MORPHOGENESIS AND REPRODUCTIVE BIOLOGY OF SACRED LOTUS
(*Nelumbo nucifera* Gaertn.)

By

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THESIS

Submitted in partial fulfilment of the requirement for the degree of

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DECLARATION

I, hereby declare that this thesis entitled **"Morphogenesis and Reproductive Biology of Sacred Lotus (*Nelumbo nucifera* Gaertn)"** is a bonafide record of research work done by me during the course of research and that 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.

7/7/04

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Vellanikkara,
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CERTIFICATE

Certified that this thesis entitled **"Morphogenesis and Reproductive Biology of Sacred Lotus (*Nelumbo nucifera* Gaertn)"** is a record of work done independently by Ms. Minimol, J.S. under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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We, the undersigned members of the Advisory Committee of Ms. Minimol, J.S., a candidate for the Degree of Doctor of Philosophy in Agriculture, agree that this thesis entitled **"Morphogenesis and Reproductive Biology of Sacred Lotus (*Nelumbo nucifera* Gaertn)"** may be submitted by Ms. Minimol, J.S. in partial fulfilment of the requirement for the degree.

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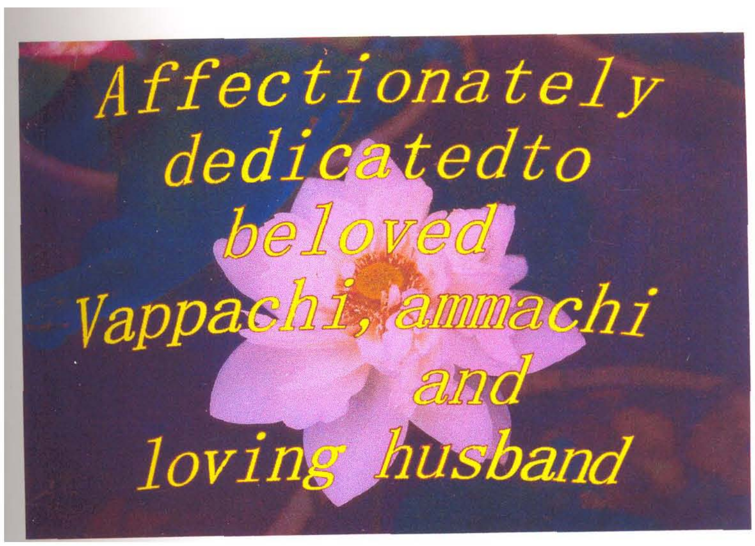
Affectionately
dedicatedto
beloved
Vappachi,ammachi
and
loving husband

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Introduction

1. INTRODUCTION

Sacred lotus (*Nelumbo nucifera* Gaertn) is an aquatic perennial herb with creeping rhizomes embedded in mud. It is the only genera belonging to the family Nelumbonaceae. Lotus blossoms are sacred to Hindus and Buddhists because of their ancient shamanic function. Lotus is historically and culturally significant. Lotus, serving as the seat of deities, signifies their divineness and purity, which is described as 'padmasana'. This legendary flower has at least 6000 years old association with Indian culture and religion. Owing to its very long and close association with history, culture, religion, literature and arts, it is choosen as our National flower. Lotus has figured in cave murals, paintings, temple carvings, postage stamps etc. The honours awarded by Government on republic day are named Padmasree, Padmabhushan, Padmavibhushan etc. The award given to best feature film is known as 'Golden lotus prize'. Thus lotus and its synonyms seen appearing in fields, through entirely different, signifies the prominence enjoyed by lotus.

Lotus which forms an important constituent of aquatic flora possesses immense therapeutic, ornamental and vegetable value as well (Annexure I, II & III).

Despite its immense potentialities, lotus has received only very little attention of crop improvement workers. The information on developmental pattern, reproductive biology and seed physiology, which is fundamental to an understanding of the dynamics of natural population is lacking in this plant. Being a crop with tremendous potential, the present investigation entitled "Morphogenesis and Reproductive biology of Sacred lotus (*Nelumbo nucifera* Gaertn)" was undertaken with the following objectives.

1. To evaluate the growth and development pattern of leaf, flower and seeds in sacred lotus

2. To elucidate the flowering pattern and reproductive biology in sacred lotus.

Review of Literature

2. REVIEW OF LITERATURE

Sacred lotus (*Nelumbo nucifera* Gaertn) belongs to the family Nelumbonaceae. *Nelumbo* is the only genera of this family. The earliest literary reference of lotus was made by Aryans in Rig Veda (2000-1500 BC). Wigand and Dennert published a monographic study on *Nelumbo nucifera* in the year 1888. Most of the available literature are concerned with the relations of *Nelumbo* to the Nymphaeaceae (Li, 1995, Khanna, 1965 and Simon, 1970). The floral anatomy, the peculiar gynoecium, the occurence of primitive and monocotyledonoid characters in *Nelumbo* (Gupta and Ahjua, 1967). and their phylogenetic relationships (Ito, 1986),had received special attention of the workers.

Borsch and Barthlott (1996) based on investigations in an extensive range of materials have shown that new world taxon is a sub-species of *Nelumbo nucifera* and is represented as *Nelumbo nucifera* subsp. *lutea.*

Data derived from high resolution scanning electron microscopy and chemical analysis of epicuticular wax have revealed that *Nelumbo* is closely related to Ranunculaceae. The occurence of aporphin alkaloids nuciferin and nornuciferin common in Ranunculaceae in cuticular waxes is a unique feature of *Nelumbo* (Barthlott *et al*., 1996).

Presannakumari *et al.* (2002) grouped 24 ecotypes of *Nelumbo* into three clusters by unweighted pair group method of cluster analysis based on anatomical and morphological characters.

2.1. Growth pattern of leaf

The leaves of all the ecotypes of sacred lotus are simple with long petiole and peltate lamina

Zuo- Baoyu *et al.* (1991) have studied the ultra structure development of chloroplasts in the embryo of sacred lotus.

Zuo- Baoyu *et al.* (1992) observed the changes that occur in thylakoid membrane stacks and chlorophyll a/b ratio of the chloroplast.

Welsh (1998) described lotus leaf as petiolate, two meter or more with leaf diameter sixty cm or more.

Watson and Dallwitz (2000) reported that in lotus, leaves are medium sized or large, with long petiole, simple, peltate, entire, palmately veined and lamina with anomocytic stomata.

2.2. Growth in relation to propagule

Garton (2000) reported that growth of healthy lotus started from a healthy tuber with at least two nodes, each with a leaf sheath and axillary bud.

Katori *et al.* (2002) reported that leaves appeared two to seven days after transplanting in both methods of propagation using rhizome straps and enlarged rhizomes. The days to flowering was significantly shorter in the rhizome strap method than in the enlarged rhizome method. Plant generated by the rhizome strap method produced significantly larger flowers.

2.3. Floral morphology

Pearl (1906) reported that number of carpels in a flower varied to a great extent even within a single population of *Nelumbo nucifera*.

Flowers of lotus were described by Welsh (1998) as large, solitary, with caducous sepals, petals with pink, pink-tinged or fading to white colour, 1 to 13 cm long. Anthers were one to two cm long.

Lotuses possess large, solitary flowers, held above water with colours pink, rose or white. Fruits develop above the water on a conical structure. Each fruit is kept in a socket. (Kasumi and Sakuma, 1998).

Hayes *et al.* (2000) from their studies reported that *Nelumbo nucifera* is characterised by polysymmetric floral development originating spirally with stamen and carpel in simultaneous whorls.

2.4. Pollination biology

Shomer and Sefton (1978) in their study on reproductive biology of *Nelumbo pentapetala* (Nelumbonaceae) found that various syrphid flies and bees visited the flowers. The beetles and honey bees visiting flowers were observed to carry large amount of pollen grains to another flower indicating cantharophilly.

Ke *et al.* (1987) in their study on pollination in lotus by caging with honey bees have found high percentage of seed set in plants caged with honey bees than in plants caged without honey bees.

Flowers are entamophillous in nature (Kasumi and Sakuma, 1998).

Garton (2000) stated that, stigma become receptive to pollen during maturation of the flower bud and pollen was dehisced from anther only after buds were completely opened. It was also reported that there was a discrete period between the stigma surface receptivity and the production of pollen by the anthers.

2.5. Thermogenesis

Thermogenesis is the mechanism of raising the temperature inside the bud prior to flower opening and continues through out the day until the petals opens slightly.Thermogenesis corresponds with stigma receptivity and it may reward to insect pollination.

Comi (1939) was the first to discover the dissimilation of starch stored in the stamen appendages during thermogenesis in *Nelumbo nucifera.*

Schneider and Buchanan (1980) reported 5 to 10°C rise in temperature in *Nelumbo lutea* flowers during thermogenesis.

Gottsberger (1992) interpreted that thermogenesis is a typical characteristics of cantharophillous flowers, which also facilitates the dispersal of olfactory chemicals.

Skubatz *et al.* (1992) reported that the *Nelumbo* flowers actively raise their inside temperature and CN intensive photosynthetic pathway was proved to take place in the appendages, the same mechanism that causes thermogenesis in Araceae.

Seymour *et al.* (1998) reported that *Nelumbo nucifera* maintains receptacle temperature between 30 to 36°C during their 2-4 days sequence of anthesis by increasing the rate of heat production.

An increase in temperature begins in lotus before petal opening and continues throughout the period of stigma receptivity as reported by Seymour and Schultze Motel (1998). They also stated that the temperature regulation favour insect pollination with a warm environment.

Seymour reported thermogenesity in arum lilly (*Philodendron selloum*) in 1999. He observed that inflorescence of arum lilly were strongly thermogeneic for two days during anthesis. Spadix temperature ranged from 38 to 42°C while outer temperature ranged from 25 to 36°C. Thermoregulation is also reported to facilitate the beetle activities.

Study conducted by Seymour and Blaylock (1999) in Skunk Cabbage (*Symplocarpus foetidus*) revealed that there is an increase in spadix temperature well above the ambient temperature, in inflorescence which are in the receptive female or early pollen bearing stage.

Gibernae and Barabe during 2000 studied thermogenesis in three *Philodendron* species of French Guiana and reported that irrespective of the species, there is an increase in temperature inside the fully developed spadix before it's opening.

2.5. Palynology

Nelumbo pollen is tricolpate (Erdtman, 1952).

Nelumbo pollen is sub porate and has a length of 55 to 70 µm with striate reticulate sculptures (Borsch and Barthlott, 1996). No significant difference was observed by them in the size of the pollen grains of *Nelumbo nucifera* and *Nelumbo leutea.*

Kreunen and Osborn (1999) in their studies reported that majority of pollen grains in *Nelumbo* are tricolpate.

2.6. Seed physiology

Lotus seeds about 466 years old were found to be viable (Priestely and Prosthumus, 1982).

Germination percentage of lotus seeds was found to be negatively correlated with depth of sediment. Seeds placed above sediment surface showed 100 percent germination (NBRI, 1996).

Lotus fruit is a non-fleshy aggregate with individual carpels sunken in the spongy receptacle. Fruiting carpel indehiscent, fruit loosely enclosed within spongy, swollen receptacle, finally released by decay, one seeded, non-endospermic, cotyledons two and embryo chlorophyllous (Watson and Dallwitz, 2000).

Presannakumari *et al.* (2000) found that dormancy in lotus seeds is non-embryonic.

Materials and Methods

3. MATERIALS AND METHODS

The investigation entitled 'Morphogenesis and reproductive biology of Sacred lotus (*Nelumbo nucifera* Gaertn)' was carried out in the Department of Plant Breeding and Genetics at the College of Horticulture, Vellanikkara during the period from December 1999 to March 2003.

A. Materials

Six different genotypes collected from diverse ecological conditions namely Nagarkovil (pure water) from Kanyakumari district of Tamil Nadu, Bramangalam (clay) from Ernakulam district, Nelliyampathy (high altitude), Chitoor (laterite tracts), both from Palakkad district and Chemmanda (kole area) from Thrissur district Chandiroor (coastal clay) from Alleppy district, were used for the study. These ecotypes representing diverse ecological situations were evaluated under *ex situ* conditions in cement tanks of two feet diameter and three feet high. Clay and water levels were retained at uniform height throughout the experiment period.

B. Methodology

3.1. Growth pattern of leaf and periodicity of leaf development

The growth and development pattern of leaf in all the selected ecotypes were studied by taking observations on various morphological and biometric characters at regular intervals right from leaf initiation till abscission all throughout an year. The observations on the following biometric characters were taken from ten different plants in each ecotype:

a) Longevity of leaves (days from visual appearance stage to abscission)
b) Petiole length in c.m.
c) Length and breadth of lamina at full expansion in c.m.
d) Length and breadth of lamina at abscission in c.m.
e) Mean number of days from visual appearance to full expansion of lamina
f) Stomatal frequency and type
g) Number of leaves produced
h) Frequency of leaf formation

The frequency of stomata per unit area of the leaf in each ecotype was estimated. The stomatal type was described following the classification proposed by Van Cotthem, 1970.

These data were correlated with weather data to know the influence of weather on growth parameters and development of leaf. The seasonal effect on various growth parameters of leaf was also computed by standard statistical methods.

For convenience, the whole year was divided into four seasons ie., December-February representing winter; March-May representing summer;

June-August representing rainy and September-November representing spring seasons.

3.2. Growth pattern in relation to propagule

Different propagules viz., three noded rhizome bits representing three age groups (fully mature, partially mature and tip portions) from each ecotypes were planted separately in cement tanks. Each treatment was replicated six times. Harvesting was done one year after planting and the rhizome yield was recorded. The nutrient composition of rhizomes was analysed following standard procedures. Based on the performance, the best propagule was selected.

3.3. Flowering biology

3.3.1. Growth pattern of flower bud

Five flower buds from each type were tagged soon after their appearance at the surface of the mud. The growth of flower bud from visual appearance stage till opening was studied at periodic intervals in all the ecotypes selected. The time taken for opening from visual appearance of flower was also recorded.

3.3.2. Periodicity of flowering

The number of flowers produced in each month for each ecotype was recorded and expressed as percentage of total number of flowers produced per year. The seasonality of flowering was then computed. For convenience of analysing the seasonal effect on flowering, the whole year was divided into four season. December-February representing winter, March-May representing summer, June-August, representing rainy season and September-November representing spring season. The succession of flower formation in the peak season was also observed.

3.3.3. Floral morphology

The description of morphological features of flowers of different ecotypes were made after examining the fresh flowers.

3.3.4. Anther dehisence and stigma receptivity

The colour and appearance of anthers were examined with hand lens at bihourly intervals in five fully matured flower buds of each type to find out the time of anther dehiscence.

Stigmatic surfaces were also examined for change in colour and appearance in the same buds at same intervals of time to find out stigma receptivity. Different insects visiting the flowers were also recorded.

3.3.5. Thermogenesis

The thermogenesity in fully mature buds was estimated at periodic intervals starting from two days prior to flower opening. The temperatures within and out side the flower bud were recorded. Five buds from each ecotypes were selected for recording the observations.

3.3.6. *Palynology*

The morphology, size and fertility of the pollen grains of each ecotype were determined following standard procedures using pollen collected from newly opened flower. The pollen grains were acetolysed according to the method described by Erdtman (1960) and the sculpturing was examined under microscope. Classification was done following the procedure suggested by Moore and Webb (1978). Fertility of pollen was assessed on the basis of staining with acetocarmine-glycerin mixture (Radford *et al.*, 1974). Pollen grains for this study were collected from newly opened flowers and stained in a drop of acetocarmine-glycerin mixture on a clean slide and kept aside for one hour. Pollen grains which are well filled and stained were counted as fertile and others sterile. Observations were taken from two fields of each of the five slides prepared for each ecotype. The values were expressed as percentage. The pollen diameter was measured using an ocular micrometer after calibration. The observations were taken from 100 pollen grains of each ecotype and mean was computed.

3.3.7. *Pollination biology*

Sets of ten fully matured buds from each ecotype were kept protected until completion of anthesis. The buds were kept protected by tieing a thread around the bud two days prior to anthesis. Another set was emasculated but kept unprotected. A third set was kept as control. The extend of fruit set in protected buds, unprotected buds and emasculated but kept unprotected bud were recorded. Observations were taken from five buds in each ecotype for each treatment. Different insects visiting the flowers were also recorded.

3.3.8. Seed physiology

Observations on time taken for maturity, moisture content, 100 seed weight, seed density and developmental changes were observed for each ecotype. Ten samples were observed in each case. For estimating seed density, known weight of seeds were immersed in distilled water taken in a measuring cylinder. The water displaced by the seeds was measured and seed density was calculated according to the formula.

$$\text{Seed density} = \frac{\text{Weight of seed in air}}{\text{Weight of water displaced}} \times \text{Specific gravity of water}$$

Moisture content was estimated by gravi-metric method. Nutrient status of seeds was also estimated by adopting standard procedure.

Lotus seeds are considered to have the longest period of dormancy (Priestly and Prosthumus, 1982). Germination tests were carried out with fully matured seeds after giving different pre-treatments to find out the factors contributing to dormancy. Untreated control was also used for comparison.

Pre-treatments tried

1. Pre-washing intact seed in water for 12 hrs.
2. Pre-washing intact seed in water for 24 hrs.
3. Pre-washing intact seed in water for 48 hrs.
4. Dipping the seed in cowdung slurry for 12 hrs followed by washing in water
5. Dipping the seed in cowdung slurry for 24 hrs followed by washing in water
6. Dipping seed in cowdung slurry for 48 hrs followed by washing in water
7. Mechanical scarification followed by leaching in water for 12 hrs.
8. Mechanical scarification followed by leaching in water for 24 hrs.
9. Mechanical scarification followed by leaching in water for 48 hrs.
10. Excised intact embryo alone
11. Intact seed

100 seeds of each type were used for the study. Water was used as the medium. The soaking water was changed daily. Total number of seeds germinated within 15 days were counted and expressed as percentage. From the results, the factors contributing to seed dormancy was elucidated.

The germination behaviour of the seeds were observed and recorded.

The anatomical changes in the fruit wall during the course of development was studied by taking transverse sections during various stages of development. The sections were made permanent following the procedure described by Prasad and Krishnaprasad (1970).

The total phenol content of seeds was estimated by Folin-Ciocalteau method (Malick and Singh, 1980).

Appropriate statistical analysis was carried out wherever necessary.

Results and Discussion

4. RESULTS AND DISCUSSION

The results of the study 'Morphogenesis and reproductive biology of sacred lotus' carried out in the Department of Plant Breeding and Genetics, College of Horticulture, Vellanikkara during 1999-2003 using six different ecotypes are presented below.

4.1. Growth pattern of leaf and periodicity of leaf development

The leaves of all the ecotypes of sacred lotus are simple with long petiole and peltate lamina. Leaves are either floating or held above water surface. The lamina remained in a rolled condition at the time of its appearance above water surface (Plate 1). The observations on various leaf characters like length and breadth of leaves at full expansion and at abscission stage, petiole length at full expansion of lamina, longevity of leaves and days taken for the lamina to open fully in six different ecotypes selected for the study are presented in Table 1 and Plate 2.

From the Table 1 it is clear that significant variability was observed among the ecotypes for all the leaf characters studied. Maximum leaf longevity of 28 days was observed in Bramangalam. The ecotypes Chittoor and Nagarkovil registered the minimum value of 24 days. In the case of petiole length, the lowest value of 25.93 cm was registered by Chittoor and the highest value of 34.91 cm by Chemmanda. The ecotypes Nagarkovil and

Table 1 Variability in leaf characters of six ecotypes

Ecotypes	Longevity of leaves (days)	Petiole length (cm)	Length of lamina at full expansion (cm)	Length of lamina at the time of abscission (cm)	Breadth of lamina at full expansion (cm)	Breadth of lamina at the time of abscission (cm)	Mean number of days for the leaf to open fully
Nagarkovil	23.63	33.21	13.88	14.56	18.58	18.23	3.90
Bramangalam	28.39	32.47	12.40	12.38	16.11	14.95	4.40
Chemmanda	27.27	34.91	13.04	12.73	16.05	16.46	4.59
Nelliyampathy	27.35	33.77	14.53	15.21	17.92	17.73	4.27
Chittoor	24.00	25.93	10.29	10.67	13.63	13.15	4.10
Chandiroor	24.86	30.79	12.86	12.80	15.51	14.41	4.65
CD (0.05)	0.57	1.19	0.68	0.65	0.84	0.70	0.28
CV (%)	3.11	5.27	7.55	6.30	7.27	6.24	9.05

Plate 1 Stages of unrolling of lamina in sacred lotus

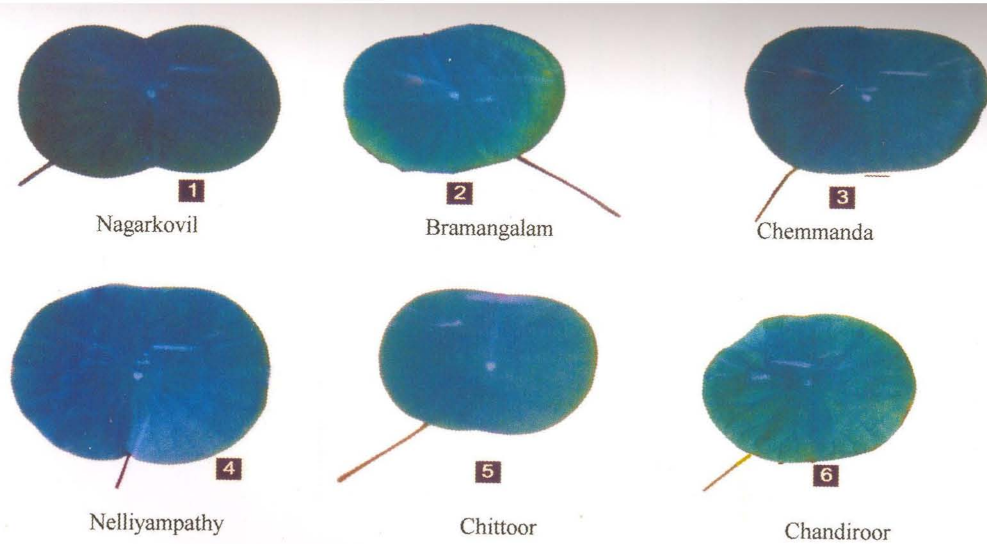


Plate 2 Leaves of different ecotypes of sacred lotus

Nelliyampathy were having the largest leaves. Among the ecotypes evaluated, Chittoor was having the smallest leaves with short petiole and low longevity. The growth pattern of leaves from different ecotypes based on length and breadth of lamina during peak season is presented in Table 2 and Fig.1 & 2.

Since the unrolling of lamina occurred only after 4-6 days of it's appearance on the surface of mud, breadth measurements were recorded only after that. Fluctuation was observed in both length and breadth of lamina in all the ecotypes. These fluctuations did not follow a regular pattern and was observed to be highly irregular as it is evident from Table 2.

The correlation of various growth parameters of leaf in different ecotypes with weather parameters viz., mean maximum, mean minimum, high maximum and low minimum temperatures, mean relative humidity, rainfall, number of rainy days, evaporation, mean sun shine hours and wind speed are presented in Table 3 to 11.

All the ecotypes except Chitoor and Chandiroor showed significant negative correlation for the character longevity of leaves with temperature (Table 3). Longevity showed no significant correlation with any other weather parameters. Nelliyampathy showed significant negative correlation with evaporation.

