Characterization and hybridization of *Nymphaea* spp.

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MANJU A

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DECLARATION

I hereby declare that this thesis entitled "Characterization and hybridization of *Nymphaea* spp." is a bonafide record of research done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis, entitled "Characterization and hybridization of *Nymphaea* spp." is a record of research work done independently by Ms. Manju A. (2016-11-079) 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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EXTERNAL EXAMINER

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LIST OF ABBREVIATIONS AND SYMBOLS USED

%	Per cent
ANOVA	Analysis of Variance
C.V	Coefficient of Variation
CD	Critical difference
Cm	Centimetre
et al.	Co- workers/ Co-authors
g	Gram
GA_3	Gibberellic acid
GCV	Genotypic coefficient of variation
GG	Genetic gain
H^2	Heritability
i.e.	that is
IAA	Indole acetic acid
KAU	Kerala Agricultural University
MS	Murashige and Skoog
PCR-RFLP	Polymeric chain reaction-Random fragment length polymorphism
PCV	Phenotypic coefficient of variation
TDZ	Thidiazuron
UPOV	The International Union for the Protection of New Varieties of Plants
viz.,	Namely

Introduction

1. INTRODUCTION

The genus *Nymphaea* L. coming under the family Nymphaeaceae, commonly known as waterlilies is one of the few fascinating groups of aquatic plants recently being delighted by the plant growers in India and cultivated in gardens and nurseries for its much attractive flower colours. Nymphaeaceae is a primitive group and considered as a missing link in the evolution of flowering plants. Waterlily is the national flower of Sri Lanka and Bangladesh. In Malayalam they are famous in the name 'Ambal'. The genus *Nymphaea* consist of about 50 species found in tropical and temperate climates of both hemispheres. It has been long valued as a garden flower to decorate ponds and gardens. The tuberous rootstock and stem are submerged in the water and only the leaves are seen floating on the water surface punctuated by the fairly large and perfectly built red, pink, blue, yellow or white flowers. There are tropical and hard waterlilies, in which tropical types are scented and include several varieties that flower at night.

Waterlilies have been known for their economic importance and aesthetic value since ancient times. They form an important component of wetland ecosystem which is one of the most threatened ecosystems. They not only serve as a good habitat for aquatic organisms by nourishing, protecting and keeping water cool but also perform a very important function of filtering and detoxification of water by absorbing heavy metals (Shuaibu and Nasiru, 2011). They are important to ensure the flow of energy throughout the ecosystem. Waterlily flowers are most commonly used for the traditional and cultural festivals. Their starch rich rhizome, root, fruit, leaf, petiole, flower, tuber, seeds etc. are edible parts. Many species of *Nymphaea* are reported in Indian Ayurveda and Siddha systems of medicine as chief ingredient for the treatment of diabetics, lung and stomach disorders, and even for infertility.

Water lily can be propagated in three ways; asexually by splitting the rhizome and at the center of mature leaves (some of the tropical varieties), and sexually by seed. The propagation by seed is difficult due to dormancy. Besides their morphological similarity blurs the boundaries between species and high plasticity adds to ambiguity in identification. Ansari and Jeeja (2009) while examining quite large number of specimens kept in the Indian herbaria noted that many species exhibit a lot of intraspecific morphological variations even within the collections of same locality.

Even though water lily has tremendous potentialities in various fields, they got only little consideration in crop improvement. Most of the researches have been concentrated on the diversity and phylogeny studies both at morphological as well as molecular level as evidenced by the literature available. In order to develop water lily as a commercial crop, it is important to study reproductive biology and propagation methods of the crop. In Kerala Agricultural University two studies were taken up in waterlily. Fahida (2012) conducted a study on the reproductive biology of two flower colour variants of *N. nouchali* and Tom (2015) did a comparative study on day and night blooming types of *Nymphaea* sp., collected from Thrissur district. The knowledge of reproductive and seed biology in most of the species reported from India is limited.

In this background the present study was undertaken to explore and collect *Nymphaea* species from Northern parts of Kerala and to investigate the reproductive biology and hybridization in few selected types which will form a basic document for further crop improvement work. The present study was undertaken with the following objectives:

- To study the morphology, reproductive biology, seed biology and methods of propagation of selected species of *Nymphaea.*
- Hybridize the seed producing types to develop different colour variants.

Review of literature

2. REVIEW OF LITERATURE

2.1 TAXONAMIC CLASSIFICATION AND DISTRIBUTION

Waterlily is one of the fascinating groups of aquatic plant which produces beautiful and spectacular flowers with different colours and fragrance. It comes under the family Nymphaeaceae consisting of eight different genera and it has more than 50 species distributed in temperate and tropical regions and absent in Antarctica (Woods *et al.*, 2005).

One of the earliest classifications of genus *Nymphaea* has been done by Conard (1905). He subdivided *Nymphaea* into five subgenera: Anecphya (7-10 sp.), Brachyceras (14-16 sp.), Hydrocallis (14 sp.), Lotos (2-3 sp.) and Nymphaea (8 sp.). In these Nymphaea subgenus was again divided into three sections as Chamaenymphaea, Nymphaea and Xanthantha.

According to Van-Royen (1962) the genus *Nymphaea* comprises about 40 species and numerous forms. Plants in the genus *Nymphaea* can be divided into two groups: Apocarpiae and Syncarpiae. The Apocarpiae group consists of three subgenera: Anecphya (an Australian tropical waterlily), Confluentes (an Australian tropical waterlily) and Brachyceras (day blooming tropical waterlily). The Syncarpiae group also consists of three subgenera: Hydrocallis (night blooming tropical waterlily), Lotos (night blooming tropical waterlily) and Nymphaea (hardy waterlily).

Rodriguez (2007) mentioned the tropical and hardy waterlilies. The tropical waterlilies were further divided into day and night blooming types while the hardy waterlilies are day bloomers. Huang *et al.* (2009) conducted a valid grouping in water lilies into two categories in accordance with the ecological characteristics: hardy water lilies and tropical water lilies. Hardy water lilies are further divided

into five types on the basis of their tuber characteristics: marliac, odorata, tuberosa, and finger types as well as the underground rhizome.

The Nymphaea subgenus distributed throughout the temperate regions of northern hemisphere, Lotos is restricted to the palaeotropic regions, Hydrocallis is Neotropical, Brachyceras has pantropical range and Anecphya is restricted to Australia and New Guinea (Conard, 1905). Among the *Nymphaea* genus, *N. tetragona* has the most widespread distribution, extending to sub-boreal and boreal regions of northern hemisphere in ponds or lakes starting from sea level to 4000 m altitude (Fu and Wiersema, 2001). Dkhar *et al.* (2013) mentioned the distribution *N. rubra, N. lotus and N. pubescence* were mostly in Asia.

Ten *Nymphaea* species are reported from India. Six of them are wild species (*N. alba, N. candida, N. nouchali, N. pubescens, N. rubra*, and *N. tetragona*) and four are cultivated (*N. alba* var. *rubra, N. caerulea, N. marliacea*, and *N. micrantha*). The species *N. nouchali, N. pubescens and N. rubra* are distributed throughout the plains of India whereas *N. alba, N. candida* and *N. tetragona* are confined to some particular areas. *N. alba* and *N. candida* are restricted to the state of Jammu and Kashmir (Mitra, 1990). Two additional species *N. mexicana* and *N. omrana* found in Kashmir and Kozhikode respectively were also reported from India, (Ansari *et al.*, 2005). In India *N. rubra, N. lotus and N. pubescence* were mainly distributed in wide spread areas of Odisha and west Bengal India (Devi *et al.* (2015).

2.2 EVOLUTIONARY STATUS

Polyploidy, structural chromosome aberrations and gene mutations have played a key role in the evolution of *Nymphaea* species. Chromosome number of the *Nymphaea* genus indicated a base number of x=14, with polyploidy evident in all subgenera. The Nymphaea subgenus has 2n= 56, 84, 112 (Gupta, 1978 and 1980).

Huang *et al.* (1997) reported that waterlilies are basal angiosperms which have importance in aquatic landscaping and also as a source of vegetable. The *Nymphaea* sp. considered as primitive in the evolutionary chain of angiosperm and hence form an important model for many angiosperm families according to Barkman *et al.* (2000). Nymphaeaceae is classified under the order Nymphaeales in the group of basal families in the recent studies of molecular-based angiosperm phylogeny (Judd *et al.*, 2002). The investigations of Friis *et al.* (2005) revealed that Nymphaceae is a primitive family and the fossil records suggest the existence of them in the Cretaceous period.

Chukiathman (2006) conveyed that the Nymphaceae family is the oldest group of plants on earth. In recent studies botanist have split this family into two groups. Nymphaeales (Cabombaceae, Nymphaeaceae and Hydatellaceae) is an ancient aquatic lineage that in most recent analyses is sister to all angiosperms except *Amborella trichopoda* in the family Amborellaceae (Moore *et al.*, 2007; Saarela *et al.*, 2007 and Soltis *et al.*, 2009). According to Maia *et al.* (2014) and Ruhfel *et al.* (2014). Nymphaeales are one of the most ancient angiosperm lineages, forming the sister group of all flowering plants.

2.3 ECONOMIC IMPORTANCE AND MEDICINAL USES

Waterlilies have importance in both aspects of ecology and economy. Besides adding beauty, the shade of leaf also helps to reduce the luxurious algal growth and provide shelter to aquatic organisms like fish (Brickell, 1989). They are important to ensure the flow of energy throughout the ecosystem. Its rhizome (rich in starch), root, fruit, leaf, petiole, flower, tubers, seeds etc. are used as edible parts (Raja *et al.*, 2010). It nourishes, protects, keeps water cool and serves as a good habitat for aquatic organisms. The most important function of waterlily is to filter and detoxify water by absorbing heavy metals (Shuaibu and Nasiru, 2011).

Many species in *Nymphaea* are important medicinal plant used in Ayurveda and Siddha systems of medicine for the treatment of stomach disorder, nerve disorder, inflammation, liver disorder, urinary infection, heart disease, menstrual problems, infertility, diabetes etc. and researches have proven that different parts of waterlily plants have hepato-protective, anti-inflammatory, and particularly antidiabetic activities. Nymphayol, a steroid isolated from waterlily flowers has been scientifically evidenced to be responsible for the antidiabetic activity and it reverses the damaged endocrine tissue and stimulates insulin secretion in the β-cells (Raja *et al.*, 2010).

Waterlily is the chief ingredient in Ashokarishta, Aravindasana, Chandanasava, Usirasava, Kanaka thaila, Chandanadi lauha, Thriphala ghita etc. (Raja *et al.*, 2010). Leaf and flower produce antioxidants which are used in the polyherbal formulation for anti-ageing and rejuvenation of cells. Ethanoic extract from the plant has antimicrobial activity (Biplab *et al.*, 2013).

2.4 LEAF MORPHOLOGY AND GROWTH PATTERN

The leaves of *N. tetragona* were about five inches long and elliptic-oval in shape (Hulten, 1968). Dalton and Novelo (1983) found that the petiole length varied according to the water depth in *N. odorata*. Wooten (1968) observed that plants with many floated leaves had longer petiole length with increasing water depth. The stomata of *Nymphaea* leaf helped to absorb nutrients and gas exchange, but they did not have any protective function against transpiration loss, as aquatic plants did not face desiccation. Dassanayake (1996) observed the leaves of *N. nouchali* as shiny green on the upper side and dusky purplish green on under surface. The petiole was terete and purplish green in colour.

The presence of thin cuticle on leaves helps to repel water from floating leaves as the stomata are arranged on the adaxial side (Gonzalez, 2002). According to Rossow and Charboneau (2006), the waterlily plants grow at the base of the pond and produce leaves and flower to the water surface. They have

a dense floating mat of leaf, avoiding light penetration for native water plants. Voeseneck *et al.* (2006) noticed the changes in juvenile leaf morphology with increased shade responses. Grob *et al.* (2006) explained that in *N. prolifera* the leaves were arising from the rooted tuber and their petiole reached up to 120 cm. Deviprasad (2009) reported the superiority of *N. rubra* over *N. alba* and *N. stellata* for leaf length and longevity.

Etnier and Villani (2007) observed that the leaves *of N. odorata* exhibited heterophylly where a single plant may have submerged, floating or aerial leaves. The growth of petiole above water level might be an adaptive response to shielding and allowing the aerial leaves to rise above the crowded water surface.

2.5 FLOWERING MORPHOLOGY

Waterlilies naturally have solitary-perfect flowers (Vandaveer, 2003). Krishnan *et al.* (2004) mentioned the role of the circadian rhythm in the growth of flower bud and flower opening in waterlily.

The intensity of blooming of *Nymphaea* depends on the temperature and air (Volkova *et al.*, 2001). Slocum (2005) found that some *Nymphaea* sp. do not flower in high-temperature regions. Astle (2006) reported that bud development and flowering of waterlily depends on the intensity of sunlight. Songpanich (2007) mentioned the two main factors which influence the growth and development of hard waterlily flower grown in tropics are light and temperature. He also observed that the maximum number of flowers produced in *Nymphaea gloriosa* was 163 blooms per year and minimum by Ferry's fire opal *Nymphaea* sp. was 28 blooms per annum.

Deviprasad (2009) studied the growth pattern of flower bud in three *Nymphaea* sp. namely *N. alba*, *N. rubra* and *N. stellate*. Early and maximum flowering was observed in *N. stellate* and also the complete opening of flower

from bud took minimum time. Length of the flower bud, size and longevity of flower were found to be maximum in *N. rubra.*

2.6 FLORAL BIOLOGY

Dalton and Novelo (1983) reported that the flowers of *N. odorata* were up to 6 inch wide. However, the flowers of *N. rubra* were large and 4-10 inches across (Biswas and Calder, 1984). Soltis and Soltis (2004) observed that the flower size is varying widely among the genera, ranging from small simple monocot to very large showy flowers.

Wiersema (1988) reported the general floral biology in *Nymphaea* sp. as the flower contain 4 sepals, 7- 40 petals, 20-700 stamens and 5-47 carpels. The flowers of subgenus Hydrocallis are characterised by the presence of completely fused carpels, swollen ovary and tetramerous arrangement of petals as well as outer stamens.

Jokla and Mussob (2000) observed that *N. alba* is a white flowering species with four lanceolate sepals and yellow stigmatic disc. There were 15-30 petals, which gradually turns into stamens. Hossain *et al.* (2007) reported that *N. rubra* shared some similar morphological features with *N. pubescens.* However, the two species differed considerably in size and vein pattern of the leaf as well as the flower size, petal colour, number and size of the stamens.

Nymphaea flower has four sepals, initiating unidirectional and alternate with basal petals. The dome-shaped floral apex continued to expand and produce more petal and stamen primordia. The remaining petals and all stamens are initiated in spirals or whorls. Later the periphery of the floral apex grows more quickly than the centre and results in a depression in the centre of the apex, after all stamens have been initiated. Carpels are simultaneously initiated in a cycle at the periphery of the depression. After all organs have been initiated, the centre of

the depression on the floral apex develops into a globular structure. The connected inferior ovary, stigma caps and the globular floral apex together form an extra gynoecial compitum (Hu *et al.*, 2009).

Begum *et al.* (2010) reported that *N. rubra* has four ovate-lanceolate sepals which were reddish-green as well as ribbed outside and crimson-red inside. The petals, stigmatic appendages and anthers were also crimson-red in colour. The long stigmatic appendages were densely arranged covering the stigma completely.

According to Fahida (2012) the flowers of white and blue colour variants of *N. nouchali* were found to be complete with various floral whorls in spiral fashion around the floral axis. The outer whorls of stamens were observed as slight petaloid in nature.

Tom (2015) observed that the night bloomers differed from the day bloomers in having larger flowers with stamens devoid of appendages. In addition to that the sepals of night bloomers were observed to have five to six prominent nerves on both sides.

2.7 ANTHESIS AND POLLINATION

Watson (1884) was the first to report a disparity in the opening and closing time in various *Nymphaea* species. Some species, such as *N. nouchali* and *N. odorata,* are diurnal, opening in the morning and closing about after noon, while others are nocturnal, opening at evening, remaining open during night and closing around noon on the next day. He noticed the presence of clear sweet nectar in the stigmatic cup, only during the first day of anthesis.

Robertson (1889) also observed the presence of fluid in the stigmatic bowl of *N. odorata* flower, but supposed it to be water since he sensed no taste and saw no insects visit the fluid. He observed insects searching for pollen in second and

third day of flower opening and then flying to pollen receptive first day flowers where they effected cross-pollination.

Some *Nymphaea* species open only for two or three consecutive days while other species open on as many as six or seven successive days (Conard, 1905). Knu (1908) stated that *Nymphaea* flowers are homogamous or slightly protogynous in nature with the stigma being receptive for several days and reported the evidence of self - pollination in waterlily.

The best and most complete work on the pollination process of water lilies is still that of Schmucker (1935) who worked with a number of tropical species (*N. citrina, N. colorata, N. gigantea, N. micrantha, N. zanzibariensis)* and the hybrid *Nymphaea pennsylvanica*. He agreed that the flowers are protogynous and an impressive stigmatic pool is present. He claimed that the fluid remains present for several days, and that it is a 1% to 2% solution of glucose.

The studies of Prance and Anderson (1976) and Meeuse and Schneider (1980) mainly concentrated on protogynous nature and pollination mechanism of *Nymphaea* species. The protogynous phenomenon in the genus *Nymphaea* is the event in which the stigma is receptive for accepting the pollen from a different flower before the pollen in the same flower is ready to shed. The stigma of the genus *Nymphaea* becomes ready to be pollinated during the second and third day of blooming. However, exceptions exist for some species where the pollen is ready to shed for fertilization on the first day at the time of the stigma receptivity, enabling fertilization to occur within a same single flower. Capperino and Prance (1980) reported that the main pollinating agent of *N. amazonica*, a nocturnally flowering waterlily is beetles, while bees are found to be the pollinators of some of the diurnally flowering waterlilies.

On the first day of anthesis, flowers are partially open with abundant stigmatic fluid and immature anthers. In second-day flowers, stigmatic fluid is absent and the innermost anthers are dehiscent shortly after flower opening,

whereas outermost anthers do not open until the third day. The upper, outer margin of each carpel extends to form a 'stylar process,' which is erect during the female phase and then arches over the stigmatic cup during the male phase. On each day of anthesis, flowers open at approximately 9:00 h, then close and submerge after the fourth day (Schneider and Chaney, 1981).

Schneider (1982) observed the emission of strong, sweet odour which, together with the blue, pale-violet corolla, attracts pollinators in *N. elegans.* Schneider (1986) reported that beetles, honey bees and flies are the insect pollinators in *N. capensis.* According to Wiersema (1987 and 1988) the flowers of night-blooming water lilies were protogynous. In these species, separate female and male phases of anthesis occurred on the consecutive evenings and were characterized by intense volatilization of pollinator attractive floral scents.

According to Slocum (2005) waterlilies usually open for three consecutive days by opening during the day and closing during the night. The blooming time and duration was different for each subgenus.

Fahida (2012) has reported that the opening time of the flower in the two colour variants of *N. nouchali* varied from 7.30 am to 6.30 am and closing time varied from 5.15 pm to 6.15 pm. She observed the honey bees visiting on the flowers might be their pollinator.

Tom (2015) conducted detailed study on reproductive biology of three night and two day blooming waterlily cultivars and observed that, the process of blooming began with the opening of the sepals and the process of opening and closing repeated consecutively for four days. The time required to complete the process of full blooming was 20-30 minutes and for complete closing was 15-20 minutes. The presence of honey dew like secretion in the stigmatic cup indicated the initiation of receptivity of the stigma. Anther dehiscence started from the outermost to the inner most whorls. She also reported the cross pollinating

nature of water lily due to the presence of insect predominantly honey bees and stingless bees.

2.8 POLLEN MORPHOLOGY

Determination and comparison of pollen morphology and viability among different cultivars and species are essential to extend the knowledge about reproductive biology (Asma, 2008).

Pollination biology, pollen development and morphology of *Nymphaea* have been studied or compared in many species such as *N. alba, N. candida, N. mexicana, N. odorata, and N. tetragona.* The pollen grains are bilaterally symmetrical and are generally single aperturate in the other five genera of the family *viz.*, Brasenia, Cabomba, Eurayle, Nymphaea and Victoria. Singh *et al.* (1969) revealed that the cultivated varieties of *Nymphaea* have operculate spheroidal and one colpate elongate pollen.

Poddubnaya-Arnol (1976) perceived that structure, size and shape of pollen grains might vary considerably within one species, even though these are diagnostic characters. Gabarayeva and El-Ghazaly (1997) reported that the pollen grains of *N. Mexicana* have a verrucate proximal surface.

The studies of Kupriyanova (1976), Muntendam *et al.* (1996) and Uotila (2001) publicized that pollen grains of *Nymphaea* were characterized by high morphological stability and can be used for species identification on the basis of size, shape and exine sculpture of pollen grains. Monila *et al.* (1996) opined that if the anther length and pollen size are small, pollen production by the concerned taxon is lowest.

According to Bhunia and Mondal (2012), the pollen grains of *Nymphaea*ceae are mostly medium and rarely small sized, monosulcate and spheroidal with granular membranes. *N. nouchali, N. pubescence, N. rubra* and *N. stellate* are high pollen producing types. Among these *N. pubescence* showed

highest pollen production and *N. rubra* the lowest. Fahida (2012) conducted a study on reproductive biology on two flower colour variants of *N. nouchali* and reported that the pollen grains are round, yellow, monocoptile with reticulate exine in both.

2.9 POLLEN FERTILITY AND VIABILITY

Volkova and Shipunov (2007) mentioned the crucial role of sucrose concentration under optimized environmental condition (32^0 C in dark). Thien *et al.* (2009) reported that the concentration of sucrose in the stigmatic fluid of *Nymphaea* flowers was less than 5% therefore the pollen grains would not possess any problem for germination under natural conditions.

According to Tang *et al.* (2009), pollen grains are considered as viable when the pollen tube length is longer than the diameter of the pollen grain. Sun *et al.* (2011) reported that low number of viable pollen grains and the abnormal growth of most of the pollen tubes might be the main causes of the failure of seed set.

Bodhipadmaa *et al.* (2013) studied two forms of *N.nouchali* var. *Versicolor,* and reported similar pollen morphology and 95% pollen viability in both. *In vitro* germination of pollen grains showed maximum germination at the concentration of 5% sucrose.

Tom (2015) reported that the *Nymphaea* pollen grains are viable even after 10 hours of its dehiscence.

2.10 FRUIT AND SEED CHARACTERS

Fruit of *Nymphaea* is a capsule, irregularly dehiscent and many seeded. Seeds are mostly arillate with a small embryo, little endosperm, abundant perisperm and fleshy cotyledons (Wiersema, 1987). Hitchcock and Cronquist (1990) and Stone (1993) described the fruit as a berry like capsule of one inch diameter and having numerous small seeds (1-2 mm long). Each seed is partially

covered with a companulate outgrowth of funiculus called aril. Presence of mucilage and gas bubbles in the aril tissues aids in floating and dispersal of seeds (Conard, 1905; Valla and Martin, 1976 and Collinson, 1980). According to the morphology of integument, *Nymphaea* ovules were classified into two groups, with hood shaped (Khanna, 1967 and Richardson, 1969) and cup shaped outer integument (Schneider, 1986).

In Mexico, *N. mexicana* had the largest seeds among the species of subgenus *Nymphaea.* And the subgenus Hydrocallis had the species with smallest seeds. In contrast seeds of Victoria being 8-10 mm long were the largest within the *Nymphaea*ceae family. *Nymphaea nouchali* flowers remain open functionally for 3-4 days after the peduncle sinks to water and develops into fruits. The fruit ripens under submerged water and covered with persistent calyx (Tetali *et al.*, 2008). Begum *et al.* (2010) reported excellent fruit set and ellipsoidal seeds in *N. pubescence* and *N. nouchali*, and absence of natural seed set in *N. rubra*. In India *N. caerulea* do not set fruits in normal condition (Ansari *et al.*, 2005).

2.11 SEED DORMANCY AND GERMINATION STUDY

Multiplication of plants through seed is the effective method of conservation, creation and maintenance of genetic variability which is unsuccessful under vegetative propagation (Smits *et al.*, 1995). Conard (1905) reported the difficulties of waterlily seeds to germinate due to the development of dormancy with the passage of time. However germination can be induced by submerging seeds in 5-30 cm of water.

Else and Riemer (1984) observed that, the dormancy of *N. odorata* seeds broken when large number of seeds crowded in a small container. The seeds started themselves to produce a substance which promoted its germination. Freezing and drying for short period up to one day strongly inhibited the germination of Nymphaea seeds.

Germination of *Nymphaea* seeds were enhanced by sunlight as well as cold scarification for several months. Seedlings are rarely observed when adult population is high in the field (Else and Riemer, 1984; Bonilla-Barbosa *et al.*, 2000; Tomaso and Healy, 2003)

Highest germination of fresh seeds of *N. alba* observed on MS medium containing 1 mg/l BAP+0.1 mg/l IAA. However, the seeds failed to germinate, even after three months in the same medium either alone or with sucrose, IBA and GA_3 in different concentration. But 2 mg/l TDZ could be used effectively for its germination (Sumlu *et al.*, 2010).

Leaching treatment and acid scarification (H_2SO_4) enhanced the speed of seed germination (Tom, 2015).

2.12 ASEXUAL REPRODUCTION

In waterlily, the easiest method of propagation is division. Plants are left in one place for two years, divided just before new growth commences in the spring and planted in fresh soil. The fleshy roots are pulled or cut and replanted immediately in a fresh soil medium. Each new plant should have at least one bud at the tip of the rhizome (Soyza, 1936; Irvine and Trickett, 1953).

Wiersema (1988) described various means of propagation in waterlily which are detachable tubers, stolon, the proliferation of floral and foliar tissues and seed. Horizontal rhizomes are the main propagule in temperate species like *N. alba, N. odorata, N. tuberose* and *N. candida.* In tropical species autogamy is common. Stolons in *N. nouchali,* foliar proliferation in *N. micrantha,* floral propagation in *N. prolifera* and *N. lasiophylla* are other methods. Fahida (2012) reported that leaf propagation is common among two flower colour variants of *N. nouchali* and fruit and seed set are absent due to some incompatibility mechanisms.

Yakandawala *et al.* (2017) reported the ability of Sri Lankan violet *Nymphaea* to reproduces by developing epiphyllous plantlets or vivipary. Once detached from the deteriorated lamina, it is a miniature of the parent and is capable of floating away and establishing as an independent plant. Further blooming of these plants while still attached to the mother plant has also been observed.

2.13 HYBRIDIZATION

2.13.1 Natural hybrids

Ward (1977) mentioned the first naturally occurring *Nymphaea* hybrids involving species *N. alba* and *N. candida.* An alleged natural hybrid, *N. thiona* was assumed to have originated through the cross between *N. odorata* and *N. mexicana.* Wiersema (1987) reported sterile natural hybrids involving *N. mexicana* and *N. odorata* subsp. *odorata*, and fertile natural hybrids between *N. odorata* subsp. *odorata* and *N. odorata* subsp. *tuberosa.* According to Wiersema (1988), *Nymphaea alba* is one of the waterlily species that can self-fertilise. Padget *et al.* (1998) reviewed criteria for evaluating natural plant hybrids in the waterlily genus *Nupar.* Naturally occurring hybridization in *N. alba* is reported by Poczai *et al.* (2011).

2.13.2 Artificial hybrids

In addition to the natural hybrids, a large number of artificially raised varieties have been increasing the list of *Nymphaea.* Since hybridization of waterlily was initiated early in the 19th century, claims of successful crosses have been challenged repeatedly due to poor documentation or lack of evidence (Conard, 1905 and Swindells, 1983). Most of the *Nymphaea* hybrids involve interspecific hybridization between closely related species.

Latour-Marliac (1893) crossed hardy waterlilies with tropical waterlilies but the cross was unsuccessful in creating a blue flower *Nymphaea* hybrid. Notably, several inter-subgeneric crosses in *Nymphaea* were reported by Ames (1900) and Grey (1900). However, Conard (1905) dismissed the validity of these claims due to a lack of evidence of pollen parentage and, in some cases strong indications that traits were inherited from related species within the same subgenus.

Nymphaea hybrids have been shown to grow more vigorously than the parental species, but they are usually sterile. The reason for the sterility is that different stages of ploidy may be present within one species, together with aneuploid forms (Gupta, 1978, 1980; Heslop-Harrison, 1955).

Reditt (1989) reported on the continuing attempt of researchers to create the blue hardy waterlily. The biggest obstacle for the development of inter sub-generic Nymphaea hybrid is the failure in developing mature zygotes, due to the difference in chromosome numbers between subgenus of tropical waterlily and that of hardy waterlily (Gupta, 1980; Hossain *et al.*, 2007).

Donald *et al.* (2004) provided the genetic confirmation of first successful synthetic inter-subgeneric hybrid in *Nymphaea* from a cross of a white-flowered variant of (subgenus Brachyceras Casp.) as pollen parent and a cultivar of *N. gigantea* (subgenus Anecphya Casp.) as ovule parent. Morphologically, the hybrid possesses some characteristics of the parents, some intermediate features and some unique traits. The cross was confirmed using DNA sequencing and molecular cloning techniques to compare bi-parentally inherited nuclear genetic markers in the parents and hybrid plant.

