division in Wayanad district

vested forest. Altitude of the division varies from 700m to 2300m above MSL. Temperature varies between 13^0C and 35^0C. Average annual rainfall ranges from 3000mm to 3500mm. The region receives pre-monsoon showers during April- May and monsoon shower in the months October- November apart from the monsoon shower during June- August. Major forest types in the region are west coast tropical evergreen forest (1A/C4), west coast semi-evergreen forest (2A/C2), and southern tropical moist deciduous forest (3B/C1). The region selected in Meppadi range was selected for the study which is dominated by *D. longan*.

3.3 PLUS TREE SELECTION FROM SELECTED LOCATION

Subjective selection method was adopted to select sound and healthy trees from six locations. Minimum of 15 trees were evaluated based on tree and foliar characteristics to select five superior trees from each study location (Table 2). Subjective method was used to locate superior trees because of excess environmental noises in study areas. Tree height, girth at breast height and crown shape were considered to select the healthy superior trees among the longan population. The trees selected were 50m apart from each other.

Observations were taken from each selected trees. *D. longan* is closely related to litchi and also share many similarities in characters like physiology, origin, distribution range *etc.* with *Litchi chinensi*s Sonn., both belonging to same family Sapindaceae (Wall, 2006; Rymbai, Jha and Paul, 2018). Observations on the tree characters, floral and fruit characters and other quality attributes were documented based on the descriptor for litchi authorized by IPGRI (2002) since no description was available for *D. longan* and it is known to be a close relative of *L. chinensis* (Sapindaceae) in terms of morphological characters.

Table 2. Morphological parameters of 15 trees from each selected locations

Sample	Crown shape	Branching pattern	GBH (m)	Tree Height (m)	Height of First branch (m)
C1	Irregular	Irregular	1.397	8.00	3.00
C2	Irregular	Irregular	1.17	5.80	3.50
C3	Irregular	Irregular	1.245	6.50	2.10
C4	Irregular	Irregular	1.582	14.80	2.50
C5	Irregular	Irregular	1.885	13.50	3.40
C6	Irregular	Irregular	1.24	6.50	2.10
C7	Irregular	Irregular	1.023	6.00	2.40
C8	Irregular	Irregular	1.306	5.50	2.60
C9	Irregular	Irregular	1.706	13.10	2.70
C10	Irregular	Irregular	1.501	11.90	4.10
C11	Irregular	Irregular	1.38	4.80	3.40
C12	Irregular	Irregular	1.143	8.30	2.50
C13	Irregular	Irregular	1.409	15.40	2.90
C14	Irregular	Irregular	1.122	5.70	2.10
C15	Irregular	Irregular	1.08	6.50	3.40
L1	Irregular	Irregular	1.408	7.40	2.50
L2	Irregular	Irregular	1.219	6.50	2.80
L3	Irregular	Irregular	1.236	5.60	2.50
L4	Irregular	Irregular	1.052	7.60	3.00
L5	Irregular	Irregular	1.38	6.50	2.80
L6	Irregular	Irregular	1.344	6.30	3.50
L7	Irregular	Irregular	1.36	6.00	3.60
L8	Irregular	Irregular	1.99	14.20	8.10

L9	Irregular	Irregular	1.085	6.30	3.20
L10	Irregular	Irregular	1.189	5.80	2.60
L11	Irregular	Irregular	1.511	9.40	4.00
L12	Irregular	Irregular	1.632	11.70	4.10
L13	Irregular	Irregular	1.62	8.20	2.40
L14	Irregular	Irregular	1.074	5.20	2.10
L15	Irregular	Irregular	1.624	13.80	2.90
M1	Oblong	Irregular	1.553	10.00	4.50
M2	Oblong	Irregular	2.207	9.50	5.60
M3	Semi-circular	Irregular	1.7	8.90	4.80
M4	Irregular	Irregular	1.724	10.50	5.00
M5	Oblong	Irregular	1.45	11.00	6.80
M6	Oblong	Irregular	1.405	11.20	6.70
M7	Oblong	Irregular	1.524	9.30	4.60
M8	Irregular	Irregular	1.667	13.30	7.30
M9	Irregular	Irregular	1.846	18.10	8.50
M10	Oblong	Irregular	1.621	8.50	4.10
M11	Irregular	Irregular	1.889	16.10	5.60
M12	Semi-circular	Irregular	1.345	6.70	3.60
M13	Semi-circular	Irregular	1.898	8.60	4.70
M14	Oblong	Irregular	1.477	8.50	5.00
M15	Oblong	Irregular	1.706	9.40	4.10
K1	Broadly pyramidal	Irregular	1.452	8.00	3.00
K2	Broadly pyramidal	Irregular	1.41	5.80	3.50

K3	Broadly pyramidal	Irregular	1.337	6.50	2.20
K4	Broadly pyramidal	Irregular	1.293	4.80	2.50
K5	Broadly pyramidal	Irregular	1.315	6.50	3.40
K6	Broadly pyramidal	Irregular	1.543	7.70	3.40
K7	Irregular	Irregular	1.189	5.80	2.60
K8	Irregular	Irregular	1.243	5.60	4.00
K9	Irregular	Irregular	1.132	5.70	4.40
K10	Irregular	Irregular	1.345	6.40	3.20
K11	Oblong	Irregular	1.411	5.40	3.60
K12	Irregular	Irregular	1.117	5.30	4.00
K13	Irregular	Irregular	1.278	5.70	3.90
K14	Broadly pyramidal	Irregular	1.335	6.80	3.20
K15	Irregular	Irregular	1.288	4.90	2.70
P1	Irregular	Irregular	1.405	6.90	1.90
P2	Semi-circular	Irregular	1.483	7.40	2.50
P3	Semi-circular	Irregular	1.376	5.60	3.00
P4	Irregular	Irregular	1.45	7.60	3.10
P5	Irregular	Irregular	1.504	6.50	4.50
P6	Irregular	Irregular	1.524	6.10	3.20
P7	Irregular	Irregular	1.332	5.40	2.70
P8	Irregular	Irregular	1.204	8.00	5.30
P9	Oblong	Irregular	1.142	8.60	4.10

P10	Irregular	Irregular	1.217	5.70	3.10
P11	Irregular	Irregular	1.402	6.10	2.40
P12	Irregular	Irregular	1.33	5.10	1.70
P13	Irregular	Irregular	1.282	4.30	2.50
P14	Irregular	Irregular	1.17	5.80	3.50
P15	Broadly pyramidal	Irregular	1.245	6.50	2.10
W1	Pyramidal	Irregular	1.6	10.00	7.50
W2	Pyramidal	Irregular	2.1	12.60	6.80
W3	Oblong	Irregular	2.028	11.70	6.10
W4	Pyramidal	Irregular	1.88	12.00	7.00
W5	Broadly pyramidal	Irregular	1.905	11.80	7.40
W6	Pyramidal	Irregular	1.653	10.20	4.10
W7	Pyramidal	Irregular	2.203	9.50	5.10
W8	Pyramidal	Irregular	1.742	8.90	4.30
W9	Pyramidal	Irregular	1.794	10.80	5.10
W10	Pyramidal	Irregular	1.356	11.30	6.40
W11	Pyramidal	Irregular	1.845	15.20	6.70
W12	Pyramidal	Irregular	1.923	17.30	7.60
W13	Pyramidal	Irregular	1.767	9.30	3.90
W14	Pyramidal	Irregular	2.346	19.10	9.20
W15	Pyramidal	Irregular	1.953	15.40	5.30

(C1 to C15 are trees from Chimmony range, L1 to L15 are those from Lakkom under Eravikulam range, M1 to M15 are from Mankulam range, K1 to K15 are trees from Kakkayam range, P1 to P15 from Pookode under Kalpetta range and W1 to W15 are from 900-Kandi under Meppadi range)

3.4 MORPHOLOGICAL DIVERSITY ASSESSMENT

3.4.1 General tree character

Trees having GBH above 100cm was selected for the study. Following observations were done and recorded to identify the variability in the tree charters among different *D. longan* population.

3.4.1.1 Shape of tree canopy

Crown shape is classified into seven groups (Figure 8) according to the descriptor for litchi, namely,

1. Pyramidal
2. Broadly pyramidal
3. Spherical
4. Oblong
5. Semi-circular
6. Dome shaped
7. Irregular

3.4.1.2 Girth at Breast Height

Girth was measured at 50cm above the ground. GBH of trees in a slope were measured from uphill side and was expressed in centimeters.

3.4.1.3 Branching pattern

Based on the descriptor for litchi, the branching pattern on *D. longan* were observed and classified into five groups (Figure 9) namely,

1. Erect
2. Opposite

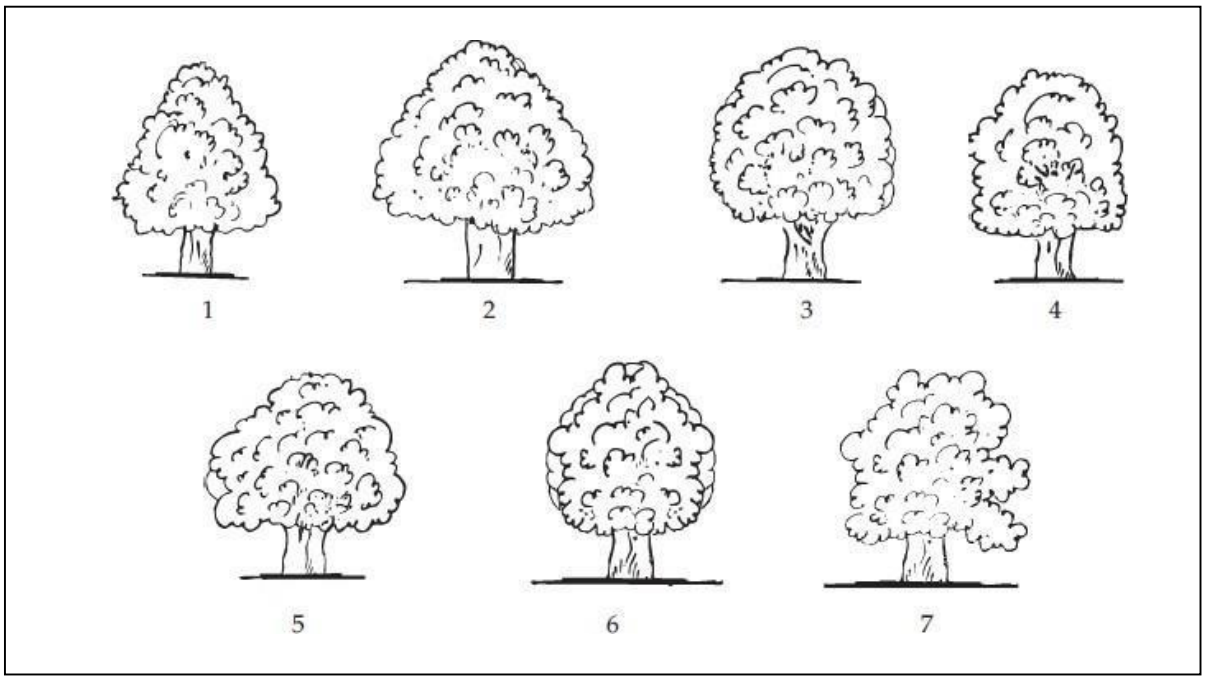


Figure 8. Different crown shape from descriptor of litchi, IPGRI

1. Pyramidal
2. Broadly pyramidal
3. Spherical
4. Oblong
5. Semi-circular
6. Dome shaped
7. Irregular

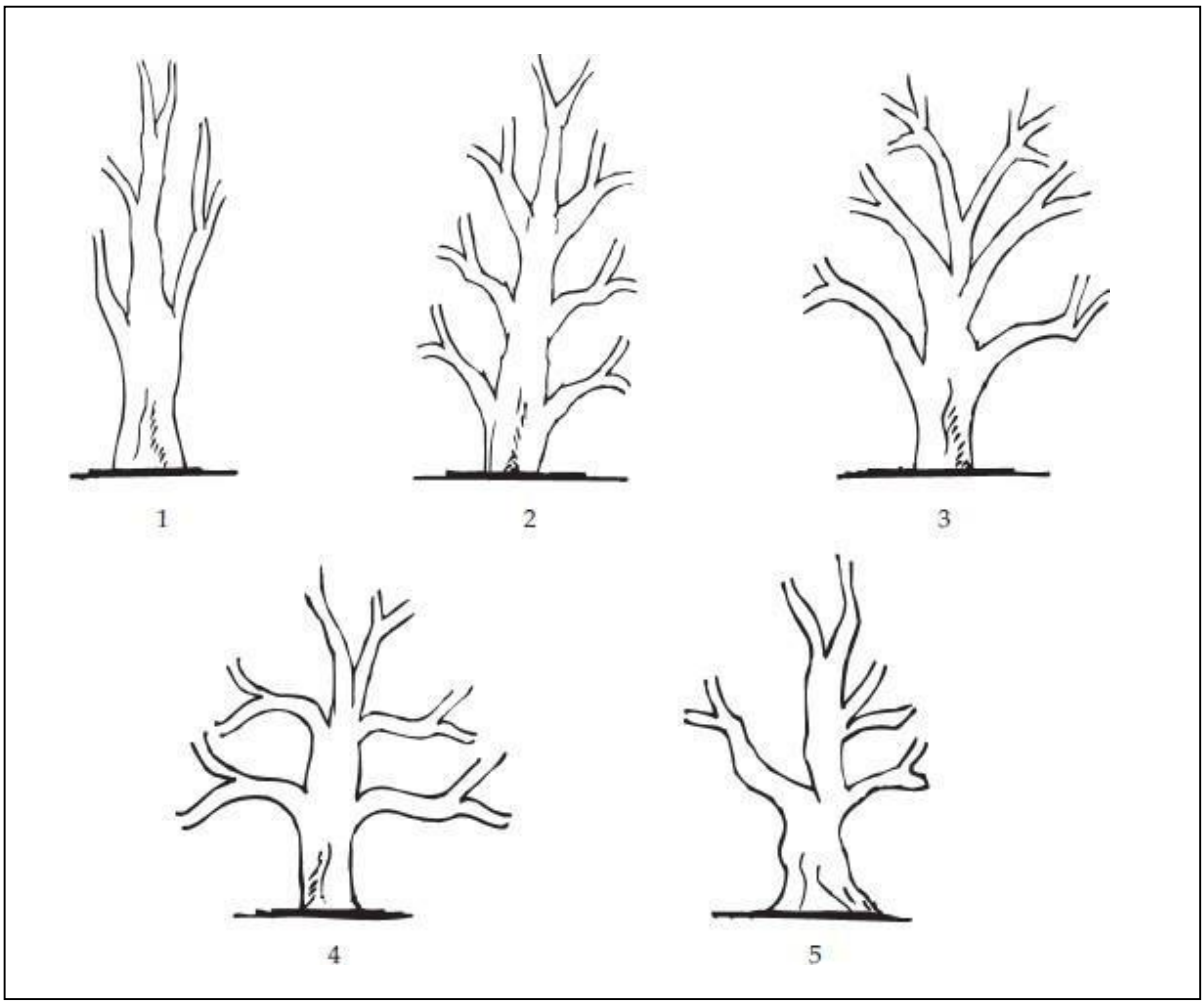


Figure 9. Different branching pattern from descriptor of litchi, IPGRI

1. Erect
2. Opposite
3. Verticillate
4. Horizontal
5. Irregular

3. Verticillate
4. Horizontal
5. Irregular

3.4.1.4 Height of the tree

Total height of the tree was measured using laser hypsometer and was recorded in meter (m).

3.4.1.5 Height of first branch

Bole length was measured using laser hypsometer and was recorded in meter.

3.4.2 Variability of leaf character

3.4.2.1 Leaf length

Leaf length was measured using a regular scale and was recorded in centimeter (cm) (Plate 2).

3.4.2.2 Leaf width

Leaf width was measured using regular scale and was recorded in centimeter (cm) (Plate 3).

3.4.3 Fruit characters

Fruits were collected from the Mankulam forest range apart from the selected plus trees near Kurishupara region.

3.4.3.1 Fruit weight

Weight of 10 fruits were measured using electronic weighing balance and average weight was calculated.

Plate 2. Measuring leaf length using ruler

Plate 3. Measuring leaf width using ruler

Plate 4. Measuring diameter of longan fruit using digital vernier caliper

3.4.3.2 Fruit characters

Fruits characters such as size (diameter), shape and color were recorded from the collected sample. Diameter of fruits was measured using digital vernier caliper (Plate 4).

3.4.4 Seed characters

Seed characters such as size (diameter) and color were recorded from the collected sample. Digital Vernier caliper was used to measure seed diameter.

3.5 MOLECULAR DIVERSITY ANALYSIS

Molecular analysis for 30 plus trees of *D. longan* from six different locations (southern Western Ghats region of Kerala), five from each location, were carried out using ISSR molecular marker system.

3.5.1 Genomic DNA extraction

The mature leaves from individual trees (each accessions) were collected from selected plus trees from different locations and was brought to laboratory in generic icebox, wrapped in aluminium foil paper in a sealed cover. The leaves samples were stored in deep freezer at -20^0C (Rotek 1907) in the laboratory. Before use for DNA extraction, surface of leaves were wiped with 70% alcohol. DNA was extracted from the 30 samples using CTAB method given by Rogers and Bendich (1994) with some modifications.

3.5.1.1 Reagents

Procedures for preparation of different reagents used for extraction of DNA are given in Appendix I.

3.5.1.2 Procedure for DNA extraction

Healthy and mature leaf lamina (250mg) was weighed avoiding the large veins and ground into fine powder in a pestle and mortar using liquid nitrogen and a pinch of PVP

(50mg) was added at the time of grinding. 2mL pre-warmed (60^0C) sucrose extraction buffer was added into the powder and ground well and the obtained green viscous liquid was then transferred to a 2mL micro centrifuge tube. 50 µL β-mercaptoethanol was added into it and is mixed well. The homogenate in the tubes were then incubated in water bath at 65 ^{0}C on a floating rack for 1 hour with intermittent inversion in every 10 minutes. After incubation tubes were centrifuged at 10000 rpm for 10 minutes at 4 ^{0}C. The supernatant was carefully pipetted out to a new micro centrifuge tube. 2mL pre-warmed CTAB (2%) extraction buffer was added into the tube and mixed well by gentle inversion. The tubes were then incubated in water bath at 65^0C for 30 minutes with recurring gentle inversion in every 5 minutes. After incubation, tubes were centrifuged at 10000 rpm for 10 minutes at 4^0C. Clear green aqueous phase supernatant solution thus obtained was pipetted out to a new micro centrifuge tube and 20 µL RNase (10mg/mL) was added into the tube. It was thoroughly mixed through gentle inversion and was kept in the water bath on a floating rack at 37^0C for 30 minutes. The solution in the tube was then added with equal volume of Phenol: Chloroform: Isoamyl alcohol (25:24:1 v/v) and was mixed well followed by centrifugation at 10,000 rpm for 10 minutes at 4^0C to form three layers. The topmost aqueous layer (light greenish tinge) was transferred to a 1.5 mL microcentrifuge tubes carefully without interfering with the thin whitish interphase layer. Equal volume of Chloroform: Isoamyl alcohol (24:1, v/v) was then added to the 1.5 mL microcentrifuge tubes and emulsified by shaking the tubes. These tubes were centrifuged at 10000 rpm for 10 minutes at 4^0C to form three layers again and top aq. layer was carefully pipetted out to a fresh 1.5mL microcentrifuge tube and equal volume of Chloroform: Isoamyl alcolohol (24:1, v/v) was added and properly mixing. These tubes were centrifuged over again (10,000 rpm; 10 minutes; 4^0C). The top clear aqueous layer was transferred to fresh 1.5mL microcentrifuge tubes and add 1/3rd volume of ice-cold isopropanol and mixed by gently inverting to cause the precipitation of DNA. The tubes were kept under cooling condition (-20^0C) in a deep freezer (Rotek 1907 Deep freezer)
for about 20-30 minutes. Tubes were taken off from the freezer and were centrifuged at10000 rpm for 10 minutes at 4^0C. Supernatant was poured out carefully leaving behind

the DNA pellets in the tubes and 70% ethanol was used for washing the DNA pellets for removing the remains of any salts from the extraction buffer. 50 µL 70% ethanol was added to and were centrifuged at 10000 rpm for 5 minutes at 4^0C. Ethanol wash was done twice. After centrifugation, ethanol was drained off completely carefully by gently tapping on a piece of sterile tissue paper. The tubes were then air dried for 30-40 minutes to remove the remnants of any ethanol content. Followed by drying, the DNA pellets were dissolved in 50 µL of TE buffer by gently tapping at the bottom of the centrifuge tube for fastening the dilution. The extracted DNA were stored at -20^0C.

3.5.2 Assessing the quality of DNA by Electrophoresis

The quality of extracted DNA was assessed through agarose gel electrophoresis method (Sambrook *et al.*, 1989).

3.5.2.1 Reagents and Equipments

Procedure for the preparation of buffers and details of composition of reagents are mentioned in Appendix II.

0.8% agarose gel was used for the electrophoresis. Image of gel was then documented using Molecular Imager Gel Doc^{TM} XR+ imaging system (BIO-RAD).

3.5.3 Assessing the quality and quantity of DNA by Nanodrop Spectrophotometer

The quality and quantity of extracted DNA was estimated using the Nanodrop spectrophotometer (Jenway- Genova Nano). OD at 260 and OD at 280 were taken and $OD_{260/280}$ ratio were calculated for finding the purity of DNA. Quantity of DNA was computed using formula $OD_{260} = 1$, equivalent to 50µg double stranded DNA/mL sample. 1 OD at 260nm = 50µg DNA/mL

Therefore, $OD_{260} \times 50$ = Quantity of DNA (µg/ mL)

3.6 MOLECULAR MARKERS USED FOR STUDY

ISSR (Inter-Simple Sequence Repeats) marker was used to analyze the genetic diversity among the 30 accessions of *D. longan*. DNA from all selected accessions were amplified separately using all selected ISSR primers (table 3).

3.6.1 Standardization of PCR condition

The PCR conditions necessary for the efficient amplification of DNA using ISSR markers include suitable proportion of components in PCR mixture, number of PCR cycles, time and denaturation and extension temperature. Ready to use Emerald master mix (Takara) was used in the study instead of separate PCR master mix components. The reaction mixture comprised of PCR Emerald master mix, template DNA, ISSR primer and nuclease-free water. PCR was carried out using Nexus gradient Thermal cycler (Eppendorf Master cycler). The reaction mixture was dispensed in 0.2mL micro centrifuge tubes. The thermal cycler was programmed for desired temperature (denaturation, annealing and extension), time and number of cycles. Standardization of
PCR conditions were done by changing different temperature and time for steps using UBC 809 primer.