Irrespective of ecotypes, petiole length showed significant negative correlation with mean maximum and high maximum temperatures,

Table 2 Growth pattern of leaf in six different ecotypes of sacred lotus based on length and breadth of lamina

Days	Nagarkovil		Bramangalam		Chemmanda		Nelliyampathy		Chittoor		Chandiroor	
	Length (cm)	Breadth (cm)	Length (cm)	Breadth (cm)	Length (cm)	Breadth (cm)	Length (cm)	Breadth (cm)	Length (cm)	Breadth (cm)	Length (cm)	Breadth (cm)
1 day	3.8	-	3.9	-	5.0	-	3.7	-	2.8	-	4.0	-
2 day	5.7	-	5.0	-	6.9	-	12.4	-	5.3	-	5.8	-
3 day	8.4	-	7.0	-	9.0	-	14.5	-	6.0	-	9.9	-
4 day	9.7	-	8.3	-	11.0	-	16	-	7.8	-	12.1	-
5 day	12.2	15.0	11.6	-	13.7	-	20.1	-	9.2	-	14.5	-
6 day	13.4	16.7	14.1	17.5	15.0	-	23.9	29.0	10.6	-	17.0	-
7 day	13.8	17.1	16.5	21.5	17.7	20.2	25.2	33.0	12.2	14.2	19.4	21.5
8 day	14.0	17.2	18.4	24.0	19.4	22.5	27.0	35.7	13.8	17.3	20.8	26.3
9 day	14.0	17.2	18.5	24.7	19.7	23.6	27.0	35.8	14.4	19	22.3	27.2
10 day	14.0	17.2	18.5	24.8	19.7	24.0	27.0	35.8	14.5	19.6	22.3	27.5
11 day	13.9	17.2	18.5	24.8	19.7	23.8	27.0	35.8	14.6	19.7	22.4	27.5
12 day	13.9	17.2	18.5	24.8	19.7	23.8	27.0	35.8	14.6	19.7	22.4	27.5
13 day	13.9	17.2	18.5	25.0	19.7	23.8	27.0	36.0	14.6	19.7	22.4	27.5
14 day	13.8	17.3	18.5	25.0	19.7	23.8	27.0	36.0	14.6	19.7	22.4	27.7
15 day	13.8	17.3	18.6	25.0	19.6	23.7	27.0	36.0	14.7	19.7	22.5	27.7
16 day	13.8	17.3	18.6	25.0	19.6	23.7	27.0	36.0	14.7	19.7	22.5	27.7
17 day	13.8	17.2	18.6	25.0	19.6	23.7	26.8	35.9	14.7	19.8	22.5	27.5
18 day	13.8	17.2	18.5	25.0	19.5	24.0	26.8	35.9	14.7	19.8	22.5	27.5
19 day	13.8	17.2	18.5	25.0	19.5	24.0	26.8	35.9	14.7	19.8	22.5	27.5
20 day	13.8	17.2	18.5	25.0	19.5	24.0	27.0	35.9	14.7	19.8	22.3	27.6
21 day	13.8	17.2	18.5	26.0	19.5	23.7	27.0	35.9	14.7	19.7	22.3	27.6
22 day	13.8	17.2	18.5	26.1	19.5	23.7	27.0	35.9	14.6	19.7	22.3	27.6
23 day	13.8	17.2	18.5	25.0	19.5	23.7	27.0	35.8	14.6	19.7	22.3	27.6
24 day	13.8	17.2	18.5	25.0	19.6	23.7	27.0	35.8	14.6	19.7	22.3	27.6
25 day	13.8	17.2	18.5	25.0	19.6	23.7	27.0	35.8	14.6	19.8	22.3	27.6
26 day	14.4	17.4	18.5	25.0	19.7	23.8	27.0	35.8	14.6	19.8	22.3	27.6
27 day	15.0	17.4	18.5	25.0	19.7	23.8	27.0	35.8	14.7	19.8	22.5	27.5
28 day	15.6	17.9	18.5	25.0	19.7	23.8	27.0	35.8	14.7	19.8	22.5	27.5

29 day	15.8	18.6	18.5	25.0	19.7	23.8	27.0	35.8	14.7	19.8	22.5	27.5
30 day	16.2	20.0	18.8	25.0	19.7	23.8	27.0	35.8	14.7	19.8	22.5	27.5
31 day	17.0	21.2	18.8	24.9	19.8	23.8	27.0	35.8	14.7	19.8	22.5	27.5
32 day	17.0	21.5	18.8	24.9	20.0	23.8	27.0	35.8	14.7	19.8	22.5	27.7
33 day	17.0	21.5	19.0	24.9	20.0	23.9	27.0	35.8	14.5	19.8	-	-
34 day	17.0	21.5	19.0	25.0	20.0	23.9	27.0	35.8	14.5	19.8	-	-
35 day	17.1	21.5	19.0	25.0	20.0	23.9	27.0	35.8	14.5	19.7	-	-
36 day	17.1	21.5	19.0	25.0	20.0	23.9	27.0	35.8	14.5	19.7	-	-
37 day	17.1	21.5	19.0	25.0	20.0	23.9	27.0	35.8	14.5	19.7	-	-
38 day	-	-	19.0	25.0	19.8	23.9	27.0	35.8	14.5	19.7	-	-
39 day	-	-	19.0	25.0	19.8	23.9	27.0	35.8	-	-	-	-
40 day	-	-	18.7	25.0	19.8	23.9	27.0	35.8	-	-	-	-
41 day	-	-	18.7	25.0	19.8	23.9	-	-	-	-	-	-
42 day	-	-	18.7	25.0	19.8	23.9	-	-	-	-	-	-
43 day	-	-	18.7	25.0	19.8	23.9	-	-	-	-	-	-
44 day	-	-	19.0	25.0	19.8	23.7	-	-	-	-	-	-
45 day	-	-	19.0	25.0	19.5	23.7	-	-	-	-	-	-
46 day	-	-	19.0	25.0	19.5	23.7	-	-	-	-	-	-
47 day	-	-	19.0	25.0	-	-	-	-	-	-	-	-
48 day	-	-	19.0	25.0	-	-	-	-	-	-	-	-
49 day	-	-	19.0	25.0	-	-	-	-	-	-	-	-

Fig.1 Leaf expansion pattern of different ecotypes based on leaf length

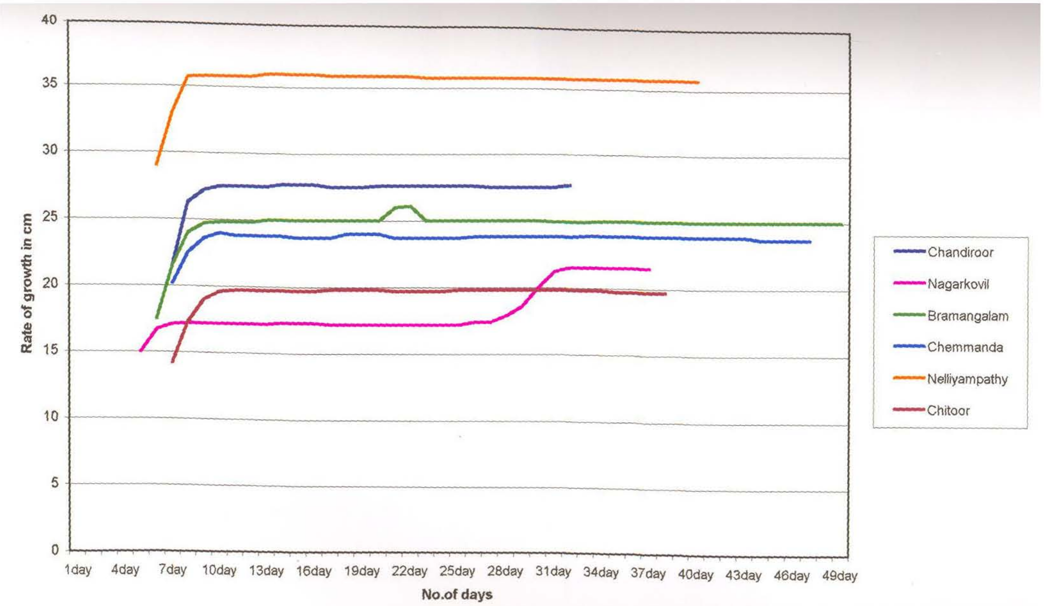


Fig.2 Leaf expansion pattern of different ecotypes based on leaf breadth

Table 3 Correlation of weather parameters with longevity of leaves

Ecotypes	Mean_Max Temperature	Mean_Min Temperature	High_Max Temperature	Low_Min Temperature	Mean Relative humidity	Rainfall	Rainy days	Evaporation	Mean sun shine hours	Wind speed
Nagarkovil	-0.192	-0.271*	-0.282*	-0.017	-0.091	-0.283*	-0.139	0.095	0.071	0.244
Bramangalam	-0.355**	-0.353*	-0.441**	-0.033	0.126	-0.03	0.113	-0.157	-0.186	0.07
Chemmanda	-0.283*	-0.274*	-0.309*	-0.026	0.081	-0.169	-0.013	-0.157	-0.078	0.077
Nelliyampathy	-0.434**	-0.324*	-0.458**	-0.041	0.202	0.031	0.173	-0.263*	-0.229	-0.014
Chitoor	-0.189	0.242	-0.15	0.493**	0.243	0.033	0.157	-0.089	-0.147	-0.178
Chandiroor	0.007	-0.007	-0.113	0.208	0.037	-0.03	0.047	-0.005	-0.067	-0.008

** Correlation significant at the 0.01 level
* Correlation significant at the 0.05 level

evaporation, mean sunshine hours and wind speed. Positive correlation was registered with low minimum temperature, relative humidity and rainfall (Table 4)

It is evident from the Table 5 that lamina length at the time of full expansion in Chemmanda and Nelliyampathy had significant negative correlation with mean maximum and high maximum temperature and evaporation. Same ecotypes showed significant positive correlation with mean relative humidity, rainfall and rainy days. In Chandiroor, significant negative correlation was observed for leaf lamina length at the time of abscission with mean maximum and high maximum temperature, evaporation and mean sun shine hours. Positive correlation for same ecotypes were observed with low minimum temperature, means relative humidity and number of rainy days.

Lamina breadth at the time of full expansion and lamina breadth at the time of abscission (Table 7 &8) showed significant negative correlation with mean maximum and high maximum temperature, evaporation rate and mean sun shine hours in ecotypes Chemmanda, Nelliyampathy and Chandiroor . The same ecotypes registered significant positive correlation for the same cha-acter with mean relative humidity, rainfall and number of rainy days.Nagar-kovil registered positive correlation with mean maximum, mean minimum temperatures and negative correlation with wind speed. Bramangalam and Chitoor showed significant positive correlation with low

Table 4 Correlation of weather parameters with petiole length

Ecotypes	Mean_Max Temperature	Mean_Min Temperature	High_Max Temperature	Low_Min Temperature	Mean Relative humidity	Rainfall	Rainy days	Evaporation	Mean sun shine hours	Wind speed
Nagarkovil	-0.438**	0.196	-0.370**	0.570**	0.796**	0.778**	0.652**	-0.815**	-0.776**	-0.632**
Bramangalam	-0.451**	0.238	-0.409**	0.614**	0.779**	0.463**	0.611**	-0.652**	-0.614**	-0.636**
Chemmanda	-0.517**	0.121	-0.404**	0.468**	0.817**	0.751**	0.654**	-0.823**	-0.786**	-0.635**
Nelliyampathy	-0.465**	0.152	-0.426**	0.521**	0.802**	0.680**	0.576**	-0.761**	-0.730**	-0.646**
Chitoor	-0.610**	-0.205	-0.574**	0.249	0.697**	0.666**	0.529**	-0.726**	-0.755**	-0.519**
Chandiroor	0.586**	0.048	-0.553**	0.489**	0.826**	0.766**	0.703**	-0.824**	-0.824**	-0.562**

** Correlation significant at the 0.01 level
* Correlation significant at the 0.05 level

Table 5 Correlation of weather parameters with leaf lamina length at full expansion

Ecotypes	Mean_Max Temperature	Mean_Min Temperature	High_Max Temperature	Low_Min Temperature	Mean Relative humidity	Rainfall	Rainy days	Evaporation	Mean sun shine hours	Wind speed
Nagarkovil	0.226	0.335**	0.220	0.167	0.131	-0.002	0.096	-0.029	0.052	-0.303*
Bramangalam	0.215	0.154	0.135	0.264	0.157	-0.054	-0.123	-0.080	-0.013	-0.342*
Chemmanda	-0.577**	-0.272*	-0.561**	0.087	0.356**	0.552**	0.472**	-0.416**	-0.585**	-0.064
Nelliyampathy	-0.532**	-0.261*	-0.557**	0.098	0.461**	0.536**	0.494**	-0.481**	-0.608**	-0.282*
Chitoor	0.026	0.107	0.014	0.340*	-0.041	-0.143	-0.099	0.168	0.045	-0.016
Chandiroor	-0.532**	-0.178	-0.545**	0.338**	0.421**	0.336**	0.427**	-0.374**	-0.524**	-0.150

** Correlation significant at the 0.01 level
* Correlation significant at the 0.05 level

Table 6 Correlation of weather parameters with leaf lamina length at the time of abscission

Ecotypes	Mean_Max Temperature	Mean_Min Temperature	High_Max Temperature	Low_Min Temperature	Mean Relative humidity	Rainfall	Rainy days	Evaporation	Mean sun shine hours	Wind speed
Nagarkovil	0.278*	0.305*	0.256*	0.094	0.102	-0.039	0.058	-0.021	0.091	-0.314*
Bramangalam	0.236	0.177	0.165	0.271	0.148	-0.056	-0.136	-0.073	-0.002	-0.344*
Chemmanda	-0.577**	-0.261	-0.558**	0.103	0.369**	0.562**	0.478**	-0.421**	-0.595**	-0.079
Nelliyampathy	-0.534**	-0.253	-0.559**	0.114	0.471**	0.530**	0.494**	-0.486**	-0.610**	-0.291*
Chitoor	0.028	0.10	0.014	0.333*	-0.044	-0.143	-0.106	0.168	0.046	-0.017
Chandiroor	-0.533**	-0.179	-0.546**	0.340**	0.422**	0.336**	0.427**	-0.376**	-0.525**	-0.152

** Correlation in significant at the 0.01 level
* Correlation in significant at the 0.05 level

Table 7 Correlation of weather parameters with leaf lamina breadth at full expansion

Ecotypes	Mean_Max Temperature	Mean_Min Temperature	High_Max Temperature	Low_Min Temperature	Mean Relative humidity	Rainfall	Rainy days	Evaporation	Mean sun shine hours	Wind speed
Nagarkovil	0.316*	0.368**	0.293*	0.202	0.150	-0.043	0.022	-0.058	0.084	-0.384**
Bramangalam	0.122	0.152	0.054	0.308*	0.253	0.029	-0.023	-0.172	-0.107	-0.398**
Chemmanda	-0.591**	-0.278*	-0.585**	0.134	0.415**	0.555**	0.469**	-0.475**	-0.622**	-0.117
Nelliyampathy	-0.525**	-0.167	-0.547**	0.200	0.527**	0.531**	0.537**	-0.518**	-0.614**	-0.347**
Chitoor	-0.059	0.107	-0.062	0.384**	0.090	-0.060	-0.015	0.038	-0.0510	-0.132
Chandiroor	-0.525**	-0.127	-0.528**	0.401**	0.516**	0.394**	0.452**	-0.454**	-0.578**	-0.258

** Correlation significant at the 0.01 level
* Correlation significant at the 0.05 level

Table 8 Correlation of weather parameters with lamina breadth at the time of abscission

Ecotypes	Mean_Max Temperature	Mean_Min Temperature	High_Max Temperature	Low_Min Temperature	Mean Relative humidity	Rainfall	Rainy days	Evaporation	Mean sun shine hours	Wind speed
Nagarkovil	0.300*	0.355**	0.275*	0.196	0.161	-0.032	0.036	-0.074	0.069	-0.389**
Bramangalam	0.129	0.154	0.061	0.305*	0.243	0.026	-0.031	-0.163	-0.101	-0.388**
Chemmanda	-0.588**	-0.269*	-0.579**	0.143	0.415**	0.559**	0.475**	-0.473**	-0.623**	-0.117
Nelliyampathy	-0.528**	-0.168	-0.549**	0.204	0.527**	0.534**	0.538**	-0.520**	-0.618**	-0.345**
Chitoor	0.008	0.129	-0.002	0.376**	0.055	-0.098	-0.063	-0.081	0.002	-0.127
Chandiroor	-0.516**	-0.120	-0.518**	0.406**	0.499**	0.386**	0.453**	-0.446**	-0.567**	-0.239

** Correlation significant at the 0.01 level
* Correlation significant at the 0.05 level

Table 9 Correlation of weather parameters with days taken for the leaf to open fully

Ecotypes	Mean_Max Temperature	Mean_Min Temperature	High_Max Temperature	Low_Min Temperature	Mean Relative humidity	Rainfall	Rainy days	Evaporation	Mean sun shine hours	Wind speed
Nagarkovil	-0.381**	-0.321*	-0.324*	-0.071	0.217	0.264*	0.121	-0.369**	-0.324*	-0.040
Bramangalam	-0.581**	-0.291*	-0.551**	-0.010	0.435**	0.313*	0.398**	-0.499**	-0.461**	-0.175
Chemmanda	-0.560**	-0.577**	-0.591**	-0.194	0.224	0.256	0.153	-0.297*	-0.426**	0.080
Nelliyampathy	-0.240	-0.241	-0.279*	-0.039	0.191	0.047	-0.052	-0.213	-0.200	-0.113
Chitoor	-0.402**	-0.035	-0.390**	0.258	0.609**	0.324*	0.423**	-0.558**	-0.457**	-0.531**
Chandiroor	-0.393**	-0.304*	-0.443**	-0.042	0.378**	0.240	0.141	-0.434**	-0.379**	-0.227

** Correlation significant at the 0.01 level
* Correlation significant at the 0.05 level

Table 10 Correlation of weather parameters with periodicity of leaf formation

Ecotypes	Mean_Max Temperature	Mean_Min Temperature	High_Max Temperature	Low_Min Temperature	Mean Relative humidity	Rainfall	Rainy days	Evaporation	Mean sun shine hours	Wind speed
Nagarkovil	0.007	-0.049	0.025	0.019	-0.240	-0.207	-0.183	0.230	0.163	0.362**
Bramangalam	0.072	-0.063	0.050	-0.142	-0.185	-0.090	-0.104	0.168	0.112	0.200
Chemmanda	0.167	-0.128	0.079	-0.160	-0.286*	-0.280*	-0.287*	0.317*	0.233	0.198
Nelliyampathy	-0.042	-0.022	0.029	-0.025	-0.038	0.119	-0.026	0.014	-0.068	0.090
Chitoor	0.155	0.016	0.130	0.012	0.052	0.015	-0.129	-0.085	-0.005	-0.170
Chandiroor	-0.112	-0.086	-0.092	-0.143	0.024	0.065	0.038	-0.047	-0.066	0.002

** Correlation significant at the 0.01 level
* Correlation significant at the 0.05 level

Table 11 Correlation of weather parameters with number of leaves produced

Ecotypes	Mean_Max Temperature	Mean_Min Temperature	High_Max Temperature	Low_Min Temperature	Mean Relative humidity	Rainfall	Rainy days	Evaporation	Mean sun shine hours	Wind speed
Nagarkovil	0.387**	0.489**	0.465**	0.275*	0.199	0.076	0.086	-0.033	0.036	-0.541**
Bramangalam	0.468**	0.169	0.590**	-0.044	-0.336**	-0.109	-0.333**	0.385**	0.258*	0.114
Chemmanda	-0.278*	0.471**	-0.129	0.602**	0.696**	0.708**	0.768**	-0.622**	-0.613**	-0.602**
Nelliyampathy	0.451**	0.678**	0.490**	0.281*	0.161	0.043	0.238	-0.054	0.115	-0.403**
Chitoor	0.107	0.066	0.141	0.277*	0.047	0.172	-0.069	0.113	-0.121	-0.061
Chandiroor	0.483**	0.534**	0.521**	0.325*	0.079	-0.038	-0.049	0.139	0.129	-0.437**

** Correlation significant at the 0.01 level
* Correlation significant at the 0.05 level

minimum temperature. Bramangalam exhibited negatives correlation with wind speed.

Among the different ecotypes periodicity of leaf formation exhibited significant positive correlation with wind speed in Nagarkovil. In Chemmanda, periodicity of leaf formation showed significant negative correlation with relative humidity, rainfall and rainy days.

Number of leaves produced showed positive correlation with mean maximum and mean high temperature in all ecotypes except Chitoor and Chemmanda. Chemmanda registered negative correlation with mean maximum temperature. In Chitoor, positive correlation with low minimum temperature was recorded. Chandiroor, Nagarkovil and Nelliyampathy showed positive correlation with low minimum temperature.In the ecotype Bramangalam, the number of leaves produced showed significant negative correlation with mean relative humidity and number of rainy days. All ecotypes except Chitoor and Bramangalam showed negative correlation with wind speed.

The influences of season on various leaf characters of lotus are presented in Table 12.

The study has revealed that seasonal effects on leaf characters are significant. Longevity of the leaves and days taken for the leaves to open fully were the highest in spring season followed by rainy season and the least

Table 12 Effect of season on various leaf characters

Seasons	Longivity of leaves (days)	Petiole length (cm)	Length of lamina at full expansion (cm)	Lamina length at the time of abscission (cm)	Leaf lamina breadth at full expansion (cm)	Leaf lamina breadth at the time of abscission (cm)	Days taken for the leaves to open fully
Winter (Dec – Feb)	24.94	19.90	11.25	12.04	13.50	12.48	3.82
Summer (Mar – May)	23.21	29.36	11.73	12.06	14.78	15.40	3.71
Rainy (Jun – Aug)	27.38	39.96	15.47	15.28	19.82	19.02	4.76
Spring (Sep – Nov)	28.21	38.17	12.88	12.84	17.09	16.39	4.98
CD (0.05)	0.47	0.97	0.56	0.48	0.68	0.57	0.23
CV (%)	3.11	5.27	7.55	6.30	7.27	6.24	9.05

in summer. However, the petiole length and size of leaf as represented by length and breadth of lamina were the highest during rainy season (June – August) followed by spring season (September-November). Rainy season favoured the growth in size of leaves and spring season favoured the longevity and days taken for leaves to open fully.

The periodicities of leaf formation in different ecotypes in different seasons are presented in Table 13.

Table 13. Periodicity of leaf formation in different ecotypes of sacred lotus in different seasons(days)

Sl. No.	Ecotype	Seasons				Mean
		Winter Dec-Feb	Summer Mar-May	Rainy Jun-Aug	Spring Sep-Nov	
1	Nagarkovil	2.03	1.83	1.78	2.03	1.91
2	Bramangalam	2.15	2.03	1.70	1.95	1.96
3	Chemmanda	2.30	2.08	1.90	1.98	2.06
4	Nelliyampathy	2.05	1.78	1.95	1.85	1.91
5	Chitoor	2.03	1.88	2.05	2.15	2.02
6	Chandiroor	1.93	1.80	1.83	1.88	1.86
	Mean	2.08	1.90	1.87	1.97	

CD (0.05) for seasons = 0.08 CD (0.05) for ecotypes = 0.022
CD (0.05) for interaction = 0.202 CV(%) = 17.31

From Table 13 it is evident that the periodicity of leaf formation varied with the ecotype as well as season. Leaf production was very low during December to February period representing winter season as indicated by the higher number of days between successive leaf formation.

The stomatal count/unit area in different ecotypes of lotus is presented in Table 14. The stomata are found to be anomocytic or ranunculaceous in all the ecotypes (Plate 3). Borsch and Barthlott (1996) has also reported ranunculaceous stomata in *Nelumbo* genus. Leaves of all the ecotypes evaluated were epistomatic and did not differ significantly in the number of stomata per unit area.

Table 14. Stomatal count in different ecotypes of lotus

Ecotype	Stomatal count / mm^2
Nagarkovil	520
Bramangalam	490
Chemmanda	510
Nelliyampathy	510
Chitoor	500
Chandiroor	490
CD (0.05)	NS
CV (%)	4.08

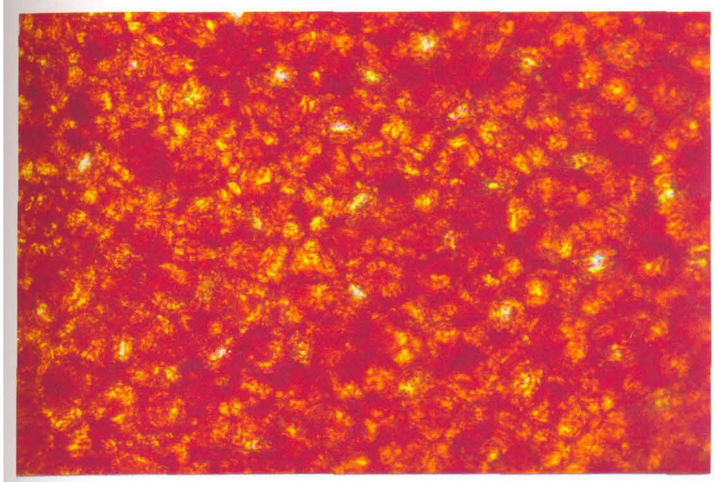


Plate 3 Stomata of sacred lotus (×400)

4.2. Growth in relation to propagule

The rhizome yield obtained by planting different propagules (Plate 4) viz., fully mature, partially mature and tip portions of rhizomes are presented in Table 15.

Table 15 Rhizome yield from different propagules of sacred lotus

Propagule	Rhizome yield (kg/m^2)
Tip portion	0.63
Partially matured rhizome	0.52
Fully matured rhizome	0.12
CD (0.05)	0.08
CV (%)	12.40

A comparison of the rhizome yield from different propagules revealed that three noded tip portion is the best propagule. Fully matured portion of the rhizome when used as propagule was showing very poor performance (Table 15). Katori *et al* (2002) has also reported that plants produced by rhizome straps are superior to enlarged rhizomes.

Table 16 shows rhizome yield in different ecotypes under evaluation.Among the different ecotypes evaluated, Chandiroor produced the highest yield of rhizomes. This was found to be on par with Nelliyampathy. Chittoor was found to be the lowest yielder among the ecotypes studied.

Plate 4 Different propagules of sacred lotus

Plate 5 Excentric starch grains in rhizome of sacred lotus (×400)

Table 16 Rhizome yield in relation to ecotypes

Ecotypes	Yield (kg/m^2)
Nagarkovil	0.58
Bramangalam	0.60
Chemmanda	0.58
Nelliyampathy	0.61
Chittoor	0.51
Chandiroor	0.63
CD (0.05)	0.02
CV (%)	12.40

Nutrient composition of rhizomes of different ecotypes are presented in Table 17.

Table 17 Nutrient content of rhizomes

Ecotypes	Starch (%)	Crude protein (%)	Crude fiber (%)
Nagarkovil	73.2	14.8	5.3
Bramangalam	71.4	14.9	5.8
Chemmanda	72.5	15.1	5.4
Nelliyampathy	71.8	14.4	5.6
Chittoor	72.2	14.5	5.4
Chandiroor	72.5	15	5.3

Neither the ecotype nor the propagule had any significant effect on the nutrient composition of rhizome. The result of the nutrient analysis revealed that starch is the main component accounting 71.5 per cent to 73.2 per cent of the dry weight of the rhizome. The microscopic examination of the starch grains revealed that they are excentric in nature (Plate 5)

4.3. Flowering biology

4.3.1. Growth pattern of flower bud

The growth pattern of flower buds of six different ecotypes (Plate 6) viz., Nagarkovil, Bramangalam, Chemmanda, Nelliyampathy,Chitoor and Chandiroor as represented by mean number of days for flower opening from their appearance at the surface of mud, mean length of pedicel at the time of flower opening and at fruit maturity, mean length of bud at the time of emergence at the surface of water and at maturity, diameter of fully opened flower and blossom life are presented in Table 18.

The mean number of days to flower opening from the appearance of bud at mud surface varied from 12 days in Bramangalam to 21 days in Chandiroor. However, Bramangalam alone was found to differ significantly from the other ecotypes which were on par. The pedicel length varied from 71.32 cm in Bramangalam to 96.8 cm in Nelliyampathy. A slight increase in pedicel length ranging from 2-4 cm was observed in different ecotypes at the time of maturity of fruit indicating that elongation of pedicel continues even after flower opening. Bramangalam, though was having the biggest bud at the

Table 18 Growth pattern of flower buds of different ecotypes

Ecotypes	Days to flower opening	Mean pedicel Length		Mean length of bud		Diameter of flower (cm)	Blossom life (days)
		At flower opening (cm)	At fruit maturity (cm)	Emergence (cm)	Maturity (cm)		
Nagarkovil	17	90.56	94.86	1.16	12.38	20.90	3
Bramangalam	12	71.32	75.94	1.62	10.04	17.72	3
Chemmanda	20	76.00	78.50	1.30	10.60	19.80	3
Nelliyampathy	20	96.80	99.00	1.10	11.90	20.00	3
Chittoor	18	90.60	94.82	1.18	11.00	19.85	3
Chandiroor	21	72.00	75.85	1.10	9.00	16.30	3
CD (0.05)	4.39	5.82	2.58	0.24	1.40	1.57	-
CV (%)	23.50	20.20	18.15	12.53	13.99	12.49	-

Plate 6   Growth pattern of flower bud in sacred lotus

time of emergence did not retain that superiority at full maturity of bud. The biggest fully matured buds (12.38 cm) and fully opened flowers (20.90 cm) were observed in the ecotype Nagarkovil. The flower size of Chemmanda, Nelliyampathy and Chittoor were on par with that of Nagarkovil. Irrespective of ecotypes, blossom life was only three days. Hence, for production of large flowers, the ecotypes Nagarkovil, Nelliyampathy, Chittoor and Chemmanda can be preferred.