A successful cross reported by Les *et al.* (2004) from a controlled cross between the subgenera Anecphya and Brachyceras, has been verified using molecular genetic techniques. This novel hybrid described as *Nymphaea* 'William

Phillips' represents the first proven inter sub-generic cross in the genus, and also is a tropical water-lily with highly desirable ornamental qualities. The hybrid was distinct from the parents by its novel petal and anther colours and by its intermediate flower and petal shapes.

Most of the inter-subgeneric hybrids can be easily identified morphologically. However, certain white-flowering hybrids of *N. alba* with *N. odorata* cannot be distinguished based on morphological characters alone (Slocum, 2005). The broad morphological variation of *N. alba* makes it difficult to distinguish specimens of the true species from white flowering hybrids (Heslop-Harrison, 1955; Volkova and Shipunov, 2007).

Songpanich and Hongtrakul (2010) obtained successful crosses from the blue-flowered subgenera Anecphya and Brachyceras. Good pod setting types were used as pollen parent for transferring blue-flowered characteristic to the hardy waterlily. The hybrids were categorized into two groups, pink-flowered group with 17 hybrids and a blue- flowered group with 3 hybrids. A prominent blue-purple flowered in the blue-flowered group was determined by PCR-RFLP markers to be an intersub-generic hybrid and it has been named *Nymphaea* 'Siam Blue Hardy'.

Dkhar *et al.* (2011) DNA sequence analyses indicate *N. rubra* is an interspecific hybrid involving *N. odorata* as the maternal parent, and *N. alba* as the paternal parent. However the recent study has found that *N. rubra* is a hybrid produced by a cross between *N. lotus* and *N. pubescens* as a parental species (Dkhar *et al.*, 2013; Devi *et al.*, 2015). Sequencing signals of the biparentally inherited ITS marker and sequence matching of the chloroplast trnK intron, matK and rbcL gene of an Indian plant identified as *Nymphaea alba* var. *rubra* contradict its identity. Additional signals depicted in chromatograms of the ITS region and the exact match of the maternally inherited chloroplast DNA sequences suggest that the Indian material is a hybrid of *N. alba* and *N. odorata.*

Materials and methods

3. MATERIALS AND METHODS

The investigation entitled 'Characterization and hybridization of *Nymphaea* spp.' was carried out in Department of Plant Breeding and Genetics, College of Agriculture, Padannakkad during the academic year 2016 September - 2018 July. The details regarding the experimental materials and methodologies adopted for the study are given below.

3.1 EXPERIMENT 1: EXPLORATION AND COLLECTION OF GERMPLASM OF *NYMPHAEA* SPP.

3.1.1 Materials

A survey was conducted in different *Nymphaea* growing regions of North Kerala and fourteen accessions were collected. The planting materials were collected in the form of rhizome and a collector's number was given to identify the accessions. The plants were maintained in cement pot having a diameter of 60 cm and height of 45 cm. The potting mixture prepared with sand, soil and cow dung in the ratio 1:1:1 and a water level of 20-25 cm were maintained in the pots during the course of the experiment.

3.1.2 Methodology

Preliminary evaluation for flower colour, blooming nature and mode of reproduction were carried out in the collected accessions. Among the collected fourteen accessions ten were carried over to morphological characterization and detailed evaluation for reproductive and seed biology and mode of propagation in experiment 2. The selection of ten accessions was made in accordance with the availability of flowers for evaluation.

3.2 EXPERIMENT 2: EVALUATION OF COLLECTED TYPES

3.2.1 Materials

Ten accessions (Acc. 1 to Acc. 10) collected from different locations in the form of rhizome which included day as well as night blooming types, seed setting leaf and root proliferating and rhizome propagating types.

3.2.2 Methodology

The experiment was laid out in pot culture in CRD with three replications. These were subjected to detailed evaluation and characterization based on leaf and floral biology. Fruit and seed development and seed germinability with physical, chemical and mechanical treatments in seed setting accessions were studied. Methods of asexual reproduction were also examined in the ten accessions. The main items of observations made in the field are detailed below.

3.3 OBSERVATIONS

3.3.1 Leaf Characters

The growth and developmental pattern of leaf were observed by taking observations of six leaves from each replication of every single accession. Various qualitative and quantitative characters were recorded from all selected types.

3.3.1.1 Growth, Developmental Pattern and Biometric Characters of Leaf

The following biometric characters were observed in each accession.

3.3.1.1.1 Days to reach leaf on water surface

Days counted by tagging a leaf, from its visual appearance on the soil surface to reach on the water surface after complete unrolling of the leaf lamina.

3.3.1.1.2 Length of petiole

The length of petiole of a fully opened leaf was measured in cm.

3.3.1.1.3 Length of leaf

Leaf length was measured in cm on the longest segment of the fully opened leaf.

3.3.1.1.4 Breadth of leaf

Breadth of leaf was measured in cm on the broadest segment of the fully opened leaf.

3.3.1.1.4 Longevity of leaf

It was determined by counting the days from visual appearance of leaf on water surface till decay initiation.

3.3.1.2 Qualitative Characters of leaves

For the assessment of qualitative characters the following features were considered.

3.3.1.2.1 Sinus overlap

Sinus is the area in between the lobes of leaf. The presence and absence of its overlap in each accession was recorded.

3.3.1.2.2 Shape of leaf tip

The shape of leaf tip was recorded with reference to the following descriptor (Nix, 2017) (Figure 1).

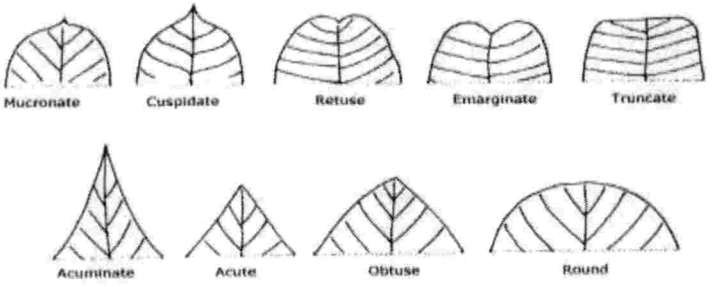


Figure 1: Descriptor for shape of leaf tip

3.3.1.2.3 Shape of leaf margin

The shape of leaf margin was decided by the following descriptor (Nix, 2017) (Figure 2).

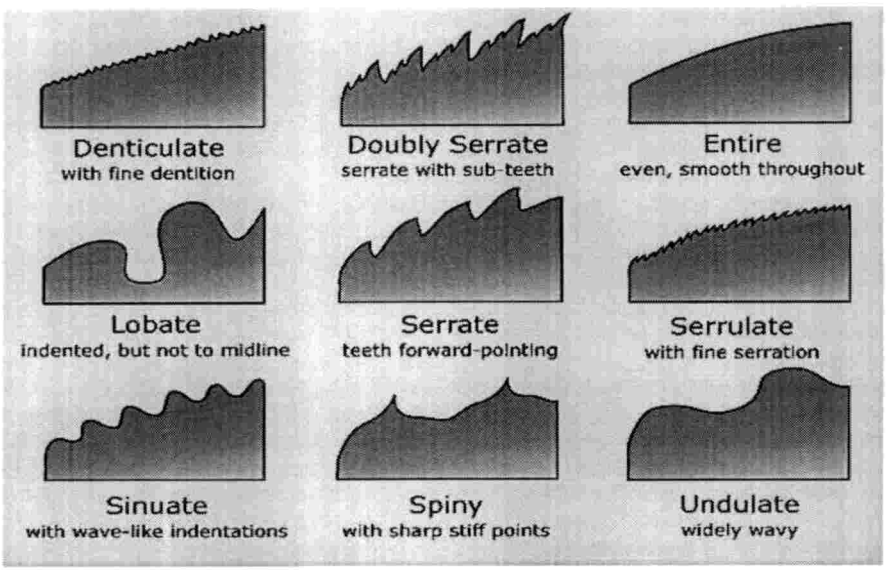


Figure 2: Descriptor for shape of leaf margin

3.3.1.2.4 Appearance of veins on abaxial surface

Based on appearance of primary and secondary veins on the abaxial surface, the leaves are classified as not prominent, slightly prominent and prominent.

3.3.1.2.5 Leaf colour on both abaxial and adaxial surface

The leaf colour was decided with reference to the International Union for the Protection of New Varieties of Plants colour chart (RHS colour chart, 2006).

3.3.2 Flowering Biology

3.3.2.1 Growth pattern of flower bud

Six flower buds from each replication of every accession were tagged immediately after their visibility on the surface of the mud. The growth of

flower bud from visual appearance till decaying or sinking into the water was studied by taking observations at regular intervals according to the availability of flower opening. The following characters were recorded for evaluation.

3.3.2.1.1 Length of flower bud

Length of flower bud was measured in cm on previous day of flower opening.

3.3.2.1.2 Circumference of flower bud

The circumference of flower bud was measured in cm with the help of a twine by placing it around the base of bud where it is more bulged on previous day of flower opening.

3.3.2.1.3 Days taken by the flower bud to reach on the water surface.

The number of days was counted from the visual appearance of bud on the mud to reach on the water surface by tagging a flower bud

3.3.2.1.3 Days taken by the flower bud to open after reaching water surface

This observation was taken from the same flower bud tagged for the previous observation.

3.3.2.1.4 Diameter of fully opened flower

Diameter measured across the fully opened flower on the second or third day of flowering

3.3.2.2 Flower Morphology

The observations on floral features recorded on the first day of flower opening are listed below.

3.3.2.2.1 Flower colour

The flower colour was decided with reference to the International Union for the Protection of New Varieties of Plants colour chart (RHS colour chart, 2006).

3.3.2.2.2 Number of sepals per flower

Number of sepals was counted from dissected flower.

3.3.2.2.3 Number of petals per flower

Number of petals was counted from dissected flower.

3.3.2.2.4 Number of stamens per flower

Number of stamens was counted from dissected flower.

3.3.2.2.5 Number of carpels per flower

Number of carpels was estimated by counting from dissected flower.

3.3.2.2.6 Number of stigmatic appendages

Number of stigmatic appendages was counted from dissected flower.

3.3.2.2.7 Length of sepals

Length was measured in cm from the longest region of sepal of a dissected flower.

3.3.2.2.8 Length of petals

The longest segment of petal length was measured in cm from the outermost whorl of a dissected flower.

3.3.2.2.9 Length of stamen

Stamen length was measured in cm from the outermost whorl of a dissected flower.

3.3.2.2.10 Length of carpels

Carpel length was measured in mm on the first day of flowering.

3.3.2.3 Successive Increase in Growth of Pedicel

Successive growth of pedicel was observed by measuring the length of pedicel on each day from the initiation of flower bud to the date of flower sinking. The pedicel length at the time of flower opening and after flower sinking was noted separately.

3.3.3 Pollination Biology

3.3.3.1 Anthesis

Time and duration of opening and closing of flowers, periodicity and longevity of flower in all accessions were examined.

3.3.3.2 Anther Dehiscence and Stigma Receptivity

Stigmatic surface was examined at hourly interval from 7 am on the previous day of flower opening for the change in colour and appearance. The male reproductive nature was analysed in respect of time and duration of anther dehiscence and bending of stamens.

3.3.3.3 Nature of Pollination

Three sets of three flower buds from each replication were used for the study of pollination biology. In one set the mature flower buds were protected by bagging with butter paper till the completion of anthesis. The second set was emasculated and unprotected and third set kept as control by keeping in open condition with neither emasculation nor bagging. Bagging and emasculation were done two days before flower opening. Various insects visiting the flower were recorded to confirm the mode of pollination.

3.3.3.4 Pollen Morphology and Fertility

Nymphaea accessions of each type were studied by collecting flowers on the second day of blooming. The pollen grains were extracted to glass slides with the help of a brush. Pollen colour was examined with the help of a hand lens. The pollen fertility was assessed on the basis of stainability by staining the collected pollen grains with a drop of 1% safranin dye on a clean slide for ten minutes. All the pollen grains that were well filled and stained were counted as fertile and others as sterile. Five fields of three different slides prepared from each accession were observed under a microscope and the values expressed as percentage. Morphological observations and measurements of fertile pollen were made with an image analyser using a 40 x eyepiece. Size of fertile pollen was measured in micrometre as two components namely polar diameter (p) and equatorial diameter (e). Maximum length in the vertical direction was considered as polar diameter and that in the equator region as equatorial diameter.

Pollen shape was determined by calculating *p/e* ratio. According to the method suggested by Wodehouse (1935), pollen with *p/e* ratio less than one was classified as oblate spheroidal, greater than one as prolate spheroidal and one as spheroidal. Pollen dimension was determined in accordance with the *p* x *e* value. Pollen grain with less than 1000 *p* x *e* value was categorized as small and more than 1000 as medium size (Bhunia and Mondal, 2012). The data of pollen size and fertility percentage were subjected to ANOVA and Duncan's Multiple Range Test by CRD for mean comparison.

3.3.3.5 In vitro Viability or Germinability of Pollen Grain

Pollen viability study was conducted by examining the germinability of pollen grains on different concentrations of sucrose solution. On the second day of flowering, freshly dehisced anthers were collected carefully from each accession by cutting the stamens and kept in 5%, 10% and 15% of sucrose solution under room temperature and natural light for 30 hours.

Slides were prepared from each sample by taking a drop of the germinating medium containing pollen grain. The germinated pollen grains were counted from five different fields per treatment under a stereo microscope and the percentage of germination was calculated.

3.3.4 Fruit and Seed Development

The fruit development studies were carried out in normal fruit setting accessions under open condition, as well as under artificial pollination, after the submergence of flower into the water. The main items of observations made about fruit and seed characters are listed below.

3.3.4.1 Length and Circumference of Fruit

Size of fruit measured in cm on 2-4 days before seed dispersal. Length of fruit measured with a scale (cm) and the circumference with the help of a twine by placing it around the base of the fruit.

3.3.4.2 Weight of Fruit

Weight of fruit measured in gram.

3.3.4.3 Number of Days Taken from Flower Opening to Seed Dispersal

Days taken from flower opening to seed dispersal were counted from a tagged flower.

3.3.4.6 Size of Seed

Size of seed was measured in image analyser

3.3.4.7 100 Seed Weight

Weight of 100 seeds was measured in gram.

3.3.5 Seed Germination Study

The seeds were collected by tying a muslin bag around the ripening pod. In this way after it bursts, the seeds cannot float away. After bursting of

the capsule the seeds were wiped with a tissue paper to remove the mucilaginous coating and immediately subjected to germination study. The germination test was carried out by using hot water, mechanical scarification and different concentrations of H_2SO_4, Gibberellic acid as well as Ethrel at room temperature and natural light. Three replications were maintained for every treatment with 50 seeds in each replication. In each experiment seeds were treated for three minutes with the specified concentration of the medium and transferred to a petri plate containing 25-30 ml tap water for germination.

3.3.5.1 Hot Water Treatment

Seeds were treated with 60^0 C of hot water for three minutes and kept for germination.

3.3.5.2 Mechanical Scarification

The seeds were scarified by rubbing with sand paper and kept for germination in tap water.

3.3.5.3 Treatment with H_2SO_4

Seeds were treated with 1% and 5% H_2SO_4 for three minutes and kept for germination.

3.3.5.4 Treatment with Gibberellic acid

Seeds were treated with different concentrations of GA_3 *viz.*, 50 ppm and 100 ppm.

3.3.5.5 Treatment with Ethrel

Seeds were treated as two lots with 50 ppm and 100 ppm of Ethrel taken in petri plate and tightly capped for three minutes to prevent the escape of ethylene gas.

One set of seeds treated with distilled water was taken as control. All seeds with a protruding epicotyl were considered to be germinated. Observations were taken by counting the germinated seeds at 10, 20 and 30 days after treatment and presented in percentage.

3.3.6 Asexual Reproduction

The ten accessions were thoroughly examined for following asexual mode of reproduction. Time taken for the development of first leaf, root and flower bud were recorded from each accession. The size of flower in terms of diameter as well as total number of flowers produced in first one month in rhizome propagated, leaf proliferated and root proliferated progenies were recorded and compared between the accessions.

3.3.6.1 Rhizome as Natural Propagule

Rhizome propagation was studied by using the same experimental material of experiment 2 in pot culture using experimental design CRD. A two month old rhizome placed in a pot filled with water alone. After the emergence of leaves and roots the rhizome planted in pot filled with optimum level of potting mixture and water.

3.3.6.2 Leaf as Natural Propagule

In leaf proliferating accessions, observations were recorded from 13 days old mature leaves.

3.3.6.3 Root as Natural Propagule

Two month old plant of root proliferating accession kept in water. After the emergence of leaves and roots the tiny plantlets were transferred to pot filled with optimum level of potting mixture and water.

3.4 STATISTICAL ANALYSIS

The recorded data were used for mean comparison of the accessions by ANOVA and Duncan's Multiple Range Test. The genotypic and phenotypic variances were calculated as described by Burton and De-Vane (1953) and these were used for the estimation of the genotypic and phenotypic coefficient of variation according to the method suggested by Singh and Chaudhary (1977). Heritability and expected genetic advance for each character were calculated according to the formula developed by Allard (1960).

3.5 EXPERIMENT 3: HYBRIDIZATION IN SEED PRODUCING *NYMPHAEA* SPP.

3.5.1 Materials

Among the collected accessions five were seed-producing type (*viz.*, Acc. 3, Acc. 4, Acc. 5, Acc. 6 and Acc. 7)

3.5.2 Methodology

All possible crosses (20 crosses) including direct and reciprocal were conducted between these seed setting accessions as per the hybridization procedure in plate 1. Protected flower buds of ovule parent were emasculated on the morning hours of first day of flowering before anther dehiscence. Pollen grains were collected from freshly dehisced anthers of male parent on the second day of flowering. Hand pollination was carried out in between 9.00- 11.00 am by dusting the pollen grains collected from male parent to the stigmatic fluid of female parent flower on its first day of flowering. After bagging and tagging of the artificially pollinated flowers, were examined for fruit set and seed development.

The observations on leaf and floral characters, pollen morphology and seed characters were recorded from the hybrid progeny and compared with their parent plants.

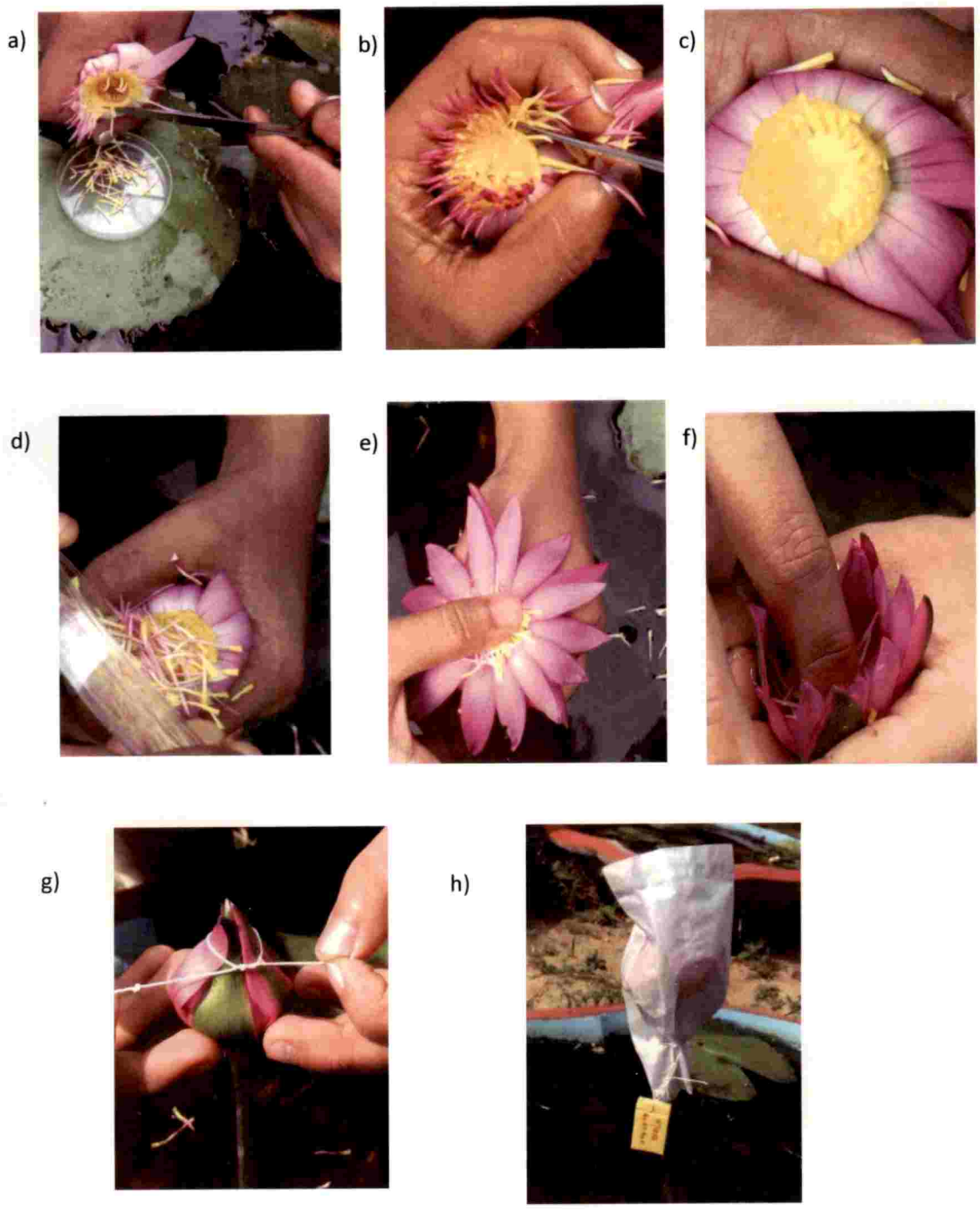


Plate 1: Hybridization procedure in *Nymphaea* sp.

a) Collecting pollen from pollen parent. b) Emasculation pf ovule parent. c) emasculated flower. d,e and f) Artificial pollination on emasculated flower. e) Tying the pollinated flower. f) Tagging and bagging of pollinated flower.

Results

4. RESULTS

The research entitled "Characterization and hybridization in *Nymphaea* spp." carried out in the department of Plant Breeding and Genetics, College of Agriculture, Padannakkad in the academic year 2016-18. The study was conducted as three experiments *viz.*

Experiment 1: Exploration and collection of germplasms of *Nymphaea* spp.

Experiment 2: Evaluation of collected types

Experiment 3: Hybridization in seed producing *Nymphaea* spp.

The results of investigation are presented under these headlines.

4.1 EXPERIMENT 1: EXPLORATION AND COLLECTION OF GERMPLASMS OF *NYMPHAEA* SPP.

The survey conducted in different natural growing tracts of *Nymphaea* in North Kerala revealed the presence of mostly white, purple and pink coloured waterlilies. Based on the variation in flower colour, seven types were collected from their natural habitat. One waterlily maintained at Instructional farm, College of Agriculture, Padannakkad and two from previous study of Tom (2015) under Kerala Agricultural University were also used in the study. Since sufficient variability could not be obtained from the natural habitat, four more types were collected from Mekattil Nursery, Thrissur. Details regarding the flower colour, blooming nature, place of collection in terms of latitude, longitude and height above mean sea level of fourteen different accessions are presented in Table 1. Rhizome was used as the propagule for the initial establishment of all the waterlily plants. Ten among the fourteen collected accessions were carried over to detailed evaluation and characterization, based on flower colour variation, blooming nature and mode of propagation (Plate 2). Among the selected ten, two were night blooming and eight were day blooming types. Though rhizome was the common propagule, two accessions showed leaf proliferation while one showed root tip proliferation and five showed seed setting (Table 1).

Table 1: Details of waterlily cultivars collected during the survey

Acc. No.	Flower colour	Blooming nature	Mode of propagation	Place of collection	Latitude and Longitude	Height from MSL (m)
Acc.1	Light pink	Day	Rhizome, leaf proliferation	Kizhisseri (pond)Malappuram	$11^0$18'N,$76^0$01 E	42
Acc.2	Pink	Day	Rhizome	Mekkattil nursery, Thrissur	$10^0$54'N,$76^0$33 E	126
Acc.3	Violet Blue	Day	Rhizome, seed	College of Horticulture, Vellanikkkara	$10^0$54'N,$76^0$28 E	40
Acc.4	White	Day	Rhizome, seed	Teerthankara, Padannakkad	$12^0$25' N,$75^0$11 E	11
Acc.5	White	Night	Rhizome, seed, root tip proliferation	College of Horticulture, Vellanikkkara	$10^0$54'N,$76^0$28 E	40
Acc.6	Dark pink	Night	Rhizome, seed	Vadakara, Kozhikode	$11^0$63' N,$75^0$70' E	25
Acc.7	Violet	Day	Rhizome, seed, leaf proliferation	Instructional farm, college of agriculture, Padannakkad	$12^0$25' N,$75^0$11 E	11
Acc.8	Violet blue	Day	Rhizome	Mekkattil nursery, Thrissur	$10^0$54'N,$76^0$33 E	126
Acc.9	Pink	Day	Rhizome	Temple pond, Kondotty, Malappuram	$11^0$14' N,$75^0$98' E	89
Acc.10	Pink	Day	Rhizome	Vadakara (pond)	$11^0$63' N,$75^0$70' E	25
Acc. 11	White	Day	Rhizome	Ramanattukara (pond), Kozhikkode	$11^0$19' N,$75^0$88' E	19
Acc.12	Purple pink	Night	Rhizome	Mekkattil nursery,Thrissur	$10^0$54'N,$76^0$33 E	126
Acc.13	Pink	Day	Rhizome	Anakkayam, Malappuram	$11^0$12' N,$76^0$12'E	41
Acc.14	Yellow	Day	Rhizome	Mekkattil nursery,Thrissur	$10^0$54'N,$76^0$33 E	126

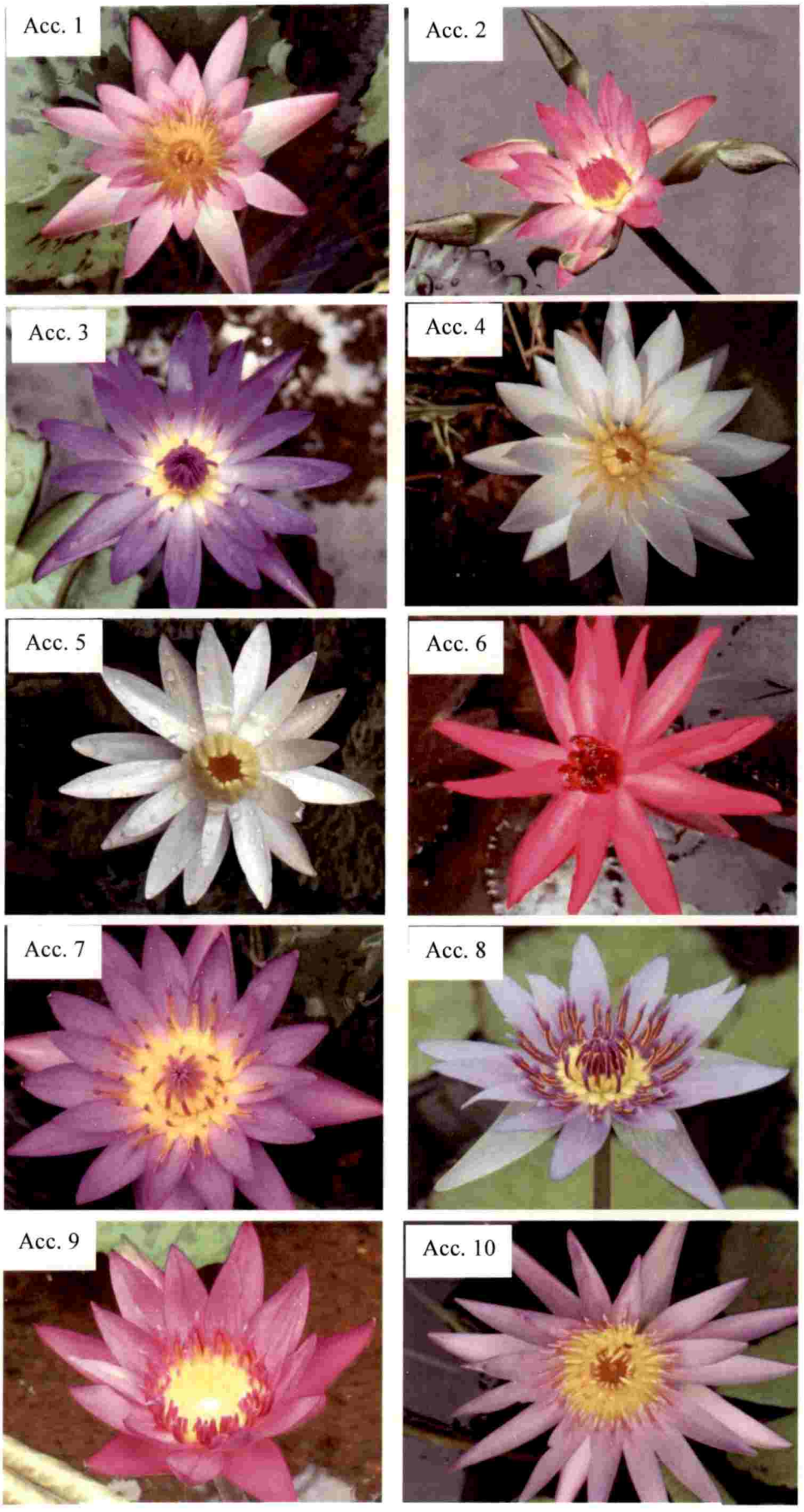


Plate 2: Flowers of ten *Nymphaea* accessions

4.2 EXPERIMENT 2: EVALUATION OF COLLECTED TYPES

The evaluation based on the leaf, flower, pollen grain, fruit and seed exhibited distinct morphological variations in the ten selected accessions. The results are presented below.

