

**CHARACTERIZATION AND COMPATIBILITY STUDIES IN WATERLILY
(*Nymphaea* spp.)**

by

AISWARYA P.

(2021-12-025)

THESIS

**Submitted in partial fulfilment of the
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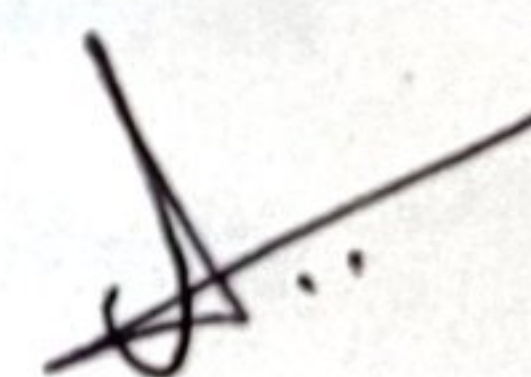
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DECLARATION

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

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LIST OF ABBREVIATION

am	Ante meridiem
ANOVA	Analysis of Variance
CD	Critical difference
cm	Centimeter
CRD	Completely Randomized Design
CV	Coefficient of Variation
Df	Degree of freedom
e	Equatorial diameter
<i>et al.</i>	Co- workers/ co- authors
Fig	Figure
FYM	Farm yard manure
g	Gram
GCV	Genotypic Coefficient of Variation
hr	Hour
IWGS	International Waterlily & water Gardening Society
KAU	Kerala Agricultural University
kg	Kilogram
m	meter
min	Minutes
mm	Millimeter
<i>N.</i>	<i>Nymphaea</i>
No.	Number
p	Polar diameter
PCV	Phenotypic Coefficient of Variation
pm	Post meridiem
RHS	Royal Horticultural Society
Sl.	Serial
spp.	Species
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
<i>viz</i>	Namely

LIST OF SYMBOLS

%	Per cent
°C	Degree Celsius
=	Equal to
μ	Micro
/	Per

1. INTRODUCTION

Waterlily (*Nymphaea* spp.) is an important aquatic ornamental plant belonging to the family Nymphaeaceae with about seventy species in the genus which are distributed in all the continents except Antarctica (Lohne *et al.*, 2008). *Nymphaea* varieties differ in their shape, size, colour, fragrance, blooming pattern, and temperature tolerance. The floral morphology of this group has been investigated systematically and is considered to represent one of the earliest groups of angiosperms.

Waterlily is the national flower of Sri Lanka and Bangladesh having year-round flowering and extensive spectrum of flower colours (Guruge *et al.*, 2016). It is a perennial aquatic plant which is an indispensable and important material in garden waterscapes. The flowers were observed as solitary, pedicellate and with radial symmetry. The tuberous rootstock and stem are submerged beneath the water, with only the leaves and flowers emerging and floating on the water's surface. *Nymphaea* species are categorized into two groups according to their temperature preferences for survival as tropical water lilies and hardy water lilies (Yu *et al.*, 2018). In general, tropical water lilies are more valuable as ornamental and commercial plants than hardy varieties. The intricate colour composition of tropical water lily blossoms includes shades of red, yellow, white, blue, purple, and other hues. Hardy water lilies, on the other hand, only have three petal colours: red, white, and yellow.

The flowers and pads of water lilies play a role in providing shade, thereby keeping the water cooler and preventing the excessive growth of algae, which tends to thrive in warmer temperatures. Additionally, water lilies serve as a source of food and shelter for fish, offering protection from predatory birds and shielding them from the direct heat of the sun. Waterlilies also play a critical role in filtering and detoxifying water by absorbing heavy metals (Shuaibu and Nasiru, 2011; Khan *et al.*, 2016) and thus aid in phytoremediation of polluted water bodies. Numerous species of *Nymphaea* have medicinal uses like antibacterial, antimicrobial, antioxidant, and anti-diabetic activities. The various parts such as

the starch-rich rhizome, root, fruit, leaf, petiole, flower, tuber, and seeds are edible. Many species in *Nymphaea* are medicinally important, forming major constituents of Ayurveda and Siddha systems of medicine (Raja *et al.*, 2010).

The cultivation of water lilies has high demand due to its ease of growing in containers on balconies and terraces. The waterlily business is thriving with the introduction of novel varieties in the market. In addition to gardeners, small scale farmers are attracted to the water lily business because of the reasonable income they can generate through the sale of planting materials. Although water lilies possess considerable potential across various fields, they have received limited attention in terms of crop improvement (Tom, 2015). Natural cross pollination or hybridization between cultivars has yielded many interesting new water lily cultivars. However, for developing a cultivar with desired characteristics, controlled pollination is necessary. Considering the aesthetic, economic, medicinal, and ecological merits associated with *Nymphaea* spp. it is essential to further study its flowering characteristics including flowering phenology and floral traits and to comprehend its reproductive biology, which is vital for future crop improvement studies.

Breeding programs require the acquisition and evaluation of germplasm. Varieties that can adapt to the changing consumer demands are essential, as novelty is a key requirement for the ornamental flower industry. As a supportive requisite for the floriculture production sector in Kerala, the present research programme is undertaken with the aim of evaluation of *Nymphaea* genotypes for growth, flowering and floral attributes, characterizing them using morphological descriptors and assessment of their compatibility with cultivars having breeding potential.

2. REVIEW OF LITERATURE

Waterlily is one of the most perennial aquatic landscaping plants belonging to Nymphaeaceae family having approximately 70 species with 6 genera. They bloom extensively in tropical and temperate regions on every continent, excluding Antarctica and large deserts. The plant holds economic significance in various sectors, such as medicine, aquatic landscaping, industry, food, and cosmetics. The crop differs highly in their shape, size, colour, fragrance, blooming pattern, temperature tolerance and have high genetic potential which is yet to be exploited. So, the present study was carried out to evaluate the performance of waterlily varieties and a brief review which is relevant to the study is given in this chapter.

2.1. CLASSIFICATION

Water lilies belong to the genus *Nymphaea* and the family Nymphaeaceae. They are the most brilliant aquatic blooming plants because of their broad variety of petal colours (Chen *et al.*, 2017). White water lily is the national flower of Bangladesh and state flower of Andhra Pradesh, India while blue water lily is the national flower of Sri Lanka (Yakandawala *et al.*, 2017).

Borsch (2008) proposed Nymphaeales lineage which includes water lilies as one of the first-diverging branches of the angiosperms. The order Nymphaeales is cosmopolitan in distribution with three families Cabombaceae, Nymphaeaceae and Hydatellaceae (Pareek *et al.*, 2016). Majority of the diversity within the Nymphaeales order was reported to be centralized in the *Nymphaea* genus (Gruenstaeudl *et al.*, 2017).

Several workers reported that there are 45–50 species of perennial aquatic plants in the genus *Nymphaea*, which is globally distributed with five subgenera: *Nymphaea* subg. *Anecphyra* (7 to 10 spp.), *N.* subg. *Brachyceras* (14 to 16 spp.), *N.* subg. *Hydrocallis* (14 spp.), *N.* subg. *Lotos* (2 to 3 spp.), and *N.* subg. *Nymphaea* (8 spp.) (Conard, 2005; Borsch *et al.*, 2011; Povilus *et al.*, 2015; Selvakumari *et*

al., 2016; Singh and Jain, 2017). Dkhar *et al.* (2019) later reported that due to their partly fused carpel walls, subgg. *Anecphya* and *Brachyceras* were classified as *Apocarpiae* and subgg. *Hydrocallis*, *Lotos*, and *Nymphaea* as *Syncarpiae* with more thorough carpel wall fusion.

Gupta (1980) reported that the evolution of *Nymphaea* species was significantly influenced by gene mutations, structural chromosomal abnormalities and polyploidy. The chromosome number for the genus *Nymphaea* showed a base number of $x=14$, with polyploidy being evident in all subgenera and the subgenus of *Nymphaea* has $2n= 56, 84, 112$. *N. colorata* Peter possessed a comparatively modest genome size, with a chromosome count of $2n = 28$ and an estimated genome size of around 400 Mb (Zhang *et al.*, 2020).

The earliest fossil evidence for the *Nymphaeaceae* family dated back to the early Cretaceous epoch (Friis *et al.*, 2001). Chukiattman (2006) described that the earliest class of plants on earth were those belonging to the *Nymphaeaceae* family and is represented in India by the native genera *Euryale* and *Nymphaea* (Ansari and Jeeja, 2009). The *Nymphaeaceae* family is distinguished by its big blooms, multiple ovules in each carpel, non-floating stem, and leaves linked by rhizomes (Singh and Jain, 2017).

Chukiattman (2006) and Deviprasad (2009) reported that *Nymphaeaceae* are plants having soft petiole and peduncle, while *Nelumbonaceae* are those plants having rigid petiole and peduncle. Perera *et al.* (2021) reported that with rhizomes embedded in mud and growing in open water bodies, the *Nymphaea* species were either annuals or perennials.

Borsch *et al.* (2007) observed that the waterlilies had enormous spherical floating leaves and rhizomes and year-round blossoming, which made them one of the most attractive groups of aquatic plants (Guruge *et al.*, 2016). Ban *et al.* (2021) reported that the water lilies are also one of the popular cut flowers on the marketplaces across the world.

According to Huang *et al.* (2009) the genus was classified into two ecological types as the hardy water lily and the tropical water lily, depending on the temperature it needs to survive. The former is naturally dispersed mostly in temperate and subtropical regions, whereas the latter is primarily distributed in tropical areas. According to the features of their tubers, hardy water lilies were further categorised into five types: marliac, odorata, tuberosa, finger, and subterranean rhizome types.

According to Rodriguez (2007) and Zhang *et al.* (2022) the tropical waterlilies comprised of both day and night bloomers, whereas hardy waterlilies are only day bloomers.

2.2. DISTRIBUTION

Maia *et al.* (2014) the *Nymphaea*. subg. *Nymphaea* is found throughout the Northern Hemisphere in temperate regions; *N.* subg. *Brachyceras* was reported to have a Pantropical range, and *N.* subg. *Anecphya* is restricted to Oceania.

Dąbrowska *et al.* (2015) opined that the *Nymphaeaceae* is a family of hydrophytes that exist in water reservoirs all around the world. *Nymphaea* is found worldwide in temperate and tropical regions, primarily in shallow freshwater ecosystems (Sun *et al.*, 2018). Crane and Friis (2020) documented the presence of waterlily along the borders of ponds, lakes, and streams.

Mitra *et al.* (1993) reported ten species of the genus *Nymphaea* from India, six of which were wild and the other four were cultivars. In India *N. mexicana* and *N. omrana* were reported from Kashmir and Kozhikode, respectively (Ansari *et al.*, 2005). Pimentel *et al.* (2005) recorded that the *N. mexicana* as the most troublesome plant in Dal Lake in Kashmir. Fischer and Rodriguez (2010) found that every continent, except for Antarctica and the Great Dessert have members of the *Nymphaeaceae* family.

Fischer (1988) reported that *N. thermarum* was initially recorded in a limited thermal spring environment in Rwanda. Mitra *et al.* (1993) reported the presence of *N. rubra* across the Indian plains, as well as in Sri Lanka, Laos, Cambodia, Vietnam, Indonesia, Myanmar, Malaysia, Thailand and Philippines.

Slocum (2005) reported that the *N. stellata* was typically found in the warmer regions of India, especially in the Eastern ghats and Raja *et al.* (2010) reported that it was distributed in Afghanistan, Pakistan, Bangladesh, Nepal, Philippines, Borneo, Sri Lanka, Myanmar, Cambodia, Malaysia, Laos, Thailand, Australia and Africa. According to Tandon *et al.*(2010) in India *N. tetragona* was found exclusively in the states of Jammu & Kashmir and Meghalaya. *N. pubescens*, was found all throughout the island of Sri Lanka and was particularly prolific in the arid zone (Guruge, 2014).

Robson *et al.* (2016) reported the presence of *N. Lorianana* at Boreal Plains and Boreal Shield regions of Manitoba and Saskatchewan. *N. odorata* was recognised in the USA, Australia, Brazil, and India, *N. alba* was reported from Europe to the Western Himalaya (Hassan *et al.*, 2020) and *N. lotus* L. is indigenous to Asia, Africa and some specific areas in Europe (Tungmunnithum *et al.*, 2020).

Nzei *et al.* (2021) reported that due to temperature and precipitation factors, the distribution of *Nymphaea* species in South America were noticed. He also mentioned that the amazon ecosystem was initially the most suitable habitat for *Nymphaea* species but later it was limited due to increased incidence of climatic events like flood and drought which destroyed the vegetation. Nzei *et al.* (2022) documented that in West Australia, a lower distribution of waterlilies was observed in comparison to the northern, eastern, and southern regions of the continent.

Pandey and Diwakar (2008) observed that due to the low number of freshwater aquatic environments in the Andaman and Nicobar Islands, proportionately a smaller number of species of *Nymphaea* were reported from

there. So far, records from the Andaman and Nicobar Islands have documented *N. nouchali* and *N. pubescens*. Also identified eighteen *Nymphaea* species from Brazil.

2.3. ECONOMIC IMPORTANCE

Several workers reported the economic values of *Nymphaea* in many fields including medicine (Che *et al.*, 2015; Zhang *et al.*, 2017), industry (Zhou *et al.*, 2018), food (Zhou and Zhu, 2018) and cosmetics (Sun *et al.*, 2018).

Anbumani *et al.* (2022) reported that the diverse nature, vibrant hues and their lovely scents made them an excellent choice for landscape purpose.

Several cultivars or wild species of *Nymphaea* were utilised in water gardens (Sharma, 2001). *N. micrantha* is a well-known tropical day-blooming viviparous cultivar which is popular in aquatic landscapes (Knotts, 2003). Al-Menaie *et al.* (2011) reported that the *Nymphaea* was utilized to enhance the aesthetic value of the urban environment. The large eye-catching flowers of the violet water lily are used in aquatic cut flower industry and the aquatic landscapes and are also offered at Buddhist temples (Yakandawala *et al.*, 2017).

Jirapong *et al.* (2012) reported that the flowers are frequently utilised in India for religious rituals, worship, and the creation of various medications by herbalists. Jha (2013) reported that several bird species were reported to consume the seeds and fruit of *N. nouchali* and *N. rubra*. Additionally, *Nymphaea* species in freshwater habitats were reported to provide food and shelter for a variety of herbaceous organisms as well as lowering water turbidity and stabilising in-stream silt (Leito *et al.*, 2016; Ranjan and Prakash, 2019; Dalziell *et al.*, 2020).

Yin *et al.* (2015) reported that the fragrance present in waterlilies can be used in aromatherapy and the stamens of the *N. lotus* have potentially rich source of flavonoid antioxidants, which is used for creating cosmetic products. Pareek and Kumar (2016) reported the diverse range of pharmacological properties in the

leaves, roots, and flowers of *Nymphaea and* were used to cure conditions including diabetes, infections of the urinary tract, liver diseases and eruptive fevers.

The flowers and roots were reported to have sedative effect and frequently used to increase mental alertness (Kirtikar and Basu, 2001). Seeds were considered as cooling medicine which treat skin conditions like eczema and also they are used as appetizer (Panda and Mitsra, 2011). According to Mohammed *et al.* (2012) and Abarike *et al.* (2015) considerable protein and mineral content in waterlily seeds suggested the potential utilization of these substances in poultry diets.

Nazir (2015) found that the flower extracts of the *Nymphaea* genus were reported to have antineoplastic effects in different types of cancer, including leukaemia, lung cancer, colon cancer and prostate cancer. Bello *et al.* (2016) observed that the rhizome of the *N. lotus* were included in the treatment of circulatory problems, digestive problems like diarrhoea and dysentery, genitourinary problems like diuretics, and mental problems like insanity, hypsomnia, leprosy, infections, and inflammations.

Mohammed *et al.* (2010) observed that the moisture content of the rhizome was high compared to seeds. Highest crude protein was obtained from the leaves while seeds due to its oily nature had the maximum amount of fat. The tuber and rhizome were roasted and boiled for consumption. Devi *et al.* (2015) in a study reported that the protein content of leaf as 23.88%, fibre and ash content in the peduncle as 43.5% and 20.5% respectively. Carbohydrates content of rhizome were 63.14%. Zhang *et al.* (2022) reported the presence of potent active compounds in waterlily which are employed in foods and drinks. He also mentioned the antimicrobial, anti-anxiety, and anti-hepatotoxicity properties.

Jung *et al.* (2003) and Tungmunnithum *et al.* (2018) reported that the stamen of *N. nucifera* is utilized in the form of dried or powdered form for making herbal tea and served as a primary ingredient in various traditional remedies. Tea

made from the roots of *N. odorata* is employed in the treatment of diarrhea, sores, sore throats, and rashes.

Mohamed and Serag (2003), Huang *et al.* (2009) and Drisy and Ashokan (2021) reported that the *Nymphaea* plants are helpful in the treatment of sewage, heavy metals, water turbidity, conductivity, Total Dissolved Solids, chloride, nitrate, Biological Oxygen Demand and Chemical Oxygen Demand.

All parts of the *N. nouchali* are edible and high in starch (Raja *et al.*, 2010). Nagavani and Rao (2010) described about the non enzymatic, enzymatic and antioxidant potential of methanol, ethanol and aqueous extract of dry and fresh blooms.

Flowers and flower stalks of *N. stellata* are utilised as vegetables, green manure, and fodder, while rhizomes are employed in medicine (Bhandarkar and Khan, 2004). Rakesh *et al.* (2010) reported that the Hydroalcoholic extracts of dried *N. stellata* Willd. flowers have been shown to have free radical scavenging abilities. Abd Rasid *et al.* (2019) reported that *N. nucifera* has a strong phytoremediation system because of the anatomy of leaves, stem, and rhizomes

2.4. LEAF CHARACTERS

Brickell (1989) and Rossow and Charboneau (2006) reported that the waterlilies develop at the pond's bottom and send leaves and blossoms to the surface. They serve as beneficial leaf cover that inhibits the luxuriant growth of algae, place for fish to hide and blocks the native aquatic plants from receiving light.

Singh and Jain (2017) reported that the leaves of waterlily are easily distinguished by their considerable size and a clear, flat, rounded, or oval shape. They also have features such as alternation, the ability to float, pinnate venation, elongated petioles, or a distinctive cordate (heart-shaped) structure.

Kaul (1976) observed the epidermis of floating Nymphaeaceae leaves having variable stomatal densities and an obvious thin cuticle that helps to repel water from the surface of floating leaves. The ribs on the underside of the leaf become more noticeable as it becomes larger. The bigger leaves of certain tropical *Nymphaea* species have crenate or dentate edges, while the smaller leaves are entire-margined.

Su *et al.* (2022) reported that leaf tissue of *Nymphaea* exhibited viviparous property and there are four developmental stages for viviparous species. The stage 1 having the submerged and completely rolled leaves which are 1-2 cm in length and 4- 6 cm when the leaves are at stage 2. In stage 3 partially rolled leaves with tip in contact with water surface. The viviparous structure is evident in stage 4 when the leaves are completely unfolded, revealing prominent viviparous traces or marks in the centre.

Wei-Pei and Si-Mei (1992) reported the absence of actual vessels in six *Nymphaea genera* and the uppermost side of the margin are gently rolled inward ensuring the buoyancy of the plant. Junluthin *et al.* (2021) and Gee *et al.* (2022) reported that the amazing buoyancy in the leaves of the gaint waterlily is due to the presence of spider-web like tall supporting ribs of thickness less than 1 cm and a height of up to 7.5 cm.

Klok and van der Velder (2017) reported that the leaf development in *Nymphaea* is highly dependent on factors such as water temperature, acidity and the wind action. The ratio of leaves to flowers for complete plants varies from 1:1 to 4:1 in *Nymphaea spp.* and also the shoot apical meristem often produces more leaves than flowers (Weidlich, 1976).

Villani and Etnier (2008) reported that the leaves of *N. odorata* exhibits strong heterophylly due to the change in the light environment. The phenotypic variations of *N. gardneriana* that result in fully submerged leaves indicate that this species adjusts leaf development in response to physical variations in the environment (Catian and Scremin-Dias, 2015).

Deviprasad (2009) found that *N. rubra* exhibited better performance in terms of leaf length and longevity compared to *N. alba* and *N. stellata*. Anbumani *et al.* (2022) reported that the difference in leaf length demonstrates that the hardy waterlilies develop more leaves than the tropical waterlilies.

Wiersema (1988) recorded that with rising water depth, the floating leaf of waterlily plants develop longer petiole. The response to variations in light, petiole of lily pad exhibit a significantly distinctive developmental flexibility like aerial petioles get shorter, thicker, and ultimately stiffer.

Dassanayake and Clayton (1996) reported that the petiole of *N. nouchalli* is terete with purple green shade. The petiole tip in *N. amazonum* have the non-glandular trichomes (Etnier and Villani, 2007). When compared to *N. candida*, the petiole of *N. alba* is cylindrical and 40% thicker (Sarbu *et al.*, 2020). Ismuhajarah *et al.* (2022) reported that as the water depth increased, the petiole's length, as well as the volume of both the petiole and air canal, also increased.

Yang (2022) reported that the leaf of *Nymphaea* has bifacial development and the top epidermis is thinner than the lower epidermis, with scattered stomata. *N. alba* has the maximum cold tolerance, followed by *N. potamophila*. *N. minuta* have least cold tolerance. He also reported that the leaf thickness varies significantly as *N. alba* had the loosest leaf structure while *N. potamophila* had the most compact leaf structure.

According to Catian and Scremin-Dias (2015) the leaf size, flexibility, colour, and leaf architecture of *N. gardneriana* have an impact on the flow, nutrients, water turbidity, and light availability. *N. tetragona* Georgi that grow in northern Russia has small leaves measuring from 5 to 9 cm in length (Gee *et al.*, 2022).

(Pott (1998) reported that the leaves of *N. tetragona* were elliptic-oval shape with five inches long. *N. gardneriana* has red membranous leaves when it is fixed as a submerged aquatic plant, while it exhibits elliptical to suborbicular

shaped green subcoriaceous floating leaves when fixed as a floating aquatic plant. De lima and Guilietti (2013) reported that the leaves of *N. vanildae* can be elliptic, membranaceous to subcoriaceous, green chestnut brown coloration with vinaceous spots. Sharaibi *et al.* (2015) reported that the leaves of *Nymphaea lotus* are various shades of green to reddish brown, with many purple dots.

Catian and Scremin-Dias (2013) collected seven species of *Nymphaea* and observed that their petiole has bicollateral bundle, except for *N. oxypetala*, which have the presence of angular collenchyma that makes it different from others. They also reported that the only species with bicollateral bundles in the leaf blade is *N. lingulata*.

2.5. FLORAL BIOLOGY

Wiersema (1988) conducted deep morphological investigations of the family Nymphaeaceae. The waterlily flowers have bitter-sweet, acrid flavour and huge variation in petal shade.

Soltis and Soltis (2004) reported the floral characteristics of the several genera vary greatly, ranging from the tiny, straight forward monocot-like blooms of the Cabomba to the enormous, extravagant flowers of the *Nymphaea* and *Victoria*.

Grob *et al.* (2006) reported that the Nymphaeales have solitary flowers, rhizomatous stems, radial floral symmetry, simultaneous initiation of the carpels in a whorl, acropetal initiation of floral organs, and formation of a vascular plexus at the bottom of the flower.

Schneider (1982) observed that the flowers of *Nymphaea* exhibit protogynous nature and the common floral biology contain 4 sepals, 7 to 40 petals, 5 to 47 carpels and 20 to 700 stamens.

According to Tom (2015) the night blooming flowers have large blooms than the day bloomers with stamens lacking appendages. Also, there are 5 to 6

prominent nerves on both sides of sepals in night bloomers. Zini *et al.* (2019) reported that the night-flowering species *N. gardneriana*, *N. prolifera*, *N. amazonum*, and *V. Cruziana* exhibit reddish hue appendages while in the day-flowering *N. caerulea* possess a yellow colour appendage.

Ito (1983) and Luo *et al.* (2011) observed a blend of sepaloid petal and petaloid stamens which are arranged in a spiral pattern along the wall of the gynoecium. Kirie *et al.* (2020) reported that the flowers have abundant tepals but lack stamens in *N. King of Siam* and *N. Midnight*.

Hassan *et al.* (2020) reported the stamens of *Nymphaea* have linear, introrse anthers which is longitudinally divided.

Den Hartog (1970) reported that in *N. rubra* and *N. pubescens* the anther lobes open and close in response to the spreading of petals but in *N. nouchali*'s anther lobes never close. Doran *et al.* (2004) reported that the age causes filaments to change from their initial yellow colour to a greenish white colour with a violet blue border. Tetali (2008) observed 140 to 150 stamens of the *N. nouchali* are grouped into 9 to 11 spiral rows.

Fahida (2012) observed clavate appendages with a small inward curl along the rim of the stigmatic cup. She also reported that both the white and the blue colour variations the number of stigmatic appendages was equal to the number of carpels. Zini *et al.* (2019) found that the *N. prolifera*, *N. amazonum*, and *V. cruziana* exhibit clavate appendages, *N. gardneriana* display linear and *N. caerulea* have conical appendages. In *N. caerulea*, the tip of the carpellary appendage exhibited staining with neutral red.

Schneider (1982) reported that in *N. elegans*, a cone-shaped structure is formed above the dry stigmatic cup due to its numerous yellow carpellary styles and stamens. Also in *N. alba*, the stigma is flat, while in *N. candida*, it is clearly concave (Sarbu *et al.*, 2020).

According to Meeuse and Schneider (1980) the sugar content of stigmatic exudate was between 1 and 1.5%, the boron concentration was 5 ppm, and the ionic composition in terms of Ca, K, and Mg was almost at the ideal level for pollen germination.

Mohamed and Serag (2003) described that the *N. alba* has a yellow stigmatic disc and white flowers. The four sepals have lance-shaped shapes and have 15 to 30 petals, which developed into stamens throughout time. The outermost petals of *N. alba* often have tinged green centre, with a pink line at the base. Nierbauer *et al.* (2014) observed that the ovary displays syncarpous features, having multiple locules with numerous ovules in each ovary. Placentation occurs superficially, and within each locule, there are many bitegmic and anatropous ovules. Also, their flower pedicels and leaf petioles have more blunt ends at leaf blade lobes.

Seaton (1908) reported that *N. advena* have six sepals that exhibit trimerous perianth and the flower of *N. odorata* has white centripetal petals and four lance-shaped sepals, 72 to 107 stamens, and 15-20 carpels (Schneider and Chaney, 1981).

Stephens and Dowling (2002) and Grob *et al.* (2006) reported that in *N. nouchalli* the flower stalks may grow to a length of 1.5 metres, and the curved sepals are roughly 4 to 15 cm in diameter and 5 to 20 cm above the water. Sarbu *et al.* (2020) reported that in *N. candida*, the base of the bloom has four obtuse edges while in *N. alba* have four rounded edges.

2.6. FLOWERING AND FACTORS AFFECTING FLOWERING

Dalton and Novelo (1983) described that early in the morning, *N. odorata* buds opened, revealing the magnificent, waxy, white blooms that may reach a width of 6 inches while some petals close at afternoon. Biswas and Calder (1984) reported that *N. rubra* had huge, sweet-smelling blooms that were 4–10 inches

wide. *Nuphar lutea*, had blossomed both during the day and at night, with no flower parts shutting during anthesis (Velde and Vander, 1986).

Walker- Larsen and Harder (2000) observed the large solitary, androgynous blooms with a double perianth and a lengthy peduncle on *N. Alba*. Krishnan *et al.* (2004) reported that the majority of night-blooming species of the subgenera *Hydrocallis* and *Lotos*, however, have a similar white or very white flower-coloured petals.

Muthukulam (2006) reported that during summer, the double-red blooms of *N. rubra* opened at night. Songpanich and Hongtrakul (2010) reported that the temperature and day duration were two important elements for optimal bloom output in tropical and hardy waterlilies. He also reported that *N. Gloriosa* have maximum number of flowers (163 blooms) per year while *N. Perry's Fire Opal* had minimum number of flowers (28 blooms) per year.

The flowering of both day and night blooming varieties is highly influenced by the circadian cycle. Flowering in *N. pubescens* and *N. nouchalli* takes place from May to November whereas in *N. rubra* flowering takes place throughout the year (Begum *et al.*, 2010)

Songpanich and Hongtrakul (2010) and Peter *et al.* (2011) reported that in the daylight (600–1400 hr), the subgenera *Anecphya*, *Brachyceras*, *Nymphaea*, and *Confluentes* bloom, whereas *Lotos* and *Hydrocallis* bloom at night (1900–1000 hr). Water lily is a tropical plant known for flourishing in locations with ample sunlight (Yu *et al.*, 2018).

Junluthin *et al.* (2021) described that the Gaint waterlily blossoms can take up to 48 hours to completely open in their native environment after the sun sets. Each plant will produce blooms throughout the growing season, and these blossoms have a diameter of up to 40 cm.

Zhang *et al.* (2021) observed the *N.* hybrid population had a blooming cycle of around 160 days with the full bloom period of 135 days, lasting from late May to mid- november. *N.* hybrid flowers displayed characteristics of inverse herkogamy and protogyny. On the first day of floral anthesis, stigma receptivity was at its highest level, and on the second day, between 9:30 and 11:30 a.m., pollen viability peaked. Blue water lilies have blooming time of 36 months (Kiranmai *et al.*, 2023).

Hao *et al.* (2022) reported that the blooms of hardy waterlily can survive in cold winter while it is difficult for the tropical waterlily to bloom over 25° C.

2.7. ANTHESIS

Watson (1884) became the first to note variations in the opening and closing times among different *Nymphaea* species. He found that certain *Nymphaea* species display a short blooming period, lasting merely two or three consecutive days, while others have a more extended duration, remaining open for as many as six or seven successive days.

Capperino and Schneider (1985) reported that *N. mexicana* exhibits diurnal anthesis, with flowers opening and shutting for two consecutive days. Flowers are functionally female during the first day of anthesis as their stigmatic papillae are secreting a little amount of fluid. Velde and Van der (1986) reported that the initiation of the anthesis phase in *Nymphaea* was marked by the appearance of stigmatic fluid in the stigmatic cup, accompanied by the absence of anthers. Also, distinct variations in the duration of anthesis were noted across various *Nymphaea* species due to variation in the ripening of the rows of anthers.

Orban and Bouharmoni (1995) observed that flower of *N. capensis* showed anthesis for three days. Also, the outermost anthers of *N. capensis* are dehiscent on the initial day while on second day of flowering the middle anthers dehiscent. Hirthe and Porembski (2003) reported that *N. lotus* have four to five days of anthesis.

Krishnan *et al.* (2004) stated that the flower opening and flower bud growth in water lily is influenced by the circadian cycle. In areas with high temperatures, certain species of *Nymphaea* do not blossom (Slocum, 2005). Astle (2006) concluded that the intensity of sunshine affects the waterlily's bud growth and flowering.

Zhou and Fu (2008) reported that in *Nuphar pumila* flower anthesis lasts for four days. Begum *et al.* (2010) reported that the distinct species of *Nymphaea* that were collected from Bangladesh exhibit tremendous variety in their anthesis. *Nuphar lutea* flowered day and night without the flower parts shutting during anthesis, in contrast to the *N.alba* and *N.candida*, which exhibited day blooming.

Fahida (2012) has mentioned the opening and closing time of two-coloured varieties of *N. nouchali* ranged from 7.30 am to 9.45 am and 5.15 pm to 6.15 pm, respectively. The flower of blue type exhibited three days of anthesis whereas it was four days in white type. Lima and Guilietti (2013) reported protogynous flowers with two nocturnal anthesis in *N. vanildae*. The flowers bloomed on the first night about 8:00 pm and closed at 11:00 pm.

Povilus *et al.* (2015) reported that two days prior to the opening of the flower, the anthers undergo an increase in yellow pigmentation. Simultaneously, the papillate stigmatic surfaces become fully exposed. Tom (2015) reported that the process of flowering commenced with the unfurling of the sepals and the process of anthesis is repeated consecutively over a span of four days. It took approximately 20-30 minutes to accomplish the process of full blossoming, while the process of complete closure required around 15-20 minutes.

Zepeda-Gómez *et al.* (2020) reported that in *N. nouchali*, anthesis occurred over three days and the burst of various whorls occurred progressively from the outermost to the innermost whorl while *N. gracilis* exhibits diurnal anthesis lasting for 4-6 days.

Zhang *et al.* (2021) reported the blooming of an individual flower exhibited significant variation across different seasons. During the summer months (June–August), the duration of the blooming period spanned between 3 to 5 days, while in autumn (September–November), it extended for a period of 5 to 10 days.

2.8. STIGMA RECEPTIVITY

Hao *et al.* (2022) reported that the stigma's receptivity during anthesis is limited to a short period, typically only a few hours and this short time frame is crucial for the success of pollination. The stigma exhibited receptivity 17 hours prior to flower opening and retained the receptivity upto 20 hours after bloom opening (Fahida, 2022).

Heslop-Harrison and Shivanna (1977) and Krishnan *et al.* (2004) reported that the waterlilies are characterized by having typical wet stigmas and are capable of producing significant volumes of secretion on the first day of floral anthesis and however scatter pollen grains on the second and third day of floral anthesis.

Schneider (1982) reported that in *N. gigantea*, the fluid released from the stigma filled the cup-shaped gynoecium at the base. Velde and Vander (1986) reported that *N. candida*, *N. alba*, *Nuphar lutea* are all protogynous *N. capensis* flowers are homogynous, and the gynoecium is distinguished by a wet-papillate stigma, a small hollow style, and secretory cells on the ventral side of the ovary (Orban and Bouharmont, 1995).

Ye (2007) founded stigma receptivity using a benzidine-hydrogen peroxide reaction solution (1% benzidine, 3% hydrogen peroxide, and water by volume ratio of 4:11:22). He concluded the presence of receptivity if the solution surrounding the stigma turns to blue or blue-black with several bubbles emerging simultaneously.

Chen *et al.* (2013) reported the study of stigma receptivity often involves the measurement of specific enzyme activities, including esterases, peroxidases, acid phosphatases, and dehydrogenases on the stigma.

The stigma was receptive before the anther dehisced revealed that flowers were protogynous (Khaenthong *et al.*, 2019; Moschin *et al.*, 2021; Uribe, 2021; Zhang *et al.*, 2021; Li *et al.*, 2021; Parveen *et al.*, 2022).

Tetali *et al.* (2008) reported that the stigmatic cup contains 2.2–3 ml of an acidic (pH 4.5) watery solution. A row of 3mm long, clavate-shaped appendages that are smooth and glabrous which is formed at the tip of locules encircle the lip of the cup. Begum *et al.* (2010) observed very little stigma receptivity during the third of anthesis. The stigmatic receptivity of *N. gracilis* is observed to be at its peak during the first two days of flowering (Zepeda-Gómez *et al.*, 2020).

2.9. PALYNOLOGY

The study of pollen and spores is known as palynology (Bhunia and Mondal, 2012).

Mondal (1989) observed that aquatic plants often produce monad-type pollen grains. Dicotyledonous pollen grains are either triporate or tricolporate type except in the primitive family Nymphaeaceae.

Bhunia and Mondal (2012) reported that the genus *Nymphaea* exhibits high pollen grain production due to self-pollination through entomophily. The pollen were also characterized as bilateral, isopolar or heteropolar medium sized and monad. Apertures in *N. micrantha*, *N. pubescens*, *N. rubra*, *N. stellata*, and *Nelumbo nucifera* were monocolpate.

Bhowmik and Datta (2023) The pollen grains of *Nymphaea* are bicellular, having two cells, both within dehiscent anthers and upon reaching the stigma they exhibit a zonosulcate structure, characterized by furrows arranged in a zone-like pattern, and possess a notably large aperture area.

According to Walker (1974), Nymphaeaceae pollen lacks distinct columellae and instead displays a granular layer between the nexine and tectum similar to gymnospermous pollen. The triaperturate pollen of *Nelumbo* and

monoaperturate pollen grains of most *Nymphaeales* are reported by Osborn *et al.* (1991).

Murthy (2000) reported that except for *Nelumbo*, all members of family Nymphaeaceae have bilaterally symmetrical pollen grains and contain a single opening. He also identified the encircling aperture in 6 *Nymphaea* species as "zonosulcate." and found different types of ornamentation like granulate (*N. tetragona*, *N. rubra*), psilate (*N. pubescens*, *N. nouchali*), baculate (*N. candida*) and gemmate (*N. Alba*).

Dkhar *et al.* (2013) observed that the pollen of *N. pubescens* and *N. rubra* are white in colour while *N. tetragona*, *N. caerulea*, *N. nouchali*, *N. alba* var. *rubra* exhibited yellow hue.

Volkova and Shipunov (2007) reported small pollen is found on plants with the *N. alba* and *N. tetragona* morphotypes. Pollen grain of *Nelumbo nucifera* were sub globose, radial symmetry with 4- 6 flowering period (Shubharani *et al.*, 2013). Gandhi (2014) observed that pollen often has a spheroidal or oblate-spheroidal form, while it can also be sub-prolate (*Nelumbo nucifera*) and boat shaped (*N. pubescens*).

A study conducted by Bodhipadma *et al.* (2013) in two distinct forms (Bua Phan and Bua Phuean) of *N. nouchali* var. *Versicolor* and found that the shape of viable pollen was nearly spheroidal and have no correlation with size of the flower. Presence of a ring-like sulcus called zonosulculus around the equator. The pollen grains were ellipsoidal, round in one perspective while boat-shaped from another angle. The germination of pollen is influenced by temperature.

Pollen grains with less viability when transferred to the stigma and increase unsuccessful pollination and reduce the quantity of produced seeds (Deng *et al.*, 2017). The percentage of viable pollen grains was 17.3% for the cultivar 'Peter Slocum', 19.3% for *N. colorata*, 10.3% for the species *N. micrantha*, and 17.6% for the species *N. gigantea* (Sun *et al.*, 2018).

Tom (2015) reported that even ten hours after dehiscence, *Nymphaea* pollen grains are still alive. After 2 hours of culture on the germination medium, pollen grains started to germinate, and the germination peak happened 6 hours later (Sun *et al.*, 2018).