3.6.2 Amplification using ISSR primers

The good quality DNA (100ng/µL) isolated from different accessions of *D. longan* and litchi was used for ISSR analysis. ISSR primers (obtained from Sigma Aldrich) with good resolution was chosen for the analysis. Initial screening was carried out to select suitable primers.

Table 3. Melting temperature and annealing temperature of ISSR primers

ISSR Primer	Sequence (5'-3')	Tm^0C	Ta^0C
UBC 809	AGAGAGAGAGAGAGAGG	46.6	43.2
UBC 810	GAGAGAGAGAGAGAGAT	42.9	40.0
UBC 816	CACACACACACACACAT	51.2	47.2
UBC 823	TCTCTCTCTCTCTCTCC	47.5	45.1
UBC 864	ATGATGATGATGATGATG	51.3	49.0
UBC 878	GGATGGATGGATGGAT	56.0	53.8
UBC 881	GGGTGGGGTGGGGTG	66.5	63.5
UBC 884	HBHAGAGAGAGAGAGAG	35.0	32.4
UBC 890	VHVTGTGTGTGTGTGTG	51.8	47.2
UBC 891	VHVGTGTGTGTGTGTGT	51.8	47.5
L1	AGAGAGAGAGAGAGAGGT	48.3	40.3
L3	CCAGTGGTGGTGGTG	57.4	55.0
L4	DBDCACACACACACA	36.5	33.6
L9	GAGAGAGAGAGAGAGAGAC	48.5	40.3
L10	GAGAGAGAGAGAGAGAGAT	48.1	40.3

PCR amplification was done in a 20µl reaction mixture and the composition of reaction mixture include

a. Genomic (template) DNA (100ng/µl) : 2µl
b. Emerald Mastermix (Takara) : 10µl
c. Nuclease free water : 6µl
d. Primer (2µM) : 2µl

Total volume : 20µl

The PCR amplification was performed using following programme,

Step	Temperature	Time	
Hotstart	95^0C	3 min	
Denaturation	95^0C	45 sec	39 cycles
Annealing	Ta	1min	
Final extension	72^0C	10min	
Hold	4^0C	∞	
Extension	72^0C	2min	

3.6.2.1 Screening and analysis of ISSR primers

Screening of primers were done using 19 ISSR primers (Sigma-Aldrich). Out of 19 primers 15 primers were selected for the analysis based on its reproducibility and polymorphism for analysis. Annealing temperature for the selected primers were fixed by running gradient PCR for each primer. The sequence and annealing temperature of selected primers are given in table 2.

The amplified DNA products were run on 2% agarose gel employing 1X TAE buffer along with ethidium bromide added in the gel. The amplified products are loaded in the gel along with a marker 100bp ladder. The gel profile was viewed under UV Trans illuminator and was documented using Molecular Imager Gel Doc^{TM} XR+ imaging system (BIO-RAD). The documented ISSR marker gel profile was evaluated in detail for the amplification. Number of polymorphic and monomorphic bands in the profile were recorded for further analysis.

3.7 DIVERSITY ANALYSIS USING ISSR MARKERS

For ISSR analysis to evaluate the genetic diversity among the accessions of *D. longan* 15 ISSR primers were selected.

3.7.1 Scoring of bands and data analysis

Scoring of bands was done from the documented gel image. 100bp ladder was used as molecular weight size marker for each gel along with DNA samples. Only well distinct and resolved DNA fragments were scored for analysis. The resulted data was further analyzed using software packages PAST, DARwin and GRAPES. The bands were scored as 1 and 0 for the presence and absence of bands respectively. Jaccard's coefficient of similarity was measured and a dendrogram based on similarity coefficient was generated using UPGMA (Unweighed Pair Group Method with Arithmetic mean) using R software.

4. RESULT

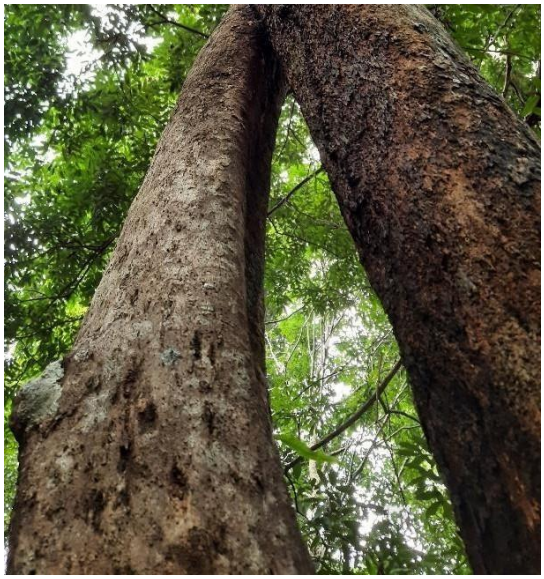
The study entitled "Genetic diversity of *Dimocarpus longan* Lour., in Southern Western Ghats" was done at Department of Forest Biology and Tree Improvement, College of Forestry in Kerala Agricultural University during the period from November 2019 to September 2021. The research work was instigated with the objective to identify the natural population of *Dimocarpus longan*, in Western Ghats regions of Kerala and analyze the diversity present among the population at morphological, biochemical and molecular level. Thirty plus trees were identified, characterized and analyzed for genetic variability. The morphological characters were described and recorded according to the litchi descriptor from IPGRI (International Plant Genetic Resources Institute).

4.1 MORPHOLOGICAL DIVERSITY OF Dimocarpus longan

D. longan is commonly distributed in the evergreen forests of Western Ghats (Plate 5). It is huge tree occurring up to an altitude of 2300m. The tree sometimes form buttress. Bark was brown in color with irregular flakes (Plate 6a). It has a compound leaf with 7- 8 leaflets, often imparipinnate and sometimes paripinnate. Leaflets are elliptic with acute leaf apex with wavy entire leaf margin. Leaflets have 10-20 secondary veins which are straight but curved near the margin. It has asymmetric leaf base which is acute to obtuse in shape (Plate 6 b). Fruits have reddish- brown pericarp, which is tubercle. Fruits are globose in shape and are single seeded. Seeds are spherical to oval with dark brown color (Plate 6 (c)).

Various observations on morphological characters like crown shape, branching pattern, tree height, girth at breast height, height of first branch, leaf length and width, season of arrival of new flush were recorded from the five selected superior trees from each location (Table 4). These data were analyzed and descriptive statistics of minima, maxima, range, average, standard deviations and standard error mean and coefficient of variation (CV) are given in Table 5.

Plate 5. *Dimocarpus longan* from Chimmony forest range



a. Trunk of *Dimocarpus longan*

b. Leaf of *Dimocarpus longan*

c. Fruit of *Dimocarpus longan*

d. Pulp of the fruit

Plate 6. Morphological description of *Dimocarpus longan*

4.1.1 Tree Girth

Tree girth ranged from 1.05m to 2.20m. Maximum girth was recorded for L1 from Lakkom followed by W2 from Meppadi (2.10m), W3 from Meppadi (2.02m), and W5 in Meppadi (1.90m). On an average, population from Meppadi range (South Wayanad Division) showed the highest GBH of 1.90m, followed by Mankulam range (1.72m), Pookode region from Kalpetta range (1.44m), Kakkayam range (1.36m), Eravikulam range (1.25m) and Chimmony range (1.25m) (Table 4). GBH shows a CV of 19.11. The average value of girth obtained from each locations is depicted in figure 10.

4.1.2 Tree Height

Height of the tree ranged from 4.8m to 12.6m with a mean height of 7.87m. Maximum height was observed in W2 from Meppadi population (4.8m) followed by W4 (12m), W5 (11.8m), W3 (11.7m) and minimum was recorded from K2 from Kakkayam population (4.8m). Population from Meppadi range showed highest average tree height of 11.62m, followed by Mankulam range (9.98m), Pookode region from Kalpetta range (6.8m), Eravikulam range (6.72m), Kakkayam range (6.32m) and Chimmony range (6.32m). CV of tree height was 28.20. Trees from Wayanad had the highest mean value of 11.62m followed by trees from Mankulam (9.98m), Lakkom (6.72m), Chimmony and Kakkayam (6.32m), and least was for population from Pookode (6.26m) (figure 11).

4.1.3 Height of First Branch

Height of first branch greatly range from 1.9m to 7.5m. Maximum HFB was recorded from Wayand range for W1 (7.5m) and minimum was reported from Pookode range for P1 (1.9m) with a grand mean of 3.9m. HFB showed a CV of 41.07. In general, highest average HFB was showed by the longan population in Meppady range (6.36m), followed by Mankulam range (5.37m), Pookode region from Kalpetta range (3m), Kakkayam range (2.92m), Chimmony range (2.9m) and Eravikulam range (2.72m). Average value of first branch height of different locations are shown in figure 12.

Table 4. Recorded morphological parameters of selected plus trees from selected locations

Plus tree	Crown shape	Branching pattern	GBH (m)	Tree Height (m)	Height of First branch (m)	Leaf length (m)	Leaf width (m)	Season of new flush
C1	Irregular	Irregular	139.7	8	3	16.5	4.1	mid March-April
C2	Irregular	Irregular	117	5.8	3.5	16.6	4.2	mid March-April
C3	Irregular	Irregular	124.5	6.5	2.1	16.9	4.9	mid March-April
C4	Irregular	Irregular	138.2	4.8	2.5	17.2	5.1	mid March-April
C5	Irregular	Irregular	108	6.5	3.4	18.5	5	mid March-April
L1	Irregular	Irregular	140.8	7.4	2.5	17.2	5	April-May
L2	Irregular	Irregular	121.9	6.5	2.8	18.9	5.5	April-May
L3	Irregular	Irregular	123.6	5.6	2.5	17.6	5.3	April-May
L4	Irregular	Irregular	105.2	7.6	3	13.6	5	April-May
L5	Irregular	Irregular	138	6.5	2.8	15.8	5.7	April-May
M1	Oblong	Irregular	155.3	10	4.5	20.4	5.6	March - April
M2	Oblong	Irregular	220.7	9.5	5.6	21.5	5.6	March - April
M3	Semi-circular	Irregular	170	8.9	4.8	20.6	4.8	March - April
M4	Irregular	Irregular	172.4	10.5	5	19.1	5.5	March - April

M5	Oblong	Irregular	145	11	6.8	18.5	4.7	March - April
K1	Broadly pyramidal	Irregular	145.2	8	3	16.9	4.1	March-mid April
K2	Broadly pyramidal	Irregular	141	5.8	3.5	17.2	4.2	March-mid April
K3	Broadly pyramidal	Irregular	133.7	6.5	2.2	17.8	4.9	March-mid April
K4	Irregular	Irregular	129.3	4.8	2.5	16.9	4.4	March-mid April
K5	Broadly pyramidal	Irregular	131.5	6.5	3.4	19.4	6	mid February-March
P1	Irregular	Irregular	140.5	6.9	1.9	16.6	5.8	mid February-March
P2	Semi-circular	Irregular	148.3	7.4	2.5	17.2	6.2	mid February-March
P3	Semi-circular	Irregular	137.6	5.6	3	16.5	6	mid February-March
P4	Irregular	Irregular	145	7.6	3.1	17	6.2	mid February-March
P5	Irregular	Irregular	150.4	6.5	4.5	17.5	4.1	March-April

W1	Pyramidal	Irregular	160	10	7.5	19	5	March-April
W2	Pyramidal	Irregular	210	12.6	6.8	16.1	5	March-April
W3	Oblong	Irregular	202.8	11.7	6.1	14.9	4.4	March-April
W4	Pyramidal	Irregular	188	12	7	17.8	4.6	March-April
W5	Broadly pyramidal	Irregular	190.5	11.8	7.4	16	4.4	March-April

(C1 to C5 - trees from Chimmony range, L1 to L15 - from Lakkom, M1 to M15 - trees from Mankulam range, K1 to K15 - from Kakkayam range, P1 to P15 from Pookode under Kalpetta range and W1 to W15 are from 900-Kandi under Meppadi range)

Table 5. Summary of morphological data

Parameters	Maximum	Minimum	Range	Average	SD	CV
GBH	220.7	105.2	115.5	149.1	28.5	19.11
TH	12.6	4.8	7.8	7.8	2.24	28.20
HFB	7.5	1.9	5.6	3.97	1.72	43.34
LL	21.5	13.6	7.9	17.5	1.64	9.37
LW	6.2	4.1	2.1	5	0.64	12.73

Table 6. Genotypic parameters of selected plus trees

Parameters	Max. value	Min. value	SEm	EV	GV	PV	E CV	G CV	P CV	H2	GA
GBH	220.7	105.2	7.6	288.82	649.57	938.4	11.39	17.08	20.54	0.69	43.68
TH	12.6	4.8	0.44	0.98	4.94	5.93	12.49	27.94	30.61	0.83	4.18
HFB	7.5	1.9	0.23	0.28	3.05	3.33	13.3	44.00	45.9	0.91	3.44
LL	21.5	13.6	0.6	1.84	1.25	3.09	7.74	6.38	10.04	0.4	1.46
LW	6.2	4.1	0.28	0.39	0.09	0.48	12.42	6.11	13.84	0.19	0.28

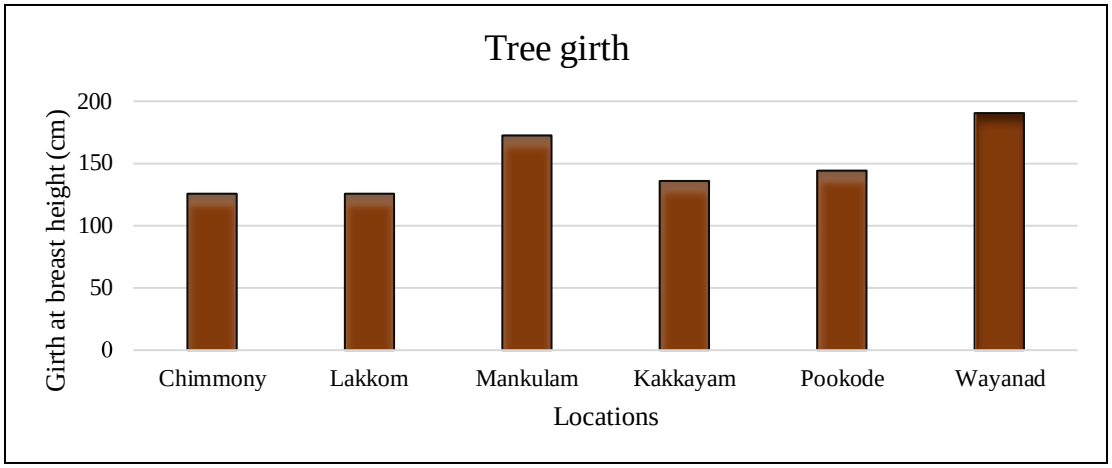


Figure 10. Mean value of GBH from different locations

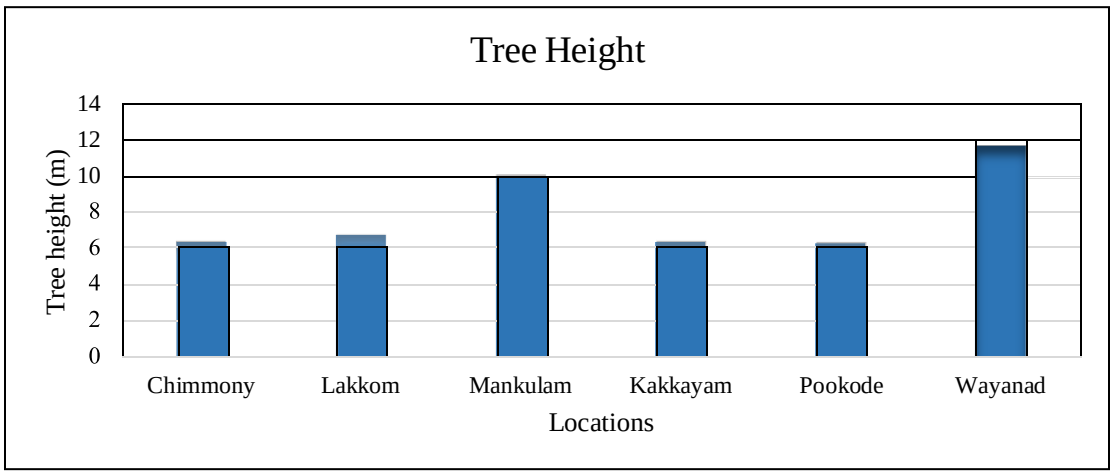


Figure 11. Average value of tree height from various locations

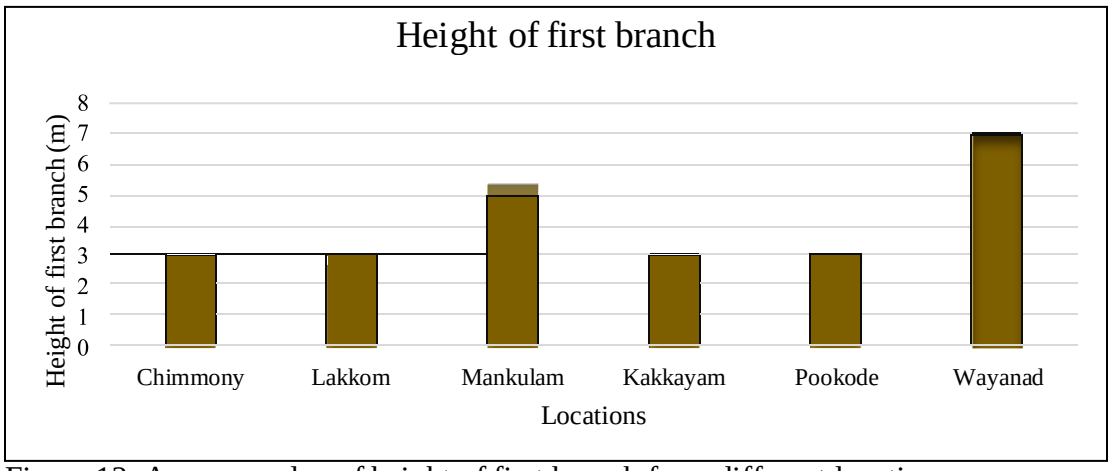


Figure 12. Average value of height of first branch from different locations

4.1.4 Leaf Length

Leaf length ranged from 0.13m to 0.21m. Maximum leaf length observed for L1 from Lakkom population (0.21m), followed by M3 (0.20m), M1 (0.20m) and K5 (0.19m). Minimum was observed for L4 from Lakkom (0.13m). Mean leaf length calculated was 0.17m. Calculated CV of leaf length was 9.37. On an average, population from Mankulam showed highest leaf length (0.20m), followed by population from Kakkayam (0.17m), Chimmony (0.17m), Pookode region (0.16m), Meppadi range (0.16m) and Eravikulam range (0.16m) (figure 13).

4.1.5 Leaf Width

Leaf width ranged from 0.041cm to 0.062cm. P1 from Pookode region had the maximum leaf width (0.06m) and C1 had the minimum leaf width (0.04m). Highest average leaf width was recorded from population in Pookode region (5.66cm) followed by Eravikulam range (0.05m), Mankulam (0.05m), Kakkayam range (0.047m), Meppadi range (0.046m) and Chimmony range (0.046m) (figure 14). Leaf width showed a CV of 12.73.

4.1.6 Crown Shape

Mainly five crown shapes were observed among different locations *viz.* irregular, pyramidal, broadly pyramidal, oblong and semi-circular. Crown shape noticed in majority was irregular followed by broadly pyramidal, oblong, pyramidal and semi- circular shape. Out of total 30 plus trees selected, 14 trees were irregular and spreading in nature, six were broadly pyramidal, four were oblong, three were semi-circular and three were pyramidal. All selected trees from Chimmony and Lakkom had irregular crown shape. Oblong, semi-circular and irregular crown shape were observed from Mankulam, irregular and broadly pyramidal crown shape were observed in populations in Kakkayam, irregular and semi-circular crown shapes were obtained from the populations in Pookode while pyramidal, broadly pyramidal and oblong were observed in the population from Meppadi.

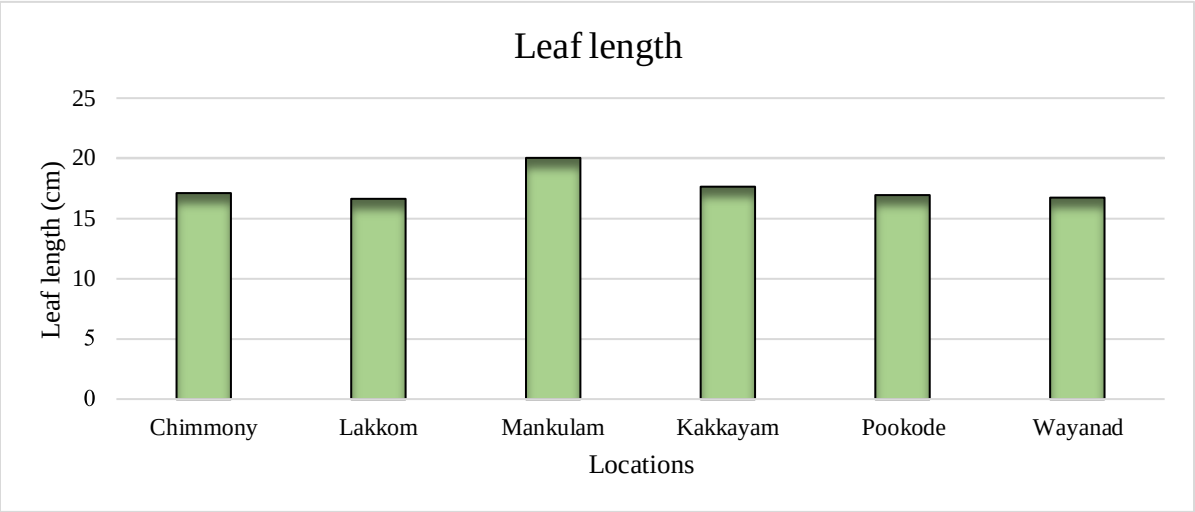


Figure 13. Mean leaf length from different location

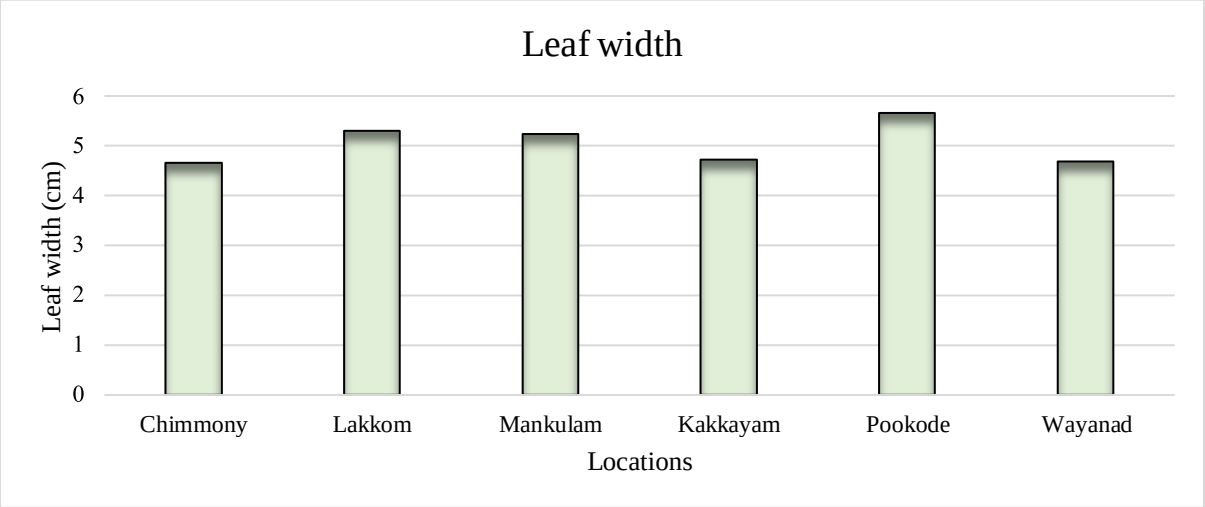


Figure 14. Mean leaf width from different locations

4.1.7 Branching pattern

Branching pattern noticed was irregular in all selected plus trees in all the locations.

