MORPHOGENESIS AND REPRODUCTIVE BIOLOGY OF WATER LILY (*Nymphaea* spp.)

BY

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THESIS

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DEPARTMENT OF PLANT BREEDING AND GENETICS
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DECLARATION

I hereby declare that this thesis entitled **"Morphogenesis and reproductive biology of water lily (*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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Introduction

1. INTRODUCTION

Water lilies, belonging to the angiosperm family Nymphaeceae and genus *Nymphaea*, form a popular and attractive group of aquatic plants. They are native to Egypt and are significant in Egyptian religion and history. The generic name *Nymphaea* is derived from the Greek word 'Nymphala' referring to water nymphs (Wiart, 2006). The colourful and showy flowers as well as the unusual floating leaves of water lilies make them the focal point of many aquatic gardens.

The symbolism of water lily can be traced back to antiquity. Historically water lily has a prominent place as it is associated with various cultures across the globe. They occupied a prominent place in the ancient Egyptian and Mayan art. The ancient Egyptians regarded water lily as a representation of Sun and as a symbol of rebirth. The pillars of the temples in ancient Egypt were in the form of water lily flowers blossoming from soil. Water lily is the national flower of Bangladesh and Srilanka and is commonly called as 'Shapla'. There are several mythological stories associated with this beautiful aquatic flower. Water lily is considered to be sacred by Buddhas and Hindus. According to Buddhist mythology, this flower was one of the 108 auspicious signs found on prince Siddhartha's footprint. It is also a symbol of purity as, even though the plant grows in mud, the flowers are pure and free from blemishes.

The rhizomes, flowers and seeds form an important ingredient of many Ayurvedic formulations and Siddha medicines. The hypoglycemic and hepato-protective effects of rhizomes are well confirmed. The tender leaves and peduncles are also valued as food. The dried plants, collected from ponds and tanks during dry season are used as animal forage. Rhizomes are used for brewing and also as a mordant for dyeing and tanning.

The genus *Nymphaea* which includes nearly 70 species, manifests as a complex and entangled group owing to its diversity in species and intra specific variations in flower colour. This genus also maintains species specificity of photoperiodism in flowering. Thus, two phenological groups based on flowering *viz.*, diurnals (those that open during day time) and nocturnal (those that open during night time) are recognized in this genus. Despite its immense potentialities, *Nymphaea* spp. has received only very little attention from the crop improvement workers.

In water lilies, a crop of tremendous potential, improvement with respect to economic and aesthetic aspect is of great significance. Study on taxonomy, morphology and reproductive biology are of utmost importance prior to taking up genetic improvement work in any crop. A thorough knowledge of the flowering biology which includes floral morphology, anther dehiscence, stigma receptivity and the developmental pattern of flowers and fruits, is fundamental to an understanding of the dynamics of natural population. Information on the developmental pattern and reproductive biology is also essential for the success of any crop improvement programme particularly those involving hybridization. However, most of these information is lacking in different phenological groups of this genus. It was in this background, the present investigation entitled "Morphogenesis and reproductive biology of water lily (*Nymphaea* spp.)" was taken up with the following objectives:

1. Evaluate the developmental pattern of leaves, flowers and fruits in day blooming and night blooming types of *Nymphaea* species
2. To elucidate and compare the reproductive biology of two day blooming and two night blooming types of *Nymphaea* species

Review of Literature

2. REVIEW OF LITERATURE

The genus *Nymphaea*, commonly known as water lilies, is one of the fascinating group of aquatic plants which give aesthetic view to the Indian waters because of their exquisite blooms of various colours. Water lilies are perennial plants, with erect or horizontal underground stems and floating, elliptic to orbicular leaf blades with a sagittate base. The solitary flowers are emergent, with many sepals, petals, stamens and carpels. The fragrant, showy blossoms range from white to numerous shades of green, orange, red, blue and purple (Lima *et al.*,2012). With the numerous types having attractive blooms and foliage, water lilies are considered as "Table Jewels of Water garden" (Muthukulam, 2006).

In India, the family Nymphaeaceae is represented by two indigenous genera *Euryale* and *Nymphaea* in addition to the genus *Victoria* to which the Amazon Giant water lily belong. Among these three genera, the genus *Nymphaea* remains incompletely defined due to the lack of a consolidated taxonomic revision, while the plants of monotypic genus *Euryale* and exotic genus *Victoria* are easily recognizable due to their well defined morphogenic features (Ansari and Jeeja, 2009).

2.1. Biodiversity and distribution

The number of genera and species included in the family Nymphaeaceae are found to vary according to the authors. Among the different genera *Nymphaea* is the largest and geographically most diverse genus in the family Nymphaeaceae.

According to Mabberley (1997), the family Nymphaeaceae is cosmopolitan with about six genera and 75 species. They can be seen in luxuriant growth inhabiting ponds, lakes and quiet back waters (Everett, 1981).

Gandolfo *et al.* (2004) described Nymphaeales as comprising of eight genera and approximately 70 species of aquatic plants with a world wide distribution in tropical and temperate region. As reported by Woods *et al.* (2005), *Nymphaea* is the most diverse genus in the family Nymphaceae containing over 50 species distributed in the tropical and temperate regions

The family Nymphaeaceae, consisted of six genera *viz.*, Barclaya, Euryale, Nuphar, Nymphaea, Ondinea and Victoria. Plants in the genus Nymphaea could be divided into two groups: Apocarpiae and Syncarpiae. The Apocarpiae group consisted of three subgenera: Anecphya (an Australian tropical water lily), Confluentes (an Australian tropical water lily) and Brachyceras (day blooming tropical water lily). The Syncarpiae group also consisted of three subgenera: Hydrocallis (night blooming tropical water lily), Lotus (night blooming tropical water lily) and Nymphaea (hardy water lily). Water lilies of Nymphaea genera usually bloom for three days with different blooming times for each subgenus. The sub genera Anecphya, Brachyceras, and Nymphaea are day bloomers (6 am –2 pm), while Hydrocallis and Lotus are night bloomers (7 pm –10 am) (Slocum, 2005; Jacobs and Porter, 2007).

Nymphaeaceae, the family to which water lilies belong, are distributed world-wide in temperate and tropical wet lands (Borsch et al., 2007) and are important indicators of deep wetland habitats (Cronk and Fennessy, 2001; Mitsch and Gosselink, 2007; Lohne *et al.*, 2008).

Borsch *et al.* (2008) described Nymphaeaceae as comprising of seven genera and 70 species. Judd *et al.* (2008) reported eight genera and 70 species in this family. According to Lohne *et al.* (2009), Nymphaeaceae included six genera and about 70 species.

Guruge (2014) reported that *N. pubescens*, commonly known as "Olu" were distributed throughout the Sri Lankan island and was abundant in the dry zone.

2.2. Evolutionary status

Huang *et al.* (1997) reported water lily (*Nymphaea* spp.) as the basal angiosperm plant which is important in aquatic landscaping and also as a source of vegetable as well as medicinal compounds.

The genus *Nymphaea* is believed to be relatively primitive in the evolutionary chain of angiosperms and hence forms an interesting model for many angiosperm families according to Barkman *et al.* (2000).

Nymphaeaceae is classified under the order Nymphaeales in the group of the "basal families" in the recent molecular based angiosperm phylogeny (Judd *et al.*,2002; Anon, 2003).

Friis *et al.*(2005) reported that Nymphaeaceae is a primitive family and the fossil record goes back to the early cretaceous period.

Chukiatman (2006) described that the members of the family Nymphaeaceae are the oldest group of plants on the earth. Recently, the botanists have split this family into two. Those with rigid petiole and peduncle are grouped under Nelumbonaceae. Others with soft petiole and peduncle are kept under Nymphaeaceae itself.

Maia *et al.* (2014) and Ruhfel *et al.* (2014), have reported that Nymphaeales is one of the most ancient angiosperm lineages, either sister to all flowering plants except Amborella or sister to Amborella and together forming the sister group to all other flowering plants.

2.3. Leaf characters

Besides adding beauty to the pool, the shade formed by the leaves helps in reducing the luxuriant growth of algae and provide shelter to fish (Brickell, 1989).

Hulten (1968) observed that the leaves of *N. tetragona* were elliptic-oval and about five inches long. In *N. odorata* the petiole length varied according to the water depth (Dalton and Novelo, 1983). Wooten (1986) found that many floating leaved plants had increasingly longer petioles with increasing water depth.

Fahn (1990) stated that stomata helped to absorb nutrients and exchange gas, but they did not have a protective function against transpiration in these plants, as aquatic species do not face desiccation.

Dassanayake (1996) described the leaves of *N. nouchali* as glossy bright green on upper surface and dark purplish green beneath. The leaves were glabrous on both surfaces with green prominent veins on the lower surface. The petiole was terete and purplish green in colour.

According to Gonzalez (2002), the presence of an evident thin cuticle on leaves helped to repel water from the surface of floating leaves as the stomata were arranged on the adaxial surface. The water lily tends to form dense floating mat of vegetation, preventing light penetration for native aquatic plants (Washington Department of Ecology, 2005).

According to Rossow and Charboneau (2006), water lilies grow at the base of the pond and produce leaves and blooms to the surface of water. They also provide valuable leaf cover, which reduces algal growth.

According to Voesenek *et al.* (2006), the changes in juvenile leaf morphology with increased depth resembled shade responses consistent with the reduction in light intensity seen with increased water depth.

Grob *et al.* (2006) observed that in *N. prolifera* the leaves were arising from the rooted tuber and the petioles were up to 120 cm long. Etnier and Villani (2007) reported that *N. odorata* exhibited heterophylly where a single plant may have leaves that are submerged, floating, or above (aerial) the surface of the water. Lily pads were placed in a unique situation because each leaf form was exposed to a distinctly different set of mechanical demands. The aerial petioles were loaded in tension under conditions of wind or waves because they must support the weight of the lamina. They also described that in *N. odorata*, the growth of aerial petiole might be an adoptive response to shading, allowing the aerial leaves to rise above the crowded water surface.

Deviprasad (2009), based on the studies on three *Nymphaea* species *viz.*, *N. alba*, *N. rubra*, and *N. stellata* reported that *N. stellata* was superior to other two in all the vegetative parameters, except leaf length and longevity which were higher in *Nymphaea rubra*

Laminae produced by juvenile and adult plants had different allometric relations, with submerged juvenile leaf blades longer than wide. However, the floating adult lamina were round (Richards *et al.*, 2011).

N. vanildae was characterized by petioles of 30–150 cm length and 0.3–0.5 cm diameter, green to chestnut-brown in colour, glabrous and with four median air canals surrounded by eight minor peripheral ones. Leaf blades were membranous to subcoriaceous, elliptic, with obtuse apices. The adaxial surfaces were green with red tonality and veinaceous spots and the abaxial surfaces slightly chestnut-brown. The leaf margins were entire, with slightly prominent central veins (Lima and Guilietti, 2013).

The leaves of *N. pubescens* had sharply dentate margin with pubescent abaxial surface where as the leaves of *N. nouchali* were linear to lanceolate with upper surface light green and lower surface dark purple colour (Guruge, 2014).

The studies on *N. gardneriana* (Nymphaeaceae) by Catian and Scremin-Dias (2015) revealed that the size, flexibility, color, and leaf anatomy were influenced by factors such as flow, nutrients, turbidity of the water and light availability. The phenotypic variations in fully submerged leaves compared to the floating ones of *N. gardneriana* indicated that this species adjusted leaf development in response to physical variations in the environment.

2.4 Flowering biology

Studies on three *Nymphaea* species, *N. alba*, *N. rubra*, and *N. stellata* by Deviprasad (2009) revealed that early flowering and maximum flower production were observed in *N. stellata* in which duration from bud emergence to complete flower opening was minimum. Length of bud, flower size and longevity of flowers were found to be maximum in *N. rubra.*

2.4.1 Floral morphology

Detailed morphological studies have been carried out in the family Nymphaeaceae (Khanna, 1967).

Dalton and Novelo (1983) found that the flowers of *N. odorata* were up to 6 inch wide. However, the flowers of *N. rubra* were large, sweet scented and 4-10 inches across (Biswas and Calder, 1984).

The flowers of *Nymphaea* subgenus *Hydrocallis* are characterised by the presence of completely fused carpels, swollen carpel appendages and tetramerous arrangement of perianth as well as outer stamens (Wiersema,1987).

Jokla and Mussob (2000) observed that *N. alba* is a white flowering species with yellow stigmatic disc. The four sepals are lanceolate. There were 15-30 petals, which gradually turn into stamens. They also noticed a gradation in outward direction from conventional stamens to staminodes and petals.

Hossain *et al.* (2000) observed that *N. rubra* shared similar morphological characters with *N. pubescens*. Hence, *N. rubra* flower is commonly known as "Ratu Olu". However, the two species differed significantly in the size and venation pattern of the leaf as well as the size of the flower, colour of the petals, number and size of the stamens

Walker-Larsen and Harder (2000) reported that *N. alba* had large solitary androgynous flowers with double perianth and long peduncle.

The level of transition between petals and stamens is assumed as an important diagnostic character in *Nymphaea* taxonomy. The outer perianth whorls are composed of three to seven (usually four) sepals. The abaxial side of sepal is green and the adaxial one is greenish-white. The transitional region of the flower is placed inwards to the typical petals. Stamens are numerous, more or less spirally arranged with long connective (Uotilla, 2000). Gynoecium consists of 5–35 laterally connate carpels with sessile stigma (Heslop-Harrison, 1955; Moseley, 1961; Uotilla, 2000; Schneider *et al.*, 2003).

In *N. prolifera* the rooted tubers produce mother flowers with flower stalks nearly 80 cm long. However, the stalks of daughter flowers are usually less than 20 cm in length (Grob *et al.*, 2006).

Studies on three *Nymphaea* species *N. alba*, *N. rubra* and *N. stellata* by Deviprasad (2009) revealed that *N. stellata* was superior to the other two in all the vegetative parameters, except leaf length and longevity which were higher in *N. rubra*. Early flowering and maximum flower production were also observed in *N. stellata* in which duration from bud emergence to complete flower opening was minimum. The length of flower bud, size of flower and longevity of flower were found to be maximum in *N. rubra*.

Hu *et al.* (2009) studied the floral development in *N. tetragona*. They observed that the four sepals were initiated uni-directionally. The basal petals also followed uni-directional initiation and they alternated with the sepals. The dome-shaped floral apex continued to expand and produced more petal and stamen primordia. The remaining petals and all the stamens were initiated in spiral or whorl fashion. Subsequently, after the initiation of all the stamens, the periphery of the floral apex grew faster than the centre and resulted in a depression at the centre of the apex. Carpels were simultaneously initiated in a cyclic fashion at the periphery of the depression. After all the floral whorls had been initiated, the centre of the depression on the floral apex developed into a globular structure. The floral ontogeny of *Nymphaea* was found to be very similar to that of *Euryale* and *Victoria* of family Nymphaeaceae,

According to Begum *et al.* (2010) *N. rubra* were characterized by the presence of four sepals which were ovate-lanceolate, reddish-green as well as ribbed outside and crimson-red inside. Petals were also crimson-red in colour. Anthers and stigmatic appendages were deep crimson-red in colour. The long stigmatic appendages were densely arranged covering the stigma completely. No fruit was formed naturally in *N. rubra.*

The flowers of *N. nouchali* were found to be solitary, pedicellate and complete with various floral whorls in spiral fashion on the floral axis (Fahida, 2012).

Guruge (2014) noticed that the flower colour varied from white, pink or yellow in *N. pubescens*. Flowers were having 8-30 petals and 30-90 stamens with yellow anthers devoid of any appendage.

N. nouchali , the Sri Lankan national flower, has three flower colour variants *viz.*, white, pink and violet blue and are commonly known as "Manel". The field observations revealed that pink flowered types were not widely distributed as the other colour variants. The common type of *N. nouchali* is characterized by the presence of violet blue flowers having a diameter of 20- 30cm. There are 8-30 petals having an average length of 3-6cm and width of 0.7- 1.5cm. The sepals are four in number with purple streaks. The number of stamens vary from 8-40 (Guruge, 2014).

2.4.2. Blooming

Rodriguez (2007) described that there are two types of water lilies, tropical and hardy. Tropical water lilies are divided into day and night bloomers. Hardy water lilies are all day bloomers and some of the hardy water lily flowers change colour over the life of the bloom. According to Henry (2008), water lilies were the most exquisite and colourful plants in the water garden which could give months of pleasure in summer.

According to Prance and Anderson (1976) and Schneider and Chaney (1981), species belonging to three subgenera under *Nymphaea viz.*, Nymphaea, Brachycera and Anecphya were day-blooming and were characterized by brightly coloured flowers that were sought as a pollen and nectar source by unspecialized anthophilous insects, mainly bees and flies.

According to Dalton and Novelo (1983), *N.odorata* buds opened early in the morning exposing the spectacular white, waxy flowers floating on the surface of water. Some petals were found to close in the afternoon.

The *N.alba* and *N.candida* studied by Velde and Vander (1986) were day-flowering only, where as *Nuphar lutea* flowered during the day and night without any closing movement of flower parts during anthesis.

N. nouchali Burm. is the only day-blooming water lily native to Thailand (Dezhi and Wiersema, 2001 ; Chomchalow and Chansilpa, 2007). Hasan and Chakrabarti (2009) reported *N. nouchali* as an emergent aquatic macrophyte which is commonly known as a blue water lily.

Flowers in *N. lotus* open at sunset and close in the morning hours, thereby revealing high variability in timing (Hirthe and Porembski, 2003). Krishnan *et al.*(2004) observed that the night blooming flowers are white in colour did not seem to be true in water lilies. The circadian rhythm of flower opening was also seen in water lilies.

The flowers of *N. rubra* were double red in colour and opened at night during summer (Muthukulam, 2006). Henry (2008) also recorded that water lilies were either day blooming or night blooming.

2.4.3. Factors affecting blooming

Opening and closing time of *Nymphaea* flowers were very much influenced by the intensity of sunlight and hence, temperature also. According to Prance and Anderson (1976), temperature was more effective than sunlight for the opening and closing of *Nymphaea* flowers.

Volkova *et al.* (2001) observed that the intensity of blooming of *Nymphaea* depended upon the temperature of the air and water. Slocum (2005) found that some *Nympheae* species did not bloom during the period of high temperature

Astle (2006) reported that water lilies required full sunlight as the development of bud was dependent on amount of sunlight.

Day length and temperature were the two main factors influencing the flower production of hardy water lilies when grown in tropics (Songpanich, 2007). Factors changing during the whole day regulated the amplitude of flower opening of *Nymphaea* and its immersion.

Songpanich (2007) reported that besides good care and maintenance, day length and temperature were the two major factors for good production of flowers in water lilies in tropical climate. Long day length increased flowering and short day length decreased flowering.

Flowering in *N. rubra* occured round the year, while in *N. pubescens* and *N. nouchali* flowering occured from May to November. They also described that opening and closing time of flowers were very much influenced by the intensity of sunlight and hence temperature also. The optimum temperature for growth and flowering ranged from 31 to 36° C (Begum *et al.*, 2010).

Fahida (2012) observed that bright and sunny days favoured early opening of flowers in *N. nouchali.*

2.4.4. Anthesis

Prance and Anderson (1976) observed fluid in the stigmatic cup of mature buds of *N. ampla*.

Meeuse and Schneider (1980) found that the sugar level was around 1 - 1.5%, boron concentration around 5 ppm and ionic composition in terms of Ca, K and Mg very close to optimal for pollen germination in the stigmatic exudate. The presence and role of exudate were observed by others like Heslop-Harrison and Shivanna (1977); Mosely (1961) and Schneider (1982).

Flowers of *N. odorata* were protogynous, opening as females in the morning of the first day, closing in the afternoon and re-opening on the next day in the male phase. The male phase was found to last for 1–2 days. The flowering process hence was completed in 2–3 days (Schneider and Chaney, 1981).

The flowers of *N. gigantea* growing in Western Australia bloomed for at least four consecutive days. In *N. gigantea* the flowers opened by 9.30 in the morning and closed by 5.00 in the evening. The first day flowers were 20 cm above the water level, odorous and with dark purple-violet corolla and receptive stigma. On the succeeding days, the flowers were raised to 30 cm above the water level, and the corolla faded to light purple-violet. The flowers were then functionally staminate and the stigmatic surface was dry and non-receptive (Schneider, 1982).

Schneider (1982) described various aspects of the pollination biology and floral behavior of *N. elegans*. Flowers opened each morning for three successive days. First-day flowers were protogynous with the stigmata secreting a fluid which filled the perigynous cup. The insects were seen fallen into the stigmatic fluid. The stigmatic fluid washed pollen from the insects and pollination was achieved. Second- and third-day flowers were functionally staminate, lacking the stigmatic fluid

Capperino and Schneider (1985) reported that in *N. mexicana* anthesis was diurnal with flowers opening and closing for two consecutive days. Flowers on the first day of anthesis were functionally female with the stigmatic papillae secreting a small quantity of fluid. The total dissolved solids in the stigmatic fluid ranged from 3 to 4%. Glucose and fructose, in all most equal concentrations, along with a large number of free amino acids were present in the stigmatic fluid. The anthers dehisced on the second day of anthesis and simultaneously the stigmatic fluid disappeared indicating the loss of stigma receptivity. Since protogyny was complete, flowers were obligatorily entomophilous.