4.3.2. Periodicity of flowering

The flowers produced by each ecotype of lotus in each season expressed as the percentage of total number of flowers produced per year is given in Table 19.

Table 19 Seasonal effect on flower production

Ecotypes	Proportion of flowers produced (%)			
	Winter (Dec – Feb)	Summer (Mar – May)	Rainy (Jun – Aug)	Spring (Sep – Nov)
Nagarkovil	8.5	-	31.5	60.0
Bramangalam	7.2	-	37.2	55.6
Chemmanda	7.5	-	38.0	54.5
Nelliyampathy	8.3	-	36.0	55.9
Chittoor	7.9	-	35.9	56.2
Chandiroor	8.1	-	37.7	54.2

It can be seen that flower production started with the onset of Monsoon and reached the peak in September to November representing spring season and then declined. There was practically no flower production during March to May representing the summer season (Table 19).

The succession of flower formation in each ecotype during the peak period is presented in Table 20.

Table 20 Periodicity of flower production in different ecotypes in peak season

Ecotype	Periodicity (days)
Nagarkovil	12
Bramangalam	15
Chemmanda	14
Nelliyampathy	15
Chittoor	14
Chandiroor	14
CD (0.05)	NS
CV (%)	16.80

During peak flowering season, flowers were produced at 12-15 days interval and there was no significant difference between ecotypes in the periodicity of flower production.

4.3.3. Floral morphology

The flowers were found to be solitary, ebracteate, pedicellate, actinomorphic and complete with floral formula

$$\oplus\ ⚥\ K_{3-5}\ C_{\propto}\ A_{\propto + \propto\ \text{staminode}}\ G_{7-13}$$

Pedicels were armed with spines (Plate 7).

The comparison of morphological features of flowers of the six different ecotypes are presented in Table 21to 25 and depicted in Plates 8 to 12 and Fig.3.

Sepal characters of different ecotypes of sacred lotus are presented in Table 21.

Table 21 Sepal characters of six different ecotypes

Ecotypes	Mean No. of sepals/flower	Mean length (cm)	Mean breadth (cm)
Nagarkovil	5	1.69	1.11
Bramangalam	3	1.54	0.92
Chemmanda	5	1.28	0.78
Nelliyampathy	5	1.42	0.64
Chittoor	5	1.45	0.62
Chandiroor	5	1.28	0.58
CD (0.05)	-	0.26	0.20
CV (%)	-	12.53	12.89

Plate 7 Spines on the pedicel

Five sepals greenish in colour (Plate 8) were present in all the ecotypes except Bramangalam. The ecotype Bramangalam registered only three sepals. The mean length of sepals ranged from 1.28 cm in Chemmanda and Chandiroor to 1.69 cm in Nagarkovil and mean breadth from 0.58 cm in Chandiroor to 1.11 cm in Nagarkovil. Among the ecotypes studied, Nagarkovil was having the biggest sepal with 1.69 cm length and 1.11 cm breadth (Table 21).

The petal characters of different ecotypes of lotus are presented in Table 22.

Table 22 Petal characters of six different ecotypes

Ecotypes	Colour	Mean No./ flower	Mean length (cm)	Mean breadth (cm)	Angle at tip
Nagarkovil	Pink	36	10.77	5.37	108°
Bramangalam	Pink with dark pink at the edge	18	9.07	5.00	111°
Chemmanda	Pink	24	8.98	4.52	112°
Nelliyampathy	Light pink	30	10.14	5.62	102°
Chittoor	Light pink	12	10.04	4.48	101°
Chandiroor	Pink	15	6.70	4.50	118°
CD (0.05)	-	10.19	1.76	1.04	NS
CV (%)	-	27.34	13.99	20.20	24.45

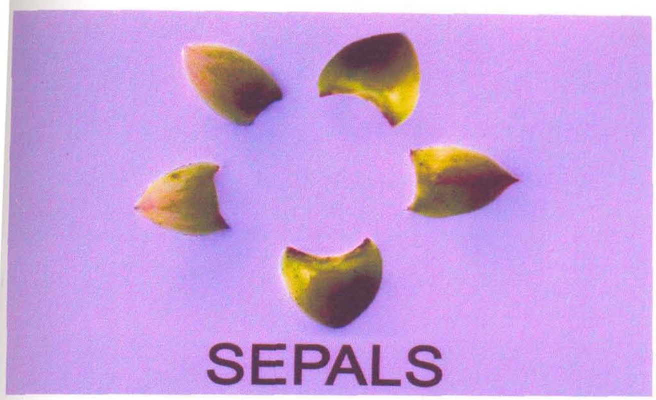


Plate 8 Sepals of sacred lotus

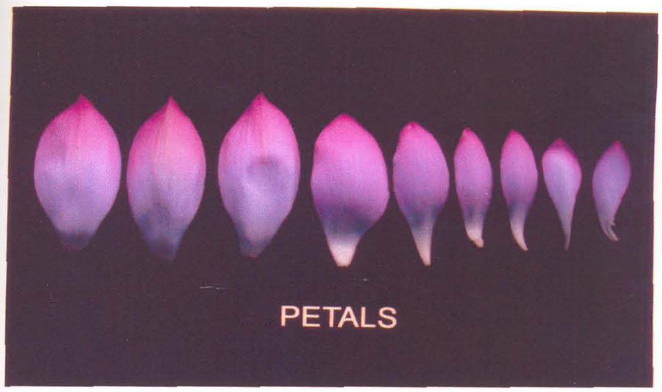


Plate 9 Petals of sacred lotus

The petals are obovate and slightly boat shaped was arranged in a spiral fashion on the floral axis. Whorls showed gradation in size of petals with the outer most whorl having large petals and inner most whorl having short petals (Plate 9). The mean petal size was the highest in Nagarkovil (10.77 cm length and 5.37 cm breadth). Nagarkovil also showed the highest number of petals (36/flower).

The character of transitional petals are furnished in Table 23.

Table 23 Transitional petal characters of six different ecotypes

Ecotypes	Mean No./ flower	Mean length (cm)	Mean breadth (cm)	Angle at tip
Nagarkovil	72	7.50	2.54	85.80°
Bramangalam	Nil	-	-	-
Chemmanda	47	7.36	2.16	91.40°
Nelliyampathy	52	7.36	2.52	97.80°
Chittoor	95	6.50	2.18	91.30°
Chandiroor	68	7.28	2.60	92.00°
CD (0.05)	17.80	0.27	0.22	NS
CV (%)	19.57	10.18	9.92	18.58

Transitional petals representing sterile stamens are also found in all the ecotypes except Bramangalam (Table 23 and Plates 10 & 11).

Plate 10 Transitional petals of sacred lotus

Plate 11 Ecotype Bramangalam without transitional petal

Chittoor exhibited the highest numbers of transitional petals.However, the biggest transitional petals were observed in Nagarkovil (Table 23).

Table 24 shows the staminal characters of different ecotypes of sacred lotus.

Table 24 Androecium character of six different ecotypes

Ecotype	Mean No. of stamen/ flower	Mean length of filament (cm)	Mean length of anther lobe (cm)	Mean length of appendage (cm)	No. of carpels/ receptlcle
Nagarkovil	137	1.50	1.02	0.37	13
Bramangalam	147	1.58	1.06	0.39	12
Chemmanda	85	1.32	1.00	0.30	7
Nelliyampathy	131	1.40	1.06	0.44	7
Chitoor	130	1.50	1.02	0.37	12
Chandiroor	120	1.58	1.06	0.30	13
CD (0.05)	7.56	0.18	NS	NS	2.3
CV (%)	18.90	7.10	8.50	9.80	14.50

Numerous stamens, ranging from 85 in Chemmanda to 147 in Bramangalm were observed in each flower. Each stamen consisted of a filament, long bilobed, basi fixed yellow anther lobe and a white coloured connective extending beyond the length of anther lobe (Fig.3). The connective distally has a peal coloured club shaped appendage. The differences in the length of stamens among the ecotypes is due to difference in the length of filaments (Table 24 and Plate 12).

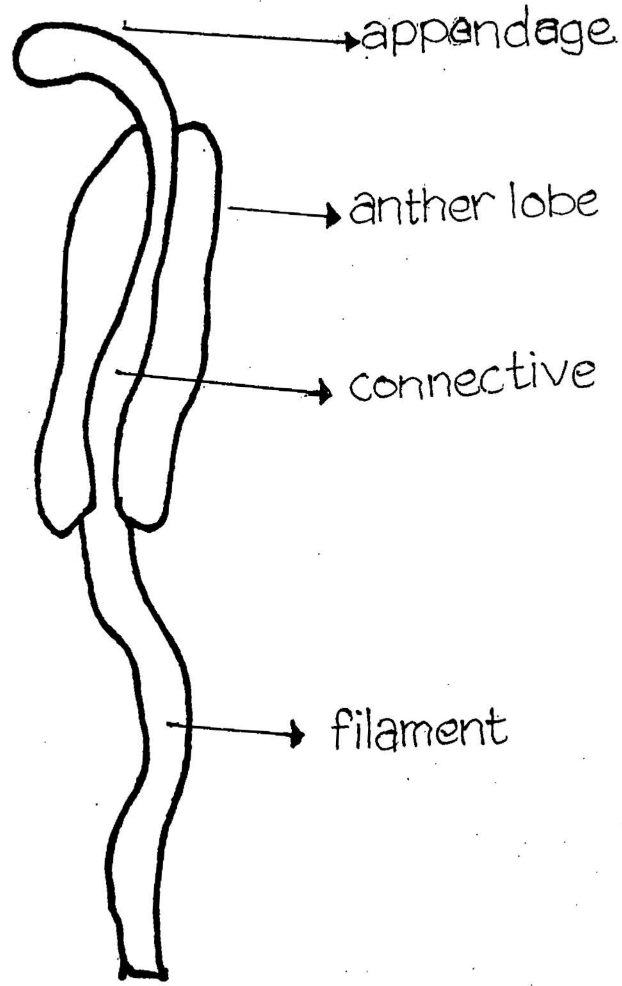


Fig.3 Stamen of sacred lotus

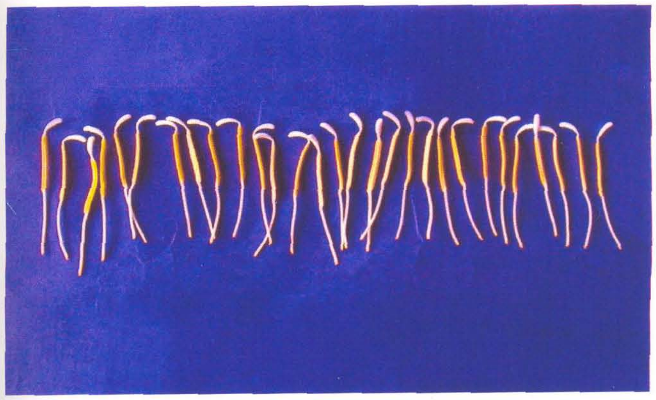


Plate 12 Androecium of sacred lotus

Plate 13   Carpel of sacred lotus

The characters of gynoecium in different ecotypes of lotus are presented in Table 25.

Table 25 Gynoecium characters of six different ecotypes

Ecotypes	No. of carpels/ receptacle (cm)	Diameter of receptacle (cm)	
		Just after anthesis	At maturity
Nagarkovil	13	2.62	5.10
Bramangalam	12	1.66	4.32
Chemmanda	7	1.20	4.00
Nelliyampathy	7	1.80	3.70
Chittoor	12	1.26	3.90
Chandiroor	13	1.58	4.10
CD (0.05)	2.3	0.48	0.98
CV (%)	14.5	12.58	12.50

Gynoecium apocarpous with several uniovulate carpels placed separately in deep cavities in the receptacular tissues (Plate 13). The carpels ranged from seven to thirteen per flower depending upon the ecotype. The stigma projects slightly above the receptacle. It is round in outline and with a depression in the middle. The size of the receptacle also varied with the ecotype. Nagarkovil was having the largest receptacle with a diameter of

5.10 cm at maturity. In all the ecotypes, a two to three fold increase in diameter of the receptacle was observed after anthesis (Table 25).

4.3.4. Anther dehiscence and stigma receptivity

The process of anthesis in sacred lotus was observed to be completed in stages, which lasted for three days in all the ecotypes.

The flower opening started at 10-15 days after the appearance of bud. On the first day of flower opening, the floral whorls just loosened, keeping the flowers in half open condition. This loosening of whorls occurs by a sudden jerting movement of the petals which took place between 8.00 am to 8.30 am .The flower remained in that condition upto 10.30 am to 11.00 am and closed again (Plate 14).

On the next day, it opened fully and the opening started by 5.30 am and was completed by 7.00 am. (Plate 15).The anther dehiscence was found to occur between 7.15 am and 7.30 am on the second day of flower opening in all the ecotypes. The anther dehisce through by longitudal slits, starting from the inner most whorl (Plate 15). The pollen grains remained viable only for 30 to 35 minutes.

The details of stigma receptivity in different ecotypes are presented in Table 26.

Plate 14 Flower on the first day of anthesis

Plate 15 Flower on the second day of anthesis

Table 26 Stigma receptivity in six different ecotypes

Ecotypes	Time of start of receptivity	Total duration of receptivity
Nagarkovil	32 hours before flower opening	83 hours
Bramangalam	32 hours before flower opening	82 hours
Chemmanda	32 hours before flower opening	82 hours
Nelliyampathy	32 hours before flower opening	83 hours
Chittoor	32 hours before flower opening	82 hours
Chandiroor	32 hours before flower opening	82 hours

The stigma was found to be receptive 32 hours before flower opening and the receptivity was retained until third day of flower opening upto 11 am. The peripheral lobes became receptive first. A honeydew like secretion was found on the stigmatic surface during the receptive period (Plate 16). The loss of receptivity could be identified by the blackening of stigmatic surface (Plate 17). The loss of receptivity also proceeded from the periphery to the center.

Withering of the floral parts started on the third day of flower opening (Plate 18).

Wide range of insects, mainly beetles and bees were found visiting the flowers on the first and second day of flower opening (Plate 19). The flowers

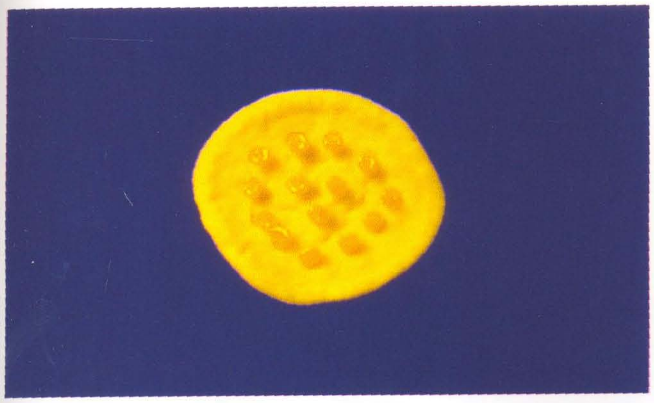


Plate 16 Receptive stigma

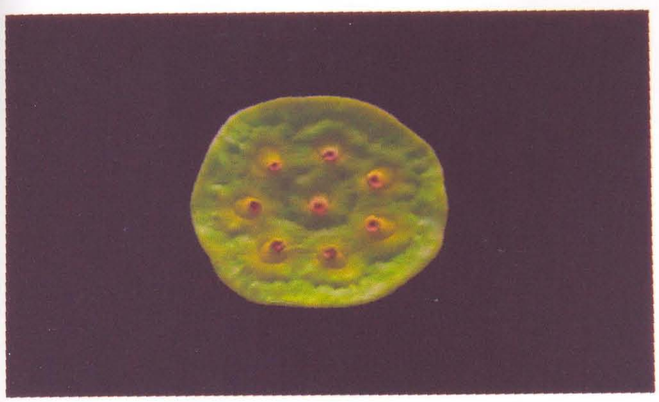


Plate 17 Loss of receptivity

Plate 18 Flower on third day of anthesis

can be considered as cantharophillous. Ke *et al.* (1987) also reported cantharophillous nature of the flower.

4.3.5. Thermogenesis in flowers

The thermogenesity in fully matured buds of the six different ecotypes recorded (Plate 20) at six hourly intervals starting two days prior to flower opening depicted in the Table 27 and Fig.4.

From the Table 27, it is clear that receptacular temperature of the flower is maintained between 30 to 35°C despite the changes in environmental temperature between 27 to 34°C. However Schneider and Buchanan (1980) reported 5 to 10°C higher temperature in *Nelumbo lutea* flowers in fully matured flower bud before anthesis. The temperature regulation is apparent at two previous nights of flower opening and disappeared after the opening of the flower. The thermo regulation begins in the bud when petals are tightly closed and continues throughout the day until the petals open slightly to reveal the stigma. The thermo regulation was found to correspond with the receptivity of stigma. This may be a reward to insect pollinators. Seymour and Schultze Motel (1998) has considered this as a floral adaptation for cross pollination.

4.3.6. Palynology

The morphology, size and fertility of the pollen grains of six ecotypes under evaluation are presented in Table 28 and Plate 21.

Table 27 Thermogenesis in sacred lotus flowers

Ecotypes	Temperature °C										
	Two days before anthesis				A day before anthesis				On the day of anthesis		
	6 AM	12 PM	6 PM	12 AM	6 AM	12 PM	6 PM	12 AM	6 AM	12 PM	6 PM
V_1	29.5	34.2	30.5	32.0	32.0	33.0	32.0	33.8	33.8	34.0	29.1
V_2	29.5	34.5	30.0	32.0	31.8	33.5	31.4	33.8	33.8	33.8	29.5
V_3	29.5	34.3	30.5	31.5	32.0	34.2	32.0	34.0	33.6	33.6	29.8
V_4	29.8	34.0	30.5	32.0	32.8	33.5	31.8	33.6	33.1	33.1	29.8
V_5	29.6	34.1	31.8	31.8	32.4	34.0	32.0	34.0	33.2	33.2	29.9
V_6	29.5	34.0	30.0	32.0	33.0	34.0	32.0	34.0	33.0	33.0	30.0
Outside temperature	29.0	33.0	28.0	27.0	29.0	33.0	29.0	27.0	28.0	33.5	29.0

Plate 19 Insect visiting

Plate 20 Thermogenesity

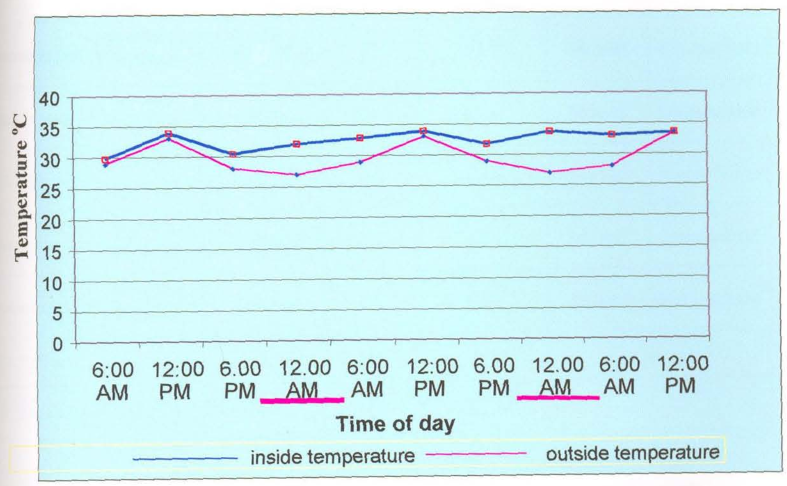


Fig. 4. Thermogenesity

Table 28 Pollen characters of six different ecotypes

Ecotypes	Type	Colour as appeared to naked eye	Mean fertility (%)	Mean size (μm) x 100	Pollen unit	Nuclear condition
Nagarkovil	Round, triporate	Yellow	95	62.4	Monad	Uninucliate
Bramangalam	Round, triporate	Yellow	95	63.2	Monad	Uninucliate
Chemmanda	Round, triporate	Yellow	96	63.4	Monad	Uninucliate
Nelliyampathy	Round, triporate	Yellow	96	62.8	Monad	Uninucliate
Chittoor	Round, triporate	Yellow	95	62.6	Monad	Uninucliate
Chandiroor	Round, triporate	Yellow	96	62.4	Monad	Uninucliate
CD (0.05)	-	-	NS	NS	-	-
CV (%)	-	-	9.8	15.6	-	-

Irrespective of ecotype, the pollen grains were found to be round, triporate and yellow in colour with reticulate sculpturing on the exine. Very high pollen fertility (95 to 96per cent) was observed in different ecotypes. The ecotypes did not differ significantly in the size of pollen grains. The mean size ranged from 62.4 μm to 63.4 μm. Borsh and Barthlott (1996) also reported the reticulate sculpturing on the exine.

4.3.7. Pollination biology

The extend of fruit set in protected buds, unprotected buds and emasculated but unprotected buds are furnished in Table 29 and Plate 22.

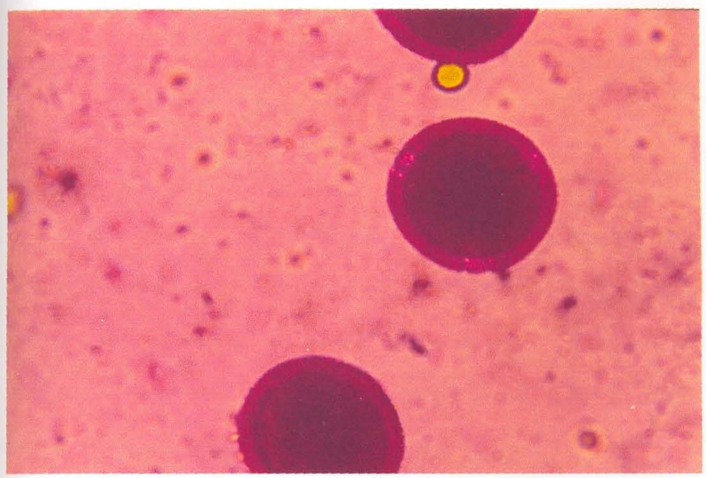


Plate 21 Pollen grains (×1000)

Table 29 Extend of fruit set in different ecotypes under different treatment

Ecotypes	Mean fruit set (%)		
	Protected	Unprotected	Emasculated but unprotected
Nagarkovil	0	44.40	46.20
Bramangalam	0	36.10	36.50
Chemmanda	0	88.80	87.40
Nelliyampathy	0	38.20	36.80
Chittoor	0	41.66	42.00
Chandiroor	0	50.67	49.60

From the Table 29, it can be seen that the extend of fruit set varied in protected and unprotected buds. No seed set was observed in protected buds. However, in the case of unprotected buds as well as emasculated but unprotected buds, high fruit set was recorded. From this, it can be concluded that sacred lotus is adapted to cross pollination and no self pollination takes place even though the flowers are bisexual and fertile. The protogynous nature of the flowers further supports cross pollination. Some self incompatibility mechanism may also be working in the flower which needs further detailed investigation.

4.3.8. Seed physiology

The observations on seed volume, 100 seed weight, seed density, moisture content, mean length and diameter of the seed, shape of the seed and

Plate 22 Protected bud

Plate 23 Seeds of sacred lotus

time taken for maturity of the seed in six different ecotypes are presented in Table 30 and Plate 23 and 24.

Table 30 Seed characters of six different ecotypes

Ecotype	Volume (ml)	100 seed weight (g)	Density	Moisture content (%)	Mean length (cm)	Mean diameter (cm)	Time taken for maturity (days)
Nagarkovil	0.86	76.25	0.87	4.32	1.88	1.16	30
Bramangalam	0.88	83.80	0.96	13.86	1.80	1.20	30
Chemmanda	0.88	127.75	1.44	35.91	2.50	1.40	30
Nelliyampathy	0.92	129.00	1.41	32.80	2.90	1.56	30
Chittoor	0.72	75.90	1.05	4.11	1.80	1.35	30
Chandiroor	0.88	93.00	1.05	7.46	1.82	1.20	30
CD (0.05)	NS	21.30	0.198	-	0.41	NS	-
CV (%)	22.80	28.20	29.10	-	18.90	23.80	-

As evident from the above Table mean seed volume and mean fruit diameter did not show any significant difference among the ecotypes. However, the ecotypes differed significantly in 100 seed weight and moisture content.The ecotypes Chemmanda and Nelliyampathy registered the highest values for 100 seed weight and moisture content. The high moisture content of the seeds may be responsible for the high 100 seed weight registered by these ecotypes.

The carpels mature into egg shaped fruits. Irrespective of ecotype the time taken for maturity of the fruit was found to be 30 days (Plate 24). Each fruit is single seeded with two cotyledons and a chlorophyllous embryo. Fruit wall and seed coat are fused. Hence fruit as such is taken as the 'seed'.

The seeds are placed in cavities in the receptacle. The cavity was observed to be narrow at receptacular surface at the time of initiation. Stigmas projected slightly above the receptacle area. In fully matured stage of carpel, the opening of the cavity widened and seeds were shed into water by slight drooping of the flower stalk (Plate 25).

The nutrient status of the fully matured seeds are presented in Table 31 and Fig.5.

