

**GENETIC DIVERSITY AND POPULATION STRUCTURE OF TWO
DISJUNCT NATURAL POPULATIONS OF *Syzygium travancoricum*
GAMBLE.**

**By
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(2014-17-105)**

THESIS

Submitted in partial fulfilment of the requirement for the degree of

MASTER OF SCIENCE IN FORESTRY

Faculty of Forestry

Kerala Agricultural University



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KERALA, INDIA

2016

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DECLARATION

I hereby declare that this thesis entitled “**Genetic diversity and population structure of two disjunct natural populations of *Syzygium travancoricum* Gamble**” is a bonafide record of research done by me during the course of research and the thesis has not previously formed the basis for the award to me of any degree, diploma, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis, entitled “**Genetic diversity and population structure of two disjunct natural populations of *Syzygium travancoricum* Gamble**” is a record of research work done independently by **Mr. Mohammed Anees P. V. (2014-17-105)** under my guidance and supervision and it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to him.

Vellanikkara

Dr. A. V. Santhoshkumar
Chairman
Advisory Committee

ACKNOWLEDGEMENT

*It is with utmost and great devotion, I place on record my deep sense of gratitude and indebtedness to my project advisor **Dr. A.V. Santhoshkumar**, Professor and Head, Department of Tree Physiology and Breeding, whose valuable guidance, timely help and moral support, friendly co-operation and warm concern throughout the study period made my thesis work an easy task.*

*I owe my sincere thanks to **Dr. K. Vidyasagaran**, Dean, College of Forestry and member of advisory committee for his immense help in a way of extending the facilities available in the college for conducting the present study and valuable suggestions he has provided throughout the course of my research. I express my heartfelt gratitude to **Kerala Agricultural University** for extending financial and technical support for pursuance of my study and research.*

*I am deeply indebted to my advisory committee members **Dr. E. V. Anoop**, Professor and Head, Dept. of Wood Science, College of Forestry, **Dr. P. S. Abida**, Associate Professor, Regional Agricultural Research Institute (RARS), Pattambi, and **Dr. J.S. Minimol**, Assistant Professor, Cocoa Research Centre, College of Horticulture, Vellanikkara, for their constant encouragement and constructive suggestions throughout the study period, and also for their critical evaluation of the manuscript.*

*I am whole heartedly obliged to **Dr. P.O. Nameer**, Professor and head, Dept. of Wildlife Sciences, **Dr. T. K. Kunhamu**, Professor and Head, Dept. of Silviculture and Agroforestry, **Dr. S. Gopakumar**, Professor and Head, Dept. of Forest Management and Utilisation, and **Dr. Binu. N. K.** Assistant Professor, Dept. Of Tree Physiology and Breeding, College of Forestry, for kindly providing me valuable advice and various facilities for the conduct of the study.*

*I take this opportunity to render my sincere gratitude to **Mr. K. Shanavas**, Wildlife Warden, Shendurney Wildlife Sanctuary, for providing field staff during the field works. I owe my heartfelt gratitude to **Ms. Sangeetha. S**, my second half, for her timely help, suggestions and back up whenever I was in need, which gave me enough mental strength to complete the work in time. Special thanks to **Mrs. Mini, Mrs. Jyothi, Mrs. Remya and Mrs. Seena Cherian** for their help during the field work and thesis preparation.*

Words cannot really express the true friendship that I realized from Mr. Toji Antony, Mr. Alex K, George, Mr. Jobin Kuriakose, Mr. Arun K, R, Mr. Sachin Krishna, Mr. Hashim K, P, Mr. Anees Ahmed, Mr. Sreejith M. M, Mr. Subu. R, Unnithan, Mr. Nithin Mohan, Ms. Alida C. K, Ms. Anshee Antony, Ms. Devipriya K, S, Ms. Reshma M. Raj, Mr. Harilal K, Ms. Gayathri M, Ms. Bineesha K, Ms. Sana. M. Ali and Ms. Kavya. S. R, for their valuable mental and physical support during the course of the study. Also the co-operation and help extended by Mr. AnooB P is remembered with gratitude.

At this juncture, I express my deep love to my parents and sisters without whose moral support, blessings and affection this would not have been a success.

*Above all I bow my head before **GOD ALMIGHTY** whose blessings and care enabled me to undertake this venture successfully.*

Mohammed Anees. P. V

1. INTRODUCTION

The Western Ghats, which influences the ecology and biogeography of Peninsular India, have more than 3500 flowering plants of which about 1500 are endemics (Gopalan and Henry, 2000). Most of the endemic flora of Western Ghats are rare, facing threat from extinction. As Volga et al. (2013) reported, the endemic plants of Western Ghats are on the verge of extinction because, more than thousand species have highly uneven and scattered population. The chances of survival of endemic trees at Western Ghats are very low at present, though small patches are available at remote pockets. These rare and endemic trees deserve special conservation strategies due to their restricted distribution.

The genus *Syzygium* is one of the most important genus of forest trees in Western Ghats, which includes several endemic and endangered species. Studies revealed that 55 taxa are recorded so far from India and the Western Ghats has the highest concentration of the genus in India (Govaerts *et al.*, 2008) of which 20 taxa are endemic. Latest taxonomic investigations about the genus revealed that 39 taxa occur in the Western Ghats region of Kerala (Nayar *et al.*, 2006), among which, 17 are endemic to Western Ghats.

Syzygium travancoricum Gamble is one of the most important, critically endangered tree endemic to the South Western Ghats, India. According to IUCN Red list 2014.2, around 200 trees of the species are found in Western Ghats, even though more numbers are being identified. *S. travancoricum* is an evergreen tree growing up to 25 m in height, found in evergreen, semi-evergreen forests and a few sacred groves. *S. travancoricum* is a species having high medicinal value. The bark and leaf of the tree was used in traditional medicine. The *Syzygium* genus is used in treating diabetes, haemorrhage and derangement of arthritis (Prajapati *et al.*, 2006). Generally *S. travancoricum* grows in the swampy habitats such as the Myristica swamps of Kerala and Karnataka. But, recently a population of the species has reported from a sacred grove at Kalasamala near Kunnamkulam. The study on the reproductive biology of *S. travancoricum* Gamble and the variation among two populations of the species leads to extend knowledge on the protection and restoration of this critically endangered species.

There are several causes for the rarity of a species. These include, factors such as the taxon's evolutionary history, ecological interactions, genetics, reproductive biology, population dynamics and factors that are the outcome of human activities such as habitat conversion, land management and harvesting. Reproductive biology is one of the most important factors which influence the distribution of a species. So, understanding the reproductive biology of an endangered species will help to find out the discrepancies between the rare species and its common congeners, and also to explore to the factors affecting the rarity of the species. Whether there are discrepancies between rare and common species or not, the knowledge gained from such studies is useful for their management. An understanding of a rare taxon's general life history characteristics and reproductive biology is accepted as fundamental for the protection and restoration of the species.

The objectives of the current study are, (a) to observe the reproductive biology of *S. travancoricum* in order to find out the constraints for seed production and (b) to compare the variation between the two disjunct natural populations of the species, at Kalasamala and in the Myristica swamps of Kulathupuzha.

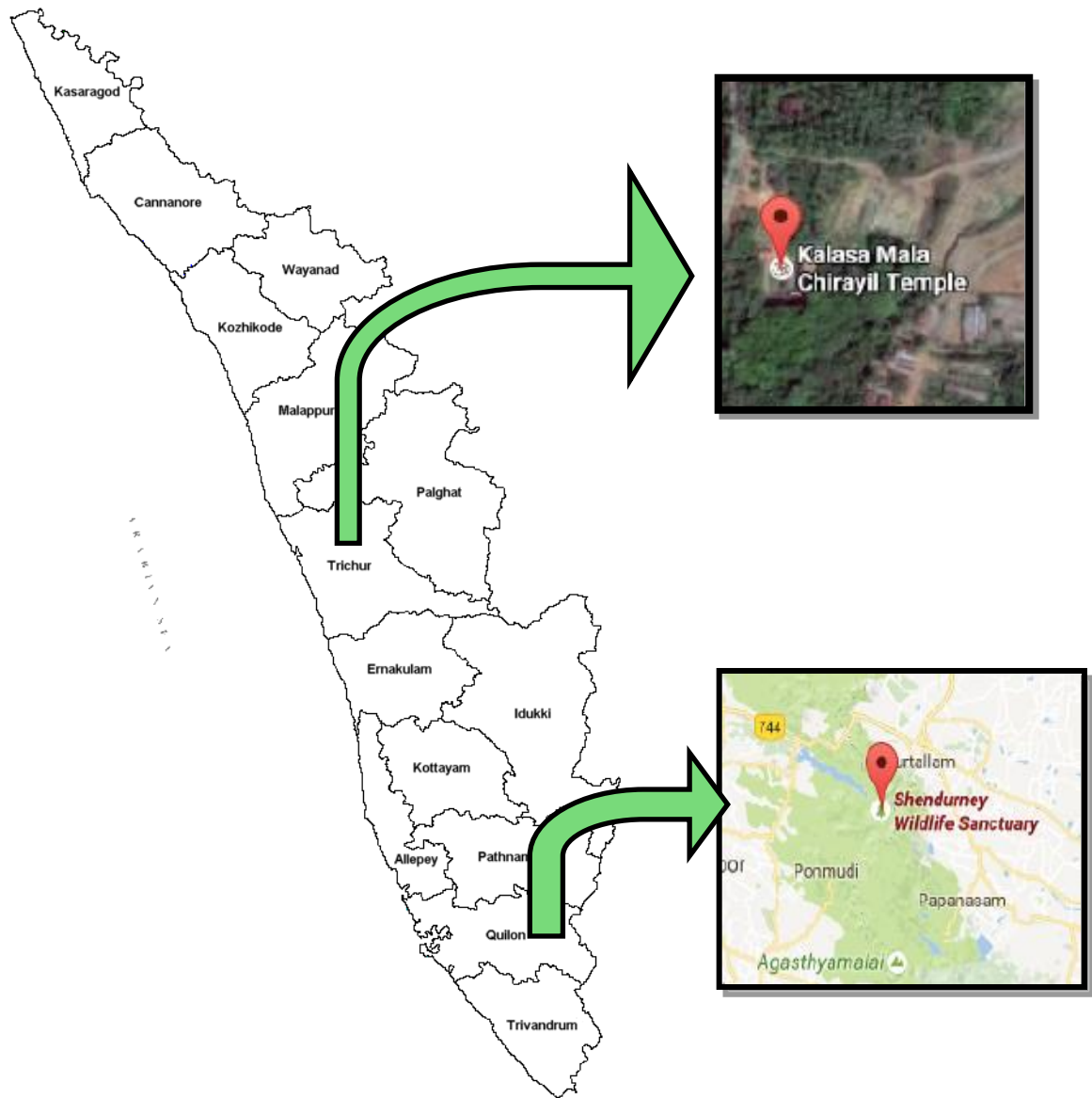


Plate 1. Location map of the study areas, Kalasamala and Kattilappara

2. REVIEW OF LITERATURE

The Western Ghats is a habitat for several endemic and endangered species. The genus *Syzygium* is one of the most important genus in the Western Ghats which have a lot of endangered species and most of them are endemic to Western Ghats. *S. travancoricum* is such a critically endangered species which is endemic to Western Ghats. The study with respect to the reproductive biology of the species, and the comparison of variation between two disjunct populations of the species will advantageous in conservation and protection of the species.

2.1. ENDEMISM

2.1.1. Definition

The word *endemic* is formed from the Greek word *endemos* which means “native”. Endemism is the ecological state of a species being unique to a defined geographic location, such as an island, nation, country or other defined zone, or habitat type; organisms that are indigenous to a place are not endemic to it if they are also found elsewhere (MacCaughey and Vaughnaun, 1917). According to Chitale *et al.* (2014), the plant species, which are unique to a distinct geographic unit such as an island/nation or habitat type and are not found elsewhere, are known as endemic plant species. Endemism is an ecological word, which means that a plant or animal lives only in a particular site, such as a specific island, habitat type, nation or other defined zone, or endemism is the association of a biological organism with a unique and well-defined geographic area (Malik, 2015). The opposite of endemism is cosmopolitan which refers to a taxon which is widespread in many parts of the world.

According to Primack (2006), a species is considered to be rare if it shows any one of the following attributes: (1) naturally occupies a narrow geographic region, (2) occurs in only one or a few specialised habitats, and (3) makes only small populations in its range. But,

according to the same author, an “endemic” species grows naturally in a single geographical area, the size of which could be either narrow or relatively large. All endemic species may not be rare.

Isik (2011), listed a few attributes which are exhibited by many rare and/ or endemic species. They are: (1) narrow and single geographic range, (2) only one or a few populations, (3) small population size and a little genetic variability, (4) over-utilisation by people, (5) diminishing population sizes, (6) low reproductive potential, (7) need for specialised ecological niches and (8) growth that requires stable environment. When habitats of a rare and/or endemic species are spoiled and/or fragmented by several human actions, the distribution range and population size of the species will be declined, leaving them vulnerable to extinction at a much higher rate than other comparable species.

Frankham (2005) reported that, depending on the scale of the geographic range, endemic species are known by various names. For example, an endemic species may be restricted only to a local geographic area, in which case it is called a “local endemic.” If an endemic species is restricted within the borders of a province, it may be labelled as “provincial endemic”, like that, “national endemic” “regional endemic” and “continental endemic” are the other types of endemic species. .

2.1.2. Factors affecting rarity and endemism

The query of why some species are rare and some species are common has long interested biologists and in recent times has developed as an important area of research for the discipline of Conservation Biology (Fiedler and Ahouse, 1992; Kunin and Gaston, 1997). In the last two decades substantial progress has been made towards understanding why some plant species are rare and some are common (Pate and Hopper, 1993; Fiedler, 1995).

Species that are limited in numbers or spatial existence are considered to be rare, relative to the distribution and richness of other species making up the pool of interest (Flather and siege, 2013). An important feature of this definition is that rarity is a relative, rather than an absolute, concept. Rarity is most frequently explained by two aspects: species' distribution and its abundance.

There are many causes of rarity, some of which, but not necessarily all, play a significant role to retain a rare taxon's distribution and abundance. They can be classified into two broad categories: (1) *natural* or *intrinsic* causes defined by a species' innate biological or ecological characteristics; and (2) *anthropogenic* or *extrinsic* causes defined by detrimental human activities that have given rise to restricted distribution and abundance, independent of their biology (Partel *et al.* 2005). The intrinsic factors include habitat specificity (species. trait), availability of suitable habitat (ecosystem trait), and dispersal capability (species trait) which will together affect the potential rarity of a species (Kruckeberg and Rabinowitz 1985). The factors affecting rarity include factors such as the taxon's geological and evolutionary history, reproductive biology, ecological interactions, population dynamics, genetics and factors that are the result of human activities (extrinsic factors) such as habitat alteration, land management and harvesting (Fiedler and Ahouse, 1992).

In plants, traits associated with breeding system, reproductive outlay, dispersal and competitive ability have been considered important (Gaston and Kunin, 1997). Some authors have claimed that because species abundances are not allocated at random, rare species should have on average, excessively higher occurrences of particular traits compared to common species (Gaston and Kunin, 1997). Generally speaking, there is some proof to advocate that rare taxa compared with their commoner counterparts have inferior levels of self-incompatibility, an inclination towards asexual reproduction, lesser overall reproductive power and poorer dispersal ability (Kunin and Gaston, 1993). Also, among a group of ecologically similar species, those that are rare will have a greater risk of extinction than those that are common (Johnson 1998; Matthies *et al.* 2004). Small populations are more likely to be affected by unexpected demographic and environmental events, such as failure to reproduce, diseases, fires, and floods (Boyce, 1992). Moreover, the genetic simplification that often escorts severe population declines can diminish a species' ability to acclimatize to varying environmental circumstances, lead to higher rates of inbreeding depression (Lande, 1995). Because of these reasons, conservation science has become engrossed with identifying at-risk species and concentrating conservation efforts on those which are on the verge of extinction (Flather and siege, 2013).

According to Kruckerberg and Rabinowitz (1985), there are multiple causes of rarity and endemism. Three primary factors i.e. geographical area, ecological breadth and isolation defines the distribution of endemics. More inquisitive is the well-known fact, first identified by Charles Darwin that the quantity and quality of endemism differ among the major geographic, topographic, and vegetation types (Harper, 1977). For instance, while species number is smaller for islands than for areas of comparable size on continents, the islands have higher proportions of endemics (Carlquist, 1974). Most isolated oceanic islands are far richer in endemics than islands near continents, and by virtue of their small size, their endemics would prefer to be narrowly distributed.

Neither genetics, ecology, nor history alone will suit to explain the origin of endemic taxa (Stebbins, 1980). The interaction among various causal factors will differ in intensity, depending on the specific taxon under inspection. Stebbins (1942) proposed the gene pool/niche interaction theory to describe origin of rarity and endemism. Next to climatic and edaphic factors, those intrinsic characteristics in the gene pool of the population are of grave importance. They include the total amount of variability, the amount of variability that can be released at any one time, and the amount of variation that can be generated with respect to those particular characteristics that affect most strongly the establishment of new populations (Malik, 2015).

Whether there are dissimilarities between rare and common species or not, the information acquired from such studies are useful for their management (Pavlik, 1994). An understanding of an uncommon taxon's general life history physiognomies and reproductive biology is acknowledged as vital for the protection and restoration of the species (Elliott *et al.*, 2002).

2.1.3. Endemism in Western Ghats

The Western Ghats are a chain of mountains of 1600 Km length, running parallel to West coast of Peninsular India from the river Tapti to Kanyakumari. Western Ghats is one of the 33 recognized ecologically sensitive zones in the World, which is home to 1500 flowering plants, at least 84 amphibian species, 16 bird species, 7 mammals and, which are restricted to specific habitat niches (Volga *et al.*, 2013). The Western Ghats retain a high percentage

of endemic species, about 48 per cent of 4000 species seen in this region are endemic (Gopalan and Henry, 2000). The Western Ghats, which is considered as the regulator of the ecology and bio-geography of Peninsular India, have more than 3500 flowering plants of which about 1500 are endemics (Subrahmanian and Nayar, 1974). It is estimated that there are four thousand species of flowering plants identified from the Western Ghats and 1,500 (nearly 38 percent) of these are endemic (Nair and Daniel, 1986).

The evergreen forests of the Western Ghats are flooded by a very high population of species endemic to the region. According to Ramesh and Pascal (1991), there are 490 arborescent taxa occur in the low and medium elevation evergreen forests of Western Ghats, among which 308 (60.8 per cent) are endemic to the region. Approximately 63 percent of India's woody evergreen taxa are endemic to the Western Ghats (Johnsingh, 2001). Among the evergreen tree species, 56% are endemic. Therefore, the Western Ghats are included in the list of biodiversity hot spots of the world (Myers, 1988).

The southern part of Western Ghats is the richest area with respect to floristic diversity and concentration of endemic taxa (Nayar, 1996). Kerala form a major species rich part of Southern Western Ghats harbouring a total of 4679 flowering plants in which 1637 are endemics and 483 are listed as threatened (Volga *et al.*, 2013). A total of 1016 tree species are reported to be present in Kerala, out of which 319 are endemic to Western Ghats and 171 listed as threatened (Sasidharan, 2004). The taxonomic studies done by Singh and Subramanian, (1991) revealed that there are over 100 taxa including herbs endemic to Kerala region alone.

The Western Ghats are on the rim of endemic plant collapse, about 1500 species have a highly fragmented population and at least 50 endemic species have not been relocated after repeated surveys (Nayar, 1998). Nair (1986) described the importance of the rare and endemic species of Western Ghats and the need for conservation. He found out that survival chances of endemic trees in the Western Ghats are very low at present, though small patches available in isolated pockets do not contain a very few endemics today. These endemic trees are the first to be taken into consideration since they are better adapted than many of their co-evolved counter parts (Nair, 1986). Endemic species of any geographical region, lead us to discover the biogeography of the area, areas of extinction and evolution of the flora. Identification and documentation of Rare

Endangered and Threatened species is important in the conservation of biodiversity as these have specific ecological niches (Prasad and Raveendran, 2012).

2.2. The genus *Syzygium*

The genus *Syzygium* (Myrtaceae) is native to the tropics, particularly to Australia and tropical America (Raju *et al.*, 2014). It has a universal, although very irregular, distribution in tropical and subtropical areas. It is recorded from many countries including Australia, South Africa, South America, and South East Asia (Chantaranothai and Parnell, 1994). Hyland (1983) reported that *Syzygium* are extensively distributed, in Africa, mainland Asia, Malaysia, New Zealand, the western Pacific, and Australia. According to Raju *et al.* (2014) and Shareef *et al.* (2014), the genus contains about 1,100 species, and has a native range that spreads from Africa and Madagascar through southern Asia and to the east through the Pacific. According to Parnell *et al.* (2007), the *Syzygium* genus encompasses more than 1200 species scattered mostly in the tropics from Africa to the West Pacific with a major concentration in Malaysia. The most interesting fact about the genus is that many of the species coming under the genus are very poorly known to the world and a lot of them have not been taxonomically described yet. There are numerous species under the genus which are yet to be identified by the botanists. Wrigley and Fagg (2003) reported that the highest levels of diversity of the genus *Syzygium* occur from Malaysia to north-eastern Australia, where several species are unknown to the scientific world and many more have not been described taxonomically.

The genus *Syzygium* includes a wide variety of species ranging from shrubs to large trees. Some species can be dominant in the rainforest canopy (*S. luehmannii*, *S. corynanthum*) while other species (*S. wilsonii*) may be largely understorey shrubs (Woodall *et al.*, 1998). This genus is of commercial importance with a wide variety of useful species. There are timber yielding plants such as *S. bracteatum* and fruit trees such as *S. cuminii* and *S. aqueum*. The species like *S. aromaticum* are used as spices. The fruits of many plants are edible and found to be used in local medicine (Raju *et al.*, 2014). Most of the species under this genus show a very high medicinal quality such as *S. cumini*, *S. alternifolium*, *S. travancoricum* etc. Jamun (*Syzygium*) fruits are excellent sources of iron, calcium, phosphorus, sodium, potassium, vitamin-C and carotene, and have been reported

to alleviate heart and liver ailments (Sivasubrahmaniam and Selvarani, 2012). The leaf essential oils of the species like *S. travancoricum* and *S. cumini* also possess anti-microbial properties (Shafi *et al.*, 2002).

Sanewski (2010) stated that the explored species of *Syzygium* for their reproductive ecology indicate that both self-compatible and self-incompatible species exist in this genus, but the self-compatible species are most common. The detailed studies on reproductive and pollination biology of most of the *syzygium* species are not done yet. The pollination in *Syzygium* generally occurs by means of nectar feeding animals, butterflies and other insects (Williams and Adam, 2010) and also by bats (Ganesh, 1996). In India, only *S. mundagum* and *S. cuminii* have been studied for their pollination biology. *S. mundagum* in the Western Ghats is pollinated and dispersed exclusively by bats (Ganesh 1996). *S. cuminii* is pollinated by wind, insects and gravity (Misra and Bajpai 1984; Bajpai *et al.* 2012).