There is no overlapping of male and female phases in *N. odorata* and this seems to be true for other temperate zone *Nymphaea* spp. (Capperino and Schneider, 1985; Wiersema, 1988). Male phase began with the dehiscence of inner anthers on second-day of blooming in *N. odorata* flowers. In contrast, in at least two tropical species of *Nymphaea*, the outer anthers were found to open first and that too on the first day of blooming (Prance and Anderson, 1976; Orban and Bouharmont, 1995; Endress, 2001).

Specific differences in the duration of anthesis observed by Velde and Vander (1986) in different species of *Nymphaea* appeared to be due to the variation in the ripening of the rows of anthers and could be related to the considerable differences in the number of stamens in individual flowers. The male phase of flowering was found to be prolonged in all the species.

According to Wiersema (1987), the flowers of night-blooming water lilies were protogynous. In these species, separate female and male phases of anthesis had taken place on the consecutive evenings and were characterized by intense volatilization of pollinator attractive floral scents (Wiersema, 1988; Bernhardt, 2000). Unlike the flowers of day-blooming species from the subgenera *Nymphaea*, which were moderately fragrant, the night-blooming species were intensely fragrant. This aided in attracting the scent-oriented scarab beetles involved in pollination (Wiersema, 1988).

The stigmatic cup of *Nymphaea* was found to hold 2.2-3 ml of watery fluid which was acidic (pH 4-4.5) in nature. The rim of the cup was surrounded by a row of 3mm long clavate appendages which were curved inwards (Tetali *et al.*, 2008).

Williams *et al.* in (2010) reported that male phase flowers open slightly earlier than female-phase flowers of *Nymphaea* on each day.

Anthesis showed great variation among different species of Nymphaea. *N. pubescens* and *N. nouchali* showed anthesis for three consecutive nights. In *N. rubra* it was for four consecutive nights. The anthers were introrse and split longitudinally in all the species. The anther lobes opened for ever in *N. nouchali* but in *N. rubra* and *N. pubescens* the opening and closing corresponded to the spreading of petals. In all the species anthesis for the second time was found to take place about half an hour earlier (Begum *et al.*, 2010). The flowers of all species of *Nymphaea* had their stigmatic cup full of exudates on the first anthesis. On the second day of anthesis, the stigmatic cup of of *N. rubra* dried up. However, in *N. nouchali* and *N. pubescens* the stigmatic cup remained sufficiently moist (Begum *et al.*, 2010).

Studies conducted by Begum *et al.* (2010) revealed that in *N. nouchali*, the bursting of anthers of different whorls proceeded gradually from the outermost to the innermost whorl during successive days of anthesis. But, in *N. pubescens* and *N. rubra* all the anthers were observed to burst simultaneously. On the second day of anthesis, the tip of stamens gradually bent inwards in *N. pubescens* and *N. rubra*. The bending formed a cone and completely covered the stigma in *N. rubra*. But, in *N. pubescens*, the loose cone of anthers left a small terminal opening. The case was reverse in *N. nouchali*, where the non-dehiscent inner whorl bent inside and the dehisced anthers bent outwards. Stigmatic appendages remained erect on first day of anthesis. However, they started benting inwards from the second day of anthesis. In *N. rubra*, long stigmatic appendages completely covered the stigma inhibiting pollination.

Fahida (2012) has reported that the opening time of the flower in the two colour variants of *N. nouchali* varied from 7.30am to 6.30am and closing time varied from 5.15pm to 6.15 pm.

N. vanildae had protogynous flowers with two nights of anthesis. On the first night the flowers opened around 8.00 pm and closed by 11.00 pm. The flowers emitted a strong odour of acetone and the receptive stigmatic region appeared bright and moist (Lima and Guilietti, 2013).

N. thermarum, a self-fertilizing species, was also capable of out crossing and it reproduced profusively by seed. It had a genome size which was on par with the other established flowering plant model systems and roughly twice as large as the genome of *A. thaliana* (Pellicer *et al.*, 2013). Early male and female functions indicated that *N. thermarum* was predisposed towards self-pollination. The formation of distinct micropylar and chalazal developmental domains in the endosperm, along with a copious perisperm characterized the seeds of most members of the Nymphaeales though the seed ontogenies varied among the constituent families. Floral biology, life history and small genome size make *N. thermarum* uniquely promising as an early-diverging angiosperm model system for genetic and molecular studies (Povilus *et al.*, 2014)

2.5. Pollination biology

Though hybridisation of water lilies (*Nymphaea* sp.) was initiated in the early 19th century, claims of successful crosses had been repeatedly challenged due to poor documentation or lack of compelling corroborative evidences (Conard,1905; Swindells, 1983). The first hybrid *Nymphaea* was produced in 1804 by a cross between *N. dendata* and *N. rubra*. However, this was undocumented (Henkel *et al.*,1907). A similar cross between *N. rubra* and *N. lotus* was reported by Paxton (1852).

Prance and Arias (1975) reported that pollination of the Amazon water lily *Victoria amazonica was* associated with Cyclocephala beetles.

Beetles and bees had been observed visiting the flowers of water lily. Dead insects were frequently found in flowers of *Nymphaea odorata* when pollination studies were conducted (Schneider and Chaney 1981). The honeybee was considered as the most common insect visitor to flowers. Schneider and Chaney (1981) viewed this non-native bee as an ineffective pollinator of *N. odorata* as it primarily visited male-phase flowers alone to collect pollen. However, transfers from male to female phase flowers by the honeybee were frequent in the related *N. mexicana Zucc.* (Capperino and Schneider, 1985)

Capperino and Schneider, (1985) noticed that *N. mexicana* flowers were primarily visited by *Dialictus* bees, flies and beetles. These insects typically landed on the wet stigmatic surface where the secretion loosens pollen from the insect's body.

Day-blooming water lilies are usually pollinated by Diptera and Hymenoptera (Wiersema,1988).

In the neotropics, it was estimated that 900 species of thermogenic plants were pollinated by the scarab beetle genus *Cyclocephala* alone (Schatz, 1990).

Bernhardt (2000) reported that insects attracted to flowers in female phase often remained in them until the male phase.

Honey bees (*Apis mellifera L.*) were the predominant pollinators in water lily but were mostly seen collecting pollen from second- and third-day flowers. Flowers were often visited, by many bees at a time, and visit was the heaviest mid-morning (Endress, 2001).

2.6. Palynology

Molina *et al.* (1996) found that if the pollen size and the anther length were small, the pollen production by the particular taxon was the lowest.

In contrast to *N. nouchali*, the pollen grains of *N. mexicana* had a psilate distal surface, a verrucate proximal surface,and were anazonasulculus (Gabarayeva and El-Ghazaly,1997).

Volkova and Shipunov (2007) also found morphological variations for pollen grains among some *Nymphaea* species. Under the optimized environmental conditions (in the dark at 32 °C), sucrose concentration played a crucial role in the *in vitro* germination of the pollen grains of the two forms of *N. nouchali* var. *versicolor*. However, sucrose concentration above 5% reduced the germination of pollen (Bodhipadmaa *et al.*, 2013).

Volkova (2008) reported that acetolysis of pollen grains did not significantly change pollen size or the characters of exine sculpture. *Nymphaea tetragona* differed from *N. alba* and *N. candida* by the sculpture of the exine at the proximal part of the pollen grain. But, the latter species could not be differentiated by pollen characters.

Thien *et al.* (2009) reported that the sucrose concentration in the stigmatic fluid of open flowers of *Nymphaea* spp. was lower than 5% and therefore would not pose a problem for pollen germination under natural conditions.

Bhunia and Mondal (2012) described that in Nymphaeceae the pollen grains were mostly medium sized and very rarely small sized, spheroidal and monosulcate with granular membranes. The exine was found to be verrucate and psilate with 1.82-3.78μm thickness. *N. pubescens, N. nouchali, N .stellate and N. rubra* were basically high pollen producing types. The highest pollen production was observed in *N. pubescens* and the lowest in *N. rubra.*

According to Bodhipadmaa *et al.* (2013), the two forms of *N. nouchali* var. *versicolor,* had similar pollen morphology. The pollen grains were nearly ellipsoidal, hetero-polar, round-shaped from one side and boat-shaped from the other side. They also had a smooth surface and a ring-like aperture around the equator. There was no significant difference between the pollen viability (95%) of the two forms of *N. nouchali* var. *versicolor* examined after acetocarmine staining.

2.7. Fruit and seed development

Multiplication of plants through seeds is an effective means of conserving of species, as it helps to maintain genetic variability, which is not possible under asexual methods of propagation, a common practice for *N.alba.* Smits *et al.* (1995) reported that production of white water lily through seeds has proved difficulties because of the development of dormancy in seeds with the passage of time. Conard (1905) reported that seeds of water lilies had to be submerged in 5–30 cm of water for germination.

Fruit is capsule, many seeded, irregularly dehiscent. Seeds mostly arillate with little endosperm, abundant perisperm, small embryo and fleshy cotyledons (Wiersema,1987). The fruit is a berry-like capsule about one inch across with numerous small seeds up to two mm long (Hitchcock and Cronquist 1990; Stone 1993). Aquatic and semi-aquatic insects use this species both for habitat and food (Cronin *et al.*, 1998; Dorn *et al.*, 2001). The aril of the seed is a campanulate or sacciform outgrowth of the funiculus that at least partially covers the seed. Because, of the presence of mucilage and stored gas bubbles in the aril tissue it is believed that aril aids in seed flotation and dispersal (Conard, 1905; Valla and Martin;1976; Collinson, 1980).

The ovules of Nymphaeales were classified into two groups with respect to the morphology outer integument. One group had hood-shaped outer integument (Khanna, 1967; Richardson, 1969) where as the other had cup-shaped outer integument (Schneider, 1976).

Seed size was distinct among the three subgenera of *Nymphaea*, common in Mexico. *N. mexicana* had the largest seeds among the species of subgenus Nymphaea. Species of subgenus Hydrocallis had the smallest seeds. In contrast, seeds of Victoria were the largest within the family Nymphaeaceae, being 8–10 mm in length (Valla and Martin, 1976).

Germination was enhanced by cold stratification for several months. Seedlings are rarely observed in the field, when the adult population is high. However, a large number of seeds germinated after the removal of adult plants. Light breaks dormancy and stimulates germination (Else and Riemer, 1984; Bonilla-Barbosa *et al.*, 2000; Di Tomaso and Healy, 2003).

Sumlu *et al.* (2010) described that fresh seeds of *N.alba* gave the highest germination on MS medium containing 1 mg/l BAP + 0.1 mg/l IAA. However, the seeds that were stored for five months at 40 C failed to germinate in medium containing 1 mg/l BAP + 0.1 mg/l IAA, either alone or combined with sucrose, IBA and GA3 in different concentrations. After five months, these seeds could be germinated only on germination medium that contained 0.05 to 4 mg/l TDZ. The highest germination was in the medium that contained 2 mg/l TDZ. Hence, TDZ could be used effectively to propagate *N. alba* from seeds which help to conserve and multiply this species in its natural habitat.

Baskin and Baskin (1998) reported that seeds of *N. odorata* had physiological dormancy that can be overcome by cold treatment. Richards and Cao (2012) described that the seeds could germinate under water and grow to the surface through water up to 90 cm depth.

Seed germination of Nymphaea was studied in *N. odorata*, fragrant water lily (Else and Riemer, 1984), *N. lotus* (Mohammed and Awodoyin, 2008) and *N. alba*, white water lily (Estrelles *et al.*, 2004; Sumlu *et al.*,2010). One of the unique characters of Nymphaea seeds is the whitish or translucent aril (Bonilla-Barbosa *et al.*, 2000).

Else and Riemer (1984) reported that in *N. odorata* seeds were dormant at the time of release and no after ripening treatment was required. Mechanical puncturing of seed coat had no effect on germination. Seeds germinated when large number of them were crowded in a small container and it was thought that the seeds themselves produced something that promoted their germination. Germination was strongly inhibited by periods of freezing or drying as short as one day.

Yamada *et al.* (2001) reviewed that in Nymphaeaceae the outer integument was annular at an early stage and then cup-shaped. However, it was semi annular at initiation and then hood shaped in *Nupar japonicum* and *Nymphaea alba.* The micropyle and hilum were separated by an intervening testa. The developmental pattern of the outer integument from semi annular to hood-shaped or from annular to cup-shaped is useful for inferring the morphology of the outer integument from the relative position of the micropyle to the hilum in seed fossils.

Materials and Methods

3. MATERIALS AND METHODS

The investigation entitled 'Morphogenesis and reproductive biology of water lily (*Nymphaea* spp.) was carried out in the Department of Plant Breeding and Genetics, College of Horticulture, Vellanikkara, during the period from August 2013 to June 2015. The details regarding the experimental materials and methodology adopted for the study are presented below.

A. Materials

A survey was conducted in the water lily growing tracts in Thrissur district. The ecotypes collected during the survey were multiplied and maintained under uniform *ex situ* condition. The flower colour was decided by following the Royal Horticultural Society colour chart (UPOV colour groups, 2001).

Five among the collected types were selected based on blooming time and seed set for detailed evaluation. The selected types, three day bloomers and two night bloomers, were grown in cement tanks having a diameter as also height of two feet with five replications for each type. Clay and water level were retained at uniform height in all the tanks throughout the experimental period.

B. Methodology

The collected types were subjected to a preliminary evaluation based on leaf and floral characters. Data collected were subjected to ANOVA and Duncan's Multiple Range Test was used for mean comparison and grouping of collected types. The phenotypic and genotypic variances were estimated according to the methods suggested by Burton and De Vane (1953) and these components of variance were used for the estimation of coefficients of variation (PCV, GCV) as described by Singh and Chaudhary (1977). Heritability and expected genetic advance (K=2.06 at 5% selection intensity) were computed for each character based on the formula developed by Allard (1960).

The growth and developmental pattern of leaves and flowers as well as the flowering biology were critically evaluated in the five selected types under *ex situ* condition.

3.1. Leaf characters

3.1.1. Growth and developmental pattern of leaf

The growth and developmental pattern of leaf in selected types were studied by taking observations on various biometric characters at regular intervals right from visual appearance stage of leaf bud till decaying. Observations were recorded from five leaves, replication wise of the selected types. The biometric characters considered were days to full expansion of lamina from visual appearance stage, longevity of leaves, rate of elongation of the petiole and frequency of leaf formation.

3.1.2. Morphological evaluation

Both qualitative and quantitative characters were considered for morphological evaluation. The evaluation was done by observing five fully opened leaves from each replication of each type evaluated. For qualitative evaluation colour and shape of the leaf were considered. The various biometric characters of the leaf *viz.*, length of petiole, length of sinus, length and breadth of lamina were considered for quantitative evaluation of the types. The length and breadth of lamina were measured at the biggest segment.

3.2. Flowering biology

3.2.1. Growth pattern of flower bud

Five flower buds per replication were tagged immediately after their appearance on the surface of the mud. The growth of flower bud from visual appearance stage till sinking in water was studied at periodic intervals in all the types. The days taken by the flower bud from initiation to reach the water surface as well as to opening were also noted. The rate of elongation of pedicel, length of pedicel, size of fully mature flower bud as indicated by its length and circumference, diameter of fully opened flower and longevity of flower in all the types were also observed. The succession of flower formation in each type was also observed.

3.2.2. Anthesis

The process of opening and closing of the flower was closely monitored in all the types.

3.2.3. Floral morphology

The floral features of all the five types were listed after examining fresh flowers on the first day of blooming. The observations on the following biometric characters of the flower were also recorded.

1. Number of sepals / flower
2. Length and breadth of sepal
3. Angle at the tip of sepal
4. Number of petals / flower
5. Length and breadth of petal (Measured at the biggest segment)
6. Angle at the tip of petal
7. Length of filament, anther as well as appendage of the stamen
8. Number of carpels / flower
9. Number of stigmatic appendages / flower
10. Diameter of the stigmatic cup

The breadth of the sepal as well as petal was measured along the broadest segment. If several whorls of petals and stamens were present, observations were recorded from each whorl.

3.2.4. Determination of anther dehiscence

The colour and appearance of anthers were observed with hand lens at hourly intervals from 6 am on the previous day of flower opening onwards in ten fully mature flower buds until the dehiscence of pollen grains, in each type, to find out the time of anther dehiscence (Prasad and Krishnaprasad, 1994).

3.2.5. Determination of stigma receptivity

The stigmatic surface was also observed for any change in colour or appearance in the same buds used for the above study at hourly intervals to find out the onset of stigma receptivity. Duration of stigma receptivity was also estimated as per standard procedures (Radford *et al.,* 1974). The presence of exudates in the stigmatic cup or its moist condition was considered as indication of stigma receptivity.

3.3. Pollination biology

Three sets of five flowers each from each type were used for this study. First set of five mature flower buds in each type was protected until the completion of anthesis. The buds were protected by covering them with butter paper cover and tying at the base two days prior to opening. Another set from each type was emasculated but kept unprotected. The third set was taken as control. The extent of fruit set was recorded in all the sets under evaluation. Various insects visiting the flowers were also observed.

3.4. Palynology

Pollen grains were acetolysed as per the procedures suggested by Nair (1970). The acetolysed pollen grains were microscopically examined to describe the shape, presence of aperture, exine sculpturing and other special features. Pollen size was measured using phase contrast microscope.

Fertility of pollen was assessed on the basis of staining with acetocarmin-glycerin mixture (Moore and Webb, 1972). The pollen grains which were well filled and stained were classified as fertile and others as sterile. Observations were taken from ten different fields for each type using microscope. The values were expressed as percentage

3.5. Fruit and seed characters

The fruit development pattern was studied in the fruit forming types. Fruit weight was recorded in such cases. Seed size, germinability and 100 seed weight were recorded from all the selected accessions.

3.6. Correlation with weather parameters

The data were correlated with weather parameters to know the influence of weather on growth and development of leaf and flower.

Appropriate statistical analysis was carried out where ever necessary.

Results and Discussion

4. RESULTS AND DISCUSSION

The results of the study "Morphogenesis and reproductive biology of water lily (*Nymphaea* spp.)" carried out in the Department of Plant Breeding and Genetics, College of Horticulture, Vellanikkara during 2013-2015 are presented below.

4.1. Survey in water lily growing tracts

The survey conducted in the natural water lily growing tracts in Thrissur district revealed the presence of only purple red, violet, and white colour variants of *Nymphaea* ((Plate1a-1b). Since sufficient variability was not obtained for blooming time and seed set in the natural growing habitats in Thrissur district, types were also collected from reputed nurseries. The details of the twelve different types thus collected are presented in Table1. The types collected were serially numbered as Acc. 1 to 12 (Plate 2a-2f).

Among the 12 accessions collected, ten were day bloomers and two were night bloomers. The types collected differed in flower colour also. None of the night blooming types collected set seeds. However, five among the day bloomers were seed setting types. From the collected accessions five accessions were selected based on the blooming time as well as the ability to set seeds for detailed evaluation.

The collected accessions were also subjected to a preliminary evaluation based on leaf as well as floral biometric characters *viz*., width as well as length of lamina, length of notch on lamina, length of mature flower bud, circumference of mature flower bud, diameter of fully opened flower, number of petals and number of stigmatic appendages. Analysis of variance revealed significant difference among the12 accessions for all the characters evaluated. The descriptive statistics of the eight leaf and floral characters observed on the 12 accessions of water lily are presented in Table 2.

Plate1a.Survey in water lily growing tracts at Kanimangalam and Anthikkad

Plate1b.Survey in water lily growing tracts at Kanjani

Plate 1c.Survey in reputed nurseries

Plate 2a. Acc. 1 and Acc. 2

Plate 2b. Acc. 3 and Acc. 4

Plate 2c. Acc. 5 and Acc. 6

Plate 2d. Acc. 7 and Acc. 8

Plate 2e. Acc. 9 and Acc. 10

Plate 2f. Acc. 11 and Acc. 12

The genotypic coefficient of variation ranged from 8.06 % for circumference of mature flower bud to 28.53 % for length of lamina (Table 2). Among the leaf and floral characters evaluated, the length of lamina exhibited maximum variability as indicated by its high PCV and GCV. The characters *viz.*, circumference of flower bud, number of petals and length of fully mature flower bud exhibited low GCV of 8.06%, 9.07% and 9.83% respectively.

The high h^2 values for all the characters indicated the low influence of environment on these characters. The high amount of variability coupled with high heritability (99%) for length of lamina indicates the scope for selection based on this character. It was also observed that such a selection can bring about nearly 58.55% (Table 2) improvement in the population.

Grouping of the collected accessions were done based on the results of post hoc test; Duncan's Multiple Range Test (DMRT), for the two most decisive characters of water lily in ornamental gardening *viz.*, diameter of fully opened flower as well as size of mature flower bud as indicated by its length and circumference. The groups formed and accessions falling in each group are presented in Table 3.