4.2.1 Leaf Characters

The selected accessions were evaluated on the basis of growth and development as well as morphological features of leaves.

4.2.1.1 Growth, Developmental Pattern and Biometric Characters of Leaf

In the initial stage the leaves were submerged in water with rolled leaf lamina and the unrolling started after reaching water surface. The observations on the growth pattern of the leaf were recorded from the visual appearance of leaf over mud surface and presented in Table 2. The data exhibited a significant difference between the ten accessions for the leaf characters such as, days to reach on the water surface, length and width of lamina, length of petiole and longevity of leaf.

The leaf lamina took a mean of 4.9 days to reach on the water surface and it differed significantly in each accession. Among these, the leaves of Acc. 5 took longest time (7 days) whereas Acc. 7 took minimum number of (3.5) days to reach on the water surface. The width and length of leaf at full expansion stage in the different accessions of *Nympaea* showed the maximum value for leaf length in Acc. 2 and maximum width in Acc. 1 whereas Acc. 8 showed the minimum value for these characters. The ratio of leaf length and width showed no variation among the accessions. The longevity of leaf was highest in Acc. 2 (25.36 days) and lowest in Acc. 5 (16.3 days).

Leaf petiole was long, slender and submerged in water with lamina floating on the water surface. There was wide variation among the accessions for petiole length. The leaves of Acc. 6 had longest petiole (41.2 cm) and Acc. 5 had shortest (20.9 cm). All the remaining accessions showed almost similar petiole length.

Table 2: Growth pattern and biometric characters of leaves of ten *Nymphaea* accessions

Acc. no.	**Days to reach leaf on water surface**	**Length of lamina (L) (cm)**	**Width of lamina (W) (cm)**	**L/W ratio**	**Length of petiole (cm)**	**Days from visual appearance to decay initiation**
Acc.1	4.90^{c}	18.85^{ab}	18.49^{a}	1.01	35.43^{ab}	23.44^{b}
Acc.2	3.50^{e}	20.50^{a}	16.68^{c}	1.23	37.03^{ab}	25.36^{a}
Acc.3	6.06^{b}	18.63^{ab}	15.81^{d}	1.17	35.80^{ab}	20.83^{c}
Acc.4	4.80^{c}	17.87^{bcd}	14.35^{f}	1.24	38.86^{ab}	17.98^{f}
Acc.5	6.96^{a}	13.06^{e}	13.00^{g}	1.00	20.91^{c}	16.30^{g}
Acc.6	4.13^{d}	16.69^{cd}	14.92^{ef}	1.12	41.20^{a}	19.50^{de}
Acc.7	3.53^{e}	16.27^{d}	15.26^{de}	1.06	34.00^{ab}	22.50^{b}
Acc.8	5.66^{b}	10.33^{f}	10.21^{h}	1.01	32.00^{ab}	19.36^{e}
Acc.9	4.66^{cd}	19.09^{ab}	17.17^{bc}	1.11	29.46^{ab}	20.46^{cd}
Acc.10	4.80^{c}	18.24^{abc}	17.69^{ab}	1.03	27.46^{b}	22.53^{b}
Mean	**4.9**	**16.87**	**15.21**	**1.09**	**34.08**	**20.82**
CV	**6.97**	**6.109**	**2.76**	**-**	**23.37**	**2.72**
CD	**0.58**	**1.75**	**0.71**	**-**	**12.83**	**0.98**

4.2.1.2 Morphological Evaluation Based on Qualitative Characters

The general leaf morphology exhibited by all the ten *Nymphaea* accessions was simple, orbicular with sub-peltate lamina and deeply cleft to the leaf base (sinus). The qualitative characters of leaf *viz.*, sinus overlap, shape of leaf tip and leaf margin, appearance of veins on abaxial surface and leaf colour of ten accessions are presented in Table 3 and Plate 3. The leaf margin was smooth or wavy or irregularly toothed (spiny and sinuate) in different accessions. Primary veins numbering 14-18 were clearly prominent in Acc.4, Acc. 5, Acc.6 and Acc.9 and slightly prominent in Acc1and Acc. 3. The tip of leaf was obtuse in most of the accessions although, truncate, round and retuse leaf tip were also observed. The leaf base was found to be glabrous on both the surfaces except in Acc. 5 and Acc. 6 where the abaxial surface of leaf was hairy. Sinus overlap was present in Acc. 5 only.

The leaf colour varied from dark green to light green with irregular violet spots or patches on the adaxial surface and light green to violet on the abaxial surface.

In Acc.1, the adaxial surface of leaf was dark green and the abaxial surface was light green. Violet irregular spots were found on the both surface of leaf, but these were more prominent on younger leaves and became faint purple on maturity. The primary veins radiating from the petiole base on the abaxial surface were slightly prominent. The margin of the leaf was undulating and irregularly sinuates. There was no sinus overlap and leaf tip was obtuse. The petiole was greenish in colour.

Large purple patches were observed on the dark green adaxial leaf surface of Acc.2. On its abaxial surface, both purple patches and spots were present on light green back ground. The purple patches on both surfaces did not disappear till decay. The primary veins on the abaxial surface were not prominent. The leaf tip

was obtuse and leaf margin was undulating and irregularly sinuates. The petiole was green in colour and no sinus overlap was observed.

In Acc.3 both the adaxial and abaxial surfaces of the leaf was shiny light green without any spot or patches. The primary veins on the abaxial side were slightly prominent. The leaf was with undulating and irregularly sinuate margin without sinus overlap. The leaf tip was truncate and the petiole was light green in colour.

In the case of Acc.4, the adaxial surface was dark green and abaxial surfaces uniformly violet or coffee brown. Although small faint purple spots were present on the adaxial side in young stages, they vanished on maturity. The abaxial surface was characterized by the presence prominent primary and secondary veins. Sinus overlap was absent. The leaves have round tip and wavy margin. The petiole was green in colour.

The adaxial surface of the leaf of Acc.5 was dark green. In the younger leaves small prominent purple patches were radiating from the point of petiole joining to mid rib and it became faded during maturity. The abaxial surface was brownish in colour with prominent primary and secondary veins. The tip of the leaf was obtuse and the margin was spiny. Sinus overlap was observed. The petiole was coffee brown in colour. Hairs were petiole and abaxial leaf surface were also observed.

In Acc.6, the adaxial side of leaf was brownish green and abaxial surfaces was chocolate brown. The primary veins on the abaxial side were prominent. The leaf was with undulating and irregularly sinuate margin without sinus overlap. The leaf tip was obtuse and the petiole was pinkish brown in colour.

In the case of Acc.7, the adaxial surface was shiny green and the abaxial surface was light green with irregular violet spots. The abaxial surface was

characterized by the presence prominent primary and secondary veins. Sinus overlap was absent. The leaf was having obtuse tip and irregularly sinuate margin. The petiole was green in colour.

The adaxial surface of the leaf of Acc.8 was shiny green and the abaxial surface was greenish violet with slightly prominent primary and secondary veins. The tip of the leaf was round and the margin was smooth. Sinus overlap was absent. The petiole was green in colour.

In Acc.9, the adaxial surface of leaf was dark green with violet patches and the abaxial surface was purple brown with green colour towards the midrib. The primary veins radiating from the petiole base on the abaxial surface were prominent. The margin of the leaf was irregularly sinuate. There was no sinus overlap and leaf tip was retuse. The petiole was dark green in colour.

Light green lamina with violet patches observed on the adaxial leaf surface of Acc.10. Its abaxial surface was pinkish brown and light colour towards the midrib. The primary veins on the abaxial surface were prominent. The leaf tip was obtuse and leaf margin was undulating and irregularly sinuate. The petiole was green in colour and no sinus overlap was observed.

Table 3: Qualitative characteristics of leaf of ten *Nymphaea* accessions

Acc. no.	Sinus overlap	Leaf tip	Veins on abaxial side	Leaf margin	Leaf colour	
					Adaxial surface	Abaxial surface
Acc.1	Absent	Obtuse	Slightly prominant	Irregularly sinuate	Dark green with irregular violet spots	Light green with violet irregular spots
Acc.2	Absent	Obtuse	Not prominent	Irregularly sinuate	Dark green with violet patches	Light green with irregular violet spots
Acc.3	Absent	Truncate	Slightly prominent	Irregularly sinuate	Shiny green	Light green
Acc.4	Absent	Round	Prominent	Wavy	Dark green with irregular violet spots	Violet
Acc.5	Present	Obtuse	Prominent	Spiny	Dark green with irregular violet spots	Violet
Acc.6	Absent	Obtuse	Prominent	Irregularly sinuate	Dark green	Violet
Acc.7	Absent	Obtuse	Slightly prominent	Irregularly sinuate	Shiny green	Light green
Acc.8	Absent	Round	Slightly prominent	Smooth	Shiny green	Greenish violet
Acc.9	Absent	Retuse	Prominent	Irregularly sinuate	Dark green with violet patches	Pinkish violet
Acc.10	Absent	Obtuse	Prominent	Irregularly sinuate	Light green with violet patches	Pinkish violet

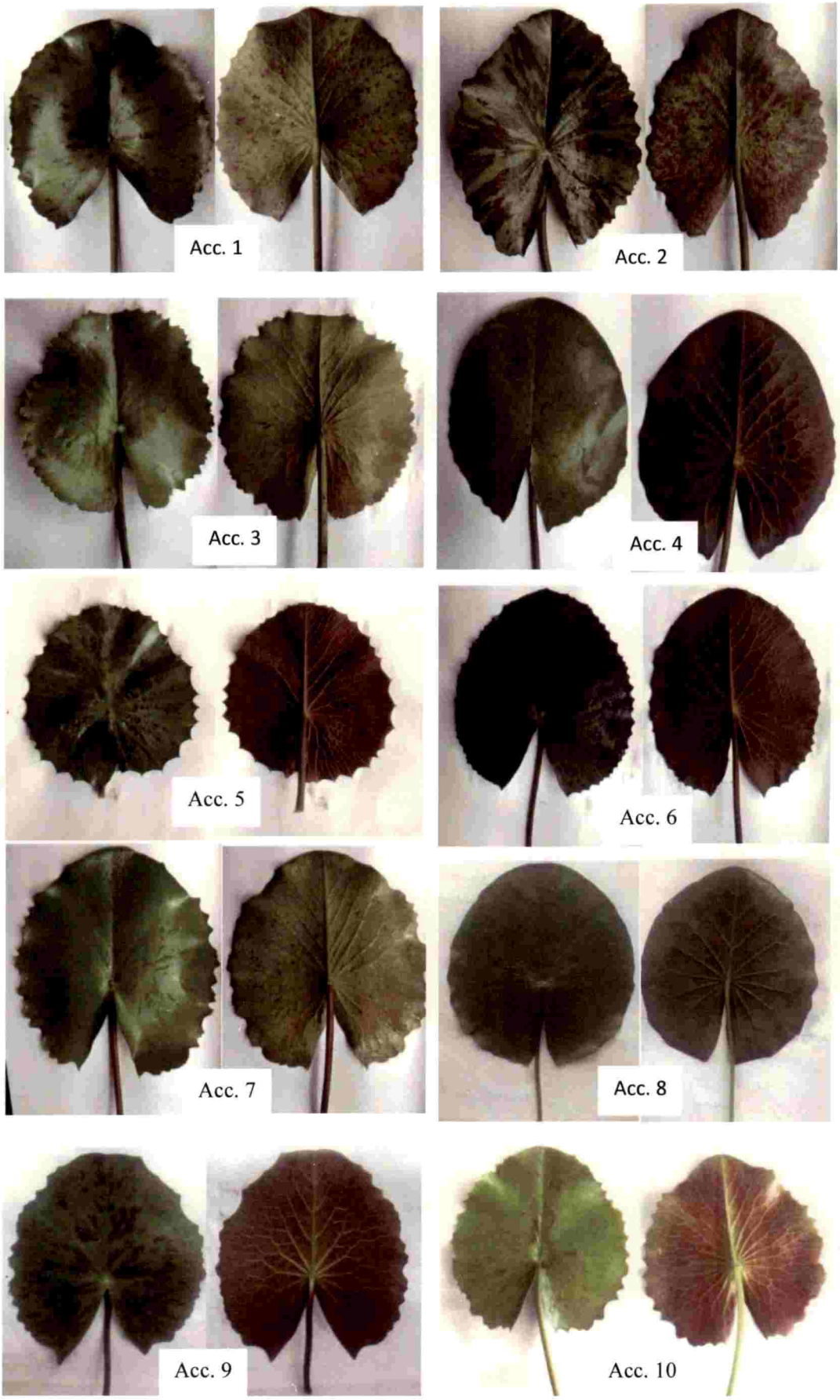


Plate 3: Adaxial and abaxial surface of leaves of ten *Nymphaea* accessions

4.2.2 Reproductive Biology

The reproductive biology in the collected accessions was studied in detail and the results are presented under the two subheads sexual and asexual reproduction.

4.2.2.1 Sexual Reproduction

The sexual mechanism of reproduction was studied by examining in detail the floral biology, fruit and seed development and seed germination study in the collected accessions.

4.2.2.1.1 Floral biology

4.2.2.1.1.1 Growth pattern of flower bud

The flower buds were emerged from the leaf axil under submerged condition. It took 3-6 days for the bud to reach the water surface. The growth pattern of flower buds as indicated by the mean days taken to reach on the water surface, mean days to open, length and circumference of mature flower bud and diameter of fully opened flower are presented in Table 4. The result revealed that the accessions differed significantly in all the examined characters.

The flower buds of Acc. 6 took maximum number of days to reach on water surface (6.76) and were on par with Acc. 5 (6.5 days). The minimum number of days was taken by Acc. 3 (3.73) and was on par with Acc. 8. The Acc. 5 took maximum days for blooming (to open the flower bud from its appearance on water surface) followed by Acc. 6 (13.9 days) and minimum number of days was taken by Acc. 10 (10.31 days). In case of length, circumference of flower bud and diameter of fully opened flower, both the night blooming accessions (Acc. 5

and Acc. 6) showed superiority over all other accessions with maximum values for these characters. Acc. 8 exhibited lowest values for these characters.

Table 4: Growth pattern of flower buds of ten *Nymphaea* accessions

Acc. No.	Days taken to reach bud on water surface	Days taken to open bud after reaching water surface	Length of flower bud (l) (cm)	Circumference of flower bud (c) (cm)	Diameter of fully opened flower (cm)
Acc.1	3.83^{d}	7.30^{c}	4.90^{b}	5.11^{c}	9.45^{b}
Acc.2	4.76^{cd}	6.64^{d}	4.11^{e}	4.78^{d}	8.97^{c}
Acc.3	3.73^{d}	6.59^{d}	4.52^{c}	5.16^{c}	9.01^{c}
Acc.4	4.23^{cd}	6.55^{d}	4.31^{d}	4.45^{ef}	8.66^{d}
Acc.5	6.50^{a}	7.67^{bc}	5.65^{a}	5.7^{a}	11.86^{a}
Acc.6	6.76^{a}	8.65^{a}	5.20^{a}	5.60^{ab}	10.98^{a}
Acc.7	5.00^{bc}	7.76^{bc}	3.92^{f}	4.72^{d}	8.40^{d}
Acc.8	3.76^{d}	8.65^{a}	3.49^{g}	3.49^{f}	7.63^{e}
Acc.9	5.03^{bc}	7.39^{bc}	4.09^{ef}	4.69^{de}	8.70^{d}
Acc.10	5.96^{ab}	7.93^{b}	4.47^{cd}	5.42^{b}	9.38^{b}
Mean	4.95	7.51	4.42	4.99	9.19
CV	**12.82**	**4.48**	**2.54**	**2.94**	**1.66**
CD	**1.08**	**0.57**	**0.19**	**0.25**	**0.26**

4.2.2.1.1.2 Flower morphology

In general the waterlily flowers are solitary, pedicellate, complete, actinomorphic and bisexual with the floral formula

$\oplus \varphi \nearrow K_{(4)}\, C_{(10\text{-}24)}\, A_{(20\text{-}120)}\, \underline{G}_{(10\text{-}23)}$

The floral characters studied are presented in the Table 5.

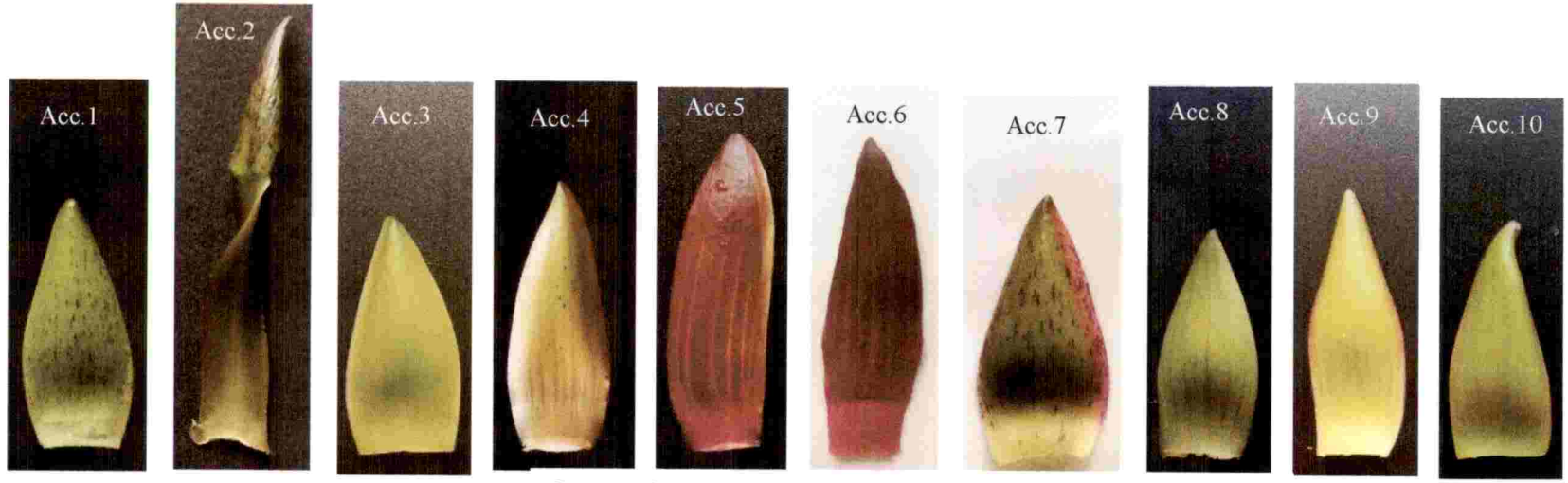
4.2.2.1.1.2.1 Calyx

All accessions had four sepals except Acc. 2 (5-7 sepals) showing acute tip and imbricate aestivation. The number of other floral parts *viz*., petals, stamens and carpels differed significantly between each accession and the mean values are presented in Table 5. The colour of sepal varied among the accessions on both sides (Plate 4).

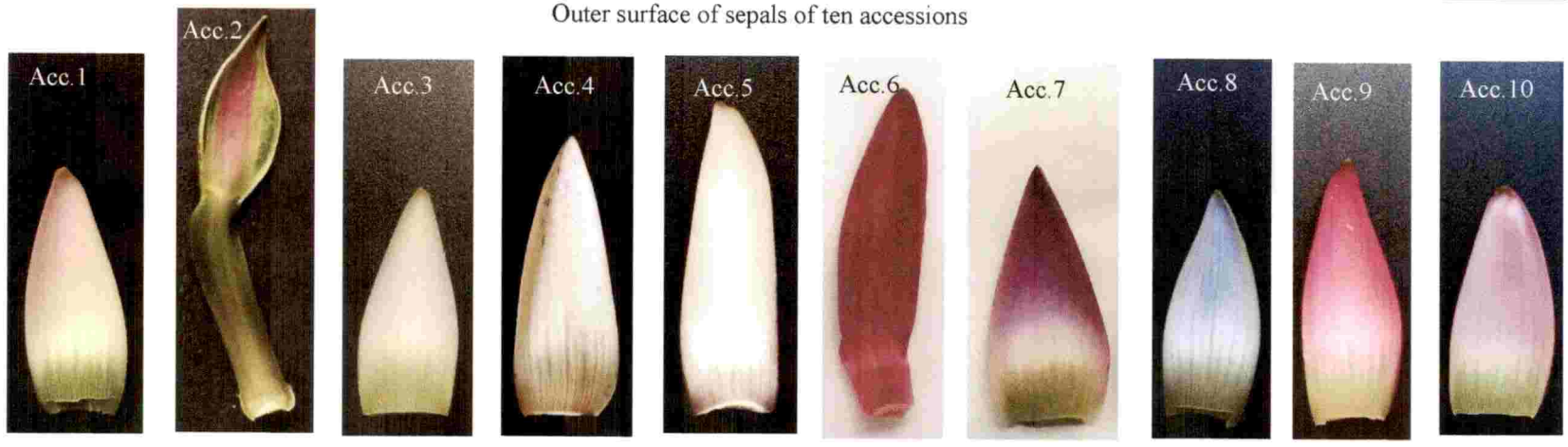
In Acc. 1 the sepals were green with small brown narrow steaks scattered along the length of outer surface. The inner surface was white with a pink tinge on the top and green shade on the basal portion. In Acc. 2 the sepal modified to a feather like structure and was slightly twisted. The long narrow basal portion on both the sides was dark green colour. The broad upper part was dark green with brown streaks along the length on the outer surface and a shade of pink on the inner side.

In Acc. 3 the outer surface of sepal was light green while the inner side was white with a light violet shade on the tip. The sepals of Acc. 4 were characterized by the presence of pale green colour on the outer side and white shades on the inner side. Small brown lines were present on the upper portion of both sides.

In the night blooming accessions Acc. 5 and Acc. 6 five to seven veins running parallel to the length of sepals were prominent on both surfaces. In Acc.5 the outer surface was chocolate brown with pink nerves and white colour on the inner side. The sepal of Acc. 6 was brownish pink with pink veins on the outer surface and uniform pink on the inner surface. Acc. 7 have green outer surface with a pink tinge on one side and brown narrow streaks scattered along the length of sepal. Its inner side was characterized by violet shades on top and greenish white on the basal portion.



Outer surface of sepals of ten accessions



Inner surface of sepals of ten accessions

Plate 4: Sepals of ten *Nymphaea* accessions

The outer side of sepals of Acc. 8, Acc. 9 and Acc. 10 were uniformly distributed with different shades of green. Acc. 9 was greenish yellow with a line of pink shade on both margins along the length of sepal. The inner surface was characterized by the presence violet, bright pink and pale pink in Acc. 8, Acc. 9 and Acc.10 respectively. The result revealed that there was no significant difference for the sepal length between the accessions with a mean length 4.9 cm.

4.2.2.1.1.2.2 Corolla

The petals were boat shaped, elliptic, and acuminate at apex and arranged spirally on the floral axis with two or three whorls. There was variation among the accessions with respect to number, colour and length of petals. As presented in the Plate 5, the intensity of colour of petal in each accession was more towards the tip.

In Acc. 1 the petals are pale pink in colour at the apex and it gradually diminishes to white towards the basal portion. A single central vein is prominent along the length of the sepals of outer whorl. In Acc. 2 three fourth part of the sepal from tip is pink in colour and basal part is yellow. Light blue sepals are present in Acc. 3. The blue colour was observed at the tip with its maximum intensity and gradually merges with white at the base of the sepal. Clear white petals are observed in Acc. 4 and Acc. 5 with three to five veins running parallel to its length. Petals of Acc. 6 were dark pink or red on both the surfaces and are hook like towards the apex. Purple coloured petals with four central veins are arranged in three whorls in Acc. 7. In A cc. 8 three fourth of the upper part of sepal was violet colour and it merged with white at the basal end. Acc. 9 have petals with violet pink colour with a white shade at the base and single central vein. In Acc. 10 petals are uniform light pink with a light yellow shade at basal end.

Plate 5: Petals of ten *Nymphaea* accessions

The flowers of Acc. 9 produced maximum number of petals (20. 7) and Acc. 2, Acc.3 and Acc. 4 were on par to it. Acc.8 produced lowest number of petals (11.8). The Acc. 5 and Acc. 6 were also on par with an average of 15.1 and 15.9 petals per flower (Table 5).

4.2.2.1.1.2.3 Androecium

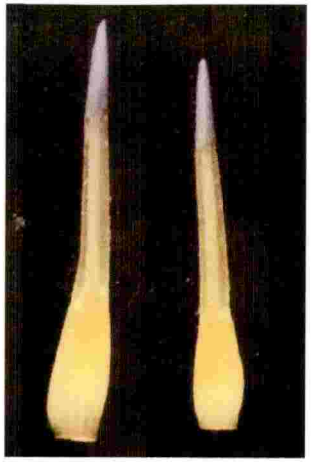
Numerous stamens are arranged in a spiral fashion on two or three whorls in all accessions. Each stamen consists of a filament, anther and a sterile appendage on the tip in all the Accessions except Acc. 5 and Acc. 6. These exceptions are devoid of the terminal appendage. There was a progressive decrease in anther size from outer most to the inner most whorls. The mean number of stamen was highest in Acc. 3 (115.16) followed by Acc. 9 (104.8) and lowest in Acc. 6 (23.05) as presented in Table 5. Variability was observed in the colour of terminal appendage between accessions (Plate 6). The appendage was pink in Acc. 1, Acc. 2, Acc. 9 and Acc.10, blue in Acc. 3, Acc. 7 and Acc. 8 and white colour in Acc. 4. The filament and anther lobe was violet in Acc. 8 and brownish pink in Acc. 6. Acc. 5 had yellow anther lobe and white filament was in Acc. 5. All the remaining accessions possessed yellow filament and anther lobe. The length of stamen was measured from the outer whorls of each accession and it significantly differed between each other with a mean length 2.28 cm. Acc. 5 (2.48 cm) exhibited highest stamen length followed by Acc. 3 (2.38 cm). The lowest length of stamen was observed in Acc. 6 (2.03 cm).

4.2.2.1.1.2.4 Gynoecium

The gynoecium was syncarpous with prominent stigmatic cup in all accessions. The stigmatic cup was yellow in all the accessions except in Acc. 6 which was creamy white with a pink tinge at centre (Plate 7). A small knob like receptacle tissue was found at the centre of the cup and clavate appendages around the rim of the stigmatic cup in all the accessions. The number of stigmatic

Accession 1

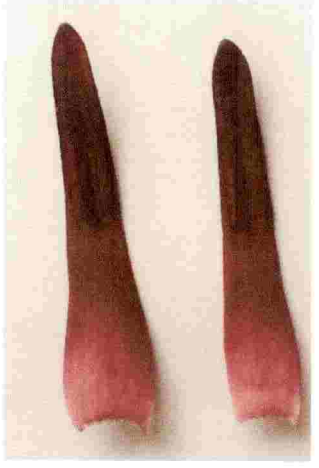
Accession 2



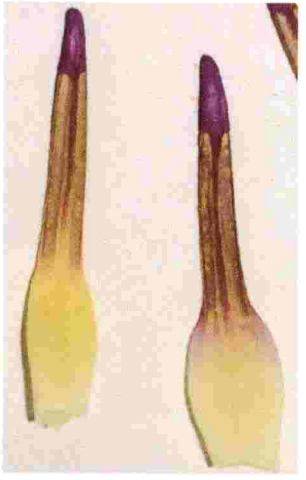
Accession 3

Accession 4

Accession 5



Accession 6



Accession 7

Accession 8

Accession 9

Accession 10

Plate 6: Stamens of ten *Nymphaea* accessions

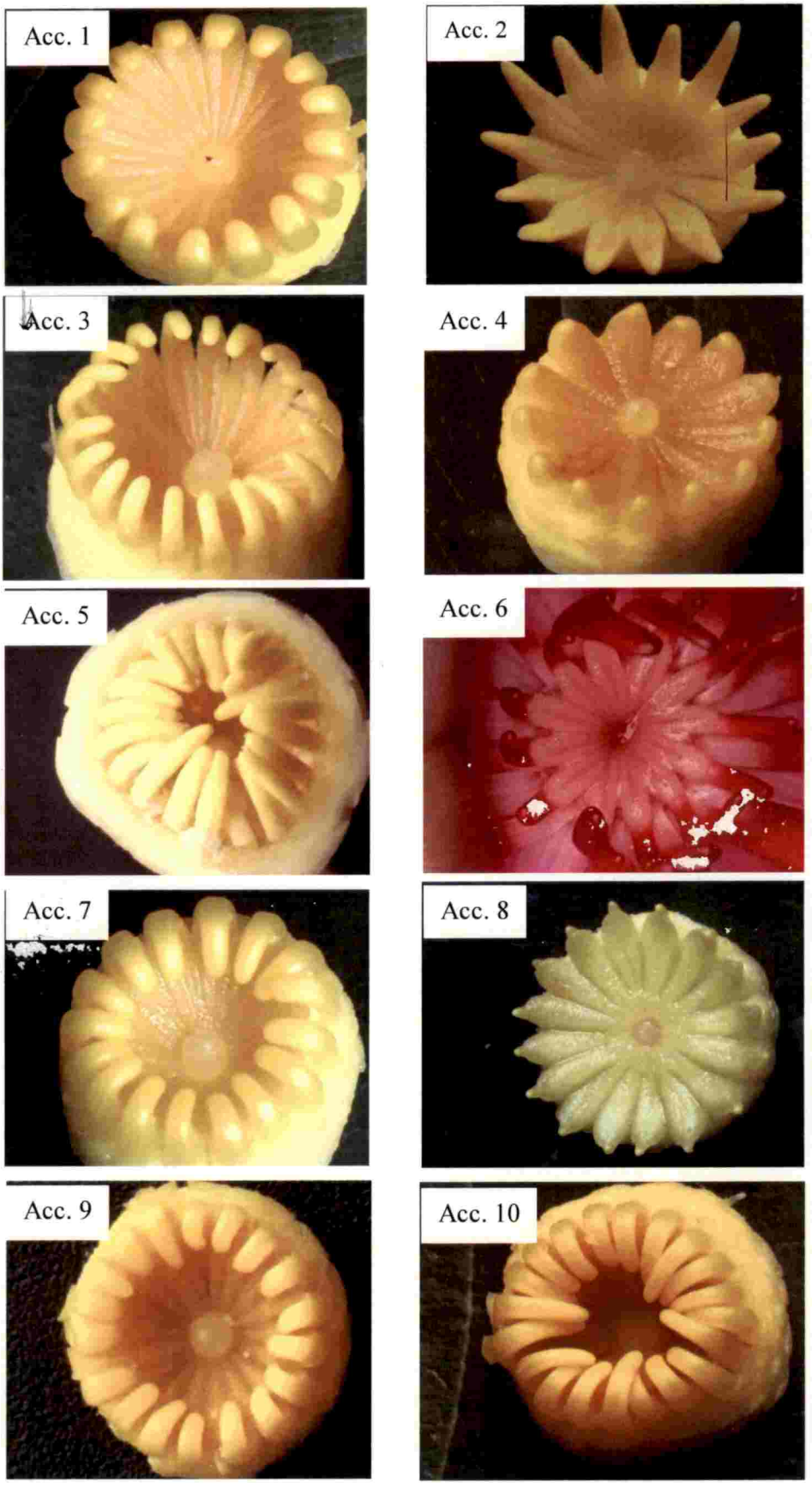


Plate 7: Carpel and stigmatic appendage of ten *Nymphaea* accessions

appendages was found to be equal to the number of carpels since they are continued as an extension to the carpels.