Recent compilation studies revealed that in India, 55 species have been reported so far under the genus *Syzygium* (Govaerts *et al.* 2008; Shareef *et al.* 2012; Shareef *et al.* 2013; Krishnaraj and Shareef 2013). According to Reddy and Reddy (2008), there are 75 species of *Syzygium* recorded from India. A list of 18 *Syzygium* species is incorporated in the International Union for the Conservation of Nature (IUCN) Red List Plants of India (Reddy and Reddy 2008). They are:

1. *S. andamanicum*
2. *S. courtallense*
3. *S. myhendrae*
4. *S. benthamianum*
5. *S. parameswaran*
6. *S. beddomei*
7. *S. chavaran*
8. *S. bourdillonii*
9. *S. densiflorum*
10. *S. occidentale*
11. *S. palghatense*
12. *S. manii*
13. *S. ramavarma* (Vulnerable)

14. *S. utilis* (Data deficient)
15. *S. travancoricum* (Critically Endangered)
16. *S. mycrophyllum* (Endangered)
17. *S. stocksii* (Endangered)
18. *S. gambleanum* (Extinct).

Even though the richness of *Syzygium* species is studied in India, the reproductive biology of most of the species has not been explored so far (Raju *et al.*, 2014).

The genus *Syzygium* is one of the most important genus of forest trees in Western Ghats. A new compilation study done by Goverts *et al.* (2008) discovered that 55 taxa are recorded so far from India and the Western Ghats stands the highest concentration of the genus with 50 taxa (Sheeba *et al.*, 2003; Murugan and Manickam, 2004; Viswanathan and Manikandan, 2008) of which 20 taxa are endemic (Shareef *et al.*, 2012; Sujanapal *et al.*, 2013). Latest taxonomic investigations about the genus revealed that 39 taxa occur in the Western Ghats region of Kerala (Nayar *et al.*, 2006), among the 17 are endemic to Western Ghats. Recent taxonomic enumeration of the genus revealed that 43 taxa in Kerala state alone, including four exotics. (Nayar *et al.* 2006; Shareef *et al.*, 2012). Most of the species of this genus are seen in the evergreen forests of Kerala, some of them are in the shola forests and some species like *S. travancoricum* are seen in the sacred groves (Thomas *et al.*, 2014). Just like the other parts of the world, a wide range of new *Syzygium* species are identified day by day in the Western Ghats also. Some of the recent additions to the checklist are *S. sasidharani* (sujanapal *et al.*, 2013), *S. munnarensis* (Shareef *et al.*, 2014), *S. dhaneshiana* (Narayanan *et al.*, 2014) and *S. sahyadricum* (Sujanapal *et al.*, 2014). An extensive collection of *Syzygium* species are there in the Western Ghats which are yet to be identified by the taxonomists.

Syzygium travancoricum

Syzygium travancoricum (Gamble) is a critically endangered tree endemic to the South Western Ghats, India. According to IUCN Red list 2013 only 200 trees are found in Western Ghats. *S. travancoricum* was first discovered in the swampy low lands (altitude less than 65 m) of Travancore by Bourdillon in 1894. Gamble (1935) described it in 1918 in the Kew Bulletin and in the Flora of the Presidency of Madras. Nayar and Sastry, (1987)

described *Syzygium travancoricum* Gamble as an endangered taxa endemic to the southern Western Ghats of the Indian subcontinent. *S. travancoricum* are present in evergreen, semi-evergreen forests and a few scared groves in Thiruvanthapuram, Kollam, Pathananthitta, Alapuzha and Thrissur districts (Sasidharan, 2006). He also reported the species from freshwater swamps dominated by trees belonging to the family Myristicaceae (Myristica Swamps). According to Thomas *et al.* (2014), *S. travancoricum* can be seen in some of the sacred groves of Kerala.

Anand *et al.* (2014) described the morphology of *S. travancoricum* as evergreen trees of about 25 m high, with greyish-brown bark surface which is longitudinally fissured and peeling off in thin irregular flakes. The leaves are simple, opposite, estipulate with the petiole having a length of 10-20 mm long. The lamina is glabrous, ovate or ovate-oblong with a narrow base. The apex of the leaf is acuminate, obtuse, and the margin is entire. The leaves have lateral nerves in 10-15 pairs, parallel but very irregular looped near the margin forming indistinct intra-marginal nerve. The leaves also have a characteristic odour. They also studied the flowers of the species and described them as bisexual, white, mostly in axillary cymose or corymbose inflorescence. The petals are white, and the stamens numerous and free. The ovary is inferior, 2-celled with many ovules. Fruit is a berry, oblong-obtuse on both sides having a deep violet colour with juicy pericarp and single seed.

Syzygium travancoricum is known for its medicinal value. Traditionally this plant species has been used for curing diabetes and arthritis by local people. The species is well known for its astringent, hypoglycemic, bactericidal, antifungal and neuro-psychopharmacological effects (Anand *et al.*, 2014). It is also a source of essential oil. Shafi *et al.* (2002) reported that the essential oil extracted from the leaves of *Syzygium travancoricum* shows a very high anti-microbial property.

A major threat faced by the trees is that the swampy wetland habitat has been widely drained and converted into paddy fields (Faizal *et al.*, 2014). Another threat faced by the tree as reported by the IUCN is the very poor natural regeneration of the seeds. According to Anand *et al.* (2014), habitat loss is the main threat to the survival of *Syzygium travancoricum*. Other threats like inferior germplasm, high rate of outbreeding and habitat specificity, which accounts for poor regeneration and seed

viability are also described as threats to the existence of the species by the same author. Clonal propagation could be a viable alternative for mass multiplication of this tree species which are hard to multiply through conventional breeding and other methods. The trees, which grow in freshwater swamps suffers a lot of threats (Anand *et al.*, 1999).

Roby *et al.* (2013) proposed that while steps should be taken to ascertain the true population of *S. travancoricum* and all efforts *in-situ* and *ex-situ* be taken to ensure the continuity of this species, conserving the habitat in its pristine form should also be prioritized.

2.3. The Myristica swamps

Freshwater swamps can be seen in many parts of the world. In India, freshwater swamps are recorded from the Siwalik and Doon Valley and the Brahmaputra Valley (Rao, 1994), from the foothills of the Himalayas (Dakshini, 1960; Gupta *et al.*, 2006) and Kalakkad Mundanthurai (Ganesan, 2002). The Western Ghats, one of the renowned global epicentres of biodiversity and endemism (Myers, 1998), are home to an exceptional kind of Myristica swamps. *Myristica* swamps from the Travancore region of South Western Ghats were first encountered by Krishnamoorthy (1960) and were classified under a freshly introduced category 'Myristica Swamp Forests', under the Sub Group 4C by Champion and Seth (1968). Before Krishnamoorthy (1960), none ever mentioned about such endangered habitats, nor was anything remarkable added thereafter, and the swamps of central Western Ghats, with their specific biota remained unfamiliar until the recent work in Uttara Kannada district of Karnataka by Chandran *et al.* (1999) and Chandran and Mesta (2001). Their study revealed 51 swamp patches and investigated the evolutionary and ecological characters of this distinctive ecosystem. Swamp forests connected with a sacred grove in the Satari taluk of Goa were reported by Santhakumaran *et al.*, (1995). The edaphic and floristic features of fresh water swamp forests in Southern Kerala were studied by Varghese (1992). Jayarajan (2004), studied the Sacred Groves of North Malabar which had a small population of Myristicaceae.

The *Myristica* swamp forests are one of the most threatened ecosystems in the Western Ghats of India (Forest Type 4C/FS1 of Champion and Seth, 1968). These swamps occur at less than 300 m altitude in flat-bottomed valleys due to submergence during rainy

season. They are peculiar form of evergreen forest and due to the specific requirements, are seen only in few places. In the past, due to the increased demand for land, some of the valleys were converted to paddy fields. Only small fragments of this ecosystem remain in localities in Kulathupuzha, Anchal and Shendurney ranges in southern Kerala (Nayar *et al*, 2007).

Any patch of wood having the occurrence of *G. Farquhariana* or *M. fatua* var. *magnifica* or both can be considered as a Myristica swamp (Chandran *et al*., 1999). Varghese and Kumar (1997) distinguished between two types of swamps having Myristicaceae members, in the Travancore region: 1. Myristica swamp forest, limited to below 300 m, fringing sluggish streams. 2. Tropical sub-montane hill valley swamp forest-established as narrow strips of water-logged areas. While the former has *M. fatua* as well as *G. Farquhariana*, in the latter, *G. Farquhariana* is found along with *Mastixia arborea* and several others.

The distribution maps of the swamp species was subjected to, sporadic field observations by various workers. Varghese and Menon (1999), Varghese and Kumar (1997) and Varghese and Krishnamoorthy (2006) have done studies concerned with the vegetation in swamp forests of the Western Ghats. The ecological history of the Western Ghats and studies by Chandran *et al* (1999) and Chandran & Mesta (2001) in Uttara Kannada district indicated that the Myristica swamps at past would have formed a network along the water ways through the evergreen forests of the Western Ghats. Most of the swamps were evidently perished in the past on being transformed into rice fields (Pascal, 1988), or gardens of areca, coffee or rubber, or inundated under hydel and irrigation projects, or destroyed by the fires set on by shifting cultivators (Chandran and Mesta, 2008). Swamps, which sheltered only soft-wooded evergreens would be destroyed by various kinds of forestry operations.

The exclusive feature of the Myristica swamps is the abundance of trees belonging to the family Myristicaceae, particularly two species viz. *Myristica magnifica* and *Gymnacranthera farquhariana* (Senthilkumar *et al*, 2014). Other Myristica species found (although less frequently) are *Myristica malabarica* and *Knema attenuata*. A specific

character of these forests is the presence of pneumatophores or breathing roots, which are necessary for survival of trees in waterlogged conditions (Bhat and Kaveriappa, 2009). The superficial lateral roots emerge into the air and loop back into the soil and form the breathing roots. Knee roots and vivipary also are some specific adaptations of the trees of Myristica swamps (Chandran and Mesta, 2008). Undergrowth is usually not dense and consists of spiny plants of genera *Pandanus* and *Calamus* and herbs of *Legenandra ovata*, *Selaginella* sp., *Ochlandra* sp., *Gnetum* sp., *Helicteres* sp., *Bauhinia* sp., and creepers such as *Chilocarpus* sp., *Kunstalaria* sp., *Alpinia malaccensis*, *Phrynium* sp., *Desmodium motorium*, *Piper* sp., etc (Senthilkumar *et al*, 2014).

Rodgers and Panwar (1992) emphasised the vegetation of Myristica swamps as most critically needing conservation. With their obscure biota, the Myristica swamps are apparently live museums of ancient life of great interest to biologists. The swamps have very much importance in the life and existence of a lot of flora and fauna, and because of that the last fragments of these valuable patches of unique vegetation should be protected with utmost care, which are on the verge of extinction.

2.4. Reproductive Biology

The reproductive biology of flowering plants is essential for defining barriers to seed and fruit set, for conservation, and for understanding pollination and breeding systems that control the genetic structure of populations. Zobel and Talbert (1984) have opined that knowing the biology of tropical trees is critical before initialising any tree improvement programme. Knowledge on reproduction is one important aspect which needs much attention, also it helps to know the amount of genetic variation in a species (Costich, 1995). Detailed studies on reproductive biology of Indian plant species, especially trees, are limited. In tropical trees many reproductive biology studies have been made on ecology and evolutionary terms (Bawa *et al.*, 1985) however, only a very few applied studies are available (Venketesh and Sharma (1975), Egenti (1976), Veerendra and Ananthapadhmanaba (1996)).

Some important works related to the reproductive biology of tropical trees include the enquiry of reproductive biology of *Azadirachta indica* by Vikas and Tandon (2001), the study on reproductive biology of *Butea monosperma* by Tandon *et al.* (2003) and the observations on phenology and reproductive biology of *Aegle marmelos* by Singhal *et al.* (2010). Nagarajan *et al.* (2011) investigated the reproductive biology of *Tamarindus indicus* in connection with the tree improvement programmes. Rocha and Aguilar (2001) explored the reproductive biology of *Enterobium cyclocarpum*, in order to analyse the variation in trees growing in natural forests and pasture lands. A detailed study on the reproductive biology of a critically endangered shrub, *Grevillea althoferorum* was done by Burne *et al.* (2002), in order to compare it with two closely related more common congeners.

2.4.1. Phenology

Phenology is the science that measures the timing of life cycles events for plants, animals, and microbes and detects how environment influences the timing of these events. In case of flowering plants, these life cycle events include leaf bud burst, first flower, last flower, first ripe fruit and leaf shedding. (Haggerty and Mazer, 2008). Leith (1974) described phenology as the study of the rhythm of repetitive biological events, causes of these events, (biotic and abiotic) and also the relationship between phenophases for different species. To understand the dynamics of a plant species, the study of phenology is very important. Information on phenology or periodic events in trees is an essentiality for successful reproductive biology research. Information on phenology of a wide spectrum of plants were attributed to the studies and the observations of some keen investigators. The pioneers in this field include Croat (1969), Burger (1974), and Sasaki *et al.* (1980). Works on periodic events of trees in humid tropics were also conducted by Foster (1973).

Most widely known concept of forest restoration for biodiversity conservation, watershed protection and carbon sequestration requires in depth knowledge of plant phenology (FORRU, 2005). Phenological observations on tree species could be effectively used as an aid for seed collection (Mahadevan, 1991). Pushpakumara (2006) opined that the study of reproductive phenology can provide important information to

ensure adequate sampling for seed collection programmes, for tree improvement and ex-situ conservation. From the phenological studies conducted by Mulik and Bhosale (1989), they conclude that these studies were helpful in combating afforestation, plant management, studying floral biology and estimation of reproduction and regeneration. Knowledge regarding timing of phenological events can contribute to planning, organizing and timely execution of certain agricultural and forestry activities (Ruml and Vulic, 2005). They can also describe how populations, species, communities and biomes respond to the changes in regional and global climate (Menzel, 2003).

Flowering pattern is not the same in all kind of trees. Flower initiation differs with respect to forest types. In evergreen trees, flowering starts during the winter season (Sundarapandian *et al.*, 2005 and Nanda *et al.*, 2011.) whereas in deciduous forests, Desai and patel (2010) reported that maximum flowering occurs during April to May and lower during October to January. Within the same type of trees, phenological calendar may be different. The timings of the phenological events were reported to be different for various evergreen trees. In the same genus, there may be variations in the patterns of flowering and fruiting. Raju *et al.* (2014) and Jose *et al.* (2009) have reported the variations in phenological calendar of *S. alternifolium* and *S. mundagom*.

Variations can happen in the flowering phenology of a species. The same species standing at two different populations may sometimes show changes in the pattern of phenological events. Troup and Bor (2009) reported that the seasonal patterns of flowering and fruiting can be different in the same species if the species is standing in different populations. Thakur (2013) stated that there were variations in the phenological events of *Artocarpus hirsutus* growing in two different altitudinal zones. It is a well-known fact that plants are finely tuned to the seasonality of their environment (Cleland *et al.*, 2006). Variation among species in their phenology is an important mechanism for maintaining species co-existence in diverse plant communities by reducing competition for pollinators and other resources (Rachke and Lacy, 1985). Timing of the switch between vegetative and reproductive phases that occurs in concert with flowering is crucial to optimal seed set for individuals and populations (Bernier, 1988). The timing of growth onset and senescence also determine growing season length, thus driving annual carbon uptake in terrestrial ecosystems (Gu *et al.*, 2003).

Phenological expressions are showing tremendous variation, as periodic events in plants were controlled by endogenous and exogenous factors. These variations are accounted to many climatic, genetic, and environmental factors. Jackson and Sweet (1972) reported that the periodicity of vegetative growth, flowering, fruiting and leaf fall of 33 species varied considerably with respect to the change of climatic factors. The data fit the hypothesis that the exogenous control of vegetative flush by climatic factors is related with leaf ageing and exogenous leaf inhibitors. Role of different climatic factors in determining the phenology were studied by Sarvas (1962) and found that the temperature was the most important parameter which determined the late or early occurrence of a phenological event. Similar observations were also made by Weinstien (1982) on *Pinus helpeuris* in Israel.

Phenology shifts at species-level has been the subject of inquiry by many workers such as Sparks and Carey (1995) and Walther (2003). Parmesan and Yohe (2003) described that shifting plant phenology over the last several decades delivers gripping evidence that natural ecosystems are already reacting to human-induced environmental changes. Shifts in the timing of flowering and other developmental events in recent decades establishes that ecosystems and species are already responding to global environmental change (Root *et al.*, 2003). Earlier flowering and a prolonged period of active plant growth across much of the northern hemisphere have been deduced as responses to warming (Thuiller *et al.*, 2005). Cleland *et al.* (2006) reported that warming, elevated CO₂, nitrogen (N) deposition, and increased precipitation have a great role to play in the flowering phenology of trees. As the increase in atmospheric CO₂ is a key driver of changes in temperature and precipitation, this could also alter phenology directly

In tropical ecosystems, phenology will be less sensitive to temperature and photoperiod, and more tuned to seasonal shifts in precipitation (Reich, 1995), which will vary from region to region. In addition to shifting phenology, species have begun to adapt to recent climatic changes through altered species ranges (Parmesan, 2006). Species within communities are often remarkably vary in their phenology, and we are just beginning to understand, how potential shifts in phenological complementary, might feedback to ecosystem structure and function (Cleland *et al.*, 2006).

2.4.2. Floral Morphology

Floral morphology of various tree species have been studied by various scientists. The floral morphological studies includes the detailed investigations on the morphological characters of various parts of the flowers. Several scientists such as, Veerendra and Ananthapadmanaba (1996); Arathi *et al.* (1999) and Burne *et al.* (2002) have observed the floral morphology of various tree species. The morphological characters of flowers of *Azadirachta indica* was recorded by Vikas and Tandon (2011). The flowers of *Tamarindus indica* was subjected to detailed study by Nagarajan *et al.* (2011), for investigating the floral morphology. The floral morphology of *Butea monosperma* was examined by Tandon *et al.* (2003). Comprehensive studies on the flowers of *Aegle marmelos* was done by Singhal *et al.* (2010).

on the floral biology of various species under the genus *Syzygium* were recorded by some botanists. According to Raju *et al.* (2014), analysing the floral morphology of *Syzygium* species is very much important, as there are several species under this genus which are yet to be identified, and they differ only in some minute floral morphological characters. Botanists like Arathi *et al.* (1999); Falcao *et al.* (2002); Kaiser *et al.* (2008) and Bajpai *et al.* (2012) have made thorough investigations on the floral morphology of various species of *Syzygium*. Detailed studies on all the floral characteristics of one of the most common species of this genus, *S. cumini* have already been recorded by various scientists. (Arathi *et al.*, 1999; Falcao *et al.*, 2002 and Bajpai *et al.*, 2012). The floral morphology of *S. alternifolium* has been observed by Raju *et al.* (2014). Comprehensive analysis of the floral features of *S. mundagom* has done by Jose *et al.* (2009).

Several very rare species under the genus *Syzygium* are getting identified recently. Shareef *et al.*, (2014), conducted detailed observations on the floral biology of a newly discovered species *S. munnarensis*. The floral morphology of another newly discovered species *S. dhaneshiana* was described by Narayanan *et al.* (2014).

In most of the studies, the major morphological characters of flowers subjected to study were, the number and condition of the four whorls. Number, length and breadth of sepals and petals, length of inflorescence and peduncle, number of stamen, type of stamen, length of filament, length of style etc. (Sanewski, 2010; Saju, 2000).

2.4.3. Reproductive Potential

Reproductive potential of a tree means the total number of flowers produced per an individual tree. Estimating the reproductive potential of a tree is very much important in the study of reproductive biology of the species (Bawa, 1974). There are a lot of studies in which the reproductive potential of various tree species are studied. Burne *et al.* (2002) studied the total number of flowers produced per individual tree of *Grevillea althoferorum* as a part of their studies to compare the reproductive biology of the species, with its two common congeners. Saju (2000) conducted a study on the reproductive biology of four *Terminalia* species of the tropical deciduous forests and estimated the reproductive potential of those species. Puttaswamy (2008), assessed the reproductive potential of *Jatropha curcas*. Yates and Linda (2002), estimated the reproductive potential of *Acacia aprica* and *A. cochlocarpa*, as a part of their study on comparison of population growth of these two species.

Angiosperm flowers are morphologically more complex than those of gymnosperms. The transition of flowering in angiosperm occurs when the shoot apex ceases to produce leaves and starts to produce floral parts. (Lord and Eckard, 1987). There is a wide variation in the structure of floral buds of tree crops. Floral buds may be produced either terminally or in the leaf axils. In avocado, the terminal meristem of floral bud generally remains vegetative, and floral initiation occurs in the axillary meristem of the floral bud (Scholefield *et al.*, 1985). According to Jackson and sweet (1972), citrus is one of the most variable crops with regard to bud structure. The buds can be terminal, or axillary. Some tropical tree crops show the phenomenon of cauliflory, in which the flower buds appear directly from the trunk with no accompanying leaves or shoots (Sinha, 1975).

The flowering pattern in forest trees shows great levels of variation. Detailed investigations on flowering patterns of forest trees are scarce. There are some works on fruit crops such as mango (Singh, 1960), jack (Sinha, 1975) and nutmeg (Nazeem *et al.*, 1981). There are marked variations observed among trees in expressing flowering patterns. Even individuals of the same species are said to exhibit marked variations. Bawa (1983) and Primark (1985) reported that this variability can be seen in populations and ecosystems and also according to the climatic conditions. Majority of the tropical trees are showing flowering patterns between two extreme conditions. (Janzen, 1971). At one extreme are the species with mass flowering individuals producing small number of new flowers daily or for many weeks. At the opposite extreme are the species with steady state individuals producing large number of new flowers daily or for many weeks. Details of flowering season of indian trees were given by Troup (1986). Observations in Costarican forest trees by Baker *et al.* (1983), showed a bimodial distribution in flowering pattern. Medway (1972) and Jansen (1074) reported that dipterocarps flowered synchronously once in 5 to 13 years. Teak, one of the principal timber species in India, also showed marked variations in flowering patterns (Ramprasad *et al.*, 1990).

Some botanists have already studied the reproductive potential of some of the trees in the genus *Syzygium*. According to Raju *et al.* (2014), *Syzygium* species generally exhibits two types of flowering- mass flowering and short-period steady state flowering. But, most of the studies on various *Syzygium* species states that, mass flowering is the common mode of flowering in *Syzygium*. Reddy and Rangaiah (2000) and Arathi *et al.* (1999) has reported mass flowering in *S. cumini*.

Mass flowering was reported in *S. aqeum* by Tarai and Kundu (2008), and in *S. leuhmani* by Sanewski (2010). This type of mass flowering is a technique to attract more number of pollinators at a time (Janzen, 1967). Most of the mass blooming *Syzygium* species such as *S. leuhmani* (Sanewski, 2010) and *S. aqeum* (Tarai and Kundu, 2008) showed flowering twice a year. But there are also reports of *Syzygium* species such as *S. sayeri*, which flowers only a single time per year (Williams and Adam, 2010).

Several botanists have used various techniques for estimating the reproductive potential of trees. Burne *et al.* (2002) assessed the reproductive potential of *Grevellia althoferorum* by marking the inflorescences with coloured beads. Saju (2000) has kept wooden frames of one square meter and estimated the number of flowers per m², and

calculated the total number of flowers per tree by multiplying with the crown area of the tree.

2.4.4. Fruit Production

Estimating the number of fruits produced per tree and the percentage of fruit set is a common part of reproductive biology studies. According to Burne *et al.* (2002), the number of fruits and the fruit set percentage of the tree can be estimated in order to find out the constraints in seed production and it is a must in the case of studies related to endangered taxa. The term fruit set is used rather loosely in angiosperm literature and it refers either initial or final fruit set. Final fruit set is the number of seeds remain on the tree at fruit and seed maturity. An analysis of the proportion of flowers setting fruits for 447 species of angiosperm has shown that the mean for woody perennials is significantly lower than that for annuals and herbaceous perennials (Southwick and Davenport, 1986). According to Nagarajan *et al.* (1996), very low fruit set in tropical trees is not a rare phenomenon.