Among the five groups, group I and II included single accession each. Group IV was the biggest with six accessions. The ratio of flower diameter to length of mature flower bud as well as circumference of mature flower bud in the five different groups is presented in Table 4. There was a steady decline in the flower diameter: bud length ratio from group I to group V. Similar pattern was observed in the ratio of flower diameter to width as well as length of lamina (Table 5).

From the results it can be seen that there is correlation between leaf size (width and length of lamina) and flower size as well as flower bud length and flower size.

Table1. Details of water lily types collected during survey

Types	Flower colour	Blooming time	Seed set	Place of collection
Acc. 1	White	Day	Yes	Puzhakkal
Acc. 2	White	Day	No	Kanjani
Acc. 3	Violet blue	Day	Yes	Mekkattil nursery, Vazhukkumpara
Acc. 4	Purple red	Night	No	Kanjani
Acc. 5	White	Night	No	Kanimangalam
Acc. 6	White	Day	No	Anthikkad
Acc. 7	Bluish pink	Day	Yes	Mekkattil nursery, Vazhukkumpara
Acc. 8	Violet blue	Day	Yes	Mekkattil nursery, Vazhukkumpara
Acc. 9	Bluish pink	Day	Yes	Ally gardens, Aaramkallu
Acc. 10	Violet	Day	No	Kanjani
Acc. 11	Light yellow brown	Day	No	Swapnachitra nursery, Koratty
Acc. 12	Yellow green	Day	No	Swapnachitra nursery, Koratty

Table 2. Descriptive statistics for various characters of water lily accessions

Character	Mean	Range		SD	PCV (%)	GCV (%)	h^2 (%)	GG (%)
		Minimum	Maximum					
Lamina width (cm)	3.98	2.57	4.92	0.58	15.12	14.91	97	30.25
Lamina length (cm)	3.16	2.18	3.81	0.40	28.67	28.53	99	58.55
Notch length (cm)	2.76	2.02	3.51	0.39	14.54	14.17	95	28.37
Length of flower bud (cm)	2.25	2.03	2.66	0.22	10.23	9.83	93	19.49
Circumference of flower bud (cm)	2.42	2.18	2.88	0.20	8.47	8.06	91	15.80
Diameter of flower (cm)	2.78	2.47	3.83	0.39	14.34	13.84	93	27.54
Petal number	4.38	3.67	4.89	0.42	9.95	9.07	83	17.07
No. of stigmatic appendages	4.13	3.11	5.06	0.67	16.53	12.93	61	20.81

*PCV & GCV (Sivasubramanian and Menon , 1973) - Low: less than 10%, Moderate: 10-20%, High: more than 20%

*h^2 (Johnson *et al.,* 1955) - Low: less than 30%, Moderate: 30-60%, High: more than 60%

*GG (Johnson *et al.,* 1955) - Low: less than 10%, Moderate: 10-20%, High: more than 20%

Table 3. Groups formed and members in each group

Groups	Accessions
Group I	Accession 4
Group II	Accession 5
Group III	Accession 8, Accession 9
Group IV	Accession1, Accession 2, Accession 3, Accession 6, Accession 7, Accession 10
Group V	Accession 11, Accession12

Table 4. Ratio of flower diameter to bud length and circumference in different groups

Groups	D/BL ratio	D/BC ratio
Group I	2.16	1.81
Group II	1.80	1.42
Group III	1.65	1.45
Group IV	1.58	1.31
Group V	1.11	1.06

D - Diameter of flower BL - Bud length BC – Circumference of bud

Table 5. Ratio of flower diameter to width and length of lamina in different groups

Groups	D/WL	D/LL
Group I	0.60	1.01
Group II	0.53	0.89
Group III	0.44	0.80
Group IV	0.46	0.72
Group V	0.39	0.60

D - Diameter of flower WL – Width of lamina LL – Length of lamina

The five accessions selected (Plate 3) for detailed evaluation along with their specific features are depicted in Table 6. Three among the selected accessions were found falling in group IV and the other two in group I and II respectively.

Table 6. Accessions selected for detailed evaluation

Accession No.	Parental group	Blooming time	Seed set	Flower colour
Acc.1	Group IV	Day	Present	White
Acc.2	Group IV	Day	Absent	White
Acc.3	Group IV	Day	Present	Violet blue
Acc.4	Group I	Night	Absent	Purple red
Acc.5	Group II	Night	Absent	White

4.2. Leaf characters

The selected accessions were evaluated based on the growth and developmental pattern as well as morphological features of the leaves.

4.2.1. Growth and developmental pattern of leaf

The observations on the growth pattern of leaf were recorded from the visual appearance on the surface of mud and presented in Tables 7 and 8. The lamina was found to be in the rolled condition when the leaf was under the water surface (Plate 4). The rolled appearance of lamina continued till it was close to the water surface (Plate 4). Unrolling started soon after its emergence from water and it took 3-7 days depending on the accession, for the leaf to reach the water surface (Table 7). Among the accessions, Acc. 4 took the highest number of days for the leaf to reach the water

Acc.1

Acc. 2

Acc.3

Acc.4

Acc. 5

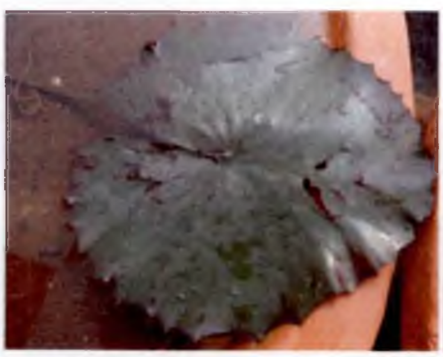
Plate 3. Accessions selected for the detailed study

Stage 1

Stage 2

Stage 3

Stage 4



Stage 5

Stage 6

Plate 4. Growth and developmental pattern of leaf

surface. There was no significant difference among the accessions in the number of days taken for the full expansion of lamina after unrolling (Table 7). The longevity of the leaf, i.e. the days from the visual appearance stage to decaying, was the highest in Acc.1 (21.20 days). The life span of the leaf was the lowest in Acc. 2 (15.8 days).

Table 7. Growth pattern of leaf in selected accessions of *Nymphaea*

Accession No.	Days to reach water surface	Days from unrolling to full expansion of lamina	Days from visual appearance till decaying
Acc.1	4.00 (2.12)	17.2 (4.19)	21.2 (4.65)
Acc.2	3.40 (1.97)	12.4 (3.58)	15.8 (4.03)
Acc.3	4.40 (2.21)	12.4 (3.58)	16.8 (4.19)
Acc.4	7.60 (2.84)	13.4 (3.71)	21.00 (4.63)
Acc.5	4.00 (2.11)	12.4 (3.58)	16.40 (4.10)
CD (0.05)	0.19	NS	0.38
CV (%)	6.28	6.35	6.75

*Values in parenthesis indicate square root transformed values.

The mean increment in the growth of petiole on the consecutive days from visual appearance stage till the decaying of leaf in the selected accessions is presented (Table 8 and Fig.1a to1e). The data revealed that the rate of elongation was high when the leaf was under water. The elongation rate gradually declined with the emergence of lamina from water. From the data it can also be seen that the rate of elongation was comparatively low in Acc.4. It may be the reason for it's the slow emergence from water by taking more number of days (Table 7) when compared to the other accessions.

Table 8. Rate of elongation of petiole in selected accessions of *Nymphaea*

Accession No.	Increment in growth of petiole on each day (cm)																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Acc.1	0.5	2.1	3.9	2.3	2.3	0.3	1.0	0.3	1.1	1.0	1.8	0.7	0.8	1.5	1.2	2.0	3.5	1.8	0.5	2.2	0.8	0
Acc.2	1.3	2.4	6.8	4.5	3.5	1.9	0.6	3.0	2.5	2.7	1.8	0.2	0.3	2.5	1.5	0	0	0	0	0	0	0
Acc.3	0.5	0.8	1.5	3.2	2.5	1.1	1.4	1.0	0.7	1.8	2.0	2.0	2.0	1.5	2.7	0.8	1.0	2.0	1.0	0	0	0
Acc.4	0.5	1.6	1.4	2.0	1.9	0.8	1.0	6.0	2.1	1.2	2.3	0.7	1.0	2.0	0.5	3.1	1.8	0.6	0.5	0.6	0	0
Acc.5	0.8	1.0	3.0	4.5	2.5	2.0	2.0	2.5	3.0	3.5	0.5	1.3	0.7	1.0	1.5	0.8	0	0	0	0	0	0

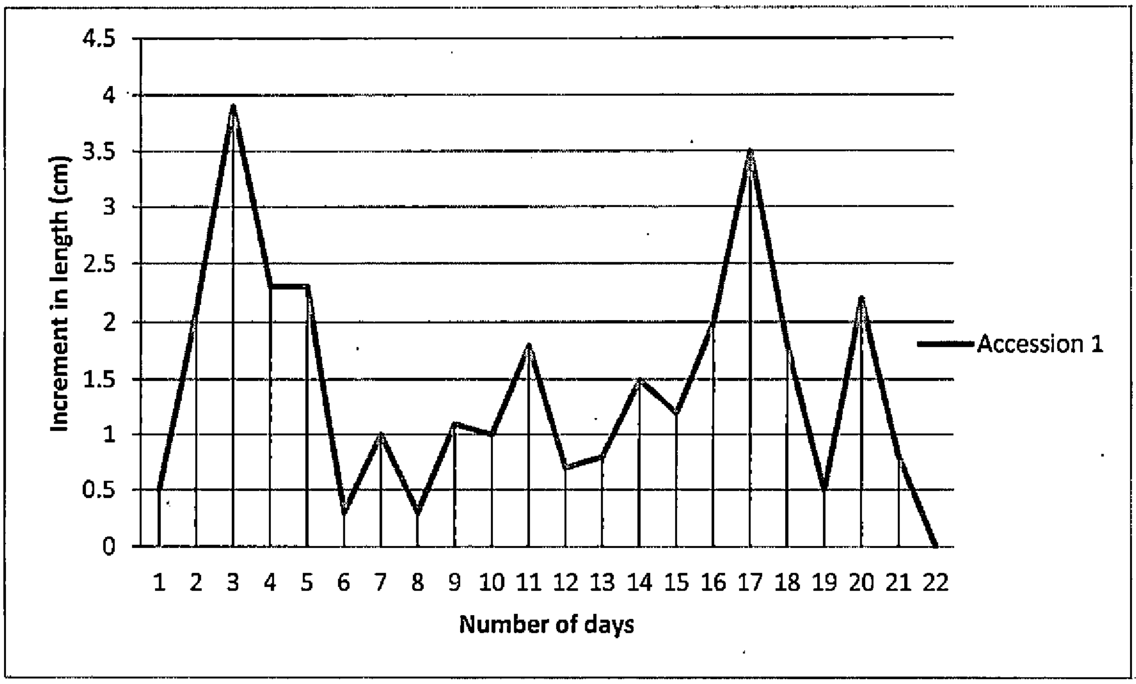


Fig. 1a. Rate of elongation of petiole in Acc.1

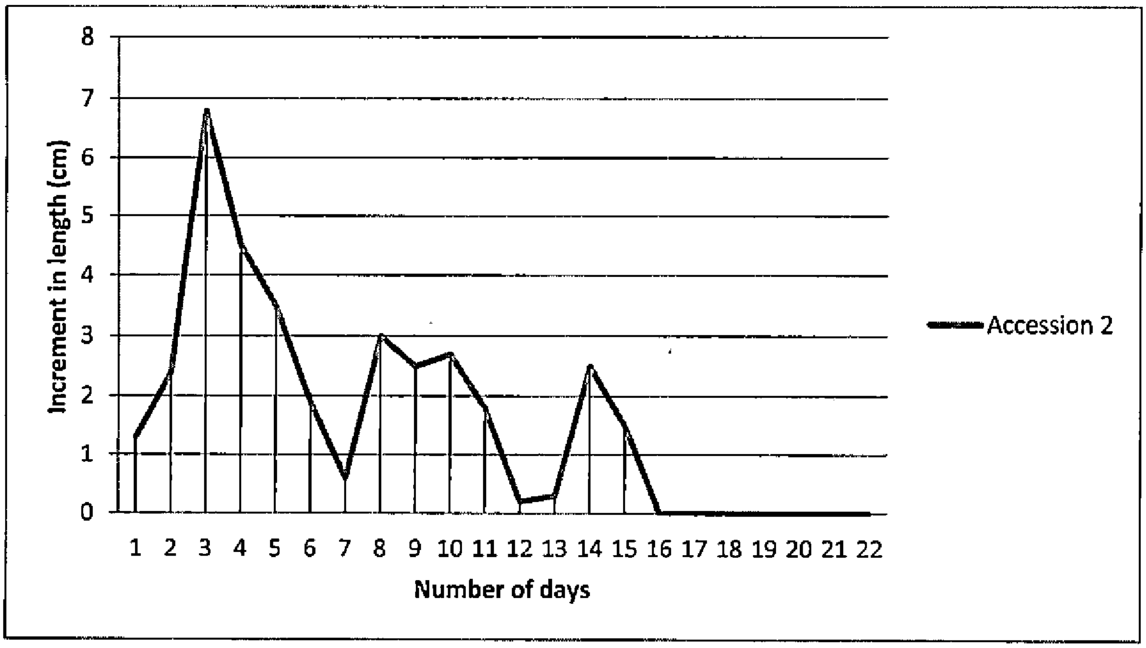


Fig. 1b. Rate of elongation of petiole in Acc.2

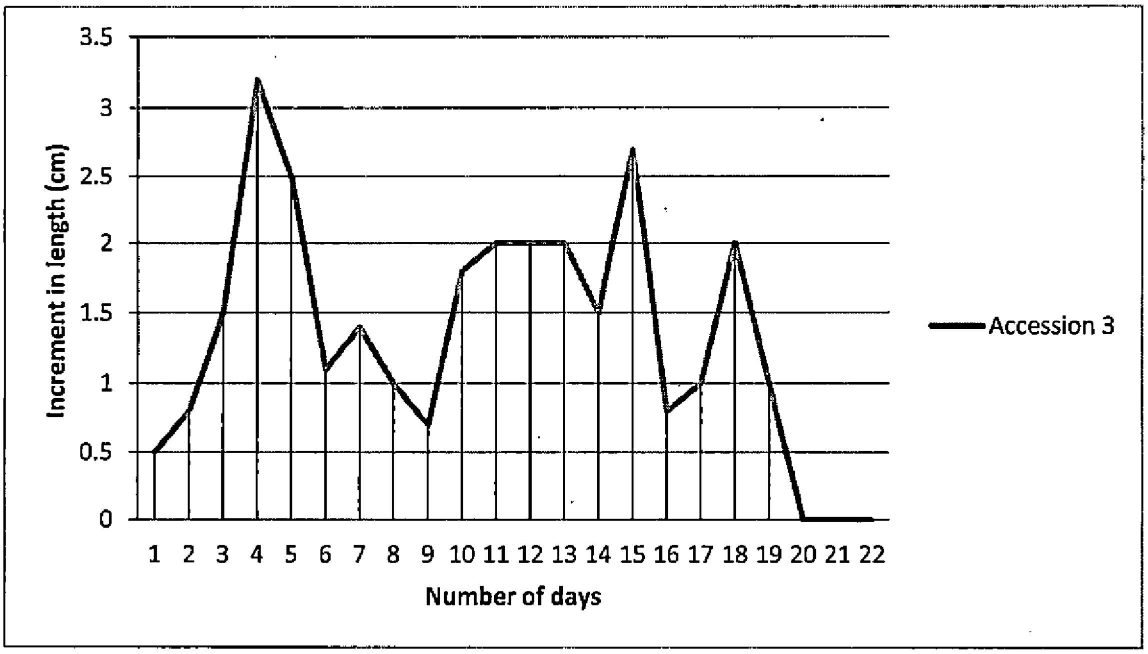


Fig.1c. Rate of elongation of petiole in Acc.3

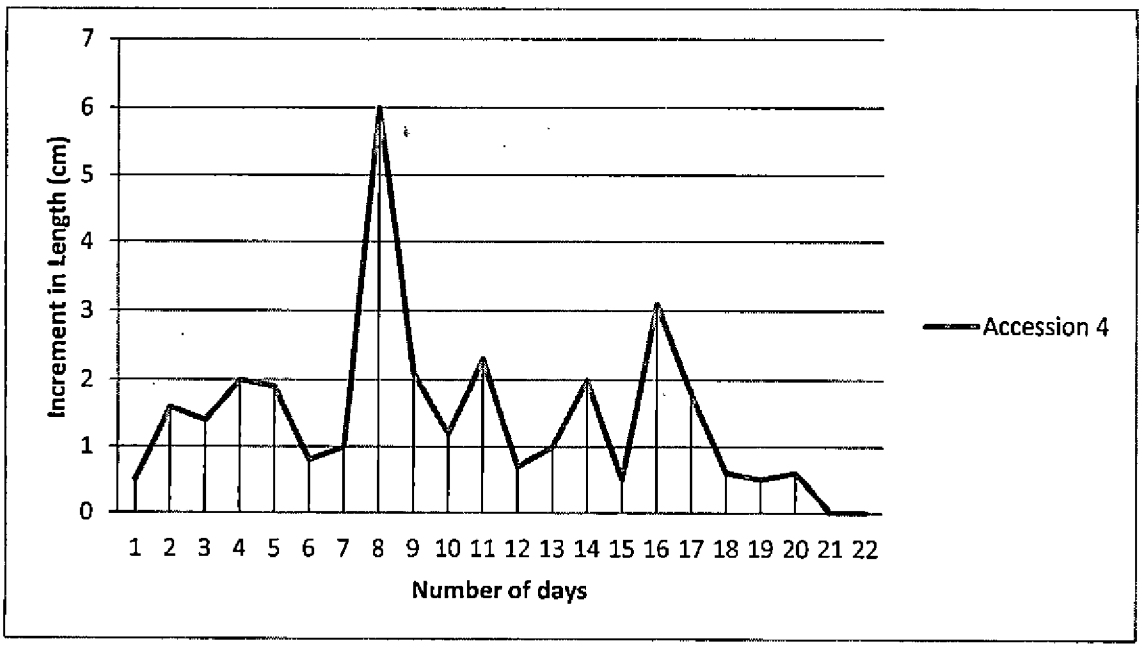


Fig. 1d. Rate of elongation of petiole in Acc.4

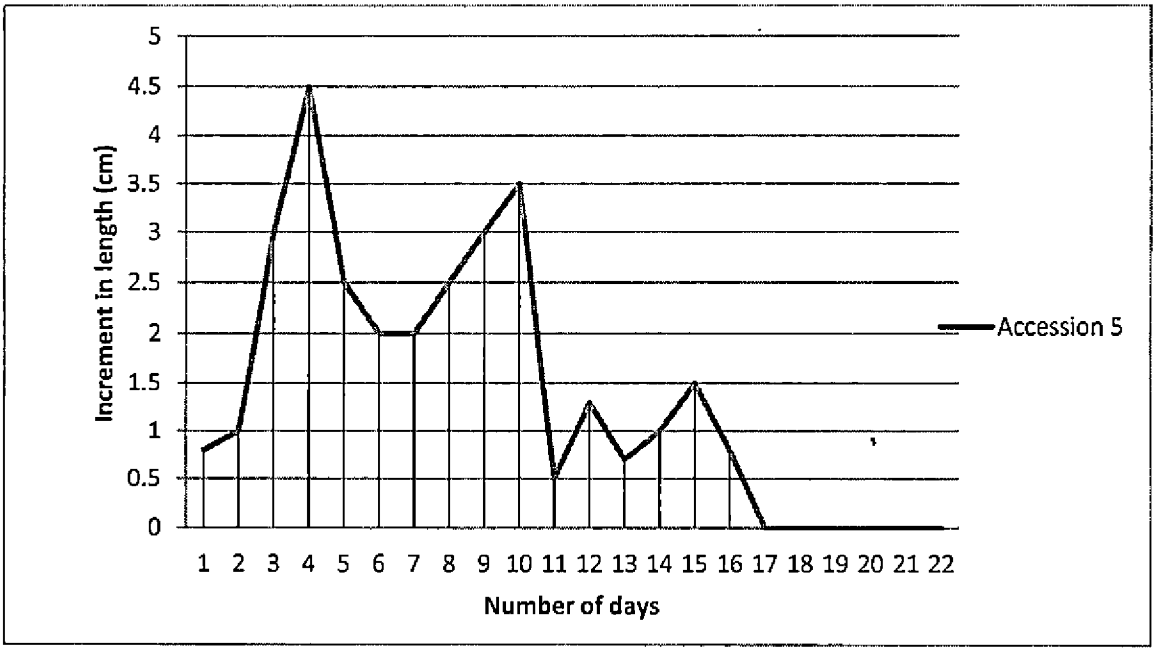


Fig. 1e. Rate of elongation of petiole in Acc.5

The frequency of leaf formation which indicates the days taken for the formation of successive leaves in the five accessions of *Nymphaea* are presented in Table 9.