4.1.8 Time of new flush emergence

At different locations new flush emerged in different period. *Dimocarpus longan* has ferruginous young leaves. New flushes were observed during the period of mid-April to May among the population in Chimmony, months of April and May among population in Lakkom (Eravikulam range), March to April in Mankulam, March to mid-April in Kakkayam and Meppadi range and mid-February to March in Pookode region.

4.1.9 Genetic parameters of *Dimocarpus longan*

4.1.9.1 Genetic variance of biometric parameters

Value of phenotypic coefficient of variance (PCV), genotypic coefficient of variance (GCV), environmental coefficient of variance (ECV), broad sense heritability (H^2), genetic advance (GA) of different biometric characters are given in table 6.

Highest GCV value was recorded for HFB (44.00), followed by tree height (27.94), GBH (17.08), leaf length (6.38) and leaf width (6.11). Maximum PCV was observed for HFB (45.9), followed by tree height (30.61), GBH (20.54), leaf width (13.84) and least for leaf length (10.04). ECV value was found to be highest for HFB (13.3), followed by tree height (12.49), leaf width (12.42), GBH (11.39) and leaf length (7.74). Highest value of broad sense heritability was observed in HFB (0.91), followed by tree height (0.83), GBH (0.69), leaf length (0.4) and lowest leaf width (0.19). The genetic advance was highest for GBH (43.68), followed by tree height (4.18), HFB (3.44), leaf length (1.46) and least for leaf width (0.28).

The summary of the morphological data collected are presented in table 4a.

4.1.10 Fruit character

Due to the pandemic situation and lock down that was prevailed during the period of study, the study confronted with various challenges due to the restrictions that were imposed on the entry into different forest areas and we couldn't follow up with the flowering and fruiting season during the months of May to September, 2020 and March to July, 2021. None of the selected plus trees were flowered during the research period (May 2020- September 2021). This was based on the unofficial information since the entry was restricted. Fruiting was observed in a tree from a different location apart from study area, in Mankulam range, near Kurishupara. Fruiting was observed during the month of mid-September to October. Various morphological parameters were recorded from the collected fruit samples from the single tree (table 7).

Observations were taken from 12 mature fruit samples collected from Mankulam (Kurishipara). Summary of each fruit measurements are presented in table 8. Fruits were collected from a single tree for taking observation. Longan had cymose inflorescence. Fruit was drupe, schizocarp, globose and tubercled with reddish to brownish pericarp. Seed was dark brownish in color with spherical to oval shape. Diameter of the fruit ranged from 13.26mm to 14.81mm with an average of 14.17mm. CV of fruit diameter was found to be 3.26. The total fruit weight in grams varied from 0.94g to 1.725 g with an average of 0.785g. Total fruit weight showed a CV of 20.34. Weight of longan seed ranged from 0.44g to 0.89g with an average of 0.63g. Seeds where nearly oval in shape with average diameter of 10.52mm for major axis and 9.66mm for minor axis. CV of seed weight was measured as 21.38. Weight of the fruit pulp varied from minimum of 0.07g to maximum of 0.24g. Calculated average fruit pulp was 0.15g with a CV of 40.03.

Table 7. Morphological parameters of collected fruits

Diameter (mm)	Weight				Diameter of seed	
	Fruit (g)	Seed with pulp (g)	Seed (g)	Pulp (g)	Major axis (mm)	Minor axis (mm)
14.61	1.725	1.07	0.89	0.18	12.06	10.36
14.08	1.3	0.76	0.65	0.11	10.82	9.98
14.81	1.58	1.06	0.84	0.22	11.4	10.13
14.5	1.37	0.89	0.73	0.16	10.76	9.88
13.26	1.01	0.61	0.44	0.17	9.35	8.46
14.58	1.38	0.93	0.69	0.24	11.31	10.02
14.17	1.15	0.73	0.62	0.11	10.67	9.6
14.19	1.3	0.82	0.59	0.23	10.31	0.62
14.23	0.95	0.62	0.52	0.1	9.41	9.29
14.49	1.04	0.71	0.64	0.07	10.39	9.76
14.31	1.15	0.81	0.59	0.22	10.49	9.68
13.39	0.98	0.61	0.51	0.1	10.15	9.67

Table 8. Summary of different morphological characters of fruit

Parameter	Max. value	Min. value	Range	Average	SD	CV
Diameter (mm)	14.81	13.26	1.55	14.17	0.46	3.26
Fruit (g)	1.725	0.94	0.785	1.22	0.25	20.34
Seed with pulp (g)	1.07	0.55	0.52	0.78	0.17	21.63
Seed (g)	0.89	0.44	0.45	0.63	0.13	21.38
Pulp (g)	0.24	0.07	0.17	0.15	0.06	40.03
Diameter of seed Major axis (mm)	12.06	9.35	2.71	10.52	0.79	7.51
Diameter of seed Minor axis (mm)	10.36	8.46	1.9	9.66	0.48	5.04

Analysis of the morphological data of selected trees obtained from the field was done using two softwares; PAST software and GRAPES software (KAU). Cluster analysis for morphological data *viz.* branching pattern, crown shape, total tree height, GBH, height of first branch, leaf length and leaf width was carried out with PAST software. The software was used to generate a hierarchical dendrogram using UGPMA method for tree morphological characteristics (figure 15).

Based on the morphological parameters, majorly two clusters were formed. The cluster I contains samples from the locations, Chimmony, Lakkom, Kakkayam and Pookode. The cluster II comprise the samples from locations Mankulam and Meppadi due to their morphological similarity which can be attributed to the edaphic factors and climatic factors prevailing in the regions.

Principle Component Analysis (PCA) was performed to recognize the phenotypic parameters that contribute to PV (total phenotypic variance) existing in the population.

Seven morphological parameters were considered for PCA in GRAPES software. Scree plot was used to identify the principle component affecting the PV (figure 16). PC1 describes 50.8% of the total phenotypic variance while PC2 and PC3 explains 21.20% and 12.50% of PV respectively. PC4, PC5 and PC6 contribute only negligible amount on the phenotypic variance of the population and can be excluded from considering as principle component.
Correlation between different principle components and morphological parameters were calculated using GRAPES software (table 9). Based on table 6, the parameters GBH, tree height, height of first branch and leaf length have positive effects on expression of character for PC1. Branching pattern shoes a negative correlation with all the principle components considered and is not considered for correlation analysis. With respect to PC1, crown shape and leaf width are negatively related. With reference to PC2, all variables except height of first branch are positively correlated. All parameters omitting

in Kakkayam, irregular and semi-circular crown shapes were obtained from the populations in Pookode while pyramidal, broadly pyramidal and oblong were observed in the population from Meppadi.

4.1.7 Branching pattern

Branching pattern noticed was irregular in all selected plus trees in all the locations.

4.1.8 Time of new flush emergence

At different locations new flush emerged in different period. *Dimocarpus longan* has ferruginous young leaves. New flushes were observed during the period of mid-April to May among the population in Chimmony, months of April and May among population in Lakkom (Eravikulam range), March to April in Mankulam, March to mid-April in Kakkayam and Meppadi range and mid-February to March in Pookode region.

4.1.9 Genetic parameters of *Dimocarpus longan*

4.1.9.1 Genetic variance of biometric parameters

Value of phenotypic coefficient of variance (PCV), genotypic coefficient of variance (GCV), environmental coefficient of variance (ECV), broad sense heritability (H^2), genetic advance (GA) of different biometric characters are given in table 6.

Highest GCV value was recorded for HFB (44.00), followed by tree height (27.94), GBH (17.08), leaf length (6.38) and leaf width (6.11). Maximum PCV was observed for HFB (45.9), followed by tree height (30.61), GBH (20.54), leaf width (13.84) and least for leaf length (10.04). ECV value was found to be highest for HFB (13.3), followed by tree height (12.49), leaf width (12.42), GBH (11.39) and leaf length (7.74). Highest value of broad sense heritability was observed in HFB (0.91), followed by tree height (0.83), GBH (0.69), leaf length (0.4) and lowest leaf width (0.19). The genetic advance was highest

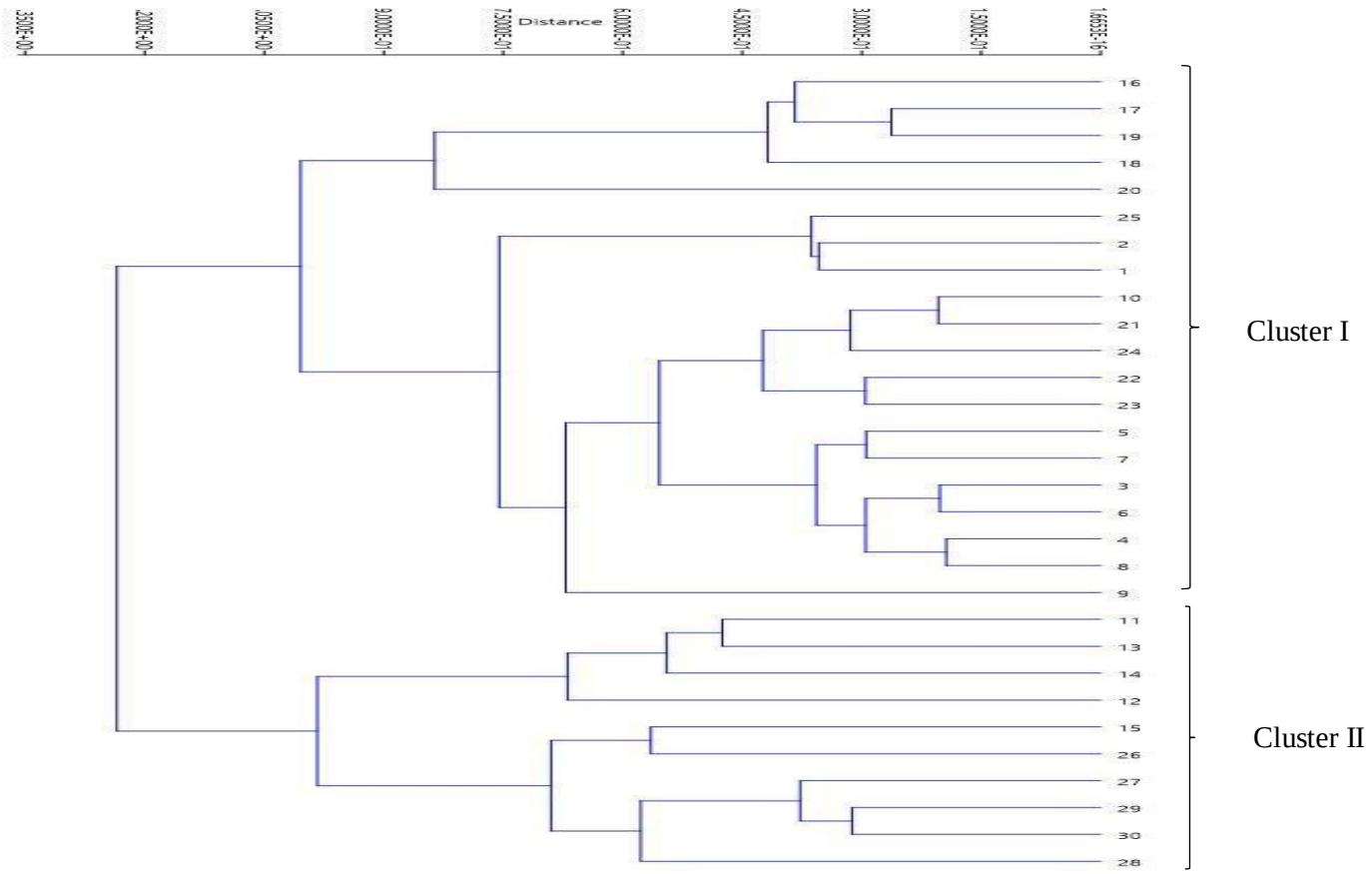


Figure 15. Hierarchical dendrogram based on morphological parameters

(1-5: trees from Chimmony, 6-10: trees from Lakkom, 10-15: trees from Mankulam, 16-20: trees from Kakkayam, 20-25: trees from Pookode, 25-30: trees from Meppadi)

Table 9. Correlation between variables and PC s influencing phenotypic trait

Variables	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC6**	**PC7**
Crown shape	-0.688	0.119	0.427	0.574	-0.021	0.028	0
Branching pattern	-Inf.	-Inf.	-Inf.	-Inf.	-Inf.	-Inf.	-Inf.
GBH	0.874	0.145	0.207	0.02	-0.412	0.048	0
Tree Height	0.905	0.008	0.308	0.102	0.154	-0.227	0
Height of first branch	0.933	-0.038	0.061	0.138	0.243	0.216	0
Leaf length	0.24	0.767	-0.521	0.285	0.003	-0.039	0
Leaf width	-0.249	0.804	0.397	-0.355	0.084	0.036	0

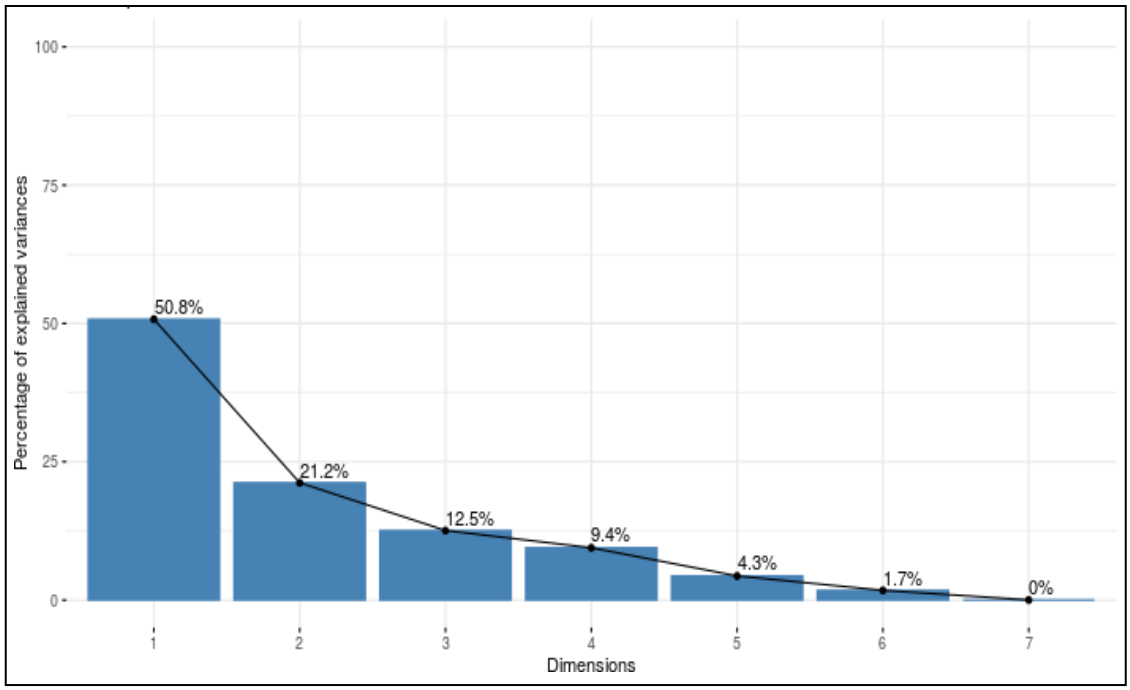


Figure 16. Scree plot of principle component contributing to total phenotypic variance

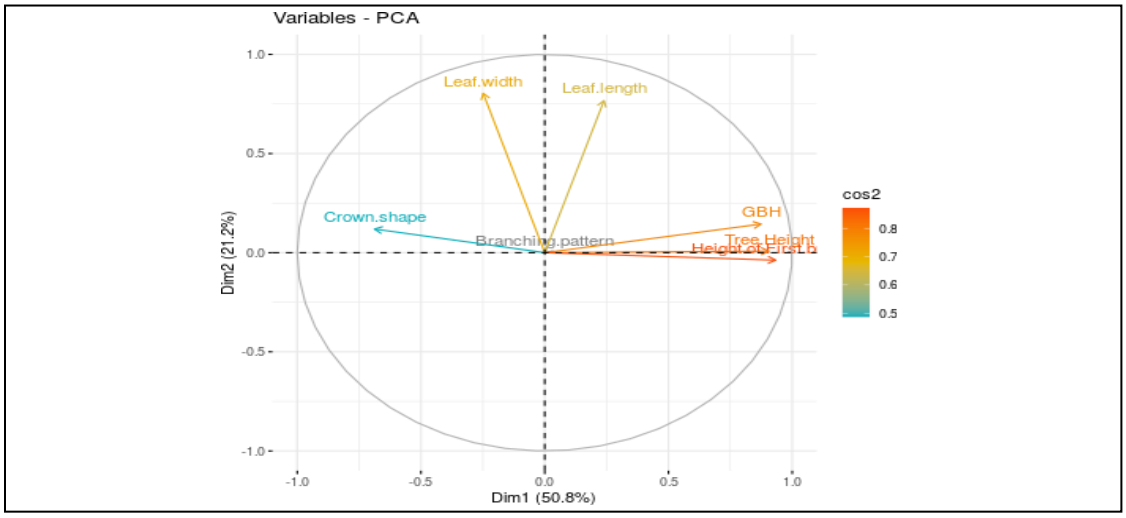


Figure 17. Phenotypic parameters based on PCA with PC1 in Y axis and PC2 in X axis

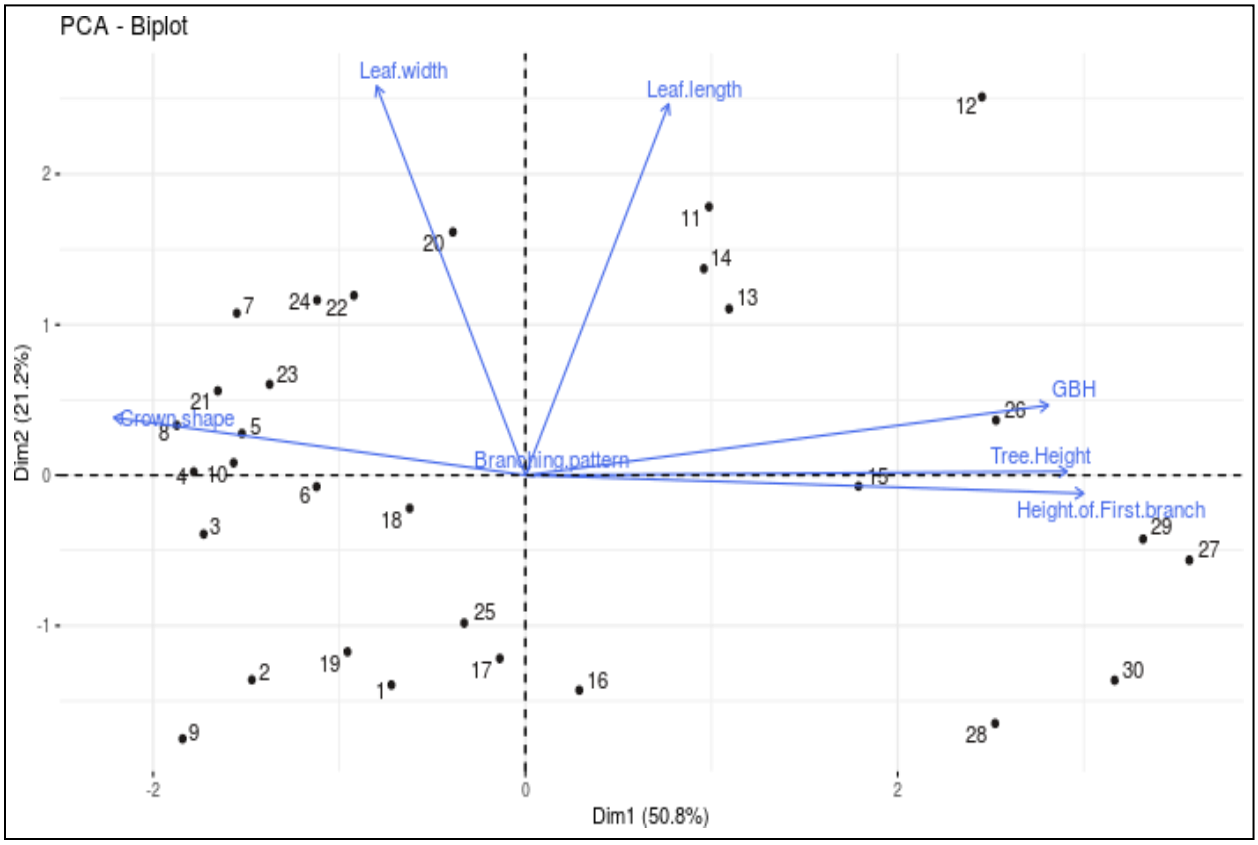


Figure 18. PCA biplot based on morphological parameters

the leaf length, shows positive effect on expressing phenotype for PC3. With respect to PC4, all factors except leaf width and branching pattern are positively correlated. According to figure 16, tree height and height of first branch influence more to the phenotypic variance than other factors. Biplot diagram of PCA based on the morphological parameters of selected trees are given in figure 18. It shows the distribution of samples on the basis of the PCA worked out (figure 17).

