UNIVERSITI PUTRA MALAYSIA

VEGETATIVE AND REPRODUCTIVE PROPAGATION OF NELUMBO NUCIFERA GAERTN

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VEGETATIVE AND REPRODUCTIVE PROPAGATION OF NELUMBO NUCIFERA GAERTN

By

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A Project Report Submitted in Partial Fulfilment of the Requirement
for the Degree of Bachelor of Bioindustry Science in the
Faculty of Agriculture and Food Sciences
Universiti Putra Malaysia Bintulu Sarawak Campus

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I dedicate this book to my beloved family, dad, mom, and sisters they are source of unconditional love and support. Thanks a lot for your encouragement, understanding, support, patience and wonderful guidance for me to complete this book.

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ABSTRACT

Nelumbo nucifera (sacred lotus) is a perennial aquatic angiosperm that has economic and ornamental importance throughout the world. The present study was conducted to observe vegetative and reproductive morphology and propagation. The study was conducted in order to identify important features of vegetative and reproductive structures including leaves, flowers and fruits of *N. nucifera* from temporary wetland, Bintulu Old Airport. *Nelumbo nucifera* from this location are distributed in slow moving water flow at the open environment, simply adapted to hot weather at the water temperature 25.2-29.7°C, while water pH slightly acidic (5.99-6.23). This species is associated with various flora and fauna. Mature leaves are dark green in colour and 30.47±0.48 cm in diameter. Adaxial (above) and abaxial (beneath) surface of leaves are netted and waxy with smooth margin. Stomata only present on the adaxial surface of leaves. Petiole is green in colour, shiny surface with spines, 102.8±0.95 cm in length and 0.91±0.08 cm diameter. Rhizomes are oval shaped, light yellow smooth surface and black spots, being 18.35±0.26 x 5.23±0.20 cm. Roots of this species are fibrous. Flower blossom are pinkish white 14.80±0.22 cm wide. Oval shaped petals have pink surface with white base, 8.90±0.14 cm in length and 3.08±0.08 cm width. Waxy surface of petals are patterned by parallel veins and present with stomata. Numbers of petals per flower are 13-17. Peduncle morphology is similar to petiole and only could differentiate through the numbers of lacunae. Peduncle is 97.13±3.42 cm in length and 0.54±0.01 cm diameter. Sepals are oval shaped, green in colour like small leaf but the size is smaller than petals. Stomata also observed in sepals. Elongated stamens consist of white filament, 1.18±0.01 cm in length, yellow anther 1.06±0.01 cm in length and rounded vegetative tips 0.30±0.01 cm in length. Stamens per flower range from 98-143. Fruits or seed pod are funnel shaped, dark brown when matured and possess 4.45±0.74 cm in length and 5.83±0.13 in diameter. Various shaped carpels occur in seed pod and 1.50±0.01 cm in diameter. Numbers of carpels per fruit or seed pod are range from 7-20. Seed are embedded in carpels 1.41±0.04 cm in length and 1.01±0.01cm diameter. Reproductive propagation was performed through scarified and non-scarified seeds. Germination was 100% for scarified seeds and 96% for non-scarified seeds. Reproductive propagation through seeds is more effective in this study because the emergence of first leaf is earlier than leaf from rhizome. First leaf through vegetative propagation was produced after 42 days, while only 36 days through seeds. The rhizome development takes around 180 days to produced 4 leaves, roots and secondary rhizome, but with seeds only 73 days.

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ABSTRAK

Nelumbo nucifera (teratai) adalah tumbuhan angiosperma akuatik berbunga dan berbuah sepanjang tahun yang mempunyai kepentingan dari segi ekonomi dan hiasan di seluruh dunia. Kajian ini telah dijalankan untuk memerhati morfologi dan pembiakan bagi struktur vegetatif dan reproduktif. Ciri-ciri penting struktur vegetatif dan reproduktif termasuk daun, bunga dan buah bagi *N. nucifera* dari kawasan tanah lembap sementara, Lapangan Terbang Lama Bintulu telah dikenalpasti. *Nelumbo nucifera* dari kawasan ini hidup di dalam parit yang mempunyai aliran air yang perlahan, suhu sekitar 25.2-29.7°C, pH sedikit berasid (5.99-6.23) dan di kawasan terbuka yang terdedah pada cuaca persekitaran yang panas. Spesies ini berasosiasi dengan pelbagai flora dan fauna. Daun yang matang berwarna hijau gelap, berukuran 30.47±0.48 cm diameter. Permukaan atas dan bawah daun berlilin dan mempunyai urat berjaringan. Stomata hanya hadir pada permukaan atas daun. Petiol berwarna hijau, berduri serta mempunyai permukaan berkilat, 102.8±0.95 cm panjang dan berdiameter 0.91±0.08 cm. Rizom berbentuk bujur berwarna kuning muda, berbintik hitam serta permukaan yang licin. Rizom mempunyai 18.35±0.26 cm panjang dan berdiameter 5.23±0.20 cm. Spesies ini mempunyai akar serabut. Bunga yang kembang berwarna merah jambu keputihan dan lebarnya adalah 14.80±0.22 cm. Petal berwarna merah jambu berbentuk bujur dengan bahagian bawahnya berwarna putih, panjang 8.90±0.14 cm dan lebar 3.08±0.08 cm. Permukaan petal adalah berlilin, mempunyai urat selari dan stomata. Bilangan petal bagi setiap bunga adalah 13-17. Permukaan morfologi tangkai bunga adalah sama seperti petiol dan hanya dapat dibezakan melalui bilangan ruang udara. Panjang tangkai bunga adalah 97.13±3.42 cm dan berdiameter 0.54±0.01 cm. Sepal berbentuk bujur dan berwarna hijau seperti daun kecil, tetapi saiznya lebih kecil daripada petal. Stomata juga diperhatikan pada permukaan sepal. Stamen memanjang dan terdiri dari filamen putih, 1.18±0.01 cm panjang, anter kuning 1.06±0.01 cm panjang dan hujung vegetatif adalah bujur 0.30±0.01 cm panjang. Bilangan stamen bagi setiap bunga adalah 98-143. Buah atau kelongsong biji adalah berbentuk kon dan berwarna coklat gelap apabila matang, 4.45±0.74 cm panjang dan berdiameter 5.83±0.13 cm. Pelbagai bentuk karpel terbentuk di dalam kelongsong biji dan berdiameter 1.50±0.01 cm. Bilangan karpel bagi setiap buah atau kelongsong biji adalah di antara 7-20. Biji terbenam dalam karpel dan berukuran 1.41±0.04 cm panjang dan 1.01±0.01 cm diameter. Propagasi secara biji benih dijalankan dengan menggunakan biji yang kulit luarnya telah dikeluarkan dan tanpa dikeluarkan. Percambahan biji benih adalah 100% bagi biji yang kulit luarnya telah dikeluarkan dan 96% bagi biji biasa. Propagasi secara biji benih lebih efektif berbanding propagasi secara vegetatif melalui rizom kerana daun pertama tumbuh daripada biji dalam masa 36 hari, manakala 42 hari bagi propagasi menggunakan rizom. Rizom mengambil masa 180 hari untuk menghasilkan 4 daun, rhizom sekunder dan akar, tetapi biji menghasilkan semuanya dalam masa 73 hari.

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APPROVAL

I certify that this research project report entitled "Vegetative and Reproductive Propagation of *Nelumbo nucifera*" has been examined and approved as a partial fulfillment of the requirement for the degree of Bachelor Science in the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Campus.

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CHAPTER 1

INTRODUCTION

Aquatic macrophytes are plants that dominate wetlands, shallow lakes and stream with rooted in shallow water those usually less than fifteen feet deep. Cronk and Fennessy (2001) reported aquatic macrophytes including aquatic angiosperm (flowering plants), pteridophytes (ferns) and bryophytes (mosses). Aquatic macrophytes can be classified into four categories depending on their growth, such as floating unattached, floating attached, submerged and emergent (Jones and German, 1993). Emergent refer to those species which occur on saturated soils or on soils covered with water for most of the growing season and the foliage of emergent aquatic plants is partly or entirely borne above the water surface (Jones and German, 1993).

Among all the type of aquatic plants emergent plants are most similar to terrestrial species, relying on (aerial above the water) reproduction and soil as their exclusive source of nutrient (Cronk and Fennessy, 2001). *Nelumbo nucifera* or sacred lotus is defined as emergent plant rooted in the soil with basal portion that typically grow beneath the surface of the water, but whose leaves, petiole (photosynthetic parts), and reproductive organs are aerial (Jones and German, 1993).

Jones and German (1993) grouped *N. nucifera* as herbaceous plant that have large green leaves, raised high above water on stout petioles, bear solitary flower typically pink but also have white, red and striped varieties with numerous stamens, funnel shaped 'seed pods' on long peduncle (Jones and German, 1993). This species

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occur in temperate, sub-temperate, subtropical and tropical regions in South East Asia (Sharma and Goel, 2000).

The name *Nelumbo* is derived from Sri Lanka (Jones and German, 1993). The ancient Egyptians revered *N. nucifera* as symbol of immortality (Green, 2002; Jones and German, 1993) and used in many ancient drawings or architecture on tombs and temple walls (Wan, 2006). The flower is sacred to Buddhists and Hindus which is symbol of purity and strength, therefore the lotus motif has been used extensively for prayers and it was taken to Egypt by man about 500 years B.C. (Jones and German, 1993; Soepadmo, 1998). However Lei *et al.* (2000) reported that sacred lotus have many economic and ornamental importance in China and many other countries.

Greulach and Adams (1962) state that propagation can be performed through reproduction by seeds and vegetative by using rhizomes (underground creeping stem). Plants practically are propagated sexually through seeds, but very large portion of herbaceous perennial plants such as *N. nucifera* are commonly propagated vegetatively (Greulach and Adams, 1962). Propagation of sacred lotus is emphasize all around the world because of its economic importance as ornamentals (Jones and German, 1993), edible rhizomes and seeds (Simpsom, 2006), medicinal value (Chapman and Wang, 2002; Daniel, 2006; Shrivastava, 2006) and religious rites for Buddhist and Hindus. Many sacred lotuses were hybridized in Honululu, some of the hybrids are very fine and should be tried to cultivate in Malaysian climate (Simpsom, 2006).

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Studies of evolution, population and ecology (Greg and Speichert, 2000), genetics (Lei *et al.*, 2000) and seed germination (Pagels, 2001) of this species are abundant in China, India, Japan, Florida and some other Asian countries that have contributed significant information about *Nelumbo nucifera*, but little is known about propagation and seed germination of this species in Malaysia. Therefore, there is a need to conduct a study on the propagation and seed germination of *N. nucifera* in this country. Consequently, a study was initiated on vegetative and reproductive propagation of *Nelumbo nucifera* in Bintulu, Sarawak, Malaysia.

The objectives of this study are to determine the vegetative and reproductive morphology of *Nelumbo nucifera*, determine effective and efficient propagation between vegetative and reproductive and to study seed germination of *Nelumbo nucifera.*

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CHAPTER 2

LITERATURE REVIEW

2.1 Taxonomy

Classification of *Nelumbo nucifera*

Kingdom	:	Plantae
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Order	:	Proteales
Family	:	Nelumbonaceae
Genus	:	*Nelumbo*
Species	:	*nucifera*
Common name	:	Seroja, Teratai or Telipok

(http://www.itis.gov/servlet/singleRpt/singleRpt?search_topic=TSN&search_value=503949)

Nelumbo nucifera GAERTN. belong to monogeneric family Nelumbonaceae and is also known as *Nelumbium nelumbo*, *Nelumbium nucifera*, *Nelumbiun speciosum* and *Nelumbo nelumbo* (Heywood *et al.*, 1986; Goel *et al.*, 2001). The family Nelumbonaceace is a small family of aquatic angiosperms which consists of two species, *Nelumbo nucifera* (Asian lotus) usually has white or pink, single, semidouble or double blossom flower and *Nelumbo lutea* (American lotus) usually white creamy yellow flower (Mukherjee, 1996; Takhtajan, 1997; Greg and Speichert, 2000).

Jones and German (1993) stated that these species classified as water lily under family Nymphaeacea, or Nelumbonaceae. Porter (1967) and Takhtajan (1997) placed the family Nelumbonaceae in the sub-class Nelumbonidae and setting sacred lotus apart

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from water lily family but Graham *et al.* (1988) was reported DNA data has revealed that the *N. nucifera* is not related to other water lily family because *N. nucifera* is significantly different from water lily.

Hegnauer (1969) compared *Nelumbo* and Nympheaceae, and reported, that there is an inappropriate presence of criterion with chemical evidence that possess sharp dichotomy differences between *Nelumbo* and Nymphaeceae but according to Heywood and Moore (1984) the endosperm or perisperm of *Nelumbo* seeds suggest a particular relationship with the genus Nymphaeceace. However, evidence from reproductive biological studies; suggest that there is great similarity between *N. lutea* and *N. nucifera*, therefore only these two species are in the same genus (Barthlott *et al.*, 1996).

Nelumbo nucifera have now been assigned to its own family, Nelumbonaceae and is locally known as seroja, teratai or telipok (Soepadmo, 1998; Green, 2002; Kamarudin and Latiff, 2002).

2.2 Vegetative and reproductive morphology of *Nelumbo nucifera*

Nelumbo nucifera are emergent plants that grow in submersed soil but whose leaves, stems and flowers rise above the water surface (Green, 2002). According to Muhlberg (1982), Jones and German (1993), Cronk and Fennessey (2001), Kamarudin and Latiff (2002) and Narayanan *et al.* (2003) this species has floating leaves while Daniel (2006) and Simpsom (2006) stated that the leaves are emergent.

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Leaves are spiral, peltate, petiolate, simple, undivided, orbicular and net-veined (Jones and German, 1993; Mukherjee, 1996; Cronk and Fennessey, 2001; Narayanan *et al.*, 2003; Daniel, 2006; Simpsom, 2006). According to Green (2002) leaves are round, plate shaped and blue-green in color while Greg and Speichert (2000) reported leaves are bowl-like shaped. The width of the leaves has been reported to be 30-60cm (Muhlberg, 1982; Kamarudin and Latiff, 2002) or two feet (Greg and Speichert, 2000).

Leaves have smooth margin with waxy surface. Wax is deposited in the cutinized layer of the cell wall and becomes particularly impermeable to water makes the leaf unwettable, and allow it to float or emerge above the water surface (Denffer *et al.*, 1965; Muhlberg, 1982; Narayanan *et al.*, 2003). Petiole are long aculeate inserted in the middle of leaves and could be five to six feet in tall (Jones and German, 1993; Greg and Speichert, 2000; Kamarudin and Latiff, 2002; Narayanan *et al.*, 2003). The rhizome is shaped like long sausages with hollow portions (Mukherjee, 1996).

Muhlberg (1982) stated *Nelumbo nucifera* blossoms are pale red, but some studies reported this species has white or dark pink solitary blossom flowers (Jones and German, 1993; Mukherjee, 1996; Greg and Speichert, 2000; Kamarudin and Latiff, 2002; Narayanan *et al.*, 2003). Green (2002) claims the flowers are tulip-shaped but according to Klein (1987) the flower is cup shaped with yellow color in the middle. The flower is almost 4 -12 inches in diameter (Mukherjee, 1996 and Green, 2002). The range of petal per flower is 16 to 160 (Narayanan *et al.*, 2003).

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The stamens are numerous, spiral, apostemonous with filaments that are thin or narrow while anthers are latrorse to introrse that are elongated, have vegetative tip appendage and are tetrasporangiate with a laminar connective (Schneider *et al.*, 1990; Jones and German, 1993; Daniel, 2006; Simpsom, 2006). Centre of the flower has flat-topped, conical receptacle that enlarged and spongy, with numerous sunken cavities containing individual pistils (Lawrence, 1951; Porter, 1967; Klein, 1987 and Simpsom, 2006). The style and stigma are solitary and terminal, placentation is apical; gynoecium is apocarpous, with 12-40 loculed on superior or hypogenous ovaries, ovules are anatropous and bitegmic one per ovary, while pistils partially embedded within and expanded receptacle (Simpsom, 2006).

Receptacle develops into brown, cone-shaped (Mukherjee, 1996) or funnel shaped seed pod with 12 large holes that hold smooth, brown, long and oval shaped seeds (Klein, 1987; Jones and German, 1993). The seeds are also nut-like achenes, and blackish brown when ripe (Porter, 1967; Narayanan *et al.*, 2003; Simpsom, 2006 and Daniel, 2006).

Seeds of this species are exalbuminous due to the nucellar tissue which persists and increases in volume to form endosperm or perisperm (Fahn, 1990 and Simpsom, 2006). Heywood *et al.* (1986) stated that the seed contains a large embryo with large fleshy cotyledons and a well-developed plumule without radical but Lawrence (1951), on the other hand reported that seeds are leathery or tardily dehiscent berry, which means that seeds have straight embryo and the starchy endosperm are absent in *Nelumbo nucifera*. Seed pod usually contain 20-31 edible seeds (Bidlack *et al.*, 2006; Daniel,

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2006; Wan, 2006) on long peduncle almost 3-6 feet (Greg and Speichert, 2000; Sharma and Goel, 2000 and Green, 2002).