Table 9. Frequency of leaf formation in selected *Nymphaea* accessions

Accession No.	Days taken for successive leaf formation
Acc.1	2.20 (1.60)
Acc.2	1.40 (1.37)
Acc.3	1.20 (1.30)
Acc.4	1.80 (1.49)
Acc.5	3.00 (1.82)
CD (0.05)	0.31
CV (%)	20.63

*Values in parenthesis indicate square root transformed values

The Table 9 shows that the number of days taken for the formation of successive leaves varied with the accession. In accession 5, the leaves were formed once in three days whereas they were formed almost once in a day in all the other accessions.

4.2.2. Morphological evaluation

The leaves in all the selected accessions were simple orbicular with subpeltate lamina which is deeply cleft near to the petiole base dividing . The cleft is known as sinus. The tip of the leaf was emarginate or obtuse. The accessions evaluated however, differed in the colour of leaf, sinus overlap, nature of leaf margin as well as veins. In Acc.1, the adaxial surface of leaf was pale green. The abaxial surface was green but, darker towards the midrib. The primary veins radiating from the petiole base on the abaxial surface were not prominent. The notch was dark green in colour. The margin of the leaf was undulating and irregularly sinuate. There was no sinus overlap and leaf tip was emarginate. The petiole was greenish in colour and the younger leaves were characterized by the presence of faint purple spots on the adaxial surface which disappeared on maturity (Plate 5a and Plate 5f).

Large purple patches were present on the adaxial surface of the leaf in Acc.2. On the abaxial surface both purple patches and spots were present. However, the patches on the abaxial surface disappeared two to three days prior to senescence (Plate 5f). The primary veins on the abaxial surface were not prominent. The leaf margin was undulating and irregularly sinuate and leaf tip was obtuse. The petiole was green in colour and sinus overlap was present (Plate 5b).

In Acc.3 the adaxial surface of the leaf was green and the abaxial surface purple with greenish patches towards the midrib region. Though faint purple spots were present on the adaxial side in young stages, they disappeared on maturity (Plate 5f). The primary veins on the abaxial side were not prominent. The leaf was with undulating and irregularly sinuate margin with sinus overlap. The leaf tip was emarginate. The petiole was coffee brown in colour. However, the portion of the petiole close to the lamina was green (Plate 5c).

In the case of Acc.4, both the adaxial and abaxial surfaces were green. However, the juvenile leaves had a brownish tinge. The abaxial surface was characterized by the presence prominent primary and secondary veins. Sinus overlap was present. The leaf was having obtuse tip and sinuately dentate margin. The petiole was coffee brown in colour (Plate 5d).

The adaxial surface of the mature leaf of Acc.5 was dark green with faint purple patches. The abaxial surface was brownish in colour with prominent primary and secondary veins. In young leaves the adaxial surface was green, marked with the

Plate 5a. Adaxial and abaxial surface of Acc. 1

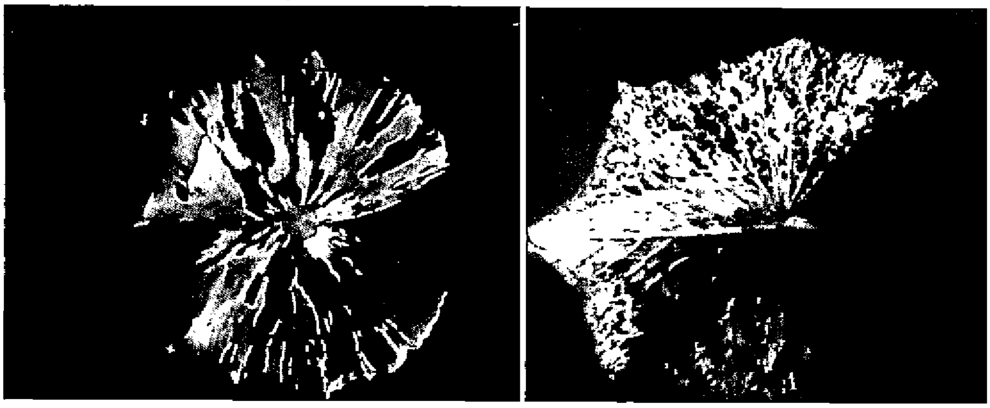


Plate 5b. Adaxial and abaxial surface of Acc. 2

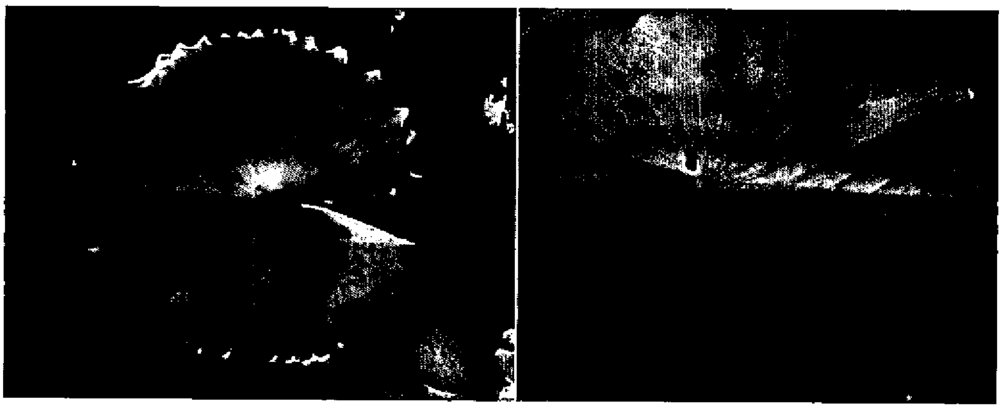


Plate 5c. Adaxial and abaxial surface of Acc. 3

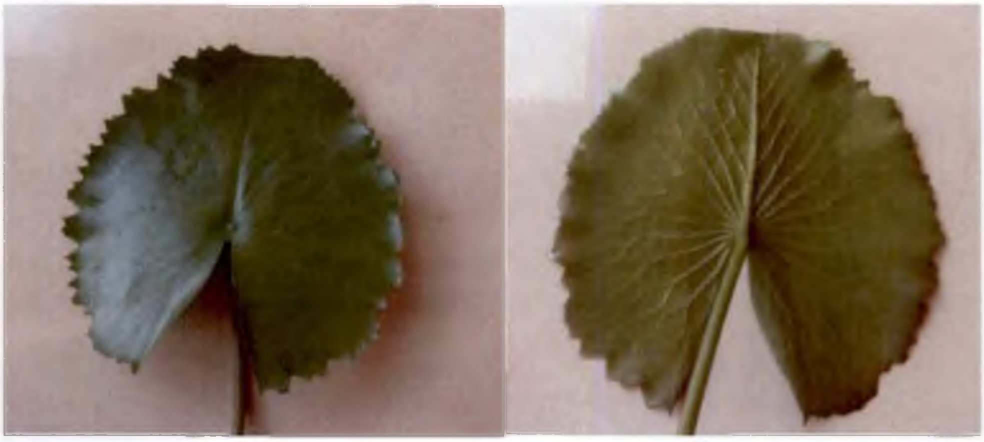


Plate 5a. Adaxial and abaxial surface of Acc. 1

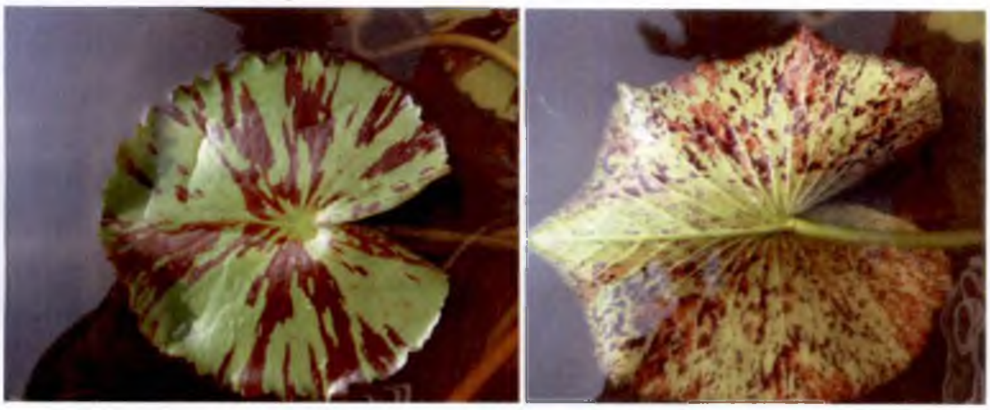


Plate 5b. Adaxial and abaxial surface of Acc. 2

Plate 5c. Adaxial and abaxial surface of Acc. 3

Plate 5d. Adaxial and abaxial surface of Acc. 4

Plate 5e. Adaxial and abaxial surface of Acc. 5

Plate 5f. Immature leaf of Acc. 1 & mature leaves of Acc. 2 and 3

presence of small but prominent purple patches. The tip of the leaf was obtuse and the margin was sinuately dentate. The petiole was coffee brown in colour and there was sinus overlap (Plate 5e).

A comparison of the leaf qualitative characters of the five accessions is presented in Table 10

Table 10. Qualitative characters of leaf in selected *Nymphaea* accessions

Accession No.	Sinus overlap	Leaf tip	Leaf margin	Veins on abaxial surface	Petiole colour
Acc.1	Absent	Emarginate	irregularly sinuate	Not prominent	Green
Acc.2	Present	Obtuse	irregularly sinuate	Not prominent	Green
Acc.3	Present	Emarginate	irregularly sinuate	Not prominant	Coffee brown
Acc.4	Present	Obtuse	sinuately dentate	Prominent	Coffee brown
Acc.5	Present	Obtuse	sinuately dentate	Prominent	Coffee brown

The observations on various biometric characters of the leaf *viz.,* width and length of leaf as well as length of sinus at full expansion stage in the five different accessions of *Nympaea* are presented in Table 11.

From the Table 11 it can be seen that leaf width was the highest in Acc.4 (23.68±0.48cm) followed by Acc.2 (19.56±0.40 cm) and the lowest in Acc.1 (14.00±0.46 cm) which was on par with Acc.3 (14.66±0.37 cm). The highest mean leaf length was also recorded by Acc.4. (13.98±0.27 cm) followed by Acc.2 (12.26±0.34 cm). The lowest value was recorded by the Acc.1 (8.82±0.12 cm). The mean sinus length, ranged from 11.82±0.27 cm in Acc.4 to 6.44±0.14 cm in Acc.1.

With respect to the biometric characters of the leaf, Acc.4 was found to be superior and Acc.1 to be inferior.

Table 11. Biometric characters of leaf in selected accessions of *Nymphaea*

Accession No.	Mean leaf width (cm)	Mean leaf length (cm)	Mean length of sinus (cm)
Acc.1	14.00±0.46	15.26±0.23	6.44±0.14
Acc.2	19.56±0.40	21.60±0.56	9.34±0.33
Acc.3	14.66±0.37	16.12±0.53	6.56±0.34
Acc.4	23.68±0.48	25.80±0.51	11.82±0.27
Acc.5	18.06±0.17	19.54±0.60	8.82±0.27
CD (0.05)	1.15	1.50	0.826
CV(%)	4.90	5.75	7.29

4.3. Flowering biology

4.3.1. Growth pattern of flower bud

The growth pattern of the flower bud (Plate 6) as indicated by the mean number of days to reach the water surface from the visual appearance stage of flower bud, mean number of days to flower opening after reaching the water surface, mean pedicel length at the time of flower opening and after sinking into water as well as blossom life are presented in Table 12. The flower buds were found to be developing from the leaf axils.

The results revealed that the accessions differed significantly in the days taken by the flower bud to reach the water surface, days taken by the flower bud to open after reaching the water surface, mean pedicel length at flower opening and after

Stage 1

Stage 2

Stage 3

Stage 4

Stage 5

Stage 6

Plate 6. Growth and development pattern of flower bud

sinking into water. Among the accessions evaluated, Acc.4 recorded the highest number of days for the flower bud to reach the water surface (8.8 days) after its emergence on the surface of the mud. The lowest number of days was taken by Acc.2 (3.80 days). It can also be seen from Table 12 that night blooming types took more number of days for the flower bud to reach the water surface when compared to the day blooming ones.

The number of days taken by the flower bud to open after reaching the water surface is also presented in Table 12.There was significant difference among the accessions with respect to the number of days taken by the flower bud to open after reaching the water surface. Accession 4 took the highest number of days for blooming (2.6 days). Acc.1, 3 and 5 were on par with respect to this character. The lowest number of days for the flower bud to open was recorded by Acc.2 (1.2 days).

There was significant difference among the accessions in the mean pedicel length at flower opening stage and after sinking into water. The flowers of Acc.4 had the longest pedicel (19.88 cm). It can also be seen from the Table 12 that even after flower opening the pedicel elongation continued in all the types except in Acc. 2 to an extent of nearly five cm.

The blossom life was uniform in both day blooming and night blooming types and the different stages of growth of flower bud in the selected accessions are shown in Plate 7.

The increment in pedicel elongation on consecutive days of flower bud development till it plunged into water is presented in Table 13 and Fig. 2a to 2e. It can be seen that the different accessions studied followed the same pattern with maximum growth rate just prior to the flower bud reaching the water surface. Even though the growth of pedicel continued even after flower opening, the elongation rate slowed down and stopped with the sinking of flower into water. The highest rate of

Acc.1

Acc.2

Acc.3

Acc.4

Acc. 5

Plate.7 Stages of growth of flower bud in different accessions

Table 12. Growth pattern of flower bud in selected accessions of *Nymphaea*

Accession No.	Days to reach water surface	Days to open after reaching water surface	Mean pedicel length at flower opening (cm)	Mean pedicel length after sinking (cm)	Blossom life (days)
Acc.1	5.40 (2.43)	1.80 (1.51)	7.94±1.15	12.66 ±1.04	4
Acc.2	3.80 (2.07)	1.20 (1.30)	13.38±0.52	14.91 ±1.03	4
Acc.3	6.80 (2.69)	2.00 (1.58)	8.96±0.55	13.16 ±0.37	4
Acc.4	8.80 (3.05)	2.60 (1.75)	14.4±1.30	19.88 ±1.18	4
Acc.5	7.80 (2.88)	2.00 (1.58)	9.48±0.20	14.66 ± 0.35	4
CD (0.05)	0.19	0.16	2.51	2.65	-
CV (%)	5.62	8.00	17.60	13.34	-

*Values in parenthesis indicated square root transformed values pedicel elongation was observed in the case of Acc.2. It may be the reason for the appearance of flower bud above water level in a short span of 3.80 days in Acc.2.

The data on various floral biometric characters *viz.*, the length and circumference of fully mature bud as well as the diameter of the fully opened flower are presented in Table 14. The Table shows that the length of mature flower bud was the highest in Acc.4 (6.56±0.15 cm) and the lowest in Acc.1 (3.66±0.22 cm).

The circumference of the mature flower bud was the highest in Acc.4 (7.82±0.19 cm) followed by Acc.5 (6.70±0.08 cm). A similar pattern was observed in the case of diameter of the fully opened flower also. The Acc.4 produced the biggest flowers with a mean diameter of 14.12±0.56 cm. This can be attributed to the superiority of this accession in length of mature flower bud. Hence, it can be seen that the diameter of flower is proportional to the length of flower bud.

The Acc.1 was found to be inferior with respect to the flower bud characters depicted in Table 14. The length and circumference of the flower bud as well as the diameter of the fully opened flower were higher in night blooming types, Acc.4 and 5 (Table 14). Hence, it can be concluded that the night blooming types are superior to the day blooming types as regards the above characters.

Table 13. Rate of elongation of pedicel in selected accessions of *Nymphaea*

Accession No.	Increment in growth of pedicel on each day (cm)											
	1	2	3	4	5	6	7	8	9	10	11	12
Acc.1	0.3	0.4	0.9	1.2	1.9	2.1	0	0	0	0	0	0
Acc.2	1.1	1.9	4.1	0	0	0	0	0	0	0	0	0
Acc.3	1.1	0.4	0.7	1.1	1.4	1.9	2.5	0	0	0	0	0
Acc.4	0.7	0.7	1.1	1.6	0.7	1.5	1.5	1.4	3.5	0	0	0
Acc.5	0.1	0.1	1.3	0.3	0.7	1.0	1.2	3.1	0	0	0	0