The morphological characters of cultivated longan cultivar was also recorded during the research study. The cultivated longan variety, e-daw, was taken for the study and was obtained from a fruit nursery in Vellanikkara. The tree was three years old with 1.5m height. The leaf of longan cultivar was elliptical with entire margin. Total leaflet length ranged from 10.00cm- 15.50cm and total leaflet width ranged 4.00 – 5.50 cm. Leaflet had asymmetric leaf base which was obtuse to elliptic. Inflorescence was cyme. Fruit was globose with smooth yellowish- brown pericarp. Diameter of fruit ranged from 3.50cm to 5.00cm.

4.2 MOLECULAR CHARACTERIZATION

Five plus trees from each six different locations were selected to estimate the genetic diversity among and within the population. In addition to this, a single sample of wild longan from Nelliyampathy forest range (Palakkad, south of Palghat gap) was also selected to analyze the genetic diversity among the populations. Two types of commercially cultivated longan varieties commonly known as white longan and longan E-daw were also taken in order to study the extent of genetic variability existing among the wild longan population and commercially cultivated longan population. Apart from estimating the genetic variability among different longan populations, the genetic relation existing among wild and commercial longan and litchi, a close relative of *D. longan* was also evaluated in the study.

4.2.1 Standardization of DNA extraction

Mature leaves were more preferred for quality DNA extraction. The stage of leaf maturity is crucial for obtaining good quality DNA with sufficient quantity. Polyphenol interference was more in the DNA extracted from both young tender leaves (reddish to light greenish tinge) and over matured leaves. Good quality DNA was yielded from the mature leaves.

Sucrose isolation buffer was used to supplement osmotic pressure during cell wall breakage or lysis. More intact DNA was obtained when the sucrose solution was used in samples of *D. longan*. Adding PVP and β mercaptoethanol to the extraction buffer prevented browning of the pellets. Phenol: chloroform: isoamyl alcohol (25:24:1) was employed to reduce the protein contamination in the extracted DNA (Vandrey and Stutz, 1973).

Isolated DNA from all six populations was apparently pure. The extracted DNA given an $OD_{260/280}$ value ranging from 1.5 to 2.2 (Table 10) in Nanodrop Spectrophotometer. Quantity of DNA extracted ranged from 434.82 ng μL^{-1} to 4538.0 ng μL^{-1}.

4.2.2 Gel Electrophoresis

Quality of isolated DNA was verified through gel electrophoresis using 0.8 percent agarose gel. DNA obtained appeared as a single sharp band without much shearing. All samples were electrophoresed and more or less single sharp bands were obtained for all samples.

4.2.3 Standardization of PCR conditions

Conditions for the Polymerase Chain Reaction (PCR) were standardized using a single DNA sample (C2) with primer L1 which were selected randomly. Conditions were optimized to obtain reproducible clear and consistent bands under suitable cycle duration. Good amplicons were obtained while using emerald master mix (Takara) in the reaction

Table 10. Quantity of extracted DNA obtained using Nano Drop spectrophotometer

Sl. No.	Location	Sample	OD $_{260/280}$	Quantity of DNA (ng μL^{-1})
1.	Chimmony (Thrissur)	C1	1.89	1552.3
2.		C2	1.75	2376.1
3.		C3	1.91	1360.2
4.		C4	1.66	4538.0
5.		C5	1.82	1533.5
6.	Lakkom (Idukki)	L1	1.98	459.82
7.		L2	1.97	476.62
8.		L3	1.80	659.71
9.		L4	1.58	912.30
10.		L5	2.10	741.89
11.	Mankulam (Idukki)	M1	1.70	2891.63
12.		M2	1.86	2682.71
13.		M3	1.60	1751.63
14.		M4	1.94	2591.80
15.		M5	2.20	1910.66
16.	Kakkayam (Kozhikode)	K1	1.83	660.68
17.		K2	1.78	540.10
18.		K3	2.19	515.65
19.		K4	1.81	487.77
20.		K5	1.99	434.82
21.	Pookode (Wayanad)	P1	2.00	505.93
22.		P2	1.97	601.88
23.		P3	1.71	805.99
24.		P4	1.83	945.61
25.		P5	1.69	598.62

26.	Meppadi (Wayanad)	W1	1.86	1960.11
27.		W2	1.51	1892.90
28.		W3	1.80	2118.01
29.		W4	2.02	997.06
30.		W5	1.62	1006.98
31.	Nelliyampathy	BG	1.71	532.98
32.	Vellanikkara	Ruby longan	2.08	1519.6
33.		White longan	1.86	1216.36
34.	COF	Litchi	1.93	944.66

mixture. PCR gradient was carried out to standardize the annealing temperature for each primer.

4.2.4 Primer screening

19 ISSR primers were used for screening. 15 primers produced good amplified products. Sample C3 was used to screen all the 19 primers. Annealing temperature of each primers were standardized and is given in table 2. Molecular characterization of longan was done using 15 ISSR primers, namely, UBC 816, UBC 884, UBC 823, UBC 864, UBC 891,
UBC 809, UBC 890, UBC 810, UBC 881, UBC 878, L1, L9, L10, L3 and L4.

4.3 GENETIC DIVERSITY AMONG *D. longan* POPULATIONS IN SOUTHERN WESTERN GHATS, KERALA

Five to fourteen bands were produced by each primer. Maximum number of bands were obtained from ISSR primer UBC 810 (14 bands) and least number of bands were obtained for UBC 816 (4 bands). Number of polymorphic bands ranged between four and eleven. Highest number of polymorphic bands were obtained for UBC 810 (11 bands) and least for UBC 816 and L1 (4 bands). Maximum percentage polymorphism was observed for primers L3 and L9 with polymorphism percentage 88.89% and least was observed for UBC 816 (40%). Average polymorphism observed was 69.51%. In total, 135 distinct amplicons were produced. Total amplicons produced by each primers and details of polymorphism are depicted in table 11.

UBC 809

Amplification employing UBC 809 yielded 11 bands in total with 72.73% polymorphism. Out of 11 amplicons, eight were polymorphic and three were monomorphic bands. It identifies the genetic relation existing among the populations in South and North of Palghat gap. It differentiate L1 from Lakkom from other samples in Lakkom since it Table 11. showed a distinct band in L1. It distinguished K3 from other Kakkayam population. It differentiate the populations from Lakkom and Mankulam (Plate 7a).

Table 11. Total number of bands and polymorphism exhibited by different primers in 30 samples

Primer	Total bands	Polymorphic bands	Monomorphic bands	Percentage polymorphism
UBC 809	11	8	3	72.73
UBC 810	14	11	3	78.57
UBC 816	5	4	1	80.00
UBC 823	7	5	2	71.43
UBC 864	7	5	2	71.43
UBC 878	10	7	3	70.00
UBC 881	7	5	2	71.43
UBC 884	9	6	3	33.33
UBC 890	9	7	2	77.78
UBC 891	9	5	4	55.56
L1	8	4	4	50.00
L3	9	8	1	88.89
L4	10	6	4	60.00
L9	9	8	1	88.89
L10	11	8	3	72.73

UBC 810

Amplification with UBC 810 produced the highest number of bands (14 amplicons), out of which 11 were polymorphic and rest were present in all samples. Polymorphic percentage observed for the primer was 78.57% and was used to distinguish genetic relation among the population in North and South of Palghat gap. Banding pattern obtained for Pookode and Meppadi were similar and cannot be distinguished using UBC 810. Distinct amplicon present in Kakkayam distinguish population of Kakkayam from other longan populations. It can differentiate Chimmony population from rest of the longan populations (Plate 7b).

UBC 816

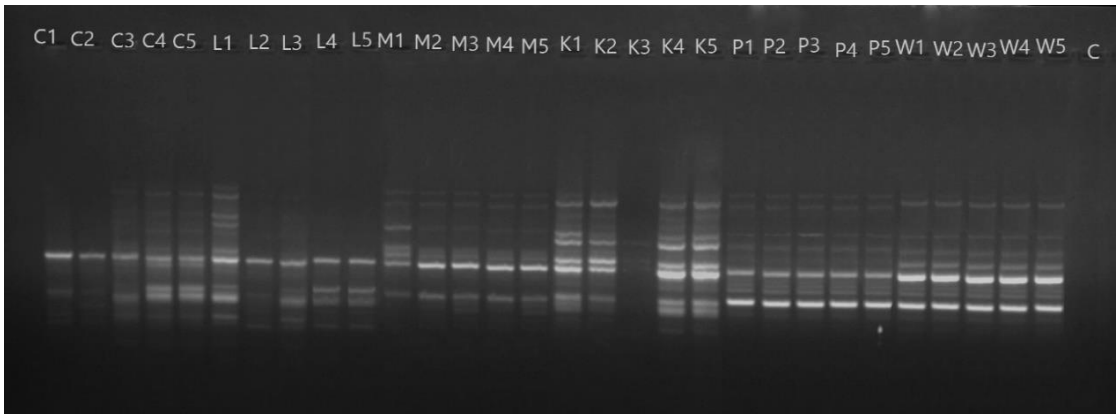
Five amplicons were obtained in total after the PCR with UBC 816. The primer showed 80% polymorphism. Four bands were polymorphic while one was monomorphic band. Distinct amplicon in Kakkayam population distinguished Kakkayam from other populations (Plate 7c).

UBC 823

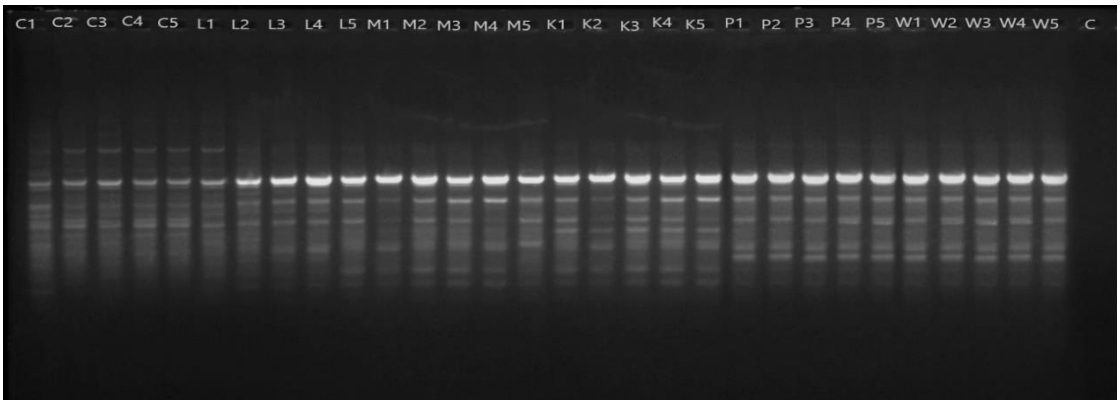
Seven amplicons were produced in total. Five bands produced were polymorphic while two were monomorphic bands owing to 71.43 % polymorphism. UBC 823 primer clearly differentiated Lakkom population from rest of the population due to the presence of two distinct bands. UBC 823 differentiated C4 from rest of the Chimmony samples due to the absence of one amplicon. It could also differentiate Kakkayam population from other populations. The primer cannot differentiate Pookode and Meppadi populations since they produced same banding pattern (Plate 8a).

UBC 864

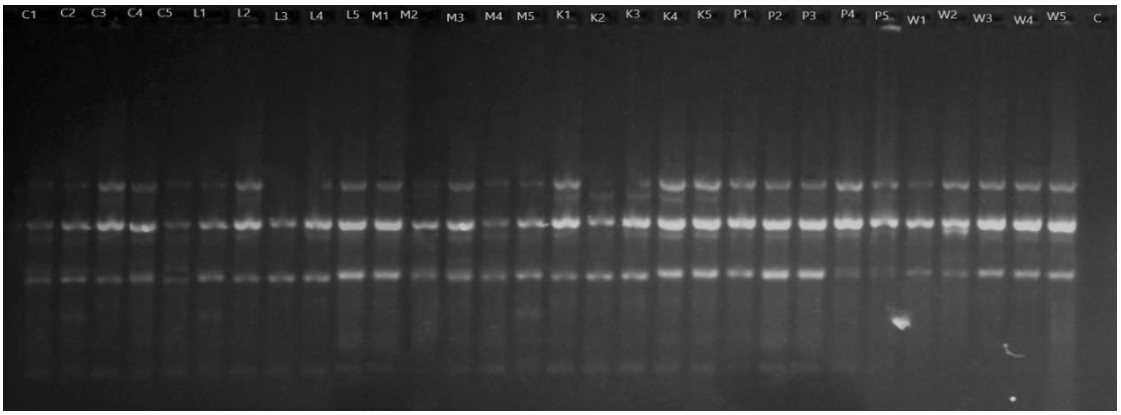
DNA amplification using UBC 864 generated seven amplicons out of which five were polymorphic whereas two were monomorphic bands. Polymorphic percentage observed



a. ISSR primer UBC 809



b. ISSR primer UBC 810



c. ISSR primer UBC 816

Plate 7. Amplification profile of ISSR primers for samples of longan from six selected locations

for UBC 864 was 71.43%. It cannot differentiate Chimmony and Lakkom population but can differentiate them from other longan populations. It differentiated K2 and K4 ok Kakkayam from other samples of Kakkayam. UBC 864 can distinguish population of Meppadi and Pookode (Plate 8b).

UBC 878

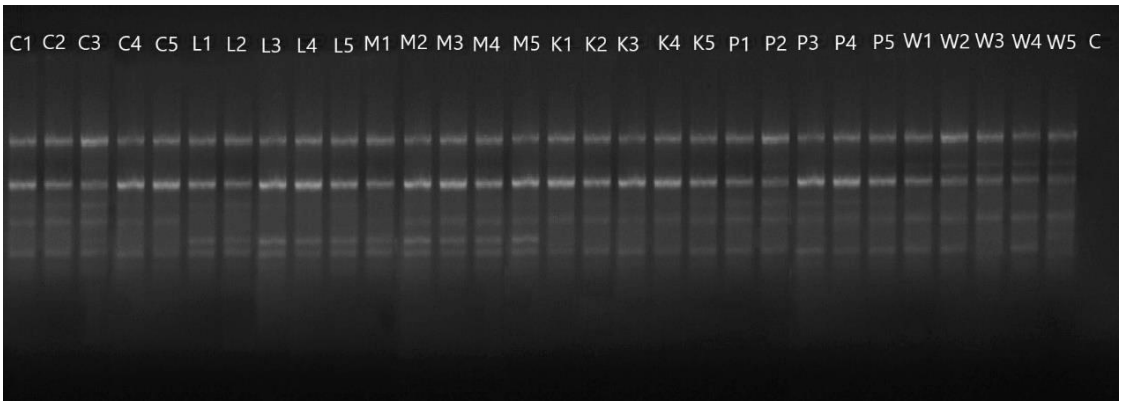
Observed polymorphism percentage in UBC 878 was 70%. Ten bands were produced by the primer in whole profile in which seven bands were polymorphic. It produced distinct bands for C3 and C4 which were absent in rest of Chimmony samples. This primer cannot differentiate populations from Meppadi and Pookode since bands produced was similar but distinguished them from other populations due to the presence of a distinct band. A distinct amplicon produced in Mankulam population differentiated them from other populations in South of Palghat gap. Out of seven polymorphic bands a single band was absent in K3 that distinguished the sample from other samples. Population of Kakkayam is differentiated from Pookode and Wayanad (Plate 8c).

UBC 881

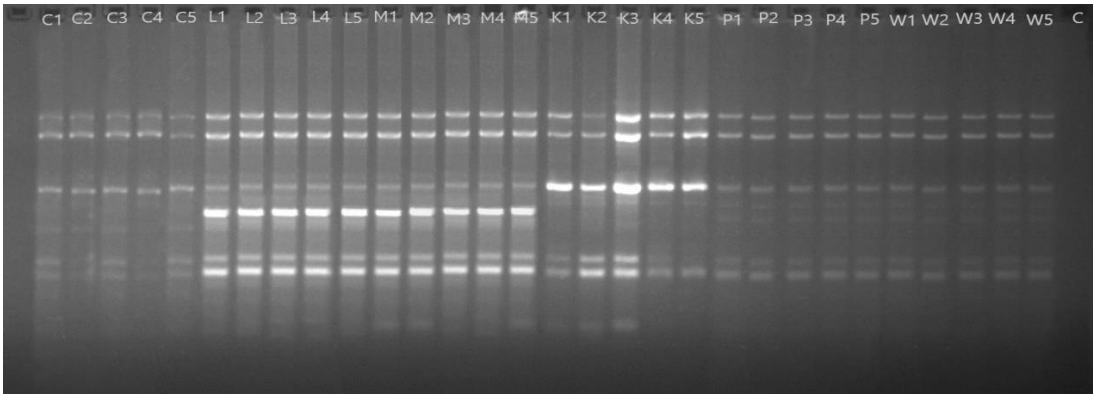
In total, seven amplicons were observed in the amplification profile. Five bands were polymorphic and two are monomorphic bands accounting to 71.43% polymorphism. Out of five polymorphic bands one was absent in Lakkom population. UBC 881 can be used to draw distinction in population from Pookode and Meppadi. It differentiated Lakkom and Mankulam population from other longan populations (Plate 9a).

UBC 884

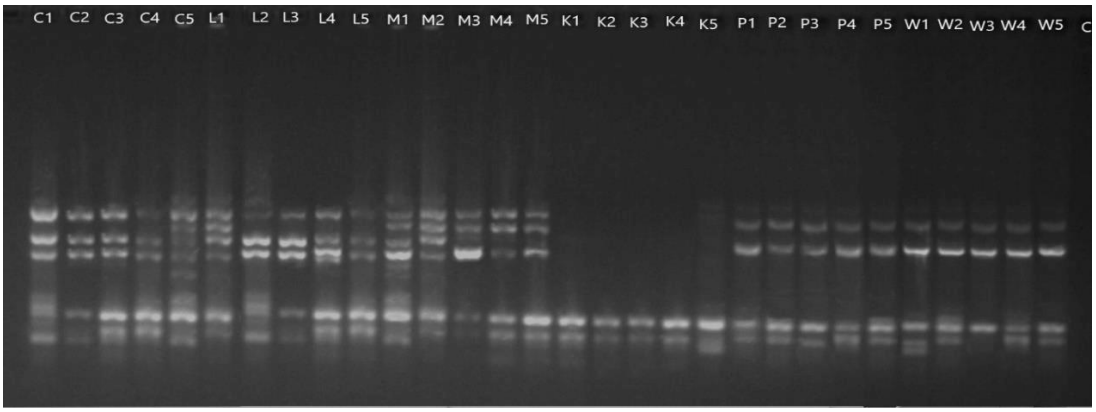
b. In total, nine amplicons were produced out of which six were polymorphic and three were monomorphic bands. Most of the bands produced were present in majority of the samples. The primer cannot differentiate Lakkom and Mankulam population since they produced similar bands. The primer cannot differentiate populations from Pookode and



a. ISSR primer UBC 881



b. ISSR primer UBC 884



c. ISSR primer UBC 890

Plate 9. Amplification profile of ISSR primers for samples of longan from six selected locations

Meppadi. Absence of a band differentiated populations of Chimmony and Kakkayam from rest of the population (Plate 9b).

UBC 890

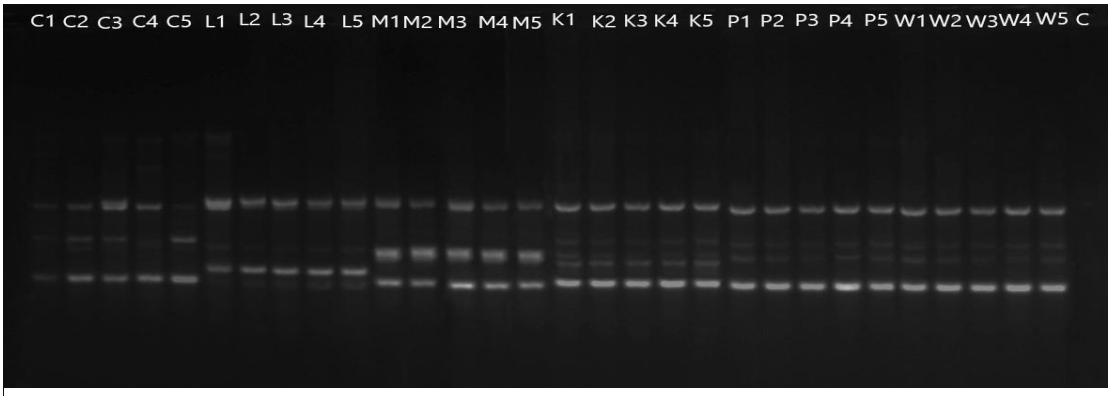
Nine amplicons were generated in total by UBC 890. Out of nine, seven bands showed polymorphism with a polymorphic percentage of 77.78%. Primer was used to differentiate among longan population. Kakkayam had a different banding patter from other samples from North of Palghat gap. It clearly distinguished populations from south and north of Palghat gap. A distinct band produced which differentiated W1 of Meppadi from other samples of Meppadi and W3 from other Meppadi population due to the absence of an amplicon (Plate 9c).

UBC 891

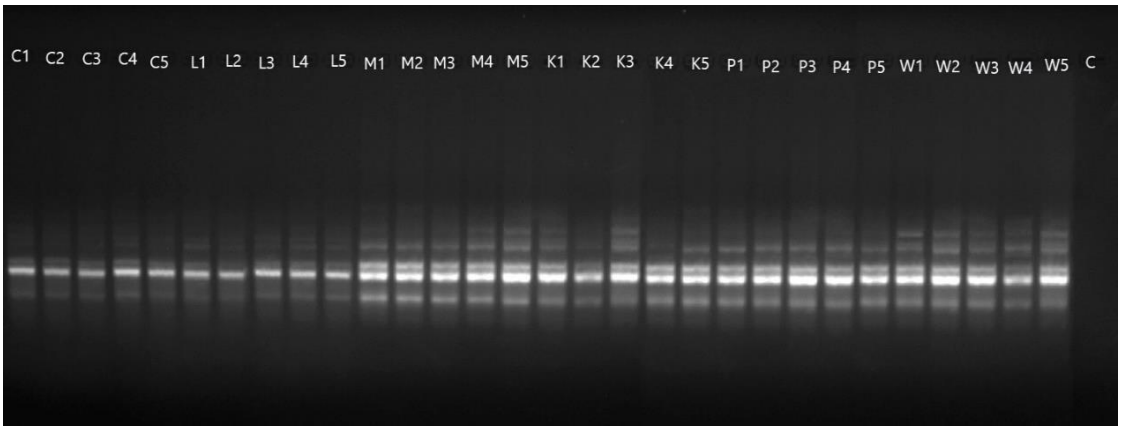
Out of nine amplicons, five were polymorphic and four were monomorphic bands. UBC 891 showed 55.56% polymorphism. It differentiated Meppadi population from rest of the longan populations due to the absence of an amplicon. UBC 891 produced a distinct amplicon for the Kakkayam population that help to distinguish samples from Kakkayam. It differentiated plus trees within Chimmony population. It also differentiated plus trees within longan population in Mankulam due to the presence of distinct amplicon in M5 (Plate 10a).