Tungmunnithum (2020) conducted pollen analysis of *N. nucifera* and *N. lotus* and revealed that the pollen grains of both species exhibit a furrow-shaped structure. *N. lotus* possesses smaller pollen grains compared to *N. nucifera*. Furthermore, *N. lotus* has 2-colporate pollen grains, while *N. nucifera* features 3-colporate pollen grains. The pollen surface of *N. lotus* is characterized by verrucated ornamentation with microgranules, whereas *N. nucifera* displays regulated ornamentation without microgranules.

Uribe (2021) reported that secondary metabolites in the nectar of *N. ampla* promote pollen deposition by altering pollinator behaviour.

Zhang *et al.* (2022) reported that the tropical water lilies had pollen grains of oval to nearly spherical shape, were they are almost circular in the polar view, and oval or navicular in the equatorial view. The pollen exine ornamentation was psilate or verrucate. Also pollen size and shape from three hardy water-lilies, *N.* ‘Rose Arey’, *N.* ‘Perry’s Fire Opal’, and *N.* ‘Peter Slocum’ were found to be medium sized and oblate shaped. *N. mexicana* is characterized by dome-shaped pollen grains, which is 34-38 μm long and having a furrow ring.

Wodehouse (1965) reported that when the pollen grains are wet they appear broadly ovate with partially flattened ventral surfaces and appears to be a "round-topped bun" when dry since the furrow ring pull the operculum up towards the dorsal hemisphere. Buitink *et al.* (2000) reported that among the environmental elements, it is evident that temperature affects pollen germination, speeding up pollen ageing and germination rate (Young *et al.*, 2004). Tang *et al.* (2009) reported that when the length of the pollen tube exceeds the diameter of the pollen grain, the pollen grain is viable.

2.10. POLLINATION BIOLOGY

Begum *et al.* (2010) reported that anther dehiscence and the existence of stigma appendages are pivotal factors in the pollination process.

Schneider (1979) and Meeuse and Schneider (1980) reported that in *Nymphaea*, insects initially go to the pollen-releasing blooms before they enter the pollen-receptive flowers.

Bernhardt (2000) reported inflorescences that are primarily pollinated by beetles are characterised by intense thermogenesis. Seymour and Mathews (2006) investigated the function of thermogenesis in the pollination biology of the amazon waterlily and resulted that warmer beetles increase pollination efficiency. Sun *et al.* (2018) reported that on bright days, the optimal period for pollination is between the hours of 800 hr and 1000 hr.

Tetali (2008) reported that through continual examination of dead insects in their stigmatic cups, the insectivorous nature of *N. nouchalli* and *N. ceruna* was determined. Also *N. stellata* possess primitive form of insectivory (Raja *et al.*, 2010).

Cramer *et al.* (1975) reported that the biology of pollination in nocturnally blossoming *Nymphaea* species has received less attention, although cantharophily have been already reported. Prance (1980) found that cyclocephala beetles is related to the pollination of nocturnal flowering *Victoria amazonica*. Ervik and Knudsen (2003) reported that the beetles pollinate night-blooming varieties of Lotos and Hydrocallis.

Capperino (1985) reported that *N. mexicana* is commonly visited by the members of the orders Coleoptera, Diptera, and Hymenoptera. The activity of these insects normally started at 11:00 in the morning, peaked around noon, and ended by 3:30 in the afternoon.

Hirthe and Porembski (2003) reported that the beetles were less successful pollinators than some bee species, which visited flowers in the morning. Maia *et al.* (2014) reported that in *N. lotus* is suited for pollination by both nocturnal beetles and daytime bees and also *Ruteloryctes morio*, an indigenous dynastid beetle, visited the flowers of *N. lotus* throughout the night.

Chalegre *et al.* (2020) found that the main pollinators noted were honey bees (*Apis mellifera* L.), primarily focused on gathering pollen from flowers in their second and third days of bloom. The highest level of visitation activity was observed during mid-morning.

2.11. HYBRIDIZATION

Huxel *et al.* (1999) reported that natural hybridization occurs often in the *Nymphaea* species and several artificial hybrids have been created as ornaments (Yakandawala *et al.*, 2017).

Les *et al.* (2004) reported *N.* 'William Philip' is the first proven inter subgeneric cross between *N. colorata* Peter as pollen parent and *N. gigantea* Hook. Andre leu as ovule parent with numerous ornamental properties.

Cheng *et al.* (2022) reported that *N.* 'Midnight' is the first known hybrid where stamen becomes petals and it is characterized by a double-flowered waterlily with deep purple star-shaped blooms and pads with slight flecks.

Wiersema (1988) reported that the hybrids of *Nymphaea* have been shown to develop more quickly than their parent species, although they are often sterile. *N.* 'Woods Blue Goddess' is a tropical waterlily which features star-shaped petals in blue hue and vibrant green pads. Nierbauer *et al.* (2014) reported that the varieties crucial for breeding included the red-flowered *N. alba* var. *rubra* originating from Sweden, along with pink- and white-flowered variations of *N. odorata* and *N. colorata* due to its blue petals.

Stigma pollen incompatibility and embryo abortion are the pre and post-fertilization barriers that can lead to failure of hybridization (Spielman and Scott, 2008). To get beyond pre fertilization obstacles in water lily interspecific hybridizations, certain unusual pollination techniques may be applied, including mentor pollen, delayed pollination, and gibberellic acid treatment (Sun *et al.*, 2018).

Songpanich and Hongtrakul (2010) conducted inter subgeneric hybridization between tropical and hardy waterlily to develop blue hardy waterlilies. The inability to achieve the maturation of intersubgeneric zygotes is attributed to difficulties arising from the differing chromosome numbers between the subgenus of tropical water lilies and hardy water lilies. Later by cross pollination between hardy waterlily with blue purple flowered tropical waterlily became the first blue hardy waterlily in the world.

Zhang *et al.* (2021) reported that majority of hybrids can be readily identified based on their morphological characteristics but some white-flowering hybrids of *N. alba* with *N. odorata* are difficult to distinguish based solely on morphological characteristics.

Kumari *et al.* (2022) reported that one of the species of *Nymphaea* that may self-fertilize is *N. Alba* and also reported that in the hybrid breeding process, determining the pollen activity with accuracy is a crucial stage.

2.12. FRUIT AND SEED CHARACTERS

Tackholm (1974) reported that the fruit is a berry that matures underwater and has numerous seeds surrounded by pulp. The fruit exhibited a lateral mode and terminal mode of dehiscence within the varieties.

Tom (2015) reported that fruit is a dehiscent capsule with many seeds which developed under the water. Also in mature fruit, it was observed that all the floral whorls persistent and took 20 to 25 days for the maturation of fruit.

Weberbauer (1894) observed a notable feature of *Nymphaea* seeds is the presence of an aril, characterized by its whitish or translucent appearance. In *N. ampla*, *N. elegans*, *N. gracilis*, and *N. pulchella* of subg. *Brachyceras*, the aril covers approximately 50–75% of the seed. In subgenera *Hydrocallis* and *Nymphaea*, the aril extends up to 25% beyond the length of the seed

Shui *et al.* (2001) reported that the seeds are horizontally ridged, ellipsoid-globose, 1-1.5 m long, greyish-white, and buoyed by a mucous membrane that resembles vesicles that is surrounded by air sacs. The seeds are small and have a translucent white aril. The fruit ripens underwater and is covered by a persistent calyx (Tetali, 2008).

Zhang *et al.* (2021) reported that *N.hybrid* is protogynous and depends on pollinators to produce fruits by cross-pollination. Also under natural circumstances, the *N. hybrid* had a poor rate of seed setting.

Valla and Martin (1976) and Conard (1905) founded that seed size in *Nymphaea* exhibits distinct differences among its subgenera. *N. mexicana*, belonging to subg. *Nymphaea*, features the largest seeds, whereas species within subg. *Hydrocallis* have the smallest seeds. The seeds of subgenus (subg.) *Brachyceras* are spherical, while those of subg. *Nymphaea* are ovoid. In contrast, the seeds of subg. *Hydrocallis* range from spherical to ellipsoid in shape.

Capperino and Schneider (1985) reported that the number of seeds in the fruit shows an inverse relationship with its size, and the colour can vary from light to dark green. Mature seeds in all species exhibit a characteristic reddish-brown tint (Bonilla-Barbosa *et al.*, 2000).

Richards and Cao (2012) reported that *N. odorata* plants have the capacity to germinate from seeds in water depths of up to 90 cm and crowding of seeds has been shown to enhance germination. Tom (2015) reported that seed germination can be enhanced by acid scarification as well as leaching treatment.

Dalziell *et al.* (2020) conducted a study in four *Nymphaea* species of native Australian and recorded that the germination of seeds was noticeably hindered with the increasing concentrations of NaCl to which they were exposed. The germination time of waterlily seeds was observed to decrease when exposed to nanosilver (5ppm), as it facilitated the breaking of the seed coat (Ban *et al.*, 2021).

3. MATERIALS AND METHODS

The investigation entitled 'Characterization and compatibility studies in waterlily (*Nymphaea* spp.) was carried out in the department of Floriculture and landscaping, College of Agriculture, Vellayani, during the period from November 2022 to October 2023. The details regarding the experimental materials and methodology adopted for the study are presented below.

3.1. EXPERIMENT 1: COLLECTION AND CHARACTERIZATION OF NYMPHAEA GENOTYPES

3.1.1. Climate

Vellayani is located at an altitude of 29 m above mean sea level and between 76.9°E longitude and 8.5°N latitude. The meteorological data during the observation period recorded at the meteorological observatory at the College of Agriculture, Vellayani is presented in Appendix I.

3.1.2. Experimental Material

Near flowering size plants of thirty *Nymphaea* genotypes were collected from all over India for studying their vegetative and floral quantitative and qualitative characters for a period one years (Plate 1).

The details of the genotypes used in the study are given in Table 1.

3.1.3. Details of Experimental Design

The selected 30 varieties were raised in tubs and the experiment was conducted using completely randomized design with thirty treatments and three replications each (Plate 2).

3.1.4. Layout and conduct of the experiment

The experimental plants were grown in tubs of diameter 24 inches for a period of two years at the Instructional farm, College of Agriculture, Vellayani. The tubs were filled with potting mixture containing 2 kg FYM, 300 g bone meal and 10 kg garden soil. Water level was maintained throughout the growth phase. All plants received uniform cultural practices. The plants were kept at a location that receive maximum sunlight. To boost up the growth, the plants were fertilized

with a monthly application of Mono Ammonium Phosphate (4g) and Di Ammonium Phosphate (6g) alternatively. The fertilizer was wrapped in small papers and inserted deep into the soil near to the root zone.

Observations were recorded on the following characters.

3.1.5. Vegetative parameters

3.1.5.1. Number of days from visual appearance to full expansion of leaf lamina (days)

Leaves when appeared on the surface of the mud were tagged and the number of days taken till unfurling was counted.

3.1.5.2. Length of lamina (cm)

Length of leaf was measured from the bottom part of the leaf to the tip using a scale.

3.1.5.3. Width of lamina (cm)

Width of leaf was measured from the broadest horizontal part of the leaf using a scale.

3.1.5.4. Number of leaves/ plants

Total number of leaves present in the plant were counted during a particular date of every month. Young developing, diseased and old drying leaves were not counted.

3.1.5.5. Longevity of leaves

Counting the days from visual appearance of leaf on water surface till decay initiation.

3.1.5.6. Petiole circumference (cm)

Circumference of petiole were measured using a thread and scale.

3.1.5.7. Petiole diameter (cm)

Petiole diameter was taken from lower, middle and highest point using a scale.

3.1.5.8. Petiole length (cm)

Length of the petiole was measured from the bottom to the point of attachment of the leaf to the petiole using a scale.



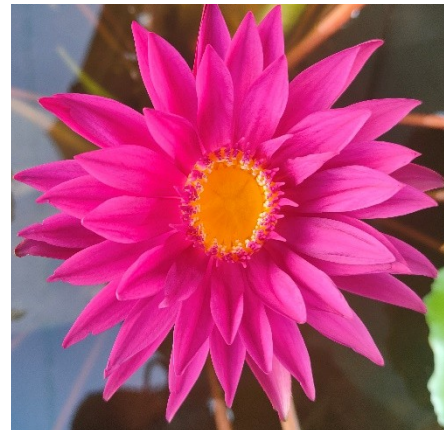
Micrantha



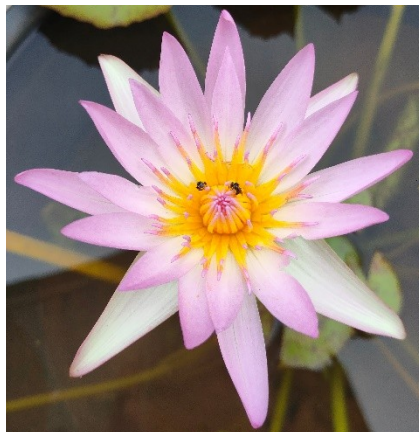
Chompu



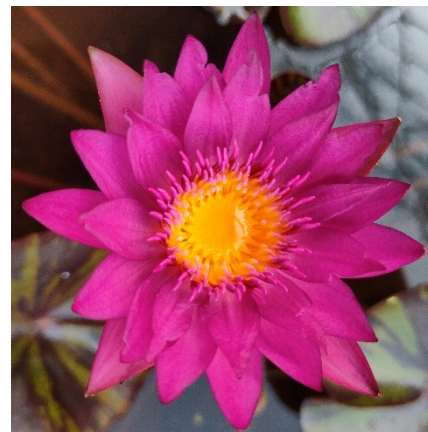
Taweekan



Bull's Eye



Hilary

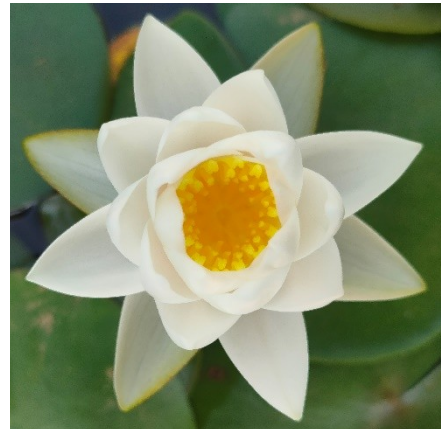


Doris Holt

Plate 1. *Nymphaea* varieties used for the study



Carla's Sunshine



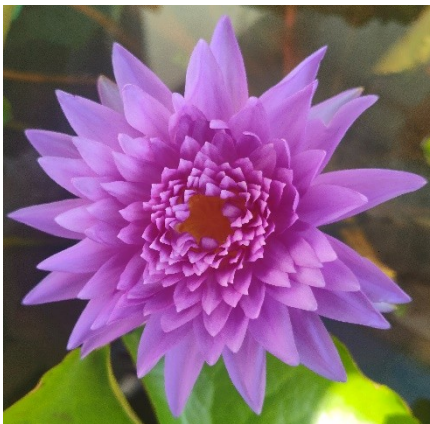
Siam Jasmine



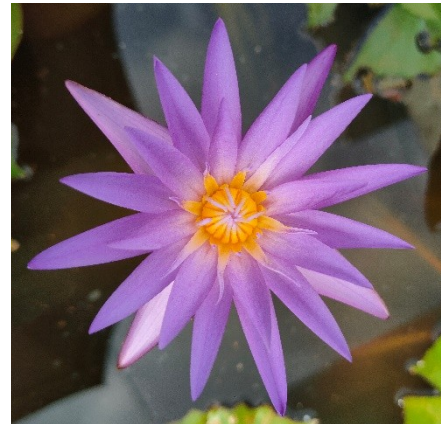
Mexicana



Wanvisa



King of Siam



Blue Whistle

Plate 1. *Nymphaea* varieties used for the study (Continued)



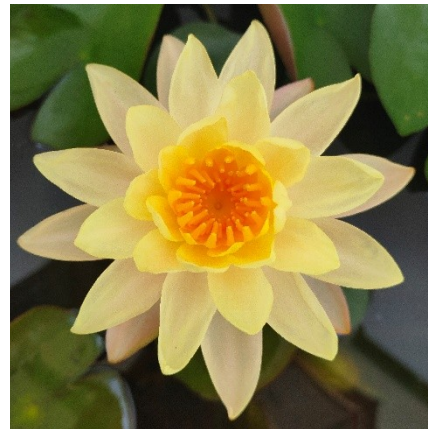
Purple Joy



Dauben



Poosub



Clyde Ikins



Virginalis



Peach Blow

Plate 1. *Nymphaea* varieties used for the study (Continued)



Islamorada



Perry's Baby Red



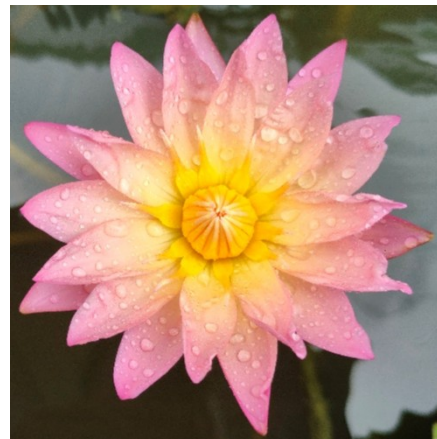
Supranee Pink



Siam Pink



Jalueang Sub



Tropic Sunset

Plate 1. *Nymphaea* varieties used for the study (Continued)



Miami Rose



Colorado



Paranne



Siam Amber

Plate 1. *Nymphaea* varieties used for the study (Continued)

3.1.5.9. Shape of leaf

Based on visual observations, the leaves were categorized into round or ovate shape.

3.1.5.10. Shape of leaf margin

Based on visual observations, the leaf margins were categorized into toothed or smooth margin.

3.1.5.11. Overlapping of leaf base

Presence or absence of overlapping between the lobes(sinus) of leaf was recorded.

3.1.5.12. Colour of upper and lower surface of the leaf

The colour was decided as per RHS colour chart, 2006.

3.1.5.13. Striations/ markings on the leaf

Striations on adaxial surface of the leaf were recorded as present/ absent.

3.1.5.14. Leaf venation on lower surface

Leaf venation on the lower surface was recorded as prominent or non-prominent.

3.1.5.15. Leaf hairs on lower surface

Leaf hairs on the lower surface were recorded as long and short. It is observed using a hand lens.

3.1.5.16. Petiole colour

Petiole colour was decided using RHS colour chart.

3.1.5.17. Hairs on petiole

Hairs on petiole were recorded as present or absent.

3.1.6. Floral parameters

Twenty-one varieties were used for calculating the floral quantitative characters whereas twenty eight varieties were used for observing the floral qualitative characters based on flower production.

3.1.6.1. Days to appearance of flower bud

Number of days taken from visual appearance of the bud on the surface of mud to its appearance on water surface was recorded by tagging the flower buds.

3.1.6.2. Length of flower bud (cm)

Length of the flower bud was measured in the previous day of flower opening using a scale.

3.1.6.3. Days to flower opening

Number of days taken by the flower bud to open fully after reaching the water surface.

3.1.6.4. Flower diameter (cm)

Diameter of flower was measured using a scale on the second day of flowering.

3.1.6.5. Pedicel circumference (at the joint of receptacle)

Pedicel diameter was measured using a thread and scale from the joint of receptacle. It is expressed in cm.

3.1.6.6. Pedicel diameter (cm)

Pedicel diameter was taken from lower, middle and highest point using scale.

3.1.6.7. Pedicel length (cm)

Length of the pedicel was measured from the bottom to the point of attachment of the flower with the pedicel.

3.1.6.8. Pedicel colour

Pedicel colour was decided based on RHS colour chart.

3.1.6.9. Number of sepals

Number of sepals were counted from a dissected flower.

3.1.6.10. Length of sepal (cm)

Length of sepals were measured from the longest vertical region of the sepal.

3.1.6.11. Width of sepal (cm)

Breadth of sepals were measured from the broadest horizontal region of the sepal.

3.1.6.12. Number of petals

Number of petals were counted from a dissected flower.

3.1.6.13. Length of petal (cm)

Length of petal was measured from the longest vertical region of the petal.

3.1.6.14. Width of petal (cm)

Breadth of petal was measured from the broadest horizontal region of the petal.

3.1.6.15. Number of stamens

Number of stamens were counted from a dissected flower.

3.1.6.16. Length of stamen (cm)

Length of stamen was measured from the outermost whorl.

3.1.6.17. Stamen colour

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

3.1.6.18. Number of flowers/plants/months

The total number of flowers produced by the plants each month were recorded.

3.1.6.19. Field life of flower

Field life of flower was recorded as the number of days the flower remain open in plant.

3.1.6.20. Sepal shape

Shape of the sepal was observed as linear lanceolate or ob lanceolate.

3.1.6.21. Flower colour

Inner and outer petal colour was decided with reference to the International Union for the Protection of New Varieties of Plants colour chart (RHS colour chart, 2006)

3.1.6.22. Petal apex

Tip of the petal was observed as acute or obtuse.

3.1.6.23. Anthesis time

Time and duration of opening and closing of the flower was observed at hourly intervals on the first day of anthesis. The observations was expressed in minutes.

3.2. CROP IMPROVEMENT STUDIES

3.2.1. Palynology

Pollen grains were acetolysed as per the procedure suggested by Nair (1970). The pollen grains were collected during the second day of anthesis and stained with 1:1 glycerin – acetocarmine stain (2%). Morphological observations of fertile pollen were made using a compound microscope with binocular lens of 40x eyepiece. Size and shape of fertile pollen was measured as polar diameter (p) and equatorial diameter (e) and expressed in micrometers. Maximum length in the vertical direction was considered as polar diameter and that in the equator region as equatorial diameter. Pollen shape was determined by calculating p/e ratio. According to the method suggested by Wodehouse (1935), pollen with p/e ratio less than one was classified as oblate spheroidal, greater than one as prolate spheroidal and one as spheroidal. Pollen size was determined in accordance with the p x e value. Pollen grain with less than 1000 p x e value was categorized as small and more than 1000 as medium size (Bhunja and Mondal, 2012).

Two slides were made for each variety and from each slide, four microscopic fields were scored, and the data recorded. Pollen grains that were unfilled, small, partly stained, and shrivelled were rated as sterile, whereas pollen grains that were evenly filled and stained were scored as fertile. Fertility of each variety was estimated as percentage of the number of fertile pollen grains to the total number of pollen grains scored. The palynology study was carried out at Department of Microbiology, College of Agriculture, Vellayani.

$$\text{Pollen fertility (\%)} = \frac{\text{No: of uniformly stained and completely filled pollen grains}}{\text{Total number of pollen grains}} \times 100$$

3.2.2. Determination of stigma receptivity

The duration of stigma receptivity was determined by observing the presence of stigmatic fluid in the stigmatic cup starting from the previous day of anthesis and expressed in minutes. The stigmatic surface was also observed for any change in colour and the colour was expressed using RHS colour chart. The



Plate 2. General view of the experimental plot

presence of exudate in the stigmatic cup or its moist condition was considered as indication of stigma receptivity. Diameter of stigmatic cup was measured using scale and expressed in millimetre. The number of stigmatic appendages per flower was counted on the first day of bloom emergence.

3.2.3. Hybridization and compatibility studies

A preliminary hybridization was conducted among the genotypes based on flower availability. Flowers for hybridization were taken from first day of anthesis and covered using net bag to prevent the entry of pollinators. Pollen grains were taken from a fully opened second day flower and dusted on the stigmatic fluid of a female flower. The pollination was carried out between 9:00 am to 11:00 am. Each pollinated flower was labelled showing the parentage and date of crossing.

Based on the palynology and stigma receptivity five best pollen and ovule parents were selected for the hybridization and compatibility studies.

3.2.3.1. Number of flowers pollinated

The total number of flowers subjected to pollination were recorded.

3.2.3.2. Number of successful crosses

The total number of successful crosses obtained after pollination.

3.2.3.3. Percentage of fruit set

$$\text{Percentage of fruit set} = \frac{\text{Number of successful cross}}{\text{Total number of crosses performed}} \times 100$$

3.2.3.4. Number of days taken for fruit set

The number of days from hybridization to fruit set were recorded.

3.2.3.5. Fruit weight(g)

Weight of individual fruit was measured in gram.

3.2.3.6. Fruit development pattern

The fruit development was studied after hybridization. Post fertilization changes in the flower and pedicel were observed.

3.2.3.7. Number of seeds

The total number of seeds present inside the fruit were counted.

3.2.3.8. Seed size (mm)

Seed size was measured using image analyser microscope.

3.2.3.9. Number of days taken for germination

Total number of days taken from sowing till germination was recorded.

3.2.3.10. Germination percentage (%)

$$\text{Germination percentage (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100$$

3.3 STATISTICAL ANALYSIS

The data collected were subjected to Analysis of Variance (ANOVA) (Panse and Sukhatme, 1967), variance component analysis and cluster analysis (Singh and Chaudhary, 1985) GrapesAgril software was utilised for the analysis of data (Gopinath *et al.*, 2021).

3.3.1 Analysis of Variance

Analysis of variance with two variables X and Y was measured in genotypes 'g' developed in completely randomised design with 'r' replication and is given as below.

Source	Degrees of freedom	Mean Square		
		X	Y	XY
Between genotypes	(g-1)	G _{XX}	G _{YY}	G _{XY}
Error	(r-1)(g-1)	E _{XX}	E _{YY}	E _{XY}

3.3.2. Coefficient of Variation

Variability that existed in the population for various characters were apportioned using the estimates of coefficient of variation.

For the character X_i,

$$\text{Phenotypic coefficient of variation, PCV} = \frac{\sigma_{pi}}{\bar{x}_i} \times 100$$

Genotypic coefficient of variation, $GCV = \frac{\sigma_{gi}}{\bar{x}_i} \times 100$

Environmental coefficient of variance, $ECV = \frac{\sigma_{ei}}{\bar{x}_i} \times 100$

Where σ_{pi} , σ_{gi} and σ_{ei} are the phenotypic, genotypic and environmental standard of deviation respectively.

The PCV and GCV were categorized as Low (<10), moderate (10- 20) and high (>20) (Sivasubramanian and Menon, 1973).

3.3.3. Heritability Coefficient

Jain (1982) proposed the mathematical relationship of variance estimates on computation of heritability, which is usually expressed as a percentage.

Heritability (broad sense), $H^2 = \frac{\sigma_{gi}^2}{\sigma_{pi}^2} \times 100$

The heritability % was categorized as low (<30), moderate (30-60) and high (>60) (Johnson *et al.*, 1995).

3.3.4. Genetic Advance

Genetic advance as percentage of mean was calculated as per the formula given by Lush (1949).

Genetic advance, $GA = \frac{kH^2 \sigma_{pi}}{\bar{x}_i} \times 100$

H^2 - heritability in broad sense

σ_{pi} - phenotypic standard deviation

k - selection differential that is 2.06% in case of 5% selection in larger samples (Miller *et al.*, 1958).

Genetic advance as percentage were categorized into low (<10), moderate (10-20) and high (>20) as suggested by Johnson *et al.* (1955).

3.3.5. Correlation Analysis

The correlation coefficients (phenotypic and genotypic) between two characters denoted as i and j were worked out as

$$\text{Genotypic correlation } (r_{gij}) = \frac{\sigma_{gij}}{\sigma_{gix}\sigma_{gj}}$$

$$\text{Phenotypic correlation } (r_{pij}) = \frac{\sigma_{pij}}{\sigma_{pix}\sigma_{pj}}$$

$$\text{Environmental correlation } (r_{eij}) = \frac{\sigma_{eij}}{\sigma_{eix}\sigma_{ej}}$$

Where σ_{gij} , σ_{pij} and σ_{eij} are the genotypic, phenotypic and environmental covariances between the characters i and j.

3.3.6. Cluster Analysis

Genetic divergence was studied using Average method and Euclidean Distance measures.

Table 1. Details of *Nymphaea* genotypes used in the study.

Sl. No.	Abbreviations	<i>Nymphaea</i> varieties
1	MC	Micrantha
2	CP	Chompu
3	TW	Taweekan
4	BE	Bull's Eye
5	HY	Hilary
6	DH	Doris Holt
7	CS	Carla's Sonshine
8	SJ	Siam Jasmine
9	PK	Puttaraksa
10	MX	Mexicana
11	WV	Wanvisa
12	KOS	King of Siam
13	BW	Blue Whistle
14	PJ	Purple Joy
15	DB	Dauben
16	PS	Poonsub
17	CI	Clyde Ikins
18	VG	Virginalis
19	PB	Peach Blow
20	IM	Islamorada

Sl. No.	Abbreviations	<i>Nymphaea</i> varieties
21	PBR	Perry's Baby Red
22	SK	Supranee Pink
23	SP	Siam Pink
24	JS	Jalueang Sub
25	TS	Tropic Sunset
26	MR	Miami Rose
27	CD	Colorado
28	AB	Almost Black
29	PR	Paranee
30	SA	Siam Amber

4. RESULTS

The results of the investigation entitled ‘Characterization and compatibility studies in waterlily (*Nymphaea* spp.)’ are represented in this chapter.

4.1. COLLECTION AND CHARACTERIZATION OF *Nymphaea* GENOTYPES

The performance of thirty *Nymphaea* genotypes was evaluated for one year and the data were statistically analysed. The results are represented in the following sub heads.

4.1.1. Vegetative quantitative characters

The average performance of all the genotypes for the vegetative quantitative characters *viz.* number of days from visual appearance to full expansion of leaf lamina (days), length of the lamina (cm), width of the lamina (cm), number of leaves per plant, longevity of leaves (days), petiole diameter (cm) are presented in Table 2.

4.1.1.1. *Number of days from visual appearance to full expansion of leaf lamina (days)*

The genotypes varied significantly with respect to the number of days from visual appearance to full expansion of leaf lamina (Plate 3). The variety Puttaraksa took the lowest period (6.5 days) whereas the variety Peach Blow leaves took the highest period (13.2 days).

4.1.1.2. *Length of lamina (cm)*

Variety Siam Pink (21.28 cm) recorded the highest value and Wanvisa (10.84 cm) recorded the lowest value for the length of leaf lamina and differed significantly from other varieties.

4.1.1.3. *Width of lamina (cm)*

The variety Purple Joy (20.59 cm) had the longest lamina width while Wanvisa (9.87 cm) had the shortest lamina width and it was on par with Paranee (10.04 cm).

4.1.1.4. Number of leaves per plant

The monthly leaf production per plant varied, with a mean of 18.43 numbers. The minimum number of leaves per plant was displayed by Bull's Eye (10.05 No.) and was on par with Carla's Sonshine (10.13 No.). While significantly high numbers of leaves were exhibited by Wanvisa (29.4 No.).

4.1.1.5. Longevity of leaves (days)

Days from visual appearance to initiation of decay in leaves showed significant variation with a mean of 26.8 days. Jalueang Sub (21.3 days) showed the lowest longevity while Wanvisa (35.2 days) showed the highest.

4.1.1.6. Petiole circumference (cm)

The lowest petiole circumference was exhibited by Mexicana (1.19 cm) and the highest by Tropic Sunset (2.39 cm).

4.1.1.7. Petiole diameter (cm)

The lowest petiole diameter was exhibited by Supranee Pink (0.36 cm) and the highest by King of Siam (0.92 cm).

4.1.1.8. Petiole Length (cm)

The lowest petiole length was exhibited by Mexicana (15 cm) and the highest by Siam Pink (38.5 cm).

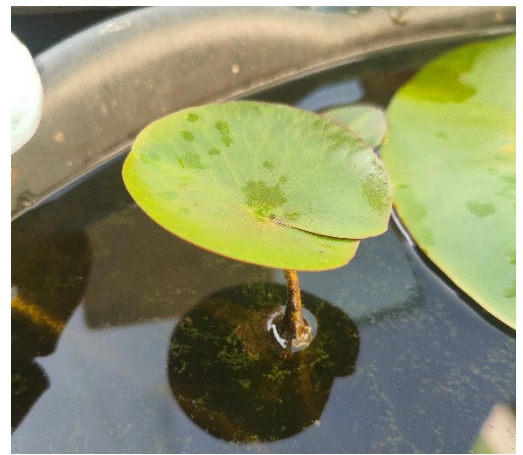


Plate 3. Different stages of leaf emergence

Table 2. Variability in vegetative quantitative characters of thirty *Nymphaea* varieties

Variety	Number of days from visual appearance to full expansion of leaf lamina (days)	Length of lamina (cm)	Width of lamina (cm)	Number of leaves per plant	Longevity of leaves(days)	Petiole circumference (cm)	Petiole diameter (cm)	Petiole length (cm)
MC	8.22±0.50 ^{cde}	15.84±0.10 ^g	13.73±0.31 ^{ij}	20.00±0.22 ^{def}	24.33±2.72 ^{defgh}	1.58±0.10 ^{ij}	0.54±0.08 ^{defgh}	24.23±0.15 ^k
CP	9.22±1.38 ^{bcd}	14.91±1.01 ^{hi}	12.30±0.20 ^{mno}	14.94±1.83 ^{ijk}	28.55±5.09 ^{bcdef}	1.98±0.04 ^d	0.45±0.08 ^{ghij}	28.83±0.11 ^{ef}
TW	9.22±1.95 ^{bcd}	14.01±0.27 ^{jk}	13.64±0.19 ^{ij}	14.36±1.31 ^{ijkl}	30.00±4.72 ^{abcd}	2.27±0.06 ^b	0.84±0.10 ^a	26.53±0.25 ^g
BE	8.22±1.07 ^{cde}	13.64±0.37 ^{kl}	13.31±0.18 ^{ijk}	10.05±0.83 ^m	22.66±7.81 ^{fgh}	1.98±0.06 ^d	0.56±0.05 ^{cdefg}	21.16±0.15 ⁿ
HY	9.22±1.26 ^{bcd}	18.77±0.29 ^d	17.56±0.02 ^{cd}	16.27±1.07 ^{fghi}	23.22±0.69 ^{efgh}	2.10±0.07 ^c	0.66±0.05 ^{bc}	29.90±0.20 ^d
DH	8.33±0.33 ^{bcd}	16.86±0.18 ^f	16.21±0.34 ^{fg}	19.77±1.72 ^{defg}	23.88±2.26 ^{defgh}	2.13±0.09 ^c	0.63±0.10 ^{bcd}	29.00±0.30 ^e
CS	9.11±2.16 ^{bcd}	19.14±0.54 ^{cd}	16.25±0.28 ^{efg}	10.13±0.83 ^m	25.55±4.88 ^{defgh}	1.99±0.01 ^d	0.54±0.08 ^{defgh}	29.56±0.35 ^d
SJ	8.11±0.50 ^{cde}	11.95±0.27 ^{nop}	12.60±0.13 ^{lmn}	19.19±2.99 ^{defgh}	29.11±7.34 ^{abcde}	1.81±0.05 ^{ef}	0.53±0.08 ^{efgh}	17.43±0.15 ^s
PK	6.55±0.38 ^e	12.59±0.82 ^{mn}	11.96±0.84 ^{nop}	19.88±1.81 ^{def}	24.44±1.50 ^{defgh}	1.36±0.07 ^m	0.52±0.10 ^{efghi}	21.76±0.25 ^m
MX	9.44±1.50 ^{bcd}	11.37±0.37 ^{pqr}	11.40±0.68 ^{pq}	20.33±1.01 ^{de}	23.44±2.83 ^{efgh}	1.19±0.06 ⁿ	0.38±.04 ^j	15.00±0.20 ^t
WV	10.22±1.38 ^{bc}	10.84±0.49 ^r	9.87±0.46 ^r	29.44±1.93 ^a	35.22±2.77 ^a	1.49±0.09 ^{ikl}	0.38±0.04 ^j	17.70±0.20 ^{rs}

Table 2. Variability in vegetative quantitative characters of thirty *Nymphaea* varieties (Continued)

Variety	Number of days from visual appearance to full expansion of leaf lamina (days)	Length of lamina (cm)	Width of lamina (cm)	Number of leaves per plant	Longevity of leaves(days)	Petiole circumference (cm)	Petiole diameter (cm)	Petiole length (cm)
KOS	9.11±2.36 ^{bcd}	20.16±0.37 ^b	19.18±0.37 ^b	16.52±1.18 ^{fghi}	32.44±1.07 ^{ab}	2.14±0.05 ^c	0.92±0.07 ^a	25.03±0.25 ⁱ
BW	8.33±1.45 ^{bede}	19.19±0.09 ^{cd}	16.57±0.59 ^{efg}	24.38±4.10 ^{bc}	28.33±4.63 ^{bcdefg}	1.85±0.02 ^e	0.54±0.11 ^{defgh}	28.56±0.20 ^f
PJ	9.11±2.36 ^{bcd}	19.65±0.40 ^{bc}	20.59±0.92 ^a	15.66±0.87 ^{hij}	25.22±0.83 ^{defgh}	2.10±0.03 ^c	0.63±0.08 ^{bcde}	25.70±0.36 ^h
DB	10.66±1.76 ^b	17.64±0.39 ^e	15.93±0.44 ^g	25.47±3.33 ^{bc}	25.44±6.86 ^{defgh}	1.68±0.002 ^{gh}	0.54±0.11 ^{defgh}	24.80±0.20 ^{ij}
PS	8.66±1.85 ^{bede}	17.28±0.18 ^{ef}	13.15±0.07 ^{jkl}	11.83±1.45 ^{klm}	28.33±2.90 ^{bcdefg}	1.70±0.04 ^{gh}	0.46±0.03 ^{ghij}	24.53±0.15 ^{ik}
CI	9.88±1.95 ^{bcd}	12.63±0.65 ^{mn}	12.94±0.29 ^{klm}	10.89±2.49 ^{lm}	25.88±3.83 ^{cdefgh}	1.34±0.04 ^m	0.43 ^{hij}	24.50±0.10 ^{ik}
VG	8.33±0.33 ^{bede}	13.16±0.39 ^{lm}	11.42±0.32 ^{pq}	14.83±0.94 ^{ijk}	27.33±7.21 ^{bcdefgh}	1.82±0.07 ^{ef}	0.45±0.01 ^{ghij}	19.60±0.30 ^p
PB	13.22±2.14 ^a	15.15±0.27 ^{ghi}	14.49±0.70 ^b	18.11±2.06 ^{efghi}	26.55±4.00 ^{bcdefgh}	2.10±0.04 ^c	0.52±0.07 ^{efghi}	21.80±0.20 ^m
IM	8.77±1.83 ^{bede}	14.53±0.25 ^{ij}	11.07±0.30 ^q	19.52±2.89 ^{defg}	32.00±4.17 ^{abc}	1.60±0.02 ^{hi}	0.51±0.05 ^{efghi}	26.03±0.35 ^h
PBR	9.55±0.38 ^{bcd}	11.60±0.32 ^{opq}	11.50±0.17 ^{pq}	15.50±3.18 ^{hijk}	25.33±2.40 ^{defgh}	1.55±0.02 ^{ijk}	0.50 ^{fghi}	18.90±0.20 ^q
SK	9.00±1.52 ^{bcd}	15.50±0.34 ^{gh}	16.44±0.10 ^{efg}	19.08±1.01 ^{defgh}	27.88±2.14 ^{bcdefg}	1.47±0.08 ^{kl}	0.36±0.03 ^j	26.06±0.25 ^h
SP	7.77±0.69 ^{de}	21.28±0.26 ^a	19.68±0.43 ^b	11.91±2.20 ^{ijklm}	22.22±1.95 ^{gh}	1.84±0.01 ^e	0.65±0.10 ^{bcd}	38.50±0.30 ^a
JS	9.77±1.07 ^{bcd}	20.06±0.45 ^b	17.65±0.50 ^c	20.50±3.17 ^{de}	21.33±0.33 ^h	2.25±0.02 ^b	0.70 ^b	31.86±0.25 ^c

Table 2. Variability in vegetative quantitative characters of thirty *Nymphaea* varieties (Continued)

Variety	Number of days from visual appearance to full expansion of leaf lamina (days)	Length of lamina (cm)	Width of lamina (cm)	Number of leaves per plant	Longevity of leaves(days)	Petiole circumference (cm)	Petiole diameter (cm)	Petiole length (cm)
TS	9.22±1.07 ^{bcd}	18.81±0.50 ^d	16.92±0.43 ^{de}	16.11±2.41 ^{ghi}	27.11±1.38 ^{bcdefgh}	2.39±0.06 ^a	0.58±0.06 ^{cdef}	20.53±0.15 ^o
MR	9.88±1.64 ^{bcd}	16.68±0.57 ^f	16.66±0.20 ^{ef}	27.80±4.37 ^{ab}	28.33±2.64 ^{bcdefg}	1.72±0.05 ^{fg}	0.58±0.06 ^{cdef}	22.30±0.10 ^l
CD	10.33±0.33 ^{bc}	12.59±0.43 ^{mn}	13.96±0.26 ^{hi}	26.64±0.46 ^{ab}	26.55±1.57 ^{bcdefgh}	1.51±0.04 ^{ijkl}	0.40±0.05 ^{ij}	18.00±0.30 ^r
AB	9.44±1.50 ^{bcd}	13.27±0.09 ^{lm}	11.87±0.28 ^{op}	17.03±2.11 ^{efghi}	27.55±2.00 ^{bcdefgh}	1.42±0.03 ^{lm}	0.42±0.05 ^{hij}	32.13±0.25 ^c
PR	8.77±2.21 ^{bcd}	11.05±0.04 ^{qr}	10.04±0.08 ^r	24.83±2.59 ^{bc}	29.77±3.59 ^{abcd}	1.58±0.08 ^{ij}	0.36±0.05 ^j	20.40±0.30 ^o
SA	9.55±1.38 ^{bcd}	12.22±0.13 ^{no}	12.20±0.25 ^{no}	21.91±4.20 ^{cd}	25.88±1.71 ^{cdefgh}	1.77±0.12 ^{efg}	0.50 ^{fghi}	33.86±0.35 ^b
SE(m)	0.863	0.243	0.238	1.333	2.218	0.036	0.041	0.14
Mean	9.178	15.41	14.37	18.43	26.8	1.79	0.538	24.8
SE(d)	1.221	0.344	0.337	1.885	3.137	0.051	0.058	0.198
CD (5%)	2.442	0.687	0.674	3.77	6.275	0.101	0.115	0.396
CV	16.292	2.73	2.87	12.524	14.336	3.462	13.122	0.977

4.1.2. Vegetative qualitative characters

Data related to the qualitative characters *viz.*, the shape of the leaf, shape of leaf margin, overlapping of leaf base, the colour of the upper and lower surface of the leaf, striations on the leaf, leaf venation on the lower surface, leaf hairs on the lower surface, petiole colour and hairs on petiole are represented in Table 3.