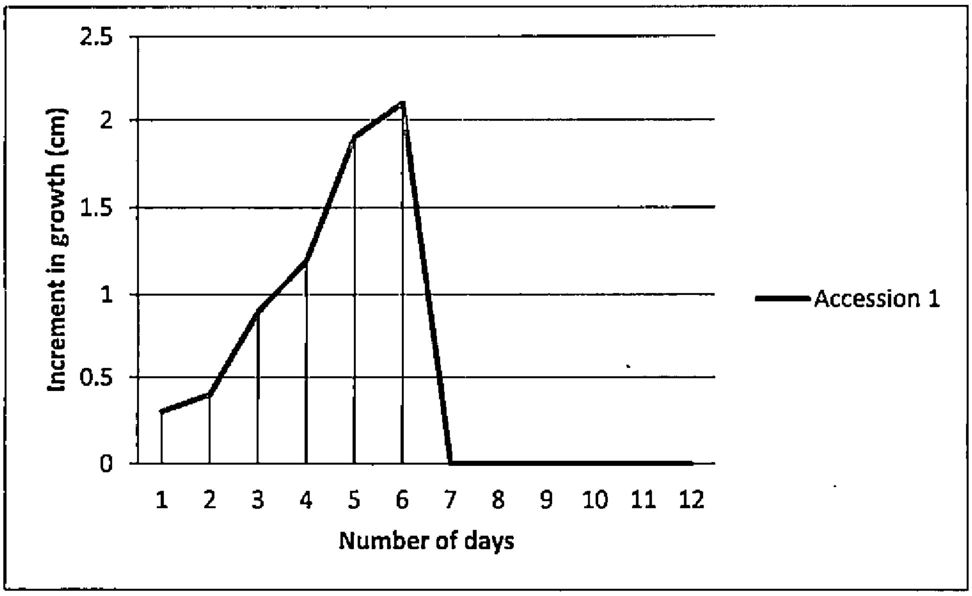


Fig. 2a. Rate of elongation of pedicel in Acc.1

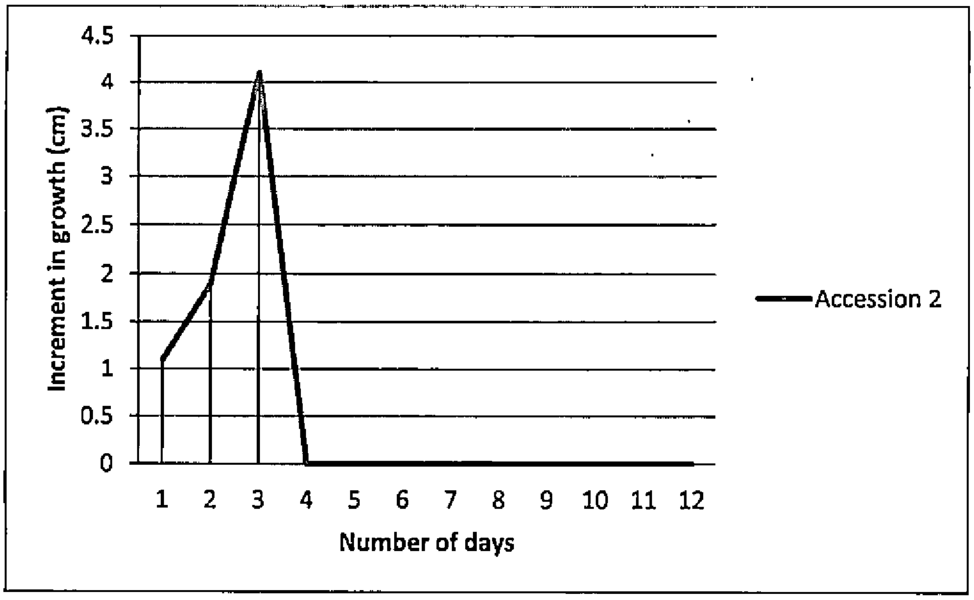


Fig. 2b. Rate of elongation of pedicel in Acc.2

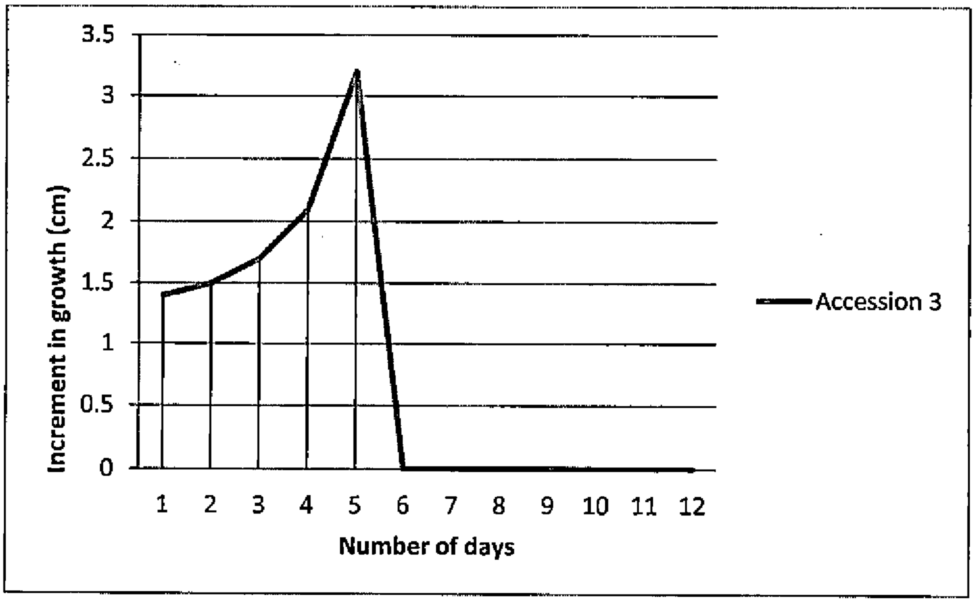


Fig. 2c. Rate of elongation of pedicel in Acc.3

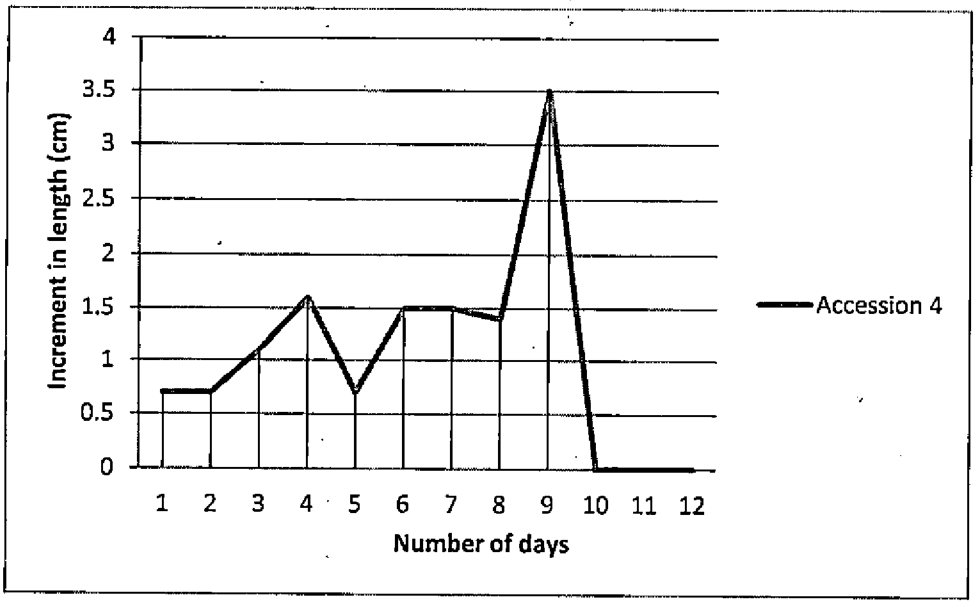


Fig. 2d. Rate of elongation of pedicel in Acc.4

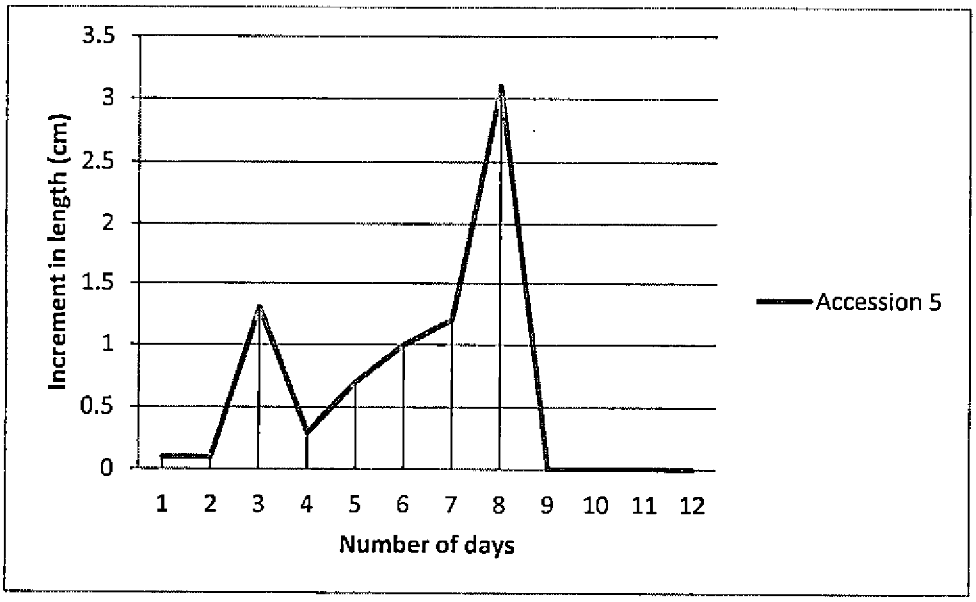


Fig. 2e. Rate of elongation of pedicel in Acc.5

Table 14. Biometric characters of flower bud and blossom life of selected accessions of *Nymphaea*

Accession No.	Length of bud (cm)	Circumference of bud (cm)	Diameter of fully opened flower (cm)
Acc.1	3.66 ± 0.22	4.48 ± 0.28	6.12 ± 0.24
Acc.2	4.26 ± 0.12	4.84 ± 0.15	6.62 ± 0.28
Acc.3	4.12 ± 0.04	5.16 ± 0.12	6.16 ± 0.22
Acc.4	6.56 ± 0.15	7.82 ± 0.19	14.16 ± 0.56
Acc.5	5.28 ± 0.07	6.70 ± 0.08	9.5 ± 0.39
CD (0.05)	0.41	0.53	1.06
CV (%)	6.41	6.90	9.46

The succession of flower formation in all the five accessions of *Nymphaea* evaluated is presented in Table 15.

Table 15. Frequency of flower formation in selected *Nymphaea* accessions

Accession No.	Blooming time	Days for the formation of successive flowers
Acc.1	Day	2.40 (1.69)
Acc.2	Day	1.40 (1.37)
Acc.3	Day	2.20 (1.63)
Acc.4	Night	1.20 (1.30)
Acc.5	Night	6.60 (2.64)
CD (0.05)	-	0.32
CV (%)	-	14.41

*Values in parenthesis indicated square root transformed values

There was significant difference among the accessions for the periodicity of flower production. The frequency of flower formation was the lowest in Acc.5 (6.60days). However, the flowers were produced almost every day in Acc. 2 and 4 (1.40 and 1.20 respectively). With respect to frequency of flower formation, distinction could not be made between the night blooming and day blooming types (Table 15).

4.3.2. Anthesis

The anthesis in all the evaluated accessions lasted for a few days. In day blooming types, the flowers opened in the morning and closed in the evening. In night blooming types the flower opening was in the evening hours and closing in the morning hours. The process was repeated consecutively for four days in all the accessions (Plate 8- 12). The day blooming types *viz.*, Acc.1, 2 and 3 were highly fragrant and night blooming types *viz.*, Acc.4 and 5 were lightly fragrant. After the closure of the flower on the fourth day the pedicel started bending downwards. On the fifth day the flower sank completely into water along with the pedicel (Plate 8-12). The floral parts did not wither as in the case of other flowers. The floral parts decayed completely in 5 to 6 days if there was no seed set. However, if there was seed set, all the floral parts could be seen persisting on the fruit. All the accessions studied followed the same pattern except for the time of time of opening and closing..

The process of blooming began with the opening of the sepals. In the day blooming types *viz.*, accessions 1, 2 and 3 opening time varied from 6.30 am to 7.15 am on bright sunny days. On cloudy or rainy days, the opening was delayed up to 8.30 am-9.45 am. In the night blooming types *viz.*, accessions 4 and 5 flowers opened between 6.30pm- 6.50 pm. It took 20 to 30 minutes for full blooming. In day blooming ones all the whorls of petal were wide open on the first day itself (Plate 8-10). However, in night blooming types on the first day of flower opening all whorls of petal except the inner most whorl was wide open (Plate 11-12). The closing time in day blooming types fell in the interval 4.30 pm – 5.45 pm in the evening where as for night blooming types it was 10.30am -12.00 pm. The closing process was completed in 15 to 20 minutes.

Begum *et al.*, (2010) reported that depending upon the weather conditions the opening time varied. She also reported that the opening and closing of flowers were very much influenced by sunlight and temperature. According to Prance and Anderson (1976), temperature was more effective than sunlight in the opening and closing of *Nymphaea* flowers. According to Jacobs and Porter (2007), in the subgenera *Hydrocallis* which included the night blooming tropical water lilies, the opening and closing times were 7 pm and 10.00 am respectively.

First day

Second day

Third day

Fourth day morning

Fourth day evening

Fifth day

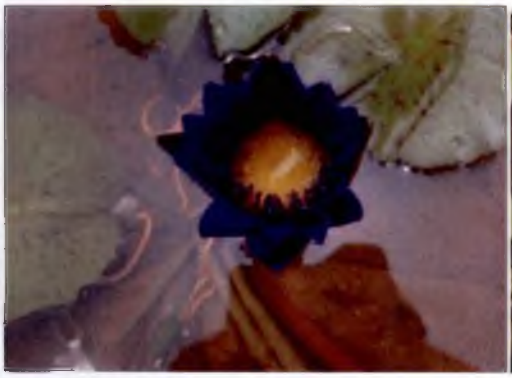
Plate 8. Consecutive days of flower opening in Acc. 1


First day Second day


Third day Fourth day morning


Fourth day evening Fifth day

Plate 9. Consecutive days of flower opening in Acc. 2



First day

Second day

Third day

Fourth day morning

Fourth day evening

Fifth day

Plate 10. Consecutive days of flower opening in Acc. 3

First day

Second day

Third day

Fourth day

Fifth day

Plate 11. Consecutive days of flower opening in Acc. 4

First day

Second day

Third day

Fourth day

Fifth day

Plate 12. Consecutive days of flower opening in Acc. 5

4.3.3. Floral Morphology

The water lily flowers are solitary, pedicellate, actinomorphic and complete with floral formula

$$\text{♀} \; K_4 \; C_{(13\text{-}23)} \; A_{(\alpha)} \; \underline{G}_{(16\text{-}26)}$$

Irrespective of accession four sepals, acute and in imbricate aestivation were present. However, the colour of sepals varied with the accession. In Acc.1, outer surface of the sepal was light green and the inner surface white (Plate 13a).

The sepals were dark green with purple streaks along the entire length on the outer surface in Acc.2. The inner surface was white in colour. However, a pinkish tinge appeared at the tip of the inner side of the sepal from the second day of flower opening (Plate 13b). The outer surface of the sepals was dark green with a light green tinge at the basal part in Acc.3. The inner surface was violet blue in colour (Plate 13c).

In the night blooming types, sepals were characterized by the presence of five to six prominent nerves on either side. The outer surface of the sepal was greenish in colour with a rose shade along the margin in Acc.4. However, the inner surface was completely purple red (Plate 13d). In accession 5 the outer surface of the sepal was dark green whereas the inner surface was white in colour (Plate 13e). The nerves were pinkish in colour.

The biometric characters of sepals are presented in Table 16. The accessions differed significantly in the mean length and breadth of the sepals as well as the tip angle of the sepal.

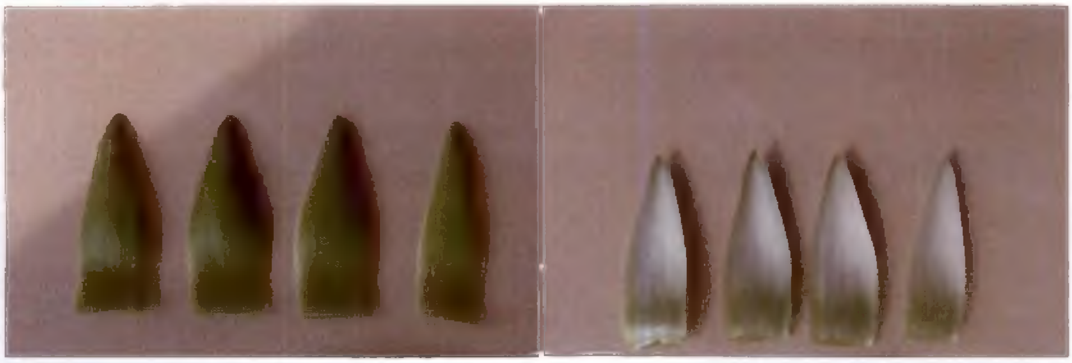


Plate 13a. Sepal characters in Acc. 1

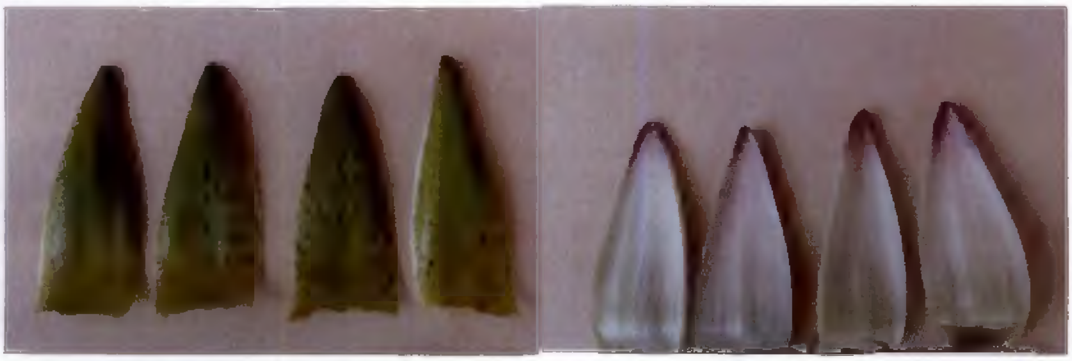


Plate 13b. Sepal characters in Acc. 2

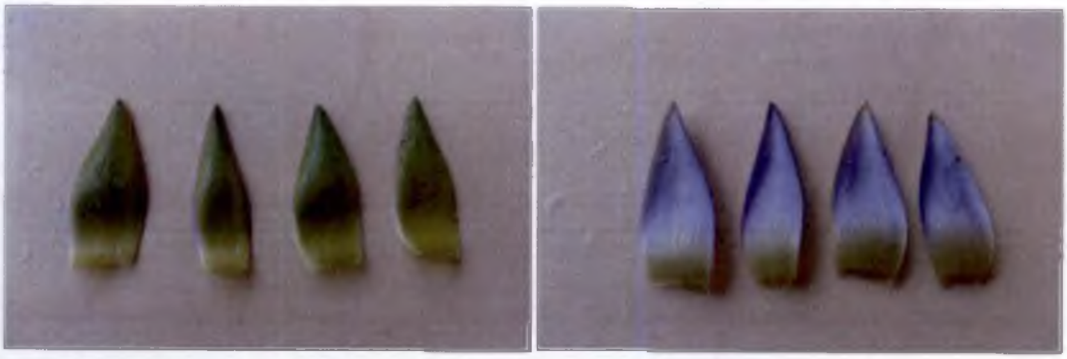


Plate 13c. Sepal characters in Acc. 3

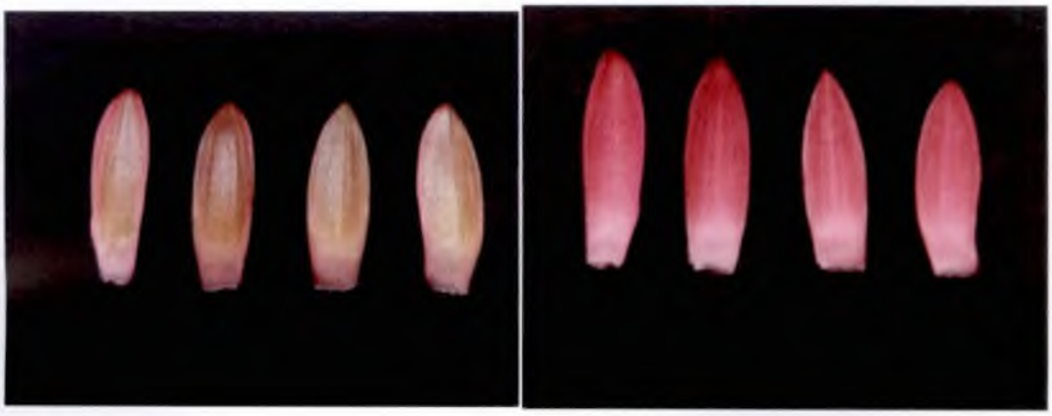


Plate 13d. Sepal characters in Acc. 4

Plate 13e. Sepal characters in Acc. 5

Table 16. Biometric characters of sepal in selected accessions of *Nymphaea*

Accession No.	Mean number/flower	Mean length (cm)	Mean breadth (cm)	Angle at the tip (°)
Acc.1	4	3.39±0.09	1.16±0.02	61.00±0.96
Acc.2	4	3.25±1.30	1.14±0.01	59.30±1.81
Acc.3	4	3.55±0.19	1.59±0.04	70.50±1.51
Acc.4	4	6.30±0.29	2.10±0.11	83.00±4.08
Acc.5	4	5.00±0.29	1.47±0.11	57.25±2.25
CD (0.05)	-	0.65	0.21	7.02
CV (%)	-	11.30	10.95	8.02

The length and breadth of sepals were the highest in Acc.4 (6.30±0.9cm and 2.10±0.11cm respectively). The day blooming ones evaluated were on par with respect to sepal length. The night blooming types were having sepals significantly longer than day bloomers. Significant difference was also observed among the accessions for the angle at the tip of the sepal. The highest tip angle was recorded by Acc.4 (83.00±4.08^0) and the lowest by Acc.5 (57.25±2.25) (Table 16).

The petals were boat shaped and arranged in a spiral fashion on the floral axis. There was variation among the accessions with respect to the colour of petals. Acc.1, 2 and 5 were having white petals (Plates 14a, 14b and 14e). The petals were violet blue in Acc.3 (Plate 14c) and purple red in Acc.4 (Plate 14d). There was a reduction in the intensity of blue colour towards end of blossom life in Acc.3. The intensity of colour was more on the inner side of petals of Acc.4 (Plate 14d).

In all accessions except 4, a green shade was observed on the outer surface of the outer whorl of petals, extending from the base to the tip along the middle. In Acc.2, pinkish tinge developed at the tip of petal from the second day of flower opening.