Table 31 Nutrient compositions of seeds of different ecotypes

Ecotypes	Crude protein (%)	Crude fat (%)	Crude fiber (%)	Total ash (%)	Acid soluble ash (%)	Phenol content mg/g
Nagarkovil	19.6	7.2	4.2	4.6	0.15	3.5 – 4
Bramangalam	19.2	7.3	4.3	4.5	0.15	3.5 – 4
Chemmanda	19.8	7.2	4.2	4.2	0.14	3.5 – 4
Nelliyampathy	19.6	7.2	4.3	4.5	0.15	3.5 – 4
Chittoor	18.9	7.4	4.2	4.6	0.14	3.5 – 4
Chandiroor	18.8	7.2	4.2	4.2	0.15	3.5 – 4

Plate 24 Different stages of seed development

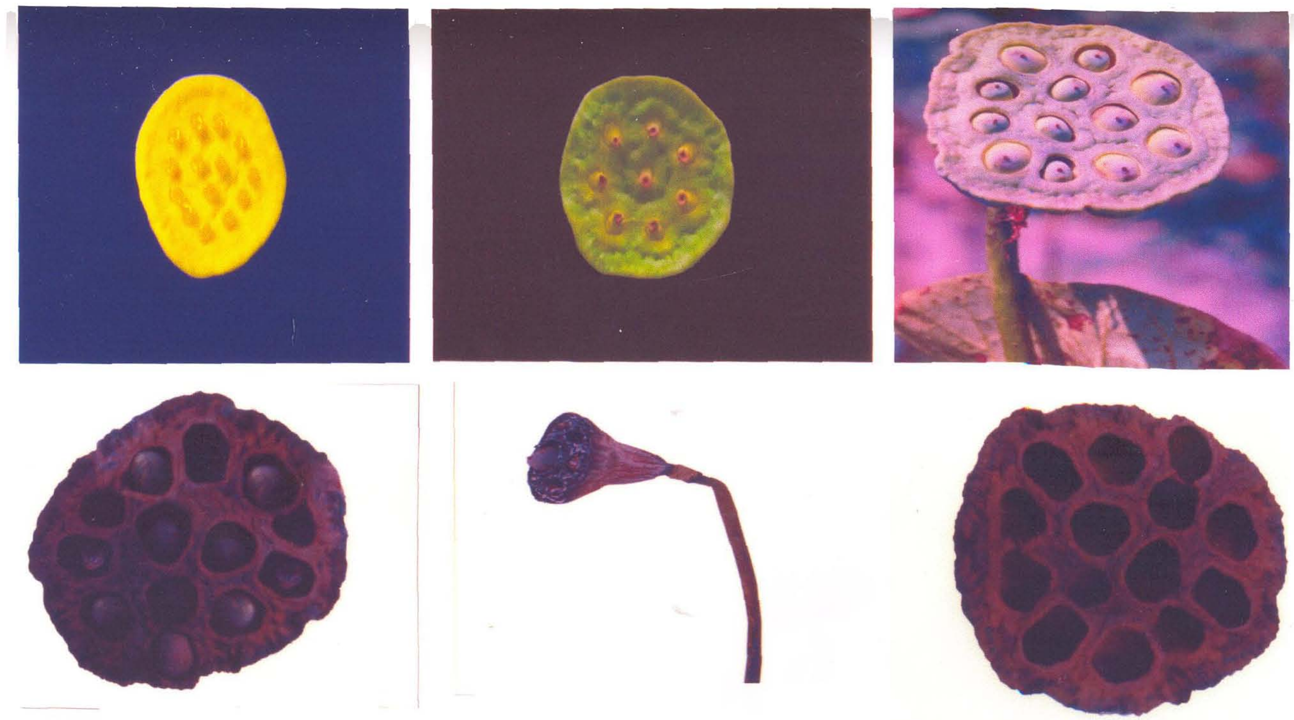


Plate 25 Different stages of development of carpel

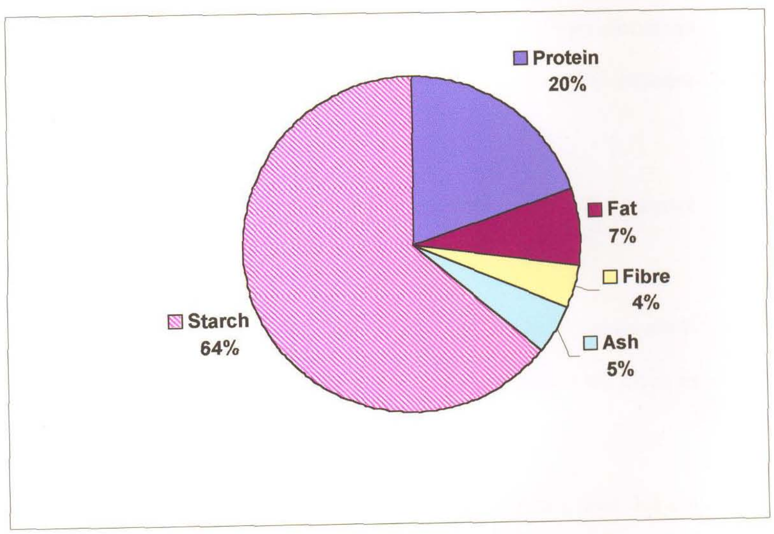


Fig. 5 Nutrient status of lotus seeds

The ecotypes did not differ in the content of starch, crude protein, fat, fibre and ash. The protein content of the seeds ranged from 18.8 per cent to 19.6 per cent. It can be seen that in addition to starch, seeds contain a substantial amount of protein also.

The total phenol content of the seed estimated by Folin-ciocalteau method revealed that it's content ranged from 3.5-4 mg/g of seed in different ecotypes of lotus.

Lotus seeds are considered to have the longest period of dormancy (Priestly and Posthumus, 1982).

The results of the germination test carried out with fully mature seeds after giving different pre-treatments along with untreated control are given in Table 32 .

From the Table 32, it can seen that pre-washing intact seed did not improve germinability. However, dipping them in cowdung slurry for varying periods recorded slight improvement in germination. Mechanical scarification followed by leaching for varying periods, 12 hours, 24 hours and 48 hours were found to be equally effective in improving germinability. Hence it is clear that fruit wall is playing a significant role in delaying germination by acting as a barrier. Since leaching improves germinability, it can be concluded that some water soluble factors present in the seeds are contributing to seed dormancy. High content of total phenol also contribute to dormancy.

The excised embryo was giving high germinability indicating that embryo as such is non-dormant and factors contributing to dormancy are residing in some part of the seed other than the embryo (Table 32 and Plate 26). Presannakumari *et al.*(2000) has reported that embryo of lotus seeds is nondormant.

Table 32 Effect of different pre-treatments on germinability

Sl. No.	Pre-treatment	Germination (%)
1	Pre-washing intact fruit in water for 12 hours	0
2	Pre-washing intact fruit in water for 24 hrs.	0
3	Pre-washing intact fruit in water for 48 hrs.	0
4	Dipping the fruit in cowdung slurry for 12 hrs followed by washing in water	45
5	Dipping the fruit in cowdung slurry for 24 hrs followed by washing in water	68
6	Dipping the fruit in cowdung slurry for 48 hrs followed by washing in water	62
7	Mechanical scarification followed by leaching in water for 12 hrs.	92
8	Mechanical scarification followed by leaching in water for 24 hrs.	91
9	Mechanical scarification followed by leaching in water for 48 hrs.	92
10	Excised intact embryo	80
11	Intact seed	0

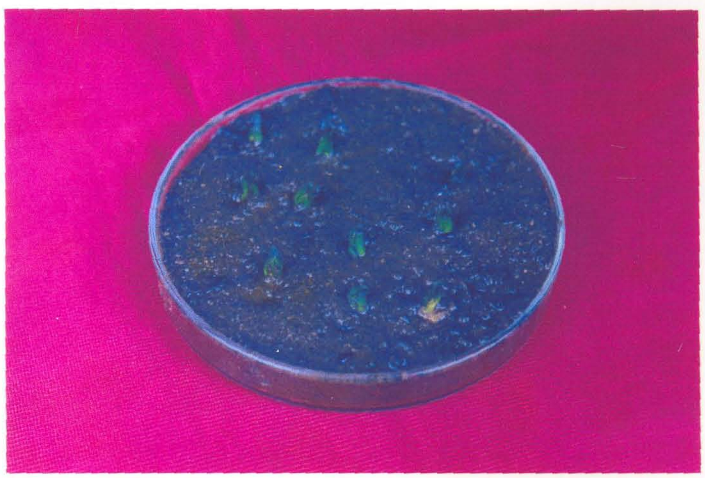


Plate 26 Germination of excised embryo

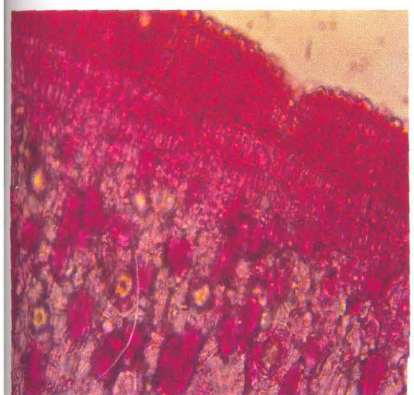
The anatomical changes that takes place in the seed during the course of development are given in Table 33 and Plate 27.

Table 33 Fruit wall thickening at varying days after fertilization

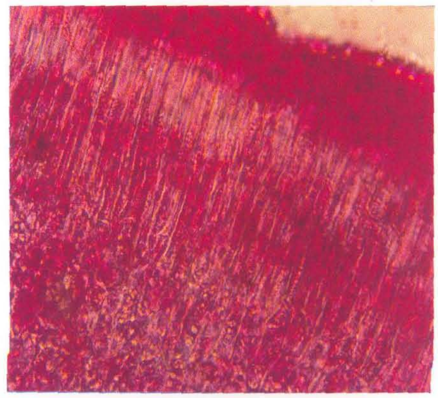
Part of fruit wall	Thickness mm (x 400)					
	5th day	10th day	15th day	20th day	25th day	30th day
Epicarp	0.051	0.058	0.058	0.062	0.064	0.064
Mesocarp	0.063	0.128	0.128	0.132	0.188	0.188

The anatomical studies of the seed revealed the presence of thick waxy layer on the surface of fruit wall. A progressive thickening of the mesocarp of the fruit during the developmental process was observed. The results also revealed that (Table 33) a three fold increase in the thickness of mesocarp in the fully matured seed of 30 days development was observed. The mesocarp region of a fully mature seed was represented by double layered compactly arranged macrosclerieds which served as an impermeable layer (Plate 27). The thick waxy coating of the seed coupled with the compactly arranged macroscleried layer, acting as mechanical barrier contributes to dormancy of lotus seed.

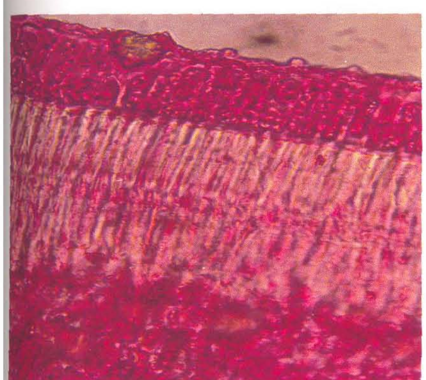
The dormancy in lotus seeds can be attributed to the presence of



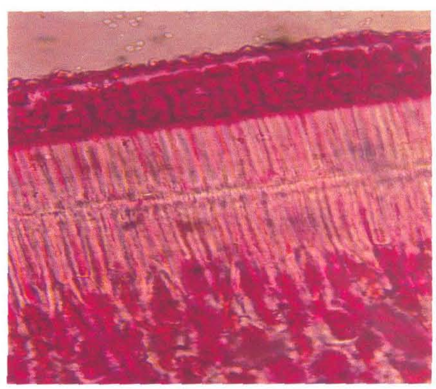
Five days after fertilization



Fifteen days after fertilization



Twenty days after fertilization



Thirty days after fertilization

Plate 27 Anatomical changes during the course of seed development (x400) water soluble inhibitors coupled with thick waxy coating on the surface of the fruit and compactly arranged macroscleried layer (Fig.6).

The germination behaviour of the seeds was observed to be quite different from normal dicots and the results are depicted in Table 34 and Plate 28.

Table 34 Germination behaviour of sacred lotus

Plumule emergence	5 – 7 days
Formation of second leaf	10 – 12 days
Expansion of rolled first and second leaf	18 – 20 days
Root development	20 – 22 days
Type of root	Adventitious

Unlike other plants, plumule emerged first. The radicle was aborted. The adventitious roots are found anchoring the plant in mud. Adventitious roots developed from the nodes of first and second leaves by 20 to 22 days after sowing, that is after the unrolling of lamina of these leaves.

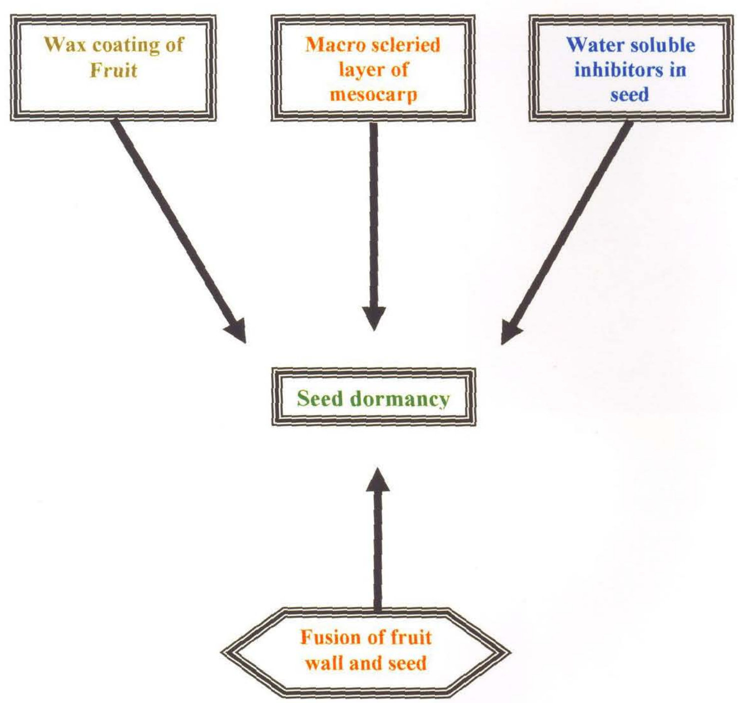


Fig. 6 Factors of seed dormancy in lotus

Plate 28 Germination behaviour of sacred lotus

5. SUMMARY

An investigation entitled "Morphogenesis and reproductive biology of sacred lotus (*Nelumbo nucifera* Gaertn) was carried out in the Department of Plant Breeding and Genetics, College of Horticulture, Vellanikkara during the period from December 1999 to March 2003.

Six different ecotypes collected from diverse ecological conditions namely Nagarkovil, Bramangalam, Chemmanda, Nelliyampathy, Chittoor and Chandiroor were evaluated under *exsitu* conditions. The salient findings of the study are summersised below:

- Significant variability was observed among the ecotypes for various leaf characters like petiole length, longevity of leaves and size of leaves.

- Seasonal effect on different leaf characters was found to be significant. Longevity of the leaves and days taken for the leaves to open fully were the highest in spring season followed by rainy season and the least in summer. However, the petiole length and size of leaf as represented by length and breadth of lamina were the highest in rainy season followed by spring.

- Significant variability was observed in various floral characters among the ecotypes.

- Flower production started with the on set of Monsoon and reached the peak in spring and then declined with no production of flower in summer months.
- Flowers of lotus were found to be solitary, ebracteate, antinomorphic and complete with various floral whorls in spiral fashion on the floral axis.
- The stigma receptivity started 32 hours before flower opening and receptivity was retained for 52 hours, even after flower opening.
- Pollen grains dehisced only after the opening of flower.
- The flowers are protogynous and the process of flower opening was completed in stages lasting for three days.
- Thermogenesity was observed in fully mature flower buds of lotus. The thermo regulation was found to correspond with stigma receptivity.
- The lotus flowers were observed to be cantharophillous
- Pollen grains were found to be fertile, triporate and with reticulate sculpturing.
- Lotus flowers are adapted to cross pollination
- The dormancy in lotus seeds can be attributed to thick waxy coating on the fruit wall, presence of water soluble inhibitors, presence of double layered macrosclereids in the mesocarp of fruit.

- The embryo as such is non-dormant and mechanical scarification followed by leaching improves germinability.

- Lotus though dicot ,germination behaviour is like that of monocot ie., plumule emerges first and radicle is aborted.

REFERENCES

Barthlott, W., Neinhuis, C., Jetter, R., Bouranel, T. and Riederer, M. 1996. *Nelumbo* – Relation with Ranunculiflorae. *Flora* **191**: 169-174

Borsch, T. and Barthlott, W. 1996. Classification and distribution of the genus *Nelumbo*. *Beitr. Biol. Pflanzen.* **64**: 421-450

Comi, C. 1939. Ricerche Sulla appendice elavata nell anthera del *Nelumbo nucifera*. *Nuova G.Bot.Ital.* **46**: 600-610

Erdtman, G. 1952. *Pollen morphology and Plant Taxonomy. Angiosperms.* Almqvist and Wiksell, Stockholm, 432 p.

Erdtman, G. 1960. The acetolysis method. A revised description. *Svensk. Bot. Tidsk.* **54**(4): 561-564

Garton, S. 2000. Hybridizing lotuses. *Accent aquat.* **55**(4): 74-77

Gibernae, M. and Barabe, D. 2000. Thermogenesis in three *Philodendron* species of French Guiana. *Can. J. Bot.* **78**: 685-689

Gottsberger, G.1992. Reproductive biology of angiosperms. *Stapfia*. **28**:11-27

Gupta,S.C. and Ahuja, R. 1967. Is *Nelumbo* a monocot? *Naturwissenschaften.* **54**: 498

Hayes, V., Schneider, E.L. and Carlquist, S. 2000. Floral development of *Nelumbo nucifera* (Nelumbonaceae) (eds. Endress, P.K., Friis, E.M., Qiu-Yin Long and Zimmer, E.A.). *Int. J. Plant Sci.* **161**(6): 183-191

Ito, M. 1986. Studies on the floral morphology and anatomy of *Nelumbo nucifera* Gaertn. *Acta Phytotax. Geobot.* **37**: 82-96

Kasumi, M. and Sakuma, F. 1998. Flowering, pollination, fertilization and seed formation in lotus rhizome plant. *J. Jap. Soc. Hort. Sci.* **67**(4): 594-599

Katori, M., Nomura, K. and Voneda, K. 2002. Propagation of flowering lotus (*Nelumbo nucifera* Gaertn) by rhizome straps, without enlarged rhizomes. *Jap. J. trop. Agric.* **41**(3): 195-197

Ke, X., Zhang, W., Zhang, H., Xu, D. and Jinag, Z. 1987. Experiments in the use of honey bees for the pollination of lotus seed crops. *J. fujian agric. coll.* **16**(2): 169-171

Khanna, P. 1965. Morphology and embryological studies in Nymphaeaceae.II *Brasenia schreberi* Gmel. and *Nelumbo nucifera* Gaertn. *Aust. J. Bot.* **13**: 379-387

Kreunen, S.S. and Osborn, J.M. 1999. Pollen and anther development in *Nelumbo* (Nelumbonaceae). *Am. J. Bot.* **86**(12): 1662-1667

Li, H.L. 1995. Classification of phylogeny of Nymphaeaceae and allied families. *Am. Midl. Nat.* **45**: 33-41

Malick, C.P. and Singh, M.B. 1980. *Plant Enzymology and Histo-Enzymology*. Kalyani Publishers, Ludhiana, 431 p.

Moore, P.D. and Webb, J.A. 1978. *All Illustrated Guide to Pollen Analysis*. Hodder and Stoughton, London, 160 p.

NBRI, 1996. Annual Report. National Botanical Research Institute, Lucknow, 86 p.

Pearl, R. 1906. Variation in the number of seeds of lotus. *Am. nat.* **40**: 757-768

Prasad, M.K. and Krishnaprasad, M. 1970. *Outlines of Microtechniques*. Emkay Publishers, New Delhi, 203 p.

Presannakumari, K.T., Suma, V.A. and Girija, T. 2000. Investigation on seed dormancy in sacred lotus. Proceedings of National Seminar in Plant Biology. Central Plantation Crop Research Institute, Kasargod, p.77

Presannakumari, K.T., Suma, V.A. and Minimol, J.S. 2002. Evaluation of morphoanatomical variations in sacred lotus. Proceedings of XII Swadeshi Science Congress, Central Tubercrop Research Institute, Thiruvananthapuram, pp.233-235

Priestely, D.A. and Prosthumus, M.A. 1982. Extreme longevity of lotus seeds from Pulantien. *Nature*. **299**(5879): 148-149

Radford, A.E., Dickison, W.C., Massey, J.R. and Bell, C.R. 1974. *Vascular Plant Systematics*. Happer and Rao Publishers, London, 466 p.

Schneider, E.L. and Buchanan, J.D. 1980. Morphological studies of the Nymphaceae.II. The floral biology of *Nelumbo pentapetala*. *Am. J. Bot.* **67**: 182-193

Seymour, R.S. 1999. Pattern of respiration by intact infloresence of the thermogenic arum lilly (*Philodendron selloum*). *J. Exp. Bot.* **50**(335) : 845-852

Seymour, R.S. and Blaylock, A.J. 1999. Switching of the heater: Influenze of ambient temperature on thermoregulation by eastern Skunk cabbage (*Symplocarpus foetidus*). *J. Exp. Bot.* **50**(338): 1525-1532

Seymour, R.S. and Schultze Motel, P. 1998. Physiological temperature regulation by flowers of sacred lotus. *Biol. Sci.* **353**: 935-943

Seymour, R.S., Schultze Motel, P. and Lamprecht, I. 1998. Heat production by sacred lotus depends on ambient temperature not light cycle. *J. Exp. Bot.* **49**(324): 1213-1217

Shomer, S.H. and Sefton, D.F. 1978. The reproductive biology of *Nelumbo pentapetala* (Nelumbonaceae) on the Upper Mississippi river.II.. The insect associated with the transfer of pollen. *Brittonia*. **30**(3): 355-364

Simon, J.P. 1970. Comparative serology of the order Nymphaeales.I.. Preliminary survey on the relationship of *Nelumbo*. *Aliso*. **7**: 243-261

Skubatz, H., Williamson, P.S. and Schneider, E.L. 1992. Cyanide insensitive respiration in thermogenic flowers of *Nelumbo* and *Victoria*. *J. Exp. Bot.* **41**: 1335-1339

172436

Van Cotthem, W.R. 1970. A classification of stomatal types. *Bot. J. Linnean Soc.* **63**: 235-246

Watson, L. and Dallwitz, M.J. 2000. The families of flowering plants: Description, Illustration, Identification and Information retrieval. Version: 14th December, 2000, 322 p.

Welsh, S.L. 1998. *Flora societensis: A summary revision of the flowering plants of the society islands*. E.P.S. Inc. Orem. Utah, 205 p.

Wigand, A. and Dennert, E. 1988. *Nelumbium speciosum* W. Eine monographische studie. *Biblioth. Bot.* **11**: 1-68

Zou-Baoyu, Jiang-Guizhen and Yu-Yanli 1991. Ultrastructure development of chloroplast in sacred lotus embryo bud under inersible light. *Acta Bot. Sinica.* **33**(3): 169-175

Zou-Baoyu, Li-Guoging and Tang-Chonggin. 1992. Changes of thylakoid membrane stocks and a/b ratio of chloroplast from sacred lotus (*Nelumbo nucifera*) seeds during their germination under light. *Acta Bot. Sinica.* **34**: 645-650

MORPHOGENESIS AND REPRODUCTIVE BIOLOGY OF SACRED LOTUS
(*Nelumbo nucifera* Gaertn.)

By

MINIMOL, J. S.

ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the requirement for the degree of

Doctor of Philosophy in Agriculture

Faculty of Agriculture
Kerala Agricultural University

Department of Plant Breeding and Genetics
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VELLANIKKARA, THRISSUR - 680656
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2004

ABSTRACT

Sacred lotus (*Nelumbo nucifera*) belonging to Nelumbonaceae is the only genera in that family. This legendary flower has very long and close association with history, culture, religion, literature and arts. Hence it is chosen as our national flower. Despite its immense potentialities as medicinal, ornamental and vegetable crop, this plant has received only very little attention of crop improvement workers. It was in this background the present investigation entitled 'Morphogenesis and Reproductive Biology of Sacred Lotus' was under taken with the objectives of evaluating the growth and development pattern of leaf, flower and seed and elucidating the reproductive biology.

Six different genotypes collected from diverse ecological conditions were evaluated under *ex situ* conditions during December 1999 to March 2003 at College of Horticulture, Vellanikkara.

Variability was observed in different biometric characters like size of lamina, longevity of leaves, petiole length etc. among the ecotypes evaluated. Study of the seasonal effect on these characters revealed that rainy season favoured growth in size of leaves and spring season favoured longevity. The growing tip of the rhizome was found to be the best propagule with respect to rhizome yield.

Significant variability was observed in various floral characters among the ecotypes. Flower production started with the onset of Monsoon and reached the peak in Spring season and then declined with no flower production at all in Summer season.

Flowers were found to be solitary, ebracteate, actinomorphic and complete with the different whorls arranged in a spiral fashion on the floral axis. Stigma became receptive 32 hours before flower opening and the receptivity was retained for 52 hours even after flower opening. Pollen dehiscence occurred only after complete opening of the flower bud. Pollen grains of lotus were found to be fertile, round and triporate. However, no seed set was obtained in protected buds indicating cross pollination. The temperature inside the flower bud remained between 30 to 35°C till the fourth day during the period of anthesis despite the changes in environmental temperature between 27 to 33°C. The period of this thermoregulation corresponded to receptivity of stigma. This is considered as a floral adaptation favouring cross pollination (Seymour and Schultze-Motel, 1998).

Unlike other dicot, in sacred lotus, plumule emerged first and radicle was aborted. Adventitious roots were found anchoring the plant in mud.

The seeds matured in 30 days after fertlization. Lotus seeds are reported to have the longest period of dormancy. The germination trials conducted after giving different pretreatments revealed that embryo as such is nondormant. Mechanical scarification followed by leaching improved germinability indicating that hard fruit wall along with thick waxy coating and water soluble inhibitors are responsible for dormancy.

Annexure I

Lotus parts and their uses

Part		Use
Rhizomes	-	Vegetable called Kamalkakkadi Thamaravalayam (Kondattoms) Source of starch – Medicinal purpose
Leaves	-	as plates
Flowers	-	Ornamental purpose as cut flowers in temples and for religious purpose
Stamens	-	Medicinal purpose
Seeds	-	Medicinal purpose In dishes after cooking

Annexure II

Cost of lotus parts/products in the market

Part		Cost
Leaf (bundles of 100)	-	Rs.7 to Rs.10
Fresh rhizomes	-	Rs.20 / kg.
Thamaravalayam	-	Rs.200 / kg.
Starch	-	Rs.30 / kg.
Thamarayalli	-	Rs.350 / kg.
Flowers	-	Rs.3 to 7 / flower
Seed	-	75 paise / seed

Annexure III

Ayurvedic products with lotus parts as an ingredient

Product		Parts
Drakshadikashayam	-	Rhizome Stamens
Manjishtadithylam	-	Rhizome
Aravindasavam	-	Flowers
Triphaladithylam	-	Rhizome
Saraseejamakarandadi choornam	-	Stamens
Sethubandham	-	Seeds
Ayushgutty	-	Seeds

MORPHOGENESIS AND REPRODUCTIVE BIOLOGY OF SACRED LOTUS
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KERALA, INDIA

2004

DECLARATION

I, hereby declare that this thesis entitled **"Morphogenesis and Reproductive Biology of Sacred Lotus (*Nelumbo nucifera* Gaertn)"** is a bonafide record of research work done by me during the course of research and that 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.

7/7/04

MINI MOL, J.S.