Table 5: Characteristics of floral parts of ten *Nymphaea* accessions

Acc. No.	Length of sepal (cm)	No of petals	Length of petal (cm)	No of stamens	Length of stamen (cm)	No. Of carpels & No. of stigmatic appendages
Acc.1	4.59	16.94^{b}	4.37^{b}	68.61^{e}	2.28^{cd}	18.55^{b}
Acc.2	6.22	19.94^{a}	4.17^{bc}	80.33^{d}	2.22^{d}	13.44^{d}
Acc.3	4.35	19.83^{a}	3.95^{cd}	115.16^{a}	2.38^{b}	20.72^{a}
Acc.4	4.18	19.88^{a}	3.74^{d}	47^{g}	2.21^{d}	13.50^{d}
Acc.5	5.30	15.11^{c}	4.80^{a}	52.94^{f}	2.48^{a}	20.44^{a}
Acc.6	5.30	15.94^{c}	5.01^{a}	23.05^{h}	2.03^{e}	13.33^{d}
Acc.7	4.30	13.16^{d}	3.95^{cd}	72.27^{e}	2.26^{cd}	17.05^{c}
Acc.8	4.1	11.83^{e}	3.75^{d}	52.66^{f}	2.26^{cd}	14.33^{d}
Acc.9	4.46	20.77^{a}	3.85^{cd}	104.88^{b}	2.32^{bc}	20.33^{a}
Acc.10	4.58	16.05^{b}	4.19^{bc}	99.49^{c}	2.32^{bc}	20.83^{a}
Mean	**4.9**	**17.15**	**4.18**	**71**	**2.28**	**17.25**
CV	**24.66**	**3.25**	**5.33**	**3.61**	**2.32**	**4.01**
CD	**NS**	**0.954**	**0.381**	**4.403**	**0.093**	**1.175**

The highest number of carpel was found in Acc. 10 (20.8) and Acc.3, Acc.5 and Acc. 9 were on par to this while the lowest number was observed in Acc. 2 (Table. 5). In all the accessions, the length of carpel was in a range of 4-5.5 mm. The carpels and stigmatic appendages of all the ten accessions were shown in Plate 6. The lengths of sigmatic appendages within a flower were uniform in each accession with an exception in Acc. 10. However between accession variation in the appendage length was observed. In Acc. 4 and Acc. 8 appendages are observed as small protuberance from the carpel rim hence they can't cover the

stigma surface after first day of anthesis. Among the day blooming types Acc. 10 exhibited longest appendages. In Acc. 5 and Acc.6 which are night blooming types, they are longer than other accessions and completely cover the stigmatic cup on second day of anthesis.

4.2.2.1.1.3 Successive increase in growth of pedicel

The increment in pedicel elongation on consecutive days of flower bud development till it plunged into water after flower opening were represented in the Table 6. The growth of pedicel continued even after flower opening and was maximum in Acc. 3.

The length of pedicel at flower opening and after flower sinking was presented in Table 7. Highest length of pedicel at the time of flower opening was in Acc. 1 (33.8 cm) and Acc. 9 (33.24 cm) and the shortest was in Acc. 5 (20.68) and Acc. 8 (21.93 cm). The maximum and minimum length of pedicel after flower sinking was observed in Acc. 9 (35.74 cm) and Acc. 5 (21.63cm) respectively.

Table 6: Successive increase in growth of pedicel of ten *Nymphaea* accessions

Acc. no. \ No. of days	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Acc.1	2	4	6.3	8.9	10	14	18	23	25.4	28	30	32	33.2	33.9	34.2	34.3	34.3	34.3	34.3
Acc.2	2.5	4.8	6.6	9	11	14.5	19	24	27	28	29	29.3	29.6	31	31.5	32	32	32	32
Acc.3	2.5	4	5.8	8	11	13	15.6	19.3	23	26	30	33	33.5	33.5	34	34	34	34	34
Acc.4	2	4.3	6.2	9	12	15.6	19	25	28	29.6	31.5	32	32.4	32.6	32.8	32.8	32.8	32.8	32.8
Acc.5	2	2.5	3.3	4	6	8.6	10.2	11	12	14.6	16.5	18.3	19	19.6	20	20.4	20.8	21	21.3
Acc.6	2.5	3.5	5.8	8	10	12.3	14.3	17	20.5	23	25	26.8	27.6	28.2	29	29.5	30	30.3	30.3
Acc.7	2	3	3.7	4.2	6.8	9.2	13	15	16	20	25	28.5	29.5	30	30.4	31	31.6	32	32
Acc.8	2	3	4	5.2	7.8	12	15.6	19	23	26	26.4	27	28	28	28.4	28.4	28.8	28.8	28.8
Acc.9	2.5	3	3.8	5.6	8.5	13	16	21	23	26	30	33	34	35	35	35	35.7	35.7	35.7
Acc.10	2	3	4.5	6	7.8	9.5	11.9	13.6	16	18.5	20	21	22	22.5	23	23.5	23.8	23.8	23.8


First day of flower opening

Table 7: Length of petiole at flower opening and after flower sinking

Accessions	Length of pedicel at flower opening (cm)	Length of pedicel after flower sinking (cm)
Acc.1	33.80^{a}	34.12^{b}
Acc.2	31.02^{c}	32.24^{c}
Acc.3	33.00^{ab}	34.03^{b}
Acc.4	31.72^{bc}	32.88^{bc}
Acc.5	20.68^{e}	21.63^{g}
Acc.6	29.42^{d}	30.32^{d}
Acc.7	29.26^{d}	32.22^{c}
Acc.8	21.93^{e}	28.82^{e}
Acc.9	33.24^{a}	35.74^{a}
Acc.10	22^{e}	23.82^{f}
Mean	**29.27**	**30.58**
CV	**2.85**	**2.65**
CD	**1.42**	**1.38**

4.2.2.1.1.4 Periodicity of flowering

The days for successive flower formation and total number of flowers produced in one month are presented in the Table 8 and they differed significantly among the ten accessions. The frequency of flower formation was lowest in Acc. 6 with about 16 day interval in between two successive flowering followed by the night blooming Acc. 5 with 12.7 days. However the flowers were produced almost all days in Acc. 1 and Acc. 3 at a frequency of 1.68 and 1.4 days respectively. Therefore the number of flowers produced in a month was maximum in Acc. 3 (21.33) followed by Acc. 1 (17) and Acc. 4 (14.6) and minimum was in Acc. 5 (2.6) and Acc. 6 (1.6). After fourth day of flowering, the floral parts *viz.*, sepals, petals and stamens decayed completely into a dark mucilaginous mass in Acc. 1, Acc. 2, Acc. 8, Acc. 9 and Acc. 10 on next 6-8 days. In the remaining

accessions *viz.*, Acc. 3, Acc. 4, Acc. 5, Acc.6 and Acc. 7 the flower sank into water and developed fruit.

Table 8: Periodicity of flowering of ten *Nymphaea* accessions

Accessions	**Days for successive flower formation**	**Total no. of flowers produced in one month**
Acc.1	1.68^{ef}	17^{b}
Acc.2	3.00^{e}	11^{d}
Acc.3	1.4^{f}	21.33^{a}
Acc.4	2.00^{ef}	14.66^{c}
Acc.5	12.77^{b}	2.66^{g}
Acc.6	16.22^{a}	1.93^{g}
Acc.7	7.66^{c}	4.66^{f}
Acc.8	5.66^{d}	5.66^{ef}
Acc.9	5.66^{d}	7.00^{e}
Acc.10	5.00^{d}	6.00^{ef}
Mean	**6.15**	**9.19**
CV	**11.73**	**9.53**
CD	**1.23**	**1.49**

4.2.2.1.2 Pollination biology

4.2.2.1.2.1 Anthesis

The blossom life was uniform and found to be four days in all accessions except Acc. 6, in which the flower opened for five days. The blooming process started with the opening of sepals. Among the ten accessions, Acc. 5 and Acc. 6 were night blooming type and the remaining accessions were day blooming type. The result of observations of time and duration of anthesis in each accession was presented in Table 9.

The flowers of all the day blooming accessions opened in morning Hrs between 7.00 - 9.00 am and closed in evening from 4.00 - 5.00 pm, while in the night blooming types (Acc. 5 and Acc. 6) the flower opening started from evening 7-7.30 pm and closed at 9 am -12.30 pm on second day. The same sequence is repeated consecutively for subsequent four days in Acc. 6 and three days in all remaining accessions.

The opening time varied and it took about 20-30 minutes for complete opening and 15-20 minutes for complete closing of the flowers irrespective of the accession. The flower of all accessions except Acc. 5 started bending downwards on the fourth day and completely sank in to the water on fifth day, while in Acc. 6 the same process occurred on fifth and sixth day respectively.

4.2.2.1.2.2 Anther dehiscence and stigma receptivity

Nymphaea accessions displayed strong protogyny, being receptive to pollen on the first day of flower opening whereas, shedding their own pollen on the second and third day of flower opening. Stigma receptivity was indicated by the presence of clear water on the bowl shaped stigmatic cup (Plate 8a) and was uniform in all the accessions. Any chance removal of the fluid is replaced partially during the course of opening on the first day. Drying and darkening of the stigma surface and inward curling of stigmatic appendages to the stigma cup indicates the loss of receptivity and it occurred on the second day of anthesis itself in all the ten accessions (Plate 8b). However the receptivity time and duration was varied in each accession (Table 9).

The anther dehiscence was started only on the second day of blooming and the anthers split longitudinally in all the accessions (Plate 8c). The time and pattern of anther dehiscence differed in night and day blooming *Nymphaea* species and presented in Table 10. In day bloomers the anther of different whorls

burst gradually from outermost to innermost whorl during successive anthesis. In the two night blooming accessions *viz.*, Acc. 5 and Acc. 6 the anthers dehisced simultaneously in all the whorls on the second day of anthesis itself.

In all the day blooming accessions, stigma receptivity was observed to initiate on the previous day evening of flower opening. The exudate started to appear on the stigmatic cup from 3.00 pm to 5.30 pm on previous day of flower opening in day blooming accessions. Hence in these accessions the stigma receptivity started about 16 to 18 Hrs before the flower opening. The stigma remained receptive 18 to 21 Hrs after flower opening in the day blooming accessions.

While in the two night blooming accessions the initiation of stigma receptivity was observed on the mid-day Hrs of the same day first anthesis. In the night blooming Acc.5 and Acc. 6 stigma became receptive almost 8-9 Hrs before flower opening and remained open up to 16 Hrs after flower opening. The duration of stigma receptivity in each accession was presented in Table 9.

In all accessions the anther dehiscence occurred after the flower opening on the second day of anthesis except in Acc. 5. In Acc. 5 the anther dehisced at 7.30 am on the second day of flower opening, but it was 10-12 Hrs prior to second anthesis, since it is a night blooming accession.

Table 9: Time and duration of flowering, anther dehiscence and stigma receptivity of ten *Nymphaea* accessions

Acc. no.	Blooming nature	Blooming Time	Time of stigma receptivity	Time of anther dehiscence
Acc.1	Day	7am - 5 pm (day 1)	3 pm (day 0) - 6 am (day 1)	9 am (day 2)
Acc.2	Day	8 am - 4 pm (day 1)	3.15 pm (day 0) -7.30 am (day 1)	9.30 am (day 2)
Acc.3	Day	7am - 5pm (day 1)	3 pm (day 0) -7am (day 1)	9 am(day 2)
Acc.4	Day	8 am - 4 pm (day 1)	3pm (day 0) – 7.30 am (day 1)	9.30 am (day 2)
Acc.5	Night	7pm (day 1)-9 am (day 2)	11 am (day 1) - 12 noon (day 2)	6.30 am (day 2)
Acc.6	Night	7 pm (day 1)-12 pm (day 2)	12 pm(day 1) - 2pm (day 2)	8.30 pm (day 2)
Acc.7	Day	8 am - 4 pm (day 1)	4.15 pm (day 0)-7am (day 1)	9.30 am (day 2)
Acc.8	Day	9 am - 4 pm (day 1)	4pm (day 0) – 6 am (day 1)	10.15 am (day 2)
Acc.9	Day	8 am – 4 pm (day 1)	4 pm (day 0) – 8 am (day 1)	9.45 am (day 2)
Acc.10	Day	8 am – 5 pm (day 1)	4.30 pm (day 0) – 8 am (day 1)	9.30 am (day 2)

4.2.2.1.2.3 Nature of pollination

During the study of pollination biology seed set was observed in unprotected as well as emasculated and unprotected flowers of five accessions namely, Acc.3, Acc.4, Acc.5 and Acc. 6 and Acc. 7. No fruit and seed formation was observed in bagged flowers. This indicates the geitinogamy or cross pollination in these accessions. From the Table 10 it can be seen that the highest percentage for seed set was recorded in Acc.4 (98 and 93%) and lowest in Acc. 7 (35 and 30 %) in both the conditions. The major insects visited in the flowers of day bloomers were honey bee, stingless bee and weevils. Their number was maximum on the first and second day of flower opening. Some beetles were observed to be visiting in the night blooming types also. Insect cadavers were found on the stigmatic cup of Acc. 1, Acc. 3, Acc. 4, Acc. 7 and Acc. 10.

Table 10: Percentage fruit set in *Nymphaea* accessions under open condition

Acc. no.	**Percentage of fruit set in open condition (%)**	
	Unprotected flower	**Emasculated and unprotected flower**
Acc. 3	80	84
Acc. 4	98	93
Acc. 5	87	90
Acc. 6	85	80
Acc 7	35	30

4.2.2.1.2.4 Pollen morphology and fertility

The pollen morphology which includes size, shape, dimension and fertility of pollen from all the ten accessions were evaluated and presented in Table 11. The findings indicated that the pollen grains of different *Nymphaea* accessions were diverse in morphology. All the day blooming accessions exhibited yellow

coloured pollen grain to the naked eye. The night blooming accessions Acc. 5 and Acc. 6 possessed creamy white pollen.

Table 11: Pollen morphology and fertility percentage of ten *Nymphaea spp.* under staining with 1% safranin

Acc. No.	**Colour**	**Size of fertile pollen (µm)**		**p/e ratio**	**Pollen shape (p/e)**	**Fertility (%)**
		Polar diameter (p)	**Equatorial diameter(e)**			
Acc.1	Yellow	26.4^{f}	24.86^{i}	1.06	Prolate Spheroidal	90.53^{ef}
Acc.2	Yellow	45.8^{a}	45.8^{a}	1	Spheroidal	72.4^{g}
Acc.3	Yellow	38.54^{b}	37^{b}	1.04	Prolate Spheroidal	88.9^{f}
Acc.4	Yellow	29.64^{e}	29.64^{h}	1	Spheroidal	95.75^{a}
Acc.5	Creamy white	32^{d}	33.4^{f}	0.95	Oblate Spheroidal	90.2^{ef}
Acc.6	White	37.21^{b}	37.21^{b}	1	Spheroidal	89.3^{ef}
Acc.7	Yellow	38^{b}	36.47^{c}	1.04	Prolate Spheroidal	92.6^{cd}
Acc.8	Yellow	30.65^{e}	30.65^{g}	1	Spheroidal	91^{de}
Acc.9	Yellow	35^{c}	35^{d}	1	Spheroidal	94.5^{ab}
Acc.10	Yellow	35^{c}	33.86^{e}	1.03	Prolate Spheroidal	93.9^{bc}
Mean	-	34.4	34.13	1.01	-	89.8
CD	-	**2.90**	**0.49**	-	-	**1.13**
CV	-	**1.70**	**0.29**	-	-	**1.74**

Microscopic observations exposed two types of pollen grains for each accession such as abnormal pollen having more or less shrunken as well as irregular shape and normal pollen which swelled to a spherical shape. The fertility percentage and size of fertile pollen differed significantly between the ten accessions in this study.

The viable pollen from five types (Acc.2, Acc.7, Acc.6, Acc.8 and Acc.9) exhibited spheroidal shape, four (Acc.1, Acc.3, Acc.5, Acc.10) were prolate spheroidal and Acc.4 was oblate spheroidal shape under the light microscope, and the equatorial region surrounded by a ring-like sulcus. As per the pollen dimension (*p x e* value) the Acc.1, Acc.7 and Acc.8 can be grouped as small and others as medium.Although the result of staining pollen with 1% safranin showed a significant difference in pollen fertility between all types, a majority (more than 70%) of them were fertile in all accessions (Table 11). Pollen fertility was highest in Acc. 4 (95.75%) and lowest in Acc. 2 (72.4%).

4.2.2.1.2.5 In vitro viability or germinability of pollen grain

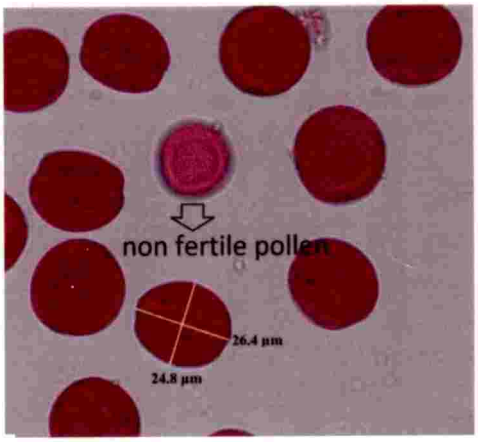
Swelling of pollen grains was observed in the germination medium for all the ten forms in the present investigation. However, different concentrations of sucrose solution resulted in varying pollen germination percentages (Table 12).

In all accessions except Acc. 8, maximum viability was observed in pollen grains kept in 10% sucrose. Among these Acc. 3, Acc. 4, Acc. 5, Acc. 6, Acc. 7 and Acc. 9 had more than 10% pollen viability. Least pollen fertility was observed in Acc. 1 (0.39%). Even though germination was observed in all the accessions, only seven accessions had a remarkable increase in the pollen tube length within 30 Hrs. The accessions *viz.*, Acc. 1, Acc.3, Acc. 4, Acc. 5, Acc. 6, Acc. 7 and Acc. 9 had pollen tube length greater than the diameter of the pollen grain (Plate 8). Among these, four accessions *viz.*, Acc.3, Acc.4, Acc.5 and Acc. 6 exhibited good seed set of 80%, 98%, 87% and 85% respectively under open condition while low

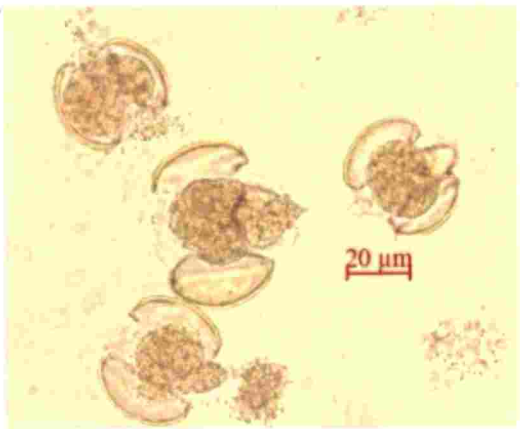
b) Receptive stigma with stigmatic fluid

a) Loss of stigma receptivity

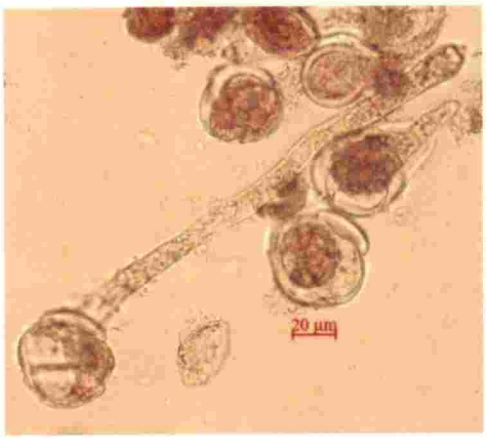
c) Anther dehiscence



d) Fertile and non-fertile pollen



e) Germinating pollen grains



f) Pollen tube growth

Plate 8: Stigma receptivity, anther dehiscence and pollen morphology of *Nymphaea* sp.

fruit set was observed in Acc. 7 with a percentage of 35. In the remaining three accessions *viz.*, Acc. 2, Acc. 8 and Acc. 10 only a few pollen grains were germinated with short pollen tube (Plate 8).

Table 12: Pollen viability percentage of ten *Nymphaea* spp. in different concentrations of sucrose solution

Acc. No.	**Viability (%)**		
	5% sucrose solution	**10% sucrose solution**	**15 % sucrose solution**
Acc.1	0.39	0.39^{h}	0
Acc.2	2.68	5^{g}	1.5
Acc.3	6.5	19.09^{d}	1.4
Acc.4	40.47	51.65^{a}	2.55
Acc.5	30.5	37^{b}	20
Acc.6	7.3	20^{d}	2.5
Acc.7	8.6	10.5e	1.7
Acc.8	7.04	3.57^{g}	0.84
Acc.9	20	26^{c}	19.2
Acc.10	6.03	8.7^{f}	8.3
Mean		**18.09**	
CV		**4.58**	
CD		**1.42**	

4.2.2.1.3 Fruit and seed development

Fruit and seed development was observed only in five accessions *viz.*, Acc. 3, Acc. 4, Acc. 5, Acc. 6 and Acc.7. The fruit development was observed under the water surface. The water lily fruit is a many seeded dehiscent capsule. All the floral parts persisted with the fruit in Acc. 3, Acc. 4 and Acc. 7 while in case of Acc. 5 and Acc.6 the sepals and petals detached and only stamens were remained. The carpels and stigmatic appendages remain attached to the fruit in all the seed setting accessions (Plate 9a-e).

Table 13: Fruit and seed characters of five seed setting *Nymphaea* accession

Acc. no.	No of days from flower opening to seed dispersal	Weight of fruit (g)	Size of fruit (cm)		Size of seed (mm)		100 seed weight (g)
			Length	Circumference	Length	breadth	
Acc. 3	22.33^{a}	13.33	5.56^{a}	5.76^{a}	1.60^{c}	1.08^{b}	0.07
Acc. 4	16.33^{bc}	13.66	4.43^{bc}	5.06^{b}	1.41^{d}	1.00^{bc}	0.08
Acc. 5	19.66^{c}	10.46	4.06^{c}	5.70^{a}	1.35^{d}	0.90^{c}	0.06
Acc. 6	25.33^{bc}	10.23	4.26^{bc}	5.93^{a}	2.70^{a}	1.96^{a}	0.05
Acc. 7	18.66^{b}	11.96	4.66^{b}	5.13^{b}	1.91^{b}	0.96^{bc}	0.05
Mean	20.66	11.93	4.6	5.52	1.79	1.18	0.05
CV	**9.69**	**18.65**	**5.02**	**3.65**	**5.96**	**8.49**	**41.23**
CD	**3.60**	NS	**0.42**	**0.368**	**0.191**	**0.181**	NS

Matured fruits were burst open and dispersed seeds into the water by about 20 days after flower opening (Plate 9f). The dispersed seeds were floating on the water surface since they are covered with thin mucilage and stored gas bubbles in the aril tissue. The biometric observations of fruit and seed were presented in Table 13. No significant difference was observed in case of fruit weight as well as 100 seed weight between the five accessions while, significant difference was observed in the size of fruit and size of seed. Maximum fruit length was noticed in Acc. 3 (5.56 cm) and minimum in Acc. 5 (4.06 cm). However maximum circumference of fruit was in Acc. 6 (5.93 cm) and minimum in Acc.4 (5.06 cm).

Highest seed size was observed in Acc. 6 and lowest in Acc. 5 in terms of both length and breadth (Plate 9g). The hundred seed weight was highest in Acc. 5 and lowest in Acc. 3.

4.2.2.1.4 Seed germination study

In all seed setting accessions except Acc. 4 the seed germination initiated within 4-6 days after dispersal, while Acc. 4 took around 17 days. The seed germination process was found to be similar in all the seed setting accessions *viz*., Acc. 3, Acc. 4, Acc.5, Acc. 6 and Acc. 7. A small portion of coleoptile emerged from the seed on the first day of germination and the green coloured coleoptile became noticeable as an awn like structure. Two to three days after emergence of coleoptile a single slender root was emerged. The root emergence was followed by the emergence of first leaf on fourth or fifth day of germination. The lamina was in unrolled condition at time of emergence (Plate 9h). The second and third leaf emerged at an interval of 10-13 and 20-25 days after the emergence of first leaf.

The results of seed germination study conducted with physical, mechanical and chemical methods in all the seed setting accessions within two days of seed dispersal were presented below (Table 14 and Table 15).

Acc. 3

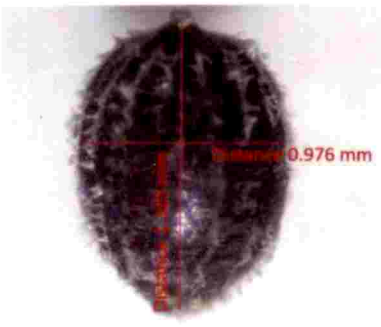
Acc. 4

Acc. 5

Acc. 6

Acc. 7

Fruit bursting and seed dispersal



Seed

Seed germination

Plate 9: Fruit and seed characteristics of five seed setting *Nymphaea* accessions

In natural condition majority of seeds of all accessions except Acc. 4 were germinated after 30 days without any treatment. Acc. 3 and Acc. 6 exhibited highest seed germination at 10 days of treatment in control. Highest germination after 30 days was observed in the seeds of Acc. 6 (71.6%) followed by Acc. 3 (68%) and Acc.5 (67%) in control. In Acc. 4 the percentage of seed germination was very low as 5.6 even after 30 days (Table 14). Both the seed lots, treated with 60^0 C water and mechanically scarified with sand paper showed an increased number of germinated seeds in the first 10 days, but a decrease in the germination percentage was observed after 30 days than the control.

Table 14: Seed germination percentage of five *Nymphaea* spp. with physical and mechanical methods

Germination percentage (%)			
	Control		
Acc. no.	10 day	20 day	30 day
Acc. 3	17	36	68
Acc. 4	0	3	5.6
Acc. 5	12	31	67
Acc. 6	17	39.6	71.6
Acc. 7	8.6	26.6	49.2
Hot water treatment			
Acc. 3	20	32	64
Acc. 4	0	2	3
Acc. 5	15	32	48
Acc. 6	10	25	45
Acc. 7	15	28	46
Mechanical scarification			
Acc. 3	23	33	54
Acc. 4	0	2	3.2
Acc. 5	14	28	43
Acc. 6	19	27	48
Acc. 7	21	30	34

The treatment with all concentrations of H_2SO_4, Ethrel and GA_3 showed an enhancement in seed germination in all the five accessions over the control (Table 15).