L1

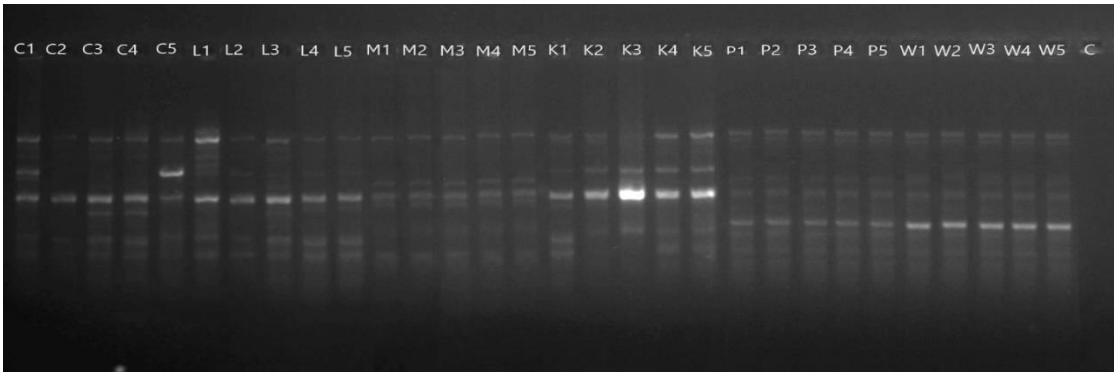
The primer showed 50% polymorphism. Out of total 8 bands produced four were polymorphic and four were monomorphic. Distinct bands were produced for Chimmony population. L1 distinguished population in Meppadi from other populations since there is distinct band in the population. It cannot differentiate Mankulam and Kakkayam population due to similar banding pattern (Plate 10b).



a. ISSR primer UBC 823

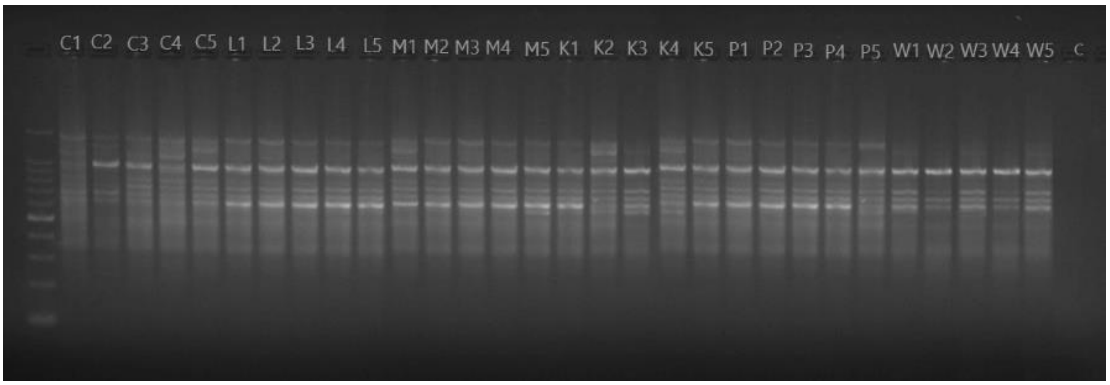


b. ISSR primer UBC 864

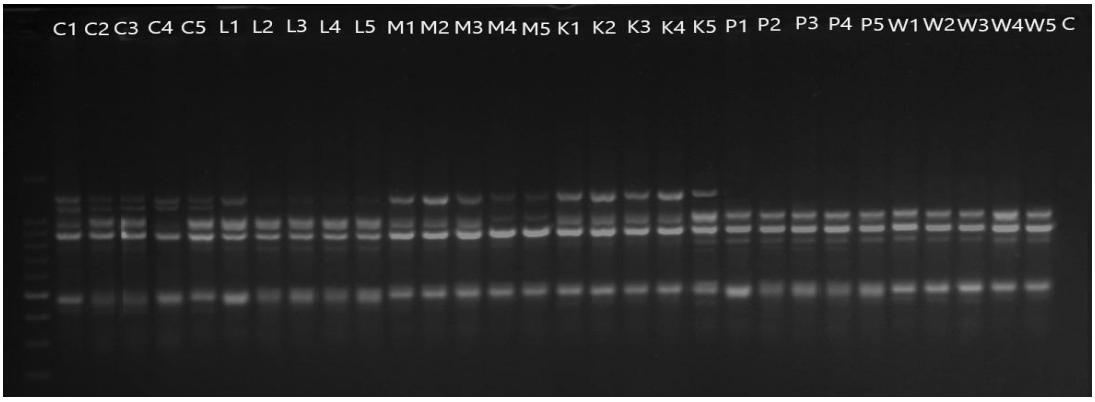


c. ISSR primer UBC 878

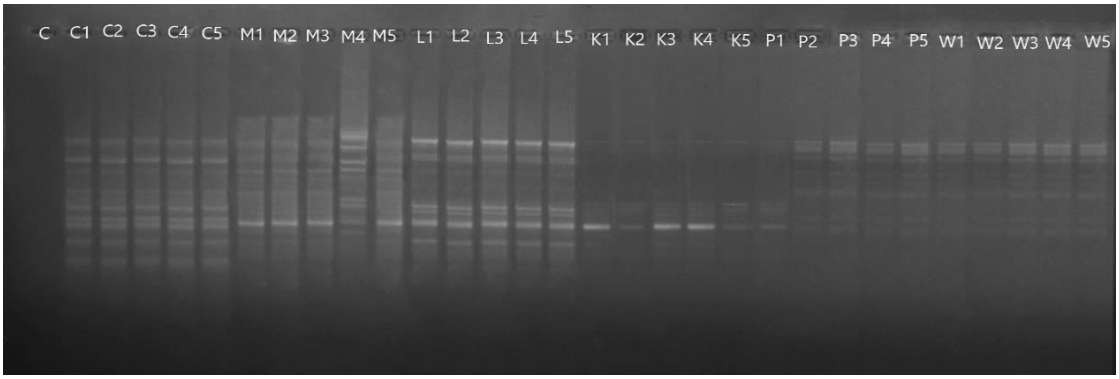
Plate 8. Amplification profile of ISSR primers for samples of longan from six selected locations



a. ISSR primer UBC 891



b. ISSR primer L1



c. ISSR primer L3

Plate 10. Amplification profile of ISSR primers for samples of longan from six selected locations

L3

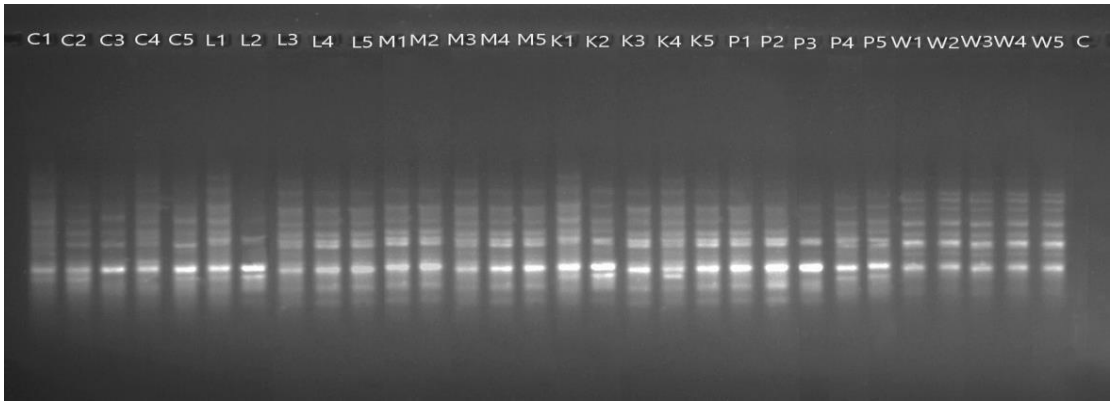
Nine bands were produced in total by L3 after amplification. Eight bands were polymorphic and observed highest amount of polymorphism giving 88.89% polymorphism percentage. Banding pattern of M4 differed from other samples from Mankulam. L3 clearly differentiate population in Kakkayam from the rest. It distinguished samples of Lakkom from other population in South of Palghat gap and Kakkayam from other populations in North of Palghat gap. It cannot differentiate population from Meppadi and Pookode but the absence of a single amplicon in P1 differentiated it from other samples in Pookode and Meppadi. Clearly differentiate the population between population from North and South of Palghat gap (Plate 10c).

L4

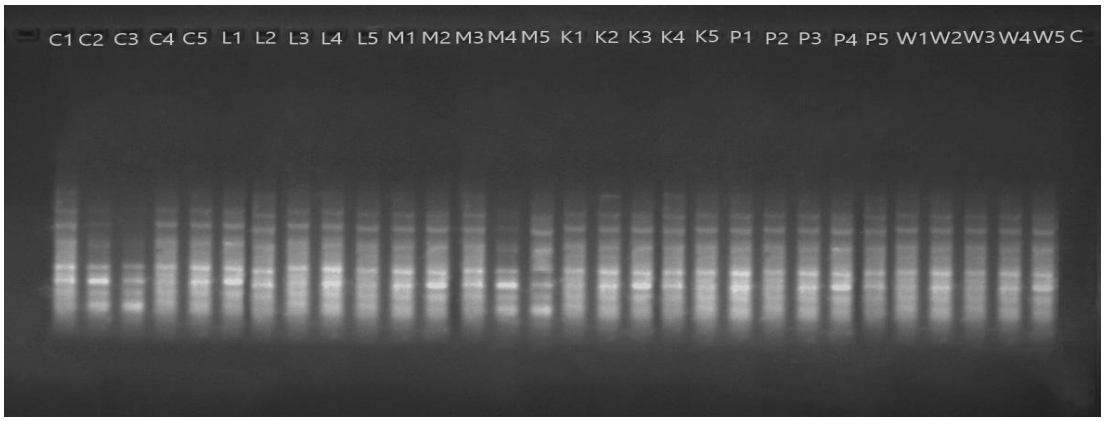
PCR using L4 generated ten amplicons in total out of which six where polymorphic bands and four bands were monomorphic bands. It was used to differentiate the population of longan from different locations. It produced distinct bands for the population in Kakkayam. It can differentiate populations from Pookode and Meppadi. It cannot distinguish populations from Lakkom and Mankulam. It revealed genetic similarity existing between K2 and K4 among Kakkayam population and distinguished K1 from other Kakkayam samples due to the presence of a distinct band (Plate 11a).

L9

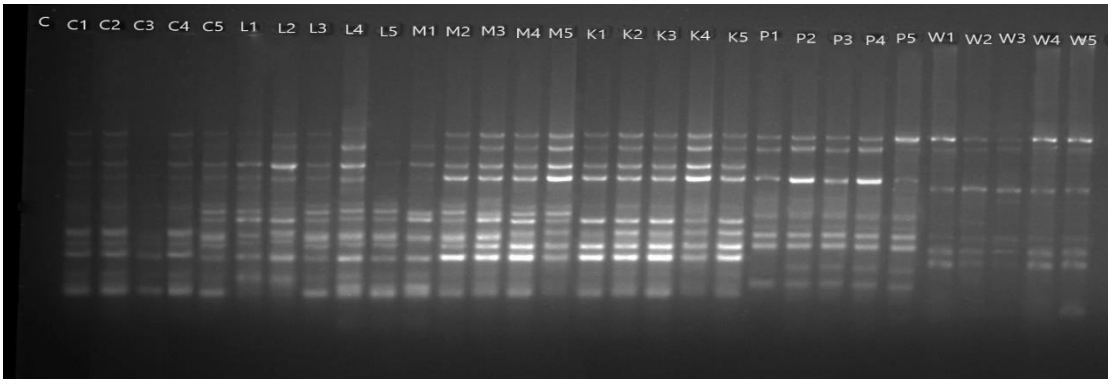
Amplification employing L9 produced nine amplicons. Four bands produced were polymorphic and five were monomorphic bands with 44.44%. Banding profile obtained by Kakkayam, Pookode and Meppadi populations are similar. It can differentiate populations in North and South of Palghat gap. It can differentiate C3 from other samples from Chimmony since an amplicon is absent in C3 (Plate 11b).



a. ISSR primer L4



b. ISSR primer L9



c. ISSR primer L10

Plate 11. Amplification profile of ISSR primers for samples of longan from six selected locations

L10

DNA amplification with L10 generated total of 11 bands. Eight amplicons were polymorphic giving 72.73% polymorphism. This primer differentiated genetic diversity within the population of Chimmony an also in Lakkom population. L10 clearly differentiate populations in South and North of Palghat gap. This primer can differentiate longan populations from Pookode and Meppadi. Out of 8 polymorphic bands, one amplicon is absent in Pookode population and thus can distinguish from other longan populations (Plate 11c).

Out of 19 primers, five ISSR primers (UBC 809, UBC 810, UBC 890, L3, L9 an L10) distinguished the populations of north and south of Palghat gap. Population of Kakkayam stood different from rest of the wild longan population in the banding profile obtained from most of the primers. ISSR primers UBC 809, UBC 881, L3 and L4 distinguished populations within south of Palghat gap and primers UBC 864, UBC 878, UBC 890, L4 and L10 differentiated populations among North of Palghat gap. L4 was comparatively best in distinguishing the longan populations from different locations.

Summary of the total amplicons and polymorphic bands produced by different selected ISSR primers are graphically represented in figure 19.

PIC (Polymorphic Information Content) value of the 15 primers (figure 20) ranged between 0.067 and 0.39. Least value was recorded for UBC 884 and highest value was observed for UBC 816 with an average of 0.24.

Binary scoring was done for scorable amplicons. Value of 0 was given for absence of DNA amplicon and 1 for presence of bands at each locus of sample. Based on this binary data, a dendrogram was created using cluster analysis on the basis of the unweighted pair group method with arithmetic mean (UPGMA).

Data analysis was done using mainly three softwares: DARwin software, PAST software and GRAPES software (KAU).

Factorial analysis of the above data were done using DARwin software for grouping related populations (figure 21). Factorial analysis resulted in four genetically related groups, group I (Chimmony), group II (Mankulam and Lakkom), group III (Kakkayam), and group IV (Pookode and Meppady).

The dendrogram grouped the wild *Dimocarpus longan* populations into two clusters on the basis the similarity coefficient (figure 22). The first cluster (Cluster I) constitute population from Chimmony, Lakkom and Mankulam. Within the first cluster, population from Lakkom and Mankulam had a common root. Cluster I accounts the population in South of Palghat gap. Within Chimmony populations, it formed 2 subclusters. Cluster II constitutes population from Kakkayam, Pookode and Meppadi. The selected plus trees from Meppadi and Lakkom shared same root.

Tree genetic distance among the samples were also calculated using DARwin software which is presented in figure 23.

PCA was worked out based on the molecular characterization using selected ISSR primer to identify the principle factors affecting the genetic variance in GRAPES software. Based on the scree plot obtained through PCA (figure 24), PC1 and PC2 are the main components that affect the genotypic variance existing, all other components can be omitted from analysis due to their very low contribution to the genetic variance.

The correlation of amplicons of different primers are depicted in a graphical representation (figure 25).

Past software was used for PCA (Principle Component Analysis) to obtain a scatter plot to find related population based on the genetic closeness.

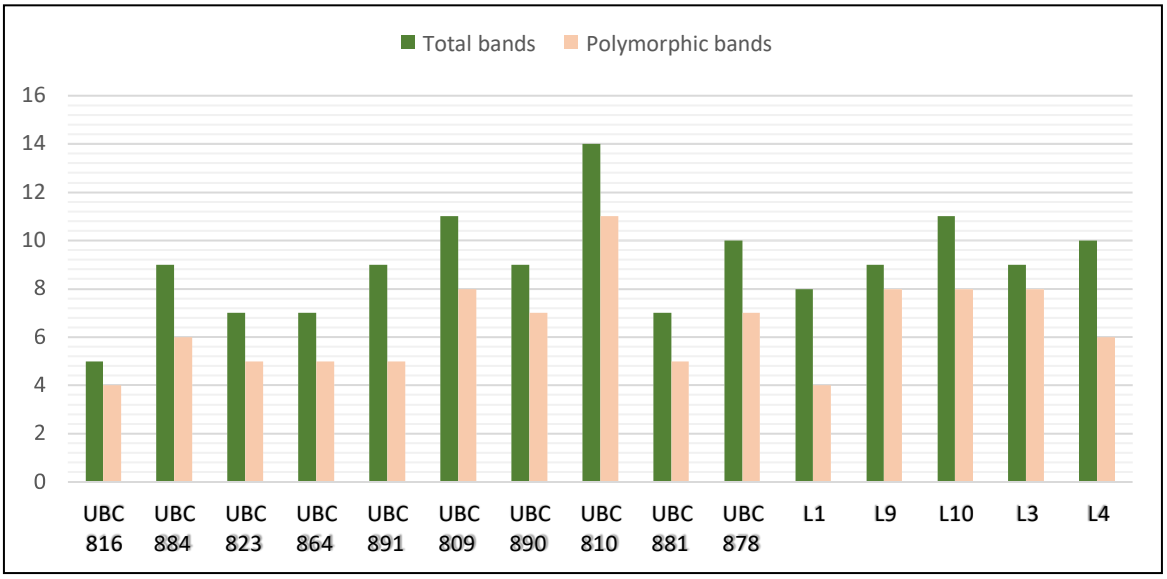


Figure 19. Summary of total amplicons and polymorphic bands produced by 15 ISSR primer in longan samples from six locations

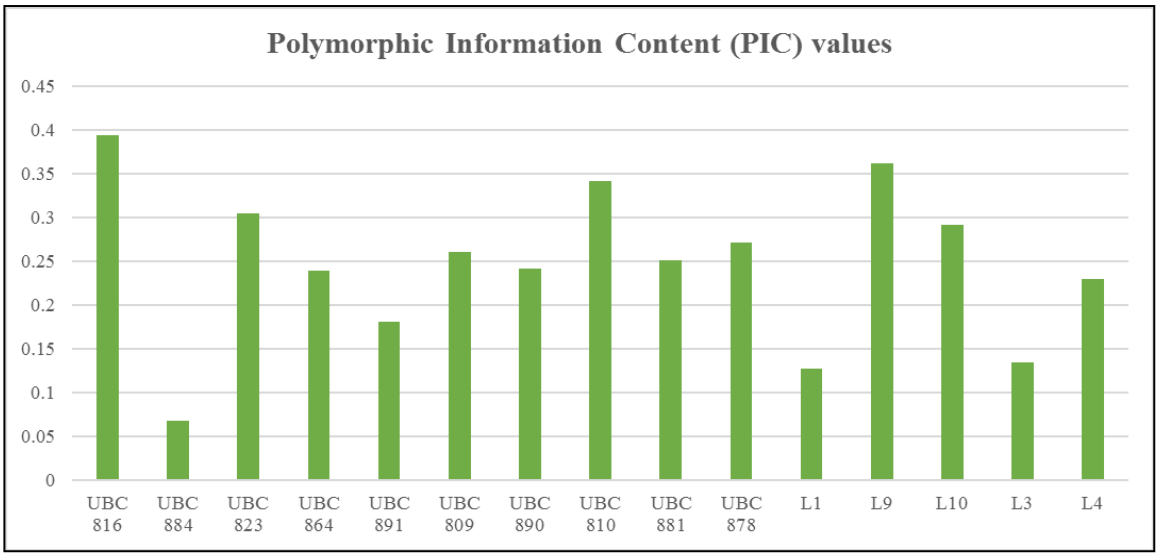


Figure 20. Polymorphic Information Content values of 15 ISSR primer in longan samples from six locations

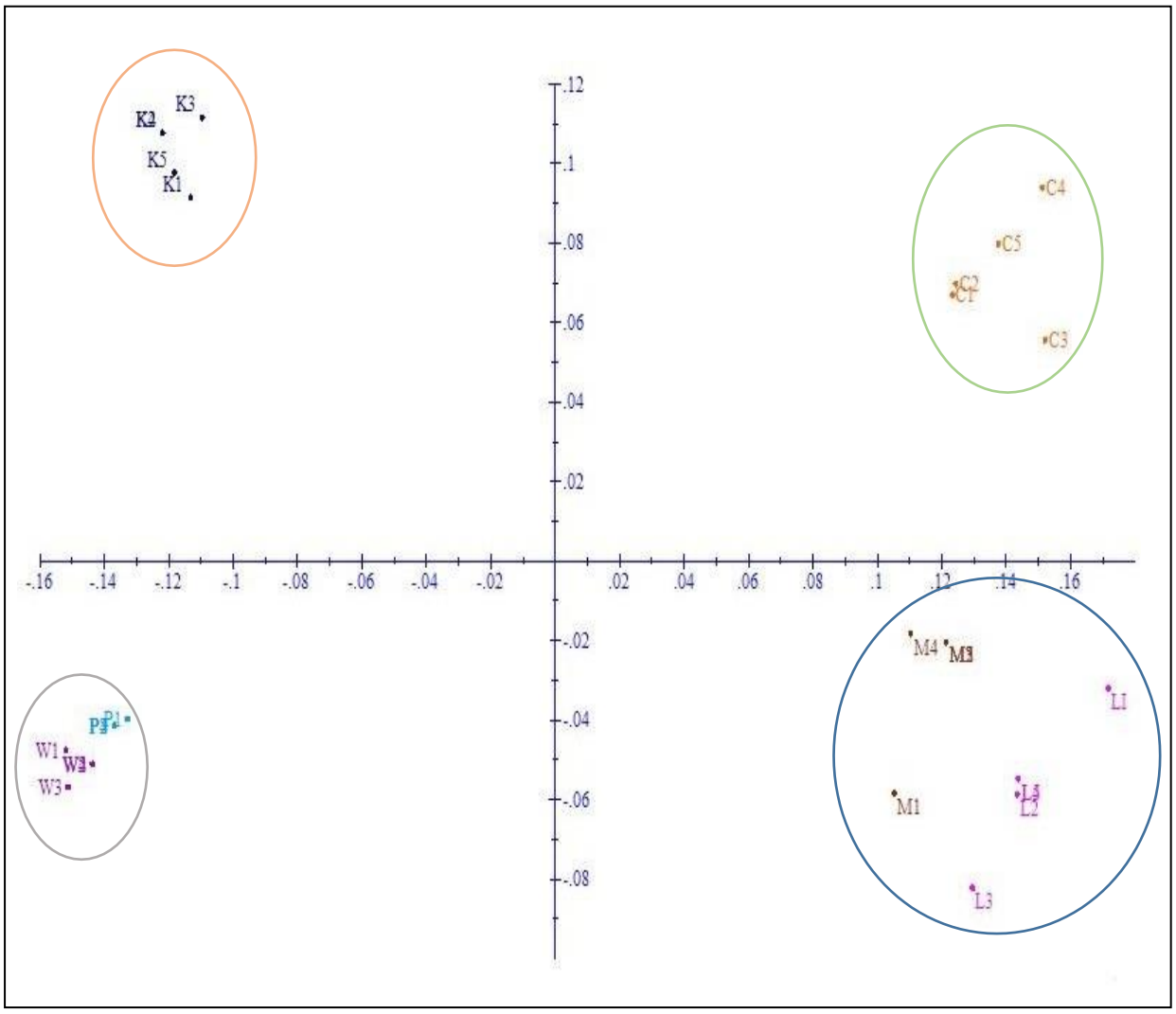


Figure 21. Clustering different locations based on factorial analysis using ISSR primer amplicons