Vellanikkara,
7.7.04

CERTIFICATE

Certified that this thesis entitled **"Morphogenesis and Reproductive Biology of Sacred Lotus (*Nelumbo nucifera* Gaertn)"** is a record of work done independently by Ms. Minimol, J.S. under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

Vellanikkara,
7.7.04

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CERTIFICATE

We, the undersigned members of the Advisory Committee of Ms. Minimol, J.S., a candidate for the Degree of Doctor of Philosophy in Agriculture, agree that this thesis entitled **"Morphogenesis and Reproductive Biology of Sacred Lotus (*Nelumbo nucifera* Gaertn)"** may be submitted by Ms. Minimol, J.S. in partial fulfilment of the requirement for the degree.

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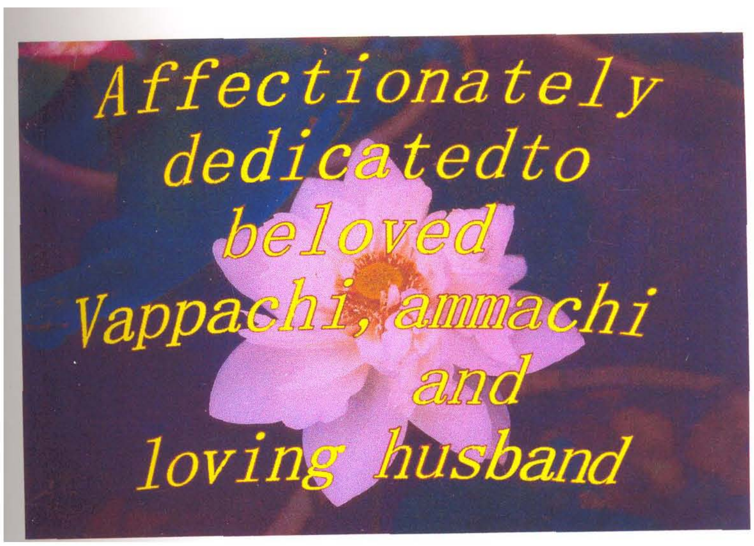
Above all, I humbly bow my head before the Almighty, who blessed me with will power and courage which enable me to complete this venture successfully.

MINI MOL, J.S.


Affectionately
dedicatedto
beloved
Vappachi, ammachi
and
loving husband

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Introduction

1. INTRODUCTION

Sacred lotus (*Nelumbo nucifera* Gaertn) is an aquatic perennial herb with creeping rhizomes embedded in mud. It is the only genera belonging to the family Nelumbonaceae. Lotus blossoms are sacred to Hindus and Buddhists because of their ancient shamanic function. Lotus is historically and culturally significant. Lotus, serving as the seat of deities, signifies their divineness and purity, which is described as 'padmasana'. This legendary flower has at least 6000 years old association with Indian culture and religion. Owing to its very long and close association with history, culture, religion, literature and arts, it is choosen as our National flower. Lotus has figured in cave murals, paintings, temple carvings, postage stamps etc. The honours awarded by Government on republic day are named Padmasree, Padmabhushan, Padmavibhushan etc. The award given to best feature film is known as 'Golden lotus prize'. Thus lotus and its synonyms seen appearing in fields, through entirely different, signifies the prominence enjoyed by lotus.

Lotus which forms an important constituent of aquatic flora possesses immense therapeutic, ornamental and vegetable value as well (Annexure I, II & III).

Despite its immense potentialities, lotus has received only very little attention of crop improvement workers. The information on developmental pattern, reproductive biology and seed physiology, which is fundamental to an understanding of the dynamics of natural population is lacking in this plant. Being a crop with tremendous potential, the present investigation entitled "Morphogenesis and Reproductive biology of Sacred lotus (*Nelumbo nucifera* Gaertn)" was undertaken with the following objectives.

1. To evaluate the growth and development pattern of leaf, flower and seeds in sacred lotus

2. To elucidate the flowering pattern and reproductive biology in sacred lotus.

Review of Literature

2. REVIEW OF LITERATURE

Sacred lotus (*Nelumbo nucifera* Gaertn) belongs to the family Nelumbonaceae. *Nelumbo* is the only genera of this family. The earliest literary reference of lotus was made by Aryans in Rig Veda (2000-1500 BC). Wigand and Dennert published a monographic study on *Nelumbo nucifera* in the year 1888. Most of the available literature are concerned with the relations of *Nelumbo* to the Nymphaeaceae (Li, 1995, Khanna, 1965 and Simon, 1970). The floral anatomy, the peculiar gynoecium, the occurence of primitive and monocotyledonoid characters in *Nelumbo* (Gupta and Ahjua, 1967). and their phylogenetic relationships (Ito, 1986),had received special attention of the workers.

Borsch and Barthlott (1996) based on investigations in an extensive range of materials have shown that new world taxon is a sub-species of *Nelumbo nucifera* and is represented as *Nelumbo nucifera* subsp. *lutea.*

Data derived from high resolution scanning electron microscopy and chemical analysis of epicuticular wax have revealed that *Nelumbo* is closely related to Ranunculaceae. The occurence of aporphin alkaloids nuciferin and nornuciferin common in Ranunculaceae in cuticular waxes is a unique feature of *Nelumbo* (Barthlott *et al.*, 1996).

Presannakumari *et al.* (2002) grouped 24 ecotypes of *Nelumbo* into three clusters by unweighted pair group method of cluster analysis based on anatomical and morphological characters.

2.1. Growth pattern of leaf

The leaves of all the ecotypes of sacred lotus are simple with long petiole and peltate lamina

Zuo- Baoyu *et al.* (1991) have studied the ultra structure development of chloroplasts in the embryo of sacred lotus.

Zuo- Baoyu *et al.* (1992) observed the changes that occur in thylakoid membrane stacks and chlorophyll a/b ratio of the chloroplast.

Welsh (1998) described lotus leaf as petiolate, two meter or more with leaf diameter sixty cm or more.

Watson and Dallwitz (2000) reported that in lotus, leaves are medium sized or large, with long petiole, simple, peltate, entire, palmately veined and lamina with anomocytic stomata.

2.2. Growth in relation to propagule

Garton (2000) reported that growth of healthy lotus started from a healthy tuber with at least two nodes, each with a leaf sheath and axillary bud.

Katori *et al.* (2002) reported that leaves appeared two to seven days after transplanting in both methods of propagation using rhizome straps and enlarged rhizomes. The days to flowering was significantly shorter in the rhizome strap method than in the enlarged rhizome method. Plant generated by the rhizome strap method produced significantly larger flowers.

2.3. Floral morphology

Pearl (1906) reported that number of carpels in a flower varied to a great extent even within a single population of *Nelumbo nucifera*.

Flowers of lotus were described by Welsh (1998) as large, solitary, with caducous sepals, petals with pink, pink-tinged or fading to white colour, 1 to 13 cm long. Anthers were one to two cm long.

Lotuses possess large, solitary flowers, held above water with colours pink, rose or white. Fruits develop above the water on a conical structure. Each fruit is kept in a socket. (Kasumi and Sakuma, 1998).

Hayes *et al.* (2000) from their studies reported that *Nelumbo nucifera* is characterised by polysymmetric floral development originating spirally with stamen and carpel in simultaneous whorls.

2.4. Pollination biology

Shomer and Sefton (1978) in their study on reproductive biology of *Nelumbo pentapetala* (Nelumbonaceae) found that various syrphid flies and bees visited the flowers. The beetles and honey bees visiting flowers were observed to carry large amount of pollen grains to another flower indicating cantharophilly.

Ke *et al.* (1987) in their study on pollination in lotus by caging with honey bees have found high percentage of seed set in plants caged with honey bees than in plants caged without honey bees.

Flowers are entamophillous in nature (Kasumi and Sakuma, 1998).

Garton (2000) stated that, stigma become receptive to pollen during maturation of the flower bud and pollen was dehisced from anther only after buds were completely opened. It was also reported that there was a discrete period between the stigma surface receptivity and the production of pollen by the anthers.

2.5. Thermogenesis

Thermogenesis is the mechanism of raising the temperature inside the bud prior to flower opening and continues through out the day until the petals opens slightly.Thermogenesis corresponds with stigma receptivity and it may reward to insect pollination.

Comi (1939) was the first to discover the dissimilation of starch stored in the stamen appendages during thermogenesis in *Nelumbo nucifera*.

Schneider and Buchanan (1980) reported 5 to 10°C rise in temperature in *Nelumbo lutea* flowers during thermogenesis.

Gottsberger (1992) interpreted that thermogenesis is a typical characteristics of cantharophillous flowers, which also facilitates the dispersal of olfactory chemicals.

Skubatz *et al.* (1992) reported that the *Nelumbo* flowers actively raise their inside temperature and CN intensive photosynthetic pathway was proved to take place in the appendages, the same mechanism that causes thermogenesis in Araceae.

Seymour *et al.* (1998) reported that *Nelumbo nucifera* maintains receptacle temperature between 30 to 36°C during their 2-4 days sequence of anthesis by increasing the rate of heat production.

An increase in temperature begins in lotus before petal opening and continues throughout the period of stigma receptivity as reported by Seymour and Schultze Motel (1998). They also stated that the temperature regulation favour insect pollination with a warm environment.

Seymour reported thermogenesity in arum lilly (*Philodendron selloum*) in 1999. He observed that inflorescence of arum lilly were strongly thermogeneic for two days during anthesis. Spadix temperature ranged from 38 to 42°C while outer temperature ranged from 25 to 36°C. Thermoregulation is also reported to facilitate the beetle activities.

Study conducted by Seymour and Blaylock (1999) in Skunk Cabbage (*Symplocarpus foetidus*) revealed that there is an increase in spadix temperature well above the ambient temperature, in inflorescence which are in the receptive female or early pollen bearing stage.

Gibernae and Barabe during 2000 studied thermogenesis in three *Philodendron* species of French Guiana and reported that irrespective of the species, there is an increase in temperature inside the fully developed spadix before it's opening.

2.5. Palynology

Nelumbo pollen is tricolpate (Erdtman, 1952).

Nelumbo pollen is sub porate and has a length of 55 to 70 µm with striate reticulate sculptures (Borsch and Barthlott, 1996). No significant difference was observed by them in the size of the pollen grains of *Nelumbo nucifera* and *Nelumbo leutea.*

Kreunen and Osborn (1999) in their studies reported that majority of pollen grains in *Nelumbo* are tricolpate.

2.6. Seed physiology

Lotus seeds about 466 years old were found to be viable (Priestely and Prosthumus, 1982).

Germination percentage of lotus seeds was found to be negatively correlated with depth of sediment. Seeds placed above sediment surface showed 100 percent germination (NBRI, 1996).

Lotus fruit is a non-fleshy aggregate with individual carpels sunken in the spongy receptacle. Fruiting carpel indehiscent, fruit loosely enclosed within spongy, swollen receptacle, finally released by decay, one seeded, non-endospermic, cotyledons two and embryo chlorophyllous (Watson and Dallwitz, 2000).

Presannakumari *et al.* (2000) found that dormancy in lotus seeds is non-embryonic.

Materials and Methods

3. MATERIALS AND METHODS

The investigation entitled 'Morphogenesis and reproductive biology of Sacred lotus (*Nelumbo nucifera* Gaertn)' was carried out in the Department of Plant Breeding and Genetics at the College of Horticulture, Vellanikkara during the period from December 1999 to March 2003.

A. Materials

Six different genotypes collected from diverse ecological conditions namely Nagarkovil (pure water) from Kanyakumari district of Tamil Nadu, Bramangalam (clay) from Ernakulam district, Nelliyampathy (high altitude), Chitoor (laterite tracts), both from Palakkad district and Chemmanda (kole area) from Thrissur district Chandiroor (coastal clay) from Alleppy district, were used for the study. These ecotypes representing diverse ecological situations were evaluated under *ex situ* conditions in cement tanks of two feet diameter and three feet high. Clay and water levels were retained at uniform height throughout the experiment period.

B. Methodology

3.1. Growth pattern of leaf and periodicity of leaf development

The growth and development pattern of leaf in all the selected ecotypes were studied by taking observations on various morphological and biometric characters at regular intervals right from leaf initiation till abscission all throughout an year. The observations on the following biometric characters were taken from ten different plants in each ecotype:

a) Longevity of leaves (days from visual appearance stage to abscission)

b) Petiole length in c.m.

c) Length and breadth of lamina at full expansion in c.m.

d) Length and breadth of lamina at abscission in c.m.

e) Mean number of days from visual appearance to full expansion of lamina

f) Stomatal frequency and type

g) Number of leaves produced

h) Frequency of leaf formation

The frequency of stomata per unit area of the leaf in each ecotype was estimated. The stomatal type was described following the classification proposed by Van Cotthem, 1970.

These data were correlated with weather data to know the influence of weather on growth parameters and development of leaf. The seasonal effect on various growth parameters of leaf was also computed by standard statistical methods.

For convenience, the whole year was divided into four seasons ie., December-February representing winter; March-May representing summer;

June-August representing rainy and September-November representing spring seasons.

3.2. Growth pattern in relation to propagule

Different propagules viz., three noded rhizome bits representing three age groups (fully mature, partially mature and tip portions) from each ecotypes were planted separately in cement tanks. Each treatment was replicated six times. Harvesting was done one year after planting and the rhizome yield was recorded. The nutrient composition of rhizomes was analysed following standard procedures. Based on the performance, the best propagule was selected.

3.3. Flowering biology

3.3.1. Growth pattern of flower bud

Five flower buds from each type were tagged soon after their appearance at the surface of the mud. The growth of flower bud from visual appearance stage till opening was studied at periodic intervals in all the ecotypes selected. The time taken for opening from visual appearance of flower was also recorded.

3.3.2. Periodicity of flowering

The number of flowers produced in each month for each ecotype was recorded and expressed as percentage of total number of flowers produced per year. The seasonality of flowering was then computed. For convenience of analysing the seasonal effect on flowering, the whole year was divided into four season. December-February representing winter, March-May representing summer, June-August, representing rainy season and September-November representing spring season. The succession of flower formation in the peak season was also observed.

3.3.3. Floral morphology

The description of morphological features of flowers of different ecotypes were made after examining the fresh flowers.

3.3.4. Anther dehisence and stigma receptivity

The colour and appearance of anthers were examined with hand lens at bihourly intervals in five fully matured flower buds of each type to find out the time of anther dehiscence.

Stigmatic surfaces were also examined for change in colour and appearance in the same buds at same intervals of time to find out stigma receptivity. Different insects visiting the flowers were also recorded.

3.3.5. Thermogenesis

The thermogenesity in fully mature buds was estimated at periodic intervals starting from two days prior to flower opening. The temperatures within and out side the flower bud were recorded. Five buds from each ecotypes were selected for recording the observations.

3.3.6. Palynology

The morphology, size and fertility of the pollen grains of each ecotype were determined following standard procedures using pollen collected from newly opened flower. The pollen grains were acetolysed according to the method described by Erdtman (1960) and the sculpturing was examined under microscope. Classification was done following the procedure suggested by Moore and Webb (1978). Fertility of pollen was assessed on the basis of staining with acetocarmine-glycerin mixture (Radford *et al.*, 1974). Pollen grains for this study were collected from newly opened flowers and stained in a drop of acetocarmine-glycerin mixture on a clean slide and kept aside for one hour. Pollen grains which are well filled and stained were counted as fertile and others sterile. Observations were taken from two fields of each of the five slides prepared for each ecotype. The values were expressed as percentage. The pollen diameter was measured using an ocular micrometer after calibration. The observations were taken from 100 pollen grains of each ecotype and mean was computed.

3.3.7. Pollination biology

Sets of ten fully matured buds from each ecotype were kept protected until completion of anthesis. The buds were kept protected by tieing a thread around the bud two days prior to anthesis. Another set was emasculated but kept unprotected. A third set was kept as control. The extend of fruit set in protected buds, unprotected buds and emasculated but kept unprotected bud were recorded. Observations were taken from five buds in each ecotype for each treatment. Different insects visiting the flowers were also recorded.

3.3.8. Seed physiology

Observations on time taken for maturity, moisture content, 100 seed weight, seed density and developmental changes were observed for each ecotype. Ten samples were observed in each case. For estimating seed density, known weight of seeds were immersed in distilled water taken in a measuring cylinder. The water displaced by the seeds was measured and seed density was calculated according to the formula.

$$\text{Seed density} = \frac{\text{Weight of seed in air}}{\text{Weight of water displaced}} \times \text{Specific gravity of water}$$

Moisture content was estimated by gravi-metric method. Nutrient status of seeds was also estimated by adopting standard procedure.

Lotus seeds are considered to have the longest period of dormancy (Priestly and Prosthumus, 1982). Germination tests were carried out with fully matured seeds after giving different pre-treatments to find out the factors contributing to dormancy. Untreated control was also used for comparison.

Pre-treatments tried

1. Pre-washing intact seed in water for 12 hrs.
2. Pre-washing intact seed in water for 24 hrs.
3. Pre-washing intact seed in water for 48 hrs.
4. Dipping the seed in cowdung slurry for 12 hrs followed by washing in water
5. Dipping the seed in cowdung slurry for 24 hrs followed by washing in water
6. Dipping seed in cowdung slurry for 48 hrs followed by washing in water
7. Mechanical scarification followed by leaching in water for 12 hrs.
8. Mechanical scarification followed by leaching in water for 24 hrs.
9. Mechanical scarification followed by leaching in water for 48 hrs.
10. Excised intact embryo alone
11. Intact seed

100 seeds of each type were used for the study. Water was used as the medium. The soaking water was changed daily. Total number of seeds germinated within 15 days were counted and expressed as percentage. From the results, the factors contributing to seed dormancy was elucidated.

The germination behaviour of the seeds were observed and recorded.

The anatomical changes in the fruit wall during the course of development was studied by taking transverse sections during various stages of development. The sections were made permanent following the procedure described by Prasad and Krishnaprasad (1970).

The total phenol content of seeds was estimated by Folin-Ciocalteau method (Malick and Singh, 1980).

Appropriate statistical analysis was carried out wherever necessary.

Results and Discussion

4. RESULTS AND DISCUSSION

The results of the study 'Morphogenesis and reproductive biology of sacred lotus' carried out in the Department of Plant Breeding and Genetics, College of Horticulture, Vellanikkara during 1999-2003 using six different ecotypes are presented below.

4.1. Growth pattern of leaf and periodicity of leaf development

The leaves of all the ecotypes of sacred lotus are simple with long petiole and peltate lamina. Leaves are either floating or held above water surface. The lamina remained in a rolled condition at the time of its appearance above water surface (Plate 1). The observations on various leaf characters like length and breadth of leaves at full expansion and at abscission stage, petiole length at full expansion of lamina, longevity of leaves and days taken for the lamina to open fully in six different ecotypes selected for the study are presented in Table 1 and Plate 2.

From the Table 1 it is clear that significant variability was observed among the ecotypes for all the leaf characters studied. Maximum leaf longevity of 28 days was observed in Bramangalam. The ecotypes Chittoor and Nagarkovil registered the minimum value of 24 days. In the case of petiole length, the lowest value of 25.93 cm was registered by Chittoor and the highest value of 34.91 cm by Chemmanda. The ecotypes Nagarkovil and

Table 1 Variability in leaf characters of six ecotypes

Ecotypes	Longevity of leaves (days)	Petiole length (cm)	Length of lamina at full expansion (cm)	Length of lamina at the time of abscission (cm)	Breadth of lamina at full expansion (cm)	Breadth of lamina at the time of abscission (cm)	Mean number of days for the leaf to open fully
Nagarkovil	23.63	33.21	13.88	14.56	18.58	18.23	3.90
Bramangalam	28.39	32.47	12.40	12.38	16.11	14.95	4.40
Chemmanda	27.27	34.91	13.04	12.73	16.05	16.46	4.59
Nelliyampathy	27.35	33.77	14.53	15.21	17.92	17.73	4.27
Chittoor	24.00	25.93	10.29	10.67	13.63	13.15	4.10
Chandiroor	24.86	30.79	12.86	12.80	15.51	14.41	4.65
CD (0.05)	0.57	1.19	0.68	0.65	0.84	0.70	0.28
CV (%)	3.11	5.27	7.55	6.30	7.27	6.24	9.05

Plate 1   Stages of unrolling of lamina in sacred lotus

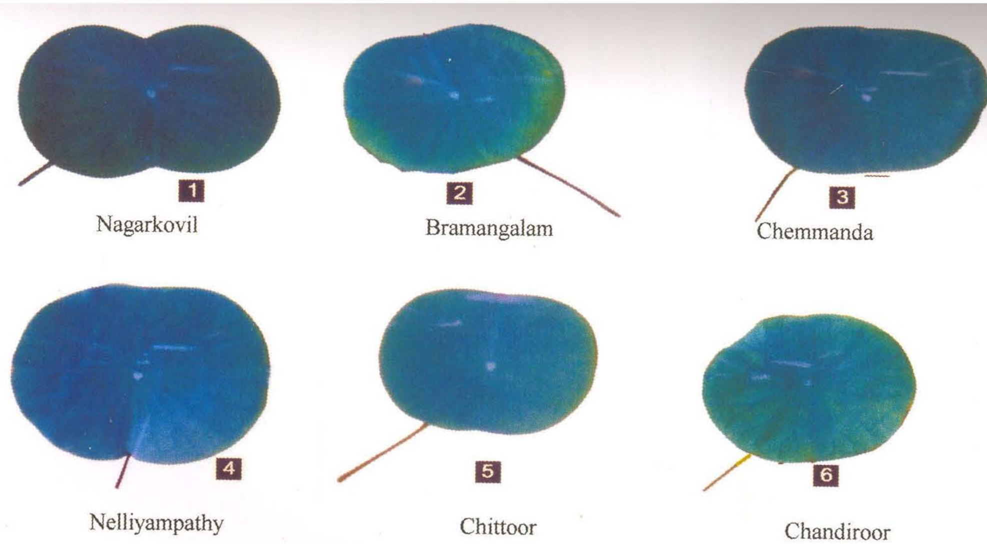


Plate 2 Leaves of different ecotypes of sacred lotus

Nelliyampathy were having the largest leaves. Among the ecotypes evaluated, Chittoor was having the smallest leaves with short petiole and low longevity. The growth pattern of leaves from different ecotypes based on length and breadth of lamina during peak season is presented in Table 2 and Fig.1 & 2.

Since the unrolling of lamina occurred only after 4-6 days of it's appearance on the surface of mud, breadth measurements were recorded only after that. Fluctuation was observed in both length and breadth of lamina in all the ecotypes. These fluctuations did not follow a regular pattern and was observed to be highly irregular as it is evident from Table 2.

The correlation of various growth parameters of leaf in different ecotypes with weather parameters viz., mean maximum, mean minimum, high maximum and low minimum temperatures, mean relative humidity, rainfall, number of rainy days, evaporation, mean sun shine hours and wind speed are presented in Table 3 to 11.

All the ecotypes except Chitoor and Chandiroor showed significant negative correlation for the character longevity of leaves with temperature (Table 3). Longevity showed no significant correlation with any other weather parameters. Nelliyampathy showed significant negative correlation with evaporation.