2.3 Ecology and Distribution

Lotuses are not tropical plants and they simply adapt to hot weather besides preferring warm temperature for soil and water, usually they revel in humid weather and not start to grow in spring until the water temperature reached 15.55 °C or 60 °F (Greg and Speichert, 2000). However, lotus plants produced a significant amount of heat during the sequence of flowering due to the stamen consist of vegetative tip appendage that has thermogenic properties which capable of raising bud and floral temperature an average of 2.3°C to 4.8°C above ambient (Schneider *et al.*, 1990). Contribution of the alternative pathway to respiration during thermogenesis in flower of sacred lotus regulates the temperature in response to raising temperature from an ambient temperature. Significant increases in alternative pathway flux were found in receptacles with temperature 16°C to 20°C above ambient temperature (Watling *et al.*, 2006).

Flower regulates the temperature by undergoing cellular respiration to form its own microclimate and flower blossoms are best at the temperature of 30°C to 35 °C, and an air temperature varying between 10°C to 30°C throughout the 2 to 4 day period (Seymour *et al.*, 1998; Sharma and Goel, 2000 and Storey and Moore, 2001). Greg and Speichert (2000) reported *N. nucifera* need temperature around 26.67°C (80°F) to bloom its flower as large as a basketball.

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Lotus can easily burn because its sensitive to pH (Greg and Speichert, 2000), but Bold and Lundell (1974) and Miller *et al.* (1995) observed the seed tissue contain abundant hardy proteins that not soluble to high temperature (110°). According to Nohara and Kimura (1996), petiole elongation respond to the amplitude of flooding and maximum rate of petiole elongation was 25cm per day at 2.4 meter water depth. They also strictly stated that 2.4m is maximum depth at which *Nelumbo nucifera* could grow and no petiole could elongate from 3m to 5m depth.

Nelumbo nucifera is distributed throughout India, Egypt, China, Japan, the Philippines, Australia, Florida, Minnesota, North California, Canada, Thailand, South Russia and the Caspian Sea (Ng *et al.*, 1991; Green, 2002). In the wild, *Nelumbo nucifera* is distributed from the Caspian Sea and Iran through India, China, Vietnam to Japan, Malaysia, Indonesia, New Guinea and Australia. This species was found in ponds, streams and lakes across Australia (Heywood *et al.*, 1986; Ng *et al.*, 1991). It is cultivated in many countries as a food crop and has naturalized in some parts of the eastern United States (Takhtajan, 1997). Steele *et al.* (2006) stated *Nelumbo nucifera* known as 'Bali Red' was probably the first so-called "tropical" lotus brought to cultivation in the US.

Paya Indah Wetland located in Dengkil Selangor was Malaysia's first tourism park offered a unique journey through rehabilitated wetland ecosystem, where *N. nucifera* used to cover the mining land (Wan, 2006). The unique ecosystem developed in Tasik Chini Pahang, is dominated by naturally present sacred lotus which covered the entire surface of the lake (Wan, 2006).

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The habitats of this species are affected to a great extent by a number of natural and anthropogenic activities such as disposal of domestic and industrial effluents, invasion of aquatic weeds, spraying of insecticides and pesticides, drainage, floods and other developmental activities, furthermore eutrophication of aquatic bodies have caused radical biochemical changes in the biomass of aquatic plant species and shrinkage of the water bodies which has threatened the habitat and distribution of the *Nelumbo* nucifera (Sharma and Goel, 2000). In Muriyad, Australia wetland *N. nucifera* is seen as a weed because it is unwanted plant in that wetland (Kumar and Thomas, 2006).

2.4 Propagation

According to Lei *et al.* (2000) *N. nucifera* is an up-right, insect pollinated which could propagated by sexual reproduction (seeds) or vegetatively as agamogenesis by rhizomes. Cultivation of plant is more convenient and practicable by vegetative propagation, even though reproduction by seeds eases to maintaining desirable combination of hereditary traits (Greulach and Adams, 1962). Vegetative propagation of lotuses is easier because planting single tuber at the beginning of spring usually ensures the production of eight or more tuber by the following spring and spread rapidly to all suitable sites (Pagels, 2001 and Green, 2002).

Some plants require several years to reach maturity when propagated by seed while some plants matured earlier when propagated vegetatively (Greulach and Adams, 1962). All the offspring propagated vegetatively have exactly the same hereditary potential as parent plant, except in cases such as mutation, whereas great hereditary

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variation occurs in sexual reproduction (Greulach and Adams, 1962). In India rhizomes are mainly used to propagate *N. nucifera* (Narayanan *et al.*, 2003).

Most propagation of *N. nucifera* have been done through seeds, because the seeds could disperse to new location and be dormant for over hundred years until hostile environmental conditions have improved (Campbell and Reece, 1998). Seeds often enter a period of dormancy once they are released from the parent plant, dormancy is a method by which seeds cope with variation in climate and resource availability and gives the seed a means of surviving prolonged unfavorable periods (Bliss and Zedler, 1998).

Lotus seeds need special treatment to germinate, even when presented with adequate water, oxygen and favorable temperature because it exhibit dormancy and usually seeds be nicked and soaked for a month for breaking it dormancy (Klein, 1987 and Green, 2000). Existing in an impenetrable seed coat and mired in an oxygen deficient mud, the seeds have intact genetic and enzymatic systems that reactivated when split open and soaked in water that lead to germination (Bidlack *et al.*, 2006).

The Asiatic *N. nucifera* produces very long-lived seeds, a 237 year old *N. nucifera* seed germinated on an herbarium sheet at the British Museum of Natural History when the museum was accidentally flooded after bombing in 1940 (Sculthorpe, 1967). The seed can be viable for many years, decades, and even centuries under favorable condition (Raven *et al.*, 2006) while there are some seeds more than 1000

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years (Denffer *et al.*, 1965; Bold and Lundell, 1974) and more than 3000 years (Simpsom, 2006).

Hallam and Osborne (1974) have been reported lotus seeds retain their plasmodesmata for more than thousand years of dry storage, means that the seeds propagation never been extinct although it dehydrated but rhizomes are part of plant that perisable and could not store for long period (Nir *et al.*, 1969). Archaeologists have found seeds of lotus in China and Japan that have an estimated age of more than a thousand years and carry ability to germinate (Nguyen, 2001). Miller *et al.* (1995) discovered seven ancient sacred lotus fruit that containing a singular seed was collected from ancient lake bed at Pulantian in Lioning Province, China were obtained from the Beijing Institute of Botany for germination test, and when combusted for radiocarbon dating, one of the germinated seeds was found to be 1,288 years.

Schutz and Grosse (2000) reported some *N. nucifera* seeds was found in a peat deposit in Manchuria and estimated by radioactive-carbon dating to be 1,400 years old, rapidly germinated (and subsequently produced flowering plants) when the seeds were filed to permit water entry. Lotus seed that found Namegawa River in Japan after World War II, estimated to be more than 2000 years old was germinated and produce flower that dark pink or almost red when is first bloom (Greg and Speichert, 2000).

Nelumbo nucifera reported to release allelopathy compound that decreasing the seeds formation, because these compound is a specialized form of interference competition in which one species inhibits the growth of neighbors by releasing

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secondary metabolite compounds (Gopal and Goel, 1993; Keddy, 2000). This compound may be from root exudates, or they may be leached from leaves (Gopal and Goel, 1993; Keddy, 2000). Allelopathics effects are manisfested in several ways including decreased germination rates of other species seeds or of their own seed, suppressed growth in neighbouring species or decreases chlorophyll content in other species and finally interference competition (Segretain, 1996).

As world climate became cooler and drier, as geological evidence shows, plants had to develop tissues that were resistant to the dryness and cold and imposed dormancy that became an important feature for herbaceous plant such as lotus (Schumann and Pippen, 1980). The herbaceous angiosperm *N. nucifera* did not evolve the necessary protective and nourishing structures found their habitats limited to those less challenging, and they often produced only seeds as the means of survival during adverse period (Schumann and Pippen, 1980). In California, seeds of *N. nucifera* are more preferred used for propagation because is almost completely impermeable to water germinated immediately (Pagels, 2001).

2.5 Importance

Lotus possesses many economic and ecological importances (Plate 1). According to Edward (1987) and Nguyen (2001) lotus has higher market demand throughout world due to its multiple uses for example, rhizomes as fresh vegetables, canned food, dessert, and starch; seeds as dessert and medicine; flowers as religious ornaments; fragnants and several parts as raw materials to produce cosmetics.

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Goel *et al.*, (2001) reported lotus possesses many medicinal, economic and neutraceutical value, in several Ayurvedic formulation as , cooling, astringent, demulcent useful in weakness, dysentery, diarrhea and also curing fewer. Flowers are used as a cardiotonic in liver, urinary and venereal disoders (Daniel, 2006; Shrivastava, 2006). Essence of lotus petal has been highly regarded as an elixin for any ailment and as having a positive effect on the entire body, therefore the essence is used to help energies the heart, liver, spleen, kidneys and intestines for tissue regeneration and to slow the ageing process (Klein, 1987).

Leaves used for diarrhea, high fever, haemorrhoids, strangury and leprosy whilst the seeds would stop vomiting or soothe irritation of the digestive tract (Goel *et al.*, 2001; Nguyen, 2001). Leaves contain active chemical isoquinoline alkaloids such as aporphine, nuciferine, roemerine and nornuciferin that used as antipyretic (Chapman and Wang, 2002 and Daniel, 2006). Ripped seeds used for medicinal purposes because these parts containing chemical components such as alkaloids, nuciferine, roemerine, protein and quercetin (Narayanan *et al.*, 2003).

Lotus embryo is preliminary used for nervous disorders, insomnia, high fever with restless and hypertension, while flower pod is used to stop bleeding and eliminated stagnant blood (Nguyen, 2001; Daniel, 2006; Shrivastava, 2006). Stamens are useful in the treatment of male sexual disorders and female leucorrhea (Shrivastava, 2006). Kamaruddin and Latiff (2002) stated *N. nucifera* cultivated in Kota Kinabalu, Sabah for medicinal purpose.

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Young leaves, petiole, petals, seeds and flower are eaten raw or cooked as vegetable (Klein, 1987; Goel *et al.*, 2001). Lotus leaves as a flavoring and used to wrap food preparations of rice, tapioca dessert and dim sum (Ruskin and Shipley, 1976 and Mukherjee, 1996). In India, the leaves are dried and used as disposable plate or the leaves used for make cups as containers to distribute food in temples (Mukherjee, 1996). In China and Japan, lotus stamens are used for make refreshing aromatic tea while rhizome are a food that sole whole or in cut piece, fresh, frozen or canned (Klein, 1987). Japan grows its own lotus for rhizome and seed production but still import 18 000 tons of lotus rhizome each year from China. Lotus seeds are high in protein and it usually demanded during 'moon cake' festivals, where China is main producer of lotus seeds (Goel *et al.*, 2001).

Sharma and Goel (2000) and Simpsom (2006) were found *N. nucifera* also being highly ornamental for landscaping lakes and ponds in India because it serves as National flower of India and used for production of perfume due to it sweet fruity aroma. Flower could also become a potential new product for the Australian ornamental market as well (Nguyen, 2001). Sacred lotuses are featured prominently in horticultural plantings in many gardens and parks, in painting and other visual arts, in architectural motifs and decoration (Klein, 1987).

The buds are used as cut flower for making floral arrangements and the dried torus is used in floral decoration and the dried plumule are use as decoration (Goel *et al.*, 2001). Buds or attractive seed pod placed among folded lotus foliage; they resemble delicate pink rosebuds and flower that already opened use for decoration (Mukherjee,

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1996 and Greg and Speichert, 2000). *Nelumbo nucifera* has beautiful flower that enhance many people to hybrid these species with other families of lilies (Green, 2002). Lotus is an important and popular cash crop in many Asian countries. Soepadmo (1998) was reported sacred lotus have eight petals that represents core of the sun and Hindus goodness Vishnu and Laksmi. Many petal of the scared lotus believed to symbolize layers of human personality, as the petals open the individual gradually reaches closer to self realization (Mukherjee, 1996).

Ecologically lotus is important because it can absorb heavy metals and may recommend for plantation in the ponds used for discharging the industrial effluents for water purification in most natural manner. The tolerance limit of aquatic macrophytes is linked with the chemical and physical quality of water (Heywood *et al.*, 1986). Lotus are important component of the plant communities of most freshwater lakes, ponds and other shallow water habitat, these plant also provide food for many type of herbivorous animals and a habitat substrate for others such as long toed birds. The flower and leaves are become a shelter for many aquatic lives such as fish, frog, snails and snakes (Goel *et al.*, 2001). Co-culture and rotated culture of lotus (*Nelumbo nucifera*) and fish in a same pond have been practiced in China for many years. The significantly lower growth and higher mortality of fish in the lotus pond result from shading by lotus leaves which could reduce phytoplankton production and cause low concentration of dissolve oxygen in ponds (Yi and Lin, 2006). Farmer reared fish in the same pond as lotus in China but with only 50% of the usual number of fish because they grew more slowly than when raised alone, instead of that farmer get two incomes (Edwards, 1987).

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Plate 1: Importance of *Nelumbo nucifera*. a- rhizome used as fresh vegetable, b- 'Moon Cake' produced from fresh seeds,c-leaves used for serve or wrap food, d-lotus seeds used for prayers, e-lotus planted surrounding Buddhist temple for ornamental purpose, f-art on wall, g-dry seed pods used for flower decoration and h- association or culture lotus in fish pond.

CHAPTER 3

METHODOLOGY

3.1 Study area

Samples were collected from temporary wetland, Bintulu Old Airport (3° 10' 34.5" N, 113° 04' 27.2"E) (Figure 1). Water pH, temperature and dissolved oxygen at study area were recorded by using pH meter and Dissolve oxygen meter.

3.2 Sampling

Samples of complete plant including leaves, petiole, rhizome, flower, peduncle, seed pod and seeds were collected using special plant hook. Each sample was then placed in different plastic bags that were labeled with date and brought to laboratory for preservation as herbarium and for microscope observation. Study area was visited every 2 weeks for *insitu* observation of flowering and fruiting season. An observation was started on August 2007 until February 2008. Some plants were tagged with water proof tags labeled as leaf, flower and seed pod. Labeled plants were observed every 2 weeks and each stage of growth were recorded. Digital images of the whole sampling area and plant samples were taken by using digital camera (BENQ 5 mega pixel).

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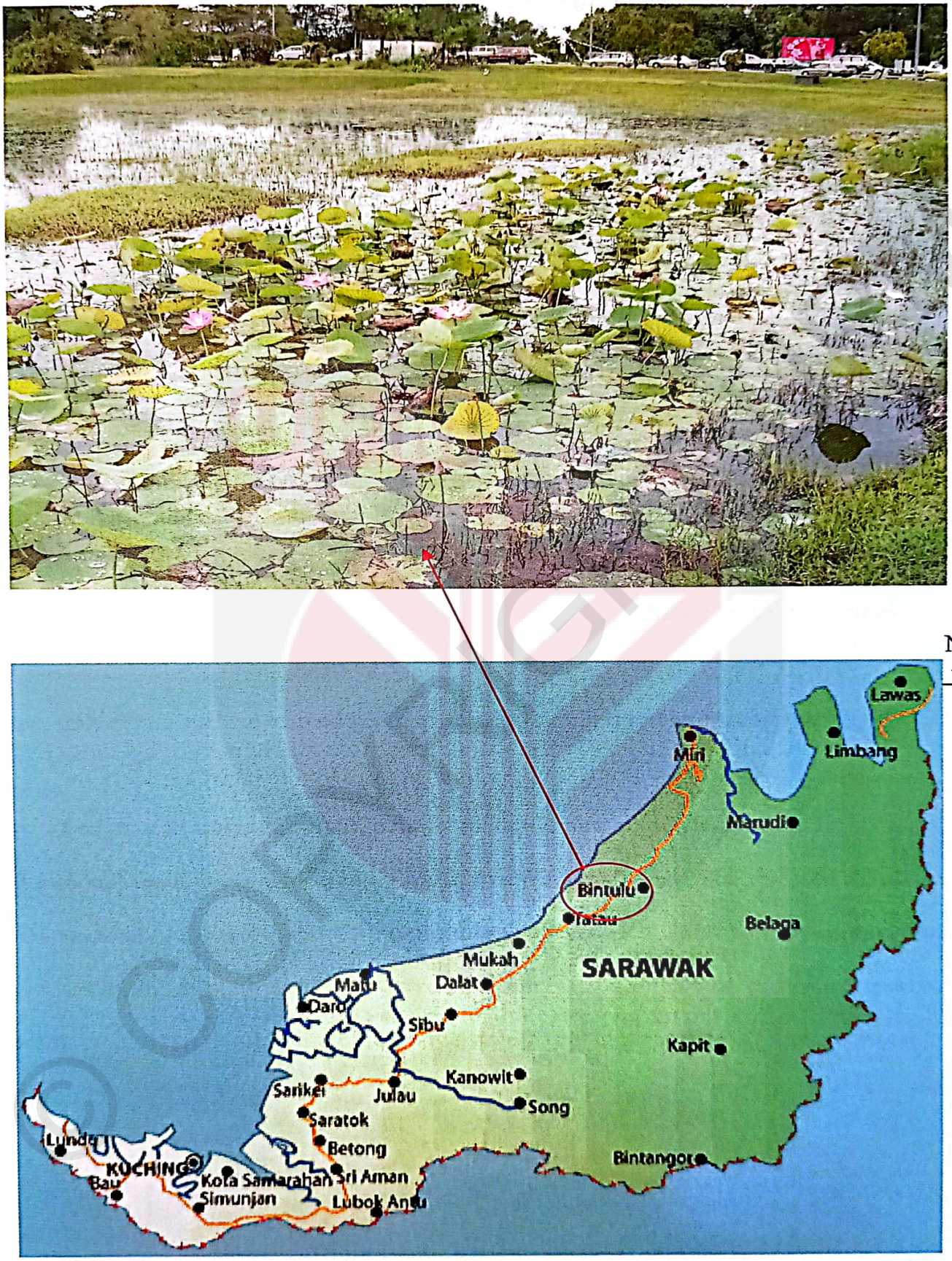


Figure 1: Sampling site of *Nelumbo nucifera* at temporary wetland, Bintulu Old Airport, Sarawak.