4.1.2.1. Shape of leaf

Two distinct leaf shapes were observed among the varieties. Micrantha, Chompu, Taweekan, Bull's Eye, Carla's Sonshine, King of Siam, Blue Whistle, Purple Joy, Dauben, Poosub, Peach Blow, Islamorada, Siam Pink, Jalueang Sub, Tropic Sunset, Miami Rose, Paranne and Siam Amber exhibited ovate leaves whereas Hilary, Doris Holt, Siam Jasmine, Puttaraksa, Wanvisa, Mexicana, Clyde Ikins, Virginalis, Supranee Pink, Colorado, Almost Black and Perry's Baby Red exhibited round leaves (Plate 4).

4.1.2.2. Shape of leaf margin

The varieties were grouped into two based on the type of leaf margin. The leaf margins of Micrantha, Chompu, Taweekan, Bull's Eye, Hilary, Doris Holt, Carla's Sonshine, King of Siam, Blue Whistle, Purple Joy, Dauben, Poosub, Peach Blow, Jalueang Sub, Tropic Sunset, and Miami Rose were toothed/serrated. The varieties like Siam Jasmine, Puttaraksa, Wanvisa, Mexicana, Clyde Ikins, Virginalis, Supranee Pink, Islamorada, Siam Pink, Colorado, Almost Black, Siam Amber, and Perry's Baby Red had smooth or entire leaf margins (Plate 5).

4.1.2.3. Overlapping of leaf base

Overlapping of leaf base was found in the varieties such as Taweekan, Bull's Eye, Doris Holt, Carla's Sonshine, King of Siam, Blue Whistle, Poosub, Siam Pink, Supranee Pink, Jalueang Sub, Tropic Sunset, Miami Rose, and Almost Black while it was absent in Micrantha, Chompu, Hilary, Siam Jasmine, Puttaraksa, Wanvisa, Mexicana, Purple Joy, Dauben, Clyde Ikins, Virginalis, Peach Blow, Islamorada, Colorado, Paranne, Siam Amber and Perry's Baby Red (Plate 6).



Round



Ovate

Plate 4. Leaf shape of *Nymphaea* varieties



Smooth



Toothed

Plate 5. Leaf margin of *Nymphaea* varieties



Sinus overlapping



No sinus overlapping

Plate 6. Overlapping of leaf base of *Nymphaea* varieties

Table 3. Vegetative qualitative characters of *Nymphaea* varieties.

Variety	Shape of leaf	Shape of leaf margin	Striations on the leaf	Hair on petiole	Overlapping of leaf base	Leaf venation on lower surfaces
MC	Ovate	Toothed	Absent	Absent	Absent	Prominent
CP	Ovate	Toothed	Absent	Absent	Absent	Prominent
TW	Ovate	Toothed	Absent	Absent	Present	Prominent
BE	Ovate	Toothed	Absent	Absent	Present	Prominent
HY	Round	Toothed	Present	Absent	Absent	Non-prominent
DH	Round	Toothed	Present	Absent	Present	Prominent
CS	Ovate	Toothed	Present	Absent	Present	Non-prominent
SJ	Round	smooth	Absent	Present	Absent	Non-prominent
PK	Round	smooth	Absent	Absent	Absent	Non-prominent
MX	Round	smooth	Present	Absent	Absent	Non-prominent
WV	Round	smooth	Absent	Absent	Absent	Non-prominent
KOS	Ovate	Toothed	Absent	Absent	Present	Prominent
BW	Ovate	Toothed	Absent	Absent	Present	Prominent

Table 3. Vegetative qualitative characters of *Nymphaea* varieties (Continued)

Variety	Shape of leaf	Shape of leaf margin	Striations on the leaf	Hair on petiole	Overlapping of leaf base	Leaf venation on lower surfaces
PJ	Ovate	Toothed	Absent	Absent	Absent	Prominent
DB	Ovate	Toothed	Absent	Absent	Absent	Non-prominent
PS	Ovate	Toothed	Absent	Absent	Present	Non-prominent
CI	Round	smooth	Absent	Present	Absent	Prominent
VG	Round	smooth	Absent	Absent	Absent	Non-prominent
PB	Ovate	Toothed	Present	Absent	Absent	Non-prominent
IM	Ovate	smooth	Absent	Absent	Absent	Prominent
SP	Ovate	smooth	Present	Present	Present	Prominent
SK	Round	smooth	Absent	Present	Present	Prominent
JS	Ovate	Toothed	Present	Absent	Present	Non-prominent

Table 3. Vegetative qualitative characters of *Nymphaea* varieties (Continued)

Variety	Shape of leaf	Shape of leaf margin	Striations on the leaf	Hair on petiole	Overlapping of leaf base	Leaf venation on lower surfaces
TS	Ovate	Toothed	Absent	Absent	Present	Non-prominent
MR	Ovate	Toothed	Present	Absent	Present	Non-prominent
CD	Round	smooth	Absent	Absent	Absent	Non-prominent
AB	Round	smooth	Absent	Present	Present	Prominent
PR	Ovate	smooth	Absent	Present	Absent	Non-prominent
SA	Ovate	smooth	Absent	Present	Absent	Non-prominent
PBR	Round	smooth	Absent	Absent	Absent	Non-prominent

4.1.2.4. Striations/ marking on the leaf

Hilary, Doris Holt, Carla's Sonshine, Wanvisa, Peach Blow, Siam Pink, Jalueang Sub, and Miami Rose had irregular moderate purplish red striations on the leaf lamina while in Micrantha, Chompu, Taweekan, Bull's Eye, Puttaraksa, Siam Jasmine, Mexicana, King of Siam, Blue Whistle, Purple Joy, Dauben, Poosub, Clyde Ikins, Virginalis, Islamorada, Supranee Pink, Tropic Sunset, Colorado, Almost Black, Paranne, Siam Amber and Perry's Baby Red striations were absent (Plate 7).

4.1.2.5. Leaf venation on lower surfaces

Micrantha, Chompu, Taweekan, Bull's Eye, Doris Holt, King of Siam, Blue Whistle, Purple Joy, Clyde Ikins, Islamorada, Siam Pink, Supranee Pink and Almost Black exhibited prominent leaf venation while Hilary, Carla's Sonshine, Siam Jasmine, Puttaraksa, Wanvisa, Mexicana, Dauben, Poosub, Virginalis, Peach Blow, Jalueang Sub, Tropic Sunset, Miami Rose, Colorado, Paranne, Siam Amber and Perry's Baby Red had non-prominent leaf venation (Plate 8).

4.1.2.6. Hairs on petiole

Hairs on petiole were not observed on most of the varieties. Siam jasmine, Siam Pink, Siam Amber, Supranee Pink, Almost Black, Clyde Ikins and Paranee exhibited small hairs on their petiole (Plate 9).

4.1.2.7. Leaf hairs on the lower surface

The presence of leaf hairs on the lower surface was not observed in most of the varieties. The varieties Siam Jasmine, Clyde Ikins, Supranee Pink, Paranne and Siam Amber exhibited short hairs under the leaf surface.

4.1.2.8. Colour of the upper surface of the leaf

The colour of the upper leaf surface is given in Table 4.

Nymphaea genotypes under the study exhibited eight colour shades on the upper surface of the leaves. Micrantha, Chompu, Bull's Eye, and Poosub are categorised into brilliant yellowish green (RHS 142B) colour, Strong yellowish green (RHS 143B) colour was exhibited by Taweekan, King of Siam, Blue Whistle, Purple Joy, Clyde Ikins, Virginalis, Siam Jasmine, Siam Amber, Paranee, Almost Black, Colorado, Tropic Sunset, Islamorada, Wanvisa, Mexicana, Peach



Striation present



Striation absent

Plate 7. Striations on the *Nymphaea* leaf



Prominent venation



Non prominent venation

Plate 8. Leaf venation on the lower surface of *Nymphaea* leaf



Presence of hair



Absence of hair

Plate 9. Petiole hairs on *Nymphaea* leaf

□

Blow and Perry's Baby Red. Hilary and Doris Holt exhibited dark purple (RHS 79B) and strong yellowish green (RHS 143B) colour. Dauben showed a deep yellowish green (RHS 141A) shade. Strong yellowish green (RHS 143B) with moderate purple spots (RHS 79D) were exhibited by Carla's Sonshine, Siam Pink and Miami Rose while Supranee Pink displayed moderate yellowish green (RHS 139B) colour. Puttaraksa exhibited strong yellowish green colour (RHS 143A) with dark purple spots (RHS 79A) while Jalueang sub showed dark red (RHS 187A) with greyish yellowish green (RHS 191B) hue.

4.1.2.9. Colour of the lower surface of the leaf

The colour of the upper leaf surface is given in Table 4.

Eight different colour shades were observed among the genotypes in the lower leaf surface. Micrantha, Chompu and Bull's Eye exhibited strong purplish red (RHS 72A) colour. Moderate yellowish green (RHS 137D) colour shade was shown by Taweekan, King of Siam, Dauben and Tropic Sunset. Light yellowish green (RHS 145B) with deep purple (RHS 79B) streaks were exhibited by Hilary, Doris Holt, Blue Whistle and Purple Joy. Poosub, Carla's Sonshine, and Clyde Ikkins exhibited a light yellowish green (RHS 142C) shade while Virginalis, Perry's Baby Red and Wanvisa exhibited strong yellowish green (RHS 144C) shade. Moderate purplish red (RHS 70A) hue was exhibited by Supranee Pink, Siam Jasmine and Jalueang sub. Greyish red (RHS 182B) colour was shown by Siam Pink, Almost Black, Colorado and Miami Rose. greyish red (RHS 182B) and dark red streaks (RHS 187A) were shown by Paranee while Islamorada exhibited deep purple (RHS 79C) hue. Moderate purplish red (RHS 185C) with dark red streaks (RHS 187A) shade was displayed by Mexicana. Siam Amber exhibited shade of greyish-yellowish green (RHS 191B) with dark purple streaks (RHS 79A).

4.1.2.10. Petiole colour

The petiole exhibited ten different colour shades and represented in Table 5. The varieties Micrantha, Chompu, Bull's Eye, Taweekan, Puttaraksa and Wanvisa exhibited moderate reddish orange (RHS 173B) shade.

Table 4. Variation in colour of upper and lower surface in *Nymphaea* leaf

Variety	Colour of the upper surface of the leaf	Colour of the lower surface of leaf
MC	Brilliant yellowish green (RHS 142B- 116)	Strong purplish red (RHS 72A- 255)
CP	Brilliant yellowish green (RHS 142B- 116)	Strong purplish red (RHS 72A- 255)
BE	Brilliant yellowish green (RHS 142B- 116)	Strong purplish red (RHS 72A- 255)
TW	Strong yellowish green (RHS 143B- 117)	Moderate yellowish green (RHS 137D- 136)
HY	Dark purple (RHS 79B- 224) and strong yellowish green (RHS 143B- 117)	Light yellowish green (RHS 145B- 119) with deep purple (RHS 79B- 224) scattered
DH	Dark purple (RHS 79A- 224) and strong yellowish green (RHS 143B- 117)	Light yellowish green (RHS 145B- 119) with dark purple (RHS 79B- 224) scattered
KOS	Strong yellowish green (RHS 143B- 117)	Moderate yellowish green (RHS 137D- 136)
BW	Strong yellowish green (RHS 141C- 131)	Light yellowish green (RHS 145B- 119) with strong purplish red scattered (RHS 71B- 255)
PJ	Strong yellowish green (RHS 143B- 117)	Light yellowish green (RHS 145B- 119) with strong purplish red scattered (RHS 71B- 255)
DB	Deep yellowish green (RHS 141A- 132)	Moderate yellowish green (RHS 137D- 136)
PS	Brilliant yellowish green (RHS 142B- 116)	Light yellowish green (RHS 142C- 119)
CS	Strong yellowish green (RHS 143B- 117) with moderate purple spots (RHS 79D- 223)	Moderate yellowish green (RHS 146C- 120) with strong purplish red (RHS 72A- 255)

Table 4. Variation in colour of upper and lower surface in *Nymphaea* leaf (Continued)

Variety	Colour of the upper surface of leaf	Colour of the lower surface of leaf
VG	Strong yellowish green (RHS 143B- 117)	Strong yellowish green (RHS 144C- 117)
SK	Moderate yellowish green (RHS 139B- 136)	Moderate purplish red (RHS 70A- 258)
SP	Strong yellowish green (RHS 141D- 117) with moderate purple spots (RHS 79D- 223)	Greyish red (RHS 182B- 19)
SJ	Strong yellowish green (RHS 143B- 117)	Moderate purplish red (RHS 185C- 258)
SA	Strong yellowish green (RHS 143A- 117)	Greyish yellowish green (RHS 191B- 122) with dark purple spots (RHS 79A- 224)
PK	Strong yellowish green (RHS 143A- 117) with dark purple spots (RHS 79A- 224)	Moderate purplish red (RHS 186A-258) with dark purple spots (RHS 79A- 224)
PR	Strong yellowish green (RHS 143A- 117)	Greyish red (RHS 182B- 19) and dark red spots (RHS 187A- 16)
AB	Strong yellowish green (RHS 143A- 117)	Greyish red (RHS 182B- 19)
CD	Strong yellowish green (RHS 143A- 117)	Greyish red (RHS 182B- 19)
TS	Strong yellowish green (RHS 143A- 117)	Moderate yellowish green (RHS 139D- 120)
MR	Strong yellowish green (RHS 141D- 117) with moderate purple spots (RHS 79D- 223)	Greyish red (RHS 182B- 19)
JS	Dark red (RHS 187A- 16) with greyish yellowish green (RHS 191B- 122)	Moderate purplish red (RHS 70A- 258)
IM	Strong yellowish green (RHS 144C- 117)	Deep purple (RHS 79C- 219)

Table 4. Variation in colour of upper and lower surface in *Nymphaea* leaf (Continued)

Variety	Colour of the upper surface of leaf	Colour of the lower surface of leaf
PBR	Strong yellowish green (RHS 141C- 131)	Strong yellowish green (RHS 141D- 117)
WV	Strong yellowish green (RHS 143B- 117)	Strong yellowish green (RHS 144C- 117)
MX	Strong yellowish green (RHS 143B- 117)	Moderate purplish red (RHS 185C 258) with dark red (RHS 187A- 16)
PB	Strong yellowish green (RHS 144C- 117)	Strong yellowish green (RHS 144C- 117) with Deep reddish-purple spots (RHS 77A- 238)

Brilliant greenish yellow (RHS 151D) colour was displayed by Hilary, Purple Joy, Virginalis and Perry's Baby Red. Doris Holt, Siam Amber, Paranee, and Almost Black exhibited a dark reddish orange (RHS 173A) hue. King of Siam and Clyde Ikins had strong yellowish green (RHS 143A) petiole colour. Poonsub exhibited a strong yellowish green (RHS 144A) while Peach Blow exhibited a strong yellowish green (RHS 143C) hue. Blue Whistle, Dauben, Carla's Sonshine, Tropic Sunset, Miami Rose and Jalueang Sub displayed moderate yellowish green (RHS 147B) shade. Brilliant yellowish green (RHS 150B) colour was observed in Supranee Pink, Siam Pink and Colorado while moderate olive brown (RHS 199A) colour was displayed by Siam Jasmine and Islamorada. A moderate reddish orange (RHS 173B) shade was exhibited by Mexicana.

4.1.3. Floral quantitative characters

Floral quantitative characters were recorded from the varieties based on the availability of flowers.

The average performance of all the genotypes for the floral quantitative characters *viz.* days to appearance of flower bud, length of flower bud, days to flower opening, flower diameter and pedicel diameter are given in Table 6a and the characters *viz.* number of sepals, length of sepal, width of sepal, number of petals, length of petal, width of petal, number of stamen and length of stamen are presented in Table 6b.

4.1.3.1. Days to appearance of flower bud

Among the twenty one varieties used in the floral characterization, the maximum number of days taken to appearance of flower bud was recorded by Carla's Sonshine (10.55 days). The minimum number of days for the appearance of flower buds was taken by Blue Whistle (7.22 days). There was no significant difference between the varieties for this character (Plate 10).

4.1.3.2. Length of flower bud

The highest value for the flower bud length was recorded by Hilary (5.04 cm) which was on par with Blue Whistle (5.03 cm). Miami Rose exhibited a

lower bud length (3.95 cm) which was on par with Jalueang Sub (3.98 cm) and Tropic Sunset (4.04 cm).

4.1.3.3. Days to flower opening

Poonsub (2.77 days) recorded maximum days for flower opening while Bull's Eye, Carla's Sonshine and Blue Whistle exhibit minimum days for flower opening (1.66 days). No significant difference was observed between the varieties for this character.

4.1.3.4. Flower diameter

The variety King of Siam (11.07 cm) recorded the highest flower diameter and Miami Rose (7.37 cm) recorded the lowest flower diameter.

4.1.3.5. Pedicel circumference (at the joint of the receptacle)

Taweekan had the highest (2.46 cm) pedicel circumference which was on par with Chompu (2.42 cm) and Bull's Eye (2.41cm). Perry's Baby Red was found to have the lowest (1.57 cm) pedicel diameter.

4.1.3.6. Pedicel diameter (cm)

Taweekan had the highest (0.97 cm) pedicel diameter which was on par with King of Siam (0.96 cm). Perry's Baby red was found to have the lowest (0.54 cm) pedicel diameter.

4.1.3.7. Pedicel length (cm)

Taweekan had the highest (31 cm) pedicel length and Carla's Sonshine had the lowest (15.7 cm) pedicel diameter.

4.1.3.8. Number of sepals

All the varieties studied exhibited four sepals except Taweekan and King of Siam which exhibited six sepals.

4.1.3.9. Length of sepal (cm)

Significantly higher sepal length was observed for the variety Peach Blow (4.97 cm) which was on par with King of Siam (4.16 cm). The minimum value was recorded for Tropic Sunset (3.56 cm) which was on par with Miami Rose (3.63cm).

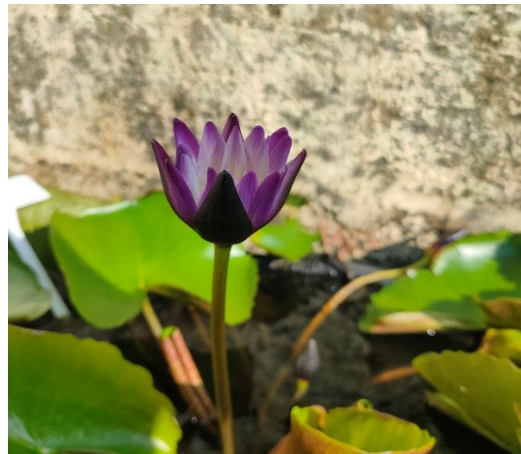
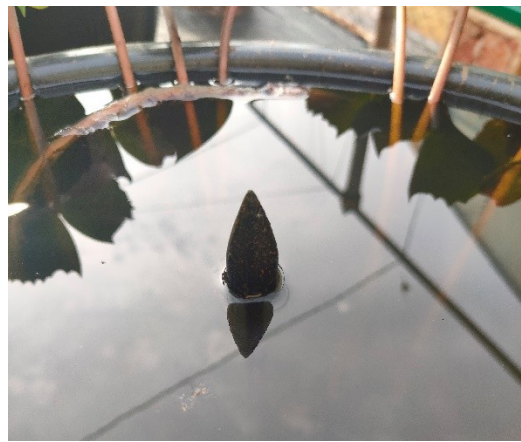


Plate 10. Different stages of bud emergence.

Table 5. Variation in petiole colour in *Nymphaea* varieties.

Variety	Petiole colour
MC	Moderate reddish orange (RHS 173B 37)
CP	Moderate reddish orange (RHS 171A- 37)
BE	Moderate reddish orange (RHS 171A- 37)
TW	Moderate reddish orange (RHS 171A- 37)
HY	Brilliant greenish yellow (RHS 151D- 98)
DH	Dark reddish orange (RHS 173A- 38)
KOS	Strong yellowish green (RHS 143A- 117)
BW	Moderate yellowish green (RHS 147B- 120)
PJ	Brilliant greenish yellow (RHS 151D- 98)
DB	Moderate yellowish green (RHS 147B- 120)
PS	Strongish yellowish green (RHS 144A- 117)
CS	Moderate yellowish green (RHS 147B- 120)
CI	Strong yellowish green (RHS 143C- 117) with hairs
VG	Brilliant greenish yellow (RHS 151D- 98)
SK	Brilliant yellowish green (RHS 150B- 116) with hairs
SP	Brilliant yellowish green (RHS 150B- 116) with hairs
SJ	Moderate olive brown (RHS 199A- 95) with hairs
SA	Dark reddish orange (RHS 173A- 38)
PK	Moderate reddish orange (RHS 173B 37)
PR	Dark reddish orange (RHS 173A- 38) with hairs
AB	Dark reddish orange (RHS 173A- 38)

Table 5. Variation in petiole colour in *Nymphaea* varieties (Continued)

Variety	Petiole colour
CD	Brilliant yellowish green (RHS 150B- 116) with hairs
TS	Moderate yellowish green (RHS 147B- 120)
MR	Moderate yellowish green (RHS 147B- 120)
JS	Moderate yellowish green (RHS 147B- 120)
IM	Moderate olive brown (RHS 199A- 95)
PBR	Brilliant greenish yellow (RHS 151D- 98)
WV	Moderate reddish orange (RHS 171A- 37)
MX	Moderate reddish orange (RHS 173B 37) with hairs
PB	Strong yellowish green (RHS 143C- 117)

*No flowering in Puttaraksa and Almost Black during the observation period

Table 6a. Variation in floral quantitative characters.

Variety	Days to appearance of flower bud	Days to flower opening	Length of flower bud (cm)	Flower diameter (cm)	Pedicel circumference (cm)	Pedicel diameter (cm)	Pedicel length (cm)
MC	8.890±1.68	2.111±0.50	4.47±0.08 ^{fgh}	7.86±0.21 ^{hi}	1.99±0.01 ^f	0.72±0.07 ^{cdef}	24.0±0.10 ^e
CP	8.443±1.70	2.000±0.66	4.51±0.04 ^{fg}	8.68±0.08 ^e	2.42±0.04 ^a	0.62±0.10 ^{efg}	18.2±0.30 ^j
TW	9.777±1.26	2.556±0.19	4.25 ⁱ	9.85±0.20 ^c	2.46±0.15 ^a	0.97±0.15 ^a	31.0±0.20 ^a
BE	8.890±1.57	1.667±0.33	4.56±0.23 ^{ef}	9.88±0.10 ^c	2.41±0.15 ^a	0.80±0.10 ^{bcd}	22.2±0.20 ^g
HY	8.780±1.16	2.222±0.38	5.04±0.11 ^a	9.26±0.12 ^d	2.33±0.07 ^{ab}	0.76±0.10 ^{cde}	26.2±0.55 ^b
DH	9.780±1.16	2.000±0.33	4.23±0.03 ⁱ	8.59±0.25 ^{ef}	1.94 ^{fg}	0.70±0.10 ^{defg}	16.9±0.40 ^k
CS	10.55±1.95	1.667±0.33	4.74±0.07 ^{cd}	7.98±0.24 ^{gh}	1.84±0.05 ^g	0.72±0.058 ^{cdef}	15.7±0.20 ^l
SJ	10.33±3.78	2.333±0.33	4.34±0.05 ^{hi}	7.97±0.16 ^{gh}	1.95±0.11 ^{fg}	0.79±0.115 ^{bcd}	19.3±0.30 ⁱ
KOS	9.333±1.85	2.333	4.76±0.07 ^{cd}	11.07±0.25 ^a	2.18±0.13 ^{cd}	0.96±0.123 ^{ab}	23.1±0.25 ^f
BW	7.223±0.95	1.667±0.33	5.03±0.15 ^a	10.02±0.17 ^{bc}	2.04±0.13 ^{def}	0.76 ^{cde}	23.9±0.15 ^e
PJ	8.667±1.20	2.444±0.83	4.95±0.10 ^{ab}	9.92±0.26 ^c	2.22±0.10 ^{bc}	0.87±0.14 ^{abc}	24.6±0.40 ^d
DB	8.333±0.33	1.889±0.38	4.77±0.02 ^{cd}	7.96±0.31 ^{gh}	2.02±0.04 ^{ef}	0.57±0.075 ^{fg}	23.3±0.45 ^f

Table 6a. Variation in floral quantitative characters (Continued)

Variety	Days to appearance of flower bud	Days to flower opening	Length of flower bud (cm)	Flower diameter (cm)	Pedicle circumference (cm)	Pedicle diameter (cm)	Pedicle length (cm)
PS	9.113±1.38	2.778±0.83	4.36±0.04 ^{ghi}	9.68±0.24 ^c	2.07±0.08 ^{def}	0.60±0.10 ^{efg}	23.5±0.15 ^{ef}
CI	10.44±2.45	2.556±0.19	4.53±0.11 ^{ef}	10.38±0.07 ^b	1.94±0.03 ^{fg}	0.68±0.068 ^{defg}	21.3±0.15 ^h
VG	9.447±2.03	2.222±0.69	4.70±0.12 ^{cde}	8.78±0.39 ^c	1.83±0.08 ^g	0.84±0.085 ^{abcd}	22.4±0.17 ^g
PB	9.00±0.33	2.111±0.38	4.81±0.05 ^{bc}	9.91±0.46 ^c	2.17±0.11 ^{cde}	0.62±0.131 ^{efg}	22.4±0.40 ^g
IM	8.330±1.73	2.667±0.33	4.62±0.12 ^{def}	9.21±0.04 ^d	1.83±0.05 ^g	0.62±0.131 ^{efg}	23.6±0.30 ^{ef}
PBR	8.667±2.51	2.111±0.38	4.22±0.05 ⁱ	8.26±0.23 ^{fg}	1.57±0.06 ^h	0.54±0.106 ^g	17.9±0.20 ^j
JS	9.333±1.33	2.111±0.50	3.987±0.12 ^j	7.48±0.06 ^{ij}	2.07±0.03 ^{cdef}	0.85±0.085 ^{abcd}	26.6±0.20 ^b
TS	9.113±3.09	2.000±0.66	4.047±0.10 ^j	7.57±0.09 ^{ij}	2.02±0.04 ^{ef}	0.71±0.115 ^{cdefg}	22.6±0.20 ^g
MR	8.777±0.50	1.889±0.19	3.957±0.09 ^j	7.37±0.29 ^j	1.95±0.08 ^{fg}	0.81±0.115 ^{abcd}	25.2±0.30 ^c
SE(m)	1.053	0.272	0.059	0.134	0.051	0.06	0.167
Mean	NS	NS	4.52	8.94	2.063	0.74	22.58
SE(d)	1.489	0.385	0.083	0.19	0.073	0.085	0.237
CD (5%)	NS	NS	0.168	0.383	0.147	0.172	0.477
CV	NS	NS	2.257	2.6	4.317	14.131	1.283

NS: Not significant

4.1.3.10. Width of sepal (cm)

The variety Clyde Ikins (2.42 cm) recorded the highest sepal width while Islamorada (1.57 cm) which was on par with Tropic Sunset (1.62 cm) had the lowest sepal width.

4.1.3.11. Number of petals

Significantly maximum petal numbers were recorded by King of Siam (96.1 No.) while Hilary exhibited the minimum number of petals (15.8 No.) which was on par with Micrantha (15.5 No.).

4.1.3.12. Length of petal (cm)

Clyde Ikins (4.83 cm) produced significantly longer petals which was on par with Blue Whistle (4.76 cm). Jalueang Sub (3.27 cm) recorded a shorter petal length which was on par with Tropic Sunset (3.32 cm).

4.1.3.13. Width of petal (cm)

Siam Jasmine (2.33cm) produced significantly longer petal width. Jalueang Sub (0.87 cm) recorded a shorter petal width which was on par with Miami Rose (0.91 cm).

4.1.3.14. Number of stamens

The number of stamens was highest in Doris Holt (138.5 No.) and lowest in Perry's Baby Red (50.3 No.).

4.1.3.15. Length of stamen (cm)

Hilary possessed the highest (2.32 cm) stamen length while Carla's Sonshine had the lowest (1.81cm) stamen length which was on par with Miami Rose (1.85 cm).

4.1.3.16. Field life of flower

All the varieties exhibited 3 to 4 days of field life. Sometimes 2 to 3 days of field life was also observed from the varieties due to the changes in the environment. The stages of flower opening is given in Plate 11.

4.1.3.17. Number of flowers per plant per month

The data on number of flowers per plant per month is given in Table 7 and Fig.1.



First day



Second day



Third day



Fourth day

Plate 11. Stages of flower opening

Only some of the varieties produced flowers regularly and hence the observations could not be statistically analysed. Maximum number of flowers per plant was observed for the varieties Dauben (9.30 No.), Blue Whistle (8.30 No.), Micrantha (8.00 No.), Tropic Sunset (7.30 No.), King of Siam (7.00 No.) and Doris Holt (6.00 No.) and the varieties Wanvisa (2.33 No.), Mexicana (1.33 No.), Siam Amber (1.33 No.), Paranee (1 No.), Siam Pink (0.66 No.), Colorado (0.66 No.), Supranee Pink (0.66 No.) and Clyde Ikins (0.66 No.) exhibited lesser number of flowers.

4.1.3.19. Flower colour (Inner and outer petal colour)

The data representing the inner and outer petal colour was represented in Table 8.

The inner and outer petal colours exhibited shades of very pale purplish blue, strong purplish red, deep purplish pink, vivid purplish red, pale purplish pink, strong purple, brilliant violet petal with light violet centre, vivid purple petal with white centre, bluish white petal, light yellowish green petal with very pale blue towards the tip, light yellowish green, pale yellow, white, deep pink, moderate purplish pink, pale orangish yellow, light pink petals with light yellow centre, vivid purplish red, strong purplish red, very pale blue with white specks, vivid purplish red, light greenish yellow and pale purplish pink. Varieties Siam Jasmine and Virginalis exhibited white colour blooms. Wanvisa, Mexicana and Paranee displayed light yellowish green petals.

4.1.3.20. Stamen colour

All the twenty-eight varieties had varied stamen colours (Table 9).

Clyde Ikins, Virginalis, Siam Jasmine, Siam Amber, Colorado, Mexicana had brilliant yellow coloured stamen (RHS 9C-83). All the other varieties exhibited stamens with two shades with brilliant yellow as the prominent colour and the stamen tip having appeared as the same colour as the petal.