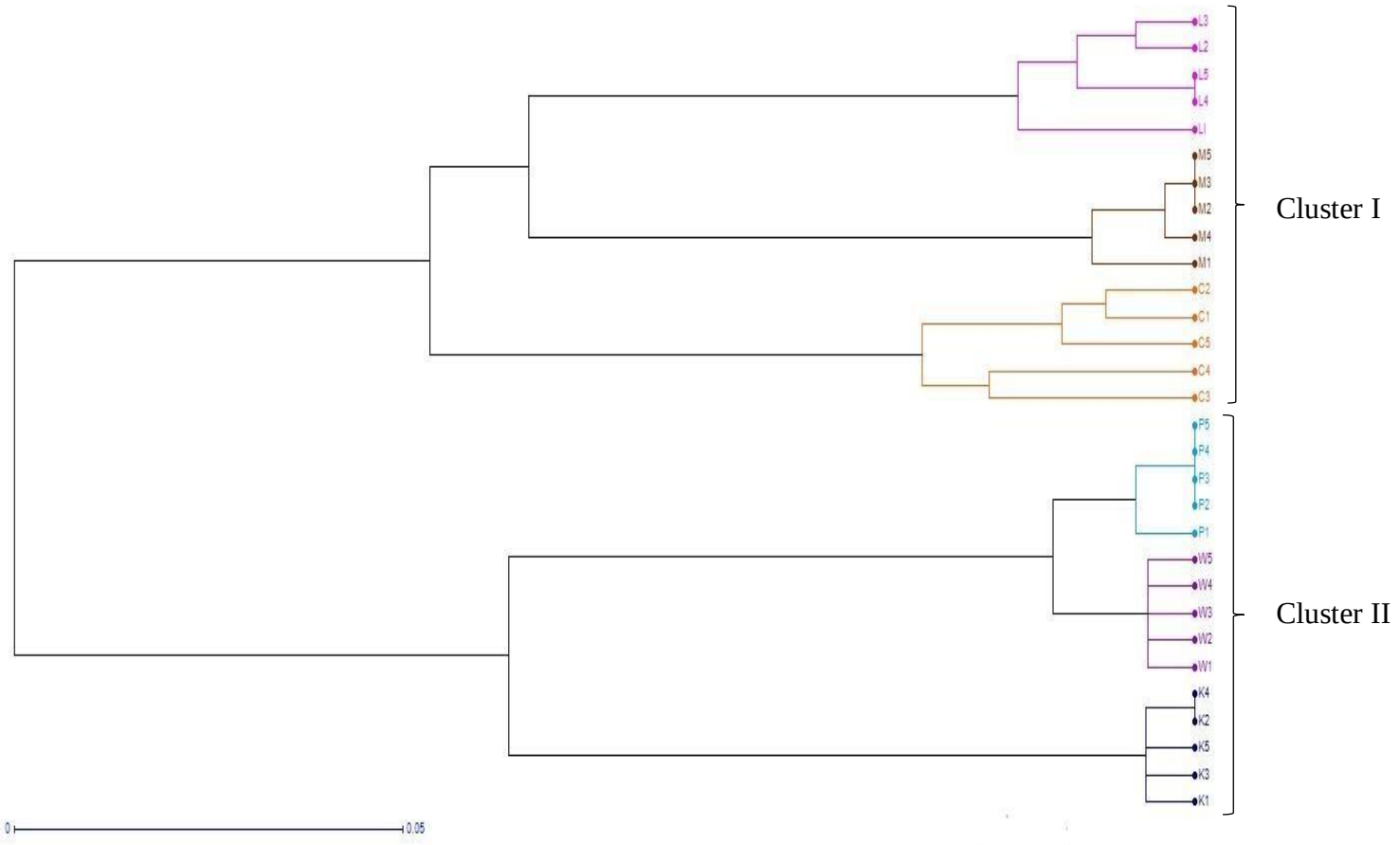


Figure 22. Hierarchical dendrogram of wild longan populations from six locations

(1-5: Samples from Chimmony, 6-10: samples from from Lakkom, 11-15: samples from Mankulam, 16-20: samples from Kakkayam, 21-25: samples from Pookode, 26-30: samples from Meppadi)

4.4 GENETIC DIVERSITY AMONG WILD LONGAN POPULATION (SOUTHERN WESTERN GHATS, KERALA), COMMERCIALLY AVAILABLE NURSERY VARIETIES AND LITCHI

The genetic relation of wild population of longan was compared with that of the commercially cultivated longan and litchi, a close relative of *D. longan*. Leaf samples of commercially cultivated longan samples were collected from a fruit tree nursery near KAU, Vellanikkara. Three samples were collected, two from a longan variety commonly known as E-daw longan and the other is white longan. Leaf samples of Litchi was obtained from College of forestry, KAU, Vellanikkara.

DNA were extracted from the samples using modified CTAB method. The quality and quantity of DNA extracted was assessed using Nanodrop spectrophotometer. Thequantity and $OD_{260/280}$ of DNA samples from Nelliyampathy (Palakkad, South of Palghatgap), 3 samples of commercially cultivate nursery varieties and Litchi are given in table 12.

Summary of total number of bands produced by each primer including the polymorphism percentage is presented in table 13. 158 amplicons were produced in total by the 15 selected primers. The percentage of polymorphism ranged from 80 to 100% with an average polymorphism percentage of 92.58%. 100% polymorphism was obtained for UBC 816, UBC 809, UBC 878, L1, L9 and L10. Least polymorphism percentage was shown by ISSR primer UBC 890. Highest number of amplicons was observed in UBC 810 (15 bands) with highest number of polymorphic bands (14 bands).

Summary of the total bands and polymorphic bands obtained by different selected ISSR primers are graphically represented in figure 26.

Units	C1	C2	C3	C4	C5	LI	L2	L3	L4	L5	M1	M2	M3	M4	M5	K1	K2	K3	K4	K5	P1	P2	P3	P4	P5	W1	W2	W3	W4
C2	0.6287																												
C3	0.6845	0.2037																											
C4	0.1754	0.6293	0.685																										
C5	0.6129	0.1321	0.1878	0.6134																									
LI	0.1818	0.6357	0.6915	0.1824	0.6198																								
L2	0.3756	0.5656	0.6214	0.3761	0.5498	0.3826																							
L3	0.5891	0.417	0.4728	0.5897	0.4012	0.5961	0.5261																						
L4	0.3599	0.4509	0.5067	0.3604	0.4351	0.3669	0.2968	0.4114																					
L5	0.3599	0.4509	0.5067	0.3604	0.4351	0.3669	0.2968	0.4114	0.1821																				
M1	0.611	0.3655	0.4213	0.6115	0.3497	0.6179	0.5479	0.3993	0.4332	0.4332																			
M2	0.6149	0.1341	0.1257	0.6155	0.1183	0.6219	0.5519	0.4033	0.4372	0.4372	0.3517																		
M3	0.6149	0.1341	0.1257	0.6155	0.1183	0.6219	0.5519	0.4033	0.4372	0.4372	0.3517	0.0561																	
M4	0.3844	0.4693	0.5251	0.3849	0.4534	0.3914	0.3213	0.4297	0.2066	0.2066	0.4516	0.4555	0.4555																
M5	0.6149	0.1341	0.1257	0.6155	0.1183	0.6219	0.5519	0.4033	0.4372	0.4372	0.3517	0.0561	0.0561	0.4555															
K1	0.5065	0.3318	0.3875	0.507	0.3159	0.5134	0.4434	0.2948	0.3287	0.3287	0.314	0.318	0.318	0.347	0.318														
K2	0.2656	0.4916	0.5474	0.2662	0.4758	0.2726	0.2385	0.4521	0.2228	0.2228	0.4739	0.4779	0.4779	0.2473	0.4779	0.3694													
K3	0.3745	0.4824	0.5382	0.3751	0.4666	0.3815	0.3115	0.4428	0.2136	0.2136	0.4647	0.4686	0.4686	0.2381	0.4686	0.3602	0.2375												
K4	0.2656	0.4916	0.5474	0.2662	0.4758	0.2726	0.2385	0.4521	0.2228	0.2228	0.4739	0.4779	0.4779	0.2473	0.4779	0.3694	0.1285	0.2375											
K5	0.4628	0.3762	0.432	0.4634	0.3604	0.4698	0.3997	0.3367	0.285	0.285	0.3585	0.3625	0.3625	0.3034	0.3625	0.254	0.3257	0.3165	0.3257										
P1	0.404	0.3174	0.3732	0.4045	0.3016	0.411	0.3409	0.2779	0.2262	0.2262	0.2997	0.3037	0.3037	0.2446	0.3037	0.1952	0.2669	0.2577	0.2669	0.1515									
P2	0.3824	0.261	0.3168	0.383	0.2452	0.3894	0.3194	0.2215	0.2047	0.2047	0.2433	0.2473	0.2473	0.223	0.2473	0.1388	0.2453	0.2361	0.2453	0.13	0.0711								
P3	0.3824	0.261	0.3168	0.383	0.2452	0.3894	0.3194	0.2215	0.2047	0.2047	0.2433	0.2473	0.2473	0.223	0.2473	0.1388	0.2453	0.2361	0.2453	0.13	0.0711	0.0148							
P4	0.3824	0.261	0.3168	0.383	0.2452	0.3894	0.3194	0.2215	0.2047	0.2047	0.2433	0.2473	0.2473	0.223	0.2473	0.1388	0.2453	0.2361	0.2453	0.13	0.0711	0.0148	0.0148						
P5	0.3824	0.261	0.3168	0.383	0.2452	0.3894	0.3194	0.2215	0.2047	0.2047	0.2433	0.2473	0.2473	0.223	0.2473	0.1388	0.2453	0.2361	0.2453	0.13	0.0711	0.0148	0.0148	0.0148					
W1	0.5435	0.298	0.3538	0.5441	0.2822	0.5505	0.4805	0.3318	0.3658	0.3658	0.2803	0.2843	0.2843	0.3841	0.2843	0.2466	0.4064	0.3972	0.4064	0.2911	0.2322	0.1759	0.1759	0.1759	0.1759				
W2	0.2885	0.4238	0.4796	0.2891	0.408	0.2955	0.2255	0.3842	0.155	0.155	0.4061	0.41	0.41	0.1795	0.41	0.3016	0.1514	0.1696	0.1514	0.2579	0.1991	0.1775	0.1775	0.1775	0.1775	0.3386			
W3	0.5223	0.3502	0.4059	0.5228	0.3343	0.5293	0.4592	0.3106	0.3445	0.3445	0.3324	0.3364	0.3364	0.3629	0.3364	0.2279	0.3852	0.376	0.3852	0.2698	0.211	0.1546	0.1546	0.1546	0.1546	0.265	0.3174		
W4	0.2885	0.4238	0.4796	0.2891	0.408	0.2955	0.2255	0.3842	0.155	0.155	0.4061	0.41	0.41	0.1795	0.41	0.3016	0.1514	0.1696	0.1514	0.2579	0.1991	0.1775	0.1775	0.1775	0.1775	0.3386	0.0836	0.3174	
W5	0.2885	0.4238	0.4796	0.2891	0.408	0.2955	0.2255	0.3842	0.155	0.155	0.4061	0.41	0.41	0.1795	0.41	0.3016	0.1514	0.1696	0.1514	0.2579	0.1991	0.1775	0.1775	0.1775	0.1775	0.3386	0.0836	0.3174	0.0836

Figure 23. Tree distance based on the UGPMA cluster analysis

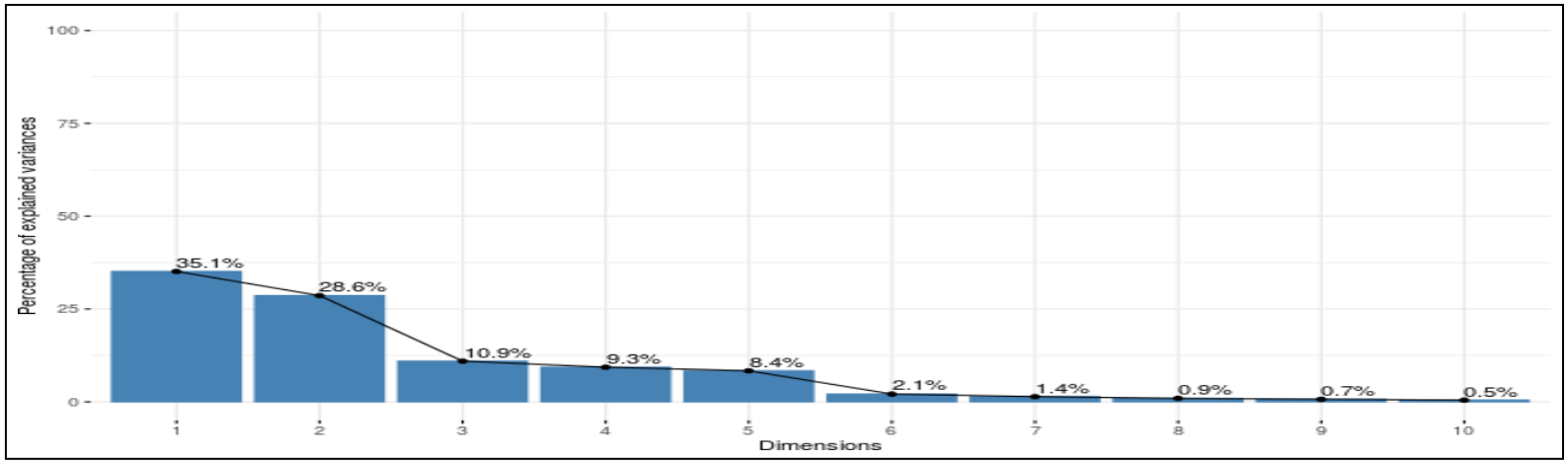


Figure 24. Scree plot based on PCA analysis based on molecular characterization of wild longan from selected locations

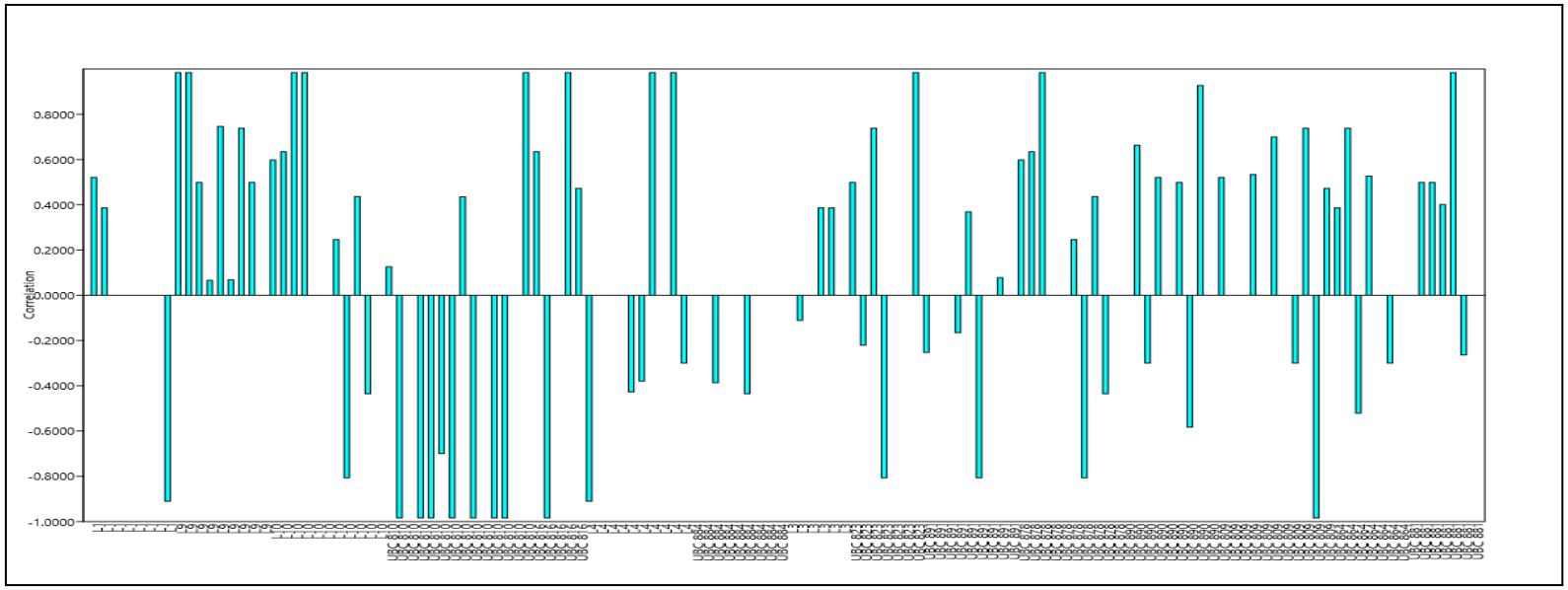


Figure 25. Correlation of DNA bands produced by the selected ISSR primer

Table 12. Quantity and quality of extracted DNA obtained using Nano Drop spectrophotometer

Sl. No.	Location	Sample	OD $_{260/280}$	Quantity of DNA (ng μL^{-1})
1.	Nelliyampathy	N	1.71	532.98
2.	Francis Garden	E1	2.08	1519.6
3.		E2	1.86	1216.36
4.		WL	1.59	845.97
5.	COF	L	1.93	944.66

Table 13. Total number of amplicons and percentage polymorphism exhibited by different primers in wild longan, cultivated longan and litchi

Primer	Total bands	Polymorphic bands	Monomorphic bands	Percentage polymorphism
UBC 809	13	13	0	100.00
UBC 810	15	14	1	93.33
UBC 816	6	6	0	100.0
UBC 823	8	7	1	87.50
UBC 864	8	7	1	85.71
UBC 878	10	10	0	100.00
UBC 881	8	7	1	87.50
UBC 884	11	9	2	81.81
UBC 890	10	8	2	80.00
UBC 891	13	12	1	92.30
L1	12	12	0	100.00
L3	9	8	1	88.88
L4	12	11	1	91.66
L9	12	12	0	100.00
L10	11	11	0	100.00
Total	158	147	11	92.58

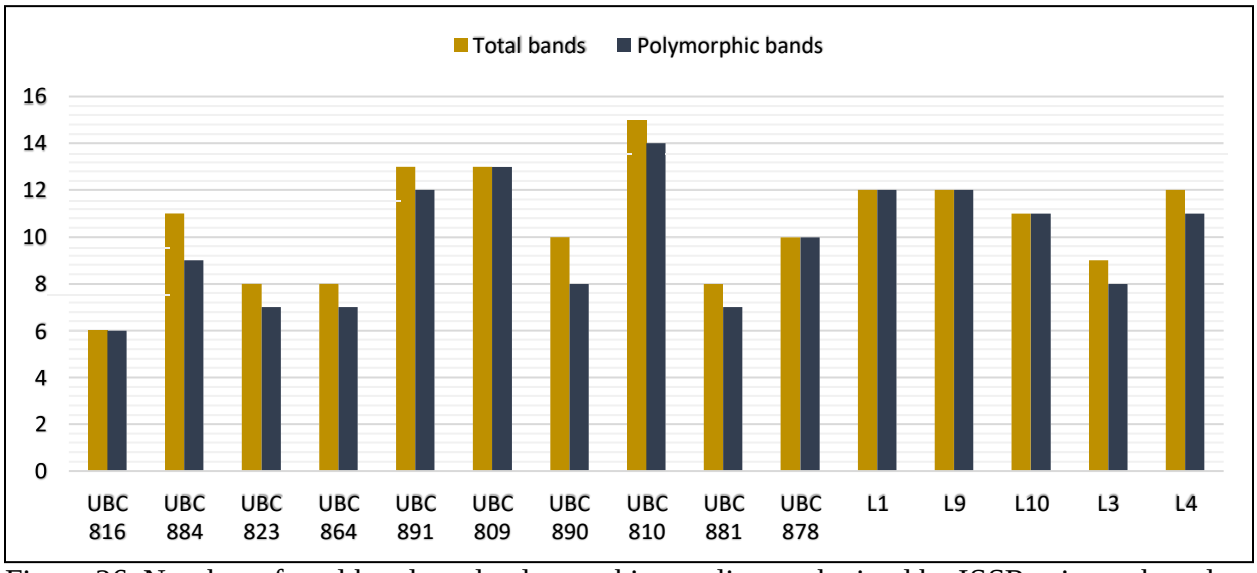


Figure 26. Number of total bands and polymorphic amplicons obtained by ISSR primers based on the molecular characterization of wild longan, cultivated varieties and litchi

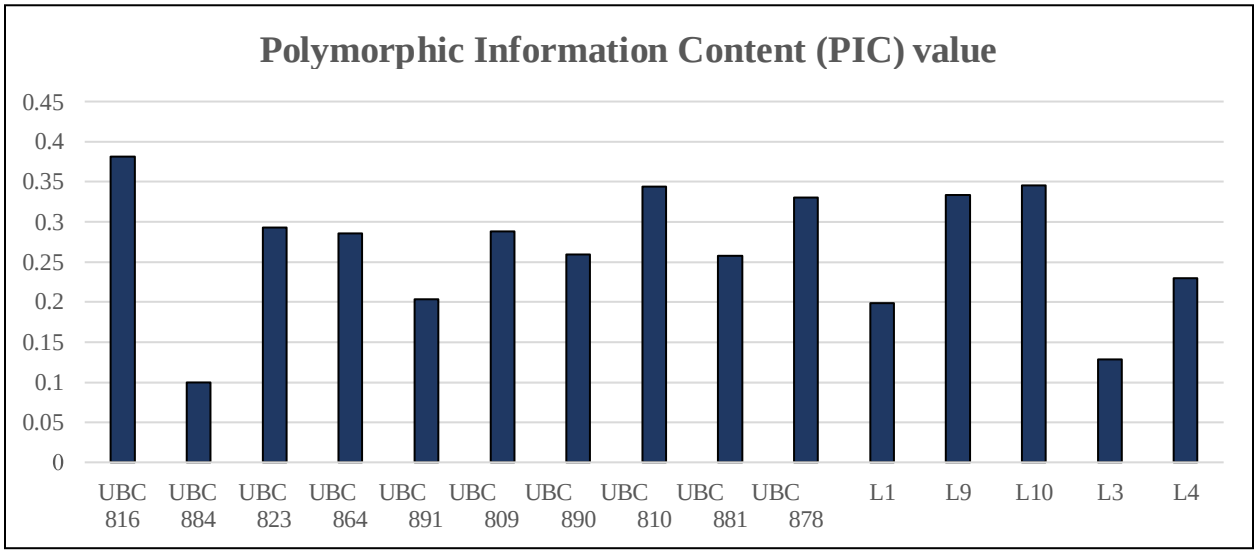


Figure 27. PIC values of selected ISSR primer based on the molecular characterization of wild longan, cultivated varieties and litchi

PIC value of used 15 primers are presented in figure 27. The PIC value varied from 0.099 and 0.38 with an average value of 0.27. Least value was observed for UBC 884 and highest value was recorded for UBC 816.