3.3 Morphology study of *Nelumbo nucifera*

The shape, margin and colour of adaxial (above) and abaxial (beneath) surface of leaves were observed and recorded. Clearing technique was performed to observe presence of stomata on the adaxial and abaxial surface of leaves. The colour, surface and special characteristic of petiole and rhizome was observed and recorded. Then, the transverse section of petiole was observed under the microscope.

Flower shape, color, petal arrangement, receptacle that contained ovary, number of sepals, petals and stamens were observed and recorded. Surface and transverse section of petal, stamen and peduncle were observed under the microscope. Transverse section of receptacle was observed under microscope to identify the type of ovary. Shape, colour and surface of seed pod and seeds were observed and recorded.

Length and width of leaves, petiole, rhizome, flower, petals, receptacle, stamens, seed pod and seeds were measured by Mitutoyo Vernier Caliper. The matured rhizomes were bought from Bintulu market for morphology observation. All the data was stored in Microsoft Excel. Digital images of leaves, petiole, rhizome, flower, petals, receptacle, stamens, seed pod and seeds were taken by using digital camera (BENQ 5 mega pixel).

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3.3.1 Clearing technique

Leaves were cleaned and soaked in 15% Natrium hydroxide (NaOH) overnight in container. The specimens were washed with distilled water twice and soaked in 15% of Clorox (Sodium hypochlorite) for 5 minutes until it stained (looses it green color). Specimens were washed again by using distilled water. The specimen were then dehydrated in 50% Tert-Butyl Alcohol (TBA) for 10 minutes and dipped in 1% safranin. Then, specimen was dehydrated again through alcohol series of TBA 50%, 70%, 80%, 90%, 95% and 100% for 15 minutes. Finally specimens were soaked in clove oil for 10 minutes and placed on a slide. It was then mounted in DPX glue and covered with cover slide then labeled and observed under microscope.

3.3.2 Herbarium

Plant samples were preserved and stored as herbarium. Samples (leaf, petiole, flower, peduncle, and seed pods) were washed with tap water and arranged properly on sheet of paper. Samples were pressed between two or more papers. Then, the samples were placed between two sheets of cardboard and two sheet of plywood and stacked with pair of rope. It was then placed in an air circulation oven to dry under temperature of 40°C for 1 week. Finally, samples were mounted on herbarium sheet using UHU glue with a label on lower right corner of the herbarium sheet.

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3.4 Propagation of *Nelumbo nucifera*

Matured rhizomes were too deep inside the soil and could not be collected from the sampling site. Only young rhizomes were collected from sampling site for the propagation and herbarium purposes. Selected young rhizome which had 4 growing point and 2 leaves (Figure 2) was used for vegetative propagation. Rhizome was collected at 38 to 43 cm soil depth by using hand from sampling site and brought to the laboratory. Rhizome was gently washed with a stream of water until free from soil. After washing the rhizome, joint section, growing point and end or tip of rhizome was identified. Then, by using sharp knife the rhizome was divided, carefully and not to breaking the growing point, because it quite fragile and plant might not sprout without it. Each division was having growing tips with first node or tubers.

Container without hole was prepared and fills with natural substrate (soil) that brought from sampling site about 10 cm depth (Figure 3). Rhizome pieces are planted horizontally 2 cm deep with sprouts on upper side and 5 cm diameter between each rhizome. Weekly observation was done to observe emergence of new leaves. When the plant starts growing and after new leaves are formed, water was slowly filled into the container. The water level in the container was maintained. Digital image for each stage of vegetative growth was taken using Digital camera (BENQ, 5 Megapixel) and the growth was recorded. Some samples were preserved as herbarium.

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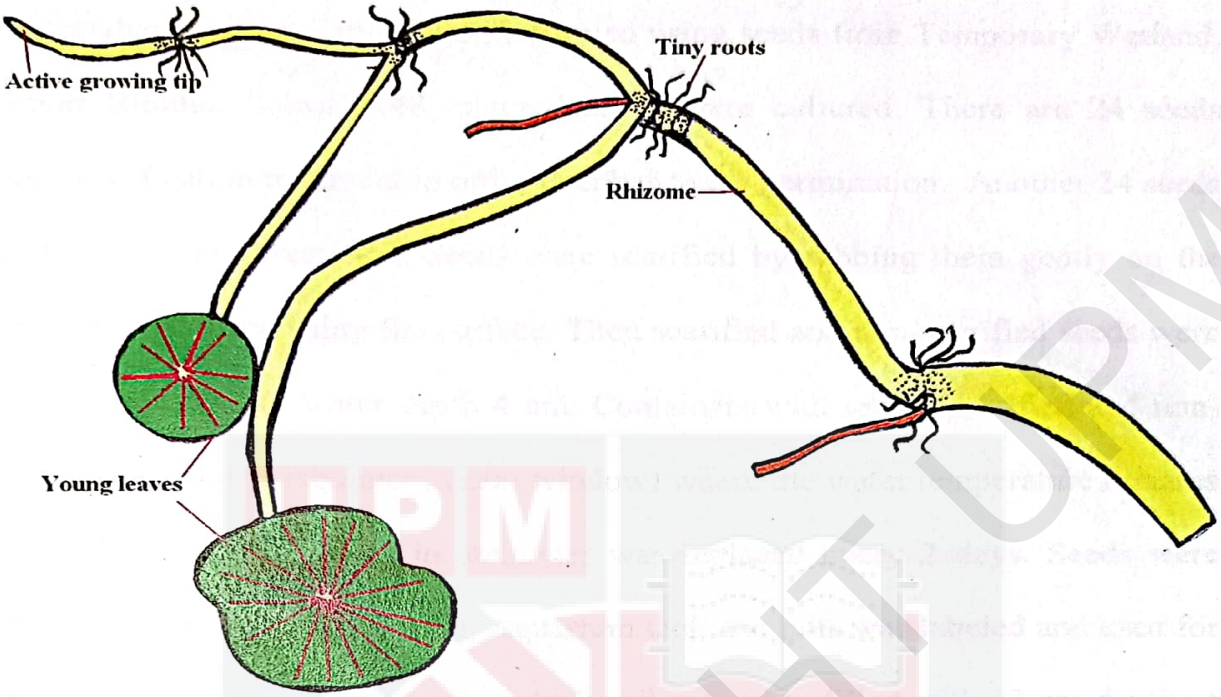


Figure 2: Selected young rhizome with tiny roots, active growing tip and leaves.

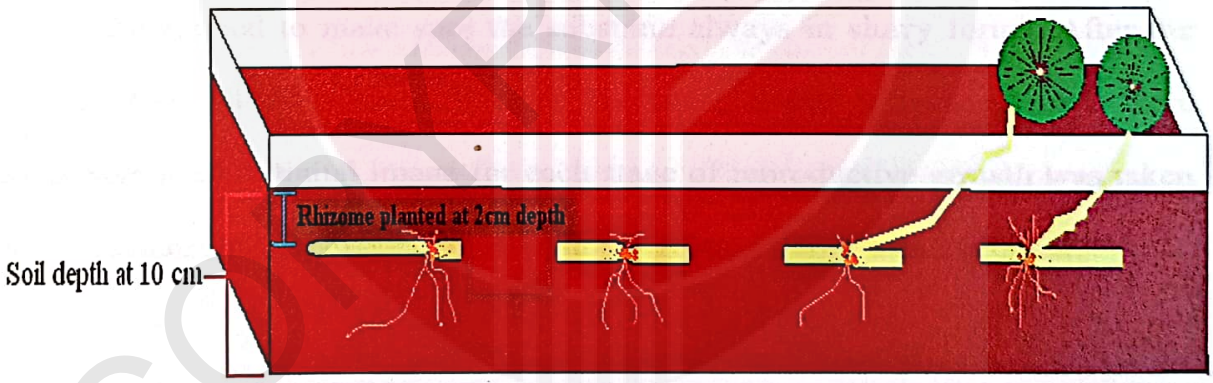


Figure 3: Container without hole was filled with soil at 10 cm depth and rhizome nodes were planted at 2cm depth with 5 cm diameter between each rhizome nodes.

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Reproductive propagation was performed using seeds from Temporary Wetland, Old Airport Bintulu. Selected 48 matured seeds were cultured. There are 24 seeds undergone scarification treatment in order to enhance the germination. Another 24 seeds cultivated without any treatment. Seeds were scarified by rubbing them gently on the sand paper until produce shiny flat surface. Then scarified and non-scarified seeds were immersed in container of water depth 4 cm. Containers with seeds (scarified and non-scarified) were placed in laboratory (near window) where the water temperature remains between 21°C to 32.2°C. Water in container was replaced every 2 days. Seeds were observed every 2 days for germinated. Aquarium tank and pots was labeled and used for transfer pre-germinated seeds. Aquarium tank and pots were filled with 13 cm depth of natural substrate (soil) that brought from sampling site. Each pre-germinated seeds were planted at 2 cm depth in aquarium tank and pots. The water level in the aquarium tank and pots was maintained to make sure the substrate always in slurry forms. After the plants produce 4 to 5 leaves, it was transfer into pond. Some germinated seeds were preserved as herbarium. Digital image for each stage of reproductive growth was taken using Digital camera (BENQ, 5 Megapixel) and the growth stages were recorded.

3.5 Data Analysis

The range, mean and standard error from morphological parameter such as length and width of leaves, petiole, rhizomes, flowers, petals, stamens, receptacle, seed pods, seeds and peduncle were analyzed. Mean for all the parameter were compared using SPSS version 14.0.

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CHAPTER 4

RESULTS

4.1 Habitat

Nelumbo nucifera is highly concentrated or growing at temporary wetland or slow moving water channel (Plate 2). The plants are significatly distributed at the deep channel areas compare to shallow areas. Flowers and fruits are continuously produced throughout the field observation (August 2007 to February 2008). This is due to seeds that dispersed into water are germinated and widely spread from it native area to other areas (Plate 2a). The seeds are initially dormant and when seeds dispersed, it's distribute to other area through water stream and starts to germinate and cause this plant widely spread to other areas. Some *N. nucifera* leaves are emerged on land (Plate 2b), this is due to the elongation of rhizome beneath soil surface until the land.

Nelumbo nucifera is associated with other aquatic and terrestrial plants including animals (Plate 3 and 4) such as *Lindernia* sp., *Tribolium* sp., *Nymphaeae pubescens*, *Limnocharis flava*, *Monochoria vaginalis*, *Eleocharis acutangula*, *Eriocaulo trancatum*, *Mimosa pudica*, *Urticularia aurea*, snail and snake. The water pH during day ranged from 5.99 to 6.23 at 25.2 to 29.7 °C temperature at sampling site and dissolved oxygen (DO) was 1.66 to 1.83 ppm.

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Plate 2: Habitat of *Nelumbo nucifera*. a-plants spread widely from native area to other areas and b-emergent of matured leaves on land.

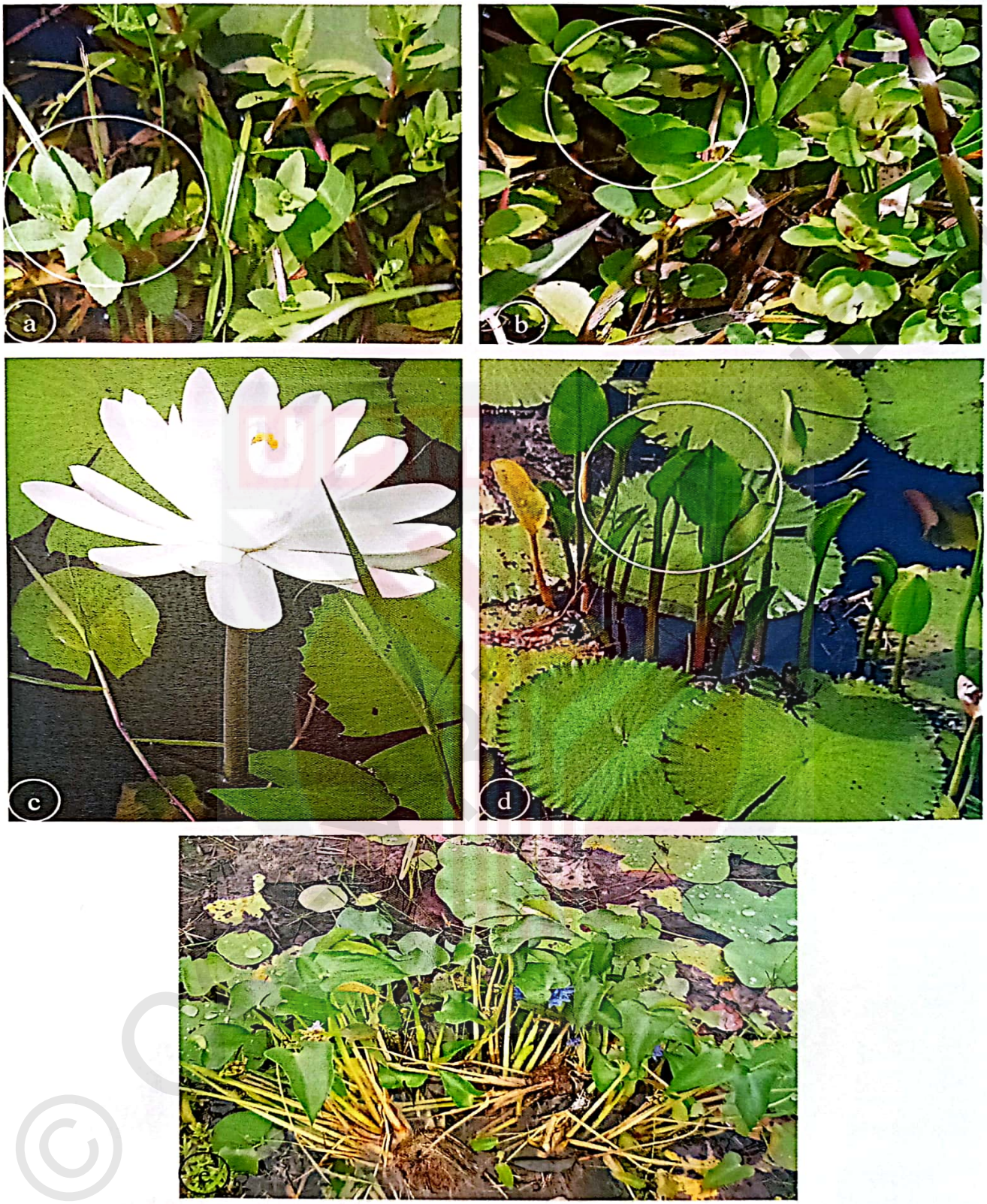


Plate 3: Plants association. a-*Lindernia* sp., b-*Tribolium* sp., c-*Nymphaea pubescens*, d-*Limnocharis flava* and e- *Monochoria vaginalis*.



Plate 4: Plants and animals association. a-*Eleocharis acutangula*, *b-Eriocaulon truncatum*, *c-Mimosa pudica*, d-*Utricularia aurea*, e-snail and f-snake.

4.2 General vegetative and reproductive morphology of *Nelumbo nucifera*

Nelumbo nucifera is perennial dicotyledonous plant which belongs to emergent aquatic macrophytes. The plants have emergent leaves, flower and fruit that exposed to open area (Plate 5). The vegetative components are leaves, petiole, rhizomes and roots, while reproductive components comprise of flowers, fruits and seeds. Summary of dimension analysis of vegetative and reproductive structures of *Nelumbo nucifera* are presented in Table 1.

4.2.1 Vegetative morphology of *Nelumbo nucifera*

4.2.1.1 Leaves and Petiole

The leaves are solitary and have round or plate shaped leaf blade (Plate 6a). Mean of matured leaves is 30.47±0.48 cm and width range from 24.98 to 35.20 cm. Adaxial (above) surface of matured leaves are waxy and dark green in colour and thick net veined. The veins are hexagon shaped (Plate 6b). Stomata are present at adaxial surface of leaves (Plate 6c). The centre of leaves is white and slightly shaped like the wing of butterfly (Plate 6d) which petiole aculeate inserted. The high numbers of stomata are observed at the middle of leaves (Plate 6e). Abaxial (beneath) surface of matured leaves is light green in color with waxy surface (Plate 7a). The abaxial surface of leaves is net veined with small pentagonal shaped (Plate 7b). There were absents of stomata at the abaxial surface. The oval shaped pink pattern is slightly visible at middle of matured leaves with scattered of pink spots (Plate 7c).