Final fruit set is generally lower than initial fruit set due to fruit drop during developmental period. The final fruit set value varies enormously between tree crops. Tropical and subtropical species which have large number of small flowers produce a few large fleshy fruits. In most of the tree crops with multiple ovules, only one will develop to seed maturity (Laun, 1986). According to Augspurger (1980), in mass flowering species like *Syzygium*, the percentage of fruit set will be more compared to the other type of species, because insect pollinators move among mass flowerings and obligate outcrossing tropical trees.

Fruit setting is controlled by some factors. Several authors have reported that fruit setting is strictly pollen-limited (Bierzychudek, 1981; Calvo, 1990; Karoly, 1992). Others have suggested that the source of pollen could influence the probability of fruit maturation (Janzen, 1977; Charnov, 1979). In addition to pollen limitation, other factors affecting fruit set include, space limitation, resource limitation, climatic variation, small population sizes and genetic load (Lamont *et al.*, 1991; Dorken and Eckert, 2001). So, the problems related to poor fruit set in some trees are because of limitation of pollinators (Calvo, 1990),

insufficient visits of pollinators or may be due to self-incompatibility which is fairly common in tropical trees (Bawa, 1974). Initial fruit set can be drastically reduced by adverse environmental conditions such as low temperature (Thompson and Liu, 1973).

Ripening is a genetically determined and regulated event which prepares the fruit or seed dispersal. But the control mechanism in ripening varies on account of the species diversity and variation in morphology of the fruit (Brady, 1987). Ripening is regarded primarily as manifestation of senescence in which inter-cellular organizations begins to break down (Sacher, 1973). The most readily apparent phenomena in the ripening processes are colour change which involves chlorophyll loss, resulting subsequent synthesis of new pigments, alternations in flavour, changes in acidity, astringency, sweetness and softening of the fleshy tissue (Rhodes, 1970). The colour change pattern is different for different species.

2.4.5. Pollen Viability

Research on pollen grains are significant in the classification of angiosperms and identification of disputed varieties or species (Nair and Mehra, 1961). Studies on pollen morphology and germination are important with regard to floral biology and taxonomy (Moti *et al.*, 1973).

Pollen viability has great importance in reproductive biology researches and hybridization works. A wide range of scientists suggested various methods for testing viability of pollen grains which include both germination and non-germination assays.

The germination assays to find out the pollen viability are more accurate than non-germination tests. Sugar solutions are commonly used as media for pollen germination (Teng and Zhang, 2009). The optimum concentration of sugar and other ingredients like agar, boric acid etc. varies with species. Previous studies have suggested that appropriate concentrations of sucrose and boron can promote the germination and growth of the pollen tube cultured *in vitro* (Pan *et al.*, 2008). Moreover, boron is one of the essential micro-nutrients in higher plants. Boron-deficient plants are characterized by dysplasia of the

reproductive organs (Liu *et al.*, 2013). Boron deficiency prevents pollination in some tropical plants. In addition to sucrose and boric acid, some scientists suggest the use of Calcium nitrate in pollen germination tests (Kumaran *et al.*, 1999).

According to many of the previous studies (Fritz and Lukaszewski, 1989; Sedgley and Harbard, 1993), the pollen viability of a species depends upon the pollen longevity, pollen dispersal and pistil receptivity. Studies were conducted by some scientists to assess the pollen viability of some of the *Syzygium* species. Sivasubramaniam and Selavarani (2012) studied the pollen viability of *S. cumini*, and Jose *et al.* (2009) assessed the pollen viability of *S. mundagom*.

2.4.6. Seed germination studies

Many tree species are propagated by seeds, but studies on seed germination are scarce due to inadequate research (Mngomba *et al.* 2007). Association of Official Seed Analysts (AOSA, 1970) defines germination as the emergence and growth from the seed of those vital structures which are indicative of the capacity to produce a normal plant under favourable conditions. In tree species, seed germination is tough due to hard seed coats and dormant seed embryos (Jaiswal, Chaudhary 2005). Seeds often fail to germinate even under favourable moisture, air and soil conditions (Urgenc, Cepel 2001).

The seed germination of a species depends upon the factors like planting substrate (Gairola *et al.*, 2011), the duration of storage (Jaiswal and Chaudhary, 2005) and the moisture content of the seeds (Richard *et al.*, 1964). Moisture content of seeds is one of the factors affecting germination, as it affects the viability of seeds (McDonald, Copeland 1999) and it also represents the seed quality and storability of seeds. According to Stephan *et al.* (2012), seeds of *S. cumini* must be kept at high moisture contents, below which viability loss is about 40 per cent. Pre-treatments like mechanical scarification i.e. soaking in cold or hot water, acid scarification with sulphuric or hydrochloric acid are well-known to overcome dormancy of seeds (Bedell 1998). Singh (1982) reported that, the seeds of *S. cumini* showed 80-90 per cent germination when pre-treated with acid scarification in sulphuric acid. Pre-soaking of seeds in growth regulator like gibberellic acid and indole acetic acid (IAA) can also boost the seed germination percentage (Chauhan, *et al* 2009).

The nature of planting substrate is another feature that impacts germination and can be species specific; therefore in nurseries significance is given to substrate for raising seedlings (Jaiswal and Chaudhary, 2005). An ideal substrate is the one which have numerous air spaces and offers adequate aeration (Thomson 1992), non-toxic, free from microorganisms, cost effective and easy to handle (Agrawal 1997).

Several botanists have assessed the seed germination percentage of various tree species. Subramaniam and Selvarani (2012), assessed the germination percentage of *S. cumini* seeds. Stephan *et al.* (2012) also conducted studies on the seed germination of *S. cumini*. In these studies, seeds showed decline in viability at low temperature. Raju *et al.* (2014) studied the germination percentage of seeds of *S. alternifolium* and Jose *et al.* (2009), observed the seed germination percentage of *S. mundagom*.

Authors like Kader *et al.* (2000) and Stephan *et al.* (2012) have reported that *S. cumini* seeds sometimes produce independent twin seedlings, having separate tap roots. The twin seedling is due to the presence of twin embryos in the seed. Polyembryony is a phenomenon of existence of more than one embryo in the embryo sac (Good, 1956; Davis, 1966) or a seed causing development of more than one seedling from it (Maheshwari, 1950). Polyembryony is reported in many species of *Syzygium* such as *S. syzygioides* (Lack and Kevan, 1984), *S. cumini* (Kader *et al.*, 2000), and *S. alternifolium* (Raju *et al.*, 2014).

2.5. Comparison of Variation among populations of a species

2.5.1. Population Structure

The studies related to population structure are important, in order to assess and compare the variation between populations of a species. Yates and Broadhurst (2002), has studied the population structure of two *Acacia* species, *A. aprica* and *A. cochlocarpa*, in order to analyse the variations among different populations of these species. Coats (2000), have noted a marked genetic variation in five different populations of *A. aprica* by studying the population structure of the species in detail. Burne *et al.* (2002), conducted

studies on the population structure of *Grevellia althoferorum*, standing in various locations, in order to compare the variations among populations.

The study of population structure of a species include the biometric characteristics like height, girth and canopy area of the tree (Coates, 1988), regeneration status of the species (Grubb, 1977), and the species associations in the population (Hobbs and Atkins, 1991).

The variations in the population structure can happen due to various reasons. According to Agren (1996), the differences in the population structure of the same species at two different localities can happen due to the dissimilarities in micro-climate, nature of the habitat, age of the trees, anthropogenic disturbances etc. In a study done by Yates and Broadhurst(2002), the population structure of two different populations of *Acacia aprica* having the same age were significantly different due to the changes in microclimatic parameters like rainfall pattern, temperature, humidity etc. In the same study the two different populations of *Acacia cochlocarpa* growing in the same microclimatic conditions had shown a significant difference in the characteristics related to population structure, only due to a change in their age. Hendrix *et al.* (2000) reported that there were significant differences in the biometric characteristics of *Phlox pilosa* only due to the variations in the habitat conditions.

Analysing the girth class distribution of a species is also an essential part of studies related to population structure. Rowland (2001) reported that, assessment of girth-class distribution of a species in a population will be helpful in understanding the stability of the species in that population. Morgan (1999) has studied the girth-class distribution of some trees as a tool for understanding the vulnerability of their populations. In the Myristica swamps of Kulathupuzha, Roby *et al.* (2013) conducted a study on the population structure and distribution of *S. travancoricum*, and they assessed the girth-class distribution in order to find out the probability of extinction of the species.

2.5.1.1. Regeneration Status

Understanding the regeneration status of a species in a population is essential, when comparing the variation among different populations of a species. Some species show very high status of regeneration while some others show very poor status of natural regeneration (Grubb, 1977). The status of natural regeneration is low in trees when compared to other smaller forms of plants such as shrubs and herbs (Hendrix, 2000). Among tropical trees, the status of natural regeneration can vary in the same species standing in different populations (Hopper, 1979).

The regeneration status of a tree species is influenced by various factors such as, micro-habitat parameters (temperature, moisture and rainfall), anthropogenic factors like habitat fragmentation (Lament *et al.*, 1991) and the issues related to seeds such as poor percentage of seed set and poor seed viability (Huber *et al.*, 1996). In some studies, changes in reproductive status of same species in different populations were reported. Rowland (2001) studied the variation between two populations of *Populus deltoides* by using morphological and population parameters. In this study, a variation in the regeneration status of the species in the two different populations were recorded. The regeneration was poor in one population and better in other.

According to Lamont *et al.* (1991), the poor regeneration status of a species can be explained by the unavailability of favourable conditions like optimum temperature and moisture, desired amount of rain fall and some anthropogenic factors like habitat conversion and fragmentation. In the case of rare and endangered species, the deprived seedling regeneration can be a result of poor percentage of seed set or production of non-viable seeds (Zimmerman *et al.*, 1988). The regeneration status of *S. travancoricum* in the Myristica swamps of Kulathupuzha was estimated by Roby *et al.* (2013), and the result showed a very poor regeneration status for the species.

2.5.1.2. Species Association

The species association studies are very much important in connection with the analysis of population structure. When the variation among different populations of a species are compared, the studies related to the associated species are essential. Hobbs and Atkins (1991), conducted a study to analyse the associations and interactions between annuals and woody perennials of Western Australia. Richardson *et al.* (1995), observed

the inter-relationship between *Banksia* species and other associated species in Southern Australia.

The species association will be different in different forest types. There are several kind of species association in moist deciduous and evergreen forests of Kerala. The evergreen forests of Kerala generally have *Mesua-Hopea-Palaquium* association (Pascal, 1988). The species association studies in evergreen and semi-evergreen forests of Kerala has done by several scientists such as Krishnamoorthy (1960), Champion and Seth (1968), and Ganesan (2002).

The *Myristica* swamp forests of the Southern- Western Ghats have a special type of species association. According to the various studies conducted in the *Myristica* swamps of Kerala and Uttara Kannada, it is evident that the major species of the *Myristica* swamps are the species of the family Myristicaceae. According to Nayar *et al.* (2007); Bhatt and Kaveriappa (2009) and Roby *et al.* (2013), the most abundant species of *Myristica* swamps are *Gymnacranthera farquhariana* and *Myristica fatua*. There are some other species of Myristicaceae, such as *Knema attenuata*, *Myristica malabarica* and *Myristica beddomii* in small numbers (Roby and Nair, 2009). The other associated species in the *Myristica* swamps include *Holigarna arnottiana*, *Vateria indica*, *Lophopetalum Wightianum*, *Xanthophyllum arnottianum* etc. (Chandran and Mesta (2001); Nayar *et al.*, (2007); Roby *et al.*, (2013) Senthilkumar *et al.*, 2014)). Scientists such as Krishnamoorthy (1960), Nayar *et al.* (2007), Nayar and Roby (2009), Roby *et al.* (2013) have reported the presence of *S. travancoricum* in the *Myristica* swamps of Kerala. Chandran and Gadgil (1993), Chandran *et al.* (1999), and Chandran and Mesta (2001) have recorded the presence of *S. travancoricum* from the *Myristica* swamps of Uttara Kannada.

2.5.1.3. Leaf Morphology

The study of leaf morphological characters of a species is always important in the case comparison of variation. Leaf morphological characters are essential to sustain photosynthesis and can impact the growth strategies of various tree species (Takayoshi *et al.*, 2001). A number of botanists have variously described changes in leaf area,

chlorophyll content, length and breadth of the leaves and length of petiole (Inomata *et al.*, 1999; Wang *et al.*, 2005; Liang *et al.*, 2009; Zhang and Dai, 2011) although no widespread, efficient studies have been carried out. In agricultural crops, Kalyan *et al.* (2012) detected a variation in foliage density, shape of lamina, leaf nature, leaf apex, colour of new and matured leaves and arrangement of major veins which can be helpful to differentiate among cultivars. Leaf morphological characters will be helpful in the studies related to variation among populations because, leaf morphological characters will change with respect to environmental factors and genetic variations (Talwara *et al.* (2013)).

It is also reported that, photosynthesis, has a robust association with chlorophyll content, production of photosynthate, sugar concentration and specific leaf weight in some plants. (Saini and Joshi, 1989; Guru *et al.*, 1999; Singh and Rajan, 2009). At the same time, leaf cuticular wax is one of the essential factors, which affect the energy balance of leaves by protecting the leaves from overheating and thereby affect photosynthesis (Armando *et al.*, 2012). However, restricted studies have been carried out on leaf morphological and physiological characteristics of trees. Such studies are not only vital for the identification of species, but also to define their genetic divergence (Rimbai *et al.*, 2014).

Leaves of many rainforest trees display an unusual form of development in which the growing leaves contain little chlorophyll, are often intensely coloured and where leaf greening and photosynthetic ability improve after full leaf expansion (Richards, 1952). Leaf colour fluctuates from red through blue and sometimes white. Red to blue colouration is due to anthocyanin pigmentation, and anthocyanin is present more in young leaves, giving a relatively reddish colour to them (Harbourne, 1993).

The study on leaf morphology is very much important in *Syzygium*, because they exhibit a wide range of variation in leaf morphology (Woodall *et al.*, 1997). According to Reddy *et al.* (2000), variations in leaf morphological characters are reported to be due to genetic divergence in some evergreen species.

2.5.2. Seed bank Analysis

Seed bank represent a sum of reproducible structures such as seed, fruit, asexual propagule and other reproducible parts of the plant. According to Roberts (1981), the term soil seed bank has been used to designate the viable seed reservoir present in a soil. For Baker (1989), this reservoir corresponds to the seeds not germinated but, potentially capable of replacing the annual adult plants, which had disappeared by natural death, diseases, disturbances, and animal consumption, including man. Simpson *et al.* (1989) defined soil seed bank as all viable seeds and fruits present on or in the soil and associated litter or humus.

The concept of seed bank was first identified by Darwin when he observed the emergence of seedlings from pond mud. The seed banks of arable weeds and their role in the rapid exploitation of disturbed ground were among the first group of plants to receive intensive study, because of its economic importance (Grime, 1981).

Soil seed bank research have important role in restoration, renovation, biodiversity preservation, vegetation succession, diffusion process and other aspects (Li, 2009). The studies on seed bank also helps in the researches related to population structure analysis and comparison of variation (Simpson *et al.*, 1989). Morgan (1999) reported about the impact of population size on seed production and seed bank. His studies on the effect of population size on seed production have found that seed production declines with decreasing population size Persistent seed banks may function as the genetic memory of a population and play an important role in community dynamics and regeneration (Hopfensperger, 2007). An understanding of persistent seed bank is the key to many aspects of vegetation management and conservation (Hegazy *et al.*, 2009).

2.5.4. Wood Anatomy

It was the work of Sanio (1872) on Scots pine (*Pinus sylvestris*) that opened new vistas in the field of wood anatomy, especially in the area of wood anatomical variation. His work prompted many researchers to search for the reasons for wood variation and their application in timber utilization in general and tree improvement in particular (Panshin and de Zeuw, 1980; Zobel and van Buijtenen, 1989).

Previously, a substantial number of works have been done in different species in order to understand the variations between clones, provenances and populations. Koubaa *et al.*, (1998), Rao *et al.*, (2002) and Pande and Singh (2005) studied anatomical variations in wood elements among clones, provenances and populations of the same species.

Vessels are unique features of hardwoods which are important cellular constituents designed to perform the function of conduction of water and mineral nutrients in the living trees (Carlquist, 1988). The site characteristics and genetic factors are the driving forces for variation in vessel morphology among populations (Pande and Singh (2009). Leal *et al.*, (2003) studied clonal and site variations of vessels in 7 year old *Eucalyptus globulus* and found that vessel varied across clones and sites. Gautam *et al.* (2008) studied the variations in vessel dimensions of *Populus deltoids* and reported extreme variation between clones and significant variation between populations. He attributed the variations to the genetic factors more than that of site factor. The variations in vessel morphology of a species is more related to genetic factors than environmental (Sreevani and Rao (2013).

Taylor (1973) in his study on *Eucalyptus grandis* found that ray and longitudinal parenchyma volume remained constant with height, and between trees variation was minor. Inter-clonal differences were significant in *populus deltoides* for fibre dimensions (Chauhan *et al* (2001). Ramirez *et al.* (2009) studied clonal variations in fibre properties of *E. globulus* and came across a narrow range of variation for fibre diameter and fibre-wall thickness. Hans *et al.* (1972) observed a marked variation among trees for fibre length in *E. grandis*.

Tyree and Zimmerman (2002) stated that, trees will develop a tendency of producing short and narrow vessel elements in plenty, when they are under stress. In a study conducted by Mahmooduzzafar *et al.* (2010), in *S. cumini* trees, not only the vessel-element dimensions but also the vessel frequency weakened under the environmental

stress, as an adaptive feature to water-stress condition. According to Gupta and Iqbal (2005), variation in wood anatomical properties of trees is attributed to environmental pollution. According to Creber and Chaloner (1990), the wood, an outcome of the vascular cambial activity, is greatly affected by the leaf-production pattern and the environmental factors of the habitat.

3. MATERIALS AND METHODS

3.1. STUDY SPECIES

Syzygium travancoricum Gamble is one of the most important, critically endangered tree endemic to the South Western Ghats, India. According to IUCN Red list 2014.2, around 200 trees of the species are found in Western Ghats, even though more numbers are being discovered. It is present in evergreen, semi-evergreen forests and a few sacred groves in Thiruvanthapuram, Kollam, Pathananhitta, Alapuzha and Thrissur districts and also in the Myristica swamps (Sasidharan, 2006).

S. travancoricum is an evergreen tree species, belonging to the family Myrtaceae, growing up to 25 m in height. The bark of the tree is longitudinally fissured and greyish brown in colour. Leaves are simple, petiolate and large in size and having characteristic odour. Flowers are small, bisexual and white in colour, seen in axillary cymose or corymbose inflorescence. Fruit is a berry of deep purple colour containing single seed.

S. travancoricum is a species having high medicinal value. The bark and leaf of the tree are used in traditional medicine. Traditionally this plant species has been used for curing diabetes and arthritis by local people. The species is well known for its astringent, hypoglycaemic, bactericidal, antifungal and neuro-psycho-pharmacological effects (Anand *et al.*, 2004). Its leaves are source of essential oil.

3.2. STUDY AREA

3.2.1. Name, Location and Extent

The study area comprised of two sites, one at a sacred grove of the Sree Siva temple at Kalasamala, near Kunnankulam, in Thrissur district and the other was at Kattilappa, in the Myristica Swamps of Shendurney in Kollam district (plate 2).

The site at Kalasamala, having an area of 1.2 ha, is a Biodiversity Heritage Site. It is situated in geo co-ordinates 8.65° N and 76. 70° E, in Porkulam panchayat in the north-east zone of Thrissur district.

Myristica swamps of Kulathupuzha and Shendurney region is located in Southern Kerala at the geo co-ordinates 8.75° to 9.0° N and 76.75° to 77.25° E. It is at the confluence of three revenue districts of Kerala namely Thiruvananthapuram, Kollam and Pathanamthitta. The swamps are scattered in three forest ranges namely Kulathupuzha and Anchal forest ranges, and Shendurney Wildlife Sanctuary. Myristica swamp forests are located along the tributaries of the west flowing rivers, Kallada and Itthikkara (Nair *et al.*, 2007). Roby and Nair (2006) have mapped sixty swamp patches having a collective area of 149.75 ha which is 0.0039% of Kerala's land area (38,864 km²) and 0.01348% of Kerala's total forest area (11,126.46 km²). They are found between elevations of 100- 200 m from sea level (Roby *et al.*, 2013). The site selected for the study was a patch known as Kattilappara having an area of 0.5 ha, which is located inside the Shendurney Wildlife Sanctuary.

3.2.2. Geology and Soil

The Kalasamala site was earlier a swamp, or part of a swamp which has been dried up over the years. Most of the water has been drained and the nature of a swamp has been lost. Now the site holds water only during the rainy season. The soil is lateritic. These soils are in general well drained, low in essential plant nutrients and organic matter. They exhibit very low cation exchange capacity and are generally acidic. (Ground water Information Booklet, 2013)

Krisnamoorthy (1960) in his pioneering paper on *Myristica* swamps, including Kattilappara region, described the rocks underlying the swamps as gneisses. All the rock formations in the area have undergone varying degrees of weathering and laterization. Fine gravel formed from the disintegration of rock is also present. The soil along the stream and river banks has been described as alluvial deposit and is deep enough to support good tree growth.

Myristica swamp of Kattilappara is composed of a specific soil type which indicates the influence of soils in the development of a particular plant community. Roby *et al* (2013) , reported that according to soil maps of Kerala (scale 1: 500,000) published by National Bureau of Soil Survey and Land Use Planning (ICAR), the soil of Myristica swamps can be classified into two categories.

1) Mapping Unit-31: Very deep well drained, gravelly loam soils on steeply sloping medium hills with thick vegetation, with moderate erosion: associated with very deep, well drained soils on moderate slopes.

2) Mapping Unit-32: Deep well drained, loamy soils on gently sloping low hills with isolated hillocks, with moderate erosion: associated with deep, well drained, loamy soils with coherent material at 100 to 150 cm on moderate slopes, severely eroded.

3.2.3. Climate

3.1.3.1. Rainfall pattern

The rainfall in and around the Kalasamala region shows the same pattern of the whole Thrissur district. The average annual rainfall ranges between 2310.1 and 3955.3 mm in the area with mean annual rainfall of 3198.133 mm (Ground water Information Booklet, 2013). The month of July experiences abundant rainfall and is the wettest month. The months of June, August, September and October also receive heavy rainfall.

At Kattilappara, generally the climate is hot and humid. The bulk of the rainfall is from the south-west monsoon between June and August. The North-East monsoon lasts for about three months from October to December. Few pre-monsoon showers are also received during April-May. The annual rainfall recorded at KFDC Nursery, Kulathupuzha during 2005-2007 is about 1300 mm per annum (Nair *et al.*, 2007).

3.2.3.2. Temperature

The maximum temperature at Kalasamala region ranges from 29.3 to 36.20C whereas the minimum from 22.1 to 24.9⁰C. The average annual maximum temperature is 32.30⁰C and minimum temperature 23.3⁰C. Generally March and April months are the hottest and November, December, January and February months are the coldest. (Ground water Information Booklet, 2013)

The temperature of Kattilappara ranges from 15° C to 36° C. The hottest months of the year are March, April and May and the coldest months are December, January and February (Nair *et al.*, 2007).