Figure 1. Number of flowers per plant per month

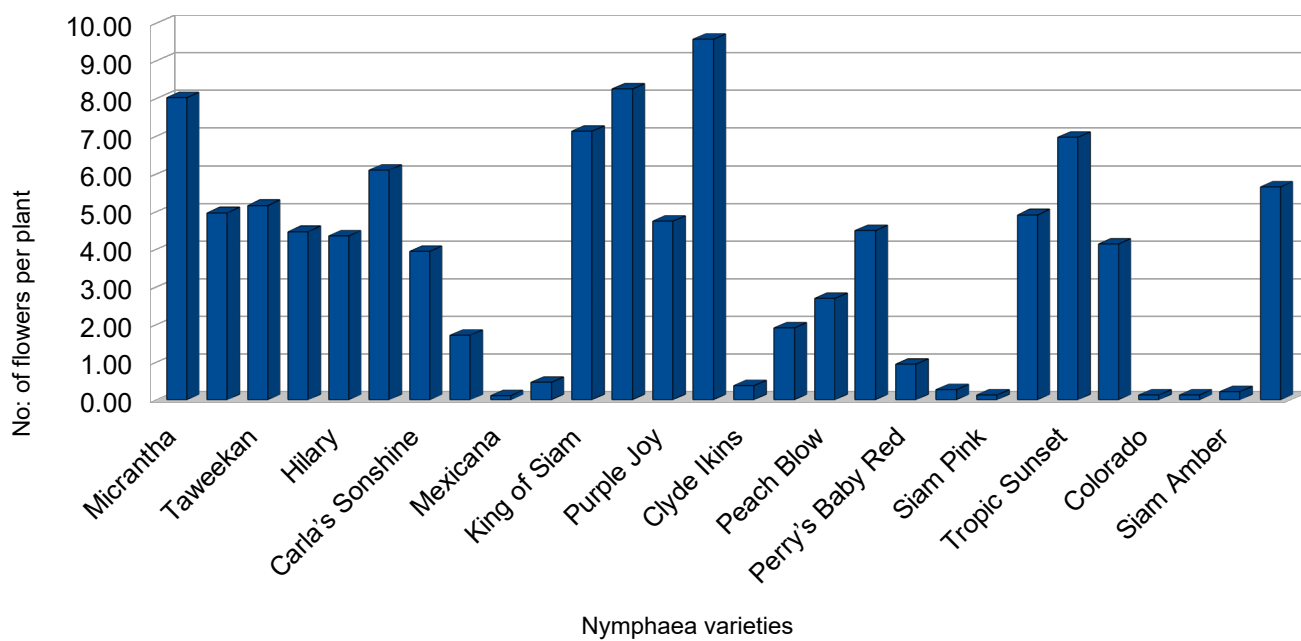


Table 6b. Variation in floral quantitative characters

Variety	Number of sepals	Length of sepal (cm)	Width of sepal (cm)	Number of petals	Length of petal (cm)	Width of petal (cm)	Number of Stamens	Length of Stamens (cm)
MC	4	4.27±0.15 ^{fg}	1.93±0.03 ^{hi}	15.58±0.38 ^{kl}	4.07±0.07 ^{ghij}	1.41±0.04 ^e	66.58±0.68 ^h	1.87±0.09 ^{hi}
CP	4	4.33±0.23 ^f	2.14±0.12 ^{cde}	19.89±0.12 ⁱ	3.92±0.05 ^{hijk}	1.25±0.02 ^f	98.41±2.02 ^e	2.02±0.04 ^{efg}
TW	6	4.24±0.07 ^{fg}	2.13±0.09 ^{de}	92.25±5.46 ^b	3.88±0.03 ^{jk}	1.04±0.10 ^h	*	*
BE	4	4.40±0.09 ^{def}	2.07±0.09 ^{defg}	41.33±0.65 ^c	3.93±0.09 ^{hijk}	1.24±0.03 ^{fg}	121.55±4.65 ^b	2.09±0.04 ^{def}
HY	4	4.61±0.11 ^{bcd}	1.92±0.02 ^{hij}	15.86±0.62 ^{kl}	4.43±0.08 ^{cd}	1.23±0.03 ^{fg}	108.53±1.64 ^{cd}	2.32±0.05 ^a
DH	4	4.00±0.10 ^{ghi}	1.88±0.07 ^{ijk}	23.33±0.96 ^h	3.90±0.08 ^{ijk}	1.16±0.05 ^g	138.50±1.00 ^a	2.15±0.02 ^{cd}
CS	4	4.06±0.16 ^{ij}	2.01±0.01 ^{fgh}	19.25±0.65 ^{ij}	3.89±0.09 ^{jk}	1.55±0.07 ^d	105.77±3.99 ^d	1.81±0.12 ⁱ
SJ	4	3.86±0.13 ^{bcd}	2.31±0.06 ^{ab}	23.94±0.55 ^h	3.82±0.16 ^k	2.33±0.08 ^a	50.86±0.77 ^{jk}	1.87±0.06 ^{hi}
KOS	6	4.61±0.03 ^a	2.18±0.04 ^{cd}	96.16±5.30 ^a	4.53±0.10 ^{bc}	1.39±0.05 ^e	*	*
BW	4	4.91±0.21 ^{bc}	2.05±0.03 ^{efg}	23.44±0.25 ^h	4.76±0.15 ^a	1.24±0.02 ^f	65.05±0.67 ^h	1.97±0.05 ^{gh}
PJ	4	4.65±0.09 ^{bc}	2.26±0.07 ^{bc}	19.66±0.38 ⁱ	4.10±0.19 ^{fghi}	1.44±0.02 ^c	85.83±0.16 ^{fg}	2.00±0.02 ^{fg}
DB	4	4.64±0.20 ^{cde}	1.81±0.07 ^{jk}	13.30±0.37 ^l	4.32±0.13 ^{de}	1.06±0.03 ^h	69.41±4.36 ^h	2.05±0.10 ^{defg}

* No stamens were observed in Taweekan and King of Siam

Table 6b. Variation in floral quantitative (continued)

Variety	Number of sepals	Length of sepal (cm)	Width of sepal (cm)	Number of petals	Length of petal (cm)	Width of petal (cm)	Number of Stamens	Length of Stamens (cm)
PS	4	4.57±0.23 ^{cde}	1.98±0.06 ^{ghi}	27.64±0.86 ^{fg}	4.29±0.14 ^{def}	1.58±0.08 ^d	94.75±2.23 ^e	2.13±0.08 ^{cde}
CI	4	4.82±0.11 ^{ab}	2.42±0.06 ^a	29.75±1.09 ^{ef}	4.83±0.06 ^a	2.17±0.02 ^b	55.14±1.39 ^{ij}	2.20±0.07 ^{bc}
VG	4	4.08±0.09 ^{gh}	2.11±0.01 ^{def}	26.28±1.00 ^{gh}	4.20±0.20 ^{efg}	1.90±0.02 ^c	57.33±1.12 ⁱ	2.06 ^{defg}
PB	4	4.97±0.07 ^a	2.01±0.09 ^{fgh}	17.13±0.17 ^{ijk}	4.70±0.09 ^{ab}	1.29±0.04 ^f	106.55±3.55 ^d	2.30±0.03 ^{ab}
IM	4	4.37±0.08 ^{ef}	1.57±0.09 ^l	16.50±0.68 ^{jk}	4.12±0.12 ^{fgh}	1.05±0.03 ^b	82.69±0.67 ^g	2.31±0.11 ^{ab}
PBR	4	3.98±0.12 ^{hi}	1.81±0.10 ^{ik}	23.69±0.41 ^h	3.80±0.03 ^k	1.59±0.03 ^d	50.36±1.89 ^k	2.08±0.02 ^{def}
JS	4	3.65±0.04 ^{jk}	1.76±0.06 ^k	38.05±1.18 ^d	3.27±0.06 ^m	0.87±0.04 ⁱ	112.02±5.83 ^c	1.89±0.04 ^{hi}
TS	4	3.56±0.10 ^k	1.62±0.07 ^l	19.52±0.61 ⁱ	3.32±0.14 ^m	1.28±0.05 ^f	86.97±3.34 ^{fg}	1.87±0.05 ^{hi}
MR	4	3.63±0.09 ^k	1.77±0.04 ^k	32.27±1.33 ^e	3.58±0.19 ^l	0.91±0.01 ⁱ	90.00±1.16 ^f	1.85±0.03 ⁱ
SE(m)	-	0.079	0.041	1.04	0.071	0.026	1.553	0.039
Mean	-	4.298	1.991	30.23	4.082	1.384	86.65	2.047
SE(d)	-	0.111	0.058	1.471	0.1	0.037	2.196	0.055
CD (5%)	-	0.225	0.118	2.968	0.202	0.075	4.446	0.11
CV	-	3.177	3.591	5.958	3.004	3.277	3.104	3.265

* No stamens were observed in Taweekan and King of Siam

4.1.3.21. Anthesis time

Anthesis time varied in all the varieties and is represented in Table 10.

The blooming time of the flowers varied from 7:00 am to 10: 00 am and the flowers closed from 1:00 pm to 6:15 pm. Varieties like Micrantha, King of Siam, Blue Whistle, Dauben, Poosub, Virginalis and Siam Jasmine showed early flower opening between 7:00 am to 7:30 am.

4.1.3.22. Sepal shape

Data on sepal shape of twenty-eight *Nymphaea* varieties are presented in Table 11.

The varieties Chompu, Taweekan, Bull's Eye, Doris Holt, Carla's Sonshine, Blue Whistle, Dauben, Clyde Ikins, Peach Blow, Islamorada, Supranee Pink, Jalueang Sub and Miami Rose exhibited linear-lanceolate shape while Micrantha, Hilary, Siam Jasmine, Wanvisa, Mexicana, King of Siam, Purple Joy, Poosub, Virginalis, Siam Pink, Tropic Sunset, Colorado, Paranee, Siam Amber and Perry's Baby Red had ob-lanceolate shape. The shape of sepal in *Nymphaea* varieties is given in Plate 12.

4.1.3.23. Petal apex

Data on petal apex of twenty-eight *Nymphaea* varieties are presented in Table 11.

Chompu, Bull's Eye, Doris Holt, Mexicana, Blue Whistle, Dauben, Poosub, Islamorada, Jalueang Sub, Tropic Sunset, Miami Rose and Siam Amber exhibited acute petal apex while Micrantha, Taweekan, Hillary, Carla's Sonshine, Siam Jasmine, Wanvisa, King of Siam, Purple Joy, Clyde Ikins, Virginalis, Peach Blow, Siam Pink, Supranee Pink, Colorado, Paranee and Perry's Baby Red had obtuse petal apex. The shape of petal apex in *Nymphaea* varieties is given in Plate 13.

Table7. Number of flowers per plant per month

Month	November	December	January	February	March	April	May	June	July	August	September	October
Variety												
MC	8	9	9.33	8.66	9.66	7.66	8	8.33	5	6.33	8	8.33
CP	5.33	3.66	5.33	7	6.33	5.66	4.33	4.66	4	5	3.66	4.66
TW	5	6.33	5	6.66	6.33	6	4.33	5	4.33	3	5.66	4.33
BE	2.66	7	4	7.33	6	5.66	3	3	3.66	3.66	4	3.66
HY	3.33	4.33	1.66	6.33	5	3.33	3.33	6	3.33	4.66	6.33	4.66
DH	5.33	5.66	5.33	6.66	8	5.33	5.66	7.66	4.33	4	8.33	7
CS	3.66	4.33	3.66	3	4.66	3.33	3.66	4.33	2.66	2.33	6.33	5.66
SJ	2	3	2	2.66	2.33	2.66	*	2.66	*	0.33	2.33	0.66
MX	*	*	*	*	*	*	*	*	*	*	1.33	*
WV	*	*	*	1.66	*	*	*	*	*	*	1.66	2.33
KOS	6.33	7.33	7.66	8	7.33	7.33	5.33	6.66	5	6.66	8.33	9.66
BW	7.33	8.33	9	9.33	8.33	7.66	7.33	7	6.33	6.66	12.67	9
PJ	4	5	2.66	6	5	5.66	5	5.33	3	4	5	6.33
DB	8.33	8.66	8.66	7	12	13	7.66	11.33	6	7.66	12.33	12.33
PS	3.33	4.66	5	3.33	5.66	4.33	5.66	3.66	1.33	2.66	3.66	4.33

* No flowering were observed

Table7. Number of flowers per plant per month (continued)

Month	November	December	January	February	March	April	May	June	July	August	September	October
Variety												
CI	*	*	0.66	*	*	*	0.33	0.33	0.33	0.33	2	0.66
VG	2.66	2.66	2	1.66	1.66	3.33	2.66	2.66	1.66	*	2	*
PB	5	4	3	3.33	6	2.66	4	4.33	*	*	*	*
IM	4.66	3	3	4.66	5.33	2.66	4.33	7.33	4	3	6.33	5.66
PBR	*	*	*	1	*	1	3	2	1.66	0.66	1.66	0.33
SK	*	*	0.33	*	*	*	0.66	0.66	0.66	*	1	*
SP	*	*	0.66	0.66	*	*	0.33	*	*	*	*	*
JS	3.33	5	5	7	7.66	5	5	3.33	3.33	2.66	6.66	5
TS	8.33	7.33	9.33	8	6.66	5	5	9.33	2.33	4	12.33	6
MR	5.33	3.33	3	5	4	4.66	3.66	7	1.33	2.66	4.33	5.33
CD	*	*	1	*	*	*	*	*	*	*	0.66	*
PR	*	*	0.33	*	0.33	*	*	*	*	*	1	*
SA	*	*	*	*	0.33	*	*	*	*	*	1	1.33

* No flowering were observed

Table 8. Variation in inner and outer petal colour of *Nymphaea* varieties

Variety	Flower colour (Inner petal colour)	Flower colour (Outer petal colour)
MC	Very pale purplish blue (RHS 97C)	Very pale purplish blue (RHS 97C)
CP	Strong Purplish Red (RHS 55B)	Deep purplish pink (RHS 55A)
BE	Strong Purplish Red (RHS 58C)	Strong Purplish Red (RHS 58C)
TW	Vivid purplish red (RHS 57D)	Vivid purplish red (RHS 57D)
HY	Pale Purplish Pink (RHS 62D)	Pale Purplish Pink (RHS 62D)
DH	Strong Purplish Red (RHS 71D)	Deep Purplish Pink (RHS 73A)
KOS	Strong Purple (RHS 83D)	Strong Purple (RHS 84A)
BW	Brilliant violet petal (RHS 86D) with light violet centre	Brilliant violet petal (RHS 86D) with light violet centre
PJ	Vivid purple (RHS 86D) petal with white centre	Vivid purple (RHS 86D) petal with white centre
DB	Bluish white petal (RHS 112D)	Bluish white petal (RHS 112D)
PS	Light yellowish green petal (RHS 2C) with very Pale Blue towards the tip (RHS 112B)	Light yellowish green petal (RHS 2C) with very Pale Blue towards the tip (RHS 112B)
CS	Light Yellowish Green (RHS 2C)	Light Yellowish Green (RHS 2C)
CI	Pale Yellow (RHS 11C)	Pale Yellow (RHS 11C)

Table 8. Variation in inner and outer petal colour of *Nymphaea* varieties (Continued)

Variety	Flower colour (Inner petal colour)	Flower colour (Outer petal colour)
VG	White	White
SK	Deep Pink (RHS 47D)	Deep Pink (RHS 47D)
SP	Moderate Purplish Pink (RHS 62B)	Light Purplish Pink (RHS 62C)
SJ	White	White
SA	Pale Orangish Yellow (RHS 16D)	Pale Orangish Yellow (RHS 16D)
PR	Light yellowish green (RHS 2C)	Light yellowish green (RHS 2C)
CD	Light yellowish pink (RHS 19B)	Light yellowish pink (RHS 19B)
TS	Light Pink (RHS 49C) petals with light yellow centre (RHS 21D)	Light Pink (RHS 49C) petals with light yellow centre (RHS 21D)
MR	Vivid purplish red (RHS 61C)	Vivid purplish red (RHS 61C)
JS	Strong Purplish Red (RHS 64C)	Strong Purplish Red (RHS 64C)
IM	Very pale blue (RHS 112C) with white specks	Very pale blue (RHS 112C) with white specks
PBR	Vivid Purplish Red (RHS 67B)	Vivid Purplish Red (RHS 67B)
WV	Light greenish yellow (RHS 4B)	Light greenish yellow (RHS 4B)
MX	Light Yellowish Green (RHS 2C)	Light Yellowish Green (RHS 2C)
PB	Pale Purplish Pink (RHS 62D)	Pale Purplish Pink (RHS 62D)

Table 9. Variation in stamen colour in *Nymphaea* varieties

Variety	Stamen colour
MC	Brilliant yellow (RHS 9C-83) and very pale violet (RHS 91D- 213)
CP	Brilliant yellow (RHS 9C-83) and light purplish pink (RHS 68D-249)
BE	Brilliant yellow (RHS 9C-83) and Deep purplish pink (RHS 68A- 248)
TW	Strong reddish purple (RHS 72C- 237)
HY	Vivid yellow (RHS 9A-82) and very pale purple (RHS 69A- 226)
DH	Brilliant yellow (RHS 9C-83) and Deep purplish pink (RHS 68A- 248)
KOS	Brilliant violet (RHS 86D-206)
BW	Brilliant yellow (RHS 9C-83) and light purple (RHS 77D- 222)
PJ	Brilliant yellow (RHS 8A- 83) and strong purple (RHS 84A- 218)
DB	Brilliant yellow (RHS 9C-83) and pale yellowish green (RHS 4D- 121)
PS	Vivid yellow (RHS 13A- 82) and light greenish yellow (RHS 8C-101)
CS	Brilliant yellow (RHS 9C-83) and light greenish yellow (RHS 8C- 101)
CI	Brilliant yellow (RHS 9C-83)
VG	Brilliant yellow (RHS 9C-83)

Table 9. Variation in stamen colour in *Nymphaea* varieties (Continued).

Variety	Stamen colour
SK	Vivid yellow (RHS 9A-82) and light greenish yellow (RHS 4B-101)
SP	Brilliant yellow (RHS 9C-83) and light purplish pink (RHS 55C- 249)
SJ	Brilliant yellow (RHS 9C-83)
SA	Brilliant yellow (RHS 9C-83)
PR	Brilliant greenish yellow (RHS 4A- 98)
CD	Brilliant yellow (RHS 9C-83)
TS	Brilliant yellow (RHS 9C-83) and light pink (RHS 49C- 4)
MR	Brilliant yellow (RHS 9C-83) and strong purplish pink (RHS 67D- 247)
JS	Brilliant yellow (RHS 9C-83) and strong reddish purple (RHS 70B- 237)
IM	Brilliant yellow (RHS 9C-83) and light purplish pink (RHS 68D- 249)
PBR	Strong purplish pink (RHS 55B- 247) and light greenish yellow (RHS 8B- 101)
WV	Brilliant greenish yellow (RHS 5B- 98)
MX	Brilliant yellow (RHS 9C-83)
PB	Brilliant greenish yellow (RHS 4A-98) and very pale purple (RHS 84D- 226)

* No stamen was observed in Taweekan and King of Siam

Table 10. Anthesis time of *Nymphaea* varieties at different seasons

Variety	November – February	Total Duration (Minutes)	March-May	Total Duration (Minutes)	June- October	Total Duration (Minutes)
MC	7:30 am to 6:15 pm	645	7:00 am to 5:45 pm	6 45	8:30 am to 2:00 pm	330
CP	9:15 am to 5:30 pm	495	8:45 am to 5:30 pm	525	9:30 am to 3:45 pm	375
BE	8:45 am to 5:45 pm	540	8:45 am to 5:45 pm	540	10:45 am to 2:15 pm	210
TW	9:45 am to 5:45 pm	480	9:45 am to 5:45 pm	480	11:15 am to 4:00 pm	285
HY	10:00 am to 5:45 pm	465	10:00 am to 5:45 pm	465	11:30 am to 2:45 pm	195
DH	8:40 am to 6:10 pm	570	8:00 am to 5:30 pm	570	9:30 am to 4:00pm	390
KOS	7:00 am to 6:15 pm	675	7:00 am to 6:15 pm	675	9:00 am to 4:45 pm	465
BW	7:00 am to 6:15 pm	675	7:00 am to 6:15 pm	675	9:00 am to 4:45 pm	465
PJ	8:45 am to 4:15 pm	450	8:45 am to 4:15 pm	450	10:45 am to 3:45 pm	300
DB	7:15 am to 5:15 pm	600	7:15 am to 5:15 pm	600	9:00 am to 4:30pm	450
P	7:15 am to 6:35 pm	680	7:15 am to 6:15 pm	660	9:45 am to 4:00 pm	375
CS	9:15 am to 5:00 pm	465	9:15 am to 5:00 pm	465	11:15 am to 4:00 pm	285
CI	*	*	*	*	7:30 am to 3:15 pm	465
VG	7:30 am to 1:15 pm	345	7:30 am to 1:15 pm	345	8:30 am to 11:45 pm	195
SK	*	*	*	*	8:15 am to 1:30 pm	315
SP	8:00 am to 2:30 pm	390	*	*	*	*
SJ	7:30 am to 1:00 pm	330	7:30 am to 1:00 pm	330	9:00 am to 12:15 pm	195

Table 10. Anthesis time of *Nymphaea* varieties at different seasons (Continued)

Variety	November – February	Total Duration (Minutes)	March-May	Total Duration (Minutes)	June- October	Total Duration (Minutes)
SA	*	*	*	*	8:45 am to 1:45 pm	300
PK	*	*	*	*	*	*
PR	*	*	*	*	8:15 am to 1:45 pm	330
AB	*	*	*	*	*	*
CD	8:15 am to 2:30 pm	375	*	*	*	*
TS	9:00 am to 3:15 pm	375	9:00 am to 3:15 pm	375	10:45 am to 2:30 pm	225
MR	9:15 am to 3:15 pm	360	9:15 am to 3:15 pm	360	10:45 am to 2:30 pm	225
JS	9:45 am to 4:15 pm	390	9:45 am to 4:15 pm	390	10:15 am to 4:00 pm	345
IM	8:00 am to 3:30 pm	450	8:00 am to 3:30 pm	450	9:30 am to 2:30 pm	300
PBR	*	*	7:45 am to 3:15 pm	450	8:50 am to 1:30 pm	275
WV	*	*	10:30 am to 3:15 pm	285	11:30 am to 3:00 pm	210
MX	*	*	*	*	7:45 am to 2:45 pm	420
PB	9:15 am to 5:30 pm	495	9:15 am to 5:30 pm	495	11:30 am to 4:15 pm	285

* No flower production was observed

Table 11. Variation in the floral qualitative character of *Nymphaea* varieties

Variety	Petal apex	Sepal shape
Micrantha	Obtuse	Ob- lanceolate
Chompu	Acute	Linear lanceolate
Taweekan	Obtuse	Linear lanceolate
Bull's Eye	Acute	Linear lanceolate
Hilary	Obtuse	Ob- lanceolate
Doris Holt	Acute	Linear lanceolate
Carla's Sonshine	Obtuse	Linear lanceolate
Siam Jasmine	Obtuse	Ob lanceolate
Wanvisa	Obtuse	Ob- lanceolate
Mexicana	Acute	Ob lanceolate
King of Siam	Obtuse	Ob lanceolate
Blue Whistle	Acute	Linear lanceolate
Purple Joy	Obtuse	Ob lanceolate
Dauben	Acute	Linear lanceolate
Poonsub	Acute	Ob lanceolate
Clyde Ikins	Obtuse	Linear lanceolate
Virginalis	Obtuse	Ob lanceolate
Peach Blow	Obtuse	Linear lanceolate
Islamorada	Acute	Linear lanceolate
Siam Pink	Obtuse	Ob- lanceolate
Suparnee Pink	Obtuse	Linear lanceolate
Jalueang Sub	Acute	Linear lanceolate
Tropic Sunset	Acute	Ob lanceolate
Miami Rose	Acute	Linear lanceolate
Colorado	Obtuse	Ob- lanceolate
Paranee	Obtuse	Ob lanceolate
Siam Amber	Acute	Ob- lanceolate
Perry's Baby Red	Obtuse	Ob lanceolate

* No flowering was observed in Puttaraksa and Almost Black during the observation period.

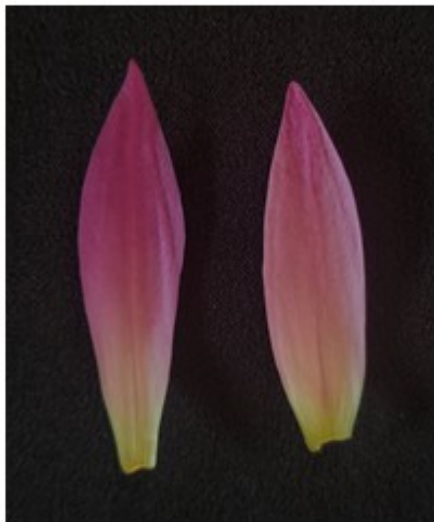


Linear lanceolate



Ob lanceolate

Plate 12. Shape of sepal in *Nymphaea* varieties



Acute



Obtuse

Plate 13. Shape of petal apex in *Nymphaea* varieties

4.1.3.24. Pedicel colour

The data containing different pedicel colours of twenty eight *Nymphaea* varieties are exhibited in Table 12.

Pedicel colour of *Nymphaea* varieties exhibited nine shades. The varieties Bull's Eye, Hilary, Poosub and Supranee Pink recorded light yellowish green (RHS 144 D). Moderate yellowish green (RHS 143D) was exhibited by King of Siam and Purple Joy. Dauben, Colorado, Jalueang Sub showed pale yellow (RHS 162D) hue. Carla's Sonshine and Tropic Sunset displayed moderate yellowish green (RHS 146C). The varieties Siam Pink, Siam Jasmine, Perry's Baby Red, Virginalis and Mexicana exhibited moderate yellowish green (RHS 147C) shade. Siam Amber and Paranee showed moderate purplish red (RHS 185C). Miami Rose and Peach Blow exhibited brilliant yellowish green (RHS 150A) colour pedicel. Islamorada exhibited greyish reddish orange (RHS 176C) hue while Wanvisa exhibited moderate orangish yellow (RHS 165C) shade.

Table 12. Variation in pedicel colour in *Nymphaea* varieties

Variety	Pedicel colour
Micrantha	RHS 185C- Moderate purplish red
Chompu	RHS 185C- Moderate purplish red
Bull's Eye	RHS 144D- Light yellowish green
Taweekan	RHS 186B- Moderate purplish red
Hilary	RHS 144D- Light yellowish green
Doris Holt	RHS 185C- Moderate purplish red
King of Siam	RHS 143D- Moderate yellowish-green
Blue Whistle	RHS 185C- Moderate purplish red
Purple Joy	RHS 143D- Moderate yellowish-green
Dauben	RHS 162D- Pale yellow
Poosub	RHS 144D- Light yellowish green
Carla's Sonshine	RHS 146C- Moderate yellowish-green
Clyde Ikings	RHS 185C- Moderate purplish red
Virginalis	RHS 147C- Moderate yellowish-green
Supranee Pink	RHS 144D- Light yellowish green
Siam Pink	RHS 147C- Moderate yellowish-green

Table 12. Variation in pedicel colour in *Nymphaea* varieties (continued)

Variety	Pedicel colour
Siam Jasmine	RHS 147C- Moderate yellowish-green
Siam Amber	RHS 185C- Moderate purplish red
Paranee	RHS 185C- Moderate purplish red
Colorado	RHS 162D- Pale yellow
Tropic Sunset	RHS 146C- Moderate yellowish-green
Miami rose	RHS 150A Brilliant yellowish green
Jalueang sub	RHS 162D- Pale yellow
Islamorada	RHS 176C- Greyish reddish orange
Perry's Baby Red	RHS 147C- Moderate yellowish-green
Wanvisa	RHS 165C- Moderate orangish yellow
Mexicana	RHS 147C- Moderate yellowish-green
Peach Blow	RHS 150A- Brilliant yellowish green

4.1.4. Morphological description of the *Nymphaea* varieties

Based on the results obtained, the thirty varieties used for the performance analysis are morphologically described following the criteria given by the International Water Gardening Society, which is the registry for *Nymphaea* varieties.

***Nymphaea* Micrantha**

Micrantha is a tropical day bloomer which exhibited an average flower diameter of 7.86 cm. It is viviparous, leaves without striations, ovate and toothed. The variety had very pale purplish blue inner and outer petals. It produced 9.66 flowers per plant per month.

***Nymphaea* Chompu**

Chompu is a tropical day bloomer which exhibited an average flower diameter of 8.68 cm. It is viviparous, leaf with no striations, ovate and toothed margins. The flowers had strong purplish red inner petals and deep purplish pink outer petals. It produced seven flowers per plant per month.

Nymphaea Taweekan

Taweekan is a tropical day bloomer which exhibited an average flower diameter of 9.85 cm. The leaf exhibited ovate shape and toothed leaf margin having no striations on it. The variety had vivid purplish red inner and outer petals. It produced 6.66 flowers per plant per month.

Nymphaea Bull's Eye

Bull's Eye is a tropical day bloomer which exhibited an average flower diameter of 9.88 cm. The leaf exhibited ovate shape and toothed leaf margin having no striations on it. The variety had strong purplish red inner and outer petals. It produced 7.33 flowers per plant per month.

Nymphaea Hilary

Hilary is a tropical day bloomer with an average flower diameter of 9.26 cm. It has viviparous leaf with striations present on it. Leaf exhibited round shape and toothed margin. The variety had pale purplish pink inner and outer petals. It produced 6.33 flowers per plant per month.

Nymphaea Doris Holt

Doris Holt is a tropical day bloomer which exhibited an average flower diameter of 8.59 cm. It is viviparous, round leaf with striations and toothed margin. The variety had strong purplish red inner petals and deep purplish pink outer petals. It produced 8.33 flowers per plant per month.

Nymphaea Carla's Sonshine

Carla's Sonshine is a tropical day bloomer which exhibited an average flower diameter of 7.98 cm. It has viviparous leaf with striations present on it. Leaf exhibited ovate shape and toothed leaf margin. The variety had light yellowish green inner and outer petals. It produced 6.33 flowers per plant per month.

Nymphaea Siam Jasmine

Siam Jasmine is a hardy day bloomer which exhibited an average flower diameter of 7.97 cm. The leaf exhibited round shape and smooth leaf margin having no striations on it. The variety had white petals. It produced 2.66 flowers per plant per month.

Nymphaea Mexicana

Mexicana is a hardy day bloomer which exhibited an average flower diameter of 8.71 cm. The leaf exhibited round shape and smooth leaf margin having no striations on it. The variety had light yellowish green inner and outer petals. It produced 1.33 flowers per plant per month.

Nymphaea Wanvisa

Wanvisa is a hardy day bloomer which exhibited an average flower diameter of 8.81 cm. The leaf exhibited round shape and smooth leaf margin with striations present on it. The variety had light greenish yellow inner and outer petals. It produced 2.33 flowers per plant per month.

Nymphaea King of Siam

King of Siam is a tropical day bloomer which exhibited an average flower diameter of 11.07 cm. The leaf exhibited ovate shape and toothed leaf margin having no striations on it. The variety had strong purple inner and outer petals. It produced 9.66 flowers per plant per month.

Nymphaea Blue Whistle

Blue Whistle is a tropical day bloomer which exhibited an average flower diameter of 10.02 cm. It has viviparous leaf with no striations on it. Leaf exhibited ovate shape and toothed leaf margin. The variety had brilliant violet petals with light violet centre. It produced 9.66 flowers per plant per month.

Nymphaea Purple Joy

Purple Joy is a tropical day bloomer which exhibited an average flower diameter of 9.92 cm. It has viviparous leaf with no striations present on it. Leaf exhibited ovate shape and toothed leaf margin. The variety had vivid purple petals with white centre. It produced 6.33 flowers per plant per month.

Nymphaea Dauben

Dauben is a tropical day bloomer which exhibited an average flower diameter of 7.96 cm. The leaf exhibited ovate shape and toothed leaf margin having no striations on it. The variety had bluish white inner and outer petals. It produced 13 flowers per plant per month.

Nymphaea Poonsub

Poonsub is a tropical day bloomer which exhibited an average flower diameter of 9.68 cm. The leaf exhibited ovate shape and toothed leaf margin having no striations on it. The variety had light yellowish green petals with very Pale blue towards the tip. It produced 5.66 flowers per plant per month.

Nymphaea Clyde Ikins

Clyde Ikins is a hardy day bloomer which exhibited an average flower diameter of 10.38 cm. The leaf exhibited round shape and smooth leaf margin having no striations on it. The variety had petals with pale yellow colour. It produced two flowers per plant per month.

Nymphaea Virginalis

Virginalis is a hardy day bloomer which exhibited an average flower diameter of 8.78 cm. The leaf exhibited round shape and smooth leaf margin having no striations on it. The variety had petals with white colour. It produced 3.33 flowers per plant per month.

Nymphaea Peach Blow

Peach Blow is a tropical day bloomer which exhibited an average flower diameter of 9.91 cm at flowering. It has viviparous leaf with striations present on it. Leaf exhibited ovate shape and toothed leaf margin. The variety had pale purplish pink inner and outer petals. It produced six flowers per plant per month.

Nymphaea Islamorada

Islamorada is a tropical day bloomer which exhibited an average flower diameter of 9.21 cm. It was viviparous leaf with no striations on it. Leaf exhibited ovate shape and smooth leaf margin. The variety had very pale blue petals with white specks. It produced 7.33 flowers per plant per month.

Nymphaea Perry's Baby Red

Perry's Baby Red is a hardy day bloomer which exhibited an average flower diameter of 8.78 cm. The leaf exhibited round shape and smooth leaf margin having no striations on it. The variety had vivid purplish red inner and outer petals. It produced 3.33 flowers per plant per month.

***Nymphaea* Supralee Pink**

Supralee Pink is a hardy day bloomer which exhibited an average flower diameter of 8.91 cm. The leaf exhibited round shape and smooth leaf margin having no striations on it. The variety had deep pink inner and outer petals. It produced 0.66 flowers per plant per month.

***Nymphaea* Siam Pink**

Siam Pink is an inter subgeneric hybrid (Hardy x Tropical) which exhibited an average flower diameter of 7.4 cm. The leaf exhibited ovate shape and smooth leaf margin having striations present on it. The variety had moderate purplish pink inner petals and light purplish pink outer petals. It produced 0.66 flowers per plant per month.

***Nymphaea* Jalueang Sub**

Jalueang Sub is a tropical day bloomer which exhibited an average flower diameter of 7.48 cm. The leaf exhibited ovate shape and toothed leaf margin having striations present on it. The variety had strong purplish red inner and outer petals. It produced 7.66 flowers per plant per month.

***Nymphaea* Tropic Sunset**

Tropic Sunset is a tropical day bloomer which exhibited an average flower diameter of 7.57 cm. The leaf exhibited ovate shape and toothed leaf margin having no striations on it. The variety had light pink petals with light yellow centre. It produced 12.33 flowers per plant per month.

***Nymphaea* Miami Rose**

Miami Rose is a tropical day bloomer which exhibited an average flower diameter of 7.37 cm. The leaf exhibited ovate shape and toothed leaf margin having striations present on it. The variety had vivid purplish red petals. It produced seven flowers per plant per month.

***Nymphaea* Colorado**

Colorado is a hardy day bloomer which exhibited an average flower diameter of 8.67 cm. The leaf exhibited round shape and smooth leaf margin having no striations on it. The variety had light yellowish pink inner and outer petals. It produced one flower per plant per month.

Nymphaea Paranee

Paranee is a hardy day bloomer which exhibited an average flower diameter of 7.81 cm. The leaf exhibited ovate shape and smooth leaf margin having no striations on it. The variety had petals of light yellowish green colour. It produced one flower per plant per month.

Nymphaea Siam Amber

Siam Amber is a hardy day bloomer which exhibited an average flower diameter of 8.31 cm. The leaf exhibited ovate shape and smooth leaf margin having no striations on it. The variety had pale orangish yellow inner and outer petals. It produced 1.33 flowers per plant per month.

Nymphaea Almost Black

Almost Black is a hardy day bloomer which exhibited an average flower diameter of 12.7 cm. It is a non-viviparous plant that exhibits dark red petals near the outside and black towards the centre.

Nymphaea Puttaraksa

Puttaraksa is a hardy day bloomer. It is a non-viviparous plant having yellow petals. The leaves are bright green with maroon marble pads.

4.1.5. Estimation of the variability components

4.1.5.1. Cluster analysis of vegetative and floral quantitative characters.

Vegetative and floral quantitative characters were subjected to cluster analysis and data is given in Table 13 and 14.

Cluster analysis was done using vegetative quantitative characters like length of lamina (cm), width of lamina (cm), number of leaves per plant, longevity of leaves and number of days from visual appearance to full expansion of leaf lamina which exhibited variability. Dendrogram (Fig.2) based on vegetative quantitative characters categorised the genotypes into four different clusters. The cluster 1 comprised of 13 genotypes (Micrantha, Hilary, Doris Holt, Carla's Sonshine, King of Siam, Blue Whistle, Purple Joy, Dauben, Suprane Pink, Siam Pink, Jalueang Sub, Tropic Sunset and Miami Rose), cluster 2 comprised of 15 genotypes (Chompu, Taweekan, Bull's Eye, Siam Jasmine, Puttaraksa,

Mexicana, Poonsub, Clyde Ikins, Virginalis, Islamorada, Perry's Baby Red, Colorado, Almost Black, Paranee and Siam Amber), cluster 3 comprised of the genotype Wanvisa and cluster 4 comprised of the genotype Peach Blow. Dendrogram based on vegetative quantitative characters is given in Figure 2.

Cluster analysis was done using floral quantitative characters like length of flower bud (cm), flower diameter (cm), length of sepal (cm), width of sepal (cm), number of petals, length of petal (cm) and breadth of petal (cm). Dendrogram (Fig.3) based on floral quantitative characters categorised the genotypes into five clusters. The cluster 1 comprised of three genotypes (Jalueang Sub, Tropic Sunset, Miami Rose), cluster 2 comprised of five genotypes (Taweekan, Micrantha, Chompu, Carla's Sonshine and King of Siam), cluster 3 comprised of seven genotypes (Dauben, Islamorada, Bull's Eye, Poonsub, Doris Holt, Perry's Baby Red and Virginalis), cluster 4 comprised of four genotypes (Hilary, Purple Joy, Blue Whistle and Peach Blow) and cluster 5 comprised of two genotypes (Siam Jasmine and Clyde Ikins). Dendrogram based on vegetative quantitative characters is given in Figure 3.

Table 13. Clustering pattern of genotypes based on vegetative quantitative characters

Cluster number	Number of genotypes	Cluster members
Cluster 1	13	Micrantha, Hilary, Doris Holt, Carlas Sonshine, King of Siam, Blue Whistle, Purple Joy, Dauben, Suprane Pink, Siam Pink, Jalueang Sub, Tropic Sunset, Miami Rose
Cluster 2	15	Chompu, Taweekan, Bull's Eye, Siam Jasmine, Puttaraksha, Mexicana, Poonsub, Clyde Ikins, Virginalis, Islamorada, Perry's Baby Red, Colorado, Almost Black, Paranee, Siam Amber
Cluster 3	1	Wanvisa
Cluster 4	1	Peach Blow

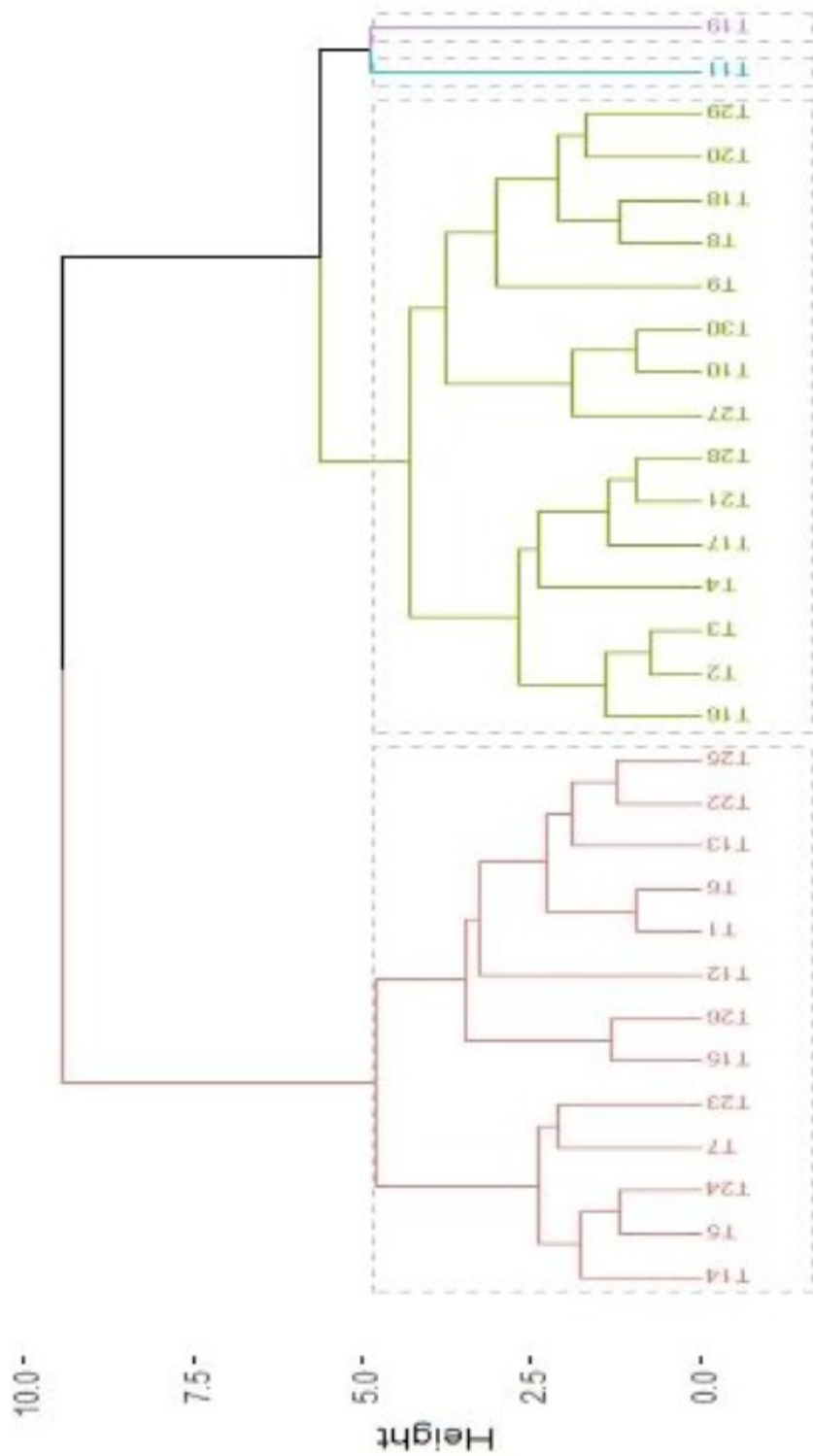


Figure 2. Dendrogram based on vegetative quantitative characters

Table 14. Clustering pattern of genotypes based on floral quantitative characters

Cluster Number	Number of genotypes	Cluster members
Cluster 1	3	Julueang Sub, Tropic Sunset, Miami Rose
Cluster 2	5	Taweekan, Micrantha, Chompu, Carla's Sonshine, King of Siam
Cluster 3	7	Dauben, Islamorada, Bull's Eye, Poonsub, Doris Holt, Perry's Baby Red, Virginalis
Cluster 4	4	Hilary, Purple Joy, Blue Whistle, Peach Blow
Cluster 5	2	Siam Jasmine, Clyde Ikins

Table 15. Cluster means of the vegetative characters of different clusters

Cluster number	Length of lamina (cm)	Width of lamina (cm)	Number of leaves/ plants	Longevity of leaves	Number of days from visual appearance to full expansion of leaf lamina
Cluster 1	18.43	17.19	18.74	25.79	9.06
Cluster 2	13.12	12.23	17.45	27.13	8.94
Cluster 3	10.84	9.88	29.45	35.22	10.22
Cluster 4	15.15	14.49	18.11	26.56	13.22

The cluster means of four vegetative quantitative characters and five floral quantitative characters were given in Table 15 and 16. Cluster 1 exhibited the highest cluster mean for length (18.43) and width of lamina (17.19). The highest cluster mean for number of leaves per plant (29.45) and longevity of leaves (35.22) were observed in cluster 3. The number of days from visual appearance to full expansion of leaf lamina (13.22) had highest cluster mean in cluster 4.