Irrespective of ecotypes, petiole length showed significant negative correlation with mean maximum and high maximum temperatures,

Table 2 Growth pattern of leaf in six different ecotypes of sacred lotus based on length and breadth of lamina

Days	Nagarkovil		Bramangalam		Chemmanda		Nelliyampathy		Chittoor		Chandiroor	
	Length (cm)	Breadth (cm)	Length (cm)	Breadth (cm)	Length (cm)	Breadth (cm)	Length (cm)	Breadth (cm)	Length (cm)	Breadth (cm)	Length (cm)	Breadth (cm)
1 day	3.8	-	3.9	-	5.0	-	3.7	-	2.8	-	4.0	-
2 day	5.7	-	5.0	-	6.9	-	12.4	-	5.3	-	5.8	-
3 day	8.4	-	7.0	-	9.0	-	14.5	-	6.0	-	9.9	-
4 day	9.7	-	8.3	-	11.0	-	16	-	7.8	-	12.1	-
5 day	12.2	15.0	11.6	-	13.7	-	20.1	-	9.2	-	14.5	-
6 day	13.4	16.7	14.1	17.5	15.0	-	23.9	29.0	10.6	-	17.0	-
7 day	13.8	17.1	16.5	21.5	17.7	20.2	25.2	33.0	12.2	14.2	19.4	21.5
8 day	14.0	17.2	18.4	24.0	19.4	22.5	27.0	35.7	13.8	17.3	20.8	26.3
9 day	14.0	17.2	18.5	24.7	19.7	23.6	27.0	35.8	14.4	19	22.3	27.2
10 day	14.0	17.2	18.5	24.8	19.7	24.0	27.0	35.8	14.5	19.6	22.3	27.5
11 day	13.9	17.2	18.5	24.8	19.7	23.8	27.0	35.8	14.6	19.7	22.4	27.5
12 day	13.9	17.2	18.5	24.8	19.7	23.8	27.0	35.8	14.6	19.7	22.4	27.5
13 day	13.9	17.2	18.5	25.0	19.7	23.8	27.0	36.0	14.6	19.7	22.4	27.5
14 day	13.8	17.3	18.5	25.0	19.7	23.8	27.0	36.0	14.6	19.7	22.4	27.7
15 day	13.8	17.3	18.6	25.0	19.6	23.7	27.0	36.0	14.7	19.7	22.5	27.7
16 day	13.8	17.3	18.6	25.0	19.6	23.7	27.0	36.0	14.7	19.7	22.5	27.7
17 day	13.8	17.2	18.6	25.0	19.6	23.7	26.8	35.9	14.7	19.8	22.5	27.5
18 day	13.8	17.2	18.5	25.0	19.5	24.0	26.8	35.9	14.7	19.8	22.5	27.5
19 day	13.8	17.2	18.5	25.0	19.5	24.0	26.8	35.9	14.7	19.8	22.5	27.5
20 day	13.8	17.2	18.5	25.0	19.5	24.0	27.0	35.9	14.7	19.8	22.3	27.6
21 day	13.8	17.2	18.5	26.0	19.5	23.7	27.0	35.9	14.7	19.7	22.3	27.6
22 day	13.8	17.2	18.5	26.1	19.5	23.7	27.0	35.9	14.6	19.7	22.3	27.6
23 day	13.8	17.2	18.5	25.0	19.5	23.7	27.0	35.8	14.6	19.7	22.3	27.6
24 day	13.8	17.2	18.5	25.0	19.6	23.7	27.0	35.8	14.6	19.7	22.3	27.6
25 day	13.8	17.2	18.5	25.0	19.6	23.7	27.0	35.8	14.6	19.8	22.3	27.6
26 day	14.4	17.4	18.5	25.0	19.7	23.8	27.0	35.8	14.6	19.8	22.3	27.6
27 day	15.0	17.4	18.5	25.0	19.7	23.8	27.0	35.8	14.7	19.8	22.5	27.5
28 day	15.6	17.9	18.5	25.0	19.7	23.8	27.0	35.8	14.7	19.8	22.5	27.5

29 day	15.8	18.6	18.5	25.0	19.7	23.8	27.0	35.8	14.7	19.8	22.5	27.5
30 day	16.2	20.0	18.8	25.0	19.7	23.8	27.0	35.8	14.7	19.8	22.5	27.5
31 day	17.0	21.2	18.8	24.9	19.8	23.8	27.0	35.8	14.7	19.8	22.5	27.5
32 day	17.0	21.5	18.8	24.9	20.0	23.8	27.0	35.8	14.7	19.8	22.5	27.7
33 day	17.0	21.5	19.0	24.9	20.0	23.9	27.0	35.8	14.5	19.8	-	-
34 day	17.0	21.5	19.0	25.0	20.0	23.9	27.0	35.8	14.5	19.8	-	-
35 day	17.1	21.5	19.0	25.0	20.0	23.9	27.0	35.8	14.5	19.7	-	-
36 day	17.1	21.5	19.0	25.0	20.0	23.9	27.0	35.8	14.5	19.7	-	-
37 day	17.1	21.5	19.0	25.0	20.0	23.9	27.0	35.8	14.5	19.7	-	-
38 day	-	-	19.0	25.0	19.8	23.9	27.0	35.8	14.5	19.7	-	-
39 day	-	-	19.0	25.0	19.8	23.9	27.0	35.8	-	-	-	-
40 day	-	-	18.7	25.0	19.8	23.9	27.0	35.8	-	-	-	-
41 day	-	-	18.7	25.0	19.8	23.9	-	-	-	-	-	-
42 day	-	-	18.7	25.0	19.8	23.9	-	-	-	-	-	-
43 day	-	-	18.7	25.0	19.8	23.9	-	-	-	-	-	-
44 day	-	-	19.0	25.0	19.8	23.7	-	-	-	-	-	-
45 day	-	-	19.0	25.0	19.5	23.7	-	-	-	-	-	-
46 day	-	-	19.0	25.0	19.5	23.7	-	-	-	-	-	-
47 day	-	-	19.0	25.0	-	-	-	-	-	-	-	-
48 day	-	-	19.0	25.0	-	-	-	-	-	-	-	-
49 day	-	-	19.0	25.0	-	-	-	-	-	-	-	-

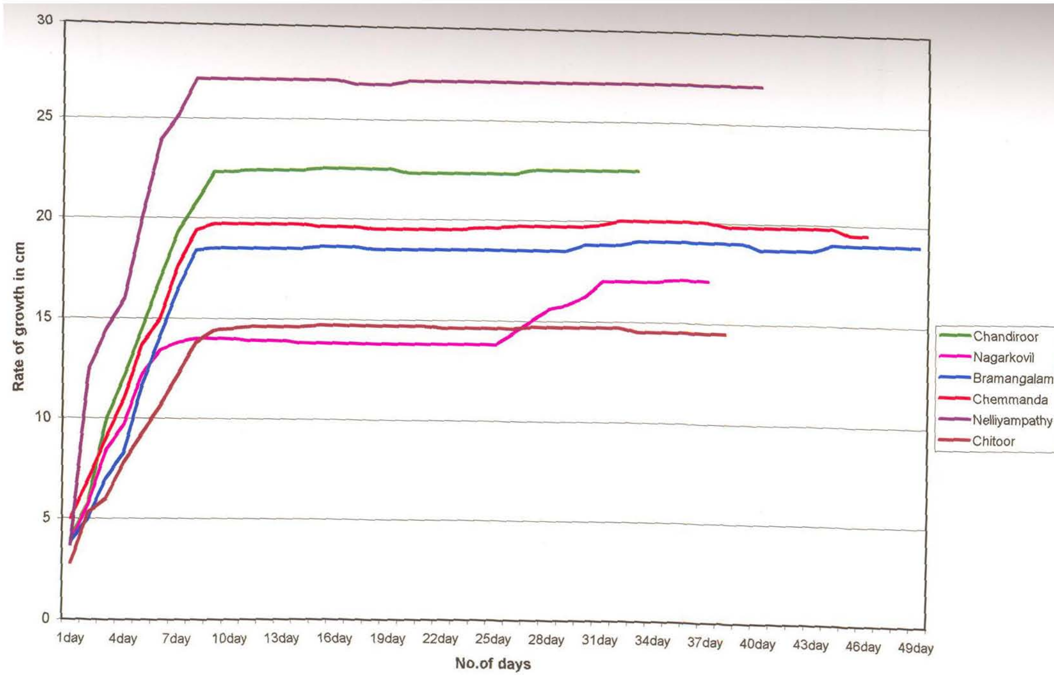


Fig.1 Leaf expansion pattern of different ecotypes based on leaf length

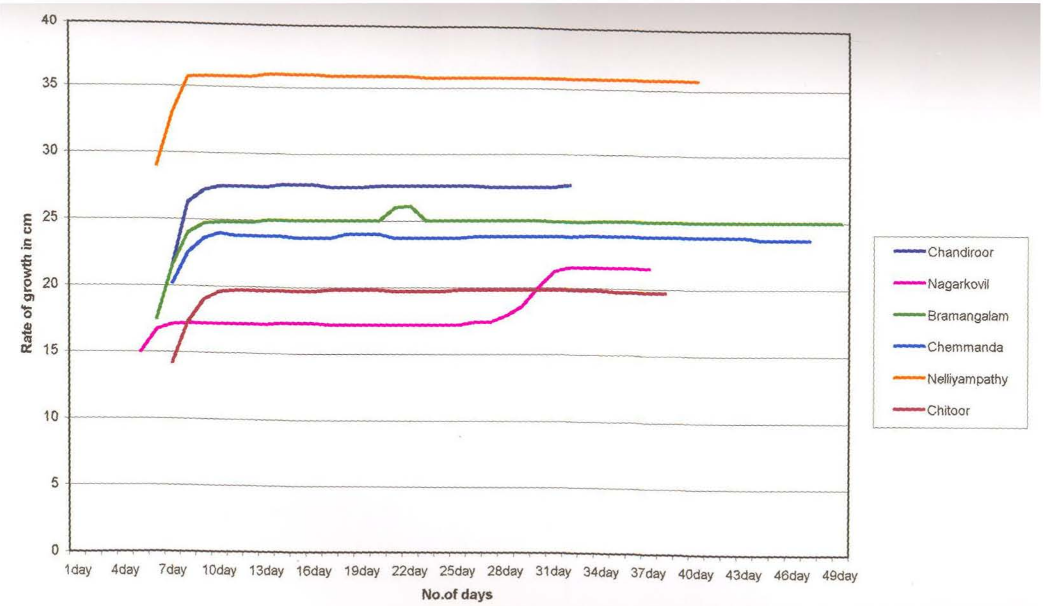


Fig.2 Leaf expansion pattern of different ecotypes based on leaf breadth

Table 3 Correlation of weather parameters with longevity of leaves

Ecotypes	Mean_Max Temperature	Mean_Min Temperature	High_Max Temperature	Low_Min Temperature	Mean Relative humidity	Rainfall	Rainy days	Evaporation	Mean sun shine hours	Wind speed
Nagarkovil	-0.192	-0.271*	-0.282*	-0.017	-0.091	-0.283*	-0.139	0.095	0.071	0.244
Bramangalam	-0.355**	-0.353*	-0.441**	-0.033	0.126	-0.03	0.113	-0.157	-0.186	0.07
Chemmanda	-0.283*	-0.274*	-0.309*	-0.026	0.081	-0.169	-0.013	-0.157	-0.078	0.077
Nelliyampathy	-0.434**	-0.324*	-0.458**	-0.041	0.202	0.031	0.173	-0.263*	-0.229	-0.014
Chitoor	-0.189	0.242	-0.15	0.493**	0.243	0.033	0.157	-0.089	-0.147	-0.178
Chandiroor	0.007	-0.007	-0.113	0.208	0.037	-0.03	0.047	-0.005	-0.067	-0.008

** Correlation significant at the 0.01 level
* Correlation significant at the 0.05 level

evaporation, mean sunshine hours and wind speed. Positive correlation was registered with low minimum temperature, relative humidity and rainfall (Table 4)

It is evident from the Table 5 that lamina length at the time of full expansion in Chemmanda and Nelliyampathy had significant negative correlation with mean maximum and high maximum temperature and evaporation. Same ecotypes showed significant positive correlation with mean relative humidity, rainfall and rainy days. In Chandiroor, significant negative correlation was observed for leaf lamina length at the time of abscission with mean maximum and high maximum temperature, evaporation and mean sun shine hours. Positive correlation for same ecotypes were observed with low minimum temperature, means relative humidity and number of rainy days.

Lamina breadth at the time of full expansion and lamina breadth at the time of abscission (Table 7 &8) showed significant negative correlation with mean maximum and high maximum temperature, evaporation rate and mean sun shine hours in ecotypes Chemmanda, Nelliyampathy and Chandiroor . The same ecotypes registered significant positive correlation for the same cha-acter with mean relative humidity, rainfall and number of rainy days.Nagar-kovil registered positive correlation with mean maximum, mean minimum temperatures and negative correlation with wind speed. Bramangalam and Chitoor showed significant positive correlation with low

Table 4 Correlation of weather parameters with petiole length

Ecotypes	Mean_Max Temperature	Mean_Min Temperature	High_Max Temperature	Low_Min Temperature	Mean Relative humidity	Rainfall	Rainy days	Evaporation	Mean sun shine hours	Wind speed
Nagarkovil	-0.438**	0.196	-0.370**	0.570**	0.796**	0.778**	0.652**	-0.815**	-0.776**	-0.632**
Bramangalam	-0.451**	0.238	-0.409**	0.614**	0.779**	0.463**	0.611**	-0.652**	-0.614**	-0.636**
Chemmanda	-0.517**	0.121	-0.404**	0.468**	0.817**	0.751**	0.654**	-0.823**	-0.786**	-0.635**
Nelliyampathy	-0.465**	0.152	-0.426**	0.521**	0.802**	0.680**	0.576**	-0.761**	-0.730**	-0.646**
Chitoor	-0.610**	-0.205	-0.574**	0.249	0.697**	0.666**	0.529**	-0.726**	-0.755**	-0.519**
Chandiroor	0.586**	0.048	-0.553**	0.489**	0.826**	0.766**	0.703**	-0.824**	-0.824**	-0.562**

** Correlation significant at the 0.01 level
* Correlation significant at the 0.05 level

Table 5 Correlation of weather parameters with leaf lamina length at full expansion

Ecotypes	Mean_Max Temperature	Mean_Min Temperature	High_Max Temperature	Low_Min Temperature	Mean Relative humidity	Rainfall	Rainy days	Evaporation	Mean sun shine hours	Wind speed
Nagarkovil	0.226	0.335**	0.220	0.167	0.131	-0.002	0.096	-0.029	0.052	-0.303*
Bramangalam	0.215	0.154	0.135	0.264	0.157	-0.054	-0.123	-0.080	-0.013	-0.342*
Chemmanda	-0.577**	-0.272*	-0.561**	0.087	0.356**	0.552**	0.472**	-0.416**	-0.585**	-0.064
Nelliyampathy	-0.532**	-0.261*	-0.557**	0.098	0.461**	0.536**	0.494**	-0.481**	-0.608**	-0.282*
Chitoor	0.026	0.107	0.014	0.340*	-0.041	-0.143	-0.099	0.168	0.045	-0.016
Chandiroor	-0.532**	-0.178	-0.545**	0.338**	0.421**	0.336**	0.427**	-0.374**	-0.524**	-0.150

** Correlation significant at the 0.01 level
* Correlation significant at the 0.05 level

Table 6 Correlation of weather parameters with leaf lamina length at the time of abscission

Ecotypes	Mean_Max Temperature	Mean_Min Temperature	High_Max Temperature	Low_Min Temperature	Mean Relative humidity	Rainfall	Rainy days	Evaporation	Mean sun shine hours	Wind speed
Nagarkovil	0.278*	0.305*	0.256*	0.094	0.102	-0.039	0.058	-0.021	0.091	-0.314*
Bramangalam	0.236	0.177	0.165	0.271	0.148	-0.056	-0.136	-0.073	-0.002	-0.344*
Chemmanda	-0.577**	-0.261	-0.558**	0.103	0.369**	0.562**	0.478**	-0.421**	-0.595**	-0.079
Nelliyampathy	-0.534**	-0.253	-0.559**	0.114	0.471**	0.530**	0.494**	-0.486**	-0.610**	-0.291*
Chitoor	0.028	0.10	0.014	0.333*	-0.044	-0.143	-0.106	0.168	0.046	-0.017
Chandiroor	-0.533**	-0.179	-0.546**	0.340**	0.422**	0.336**	0.427**	-0.376**	-0.525**	-0.152

** Correlation in significant at the 0.01 level
* Correlation in significant at the 0.05 level

Table 7 Correlation of weather parameters with leaf lamina breadth at full expansion

Ecotypes	Mean_Max Temperature	Mean_Min Temperature	High_Max Temperature	Low_Min Temperature	Mean Relative humidity	Rainfall	Rainy days	Evaporation	Mean sun shine hours	Wind speed
Nagarkovil	0.316*	0.368**	0.293*	0.202	0.150	-0.043	0.022	-0.058	0.084	-0.384**
Bramangalam	0.122	0.152	0.054	0.308*	0.253	0.029	-0.023	-0.172	-0.107	-0.398**
Chemmanda	-0.591**	-0.278*	-0.585**	0.134	0.415**	0.555**	0.469**	-0.475**	-0.622**	-0.117
Nelliyampathy	-0.525**	-0.167	-0.547**	0.200	0.527**	0.531**	0.537**	-0.518**	-0.614**	-0.347**
Chitoor	-0.059	0.107	-0.062	0.384**	0.090	-0.060	-0.015	0.038	-0.0510	-0.132
Chandiroor	-0.525**	-0.127	-0.528**	0.401**	0.516**	0.394**	0.452**	-0.454**	-0.578**	-0.258

** Correlation significant at the 0.01 level
* Correlation significant at the 0.05 level

Table 8 Correlation of weather parameters with lamina breadth at the time of abscission

Ecotypes	Mean_Max Temperature	Mean_Min Temperature	High_Max Temperature	Low_Min Temperature	Mean Relative humidity	Rainfall	Rainy days	Evaporation	Mean sun shine hours	Wind speed
Nagarkovil	0.300*	0.355**	0.275*	0.196	0.161	-0.032	0.036	-0.074	0.069	-0.389**
Bramangalam	0.129	0.154	0.061	0.305*	0.243	0.026	-0.031	-0.163	-0.101	-0.388**
Chemmanda	-0.588**	-0.269*	-0.579**	0.143	0.415**	0.559**	0.475**	-0.473**	-0.623**	-0.117
Nelliyampathy	-0.528**	-0.168	-0.549**	0.204	0.527**	0.534**	0.538**	-0.520**	-0.618**	-0.345**
Chitoor	0.008	0.129	-0.002	0.376**	0.055	-0.098	-0.063	-0.081	0.002	-0.127
Chandiroor	-0.516**	-0.120	-0.518**	0.406**	0.499**	0.386**	0.453**	-0.446**	-0.567**	-0.239

** Correlation significant at the 0.01 level
* Correlation significant at the 0.05 level

Table 9 Correlation of weather parameters with days taken for the leaf to open fully

Ecotypes	Mean_Max Temperature	Mean_Min Temperature	High_Max Temperature	Low_Min Temperature	Mean Relative humidity	Rainfall	Rainy days	Evaporation	Mean sun shine hours	Wind speed
Nagarkovil	-0.381**	-0.321*	-0.324*	-0.071	0.217	0.264*	0.121	-0.369**	-0.324*	-0.040
Bramangalam	-0.581**	-0.291*	-0.551**	-0.010	0.435**	0.313*	0.398**	-0.499**	-0.461**	-0.175
Chemmanda	-0.560**	-0.577**	-0.591**	-0.194	0.224	0.256	0.153	-0.297*	-0.426**	0.080
Nelliyampathy	-0.240	-0.241	-0.279*	-0.039	0.191	0.047	-0.052	-0.213	-0.200	-0.113
Chitoor	-0.402**	-0.035	-0.390**	0.258	0.609**	0.324*	0.423**	-0.558**	-0.457**	-0.531**
Chandiroor	-0.393**	-0.304*	-0.443**	-0.042	0.378**	0.240	0.141	-0.434**	-0.379**	-0.227

** Correlation significant at the 0.01 level
* Correlation significant at the 0.05 level

Table 10 Correlation of weather parameters with periodicity of leaf formation

Ecotypes	Mean_Max Temperature	Mean_Min Temperature	High_Max Temperature	Low_Min Temperature	Mean Relative humidity	Rainfall	Rainy days	Evaporation	Mean sun shine hours	Wind speed
Nagarkovil	0.007	-0.049	0.025	0.019	-0.240	-0.207	-0.183	0.230	0.163	0.362**
Bramangalam	0.072	-0.063	0.050	-0.142	-0.185	-0.090	-0.104	0.168	0.112	0.200
Chemmanda	0.167	-0.128	0.079	-0.160	-0.286*	-0.280*	-0.287*	0.317*	0.233	0.198
Nelliyampathy	-0.042	-0.022	0.029	-0.025	-0.038	0.119	-0.026	0.014	-0.068	0.090
Chitoor	0.155	0.016	0.130	0.012	0.052	0.015	-0.129	-0.085	-0.005	-0.170
Chandiroor	-0.112	-0.086	-0.092	-0.143	0.024	0.065	0.038	-0.047	-0.066	0.002

** Correlation significant at the 0.01 level
* Correlation significant at the 0.05 level

Table 11 Correlation of weather parameters with number of leaves produced

Ecotypes	Mean_Max Temperature	Mean_Min Temperature	High_Max Temperature	Low_Min Temperature	Mean Relative humidity	Rainfall	Rainy days	Evaporation	Mean sun shine hours	Wind speed
Nagarkovil	0.387**	0.489**	0.465**	0.275*	0.199	0.076	0.086	-0.033	0.036	-0.541**
Bramangalam	0.468**	0.169	0.590**	-0.044	-0.336**	-0.109	-0.333**	0.385**	0.258*	0.114
Chemmanda	-0.278*	0.471**	-0.129	0.602**	0.696**	0.708**	0.768**	-0.622**	-0.613**	-0.602**
Nelliyampathy	0.451**	0.678**	0.490**	0.281*	0.161	0.043	0.238	-0.054	0.115	-0.403**
Chitoor	0.107	0.066	0.141	0.277*	0.047	0.172	-0.069	0.113	-0.121	-0.061
Chandiroor	0.483**	0.534**	0.521**	0.325*	0.079	-0.038	-0.049	0.139	0.129	-0.437**

** Correlation significant at the 0.01 level
* Correlation significant at the 0.05 level

minimum temperature. Bramangalam exhibited negatives correlation with wind speed.

Among the different ecotypes periodicity of leaf formation exhibited significant positive correlation with wind speed in Nagarkovil. In Chemmanda, periodicity of leaf formation showed significant negative correlation with relative humidity, rainfall and rainy days.

Number of leaves produced showed positive correlation with mean maximum and mean high temperature in all ecotypes except Chitoor and Chemmanda. Chemmanda registered negative correlation with mean maximum temperature. In Chitoor, positive correlation with low minimum temperature was recorded. Chandiroor, Nagarkovil and Nelliyampathy showed positive correlation with low minimum temperature.In the ecotype Bramangalam, the number of leaves produced showed significant negative correlation with mean relative humidity and number of rainy days. All ecotypes except Chitoor and Bramangalam showed negative correlation with wind speed.

The influences of season on various leaf characters of lotus are presented in Table 12.

The study has revealed that seasonal effects on leaf characters are significant. Longevity of the leaves and days taken for the leaves to open fully were the highest in spring season followed by rainy season and the least

Table 12 Effect of season on various leaf characters

Seasons	Longivity of leaves (days)	Petiole length (cm)	Length of lamina at full expansion (cm)	Lamina length at the time of abscission (cm)	Leaf lamina breadth at full expansion (cm)	Leaf lamina breadth at the time of abscission (cm)	Days taken for the leaves to open fully
Winter (Dec – Feb)	24.94	19.90	11.25	12.04	13.50	12.48	3.82
Summer (Mar – May)	23.21	29.36	11.73	12.06	14.78	15.40	3.71
Rainy (Jun – Aug)	27.38	39.96	15.47	15.28	19.82	19.02	4.76
Spring (Sep – Nov)	28.21	38.17	12.88	12.84	17.09	16.39	4.98
CD (0.05)	0.47	0.97	0.56	0.48	0.68	0.57	0.23
CV (%)	3.11	5.27	7.55	6.30	7.27	6.24	9.05

in summer. However, the petiole length and size of leaf as represented by length and breadth of lamina were the highest during rainy season (June – August) followed by spring season (September-November). Rainy season favoured the growth in size of leaves and spring season favoured the longevity and days taken for leaves to open fully.

The periodicities of leaf formation in different ecotypes in different seasons are presented in Table 13.

Table 13. Periodicity of leaf formation in different ecotypes of sacred lotus in different seasons(days)

Sl. No.	Ecotype	Seasons				Mean
		Winter Dec-Feb	Summer Mar-May	Rainy Jun-Aug	Spring Sep-Nov	
1	Nagarkovil	2.03	1.83	1.78	2.03	1.91
2	Bramangalam	2.15	2.03	1.70	1.95	1.96
3	Chemmanda	2.30	2.08	1.90	1.98	2.06
4	Nelliyampathy	2.05	1.78	1.95	1.85	1.91
5	Chitoor	2.03	1.88	2.05	2.15	2.02
6	Chandiroor	1.93	1.80	1.83	1.88	1.86
	Mean	2.08	1.90	1.87	1.97	

CD (0.05) for seasons = 0.08 CD (0.05) for ecotypes = 0.022
CD (0.05) for interaction = 0.202 CV(%) = 17.31

From Table 13 it is evident that the periodicity of leaf formation varied with the ecotype as well as season. Leaf production was very low during December to February period representing winter season as indicated by the higher number of days between successive leaf formation.

The stomatal count/unit area in different ecotypes of lotus is presented in Table 14. The stomata are found to be anomocytic or ranunculaceous in all the ecotypes (Plate 3). Borsch and Barthlott (1996) has also reported ranunculaceous stomata in *Nelumbo* genus. Leaves of all the ecotypes evaluated were epistomatic and did not differ significantly in the number of stomata per unit area.

Table 14. Stomatal count in different ecotypes of lotus

Ecotype	Stomatal count / mm^2
Nagarkovil	520
Bramangalam	490
Chemmanda	510
Nelliyampathy	510
Chitoor	500
Chandiroor	490
CD (0.05)	NS
CV (%)	4.08

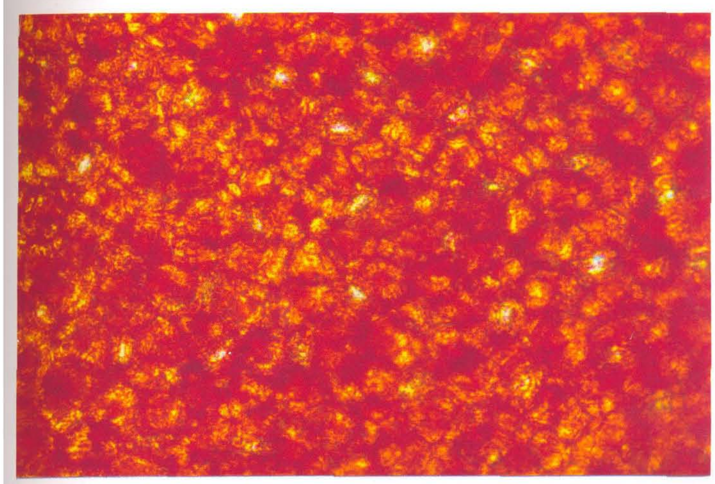


Plate 3 Stomata of sacred lotus (×400)

4.2. Growth in relation to propagule

The rhizome yield obtained by planting different propagules (Plate 4) viz., fully mature, partially mature and tip portions of rhizomes are presented in Table 15.

Table 15 Rhizome yield from different propagules of sacred lotus

Propagule	Rhizome yield (kg/m^2)
Tip portion	0.63
Partially matured rhizome	0.52
Fully matured rhizome	0.12
CD (0.05)	0.08
CV (%)	12.40

A comparison of the rhizome yield from different propagules revealed that three noded tip portion is the best propagule. Fully matured portion of the rhizome when used as propagule was showing very poor performance (Table 15). Katori *et al* (2002) has also reported that plants produced by rhizome straps are superior to enlarged rhizomes.

Table 16 shows rhizome yield in different ecotypes under evaluation.Among the different ecotypes evaluated, Chandiroor produced the highest yield of rhizomes. This was found to be on par with Nelliyampathy. Chittoor was found to be the lowest yielder among the ecotypes studied.

Plate 4 Different propagules of sacred lotus

Plate 5 Excentric starch grains in rhizome of sacred lotus (×400)

Table 16 Rhizome yield in relation to ecotypes

Ecotypes	Yield (kg/m^2)
Nagarkovil	0.58
Bramangalam	0.60
Chemmanda	0.58
Nelliyampathy	0.61
Chittoor	0.51
Chandiroor	0.63
CD (0.05)	0.02
CV (%)	12.40

Nutrient composition of rhizomes of different ecotypes are presented in Table 17.

Table 17 Nutrient content of rhizomes

Ecotypes	Starch (%)	Crude protein (%)	Crude fiber (%)
Nagarkovil	73.2	14.8	5.3
Bramangalam	71.4	14.9	5.8
Chemmanda	72.5	15.1	5.4
Nelliyampathy	71.8	14.4	5.6
Chittoor	72.2	14.5	5.4
Chandiroor	72.5	15	5.3

Neither the ecotype nor the propagule had any significant effect on the nutrient composition of rhizome. The result of the nutrient analysis revealed that starch is the main component accounting 71.5 per cent to 73.2 per cent of the dry weight of the rhizome. The microscopic examination of the starch grains revealed that they are excentric in nature (Plate 5)

4.3. Flowering biology

4.3.1. Growth pattern of flower bud

The growth pattern of flower buds of six different ecotypes (Plate 6) viz., Nagarkovil, Bramangalam, Chemmanda, Nelliyampathy,Chitoor and Chandiroor as represented by mean number of days for flower opening from their appearance at the surface of mud, mean length of pedicel at the time of flower opening and at fruit maturity, mean length of bud at the time of emergence at the surface of water and at maturity, diameter of fully opened flower and blossom life are presented in Table 18.