Repeatable and reproducible DNA amplicons were score manually in to binary forms, 0 representing absence of bands and 1 representing presence of DNA band. Dendrogram was obtained on the basis of the binary matrix using cluster analysis with UPGMA. Three softwares were used to analyze the obtained data: *viz.* DARwin software, GRAPES software (KAU) and PAST software.

Factorial analysis was done using DARwin software to differentiate the population from different locations and to categorize the *D. longan* varieties and litchi (figure 28).

Dendrogram obtained had mainly 4 clusters. Cluster 1 comprised of the wild longan population from the locations constituting South of Palghat gap (Chimmony, Lakkom, Mankulam, Nelliyampathy). Cluster II contained the wild longan populations from the locations constituting North of Palghat gap (Pookode, Meppadi, Kakkayam). Cluster III constitute the samples collected from the nursery constituting the commercially cultivated longan varieties. The two varieties collected, white longan and longan variety commonly called Edaw longan were found in a single cluster indicating that they are genetically related. The cluster IV for the litchi. Litchi is separated from rest of the samples in the dendrogram indicating that litchi is a different species. Hierarchial dendrogram using UGPMA obtained is shown in figure 29.

Tree was generated according to the genetic relation between the samples (figure 30).

Individual PCA for each primer were calculated using the software and is depicted in pictorial form in figure 31. This was worked out to examine the contribution of different ISSR primers selected to identify the genetic variability present in the wild longan population, cultivated varieties and litchi.

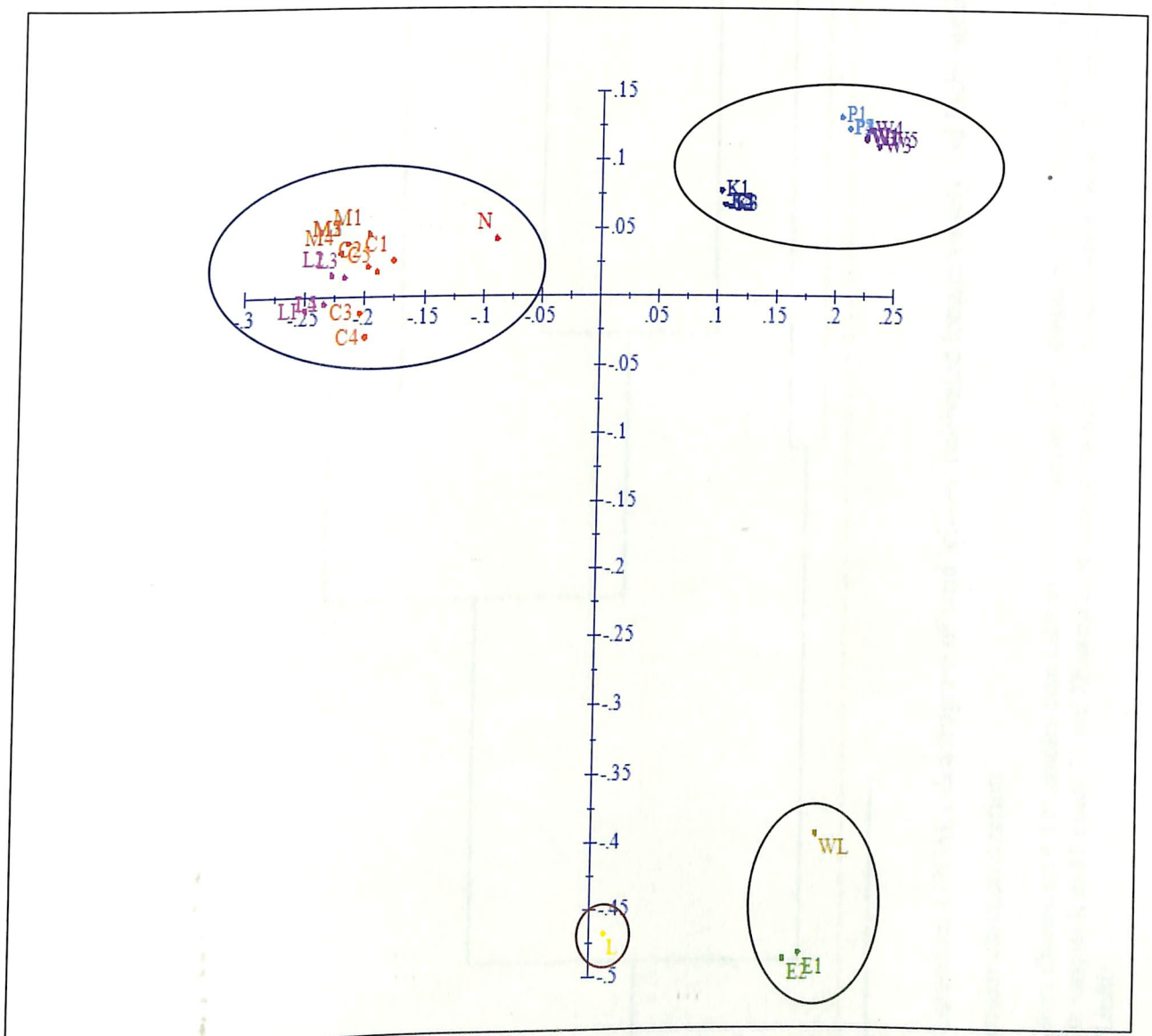


Figure 28. Factorial analysis based on molecular characterization using ISSR primer

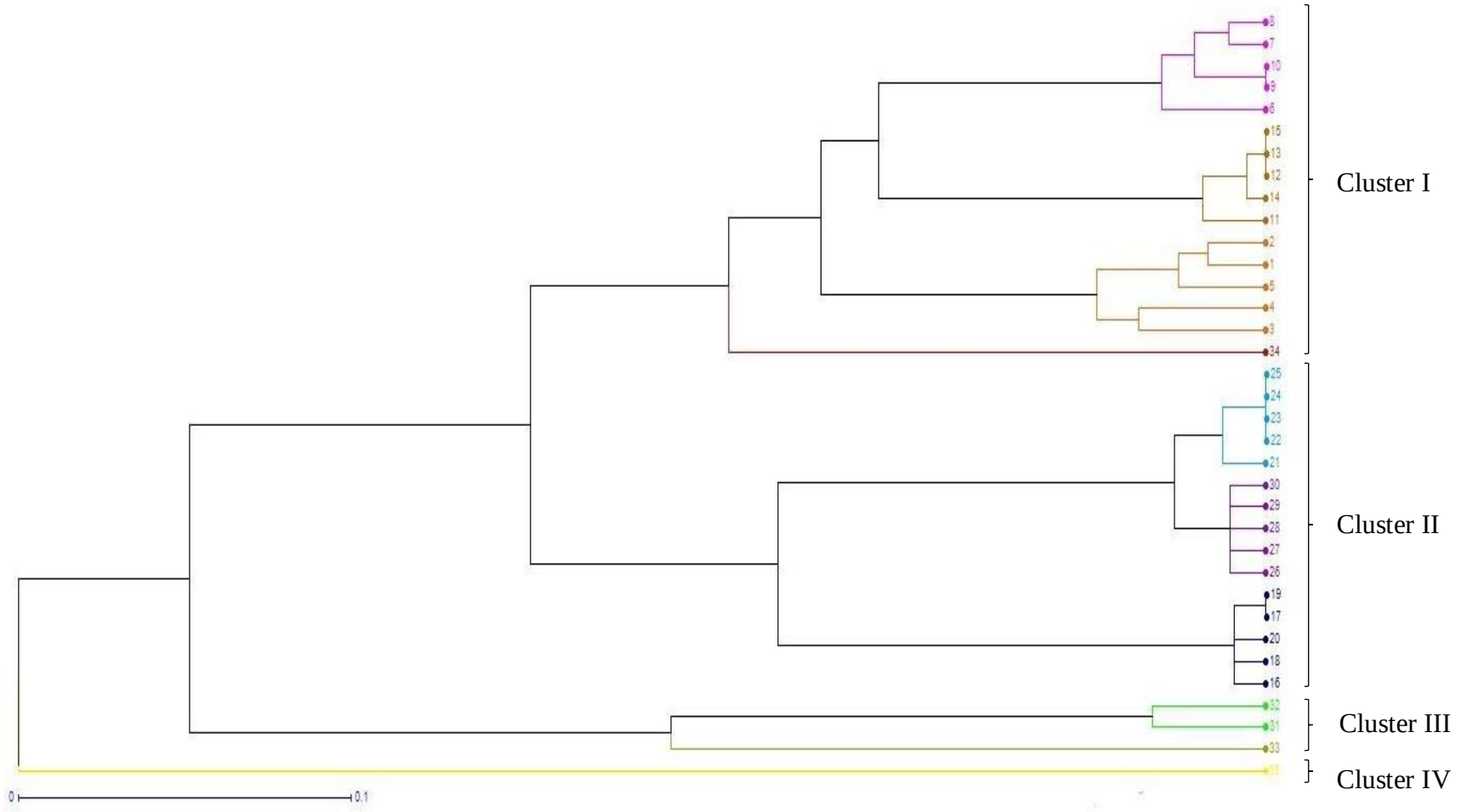


Figure 29. Hierarchial UPGMA dendrogram of wild longan, cultivated longan variety and litchi obtained through molecular characterization

(1-5: Samples from Chimmony, 6-10: samples from Lakkom, 11-15: samples from Mankulam, 16-20: from Kakkayam, 21-25: from Pookode, 26-30: samples from Meppadi, 31 and 32: samples of Edaw cultivar, 33: sample of white longan, 34: sample from Nelliyampathy, 35: sample of Litchi)

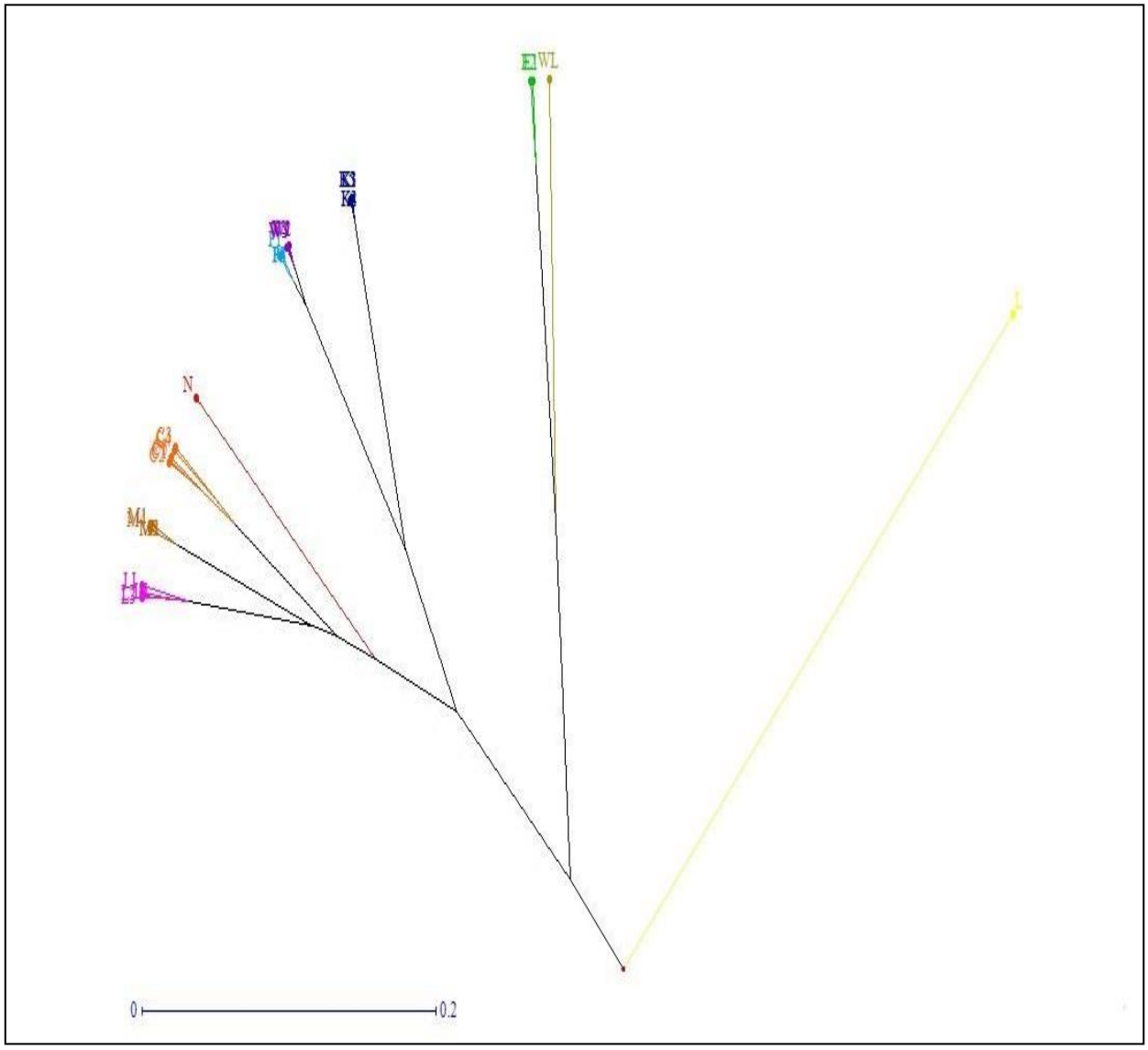


Figure 30. Hierarchical tree of wild longan, cultivated longan variety and litchi constructed using molecular characterization

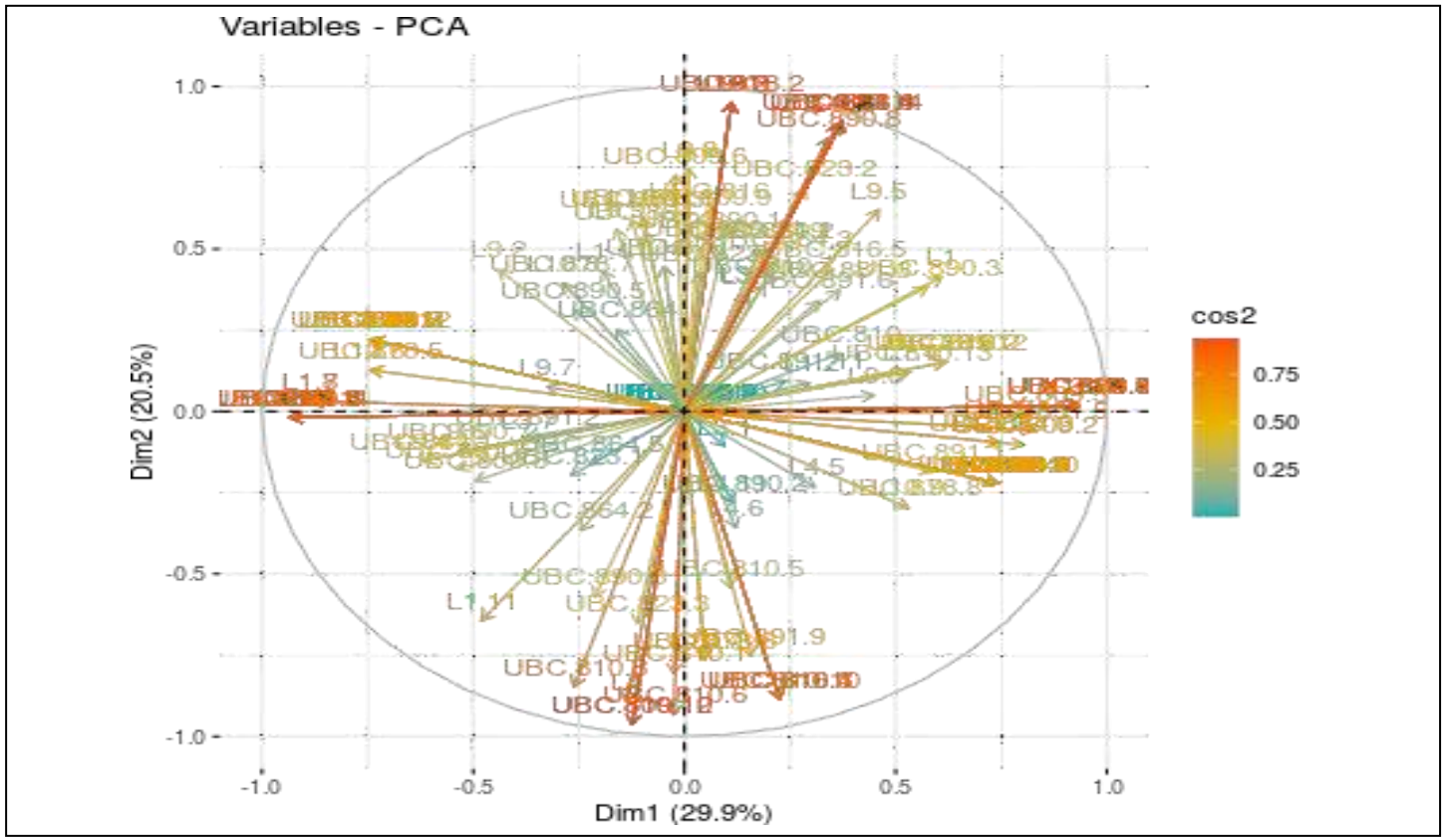


Figure 31. Principle Component Analysis of 15 ISSR primers based on molecular characterization of wild longan, cultivated longan cultivars and litchi

5. DISCUSSION

Dimocarpus longan, commonly known as 'dragon fruit' in market, is an indigenous tree species in the evergreen and semi-evergreen forests in the Western Ghats region of Kerala. It is an important commercial fruit tree that is known to originate in south China being cultivated in various countries in SE Asia. *D. longan* is reported from the Western Ghats from south of Konkon region to Tinnevelly and is described as common evergreen species up to 5000 ft. altitude (Gamble, 1935). Western Ghats is home to a wide variety of wild edible fruit trees. These edible fruits serve as a source of vitamins, minerals, carbohydrates, proteins, fats, and fibers for the local people (Karun *et al.*, 2014). Earlier *D. longan* was known by its synonyms *Euphoria longana* and *Nephelium longana* (Hooker, 1872).

The ultimate aim of a plant breeder is to develop superior plants with characters having agronomic and economic significance. It is important for a breeder to have complete data on the morphological and genetic variation present among the species population to plan the best breeding program. The success of a breeding program relies on the genetic diversity that is naturally existing within the population. Since information regarding their morphological, reproductive as well as naturally existing genetic variation is still unavailable. The improvement of *D. longan* is a challenge for Indian scientists. Different morphological parameters were used to distinguish and classify different litchi cultivars as reported by Wu *et al.* (2016). Descriptors based on morpho-taxonomic characters for *D. longan* are absent. Ke *et al.* (1988) used morphological characters such as leaf and seed size and maturity time to classify the longan varieties in Fujian. Diversity analysis based on morphological characters are not much reliable since they are highly influenced by the prevailing environmental conditions. Information obtained through DNA profiling employing ISSR markers would provide a better understanding of genetic relationship existing among different accessions. . In India, longan is less known and unexplored as a fruit tree. This research study is mainly to find the scope of

improvement of the wild longan which is indigenous to the evergreen forests of Kerala and to analyze the morphological and genetic diversity among the wild population in the southern parts of Western Ghats in Kerala. The study also focused on understanding the extent of genetic relation present between the wild population of *D. longan* in W. Ghats, commercially available nursery variety of longan and Litchi, a close relative of *D. longan*.

5.1 VARIABILITY STUDY

Evaluation of different genotypes for both morphological and molecular characteristics are crucial for tree improvement programs. Genetic variability assessment is an important tool for selecting elite trees from the naturally existing populations. ISSR markers are capable of detecting intraspecific variations and their inheritance according to Mendelian genetics (Zietkiewicz *et al.*, 1994). In addition to the ability to recognize even low levels of genetic diversity among populations, these markers allow the analysis of biogeographical patterns within populations of particular species (Deshpande *et al.*, 2001). For domesticating a plant, it is crucial to study the nature and extent of its genetic variability in the natural population. For surviving and reproducing under different environmental conditions, forest trees in general have great extent of genetic variability (Antonovicks, 1971).