For the floral characters, cluster 2 exhibited the highest cluster mean for flower diameter (10.46) and number of petals (94.21). The highest cluster mean for length of flower bud (4.96), length of sepal (4.79) and length of petal (4.5) were observed in cluster 3. The width of sepal (2.37) and width of petal (2.26) had highest cluster mean in cluster 4.

The intra and inter cluster distance between the vegetative and floral genotypes are given in Table 17 and 18 respectively.

The intra-cluster distance for vegetative characters recorded its minimum values for C 3 (0) and C 4 (0), with the highest value observed for C 1 (8.55). Maximum inter cluster distance for vegetative characters was observed between C 1 and C3 which was 18.48 while minimum distance between C 2 and C 4 which was 7.94. For the floral quantitative characters the intra-cluster distance recorded its minimum values for C 2 (4.21) and maximum value observed for C 5 (12.36). Maximum inter cluster distance among the floral characters was observed between the clusters C 2 and C 3 which was 75.19 and minimum distance between C 1 and C 3 which was 7.36.

4.1.6. Genetic parameters

4.1.6.1. Coefficient of variation

The phenotypic, genotypic and environmental variance and the phenotypic coefficient of variance (PCV) and genotypic coefficient of variance (GCV) for the vegetative (Table 19 and Fig. 4) and floral characters (Table 20 and Fig. 5)

Among the vegetative characteristics, the characters number of leaves per plant (25.34), length of leaf lamina (10.02) and width of lamina (8.45) recorded high genotypic variances. The characters number of days from visual appearance to full expansion of leaf lamina (0.54), petiole diameter (0.096) and longevity of leaves (5.02) recorded low genotypic variances.

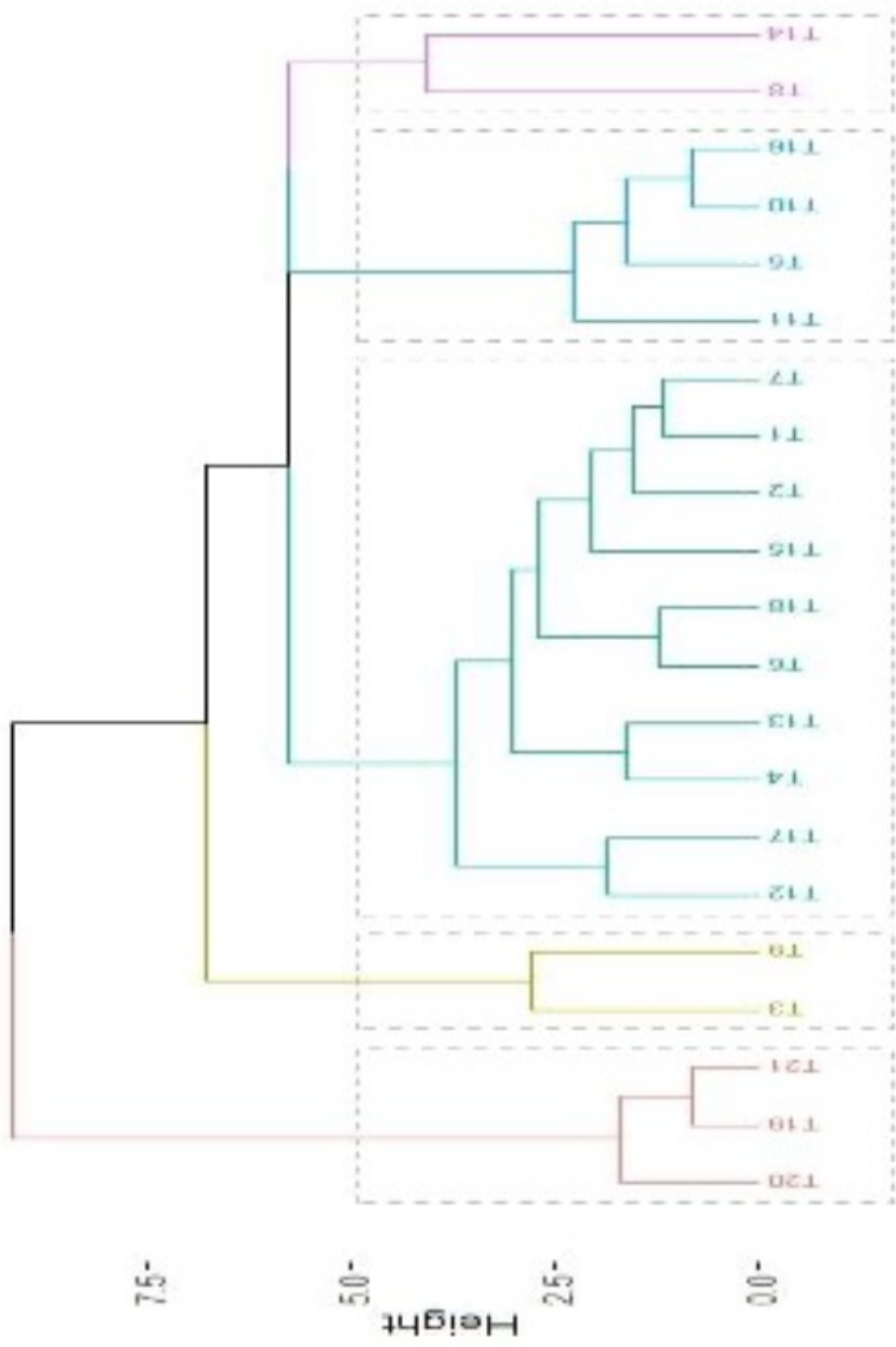


Figure 3. Dendrogram based on floral quantitative characters

Table 16. Cluster means of the floral characters of different clusters

Cluster number	Length of flower bud (cm)	Flower diameter (cm)	Length of sepal (cm)	Width of sepal (cm)	Number of petals	Length of petal (cm)	Width of petal (cm)
Cluster 1	4.52	8.69	4.27	1.93	22.68	4.05	1.38
Cluster 2	4.51	10.46	4.43	2.16	94.21	4.21	1.22
Cluster 3	4.96	9.78	4.79	2.06	19.03	4.5	1.31
Cluster 4	4.44	9.18	4.34	2.37	26.85	4.33	2.26
Cluster 5	4	7.48	3.62	1.72	29.95	3.4	1.02

Table 17. Average intercluster and intracluster distance of vegetative characters

	Cluster 1	Cluster 2	Cluster 3	Cluster 4
Cluster 1	8.556	10.92	18.48	8.970
Cluster 2	10.92	7.707	15.43	7.943
Cluster 3	18.48	15.43	0	15.92
Cluster 4	8.970	7.943	15.92	0

Bold figures in diagonals are the intra-cluster distance

Table 18. Average intercluster and intracluster distance of floral characters

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
Cluster 1	9.071	71.56	7.364	8.784	12.35
Cluster 2	71.56	4.213	75.19	67.43	64.41
Cluster 3	7.364	75.19	4.312	8.406	12.59
Cluster 4	8.784	67.43	8.406	6.447	8.727
Cluster 5	12.35	64.41	12.59	8.727	12.362

Bold figures in diagonals are the intra-cluster distance

The characters number of leaves per plant (30.67), longevity of leaves (19.78), length of lamina (10.20) and width of lamina (8.62) recorded high phenotypic variances. The characters number of days from visual appearance to full expansion of leaf lamina (2.77) and petiole diameter (0.096) recorded low phenotypic variances.

Maximum genotypic (27.31%) and phenotypic (30.04%) coefficient of variation were observed for the characters number of leaves per plant followed by length of lamina (GCV 20.53% and PCV 20.71%). The minimum GCV and PCV were observed by the character longevity of leaves as 8.36 per cent for GCV and 16.59 per cent for PCV. Number of days from visual observation to full expansion of leaf lamina also have low values of 8.03 per cent and 18.16 per cent respectively at both genotypic and phenotypic level.

The characters number of days from visual observation to full expansion of leaf lamina (10.13%) and longevity of leaves (8.23%) exhibited maximum difference between GCV and PCV. The characters, length of lamina (0.18%), width of lamina (0.2%) and petiole diameter (0.3%) recorded low difference between GCV and PCV. The PCV value ranged between 16.59 percent to 30.04 percent.

For the floral characteristics, the number of petals (504.02), number of stigmatic appendages per flower (16.82) and diameter of stigmatic cup (7.44) showed high genotypic variances. The characters, pedicel diameter (0.04), width of sepal (0.04), length of flower bud (0.10), flower diameter (1.14), width of petal (1.14), length of sepal (0.17) and length of petal (0.176) recorded low genotypic variances.

The characters the number of petals (507.27), number of stigmatic appendages per flower (17.61) and diameter of stigmatic cup (7.63) showed high phenotypic variances. The characters, width of sepal (0.052), pedicel diameter (0.055), width of petal (0.146), length of flower bud (0.104), flower diameter (1.144), length of petal (0.191) and length of sepal (0.189) exhibited low phenotypic variances.

Maximum genotypic (74.49%) and phenotypic (74.25%) coefficient of variance were observed for the character number of petals followed by width of petal (27.61% GCV and 27.42% PCV). The minimum GCV and PCV were observed by the character length of flower bud as 7.47 per cent for GCV and 7.14 per cent for PCV.

The character flower diameter (0.27%), pedicel diameter (0.859%) and length of sepal (0.521%) exhibited maximum difference between GCV and PCV. The characters diameter of stigmatic cup (0.251%), number of petals (0.239%), width of petal (0.189%) and length of flower bud (0.334%) recorded low difference between GCV and PCV.

4.1.6.2. Heritability and genetic advance

The heritability and genetic advance as percentage of mean of the vegetative and floral characters were estimated and presented in Table 21 and 22.

All the vegetative characters exhibited high genetic advance and heritability except longevity of leaf and number of days from visual appearance to full expansion of leaf lamina. Highest genetic advance was observed for number of leaves (51.14%) and lowest for number of visual observations to full expansion of leaf lamina (7.31%). The maximum heritability was observed for petiole diameter (96%) and minimum for number of days from visual appearance to full expansion of leaf lamina (19.5%).

All floral characters exhibited high genetic advance and heritability. Highest genetic advance was observed for number of petals (152.48%) and lowest for length of flower bud (14.06%). The maximum heritability was observed for number of petal (99.4%) and minimum for pedicel diameter (85.5%).

4.1.7. Correlation Studies

4.1.7.1. Phenotypic correlation

The phenotypic correlation among vegetative and floral characters were estimated and represented in Table 23 and 24.

Highly significant positive correlation was observed for length of lamina with width of lamina (0.895) and petiole diameter (0.614) and negative correlation with number of leaves per plant (-0.226) and longevity of leaves (-0.186). Width

of lamina showed positive correlation with length of lamina (0.895) and petiole diameter (0.578) while it expressed negative correlation with longevity of leaves (-0.247). Number of leaves per plant had positive correlation with longevity of leaves (0.226) and negative correlation with length of lamina (-0.226) and petiole diameter (-0.277). Longevity of leaves had positive correlation with number of leaves per plant (0.226) and negative correlation with length of lamina (-0.186) and width of lamina (-0.247). Petiole diameter had positive correlation with length of lamina (0.614) and width of lamina (0.578) while negative correlation with number of leaves per plant (-0.277). Phenotypic correlation was absent for number of days from visual appearance to full expansion of leaf lamina.

Highly positive correlation was observed for flower diameter with number of stigmatic appendages per flower (0.366), pedicel diameter (0.399), number of petals (0.46), length of flower bud (0.534), width of sepal (0.53), diameter of stigmatic cup (0.672), length of petal (0.722) and length of sepal (0.763). Length of flower bud exhibited positive correlation with breadth of sepal (0.333), diameter of stigmatic cup (0.432), flower diameter (0.534), length of petal (0.724) and length of sepal (0.751). Pedicel diameter had positive correlation with breadth of sepal (0.299), length of sepal (0.306), number of petal (0.368), flower diameter (0.399), diameter of stigmatic cup (0.458) and number of stigmatic appendages per flower (0.606) while negative correlation with width of petal (-0.324). Length of petal had positive correlation with width of petal (0.288), width of sepal (0.456), diameter of stigmatic cup (0.477), flower diameter (0.722), length of flower bud (0.724) and length of sepal (0.889). The width of sepal had positive correlation with flower diameter (0.53), length of flower bud (0.333), pedicel diameter (0.299), length of petal (0.456), width of petal (0.627), number of petal (0.278), length of sepal (0.422) and diameter of stigmatic cup (0.533). Diameter of stigmatic cup had positive correlation with flower diameter (0.672), length of flower bud (0.432), pedicel diameter (0.458), length of petal (0.477), number of petal (0.432), length of sepal (0.592), width of sepal (0.533) and number of stigmatic appendages per flower (0.424).

Table 19. Components of total variance for the vegetative characters in *Nymphaea* genotypes

Sl. No.	Characters	Genotypic variance	Phenotypic variance	Environmental variance	CV (%)	PCV (%)	GCV (%)
1	Length of lamina (cm)	10.023	10.200	0.177	2.729	20.717	20.536
2	Width of lamina (cm)	8.451	8.621	0.170	2.869	20.428	20.226
3	Number of leaves/ plants	25.347	30.676	5.329	12.523	30.047	27.313
4	Longevity of leaves	5.026	19.788	14.762	14.336	16.598	8.365
5	Petiole diameter (cm)	0.096	0.100	0.004	3.525	17.596	17.240
6	No: of days from visual appearance to full expansion of leaf lamina (days)	0.543	2.779	2.236	16.293	18.165	8.031

Table 20. Components of total variance for the floral characters in *Nymphaea* genotypes

Sl. No.	Characters	Genotypic variance	Phenotypic variance	Environmental variance	CV (%)	PCV (%)	GCV (%)
1	Flower diameter (cm)	1.144	1.198	0.054	2.599	12.243	11.964
2	Length of flower bud (cm)	0.104	0.114	0.010	2.212	7.479	7.145
3	Pedicel diameter (cm)	0.047	0.055	0.008	4.336	11.368	10.509
4	Length of petal (cm)	0.176	0.191	0.015	3.000	10.696	10.267
5	Width of petal (cm)	0.144	0.146	0.002	3.232	27.615	27.426
6	Number of petals	504.029	507.273	3.244	5.957	74.498	74.259
7	Length of sepal (cm)	0.170	0.189	0.019	3.207	10.115	9.594
8	Width of sepal (cm)	0.047	0.052	0.005	3.551	11.452	10.887
9	Diameter of stigmatic cup (mm)	7.446	7.633	0.187	3.188	20.368	20.117
10	No: of stigmatic appendages/ flower	16.832	17.614	0.782	4.996	23.713	23.181

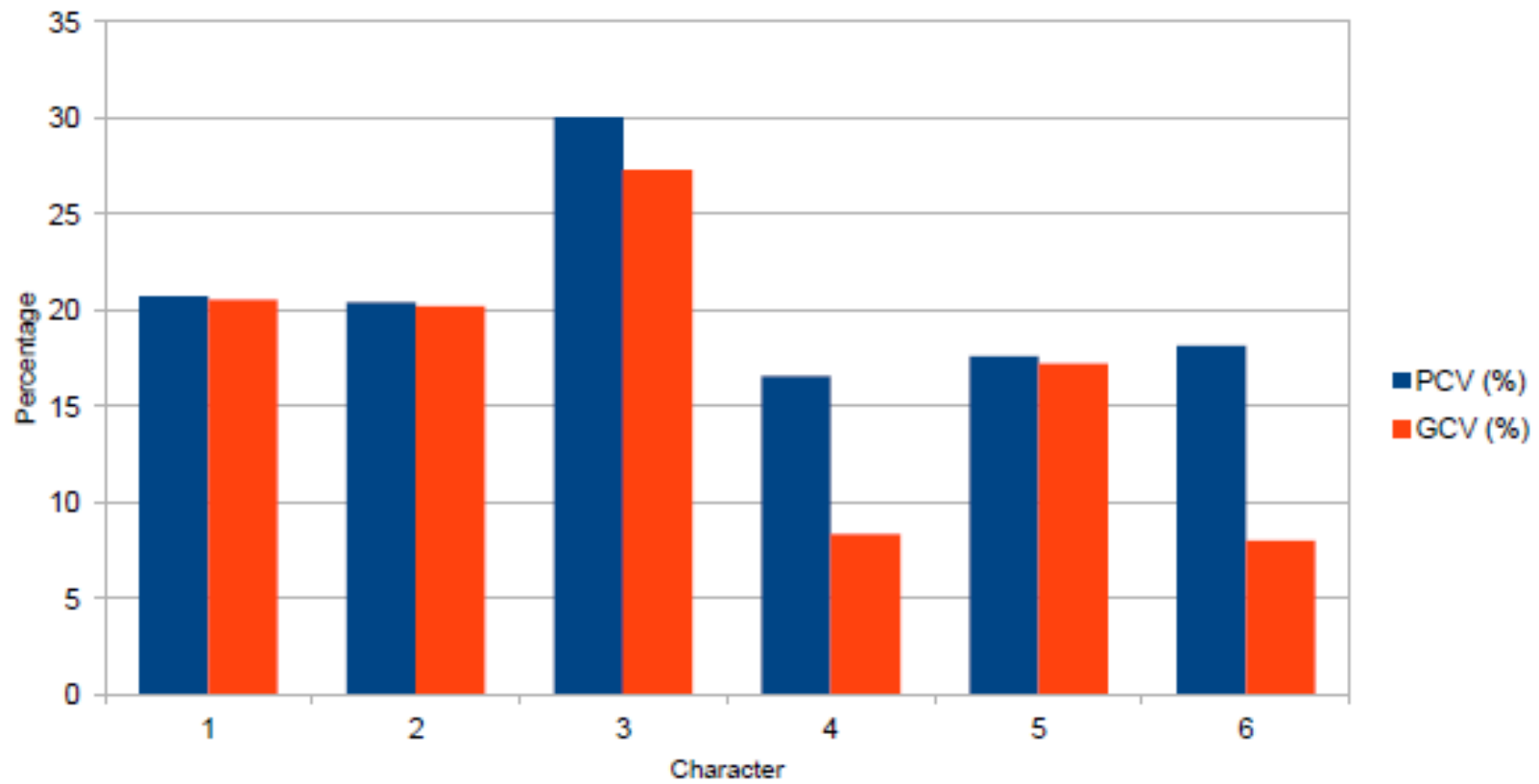


Figure 4. Phenotypic and genotypic coefficients of variation of various vegetative quantitative characters in *Nymphaea* varieties,

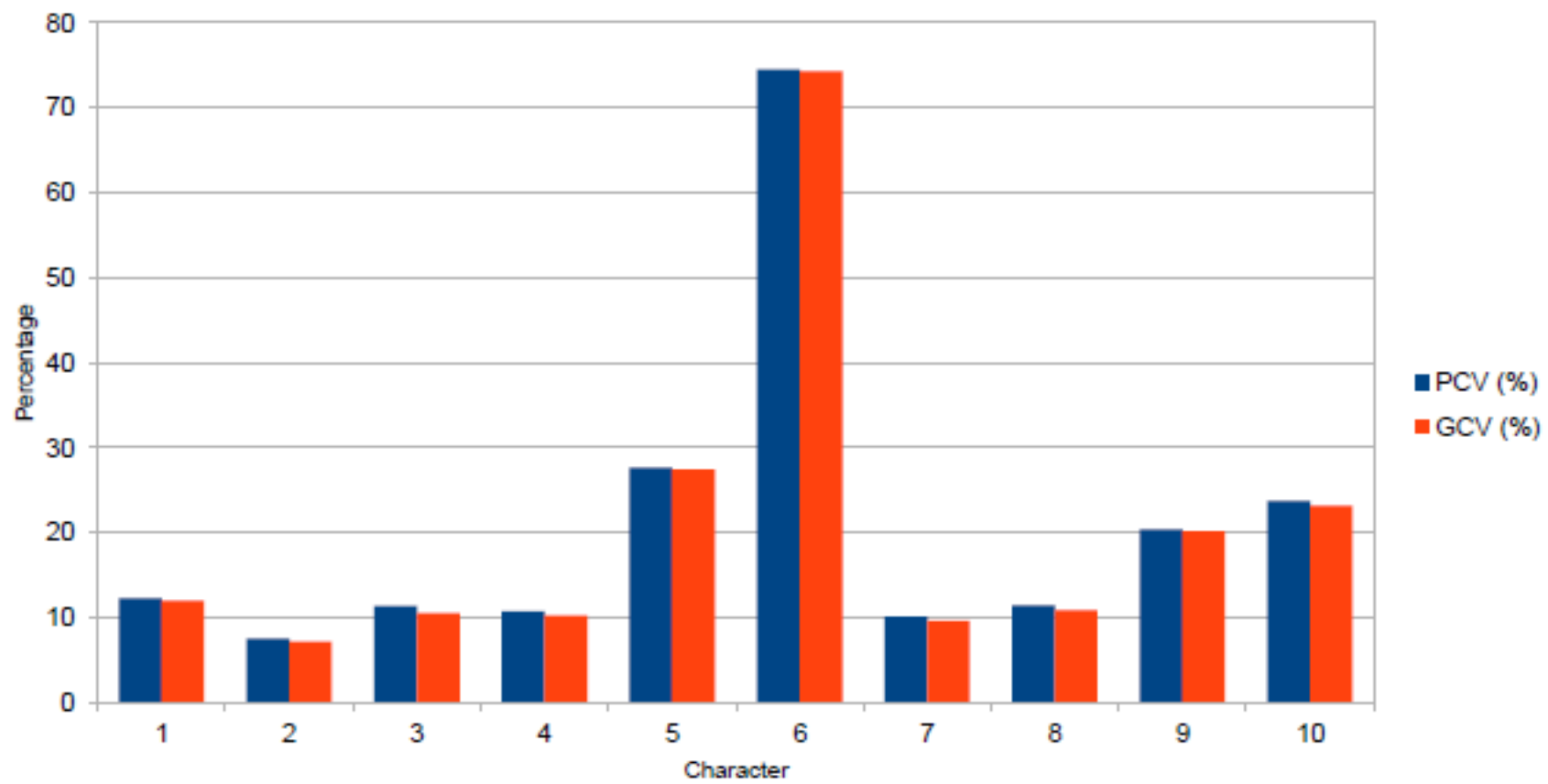


Figure 5. Phenotypic and genotypic coefficients of variation of various floral quantitative characters in *Nymphaea* varieties,

Table 21. Heritability and Genetic advance of vegetative characters of *Nymphaea* genotypes

Sl. No.	Characters	Heritability (%)	Genetic advance (as % of mean)
1	Length of lamina (cm)	98.3	41.936
2	Width of lamina (cm)	98.0	41.252
3	Number of leaves/ plants	82.6	51.144
4	Longevity of leaves	25.4	8.685
5	Petiole diameter (cm)	96.0	34.794
6	No: of days visual appearance to full expansion of leaf lamina (days)	19.5	7.315

Table 22. Heritability and Genetic advance of floral characters of *Nymphaea* genotypes

Sl. No.	Characters	Heritability (%)	Genetic advance (as % of mean)
1	Flower diameter (cm)	95.5	24.084
2	Length of flower bud (cm)	91.3	14.060
3	Pedicel diameter (cm)	85.5	20.012
4	Length of petal (cm)	92.1	20.300
5	Width of petal (cm)	98.6	56.108
6	Number of petals	99.4	152.483
7	Length of sepal (cm)	89.9	18.743
8	Width of sepal (cm)	90.4	21.322
9	Diameter of stigmatic cup (mm)	97.6	40.930
10	No: of stigmatic appendages/ plant	95.6	46.681

Table 23. Phenotypic correlation coefficient of vegetative quantitative characters in *Nymphaea* genotypes

	X1	X2	X3	X4	X5	X6
X1	1	0.894***	-0.225*	-0.184	0.615***	-0.022
X2	0.894***	1	-0.159	-0.245*	0.578***	0
X3	-0.225*	-0.159	1	0.224*	-0.276**	0.175
X4	-0.184	-0.245*	0.224*	1	-0.057	0.042
X5	0.615***	0.578***	-0.276**	-0.057	1	0.05
X6	-0.022	0	0.175	0.042	0.05	1

X1- Length of lamina(cm), X2- Width of lamina(cm), X3- Number of leaves/ plants, X4- Longevity of leaves, X5- Petiole diameter, X6- Number of days from visual appearance to full expansion of leaf lamina (Vegetative characters)

X1- Flower diameter (cm), X2- Length of flower bud (cm), X3- Pedicel diameter (cm), X4- Length of petal (cm), X5- Width of petal (cm), X6- Number of petals, X7- Length of sepal (cm), X8- Width of sepal (cm), X9- Diameter of stigmatic cup (mm), X10- Number of stigmatic appendages per plant (Floral characters)

4.1.7.2. Genotypic correlation

The genotypic correlation among vegetative and floral characters were estimated and represented in Table 25 and 26.

Length of lamina exhibited positive correlation with width of lamina (0.911) and petiole diameter (0.633) while negative correlation for number of leaves per plant (0.255) and longevity of leaves (0.385). Width of lamina had positive correlation with length of lamina (0.911), petiole diameter (0.589) while negative correlation with longevity of leaves (0.47). Number of leaves per plants was positively correlated with longevity of leaves (0.459), number of days from visual appearance to full expansion of leaf lamina (0.359) while negatively correlated with length of lamina (-0.255) and petiole diameter (-0.308). Longevity of leaves had positive correlation with number of leaves per plant (0.459) and number of days from visual appearance to full expansion of leaf lamina (0.336) while negatively correlated with length of lamina (-0.385) and width of lamina (-0.47). Petiole diameter had positive correlation with length of lamina (0.633) and width of lamina (0.589) while negative correlation for number of leaves per plant (-0.308). Number of days from visual appearance to full expansion of leaf lamina have positive correlation with number of leaves/ plant (0.359) and longevity of leaves (0.336).

High positive genotypic correlation was observed for flower diameter with length of flower bud (0.587), pedicel diameter (0.424), length of petal (0.756), number of petal (0.468), length of sepal (0.802), width of sepal (0.567), diameter of stigmatic cup (0.686) and number of stigmatic appendages per flower (0.377). Length of flower bud showed positive correlation with flower diameter (0.587), length of petal (0.797), length of sepal (0.833), width of sepal (0.387) and diameter of stigmatic cup (0.463). Pedicel diameter had positive correlation with flower diameter (0.424), number of petal (0.401), length of sepal (0.334), width of sepal (0.325), diameter of stigmatic cup (0.489) and number of stigmatic appendages per flower (0.666) while negative correlation with width of petal (-0.348). Length of petal had positive correlation with flower diameter (0.756),

length of flower bud (0.797), width of petal (0.292), length of sepal (0.946), width of sepal (0.486) and diameter of stigmatic cup (0.499). Width of petal had positive correlation with length of petal (0.292) and width of sepal (0.664) while negatively correlated with pedicel diameter (-0.348) and number of stigmatic appendages per flower (-0.629). Number of petals was positively correlated with flower diameter (0.468), pedicel diameter (0.401), width of sepal (0.297), diameter of stigmatic cup (0.427) and number of stigmatic appendages per flower (0.545). Length of sepal had positive correlation with flower diameter (0.802), length of flower bud (0.833), pedicel diameter (0.334), length of petal (0.946), width of sepal (0.446) and diameter of stigmatic cup (0.621). Width of sepal had positive correlation with flower diameter (0.567), length of flower bud (0.387), pedicel diameter (0.325), length of petal (0.486), width of petal (0.664), number of petal (0.297), length of sepal (0.446) and diameter of stigmatic cup (0.568). Diameter of stigmatic cup exhibited positive correlation with flower diameter (0.686), length of flower bud (0.463), pedicel diameter (0.489), length of petal (0.499), number of petal (0.427), length of sepal (0.621), width of sepal (0.568) and number of stigmatic appendages per flower (0.441). Number of stigmatic appendages per flower had positive correlation with flower diameter (0.377), pedicel diameter (0.666), number of petal (0.545) and diameter of stigmatic cup (0.441) while negative correlation with width of petal (-0.629).

Table 24. Phenotypic correlation coefficient of floral quantitative characters in *Nymphaea* genotypes

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10
X1	1	0.532***	0.399**	0.72***	0.148	0.459***	0.763***	0.529***	0.671***	0.365**
X2	0.532***	1	0.217	0.72***	0.139	-0.147	0.748***	0.331**	0.43***	0.077
X3	0.399**	0.217	1	0.104	-0.324**	0.367**	0.306*	0.299*	0.457***	0.604***
X4	0.72***	0.72***	0.104	1	0.288*	0.006	0.888***	0.455***	0.476***	-0.009
X5	0.148	0.139	-0.324**	0.288*	1	-0.129	0.096	0.626***	0.055	-0.608***
X6	0.459***	-0.147	0.367**	0.006	-0.129	1	0.006	0.278*	0.423***	0.534***
X7	0.763***	0.748***	0.306*	0.888***	0.096	0.006	1	0.422***	0.592***	0.163
X8	0.529***	0.331**	0.299*	0.455***	0.626***	0.278*	0.422***	1	0.532***	-0.053
X9	0.671***	0.43***	0.457***	0.476***	0.055	0.423***	0.592***	0.532***	1	0.423***
X10	0.365**	0.077	0.604***	-0.009	-0.608***	0.534***	0.163	-0.053	0.423***	1

Table 25. Genotypic correlation coefficient of vegetative quantitative characters in *Nymphaea* genotypes

	X1	X2	X3	X4	X5	X6
X1	1	0.911**	-0.255*	-0.385**	0.633**	-0.112
X2	0.911**	1	-0.181	-0.47**	0.589**	0.021
X3	-0.255*	-0.181	1	0.459**	-0.308*	0.359**

	X1	X2	X3	X4	X5	X6
X4	-0.385**	-0.47**	0.459**	1	-0.151	0.336*
X5	0.633**	0.589**	-0.308*	-0.151	1	0.168
X6	-0.112	0.021	0.359**	0.336*	0.168	1

Table 26. Genotypic correlation coefficient of floral quantitative characters in *Nymphaea* genotypes

	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10
X1	1	0.532***	0.399**	0.72***	0.148	0.459***	0.763***	0.529***	0.671***	0.365**
X2	0.532***	1	0.217	0.72***	0.139	-0.147	0.748***	0.331**	0.43***	0.077
X3	0.399**	0.217	1	0.104	-0.324**	0.367**	0.306*	0.299*	0.457***	0.604***
X4	0.72***	0.72***	0.104	1	0.288*	0.006	0.888***	0.455***	0.476***	-0.009
X5	0.148	0.139	-0.324**	0.288*	1	-0.129	0.096	0.626***	0.055	-0.608***
X6	0.459***	-0.147	0.367**	0.006	-0.129	1	0.006	0.278*	0.423***	0.534***
X7	0.763***	0.748***	0.306*	0.888***	0.096	0.006	1	0.422***	0.592***	0.163
X8	0.529***	0.331**	0.299*	0.455***	0.626***	0.278*	0.422***	1	0.532***	-0.053
X9	0.671***	0.43***	0.457***	0.476***	0.055	0.423***	0.592***	0.532***	1	0.423***
X10	0.365**	0.077	0.604***	-0.009	-0.608***	0.534***	0.163	-0.053	0.423***	1

4.2. CROP IMPROVEMENT STUDIES

4.2.1. Palynology

Pollen characters *viz.* pollen shape, pollen size and pollen fertility are given in Table 27 and Plate 14.

4.2.1.1. Pollen shape

The varieties Islamorada, Chompu, Doris Holt, Bull's Eye, Dauben, Peach Blow, Virginalis and Siam Amber exhibit prolate spheroidal shape. The varieties Micrantha, Carla's Sonshine, Hilary, Tropic Sunset, Blue Whistle had spheroidal shape and Perry's Baby Red, Poosub, Siam Jasmine, Suprane Pink and Clyde Ikins exhibited oblate-spheroidal shaped pollens.

4.2.1.2. Pollen size

The varieties showed significant differences regarding the polar and equatorial diameter of the pollen grains. Doris Holt exhibited the highest polar diameter (42.00 μ) while Siam Amber exhibited the lowest polar diameter (31.66 μ). Siam Jasmine (42.33 μ) and Perry's Baby Red (42.33 μ) exhibited maximum equatorial diameter. The varieties Micrantha, Carla's Sonshine, Doris Holt, Hilary, Perry's Baby Red, Poosub, Peach Blow, Siam Jasmine, Tropic Sunset, Suprane Pink, Clyde Ikins and Blue Whistle exhibited medium sized pollen. Small pollen size was recorded in Islamorada, Chompu, Bull's Eye, Dauben, Virginalis and Siam Amber.

4.2.1.3. Presence or absence of aperture

A ring like aperture that is slightly shifted in the direction of the distal pole was present in varieties Chompu, Hilary, Perry's Baby Red and Siam Amber. All other varieties exhibited absence of aperture in their pollen.

4.2.1.4. Exine ornamentation

The pollen cover had a prominent projecting ring which was convex in shape and arranged in a circular arch. A ring like sulcus called zonosulculus was present around the equator and had psilate ornamentation both its proximal and distal surfaces in all the varieties.