3.2.4. Water Source

The Kalasamala region gets water from the laterite aquifer present there itself which was active in all seasons earlier, but now active only in rainy season.

The major river systems along which the *Myristica* swamps are located, are the Kallada and Ithikkara rivers, both of which are west flowing and originate in the forested hills of Kulathupuzha, Shendurney and Anchal regions. The Kallada River has two main tributaries, Kulathupuzha and Shendurney Rivers.

3.2.5. Habitat and Vegetation

The Kalasamala region is a sacred grove which is composed of only *Syzygium travancoricum* trees of almost uniform size. There are about 150 trees of the species. No other vegetation is seen except some under-growth of *Holigarna arnottiana* and *Pandanus* species.

Kattilappara is a swampy area inside evergreen forests of low elevation. Champion and Seth (1968) classified the vegetation as Tropical fresh water swamp forests (4C/FS1). Most of the trees of the area are the members of the family Myristicaceae. The different forest types which border the swamp patches include Evergreen, Semi-evergreen and Moist deciduous forests. Grasslands, reed and bamboo patches and degraded forests are also present (Nair *et al.*, 2007)

3.3. METHODS

3.3.1. Comparison of Variation

The genetic variation between the two populations was compared by studying the population structure, leaf morphology, anatomy and the seed bank analysis in each population.

3.3.1.1. Population Structure

In each of the two populations, five quadrats of 20×20 m was placed at random (Burne *et al.*, 2002). Plants were tagged. Total height, bole height, girth of the trees, and the crown width were estimated. Girth and crown width was assessed using a measuring tape, and the height of the trees was assessed by using Haga Altimeter. The crown height was calculated by subtracting the bole height from the total height. The crown area was calculated using crown width and crown height. The species growing associated with *S. travancoricum* were identified and listed from each of the quadrats. The regeneration status of *S. travancoricum*, and other associated species were recorded from each of the quadrats.

3.3.1.2. Leaf Morphology

. The leaf morphology was studied by estimating the characters like leaf length, leaf width and petiole length by the common method using a measuring scale. The leaf length was measured from apex to base of leaf lamina, leaf width was taken from the broad area of leaf lamina and petiole length was measured from the base of leaf lamina to the pelvinus (Rimbai *et al.*, 2014). The colour of the flesh (young leaves) was also observed.

3.3.1.3. Anatomical Studies

3.3.1.3.1. Sample collection

Non-destructive method of sample collection was adopted. For this wood samples were taken using an increment borer (Haglof increment borer CO1512; 800 mm/32”, 12/0, 500” core diameter, 2-thread) from the selected trees at breast height.

3.3.1.3.2. Microtomy

Wood specimens of size 1.0 cm³, representing three radial positions viz., middle, pith and periphery were made out from the samples used for anatomical studies. The specimens were then softened by keeping in water bath (Rotex water bath) at 80⁰C for 10-

15 minutes. Cross and tangential sections of 10-15 μm thickness were prepared using a Leica sliding microtome (Leica SM 2000 R).

3.3.1.3.3. Maceration

Maceration of the wood samples was done using Jeffrey's method (Sass, 1971). For maceration, Jeffrey's solution was used and it is prepared by mixing equal volumes of 10 per cent potassium dichromate and 10 per cent nitric acid.

3.3.1.3.4. Staining procedure

Permanent slides of transverse and tangential sections were stained using the procedure outlined by Johansen (1940). In this, sections were stained using saffranin and later washed through a series of alcohol solutions at different concentrations (70 %, 90 % and 95 %) to ensure complete dehydration. They were subsequently dipped in acetone followed by xylene and finally mounted in DPX mountant to prepare the permanent slides.

3.3.1.3.5. Image analysis

Microscopic examination and quantification of sections was undertaken using an Image Analyser (Labomed-Digi 2; Plate 5). It consists of a microscope, digital camera and PC (Personal computer).

3.3.1.3.6. Observations

The anatomical properties listed under the International Association of Wood Anatomists (IAWA) list of card key features, such as vessel length, vessel diameter, vessel frequency, ray height, ray width, ray frequency, fibre length, fibre diameter and fibre-wall thickness were observed.

3.3.1.4. Seed Bank Analysis

Three soil samples of 15 cm x 15 cm surface area to a depth of 5 cm, from five quadrats each, from both of the population were carefully removed and put in labelled polythene bags.

The seedling emergence technique was used to determine the seed density and abundance (Roberts, 1981). At first, coarse debris were removed from the soil sample collected. Each sample was then spread on a layer of sand (2 cm thick) in seed germination trays. There were a total of 30 germination trays. All the trays were watered daily to keep the soil moist. Newly germinated seedlings were identified at the species level.

3.3.2. Phenology

The phenology was investigated on five selected trees in each of the population. Observations of flowering and fruiting were recorded from October 2014 to June 2016.

3.3.3. Reproductive Biology

The reproductive biology of the species was investigated only in the population at Kalasamala. Phenology, floral morphology, pollen viability and fruit set were studied.

3.3.3.1. Reproductive Potential

The reproductive potential was studied by estimating the total number of flowers produced on the trees marked for investigating the phenology, by using the method suggested by Burne *et al.*, 2002. The total number of flowers/plant (reproductive potential) was calculated using the following formula:

Total no: of flowers / plant = no: of inflorescences / plant × mean no: of flowers / inflorescence

The total number of inflorescences per plant was estimated by multiplying the number of inflorescences per m² by the crown area of the tree. The average number of inflorescences per m² was assessed by using a hard board frame of one square metre as explained by Saju *et al.* (2000). The frames were placed on each tree and the number of inflorescences inside the frames were counted. Then, the total number of inflorescences per tree was calculated by multiplying it with the crown area of the tree. Then, the total flowers per individual tree was estimated by using the above formula.

3.3.2.2. Floral Morphology

The floral morphological characters of the collected flowers were studied in detail using a hand lens. The morphological characters such as length of cyme, condition and number of the four whorls, length of petal, filament and style, breadth of petals, condition of ovary etc. were observed. Sections of the flowers was taken and the floral diagram of the species was prepared.

3.3.2.3. Pollen Viability

The viability of pollen was examined by using the procedure used for testing the pollen viability of other *Syzygium* species (Sivasubramaniam and Selvarani, 2012). The pollen from the collected inflorescences from the marked trees were put in a small drop of germinating medium containing a solution of 10 percentage sucrose and 100 ppm Boric acid by using the Hanging drop method. The pollen grains were allowed to germinate in high humidity. The slides were observed under the Image Analyser.

3.3.2.4. Fruit Production

The fruit production was studied based on the method suggested by Burne *et al.*, 2002. For each population the number of fruits/plant was recorded on the plants used to monitor flowering phenology. From this data, the percentage fruit set was calculated using the following formula.

$$\% \text{ fruit set} = \frac{\text{no: of fruits/plant}}{\text{no: of flowers/plant}} \times 100$$

The total number of fruits produced per an individual tree was also estimated by using the same method of using the hard board frames.

3.3.2.5. Seed Germination

Seeds were collected from the fallen fruits of *S. travancoricum*, and they were sown in trays to assess the percentage of seed germination.



Plate 2. The study areas (a) Kalasamala grove and (b) Kattilappara swamp

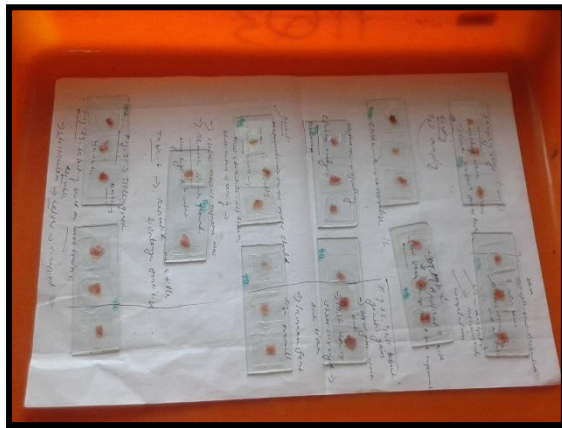


Plate 3. (a) Leica SM 2000 R microtome, (b) permanent slides prepared for analysing anatomical characters, (c) Labomed Digi-2 image analyser.



RESULTS

The results of the investigations on the reproductive biology of *Syzygium travancoricum* and the comparison of variation between two geographically disjunct populations of the species are presented in this chapter.

4.1. REPRODUCTIVE BIOLOGY

The reproductive biology of *S. travancoricum* was studied in order to find out constraints in seed production of the species, which may ultimately be the reason for the restricted distribution of the species. The phenology, floral biology, reproductive capacity, fruit production and pollen viability were observed.

4.1.1. Phenology

Observations on phenology of *S. travancoricum* from the two disjunct natural populations at Kalasamala and Kattilappara were taken during periodical intervals for a time of more than one year. All the phenological processes such as leaf shedding, flushing, flowering and fruiting were observed in detail. The observations on phenology of the species in the two different populations are represented in the phenograms (fig. 1). Each of the phenogram gives information on phenological calendar for the species in two different populations. .

Being an evergreen species, leaf shedding and leaf flushing continued throughout the year. There was a slight difference in the flowering and fruiting periods in the two populations. In the population at Kalasamala, flowering started in the first to second week of April. The blooming period was extended up to one month. The number of flowering days was 42 (Table 1). The fruiting started from the first to second week of May and extended up to the last week of June. Most of the fruits fell down at the end of June and no seeds germinated.

In Kattilappara, flowering started at the end of March. The blooming period was extended up to two months (Table 1). Fruiting began at the end of May and extended up to September. At the end of September most of the fruits were shed. Germination of the seeds followed within one month. The seeds germinated in the natural conditions below the trees, but almost all the seedlings died in the seedling stage itself.

Table 1. Period and mean number of days of flowering and fruiting of *S. travancoricum* in Kalasamala and Kattilappara.

Periodical event	Kalasamala		Kattilappara	
	Period	No. of days	Period	No. of days
Flowering	1st week of April to 3 rd week of May	42	1st week of April to 1st week of June	52
Fruiting	1st week of May to last week of June	54	1st week of June to 1st week of September	75

4.1.2. Floral morphology

The floral morphology of *S. travancoricum* was observed in detail in order to study the reproductive biology of the species. The flowers of *S. travancoricum* are bisexual, white, seen in axillary cymose. The length of the cyme is 4.5 to 7 cm. The flowers have a short calyx tube of 1mm across. The sepals are white, short and four-lobed. The petals are white in colour and four in number, which are calyptrate. The length of the petals is 0.98 to 1 cm and their breadth is 1.9 to 2.5 cm. The stamens are numerous and free, bent inwards at middle when in bud. The length of the filament is 3.4 to 3.8 cm. The ovary is inferior and two-celled. Ovules are many and only one style. The style is very slender, having a length of 4 to 4.5 cm. The stigma is simple and acute in shape. The fruit is a berry, oblong-obtuse on both sides. The size of the fruit is 1×0.5 cm, having a deep violet colour, juicy pericarp and only one seed. The morphological characters of *S. travancoricum* was compared with that of one of the most common species of the same genus, *S. cumini* as reported by Bajpai *et al.* (2012), for a detailed understanding (Table 2.)

Table 2. Morphological comparison of *S. travancoricum* with *S. cumini* as reported by Bajpai *et al*, (2012).

Characters	<i>Syzygium cumini</i>	<i>Syzygium travancoricum</i>
Habit	Medium sized to large trees , 25 meter height	Medium to large trees, more than 25 m height
Bark	Pale yellow brown, thinly flaky	Greyish-brown, longitudinally fissured, peeling off in thin irregular flakes
Blaze	Pale brown	Greyish brown
Young leaves	Bright red	Reddish brown
Leaf shape	Elliptic to ovate -lanceolate	Ovate-oblong
Leaf margin	Revolute	Entire
Lateral nerves	Elevated beneath and smooth above	10-15 pairs, irregular, prominent, looped near the margin
Intra- marginal nerve	Distinct, one tiered	Indistinct
Petiole	1-3.5cm	1.5-2 cm
Inflorescence	Lateral, rarely terminal or axillary, 12 cm long	Mostly axillary, 5-7 cm long
Petal	Elliptic to orbicular, 3- 3.5 mm.	Orbicular, 0.9 cm
Staminal filaments	4-6 mm long	3.5 -4 mm long
Fruit	Oblong, ellipsoid or globose, 3.5*2 cm, 1-5 seeded	Oblong-obtuse, 1*0.5 cm, one seeded.

4.1.3. Reproductive potential

The reproductive potential or the total number of flowers produced per tree of *S. travancoricum* was estimated, in order to study the reproductive biology of the species. The total number of inflorescence per m² and the total number of flowers per inflorescence were calculated for finding out the total number of flowers produced per tree. The details of reproductive potential of *S. travancoricum* are given in table 3.

The mean number of flowers per inflorescence was 13 with a minimum of 12 and maximum of 16. The average number of inflorescences per m² was 18 with a maximum of 22 and a minimum of 16. The average number of flowers produced per tree was 4201 with a maximum production of 5518 flowers and a minimum of 2313 flowers per tree.

Table 3. Reproductive potential of *S. travancoricum*

	No.of flowers per inflorescence	No. of inflorescence / m ²	No.of flowers / m ²	Crown area (m ²)	Total no.of flowers / individual tree
Mean	13.64	18.72	255.64	16.45	4,201.03
Std. Deviation	1.32	1.51	34.56	3.14	915.27
Maximum	16	22	352	22.33	5518.8
Minimum	12	16	192	11.34	2313.36

4.1.4. Fruit production

The total number of fruits produced per individual tree of *S. travancoricum* was estimated as a part of the enquiry of reproductive biology of the species. The percentage of fruit set of the species was also calculated in order to understand constraints to the seed production of the species. The data related to the fruit production and fruit set percentage of *S. travancoricum* is given in table 4. The average number of fruits produced per m² was 69 with a maximum of 89 and a minimum of 51. The average number of fruits produced per individual tree was 1146 with a maximum production of 1740 and a minimum of 589. The mean percentage of fruit set was estimated as 27.33 per cent with a maximum of 38 per cent and a minimum of 20 per cent.

Table 4. Fruit production and fruit set percentage of *S. travancoricum*

	No. of fruits / m ²	Crown area (m ²)	Total no. of fruits/ individual tree	Total no. of flowers / tree	Percentage of fruit set
Mean	69.56	16.45	1146.1	4201.1	27.3
S.D	14.29	3.14	327.3	915.3	5.1
Maximum	89.00	22.33	1740.9	5518.8	38.2
Minimum	51.00	11.34	589.7	2313.4	20.8

4.2.5. Pollen viability

The viability of pollen grains of *S. travancoricum* was studied as a part of the research on the reproductive biology of the species. The pollen viability of the species was estimated through pollen germination method. The mean percentage of pollen germination was 24 per cent. The maximum pollen germination was 28 per cent and minimum was 20 per cent (Table 5).

Table 5. Percentage of pollen germination of *S. travancoricum*.

	No. of germinated pollen	Total no. of pollen	Percentage of pollen germination
Mean	35.92	148.04	24.23
Std. Deviation	6.01	20.04	2.22
Maximum	46	184	27.81
Minimum	23	112	20.18

4.2.6. Seed morphology

The morphology of the seeds of *S. travancoricum* was studied. Each fruit of *S. travancoricum* contained a single seed. The colour of the seed was greyish and the shape was globose. The seeds were of 0.7 × 0.2 cm size. The morphological characters of the seeds of *S. travancoricum* were compared to that of *S. cumini* (table 6), based on the features reported by Sivasubramaniam and Selvarani (2012).

Table 6. Seed characters of *S. cumini* and *S. travancoricum*.

Seed characters	<i>Syzygium cumini</i>	<i>Syzygium travancoricum</i>
No. of seeds per fruit	1 to 5	One
Colour	Grey	Grey
Shape	Dipressed-globose	Globose
Size	1 × 0.2 cm	0.7 × 0.2 cm

4.2.7. Seed germination

The seed germination percentage of *S. travancoricum* was estimated as a part of the observations on reproductive biology. The percentage of seed germination was very poor for the species. The mean percentage of seed germination was only 16 per cent (Table 7). The maximum germination percentage was 30 and minimum was zero per cent.

Table 7. Percentage of seed germination of *S. travancoricum*.

	No. of germinated seeds	Seed germination percentage
Mean	1.6	16
Std. deviation	1.14	11.4
Maximum	3	30
Minimum	0	0

4.2. COMPARISON OF VARIATION

The variation between the two populations of *S. travancoricum* was compared by the detailed study on the population structure, leaf morphology, wood anatomy and seed bank analysis of the species.

4.2.1. Population structure

4.2.1.1. Biometric analysis

The structure of the populations of *Syzygium travancoricum* at both Kalasamala and Kattilappara was investigated to compare and evaluate the variation of the species in both of the populations. The result of the analysis are summarized in Table 8.

The analysis of height showed significant differences between the two populations. The overall mean height of trees at Kalasamala was 12.92 m and that of trees at Kattilappara was 20.75 m. The maximum height of the trees at Kattilappara was 25 m, while there were no trees at Kalasamala having a height more than 16 m.

Diameter at breast height (DBH) also significantly differed between the two populations. The mean DBH of the trees at Kalasamala was 54.67 cm and that of the trees at Kattilappara was 96.25 cm. The maximum DBH shown by the trees of Kattilappara was 124 cm, while there were no trees at Kalasamala having a DBH more than 64 cm. The comparison of height and DBH of the two populations is given in figure 2.

Table 8. Variation in biometric characters of *S. travancoricum* at Kalasamala and Kattilappara.

Location	Height (m)	Girth (cm)	Crown width (m)	Crown height (m)	Crown area (m ²)
Kalasamala	12.92±0.78	54.67 ± 3.8	2.42 ± 0.23	6.25 ± 0.41	15.24± 2.36
Kattilappara	20.75±1.18	96.25 ± 9.5	3.38 ± 0.25	8.48 ± 0.24	28.67± 2.86
t-value	12.381*	7.079*	6.28*	10.395*	8.107*

* Significant at 5 % level.

The trees of the two populations showed significant differences in the values of crown width, crown height and crown area too. The mean crown width, crown height and crown area of the Kalasamala population were 2.42 m, 6.25 m and 15.24 m² respectively and those of the population at Kattilappara were 3.38 m, 8.48 m and 28.67 m² respectively. The maximum crown area of *S. travancoricum* trees standing at Kattilappara was 37.72 m², while the maximum crown area of the trees of Kalasamala was 22.72 m². The crown area of the two populations were compared and are shown in figure 3.

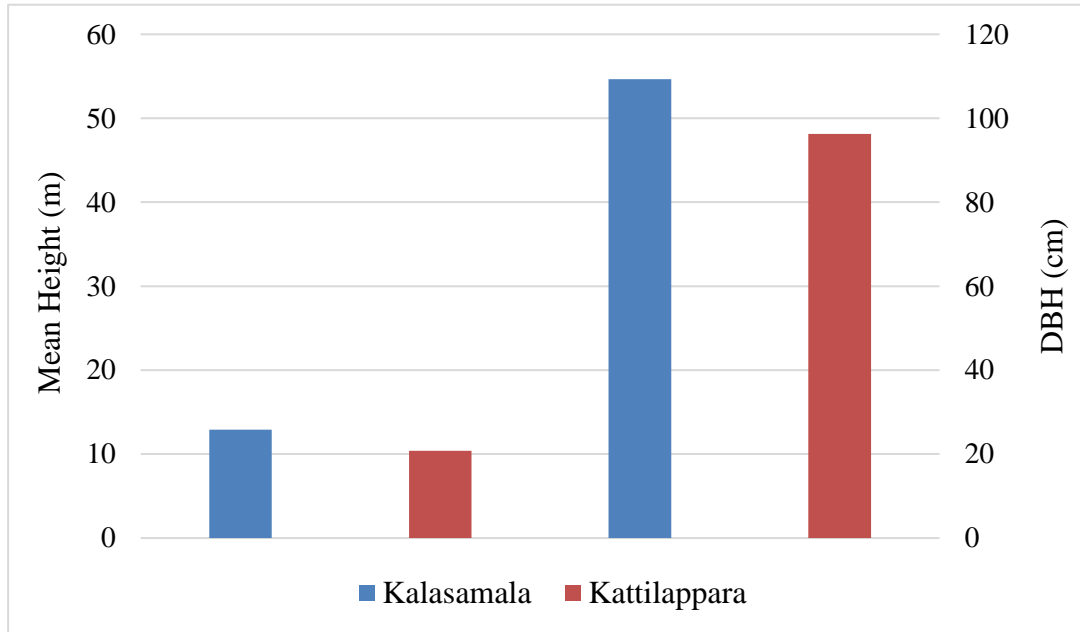


Figure 2. Variation in mean height and DBH of *S. travancoricum* trees growing at the two different populations

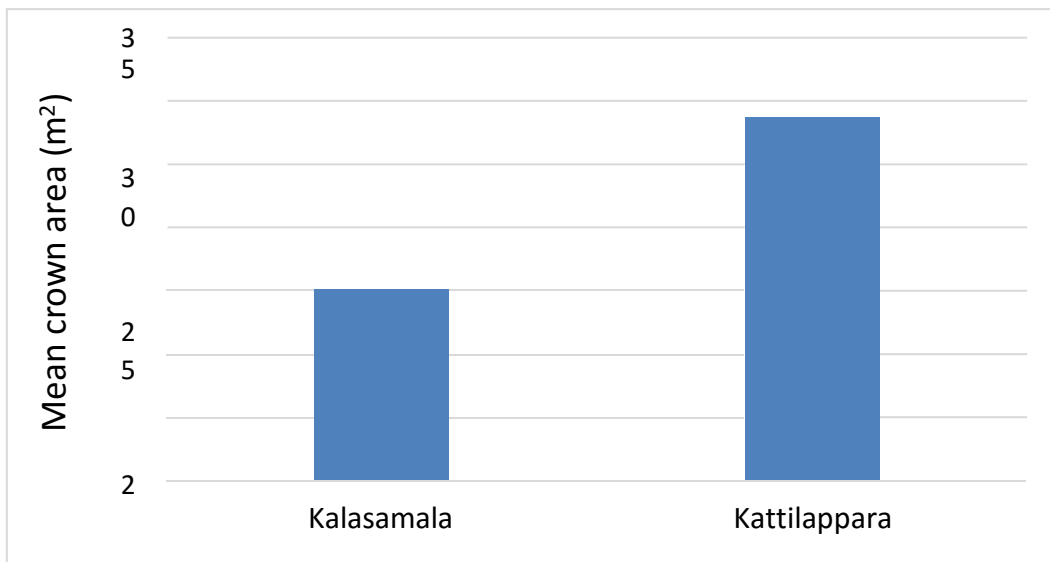


Figure 3. Variation in mean crown area of *S. travancoricum* trees growing at the two different populations

4.1.1.2. Girth class distribution

The girth class distribution of *S. travancoricum* in both of the populations were analyzed in order to study about the population structure of the trees in those populations.

The analysis of girth class distribution of *S. travancoricum* at Kalasamala reveals that most of the trees (above 80 per cent) comes under the girth class of 30-60 cm (table 9). But in contrast with that, no trees under the girth class of 10-30 cm were encountered.

Table 9. Population structure of *S. travancoricum* at Kalasamala

Diameter-class (cm)	No. of trees	% of total	% of mature and immature trees	% of decline of immature trees
90-130	nil	nil	19	Mature
60-90	19	19		Mature
30-60	77	81	81	19
10-30	nil	nil		-
Total	96	100	100	-

The girth class distribution for *S. travancoricum* at Kattilappara is shown in table 10. It shows that 58 percentage of the total trees comes under the diameter class 90-130 cm and more than 20 percent comes under 60-90 cm. A total of 80 percentage of the trees comes under the mature category. The diameter class distribution of the trees at the two populations are compared and are shown in figure 4.