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Table 1: Summary of dimension analysis (given in cm) of vegetative and reproductive structures of *Nelumbo nucifera*

Vegetative components	Numbers	Mean	Range (min-max)
Leaves diameter	32	30.47 ± 0.48	24.98 - 35.20
Petiole length	32	102.8 ± 0.95	91.63 -114.40
Petiole diameter	32	0.91 ± 0.08	0.80 -1.00
Rhizome length	15	18.35 ± 0.26	16.20 - 19.90
Rhizome diameter	15	5.23 ± 0.20	3.65 - 6.55
Reproductive components			
Flower wide	10	14.80 ± 0.22	13.40 - 15.90
Petal length	158	8.90 ± 0.14	3.22 - 11.40
Petal width	158	3.08 ± 0.08	1.02 - 5.70
Numbers of petal per flower	158	15.8 ± 0.61	13 - 17
Peduncle length	10	97.13 ± 3.42	80.50 - 110.70
Peduncle diameter	10	0.54 ± 0.01	0.50 - 0.60
Stamen length	150	2.54 ± 0.01	2.10 - 2.80
Filament length	150	1.18 ± 0.01	0.97 - 1.40
Anther length	150	1.06 ± 0.01	0.84 - 1.20
Vegetative tip length	150	0.30 ± 0.01	0.20 - 0.50
Number of stamen per flower	150	119.3 ± 5.05	98 - 143
Seed pod length	40	4.45 ± 0.74	3.40 - 7.20
Seed pod diameter	40	5.83 ± 0.13	3.40 - 7.20
Seed length	263	1.41 ± 0.04	1.20 - 1.60
Seed diameter	263	1.01 ± 0.01	0.80- 2.00
Sunken carpel diameter	525	1.50 ± 0.01	0.60 - 1.30
Number of carpel per seed pod	525	1.31 ± 0.04	7 - 20

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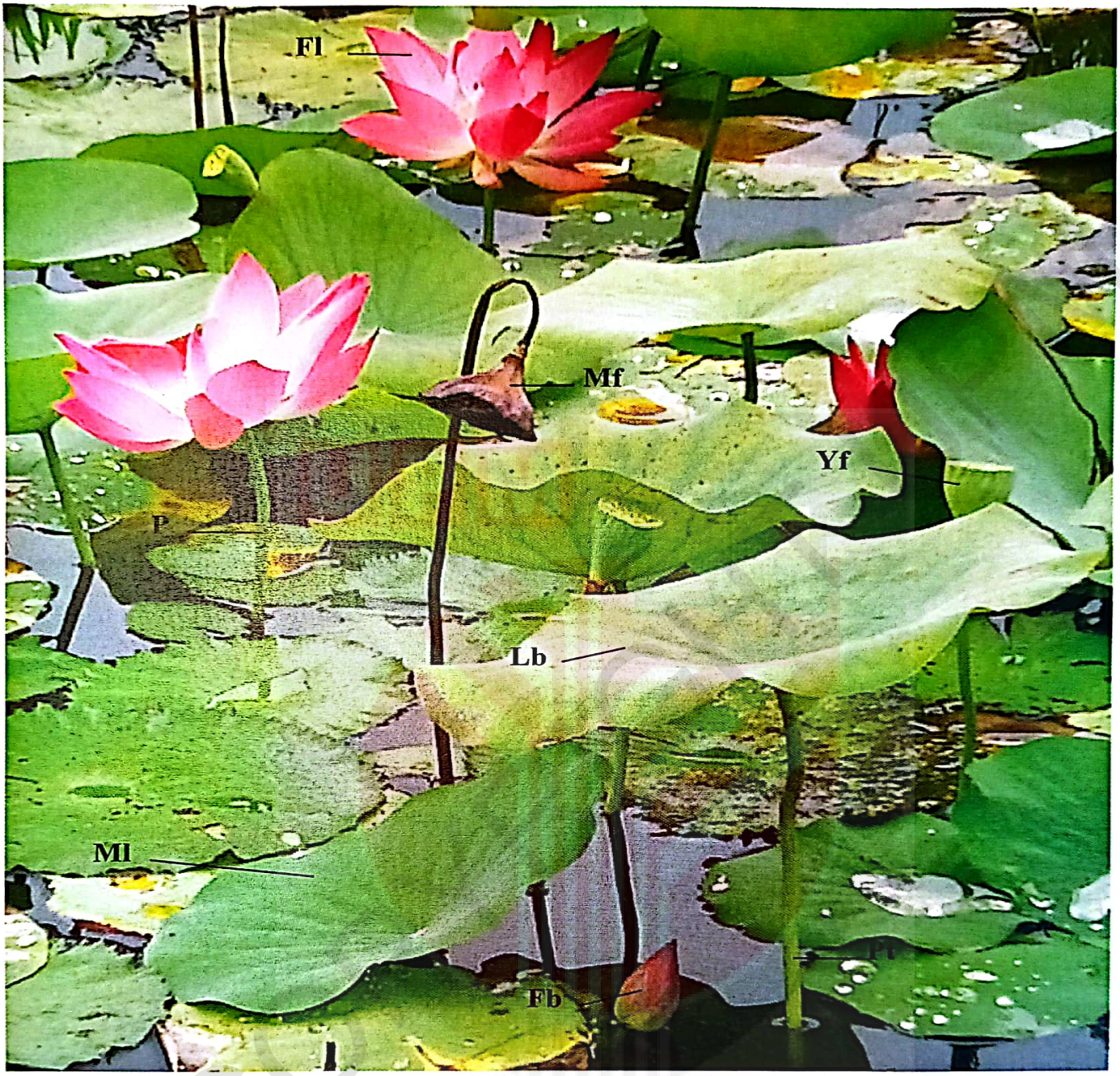


Plate 5: The external vegetative and reproductive morphology of *Nelumbo nucifera*. Ml-matured leaf, Lb-leaf blade, Pt-petiole, Fb-flower bud, Fl-flower, Mf-matured fruit, Yf-young fruit and P-peduncle.

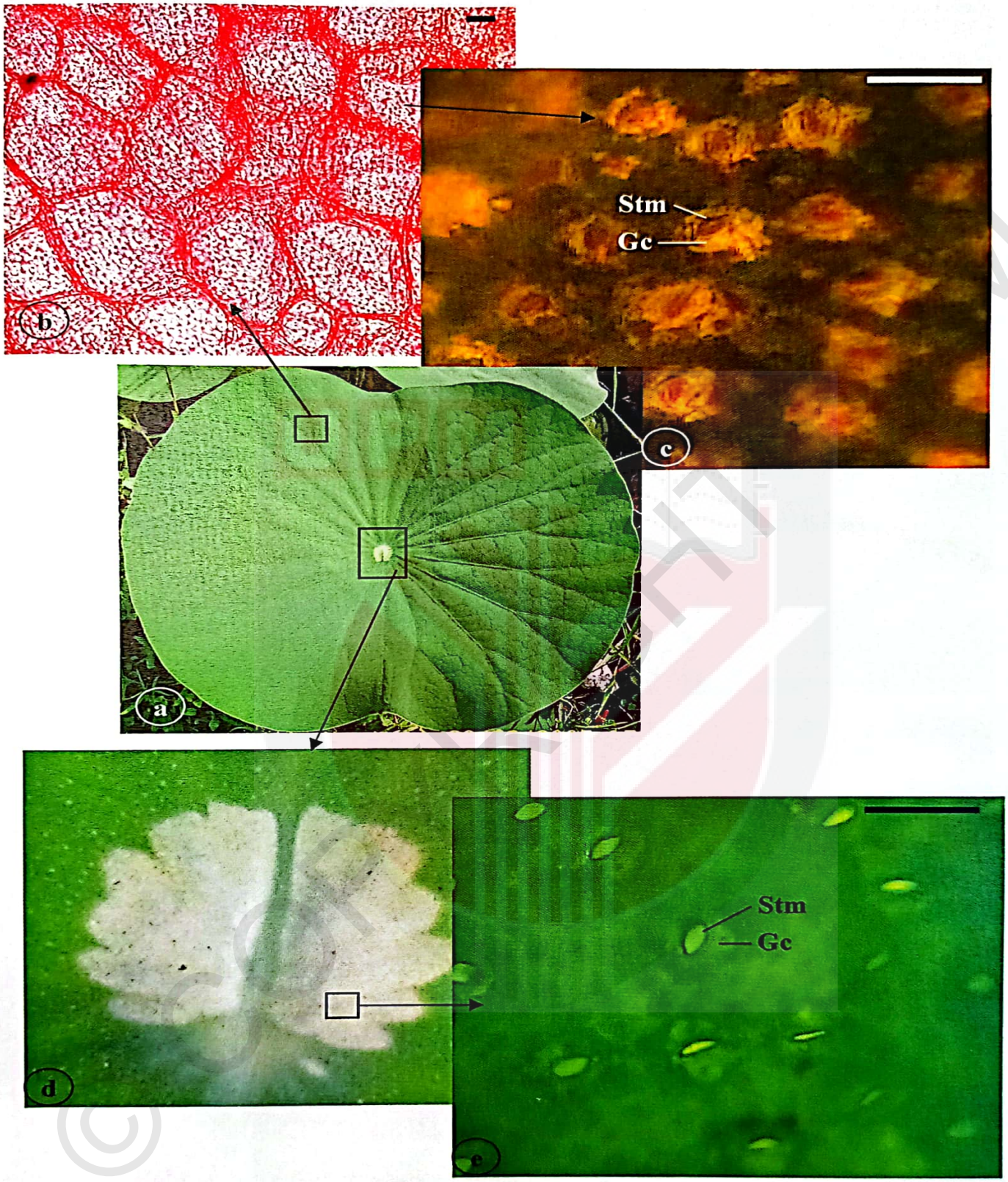


Plate 6: Adaxial (above) surface of matured leaf. a-waxy surface, b-net vein hexagon shaped (magnification 0.6X), c-stomata (magnification 2.5 X), d-middle of leaf shaped like a wing of butterfly and e-stomata (magnification 2.5X). Stm-stomata and Gc-guard cell. (Scale bar = 1mm).

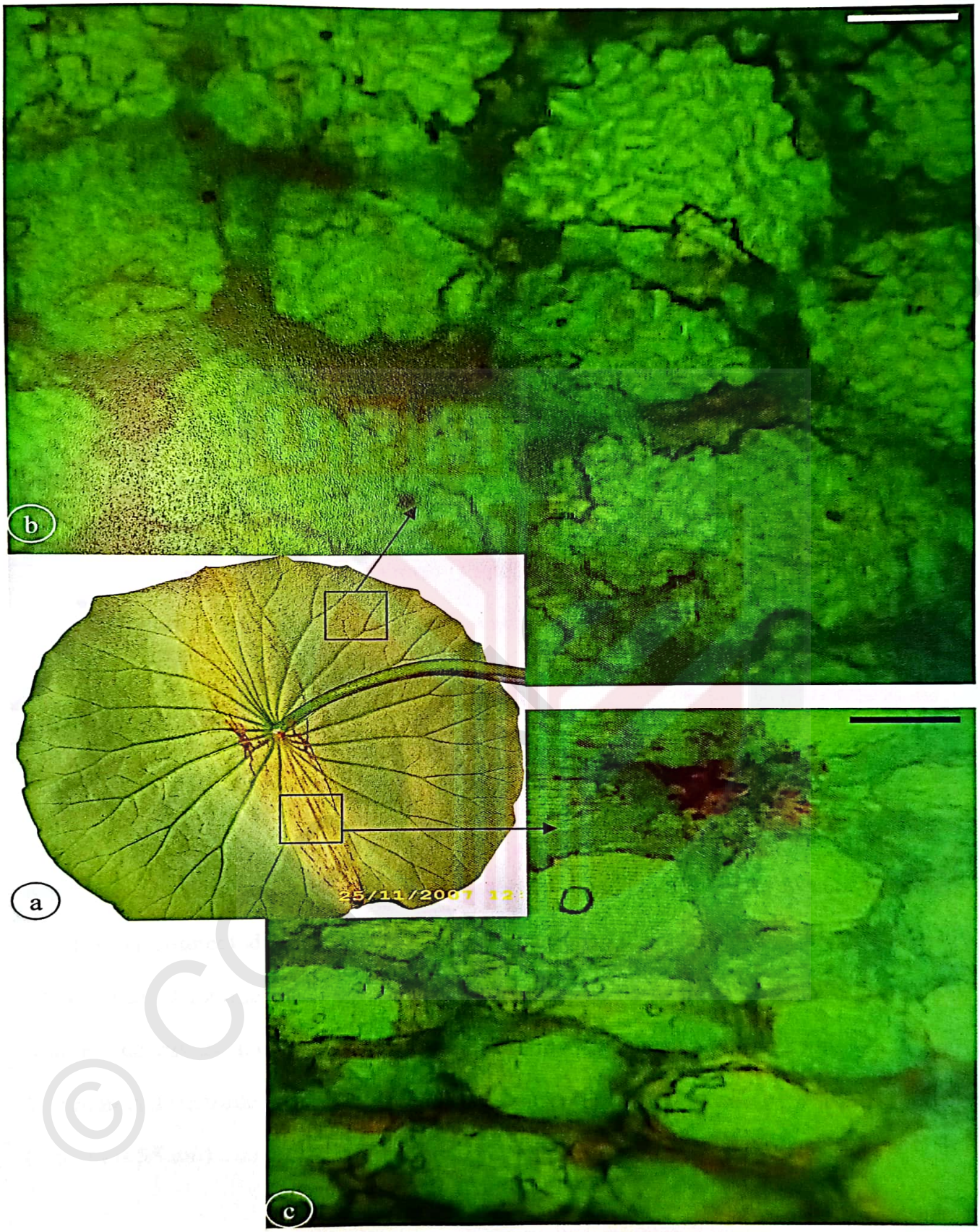


Plate 7: Abaxial (beneath) surface of matured leaf. a-abaxial (beneath) surface waxy and light green, b-net veined with small pentagonal veins (magnification 20X) and c-pink or red spot scattered (magnification 20X), Pt-petiole. (Scale bar = 1mm).

Young leaves of this species are round shaped with sharp leave tip, smooth margin and waxy surface. The adaxial (above) surface of young leaves is pale green in colour with pink net veined (Plate 8a) which makes the young leaves appear in pink color. Abaxial (beneath) of young leaves is pink colour with waxy surface. At the middle of young leaves are pink colour oval shaped pattern with red spots (Plate 8b). When the leaves dry, the leaf blade shaped like umbrella (Plate 8c). The petiole is long (Plate 9a) and has shiny green surface with spines (Plate 9b). Petiole contents white colour wax (Plate 9c). Numbers of lacunae that visible in the petiole are almost 60 and 10 of it are large. Each large lacunae surrounded by smaller air space (Plate 9d). Astrosclereid was observed in lacunae (Plate 9e). Matured plants possess petiole length 102.8±0.95 cm (91.63 to 114.4 cm) and the diameter is 0.91±0.08 cm (0.8 to 1cm). The length of petiole is depending on water depth.

4.2.1.2 Rhizome

Rhizomes are long and have smooth surface with black spots. Each segment or node of rhizome is connected by internodes (Plate 10a-10b). The surface of rhizome is netted (Plate 10c). Rhizome has 22 lacunae and 9 of it are large, which is 1.4 cm diameter. Major lacunae are more oval shaped and arranged at the edge of rhizome (Plate 10d). The matured rhizomes have length 18.35±0.26 cm (16.2 to 19.9 cm) with 5.23 ± 0.20 cm (3.65 to 6.55 cm) diameter.