Table 27. Pollen characters of *Nymphaea* varieties

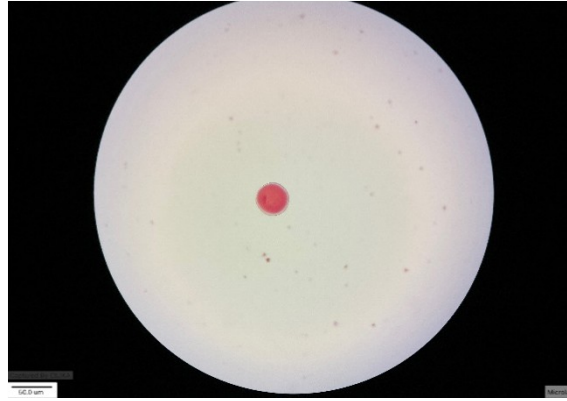
Variety	Size of fertile pollen		P/e ratio	Pollen shape	Pollen size	Pollen fertility (%)
	Polar diameter(P)	Equatorial diameter(e)				
MC	39.000 ^{abc}	39.333 ^{abcd}	1.00	Spheroidal	Medium	65 ^d
CS	35.667 ^{cdefg}	35.667 ^d	1.00	Spheroidal	Medium	27 ^{jk}
IM	34.333 ^{efg}	28.000 ^e	1.22	Prolate Spheroidal	Small	14 ⁿ
CP	34.000 ^{efg}	28.667 ^e	1.18	Prolate Spheroidal	Small	45 ^{gh}
DH	42.000 ^a	38.000 ^{bcd}	1.10	Prolate spheroidal	Medium	84 ^a
HY	36.000 ^{cdef}	36.000 ^{cd}	1.00	Spheroidal	Medium	68 ^e
PBR	38.667 ^{abcd}	42.333 ^a	0.91	Oblate spheroidal	Medium	25 ^{kl}
BE	34.333 ^{efg}	28.667 ^e	1.19	Prolate spheroidal	Small	48 ^{fg}
PS	36.000 ^{cdef}	36.667 ^{bcd}	0.98	Oblate spheroidal	Medium	43 ^h
DB	34.333 ^{efg}	28.667 ^e	1.19	Prolate spheroidal	Small	23 ⁱ
PB	40.333 ^{ab}	37.667 ^{bcd}	1.07	Prolate spheroidal	Medium	73 ^b
SJ	34.667 ^{defg}	42.333 ^a	0.81	Oblate spheroidal	Medium	55 ^e
VG	33.000 ^{fg}	30.000 ^e	1.1	Prolate spheroidal	Small	30 ^{ij}

Table 27. Pollen characters of *Nymphaea* varieties (continued)

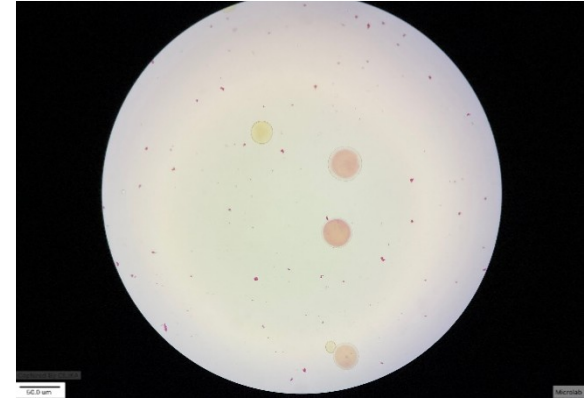
Variety	Size of fertile pollen		P/e ratio	Pollen shape	Pollen size	Pollen fertility (%)
	Polar diameter (P)	Equatorial diameter (e)				
TS	37.667 ^{bcd}	37.333 ^{bcd}	1	Spheroidal	Medium	48 ^f
SK	37.667 ^{bcd}	40.000 ^{abc}	0.94	Oblate Spheroidal	Medium	12 ⁿ
CI	36.333 ^{bcdef}	40.333 ^{ab}	0.9	Oblate spheroidal	Medium	18 ^m
SA	31.667 ^g	27.667 ^c	1.14	Prolate spheroidal	Small	18 ^m
BW	37.667 ^{bcd}	37.667 ^{bcd}	1	Spheroidal	Medium	32 ⁱ
SE(m)	1.434	1.397	-	-	-	0.01
SE(d)	2.028	1.975	-	-	-	0.014
CV	4.112	4.006	-	-	-	0.029
CD	6.842	6.857	-	-	-	5.621



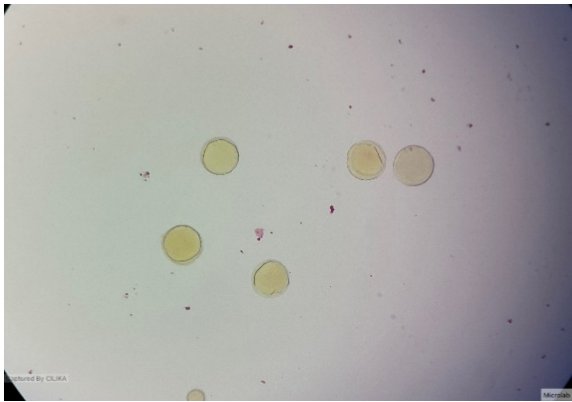
Micrantha



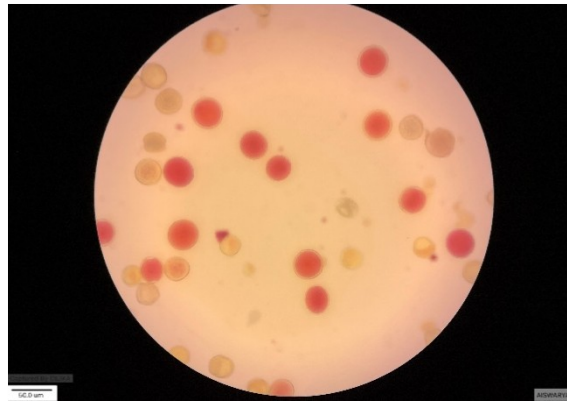
Carla's Sunshine



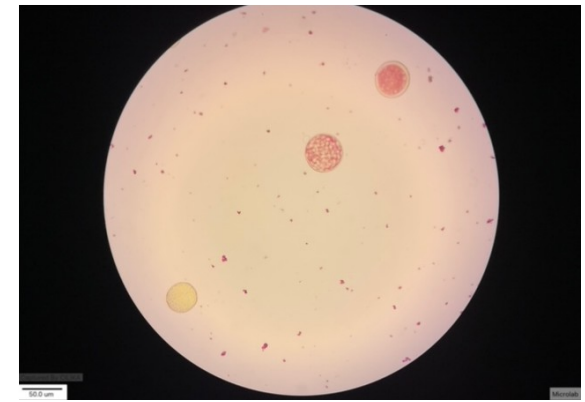
Islamorada



Chompu

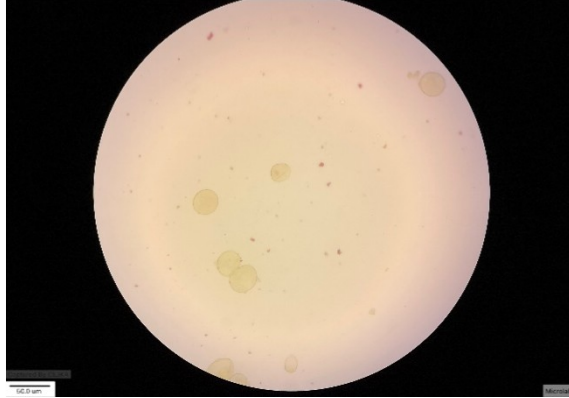


Doris Holt

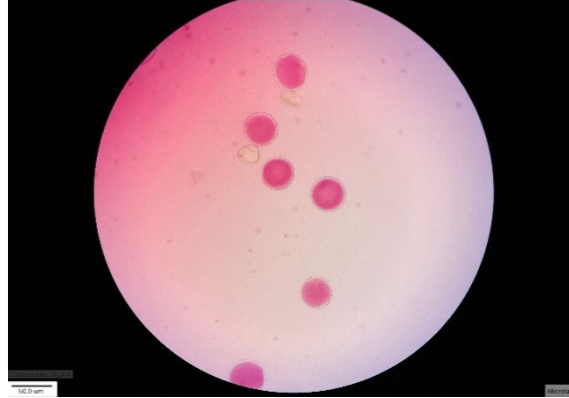


Hilary

Plate 14. Pollen grains of *Nymphaea* varieties (40X magnification)



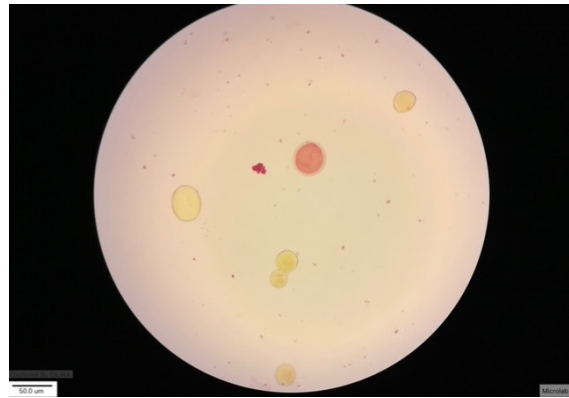
Perry's Baby Red



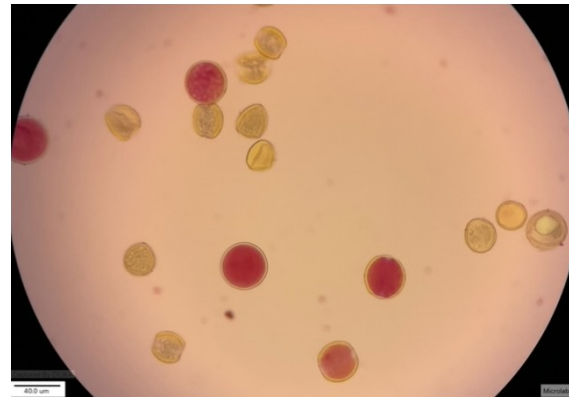
Bull's Eye



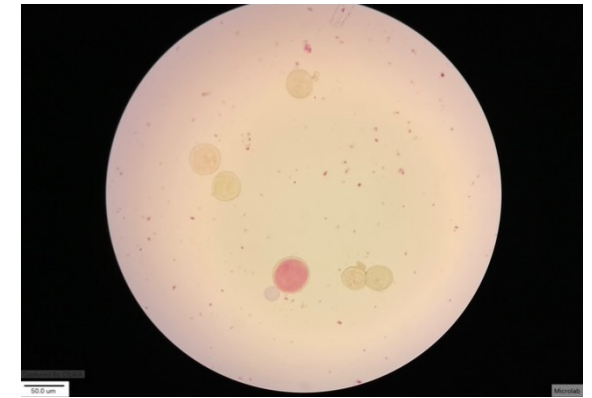
Poonsub



Dauben

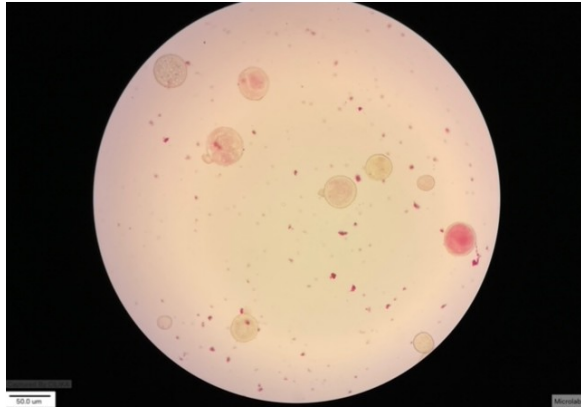


Peach Blow

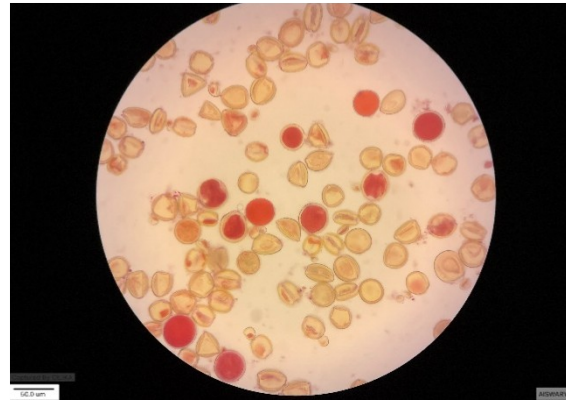


Siam Jasmine

Plate 14. Pollen grains of *Nymphaea* varieties (40X magnification)



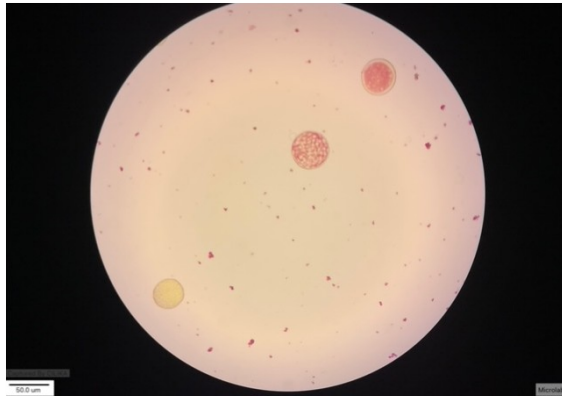
Virginalis



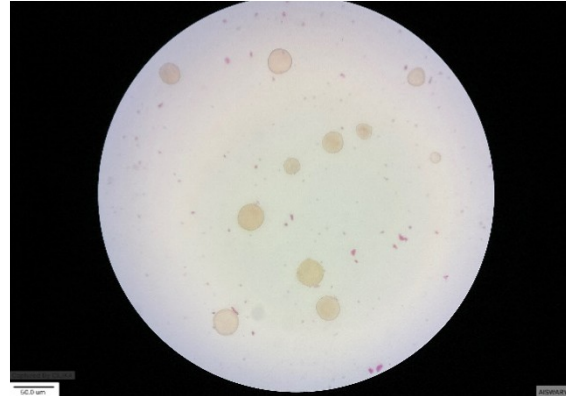
Tropic Sunset



Supranee Pink



Clyde Ikins



Siam Amber



Blue whistle

Plate 14. Pollen grains of *Nymphaea* varieties (40X magnification)

4.2.1.5. Pollen fertility

Pollen fertility value of eighteen *Nymphaea* varieties is presented in Table 27.

The variety Doris Holt had the highest pollen fertility of 84 per cent followed by Peach Blow (73%), Hilary (68%), Micrantha (65%) and Bull's Eye (48%). The lowest value was recorded for Suprannee Pink (12%), Islamorada (14%), Clyde Ikins (18%), Siam Amber (18%) and Dauben (23%). Pollen fertility of King of Siam, Taweekan, Jalueang Sub, Miami Rose, Puttaraksha, Almost Black, colorado, Wanvisa, Mexicana and Purple Joy was not calculated due to the non-availability of pollen grains during the observation period.

4.2.2. Stigma receptivity

4.2.2.1. Presence of exudates

Presence of exudate (colourless odourless stigmatic fluid) was observed in the stigmatic cup a day before the flower opening (Plate 15).

4.2.2.2. Duration of stigma receptivity

Data pertaining to the duration of stigma receptivity of twenty-one *Nymphaea* varieties are presented in Table 28. The variety Blue Whistle exhibited the maximum stigma receptivity (25 hr 30 min.) whereas the lowest was observed for Peach Blow (15 hr 45 min.).

4.2.2.3. Stigma colour

The colour of stigma in all the *Nymphaea* varieties were yellow (Plate 16).

4.2.2.4. Diameter of stigmatic cup (mm)

Data related to the diameter of stigmatic cup of twenty-one *Nymphaea* varieties are presented in Table 28.

Significantly greater stigmatic diameter was observed for the variety Bull's Eye (18.9 mm) and lowest was observed for Miami Rose (9.19 mm).



Plate 15. Exudates on stigmatic cup



Plate 16. Stigma colour of *Nymphaea* varieties

Table 28. Stigma receptivity of *Nymphaea* varieties

Variety	Duration of stigma receptivity (Initiation to end)	Duration of stigma receptivity (Total hours)	Number of stigmatic appendages/ plants	Diameter of stigmatic cup (mm)
MC	3:30 pm (Day 0) - 4:45 pm (Day 1)	25 hr 15 min.	17.05±0.58 ^c	12.80±0.19 ^{gh}
CP	4:45 pm (Day 0) - 5: 00 pm (Day 1)	24 hr 15 min.	17.19±0.37 ^c	14.44±0.09 ^e
TW	4:45 pm (Day 0) - 3:30 pm (Day 1)	17 hr 45 min	24.39±0.37 ^a	17.61±0.41 ^b
BE	5:30 pm (Day 0) - 4:45 pm (Day 1)	23 hr 15 min.	24.28±0.12 ^a	18.91±0.21 ^a
HY	5:30 pm (Day 0) - 3:45 pm (Day 1)	22 hr 15 min	17.94±0.31 ^{de}	12.02±1.25 ^{ij}
DH	4:45 pm (Day 0) - 4:50 pm (Day 1)	24 hr 5 min.	22.05±0.75 ^b	13.25±0.08 ^{fg}
CS	5:30 pm (Day 0) - 4:45 pm (Day 1)	22 hr 15 min.	17.72±1.29 ^{de}	11.80±0.31 ^{jk}
SJ	3:15 pm (Day 0) - 11:45 am (Day 1)	20 hr 30 min.	12.05±0.62 ^g	13.33±0.66 ^f
KOS	3:30 pm (Day 0) - 4:00 pm (Day 1)	24 hr 30 min.	23.94±0.61 ^a	16.53±0.34 ^c
BW	3:30 pm (Day 0) - 5:00 pm (Day 1)	25 hr 30 min.	19.75±0.44 ^c	17.86±0.12 ^b
PJ	4:45 pm (Day 0) - 5:00 pm (Day 1)	24 hr 15 min.	19.61±0.61 ^c	15.30±0.54 ^d
DB	4:45 pm (Day 0) - 3:30 pm (Day 1)	22hr 45 min.	16.80±1.79 ^e	15.66±0.16 ^d
PS	3:15 pm (Day 0) - 2:45 pm (Day 1)	23n hr 30 min.	20.11±0.86 ^c	11.80±0.19 ^{jk}

Table 28. Stigma receptivity of *Nymphaea* varieties (continued)

Variety	Duration of stigma receptivity (Initiation to end)	Duration of stigma receptivity (Total hours)	Number of stigmatic appendages/ plants	Diameter of stigmatic cup (mm)
CI	3:15 pm (Day 0) - 3:15 am (Day 1)	24 hr	9.91±0.74 ^h	15.91±0.28 ^{cd}
VG	3:15 pm (Day 0) - 2:15 am (Day 1)	23hr	10.94±0.12 ^{gh}	11.11±0.34 ^k
PB	4:45 pm (Day 0) - 1:30 pm (Day 1)	15 hr 45 min.	17.92±1.63 ^{de}	12.44±0.17 ^{hij}
IM	4:45 pm (Day 0) - 4:45 pm (Day 1)	24 hr	16.64±0.55 ^{ef}	12.33±0.08 ^{hij}
PBR	3:30 pm (Day 0) - 10:45 am (Day 1)	19 hr 15 min.	11.94±0.04 ^g	12.58±0.43 ^{ghi}
JS	5:30 pm (Day 0) - 3:45 pm (Day 1)	17hr 15 min.	19.14±1.40 ^{cd}	10.05±0.64 ^l
TS	5:30 pm (Day 0) - 5:00 pm (Day 1)	24 hr 30 min.	15.33±0.41 ^f	9.86±0.19 ^{lm}
MR	5:30 pm (Day 0)- 11:45 pm (Day 1)	18 hr 15 min.	16.91±1.55 ^e	9.19±0.41 ^m

* No flower production in Puttaraksa, Mexicana, Supranee Pink, Siam Pink, Colorado, Almost Black, Paranee and Siam Amber during the observation period.

4.2.2.5. Number of stigmatic appendages per flowers

Data related to the number of stigmatic appendages per flower of twenty-one *Nymphaea* varieties are presented in Table 28.

The lowest number of stigmatic appendages per flower was exhibited by Clyde Ikins (9.91No.) while the highest count was observed in Taweekan (24.3 No.) and was on par with Bull's Eye (24.2 No.) and King of Siam (23.9 No.).

4.2.3. Hybridization and compatibility studies

Though total number of possible crosses was 900, 316 crosses were attempted based on the simultaneous availability of receptive flowers and pollen grains. The varieties Poosub, Siam Jasmine, Siam Amber, Wanvisa and Blue Whistle with maximum fruit set were selected as female parents and Doris Holt, Peach Blow, Hilary, Micrantha and Bull's Eye based on pollen fertility were selected as male parents for the compatibility study. The data regarding the preliminary hybridization is given in Table 29.

The compatibility among the selected pollen and ovule parents are given in Table 30. Poosub was cross compatible with Doris Holt, Peach Blow and Micrantha while it was cross incompatible with Hilary and Bull's Eye. Wanvisa was cross compatible with Doris Holt, Peach Blow, Hilary and Micrantha but cross incompatible with Bull's Eye. Siam Jasmine exhibited cross-compatibility with Doris Holt, Peach Blow, Micrantha, and Bull's Eye, but it was found to be incompatible in crosses with Hilary. Siam Amber showed cross-compatibility only with Doris Holt, but it was cross incompatible with Peach Blow and Micrantha. Siam Amber could not be crossed with Hilary and Bull's Eye due to the lack of simultaneous availability of pollen and receptive stigmas. Blue Whistle showed cross-compatibility with Doris Holt and Peach Blow, but it exhibited cross-incompatibility with Hilary, Micrantha and Bull's Eye.

4.2.3.1. Number of flowers pollinated

The number of flowers pollinated was represented in Table 31.

The maximum number of flowers were pollinated in the cross Poosub x Doris Holt (7 No.) and Blue Whistle x Doris Holt (7 No.). The minimum number

of flowers were pollinated between the varieties Siam Amber x Micrantha (1 No.) and Siam Amber x Peach Blow (1 No.). Pollination was not attempted between Siam Amber x Hilary, Siam Amber x Bull's Eye and Wanvisa x Bull's Eye due to the non-availability of pollen grains and receptive stigma simultaneously.

4.2.3.2. Number of successful crosses

The number of successful crosses achieved after pollination are presented in Table 32.

The success rate of pollination was highest for the cross between Siam Amber and Doris Holt. Three flowers were crossed and fruit set was observed in all the three flowers. Five flowers were pollinated between Poonsub and Micrantha and four among them produced fruits. Similarly, Wanvisa and Doris Holt exhibited four successful crosses among the five flowers pollinated. The rate of successful crosses was low in Poonsub x Hilary, Poonsub x Bull's eye, Wanvisa x Bull's Eye, Siam Jasmine x Hilary, Siam Amber x Peach Blow, Siam Amber x Hilary, Siam Amber x Micrantha, Siam Amber x Bull's Eye, Blue Whistle x Hilary, Blue Whistle x Micrantha and Blue Whistle x Bull's Eye.

Table 29. Preliminary hybridization between *Nymphaea* genotypes (Continued)

♂	MC	CP	CI	BE	CS	PS	BW	DH	SK	PR	PB	IM	SA	SJ	PBR	MR	TS	VN	WV	DB	HY
♀																					
PBR	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1	-	-	-	-
JS	-	-	-	-	-	-	-	3	-	-	3	-	-	-	-	1	3	-	-	2	1
CI	-	-	-	1	-	1	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-
SK	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-
MR	-	-	-	-	-	-	-	3	-	-	1	-	-	-	-	1	2	1	-	-	-
TS	-	-	-	-	-	-	-	2	-	-	2	-	-	-	4	-	1	-	-	1	1
VN	-	1	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-
WV	-	-	-	-	-	1	-	1	-	-	-	1	-	-	-	-	-	-	-	-	2
DB	-	-	-	2	-	1	-	3	-	-	1	-	-	-	-	-	-	-	-	-	1

Table 30. Compatibility among selected *Nymphaea* genotypes

♂/♀	Doris Holt	Peach Blow	Hilary	Micrantha	Bull's Eye
Poonsub	CC	CC	CI	CC	CI
Wanvisa	CC	CC	CC	CC	NA
Siam Jasmine	CC	CC	CI	CC	CC
Siam Amber	CC	CI	NA	CI	NA
Blue Whistle	CC	CC	CI	CI	CI

4.2.3.3. Percentage of fruit set

The percentage of fruit set in successful crosses are represented in Table 33.

Siam Amber x Doris Holt exhibited highest percentage of fruit set (100%) followed by Poonsub x Micrantha (80%), Wanvisa x Doris Holt (80%) and Siam Jasmine x Doris Holt (66.66%). No fruit set was observed in the crosses Poonsub x Hilary, Poonsub x Bull's eye, Wanvisa x Bull's Eye, Siam Jasmine x Hilary, Siam Amber x Peach Blow, Siam Amber x Hilary, Siam Amber x Micrantha, Siam Amber x Bull's Eye, Blue Whistle x Hilary, Blue Whistle x Micrantha and Blue Whistle x Bull's Eye.

4.2.3.4. Number of days taken for fruit set

The number of days required for a successful cross to set fruit is represented in Table 34.

Maximum number of days for fruit set was observed for the cross Blue Whistle x Peach Blow (43 days) followed by Siam Amber x Doris Holt (41 days) and Poonsub x Micrantha (41 days). Minimum days for fruit set was observed in Wanvisa x Micrantha (19 days).

4.2.3.5. Fruit weight (g)

Data regarding the fruit weight is represented in Table 35. The weight of the fruit ranges from lowest for Poonsub x Micrantha (7.02 g) and highest for Siam Amber x Doris Holt (13.01 g).

4.2.3.6. Fruit development pattern

After hybridization the flower and its pedicel bend downward into the water, and within four days an 'S'-shaped pedicel formation was observed in successful crosses (Plate 17).

Table 31. Number of flowers pollinated

♂/ ♀	Doris Holt	Peach Blow	Hilary	Micrantha	Bull's Eye
Poonsub	7	4	3	5	4
Wanvisa	5	3	2	2	0
Siam Jasmine	6	4	4	5	3
Siam Amber	3	1	0	1	0
Blue Whistle	7	5	5	6	3

Table 32. Number of successful crosses

♂	Doris Holt	Peach Blow	Hilary	Micrantha	Bull's Eye
♀					
Poonsub	4	2	0	4	0
Wanvisa	4	1	1	1	0
Siam Jasmine	4	2	0	3	1
Siam Amber	3	0	0	0	0
Blue Whistle	3	2	0	0	0

Table 33. Percentage of fruit set in *Nymphaea* genotypes.

♂	Doris Holt	Peach Blow	Hilary	Micrantha	Bull's Eye
♀					
Poonsub	57.14	50	0	80	0
Wanvisa	80	33	50	50	0
Siam Jasmine	66.66	50	0	60	33.33
Siam Amber	100	0	0	0	0
Blue Whistle	42.85	40	0	0	0

4.2.3.7. Number of seeds per fruits

Data regarding the number of seeds per fruits is represented in Table 35. Maximum number of seeds were produced by Poonsub x Micrantha (295 No.) followed by Blue Whistle x Hilary (186 No.) and Hilary x Micrantha (142 No.). The minimum number of seeds per fruit was observed in Wanvisa x Peach Blow (89 No.).

4.2.3.8. Seed size

Data regarding the seed size is represented in Table 35. The length of seed was observed lowest in Blue Whistle x Hilary (1.22 mm) and highest in Siam Amber x Doris Holt (1.57 mm). Blue Whistle x Hilary exhibited lowest seed breadth (0.90 mm) while Siam Amber x Doris Holt recorded highest leaf breadth (1.06 mm).

4.2.3.9. Number of days taken for germination

Data on number of days taken by the hybrids to germinate is presented in Table 36. The hybrid 1(Poonsub x Micrantha) and hybrid 2 (Poonsub x Peach Blow) took 8 days for germination while hybrid 3 (Poonsub x Micrantha) took only 7 days for germination.

4.2.3.10. Germination (%)

Data regarding the percentage of germination is presented in Table 36.

Germination percentage of the hybrid 2 (Poosub x Peach Blow) was 16.3% while hybrid 3 (Poosub x Micrantha) had 19.3% germination. The hybrid 1 (Poosub x Micrantha) had highest germinability of 21.5%.

Even though the plant produced more number of seeds, the rate of germination was low. The hybridized flower took 25 days for fruit set and the flowering took place after 28- 45 days from seed set. Most of the characters expressed by the hybrid were intermediate of both the parents (either lower or higher than the parent). The data regarding the vegetative and floral characters of F1 hybrid (Poosub x Micrantha) and their parents are given in Table 37. and F1 hybrid (Poosub x Peach Blow) are given in Table 38.

Table 34. Number of days taken for fruit set in *Nymphaea* genotypes

♂	Doris Holt	Peach Blow	Hilary	Micrantha	Bull's Eye
♀					
Poosub	35	30	0	41	0
Wanvisa	27	24	0	19	0
Siam Jasmine	25	33	29	38	29
Siam Amber	41	0	0	0	0
Blue Whistle	39	43	0	0	0



Plate 17. Fruit development pattern of *Nymphaea* varieties



Plate 18. Viviparous production of plantlets

Table 35. Fruit and seed character of *Nymphaea* genotypes

Variety	Fruit weight (g)	No: of seeds/ fruit	Seed length (mm)	Seed breadth (mm)
Cross 1(PS x MC)	7.02	295	1.36	0.91
Cross 2 (SJ x DH)	12	95	1.46	1.02
Cross 3 (WV x PB)	11.3	89	1.48	1.03
Cross 4 (SA x DH)	13.01	107	1.57	1.06
Cross 5 (HY x MC)	8.09	142	1.29	0.93
Cross 6 (CI x DH)	10.9	96	1.43	1.03
Cross 7 (BW x HY)	8.02	186	1.22	0.90

Table 36. Germination of *Nymphaea* genotypes

Variety	Germination (%)	Number of days taken for germination
Hybrid 1 (Poosub × Micrantha)	21.5	8
Hybrid 2 (Poosub × Peach Blow)	16.3	8
Hybrid 3 (Poosub × Micrantha)	19.3	7

Table 37. Vegetative and floral characters of parental plants (Poosub x Micrantha) and F₁ hybrid of *Nymphaea*

Characters	Ovule Parent (Poosub)	Pollen Parent (Micrantha)	F1 hybrid
Shape of leaf	Ovate	Ovate	Ovate (AB)
Shape of leaf margin	Toothed	Toothed	Toothed (AB)
Overlapping of leaf base	Present	Absent	Absent (A)
Striations on leaf	Absent	Absent	Absent (AB)
Leaf venation on lower surface	Non prominent	Prominent	Non prominent (A)
Leaf hairs on lower surface	Absent	Absent	Present (D)
Hairs on petiole	Absent	Absent	Absent (AB)
Petiole colour	Strongish yellowish green (RHS 144A-117)	Moderate reddish orange (RHS 173B-37)	Moderate olive brown (RHS 199A-95) (D)
Colour of upper leaf	Brilliant yellowish green (RHS 142B-116)	Brilliant yellowish green (RHS 142B-116)	Brilliant yellowish green (RHS 150C-116) (AB)
Colour of lower leaf	Light yellowish green (RHS 142C-119)	Strong purplish red (RHS 72A-255)	Deep purple (RHS 79C-219) (D)
No: of days from visual appearance to full expansion of leaf lamina (Days)	9- 12	7- 8	8- 11(D)
Longevity of leaf (Days)	19- 32	21- 26	22- 27 (D)
Pedicel colour	RHS 144D- Light yellowish green	RHS 185C- Moderate purplish red	RHS 150A- Brilliant yellowish green (D)

Table 37. Vegetative and floral characters of parental plants (Poosub x Micrantha) and F₁ hybrid of *Nymphaea* (Continued)

Characters	Ovule Parent (Poosub)	Pollen Parent (Micrantha)	F1 hybrid
Stamen colour	Vivid yellow (RHS 13A- 82) and light greenish yellow (RHS 8C-101)	Brilliant yellow (RHS 9C-83) and very pale violet (RHS 91D- 213)	Vivid yellow (9B-82), Light blue (RHS 108D-181) (D)
Field life of flower	3-4	3-4	3-4 (AB)
Sepal shape	Ob lanceolate	Ob- lanceolate	Ob- lanceolate (AB)
Colour of inner petal	Light yellowish green with pale purplish blue towards the tip	Very pale purplish blue with white shade towards the centre	Greenish white (RHS 192D-153) with very pale blue tips (D)
Colour of outer petal	Light yellowish green with very pale purplish blue	Very pale purplish blue with white shade towards the centre	Greenish white (RHS 192D-153) with very pale blue tips (D)
Petal apex	Acute	Obtuse	Obtuse (B)
Stigma colour	Yellow	Yellow	Yellow (AB)
Anthesis time	7:15 am to 6:15 pm	7:00 am to 5:45 pm	8:45 am to 4:45 pm (D)
Stigma receptivity	3:15 pm (Day 0) - 2:45 pm (Day 1)	3:30 pm (Day 0) - 4:45 pm (Day 1)	3:45 pm (Day 0) – 12:45 pm (Day 1) (D)
Days to appearance of flower bud (days)	8- 11	7- 10	10- 11 (D)
Days to flower opening	2- 3	1- 2	2- 3 (A)
No: of stigmatic appendages/ flower	19- 23	14- 18	11-17 (D)
Stigmatic cup (mm)	10- 15	12- 15	7- 9 (D)
No: of flowers/ plant/ month	2-7	5- 9	2-5 (D)
Length of stamen (cm)	2.1- 2.6	1.7- 2.2	1.5- 2 (D)

Table 37. Vegetative and floral characters of parental plants (Poosub x Micrantha) and F₁ hybrid of *Nymphaea* (Continued)

Characters	Ovule Parent (Poosub)	Pollen Parent (Micrantha)	F1 hybrid
No: of stamen	65- 75	68- 106	57- 84 (D)
Number of Sepals	4	4	4 (AB)
Length of sepal (cm)	3.5- 5.7	3.8- 4.9	2.7- 3.5 (D)
Width of sepal (cm)	1.5- 2.7	1.7- 2.1	1.2- 1.6 (D)
Number of petals	20- 33	14- 17	14- 21 (D)
Length of petal (cm)	3.4- 5.1	3.6- 4.5	2.5- 3.3 (D)
Width of petal (cm)	1.2- 2.1	1.2- 1.8	0.7- 1 (D)
Flower diameter (cm)	7.7- 11	7.2- 9	4.8- 7.2 (D)
Pedicel diameter (cm)	1.8- 2.4	1.9- 2.5	1.4- 1.8 (D)
Length of flower bud (cm)	4- 5.3	4.3- 4.9	3.5- 4.1 (D)
Petiole diameter (cm)	1.5- 1.9	1.3- 2	1.2- 1.6 (D)

A- Character of ovule parent B- Character of pollen parent D- Unique character (Either lower or higher than the parents)

Table 38. Vegetative and floral characters of parental plants (Poosub x Peach Blow) and F₁ hybrid of *Nymphaea*

Characters	Ovule Parent (Poosub)	Pollen parent (Peach Blow)	F1 hybrid
No: of days from visual appearance to full expansion of leaf lamina (Days)	9- 12	8- 9	7- 9 (D)
No: of stigmatic appendages/ flower	19- 23	14- 21	19-20 (D)
Stigmatic cup (mm)	10- 15	12- 14	10-11 (D)
No: of flowers/ plant/ month	2-7	2-9	2-4 (D)
Length of stamen (cm)	2.1- 2.6	2.2- 2.8	1.5- 2.5 (D)
No: of stamen	65- 75	68- 109	77- 99 (D)
Number of sepals	4	4	4 (AB)
Length of sepal (cm)	3.5- 5.7	4.9- 5.6	4.4- 5.2 (D)
Width of sepal (cm)	1.5- 2.7	1.7- 2.4	1.7- 2.2 (D)
Number of petals	20- 33	15-19	26- 31 (D)
Length of petal (cm)	3.4- 5.1	4.4- 5.5	4- 5.1 (D)
Width of petal (cm)	1.2- 2.1	1.2- 1.5	1.1- 1.4 (D)

Table 38. Vegetative and floral characters of parental plants (Poosub x Peach Blow) and F₁ hybrid of *Nymphaea* (Continued)

Characters	Ovule Parent (Poosub)	Pollen parent (Peach Blow)	F1 hybrid
Flower diameter (cm)	7.7- 11	8.5- 10.5	8.3- 10.3 (D)
Pedicel diameter (cm)	1.8- 2.4	2.5- 2.6	1.7- 2 (D)
Length of flower bud (cm)	4- 5.3	4.5- 5.9	4.2- 5.8 (D)
Petiole diameter (cm)	1.5- 1.9	1.9- 2.3	1.5- 2.1 (D)
Shape of leaf	Ovate	Ovate	Ovate (AB)
Shape of leaf margin	Toothed	Toothed	Toothed (AB)
Overlapping of leaf base	Present	Absent	Present (A)
Striations on leaf	Absent	Present	Absent (A)
Leaf venation on lower surface	Non prominent	Non prominent	Prominent (D)
Leaf hairs on lower surface	Absent	Absent	Present (D)
Hairs on petiole	Absent	Absent	Absent (AB)
Petiole colour	Strongish yellowish green (RHS 144A- 117)	Strong yellowish green (RHS 143C- 117)	Strong yellowish green (RHS 141D-117) (AB)
Colour of upper leaf	Brilliant yellowish green (RHS 142B- 116)	Strong yellowish green (RHS 144C- 117)	Brilliant yellowish green (RHS 150C- 116) (A)
Colour of lower leaf	Light yellowish green (RHS 142C- 119)	Strong yellowish green (RHS 144C- 117) with Deep reddish-purple spots (RHS 77A- 238)	Deep purple (RHS 79C- 219) (D)
Pedicel colour	RHS 144D- Light yellowish green	RHS 150A- Brilliant yellowish green	Strong yellowish green (RHS 141D-117) (D)
Stamen colour	Vivid yellow (RHS 13A- 82) and light greenish yellow (RHS 8C- 101)	Brilliant greenish yellow (RHS 4A- 98) and very pale purple (RHS 84D- 226)	Strong purple (RHS 77B-218), Brilliant yellow (9C-83) (D)

Table 38. Vegetative and floral characters of parental plants (Poosub x Peach Blow) and F₁ hybrid of *Nymphaea* (Continued)

Characters	Ovule Parent (Poosub)	Pollen parent (Peach Blow)	F1 hybrid
Field life of flower	3-4	3-4	3-4 (AB)
Sepal shape	Ob lanceolate	Linear lanceolate	Linear lanceolate (B)
Colour of inner petal	Light yellowish green with pale purplish blue towards the tip	Very pale purple	Very pale purple (RHS 76D- 226) (D)
Colour of outer petal	Light yellowish green with very pale purplish blue	Very pale purple	Very pale purple (RHS 76D- 226) (B)
Petal apex	Acute	Obtuse	Acute (A)
Stigma colour	Yellow	Yellow	Yellow (AB)
Anthesis time	7:15 am to 6:15 pm	9:15 am to 5:30	9:15 am to 6:45 pm (D)
Stigma receptivity	3:15 pm (Day 0) - 2:45 pm (Day 1)	4:45 pm (Day 0) - 1:30 pm (Day 1)	3:30 pm (Day 0) – 3:15 pm (Day 1) (D)
Days to appearance of flower bud (Days)	8- 11	8- 12	10- 11 (D)
Days to flower opening	2- 3	1- 2	2-3 (A)
No: of days from visual appearance to full expansion of leaf lamina (Days)	9- 12	8- 9	7- 9 (D)
Longevity of leaf (Days)	19- 32	19- 34	21- 32 (D)
No: of stigmatic appendages/ flower	19- 23	14- 21	19-20 (D)
Stigmatic cup (mm)	10- 15	12- 14	10-11 (D)

Table 38. Vegetative and floral characteristic of parental plants (Poosub x Peach Blow) and F₁ hybrid of *Nymphaea* (Continued)

Characters	Ovule Parent (Poosub)	Pollen parent (Peach Blow)	F1 hybrid
No: of flowers/ plant/ month	2-7	2-9	2-4 (D)
Length of stamen (cm)	2.1- 2.6	2.2- 2.8	1.5- 2.5 (D)
No: of stamen	65- 75	68- 109	77- 99 (D)
Number of Sepals	4	4	4 (AB)
Length of sepal (cm)	3.5- 5.7	4.9- 5.6	4.4- 5.2 (D)
Width of sepal (cm)	1.5- 2.7	1.7- 2.4	1.7- 2.2 (D)
Number of petals	20- 33	15-19	26- 31 (D)
Length of petal (cm)	3.4- 5.1	4.4- 5.5	4- 5.1 (D)
Width of petal (cm)	1.2- 2.1	1.2- 1.5	1.1- 1.4 (D)
Flower diameter (cm)	7.7- 11	8.5- 10.5	8.3- 10.3 (D)
Pedicel diameter (cm)	1.8- 2.4	2.5- 2.6	1.7- 2 (D)
Length of flower bud (cm)	4- 5.3	4.5- 5.9	4.2- 5.8 (D)
Petiole diameter (cm)	1.5- 1.9	1.9- 2.3	1.5- 2.1 (D)

A- Character of ovule parent B- Character of pollen parent D- Unique character (Either lower or higher than the parents)

5. DISCUSSION

The present study was conducted to evaluate waterlily genotypes for growth, flowering and floral attributes, assessment of their compatibility with varieties having breeding potential and production of new *Nymphaea* varieties through inter-varietal hybridization.