5.2 GENERAL TREE CHARACTERS

Sreekumar *et al.* (2007) had reported the genetic and morphological variability existing among the population of breadfruit (*Artocarpus altilis*) in the Western Ghats employing AFLP marker. Considering the phenotypic trait, they found that the bread fruit weight was correlated with the leaf length and width. Yonemoto *et al.* (2006) have reported the existence of variability among different longan cultivars present in both morphological as well as genetic characters. Lithanatudom *et al.* (2017) reported the genetic relation among different subspecies of *D. longan* (namely, *D. longan spp. longan var. longan*, *D. longan spp. longan var. obtusus, D. longan spp. malesianus var. echinatus, D. longan spp. malesianus var. malesianus*), different Species of Dimocarpus (namely,D.australian

and *D. fumatus)* and litchi (*Litchi chinensis*) on the basis of morphological traits and multilocus molecular markers.

Trees were huge with brown to dark brown twigs, the surface of leaflets were glabrous with acute leaflet apex and flat midrib which was on par with description provided for *D. longan spp. longan var. longan* by Lithanatudom *et al.* (2017).

The species richness of longan differed among different selected locations. Phenotypic characters like crown shape, pattern of tree branching, tree height, tree girth, height of first branch, leaf length and width were considered for the diversity study. Significant difference were observed between the six populations on morphological traits *viz.* tree height, GBH, first branch height and crown shape in few locations among some trees. There were no such significant difference for the characters like leaf length, leaf width and branching pattern. Genetic variance was worked out for the five morphological traits like GBH, total tree height, first branch height, leaf width and length.

Chimmony had a combination of semi- evergreen and evergreen forest with moderate to dense canopy cover. *D. longan* was found in association with some semi-evergreen species like *Dipterocarpus indicus, Drypetes venusta, Hydnocarpus pentandra, Vateria indica, Melicope lunu-ankenda, etc*. *D. longan* was recorded from both semi- evergreen and evergreen forest patches in Chimmony, but mostly from the evergreen patches. The crown shape was more or less irregular and spreading in nature due to dense forest canopy.

Two different locations were selected from Mankulam range. Both were evergreen patches in which a portion of one location was an abandoned cardamom cultivated area and the area was predominantly distributed with *D. longan* trees and its seedlings. The *D. longan* was found in close proximity with other evergreen species namely, *Mesua ferrea, Palaquium ellipticum, Hopea parviflora, Nageia wallichiana, Holigarna beddomei, etc*. The population showed a mixture of crown shape pattern, mostly oblong

along with semi-circular crown shape. Oblong shape can be due to the close proximity of the trees with other huge tree species.

The selected location in Kakkayam range was a dense evergreen forest patch adjacent to Kakkayam dam. *D. longan* was a most common species in the evergreen patches in Kakkayam range along the road side and near the dam area. It was associated with other evergreen species like *Chionanthus mala-elengi, Heritiera papilio, Olea dioica, Symplocos racemosa, Phoebe lanceolata, etc*. Crown shape of the population in Kakkayam was commonly broadly pyramidal and some were irregular. Trees with irregular crown shape were closely distributed whereas those with broadly pyramidal crown shape were comparatively distantly located from adjacent trees.

Pookode region comes under the Kalpetta range in South Wayanad Division. The selected location was an evergreen forest patch near Pookode Lake. The area had a moderately dense canopy covering. *D. longan* was found in association with species like *Symplocos racemosa, Syzigium sp. etc.* The crown shape of the longan population in Pookode were either irregular or semi-circular. Most of the trees that were found isolated had a semicircular crown pattern.

In the Meppadi range, 900 kandi forest patches were selected as the study area which is an evergreen forest predominantly with *D. longan* trees and seedlings. Other evergreen trees in the location were mainly *Ficus nervosa, Garcinia sp.* and *Mesua ferrea*. Meppadi forest area is a vested forest region. Since the area was dominantly distributed with *D. longan*, trees were much taller and crown shapes were mostly pyramidal, some were oblong (for mainly isolated trees) and some were broadly pyramidal. There was much difference in the phenotypic characters like crown shape, tree height, first branch height and girth of the tree in Meppadi compared to other locations. Distant locations were selected within the 900 kandi forest region for selecting the superior trees.

It is common in nature for trees to display a wide variety of general tree attributes including tree height, first branch height, branching patterns, crown shapes, structural

properties of trees, GBH, *etc*. As a general thumb rule, large height, GBH and clean bole are more preferably taken as commercially important tree characters for timber species, whereas for fruit trees such as longan and litchi characters like wide spreading canopy, drooping canopy, low first branch height, low tree height, *etc.* will make it a suitable cultivar for commercial production (Lora *et al.*, 2018).

Wichaipanich (2004) identified different Daw clones of longan by analyzing morphological traits. For selecting a tree for its timber value, trees having clean bole with large height and girth are usually selected whereas for a fruit tree, trees with moderate height and girth with spreading crown are usually selected.

Remarkable variation was observed in the tree girth, value ranging between 1.05m to 2.20m. Highest GBH was observed for the Meppadi population followed by Mankulam population. The trees were not closely distributed as those in other locations.

Height of the tree showed high variation. The tree height of selected superior trees ranged from 6.32m to 11.62m. Population in Meppadi had the highest tree height. Selected region in the Meppadi range was well distributed with *D. longan* along with some other evergreen trees. In comparison with trees in other areas, the trees in the area were exceptionally tall, possibly as a result of competing for sunlight since all the tree species in the location are tall enough to provide a high competition for light interception and can also be attributed to the edaphic factors of the location. The population in Mankulam region had the second highest value for average total height. Possible reason can be the competition for light. The adjacent trees such as *Mesua ferrea*, *Hopea parviflora*, *Nageia wallichiana*, *etc*. were also at great height. The climatic as well as edaphic factors prevailing in the area may also contribute to the exceptional tree characteristics.

Height of the first branch also varied among different locations ranging from 2.72m to 6.96m. It had the same profile as that of total height with highest HFB for the population in Meppadi followed by population in Mankulam.

The difference in the certain morphological characters like tree height, girth *etc.* can be anticipated from different locations due to variations in different biophysical characters mainly, soil depth and soil nutrients.

In case of leaf length, apart from the Mankulam population, significant variations were not observed among the populations from other locations. The population in Mankulam showed a slight increase in the leaf length as compared to other populations.

The time of arrival of new flushes slightly varied with location. The phenomenon may be a result of the prevailing climatic variation in the area. Phenological activities such as season of new foliage, leaf shedding, flowering and fruiting are correlated with the seasonal events in tropical forests (Bendix *et al.*, 2006). New foliage was observed between mid- February to May which was in line with the conclusion made by Van Schaik *et al.* (1993) that peak flushing season in SE Asia for tropical trees occurs during the months of March to May. In compliance with the observation in the field, flushing was generally early in regions with comparatively higher temperature. Pookode had the earliest flushing season (mid-February to March), followed by flushing in locations in Mankulam (March to April), Meppadi (March to April), Kakkayam (March to mid- April), Chimmony (mid-March to April), and Lakkom (April-May). Seasonality in vegetation varies with topographical variation, degree of precipitation and temperature and phenological seasons reflect morphological and physiological adaptations of plant communities in appropriate utilization of resources (Prasad *et al.*, 2008).

Hierarchical cluster analysis was done to group the samples based on their morphological similarities. Based on the phenotypic characters, the trees were pooled into two major groups in which one cluster contained all the samples from Mankulam and Meppadi and the other cluster included all other samples. It is evident from the PCA analysis that the separation primarily relied on the phenotypic characteristics like tree height, height of first branch and girth which assembled the population of Mankulam (Idukki) and Meppadi (Wayanad) into a single cluster and the rest of the samples formed a separate

cluster due to their morphological similarity. PCA helps identify patterns in the data and shows them in a way that emphasizes their similarities and differences.

Sundarapandian *et al.* (2005) studied the flowering and fruiting of many evergreen fruit trees in Western regions of Karnataka for a period of 5 years. According to them, flowering and fruiting occurs in *Dimocarpus longan* once in every two years. Fruiting was not observed in any of the selected plus trees during the period of 2019-2021. Prevailing climate change can likely be the reason for difference in the fruiting season of longan in recent years. Various climatic factors like temperature, photoperiod, intensity and duration of precipitation can influence the timing, amount and intensity of flowering and fruiting of longan (Krishnan, 2002). Apart from study area, fruiting was obtained from a tree in Mankulam near Kurishupara region under Mankulam forest range during September. Flowering and fruiting was observed from July to September. Fruit characters like fruit diameter, seed diameter, weight of fruit and seed were recorded from the collected fruit samples. Many characters of the wild longan fruits significantly varied from the longan fruit that are commercially available in the market (including both imported as well as produced in the nursery varieties). Wang *et al.* (2020) studied the morphological dimensions of two *Dimocarpus longan* Lour. varieties commonly known as Chuliang and Shixia. Chuliang fruit had 26.6 ± 0.9 mm diameter with 12.44g weight where as Shixia had a diameter of 25.1 ± 1.1mm with a fruit weight of 10.28g. The fruit collected from Mankulam had an average weight of 1.22g with 14.17mm mean diameter which is far lower than the commercially available variety. The pulp that surrounds the seed had an average weight of 0.15g.

5.3 GENETIC DIVERSITY ANALYSIS

5.3.1 Standardization of DNA isolation

Mature leaves were selected for the extraction of DNA. Modified CTAB extraction protocol were tried for DNA extraction. Chances of browning of the extracted DNA was more due to high concentration of polyphenols, especially in younger leaves. The use of

sucrose extraction buffer proved effective in getting high quality DNA. Good quality DNA was yielded when extracted DNA was treated with RNase (10mg/mL) for 20 minutes in a hot water bath at 37^0C.

Molecular markers are widely used in plant science for genome mapping, identifying genes for specific characters, recognizing existing genetic variability, phylogenetic studies and even in marker- assisted breeding programs (Hayward *et al.*, 2015). PCR based molecular marker, ISSR primer was used in the present study for identifying the genetic diversity among the *D. longan* population.

Amplification of extracted DNA was done using the ISSR primers according to the procedure described by Zietkiewicz *et al.* (1994). Microsatellites are frequently used for genetic characterization because of their high repeatability and genetic polymorphism (Viruel and Hormaza, 2004). ISSR markers are quick and simple, abundant, efficient, highly reproducible and highly polymorphic (Reddy *et al.*, 2002).

Genetic diversity of the longan population in Vietnam was assessed using SSR markers by Yen *et al.* (2019). Zhu *et al.* (2013) studied genetic diversity among different ecotypes of longan germplasm using the ISSR marker and reported a 76.97% polymorphism rate. Total 19 ISSR primers were screened for the present study out of which 15 ISSR primers were selected for determining the genetic diversity among the wild population of *D. longan* in southern Western Ghats in Kerala, cultivated longan varieties and litchi. Among the selected 15 ISSR primers, 10 belonged to the UBC series and the rest 5 belonged to the L series.

5.3.2 Genetic diversity among *D. longan* populations in Southern Western Ghats, Kerala

Total 135 bands were produced with these 15 ISSR primers. Number of bands produced by each ISSR primer varied from five (UBC 816) to fourteen bands (UBC 810). Average polymorphism obtained was 69.52%. Polymorphism refers to the presence of different

alleles of same gene within a population and these differences are considered as molecular markers. The ability to identify the genetic variability depends on the percentage of polymorphism obtained using the molecular marker (Sivaprakash *et al.*, 2004). PIC value indicates the efficiency of a marker to identify the polymorphism existing within a population (Botstein *et al.*, 1980). PIC value was highest for primer UBC 816 and lowest for UBC 884.

The dendrogram obtained using UPGMA cluster analysis though molecular characterization pooled the population into two main clusters. However, the dendrogram obtained through genetic characterization was different from that obtained through phenotypic characterization. Cluster I contained the population from forests belonging to the south of Palghat gap (populations from Chimmony, Lakkom and Mankulam) and cluster II contained the longan population from areas north of Palghat gap (Kakkayam, Pookode and Meppadi). Within cluster I, populations from Mankulam and Lakkom shared a common root. Within Chimmony, C1, C2 and C5, formed a subcluster and C3 and C4, formed another subcluster. This can be because the locations from where the plus trees, C1, C2 and C3 were slightly distant and in the NE direction from the locatiom the locations of C3 and C4. In Mankulam, samples M2, M3 and M5 are very closely related which can be due to their adjacency in their location. Sample M1 was chosen

from a far area, near Kannadippara (Mankulam) and is distantly related from the rest of the population within a cluster of Mankulam population. Lakkom population shared a subcluster which contained three subgroups. Within cluster II, populations from Meppadi and Pookode showed genetic similarity which can be attributed to their closeness in the forest stretch. These populations had similarities with those from the Kakkayam region. In Kakkayam, two samples were collected from the vicinity of Kakkayam dam and are genetically closely related. When Pookode location is considered, sample P1 was collected at the Pookode lakeside and other samples were collected from nearby areas. In Meppadi, all the five samples shared single root. The selected trees in Meppadi were atleast 150m apart.

Out of selected 15 ISSR primers, L4 primer can be considered best for identifying the difference between and among the longan populations from different locations. Populations from Kakkayam was exceptionally distinct from other populations in the banding profile. This can be ascribed to the close vicinity of the samples to the Kakkayam dam.

5.3.3 Genetic diversity among wild longan population (Southern parts of Western Ghats, Kerala), commercially available longan varieties and litchi

Genetic diversity among the naturally existing wild longan population in Western Ghats, the cultivated variety and litchi using ISSR primers. Litchi and longan are the most important commercially cultivated fruit trees in the Sapindaceae family which are known to be closely related in several phenotypic as well as fruit characteristics. 158 amplicons were produced by the 15 selected primers in which 147 bands were polymorphic. Polymorphism of 92.58% was observed among the populations considered. Out of 15, six primers showed 100% polymorphism. Least polymorphism was observed for UBC 890 (80%). Polymorphism of 91.7% was observed among the litchi and *Euphoria longan* using SSR markers (Tran *et al.*, 2019). PIC value obtained varied from 0.067 to 0.39. According to Chiang *et al.* (2010), litchi cultivar 'Jhuang Yuan Hong' showed a similarity
of 44% with 24 cultivars of longan. Measured PIC varied from 0.099 to 0.39, obtained highest value for UBC 816 and lowest UBC 884.

Apart from the six wild populations from Chimmony, Mankulam, Lakkom, Kakkayam, Meppady and Pookode, single sample was chosen from Nelliyampathy forest under Nelliyampathy Forest Division, geographically occurring south of Palghat gap but near to the gap. Two cultivated varieties (white longan and E-daw) were selected for the evaluation of genetic relation with that of the wild population. Dendrogram obtained using the molecular characterization grouped the populations into 4 major clusters. Cluster I had population from south of Western Ghats, cluster II had population from north of Western Ghats. Cluster III consisted of the cultivated longan variety and cluster

IV had the litchi. Litchi and the cultivated nursery variety of longan is found to be genetically more closely related.

Determining the genetic variability using ISSR markers among the different longan populations can be further utilized for breeding programs.

6. SUMMARY

Dimocarpus longan is a fruit tree under the Sapindaceae family commonly known as longan or dragon eye. Longan is an important commercially cultivated fruit in different countries like China, Thailand *etc*. Longan, which is locally known as Chempoovam, is a commonly distributed species in the evergreen forests of Kerala. The research study entitled 'Genetic diversity of *Dimocarpus longan* Lour., in Southern Western Ghats' was conducted during the period from 2019-2021 at Department of Forest Biology and Tree Improvement, College of Forestry, Kerala Agricultural University. The objective of the research was to inspect the morphological and genetic diversity existing among the wild longan populations in the southern Western Ghats regions of Kerala.

The key features of the research study are as follows,

1. Data on morphological parameters were collected from five superior trees from six different locations along the southern Western Ghats region in Kerala to study the variability existing among the populations.
2. Eight morphological parameters, namely, tree height, GBH, height of first branch, leaf width, leaf length, crown shape, branching pattern and season of emergence of new flush were considered to study the morphological diversity.
3. Populations from Meppadi and Mankulam stood apart from rest of the population in terms of the morphological parameters like crown shape, tree height, girth at breast height, height of first branch. Differences was observed in crown shape in accordance with the microhabitat of different trees in each location. Season of new flush also varied with different location.

4. Hierarchical dendrogram was developed based on the morphological data using PAST software. The populations were clustered into two major groups. Populations from Chimmony, Lakkom, Kakkayam and Pookode formed the first cluster and populations from Mankulam and Meppadi formed a different cluster (Cluster II).

5. The procedure for genomic DNA extraction was standardized. Modifications were done in the CTAB method reported by Rogers and Bendich (1994). Phenol: chloroform: isoamyl alcohol was added in 25:24:1 ratio due to high polyphenol contamination. RNase treatment carried out to eliminate RNA contamination in the extracted DNA.

6. Quantity and quality of isolated DNA was assessed using the Nanodrop spectrophotometer and the absorbance ratio OD_{260}/OD_{280} were ranged from 1.5 to 2.2.

7 . Genetic diversity at the molecular level was estimated using Inter Simple Sequence Repeats (ISSR) primers.

8 . The protocol for PCR was standardized with varying quantities of DNA, at different PCR conditions and different reaction mixtures for amplification.

9. Out of 19 primers, 15 primers showed good amplification and were reproducible and polymorphic.

10. The obtained amplified DNA fragments were separated using electrophoresis in 1.8 per cent agarose gel containing ethidium bromide.

11. Calculated PIC value of the 15 primers ranged from 0.067 to 0.39, which indicates the suitability of primers to identify the polymorphism existing among the natural population of *D. longan* in W. Ghats region. Highest PIC value was obtained for UBC 816 and least for UBC 884.

12. The distinct, clear and reproducible amplicons produced were manually scored to form a binary matrix (1 for presence and 0 for absence of the DNA bands).

13. Dendrogram was developed with the obtained binary data using DarWIN software to pool the longan populations from different locations into clusters. The selected superior trees were grouped into two major clusters.

14. Cluster I contained populations from South of Palghat gap *viz.* Chimmony, Mankulam and Lakkom. Cluster II contained populations from North of Palghat gap *viz.* Kakkayam, Pookode and Meppadi.

Even though the project started with the objective of identifying morphological, biochemical and genetic among the longan population, since fruiting was not observed in the selected plus trees during the study period, diversity in biochemical contents in fruit were not studied.

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APPENDIX I

Reagents used for DNA extraction

Reagents

1. **Sucrose extraction buffer**

   Sucrose : 0.5M

   TrisHCl : 120mM

   EDTA : 50mM

   NaCl : 1.7M

2. **2x CTAB extraction buffer**

   CTAB : 2g

   (Cetyl trimethyl ammonium bromide)

   TrisHCl : 1.21g

   EDTA : 0.745

   NaCl : 8.18g

3. **Phenol: Chloroform: Isoamyl (25:24:1 v/v)**

   Phenol (25 parts), chloroform (24 parts) and isoamyl alcohol (1 part) were mixed properly

4. **Chloroform : Isoamyl alcohol (24:1 v/v)**

   Chloroform (24 parts) was added to isoamyl alcohol (1 part) and was mixed properly.

5. **TE buffer (pH 8, 100mL)**

   TrisHCl : 0.1576g

   EDTA : 0.0372g

APPENDIX II

Composition of buffers for gel electrophoresis

Reagents

1. **TAE Buffer 50X (1000mL)**

   TrisHCl : 242g

   Glacial acetic acid : 57.1 mL

   0.5M EDTA (pH 8.0) : 18.61g

2. **Loading Dye (6X)**

   0.25% bromophenol blue

   0.25% xylene cyanol

   30% glycerol in water

GENETIC DIVERSITY OF *Dimocarpus longan* Lour., IN SOUTHERN WESTERN GHATS

by

DEVIKA P S

(2019-17-011)

ABSTRACT

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DEPARTMENT OF FOREST BIOLOGY AND TREE MPROVEMENT

COLLEGE OF FORESTRY

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ABSTRACT

Longan (*Dimocarpus longan* Lour.), is an important commercially cultivated fruit tree, belonging to the family Sapindaceae. It is commonly known as dragon-eye. In Kerala, it is known by the names chempoovam, mullai *etc*. It is widely cultivatedin many Asian countries like China, Thailand, and Taiwan *etc*. Recently many other countries including India, Sri Lanka *etc*. have started cultivating longan tree as a commercial fruit tree. Longan is used as a traditional medicine in China due to its high medicinal and nutraceutical value.

The global demand for longan fruit has hiked rapidly due to its sweet taste and nutritional value. Fruit consist of a white edible juicy aril which is surrounded by a leathery pericarp. The fruit is rich in various bioactive polyphenols, vitamin C, volatile compounds, minerals, amino acids, proteins, fats, carbohydrates *etc*. Longan leaf, fruit pericarp, seed and pulp were used for extracting various polyphenols. Extracts from various parts of longan have shown pharmaceutical properties like antioxidance, anti-tyrosinase, anti-cancerous, anti-glycated, immunomodulatory activity, antihypertensive *etc*. Thus the importance of fruit can also be emphasized due to its richness in nutritional value.

The longan tree is a subtropical fruit tree native to the southern regions of China and Indo-Burma. *D. longan* is indigenous to India's Western Ghats, ranging from Konkan to Tinnevelly. Other distributions in India include Eastern Bengal and Western Peninsular regions. In the Western Ghats region of Kerala, longan is a species widely distributed in evergreen forests. There is a small distribution in the semi-evergreen forests of Kerala as well. The diversity of indigenous longan populations in Western Ghats has never been studied before. In this study, morphological and genetic diversity of longan populations from six different locations in the Western Ghats regions of Kerala were selected. Among these six locations, three were located in the north of Palghat gap and three were in the South

of Palghat gap. Morphological parameters like crown shape, branching pattern, tree height, tree girth, leaf length and leaf width was considered for studying the morphological diversity. Results from morphological traits revealed that the population from Meppadi region from north of Palghat gap stood different from other longan populations. Cluster analysis conducted using UGPMA method based on the morphological traits showed that population from Mankulam was closely related to the Meppadi population. The populations from six locations were divided into two major clusters. ISSR primers were used to investigate the genetic diversity existing among the six populations. 15 ISSR primers screened from total of 19 primers were used to amplify the DNA sample from different longan populations. Average polymorphism rate of 69.51% was observed. Matrix data was obtained and hierarchical dendrogram was produced using UGPMA method in NTsys pc 2.02 and DARwin software which clusters the populations into two major groups. Jaccard's dissimilarity index was calculated using R software and the values ranged from 0.00 to 0.51. Genetic relation existing between the natural populations of longan in Kerala, cultivated longan cultivars and litchi were identified. Cluster analysis using UGPMA method pooled different populations into four major clusters and study proved that litchi is genetically more related to the cultivated longan variety rather than the wild populations. This is the first report on the molecular characterization of *D. longan* from Western Ghats regions in India. The results from this research study can provide valuable information to distinguish, classify and identify the origin of longan populations in India and can be applied for future breeding programs.