The mean number of days to flower opening from the appearance of bud at mud surface varied from 12 days in Bramangalam to 21 days in Chandiroor. However, Bramangalam alone was found to differ significantly from the other ecotypes which were on par. The pedicel length varied from 71.32 cm in Bramangalam to 96.8 cm in Nelliyampathy. A slight increase in pedicel length ranging from 2-4 cm was observed in different ecotypes at the time of maturity of fruit indicating that elongation of pedicel continues even after flower opening. Bramangalam, though was having the biggest bud at the

Table 18 Growth pattern of flower buds of different ecotypes

Ecotypes	Days to flower opening	Mean pedicel Length		Mean length of bud		Diameter of flower (cm)	Blossom life (days)
		At flower opening (cm)	At fruit maturity (cm)	Emergence (cm)	Maturity (cm)		
Nagarkovil	17	90.56	94.86	1.16	12.38	20.90	3
Bramangalam	12	71.32	75.94	1.62	10.04	17.72	3
Chemmanda	20	76.00	78.50	1.30	10.60	19.80	3
Nelliyampathy	20	96.80	99.00	1.10	11.90	20.00	3
Chittoor	18	90.60	94.82	1.18	11.00	19.85	3
Chandiroor	21	72.00	75.85	1.10	9.00	16.30	3
CD (0.05)	4.39	5.82	2.58	0.24	1.40	1.57	-
CV (%)	23.50	20.20	18.15	12.53	13.99	12.49	-

Plate 6   Growth pattern of flower bud in sacred lotus

time of emergence did not retain that superiority at full maturity of bud. The biggest fully matured buds (12.38 cm) and fully opened flowers (20.90 cm) were observed in the ecotype Nagarkovil. The flower size of Chemmanda, Nelliyampathy and Chittoor were on par with that of Nagarkovil. Irrespective of ecotypes, blossom life was only three days. Hence, for production of large flowers, the ecotypes Nagarkovil, Nelliyampathy, Chittoor and Chemmanda can be preferred.

4.3.2. Periodicity of flowering

The flowers produced by each ecotype of lotus in each season expressed as the percentage of total number of flowers produced per year is given in Table 19.

Table 19 Seasonal effect on flower production

Ecotypes	Proportion of flowers produced (%)			
	Winter (Dec – Feb)	Summer (Mar – May)	Rainy (Jun – Aug)	Spring (Sep – Nov)
Nagarkovil	8.5	-	31.5	60.0
Bramangalam	7.2	-	37.2	55.6
Chemmanda	7.5	-	38.0	54.5
Nelliyampathy	8.3	-	36.0	55.9
Chittoor	7.9	-	35.9	56.2
Chandiroor	8.1	-	37.7	54.2

It can be seen that flower production started with the onset of Monsoon and reached the peak in September to November representing spring season and then declined. There was practically no flower production during March to May representing the summer season (Table 19).

The succession of flower formation in each ecotype during the peak period is presented in Table 20.

Table 20 Periodicity of flower production in different ecotypes in peak season

Ecotype	Periodicity (days)
Nagarkovil	12
Bramangalam	15
Chemmanda	14
Nelliyampathy	15
Chittoor	14
Chandiroor	14
CD (0.05)	NS
CV (%)	16.80

During peak flowering season, flowers were produced at 12-15 days interval and there was no significant difference between ecotypes in the periodicity of flower production.

4.3.3. Floral morphology

The flowers were found to be solitary, ebracteate, pedicellate, actinomorphic and complete with floral formula

$$\oplus \; \underset{+}{\overset{\nearrow}{O}} \quad K_{3-5} \quad C_{\propto} \quad A_{\propto + \propto \text{ staminode}} \quad G_{7\text{-}13}$$

Pedicels were armed with spines (Plate 7).

The comparison of morphological features of flowers of the six different ecotypes are presented in Table 21to 25 and depicted in Plates 8 to 12 and Fig.3.

Sepal characters of different ecotypes of sacred lotus are presented in Table 21.

Table 21 Sepal characters of six different ecotypes

Ecotypes	Mean No. of sepals/flower	Mean length (cm)	Mean breadth (cm)
Nagarkovil	5	1.69	1.11
Bramangalam	3	1.54	0.92
Chemmanda	5	1.28	0.78
Nelliyampathy	5	1.42	0.64
Chittoor	5	1.45	0.62
Chandiroor	5	1.28	0.58
CD (0.05)	-	0.26	0.20
CV (%)	-	12.53	12.89

Plate 7 Spines on the pedicel

Five sepals greenish in colour (Plate 8) were present in all the ecotypes except Bramangalam. The ecotype Bramangalam registered only three sepals. The mean length of sepals ranged from 1.28 cm in Chemmanda and Chandiroor to 1.69 cm in Nagarkovil and mean breadth from 0.58 cm in Chandiroor to 1.11 cm in Nagarkovil. Among the ecotypes studied, Nagarkovil was having the biggest sepal with 1.69 cm length and 1.11 cm breadth (Table 21).

The petal characters of different ecotypes of lotus are presented in Table 22.

Table 22 Petal characters of six different ecotypes

Ecotypes	Colour	Mean No./ flower	Mean length (cm)	Mean breadth (cm)	Angle at tip
Nagarkovil	Pink	36	10.77	5.37	108°
Bramangalam	Pink with dark pink at the edge	18	9.07	5.00	111°
Chemmanda	Pink	24	8.98	4.52	112°
Nelliyampathy	Light pink	30	10.14	5.62	102°
Chittoor	Light pink	12	10.04	4.48	101°
Chandiroor	Pink	15	6.70	4.50	118°
CD (0.05)	-	10.19	1.76	1.04	NS
CV (%)	-	27.34	13.99	20.20	24.45

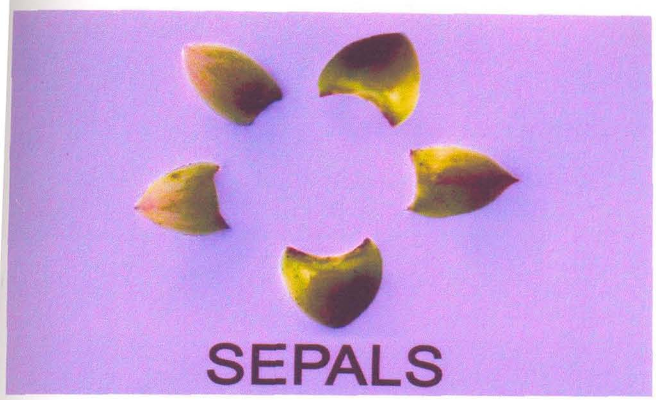


Plate 8 Sepals of sacred lotus

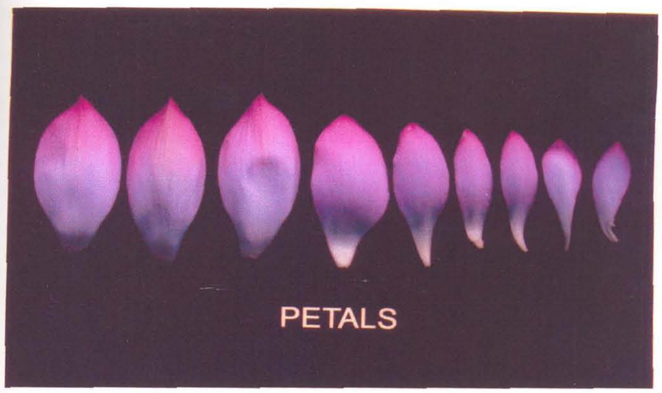


Plate 9 Petals of sacred lotus

The petals are obovate and slightly boat shaped was arranged in a spiral fashion on the floral axis. Whorls showed gradation in size of petals with the outer most whorl having large petals and inner most whorl having short petals (Plate 9). The mean petal size was the highest in Nagarkovil (10.77 cm length and 5.37 cm breadth). Nagarkovil also showed the highest number of petals (36/flower).

The character of transitional petals are furnished in Table 23.

Table 23 Transitional petal characters of six different ecotypes

Ecotypes	Mean No./ flower	Mean length (cm)	Mean breadth (cm)	Angle at tip
Nagarkovil	72	7.50	2.54	85.80°
Bramangalam	Nil	-	-	-
Chemmanda	47	7.36	2.16	91.40°
Nelliyampathy	52	7.36	2.52	97.80°
Chittoor	95	6.50	2.18	91.30°
Chandiroor	68	7.28	2.60	92.00°
CD (0.05)	17.80	0.27	0.22	NS
CV (%)	19.57	10.18	9.92	18.58

Transitional petals representing sterile stamens are also found in all the ecotypes except Bramangalam (Table 23 and Plates 10 & 11).

Plate 10 Transitional petals of sacred lotus

Plate 11 Ecotype Bramangalam without transitional petal

Chittoor exhibited the highest numbers of transitional petals.However, the biggest transitional petals were observed in Nagarkovil (Table 23).

Table 24 shows the staminal characters of different ecotypes of sacred lotus.

Table 24 Androecium character of six different ecotypes

Ecotype	Mean No. of stamen/ flower	Mean length of filament (cm)	Mean length of anther lobe (cm)	Mean length of appendage (cm)	No. of carpels/ receptlcle
Nagarkovil	137	1.50	1.02	0.37	13
Bramangalam	147	1.58	1.06	0.39	12
Chemmanda	85	1.32	1.00	0.30	7
Nelliyampathy	131	1.40	1.06	0.44	7
Chitoor	130	1.50	1.02	0.37	12
Chandiroor	120	1.58	1.06	0.30	13
CD (0.05)	7.56	0.18	NS	NS	2.3
CV (%)	18.90	7.10	8.50	9.80	14.50

Numerous stamens, ranging from 85 in Chemmanda to 147 in Bramangalm were observed in each flower. Each stamen consisted of a filament, long bilobed, basi fixed yellow anther lobe and a white coloured connective extending beyond the length of anther lobe (Fig.3). The connective distally has a peal coloured club shaped appendage. The differences in the length of stamens among the ecotypes is due to difference in the length of filaments (Table 24 and Plate 12).

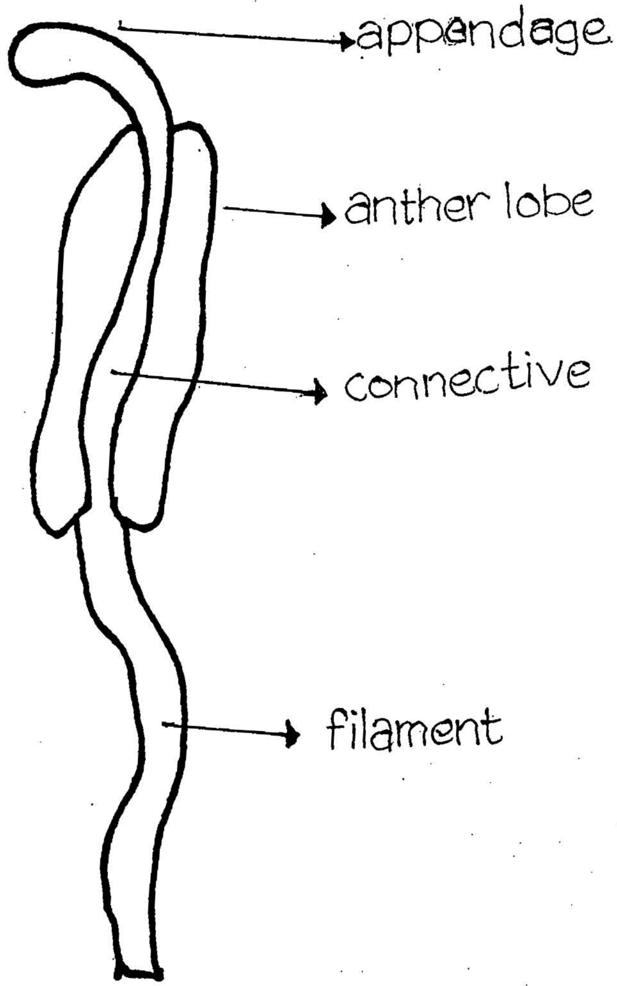


Fig.3 Stamen of sacred lotus

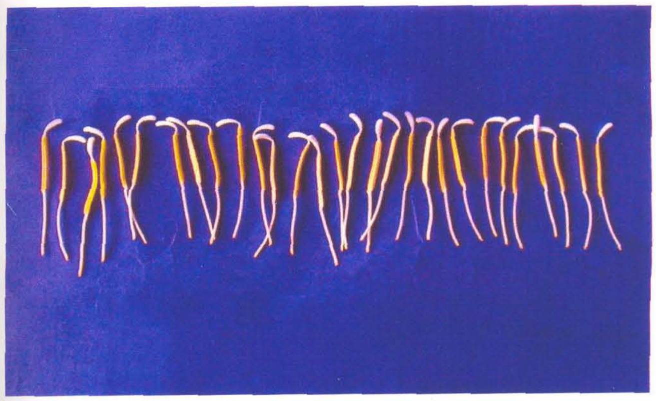


Plate 12 Androecium of sacred lotus

Plate 13 Carpel of sacred lotus

The characters of gynoecium in different ecotypes of lotus are presented in Table 25.

Table 25 Gynoecium characters of six different ecotypes

Ecotypes	No. of carpels/ receptacle (cm)	Diameter of receptacle (cm)	
		Just after anthesis	At maturity
Nagarkovil	13	2.62	5.10
Bramangalam	12	1.66	4.32
Chemmanda	7	1.20	4.00
Nelliyampathy	7	1.80	3.70
Chittoor	12	1.26	3.90
Chandiroor	13	1.58	4.10
CD (0.05)	2.3	0.48	0.98
CV (%)	14.5	12.58	12.50

Gynoecium apocarpous with several uniovulate carpels placed separately in deep cavities in the receptacular tissues (Plate 13). The carpels ranged from seven to thirteen per flower depending upon the ecotype. The stigma projects slightly above the receptacle. It is round in outline and with a depression in the middle. The size of the receptacle also varied with the ecotype. Nagarkovil was having the largest receptacle with a diameter of

5.10 cm at maturity. In all the ecotypes, a two to three fold increase in diameter of the receptacle was observed after anthesis (Table 25).

4.3.4. Anther dehiscence and stigma receptivity

The process of anthesis in sacred lotus was observed to be completed in stages, which lasted for three days in all the ecotypes.

The flower opening started at 10-15 days after the appearance of bud. On the first day of flower opening, the floral whorls just loosened, keeping the flowers in half open condition. This loosening of whorls occurs by a sudden jerting movement of the petals which took place between 8.00 am to 8.30 am .The flower remained in that condition upto 10.30 am to 11.00 am and closed again (Plate 14).

On the next day, it opened fully and the opening started by 5.30 am and was completed by 7.00 am. (Plate 15).The anther dehiscence was found to occur between 7.15 am and 7.30 am on the second day of flower opening in all the ecotypes. The anther dehisce through by longitudal slits, starting from the inner most whorl (Plate 15). The pollen grains remained viable only for 30 to 35 minutes.

The details of stigma receptivity in different ecotypes are presented in Table 26.

Plate 14   Flower on the first day of anthesis

Plate 15   Flower on the second day of anthesis

Table 26 Stigma receptivity in six different ecotypes

Ecotypes	Time of start of receptivity	Total duration of receptivity
Nagarkovil	32 hours before flower opening	83 hours
Bramangalam	32 hours before flower opening	82 hours
Chemmanda	32 hours before flower opening	82 hours
Nelliyampathy	32 hours before flower opening	83 hours
Chittoor	32 hours before flower opening	82 hours
Chandiroor	32 hours before flower opening	82 hours

The stigma was found to be receptive 32 hours before flower opening and the receptivity was retained until third day of flower opening upto 11 am. The peripheral lobes became receptive first. A honeydew like secretion was found on the stigmatic surface during the receptive period (Plate 16). The loss of receptivity could be identified by the blackening of stigmatic surface (Plate 17). The loss of receptivity also proceeded from the periphery to the center.

Withering of the floral parts started on the third day of flower opening (Plate 18).

Wide range of insects, mainly beetles and bees were found visiting the flowers on the first and second day of flower opening (Plate 19). The flowers

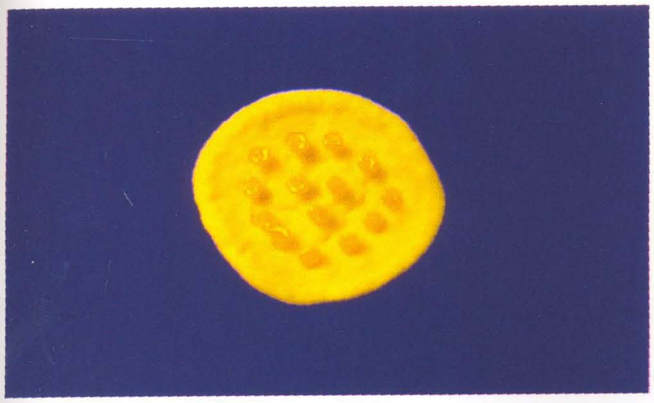


Plate 16 Receptive stigma

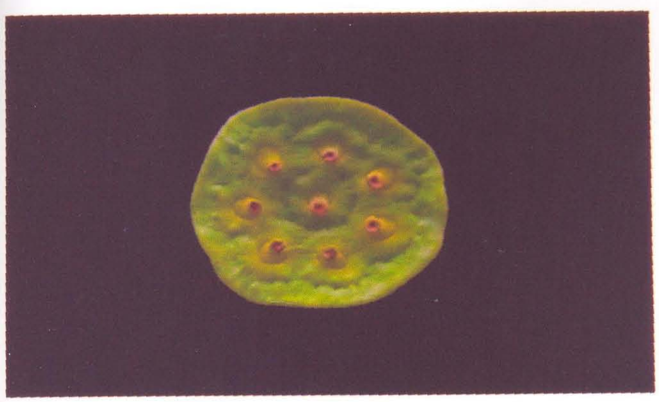


Plate 17 Loss of receptivity

Plate 18 Flower on third day of anthesis

can be considered as cantharophillous. Ke *et al.* (1987) also reported cantharophillous nature of the flower.

4.3.5. Thermogenesis in flowers

The thermogenesity in fully matured buds of the six different ecotypes recorded (Plate 20) at six hourly intervals starting two days prior to flower opening depicted in the Table 27 and Fig.4.

From the Table 27, it is clear that receptacular temperature of the flower is maintained between 30 to 35°C despite the changes in environmental temperature between 27 to 34°C. However Schneider and Buchanan (1980) reported 5 to 10°C higher temperature in *Nelumbo lutea* flowers in fully matured flower bud before anthesis. The temperature regulation is apparent at two previous nights of flower opening and disappeared after the opening of the flower. The thermo regulation begins in the bud when petals are tightly closed and continues throughout the day until the petals open slightly to reveal the stigma. The thermo regulation was found to correspond with the receptivity of stigma. This may be a reward to insect pollinators. Seymour and Schultze Motel (1998) has considered this as a floral adaptation for cross pollination.

4.3.6. Palynology

The morphology, size and fertility of the pollen grains of six ecotypes under evaluation are presented in Table 28 and Plate 21.

Table 27 Thermogenesis in sacred lotus flowers

Ecotypes	Temperature °C										
	Two days before anthesis				A day before anthesis				On the day of anthesis		
	6 AM	12 PM	6 PM	12 AM	6 AM	12 PM	6 PM	12 AM	6 AM	12 PM	6 PM
V_1	29.5	34.2	30.5	32.0	32.0	33.0	32.0	33.8	33.8	34.0	29.1
V_2	29.5	34.5	30.0	32.0	31.8	33.5	31.4	33.8	33.8	33.8	29.5
V_3	29.5	34.3	30.5	31.5	32.0	34.2	32.0	34.0	33.6	33.6	29.8
V_4	29.8	34.0	30.5	32.0	32.8	33.5	31.8	33.6	33.1	33.1	29.8
V_5	29.6	34.1	31.8	31.8	32.4	34.0	32.0	34.0	33.2	33.2	29.9
V_6	29.5	34.0	30.0	32.0	33.0	34.0	32.0	34.0	33.0	33.0	30.0
Outside temperature	29.0	33.0	28.0	27.0	29.0	33.0	29.0	27.0	28.0	33.5	29.0

Plate 19 Insect visiting

Plate 20 Thermogenesity

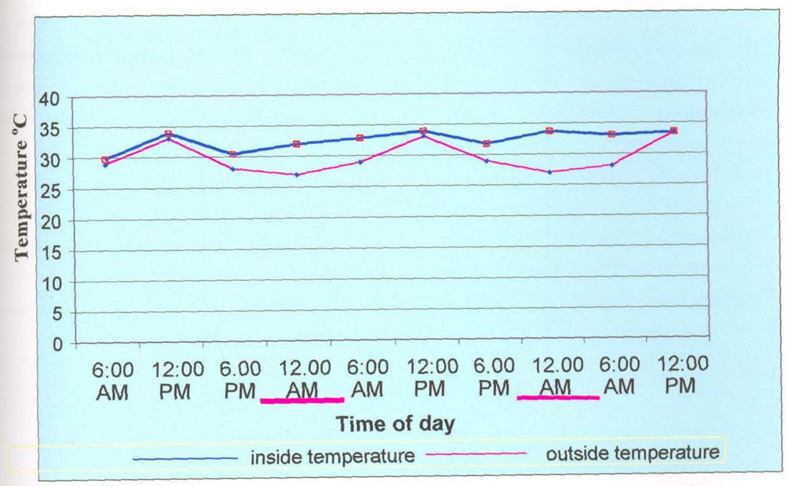


Fig. 4. Thermogenesity

Table 28 Pollen characters of six different ecotypes

Ecotypes	Type	Colour as appeared to naked eye	Mean fertility (%)	Mean size (μm) x 100	Pollen unit	Nuclear condition
Nagarkovil	Round, triporate	Yellow	95	62.4	Monad	Uninucliate
Bramangalam	Round, triporate	Yellow	95	63.2	Monad	Uninucliate
Chemmanda	Round, triporate	Yellow	96	63.4	Monad	Uninucliate
Nelliyampathy	Round, triporate	Yellow	96	62.8	Monad	Uninucliate
Chittoor	Round, triporate	Yellow	95	62.6	Monad	Uninucliate
Chandiroor	Round, triporate	Yellow	96	62.4	Monad	Uninucliate
CD (0.05)	-	-	NS	NS	-	-
CV (%)	-	-	9.8	15.6	-	-

Irrespective of ecotype, the pollen grains were found to be round, triporate and yellow in colour with reticulate sculpturing on the exine. Very high pollen fertility (95 to 96per cent) was observed in different ecotypes. The ecotypes did not differ significantly in the size of pollen grains. The mean size ranged from 62.4 μm to 63.4 μm. Borsh and Barthlott (1996) also reported the reticulate sculpturing on the exine.

4.3.7. Pollination biology

The extend of fruit set in protected buds, unprotected buds and emasculated but unprotected buds are furnished in Table 29 and Plate 22.

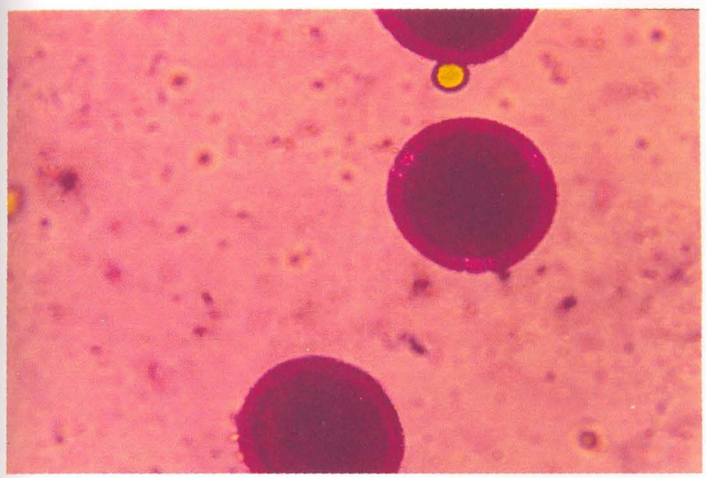


Plate 21 Pollen grains (×1000)

Table 29 Extend of fruit set in different ecotypes under different treatment

Ecotypes	Mean fruit set (%)		
	Protected	Unprotected	Emasculated but unprotected
Nagarkovil	0	44.40	46.20
Bramangalam	0	36.10	36.50
Chemmanda	0	88.80	87.40
Nelliyampathy	0	38.20	36.80
Chittoor	0	41.66	42.00
Chandiroor	0	50.67	49.60

From the Table 29, it can be seen that the extend of fruit set varied in protected and unprotected buds. No seed set was observed in protected buds. However, in the case of unprotected buds as well as emasculated but unprotected buds, high fruit set was recorded. From this, it can be concluded that sacred lotus is adapted to cross pollination and no self pollination takes place even though the flowers are bisexual and fertile. The protogynous nature of the flowers further supports cross pollination. Some self incompatibility mechanism may also be working in the flower which needs further detailed investigation.

4.3.8. Seed physiology

The observations on seed volume, 100 seed weight, seed density, moisture content, mean length and diameter of the seed, shape of the seed and

Plate 22 Protected bud

Plate 23 Seeds of sacred lotus

time taken for maturity of the seed in six different ecotypes are presented in Table 30 and Plate 23 and 24.

Table 30 Seed characters of six different ecotypes

Ecotype	Volume (ml)	100 seed weight (g)	Density	Moisture content (%)	Mean length (cm)	Mean diameter (cm)	Time taken for maturity (days)
Nagarkovil	0.86	76.25	0.87	4.32	1.88	1.16	30
Bramangalam	0.88	83.80	0.96	13.86	1.80	1.20	30
Chemmanda	0.88	127.75	1.44	35.91	2.50	1.40	30
Nelliyampathy	0.92	129.00	1.41	32.80	2.90	1.56	30
Chittoor	0.72	75.90	1.05	4.11	1.80	1.35	30
Chandiroor	0.88	93.00	1.05	7.46	1.82	1.20	30
CD (0.05)	NS	21.30	0.198	-	0.41	NS	-
CV (%)	22.80	28.20	29.10	-	18.90	23.80	-

As evident from the above Table mean seed volume and mean fruit diameter did not show any significant difference among the ecotypes. However, the ecotypes differed significantly in 100 seed weight and moisture content.The ecotypes Chemmanda and Nelliyampathy registered the highest values for 100 seed weight and moisture content. The high moisture content of the seeds may be responsible for the high 100 seed weight registered by these ecotypes.

The carpels mature into egg shaped fruits. Irrespective of ecotype the time taken for maturity of the fruit was found to be 30 days (Plate 24). Each fruit is single seeded with two cotyledons and a chlorophyllous embryo. Fruit wall and seed coat are fused. Hence fruit as such is taken as the 'seed'.

The seeds are placed in cavities in the receptacle. The cavity was observed to be narrow at receptacular surface at the time of initiation. Stigmas projected slightly above the receptacle area. In fully matured stage of carpel, the opening of the cavity widened and seeds were shed into water by slight drooping of the flower stalk (Plate 25).