5.1. EXPERIMENT 1: COLLECTION AND CHARACTERIZATION OF NYMPHAEA GENOTYPES

Nymphaea is a complex genus of aquatic plants in which limited research has been conducted. Performance evaluation of thirty *Nymphaea* genotypes collected from all over India was undertaken. The genotypes were characterized based on leaf structure, flower features, and reproductive biology. The result obtained from the study are discussed below.

5.1.1. Variability in vegetative quantitative and qualitative characters

The varieties varied significantly with respect to the vegetative quantitative characters.

All the varieties had floating leaves which were initially present under water with their lamina curled. The lamina bent over at a right angle to the petiole and unfolded when the leaves reached the water surface and emerged in a nearly upright position. The number of days from visual appearance to full expansion of leaf lamina was lowest in Puttaraksa (6.5 days) and highest in Peach Blow (13.2 days). Tom (2015) recorded 3 to 7 days for the emergence to unrolling of the leaf among waterlily accessions. The length of lamina was minimum in Wanvisa (10.84 cm) and maximum in Siam Pink (21.28 cm). The genotype Wanvisa (9.87 cm) exhibited the lowest lamina width while Purple Joy (20.59 cm) reported the maximum. The number of leaves per plant was observed lowest in Bull's Eye (10.05 cm) and highest in Wanvisa (29.4 cm).

The leaves turned yellow and decayed after a certain period. Jalueang Sub reported minimum longevity of leaves (21.3 days) while Wanvisa had the highest longevity (35.2 days). Several studies reported longevity of leaves in various

genera of the order Nymphaeales. Twilley *et al.* (1985) reported 31 days longevity for the leaves of *Nuphar advena* whereas longevity of *Nuphar japonica* leaves was reported as 40 days by Aramaki *et al.* (1989). In *Nymphaea tetragona* 21 to 55 days were reported as floating life span of the leaves by Kunii and Aramaki (1992).

Leaf shape of the varieties was either round or ovate with a cleft near the center where the petiole is attached. Round-shaped leaf was present in Siam Amber, Hilary, Doris Holt, Siam Jasmine, Puttaraksa, Wanvisa, Mexicana, Clyde Ikins, Virginalis, Supranee Pink, Colorado, Almost Black and Perry's Baby Red while ovate shaped leaves in Micrantha, Chompu, Taweekan, Bull's Eye, Carla's Sonshine, King of Siam, Blue Whistle, Purple Joy, Dauben, Poosub, Peach Blow, Islamorada, Siam Pink, Jalueang Sub, Tropic Sunset, Miami Rose and Paranee. Leaf shape of *Nymphaea mexicana* was reported as ovate to elliptic by Woods (2003).

Leaf margins were either toothed/ serrated or entire/smooth. All the tropical varieties had toothed leaf margin whereas hardy varieties had smooth leaf margins. The leaf margins of Micrantha, Chompu, Taweekan, Bull's Eye, Hilary, Doris Holt, Carla's Sonshine, King of Siam, Blue Whistle, Purple Joy, Dauben, Poosub, Peach Blow, Jalueang Sub, Tropic Sunset, and Miami Rose were toothed and the varieties Siam Jasmine, Puttaraksa, Wanvisa, Mexicana, Clyde Ikins, Virginalis, Supranee Pink, Islamorada, Siam Pink, Colorado, Almost Black, Siam Amber, and Perry's Baby Red exhibited smooth leaf margins. Conard (1905) reported sinuate-dentate leaf margin in *N. gigantea* and the Lotus group, entire margin in *N. amazonum* and *N. elegans*, deeply sinuate in *N. rudgeana*, *N. ampla*, *N. capensis*, and *N. zanzibariensis*. Tom (2015) observed sinuately dentate and irregularly sinuate leaf margin in *Nymphaea* accessions.

Leaves were two lobed and at the base, the two lobes overlapped or just touched or spread apart based on the variety. Overlapping of the leaf base was found in the varieties Taweekan, Bull's Eye, Doris Holt, Carla's Sonshine, King of Siam, Blue Whistle, Poosub, Siam Pink, Supranee Pink, Jalueang Sub, Tropic

Sunset, Miami Rose, and Almost Black while it was absent in other varieties. Sinus overlapping was observed in *N. pubescence* by Manju (2018).

Nymphaea genotypes under the study exhibited eight colour shades on the upper surface of the leaves. The shades observed are brilliant yellowish green (RHS 142B), strong yellowish green (RHS 143B), dark purple (RHS 79B), strong yellowish green (RHS 143B), deep yellowish green (RHS 141A), strong yellowish green (RHS 143B) with moderate purple spots (RHS 79D), moderate yellowish green (RHS 139B), strong yellowish green (RHS 143A) with dark purple spots (RHS 79A), dark red (RHS 187A) with a greyish yellowish green (RHS 191B) hue on the upper surface of the leaves. The lower leaf surface also exhibited eight colour shades. They are strong purplish red (RHS 72A), moderate yellowish green (RHS 137D), light yellowish green (RHS 145B) with deep purple (RHS 79B) streaks, light yellowish green (RHS 142C), strong yellowish green (RHS 144C), moderate purplish red (RHS 70A), greyish red (RHS 182B), greyish red (RHS 182B) and dark red streaks (RHS 187A), deep purple (RHS 79C), moderate purplish red (RHS 185C) with dark red streaks (RHS 187A), greyish-yellowish green (RHS 191B) with dark purple streaks (RHS 79A). Conard (1905) reported that rich chlorophyll-green is the usual colour of the upper surface of the leaves in most of the *Nymphaea* species. However, in *N.flava*, *N.mexicana*, *N.tetragona*, *N.rubra-rosea*, *N.amazonum* and *N.zanzibariensis* there were more or less brownish-red blotching, and in *N. rubra* the whole leaf was of a bronzy-red hue. The under surface of the leaf was reported as dull green in *N. Tuberosa*, reddish to dark crimson-brown in *N.odorata*, *N.alba*, *N.tetragona* and *N.flava*, dull brownish in the Lotus group, and blue-purple in *N. zanzibariensis* and *N.gigantea*. Dark brownish or blackish blotches in *N.elegans* and *N.amazonum*. Variation in the upper and lower leaf colour of *Nymphaea* accessions was reported also by Tom (2015). Abaxial leaf surface of a white colour variant of *N.nouchali* was reported as light green with purple by Fahida (2010) but it was reported as coffee brown or uniform violet by Manju (2018).

Irregular moderate purplish red striations were present on the leaf lamina of Hilary, Doris Holt, Carla's Sonshine, Wanvisa, Peach Blow, Siam Pink,

Jalueang Sub and Miami Rose while in all other varieties striations were absent. Prominent leaf venation with a midrib and pinnate branching was observed in Micrantha, Chompu, Taweekan, Bull's Eye, Doris Holt, King of Siam, Blue Whistle, Purple Joy, Clyde Ikins, Islamorada, Siam Pink, Supranee Pink and Almost Black and it was non-prominent in all other varieties. Similar to this Tom (2015) reported prominent and non-prominent leaf venation in five selected *Nymphaea* accessions.

Petioles of the genotypes were long, slender and submerged in water with lamina floating on the surface of the water. Petioles were cylindrical near their ends and top side was slightly flattened. The lowest petiole circumference was exhibited by Mexicana (1.19 cm) and the highest by Tropic Sunset (2.39 cm). Also, the lowest petiole diameter was exhibited by Supranee Pink (0.36 cm) and the highest by King of Siam (0.92 cm). Depending on the depth of water, petiole length was found to vary. The lowest petiole length was exhibited by Mexicana (15 cm) and the highest by Siam Pink (38.5 cm). Dassanayake (1996) reported similar morphology for *N. nouchali*.

Variability in the colour of the petiole was observed in the present study. The colours include moderate reddish orange, brilliant greenish yellow, dark reddish-orange, strong yellowish green, moderate yellowish green, brilliant yellowish green, moderate olive brown and moderate reddish orange. Terete, glabrous, brownish red petioles were observed in a hybrid of Sri Lankan violet waterlily by Yakandawala *et al.* (2017). Fahida (2021) reported glabrous, brownish green colour petioles in blue and white variants of *N. nouchali*.

The presence of hairs on the lower leaf surface and petioles were not observed in most of the varieties. The varieties Siam Jasmine, Clyde Ikins, Supranee Pink, Paranee and Siam Amber exhibited short hairs under the leaf surface and petiole.

Some of the varieties under study exhibited vivipary, the production of plantlets from their leaves (Plate 18). The varieties Micrantha, Chompu, Hilary, Doris Holt, Carla's Sonshine, Blue Whistle, Purple Joy, Peach Blow and Islamorada were viviparous. The viviparous plantlets matured in 20 to 30 days

and it is observed as a useful trait in the quick propagation of such varieties. Molecular basis of viviparity in *N. micrantha* was studied by Su *et al.* (2022) concluded that the variation in expression of various genes indicated their role in viviparity. Major genes involved in plant hormone signal transduction were expressed more often in *N. micrantha*. The regulation of genes related to cell division, differentiation and elongation revealed a correlation with viviparity. There was a difference in the expression of genes linked to the light harvesting chlorophyll complex, photosystem I and photosystem II between viviparous *N. micrantha* and non-viviparous *N. colorata*.

Variations were found in the quantitative and qualitative characters studied. Considerable variations in morphological characters were also reported among *Nymphaea* species by Borsch *et al.* (2007).

5.1.2. Variability in floral quantitative and qualitative characters

Three buds from each replication were tagged when they first appeared at the bottom of the plant on the surface of mud. The tagged buds were observed until they reached the water surface and the number of days taken were recorded. The floral bud appeared broad and rounded unlike the leaf rudiment which appeared in a more conical form. Flowers were produced in the axil of leaves. The rise of the flower bud from the rhizome to the surface of the water takes place in the same manner as that of the emerging to the unfurling of the leaf. The variety Blue Whistle (7.22 days) took minimum number of days while Carla's Sonshine (10.5 days) took maximum number of days for the flower bud to reach the water surface. After the visual appearance of the bud on the water surface, Bull's Eye, Blue Whistle and Carla's Sonshine took the minimum number of days (1.66) for its development into the flower whereas maximum days were taken by Poonsub (2.77 days). Povilus *et al.* (2015) reported that flower buds in *N. thermarum* originated from the shoot apical meristem situated at ground level and it took around 12 days from emergence to full bloom.

Maimi rose (3.95 cm) had the shortest bud length while Hilary (5.04 cm) possessed the longest buds. The flower diameter was lowest for Miami Rose (7.37 cm) and highest for King of Siam (11.07 cm). Though the bud size was 4.76 cm in

King of Siam, it exhibited the largest flowers. The buds were more rounded and had large number of petals and the area of stamens were occupied with petals.

A seasonal variation in flower production was observed among the varieties. For tropical waterlilies maximum flower production was observed from February to May. During the period from December to January, there was a decrease in the production of flowers. The peak flowering period of *N. micrantha* and *N. nouchali* was observed during September and November by Fahida (2022). The maximum number of flowers per plant per month was observed in the tropical varieties *viz.* Dauben (9.30 No.) followed by Blue Whistle (8.30 No.), Micrantha (8.00 No.), Tropic Sunset (7.30 No.), King of Siam (7.00 No.) and Doris Holt (6.00 No.). Fahida, 2012 reported the seasonal influence on flower production of *Nymphaea* spp. The hardy varieties were reported to produce fewer blossoms compared to tropical waterlilies (Anbumani *et al.*, 2022). In the present study also flower production of hardy varieties was low compared to tropical varieties, but their maximum flower production was observed during April, May, September and October. Hardy varieties *viz.* Siam Jasmine (2.66 No.), Siam Amber (1.33 No.), Siam Pink (0.66 No.), Wanvisa (1.66 No.), Mexicana (1.33 No.), Paranee (1 No.), Colorado (1 No.), Clyde Ikins (2 No.), Perry's Baby Red (3 No.) and Supranee Pink (1 No.) produced a lesser number of flowers. Puttaraksa and Almost Black did not produce flowers during the observation period.

All the varieties exhibited 3 to 4 days of field life for flowers. The flowers appeared to be in the female phase during the first day which was indicated by the presence of stigmatic fluid while it was in the male phase for the remaining three days which was evident by the presence of pollen grains. Fahida (2022) reported that the flowers of *N. micrantha* lasted for three days whereas in *N. nouchali* it lasted for four days.

The blooming time of the flowers started between 7:00 am to 10: 00 am and closed between 1:00 pm to 6:15 pm. Most of the hardy varieties exhibited early closing of flowers. The opening and closing behaviour of the blooms differed among the varieties and it took nearly 25 to 35 minutes for the complete opening and 30 to 40 minutes for the complete closing of the blooms. Fahida

(2012) reported that *N. nouchali* took 15 to 20 minutes for full blooming and 10 to 15 minutes for full closing. It was observed that the time and duration of anthesis was highly influenced by the environmental factors. The effect of temperature was greater than sunlight. Similar result was reported by Prance and Anderson (1976). Rainy and cloudy days from June to October caused delayed opening and early closing of the flowers. Effects of low light intensity and heavy rainfall on field life of the flowers and change in the duration of flower opening and closing was also observed by Tom (2015). Ki *et al.* (2018) studied the transcriptome profiles of the intermediate cells of petals in the waterlily, which confirmed that auxin metabolism, signaling and transport played center roles in flower opening and closure, and cell wall remodeling event regulated by auxin caused circadian cell expansion and shrinkage associated with flower movement.

Varieties Micrantha, King of Siam, Blue Whistle, Dauben and Poosub exhibited early flower opening (7:00 am to 7:30 am) and longest anthesis time. The varieties like Siam Jasmine, Virginalis, Supranee Pink, Paranee and Mexicana exhibited minimum period of anthesis. It was evident that tropical varieties exhibited long anthesis time compared to hardy varieties. Watson (1884) reported that certain *Nymphaea* species displayed a short blooming period, lasting merely two or three consecutive days, while others had an extended duration of six or seven days. Schneider (1982) documented that day-blooming *N. elegans* opened at 9 am for three consecutive days. According to Jacobs and Porter (2007), within the *Hydrocallis* subgenera, it was observed that the night blooming flowers opened at 7 pm and closed at 10 am. Manju (2018) reported that in night-blooming waterlilies the flowers opened at 7:00 pm and 7.30 pm and closed by the next day at 9:00 am and 12.30 pm respectively.

All the varieties used in the experiment exhibited four sepals except 'King of Siam' and 'Taweekan' which had six sepals. The shortest sepal was observed in Tropic Sunset (3.56 cm) and the longest in Peach Blow (4.97 cm). Lowest sepal width was recorded by the variety Islamorada (1.57 cm) and highest in Clyde Ikins (2.42 cm). The number of petals was lowest in Hilary (15.8 No.) and highest in King of Siam (96.1 No.). Clyde Ikins (4.83 cm) produced significantly

longer petals and Jalueang Sub (3.27 cm) recorded shorter petals. Siam Jasmine (2.33 cm) produced a significantly wide petal whereas Jalueang Sub (0.87 cm) recorded shorter petal width. Wiersema (1988) characterized the floral parts of *Nymphaea* species into 4 sepals, 7 to 40 petals, 20 to 700 stamens and 5 to 47 carpels.

Stamens were arranged in a spiral manner and each stamen consisted of a filament, introrse anther and a sterile appendage at the tip. Longest stamens were presented in the outer whorl and their length decreased towards the inner whorls. In the outer whorl, filaments of the stamens were flattened with slight dilation in the base. The stamens appeared to be curved over the receptacle before anthesis and assumed a somewhat sickle shape at maturity.

The number of stamens were lowest in Perry's Baby Red (50.3 No.) and highest in Doris Holt (138.5 No.). Stamens were not observed in the varieties 'King of Siam' and 'Taweekan'. Stamens of King of Siam have been reported to be modified themselves into petals creating flowers that appear to have more than 100 petals (Wu *et al.*, 2016). Flowers that possess a greater number of tepals compared to stamens exhibited a resemblance to double flowers, as observed in *Nymphaea* 'King of Siam' or *Nymphaea* 'Midnight' as reported by Kirie *et al.* (2020). Carla's Sonshine had the lowest stamen length (1.81cm) while Hilary had the highest (2.32 cm). The varieties Clyde Ikins, Virginalis, Siam Jasmine, Siam Amber, Colorado and Mexicana were recorded to have brilliant yellow coloured (RHS 9C-83) stamens. The outermost whorl was found to be dried up and dark in colour on the last day of flower opening in Blue Whistle (Plate 19). Variations were noted in the colour of the terminal appendage of the stamen among the different accessions, and these hues closely resembled the petal colour. Heslop-Harrison (1955) reported 20 to 700 stamens in the genus *Nymphaea* with outer stamens often transitional to the petals except the sub genera Lotus and Anecphyia.

The pedicel circumference and diameter were highest for Taweekan (2.46 cm and 0.97 cm respectively) and lowest for Perry's Baby Red (1.57 cm and 0.54 cm respectively). The length of pedicel was highest in Taweekan (31 cm) and lowest (15.7 cm) in Carla's Sonshine. The pedicel colour of *Nymphaea* varieties

exhibited different shades. They were light yellowish green, moderate yellowish green, pale yellow, moderate yellowish green, moderate purplish red, brilliant yellowish green, greyish-reddish orange and moderate orangish yellow.

Obtuse and acute petal apex was found in the varieties. Sepal shape was linear-lanceolate and ob-lanceolate. The inner and outer petal colours exhibited shades of cream, white, yellow, green, pink, red, purple and violet. Varieties Siam Jasmine and Virginalis exhibited white colour blooms. Stripes were present in the petals of Islamorada. Poosub had yellow petals with a light blue tinge at the tip and Purple Joy had white petals with dark purple tinge at the tip. Zhu *et al.*, 2012 observed 14 anthocyanins from hardy waterlilies and 34 flavonoids from tropical waterlilies which were responsible for different petal colours.

5.1.3. Cluster analysis of morphological and floral quantitative characters

Assessment of genetic diversity is of much importance in crop improvement programmes as far as the selection of parents for hybridisation is concerned. Cluster analysis serves as a biometric technique employed to categorize genotypes based on their similarities, forming groups where genotypes within the same cluster exhibit greater resemblance to each other than to those in other clusters. The greater the distance between two clusters the greater the divergence between genotypes belonging to the two clusters and *vice versa*. In the selection of parents for hybridisation these points mainly need to be considered. The *Nymphaea* genotypes were subjected to cluster analysis using the Average method and Euclidean Distance measures.

The genotypes are grouped into four clusters based on vegetative quantitative characters. Cluster 1 comprised of 13 genotypes (Micrantha, Hilary, Doris Holt, Carla's Sonshine, King of Siam, Blue Whistle, Purple Joy, Dauben, Supranee Pink, Siam Pink, Jalueang Sub, Tropic Sunset and Miami Rose), cluster 2 comprised of 15 genotypes (Chompu, Taweekan, Bull's Eye, Siam Jasmine, Puttaraksa, Mexicana, Poosub, Clyde Ikins, Virginalis, Islamorada, Perry's Baby Red, Colorado, Almost Black, Paranee and Siam Amber), cluster 3 comprised of the genotype Wanvisa and cluster 4 comprised of the genotype Peach Blow. Cluster 1 exhibited the highest cluster mean for the character length

of the lamina (18.43 cm) followed by width of the lamina (17.19 cm). The highest cluster mean for the number of leaves per plant and longevity of leaves were obtained in cluster 3. The number of days from visual appearance to full expansion of leaf lamina has the highest cluster mean in cluster 4.

The floral quantitative characters categorised the genotypes into five clusters. Cluster 1 composed of 3 genotypes (Jalueang Sub, Tropic Sunset and Miami Rose), cluster 2 composed of 5 genotypes (Taweekan, Micrantha, Chompu, Carla's Sonshine and King of Siam), cluster 3 composed of 7 genotypes (Dauben, Islamorada, Bull's Eye, Poosub, Doris Holt, Perry's Baby Red and Virginalis), cluster 4 composed of 4 genotypes (Hilary, Purple Joy, Blue Whistle, Peach Blow) and cluster 5 composed of 2 genotypes (Siam Jasmine and Clyde Ikins).

Wide range of mean values among the clusters were recorded for different traits. Cluster 2 showed the highest cluster mean for flower diameter and number of petals. The cluster means for the characters *viz.* length of the flower bud, length of sepal and length of petal were highest in cluster 3. The width of sepal and width of petal have the highest cluster means in cluster 4. The genetic divergence among genotypes corresponds directly to the distance between the clusters. Within a cluster, genotypes exhibit lower levels of divergence compared to the divergence observed between clusters.

In vegetative characters, the highest value for intra-cluster distance was observed for C 1 (8.55). The clusters C 3 and C 4 contained single genotype each and therefore, their intra-cluster distances were zero. The maximum inter-cluster distance (18.48) for vegetative characters was found between cluster 1 and 3 and the minimum inter-cluster distance (7.94) was observed between C 2 and C 4. In floral characters, the intra-cluster distance recorded its minimum values for C 2 (4.21) and maximum value observed for C 5 (12.36). Maximum inter-cluster distance for floral characters was observed between clusters C 2 and C 3 which was 75.19 and minimum distance between C 1 and C 3 which was 7.36. In the context of hybridization, selecting parents from diverse clusters can be highly effective. Hence, to get superior varieties with novel floral aspects, parents from cluster 2 and 3 may be selected for hybridization. The inter cluster distances were

higher than that of intra cluster distances in the present study which indicated substantial diversity among the parents and there may be a greater opportunity for obtaining rare and superior segregants from crosses between plants with more diverse genotypes. Su *et al.* (2019) classified 49 water lily resources into six clusters. The clusters consisted of tropical medium and large flower water lily varieties, tropical full double flower varieties whose stamens are all petals, cold-resistant medium and large variety group, tropical small and medium variety group, tropical large variety group and tropical giant (tropical Australian water lily) variety group.

5.1.4. Coefficient of variation

The total variability was partitioned into heritable and non-heritable components with the help of genetic parameters like genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability and genetic advance, for aiding in selection. GCV was reported to provide a valid basis for comparing and assessing the range of genetic diversity for quantitative characters and PCV was observed to measure the extent of total variation in a population. Among the vegetative characters, PCV ranged between 16.59 per cent to 30.04 per cent. The highest PCV was recorded for number of leaves and the lowest for the longevity of the leaf. The range of GCV observed was from 8.031 per cent to 27.31 per cent. The number of leaves exhibited the highest GCV and the lowest for the number of days from visual observation to full expansion of leaf lamina.

Maximum PCV and GCV were observed for the morphological characters number of leaves per plant (PCV 30.04% and GCV 27.31%) followed by length of lamina (PCV 20.71% and GCV 20.53%). Among the floral characters number of petals recorded the highest PCV (74.25%) and GCV (74.49%) followed by width of petal with PCV (27.42%) and GCV (27.61%). This indicated a great extent of variability for these characters, thereby suggesting good scope for improvement of these characters through selection.

The minimum PCV and GCV were observed for the morphological character longevity of leaves (16.59 and 8.36 per cent respectively). Number of

days from visual observation to full expansion of leaf lamina also had low values of 8.03 per cent and 18.16 per cent respectively at both genotypic and phenotypic level. The minimum PCV and GCV were observed for the floral character length of flower bud (7.14 per cent and 7.47 per cent). Therefore, improvement of these characters has only a limited scope.

The morphological characters number of days from visual observation to full expansion of leaf lamina (10.13 %) and longevity of leaves (8.23%) exhibited maximum difference between GCV and PCV whereas the morphological characters flower diameter (0.27%), pedicel diameter (0.859%) and length of sepal (0.521%) exhibited maximum difference between GCV and PCV which indicated the influence of environment on these characters was considerable. The morphological characters length of lamina (0.18%), width of lamina (0.2%) and petiole diameter (0.3%) recorded low differences between GCV and PCV. The floral characters diameter of stigmatic cup (0.251%), number of petals (0.239%), width of petal (0.189%) and length of flower bud (0.334%) recorded low difference between GCV and PCV which indicated environmental influence on these characters was less and selection on phenotypic basis will be suitable.

The highest variation at the genotypic and phenotypic levels was shown by the number of days from visual observation to full expansion of leaf lamina (16.29) followed by lamina length (2.72). For the floral characteristics, PCV values varied from 7.47 per cent to 74.49 per cent. The lowest PCV was recorded for the length of the flower bud while the highest was for the number of petals. GCV ranged between 7.14 per cent to 74.25 per cent. A similar study conducted by Tom (2015) showed that the GCV ranged from 8.06 per cent for the circumference of mature flower buds to 28.53 per cent for the length of the lamina in the leaf and floral traits. Among these characters, the length of the lamina demonstrated the greatest variability supported by its higher PCV and GCV values.

5.1.5. Heritability and Genetic Advance

The combination of genotypic coefficients and heritability estimates provides a more comprehensive understanding of the potential genetic progress in

the succeeding generation (Allard, 1960). The high h^2 values observed for all the characters suggest that the impact of the environment on these individuals is minimal. The highest genetic advance was observed for the number of leaves (51.14) and the lowest for the number of days from visual appearance to full expansion of leaf lamina (7.31). The maximum heritability was observed for petiole diameter (96.0) and the minimum for the number of days from visual appearance to full expansion of leaf lamina (19.5).

All floral characters exhibited high genetic advance and heritability. The highest genetic advance was observed for the number of petals (152.48) and maximum heritability was observed for the number of petals (99.4). High heritability and genetic advance observed for these characters indicated that they may be controlled by additive gene action and their genetic improvement could be possible through selection (Panse and Sukhatme, 1985). The significant amount of variability along with a high heritability rate of 99%, suggests considerable potential for the selection of these traits (Tom, 2015). Su *et al.* (2019) reported that variation coefficients of 16 quantitative traits of 49 introduced water lily varieties ranged from 15.67% to 89.52%.

5.1.6. Correlation studies

Correlation coefficient analysis measures the mutual relationship between various plant characters and determines the component characters on which selection is based for genetic improvement for a particular character (Robinson *et al.*, 1949). When selection is applied to a trait the population under selection is reported to be improved for not only that trait but also in respect of other associated characters (Sharma, 1994). Genetically related characters were observed to move in the same direction under selection favouring any one of such related traits. Such correlated response to selection is the basic property of quantitative traits under the control of polygenic system. The quantitative traits governed by one or a few genes do not exhibit correlated changes on selection. A positive genotypic correlation between sets of characters indicates that enhancing one trait is likely to result in improvements in the other trait as well. This ability enables the breeders to select traits that respond well to the breeding process.

Highly significant positive phenotypic correlation was observed for length of lamina with width of lamina (0.895), petiole diameter (0.614) and negative correlation with number of leaves per plant (-0.226), longevity of leaves (-0.186). Width of lamina showed positive correlation with length of lamina (0.895) and petiole diameter (0.578) while it expressed negative correlation with longevity of leaves (-0.247). Number of leaves per plant had positive correlation with longevity of leaves (0.226) and negative correlation with length of lamina (-0.226) and petiole diameter (-0.277). Longevity of leaves had positive correlation with number of leaves per plant (0.226) and negative correlation with length of lamina (-0.186) and width of lamina (-0.247). Petiole diameter had positive correlation with length of lamina (0.614) and width of lamina (0.578) while negative correlation with number of leaves per plant (-0.277). Phenotypic correlation was absent for number of days from visual appearance to full expansion of leaf lamina.

In floral phenotypic characters, highly positive correlation was observed for flower diameter with length of flower bud (0.534), pedicel diameter (0.399), length of petal (0.722), number of petal (0.46), length of sepal (0.763), width of sepal (0.53), diameter of stigmatic cup (0.672) and number of stigmatic appendages per flower (0.366). Number of petals have positive correlation with flower diameter (0.46), pedicel diameter (0.368), width of sepal (0.278), diameter of stigmatic cup (0.423) and number of stigmatic appendages per flower (0.534). The width of sepal had positive correlation with flower diameter (0.53), length of flower bud (0.333), pedicel diameter (0.299), length of petal (0.456), width of petal (0.627), number of petal (0.278), length of sepal (0.422) and diameter of stigmatic cup (0.533). High positive genotypic correlation was observed for flower diameter with length of flower bud (0.587), pedicel diameter (0.424), length of petal (0.756), number of petal (0.468), length of sepal (0.802), width of sepal (0.567), diameter of stigmatic cup (0.686) and number of stigmatic appendages per flower (0.377).

Vegetative characters *viz.* length of lamina, width of lamina and petiole diameter were correlated to each other. Among the floral characters almost all characters are correlated to each other. Positive genotypic and phenotypic

correlation thus observed indicate that improvement in any one of these characters could bring about a corresponding improvement in the other associated character. Su *et al.* (2019) reported a highly significant positive correlation ($P < 0.01$) between leaf width and leaf split depth.

5.2. Crop improvement studies

5.2.1. Palynology

Pollen morphology pattern was observed from pollens collected from the second day of anthesis. Pollen grains were collected from eighteen varieties *viz.* Micrantha, Carla's Sonshine, Hilary, Tropic Sunset, Blue Whistle, Perry's Baby Red, Poonsub, Siam Jasmine, Suprane Pink, Clyde Ikins, Islamorada, Chompu, Doris Holt, Bull's Eye, Dauben, Peach Blow, Virginalis and Siam Amber. Microscopic inspection revealed that pollen grains were spherical in shape. Most of the *Nymphaea* varieties *viz.* Islamorada, Chompu, Doris Holt, Bull's Eye, Dauben, Peach Blow, Virginalis and Siam Amber exhibited prolate spheroidal shape. The varieties Micrantha, Carla's Sonshine, Hilary, Tropic Sunset, and Blue Whistle have spheroidal shapes and Perry's Baby Red, Poonsub, Siam Jasmine, Suprane Pink and Clyde Ikins exhibit oblate-spheroidal shaped pollens. Bowmik (2012), reported that the *N. mexicana*, *N. stellata*, *N. rubra* and *N. pubesence* had heteropolar, boat shaped bilateral pollen grains. Varieties like Micrantha, Carla's Sonshine, Doris Holt, Hilary, Perry's Baby Red, Poonsub, Peach Blow, Siam Jasmine, Tropic Sunset, Suprane Pink, Clyde Ikins and Blue Whistle exhibited medium-sized pollen. A significant small pollen size was recorded by Islamorada, Chompu, Bull's Eye, Dauben, Virginalis and Siam Amber. Volkova and Shipunov (2007) observed that pollen grains of *N. alba* and *N. tetragona* have small pollen whereas *N. candida* exhibited either small or large pollen. Bodhipadma *et al.* (2013) reported that there was no significant difference in the pollen size and the fertile pollen exhibited spheroidal shape *N. nouchali* var. *Versicolor*.

A ring-like aperture that is slightly shifted in the direction of the distal pole was present in the varieties Chompu, Hilary, Perry's Baby Red and Siam Amber. All other varieties exhibited absence of aperture in their pollen. Taylor *et al.*, 2013 found that psilate to verrucate exine ornamentation in *N. ondinea*, Victoria and

Barclaya. The pollen cover had a prominent projecting ring which was convex and arranged in a circular arch. A ring-like sulcus called zonaulculus was present around the equator and had psilate ornamentation on both its proximal and distal surfaces in all the varieties. Murthy (2000) identified the encircling aperture as zonoulcate and also found different types of ornamentation like granulate (*N. tetragona* and *N. rubra*), psilate (*N. pubescens* and *N. nouchali*), baculate (*N. candida*) and gemmate (*N. alba*) in different *Nymphaea* spp. Similar observation was also recorded by Taylor *et al.*, 2015 in the order Nymphaeales.

Pollen viability plays an important role in the success and fruiting rates of plant pollination and fertilisation. Therefore, it is important to measure the pollen viability of male parents before hybridization. The variety Doris Holt had the highest pollen fertility of 84 per cent followed by Peach Blow (73%), Hilary (68%), Micrantha (65%) and Bull's Eye (48%). The lowest value was recorded for Supranee Pink (12%), Islamorada (14%), Clyde Ikins (18%), Siam Amber (18%) and Dauben (23%). Lower pollen fertility was observed in interspecific hybrids of *Nymphaea* (Heslop-Harrison, 1955; Komarov, 1970). A similar study by Sun *et al.*, 2018 recorded a pollen viability of 17.3% for Peter Slocum, 19.3% for *N. colorata*, 10.3% for *N. micrantha*, and 17.6% for *N. gigantea*. Hao *et al.*, 2022 observed pollen viability in 12 cultivars and found that Blue Beauty, Virginalis, Perry's Fire Opa, Virginalis, Marliacea Rosea, Hollandia and Almost Black as viable. They also found that Blue Beauty has the highest fertility (87.33%) in the case of tropical cultivars and Virginalis (42.22%) in the case of hardy cultivars.

5.2.2. Stigma receptivity

The appearance of honey dew-like secretion and the moist condition of the stigmatic cup signified the beginning of stigma receptivity in the varieties. The receptivity was initiated 14 to 15 hours before flower opening and was retained up to 5 to 7 hours after flower opening. Before the bloom opening, the stigma became receptive, suggesting that the flower is protogynous. On the first day of floral anthesis, stigma secretes a large amount of liquid with no flavour or colour.

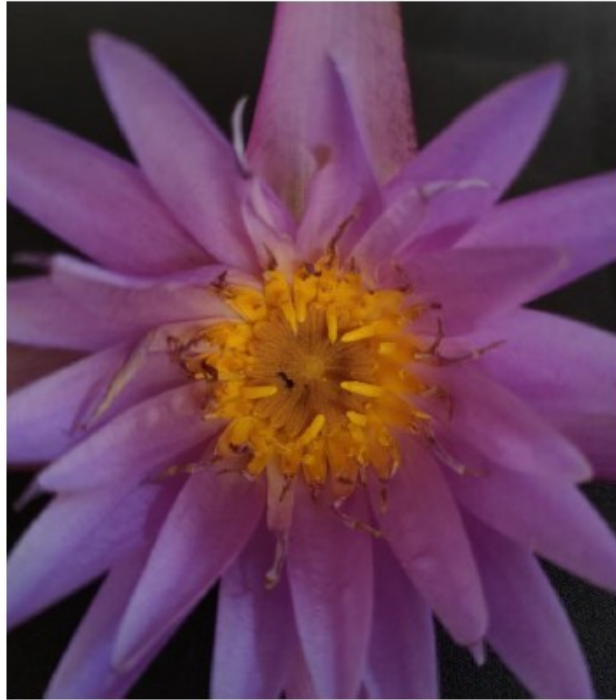


Plate 19. Dried up stamens of *Nymphaea* varieties on the last day of opening.

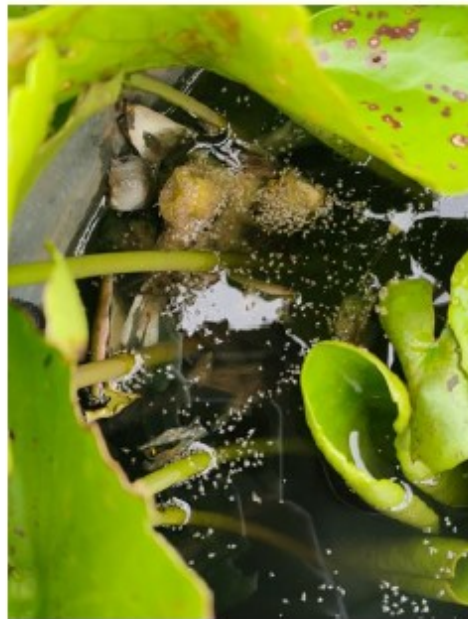


Plate 20. Seed pod at maturity

During the next two days of flowering, most of the stigmatic exudates vanished and on the third and fourth day of anthesis, the stigmatic appendages deteriorate and shrink slightly. The presence of curved stigmatic appendages on a dried stigma signified a lack of receptivity. The time and duration of stigma receptivity varied according to the variety. Blue Whistle exhibited the maximum duration of stigma receptivity (25 hr 30 min.) whereas Peach Blow had the minimum period (15 hr 45 min.). Fahida (2022) observed that the stigma receptivity took place 17 hours before flower opening and remained up to 32 hours after blossom opening in *N. nouchali*.

The colour of the stigma in all the *Nymphaea* varieties was yellow. Honeybees were observed as the natural pollinators of water lilies and the yellow colour might be an adaptation to attract the pollinators. A similar result was recorded in a study conducted by Zini *et al.*, 2019.

The gynoecium exhibits a syncarpous nature and has slightly inward curved clavate appendages around the rim of the stigmatic cup. The diameter of the stigmatic cup varied significantly. The variety Bull's Eye (18.9 mm) exhibited the highest stigmatic diameter whereas Miami Rose (9.19 mm) had the shortest stigmatic diameter. Fahida (2012) observed variation in the diameter of the stigmatic cups of the blue (2.02 cm) and the white types (1.82 cm) of *N. nouchali*. The lowest number of stigmatic appendages per flower was exhibited by Clyde Ikins (9.91 No.) while the highest count was observed in Taweekan (24.3 No.). Begum *et al.*, 2010 reported equal number of stigmatic appendages and carpels in both blue and white types of water lilies.

5.2.3. Hybridization and compatibility studies

A preliminary hybridization was conducted involving 316 crosses based on the simultaneous availability of receptive flowers and pollen grains. The varieties Poosub, Siam Jasmine, Siam Amber, Wanvisa and Blue Whistle exhibited maximum fruit set and were selected as female parents and Doris Holt, Peach Blow, Hillary, Micrantha and Bull's Eye which exhibited maximum pollen fertility were selected as male parents for the compatibility study. After

hybridization, the flower and its pedicel bend downward into the water and an 'S'-shaped pedicel formation was observed in successful crosses. A similar result was observed by Sculthorpe (1967).

Poonsub was cross-compatible with Doris Holt, Peach Blow and Micrantha while it was cross-incompatible with Hilary and Bull's Eye. Wanvisa was cross-compatible with Doris Holt, Peach Blow, Hilary and Micrantha but cross-compatible with Bull's Eye. Siam Jasmine exhibited cross-compatibility with Doris Holt, Peach Blow, Micrantha, and Bull's Eye, but it was found to be incompatible in crosses with Hilary. Siam Amber showed cross-compatibility only with Doris Holt, but it was cross-incompatible with Peach Blow and Micrantha. Siam Amber could not be crossed with Hilary and Bull's Eye due to the lack of simultaneous availability of pollen and receptive stigmas. Blue Whistle showed cross-compatibility with Doris Holt and Peach Blow, but it exhibited cross-incompatibility with Hilary, Micrantha, and Bull's Eye. Zhang *et al.*, 2022 evaluated the cross-compatibility of *Nymphaea* hybrids as female parents and three hardy water lilies as male parents and revealed that all hybrid combinations except for NH-1 × N. 'Peter Slocum', NH-2 × N. 'Peter Slocum' and NH-3 × N. 'Peter Slocum' could bear seeds. The hybrid combinations with NH-2 as the female parent had the highest seed-setting rate and the hybrid combinations with N. 'Rose Arey' as the male parent had the highest seed-setting rate.