Table 10. Population structure of *S. travancoricum* at Kattilappara

Diameter-class (cm)	No. of trees	% of total	% of mature and immature trees	% of decline of immature trees
90-130	26	58	80	Mature
60-90	10	22		Mature
30-60	6	13	20	87
10-30	3	7		93
Total	45	100	100	-

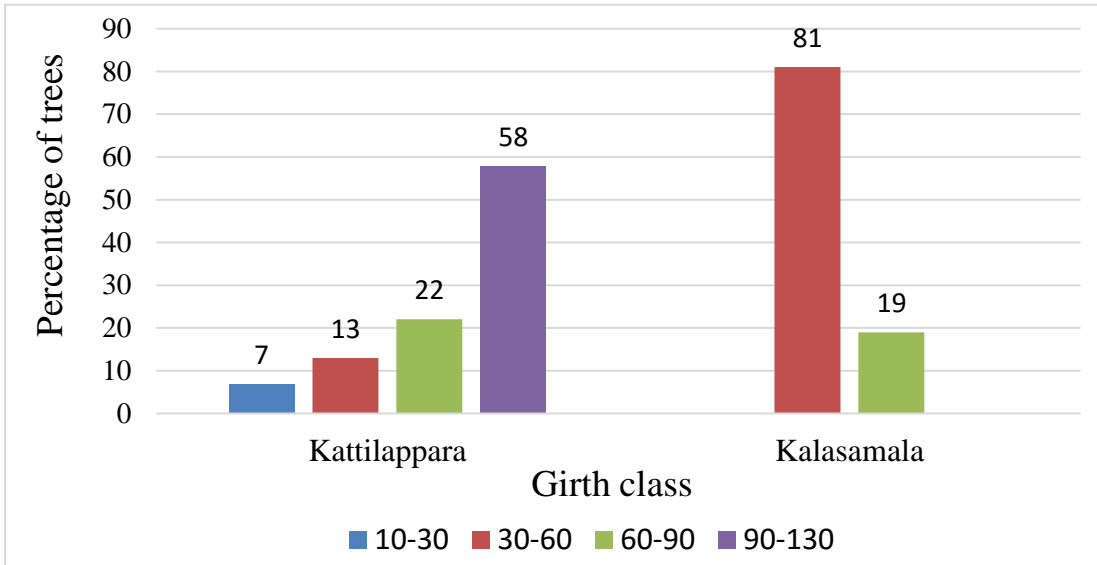


Figure 4. Girth class distribution of *S. travancoricum* at Kalasamala and Kattilappara

4.1.1.3. Species association

The species which are growing in association with the *Syzygium travancoricum* trees at both of the populations were subjected to detailed examination. In the population at Kalasamala, the relative density of the trees were worked out and *S. travancoricum* was having the highest value of 77.95. In Kattilappara, *S. travancoricum* was at the fourth position with a relative density of 18.18 (Table 11). Among the trees, the maximum relative density was for *Myristica fatua* (22.73) followed by *Gymnacranthera farquhariana* (20.20). The minimum was for *Myristica malabarica* (1.01).

In the population at Kattilappara, all the species growing associated with *S. travancoricum* were identified and the relative basal area, relative frequency, abundance and Important Value Index (IVI) of all the trees were calculated (Table 12). Distribution of species in the above area indicated that the species other than *Lophopetalum wightianum*, *Knema attenuata*, *Myristica beddomii* and *Myristica malabarica* had a relative frequency of 12.5.

Relative basal area was worked out to be highest for *Vateria indica* (11.37), followed by *Lophopetalum wightianum* (10.76), whereas lowest value was obtained for *Myristica malabarica* (9.07). The species of the study *S. travancoricum* had a relative basal area of 10.33.

Important value index were studied by taking into account the values of relative density, relative frequency and relative basal area. Maximum IVI was observed for *Vateria indica* (23.87), followed by *S. travancoricum* (22.83), whereas the minimum value was observed for *Myristica malabarica*.

Table 11. The relative density of various species at Kalasamala and Kattilappara

Sl. No.	Species	Relative Density	
		<i>Kalasamala</i>	<i>Kattilappara</i>
1	<i>Syzygium travancoricum</i>	77.95	18.18
2	<i>Holigarna arnottiana</i>	3.94	19.19
3	<i>Pandanus spp</i>	16.54	2.02
4	<i>Ochlandra spp</i>	1.57	0
5	<i>Myrsitica fatua</i>	0	22.73
6	<i>Gymnacranthera farquhariana</i>	0	20.20
7	<i>Lophopetalum wightianam</i>	0	7.58
9	<i>Knema attenuata</i>	0	2.02
10	<i>Myristica beddomii</i>	0	2.02
11	<i>Myristica malabarica</i>	0	1.01
12	<i>Vateria indica</i>	0	5.05
Total		100	100

Table 12. Structural analysis of vegetation at Kattilappara population

Sl. No	Species	RD	RF	RBA	IVI	A
1	<i>Myrsitica fatua</i>	22.73	12.5	10.01	45.24	9
2	<i>Gymnacranthera farquhariana</i>	20.20	12.5	9.80	42.50	8
3	<i>Syzygium travancoricum</i>	18.18	12.5	10.33	41.01	7.2
4	<i>Holigarna arnottiana</i>	19.19	12.5	10.23	41.92	7.6
5	<i>Lophopetalum wightianum</i>	7.58	10	10.76	28.33	3.75
6	<i>Xanthophyllum arnottianum</i>	2.02	12.5	9.44	23.96	0.8
7	<i>Knema attenuata</i>	2.02	10	9.91	21.93	1
8	<i>Myristica beddomii</i>	2.02	2.5	9.09	13.61	4
9	<i>Myristica malabarica</i>	1.01	2.5	9.07	12.58	2
10	<i>Vateria indica</i>	5.05	12.5	11.37	28.92	2

RD-Relative density, RF-Relative frequency, RBA-Relative basal area,

IVI-Important value index, A-Abundance.

The relative densities of the species growing at both of the populations were compared in figures 5 and 6.

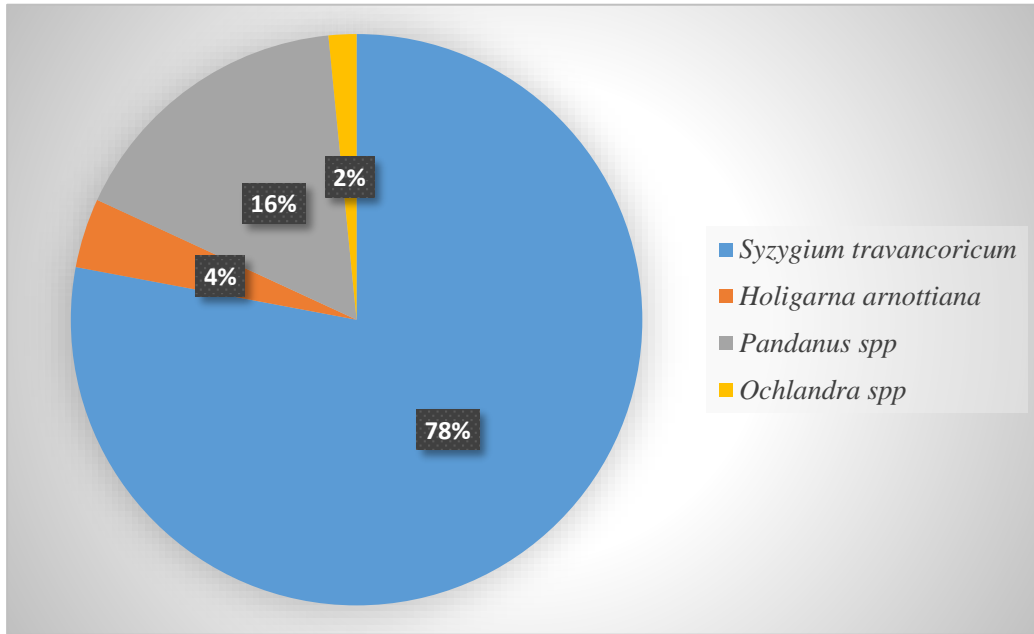


Figure 5. Relative density of various species growing in Kalasamala.

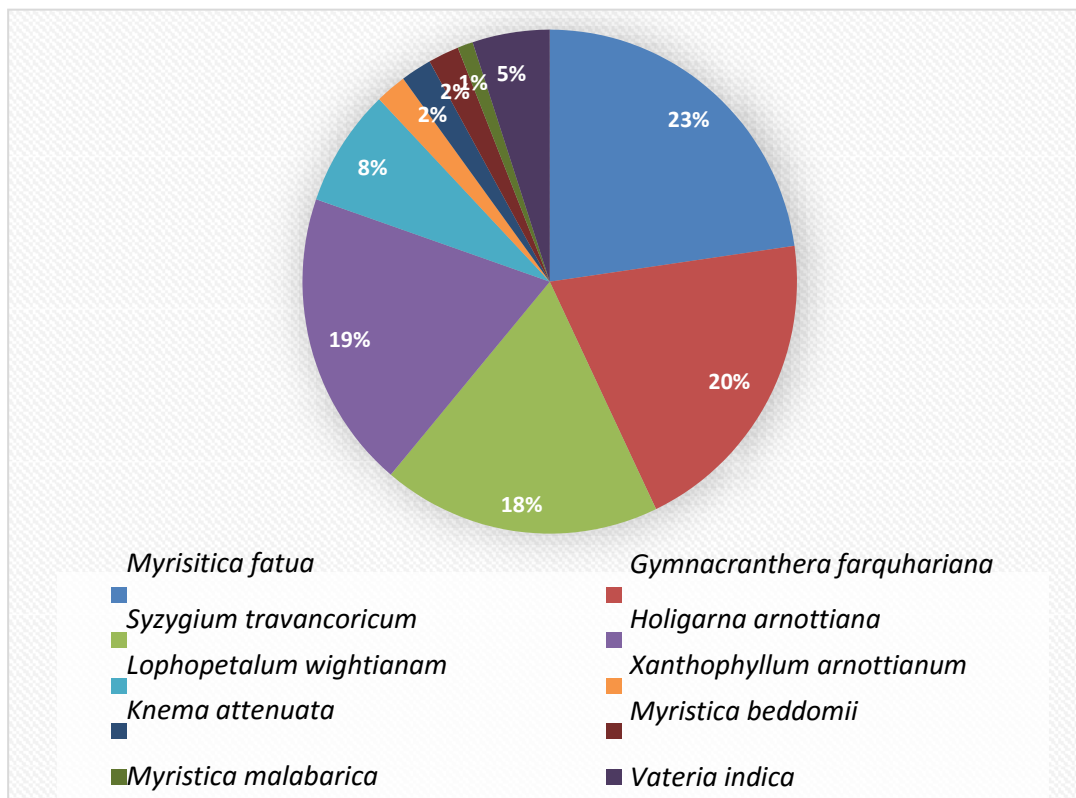


Figure 6. Relative density of various species growing in Kattilappara

4.2.1.3. Regeneration status

The regeneration status of *S. travancoricum* and the associated species were examined in detail at both of the populations. The relative density of naturally regenerated seedlings was estimated. In the population at Kalasamala, the relative density of seedlings was more for the shrub *Leea indica* (66.13), followed by *Holigarna arnottiana* (25.81) and *S. travancoricum* (8.06). No regeneration was there for any other species (Table 13).

Table 13. Regeneration status of various species at Kalasamala and Kattilappara.

Sl.No.	Species	Relative Density	
		<i>Kalasamala</i>	<i>Kattilappara</i>
1	<i>Syzygium travancoricum</i>	8.06	15.35
2	<i>Holigarna arnottiana</i>	25.81	19.09
3	<i>Leea indica</i>	66.13	0
4	<i>Myristica fatua</i>	0	17.43
6	<i>Gymnacranthera farquhariana</i>	0	16.18
7	<i>Vateria indica</i>	0	17.01
8	<i>Xanthophyllum arnottiana</i>	0	6.64
9	<i>Lophopetalum wightianum</i>	0	8.30
Total		100	100

In the population at Kattilappara, most of the tree species were showing regeneration. The relative density of naturally regenerated seedlings was more for *Holigarna arnottiana* (19.09), followed by *Myristica fatua* (17.43) and *Vateria indica* (17.01). The relative density of naturally regenerated seedlings of *S. travancoricum* was 15.35 in the population at Kattilappara (Table 13).

The relative density of various species regenerated naturally in both the populations are shown in figure 7 and 8.

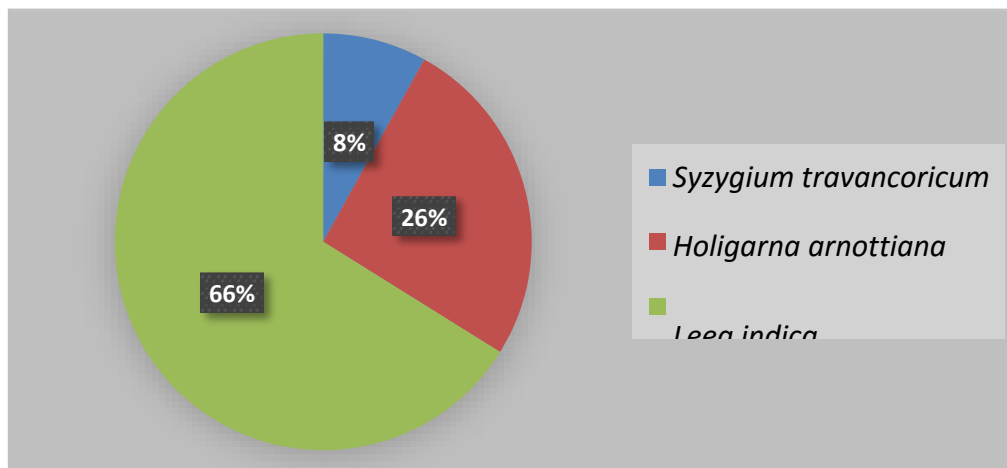


Figure 7. Regeneration status of various species at Kalasamala

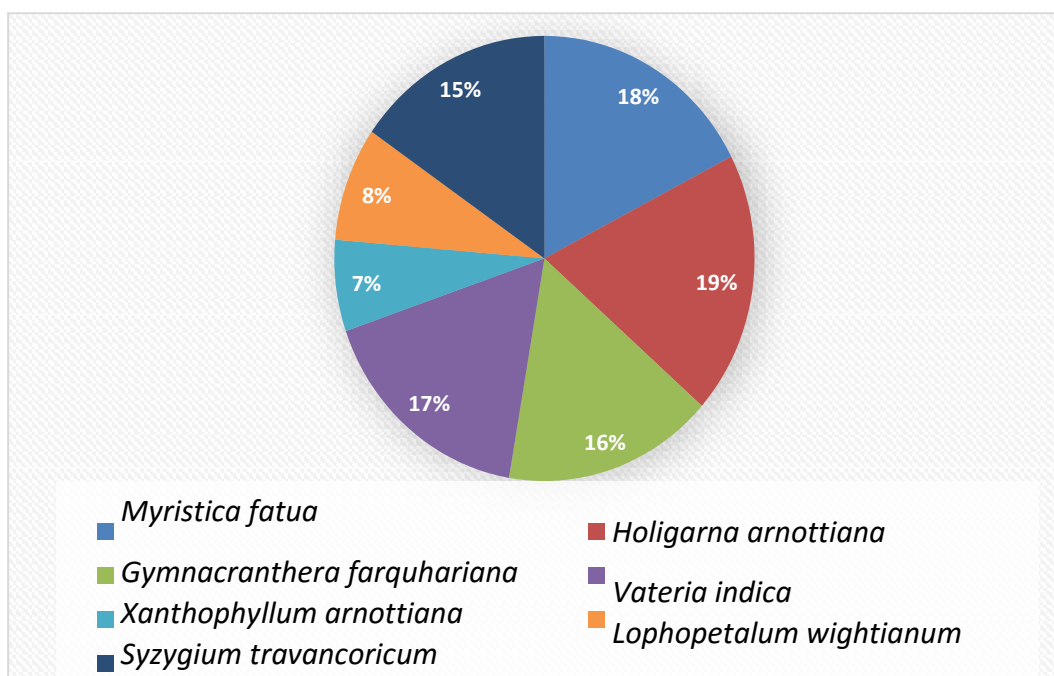


Figure 8. Regeneration status of various species at Kattilappara

4.2.2. Seed bank analysis

Seed bank analysis of both of the populations was done in order to examine the difference in the production and germination of seeds of *S. travancoricum* in both of the populations. The seeds kept in trays were examined and the seedling emergence was analyzed for each of the populations. The relative density of seedlings emerged from the seed bank was estimated for all the species.

Table 14. Relative density and habit of various seedlings emerged from seed banks of Kalasamala and Kattilappara

Sl.No	Species	Relative Density		Habit
		<i>Kalasamala</i>	<i>Kattilappara</i>	
1	<i>Syzygium travancoricum</i>	8.33	12.59	Tree
2	<i>Holigarna arnottiana</i>	16.67	16.78	Tree
3	<i>Mikania micrantha</i>	22.62	0	Climber
4	<i>Ageratum conyzoides</i>	21.43	0	Herb
5	<i>Leea indica</i>	30.95	0	Shrub
6	<i>Myristica fatua</i>	0	13.29	Tree
7	<i>Lagenandra ovata</i>	0	22.38	Herb
8	<i>Pothose scandens</i>	0	20.28	Climber
9	<i>Vateria indica</i>	0	14.69	Tree
Total		100	100	

In the Kalasamala population, the relative density of seedlings emerged was more for *Leea indica* (30.95) followed by a climber *Mikania micrantha* (22.62.). *S. travancoricum* had the least value of relative density (8.33). The relative density and habit of various species in the seed banks of Kalasamala is shown in Table 14.

The relative density of seedlings in the seed banks of Kattilappara was more for two herbaceous plants *Lagenandra ovata* (22.38) and *Pothose scandens* (20.28). *Syzygium travancoricum* was the species with the least value of relative density (12.59) (Table 14).

The relative density of seedlings emerged in the seed banks of both of the populations are shown in figure 9 and 10.

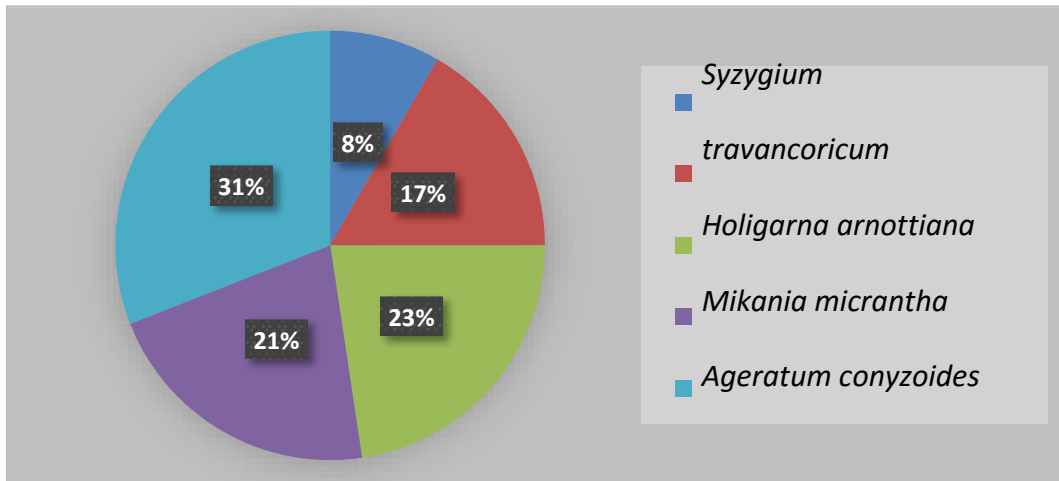


Figure 9. Seedling emergence in the seed bank of Kalasamala

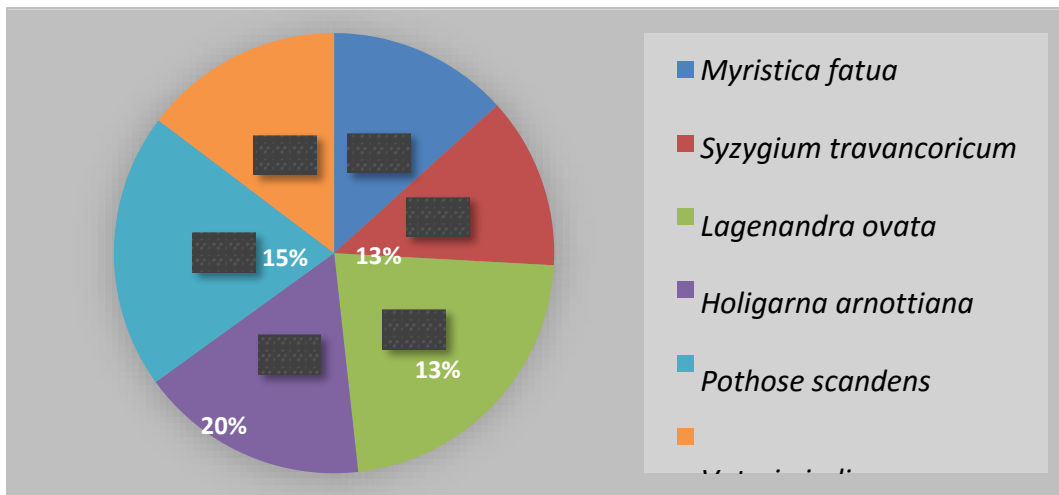


Figure 10. Seedling emergence in the seed bank of Kattilappara

4.1.3. Leaf morphology

The leaf morphological characters of *S. travancoricum* growing in the two populations at Kalasamala and Kattilappara were studied in order to find out significant difference in the characters between the two populations. The leaf morphological characters subjected to the study were leaf length, leaf width, length of the petiole and colour of the young leaves. The analysis showed significant differences between the populations in case of some of the characters.

The leaf morphological characters such as leaf length, breadth and petiole length were estimated using a meter scale. In the case of leaf length, the trees at Kalasamala showed a higher value than those at Kattilappara. The mean leaf length at Kalasamala was 17.8 cm, while that of Kattilappara was 14.6 cm. When leaf width was considered, trees at Kalasamala again had a higher mean value. The mean leaf width at Kalasamala was 8 cm whereas that of Kattilappara was 6 cm.

The mean length of the petiole was 1.7 cm in both of the populations. In the case of colour of young leaves, there was no difference between the populations. The trees in both of the populations had young leaves of reddish brown colour. The leaf morphological characters of the trees at both of the populations are given in table 15.

Table 15. Leaf morphological characters of *S. travancoricum* at Kalasamala and Kattilappara

Location	Leaf length (cm)	Leaf width (cm)	Petiole length (cm)
<i>Kalasamala</i>	17.96 ± 0.44	8.06 ± 0.34	1.66 ± 0.11
<i>Kattilappara</i>	14.66 ± 0.49	6.06 ± 0.1	1.69 ± 0.09
t-value	11.53*	13.96*	0.55 ^{ns}

*- significant at 5% level

ns- non-significant at 5 % level

4.1.4. Wood anatomy

The wood anatomical properties of *S. travancoricum* trees from both of the populations were studied in detail in order to find out variation in these properties between the two populations. Vessel morphology, ray morphology and fibre morphology of *S. travancoricum* were studied and analysed. Interestingly, all of the nine studied characters showed a significant difference between the two populations. The variation in the wood anatomical properties of *S. travancoricum* from the populations at Kalasamala and Kattilappara is shown in table 16 and figure 11.