Table 15: Seed germination percentage of five *Nymphaea* spp. with chemical treatment

Control						
	Germination percentage (%)					
Acc. no.	10 day		20 day		30 day	
Acc. 3	17		36		68	
Acc. 4	0		3		5.6	
Acc. 5	12		31		67	
Acc. 6	17		39.6		71.6	
Acc. 7	8.6		26.6		49.2	
Acc. no.	**GA_3(50 ppm)**			**GA_3(10 0ppm)**		
	10 day	20 day	30 day	10 day	20 day	30 day
Acc. 3	40	68	74	2	72	86
Acc. 4	8.6	16.6	31.6	20	44	61
Acc. 5	28	34	76	59	78	92.3
Acc. 6	24	53	76	56	76	88
Acc. 7	16	43	52	42	57	60
Acc. no.	**H_2SO_4 (1%)**			**H_2SO_4 (5%)**		
	10 day	20 day	30 day	10 day	20 day	30 day
Acc. 3	39	63	73	66	81	84.5
Acc. 4	8.6	18	36.6	20.6	34.6	54.6
Acc. 5	23	41	70	48	76	93
Acc. 6	32.6	53.6	70.6	51	69	80
Acc. 7	23	39	55	54	62	67
Acc. no.	**Ethrel (50 ppm)**			**Ethrel (100 ppm)**		
	10 day	20 day	30 day	10 day	20 day	30 day
Acc. 3	32	43	75	40	53	81
Acc. 4	4	7	24	10	32	45
Acc. 5	16	34	62	31	60	79
Acc. 6	26.6	51	71.6	41	68	79
Acc. 7	18	41	50	36	52	60

100 ppm Ethrel, 5% H_2SO_4 and 5 ppm GA_3 treated seed lots exhibited highest germination percentage in each treatment. In Acc. 3, Acc. 5, Acc. 6 and Acc. 7 the increment was observed after 10 days itself. However in Acc. 4 maximum seed germination was recorded after 30 days in each chemical

treatment. Highest germination percentage was associated with seed lots treated with 100 ppm GA_3 in all Accessions.

4.2.2.2 Asexual Reproduction

In all accessions including seed setting types the major propagule is the rhizome. Besides rhizome propagation, the asexual reproduction through vivipary was found to be prominent in some accession. The details about asexual reproduction in each accession *viz.*, days taken to produce 1st leaf, days taken for root formation, days taken for 1st flower bud, diameter of flower and total no. of flowers produced in one month from the new plantlets propagated from each method were presented in the Table 16 and 17.

4.2.2.2.1 Rhizome as a natural propagule

Rhizome propagation was more pronounced in Acc. 2 and Acc. 8 in which other propagation methods are absent. Acc. 2 took minimum days for the emergence of first leaf (13.8) and root (10.6) from the rhizome followed by Acc. 8. Flowering also initiated first in the rhizome propagated plants of Acc. 2 and Acc.8. In all the ten accessions the diameter of flower and total number of flowers produced on the new plants developed from the rhizome were slightly less than that of their mother plant during the initial months.

Table 16: Asexual reproduction in *Nymphaea* spp.; rhizome as a natural propagule

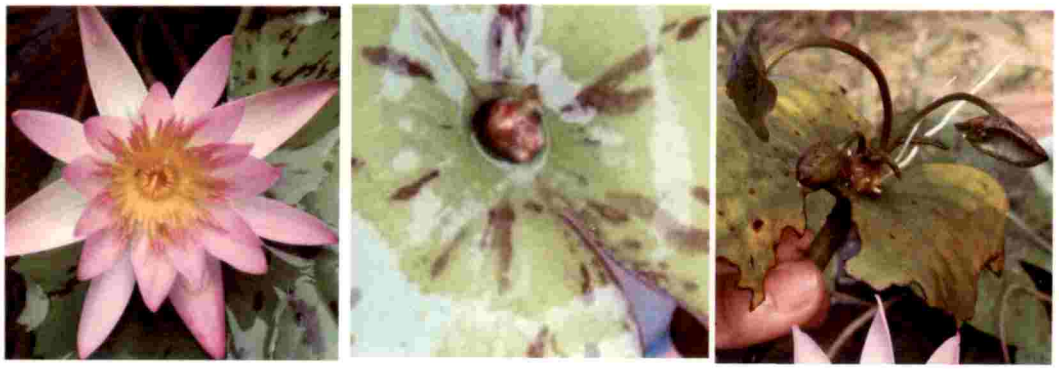
Acc. no.	**Days taken to produce 1st leaf**	**Days taken for root formation**	**Days taken for 1st flower bud**	**Diameter of flower**	**Total no. of flowers produced in one month**
Acc.1	35.00^{b}	34.00^{b}	82.33^{cd}	6.56^{abc}	13.00^{a}
Acc.2	11.33^{e}	10.66^{d}	76.00^{d}	4.36^{ef}	8.33^{c}
Acc.3	36.00^{ab}	34.33^{b}	95.66^{abc}	5.80^{cd}	14.33^{a}
Acc.4	34.66^{b}	34.33^{b}	106.33^{a}	5.13^{de}	11.33^{b}
Acc.5	42.33^{a}	42.66^{a}	107.33^{a}	7.06^{a}	3.33^{f}
Acc.6	25.00^{c}	20.66^{c}	86.00^{bcd}	6.86^{ab}	2.33^{f}
Acc.7	24.66^{c}	20.33^{c}	97.66^{ab}	5.13^{de}	5.66^{e}
Acc.8	18.00^{d}	14.00^{d}	80.66^{d}	4.00^{f}	6.00^{e}
Acc.9	35.33^{b}	36.00^{b}	101.00^{a}	6.56^{abc}	8.00^{cd}
Acc.10	34.66^{b}	33.66^{b}	100.66^{ab}	5.86^{bcd}	6.66^{de}
CV (0.5%)	**12.99**	**8.75**	**9.28**	**10.79**	**10.07**
CD	**6.57**	**4.18**	**14.71**	**1.05**	**1.35**

4.2.2.2.2 Leaf and root as natural propagules

In Acc.1 and Acc.7 new plantlets were found to be emerged from the upper portion of the mature leaf at the point of attachment of petiole (Plate 10a and 10b). There after they started growing independently. The new plantlets are emerged only when the mature leaf detached from the mother plant. In Acc. 5 root tip was observed as a natural propagule to produce new plantlets (Plate 10c). The results of observations regarding the days taken to produce first leaf, days taken for root emergence, days taken for first flower bud formation, diameter of flower and total number of flowers produced during one month in plantlets developed from leaf and root proliferation are presented in Table 17.

Acc. 1 took 2.5 days for root emergence and 6.5 days for 1st leaf formation. The first flower bud was observed after 48 days in Acc. 1. But in Acc. 7, the root emergence initiated by 5.25 days and first leaf formation by 11.5 days after detachment of the mature leaf. Acc.7 took 67 days for the formation of first flower bud in plantlet proliferated from leaf. The flower diameter was observed as 5.3 cm and 4.9 cm in Acc. 1 and Acc. 7 respectively. Acc. 1 produced around 13 flowers and Acc. 7 nearly six flowers during first one month of flowering.

In Acc. 5 the emergence of new leaves and roots were observed from the root tips of mother plant around 33 days after planting a mature rhizome. The first flower bud was observed within 85.5 days and the flower diameter was 8 cm. and nearly three flowers were produced during first month of flowering. In all the methods it could be seen that the size of flower and number of flower produced was little lower than the parent plant in the initial months of flowering in all accessions.



a) Leaf proliferation in Acc. 1

b) Leaf proliferation in Acc. 7

c) Root tip proliferation in Acc. 5

Plate 10: Asexual reproduction in *Nymphaea*

Table 17: Asexual reproduction in *Nymphaea* spp.; leaf and root as natural propagule

Acc. no.	**Days taken to produce 1st leaf**	**Days taken for root formation**	**Days taken for 1st flower bud**	**Diameter of flower (cm)**	**Total no. of flowers produced in one month**
Acc. 1 (leaf proliferation)	6.75[a]	2.50[a]	48.00[a]	5.37	12.75
Acc. 7 (leaf proliferation)	11.50[b]	5.25[b]	67.50[b]	4.90	5.75
Acc. 5 (root proliferation)	32.75[c]	32.75[c]	85.50[c]	7.90	3.00
CD (0.5%)	**10.82**	**10.62**	**7.31**	**11.88**	**8.70**
CD	**2.94**	**2.29**	**7.83**	**1.15**	**0.99**

5.2.3 Genetic Parameters of Variability

The genetic analysis of the quantitative characters of leaf, flower, fruit and seed of the ten *Nymphaea* accessions showed significant variation. The estimates for the components of variability such as phenotypic coefficient of variance (PCV), genotypic coefficient of variance (GCV), heritability (H^2) and Genetic gain (GG) of 11 leaf and flower characters observed in 10 accessions and fruit and seed characters in five seed setting accessions were presented in Table 18 and Table 19 respectively.

Among the leaf and floral characters evaluated, high H^2 value coupled with high GG was observed in lamina width, lamina length, circumference of flower bud, diameter of fully opened flower, number of stigmatic appendages, number of petals and number of stamens. In these characters the difference between GCV and PCV was very low. Among these the number of anthers exhibited maximum variation as indicated by its high GCV and PCV. The

characters *viz.*, pedicel length, length of flower bud and the length of sepal exhibited low GCV of 3.5%, 9.1% and 8% respectively. The genetic gain was high for most of the characters except pedicel length and sepal length (Table 18).

Table 18: Genetic variability of leaf and flower characters of *Nymphaea*

Character	**Mean**	**PCV**	**GCV**	**H^2**	**GG**
Lamina width	15.21	18.38	17.6	97.55	35.81
Lamina length	16.87	17.82	17.36	89	33.7
Pedicel length	15.14	3.6	3.5	94.8	7.13
Length of flower bud	4.99	3.29	9.1	88	17.4
Circumference of flower bud	9.19	10.91	10.76	97	21.8
Diameter of fully opened flower	29.27	15.75	15.49	96.84	31.36
Number of stigmatic appendages	17.25	19.43	19	95	38.55
Number of petals	17.15	18.24	17.95	96	36.38
Length of sepals	4.96	26	8	11.32	6.1
Length of petals	4.18	11.21	9.92	78.3	17.93
Number of stamens	71	40.5	40.3	99	82.75

- PCV & GCV (Phenotypic Coefficient of Variation and Genotypic Coefficient of Variation) - Low: less than 10%, Moderate: 10-20 %, High: more than 20% (Sivasubramanian and Menon, 1973)
- H^2 (Heritability) -Low: less than 30%, Moderate: 30-60%, High: more than 60% (Johnson *et al.*, 1955)
- GG (Genetic Gain)- Low: less than 10%, Moderate: 10-20 %, High: more than 20% (Johnson *et al.*, 1955)

Among the fruit and seed characters PCV, GCV and H^2 was high for length and breadth of seed. These characters can bring improvement in population about 58% and 73.7% respectively (Table 19).

Table 19: Genetic variability of fruit and seed characters of *Nymphaea*

Character	**Mean**	**PCV**	**GCV**	**H^2**	**GG**
Length of fruit	4.6	13.35	12.37	85.9	23.6
Circumference of fruit	5.52	7.79	6.7	75	12
Weight of fruit	11.93	20.18	7.7	14.6	6.09
Length of seed	1.797	31.1	30.9	92.8	58
Breadth of seed	1.18	37.9	36.84	94.4	73.7
100 seed weight	.059	3.38	2.9	75	5.23

4.3 EXPERIMENT 3: HYBRIDIZATION IN SEED PRODUCING *NYMPHAEA* SPP.

Among the 20 crosses between seed setting accessions *viz.,* Acc. 3, Acc. 4 Acc. 5, Acc. 6, and Acc. 7, only three crosses (Acc. 4 x Acc. 3, Acc. 3 x Acc.4 and Acc. 4 x Acc. 7 resulted in seed setting. 20-25 percentage fruit set was observed in these three crosses. Details regarding the size of seed, 100 seed weight, germination percentage and number of days taken for germination were presented in Table 20. About 40-50 days taken for the completion of fruit development and seed dispersal from the date of hybridization in all the three crosses. Around 250 seeds were produced in each successful cross. The seeds of all the three crosses were germinated without any scarification procedures, but the percentage of germination was low in all crosses with 15-20 % seedlings appearing over a three week period. There was no significant difference between the seeds of three crosses in case of seed size and 100 seed weight. The growth of seedlings was very slow and only four to nine seedlings survived to maturity from each cross.

Table 20: Successful crosses among seed setting *Nymphaea* accessions

Successful Crosses	**Size of seed**		**100 seed weight(g)**	**Germination percentage**
	Length (mm)	**Breadth (mm)**		
Cross 1 (Acc. 4 x Acc. 3)	1.4	1	0.09	20
Cross 2 (Acc. 3 x Acc.4)	1.41	1.02	0.09	18
Cross 3 (Acc. 4 x Acc. 7)	1.4	1	0.09	16

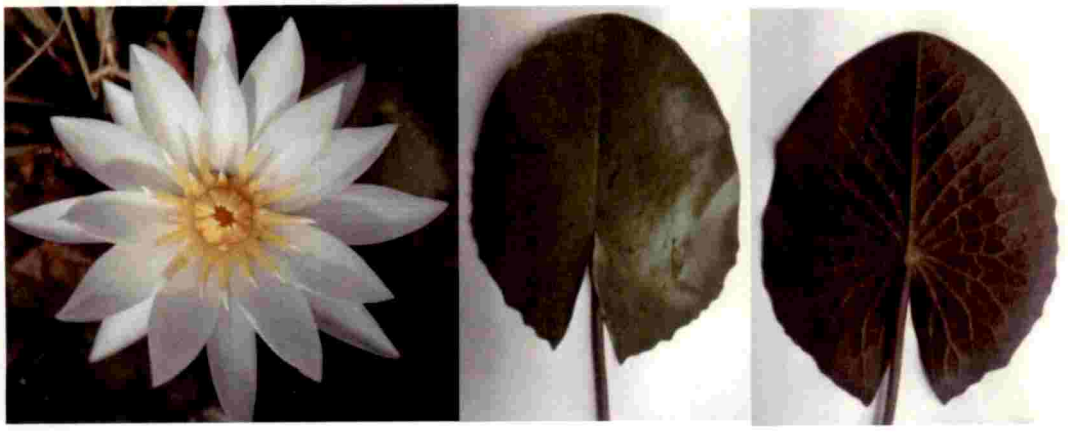
The pattern of seed germination, emergence and growth of leaves and roots were found to be similar to that of the seed setting accessions explained in previous section. The fourth and successive leaves emerged in the seedlings of cross 1was with faster rate than the seedlings from other cross seedlings. The seedlings of cross 2 and cross 3 attained its five leaf stage with a spread of 12- 17 cm even after six months. Flowering was observed after five months, in plants produced from cross 1 only.

Cross 1 was accomplished with white colour variant of *N nouchali* as ovule parent and a blue flowered hybrid as pollen parent. About 20% of seeds of cross 1 were germinated within 18 day and first root and leaf emerged on the third and fifth day of germination respectively. Among this only four plants survived after three months and initiated flowering on 20-21 weeks from germination.The seedlings of only cross 1 produced flower with in five month of seed germination. Details regarding the morphological characters of F_1 hybrid and both ovule and pollen parent were exhibited in the Table 21.

Table 21: Morphological characteristics of parental plants and F_1 hybrid of *Nymphaea*

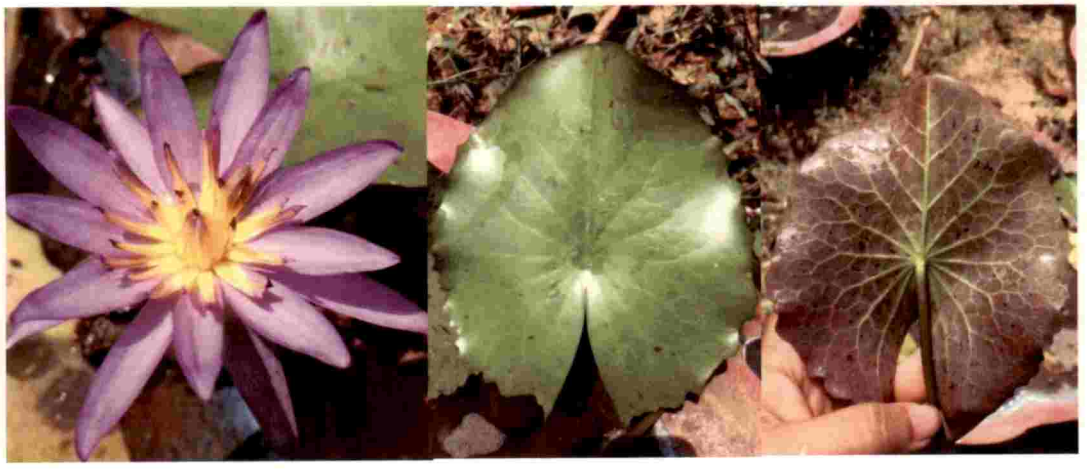
Characters	**Ovule parent (Acc. 4)**	**Pollen parent (Acc. 3)**	**F_1 hybrid**
Percentage of seed germination	3	36	18
Flower colour	White	blue	Light violet (C)
Colour of adaxial surface of leaf	Dark green with dark markings	Light green	Green with dark markings (A)
Colour of abaxial surface of leaf	Dark violet	Light green	Violet (C)
Lamina length (cm)	17-20	15.5-17	16-18 (C)
Lamina width (cm)	13-16	14-16	13-16 (A)
Leaf margin	Smooth	irregular	Irregular (B)
Leaf teeth number /10 cm	2-3	10-13	5-7 (C)
Leaf tip	Acute	Flat	Acute (A)
Length of flower bud	4-4.5	4-4.5	4-4.5 (A,B)
Circumference of flower bud (cm)	3.5-4	5-5.7	3.5-4 (A)
Diameter of flower (cm)	8-9	9-9.5	8-8.5 (C)
Number of petals	15-19	18-20	12-16 (D)
Number of stamens	45-58	98-120	34-42(D)
Number of stigmatic appendages	12-16	18-21	24-26 (D)
pollen colour	Yellow	yellow	Yellow (A,B)
Polar diameter of pollen	29.64	38.54	27.4
Equatorial diameter of pollen	29.64	37	25.8
Shape of pollen	Spheroidal	Prolate spheroidal	Prolate spheroidal (B)
Viability of pollen (%)	51.65	19.09	10 (D)

A) Feature similar to ovule parent B) Feature similar to pollen parent C) Intermediate character D) Unique character (lower or higher than both the parents)



Flower, adaxial and abaxial leaf surface of ovule parent (Acc. 4)

Flower, adaxial and abaxial leaf surface of pollen parent (Acc. 3)



Flower, adaxial and abaxial leaf surface of Hybrid progeny

Figure 11: Flower and leaf of parental *Nymphaea* accessions and hybrid progeny

The flower colour of F_1 hybrid was light violet which is intermediate to both the parents (white x blue) while the leaf shape was almost similar to the maternal parent (Plate 11). Colour of adaxial surface of leaf, lamina width, leaf tip and circumference of flower bud of the hybrid progeny was found to be similar with the ovule parent. The hybrid progeny have an intermediate character for flower colour, colour of abaxial surface of leaf, lamina length, number of leaf teeth per 10 cm length and diameter of flower. In case of the number of petals, number of stamens, number of stigmatic appendages and viability of pollen the hybrid progeny exhibited a unique range either lower or higher to both the parents. The flowers produced in the initial months were small with few petals and stamens compared to that of the parent plants.

Discussion

5. DISCUSSION

Aquatic plants often pose considerable taxonomic problems. *Nymphaea* is one such complex and still poorly understood genus deserving of a more careful and detailed consideration of its proper taxonomic classification. An exploration and collection of germplasms of *Nymphaea* was carried out to get a clear picture of the variability occurring in natural waterlily growing regions of Northrn Kerala. An attempt has been made here to characterize the collected types on the basis to leaf, flower and reproductive biology for species identification and further crop improvement through hybridization to develop new colour variants of water lily. The results obtained in the study are discussed below.

5.1 EXPERIMENT 1: EXPLORATION AND COLLECTION OF GERMPLASMS OF *NYMPHAEA* SPP.

A detailed survey was conducted in Kasaragod, Kannur, Kozhikode and Malappuram Districts and private nurseries in Thrissur. 14 types of waterlily were collected for the study, which included natural growing and popular commercially cultivated types. These were given accession numbers according to the date of collection. Based on flower colour variation, there were accessions with different shades of pink (Acc.1, Acc. 2, Acc. 6, Acc. 9, Acc. 10 and Acc. 13), violet blue (Acc. 3 and Acc. 8), white (Acc. 4, Acc. 5 and Acc. 11), violet (Acc. 7) purple pink (Acc.12) and yellow (Acc. 14).

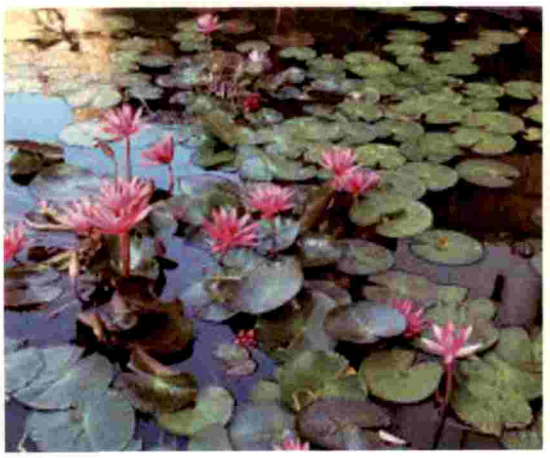
The majority of the wide spread natural growing areas of *Nymphaea* were observed in Kannur and Kasaragod district and it revealed the presence of white flowered type spread in lowlands, ponds and canals. Tom (2015) reported the presence of only purple red, violet, and white colour variants of *Nymphaea* in the natural waterlily growing tracts in Thrissur district.

In our survey, the light pink flowered Acc. 1 was observed in a single location in Malappuram district. Acc. 2, Acc. 8, Acc. 12 and Acc. 14 were collected from a private nursery in Thrissur. Two waterlily types from the previous study of Tom (2015) were also included for further evaluation as Acc. 3 and Acc 5. The white flowered Acc. 4 was collected from Teerthankara, Kasaragod. White flowered waterlilies were not known in cultivation but comes up well in large water bodies like ponds and canals (Ansari and Jeeja, 2009). In our survey the dark pink flowered water lily (Acc. 6), collected from Vadakara was found in some isolated ponds and marshy lands in Kozhikode and Kannur districts. Acc. 7 was collected from Instructional farm, College of Agriculture, Padannakkad. Acc. 9, Acc. 10, Acc. 11 and Acc. 13 were collected from different permanent ponds of Kozhikode and Malappuram districts. The Acc. 1, Acc. 3, Acc. 5 and Acc. 9 were also observed to be cultivated in stagnant water bodies in some nurseries (plate 12).

Ten among these 14 accessions were selected for further detailed evaluation in pot culture. These included seed setting types (Acc. 4, Acc. 5, Acc. 6, and Acc. 7) and viviparous types (Acc. 1, Acc. 5 and Acc. 7). Among these two were night bloomers (Acc. 5 and Acc. 6) and others were day bloomers.

5.2 EXPERIMENT 2: EVALUATION OF COLLECTED TYPES

Characterization plays a key role in the assessment of genetic diversity and assist in species identification. A number of species-specific morphological characters of waterlily were reported in previous studies (Ansari and Jeeja, 2009 and Begum *et al.*, 2010). Some of the important characters that could be considered as diagnostic features for identification of *Nymphaea* are colour and morphological variations in floral parts, as well as mature leaves. Even though the ten accessions of *Nymphaea* showed general characters of Nympheaceae, they could be identified by specific leaf and floral morphological characters.



a) Chirakkal chira, kannur

b) Instructional farm, COA, Padannakkad,

c) Teerthankara

d) Kizhisseri (house hold), Malappuram

f) Mekkattil nursery, Thrissur

Plate 12: Survey in waterlily growing regions and nurseries

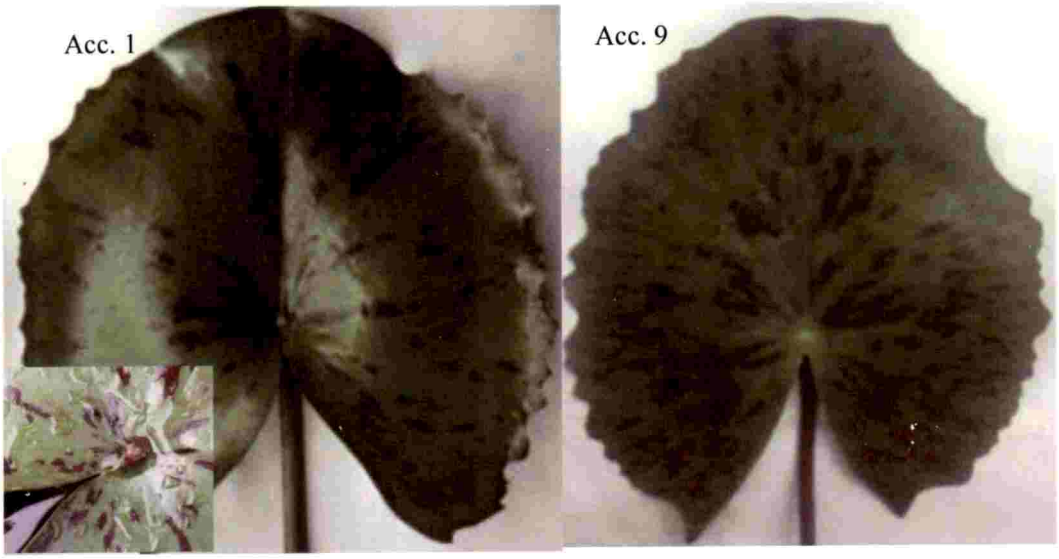
The results of preliminary evaluation showed that three among the collected accessions showed resemblance with identified *Nymphaea* species. The Acc. 6 exhibited some similarity with *N. rubra* and Acc. 7 with *N. micrantha* in terms of leaf and flower colour. Although the Acc. 4 showed similarity with white colour variant of *N. nouchali*, its flower was more close to *N. malabarica*. The results of detailed evaluation of morphology and reproductive biology are discussed below.

5.2.1 Leaf Characters

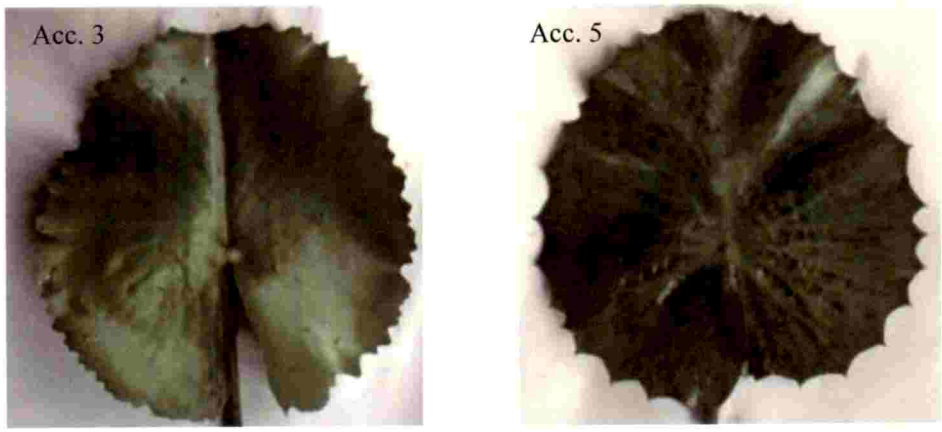
At the time of emergence, the immature leaves of all the ten accessions were in rolled condition and they complete their unrolling gradually by reaching water surface as seen in plate 13a. The immature leaves of all the accessions reached water surface within three to seven days. The unrolling pattern of leaf above the mud surface in the present study was similar to the findings of Fahida (2010) and Tom (2015). Similar observation was also was made by Minimol (2004) in the sacred lotus of the genus *Nelumbo,* coming under the Nympheaceae family.

Based on the qualitative and quantitative characters, the leaves of all the ten accessions differed from each other for one or more characters. Acc. 1 and Acc. 9 have same colour and pattern of violet spot or patches on the adaxial side of the leaf lamina. But they differed at the point of attachment of petiole to lamina (plate 13b). Acc. 1 possessed a nib like projection at this point on the adaxial surface. The truncate leaf tip in Acc. 3 (Plate 13c) and retuse leaf tip in Acc. 9 (Plate 13b) can be considered as a diagnostic character for these accessions. But Tom (2015) observed emarginated leaf tip in Acc. 3. In the detailed evaluation, spiny leaf margin and sinus overlapping was observed only in Acc. 5 (Plate 13c). This character was critical for the identification of *N. pubescence* and some intraspecific variations were reported in this species. Hence, Acc. 5 might be a variant of *N. pubescence*.

a) Stages of unrolling of leaf lamina



b) Similarity of leaves of Acc. 1 and Acc. 9



c) Leaves of Acc. 3 with truncate leaf tip and Acc.5 with spiny leaf margin

Plate 13: Leaf characteristics of *Nymphaea*

The leaf length to width ratio gives an approximate shape of leaf. Acc. 1, Acc. 5 and Acc. 8 exhibited almost similar length and width in a single leaf of each accession and their length and width ratio is 1.01, 1 and 1.01 respectively, which is close to one. Therefore the leaf shape could be assumed as round in these accessions. In Acc. 2 and Acc. 4 the ratio was 1.23 and 1.24 respectively and had more oval shaped leaf compared to others.