The maximum number of flowers were pollinated in the cross Poonsub x Doris Holt (7 No.) and Blue Whistle x Doris Holt (7 No.). The minimum number of flowers were pollinated between the varieties Siam Amber x Micrantha (1 No.) and Siam Amber x Peach Blow (1 No.). The success rate of pollination was highest for the cross between Siam Amber and Doris Holt.

The success rate of pollination and percentage of fruit set was highest for the cross between Siam Amber x Doris Holt (100%) followed by Poonsub x Micrantha (80%), Wanvisa x Doris Holt (80%) and Siam Jasmine x Doris Holt (66.66%). No fruit set was observed in the crosses Poonsub x Hilary, Poonsub x Bull's Eye, Wanvisa x Bull's Eye, Siam Jasmine x Hilary, Siam Amber x Peach Blow, Siam Amber x Hilary, Siam Amber x Micrantha, Siam Amber x Bull's Eye,

Blue Whistle x Hilary, Blue Whistle x Micrantha and Blue Whistle x Bull's Eye. Begum *et al.* (2010) documented excellent fruit set in *N. nouchali* and *N. pubescence*, while noting the absence of natural seed sets in *N. Rubra*. Manju (2018) pointed out that the process of seed set was an integrated effect of pollen pistil interaction, pollen tube growth, pollen fertility, germinability *etc.*

The fruit was a berry in which the outer wall of the fruit remained firm, but its inner portion was soft and the seeds were found embedded in a mucilage, and each surrounded with an aril. The ripening of the fruit took place underwater and dehiscence was irregular and the fruits burst open, and seeds were found floating on the surface of the water (Plate 20). Conard, 1905 reported that the opening of the fruit was due to the growth of the arils and swelling of the mucilage. During fruit ripening time various changes were reported in the floral leaves. In *Castalia* floral leaves completely rotted away and left only small scars while in *Lotus* about half an inch of the bases of the outer stamens remained attached to the fruit. In *N. amazonum* the sepals persisted, and the other parts of the flower decayed partly. In *Brachyceras* the sepals and outer four petals remained and became stiff and turgid and developed additional chlorophyll.

The maximum number of days for the fruit set was observed for the cross, Blue Whistle x Peach Blow (43 days) and the minimum days for the fruit set was observed for Wanvisa x Micrantha (19 days). The weight of the fruit ranges from 7.02 g in Poonsub x Micrantha to 13.01 g in Siam Amber x Doris Holt. The maximum number of seeds were produced by Poonsub x Micrantha (295 No.) followed by Blue Whistle x Hilary (186 No.) and Hilary x Micrantha (142 No.). The seeds obtained in cross Poonsub x Micrantha is given in Plate 20. The minimum number of seeds per fruit was observed in Wanvisa x Peach Blow (89 No.). Les *et al.* (2004) reported that the hybridization between *Nymphaea* 'Andre Leu' and *Nymphaea colorata* produced 30 seeds. A similar study was performed in *Victoria amazonica* and the number of seeds per fruit varied from 100 to 700, with an average ranging between 300 and 400 seeds per fruit (dos Santos Tozin and de Lima Corrêa-da, 2016).

The length of the seed was observed lowest in Blue Whistle x Hilary (1.22 mm) and highest in Siam Amber x Doris Holt (1.57 mm). Blue Whistle x Hilary exhibited the lowest seed breadth (0.90 mm) while Siam Amber x Doris Holt recorded the highest leaf breadth (1.06 mm). Constant variation in seed size among *Nymphaea* genotypes has been reported by several workers. Wiersema and Hellquist (1997) reported that the seeds of *N. odorata* exhibited a size variation ranging from 1.5 to 4.8 mm and *N. mexicana* seeds were large with 0.5 cm size. dos Santos Tozin and de Lima Corrêa-da (2016) noted that the average seed size for *Victoria amazonica* as 10.6 mm in length and 9.8 mm in width. Dalziell *et al.*, 2019 observed that the seeds of *N. immutabilis* and *N. macrosperma* exhibited the largest length and width, while those of *N. violacea* and *N. ondinea* were the smallest.

Among all the parents, the variety 'Poosub' can be regarded as a good female parent due to its compatibility with most of the male parents, high seed set percentage and high germination percentage. It is a good bloomer with attractive flowers too.

Out of the many crosses carried out, the germination of seeds was observed in three crosses (Plate 21 and 22). Hybrid 1 (Poosub x Micrantha) and Hybrid 2 (Poosub x Peach Blow) took 8 days for germination while Hybrid 3 (Poosub x Micrantha) took only seven days for germination. The result was similar to the study performed by Manju (2018) in water lily accessions.

The germination percentage of *Nymphaea* seeds was noted to be low. Hybrid 1 (Poosub x Micrantha) had the highest germination percentage of 21.5% followed by hybrid 3 (Poosub x Micrantha with 19.3% and hybrid 2 (Poosub x Peach Blow) with 16.3% of germination. Constant variation in germination percentage among *Nymphaea* genotypes has been reported by several workers. Richards and Cao (2012) in a similar study noted that the *N. odorata* exhibited 100% germination. dos Santos Tozin and de Lima Corrêa-da (2016) reported that most favourable temperature for germination was 25°C, regardless of the lighting conditions in *Victoria amazonica*. Also, seeds of *N. ondinea* and *N. macrosperma*



(a)



(b)

Plate 21. Seed obtained from the crosses
(a) Siam Amber x Doris Holt (b) Poosub x Micrantha

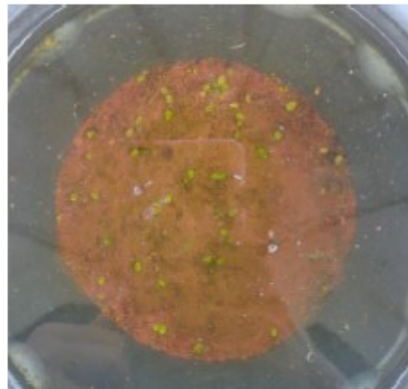


Plate 22. Germination of the seeds from the cross Poosub x Micrantha

exhibited germination at 40 °C, with a germination rate below 60%. (Dalziell *et al.*, 2019).

Among the crosses carried out during the experiment, successful flowering was observed only in three crosses. Hybrid 1 was obtained between Poosub (ovule parent) and Micrantha (male parent) and resulted in seven different F₁ hybrids (Plate 24). The Hybrid 2 was obtained between Poosub (female parent) and Peach Blow (male parent) resulting in only one F₁ hybrid (Plate 25). Hybrid 3 was a cross between Poosub (ovule parent) and Micrantha (pollen parent).

Ansari and Jeeja (2009) observed that seed-propagated waterlily plants would usually take two or three years to produce bloom after germination. Manju (2018) reported flowering within five months since its emergence. Both the findings were in contrast with the present study, where the flowering in F₁ hybrids between Poosub and Micrantha took 28-29 days for the first flowering and in the case of F₁ hybrid between Poosub and Peach Blow it took 45 days for first flowering.

The hybrids usually display a blend of morphological traits from both parental subspecies or show intermediate characteristics, making it challenging to categorize them into either subspecies (Wiersema and Hellquist, 1997). Some of the vegetative and floral characters were either similar to the ovule parent or pollen parent in the F₁ hybrid between Poosub and Micrantha. Hairs were present on the under surface of the leaves of the hybrid while it was absent in both parents. Apart from this, colour of petiole, lower leaf, stamen, inner and outer petal, number of stigmatic appendages per flower, and diameter of the stigmatic cup were the characters that exhibited differences from their parents. Characters like the shape of the leaf, shape of the leaf margin, striations on the leaf, hairs on the petiole, colour of upper leaf, field life of flower, sepal shape, stigma colour and number of sepals are identical to the characters of their parents.

The F₁ hybrid between Poosub and Peach Blow had most of the characters similar to the parents. There were only a few new characteristics developed in the progeny *viz.* leaf venation on the lower surface, leaf hairs on the lower surface, the upper colour of the leaf, the lower colour of the leaf, pedicel



Plate 23. Different stages of hybrid seedling development

colour and stamen colour. Most of the characters expressed were intermediate of both the parents (either lower or higher than the parent). Overlapping of leaf base, striations on the leaf, colour of the upper leaf, petal apex and day-to-flower opening are the characteristics of ovule parent shown by the hybrid. The pollen characters shown by the F₁ hybrid are the sepal shape and colour of the outer petal. In line with the findings of Les *et al.*, 2004 the F₁ hybrid exhibited variation only in petal colour and anther colour from their parents (*N. colorata* x *N. gigantea*).

Morphological evaluation of the thirty *Nymphaea* genotypes collected all over India revealed that tropical varieties like Micrantha, Chompu, Taweekan, Bull's Eye, Hilary, Doris Holt, Carla's Sonshine, King of Siam, Blue Whistle, Purple Joy, Dauben, Poosub, Peach Blow, Jalueang Sub, Tropic Sunset, and Miami Rose were good bloomers with long blooming period and suitable to the conditions of South Kerala. Though flowering was limited in the hardy varieties, their unique colours and shapes could be utilized for further crop improvement programmes. The performance and floral attributes of the hybrids that have flowered and which are yet to flower can be further assessed and improved if necessary. The promising hybrids can be further evaluated and selected for cultivation.



Poonsub (♀)

X



Micrantha (♂)



Hybrid 1



Hybrid 2



Hybrid 3



Hybrid 4



Hybrid 5



Hybrid 6



Hybrid 7



Hybrid 8

Plate 24. *Nymphaea* F1 hybrids obtained from the cross Poonsub x Micrantha



Poonsub (♂)

X



Peach Blow (♀)



Hybrid 1

Plate 25. *Nymphaea* F₁ hybrid obtained from the cross Poonsub x Peach Blow

6. SUMMARY

The research programme entitled ‘Characterization and compatibility studies in waterlily (*Nymphaea* spp.)’ was carried out in the Department of Floriculture and Landscaping, College of Agriculture, Vellayani, Thriruvananthapuram during the period 2022- 2023 to evaluate the performance of thirty waterlily varieties based on growth, flowering and floral attributes.

Out of the thirty waterlily varieties, flowering was observed in 28 varieties viz., Micrantha, Chompu, Taweekan, Bull’s Eye, Hilary, Doris Holt, Carla Sonshine, Siam Jasmine, Wanvisa, Mexicana, King of Siam, Blue Whistle, Purple Joy, Dauben, Poosub, Clyde Ikins, Virginalis, Peach Blow, Islamorada, Siam Pink, Supranee Pink, Jalueang Sub, Tropic Sunset, Miami Rose, Colorado, Almost Black, Paranee, Siam Amber, Perry’s Baby Red. The flower of *Nymphaea* exhibited strong protogyny, wherein they expressed stigma receptivity on the initial day, followed by the release of pollen on the subsequent two days.

Wide variability was observed between the quantitative and qualitative vegetative and floral characters. Significant differences were observed in characters like length and width of leaves, longevity of leaf, number of leaves per plant, petiole diameter and number of days from visual appearance to full expansion of leaf lamina. Leaf shape was either round or ovate and majority of the leaves were found to be ovate. Leaf margins were either toothed/ serrated or entire/smooth. All the tropical varieties had toothed leaf margin whereas hardy varieties had smooth leaf margins. Irregular moderate purplish red striations were present on the leaf lamina of Hilary, Doris Holt, Carla's Sonshine, Wanvisa, Peach Blow, Siam Pink, Jalueang Sub, and Miami Rose. The leaves were two lobed and at the base, the two lobes overlapped or just touched or spread apart based on the variety. *Nymphaea* genotypes under the study exhibited eight colour shades on the upper and lower surface of the leaves. Petioles were long, slender and submerged in water with lamina floating on the surface of the water and exhibited eight colour shades. The varieties Siam Jasmine, Clyde Ikins, Supranee Pink, Paranee and Siam Amber exhibited short hairs under the leaf surface and petiole.

Micrantha, Chompu, Hilary, Doris Holt, Carla's Sonshine, Blue Whistle, Purple Joy, Peach Blow and Islamorada exhibited vivipary, the production of plantlets from their leaves.

The floral bud appeared broad and rounded unlike the leaf rudiment which appeared in a more conical form. Flowers were produced in the axil of leaves. Maimi rose (3.95 cm) had the shortest bud length while Hilary (5.04 cm) possessed the longest buds. The diameter of the flower was found to be highest for King of Siam (11.07 cm) and lowest in case of Miami rose (7.37 cm). A seasonal variation in flower production was observed among the varieties. For tropical waterlilies maximum flower production was observed from February to May. During the period from December to January, there was a decrease in the production of flowers. The highest number of flowers per plant per month was produced by Dauben (9.3 nos.) followed by Blue Whistle (8.3 nos.), Micrantha (8 nos.), Tropic Sunset (7.3 nos.) and King of Siam (7 nos.).

All the varieties exhibited 3 to 4 days of field life for flowers. The blooming time of the flowers started between 7:00 am to 10:00 am and closed between 1:00 pm to 6:15 pm. Most of the hardy varieties exhibited early closing of flowers. The opening and closing behaviour of the blooms differed among the varieties and it took nearly 25 to 35 minutes for the complete opening and 30 to 40 minutes for the complete closing of the blooms. The rainy and cloudy days from June to October caused delayed opening and early closing of the flowers. Varieties Micrantha, King of Siam, Blue Whistle, Dauben and Poosub exhibited early flower opening (7:00 am to 7:30 am) and longest anthesis time. The varieties like Siam Jasmine, Virginalis, Supranee Pink, Paranee and Mexicana exhibited minimum period of anthesis.

Significant variability was observed among the floral quantitative characters *viz.* Length of sepal, width of sepal, number of petals, length of petals, width of petals, number of stamens, length of stamens. All the varieties used in the experiment exhibited four sepals except 'King of Siam' and 'Taweekan' which had 6 sepals. Stamens were arranged in a spiral manner and each stamen consisted of a filament, introrse anther and a sterile appendage at the tip. Stamen production

was not observed in King of Siam and Taweekan. The colour of the terminal appendage of the stamens closely resembled the petal colour and eight shades of pedicel colour were also observed. Obtuse and acute petal apex was found in the varieties and the sepal shape was linear-lanceolate and ob-lanceolate. Variation among petal colour exhibited shades of cream, white, yellow, green, pink, red, purple and violet.

The genotypes were grouped into four clusters based on vegetative quantitative characters (length of lamina, width of lamina, number of leaves per plant, longevity of leaves and number of days from visual appearance to full expansion of leaf lamina). Cluster 1 comprised of 13 genotypes, cluster 2 comprised of 15 genotypes, cluster 3 and 4 comprised of 1 genotype each. Maximum inter cluster distance with respect to vegetative characters was observed between C1 and C 3 which is 18.48 while minimum distance between C 2 and C 4 which is 7.94.

The genotypes were grouped into five clusters based on floral quantitative characters (length of flower bud, flower diameter, length of sepal, breadth of sepal, number of petals, length of petal and breadth of petal). The Cluster 1 composed of 3 genotypes, cluster 2 composed of 5 genotypes, cluster 3 composed of 7 genotypes, cluster 4 composed of 4 genotypes and cluster 5 composed of 2 genotypes. Maximum inter cluster distance among the floral characters was observed between the clusters C2 and C3 which is 75.19 and minimum distance between C1 and C3 which is 7.36. The greater the distance between two clusters the greater is the divergence between genotypes belonging to the two clusters and vice versa.

The GCV provide a valid basis for comparing and assessing the range of genetic diversity for quantitative characters and PCV measure the extent of total variation in a population. Among the vegetative characters, PCV ranged between 16.59 per cent to 30.04 per cent. The highest PCV was recorded for number of leaves and the lowest for the longevity of the leaf. The range of GCV observed was from 8.031 per cent to 27.31 per cent. The number of leaves exhibited the highest GCV and the lowest for the number of days from visual observation to full

expansion of leaf lamina. Maximum PCV and GCV were observed for number of leaves per plant (PCV 30.04% and GCV 27.31%) followed by length of lamina (PCV 20.71% and GCV 20.53%) and minimum PCV and GCV for longevity of leaves (16.59 and 8.36 per cent respectively). Among the floral characters number of petals recorded the highest PCV (74.25%) and GCV (74.49%) followed by width of petal with PCV (27.42%) and GCV (27.61%). The minimum PCV and GCV were observed for the floral character length of flower bud (7.14 per cent and 7.47 per cent).

The morphological characters number of days from visual observation to full expansion of leaf lamina (10.13 %) and longevity of leaves (8.23%) exhibited maximum difference between GCV and PCV whereas the morphological characters flower diameter (0.27%), pedicel diameter (0.859%) and length of sepal (0.521%) exhibited maximum difference between GCV and PCV which indicates that the influence of environment on these characters was considerable. The morphological characters length of lamina (0.18%), width of lamina (0.2%) and petiole diameter (0.3%) recorded low difference between GCV and PCV. The floral characters diameter of stigmatic cup (0.251%), number of petals (0.239%), width of petal (0.189%) and length of flower bud (0.334%) recorded low difference between GCV and PCV which indicated environmental influence on these characters was less and selection on phenotypic basis will be suitable.

The highest genetic advance was observed for the number of leaves (51.14) and the lowest for the number of days from visual appearance to full expansion of leaf lamina (7.31). The maximum heritability was observed for Petiole diameter (96.0) and the minimum for the number of days from visual appearance to full expansion of leaf lamina (19.5). All floral characters exhibited high genetic advance and heritability. The highest genetic advance was observed for the number of petals (152.48) and maximum heritability was observed for the number of petals (99.4).

Vegetative characters *viz.* length of lamina, width of lamina and petiole diameter were correlated to each other. Among the floral characters almost all characters are correlated to each other.

Pollen morphology pattern was observed from pollens collected from the second day of anthesis. Most of the pollen displayed a prolate spheroidal shape and fell within the medium-sized category. A ring-like aperture that is slightly shifted in the direction of the distal pole was present in the varieties Chompu, Hilary, Perry's Baby Red and Siam Amber. A ring-like sulcus called zonosulculus was present around the equator and had psilate ornamentation on both its proximal and distal surfaces in all the varieties. Highest pollen fertility was observed in the varieties Doris Holt (84%) followed by Peach Blow (73%), Hilary (68%), Micrantha (65%), and Bull's Eye (48%) and these varieties were selected as the male parent for the compatibility study.

The appearance of honey dew-like secretion and the moist condition of the stigmatic cup signified the beginning of stigma receptivity in the varieties. Blue Whistle exhibited the maximum duration of stigma receptivity (25 hr 30 min.) whereas Peach Blow had the minimum period (15 hr 45 min.). Based on preliminary hybridization, maximum stigma receptivity was observed for the varieties Poosub, Wanvisa, Siam Amber, Siam Jasmine and Blue Whistle and these varieties were selected as the female parent for the compatibility study. The colour of the stigma in all the *Nymphaea* varieties was yellow.

A preliminary hybridization was conducted involving 316 crosses based on the simultaneous availability of receptive flowers and pollen grains. The varieties Poosub, Siam Jasmine, Siam Amber, Wanvisa and Blue Whistle exhibited maximum fruit set and were selected as female parents and Doris Holt, Peach Blow, Hilary, Micrantha and Bull's Eye which exhibited maximum pollen fertility were selected as male parents for the compatibility study.

Maximum percentage of fruit set were observed when Siam Amber x Doris Holt (100%) followed by Poosub x Micrantha (80%) and Poosub x Wanvisa(80%). The fruit weight and seed size of Siam Amber x Doris Holt was larger compared to other varieties. The hybrid seeds took eight days for germination.

Out of the many crosses carried out, the germination of seeds was observed in three crosses. The parents of Hybrid 1 were between Poosub (yellow

flower) and micrantha (pale blue) while Hybrid 2 were between Poosub (yellow) and Peach Blow (pale purple). Flowering in F₁ hybrids between Poosub and Micrantha took 28-29 days for first flowering and in the case of F₁ hybrid between Poosub and Peach Blow it took 45 days for first flowering.

Some of the vegetative and floral characters were either similar to the ovule parent or pollen parent in the F₁ hybrid between Poosub and Micrantha. Hairs were present on the under surface of the leaves of the hybrid while it was absent in both parents. Apart from this, colour of petiole, lower leaf, stamen, inner petal and outer petal, number of stigmatic appendages per flower, and diameter of the stigmatic cup were the characters which exhibited differences from their parents.

The F₁ hybrid between Poosub and Peach Blow had most of the characters similar to the parents. There were only a few new characteristics developed in the progeny *viz.* leaf venation on the lower surface, leaf hairs on the lower surface, the upper colour of the leaf, the lower colour of the leaf, pedicel colour and stamen colour.

The hybrid plants are in flowering, near flowering and pre flowering stages. Vegetative and floral characters of the hybrids that flowered when compared with their parents, revealed both similarity and variations in the parameters studied. The hybrids obtained in the present study can be further assessed for floral attributes and selected for cultivation.

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APPENDIX I

Mean weather parameters during November 2022 – October 2023

Month	Temperature (°C)	Average Relative Humidity (%)	Total rainfall (mm)	Wind velocity (km/h)	Sunshine (Hours)
November	30.92	84.30	5.10	0.57	4.90
December	31.25	84.46	3.30	0.30	3.60
January	31.56	80.00	0.50	1.30	6.20
February	32.00	80.00	0.40	2.70	7.90
March	33.43	75.00	0.60	5.00	8.30
April	34.01	78.98	1.40	5.80	7.92
May	33.76	81.61	3.50	3.90	7.40
June	32.41	84.75	4.00	3.70	5.90
July	31.22	86.29	5.20	5.30	5.90
August	32.37	81.33	0.90	6.60	10.20
September	30.52	88.61	11.30	2.00	3.90
October	30.28	93.00	12.60	3.50	3.90

ABSTRACT

The research programme entitled ‘Characterization and compatibility studies in waterlily (*Nymphaea* spp.)’ was carried out in the Department of Floriculture and Landscaping, College of Agriculture, Vellayani, Thiruvananthapuram during the period 2022-2023 to evaluate the performance of thirty waterlily varieties based on growth, flowering and floral attributes.

Out of the thirty waterlily varieties, flowering was observed in 28 varieties viz., Micrantha, Chompu, Taweekan, Bull’s Eye, Hilary, Doris Holt, Carla Sonshine, Siam Jasmine, Wanvisa, Mexicana, King of Siam, Blue Whistle, Purple Joy, Dauben, Poosub, Clyde Ikins, Virginalis, Peach Blow, Islamorada, Siam Pink, Supranee Pink, Jalueang Sub, Tropic Sunset, Miami Rose, Colorado, Almost Black, Paranee, Siam Amber, Perry’s Baby Red. The flower of *Nymphaea* exhibited strong protogyny, wherein they expressed stigma receptivity on the initial day, followed by the release of pollen on the subsequent two days.

The analysis of variance revealed significant differences among the 21 genotypes for all the floral characters except for days to appearance of flower bud and days to flower opening. Significant varietal differences were observed among the thirty genotypes with respect to the vegetative characters.

Among the vegetative characters, the highest PCV was recorded for number of leaves (30.04 per cent) and the lowest for the longevity of the leaf (16.59 per cent). The number of leaves exhibited the highest GCV (27.31 per cent) and the lowest for the number of days from visual observation to full expansion of leaf lamina (8.03 per cent). Maximum PCV and GCV were observed for number of leaves per plant (PCV 30.04% and GCV 27.31%) followed by length of lamina (PCV 20.71% and GCV 20.53%) and minimum PCV and GCV for longevity of leaves (16.59 and 8.36 per cent respectively).

Among the floral characters, number of petals recorded the highest PCV (74.25%) and GCV (74.49%) followed by width of petal with PCV (27.42%) and GCV (27.61%). The minimum PCV and GCV were observed for the length of flower bud (7.14 per cent and 7.47 per cent).

The genotypes were grouped into four clusters based on vegetative quantitative characters (length of lamina, width of lamina, number of leaves per plant, longevity of leaves, petiole diameter and number of days from visual appearance to full expansion of leaf lamina). Cluster 1 comprised of 13 genotypes, cluster 2 comprised of 15 genotypes, cluster 3 and 4 comprised of 1 genotype each. Maximum inter cluster distance with respect to vegetative characters was observed between C1 and C 3 which is 18.48 while minimum distance between C 2 and C 4 which is 7.94.

The genotypes were grouped into five clusters based on floral quantitative characters (length of flower bud, flower diameter, length of sepal, breadth of sepal, number of petals, length of petal and breadth of petal). The Cluster 1 composed of 3 genotypes, cluster 2 composed of 5 genotypes, cluster 3 composed of 7 genotypes, cluster 4 composed of 4 genotypes and cluster 5 composed of 2 genotypes. Maximum inter cluster distance among the floral characters was observed between the clusters C2 and C3 which is 75.19 and minimum distance between C1 and C3 which is 7.36. The greater the distance between two clusters the greater is the divergence between genotypes belonging to the two clusters and vice versa.

All the genotypes exhibited four consecutive days of anthesis. The anthesis timings of waterlily flowers were affected by environmental factors. Cloudy and rainy weather conditions led to delayed flower opening and early flower closing.

Significant variations were observed in the number and length of stamens in all the varieties, except in King of Siam and Taweekan where stamen production was not observed.

The majority of the studied pollen displayed a prolate spheroidal shape and fell within the medium-sized category. Highest pollen fertility was observed in the varieties Doris Holt (84%) followed by Peach Blow (73%), Hilary (68%), Micrantha (65%), and Bull's Eye (48%) and these varieties were selected as the male parent for the compatibility study.

Based on preliminary hybridization, maximum stigma receptivity was observed for the varieties Poosub, Wanvisa, Siam Amber, Siam Jasmine and

Blue Whistle and these varieties were selected as the female parent for the compatibility study.

Maximum percentage of fruit set were observed when Siam Amber x Doris Holt (100%) followed by Poonsub x Micrantha (80%) and Poonsub x Wanvisa (80%).

The fruit weight and seed size of Siam Amber x Doris Holt was larger compared to other varieties. The hybrid seeds took eight days for germination and they flowered in 25 to 29 days.

The hybrid plants are in flowering, near flowering and pre flowering stages. Vegetative and floral characters of the hybrids that flowered when compared with their parents, revealed variations in the parameters studied. The hybrids obtained in the present study can be further assessed for floral attributes and selected for cultivation.

സംഗ്രഹം

'ആമ്പൽ ഇനങ്ങളുടെ സ്വഭാവ സവിശേഷതകളും സങ്കരണം നടത്തുന്നതിന്റെ അനുയോജ്യതാ പഠനങ്ങളും എന്ന വിഷയത്തിൽ ഗവേഷണ പരിപാടി തിരുവനന്തപുരം വെള്ളായണി കാർഷിക കോളേജിലെ ഫ്ലോറികൾച്ചർ വിഭാഗത്തിൽ 2022-23 കാലയളവിൽ നടത്തുകയുണ്ടായി. വളർച്ച രീതിയിലെ വ്യത്യാസങ്ങൾ, പൂവിന്റെ ഗുണവിശേഷങ്ങൾ എന്നിവയുടെ അടിസ്ഥാനത്തിൽ 30 ഇനം ആമ്പലുകളുടെ പ്രകടനം വിലയിരുത്തുക എന്നതായിരുന്നു പഠനത്തിന്റെ ലക്ഷ്യങ്ങൾ.

30 ആമ്പൽ ഇനങ്ങളിൽ മൈക്രാന, ചോമ്പു, തവീക്കൻ, ബുൾസൈ, ഹിലാരി, ഡോറിസ് ഹോൾട്ട്, കാർല സോൺ ഷൈൻ, സയാം ജാസ്കിൻ, വാൻവിസ, മെക്ലിക്കാന, കിംഗ് ഓഫ് സയാം, ബ്ലൂ വിസിൽ, പർപ്പിൾ ജോയ്, ഡൗബൻ, പൂൺസബ്, ക്ലൈഡ് ഐക്വിൻസ്, വെർജിനാലിസ്, പീച്ച് ബ്ലോ, ഇസ്ലാമോറാഡാ, സയാം പിങ്ക്, സുപ്രാനീ പിങ്ക്, ജലുയാങ്ങ് സബ്, ട്രോപിക് സൺസെറ്റ്, മിയാമി റോസ്, കോളൊറാഡോ, പരാനി, സയാം ആമ്പർ, പെറീസ് ബേബി റെഡ് എന്നിങ്ങനെ 28 ഇനങ്ങളിൽ പൂക്കൾ ഉണ്ടായതായി നിരീക്ഷിച്ചു. ആമ്പൽപ്പൂക്കൾ വിടർന്ന് ആദ്യ ദിവസങ്ങളിൽ പൂവ് പരാഗണസജ്ജമായി കാണപ്പെട്ടു തുടർന്നുള്ള രണ്ടു ദിവസങ്ങളിൽ പൂവിൽ നിന്ന് പൂമ്പൊടി പുറത്തുവരുകയും ചെയ്തു.

ഗവേഷണത്തിന്റെ അടിസ്ഥാനത്തിൽ 21 ആമ്പൽ ഇനങ്ങളിൽ പൂമൊട്ട് കണ്ടുതുടങ്ങുന്ന ദിവസങ്ങൾ മുതൽ പൂവിരിയുന്നതുവരെയുള്ള ദിവസങ്ങൾ ഒഴികെ എല്ലാതരം പുഷ്പസവിശേഷതകൾക്കും കാര്യമായ വ്യത്യാസങ്ങൾ കണ്ടെത്തി. 30 ആമ്പൽ ഇനങ്ങളിൽ സസ്യസ്വഭാവവുമായി ബന്ധപ്പെട്ട് പ്രസക്തമായ വ്യത്യാസങ്ങൾ നിരീക്ഷിക്കപ്പെട്ടു.

പഠനത്തിന് ഉപയോഗിച്ച ആമ്പൽ ഇനങ്ങളെ അവയുടെ സവിശേഷതകളെ അടിസ്ഥാനമാക്കി നാല് ക്ലസ്റ്ററുകൾ ആയി തരംതിരിച്ചിരിക്കുന്നു (ഇലയുടെ നീളം, ഇലയുടെ വീതി, ചെടിയിലെ ഇലകളുടെ എണ്ണം, ചെടിയുടെ ആയുസ്സ്, ലാമിനയുടെ പൂർണ്ണവികാസം

വരെയുള്ള ദിവസങ്ങളുടെ എണ്ണം എന്നിങ്ങനെ). ക്ലസ്റ്റർ ഒന്നിൽ 13 ആമ്പൽ ഇനങ്ങളും ക്ലസ്റ്റർ രണ്ടിൽ 15 ആമ്പൽ ഇനങ്ങളും ക്ലസ്റ്റർ മൂന്നിലും നാലിലും ഒരു ആമ്പൽ ഇനം വീതവും ഉൾപ്പെടുന്നു. സസ്യ സ്വഭാവങ്ങളുമായി ബന്ധപ്പെട്ട പരമാവധി ഇൻറർ ക്ലസ്റ്റർ ദൂരം കാണപ്പെടുന്നത് ക്ലസ്റ്റർ ഒന്നിനും ക്ലസ്റ്റർ മൂന്നിനും ഇടയിലാണ് (18.48). അതു പോലെ ക്ലസ്റ്റർ രണ്ടിനും ക്ലസ്റ്റർ നാലിനും ഇടയിൽ ഏറ്റവും കുറഞ്ഞ ദൂരവും കാണപ്പെട്ടു (7.94).

പൂക്കളുടെ സവിശേഷതകളെ അടിസ്ഥാനമാക്കി ആമ്പൽ ഇനങ്ങളെ അഞ്ച് ക്ലസ്റ്ററുകളായി തിരിച്ചിരിക്കുന്നു (പൂമൊട്ടിന്റെ നീളം, പൂവിന്റെ വ്യാസം, പൂഷ്പദളത്തിന്റെ നീളം, പൂഷ്പദളത്തിന്റെ വീതി, പൂഷ്പദളങ്ങളുടെ എണ്ണം, പൂഷ്പദളത്തിന്റെ നീളവും വീതിയും). മൂന്ന് ആമ്പൽ ഇനങ്ങൾ അടങ്ങിയ ക്ലസ്റ്റർ ഒന്നിനും, അഞ്ച് ആമ്പൽ ഇനങ്ങൾ അടങ്ങിയ ക്ലസ്റ്റർ 2, ഏഴ് ആമ്പൽ ഇനങ്ങൾ അടങ്ങിയ ക്ലസ്റ്റർ 3, നാല് ആമ്പൽ ഇനങ്ങൾ അടങ്ങിയ ക്ലസ്റ്റർ 4, രണ്ട് ആമ്പൽ ഇനങ്ങൾ അടങ്ങിയ ക്ലസ്റ്റർ 5 എന്നിവയാണവ. ക്ലസ്റ്റർ 2, ക്ലസ്റ്റർ 3 എന്നീ ക്ലസ്റ്ററുകൾക്കിടയിൽ പൂഷ്പസവിശേഷതയുടെ പരമാവധി ക്ലസ്റ്റർ ദൂരം നിരീക്ഷിക്കപ്പെട്ടു (75.19). കൂടാതെ ക്ലസ്റ്റർ 1, ക്ലസ്റ്റർ 3 എന്നിവ തമ്മിൽ ഏറ്റവും കുറഞ്ഞ ദൂരവും കാണപ്പെട്ടു (7.36). രണ്ട് ക്ലസ്റ്ററുകൾ തമ്മിലുള്ള ദൂരം കൂടുംതോറും രണ്ട് ക്ലസ്റ്ററുകളിൽപ്പെട്ട ആമ്പൽ ഇനങ്ങൾ തമ്മിലുള്ള വ്യത്യാസം വർദ്ധിക്കുന്നു.

എല്ലാ ആമ്പൽ ഇനങ്ങളിലും വിരിയുന്ന പൂക്കൾ നാലുദിവസം ചെടിയിൽ നിൽക്കുന്നതായി കാണപ്പെട്ടു. പാരിസ്ഥിതികഘടകങ്ങൾ ആമ്പലിന്റെ പൂഷ്പകാലത്തെ ബാധിക്കുന്നതായി കണ്ടെത്തി. മേഘാവൃതവും മഴയുള്ളതുമായ കാലാവസ്ഥ പൂക്കൾ വിടരുന്നത് വൈകിപ്പിക്കുകയും പൂക്കൾ നേരത്തെ അടയുന്നതിനു കാരണമാവുകയും ചെയ്തു.

പഠനകാലയളവിൽ കേസരങ്ങളുടെ ഉത്പാദനം കാണപ്പെടാത്ത ഇനങ്ങളായ കിംഗ് ഓഫ് സയാം, തവിക്കാൻ എന്നിവയിൽ ഒഴികെ എല്ലാം ഇനങ്ങളിലും കേസരങ്ങളുടെ എണ്ണത്തിലും നീളത്തിലും കാര്യമായി വ്യത്യാസങ്ങൾ കണ്ടു.

പഠനത്തിന് വിധേയമായ പുമ്പൊടികളിൽ ഭൂരിഭാഗവും ദീർഘഗോളാകൃതി കാണിക്കുകയും ഇടത്തരം വലിപ്പമുള്ള വിഭാഗത്തിൽപ്പെടുകയും ചെയ്തു. ഡോഗിസ് ഹോൾട്ട് (84%), പീച്ച് ബ്ലോ (73%), ഹിലാരി (68%), മൈക്രാന (65%), ബുൾസൈ (48%) എന്നീ ഏറ്റവും ഉയർന്ന പരാഗണ ശേഷിയുള്ളതായി കാണപ്പെട്ട ഇനങ്ങളെ സങ്കരണം നടത്തുന്നതിനുള്ള പിതൃസസ്യങ്ങളായി തിരഞ്ഞെടുത്തു.

പ്രാഥമിക ഹൈബ്രിഡൈസേഷന്റെ അടിസ്ഥാനത്തിൽ പുൺ സബ്, വാൻ വിസ, സയാം ആംബർ, സയാം ജാസ്കിൻ, ബ്ലൂ വിസിൽ എന്നീ ഇനങ്ങൾക്ക് പരമാവധി പരാഗണ സ്വീകാര്യത കണ്ടെത്തി. ഈ ഇനങ്ങളെ സങ്കരണത്തിനുള്ള അനുയോജ്യതാ പഠനത്തിനായും മാതൃ സസ്യങ്ങളായി തിരഞ്ഞെടുത്തു.

സയാം ആമ്പർ x ഡോഗിസ് ഹോൾട്ട് (100%), പുൺസബ് x മൈക്രാന (80%), പുൺസബ് x വാൻവിസ (80%) എന്നിവ എന്നിവയിൽ കൂടുതൽ ഫലങ്ങൾ ഉണ്ടായതായി കാണപ്പെട്ടു.

സയാം ആമ്പർ x ഡോഗിസ് ഹോൾട്ടിന്റെ ഫലത്തിന്റെ തുക്കവും വിത്തിന്റെ വലിപ്പവും മറ്റ് ഇനങ്ങളെ അപേക്ഷിച്ച് കൂടുതലായിരുന്നു. സങ്കരയിനം വിത്തുകൾ എട്ടു ദിവസം കൊണ്ട് മുളയ്ക്കുകയും 25 മുതൽ 29 ദിവസം കൊണ്ട് പൂവിടുകയും ചെയ്തു.

ഈ പഠനത്തിൽ ലഭിച്ച ഹൈബ്രിഡ് സസ്യങ്ങൾ വിവിധ വളർച്ച ഘട്ടങ്ങളിലാണ്. നിലവിലെ പഠനത്തിൽ ലഭിച്ച സങ്കരയിനങ്ങളെ പൂക്കളുടെ സവിശേഷതകൾക്ക് അനുസരിച്ച് കൂടുതൽ പഠനങ്ങൾ നടത്തിയതിനുശേഷം കൃഷിക്കായി തിരഞ്ഞെടുക്കാവുന്നതാണ്.