Table 16. Wood anatomical properties of *S. travancoricum* from Kalasamala and Kattilappara

Anatomical charecteristics	Kalasamala	Kattilappara	t-value
Vessel length	641.8 ± 10.21	714.2 ± 12.41	10.07
Vessel diameter	202.2 ± 3.03	222.8 ± 4.44	8.57
Vessel frequency	8.2 ± 0.84	5.4 ± 0.55	6.26
Ray height	415 ± 8.86	560.6 ± 17.81	16.36
Ray width	23.66 ± 0.88	36.06 ± 4.96	5.50
Ray frequency	15.4 ± 0.89	12.4 ± 0.55	6.40
Fibre length	1649.4 ± 11.84	1747 ± 10.84	13.59
Fibre diameter	17.62 ± 0.68	25.49 ± 3.97	4.37
Fibre-wall thickness	5.88 ± 0.31	6.8 ± 0.34	4.47

4.2.4.1. Vessel morphology

The vessel morphological properties such as vessel length, vessel diameter and vessel frequency were studied in the trees growing in the two populations. The mean values of the vessel morphological characters are shown in table 10. The trees showed significant difference in case of all the three characters. The trees from Kattilappara showed higher vessel length and vessel diameter, and therefore they showed a lesser vessel frequency than that of the trees from Kalasamala. The mean vessel length of the trees from Kattilappara was 714.2 mm while that of the trees from Kalasamala was 641.8 mm. The mean vessel diameter of the trees of Kattilappara was 222.8 mm whereas that of Kalasamala was 202.2 mm.

The trees from Kalasamala showed an average of 8.2 vessels per mm² area while that of Kattilappara was only 5.4 per mm².

4.1.4.2. Ray morphology

The ray morphology of *S. travancoricum* was analysed in both of the populations by examining the properties like ray height, ray width and ray frequency (Table 16). All these three characters were significantly different between the two populations. While ray height and width were more in the trees of Kattilappara, ray frequency was higher in the trees of Kalasamala. The mean ray height was 560.6 mm in case of the trees from Kattilappara, whereas it was 415 mm in the trees from Kalasamala. The trees from Kattilappara had a mean value of 33.06 mm for ray width, where the trees from Kalasamala had a value of 23.66 mm. The average number of rays per mm² area in the trees from Kalasamala was 2.4 while that of Kattilappara was 12.4 (Table 16).

4.1.4.3. Fibre morphology

The fibre length, fibre diameter and fibre wall thickness of the *S. travancoricum* trees from both of the populations were subjected to detailed examination in order to discover the possibilities of variation in the fibre morphology of the species, between the two populations. The trees had a significant difference between the two populations, in all the three properties. The trees from Kattilappara recorded higher values for all the three properties. The mean values of fibre length and fibre diameter for the trees from Kattilappara were 1747 mm and 25.49 mm respectively, whereas that of trees at Kalasamala were 1649.4 mm and 17.62 mm respectively. The mean fibre wall thickness was 6.8 mm and 5.88 mm in Kattilappara and Kalasamala respectively.

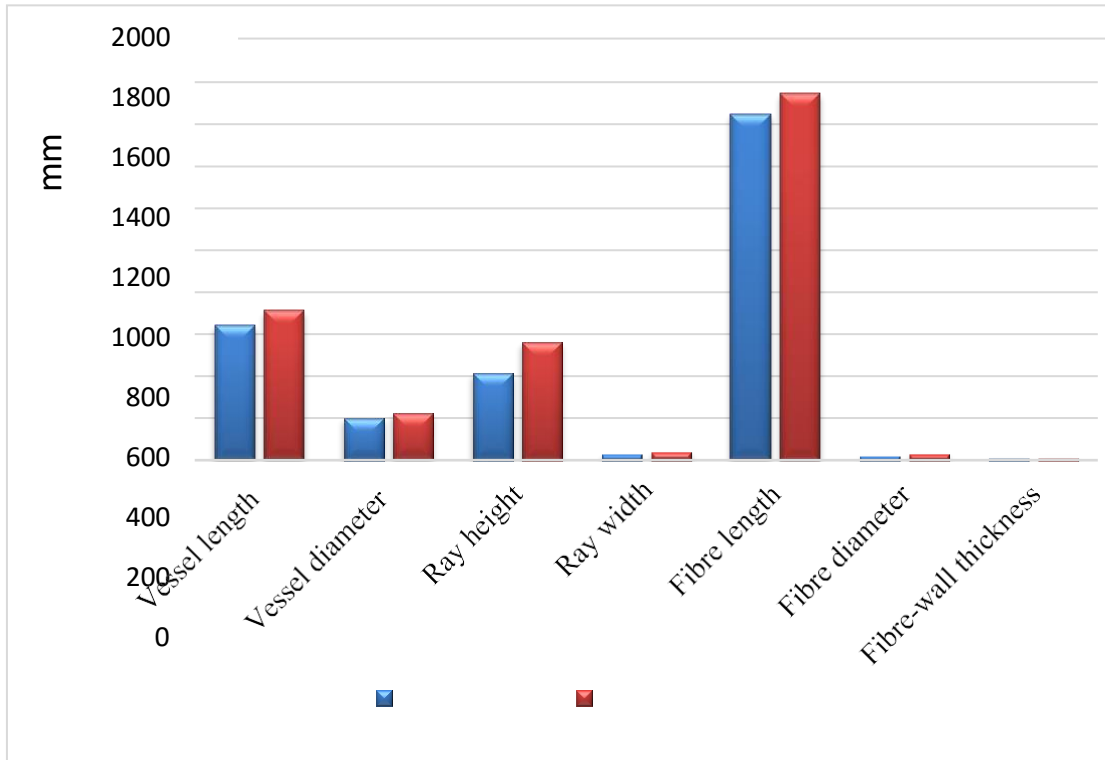


Figure 11. Variation in wood anatomical properties of *S. travancoricum* in Kalasamala and Kattilappara

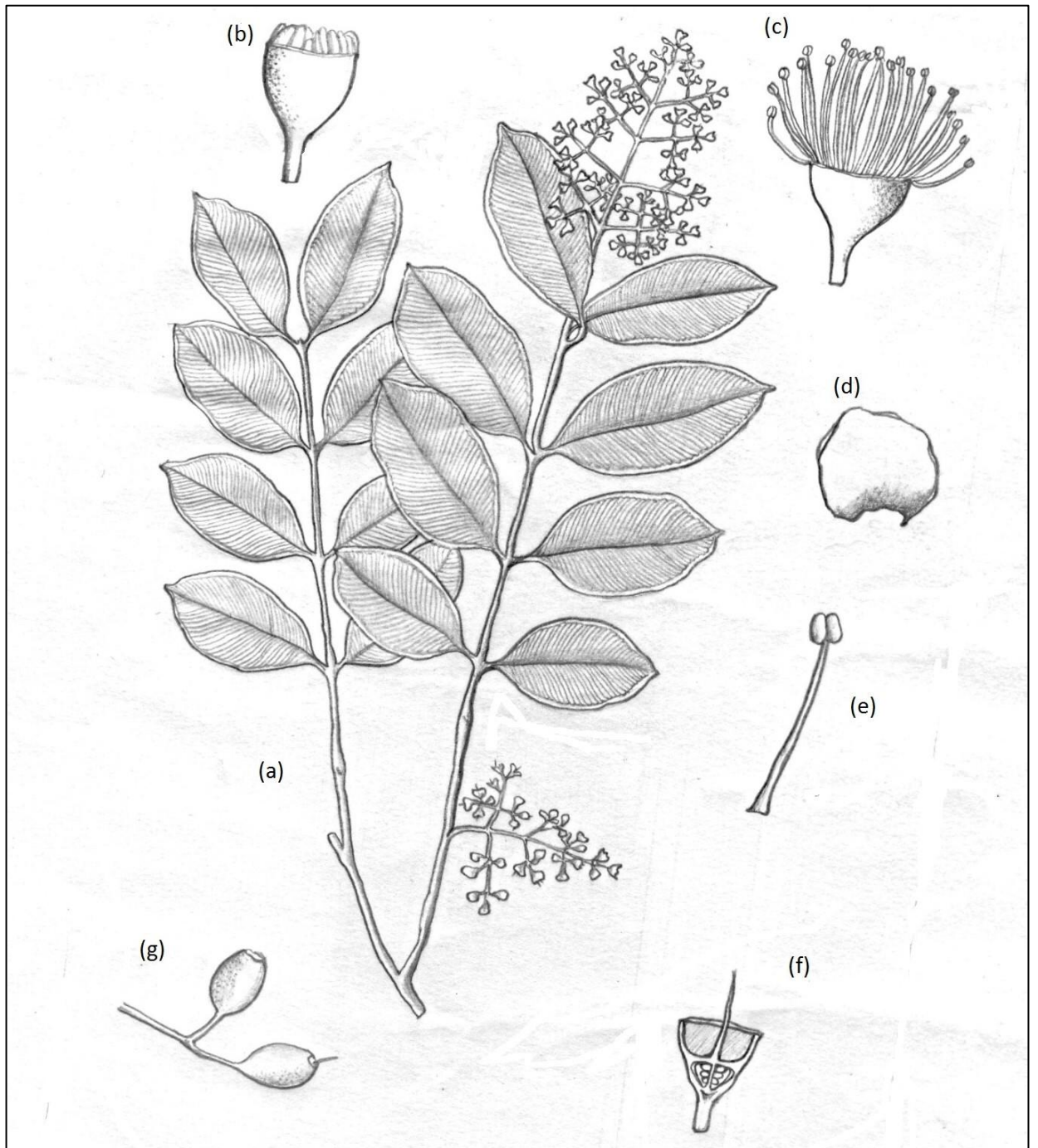
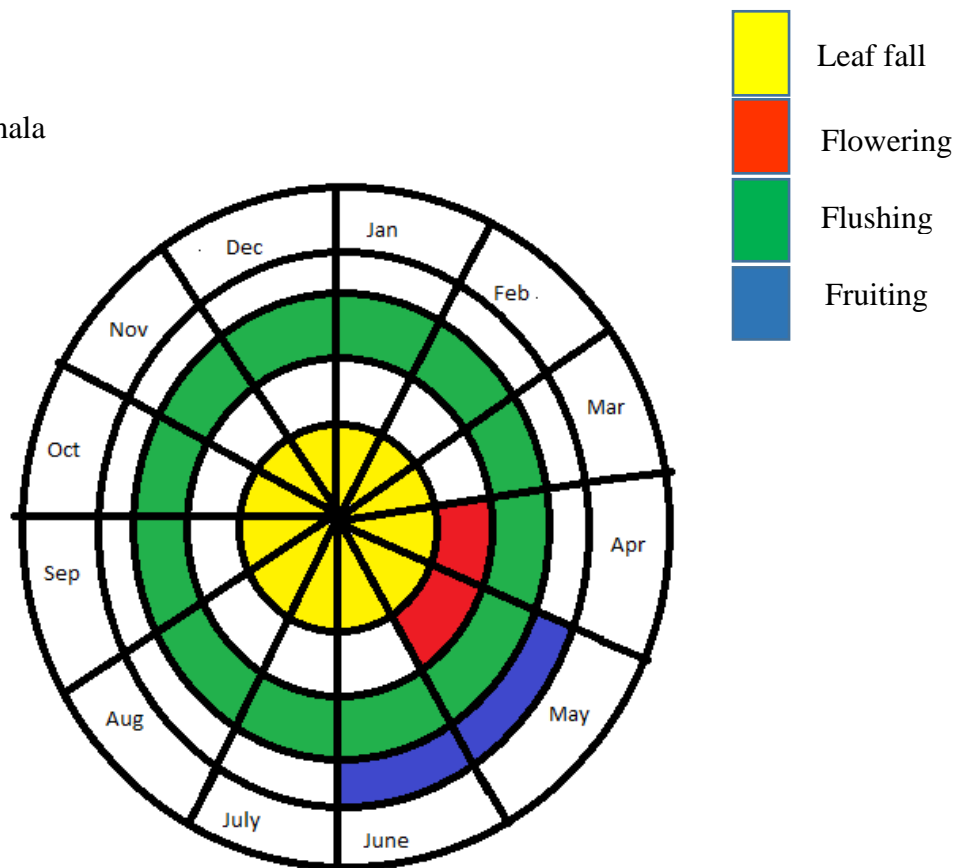


Figure 2. Floral parts of *S. travancoricum*: (a) Habit, (b) Flower bud at early anthesis, (c) Flower, (d) Petal, (e) Stamen, (f) Longitudinal section of pistil, (g) Fruit.

(a) Kalasamala



(b) Kattilappara

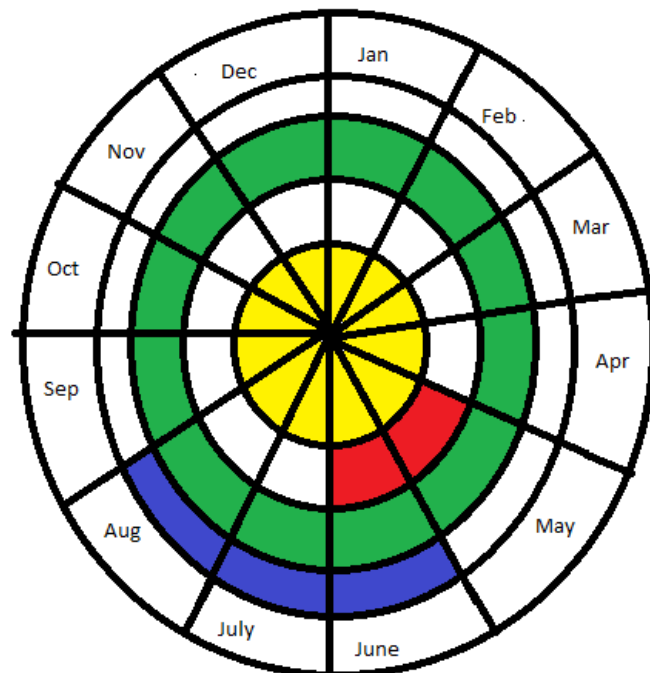


Figure.1. Phenograms of *S. travancoricum* in (a) Kalasamala and (b) Kattilappara



(a)



(b)



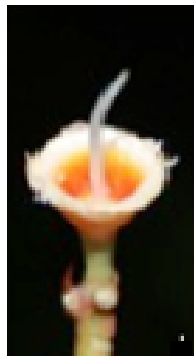
(c)



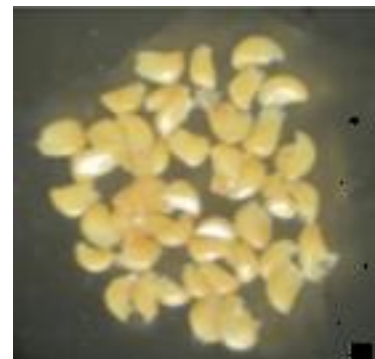
(d)



(e)



(f)



(g)

Plate 6. Floral morphology of *Syzygium travancoricum* : a- Mature buds; b-e- Different stages of anthesis; f- Calyx cup with centrally located ovary terminated with simple stigma; g- Ovules

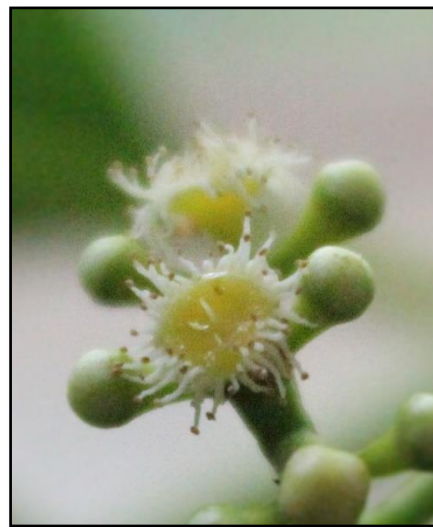


Plate 5. Flowering in *S. travancoricum*: a- Full bloom, b- Twig with inflorescence, c- Buds and flowers, d-Opened flower.



Plate 7. Fruits of *Syzygium travancoricum*: a- fruits on the tree; b- fallen fruits

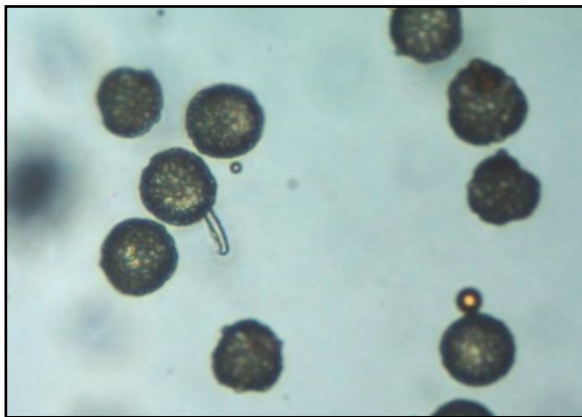
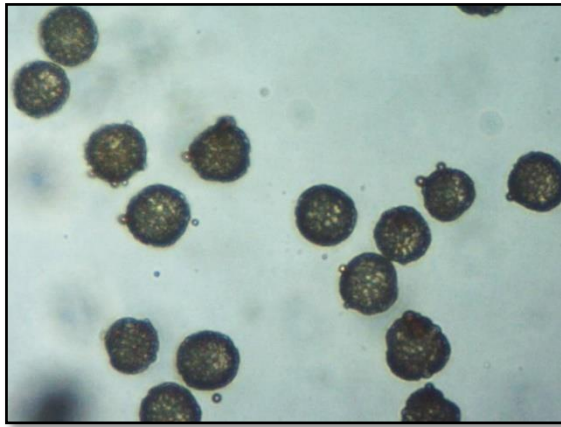


Plate 8. Various stages of pollen tube growth during the pollen viability analysis of *S. travancoricum*.



Holigarna arnottiana



Leea indica



Syzygium travancoricum

Plate 9. Naturally regenerated seedlings of various species at Kalasamala.



Holigarna arnottiana



Syzygium travancoricum



Myristica fatua



Vateria indica

Plate 10. Naturally regenerated seedlings of various species at Kattilappara.



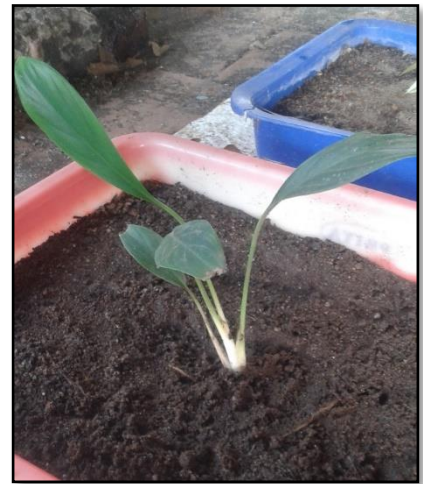
Vateria indica



Pothos scandens



Syzygium travancoricum



Lagenandra ovata

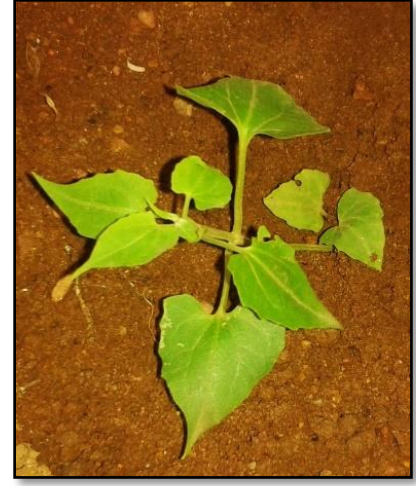


Myristica fatua

Plate 11. Seedlings of various species emerged from the Kattilappara seed bank



Ageratum conyzoides



Mikania micrantha



Leea indica



Syzygium travancoricum

Plate 12. Seedlings of various species emerged from the Kalasamala seedbank.

(a)



Plate 13. Leaf length (a) and leaf width (b) of *S. travancoricum* at Kalasamala



Plate 14. Leaf length (a) and leaf width (b) of *S. travancoricum* at Kattilappara.



Plate 15. Young leaves of *S. travancoricum* at (a) Kalasamala and (b) Kattilappara

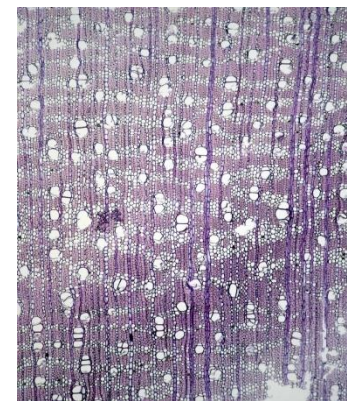
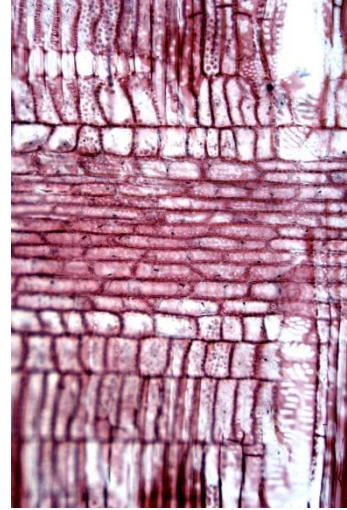
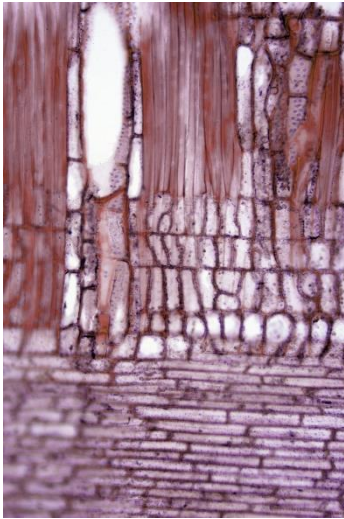


Plate 16. Wood anatomical analysis: (a), (b) &(c) - Tangential, radial and cross sections from Kalasamala; (d), (e) & (f) - Tangential, radial and cross sections from Kattilappara

5. DISCUSSION

The present study was done in order to to compare the genetic variation between two geographically isolated populations of *Syzygium travancoricum* Gamble. The study also investigated the reproductive biology of the species, to determine whether constraints to seed production can be a reason for the restricted distribution.

5.1. REPRODUCTIVE BIOLOGY

The reproductive biology of *S. travancoricum* Gamble was studied in detail in order to determine if constraints to seed production may explain the restricted distribution. The phenology, floral morphology, reproductive potential, fruit production and pollen viability of the species were observed in detail. Information about the reproductive biology of a species is a pre- requisite for undertaking conservation programmes. *S. travancoricum* being a rare and endangered species, should have to be considered for conservation strategies. Hence, the present study was taken up with the objective of understanding the phenology and reproductive biology of the species and the result of the study will provide necessary information for undertaking such conservation programmes in the species.

5.1.1. Phenology

An insight into reproductive phenology is crucial in understanding forest regeneration dynamics as the pronounced seasonality affects reproductive performances such as seed production, germination, survival and seedling growth (Augsburger, 1981). Leaf shedding, leaf flushing, flowering and fruiting are the major events in the phenological calendar of a species. Tropical plant communities show conspicuous seasonal pattern in vegetative and reproductive phenologies at

both community and species levels (Williams-Linera, 2003). According to Borchert (2005), in tropical forests, many tree species flush and flower at the same species specific time every year.