With respect to the biometric characters like length and longevity of the leaf, Acc. 2 was found to be superior over remaining accessions. Despite the similar dimensions of pots used for the study, wide variation in the petiole length was observed between accessions. This could be considered as specific to the particular accession.

Ansari and Jeeja (2009) discussed the morphological characteristics of *N. nouchali*, *N. rubra* and *N. micrantha* in detail. The leaf characters like colour on both surfaces, shape, margin and leaf tip the Acc. 4, Acc. 6 and Acc. 7 in the present study were more or less similar to specified species like *N. nouchali*, *N. rubra* and *N. micrantha* respectively. In contrast to this reference, Fahida (2010) reported that the abaxial surface of white colour variant of *N. nouchali* as light green with purple colouration but it was found to have uniform violet or coffee brown in the present study.

5.2.2 Reproductive Biology

Knowledge in reproductive biology of plants can help to assist the adaptive significance and homology of descriptive plants used in systematics. In *Nymphaea* genus this study could give insight into the delimitation of the classification of intra and inter specific hybrids. The detailed information of the reproductive biology in the *Nymphaea* species will be helpful for developing effective strategy for their conservation and utilization.

5.2.2.1 Sexual Reproduction

The results of floral biology, pollination biology, fruit and seed characteristics and seed germination of *Nymphaea* are discussed below.

5.2.2.1.1 Floral biology

5.2.2.1.1.1 Growth pattern of flower bud

The night blooming accession showed superiority over day bloomers in the flower bud characters *viz*., length and circumference of flower bud and diameter of fully opened flower. Acc. 5 had the maximum value for these characters followed by Acc. 6. The Acc. 8 exhibited lowest length and circumference of flower bud as well as diameter of fully opened flower. This revealed the inferiority of Acc. 8 with respect to all the flower bud characters considered (plate 14a). The superiority of night blooming water lily with respect to the flower bud characters was already mentioned by Tom (2015).

In contrast to this result, night bloomers were inferior with regard to the growth rate of pedicel since they took more days to reach the water surface. The number of days taken by the flower bud to reach the water surface could be reflected on the periodicity of flowering. As mentioned by Tom (2015), a direct proportionality could be observed for the diameter of fully opened flower with the length and diameter of matured flower bud in all accessions.

5.2.2.1.1.2 Flower morphology

The floral formula observed in the present investigation was $\oplus \; \overset{\nearrow}{\varphi} \; K_4 \; C_{10\text{-}24} \; A_{20\text{-}120} \; \underline{G} \; (_{10\text{-}23})$ which matched the findings of Wiersema (1988). He reported some general aspects of floral biology in *Nymphaea* sp. such as 4 sepals, 7- 40 petals, 20-700 stamens and 5-47 carpels. Considerable variation in

the petal colour of waterlily was observed. The colour of floral parts forms an important character for identifying most of the species, but intraspecific colour variants were observed in *N. nouchali* and *N. omrana* (Ansari and Jeeja, 2009).

The recognition of floral parts with certainty in *Nymphaea* plants is also often difficult since they exhibited gradual transformation from the outer parts to the inner ones. Even if some accessions showed similar petal colour, we could identify them with their outer surface of sepals, which showed varying colour and patches. In the present study, the petal colour of Acc. 1 and Acc. 10 were light pink, but the outer surface of sepals of Acc. 1 showed distinct narrow purple lines along its length and uniform green in Acc. 10. Although the petals of Acc. 7 and Acc. 9 were purple violet, the outer surface of sepal was dark green with purple lines in Acc. 7 and uniform light green with purple shade along the margin in Acc. 9. The inner surface of the sepals was almost similar to the petals in all accessions.

Sepals of Acc. 2 were distinct from all other accessions in terms of number, colour and size. The feather like sepals of Acc. 2 was five to seven in number while all others had four. Size of each one also varied in a single flower from 3.5 cm to 7 cm (Plate 14b). Each sepal was attached to the flower base in a twisted fashion. Due to this twisting, extra length and number, the flower couldn't expose fully during anthesis. Such a distinct character couldn't be observed in any identified species.

There was a reduction in the intensity of brightness of petal colour towards the end of the blossom life except in case of Acc. 4 and Acc. 5 since they are white in colour. In all the accessions, the petals showed a gradation in size from the outer whorl to the inner whorl (Plate 14c).

With respect to stamen, the day and night blooming accessions exhibited discrete variation. In day bloomers, each stamen consisted of a filament, anther

a) Flowers with highest (Acc. 5) and lowest (Acc. 8) diameter

b) Sepals of Acc. 2 showing difference in length

c) Size gradation of petals

Plate 14: Characteristics of floral parts in *Nymphaea*

lobe and a sterile appendage on the tip in all the accessions. The night blooming Acc. 5 and Acc. 6 were devoid of this terminal appendage and had wider anther lobe. In most of them, the colour of the anther lobe was yellow irrespective of the flower colour, which might help to attract the pollinators. Variability was observed in the colour of terminal appendage of stamen between accessions and they are almost similar to the flower colour. Gradual reduction in the size of filaments, anther lobes and appendages were observed from outer most to the inner whorls in all accessions (Figure 15a).

Acc. 6 possessed creamy white or light pink stigma surface in contrast to all other accessions which were yellow in colour (Plate 15b). The lengths of sigmatic appendages within a flower were uniform in each accession with an exception in Acc. 10 (Figure 15c).

5.2.2.1.1.3 Successive increase in growth of pedicel

It could be seen that the different accessions exhibited same pattern for pedicel growth. The graph plotted with the length of pedicel at each day against the number of days exhibited the maximum pedicel elongation at just prior and after to the flower bud reaching the water surface (Figure 3). The growth of pedicel continued even after flower opening, but the growth rate decreased and stopped completely with the sinking of flower into water.

A similar result was reported by Tom (2015) in five *Nymphaea* accessions collected from central Kerala and revealed the variation in the rate of pedicel elongation. The same pattern of pedicel growth was observed in sacred lotus in the Nymphaeaceae family (Minimol, 2004).

The accessions collected from different natural growing areas had larger flower bud, flower diameter and pedicel length at the location of collection. Wide difference was observed in respect of pedicel length than flower and bud

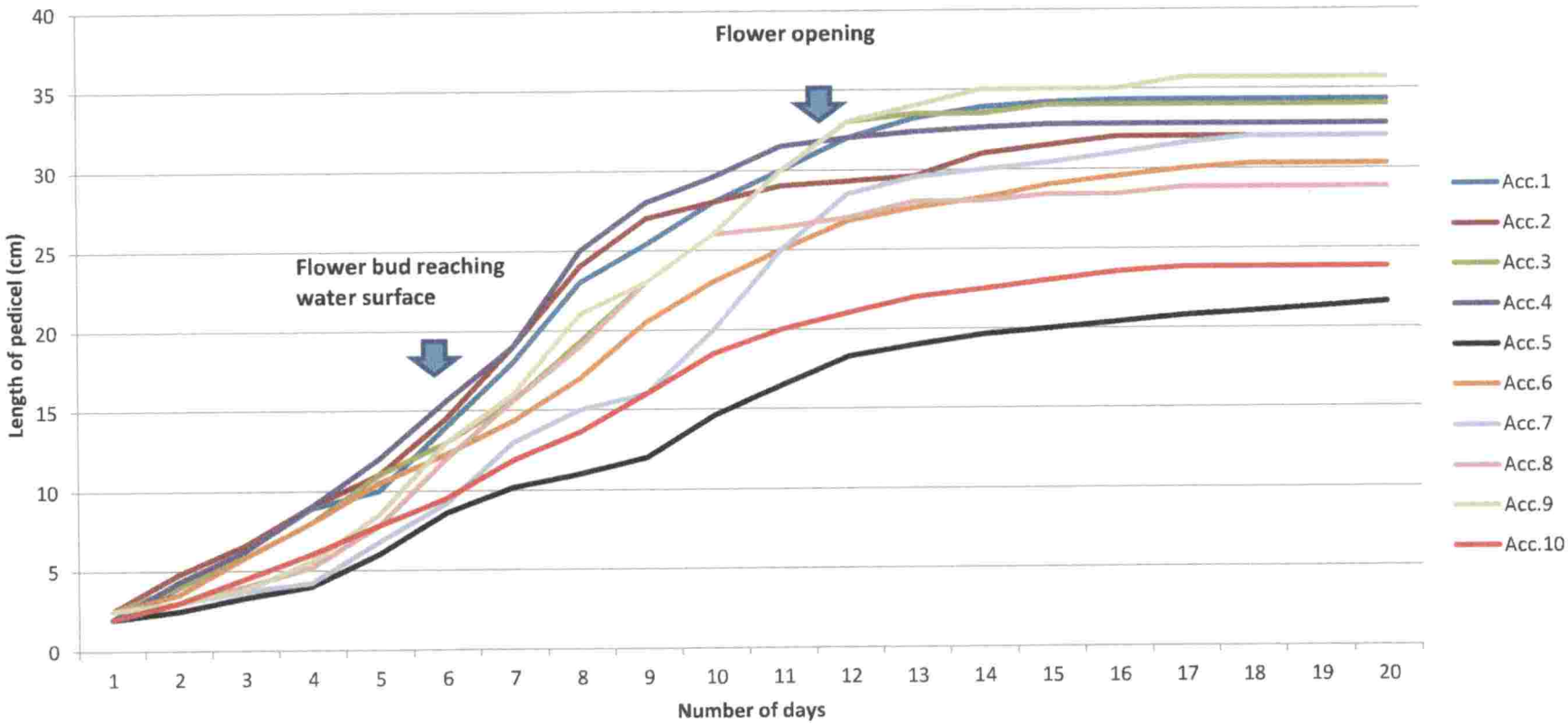


Figure 3: Successive increase in growth of pedicel in *Nymphaea* accessions

characters, which indicated the increment in the pedicel length according to the increase in water level.

5.2.2.1.1.4 Periodicity of flowering

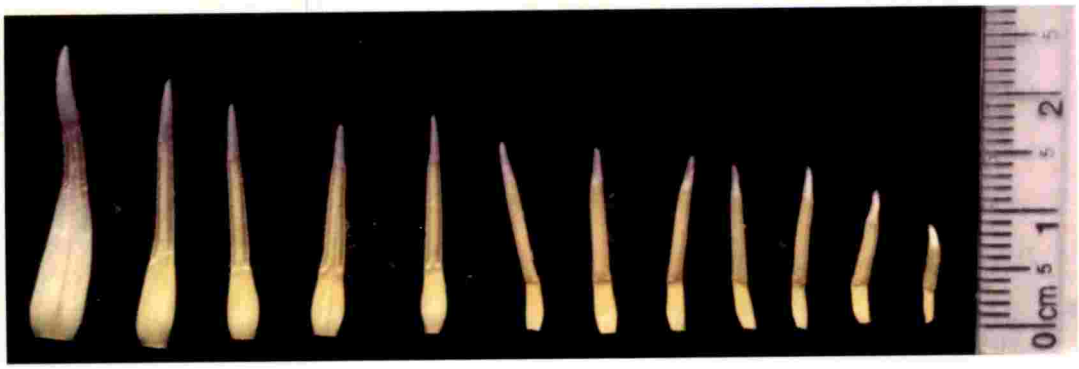
The interval for successive flower production differed significantly among the ten accessions. In this investigation, day blooming and night blooming accessions showed a greater difference in case of periodicity of flowering. The night blooming Acc. 5 and Acc. 6 showed a low frequency of flowering. Successive flowers were formed at an interval of about 12.5 and 16 days in Acc. 5 and Acc. 6 respectively. However the flowers were produced almost all days in Acc. 1 and Acc. 3 at a frequency of 1.4 and 1.68 days respectively. Three or four flowers were observed in these two accessions on a single day (Plate 15d).

It could be seen that the flowers were produced throughout the year continuously in all accessions except in Acc. 4 and Acc. 6. In these exceptions flowering was observed between May to November. During the remaining months, the plants attained a dormant condition by reducing the size of leaves and remained as rhizome under mud. According to the opinion of Mitra (1990), Ansari and Jeeja (2009) and Begum *et al.* (2010), flowering in *N. rubra* occurred round the year with up to five flowers at a time in same plant and they could not thrive in temporary water bodies. The reason for the contradictory of the flowering behaviour of *N. rubra* with Acc. 6 might be due to the habitat difference.

5.2.2.1.2 Pollination biology

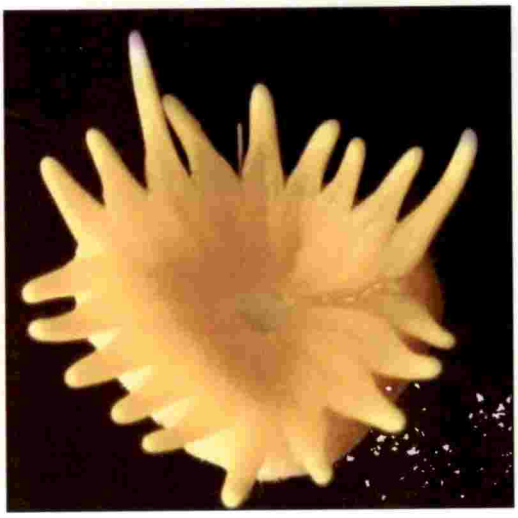
5.2.2.1.2.1 Anthesis

The process of flower opening showed great variation among different species of *Nymphaea* with respect to the time, pattern and its duration. Two accessions in the collected types were nocturnally flowering (Acc. 5 and Acc. 6)



a) Size gradation of anthers from outer most to inner most whorl

b) Creamy white coloured stigma surface of Acc. 6



c) Irregular length of stigmatic appendages of Acc. 10

d) Acc. 3 with three flowers at a time

Plate 15: Floral characteristics and periodicity of flowering in *Nymphaea*

that means, the flower opened in the evening hours and closed before noon on next day. The rest of them were diurnally flowering type in which the flower opened in the morning and closed in the evening of the same day.

The duration of flower opening of all the ten accessions was presented in the figure 4. Among this, the Acc. 6 showed maximum duration of flower opening on each day of anthesis followed by Acc. 5. The remaining accessions exhibited a short duration as compared to these night bloomers and it came under a range of 7.5 to 10 Hrs. Among the day bloomers, maximum duration of flower opening was observed in Acc. 1 followed by Acc. 3. A clear discrimination was observed between the day and night blooming accessions with respect to the duration of flower opening.

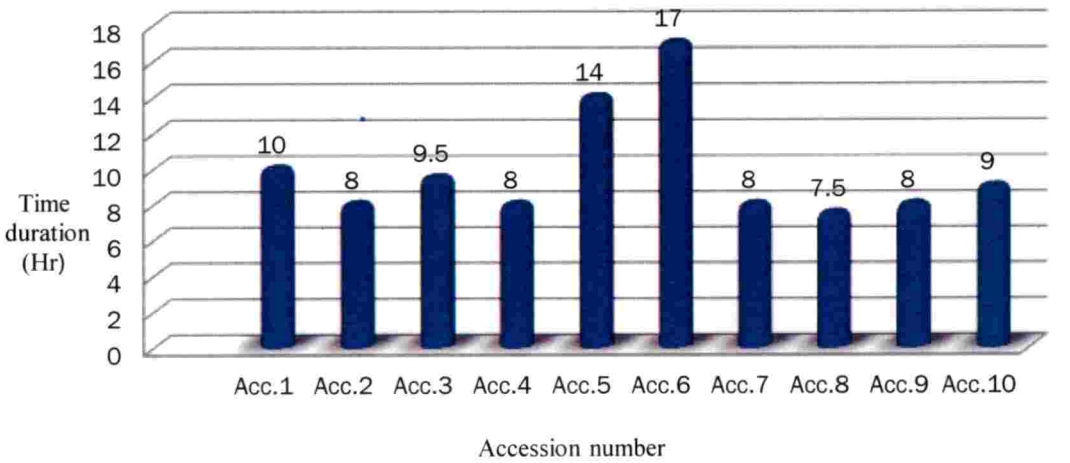


Figure 4. Duration of flower opening on each day of anthesis in ten *Nymphaea* sp.

In the present study, all day blooming accessions exhibited anthesis for four consecutive days, the night blooming Acc. 5 for four consecutive nights and Acc. 6 for five consecutive nights. On the first day of anthesis, the flower opened about three fourth of its maximum size. The complete opening was observed from the second anthesis onwards and the opening was found to take place about half an hour earlier than the first day in all types.

N. pubescens showed anthesis for four consecutive nights, *N. rubra* for five consecutive nights and in *N. nouchali* it was for three consecutive days (Bhunia and Mondal, 2012). Yakandawala *et al.* (2017) mentioned a violet waterlily with the same flowering pattern of *N. micrantha*. This is in agreement with the flower opening of Acc. 4, Acc. 6 and Acc. 7 to confirm the similarity with *N. nouchali, N. rubra* and *N. micrantha* respectively. Acc. 5 showed some similarity with the *N. pubescence* with regard to the time and duration of anthesis and blossom life.

Schneider (1982) reported the opening of day blooming *N. elegans* at 9 am for successive three days and their closing at evening. According to Jacobs and Porter (2007) in the Hydrocallis subgenera (which included night blooming tropical waterlilies), opening and closing of flower was observed at 7 pm and 10 am respectively. In the present study, night bloomers opened at 7 pm and 7.30 pm and closed at next day 9 am and 12.30 pm respectively.

As mentioned by Tom (2015) heavy rain fall and low light intensity resulted delayed opening and early closing of waterlily flowers in the present study. According to Prance and Anderson (1976) temperature was more effective than sunlight for the opening and closing of *Nymphaea* flowers.

5.2.2.1.2.2 Anther dehiscence and stigma receptivity

The results of visual observation of the flowers on successive days of flowering are discussed in detail. All the accessions displayed strong protogyny except Acc. 5. The stigma was receptive to pollen on the first day of flowering, while shedding of their own pollen was found on the second and sometimes also on the third day of opening.

On the first day, the bowl-shaped upper surface of the stigma gradually filled up with a large quantity of clear fluid, faintly yellow in colour. This indicated the receptivity of stigma and the phenomena was uniform in all the ten, but time and duration varied accordingly to the accessions.

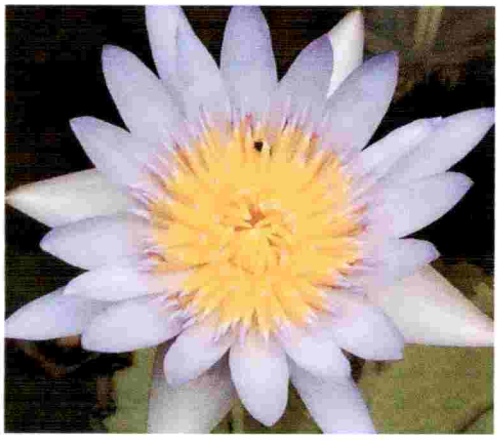
The stigma surface, position of anthers and appendages on successive days of flowering in day blooming types was presented in Plate 16. On the same day, stamens of all the whorls were compactly arranged and positioned vertically upward by facing inwards to the stigma in all the accessions. However the movement and position of anthers on each day differed between day and night blooming accessions. In day bloomers, the anthers of different whorls burst gradually from outermost to innermost whorl during successive anthesis and the non-dehiscent inner whorl bent inside and the dehisced anthers spread outside. On the second and third day, inner whorls of un-dehisced stamen formed a protective cone over the central stigmatic cup. At the same time, outer whorls of stamen dehisced the pollen and the flower was functionally staminate. This showed that there was no chance of pollen grains of either outer or inner whorls of the anthers to fall on the receptive stigma surface of the same flower. All the stamens were, bent towards the petals and completely exposed the stigma on the last day of anthesis.

The flower opening on successive days in night blooming accessions was shown in Plate17. The stigmatic surface and fluid were not fully exposed on the first day of flowering and this might be due to the uncompact arrangement of stamens around the stigma cup in night bloomers. Their anthers dehisced simultaneously on the second day of anthesis without any bending towards the petals. In Acc. 5, the anthers formed a loose cone over the stigma surface leaving a terminal hole during all the four days of flower opening.

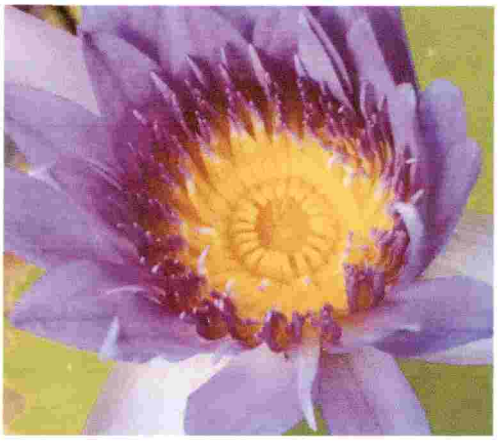
Stigma lost its receptivity on the second anthesis itself before the initiation of anther dehiscence in all types but it could be observed on the fourth anthesis in

a) First day of anthesis

b) Second day of anthesis



b) Third day of anthesis

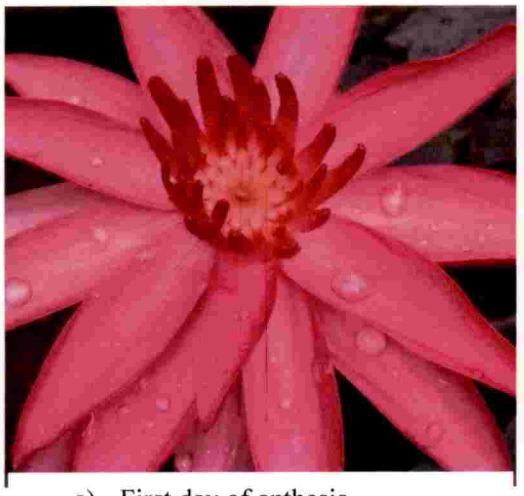


d) Fourth day of anthesis

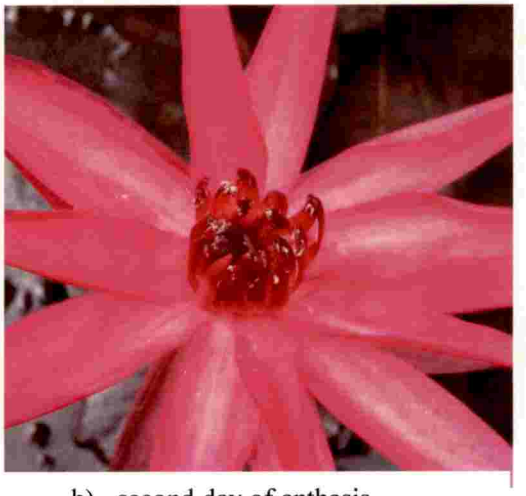
e)

Anther dehiscence pattern

Plate 16: Successive days of anthesis in day blooming *Nymphaea* accessions

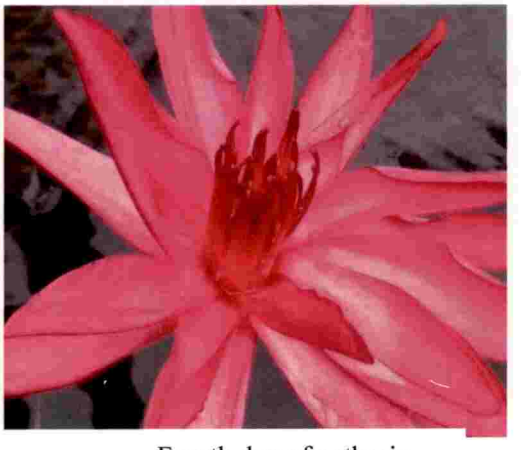


a) First day of anthesis



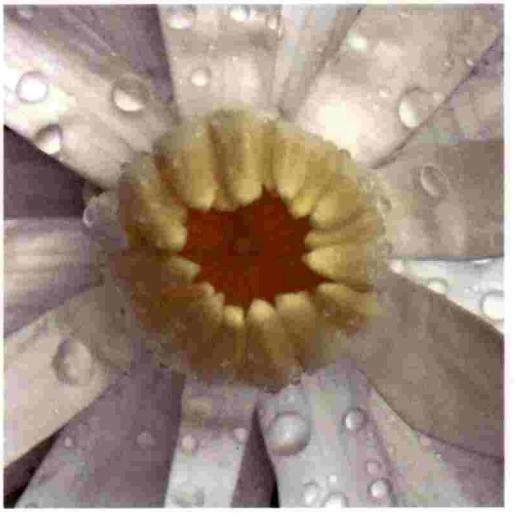
b) second day of anthesis

Third day of anthesis



Fourth day of anthesis

Patter of anther dehiscence



Acc. 5 showing a terminal small opening

Plate 17: Successive days of anthesis in night blooming *Nymphaea* accessions

day blooming accessions, when stamens uncover the stigmatic cup. Drying of stigma surface and inward curling of stigmatic appendages indicated the loss of receptivity on the second day of flowering.

This difference in the pattern of anther dehiscence in the flowers of different blooming time might be because; the day bloomers were having noticeably more number of stamens.

Similar pattern of anther dehiscence occurred from outer most to the inner most whorls in *N. capensis* (Orban and Bouharmont, 1995) and *N. nouchali* (Begum *et al.*, 2010) and Fahida, 2012) since they were diurnally flowering waterlilies. But in the case of *N. pubescence* and *N. rubra*, dehiscence occurred at the same time in anthers of all the whorls as they were night blooming type as reported by Begum *et al.* (2010).

The duration of stigma receptivity and anther dehiscence in each accession are presented in the figure 5 and 6 respectively. It could be seen that, in all the day blooming accessions the presence of stigmatic fluid was observed for about 35-38 hrs which indicated the stigma receptivity. Hence in these accessions, stigma receptivity started about 16 to 18 hours before the flower opening and it remained receptive for 18 to 21 hours even after flower opening. Tom (2015) reported that the receptivity started almost 15 hrs before flower opening and remained receptive up to 34 hrs after flower opening in the same day blooming accession of the present study (Acc. 3).

In the night blooming Acc. 5 and Acc. 6, the stigma surface remained receptive for 25-26 hrs. Here the receptivity started 8-9 hours before flower opening and remained open up to 16 hours even after flower opening. In the same accession studied by Tom (2015), the stigma receptivity started almost 6-7 hrs prior to flower opening and extended up to 18 hrs after its opening on the first

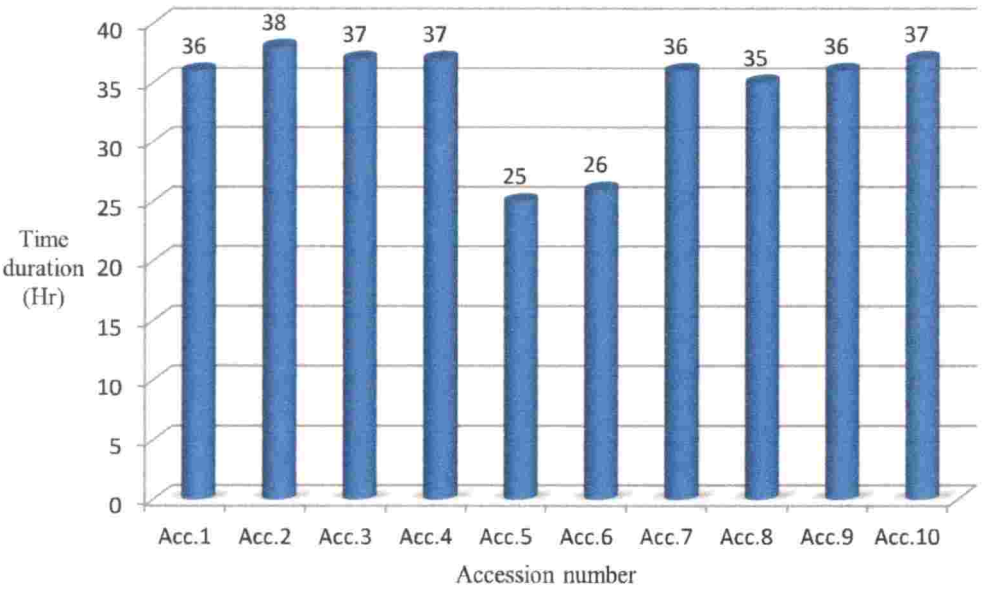


Figure 5: Duration of stigma receptivity ten *Nymphaea* accessions

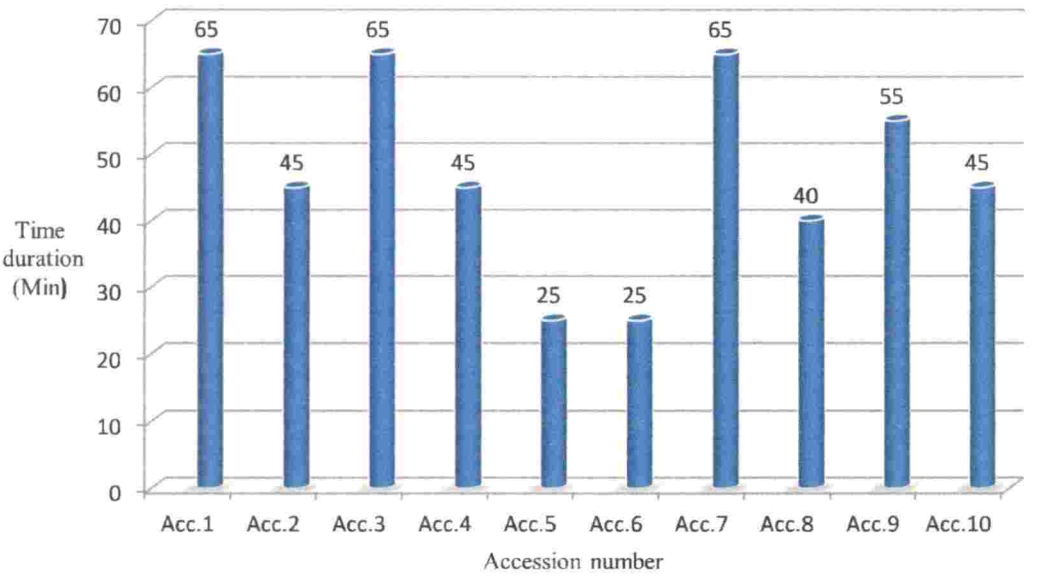


Figure 6: Duration of anther dehiscence ten *Nymphaea* accessions

day. The slight variation in the duration of receptivity might be the effect of location specific climatic condition.