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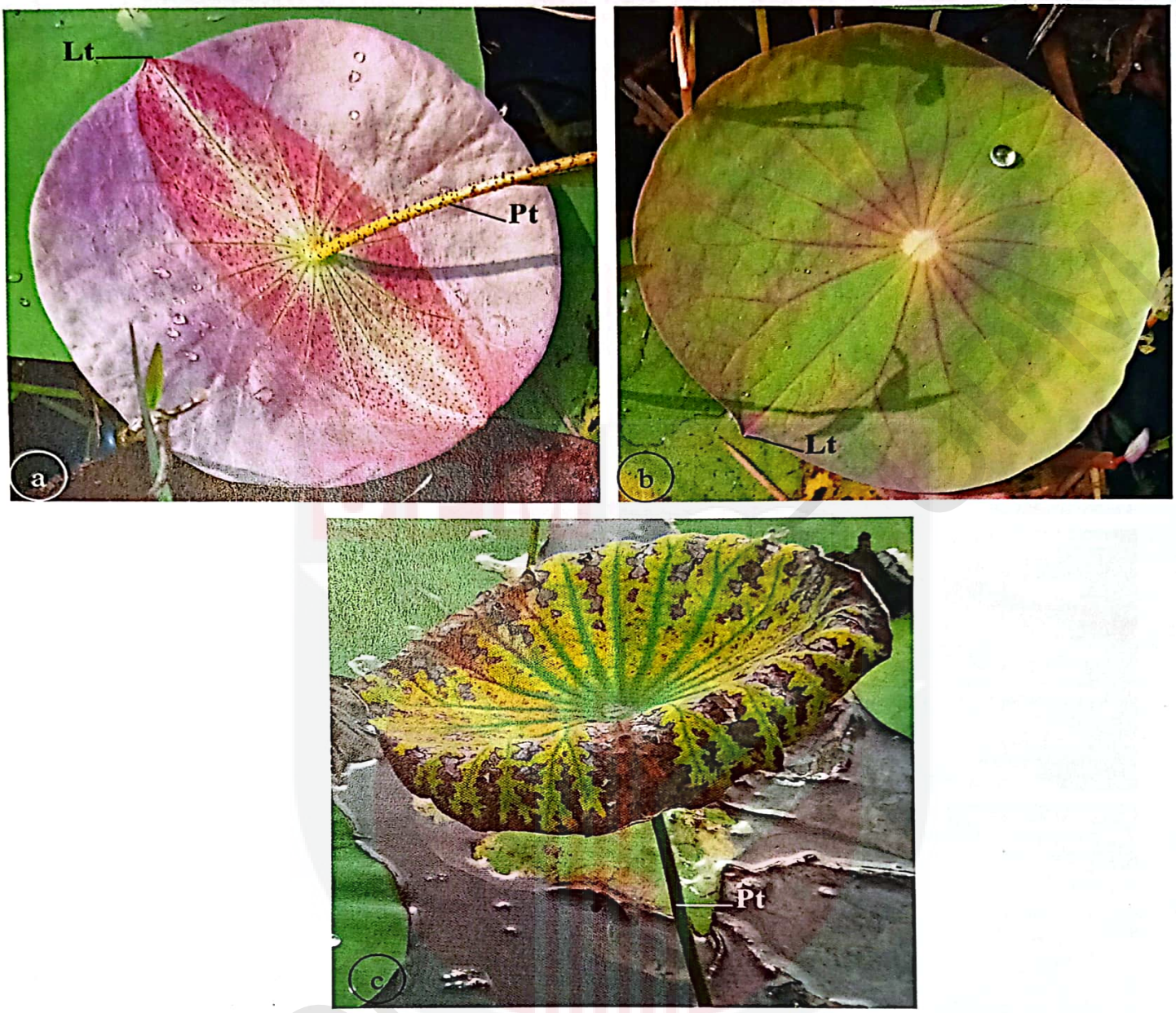


Plate 8: Young and old leaf. a-adaxial (above) surface with sharp leaf tip and pink veined, b-abaxial (beneath) surface pink colour consists of petiole and c-old leaf have umbrella shaped leaf blade. Lt-leaf tip and Pt-petiole.



Plate 9: Petiole. a-long petiole, b-smooth green surface with many spines, c-wax content (magnification 2.0X), d-major lacunae or air canal (magnification 0.6X) and e-enlarged view of lacunae (magnification 2.0X). Asc-astrosclereid, Lc-lacunae, Sn-spine and Pt-petiole. (Scale bar = 1mm).

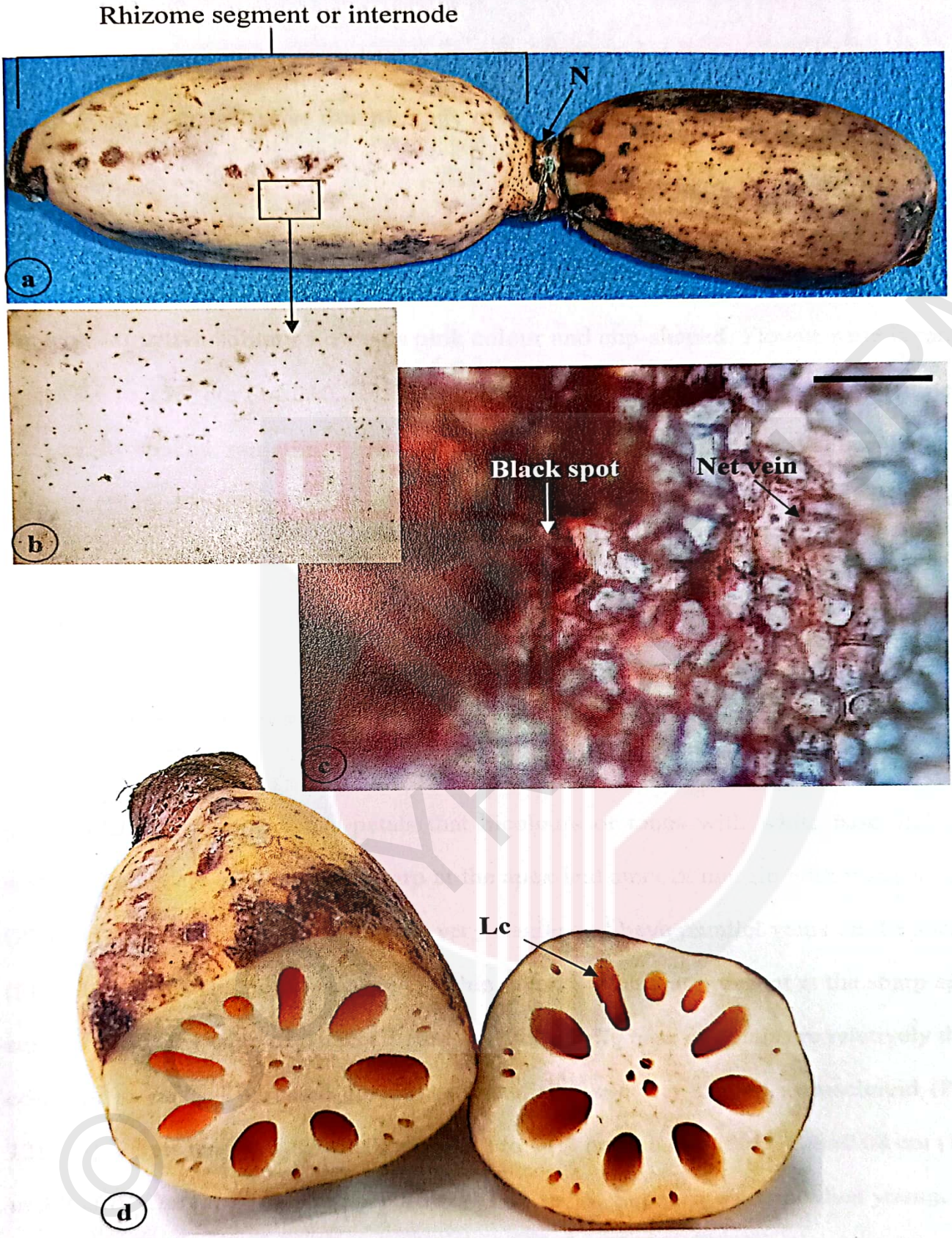


Plate 10: Rhizome of *Nelumbo nucifera*. a-two segments or internodes of rhizome connected by nodes, b-black spots, c-transverse section of rhizome surface comprises uneven rectangular veins (magnification 2.5X) and d-lacunae arranged at the edge of rhizome. Lc-lacunae and N-node. (Scale bar = 1mm).

4.2.2 Reproductive morphology of *Nelumbo nucifera*

4.2.2.1 Flower

The very attractive solitary flower is pink colour and cup-shaped. Flower wide is ranged from 13.4 to 15.9 cm (14.8±0.22 cm). The flower is made up of similarly shaped petals that equally spaced, radiating from the center of flower and surrounds the receptacle and stamens (Plate 11b). Sepal and petal are presents in this species therefore it grouped as polypetalae. Sepals are green colour outer most whorl at the flower bud (Plate 11a). The beneath part of flower has small dried sepal and peduncle inserted at the middle (Plate 11c). In this species the sepals are smaller than petals. Sepals dried and fall when the flower open. Sometimes sepals resemble as small dry leaves at the flower base.

Nelumbo nucifera has petals that bicolours or tones with white base and pink surface. Petals are oval shaped, sharp at the apex and smooth margin with waxy surface (Plate 11d). The fragranced petals are very fragile and have parallel veins on the surface (Plate 12a-12b). The petals surface is a thin layer. Stomata are present at the sharp apex, surface and at the edge of petals (Plate 12c-12e). White base of petals are relatively thick compare to surface part and content stomata, ribs and star shaped astrosclereid (Plate 12f-12i). Petals length is 8.9±0.14 cm (3.22 to 11.4 cm) and width is 3.08±0.08 cm (1.02 to 5.7 cm). The range of petal per flower is 13 to 19. Sepals are green when young, and it develop almost same like petals but smaller in size (Plate 13a). Stomata are present at the sharp apex and surface of petals (Plate 13b-13d). Ribs and star shaped astrosclereid are also observed at the sepals (Plate 13e).

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4.2.2.2 Peduncle

The peduncle is long and has shiny green surface with sharp spines (Plate 14a-14b). The spines are very sharp and hook shaped (Plate 14c). Numbers of major lacunae in the peduncle is only six and surrounded by many small lacunae (Plate 14d-14e). The peduncle is having same surface as petiole and also content wax component. Therefore, number of lacunae identified to differentiate between petiole and peduncle. The length of peduncle is depending on water depth. Matured plants possess peduncle length 97.13±3.42 cm (80.5 to 1107.2 cm) and the diameter of peduncle range from 0.5 to 0.6 cm (0.54±0.01 cm).

4.2.2.3 Stamen

Stamens surround a central, cone-shaped receptacle or also known as thalamus in which the gynoecium is embedded (Plate 15a-15b). Stamens are yellow in colour and consist of three parts that are thin filament, elongated anther and a vegetative tip (Plate 15c). The white colour, thin filaments are attached to base of receptacle and hold the anther and vegetative tip. Length of filaments is 1.18±0.007 (0.97 to 1.4 cm). Vegetative tip is at the apex of stamen, which having white and rounded apex (Plate 16a), it has length 0.30±0.005 cm (0.2 to 0.5 cm). The surface of vegetative tip highly concentrated with cusp (Plate 16b). Anther is yellow color and contains a lot of pollen grains, which are round shaped (Plate 16c-16e). Anthers possess length 1.06±0.005 cm (0.84 to 1.2 cm). Numbers of stamen per flower are 98 to 150 (119.3±5.05). The length of whole stamens is ranged from 2.1 to 2.8 cm (2.54±0.009).

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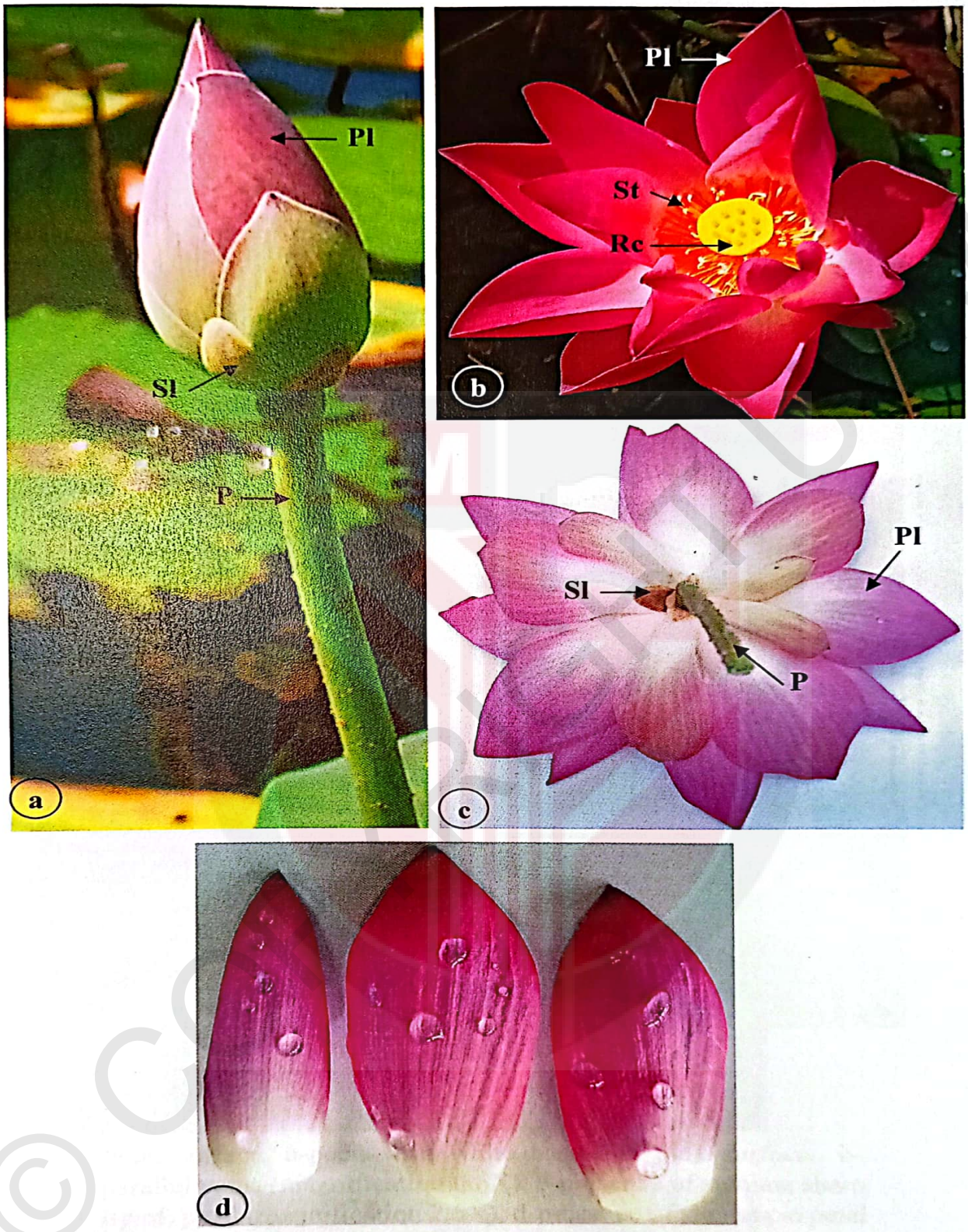


Plate 11: Flower. a-aerial peduncle hold flower bud that consist of green sepals and pink petals, b-similar shaped petals surrounds the receptacle and stamens, c-beneath part that consist of sepals and peduncle and d-oval shaped petals, sharp at the apex and waxy surface. P-peduncle, Pl-petal, Rc-receptacle, Sl-sepal and St-stamen.

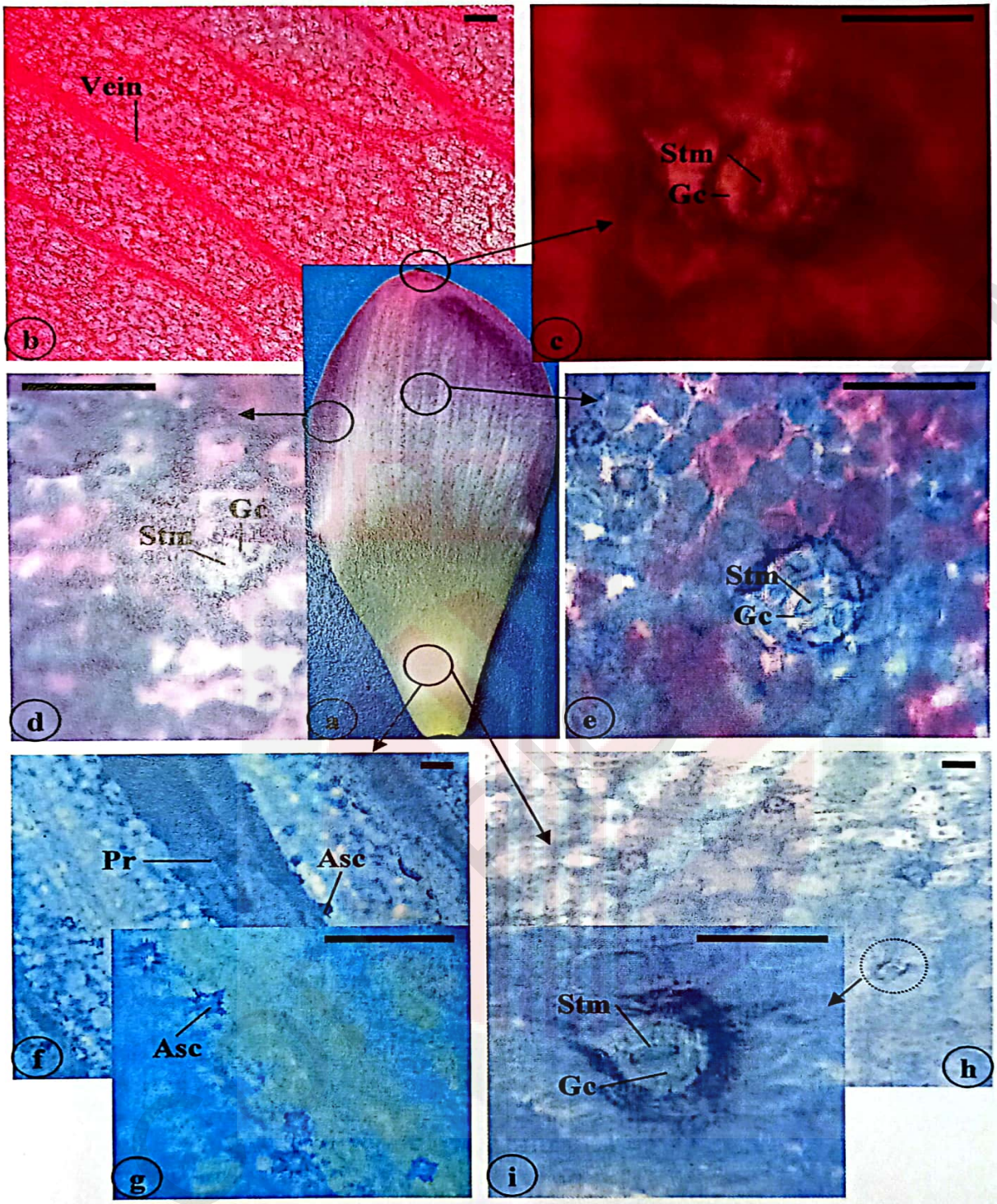


Plate 12: Petal surface. a-petal with white base and pink surface, b-parallel veins (magnification 0.6X), c-presence of stomata sharp tip of petal (magnification 2.5X), d-presence of stomata at petal edge (magnification 2.5X), e-presence of stomata at petal surface (magnification 2.5X), f-white base of petal consist of ribs and star shaped astrosclereid (magnification 0.6X), g-enlarged view of astrosclereid (magnification 2.5X), h-presence of stomata at base of petal (magnification 0.6X) and i-enlarged view of stomata (magnification 2.5X). Asc-astrosclereid, Gc-guard cell, Pr-petal rib and Stm-stomata. (Scale bar = 1mm).