Syzygium travancoricum, being an evergreen species, the leaf shedding and flushing processes could not be identified clearly and separately. Leaf fall and flushing continued throughout the year. The habit of large scale leaf shedding was evolved as a mechanism to overcome adverse conditions. In the case of deciduous species, the process is strictly followed because they have to reduce transpiration during summer months. Gopakumar (1995) and Saju (2000) have observed leaf fall during the hottest months or just after the rainfall in many deciduous tree species. Hicks (1988) suggested that the organized leaf shedding habit could be an adaptation to tide over the water stress periods in deciduous species. But in the case of evergreen species, this systematic leaf shedding process is generally not followed because they are not under that much water stress when compared to deciduous trees. *S. travancoricum* is not only an evergreen tree but a species growing in water logged conditions or near water bodies. So, there is no question of being affected by the un-availability of sufficient water. There for, *S. travancoricum* does not follow the pattern of leaf shedding in deciduous trees and that is why they start to shed their leaves in January followed by flushing in mid-february.

Trees renew their foliage in periodic flushes. The leaf renewal and leaf shedding in trees are often tuned with the climate of the area so that the species could utilize the optimum conditions for their advantage and there by achieve maximum photosynthetic production. In this study also, the trees were in its maximum foliage during the period when the moisture and sunshine were the highest. Janzen (1967) suggested that, by producing new leaves before the rainy season, the trees will be able to expose their foliage to the photosynthetically active radiation and can readily synthesise carbohydrates. In the case of the present study,

flushing of new leaves happened before the onset of rainfall, but it was not just before the rain. The flushing in *S. travancoricum* started by mid-february.

The flowering time was different in the trees at the two different populations under the study. The flowering in tropical tree species are generally influenced by moisture stress and many tropical trees have been reported to flower after the dry season (Saju, 2000). In evergreen species there occurs a synchronisation of flowering with dry season as trees are said to flower during stress period (Richards, 1952). In the present study also, the flowering in the observed species started after the start of the month of April. The findings are in agreement with those reported generally in the case of evergreen species. Drought might have acted as a stress to induce flowering in the species. Dry season flowering can also be attributed to attract the pollinators as insect activity is greatest in the months with dry periods (Schaik, 1986).

Variations can happen in the flowering phenology of a species. The same species standing at two different populations may sometimes show changes in the pattern of phenological events. Troup and Bor (2009) reported that the seasonal patterns of flowering and fruiting can be different in the same species if the species is standing in different populations. Also, Thakur (2013) stated that there were variations in the phenological events of *Artocarpus hirsutus* growing in two different altitudinal zones. In the present study also, the observed species showed variations in flowering patterns between the two populations. Rathke and Lacey (1985) had correlated a number of abiotic factors with flowering time like seasonal availability of conditions favourable for pollen transfer, availability of pollinators, competitive effects on seed set. The above mentioned reason might have contributed for variations in the flowering period. The factors related to the site, such as temperature and rainfall also can impact the phenology of a species (Cleland et al., 2006). Sarvas (1962), found that, temperature is the most important parameter

which determine the late or early occurrence of a phenological event. In the present study also, change in temperature can be a reason for the variation in flowering phenology.

Species specific variation in fruiting phenology were noticed in many trees by Sedgley and Griffin (1985). In the present study also, the fruiting phenology of *S. travancoricum* had some differences with that of other common species of the same genus. The fruiting pattern of the trees has strong bearing on its natural regeneration, and the seeds are often shed during the dry season which provide suitable conditions for their dispersal (Richard, 1975). In the present study also, this might be the reason behind the variation in fruiting period among the species of the same genus. As in the case of flowering, there were differences between the two populations of the species in the fruiting periods also. The duration of fruiting period was different in the two populations. In majority of evergreen species, fruit ripening is observed close to the onset of rainy season in order to enhance dispersal, escape predation, and avoid pathogen infection (Prasad and Hegde 1986). In the present study, flowering was followed by fruiting and continued till the end of June. Occurrence of seasonal fruiting pattern in tropical forests has been reported by Liebermann (1982). Fruiting prior to rainy seasons can be attributed to provide favourable germination conditions to the seed (Bhat, 1992). It is applicable in the present study also.

5.1.2. Floral morphology

The floral morphology of the species was studied in detail in order to understand about the reproductive biology. All the morphological characters related to all of the floral whorls were observed in detail. The characters of the flower of the species were compared to other common species of the same genus. The floral morphology of a number of *Syzygium* species is studied by scientists. A lot of new species are being discovered. In most of them, the flower appears in

cymose inflorescence, either axillary or terminal (Raju *et al.*, 2014). There are a lot of rare taxa coming under the genus *Syzygium* and most of them exhibits floral characters somewhat similar to the observed species. Mohan and Lakshmi (2000) reported that the presence of calyptra is particular character in most of the *Syzygium* species. According to a group of authors (Arathi *et al.*, 1999; Falcao *et al.*, 2002; Kaiser *et al.*, 2008 and Bajpai *et al.*, 2012), the characters such as cymose inflorescence, presence of calyptra, numerous stamens which are bent inward in bud stage are some of the common characters of the genus *Syzygium*. The observed species of the present study also exhibited flowers having calyptra and arranged in axillary cymose with numerous stamens, bent inward in bud stage.

Narayanan *et al.* (2014) explored the floral morphology of a newly identified rare species of the same genus *Syzygium dhaneshiana*. The species showed flowers almost similar to that of *S. travancoricum* except some differences in the size of the flower and length and breadth of the stamen. Raju *et al.* (2014) conducted studies on reproductive biology of *S. alternifolium* and Shareef *et al.* (2014) made investigations on a newly discovered species by them, *S. munnarensis*. They studied the floral biology of the two species. Most of the characters observed in the present study about the floral biology of *S. travancoricum* was almost similar to their observations with exceptions in only some characters such as flower colour and length of the petal.

5.1.3. Reproductive potential

Reproductive potential or the total number of flowers produced per tree of *S. travancoricum* was estimated as part of the studies on reproductive biology of the species. Tropical trees exhibits generally two types of flowering pattern, at one extreme are the species with mass flowering individuals producing large number of new flowers each day over a week or less and at the opposite extreme are the species

with steady state individuals producing small number of flowers daily or for many weeks. Studies show that, in the genus *Syzygium* also there are generally two types of flowering pattern.

In *Syzygium* genus, the flowering pattern is of two types, mass flowering and short-period steady state flowering but most species exhibit mass flowering such as *S. cumini* (Reddi and Rangaiah, 1999), *S. luehmannii* (Sanewski, 2010), *S. aqeum* (Tarai and Kundu, 2008) and *S. alternifolium* (Raju *et al.*, 2014). *S. travancoricum* is also a mass bloomer and it flowers during dry season. Flower production in large number is a mechanism to attract many type of opportunistic pollinators with density dependant foraging behaviour (Janzen, 1967). This may be the reason of mass blooming in *S. travancoricum* also. In most of the studies on reproductive biology of *Syzygium* species, the flowering occurs after partial leaf shedding and leaf flushing occurs after the completion of flowering. But in the present study, the observed species did not show the same pattern. The flowering and fruiting events occurred after new leaf formation. This finding agreed with the observation made by Mohan and Lakshmi (2000) in *S. alternifolium* that the flowering and fruiting events occur after new leaf formation in the species.

In a handful of studies done on the reproductive biology of various species in the genus *Syzygium*, there was a change in the flowering intensity of the species over years. The 3-year study on the flowering phenology of *S. alternifolium* by Raju *et al.* (2014) indicated that heavy flowering was not a regular event since the observations revealed that flowering intensity varied, it was massive in the first year and reasonable in the second and third years, and also a very little flowering on a few branches of some trees in the third year. Sanewski (2010) stated that such a flowering pattern happens in *Syzygium* species and the author linked it to rapid changes in water availability and temperature in monsoon sites. He also stated that in most tree species, sufficient starch levels are needed for the production of flowers, particularly mass-flowering species, like most of the *Syzygium*. Trees with inadequate starch levels, may not flower profoundly in that year and hence,

gradually move into an alternate bearing pattern. While environmental factors are usually the trigger, adequate starch reserves are a pre-requisite for flowering. In the present study, there may be a possibility of variation in the flowering pattern, but it could not be observed because of the time limit of the study.

In a lot of studies, mass blooming *Syzygium* species showed flowering two times a year. Tarai and Kundu (2008) reported that *S. aquem* flowers twice in a single year in India. Sanewski (2010) observed that *S. luehmannii* flowered massively in the first spell and showed minor flowering in the second spell in a single year. But, in the present study, *S. travancoricum* showed flowering only once in a year. There are reports on other mass blooming *Syzygium* species like *S. sayeri* (Williams and Adam, 2010), which showed flowering only once in an year.

The major factors affecting the flowering pattern of a species are reported to be rainfall and temperature. Several studies have accepted the influence of rainfall or water levels on the flowering intensity in mass blooming *Syzygium* species. Liao and Lin (2001), in southern Taiwan, described that *S. samarangense* displayed early mass flowering in summer after flooding. In a study done by Sanewski (2010) in *S. luehmannii*, the author observed a variation in the flowering pattern in southeast Queensland, Australia and ascribed such a flowering behaviour to the prevailing temperature and water availability. Law *et al.* (2000) stated that high rainfall in summer-autumn period caused heavy flowering in spring for most of the Myrtaceae members. Keatley *et al.* (2002) reported a substantial relationship between temperature and rainfall and flowering in eucalypts over 23 years. Therefore, water, temperature and soil nutrient status could be influencing and regulating the flowering events collectively in *S. travancoricum*.

The difference in flowering pattern is to achieve many objectives. Regulation of pollen flow, foraging behaviour of pollinators, rate of fruit development in response to resource availability and habitat affect the flowering pattern (Bawa *et al.*, 1985).

5.1.4. Fruit production

The proportion of flowers setting fruits are significantly lower in tree species (Southwick *et al.*, 1986). All non-parthenocarpic species require successful pollination and fertilization for formation of fruits and this in turn depends on many factors such as the climate, pollinators and floral biology of the species (Sedgley, 1980). In the present study, the number of fruits produced per tree and the percentage of fruit set was studied in *S. travancoricum*. The average number of fruits produced per individual tree was observed as 1683. But, when compared to the number of flowers produced per tree, this rate was low. It meant that the percentage of fruit set was poor in *S. travancoricum*. The extent of initial fruit set ranged from 20 to 27 per cent.

Generally in mass flowering species like *Syzygium*, the percentage of fruit set will be more compared to the other type of species, because insect pollinators move among mass flowerings and obligate outcrossing tropical trees (Augspurger, 1980). But, in the present study, even though *S. travancoricum* is a mass flowering species, it showed a low fruit set percentage (27.33 %).

Though plants are known to show higher rate of fruit set with cross pollen (Johnson, 1991, Young and Young 1992), studies made earlier in tropical trees have reported only low fruit setting because of the pollination techniques, flower abortions and inbreeding (Haber and Frankie 1982). According to Nagarajan *et al.* (1996), very low fruit set in tropical trees is not a rare phenomenon. The problem could be because of limitation of pollinators (Calvo, 1990), insufficient visits of pollinators or may be due to self-incompatibility which is fairly common in tropical

trees (Bawa, 1974). Some authors have stated that, fruit production is strictly pollen-limited (Karoly, 1992). There for, the low rate of fruit set percentage in *S. travancoricum* can be a result of either unavailability of viable pollen or inadequate visit of pollinating agents.

Some authors reported that, the percentage of fruit set also depends upon the environmental characteristics and site factors. According to Blum (2010), in water loving plants, fruit set and seed production was decreased by 35 per cent, when they were under water stress. In the present study also, this could be one of the reasons for poor fruit set.

The fruit development in tree species involves different developmental phases. In the case of *S. travancoricum*, these phases of fruit development and seed maturity can be easily distinguished from the colour change of fruits, like that of most of the *Syzygium* species (Raju *et al.*, 2014). It is the readily apparent phenomenon in the ripening process which involves chlorophyll loss and subsequent synthesis of pigments (Rhodes, 1970). Jackson (1986) also reported browning of pericarp as a good indices in determining seed maturity in many tree crops.

All the fruits which were set initially would not be retained until the final developmental stage, a large portion of them will be dropped before attaining the seed maturity. It means that, in a species with low fruit set percentage, the seed germination percentage will be very poor because, at the stage of seed maturity, the number of matured seeds will be further decreased due to the fruit drop. Similar to the seed maturity, only a portion of the mature seeds are germinable and will involve in the germination process. The regeneration potential of the species largely depends upon the extent of seed maturity and germination percentage (Sedgley and Griffin, 1987).

5.1.5. Pollen viability

Pollen viability is considered as an important parameter of pollen quality (Dafni and Firmage, 2000). The viability of pollen grains of *S. travancoricum* was studied by pollen germination techniques. The percentage of pollen germination observed was very low. The mean percentage of pollen germination was only 24.23 per cent.

The observed percentage of pollen germination was very much low when compared to the other common species of the same genus. In a study done by Sivasubramaniam and Selavarani (2012) to study the viability of pollen grains of *S. cumini*, they observed a pollen germination percentage in the range of 40 to 48 per cent. Jose *et al.* (2009), reported the pollen viability of *S. mundagom* fell in the range of 38-44 per cent. Most of the common species of the genus *Syzygium* generally shows a pollen germination above 40 per cent. But, in the present study, *S. travancoricum* showed only a maximum of 24 per cent. This may be one of the reasons of the rarity of the species.

According to many of the previous studies (Fritz and Lukaszewski, 1989; Sedgley and Harbard, 1993), the pollen viability of a species depends upon the pollen longevity, pollen dispersal and pistil receptivity. In the case of *S. travancoricum*, further studies are required to find out, what the causes are of decreased pollen viability.

5.1.6. Seed germination

The seed germination status of *S. travancoricum* was estimated by sowing the seeds in trays filled with sand. The observations showed that, the percentage of seed germination of *S. travancoricum* was very poor. The mean percentage of seed germination was only 16 per cent. In addition to that, all of the seedlings germinated during the study died in the seedling stage itself.

The seed germination percentage of *S. travancoricum* was very poor when compared to that of other common *Syzygium* species. Subramaniam and Selvarani (2012), reported that the germination percentage of *S. cumini* seeds was 60 to 80 per cent, without any seed pre-treatments. Raju *et al.* (2014) studied the germination percentage of seeds of *S. alternifolium* and the result was in the range of 45 to 60 per cent. According to Jose *et al.* (2009), the seed germination percentage of *S. mundagom* was 35 to 42 per cent.

The seed germination of a species depends upon the factors like planting substrate (Gairola *et al.*, 2011), the duration of storage (Jaiswal and Chaudhary, 2005) and the moisture content of the seeds (Richard *et al.*, 1964). In the present study, any of these factors can be the cause of a low rate of seed germination. Further studies are required to confirm this finding.

The death of the germinated seeds in the seedling stage itself could be due to the unavailability of a waterlogged condition. Roby *et al.* (2013) reported that, the *S. travancoricum* seedlings raised by them could not survive until they provided a waterlogged condition. The natural regeneration of *S. taravancoricum* was poor in Kalasamala, and that can also be a reason for the sudden death of the young seedlings.

5.2. COMPARISON OF VARIATION

Syzygium travancoricum trees are generally seen in the Myristica swamps of southern Western Ghats, which is only below 200 in number (Roby *et al.*, 2013). But the population of the same tree discovered recently, existing as a part of the sacred grove of Siva temple at Kalasamala, which has more than 200 trees is really interesting. The detailed studies and analysis done as a part of the comparison of variation between the trees of the two population showed that there was a significant difference between the trees of the two populations in a handful of characters which could point to a genetic variation between the two populations.

5.2.1. History of Kalasamala grove

Kalasamala Grove in Akathiyoor, in Porkkulam panchayat near Kunnamkulam is the first Biodiversity Heritage Site of the State, designated by the Kerala State Biodiversity Board (KSBDB). Lord Shiva and Lord Vishnu are the foremost deities of this sacred grove. The grove is home for the study species *Syzygium travancoricum* inside the temple premises at Kalasamala. The sacred grove has 1.2 ha of land packed with more than 150 individuals of *S. travancoricum*.

The temple authorities of Kalasamala grove are the care takers of this unique ecosystem, and play a fundamental role in the management of the bio diversity conservation system. The National Bio Diversity Authority (NRDA) and the KSBDB provide financial assistance to the temple authorities for the protection of this exceptional ecosystem. The local panchayat also allocates funds in its plan for conservation and fortification of the site. A total ban on cutting trees in the sacred grove is in place.

From the reconnaissance survey conducted in the site, it was evident that, the site was a perfect fresh water swamp at once. But, at present, the swampy nature of the site is lost forever. According to IUCN, a major threat faced by the trees is that, the swampy wetland habitat has been widely drained and converted into paddy fields.

From surveys conducted among the local people, a clear idea on the history of the grove was appeared. According to them, earlier, the deep swamps in the area could not be crossed by feet. The extant swamp extended up to nearly one acre. Most of the swamps had dried up or had been reclaimed over the years. A section of the local population depended on this critically endangered trees for firewood. Many trees were lost in the process, but some were regenerating. Then, the people there started converting the swampy wetland into paddy fields for cultivation. By this process, the water in the swamps started to get reduced due to draining. After 2005, a soil mining unit started mining the hills of Kalasamala and this lead to the death of the natural aquifers of the area. Gradually, the water level of the area got lowered and the swampy nature was completely lost.

One of the main interests of the site is, only *Syzygium travancoricum* trees are standing there just like a monoculture plantation. This may be due to various reasons. Sometimes, all other species standing in the swamp were died, after the loss of the original nature of the habitat, and only *S. travancoricum* trees survived. There can be other reasons too, and it needs further detailed investigation on the history of the swamp.

5.2.2. Population structure

5.2.2.1 Biometric analysis

As a part of comparing the variation between the two populations, the structure of the populations was examined in detail. The biometric characteristics of the trees like height, girth, crown height, crown width and crown area were

studied. In addition to that, the regeneration status of *S. travancoricum* and the species associations were also analysed. There are a lot of studies which points out that study of the population structure is important in the case of comparison between two populations.

The present study showed that there is a significant difference in the case of population structure of *S. travancoricum* between the two populations. The trees of the two populations showed significant differences in mean height, mean DBH, and the crown characteristics like crown height, crown width and crown area. The differences in the population structure of the same species at two different localities can happen due to the dissimilarities in micro-climate, nature of the habitat, age of the trees, anthropogenic disturbances etc. (Agren, 1996), apart from genetic differences. In a study done by Yates *et al.* (2002), the population structure of two different populations of *Acacia aprica* having the same age were significantly different due to the changes in the microclimatic parameters like rainfall pattern, temperature, humidity etc. In the same study, the two different populations of *Acacia cochlocarpa* growing in the same microclimatic conditions had shown a significant difference in the characteristics related to population structure only due to a change in their age. Hendrix *et al.* (2000) reported that there were significant differences in the biometric characteristics of *Phlox pilosa* attributable to the variations in the habitat conditions.

In the present study, all of the three above mentioned factors can be the cause for the changes in the population structure more than any other genetic factors. Genetic variations can also be a reason for the changes in population structure of species. In the present study, the micro-climatic characters had some differences between the two populations (Table 17). Those changes in the annual rainfall and temperature can be a cause for the variation. In the case of nature of the habitat also, there were differences between the two populations. While the Kattilappara population was a fresh water swamp, the Kalasamala grove looked like a modified swamp. The swampy nature and the water retaining capacity of the area

has been lost over years. *S. travancoricum* grows better in swampy, water logged conditions. It may be the reason for the less growth of the species at Kalasamala. The age difference between the two populations may also be a reason for the variation.

Table 17. Annual rainfall and temperature of Kalasamala and Kattilappara.

Parameter	Kalasamala			Kattilappara		
	<i>Maximum</i>	<i>Mean</i>	<i>Minimum</i>	<i>Maximum</i>	<i>Mean</i>	<i>Minimum</i>
Annual Rainfall (mm)	3955	3198	2310	2250	2950	1246
Mean Annual Temperature (°c)	36.2	29.6	24.9	36	27.9	15

The analysis of girth-class distribution of *S. travancoricum* showed substantial differences between the two populations. At Kalasamala, more than 80 per cent of the trees were immature, which means only less than 20 per cent of trees were mature. It may give a feeling that the population of *S. travancoricum* at Kalasamala is very secure. But the analysis also showed that there were no trees in the diameter class of 10-30 cm, which indicates that very poor regeneration is happening at the population. It specifies that the population at Kalasamala is going through a risk of threat. The poor regeneration status of *S. travancoricum* at Kalasamala can be explained in relation to the unavailability of favourable conditions for seed germination.

The girth class distribution analysis of the species at Kattilappara showed that about 95 per cent of the trees were mature, only less than 10 per cent of the trees were immature. It means that there are some problems related to the regeneration of the species which makes the number of species in the lower girth classes very much less. This indicates that there is a recent threat in the survival of the species. The study by Roby *et al.* (2013) on the same species in the Myristica swamps showed that, while phenological studies indicate normal patterns of fruiting and germination, regeneration enumeration shows that the problem may be in seedling recruitment. Their study also indicated a recent threat in the survival of the species.

5.2.2.2. Regeneration status

The regeneration status of *S. travancoricum* was examined in detail at both of the populations. The status of regeneration was very poor at the Kalasamala population and it was marginally better at Kattilappara. The mean number of regeneration of *S. travancoricum* at Kalasamala was only one and that at Kattilappara was 7.4 (Figure 12).

The girth class distribution of *S. travancoricum* can be explained in connection with the regeneration status. In the Kalasamala population, even though more than 80 per cent of the trees were immature, there were no trees coming under the diameter class of 10-30 cm. The regeneration studies at Kalasamala also showed that the regeneration status of the species was poor, when compared to that of other species like *Holigarna arnottiana* and *Leea indica*. The relative density of *S. travancoricum* seedlings was only 8.06 percentage. The poor regeneration can be due to poor percentage of seed set, or any other constraints to the seed germination, or the seeds may not be viable. According to Lamont *et al.* (1991), the poor regeneration status of a species can be explained by the unavailability of favourable conditions like apt temperature and moisture, desired amount of rain fall and some anthropogenic factors like habitat conversion and fragmentation. In the case of rare

and endangered species, the deprived seedling regeneration can be a result of poor percentage of seed set or production of non-viable seeds (Zimmerman *et al.*, 1988).

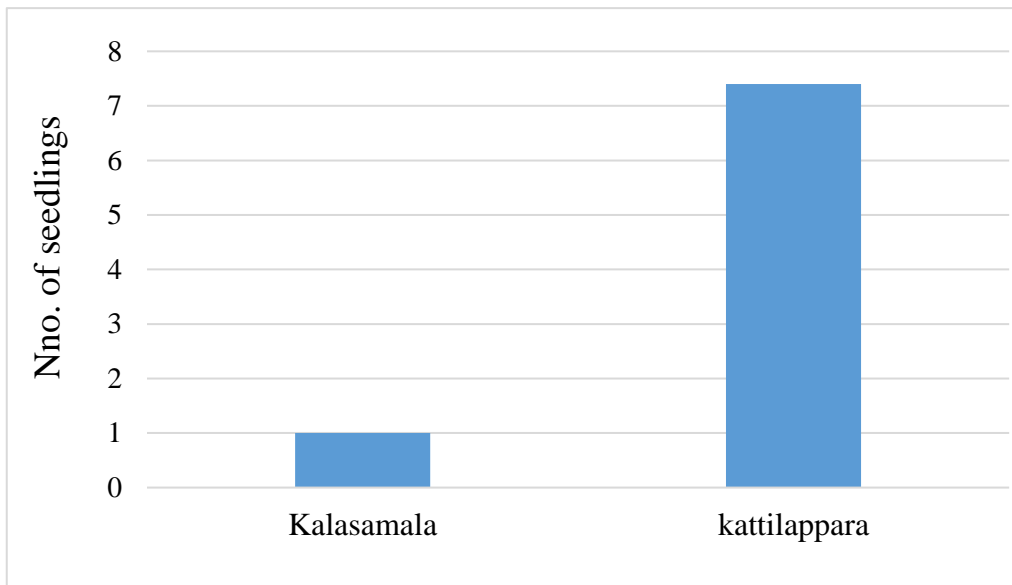


Figure 12. Regenerations status of *S. travancoricum* at Kalasamala and Kattilappara.