In case of duration of anther dehiscence also the day blooming accessions showed a superiority over the night blooming accessions as indicated in Figure 6. In the two night bloomers, all the anthers dehisced simultaneously on second day of anthesis and completed its dehiscence by 25 minutes. Among the day blooming accessions, Acc. 1, Acc. 3 and Acc. 7 showed maximum duration for dehiscence.

In the entire ten accessions, stigma became receptive before anther dehiscence indicated the protogynous nature of water lily flower and this could be an adaptation for cross-pollination. As reported by Tom (2015), the anther dehiscence started 1-1.5 hrs after the second day of flower opening in all day blooming accessions and the night blooming Acc. 6. However in Acc. 5, the dehiscence started around 12-12.30 hours prior to the second anthesis that is nearly 2.30 Hrs before the closing of flower on the first anthesis. But Tom (2015) reported the dehiscence time of Acc. 5 as 2-3 hours prior to the flower opening on the first day. This indicated that, except Acc. 5 all others exhibited protogyny since in Acc. 5 the anther dehisced before the loss of stigma receptivity.

The white flowered, night blooming *N. pubescens* also dehisced its anther in the same pattern of Acc. 5. Schneider (1982), and Prance and Anderson (1976) mentioned that *N. rubra* showed protogyny but *N. pubescens* was either protogynous or normal. The protogynous nature of *Nymphaea* flower also reported by Wiersema (1988), Begum *et al.* (2010), Fahida (2012), Tom (2015). Begum *et al.* (2010) reported the loss of receptivity of stigmatic fluid on the second day of blooming *N. rubra,* but in *N. nouchali* and *N. pubescence* the moisture of stigmatic cup retained for 4-5 hours even after the flower opening on the second day.

On the second day of blooming the appendages become completely overlapping over the stigmatic cup in Acc. 5 and Acc. 6 since, night bloomers have long stigmatic appendages. However in day blooming types, the stigmatic cup was partially exposed even after loss of receptivity as their appendages were small. In contrast to this, the day blooming Acc. 9 also possessed long appendages. In Acc. 4 and Acc. 8 the appendages are observed as small protuberance from the carpel rim hence they could not cover the stigma surface after first anthesis. In *N. pubescens* the covering was partial and loose. Horn like small stigma appendages could not cover the stigma in *N. nouchali.*

5.2.2.1.2.3 Nature of pollination

The studies on stigma receptivity and anther dehiscence indicated only the protogynous nature of *Nymphaea* accessions, but the nature of pollination in these accessions was confirmed with the protected and unprotected flowers with and without emasculation. The unprotected flowers with and without emasculation were observed as seed setting. Among these, seed set could be observed only in five accessions (Acc. 3, Acc. 4, Acc. 5, Acc. 6 and Acc. 7). At the same time no seed set was observed in the protected (selfed) buds indicating the cross-pollination or geitinogamy that is the pollen from the flowers of different plant of the same accession. Tom (2015) also mentioned the absence of seed set in selfed flowers of both day and night blooming accessions.

Despite the stigma receptivity and anther dehiscence overlapped in Acc. 5, no seed set was observed in their bagged flowers. In this accession, the cause of seed set in open condition might be due to autogamy or geitinogamy. It needs further confirmation based on artificial self-pollination or sib mating.

In contrast to the present study and Tom (2015), Prance and Arias (1975) reported that the bagged flowers were capable of producing seeds indicating that protogyny is not absolute. Knuth (1908) also stated that *Nymphaea* flowers were

slightly protogynous or homogamous in nature with the stigma surface being receptive for several days. He observed anthers dehiscing on the first and/or second day of anthesis, noting that the stamens bent over the stigma and thus effected self-pollination.

The accessions were also observed for various adaptations which favours cross pollination. The flowers of all day bloomers only were found to having fair fragrance during the blooming period. This might be an adaptation to attract insect pollinators. Ansari and Jeeja (2009) also observed the lack of fragrance in night blooming *N. rubra* which showing similarity with Acc. 6 in the present study. In contrast to this Wiersema (1988) indicated the importance of flower odour in night blooming species. In the present study, honey bees were the major visitors in the day blooming accessions. Maximum number of insects visiting the flowers was observed on the second day of anthesis. Wiersema (1988) reported that the day blooming waterlilies were pollinated by hymenopterans and the night blooming by coleopterans. Overlapping of male and female phases in Acc. 5 and absence of fragrance in both Acc. 5 and Acc. 6 were pointing towards the autogamy in these two night blooming accessions.

The presence of stigmatic fluid, slippery nature and slight incurvature of stigmatic appendages helps the flower to slide insects and trap them in the stigma cup on the first day of flower opening. The complete bending of appendages and formation of cone like structure by inner whorls of stamens prevents the escape of trapped insects. Insect cadavers were found on the stigmatic cup of Acc. 1, Acc. 3 and Acc. 4 on the last day of anthesis. Tetali *et al.* (2008) and Fahida (2012) reported the similar result of insectivorous nature of *N. nouchali*.

5.2.2.1.2.4 Pollen morphology and fertility

Application of palynology is very diverse and multidisciplinary. However, the role of pollen morphology is important in taxonomic debate for classification. Variation in pollen morphology could be observed within genera. Hence the pollen characters have proved useful for systematic purposes like species identification in various plant families.

Morphological characters of pollen grain differed considerably among the investigated *Nymphaea* accessions. As indicated by Tom (2015), the pollen colour was yellow in all the eight day blooming accessions and creamy white in night blooming accessions. The viable pollen from five types (Acc. 2, Acc. 7, Acc. 6, Acc. 8 and Acc. 9) exhibited spheroidal shape, four (Acc. 1, Acc. 3, Acc. 5, Acc. 10) were prolate spheroidal and Acc. 4 was oblate spheroidal shape under the light microscope, and the equatorial region surrounded by a ring-like sulcus. The pollen dimension observed as small in Acc.1, Acc.7 and Acc.8 and medium in the remaining accessions.

Bhunia and Mondal (2012) described the pollen grains as prolate spheroidal in *N. pubescens,* spheroidal in *N. rubra* and *N. stellate* and oblate spheroidal in *N. nouchali* pollen grains. They also reported the size of pollen as medium in *N. pubescence, N. stellate, and N.nouchali* and small in *N. rubra.* These studies direct the identity of Acc. 4 and Acc. 6 as *N. nouchali* and *N. rubra.*

As shown in the figure. 7 more than 70 % pollen grains of all the ten accessions were fertile on staining with 1% safranin after two hours of its dehiscence. Among these only the Acc. 2 exhibited low pollen fertility of 72% and is not seed producing type. The remaining accessions had 88-95 % pollen fertility and all the seed setting accessions were included in this group.

5.2.2.1.2.5 Viability or germinability of pollen grain

Pollen viability or germinability is considered as an important factor that influences fruit and seed production. There is a possibility of reduced seed production or even pollination failure if non-viable pollen grains are transported to the stigma. Germination of pollen grain was considered as an indication of viability.

In the study of *in vitro* germination of pollen grains, different concentrations of sucrose solution resulted in varying pollen germination percentages among the accessions. In each accession, the maximum germination was observed in pollen grains kept in a germinating medium of 10% sucrose solution. The pollen germination result obtained in the present study was obviously in contrast to that of other studies in which an enhancement in pollen germination was observed in 5% sucrose solution as the germinating medium (Bodhipadma *et al.*, 2013).

The result of viability percentage of pollen in 10% sucrose solution was presented in Figure 8. The Acc. 3, Acc. 4, Acc. 5, Acc. 6, Acc. 7 and Acc. 9 showed more than 10 % pollen germinability. Among these except Acc. 9, the remaining five were found to produce seeds in open condition. For *in vitro* pollen germination of waterlily species, the effects of three factors precisely sucrose concentration, light conditions and temperature were found to be important by Chomchalow and Chansilpa (2007). In many previous studies, optimization of one or two of these factors was sufficient to achieve high pollen germination rates (Lyra *et al.*, 2011). Volkova and Shipunov (2007) explained the crucial role of sucrose concentration under an optimized environmental condition, 32^0 C in dark. Sucrose concentration in the stigmatic fluids of open flowers of *Nymphaea* spp. was likely to be lower than 5% (Thien *et al.*, 2009).

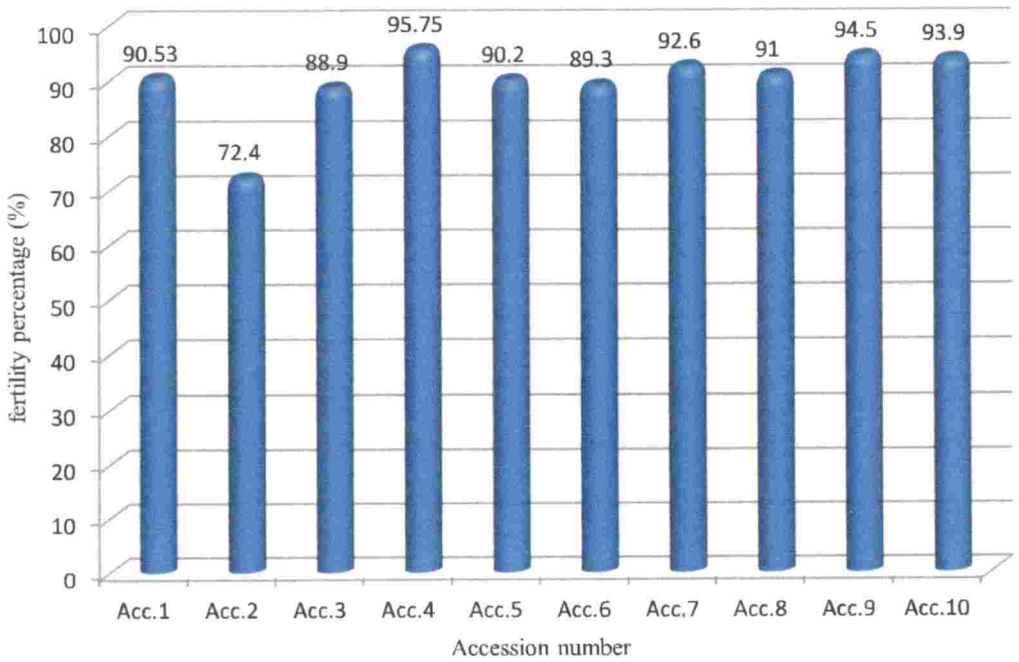


Figure 7. Percentage of pollen fertility of ten *Nymphaea* accessions on staining with 1% saffranin

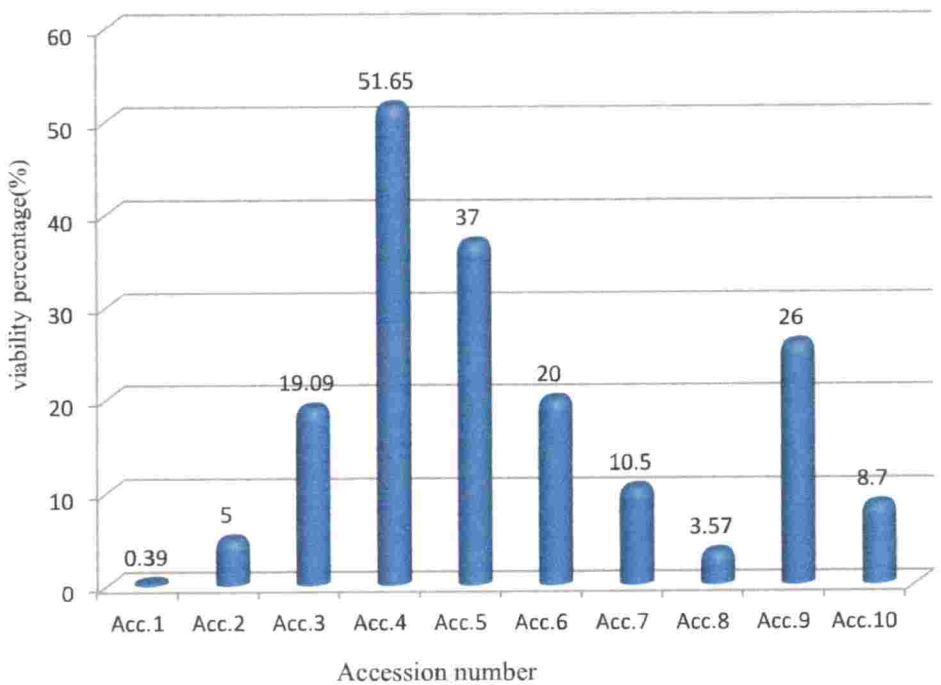


Figure 8. Percentage of pollen viability of ten *Nymphaea* accessions in 10% sucrose solution

Pollen tube growth varied significantly with respect to accessions. Among the ten accessions, a remarkable increase in the pollen tube length was observed only in seven accessions (*viz.*, Acc. 1, Acc.3, Acc. 4, Acc. 5, Acc. 6, Acc. 7 and Acc. 9) even after 30 hours of dehiscence. In these accessions the pollen tube length was greater than the diameter of the pollen grain. In the remaining three accessions *viz.*, Acc. 2, Acc. 8 and Acc. 10 only a few pollen grains germinated with short pollen tube. According to Tang *et al.* (2009), pollen grains are considered as viable when the pollen tube length is longer than the diameter of the pollen grain. Koshy and Jee (2001) disclosed that low pollen viability is one of the key factors responsible for the failure of seed set in *Bambusa vulgaris.*

Even though Acc.1 had good pollen fertility (90.53%) and pollen tube growth, their low percentage of viability (0.39%) might cause them to fail in seed setting. In case of Acc. 9 which had high pollen fertility (94.5%), better viability (29%) and good pollen tube growth, however, fail to produce fruit and seeds. The fertility of pollen could be confirmed only after determining the ploidy level of species by observing the presence of bivalents in meiotic metaphase.

After pollination, the journey of the pollen tube through the gynoecium is a complex process, which involves many interactions, including cell-cell recognition and cellular signalling (Graaf *et al.*, 2001; Franklin-Tong, 1999). In recent studies of the factors affecting seed set in several water lotus crosses and chrysanthemum, it was found that a low number of germinated pollen grains and the abnormal growth of most of the pollen tubes were the main causes of the failure of seed set (Sun *et al.*, 2011).

These results pointed out that the process of seed set was a combined effect of pollen fertility, germinability, pollen tube growth, pollen pistil interaction etc. The actual cause of lack of seed set needs detailed investigation

with artificial self-pollination and inspection at various stages of pollen tube development and fertilization.

5.2.2.1.3 Fruit and seed characteristics

Five accessions *viz.*, Acc. 3, Acc.4, Acc.5, Acc.6 and Acc.7 showed fruit and seed set under natural condition which included two night blooming (Acc.5, Acc. 6) and three day blooming (Acc. 3, Acc. 4 and Acc. 7) accessions. The fruit is a many seeded berry in *Nymphaea* species. The percentage of fruit set and number of healthy seeds were very few in the violet flowered Acc. 7. In this study, the white colour variant Acc. 4 showed similarity to *N. nouchali* in seed set, which is in contrast to that reported by Fahida (2012). The pattern of fruit development and seed dispersal observed in Acc. 3 was similar with that reported by Tom (2015) from previous study. But seed setting observed in Acc. 5 was in contrast to Tom (2015), since she reported the absence of fruit and seed production in night blooming white waterlily. The Acc. 6 assumed as *N. rubra* was seed setting in nature. Mitra (1990) mentioned that this species never set fruits but, Ansari and Jeeja (2009) had reported fruit setting *N. rubra* with fertile seeds. Seeds of all the five accessions were elliptical shape although their size differed significantly. There was no proportionality between the size of seed and 100 seed weight since the Acc. 5 with lowest seed size had highest 100 seed weight. Trichomes were present on the seeds of all the five accessions.

The exomorphic features of *Nymphaea* seeds such as shape colour, longitudinal ridges, number of rows of transverse band, etc. vary from species to species (Ansari and Jeeja, 2009). Although such variations offer characters for determination of species, it was not included in our investigation since the seeds were very small and it needs ultra-microscopic studies.

5.2.2.1.4 Seed germination study

Seed germination was observed within ten days of seed dispersal without any scarification in all the evaluated seed forming *Nymphaea* accessions except in Acc. 4. But only few seeds germinated during the initial days. This phenomenon was predominant in Acc. 4, in which the seed germination started only after 20 days. Smits and Schmitz (1995) mentioned that the production of white waterlily through seeds was difficult because of the development of dormancy. Seed dormancy in the seed forming accessions was reported by Else and Reimer (1984).

The number of seeds used for the study also influenced the germination percentage. As mentioned by Else and Reimer (1984) crowded seeds favoured germination than single seed. The reason behind the breakage of dormancy during crowding was not fully understood. However, Else and Reimer (1984) noticed the presence of ethylene in the crowded seeds, and assumed as the cause of germination.

The hard seed coat might become barrier in some water lily crosses that result in low breeding efficiency, and making it thin by various methods might result in fast germination of seeds.

5.2.2.1.4.1 Physical and mechanical scarification

The results of seed germination study in all the five seed forming accessions with physical and mechanical scarification are presented in Figure 9-13.

In all the five accessions an initial increment in the germination was observed in both hot water treated and mechanically scarified seed lots. But at the end of 30 days there was a decrease in the number of germinated seeds than

that of the control. Else and Reimer (1984) also reported that the mechanical rupture of seed coat caused failure in seed germination of waterlily.

5.2.2.1.4.1 Chemical scarification

The effect of different concentrations of H_2SO_4, GA_3 and Ethrel on germination of seeds of five accessions was presented in the Figure 14-18.

In all the five accessions, seeds treated with different concentrations of chemicals showed maximum germination within 10 days after treatment, than that of control. Maximum increment in germination was observed in the seed lots treated with 5% H_2SO_4 and 100 ppm Ethrel. H_2SO_4 makes the seed coat thin and more permeable to water. Tom (2015) also reported maximum germination of seeds treated with 5% H_2SO_4.

The growth regulators GA_3 and ethylene had germination inducing property. Ethrel enhanced seed germination by releasing ethylene gas in solution which increases the CO_2 concentration. Researches revealed significant increased seed germination with Ethrel treatment (Else and Reimer, 1984) and GA_3 (Rouhi *et al.,* 2010) in waterlily. Only chemical treatments showed a remarkable increase in the germination of *Nymphaea* seeds as compared to the physical and mechanical scarification methods.

5.2.2.2 Asexual Reproduction

Reproductive strategy is one of the several factors that contributed the distributional success. The widely distributed tropical species employ at least one reproductive alternative to total reliance of sexual reproduction. In our study three forms of asexual reproductions involving rhizome, leaf and root tip proliferation were observed. Among these most wide spread and common method was rhizome propagation in all the ten accessions. Apart from rhizome,

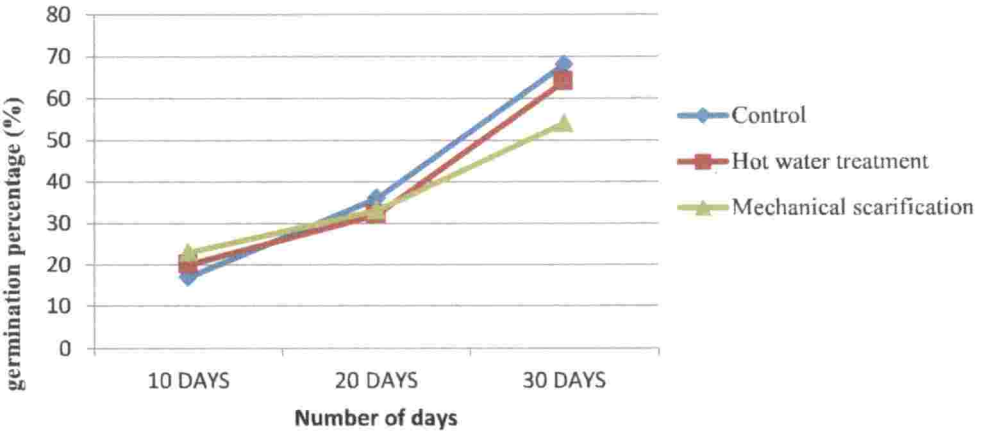


Figure 9: Seed germination percentage in Acc. 3 with physical and mechanical scarification

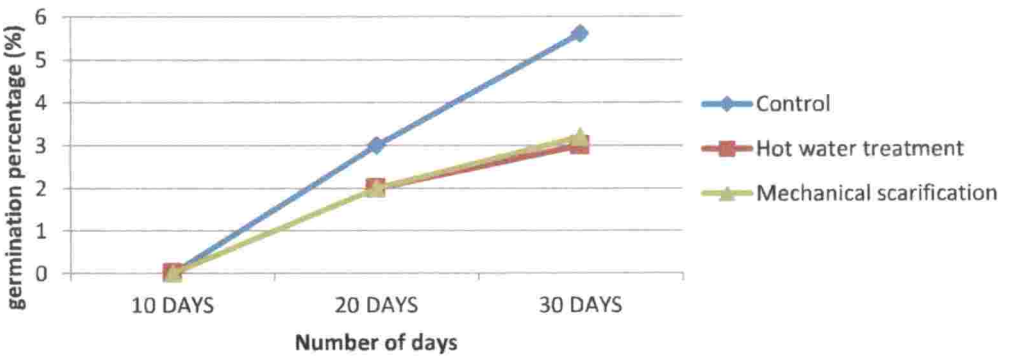


Figure 10: Seed germination percentage in Acc. 4 with physical and mechanical scarification

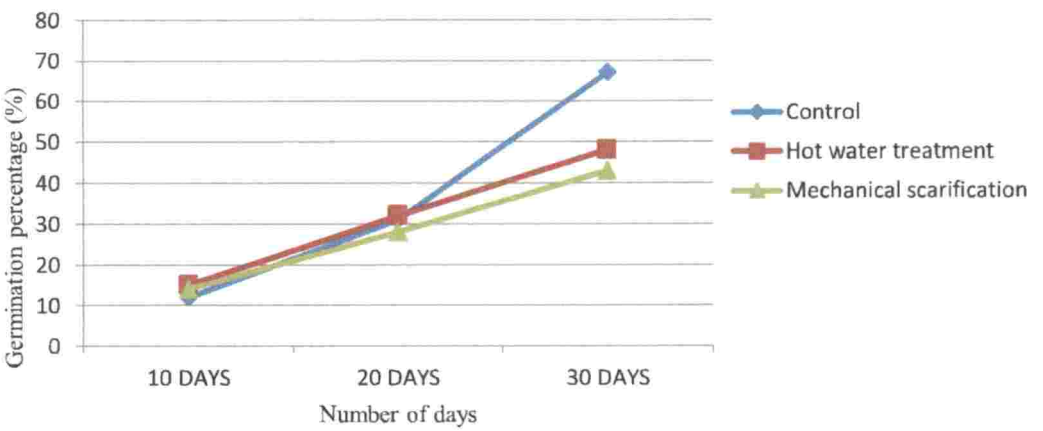


Figure 11: Seed germination percentage in Acc. 5 with physical and mechanical scarification

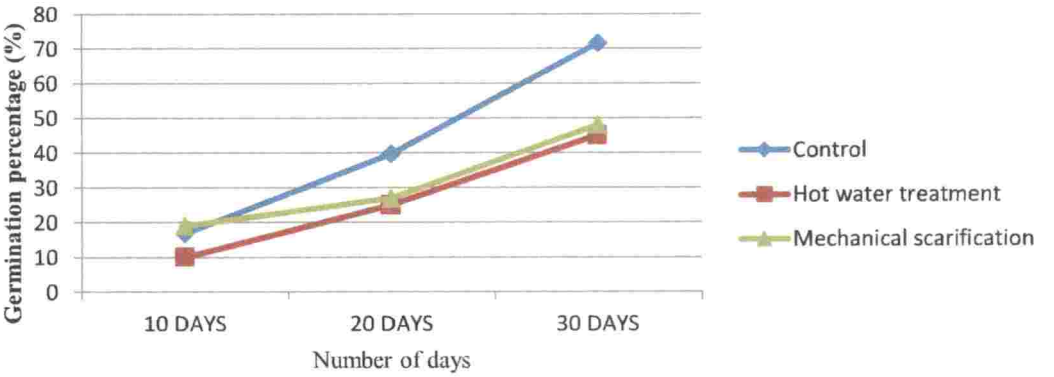


Figure 12: Seed germination percentage in Acc. 6 with physical and mechanical scarification

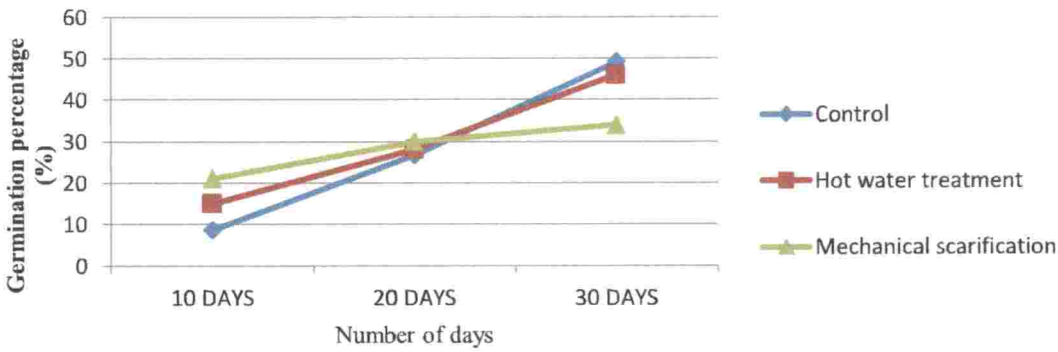


Figure 13: Seed germination percentage in Acc. 7 with physical and mechanical scarification