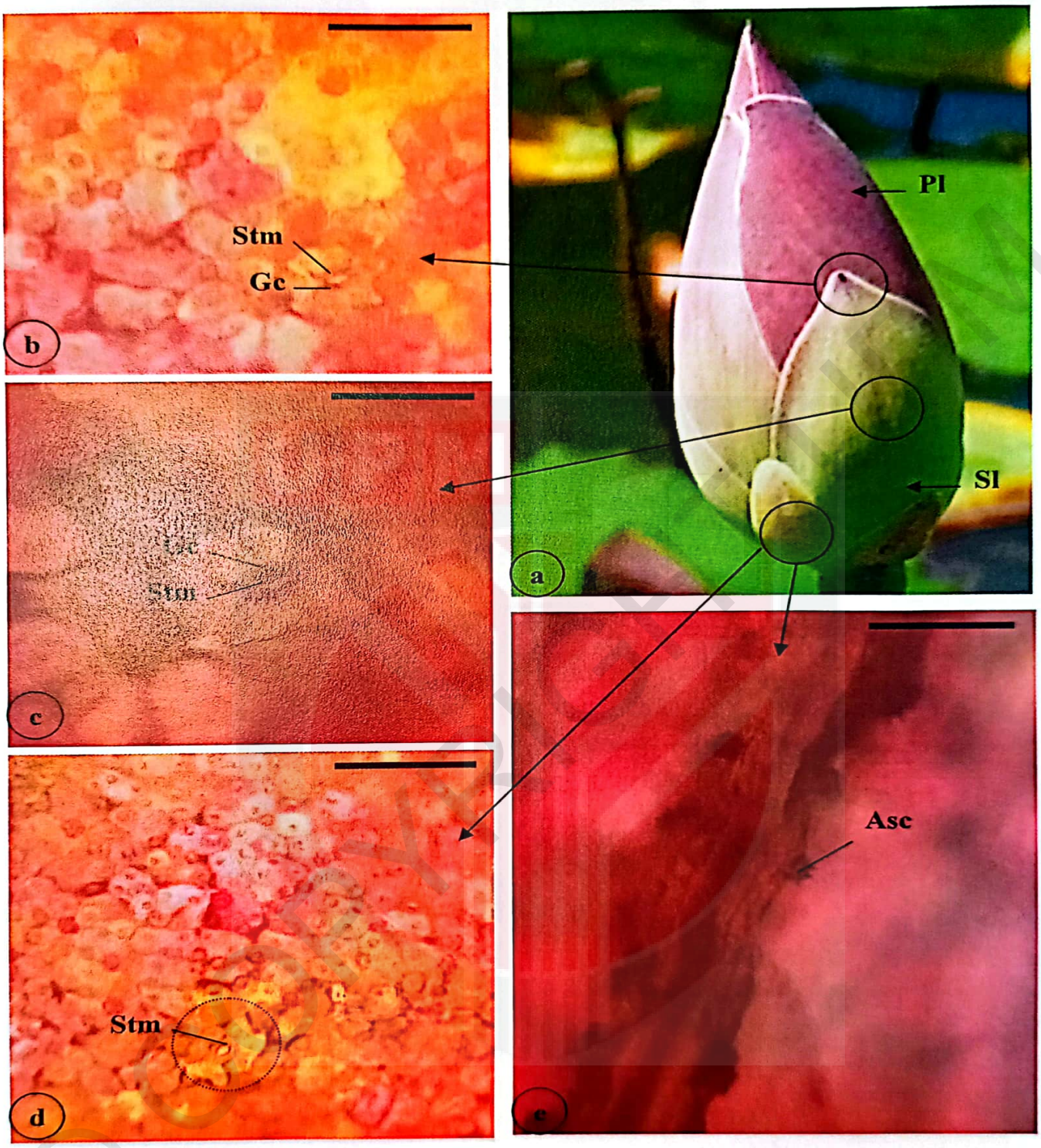


Plate 13: Sepal surface. a-green sepals at base of flower bud, b-presence of stomata at the tip of sepal (magnification 2.5X), c-presence of stomata at sepal surface (magnification 2.5X), d-stomata at surface of developing sepal (magnification 2.5X) and e-astrosclereid presence at developing sepal (magnification 2.5X). Asc-astrosclereid, Gc-guard cell, Sl-sepal and Stm-stomata. (Scale bar = 1mm).

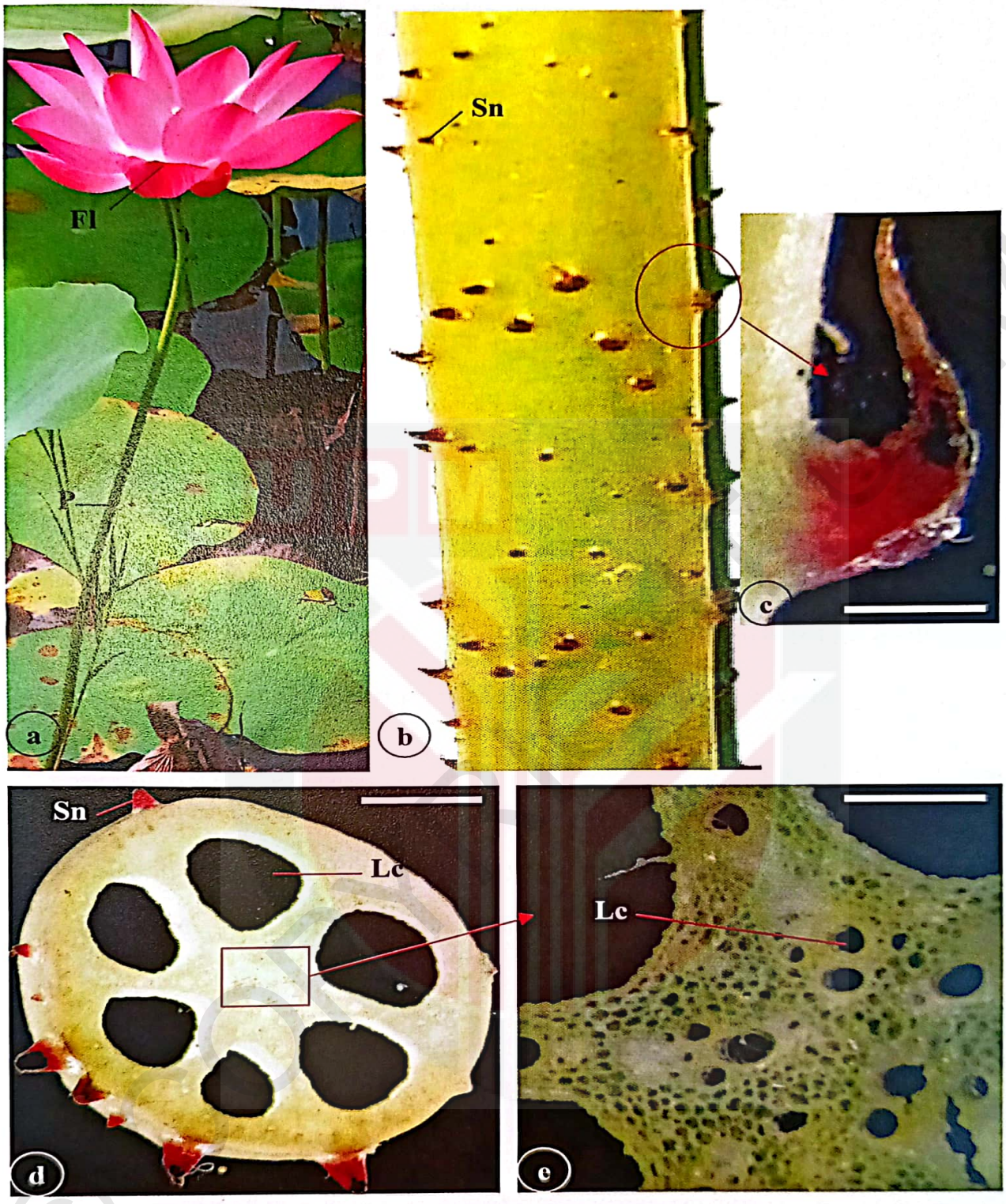


Plate 14: Peduncle.a-long peduncle hold solitary flower, b-green shiny surface with sharp spines, c-sharp spine hook shaped (magnification 2.5X), d-transverse section of peduncle has six lacunae (magnification 2.0X) and e-net like lacunae (magnification 2.5X). Fl-flower, Lc-lacunae, P-peduncle and Sn-spine. (Scale bar = 1mm).

4.2.2.4 Receptacle

The receptacles or also called as thalamus, are yellow color, spongy, flat topped and conical shaped at the middle of flower (Plate 17b), which possess length 2.25±0.06 cm (1.9 to 2.5 cm) and diameter is 19.0±0.08 cm (1.6 to 2.6 cm). Flowers are dried and fall to allow the receptacle develops into seed pods or fruit (Plate17a). Receptacle is hypogenous and having superior ovary because the base of the ovary is attached above the sepals, petals and stamens. The carpels are apocarpous due to its not joined together (Plate 17c). Each receptacle has 9 to 20 sunken pistils or carpels that contain an apical ovule. An apical ovule develops at the apex of sunken pistil and it has small stigma (Plate 17d). This apical ovule later riped and develops into nut like seeds.

When the receptacle matured it become green fruit or seed pod and contains singular seed in each carpel (Plate 18a). Only the seeds are become hard, while the fruits are remain spongy. As the fruit develop it become dark brown in colour (Plate 18b).

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Plate 15: Stamens. a- yellow stamens at the middle of flower,b-stamens arranged surround the conical shaped receptacle and c-stamen consists of filament, elongated anther and vegetative tip (magnification 2.5X). At-anther, Ft-filament, P-peduncle,Rc-receptacle, St-stamen and Vt-vegetative tip. (Scale bar = 1mm).

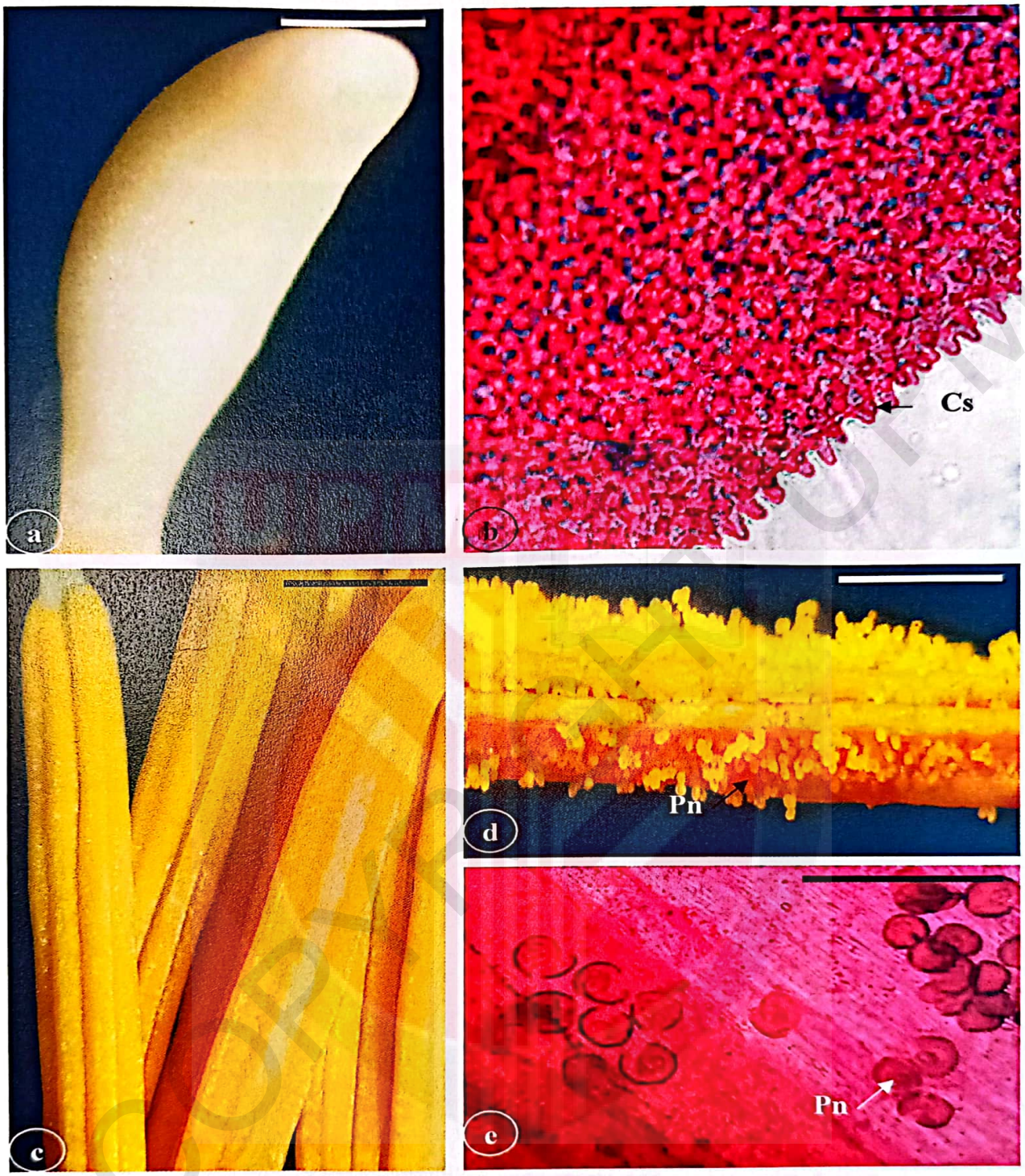


Plate 16: Component of stamen. a-vegetative tip with rounded apex (magnification 2.5X), b-surface of vegetative tip concentrated with cusp (magnification 3.2X), c-yellow colour elongated anther (magnification 2.5X),d-pollen grains on anther surface (magnification 3.2X) and d-round shaped pollen (magnification 4.0X). Cs-cusp and Pn-pollen. (Scale bar = 1mm).