In the case of Kattilappara population, more than 90 per cent of the trees were mature, with a normal fruiting and flowering pattern. Regeneration status of *S. travancoricum* in Kattilappara was better than that of the Kalasamala population. However, when compared to other common species in the population, the percentage of regeneration of *S. travancoricum* was extremely poor. The relative density of the seedlings of *S. travancoricum* was only 15 percentage. This explains that, in the Kattilappara population, the reason of poor regeneration may not be due to habitat fragmentation and unavailability of rainfall. The study done by Roby *et al.* (2013), in the Myristica swamps showed that the most of the seeds germinated within one or two weeks after fall, but they did not survive after the two-leaf seedling stage. It was presumed that fungal attack on the seeds in the intense humid

nature of the swamp conditions, due to the inundation of swamps at the time of fruit fall and seed germination may be the causes of poor germination percentage.

5.2.2.3. Species association

The species association studies made in the two disjunct populations showed that there were some differences in case of the species growing associated with *S. travancoricum*. The associated species at both of the populations were entirely different except that of *Pandanus* and *Holigarna arnottiana*. These two species were found associated with the study species in both of the populations. The density and diversity of associated species were poor at Kalasamala, whereas at Kattilappara, the associated species were more in number and they were much diverse.

In Kattilappara, the major species associated with the study species were the members of the family Myristicaceae. The most dominant species included were *Myristica fatua*, *Gynacranthera farquhariana*, *Lophopetalum wightianum*, *Holigarna arnottiana* and *Vateria indica*. Some other species like *Myristica malabarica*, *Knema attenuata*, *Xanthophyllum arnottianum*, *Pandanus spp.* were also seen. At Kalasamala, there were only a few species like *Holigarna arnottiana*, *Ochlandra* and *Pandanus* which grew in association with *S. travancoricum*.

The major difference between the two populations was that, at Kalasamala, *S. travancoricum* was the major species while at Kattilappara, it was not. In the present study, the parameters like relative density, relative frequency, relative basal area, Important Value Index (IVI), and abundance of the trees standing at Kattilappara was studied. The analysis showed that *S. travancoricum* was having fourth position in the case of relative density and abundance in the list which was topped by *Myristica fatua* and *Gynacranthera farquhariana*. In the case of IVI, *S. travancoricum* was at the second position. Roby *et al.* (2013) conducted a study in the 17 patches of Myristica swamps in order to map the potential areas for the

growth of *S. travancoricum*. In the study, they estimated the above mentioned parameters. *S. travancoricum* acquired fourth position in case of relative density. In the case of Important Value Index (IVI) *S. travancoricum* was only having the sixth position in the list in which *Myristica fatua* and *Gymnacranthera farquhariana* became the toppers. Bhat and Kaveriappa (2009), has conducted ecological studies on the Myristica swamp forests of Uttara Kannada in which they also calculated the IVI of various trees. In their study also *M.fatua* and *G. farquhariana* topped the list whereas *S. travancoricum* was in the 17th position. According to Anand *et al.* (1999), *Pandanus* is one of the major species which always grow in association with *S. travancoricum*.

5.2.3. Seed bank analysis

The seed bank analysis was done as a part of the present study in order to check and compare the variation in seed bank, between the two sites. The rate of seedling emergence was generally very less for both of the populations. The results showed that there is a significant difference between the two populations in case of seedling emergence. The mean number of seedling emergence at the Kalasamala population was 0.46 and that of the population at Kattilappara was 4.4 (figure 13). It means that, the population at Kattilappara showed better seedling growth from the seedbank compared to that of the Kalasamala population.

The seedlings of *S. travancoricum* emerged from the seedbank trays were generally less compared to a lot of other species. During the present study, the relative densities of seedlings emerged from the seed banks of both of the populations were estimated. *S. travancoricum* seedlings obtained the least value of relative density in the seedbanks of Kalasamala (8.33%), in which *Leea indica*, a shrub had the highest value (30.95%). *Holigarna arnottiana* was the tree species with highest relative density (16.67%). In the population at Kattilappara, the relative density of seedlings of *S. travancoricum* was the lowest (12.5%) where the

herb *Lagenandra ovata* had the highest value (22.38%). Among the trees, *Holigarna arnottiana* had the highest relative density. In the study conducted by Timy (2014) at Attappady, the percentage of seedling emergence from the seed bank was different in different populations for the same species. This was explained as the low density of seedling emergence from seed banks due to relatively lesser species present in the vegetation, as soil seedbanks reflect the vegetation to some degree. This explanation is applicable in the present study in the case of the population at the Kattilappara.

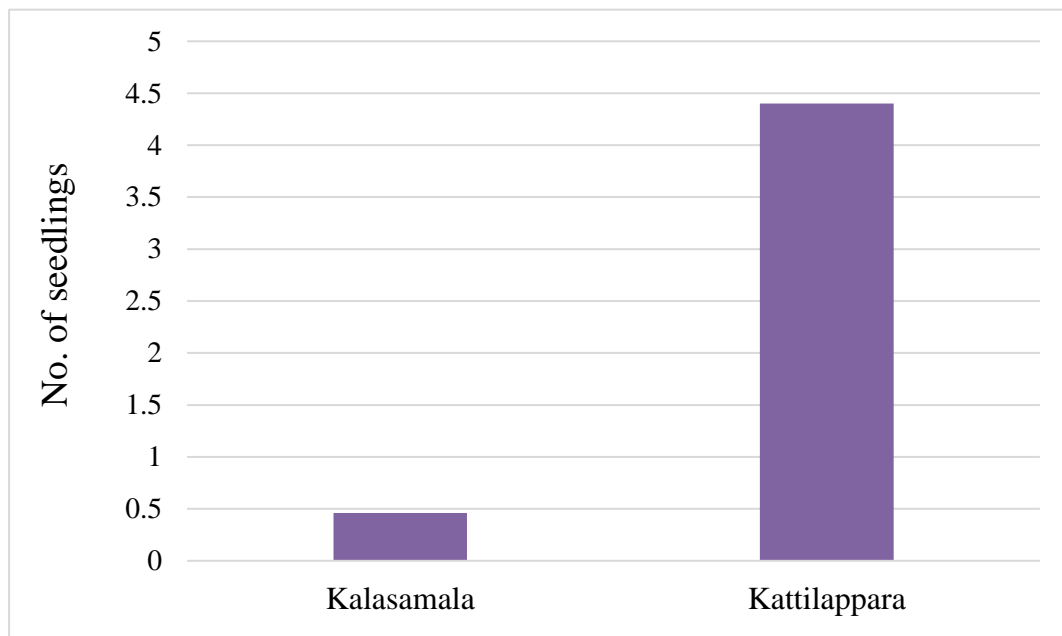


Figure 13. Seedling emergence of *S. travancoricum* from seedbanks at Kalasamala and Kattilappara.

Morgan (1999) reported about the impact of population size on seed production and seed bank. His studies on the effect of population size on seed production have found that seed production declines with decreasing population

size. So, in the present study, the low seedling emergence rate of *S. travancoricum* in Kattilappara, compared to the other species present there, can be explained by the above mentioned two factors. But in the Kalasamala population, these two factors cannot be considered as a reason for poor seedling emergence from the seedbank. Even though *S. travancoricum* was the most dominant tree species in the Kalasamala population, species other than *S. travancoricum* had higher rates of seedling emergence. In addition to that, the size of the population of *S. travancoricum* in the Kalasamala population was more than that of the Kattilappara population, but still the seedling emergence was higher at Kattilappara.

According to Schmidt (2002), the seeds of the genus *Syzygium* are generally recalcitrant in nature because of their high moisture content (25 to 35 %). So, the seeds need to be kept at a high moisture content, otherwise 40 per cent of the viability will be lost (Murty and Singh, 2009). The seed germination percentage of *S. travancoricum* was assessed as a part of the present study. The percentage of germination was poor (16 %). The low seed germination percentage also explains the poor percentage of seedling emergence from the seed banks.

Yates *et al.* (2002) explained in his study that, the poor seedling emergence of some rare species from their seedbanks is mainly a contribution of fire. In the localities which were suffered from fire earlier, the density of seedlings emerged was very less for two rare *Acacia* species. But in the present study, there were no issues of fire in any of the two populations. In another study done by Varghese and Kumar (1997), on the ecology of fresh water swamp forests of southern Kerala, they described that there are some species in the swamps which are adapted to grow in the water-logged conditions, but their seeds are adversely affected by the water logging which may cause obstructions to the germination of seeds and sometimes kills the seeds by fungal attack. This can be applicable to the present study also in case of the trees of Kattilappara. Generally, the swamps are water-logged at the fruit

fall and seeding stage of *S. travancoricum* and it may lead to the death of the seeds by fungal attack. This may be a reason for poor seedling emergence in the seedbank. Grubb (1977), made conclusion in his study on regeneration niche that, the absence of seedlings in the seedbanks of a taxa can be due to the factors associated with seed germination. Hendrix (2000) also explained that the non-availability of viable seeds does not appear to be responsible for the absence of seedling emergence, but factors associated with seed germination appear to be a more likely explanation of the decline in seedling emergence.

5.2.4. Leaf morphology

The leaf morphology is always a subject of study in the case of comparison of variation. Leaf morphological characters are essential to maintain photosynthesis and can impact the growth strategies of various tree species (Takayoshi *et al.*, 2001). In agricultural crops, Kalyan *et al.* (2012) described a variation in foliage density, shape of lamina, leaf nature, leaf apex, colour of new and matured leaves and arrangement of major veins which can be helpful to differentiate among cultivars. According to Talwara *et al.* (2013), leaf morphological characters will be helpful in the studies related to variation among populations because, leaf morphological characters will change with respect to some environmental factors and also due to genetic variations. Therefore, if there are any changes in the leaf morphological characters between two populations of the same species, then there is a possibility of genetic variability. In the present study, the leaf morphological characters like leaf length and leaf width showed significant difference between the two populations whereas the length of petiole and colour of young leaves were the same in the two populations (figure 14).

In the study done by Rimbai *et al.* (2014), they observed the leaf morphological characters among eight cultivars of mango and they reported considerable variations in the characters like leaf length, leaf width, leaf ratio and petiole length. According to Reddy *et al.* (2000), variations in leaf morphological characters are reported to be due to genetic divergence in mango cultivars. Woodall *et al.* (1997) described *Syzygium* as the genus with extreme variations in leaf morphology.

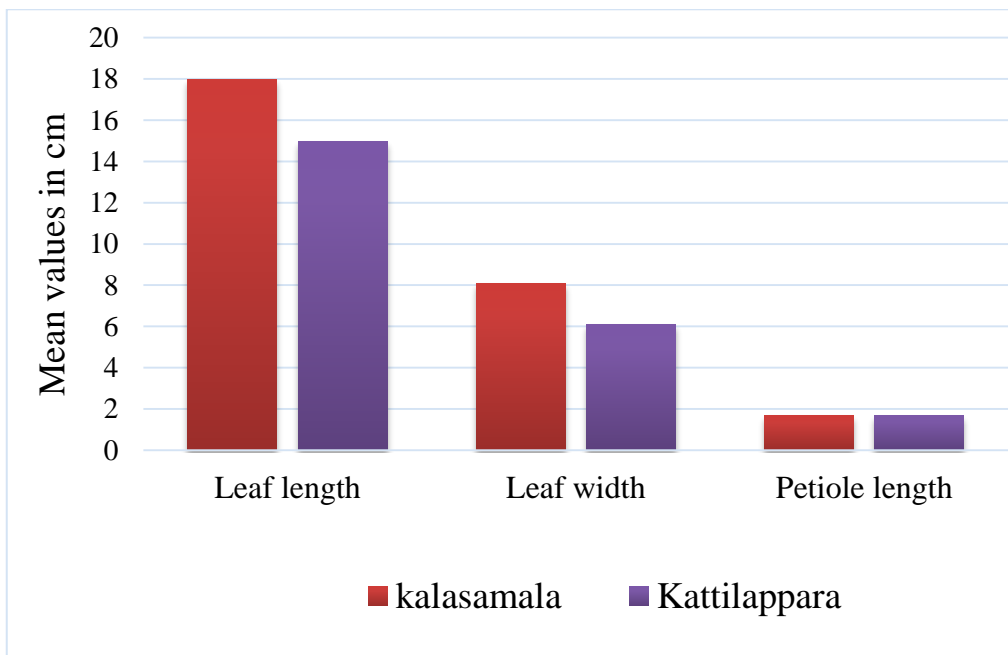


Figure 14. Leaf morphological characters of *S. travancoricum* at Kalasamala and Kattilappara

5.2.5. Wood anatomy

The study revealed that all the anatomical properties showed significant difference between the two populations. Previously, a substantial number of works have been done in different species in order to understand the variations between clones, provenances and populations. Koubaa *et al.* (1998), Rao *et al.* (2002) and Pande and Singh (2005) studied anatomical variations in wood elements among clones, provenances and populations of the same species.

5.2.5.1. Vessel morphology

Vessels are unique features of hardwoods which are important cellular constituents designed to perform the function of conduction of water and mineral nutrients in the living trees (Carlquist, 1988). The present study showed significant variation between the two populations of *S. travancoricum* in its vessel morphological characters. The morphological parameters such as vessel length, vessel diameter and vessel frequency were different for the two different populations. The trees at the Kattilappara population showed higher vessel diameter and length and lower vessel frequency than that of the trees at Kalasamala. According to Pande and Singh (2009), the site characteristics and genetic factors are the driving forces for variation in vessel morphology among populations. Significant variations in vessel frequency, vessel length and vessel diameter was reported by Ramirez *et al.* (2009) among the provenances and populations of *Eucalyptus globulus*. Leal *et al.* (2003) studied clonal and site variations of vessels in 7 year old *Eucalyptus globulus* and found that vessel varied across clones and sites. Gautam *et al.* (2008) studied the variations in vessel dimensions of *Populus deltoids* and reported extreme variation between clones and significant variation between populations. He attributed the variations to the genetic factors more than that of site factors.

The significant variation in the vessel morphological characters such as vessel length, vessel diameter, and vessel frequency of *S. travancoricum* in the

present study can be attributed the site characteristics. In Kalasamala, the size of the vessels was less and the number was more. According to Calquist (1998), trees under stress, decrease the size and increase the number of conductive tissues. This could be the reason for the lesser size and larger number of vessels in the trees of Kattilappara.

5.2.5.2. Ray morphology

In the present study, the ray morphological characteristics such as ray height, ray width and ray frequency were observed to be significantly different for the two populations of *S. travancoricum*. Ray height and ray width were higher for the trees standing in the Kattilappara population, whereas ray frequency was more in the trees of Kalasamala.

Taylor (1973) in his study on *Eucalyptus grandis* at different samplings heights found that ray and longitudinal parenchyma volume remained constant with height, and between trees variation was minor. Huda *et al* (2009), reported significant differences in ray proportion among poplar clones. Lack of literature related to inter-population variation in ray morphology limited the discussion of the same related to both genetic and environmental factors. The reduction in size and increase in number of rays in Kalasamala can also be attributed to the water stress condition of the site.

5.2.5.3. Fibre morphology

Fibre length, fibre diameter and fibre wall thickness were the parameters analysed under this topic. All these parameters showed significant differences between the two populations of *S. travancoricum*. Fibre length, fibre diameter and fibre wall thickness was more for the trees of Kattilappara population. Similar to the present study, significant variation for fibre properties among populations and clones of *E. tereticornis* was reported by Shashikala and Rao (2005). Chauhan *et al.* (2001) reported that, inter-clonal differences significant in *populus deltoides* for fibre dimensions. Ramirez *et al.* (2009) studied clonal variations in fibre properties

among 7- year old *E. globulus*. They observed a narrow range of variation for fibre diameter and fibre-wall thickness. Hans *et al.* (1972) observed a marked variation among trees for fibre length in *E. grandis*. There are a lot of studies related to the clonal variation of fibre dimensions in various species, but the inter-population variation in fibre morphology of trees is studied less.

Most of the studies related to the variation in wood anatomical characters attributes those variations to mainly two factors- site characteristics and genetic factors. Tyree and Zimmerman (2002) stated that Trees under stress, develop a trend of producing short and narrow vessel elements in plenty. The short elements are more resilient to deformation and collapse, whereas improved vessel density may help in retaining the flow of sap. In a study conducted by Mahmooduzzafar *et al.* (2010), in *S. cumini* trees, not only the vessel-element dimensions but also the vessel frequency weakened under the environmental stress, as an adaptive feature to water-stress condition. Therefore, there is a chance of variation in wood anatomical features of trees, as an adaptation to the environmental stress conditions. In the present study, this explanation can be applicable in the case of the trees at the Kalasamala population, because they suffer from water stress especially during the summer seasons.

In some studies, variation in wood anatomical features of trees is attributed to environmental pollution. In a study conducted by Gupta and Iqbal (2005), on mango trees growing under coal-smoke pollution, they have suggested that the length of vessel elements was more sensitive to pollutants than their width, or that the morphogenetic factors controlling the vessel-element length were the first to be impaired by the pollutants. The relationship of wood anatomical characters with that of pollution was also mentioned by Mahmooduzzafar *et al.* (2010). But, in the present study, there were no questions of pollution.

Variation in wood anatomy of a species standing in different populations was attributed to the leaf morphology of the trees by some authors. According to Creber and Chaloner (1990), the wood, an outcome of the vascular cambial activity, is greatly affected by the leaf-production pattern and the environmental factors of

the habitat. Magnitude of foliar surface has a great influence in wood production that needs a considerable (20-45%) part of the total seasonal production of photosynthate (Kozlowski, 1962). With increase in the amount of the foliage, wood production was amplified in the stem of *Chamaecyparis obtusa* (Satoo *et al.*, 1958), *Populus davidiana* (Satoo *et al.*, 1956) and *Zelkova serrata* (satoo *et al.*, 1959). In the present study, the variations in wood properties can be attributed to the changes in the leaf characters and pattern of leaf production, because there were significant differences in the leaf morphological characters of the species between the two populations.

Overall, we can conclude that, the variation in wood anatomical properties of *S. travancoricum* between the two populations was mainly due to the site characteristics such as the water stress. Other factors such as genetic factors and variation in leaf morphological characters could be the reason.

6. SUMMARY

The research programme entitled “Genetic diversity and population structure of two disjunct natural populations of *Syzygium travancoricum* Gamble.” was undertaken in the two populations located at Kalasamala and Kattilappara. The objective of the study was to compare the variation between two geographically isolated populations of *S. travancoricum* Gamble. The study further investigated the reproductive biology of the species, to determine if constraints to seed production could explain the restricted distribution of the species. Salient features of the study are summarized below:

- 1) *Syzygium travancoricum*, being an evergreen species, the leaf shedding and flushing continued throughout the year.
- 2) The flowering of the species was observed in the month of April, which indicates that stress can induce flowering.
- 3) There was difference in the flowering time of the species, between the two populations. This can be attributed to the change in temperature, and the availability of pollinators.
- 4) Fruiting period of *S. travancoricum* had variation from other species of the genus. Fruiting happened just before the rainy season and it can be attributed to provide favourable germination conditions for the seed.
- 5) The inflorescence of *S. travancoricum* was axillary cymose. Presence of calyptra, numerous stamens bent inward when in bud, were the other features of the flower.

- 6) The flower production per individual tree of *S. travancoricum* ranged from 2313 to 5118 with an average of 4201 per tree.
- 7) The average number of fruits per individual tree was 1146, and the percentage of fruit set was only 27 per cent. It may be due to the unavailability of viable pollen or the inadequate visit of pollinating agents.
- 8) The percentage of pollen germination was very poor (24%), and can explain the reason behind the poor fruit set percentage.
- 9) The percentage of seed germination of *S. travancoricum* was poor (16%). The germinated seeds died in the seedling stage itself, because of the lack of favourable condition.
- 10) All of the biometric characters showed a significant difference between the two populations. Changes in temperature, rainfall and moisture content can be the reasons for variation.
- 11) The girth class analysis showed that, the number of young trees of *S. travancoricum* was low in both of the populations, which indicated that there is a threat for the existence of the species.
- 12) The species association was different in the two populations. *S. travancoricum* had the highest relative density among the species at Kalasamala, whereas it was at the fourth position in Kattilappara.
- 13) The natural regeneration of *S. travancoricum* was poor in both of the populations. The relative density of naturally regenerated seedlings of *S. travancoricum* was higher in Kattilappara than that of Kalasamala.

- 14) In Kattilappara, the status of natural regeneration of *S. travancoricum* was poor when compared to other species. The inundated condition of the site during the fruit fall can be the reason for this.
- 15) The seedling emergence of *S. travancoricum* from the seed banks was better in case of the Kattilappara population than that of Kalasamala. The poor fruit set of the trees at Kalasamala can be a reason for this.
- 16) There was significant variation in the leaf morphological characters such as leaf length and leaf width, between the two populations, which indicates a genetic divergence between the populations.
- 17) The wood anatomical characters showed significant variation between the two populations. Environmental factors, genetic factors, or the variations in leaf morphological characters, can be the cause of this variation.
- 18) Overall, the reasons for the rarity of *S. travancoricum* can be the comparatively low flower production, poor fruit set, poor natural regeneration and poor seedling establishment.
- 19) There are significant variations between the two populations in biometric, morphological and anatomical characters. These differences indicate the probability of genetic and environmental variation between the two populations.

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ABSTRACT

A study was conducted to observe the reproductive biology of the critically endangered species, *Syzygium travancoricum* Gamble, and to compare the variation between two geographically disjunct populations of the species situated at Kalasamala and Kattilappara. A series of investigations on the phenology, floral biology, morphology, flowering, and fruit set were carried out to study the reproductive biology. The two populations were compared to assess the variation in terms of population structure, morphology, wood anatomy and seed bank.

The study revealed that, flowering occurred in *S. travancoricum* after a dry period and the fruiting occurred just before the onset of rain. The inflorescence of the species was axillary cymose with white, calyptrate flowers. Mass flowering was observed in *S. travancoricum*, but, when compared to other syzygium species, number of flowers produced per tree was less. The number of flowers setting to fruits was also poor. The natural regeneration from the seeds in the site too was poor. The seeds that germinated below the trees died in the seedling stage itself. The pollen viability studies revealed that the percentage of pollen germination was poor in *S. travancoricum*. The analysis of the seed germination assay indicated that seed germination was also poor in the species. The germinated seeds in lab too died at the seedling stage itself.

The observations related to the population comparison revealed that, there were a lot of variations between the two populations in most of the characters. The biometric characters like tree height, GBH, and crown area showed significant

differences between the two populations. In the case of leaf morphological characters such as leaf length and breadth, there were significant variation between the populations. The species associated with *S. travancoricum* was different in Kalasamala and Kattilappara. The relative density of the species was different in the two populations. The two populations showed significant variations in the case of wood anatomical characters. The seed banks too differed significantly between the two sites.

The study concluded that the constraints such as, lesser flower production, poor fruit set, lower seed germination, poor seed viability could be the main causes of the rarity of *S. travancoricum*. The study further showed that the two populations have considerable differences between them, either environmental or genetic. .