The nutrient status of the fully matured seeds are presented in Table 31 and Fig.5.

Table 31 Nutrient compositions of seeds of different ecotypes

Ecotypes	Crude protein (%)	Crude fat (%)	Crude fiber (%)	Total ash (%)	Acid soluble ash (%)	Phenol content mg/g
Nagarkovil	19.6	7.2	4.2	4.6	0.15	3.5 – 4
Bramangalam	19.2	7.3	4.3	4.5	0.15	3.5 – 4
Chemmanda	19.8	7.2	4.2	4.2	0.14	3.5 – 4
Nelliyampathy	19.6	7.2	4.3	4.5	0.15	3.5 – 4
Chittoor	18.9	7.4	4.2	4.6	0.14	3.5 – 4
Chandiroor	18.8	7.2	4.2	4.2	0.15	3.5 – 4

Plate 24 Different stages of seed development

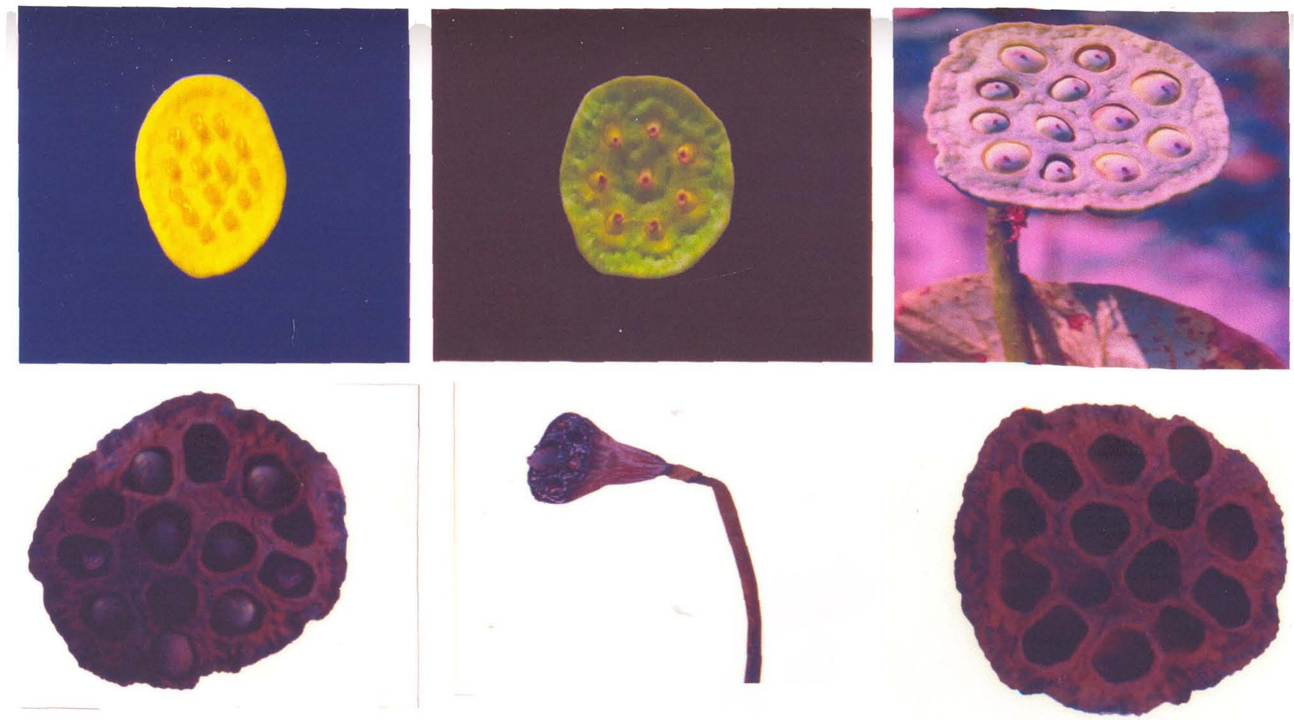


Plate 25 Different stages of development of carpel

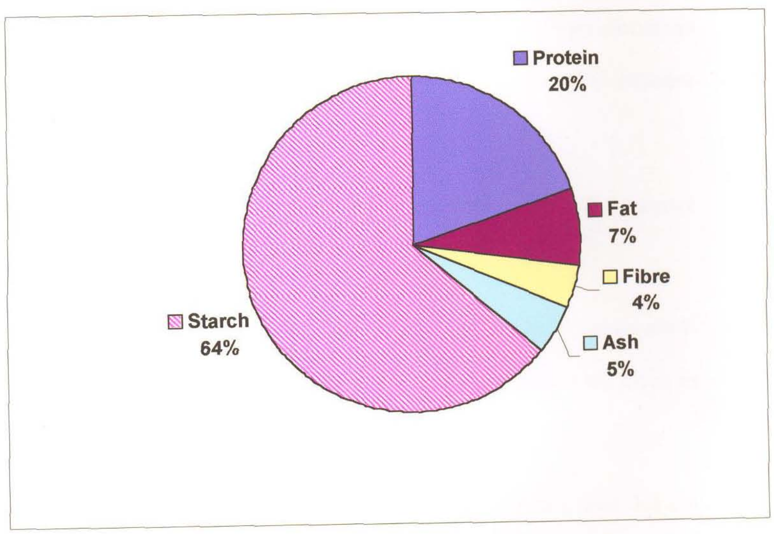


Fig. 5 Nutrient status of lotus seeds

The ecotypes did not differ in the content of starch, crude protein, fat, fibre and ash. The protein content of the seeds ranged from 18.8 per cent to 19.6 per cent. It can be seen that in addition to starch, seeds contain a substantial amount of protein also.

The total phenol content of the seed estimated by Folin-ciocalteau method revealed that it's content ranged from 3.5-4 mg/g of seed in different ecotypes of lotus.

Lotus seeds are considered to have the longest period of dormancy (Priestly and Posthumus, 1982).

The results of the germination test carried out with fully mature seeds after giving different pre-treatments along with untreated control are given in Table 32 .

From the Table 32, it can seen that pre-washing intact seed did not improve germinability. However, dipping them in cowdung slurry for varying periods recorded slight improvement in germination. Mechanical scarification followed by leaching for varying periods, 12 hours, 24 hours and 48 hours were found to be equally effective in improving germinability. Hence it is clear that fruit wall is playing a significant role in delaying germination by acting as a barrier. Since leaching improves germinability, it can be concluded that some water soluble factors present in the seeds are contributing to seed dormancy. High content of total phenol also contribute to dormancy.

The excised embryo was giving high germinability indicating that embryo as such is non-dormant and factors contributing to dormancy are residing in some part of the seed other than the embryo (Table 32 and Plate 26). Presannakumari *et al.*(2000) has reported that embryo of lotus seeds is nondormant.

Table 32 Effect of different pre-treatments on germinability

Sl. No.	Pre-treatment	Germination (%)
1	Pre-washing intact fruit in water for 12 hours	0
2	Pre-washing intact fruit in water for 24 hrs.	0
3	Pre-washing intact fruit in water for 48 hrs.	0
4	Dipping the fruit in cowdung slurry for 12 hrs followed by washing in water	45
5	Dipping the fruit in cowdung slurry for 24 hrs followed by washing in water	68
6	Dipping the fruit in cowdung slurry for 48 hrs followed by washing in water	62
7	Mechanical scarification followed by leaching in water for 12 hrs.	92
8	Mechanical scarification followed by leaching in water for 24 hrs.	91
9	Mechanical scarification followed by leaching in water for 48 hrs.	92
10	Excised intact embryo	80
11	Intact seed	0

Plate 26 Germination of excised embryo

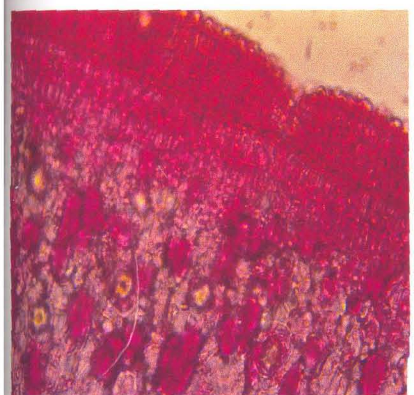
The anatomical changes that takes place in the seed during the course of development are given in Table 33 and Plate 27.

Table 33 Fruit wall thickening at varying days after fertilization

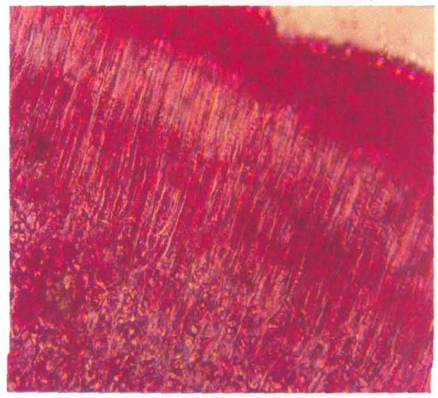
Part of fruit wall	Thickness mm (x 400)					
	5th day	10th day	15th day	20th day	25th day	30th day
Epicarp	0.051	0.058	0.058	0.062	0.064	0.064
Mesocarp	0.063	0.128	0.128	0.132	0.188	0.188

The anatomical studies of the seed revealed the presence of thick waxy layer on the surface of fruit wall. A progressive thickening of the mesocarp of the fruit during the developmental process was observed. The results also revealed that (Table 33) a three fold increase in the thickness of mesocarp in the fully matured seed of 30 days development was observed. The mesocarp region of a fully mature seed was represented by double layered compactly arranged macroscleriеds which served as an impermeable layer (Plate 27). The thick waxy coating of the seed coupled with the compactly arranged macroscleried layer, acting as mechanical barrier contributes to dormancy of lotus seed.

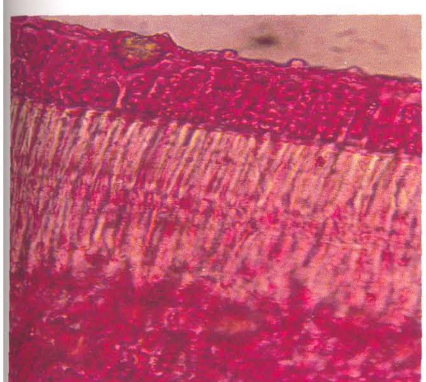
The dormancy in lotus seeds can be attributed to the presence of



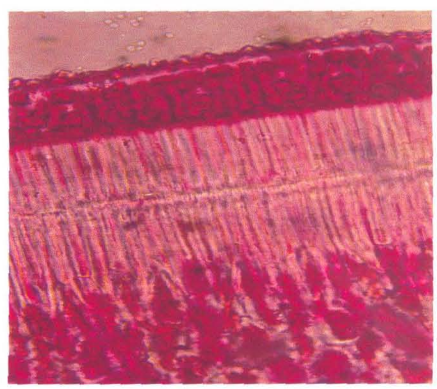
Five days after fertilization



Fifteen days after fertilization



Twenty days after fertilization



Thirty days after fertilization

Plate 27 Anatomical changes during the course of seed development (x400) water soluble inhibitors coupled with thick waxy coating on the surface of the fruit and compactly arranged macroscleried layer (Fig.6).

The germination behaviour of the seeds was observed to be quite different from normal dicots and the results are depicted in Table 34 and Plate 28.

Table 34 Germination behaviour of sacred lotus

Plumule emergence	5 – 7 days
Formation of second leaf	10 – 12 days
Expansion of rolled first and second leaf	18 – 20 days
Root development	20 – 22 days
Type of root	Adventitious

Unlike other plants, plumule emerged first. The radicle was aborted. The adventitious roots are found anchoring the plant in mud. Adventitious roots developed from the nodes of first and second leaves by 20 to 22 days after sowing, that is after the unrolling of lamina of these leaves.

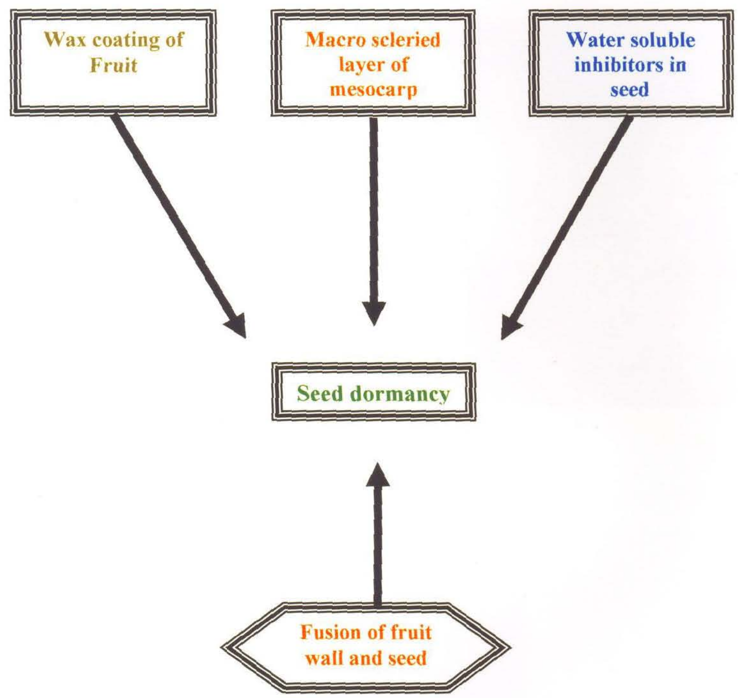


Fig. 6 Factors of seed dormancy in lotus

Plate 28 Germination behaviour of sacred lotus

5. SUMMARY

An investigation entitled "Morphogenesis and reproductive biology of sacred lotus (*Nelumbo nucifera* Gaertn) was carried out in the Department of Plant Breeding and Genetics, College of Horticulture, Vellanikkara during the period from December 1999 to March 2003.

Six different ecotypes collected from diverse ecological conditions namely Nagarkovil, Bramangalam, Chemmanda, Nelliyampathy, Chittoor and Chandiroor were evaluated under *exsitu* conditions. The salient findings of the study are summersised below:

- Significant variability was observed among the ecotypes for various leaf characters like petiole length, longevity of leaves and size of leaves.

- Seasonal effect on different leaf characters was found to be significant. Longevity of the leaves and days taken for the leaves to open fully were the highest in spring season followed by rainy season and the least in summer. However, the petiole length and size of leaf as represented by length and breadth of lamina were the highest in rainy season followed by spring.

- Significant variability was observed in various floral characters among the ecotypes.

- Flower production started with the on set of Monsoon and reached the peak in spring and then declined with no production of flower in summer months.
- Flowers of lotus were found to be solitary, ebracteate, antinomorphic and complete with various floral whorls in spiral fashion on the floral axis.
- The stigma receptivity started 32 hours before flower opening and receptivity was retained for 52 hours, even after flower opening.
- Pollen grains dehisced only after the opening of flower.
- The flowers are protogynous and the process of flower opening was completed in stages lasting for three days.
- Thermogenesity was observed in fully mature flower buds of lotus. The thermo regulation was found to correspond with stigma receptivity.
- The lotus flowers were observed to be cantharophillous
- Pollen grains were found to be fertile, triporate and with reticulate sculpturing.
- Lotus flowers are adapted to cross pollination
- The dormancy in lotus seeds can be attributed to thick waxy coating on the fruit wall, presence of water soluble inhibitors, presence of double layered macrosclereids in the mesocarp of fruit.

- The embryo as such is non-dormant and mechanical scarification followed by leaching improves germinability.
- Lotus though dicot ,germination behaviour is like that of monocot ie., plumule emerges first and radicle is aborted.

REFERENCES

Barthlott, W., Neinhuis, C., Jetter, R., Bouranel, T. and Riederer, M. 1996. *Nelumbo* – Relation with Ranunculiflorae. *Flora* **191**: 169-174

Borsch, T. and Barthlott, W. 1996. Classification and distribution of the genus *Nelumbo*. *Beitr. Biol. Pflanzen.* **64**: 421-450

Comi, C. 1939. Ricerche Sulla appendice elavata nell anthera del *Nelumbo nucifera*. *Nuova G.Bot.Ital.* **46**: 600-610

Erdtman, G. 1952. *Pollen morphology and Plant Taxonomy. Angiosperms.* Almqvist and Wiksell, Stockholm, 432 p.

Erdtman, G. 1960. The acetolysis method. A revised description. *Svensk. Bot. Tidsk.* **54**(4): 561-564

Garton, S. 2000. Hybridizing lotuses. *Accent aquat.* **55**(4): 74-77

Gibernae, M. and Barabe, D. 2000. Thermogenesis in three *Philodendron* species of French Guiana. *Can. J. Bot.* **78**: 685-689

Gottsberger, G.1992. Reproductive biology of angiosperms. *Stapfia*. **28**:11-27

Gupta,S.C. and Ahuja, R. 1967. Is *Nelumbo* a monocot? *Naturwissenschaften.* **54**: 498

Hayes, V., Schneider, E.L. and Carlquist, S. 2000. Floral development of *Nelumbo nucifera* (Nelumbonaceae) (eds. Endress, P.K., Friis, E.M., Qiu-Yin Long and Zimmer, E.A.). *Int. J. Plant Sci.* **161**(6): 183-191

Ito, M. 1986. Studies on the floral morphology and anatomy of *Nelumbo nucifera* Gaertn. *Acta Phytotax. Geobot.* **37**: 82-96

Kasumi, M. and Sakuma, F. 1998. Flowering, pollination, fertilization and seed formation in lotus rhizome plant. *J. Jap. Soc. Hort. Sci.* **67**(4): 594-599

Katori, M., Nomura, K. and Voneda, K. 2002. Propagation of flowering lotus (*Nelumbo nucifera* Gaertn) by rhizome straps, without enlarged rhizomes. *Jap. J. trop. Agric.* **41**(3): 195-197

Ke, X., Zhang, W., Zhang, H., Xu, D. and Jinag, Z. 1987. Experiments in the use of honey bees for the pollination of lotus seed crops. *J. fujian agric. coll.* **16**(2): 169-171

Khanna, P. 1965. Morphology and embryological studies in Nymphaeaceae.II *Brasenia schreberi* Gmel. and *Nelumbo nucifera* Gaertn. *Aust. J. Bot.* **13**: 379-387

Kreunen, S.S. and Osborn, J.M. 1999. Pollen and anther development in *Nelumbo* (Nelumbonaceae). *Am. J. Bot.* **86**(12): 1662-1667

Li, H.L. 1995. Classification of phylogeny of Nymphaeaceae and allied families. *Am. Midl. Nat.* **45**: 33-41

Malick, C.P. and Singh, M.B. 1980. *Plant Enzymology and Histo-Enzymology*. Kalyani Publishers, Ludhiana, 431 p.

Moore, P.D. and Webb, J.A. 1978. *All Illustrated Guide to Pollen Analysis*. Hodder and Stoughton, London, 160 p.

NBRI, 1996. Annual Report. National Botanical Research Institute, Lucknow, 86 p.

Pearl, R. 1906. Variation in the number of seeds of lotus. *Am. nat.* **40**: 757-768

Prasad, M.K. and Krishnaprasad, M. 1970. *Outlines of Microtechniques*. Emkay Publishers, New Delhi, 203 p.

Presannakumari, K.T., Suma, V.A. and Girija, T. 2000. Investigation on seed dormancy in sacred lotus. Proceedings of National Seminar in Plant Biology. Central Plantation Crop Research Institute, Kasargod, p.77

Presannakumari, K.T., Suma, V.A. and Minimol, J.S. 2002. Evaluation of morphoanatomical variations in sacred lotus. Proceedings of XII Swadeshi Science Congress, Central Tubercrop Research Institute, Thiruvananthapuram, pp.233-235

Priestely, D.A. and Prosthumus, M.A. 1982. Extreme longevity of lotus seeds from Pulantien. *Nature*. **299**(5879): 148-149

Radford, A.E., Dickison, W.C., Massey, J.R. and Bell, C.R. 1974. *Vascular Plant Systematics*. Happer and Rao Publishers, London, 466 p.

Schneider, E.L. and Buchanan, J.D. 1980. Morphological studies of the Nymphaceae.II. The floral biology of *Nelumbo pentapetala*. *Am. J. Bot.* **67**: 182-193

Seymour, R.S. 1999. Pattern of respiration by intact infloresence of the thermogenic arum lilly (*Philodendron selloum*). *J. Exp. Bot.* **50**(335) : 845-852

Seymour, R.S. and Blaylock, A.J. 1999. Switching of the heater: Influenze of ambient temperature on thermoregulation by eastern Skunk cabbage (*Symplocarpus foetidus*). *J. Exp. Bot.* **50**(338): 1525-1532

Seymour, R.S. and Schultze Motel, P. 1998. Physiological temperature regulation by flowers of sacred lotus. *Biol. Sci.* **353**: 935-943

Seymour, R.S., Schultze Motel, P. and Lamprecht, I. 1998. Heat production by sacred lotus depends on ambient temperature not light cycle. *J. Exp. Bot.* **49**(324): 1213-1217

Shomer, S.H. and Sefton, D.F. 1978. The reproductive biology of *Nelumbo pentapetala* (Nelumbonaceae) on the Upper Mississippi river.II.. The insect associated with the transfer of pollen. *Brittonia*. **30**(3): 355-364

Simon, J.P. 1970. Comparative serology of the order Nymphaeales.I.. Preliminary survey on the relationship of *Nelumbo. Aliso.* **7**: 243-261

Skubatz, H., Williamson, P.S. and Schneider, E.L. 1992. Cyanide insensitive respiration in thermogenic flowers of *Nelumbo* and *Victoria*. *J. Exp. Bot.* **41**: 1335-1339

1724 3 6

Van Cotthem, W.R. 1970. A classification of stomatal types. *Bot. J. Linnean Soc.* **63**: 235-246

Watson, L. and Dallwitz, M.J. 2000. The families of flowering plants: Description, Illustration, Identification and Information retrieval. Version: 14th December, 2000, 322 p.

Welsh, S.L. 1998. *Flora societensis: A summary revision of the flowering plants of the society islands*. E.P.S. Inc. Orem. Utah, 205 p.

Wigand, A. and Dennert, E. 1988. *Nelumbium speciosum* W. Eine monographische studie. *Biblioth. Bot.* **11**: 1-68

Zou-Baoyu, Jiang-Guizhen and Yu-Yanli 1991. Ultrastructure development of chloroplast in sacred lotus embryo bud under inersible light. *Acta Bot. Sinica.* **33**(3): 169-175

Zou-Baoyu, Li-Guoging and Tang-Chonggin. 1992. Changes of thylakoid membrane stocks and a/b ratio of chloroplast from sacred lotus (*Nelumbo nucifera*) seeds during their germination under light. *Acta Bot. Sinica.* **34**: 645-650

MORPHOGENESIS AND REPRODUCTIVE BIOLOGY OF SACRED LOTUS
(*Nelumbo nucifera* Gaertn.)

By

MINIMOL, J. S.

ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the requirement for the degree of

Doctor of Philosophy in Agriculture

Faculty of Agriculture
Kerala Agricultural University

Department of Plant Breeding and Genetics
COLLEGE OF HORTICULTURE
VELLANIKKARA, THRISSUR - 680656
KERALA, INDIA
2004

Annexure I

Lotus parts and their uses

Part		Use
Rhizomes	-	Vegetable called Kamalkakkadi Thamaravalayam (Kondattoms) Source of starch – Medicinal purpose
Leaves	-	as plates
Flowers	-	Ornamental purpose as cut flowers in temples and for religious purpose
Stamens	-	Medicinal purpose
Seeds	-	Medicinal purpose In dishes after cooking

Annexure II

Cost of lotus parts/products in the market

Part		Cost
Leaf (bundles of 100)	-	Rs.7 to Rs.10
Fresh rhizomes	-	Rs.20 / kg.
Thamaravalayam	-	Rs.200 / kg.
Starch	-	Rs.30 / kg.
Thamarayalli	-	Rs.350 / kg.
Flowers	-	Rs.3 to 7 / flower
Seed	-	75 paise / seed

Annexure III

Ayurvedic products with lotus parts as an ingredient

Product		Parts
Drakshadikashayam	-	Rhizome Stamens
Manjishtadithylam	-	Rhizome
Aravindasavam	-	Flowers
Triphaladithylam	-	Rhizome
Saraseejamakarandadi choornam	-	Stamens
Sethubandham	-	Seeds
Ayushgutty	-	Seeds

ABSTRACT

Sacred lotus (*Nelumbo nucifera*) belonging to Nelumbonaceae is the only genera in that family. This legendary flower has very long and close association with history, culture, religion, literature and arts. Hence it is chosen as our national flower. Despite its immense potentialities as medicinal, ornamental and vegetable crop, this plant has received only very little attention of crop improvement workers. It was in this background the present investigation entitled 'Morphogenesis and Reproductive Biology of Sacred Lotus' was under taken with the objectives of evaluating the growth and development pattern of leaf, flower and seed and elucidating the reproductive biology.

Six different genotypes collected from diverse ecological conditions were evaluated under *ex situ* conditions during December 1999 to March 2003 at College of Horticulture, Vellanikkara.

Variability was observed in different biometric characters like size of lamina, longevity of leaves, petiole length etc. among the ecotypes evaluated. Study of the seasonal effect on these characters revealed that rainy season favoured growth in size of leaves and spring season favoured longevity. The growing tip of the rhizome was found to be the best propagule with respect to rhizome yield.

Significant variability was observed in various floral characters among the ecotypes. Flower production started with the onset of Monsoon and reached the peak in Spring season and then declined with no flower production at all in Summer season.

Flowers were found to be solitary, ebracteate, actinomorphic and complete with the different whorls arranged in a spiral fashion on the floral axis. Stigma became receptive 32 hours before flower opening and the receptivity was retained for 52 hours even after flower opening. Pollen dehiscence occurred only after complete opening of the flower bud. Pollen grains of lotus were found to be fertile, round and triporate. However, no seed set was obtained in protected buds indicating cross pollination. The temperature inside the flower bud remained between 30 to 35°C till the fourth day during the period of anthesis despite the changes in environmental temperature between 27 to 33°C. The period of this thermoregulation corresponded to receptivity of stigma. This is considered as a floral adaptation favouring cross pollination (Seymour and Schultze-Motel, 1998).

Unlike other dicot, in sacred lotus, plumule emerged first and radicle was aborted. Adventitious roots were found anchoring the plant in mud.

The seeds matured in 30 days after fertlization. Lotus seeds are reported to have the longest period of dormancy. The germination trials conducted after giving different pretreatments revealed that embryo as such is nondormant. Mechanical scarification followed by leaching improved germinability indicating that hard fruit wall along with thick waxy coating and water soluble inhibitors are responsible for dormancy.