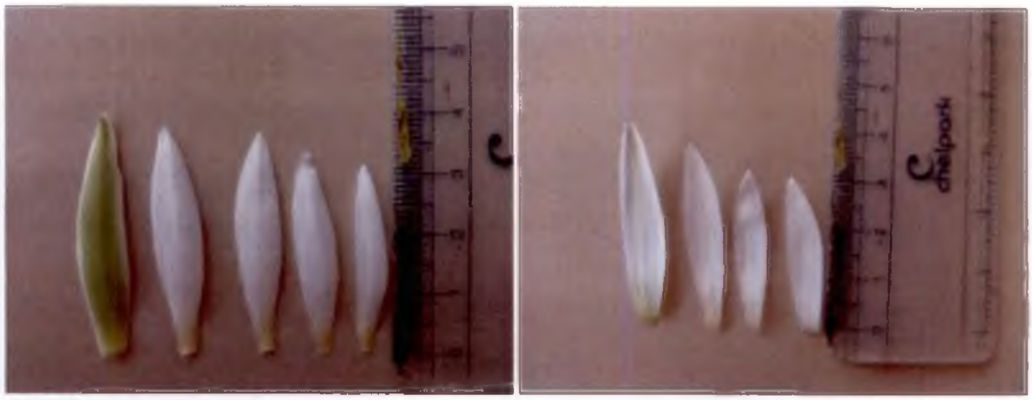


Plate 14 a. Petal characters in Acc. 1

Plate 14b. Petal characters in Acc. 2

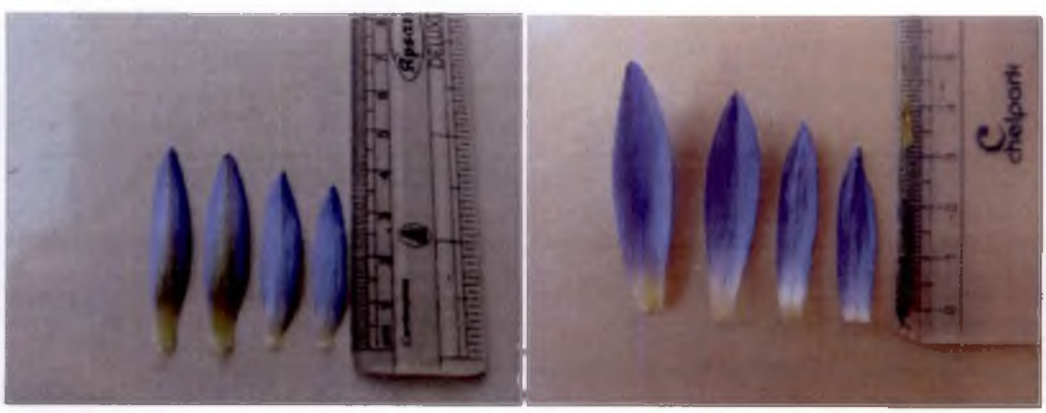


Plate 14c. Petal characters in Acc. 3

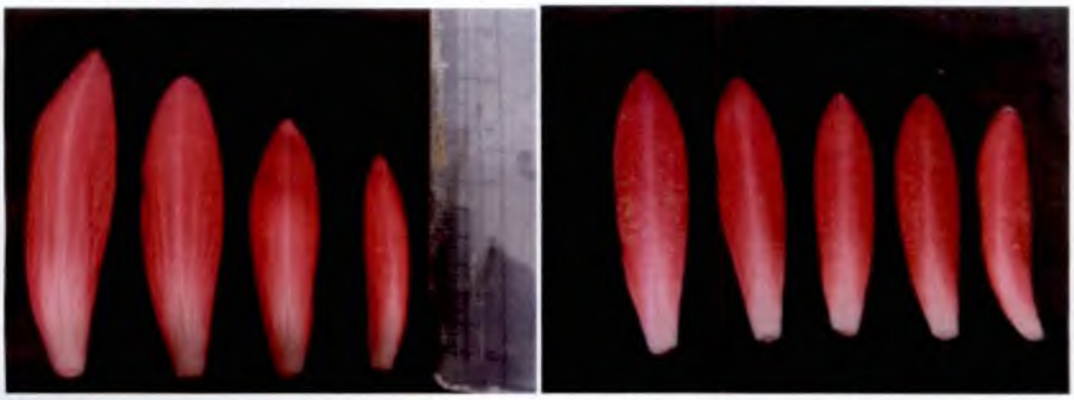


Plate 14d. Petal characters in Acc. 4

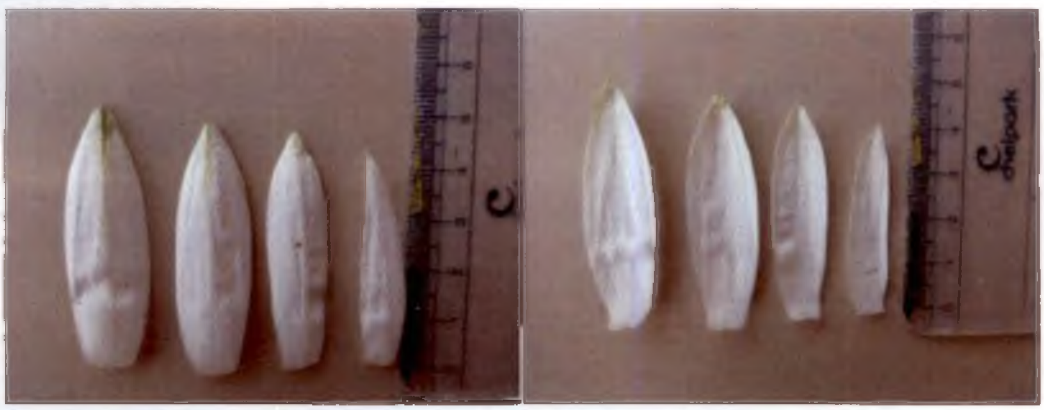


Plate 14e. Petal characters in Acc. 5

The biometric characters of petals are presented in Table 17. The mean number of petals per flower differed significantly among the accessions. The mean number of petals was the highest in Acc.4 (23.00) and the lowest in Acc.2 (13.60). In all the accessions, the petals showed a gradation in size both in length and breadth from the outer most to the inner most whorls (Plate 14a to 14e). Mean length of the petals of the outer most and innermost whorls differed significantly among the accessions. It was the highest in Acc.4 (6.41±0.27cm and 4.86±0.27cm respectively) followed by Acc.5 (4.98±0.25cm and 4.44±0.26 cm respectively) (Table 17).

The mean breadth of petals of the outermost and the inner most and whorls however, differed significantly among the accessions. The highest values for mean breadth of outermost and the inner most whorls of petals were recorded by Acc.4 (1.94±0.05cm and 1.34 ± 0.07cm respectively) and the lowest by Acc.1 (0.75±0.02 and 0.56±0.02) (Table 17).The tip angle of the petal varied with the accession and with the whorls within the accession. The tip angle of petals of the outer most whorl was the highest in Acc.4 (74.40±2.84 °) and the lowest in Acc.1 (53.84±5.21 °). In the case of inner most whorl also, the tip angle was the highest in Acc.4 (63.4±1.28 °). Acc.5 recorded the lowest value (50.80±3.01 °).

Numerous stamens were present in all the accessions and were arranged in a spiral fashion. In day bloomers each stamen consisted of filament, anther and sterile appendage. However, the night bloomers were devoid of the terminal appendage. There was a gradation in length of stamens from the outer most whorl to the inner most whorl, the outer most whorl being the longest and the innermost being the shortest (Plate 15).

Table 17. Biometric characters of petals in selected accessions of *Nymphaea*

Accession No.	Colour	Mean number/flower	Mean length (cm)		Mean breadth (cm)		Angle at tip (°)	
			Outermost whorl	Innermost whorl	Outermost whorl	Innermost whorl	Outermost whorl	Innermost whorl
Acc.1	White	17.20 (4.20)	3.14±0.19	2.82±0.06	0.75±0.02	0.56±0.02	53.84±5.21	51.20±1.98
Acc.2	White	13.60 (3.74)	3.26±0.26	3.06±0.23	0.97±0.05	0.80±0.06	61.46±3.95	59.36±1.72
Acc.3	Violet blue	19.20 (4.44)	3.36±0.15	3.14±0.14	1.00±0.02	0.76±0.02	68.60±2.62	57.56±1.10
Acc.4	Purple red	23.00 (4.84)	6.41±0.27	4.86±0.27	1.94±0.05	1.34±0.07	74.40±2.84	63.40±1.28
Acc.5	White	20.40 (4.56)	4.98±0.25	4.44±0.26	1.40±0.09	1.07±0.11	56.40±1.89	50.80±3.01
CD (0.05)		0.29	0.68	0.62	0.18	0.21	9.14	5.72
CV (%)		5.28	12.14	12.90	10.55	16.31	12.43	7.68

Variability was observed in the colour of appendages among the accessions (Plate 15). The appendage was white in Acc.1 and 2, where as it was violet blue in Acc.3. The colour of the appendage matched with that of the petal. The appendage also showed a gradation in size from the outer most to the inner most whorl, the outer most whorl being the biggest.

The colour of anther lobes also showed variability among the accessions. The anthers were dark yellow in Acc.1, 2 and 5, bluish in Acc.3 and reddish in Acc. 4 (Plate 15).

The colour of filaments also varied among the accessions (Plate 15). In Acc.1, 2 and 3 the filaments were dark yellow. Filaments of Acc. 4 were reddish in colour and Acc.5 were white in colour. The filaments of stamens of the outer most whorl were slightly petaloid (Plate 15).

The biometric characters of stamens of the five accessions are presented in Table18.

The mean filament length for the outer most and the inner most whorls were the highest in Acc.4, 1.82±0.09 cm and 0.89±0.02 cm respectively. The lowest values \were recorded by Acc.1, 0.63±0.03 cm and 0.35±0.03 cm respectively for the outermost and inner most whorls (Table 18).

The mean length of anther lobe was the highest in Acc.4, 1.90±0.09 cm and 1.16±0.05 cm respectively for the outer most and inner most whorls. The lowest were noticed in Acc.1, 0.59±0.04 cm and 0.35±0.03 cm respectively. No significant difference was noticed among the accessions for mean length of appendages (Table 18).

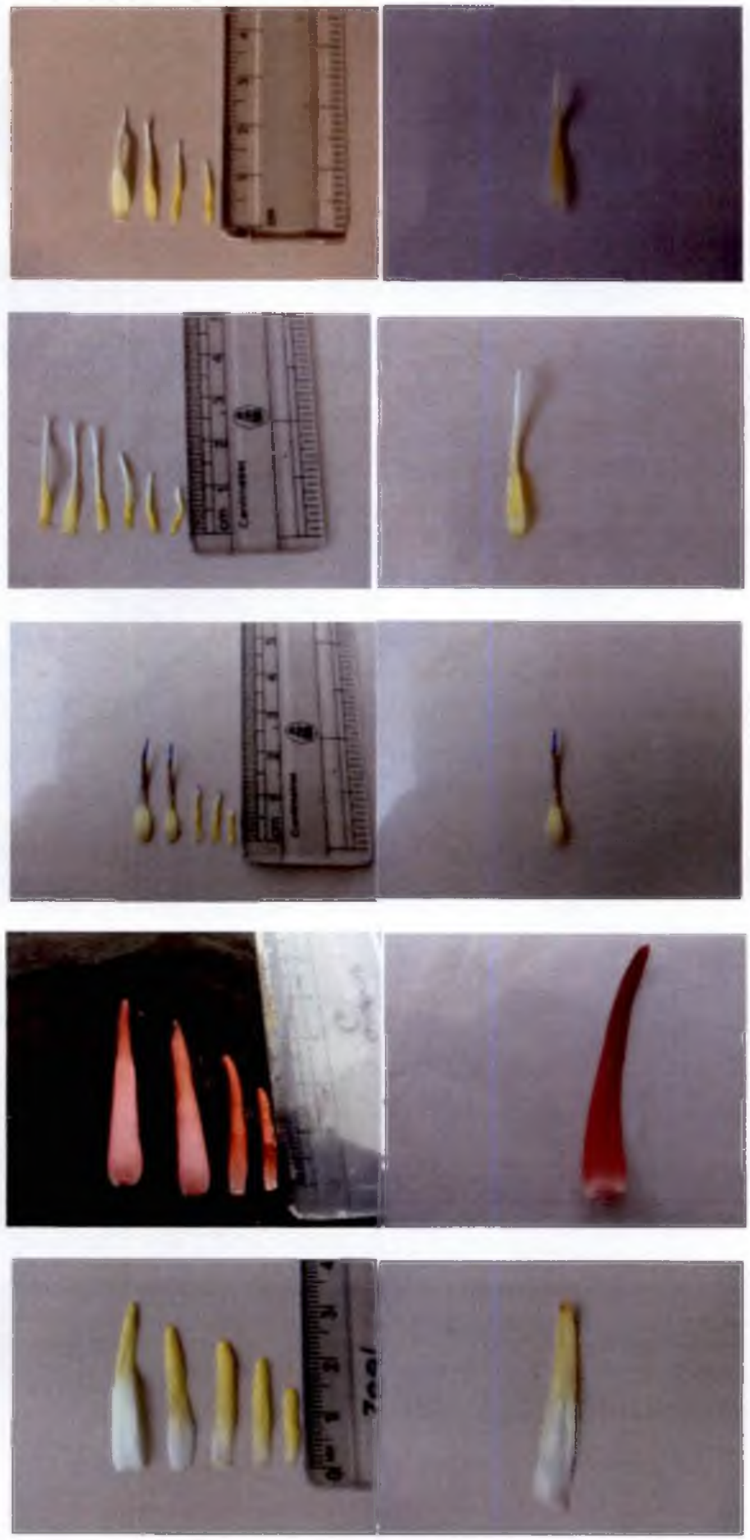


Plate 15. Stamens in Acc. 1,2,3,4 and 5

The features of gynoecium in the five different accessions evaluated are presented in Table 19. The gynoecium was syncarpous in all the accessions with prominent stigmatic cup (Plate 16). The stigmatic cup was yellow in colour in all the accessions except Acc. 4 which was creamy white (Plate 17).

A small knob like receptacular tissue was found at the centre and clavate appendages along the rim of the stigmatic cup in all the accessions (Plate 17). The number of stigmatic appendages was found to be equal to the number of carpels in all the accessions.

The diameter of stigmatic cup also varied significantly among the accessions. The stigmatic cup was the biggest in Acc.5 with a mean diameter of 1.26±0.07 cm followed by Acc.4 with a mean diameter of 1.22±0.02 cm. The Acc.2 had the smallest stigmatic cup (0.64±0.02 cm). Both the night blooming types were characterized by the presence of large stigmatic cups.

The mean number of carpels varied from 16.4 in accession 2 to 25.20 in Acc.5. The number of carpels was the highest in Acc.5 (25.20) and the lowest in Acc.2 (16.40). Begum *et al.* (2010) had observed 13.55 carpels in *N.nouchali* types from Bangladesh.

Acc. 1

Acc. 2

Acc. 3

Acc. 4

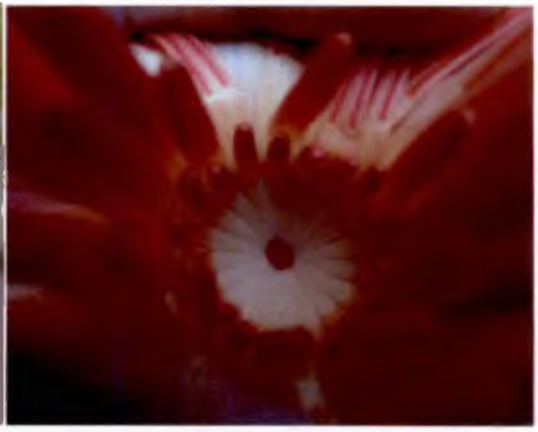
Acc. 5

Plate 16. Gynoecium in different accessions

Acc. 1

Acc. 2

Acc. 3



Acc. 4

Acc. 5

Plate 17. Stigmatic cup and appendages

Table 18. Biometric characters of stamens in selected accessions of *Nymphaea*

Accession No.	Mean number of stamen/flower	Mean length of filament (cm)		Mean length of anther lobe (cm)		Mean length of appendages (cm)	
		Outer most whorl	Inner most whorl	Outer most whorl	Inner most whorl	Outer most whorl	Inner most whorl
Acc.1	98.60 (9.59)	0.72±0.04	0.24±0.05	0.59±0.04	0.35±0.03	0.50±0.03	0.15±0.07
Acc.2	86.6 (9.27)	0.73±0.06	0.27±0.02	0.71±0.06	0.37±0.01	0.54±0.04	0.16±0.10
Acc.3	119 (10.92)	0.63±0.03	0.24±0.02	0.97±0.03	0.50±0.03	0.50±0.02	0.11±0.01
Acc.4	62.60 (7.94)	1.82±0.09	0.89±0.02	1.90±0.09	1.16±0.05	-	-
Acc.5	75.00 (8.56)	1.49±0.31	0.74±0.25	1.04±0.10	0.81±0.11	-	-
CD (0.05)	0.89	0.44	0.35	0.21	0.18	NS	NS
CV (%)	7.19	31.08	24.21	14.77	20.47	13.76	14.20

Table 19. Biometric characters of gynoecium in selected accessions of *Nymphaea*

Accession No.	Mean diameter of stigmatic cup (cm)	Number of carpels /flower	Mean length of stigmatic appendage (cm)
Acc.1	0.70±0.03	17.80 (4.28)	0.32 ± 0.0
Acc.2	0.64±0.02	16.40 (4.11)	0.58 ± 0.10
Acc.3	0.80±0.00	20.60 (4.59)	0.34 ± 0.06
Acc.4	1.22±0.02	18.20 (4.32)	1.16 ± 0.21
Acc.5	1.26±0.07	25.20 (5.06)	0.76 ± 0.14
CD (0.05)	0.12	0.23	0.08
CV (%)	9.43	4.75	9.50

4.3.4. Determination of anther dehiscence

The colour and appearance of the anthers were observed with hand lens in fully mature buds of all the accessions starting from 6.00 am on the previous day of flower opening until the completion of anther dehiscence. Anthers were introse in all the five accessions.

The anther dehiscence proceeded from the outermost whorl to the inner most whorl of the flower in day blooming types evaluated. However, among the day bloomers, no dehiscence was observed in accession 2. The dehiscence was longitudinal (Plate 18) in all the accessions.

The anthers of the outermost whorl dehisced on the second day of flower opening in Acc.1 and 3. The dehiscence time varied from 9.30am to 10.00 am in

Plate 18. Anther dehiscence in Acc. 1,3,4 and 5

these accessions depending on weather conditions. The dehiscence was completed in 30-35 minutes. The dehiscence in all the remaining whorls were completed on the third day of flower opening in both these accessions. However, dehiscence was thirty minutes earlier to that of previous day. Thus, the anther dehiscence was completed in two days in Acc.1 and 3. Staining with aceto-carmine showed that the pollen grains remained viable for 10 hours after anther dehiscence in the case of day bloomers.

In the night blooming types (Acc.4 and Acc.5) all the stamens dehisced simultaneously (Plate 18). In Acc.4, anther dehiscence was 20-21 hours after flower opening on the first day. However, in Acc.5 anthers dehisced by 4 pm on the first day, i.e. 2-3 hours prior to flower opening on the first day.

In day bloomers the anther dehiscence started on the second day of flower opening and was completed in two days. This may be because, the day bloomers were having significantly higher number of stamens. However, in night bloomers the dehiscence occurred simultaneously in all the whorls and was completed on the first day of flower opening itself.

Similar observation that anther dehiscene occurred from outer most to inner most whorl in *Nymphaea capensis* was reported by Orban and Bouharmont (1995). Fahida (2012) also made similar observation in *N. nouchali*. But in the case of *N. pubescence* and *N. rubra,* dehiscence occurred simultaneously in anthers of all the whorls according to Begum *et al.* (2010).

The stamens of all the whorls were compact and positioned vertically on the first day of flower opening in all the accessions (Plate 18). In day blooming types, Acc.1 and 3, the stamens of the outer whorls slightly refluxed out after their dehiscence on the second day of flower opening. The undehisced stamens of the remaining whorls formed a cone around the stigmatic cup (Plate 18). The day bloomers were characterized by the refluxing out of the stamens after dehiscence

In night bloomers, Acc. 4 and 5, the stamens of all the whorls however, were compact and positioned vertically even after anther dehiscence (Plate 18). The pollen grains remained viable for nearly 48 hours after dehiscence.

4.3.5. Determination of stigma receptivity

The stigmatic surface was examined for the presence of exudates at hourly intervals starting from 6 am on the previous day of flower opening. The presence of honey dew like secretion in the stigmatic cup indicated the initiation of the stigma receptivity (Plate 19a and 19b).

The exudates started appearing in the stigmatic cup between 3.15 pm-3.45 pm on the previous day of flower opening in day bloomers, Acc.1 and Acc.2. However, in Acc.3, which was also a day bloomer, the onset of stigma receptivity varied from 4.45 pm to 5.15 pm on the previous day of flower opening. Hence, in accessions 1 and 2, the receptivity started almost 15 hours before flower opening and remained receptive up to 34 hours even after flower opening. For Acc.3, receptivity started slightly late, nearly 14 hours before flower opening and retained the receptivity up to 34 hours even after flower opening.

For night blooming type, Acc.4, the receptivity started almost 8-9 hours prior to flower opening and the receptivity continued upto16 hours after flower opening on the first day. In Acc.5, which was also night blooming, the receptivity started almost 6-7 hours prior to flower opening and was extended up to 18 hours after flower opening on the first day.

Stigma becoming receptive before anther dehiscence indicated the protogynous nature of the flower. The protogynous nature of *Nymphaea* flowers were also reported by Schneider (1982), Wiersema (1988), Begum *et al.* (2010) and Fahida (2012).

Acc. 1

Acc. 1

Acc. 2

Acc. 2

Acc. 3

Acc. 3

Plate19a. Stigma receptivity in different accessions

Acc. 4

Acc. 4

Acc. 5

Acc. 5

Plate 19b. Stigma receptivity in different accessions

The loss of receptivity was indicated by drying up and slight darkening of stigmatic cup along with the inward curving of the stigmatic appendages (Plate 20). The stigmatic appendages thus play a significant role in the breeding behavior of the species. In Acc.1, 2 and 3 the loss of receptivity started around 4 pm to 5 pm on the second day of flower opening. For Acc.4 and 5 the receptivity was lost by 10 am - 12.30 pm on the first day of flower opening. The stigmatic cup was completely overlapped by the large sized appendages after the loss of receptivity, in night bloomers. However, in day bloomers the stigmatic cup was partially exposed even after the loss of receptivity as the appendages were small sized to overlap the whole cup.

Begum *et al.* (2010) has reported that on the second day of anthesis, the stigma of *N. rubra* dried up, but in *N. nouchali* and *N. pubscens* stigmatic cup remained sufficiently moist.

According to Meeuse and Schneider (1980), the stigmatic fluid consisted of 1-1.5% sugar, 5 ppm boron and low concentration of Ca, K and Mg ions and this composition was optimal for pollen germination.

4.4. Pollination Biology

Seed set was observed in unprotected (control) as well as emasculated but unprotected buds of the day blooming Acc.1 and 3 (Table 20). However, no seed set was observed in the protected buds (selfed) indicating the cross pollinating nature of these accessions.