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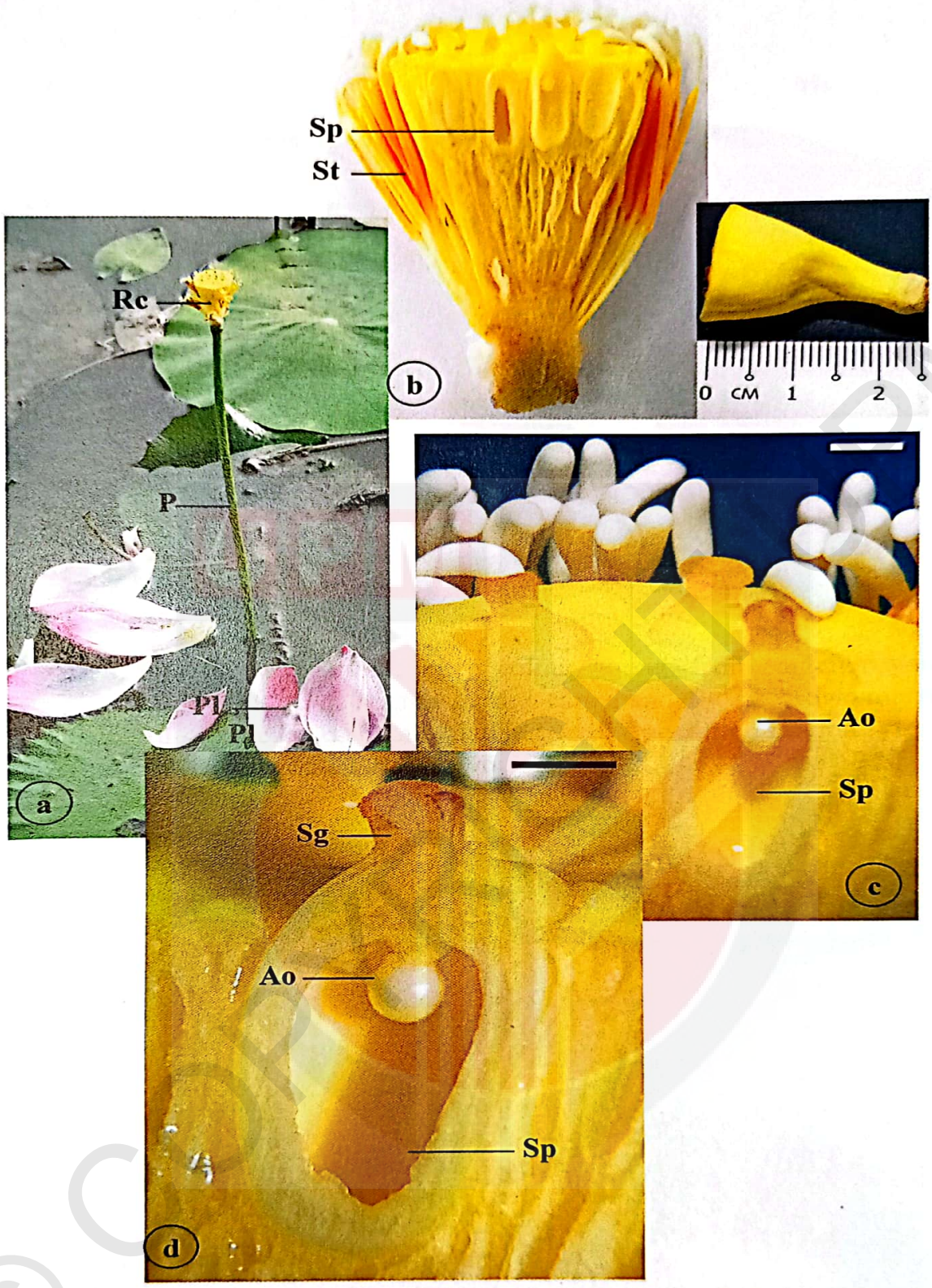


Plate 17: Receptacle or also called as thalamus. a-petal dried and falls allow receptacle develop to mature fruit, b-receptacle longitudinal section is hypogenous having superior ovary with apocarpous carpel and surrounds by stamens, c-sunken pistil (magnification 1.0X) and d-apical ovule produced in the sunken pistil or cavities that have tiny stigma (magnification 1.6X). Ao-apical ovule, P-peduncle, Pl-petal, Rc-receptacle, Sg-stigma, Sp-sunken pistil and St-stamen. (Scale bar = 1mm)

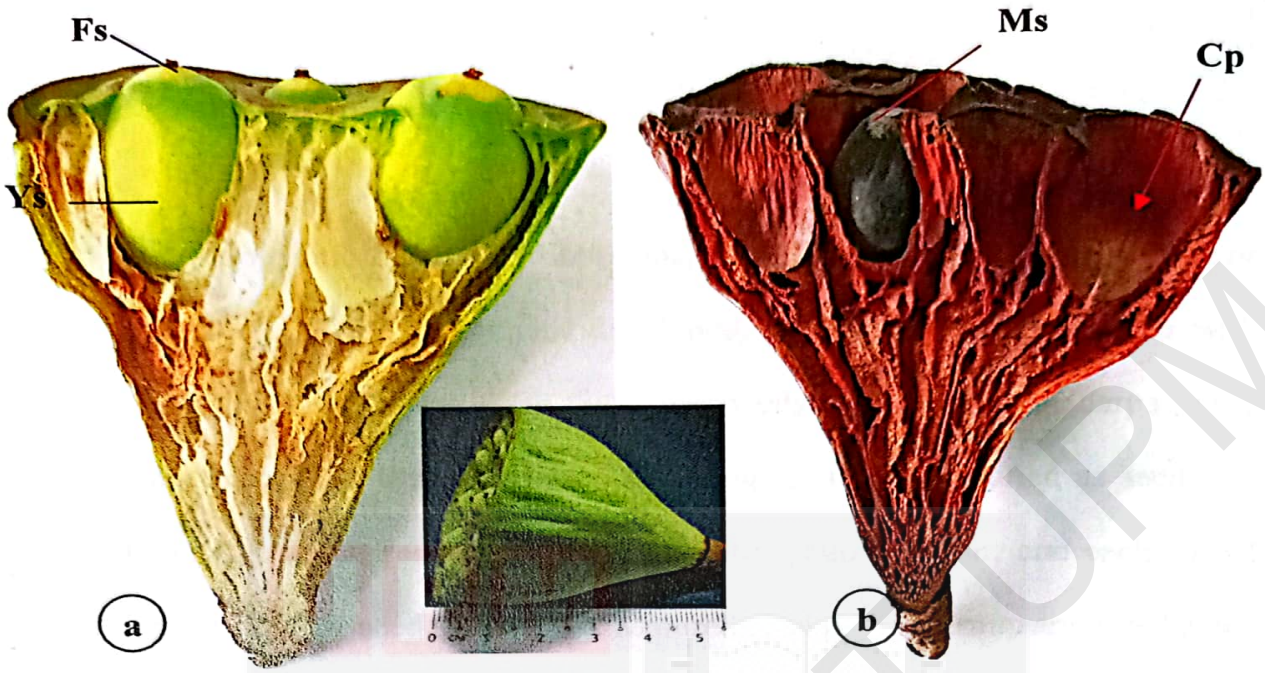


Plate 18: Fruit or seed pod. a-green colour young fruit contains young seed with floral stigma in the carpel and b-dark brown matured fruit or seed pod contain matured seed. Cp- carpel, Fs-floral stigma, Ms- matured seed and Ys-young seed.

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4.2.2.5 Seed development stages

The receptacle develops into fruits that contain matured apical ovule that become nuts or seeds (Plate 18a-18b). As the receptacles developed, it changes to green colour with slight red. When the receptacle are green the young seeds embedded in the carpel, only floral stigma are exposed at the flat surface of young fruit or also called as seed pod (Plate 19a). Later, the green seed pod turns to slightly yellow colour, and each carpel contain developing singular seed (Plate 19b). Matured seed pods or fruits are dark brown in colour which contains smooth, brown and oval shaped seeds (Plate 19c-19d). The matured seed pods are spongy with 4.45±0.74 cm (3.4 to 5.3 cm) length and 5.83±0.13 cm (3.4 to 7.2 cm) diameters. Dry stamens or sepals are resembled at the base of matured seed pod.

As the seeds matured the carpels become wider and space occurs between seeds and carpel because only the base of the seeds is attached to carpels. When the seeds matured, the peduncle dries, shrivels and weakens, turning the seed pod downwards, allowing the seeds dispersed into water (Plate 20a). The seed pods have various shaped carpels (Plate 20b). Carpels are round shaped in the young seed pods, as the seed pod and seeds are matured the carpel shape are changed. Carpel have diameter 1.5±0.01 cm (0.6 to 2.3 cm).

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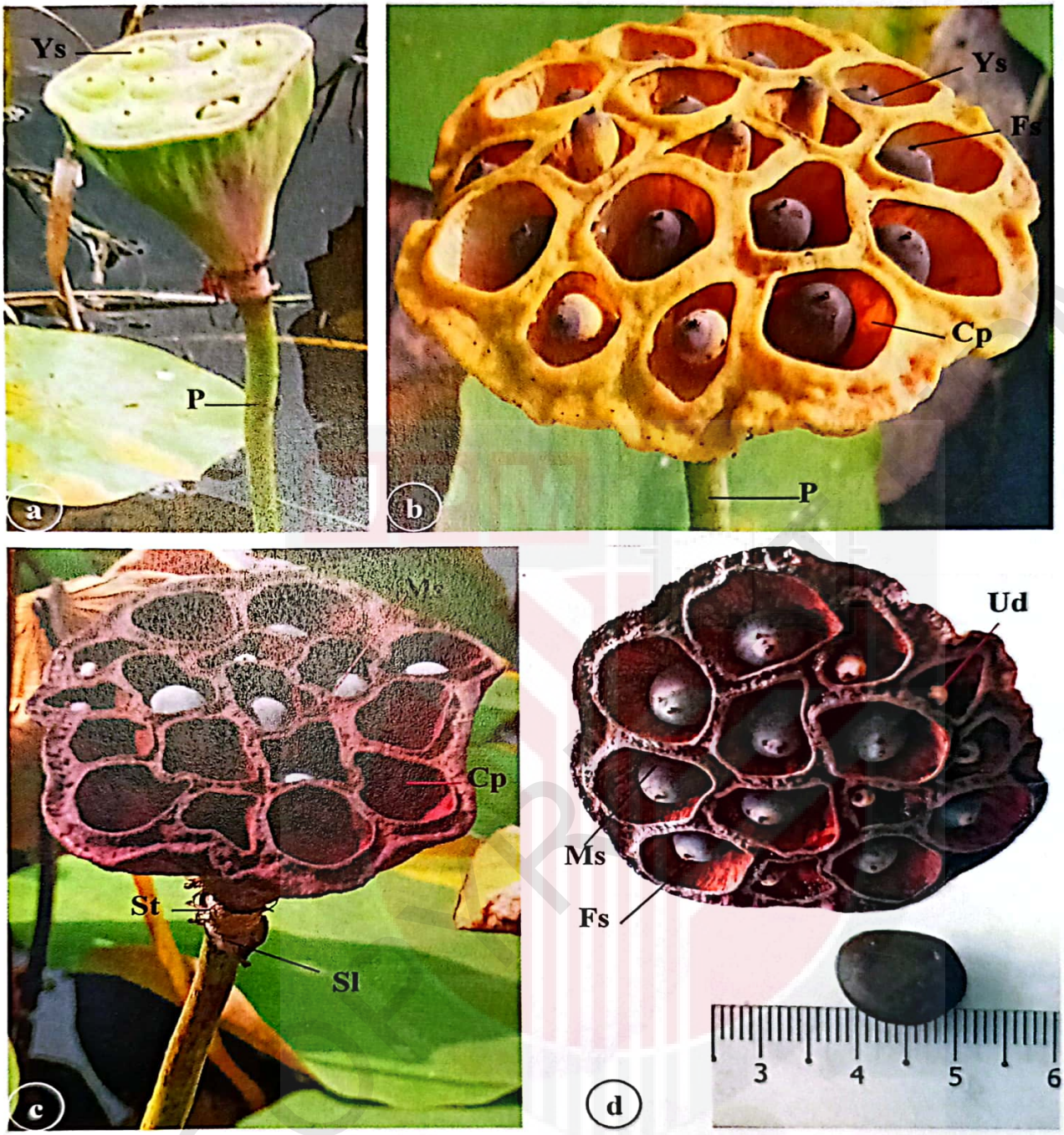


Plate 19: Seed development stages. a-peduncle hold young fruit that slightly red and young seeds embedded in the carpel, b-fruit or seed pod develop and carpel become wider and occur space between seeds and sunken pistil, c-dark brown matured fruit or seed pod, contains oval shaped matured seeds, dried stamens and sepals are resemble at the base of seed pod and d- only base of the matured seed that dimple end are attached to the carpel, while floral stigma are exposed and also some seed are undeveloped. Cp-carpel, Fs- floral stigma, Ms-matured seed, P-peduncle, Sl-sepal, St-stamen, Ud-undevelop seed and Ys-young seed.

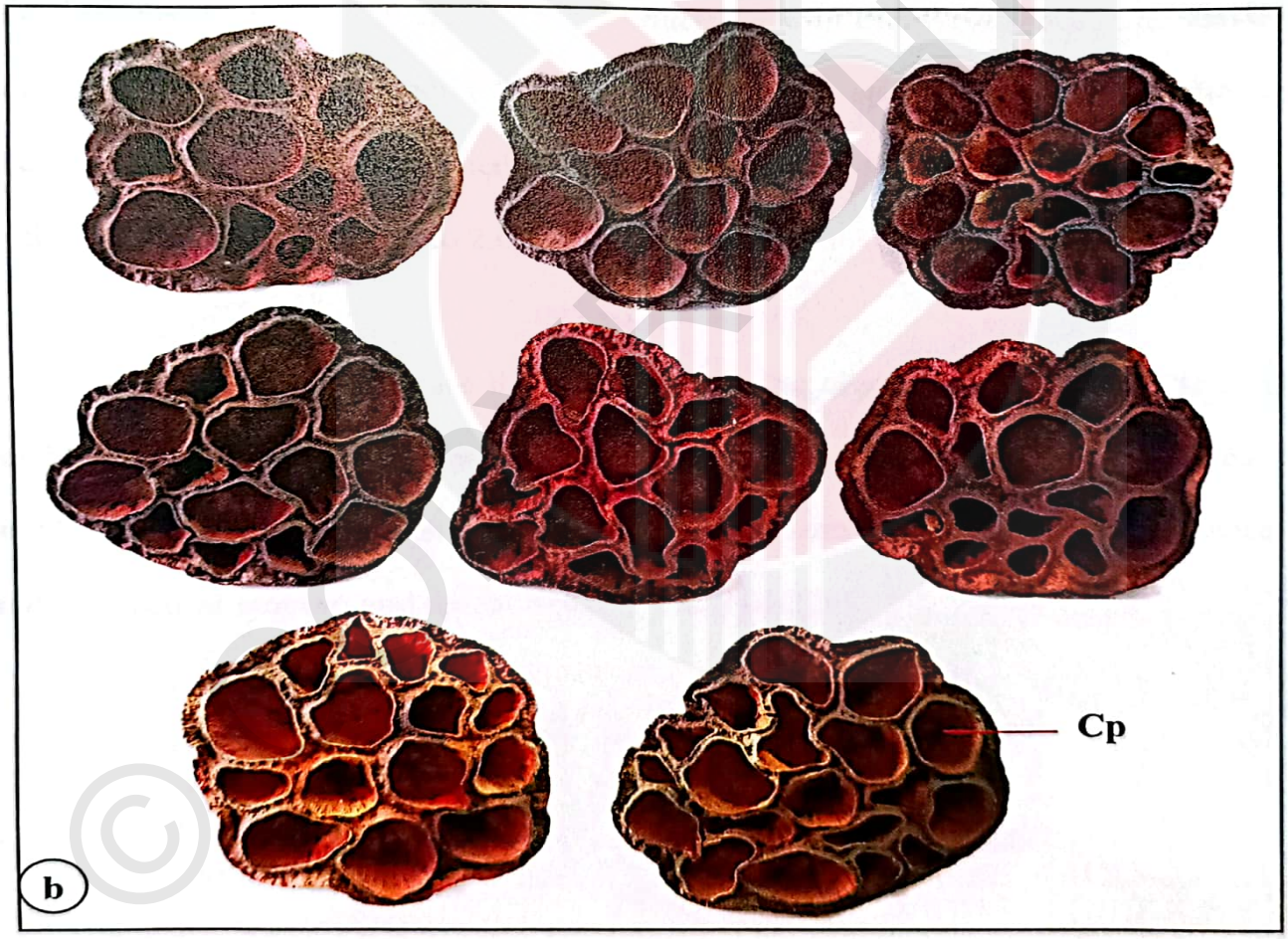


Plate 20: Seeds dispersal and carpels shapes. a-matured fruit or seed pod downward to disperse the seeds and b-matured fruits or seed pod have various carpels in term of patterns, shape and size. Cp- carpel, Mf-matured fruit, and P-peduncle.

4.2.2.6 Seed

Nelumbo nucifera seeds are nuts which round or oval shaped (Plate 21a). One end of the seed has a sharp point which is the remains of the floral stigma. On the opposite end is tiny dimple, where the seed was attached to the carpel. The seed coat can varied from gray to dark brown or black. The seed coat is very hard and consists of two layers which are tightly together. Inside the seed coat have large thick embryo with fleshy twin cotyledons that enclosing the folded plumule or two prominent enrolled leaves with attendant peduncle. This feature place *N. nucifera* into dicotyledonous. The leaves are doubled over against the peduncle because of the tight space (Plate 21b). Fertile seeds usually contain plumule. The seed length range from 1.2 to 1.6 cm (1.41±0.004 cm) and width is 1.01±0.005 cm (0.8 to 2.0 cm).

The enrolled leaves are developing since the seeds are still young. The young seeds have green colour soft seed coat and fleshy cotyledons (Plate 21c). Not all the seed in the matured fruit possess fertility. The seeds sometimes non fertile because the undeveloped of embryo and apical ovule (Plate 21d-22e).

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Plate 21: Seed. a-matured seed round or oval shaped with dark brown or black smooth surface, b-transverse section of matured seed, has very hard seed coat, cream colour cotyledon and enrolled leaves or plumule, c-young seed with floral stigma and developing plumule, d and e-undeveloped seeds. Ct-cotyledon, Fs-floral stigma, Pm-plumule and Sct-seed coat.

4.3 Vegetative propagation of *Nelumbo nucifera*

Primary or parent rhizome that have four nodes with two young leaves was planted and observe for 24 weeks (Table 2). One node of primary rhizome showed in Plate 22a. Only two nodes of the primary rhizome were produce leaves, secondary rhizome and roots. Within two to four weeks after primary rhizome planted the parent leaves were dried and removed. After the parent leaves removed, primary rhizome was produced first leaf sheath with 2.51 cm length from the first node. Plumule with 1.6 cm length was emerged from the leaf sheath within 3 days (Plate 22b). The plumule was elongates into enrolled young leaf (2.88 cm), that reddish in colour on the spiny petiole (Plate 22c). Petiole possesses 4.07 cm length. The first enrolled young leaf (2.88 cm) has develop and elongated (5.67 cm) into green colour and leaf sheath are still resemble at the petiole base.

On week six to eight, first leaf from the first node of primary rhizome was opened with 3.68 cm diameter (Plate 23a). Second leaf sheath has 1.42 cm length was produced at the same node, and the plumule with 2.03 cm length was emerged within four days and elongated into enrolled young leaf (Plate 23b). Roots emergence was identified from first node of primary rhizome (Plate 23c-23d).