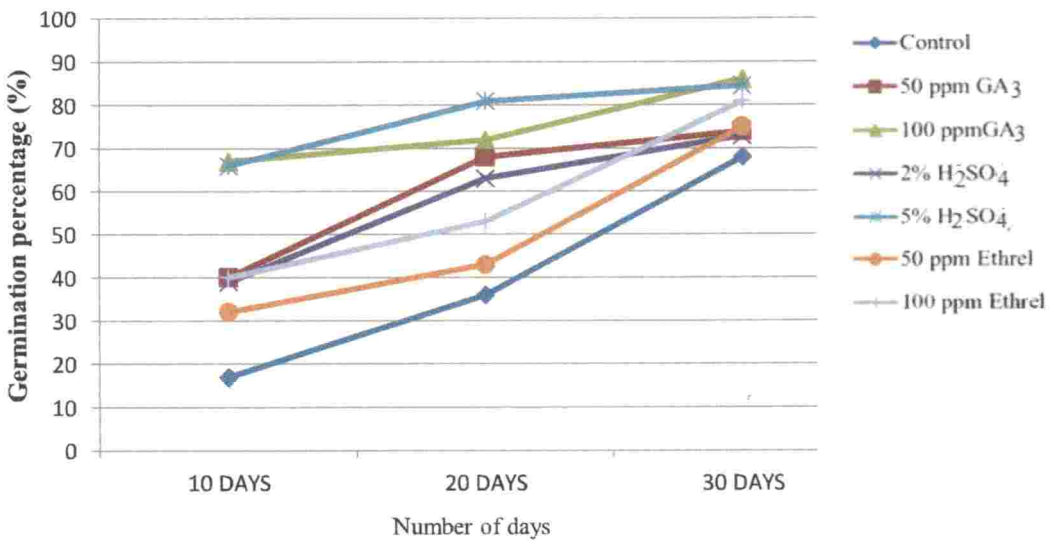


Figure 14: Seed germination percentage in Acc. 3 with chemical scarification

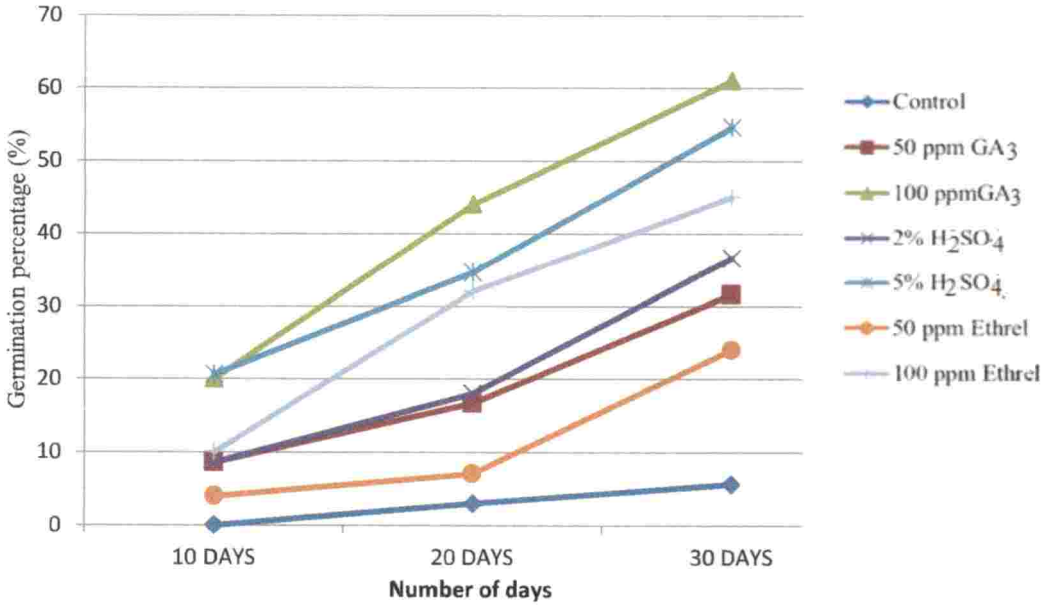


Figure 15: Seed germination percentage in Acc. 4 with chemical scarification

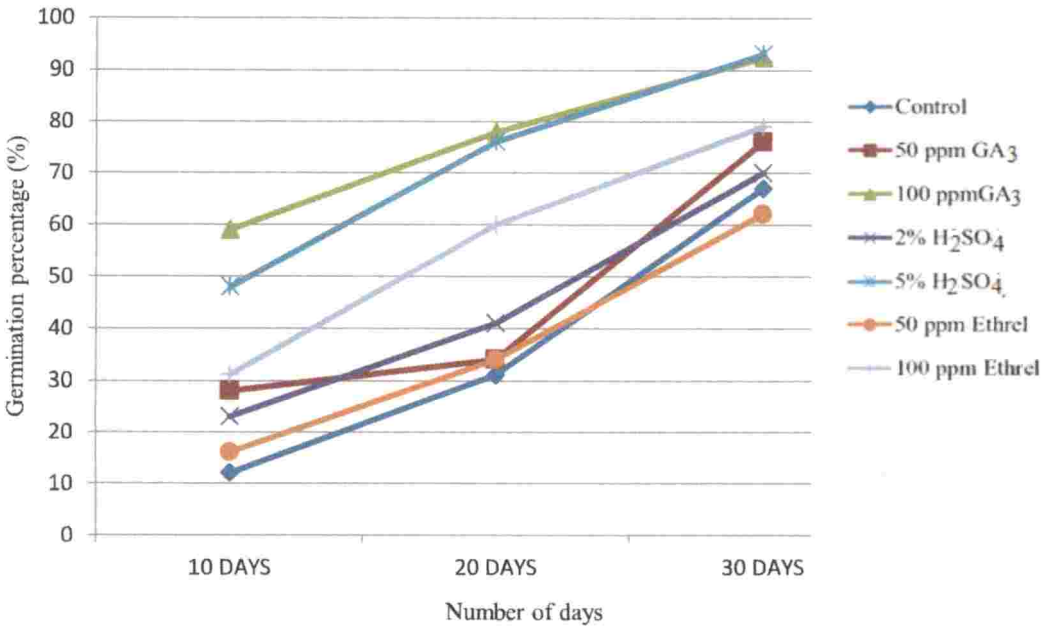


Figure 16: Seed germination percentage in Acc. 5 with chemical scarification

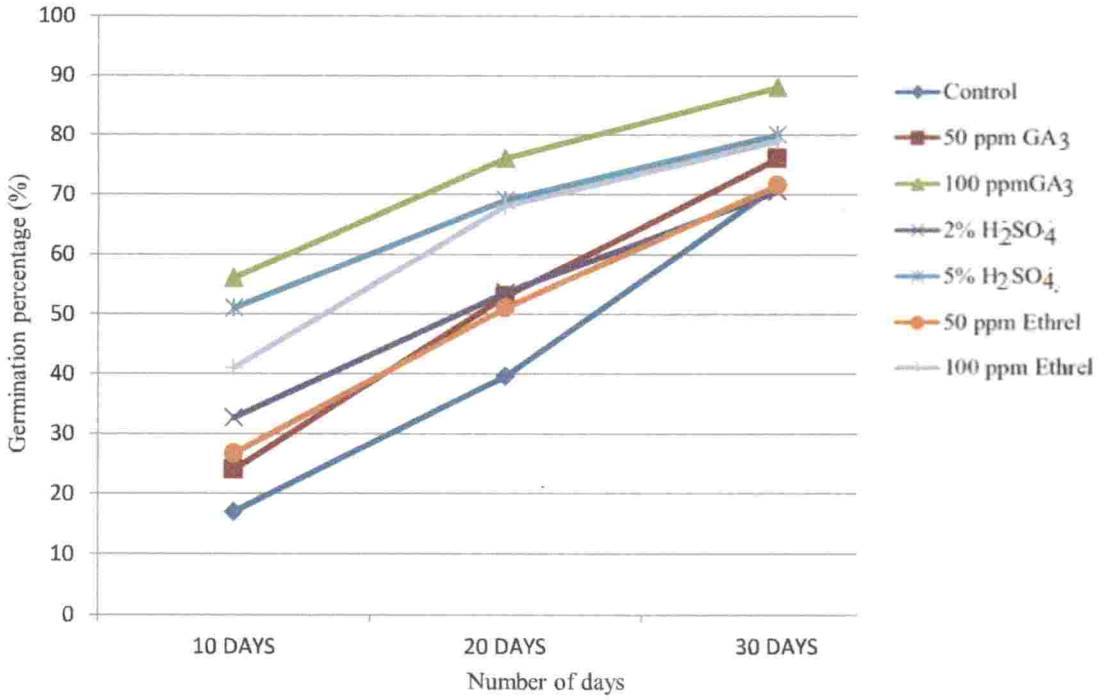


Figure 17: Seed germination percentage in Acc. 6 with chemical scarification

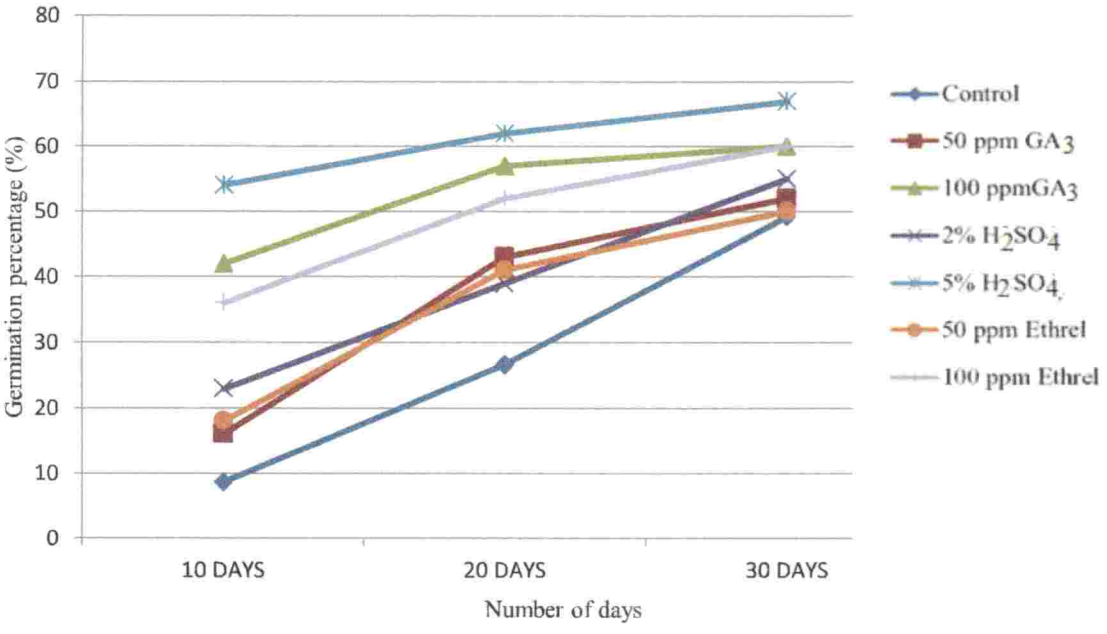


Figure 18: Seed germination percentage in Acc. 7 with chemical scarification

leaf proliferation was observed in the day blooming Acc. 1 and Acc.7 and root tip proliferation was observed in the night blooming Acc. 5.

Two month old rhizome was capable to produce new plantlets in waterlily. There were difference between the accessions in case of number of days taken for emergence of first leaf, emergence of root and first flower bud when rhizome was employed as a natural propagule (Figure 19, 20 and 21). Acc. 2 showed minimum days for the emergence of first leaf, root and flower bud from a mature rhizome followed by Acc. 8. Alternative sexual and asexual method of propagation was absent in these two accessions.

In Acc. 1 and Acc. 7 around 13 days old mature leaves were ready to proliferate new leaf and root from the nib like projection on the adaxial surface of leaf lamina, at the petiole attachment. The new plantlets grew extensively only after detaching the mature leaf from the parent plant. Among these the growth and development of plant parts and flower initiation were earlier in Acc. 1 than Acc. 7.

In Acc. 5, numerous tiny plantlets were observed as protruding through the mud surface. From a single two month old mother plant, around three new tiny plantlets were emerged within one week. The leaves and roots emerged simultaneously from the root tip of mother plant.

These alternative modes of propagation in Acc. 1, Acc.5 and Acc.7 showed comparatively higher rate of success than rhizome propagation in terms of the early formation of leaf, root and flowers.

The size of flower bud and total number of flowers produced during the initial month of flower opening in rhizome propagated plants and leaf and root proliferated plants in each accession were almost similar. It was slightly lower than that of the mother plant in the initial months.

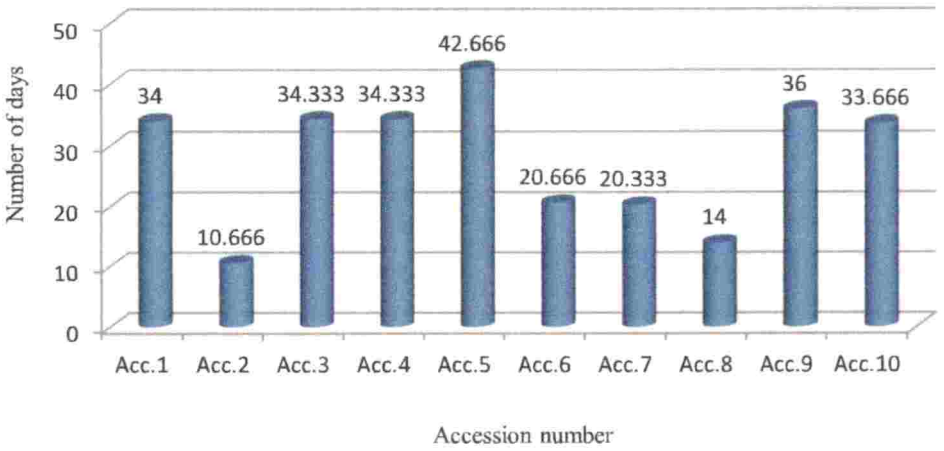


Figure19: Number of days taken for 1st leaf emergence in rhizome propagated *Nymphaea* plants

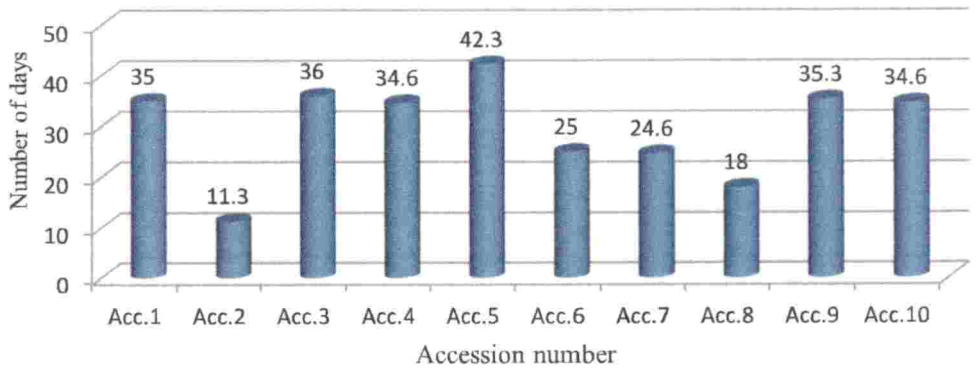


Figure 20: Number of days taken for root emergence in rhizome propagated *Nymphaea* plants

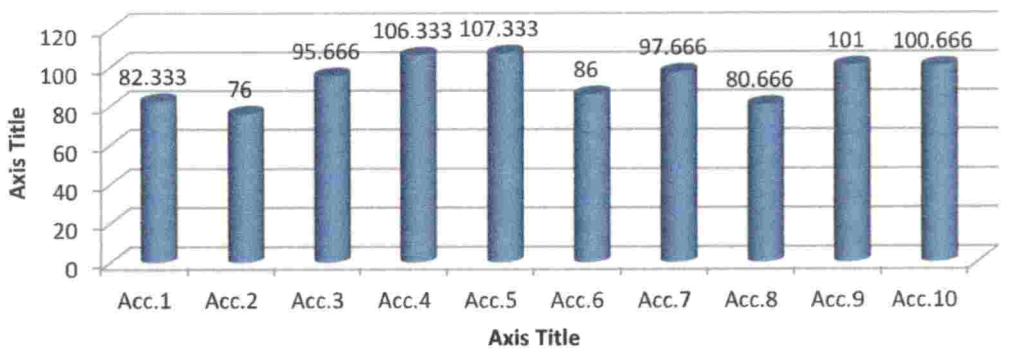


Figure 21: Number of days taken for 1st flower bud emergence in rhizome propagated *Nymphaea* plants

Monteverde (2009) standardized methods for enhancing flowering of viviparous waterlily plants in just 20 days. But in our investigation, viviparous plants produced their first flower within 48 days in Acc. 1 and 67 days in Acc.7 under natural condition. Development of epiphyllous plantlets (vivipary) were observed in tropical day blooming waterlilies such as *N. micrantha*, *N. lasiophylla*, and *N. prolifera* (Wiersema (1988), Conard (1905) and Monteverde (2009) in which the new tiny plants grew from the leaves. This is a key feature that has been incorporated during breeding of *Nymphaea* as it offered an easy mode of propagation. In the present study the day blooming Acc. 7 showing leaf proliferation, confirmed the similarity with *N. micrantha* for this feature also.

5.2.3 Genetic Parameters of Variability

Knowledge of nature and magnitude of variability existing in available germplasm is a prerequisite to choose characters for effective selection of desirable genotypes. The components of variability (such as coefficient of variation, heritability and genetic advance) and its magnitude help in deciding breeding procedures for the improvement of the trait. Genetic basis of variability was evaluated in the four quantitative traits of leaf, flower, fruit and seed and the results discussed below.

Genotypic coefficient of variance (GCV) and phenotypic coefficient of variance (PCV) only indicate the magnitude of variability, but the parameters which predict the gain under selection is heritability (H^2) and genetic gain (GG). Among the evaluated characters, the number of anthers, length and breadth of seed exhibited maximum variation as indicated by its high GCV and PCV coupled with high heritability and genetic gain. Even if the magnitude of variability was low or medium in the characters namely lamina width, lamina length, circumference of flower bud, diameter of fully opened flower, number of stigmatic appendages, number of petals, number of stamens and length of fruit

the difference between GCV and PCV was less. These have high H^2 and GG value also. This indicated the less influence of environment on the variability of these characters. The high heritability coupled with high genetic gain in all these characters indicated the scope of selection based on these characters.

Tom (2015) mentioned in her study that only the length of leaf lamina in waterlily exhibited high GCV, PCV and H^2 value with high percentage of improvement in the population.

5.3 EXPERIMENT 3: HYBRIDIZATION

In the third experiment, twenty crosses were carried out (direct and reciprocal crosses) in between the five seed setting accessions. Among this only three crosses between day bloomers were successful in fruit and seed formation. Acc. 4 was found to be a common parent in three successful crosses either as an ovule parent (cross 1 and cross 3) or pollen parent (cross 2). Although the two night blooming accessions (Acc. 5 and Acc.6) set seeds in open condition, they failed to set seed during artificial hybridization.

Viable seed production is considered as the preliminary indication that the alleged cross has been successful. Even though the ovule parent had only 3% of germinated seeds, 18 % of the hybrid seeds germinated within three weeks.

The fourth and successive leaves emerged in the seedlings of cross 1 was with a faster rate than the other cross seedlings. The seedlings of cross 2, cross 3 and all seed setting accessions were attained five leaf stage with a spread of 12-17 cm even after six months. Among the hybrid plants of three crosses, only the plants from cross 1 (Acc. 4 x Acc. 3) exhibited flowering with in five months. The adult plants emerged from the presumed hybrid seeds were compared morphologically with the assumed parent plants raised from rhizome.

The flower colour of F_1 hybrid was light violet which was intermediate to the white coloured ovule parent (Acc. 4) and blue coloured pollen parent (Acc. 3). The violet colour of anther filament and shape of petals of the off spring showed resemblance to the pollen parent. Leaf characters of hybrid plant showed more or less similarity with the ovule parent. Adaxial surface was almost similar to Acc. 4, but the violet colouration of the abaxial surface was not intense in the hybrid leaf as in Acc. 4, the ovule parent. Number of teeth on the leaf margin was intermediate to both the parents. The primary and secondary veins are more prominently visible than both the parents. Similarly, relatively weak genetic contribution was observed from Acc. 4 on the floral characters and Acc. 3 on the leaf characters.

Pollen viability of the hybrid plant was lower than that of both the parents. Meeuse and Schneider (1980) also reported the high pollen sterility of water lily hybrids.

Literature mentioned that water lily plants propagated from seed would flower usually two or more years after its germination (Ansari and Jeeja 2009). However in the present study the hybrid plant had flowered within five months since its emergence. Initially the flowers were very small with few petals and stamens compared to that of the parent plants. The periodicity of flowering also was lower than that of the parents. Six to eight flowers were produced by the hybrid parent during one month while 13 and 21 were produced by ovule parent and pollen parent respectively. The size and number of flower and floral parts might get stabilized to an optimum with the passage of time. But the leaf size with respect to lamina width and lamina length was attained almost equal and intermediate to that of parents.

Conard (1905) mentioned that, the hybrid off springs do not follow a fixed pattern of inheritance in case of morphological features. In the present investigation some characters of the hybrid plant of cross 1 exhibited similarity

with either of the parent, some intermediate to both the parental characters and some were unique to them. The novel recombination and natural variation could lead to unique phenotype in hybrid plants. Plants exhibiting such a mix of different characters can be concluded to be of hybrid origin. Donald *et al.* (2004) also reported such a pattern of expression of morphological features in the F_1 hybrid of *N. colorata* and *N. gigantic*.

There are limitations for the use of morphological evaluation for the confirmation of waterlily hybrids. Molecular characterization is essential to take a final decision with respect to the cross as well as maternally and paternally inherited characters in the hybrid plant.

Summary

SUMMARY

The research work entitled "Characterization and hybridization in *Nymphaea* spp." was carried out in the department of Plant Breeding and Genetics, College of Agriculture, Padannakkad in the academic year 2016-18. Three experiments namely, a) exploration and collection of genotypes of *Nymphaea* spp. b) evaluation for the morphological characters and reproductive biology and c) hybridization were included in the study.

- Based on the detailed survey 14 accessions were collected from Kasaragod, Kannur, Kozhikode and Malappuram districts and one nursery in Thrissur. Out of these, ten were included for detailed study. Among these two were night bloomers (Acc. 5 and Acc. 6) and others were day bloomers. These included five seed setting types (Acc. 4, Acc. 5, Acc. 6, and Acc. 7) and three viviparous types (Acc. 1, Acc. 5 and Acc. 7).

- Base on the leaf morphology, truncate leaf tip in Acc. 3, spiny leaf margin and sinus overlap in Acc. 5 and retuse leaf tip in Acc. 9 were considered as a diagnostic character for these accessions.

- In the biometric characteristics of leaf, length of lamina, width of lamina, length of petiole, days from visual appearance to decay initiation showed wide variability among the accessions. In case of length and longevity of the leaf, Acc. 2 was superior and Acc. 8 was inferior over remaining accessions.

- The floral formula observed in the present investigation was ♀ K_4 $C_{10\text{-}24}$ $A_{20\text{-}120}$ $\underline{G}_{(10\text{-}23)}$. Floral biology study revealed differences between night and day blooming accessions. The night blooming accession showed superiority over day bloomers in the flower bud characters viz., length and circumference of flower bud and diameter of fully opened flower. Sepals of Acc. 2 were distinct from all other accessions in terms of

number, shape and size. The feather like sepals of Acc. 2 was five to seven in number while all others had four. In day bloomers the stamens consisted of filament, anther and sterile appendage whereas in night bloomers terminal appendage was absent. The number and length of petal, stamens, number of carpels and number of stigmatic appendages were significantly differed in all accessions.

- All the accessions exhibited the similar pattern of pedicel growth with maximum elongation was just prior to the flower bud reaching the water surface. It continued even after flower opening. Thereafter, the growth rate decreased and stopped completely with the sinking of flower into water.

- All day blooming accessions exhibited anthesis for four consecutive days, the night blooming acc. 5 for four consecutive nights and Acc. 6 for five consecutive nights. The night blooming accessions showed maximum duration of flower opening with 17 and 14 hrs., but the day blooming accessions remain opened for about 7.5- 10 hrs. on each day of flowering. The night blooming accessions showed a low frequency of flowering compared to day bloomers.

- In general, all the accessions except Acc. 5 displayed strong protogyny, being receptive to pollen on the first day of flowering while shedding their own pollen on the second and sometimes also the third day of opening. In Acc.5 the phase of stigma receptivity and anther dehiscence were found overlapping.

- Stigma receptivity was indicated by the presence of clear water in the stigmatic cup. Drying and darkening of the stigma surface and inward curling of stigmatic appendages to the stigma cup indicated the loss of receptivity and it occurred on the second anthesis itself. In day bloomers,

the anther dehiscence started on the second anthesis and the anthers of different whorls burst gradually from outermost to innermost whorl during successive anthesis. In the night blooming accessions, the anthers dehisced simultaneously in all the whorls on the second day of blooming itself.

- The pollination biology study showed the development of fruit only in the unprotected flower buds with and without emasculation in five accessions, viz., Acc.3, Acc.4, Acc. 7 (day bloomers) and Acc.5 and Acc. 6 (night bloomers). The pattern of anther dehiscence, duration of stigma receptivity and fragrance were favoring cross pollination in day blooming accessions. The adaptations such as overlapping of stigma receptivity and anther dehiscence in Acc. 5, simultaneous dehiscence of all anthers and absence of fragrance in both the night bloomers were pointing towards autogamy in these accessions.

- More than 70 % pollen grains of all the ten accessions showed regular staining with 1% safranin after two hours of its dehiscence. Pollen morphology revealed the shape of pollen in five accessions as spheroidal shape (viz., Acc.2, Acc.7, Acc.6, Acc.8 and Acc.9) in four as (Acc.1, Acc.3, Acc.5, Acc.10) were prolate spheroidal and Acc.4 was oblate spheroidal shape. Based on size the pollens of Acc.1, Acc.7 and Acc.8 grouped as small and others as medium. The *in vitro* pollen germination revealed maximum germinating in 10% sucrose medium. A remarkable increase in the pollen tube length was observed only in seven accessions (viz., Acc. 1, Acc.3, Acc. 4, Acc. 5, Acc. 6, Acc. 7 and Acc.9) even after 30 hours of its dehiscence.

- The seeds of all five accessions showed germination without any treatments except in Acc. 4. Chemical treatments with different concentrations of H_2SO_4, GA_3 and Ethrel showed an enhancement in seed

germination as compared to the physical and mechanical scarification methods. The maximum germination was observed with 5% H_2SO_4 and 50 ppm GA_3.

- The alternative modes of propagation other than rhizome observed was, leaf proliferation in the day blooming Acc. 1 and Acc.7 and root tip proliferation in the night blooming Acc. 5. These alternative modes of propagation showed comparatively better performance than rhizome propagation in terms of the early formation of leaf, root and flowers.

- The results of detailed evaluation showed that three among the collected accessions showed resemblance with identified *Nymphaea* species. The Acc. 6 exhibited some similarity with *N. rubra* and Acc. 7 with *N. micrantha* in terms of leaf and flower colour. Although the Acc. 4 showed similarity with white colour variant of *N. nouchali*, its flower was more close to *N. malabarica*.

- The analysis of variance for various genetic parameters in all the 10 accessions revealed high heritability coupled with high genetic gain in the characters viz., lamina width, lamina length, circumference of flower bud, diameter of fully opened flower, number of stigmatic appendages, number of petals, number of stamens and length of fruit indicating the scope of selection based on these characters for crop improvement.

- Hybridization among five seed setting accessions resulted in successful seed set only in three crosses. The seedlings of cross 1 (white flowered Acc. 4 x blue flowered Acc. 3) produced flower within five months of seed germination. Colour of adaxial surface of leaf, lamina width, leaf tip and circumference of flower bud of the hybrid progeny was found to be similar with the ovule parent. While the leaf margin and shape of pollen was similar to the pollen parent. The hybrid progeny had an intermediate character for flower colour, colour of abaxial surface of leaf, lamina

length, number of leaf teeth per 10 cm length and diameter of flower. In case of the number of petals, number of stamens, number of stigmatic appendages and viability of pollen, the hybrid progeny exhibited a unique range either lower or higher to both the parents.

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Characterization and hybridization of *Nymphaea* spp.

By

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ABSTRACT

The investigation on "Characterization and hybridization in *Nymphaea* spp." was undertaken in College of Agriculture, Padannakkad in the academic year 2016-18 with the objective to study the morphology, reproductive biology and to hybridize the seed forming *Nymphaea* sp. Based on the detailed survey in Northern Kerala, fourteen accessions were collected, of which ten were selected for detailed study. These consisted of two night blooming (Acc. 5 and Acc. 6) and eight day blooming types. Based on flower colour variation, there were accessions with different shades of pink (Acc.1, Acc. 2, Acc. 6, Acc. 9 and Acc. 10), blue violet (Acc. 3 and Acc. 8), white (Acc. 4 and Acc. 5) and violet (Acc.7).

Growth and development pattern of leaves and flowers were similar in all the accessions. However, the ten accessions were distinct based on the leaf and floral characters. The leaves in all the accessions were simple orbicular with sub-peltate lamina deeply cleft near to the petiole base. There was considerable variability in the colour, margin and tip of leaf lamina. The biometric characteristics of leaf such as length and width of lamina, length of petiole and longevity of leaf showed variability among the accessions, with Acc. 2 showing maximum length and longevity of the leaf.

Flowers were pedicellate and complete with all the floral whorls in a spiral fashion on the floral axis. The process of opening and closing of flower repeated for four consecutive days, except in Acc. 6 where it continued till fifth day. Significant variation observed in flower morphology, anthesis and periodicity of flowering in all accessions with critical difference between day and night bloomers. The night blooming accessions showed superiority over day bloomers in the flower bud characters *viz.*, length and circumference of flower bud and diameter of fully opened flower. The day bloomers differed from night bloomers in having shorter stigmatic appendages and stamens with terminal appendages.

The night blooming accessions showed maximum duration for flower opening but low frequency of flowering compared to day bloomers.

In general, all the accessions displayed strong protogyny except Acc. 5, where the phases of stigma receptivity and anther dehiscence were found overlapping. The pattern of anther dehiscence, duration of stigma receptivity and fragrance were favoring cross pollination in day blooming accessions. The adaptations such as overlapping of stigma receptivity and anther dehiscence in Acc. 5, simultaneous dehiscence of all anthers and absence of fragrance in both the night bloomers were pointing towards autogamy in these accessions.

Pollen grains of all the ten accessions showed regular staining with 1% safranin. The *in vitro* pollen germination was maximum in 10% sucrose medium with seven accessions (*viz.*, Acc. 1, Acc. 3, Acc. 4, Acc. 5, Acc. 6, Acc. 7 and Acc. 9) showing a remarkable increase in the pollen tube length.

Only five accessions (Acc.3, Acc. 4, Acc. 5, Acc. 6, and Acc. 7) produced fruit and seeds in open condition with and without emasculation and no seed set under protected condition. The germinability and speed of seed germination was low without any treatments. Chemical scarification with different concentrations of H_2SO_4, GA_3 and Ethrel showed an enhancement in seed germination, with maximum germination at a concentration of 100 ppm GA_3 and 5% H_2SO_4. Besides rhizome propagation, other methods like leaf proliferation (Acc. 1 and Acc.7) and root tip proliferation (Acc. 5) were also observed. These alternative modes of propagation showed earliness in the formation of leaf, root and flowers compared to rhizome.

Hybridization among five seed forming accessions showed success only among day bloomers (Ac. 3, Acc. 4 and Acc. 7) and with seed set in three crosses. Only the seedlings of cross 1 (Acc. 4 x Acc. 3) established successfully and produced flower within five months of seed germination. The initial evaluation of

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the morphological characters of hybrid progeny showed similarity with either of the parent for some characters. The colour of flower, color and shape of leaf were intermediate to both the parents and number of petals, stamens and carpels were unique to the hybrid progeny.