Stingless bee (*Trigona irridipennis*), honey bee, house flies, weevils and flower beetles were found to be the major insects visiting the flowers (Plate 21). Among these insects stingless bee and honey bees were the predominant ones. The number of pollinators was found to be maximum on the second day of flower opening. Capperino and Schneider (1985) reported honey bees, flies and beetles as

Acc. 1

Acc. 2

Acc. 3

Acc. 4

Acc. 5

Plate 20. Loss of receptivity in different accessions

the pollinators in *N.capensis*. Fahida (2012) also observed that the major insects visiting *N. nouchali* flowers were honey bees

Table 20. Fruit set in protected and unprotected buds of *Nymphaea* accessions

	Fruit set (%)	
	Acc.1	Acc.2
Protected (Selfed)	0	0
Emasculated but unprotected	52	78
Control	50	80

Some of these insects were seen trapped in the stigmatic cup indicating the insectivorous nature of these flowers. The presence of stigmatic exudates as well as the slippery and incurved nature of the stigmatic appendages prevented the escape of the insects trapped in the stigmatic cup (Plate 22). The wet wings and body parts made swimming, flying or climbing up the anthers more difficult and the insects finally drowned. The inner whorls of stamens in day blooming type with their unique structure and shape also acted as insect retaining mechanisms.

The insect cadavers on the stigmatic cup were found to be dried up on the last day of flower opening (Plate 23) and were also seen to be embedded and retained in the floral parts even one week after submergence of the flower in water.

Schneider (1982) had reported that the stigmatic fluid washes pollen from the insect and cross pollination is achieved. Insectivorous behavior in *N. nouchali* was described by Tetali *et al.* (2008) also. They also reported that this behavior unique for *N. nouchali* and it makes *N. nouchali* as a missing link in the evolutionary history of highly evolved carnivorous families.

Plate 21. Pollinators of water lily

Plate 22. Insects trapped in stigmatic fluid

Plate 23. Insect cadavers in the stigmatic cup

4.5. Palynology

The morphology, size and fertility of pollen grains in the accessions evaluated except Acc.2 which produced pollen grains are presented in Table 21. No pollen production was observed in Acc.2. The pollen grains were almost ellipsoidal and monocolpate in all the four accessions (Plate 24) Pollen grains of the day blooming accessions appeared yellow coloured to the naked eye. However, it was white in the case of night blooming types. No significant difference was observed among the accessions with respect to pollen size (Plate 24).

Very high pollen fertility was exhibited by all the four accessions which produced pollen (Table 20). The pollen fertility was the highest in Acc. 5 (99.45%) and the lowest in Acc. 4 (82.46%). Even though there was good pollen fertility, no seed set was observed in Acc. 3 and 4. Some incompatibility mechanism may operating in these accessions which need detailed investigations.

Table 21. Morphology and fertility of pollen grains in different accessions of *Nymphaea*

Accession No.	Pollen type	Colour as appeared to the naked eye	Size of fertile pollen (20X)		Pollen fertility (%)
			Length (µm)	Breadth (µm)	
Acc. 1	Ellipsoidal	Yellow	38.38±1.24	36.84±0.95	92.67
Acc. 3	Ellipsoidal	Yellow	39.88±0.81	38.49±0.70	92.70
Acc. 4	Ellipsoidal	White	39.35±1.20	36.90±1.07	82.46
Acc. 5	Ellipsoidal	White	36.7±0.20	36.30±0.54	99.45
CD (0.05)			NS	NS	
CV (%)			5.54	5.07	

Volkova and Shipunov (2007) had reported that different *Nymphaea* species from vast populations had high pollen fertility of more than 75%.

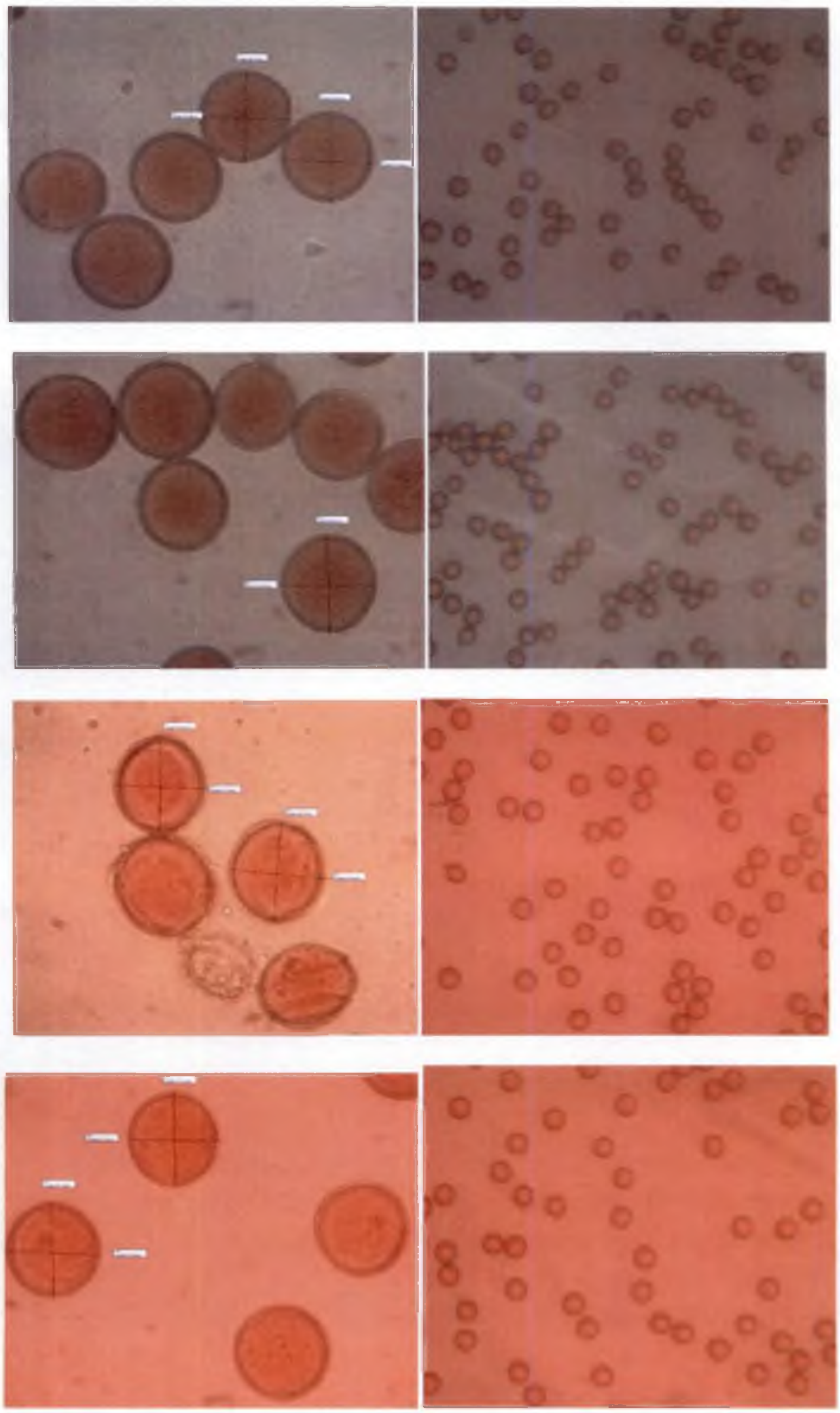


Plate 24. Pollen grains in Acc. 1,3,4 and 5 (20X and10X)

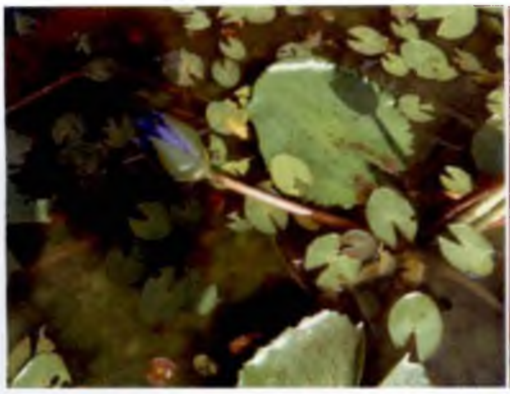
4.6. Fruit and seed characters

Fruit development was observed only in two day blooming accessions (Acc. 1 and 3) selected. The fruit development was taking place beneath the water surface (Plate 25). On the fifth day of blooming, the flower submerged into water by the bending of the pedicel. Fruits matured 20-25 days after the sinking of the flower into water. The fruit was a many seeded dehiscent capsule. All the floral whorls were persistent in the mature fruit (Plate 26a and 26b).

On maturity, the fruit burst open dehiscing the seeds into water. The dispersed seeds could be seen floating on the water surface (Plate 27). The pattern of dehiscence differed in the two accessions which set seeds. In the case of Acc.1 the dehiscence was lateral where as in Acc.3 it was terminal (Plate 28). Seeds germinated under water within 20-30 days after dehiscence under natural conditions. The floral parts decayed if there was no fruit set (Plate 29).

Seeds were arillate with a small embryo and two fleshy cotyledons. The seeds were also covered with mucilage (Plate 30). Collinson (1980) had reported that the presence of mucilage and stored gas bubbles in the aril tissue aided in the floating of the seeds and its dispersal. Richards and Cao (2012) had also described the same phenomenon in *Nymphaea* species.

The biometric characters of fruits and seeds of the two seed forming accessions are depicted in Table 22. There was no significant difference in fruit weight of the Acc.1 and 3. However, significant difference was noticed in the case of 100 seed weight. Significant difference was also noticed in seed length of Acc.1 and 3. However, no significant difference was observed for the breadth of seed.



Stage 1

Stage 2

Stage 3

Stage 4

Stage 5

Plate 25. Stages of fruit development

Plate 26a. Persistent floral parts in Acc.1

Plate 26b. Persistent floral parts in Acc.3

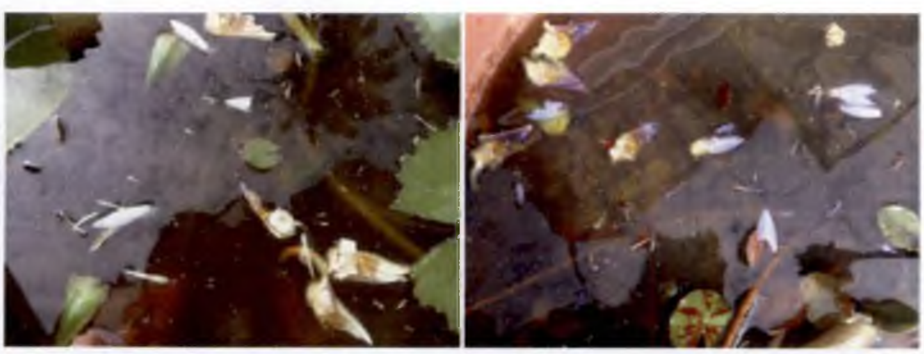


Plate 27. Fruit bursting releasing all the contents

Plate 28. Pattern of dehiscence in Acc.1 and 3

Plate 29. Decayed flower in Acc. 2, 4 and 5

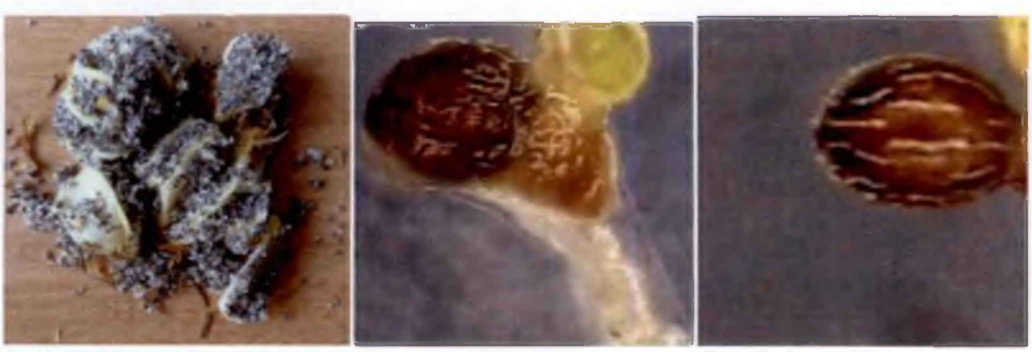


Plate 30. Seeds in *Nymphaea* accessions

Table 22. Fruit and seed characters of two accessions of *Nymphaea*

Accession No.	Fruit weight (gm)	100 seed weight (gm)	Size of seed	
			Length(μm)	Breadth (μm)
Acc.1	12.47±0.68	0.04±0.006	51.11±0.71	34.59±0.68
Acc.3	16.83±0.36	0.07±0.01	54.79±1.37	32.16±0.83
t value	NS	1.44	2.384	NS

4.6.1. Germinability of seeds

The process of seed germination was found to be similar in both seed forming accessions, Acc.1 and 3 (Plate 31) and had a resemblance to that of monocots .

On the first day of germination a small portion of the coleoptile emerged from the seed. On the second day the green coloured coleoptile became prominent as an acicular structure. Two days after emergence of coleoptile i.e. on the third day root emerged. Initially there was only a single long root. Root emergence was followed by the emergence of first leaf. The lamina was in rolled condition at time of emergence. It unrolled fully within two days of its emergence. The second leaf emerged 13-15 days after the emergence of first leaf.

The percent of seed germination at dehiscence as well as after one month of storage are presented in Table 23. The speed of germination as well as the germinability of the seeds were very low in both Acc.1 and 3 at the time of dispersal from fruit. Germination at dehiscence was higher in Acc. 1 compared to Acc.3. However, upon storage for one month germinability was reduced to zero. This may be due to acquired dormancy on storage.

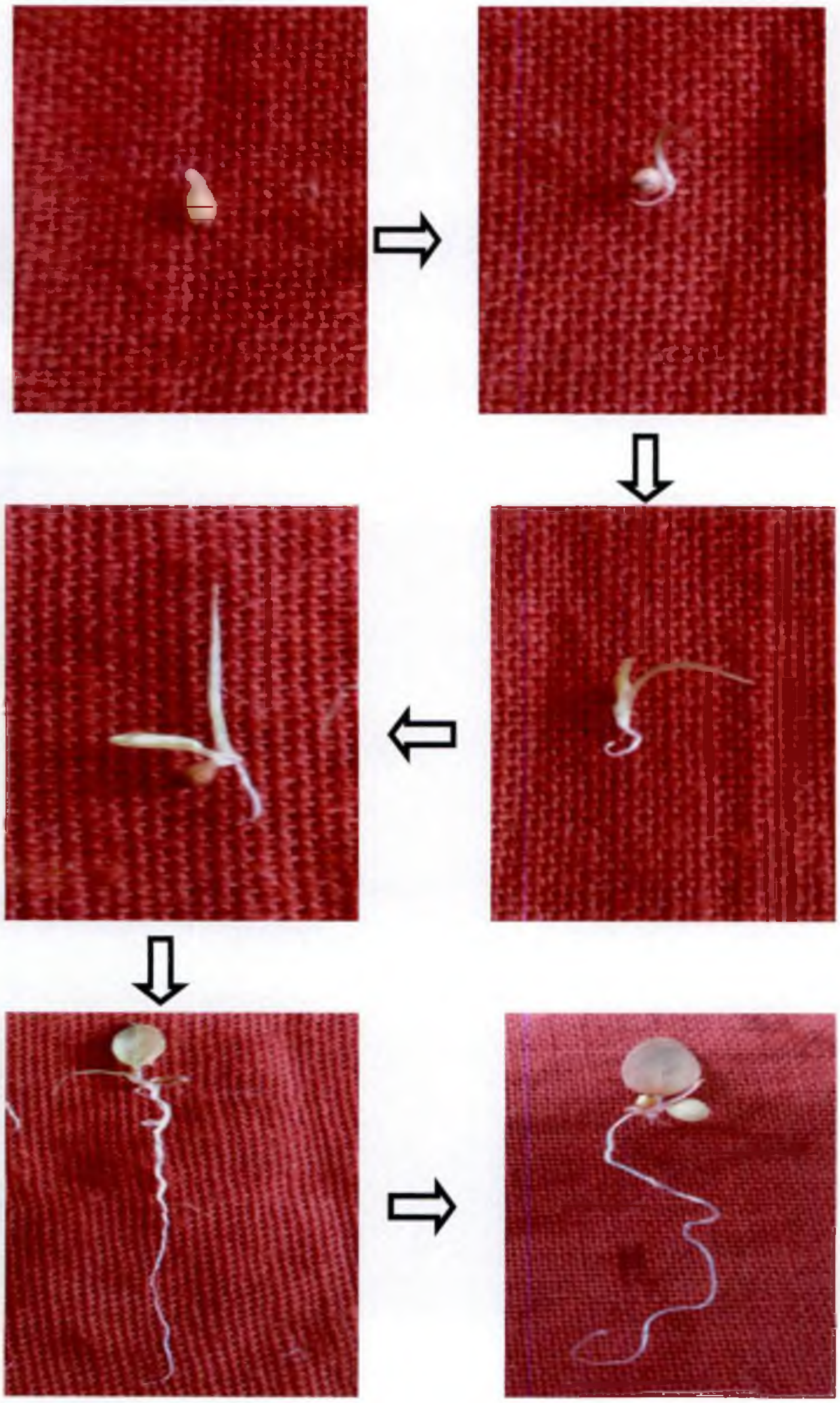


Plate 31. Process of seed germination

Seed dormancy in seed forming *Nymphaea* species was reported by Else and Riemer (1984). Smits and Schmitz (1995) reported that the production of white water lily through seeds was difficult because of the development of dormancy in seeds on storage. It was also reported that the dormancy increased with the passage of time on storage.

Table 23. Seed germinability in two accessions of *Nymphaea*

Accession No.	Germination (%)	
	At dehiscence	After one storage
Acc. 1	22.50	0
Acc. 3	7.50	0

Germinability of the seeds was observed after treating them with one per cent and five per cent sulphuric acid for varying periods *viz.*, one, two and three minutes respectively. It was observed that seeds germinated much faster on scarification with acid. Normally it took 15-20 days for the seeds of Acc.1 and 25-30 days for Acc.3 to start germinating. But, on acid scarification the number of days for initiation of germination was reduced to 10-12. On increasing the treatment time or concentration charring of the seeds occurred.

The details of germination percentage after acid treatment are presented in Table 24.

Table 24. Seed germinability in *Nymphaea* accessions after acid treatment

Accession No.	Germinability (%)					
	5% acid			1% acid		
	1 min	2 min	3 min	1 min	2 min	3 min
Acc.1	26.67	40.00	46.67	13.33	20.00	20.00
Acc.3	20.00	20.00	33.33	13.33	20.00	20.00

Seed germinability was also recorded after giving leaching treatment in running water for 6, 12 and 24 hours. The details of germination after leaching treatment for varying periods are given in Table 25.

In Acc.1 and 3, germination started 10-17 days after sowing with 12 and 24 hours of leaching treatment. A higher germination per cent was observed in these treatments. However, no germination was observed where leaching treatment was limited for 6 hours.

In both Acc. 1 and 3 germination percentage improved with 24 hours of leaching treatment. However, the number of days for initiation of germination remained the same as that with12 hours of leaching treatment. The two accessions responded differently to the different pre treatments.

Table 25. Seed germinability in *Nymphaea* accessions after leaching treatment

Accession No.	Germinability (%)		
	6 hour leaching	12 hours leaching	24 hours leaching
Acc.1	0	10	40
Acc.3	5	25	45

4.6.2. Natural propagules in selected Nymphaea accessions

In the seed forming types, Acc.1 and 3, seeds were the major propagules. Besides, bulbils and stolons also served as the propagules in these accessions. However, in the non seed forming accessions, Acc.2, 4 and 5, bulbils and stolons were the propagules (Plate 32a and 32b).

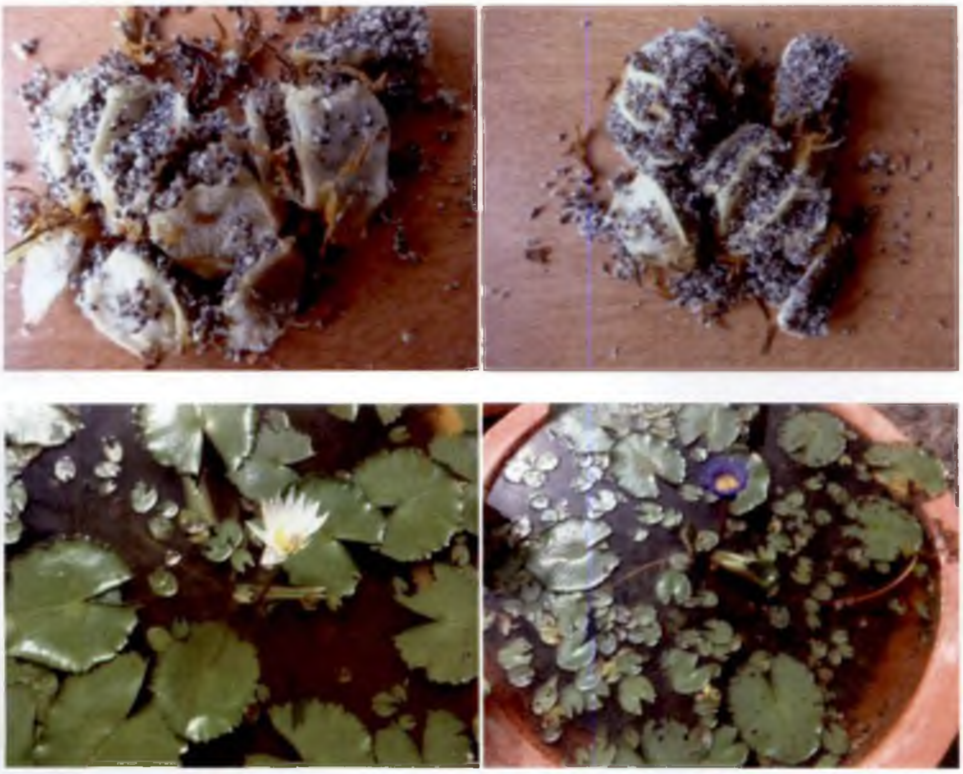


Plate 32a. Seed as major propagule in Acc. 1and 3

Plate 32b. Bulbils as propagule in Acc. 2, 4 and 5

4.7. Correlation of rate of elongation of petiole and pedicel with weather parameters

The rate of petiole and pedicel elongation were correlated with different weather parameters like sunshine hours, relative humidity (morning and evening hours), maximum and minimum temperature and rainfall. The results are presented in Tables 26.

From Table 26 it can be seen that the rate of elongation of petiole was significantly influenced by sunshine hours, relative humidity and temperature. It was observed that sunshine hours and maximum temperature had positive influence and the other parameters like relative humidity (morning and evening hours), minimum temperature and rainfall had negative influence on the rate of elongation of petiole. The effect of rainfall on the rate of elongation of petiole was not significant.

However, in the case of rate of pedicel elongation the effect of weather parameters were not significant. It was observed that sunshine hours and temperature had positive influence and relative humidity (morning and evening hours) and rainfall had negative influence on the rate of pedicel elongation.

Table 26. Correlation of petiole and pedicel elongation rates with weather parameters

	Sunshine hours	Relative humidity		Temperature		Rainfall
		Morning	Evening	Maximum	Minimum	
Petiole elongation	0.530**	-0.256**	-0.311**	0.256**	-0.540**	-0.007
Pedicel elongation	0.136	-0.055	-0.068	0.082	0.081	-0.049

4.8. Future line of work

- Reason for lack of seed set in night bloomers even though there is high pollen fertility needs thorough investigation
- Presence of female sterility and self incompatibility needs to be looked into
- Further detailed study on the seed viability and germinability in different *Nymphaea* species has to be undertaken
- Since there is variability in floral characters, the scope of water lily in cut flower industry demands detailed investigation

Summary

5. SUMMARY

The investigation entitled 'Morphogenesis and reproductive biology of water lily (*Nymphaea* spp.) was carried out in Department of Plant Breeding and Genetics, College of Horticulture, Vellanikkara, during the period from August 2013 to June 2015.

A survey was conducted in the water lily growing tracts in Thrissur district which revealed the presence of flower colour and blooming time variants. The survey led to the identification as also collection of twelve types, differing in flower colour and blooming time. Among these twelve types, ten were day blooming and two were night blooming. Night blooming types collected did not set seeds. However, five among the day bloomers set seeds.

Five accessions were selected from among the collected types based on blooming time and seed set for detailed evaluation of leaf characters as well as flowering biology. Among the five selected types three were day bloomers (Acc.1, Acc.2 and Acc.3) and two were night bloomers (Acc.4 and Acc.5). Two among the five selected accessions set seeds.

The collected accessions were subjected to a preliminary evaluation based on leaf and floral biometric characters. Grouping of the accessions was done based on the results of post hoc test, DMRT, for the two most decisive characters *viz.*, diameter of fully opened flower as well as size of mature flower bud as indicated by its length and circumference. Five groups were obtained with group I and II having single accessions each. Group IV was the biggest with six accessions. The accessions selected for detailed evaluation fell in groups I, II and IV.

Growth and developmental pattern of leaves as well as flowers were evaluated in the five selected accessions. Acc. 4 which was night blooming, took the highest number of days for the leaf and flower bud to reach the water surface. The rate of elongation of petiole was significantly influenced by sunshine hours, relative humidity and temperature. The sunshine hours and maximum temperature had positive influence and relative humidity (morning and evening hours), minimum temperature and rainfall had negative influence on the rate of elongation of petiole. The effect of weather parameters were not significant in the case of pedicel elongation. The sunshine hours and temperature had positive influence and relative humidity (morning and evening hours) and rainfall had negative influence on the rate of pedicel elongation. Number of days taken for the formation of successive leaves and flower buds varied with the accession.

The leaves in all the selected accessions were simple orbicular with subpeltate lamina which was deeply cleft near to the petiole base. The tip of the leaf was emarginated or obtuse. The night blooming types were characterized by the presence of larger leaves with prominent primary and secondary veins on the abaxial surface as well as sinuately dentate leaf margin. Biometric characters like length and width of leaf as also length of sinus were the highest in Acc.4 (night blooming) and the lowest in Acc.1 (day blooming).

The night blooming types were found to be superior to day blooming ones in flower bud characters *viz.*, length and circumference of mature bud as well as diameter of fully opened flower. The blossom life was uniform in both day blooming and night blooming types.

The process of blooming which began with the opening of the sepals was similar in all the accessions evaluated. In day blooming types, the flowers opened in the morning and closed in the evening and in night blooming types, the flowers opened in the evening hours and closed in the morning. The process of opening and closing repeated consecutively for four days. On the fourth day, the pedicel slightly bent and on the fifth day the flowers were completely immersed in water. The time required for the process of full blooming and further closing was 20-30 minutes and

15-20 minutes respectively. Day blooming types were highly fragrant and night blooming types were faintly fragrant.

All the five accessions evaluated had four sepals which were acute and in imbricate aestivation. However, the colour of sepals varied with the accession. The peals were boat shaped and arranged in a spiral fashion on the floral axis. The colour of the petals were white for accessions 1, 2, and 5, violet blue for accession 3 and purple red for accession 4. With respect to the biometric characters of sepals and petals Acc.4 was superior. Mean number of petals per flower was the highest in Acc.4 and the lowest in Acc.2.

Numerous stamens were present in all the accessions and were arranged in a spiral fashion. In day bloomers the stamens consisted of filament, anther and sterile appendage where as in night bloomers terminal appendage was absent. The colour of appendage was similar to the colour of petal. The filaments of stamens of the outer most whorls were slightly petaloid. Acc. 1, 2 and 3 had dark yellow coloured filaments. Filaments of accession 4 were purple red and accession 5 was white. The anthers were dark yellow in Acc. 1, 2 and 5, violet blue in Acc. 3 and purple red in Acc. 4. Biometric characters like length of the filament and anther was the highest in Acc.4 and the lowest in Acc.1.

There was a gradation in the size of petals and stamens from the outer most whorls to the inner most whorls, the outer most whorl being the biggest and the innermost being the smallest.

In all the accessions, gynoecium was found to be syncarpous with prominent stigmatic cup. The stigmatic cup was yellow in colour in Acc.1, 2, 3 and 5 and creamy white in Acc.4. A small knob shaped receptacular tissue was found at the center of the stigmatic cup and clavate appendages along the rim in all the accessions. The number of stigmatic appendages was found to be equal to the number of carpels in all the accessions. The biometric characters of gynoecium like diameter of stigmatic cup and the number of carpels per flower were the highest in Acc.5 and the lowest in Acc.2.

Anther dehiscence proceeded from the outermost whorl to the inner most whorl of the flower and the dehiscence was longitudinal. Among the day bloomers, no dehiscence was observed in Acc.2. However, in Acc.1 and 3 dehiscence was found to occur on the second day of flower opening. In the night blooming types (Acc.4 and Acc.5) all the stamens dehisced simultaneously. The dehiscence occurred after the opening of flower on the first day in Acc.4 where as it was prior to first day of flower opening in Acc.5. In day bloomers pollen grains retained viability for about 10 hours. The pollen grains remained viable for nearly 48 hours after dehiscence in night bloomers.

The presence of honey dew like secretion in the stigmatic cup indicated the initiation of receptivity of the stigma. The stigma became receptive before flower opening indicating the protogynous nature of the flower. The stigma receptivity started 14 to 15 hours before flower opening in day bloomers and remained receptive for about 34 hours even after flower opening. In night blooming types, receptivity started 6 to 9 hours prior to flower opening and was retained only for 16 to 18 hours after flower opening.

Stingless bee (*Trigona irridipennis*), honey bee, house flies, weevils and flower beetles were found to be the major insects visiting the flowers. Among these insects stingless bee and honey bees were the predominant ones. Some of these insects were seen trapped in the stigmatic cup indicating the insectivorous nature of these flowers.

The pollen grains were almost elliptic in all the accessions where pollen production was there. There was no pollen production in Acc.2, a day bloomer. The colour of pollen grains were yellow in day bloomers and white in night bloomers. No significant difference was observed among the accessions with respect to pollen size and fertility. Very high pollen fertility was exhibited by all the accessions.

The results of the study on fruit set in protected and unprotected buds revealed that cross pollination is the rule in *Nymphaea*. Natural fruit set was observed only in the day blooming ones, Acc.1 and 3. The fruit development was found to take place under water. Fruits matured in 20-25 days after immersion of flower into water. The fruit was a many seeded dehiscent capsule. All the floral whorls were persistent in the mature fruit. On maturity, the fruits burst open dehiscing all the contents along with the seeds into water. Seeds were arillate with a small embryo and two fleshy cotyledons. The seeds were also covered with mucilage.

The process of seed germination was found to be similar in both seed forming types evaluated and had a resemblance to that of monocots. The germination was initiated by the emergence of coleoptile. The green coloured coleoptile became prominent as an acicular structure on the second day. The root emerged two days after coleoptile emergence. Initially there was only a single long root. The first normal leaf emerged on the next day of root formation. The lamina was in the rolled condition at the time of emergence. The unrolling completed in two days. The second leaf emerged 13-15 days after the emergence of first leaf.

The germinability as well as speed of germination were low and hence, the seeds were subjected to pretreatments like acid scarification and leaching in running water. Acid and leaching treatment improved the germinability as well as speed of germination of the seeds. The germinability decreased with the storage of seeds.

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ABSTRACT

Genus *Nymphaea* forms a complicated group of aquatic perennials having immense ornamental and medicinal value. Two phenological groups based on flowering *viz.*, diurnals and nocturnals are recognized in this genus. Information on the reproductive biology and developmental features specific to the phenological groups are lacking in this genus.

The present study entitled "Morphogenesis and reproductive biology of water lily (*Nymphaea* spp.)" was taken up in this background in the Department of Plant Breeding and Genetics, College of Horticulture, Vellanikkara, during 2013-2015 for elucidating and comparing the reproductive biology and developmental pattern of leaves, flowers and fruits in day as well as night blooming types of *Nymphaea.*

A survey was conducted in the water lily growing tracts of Thrissur district and 12 accessions were collected. Five among these accessions were selected based on blooming time and seed set for further detailed evaluation.

Growth and development pattern of leaves and flowers were observed to be uniform in all the accessions. However, the accessions differed in the number of days taken by the leaf and flower bud to reach the water surface and maturity. The rate of elongation of the petiole was significantly and positively correlated with sunshine hours and maximum temperature. There was no significant correlation between pedicel elongation and weather parameters.

The leaves in all the accessions evaluated were simple orbicular with subpeltate lamina which was deeply cleft near to the petiole base. However, there was variability in the colour, margin and tip of the lamina. The night bloomers could be easily distinguished by the sinuately dentate margin and thick prominent veins on the abaxial surface of the lamina.

Flowers were pedicellate and complete with all the floral whorls in a spiral fashion on the floral axis. Significant variability in both qualitative and quantitative characters were observed among the accessions for floral characters. The night bloomers differed from the day bloomers in ... with stamens devoid of appendages. Besides, the sepals were observed to

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MORPHOGENESIS AND REPRODUCTIVE BIOLOGY OF WATER LILY (*Nymphaea* spp.)

BY

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ABSTRACT OF THE THESIS

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MASTER OF SCIENCE IN AGRICULTURE

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ABSTRACT

Genus *Nymphaea* forms a complicated group of aquatic perennials having immense ornamental and medicinal value. Two phenological groups based on flowering *viz.,* diurnals and nocturnals are recognized in this genus. Information on the reproductive biology and developmental features specific to the phenological groups are lacking in this genus.

The present study entitled "Morphogenesis and reproductive biology of water lily (*Nymphaea* spp.)" was taken up in this background in the Department of Plant Breeding and Genetics, College of Horticulture, Vellanikkara, during 2013-2015 for elucidating and comparing the reproductive biology and developmental pattern of leaves, flowers and fruits in day as well as night blooming types of *Nymphaea.*

A survey was conducted in the water lily growing tracts of Thrissur district and 12 accessions were collected. Five among these accessions were selected based on blooming time and seed set for further detailed evaluation.

Growth and development pattern of leaves and flowers were observed to be uniform in all the accessions. However, the accessions differed in the number of days taken by the leaf and flower bud to reach the water surface and maturity. The rate of elongation of the petiole was significantly and positively correlated with sunshine hours and maximum temperature. There was no significant correlation between pedicel elongation and weather parameters.

The leaves in all the accessions evaluated were simple orbicular with subpeltate lamina which was deeply cleft near to the petiole base. However, there was variability in the colour, margin and tip of the lamina. The night bloomers could be easily distinguished by the sinuately dentate margin and thick prominent veins on the abaxial surface of the lamina.

Flowers were pedicellate and complete with all the floral whorls in a spiral fashion on the floral axis. Significant variability in both qualitative and quantitative characters were observed among the accessions for floral characters. The night bloomers differed from the day bloomers in having larger flowers with stamens devoid of appendages. Besides, the sepals were observed to have five to six prominent nerves on both sides.

The flower opened in the morning and closed in the evening in day blooming types. In night bloomers the flower opening was in the evening hours and closing in the morning hours. The time of opening and closing further varied with the accessions. The process of opening and closing continued for four days and on the fifth day the flower submerged in water.

The anther dehiscence started on the second day of flower opening and it proceeded from the outermost whorl to the inner most whorl of the stamens. The dehiscence was hence completed in two days in day bloomers. However, in night bloomers the stamens of all the whorls dehisced simultaneously on the first day of flower opening.

The presence of honey dew like secretion in the stigmatic cup indicated the initiation of stigma receptivity in all the accessions irrespective of blooming time. The receptivity started 14 to15 hours before flower opening and was retained for 34 hours even after flower opening in day bloomers. In night bloomers the onset of receptivity was six to nine hours prior to flower opening and remained receptive only for 16 to 18 hours after flower opening. Stingless bee (*Trigona irridipennis*), honey bee, house flies, weevils and flower beetles were the major insects visiting the flowers. Cross pollination was the rule in all the accessions due to the protogynous nature of the flower.

Fruit set was noticed only in two of the day blooming types evaluated. Fruit was a many seeded dehiscent capsule which developed under water. All the floral whorls were persistent in the mature fruit. It took 20 to 25 days for the fruit to mature. Seeds were arillate with an embryo, two fleshy cotyledons and a mucilaginous covering. Despite being a dicot, the germination was initiated by the emergence of coleoptile. Acid scarification as well as leaching treatment enhanced the speed of germination of the seeds. Bulbils served as the propagules in the non seed forming accessions.

Appendix

Annexure 2

Weather data (June 2015-December 2015)

Date	Sunshine hours		Relative Humidity		Temperature		Rainfall (mm)
.	Sunshine hours	Mean	Morning	Evening	Maximum	Minimum	
4/6-10/6	21.6	3.1	92	81	30.4	24.5	161.4
11/6-17/6	16.9	2.4	96	75	30.7	23.8	79.1
18/6-24/6	8.4	1.2	97	79	30.5	24.2	142.9
25/6-01/7	32.2	4.6	95	72	31.1	24.6	95.7
2/7-8/7	31.4	4.4	93	70	30.8	23.5	228
9/7-15/7	1.1	0.2	97	85	28.1	22.8	243.9
16/7-22/7	10.3	1.5	95	84	29.2	22.8	182.5
23/7-29/7	4.8	0.7	97	81	29.8	23.1	166.1
30/7-5/8	2	0.3	97	84	28.0	23.4	303.1
6/8-12/8	2.8	0.4	97	79	28.4	22.5	136.1
13/8-19/8	34.4	4.9	97	69	30.6	23.8	6.1
20/8-26/8	37.7	5.4	94	74	31.0	23.6	128.2
27/8-2/9	9.2	1.3	98	78	29.2	23.0	203.0
3/9-9/9	23.8	3.4	97	94	29.8	23	47.6
10/9-16/9	52.0	7.4	97	66	30.9	23.3	22.0
17/9-23/9	51.1	7.3	94	65	31.5	23.5	1.8
24/9-30/9	42.9	6.1	89	68	33.6	23.6	90.6
1/10-7/10	30.8	4.4	93	71	32.4	23.7	59.2
8/10-14/10	26.3	3.8	96	73	31.2	23.8	29.9
15/10-21/10	26.8	3.6	90	63	32.2	23.9	32
22/10-28/10	34.3	4.9	91	68	31.8	23.7	67.4
29/10-4/11	30.5	4.4	95	66	32.0	23.1	20.5

Annexure 1

Mean values of various characters in different accessions of water lily

Accession No.	Lamina width (cm)	Lamina length (cm)	Notch length (cm)	Flower bud length (cm)	Flower bud circumference (cm)	Flower bud diameter (cm)	Petal number	Number of stigmatic appendages
1	14 (3.8^{fg})	8.82 (3.05^{ef})	6.44 (2.63^{d})	3.66 (2.04^{e})	4.448 (2.23^{ef})	6.12 (2.57^{def})	17.20 (4.28^{bc})	17.80 (4.20^{d})
2	19.56 (4.48^{c})	12.26 (3.57^{b})	9.34 (3.14^{b})	4.26 (2.18^{cd})	4.84 (2.31^{de})	6.62 (2.67^{d})	13.60 (4.11^{c})	16.40 (3.74^{ef})
3	14.66 (3.89^{efg})	9.56 (3.17^{de})	6.56 (2.65^{d})	4.12 (2.15^{cd})	5.16 (2.38^{cd})	6.16 (2.58^{def})	19.20 (4.00^{c})	17.00 (4.44^{cd})
4	23.68 (4.92^{a})	13.98 (3.80^{a})	11.82 (3.51^{a})	6.56 (2.66^{a})	7.82 (2.88^{a})	14.16 (3.83^{a})	23.00 (4.32^{bc})	18.20 (4.85^{a})
5	18.06 (4.31^{d})	10.72 (3.35^{c})	8.82 (3.05^{b})	5.28 (2.40^{b})	6.70 (2.68^{b})	9.50 (3.16^{b})	20.40 (5.06^{a})	25.20 (4.57^{bc})
6	6.10 (2.57^{c})	4.24 (2.18^{h})	3.58 (2.02^{f})	3.96 (2.11^{de})	4.24 (2.18^{f})	5.98 (2.54^{def})	13.00 (3.94^{c})	15.00 (3.67^{f})
7	12.040 (3.54^{h})	7.46 (2.82^{g})	5.42 (2.43^{e})	4.14 (2.15^{cd})	4.50 (2.24^{ef})	6.42 (2.63^{de})	18.60 (4.28^{bc})	17.80 (4.37^{cd})
8	20.70 (4.60^{b})	11.18 (3.42^{c})	9.10 (3.09^{b})	5.12 (2.37^{b})	5.50 (2.45^{c})	8.20 (2.95^{c})	20.20 (4.76^{ab})	22.20 (4.55^{bc})
9	14.96 (3.93^{efg})	8.52 (3.00^{f})	6.64 (2.67^{d})	4.42 (2.22^{c})	5.32 (2.41^{c})	7.52 (2.83^{c})	15.20 (4.32^{bc})	18.20 (3.96^{e})
10	15.16 (3.96^{e})	9.76 (3.20^{d})	7.32 (2.80^{c})	3.62 (2.03^{e})	5.36 (2.42^{c})	6.32 (2.61^{def})	20.40 (4.30^{bc})	18.00 (4.57^{bc})
11	15.68 (4.02^{e})	9.60 (3.18^{de})	6.52 (2.65^{d})	6.52 (2.65^{a})	5.46 (2.42^{c})	5.60 (2.47^{f})	21.80 (3.11^{d})	9.20 (4.72^{ab})
12	13.74 (3.77^{g})	9.30 (3.12^{de})	5.54 (2.45^{e})	3.70 (2.05^{e})	5.22 (2.40^{cd})	5.74 (2.50^{ef})	23.40 (3.11^{d})	9.20 (4.89^{a})

Annexure 2

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