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Table 2: Summary of primary rhizome development stages

Substrate	Week	Rhizome development stages (from the time primary rhizome planted until leaves, roots, secondary and tertiary rhizome emerge)	
	2-4	Parent leaves from primary rhizome removed	
		1st node of primary rhizome	**2nd node of primary rhizome**
	4-6	Produced first leaf sheath. Emergence of plumule and roots from the leaf sheath and elongates into enrolled young leaf.	-
	6-8	First leaf was opened. Second leaf sheath was produced from the same node, and the plumule was emerged within 3 days and develops into young leaf.	New leaf sheath with roots was produced. Plumule and roots was emerged within 4 days.
	8-10	Leaves were keep elongates and the second leaf was opened. New leaf sheath was produced.	The plumule elongated into enrolled young leaf.
	10-12	Third plumule was emerged and develops into enrolled young leaf.	The first leaf was open and new leaf sheath was produced.
Loam soil	12-14	Third leaf was opened, and all the leaves were elongates	Second leaf opened, produced third leaf sheath. Plumule was emerged within 4 days from the third leaf sheath and elongates into young enrolled leaf.
	14-16	Secondary rhizome was produced.	Third leaf was opened.
	16-18	All the 3 leaves develop with petiole elongation. The secondary rhizome also elongates.	The entire leaves elongate and secondary rhizome was produced
	18-20	Secondary rhizome has produced first node and possesses emergence of leaf sheath and tiny roots. Plumule was emerged within 3 days from the leaf sheath and develops into young enrolled leaf. The leaf was opened at end of week 20.	Leaf sheath was produced from secondary rhizome, the plumule emerged and develop into enrolled young leaf. First leaf from secondary rhizome was opened. The entire secondary rhizome keeps elongate.
	20-24	Secondary rhizome was produced second node. Tertiary rhizome and roots emerged from the second node.	Secondary rhizome was produced second node. Tertiary rhizome, plumule and roots emerged from the second node.

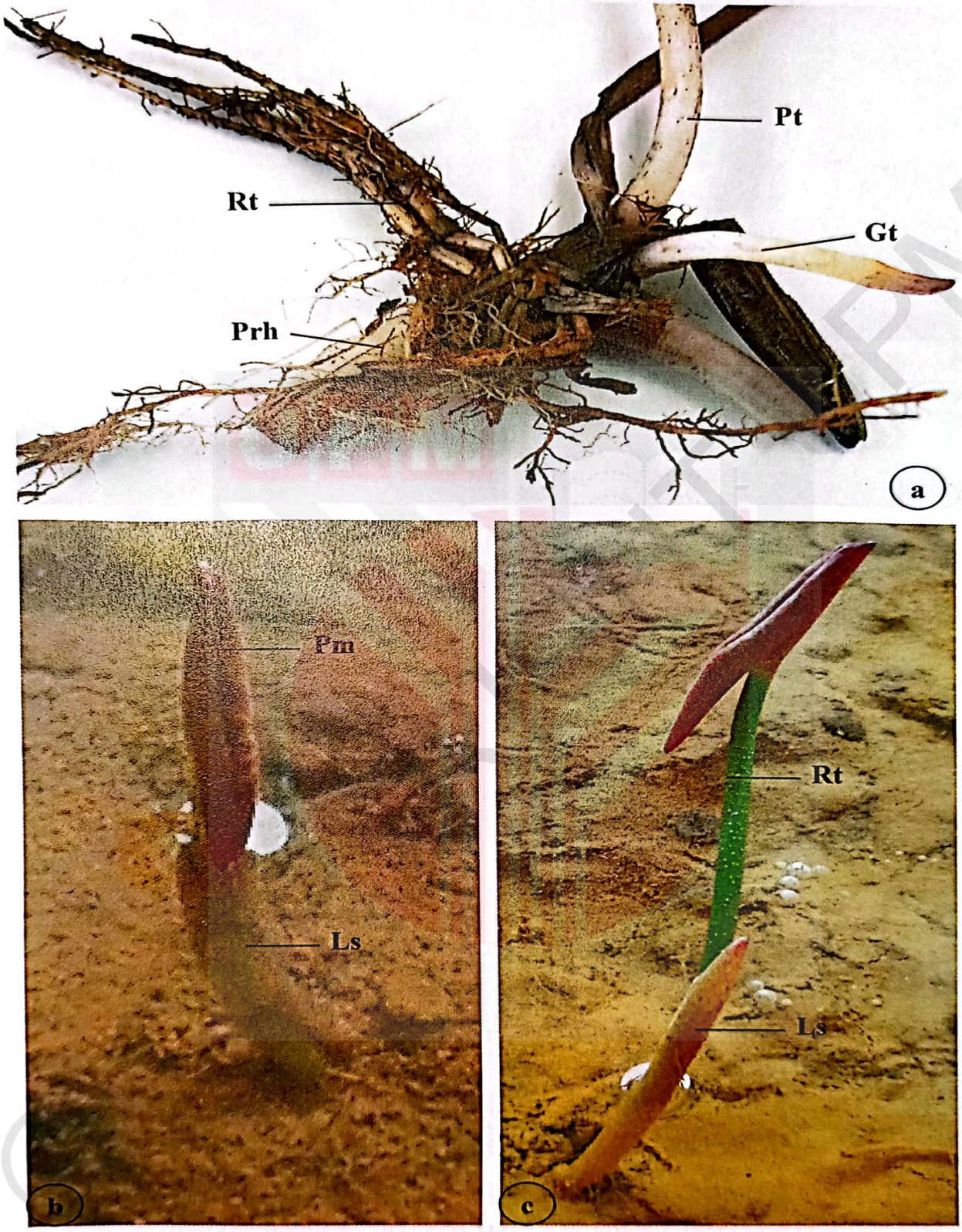


Plate 22: Stages of rhizome development. a-primary rhizome having petiole, roots and active growing tip,b-emergence plumule from leaf sheath and c-elongation of plumule into young enrolled leaf. Gt-active growing tip, Ls-leaf sheath, Pm-plumule, Prh-primary rhizome, Pt-petiole and Rt-roots.

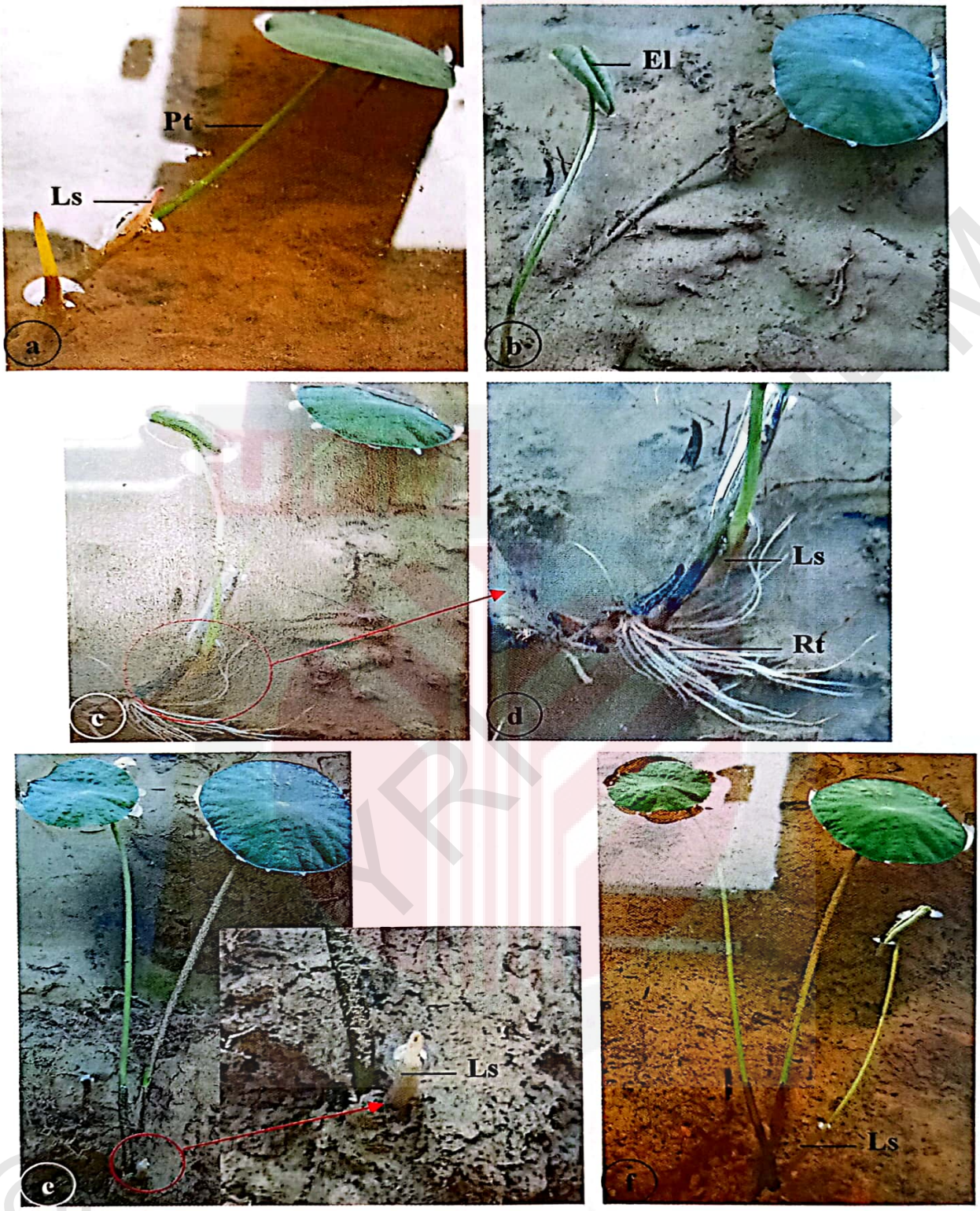


Plate 23: Emergence of leaves from first node of primary rhizome. a-first leaf open and second leaf sheath produced, b-second enrolled leaf produced, c-elongation of two leaves and roots, d-enlarged view of roots, e-third leaf sheath produced and f-elongation of leaves at first node. El-enrolled leaf, Ls-leaf sheath, Pt-petiole and Rt-root.

New leaf sheath (1.92 cm) with roots was produced at second node of primary rhizome (Plate 25a). At the following week, the first leaf from the first node of primary rhizome was elongates until 10.24 cm, while second enrolled leaf possess length 7.11 cm. At the tenth week, the second leaf was opened with 2.56 cm diameter. While, plumule was produced from leaf sheath of second node, and elongates until petiole length 6.0 cm (Plate 25b).

Third plumule was emerged from first node, and elongates into enrolled young leaf (Plate 23e-23f). First leaf with 2.18 cm diameter was opened at the second node and another leaf sheath that has 1.90 cm length also produced. Entire leaves from first node were keep elongates. During the 13 week, leaves from first node possess petiole length 9.67, 10.55 and 12.14 cm, while leaves diameters are 3.52, 4.12 and 4.79 cm respectively. Second leaf was opened with 2.9 cm diameter and new leaf sheath (1.42 cm) was produced at the second node. Within four days plumule was emerged and develops into enrolled young leaf.

Observation on week 14 to 16 shows, entire leaves from first node were elongates slightly, because the petiole lengths are 10.05, 11.3 and 12.78 cm. On the same weeks, secondary rhizome was produced from the first node and it has 2.31 cm length. While, third leaf of the second node was opened and possess 2.63 cm diameter. The observation was continued and on the 17 week leaves from first node have petiole length 12.31, 13.25 and 13.05, while leaves diameters are 5.92, 5.81 and 6.2 cm. The secondary rhizome elongates and possesses 4.11 cm length. Leaves from second node have petiole length 10.21, 11.23 and 12.22 cm with leaf diameter 3.1, 4.8 and 3.9 cm

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respectively. On week 18 secondary rhizome (2.32 cm) was produced from second node and it elongates into 6.81 cm within six days. On the following weeks, the secondary rhizome from first node has produce node and emergence of leaf sheath (1.11 cm) and tiny roots from the node. Plumule with 1.10 cm length was emerged from the leaf sheath within 3 days. The plumule elongates and develops into enrolled young leaf and the leaf was open after 13 days. Entire leaves from second node was elongate and the secondary rhizome produce node. On week 20, leaf sheath was produced from secondary rhizome of second node and the plumule emerged and develops into enrolled young leaf open after 10 days. The entire secondary rhizome at second node keeps elongate.

The observation from week 21 to 24 shows, that secondary rhizome at first node was elongate until 8.2 cm and it was produce second node with tiny roots. One week after second node produced two tertiary rhizomes were emerged with 2.02 and 2.56 cm length (Plate 24a-24c). On the week 22, secondary rhizome of second node was elongates and produced node that possess emergence of leaf sheath, roots and tertiary rhizome (Plate 25c-25d). Plumule was emerged from the leaf sheath within four days and develops into young enrolled leaf after six days. Tertiary rhizome was elongates until with 3.44 cm length. Throughout the observation only three leaves are enlarged and elongates from first and second node of primary rhizome. The secondary rhizome from first node of primary rhizome was possessed one leaf and tertiary rhizome. Secondary rhizome from second node was produced two leaves and one tertiary rhizome. On 24th week, entire leaves from first and second nodes of primary rhizome have petiole length range from 16.57 to 20.26 cm and the leaf diameter is 5.98 to 7.89 cm.

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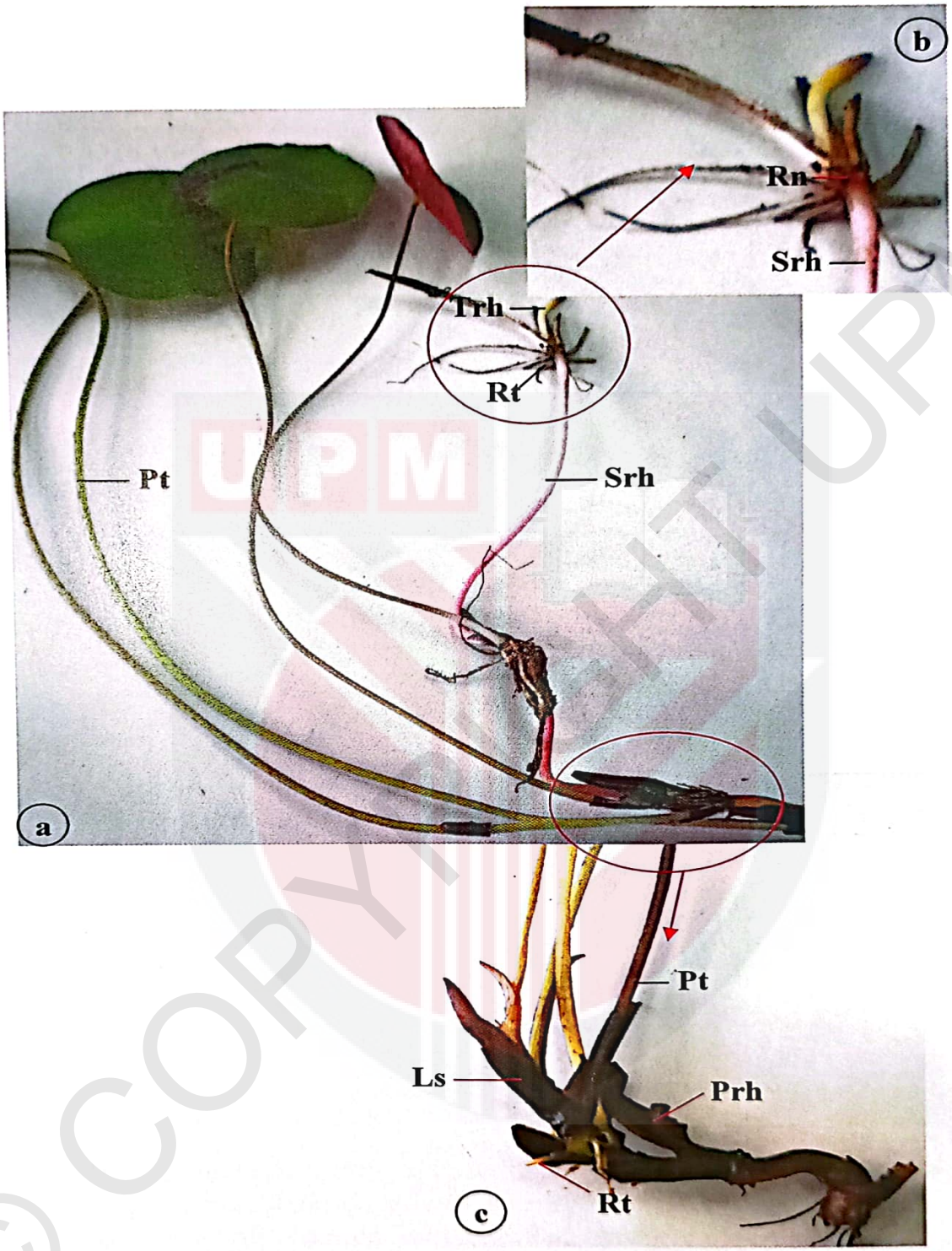


Plate 24: Emergence and elongation of leaves, roots and secondary rhizome. a-three leaves elongates from primary rhizome, secondary rhizome elongates and produced first leaf, b-tertiary rhizome and roots was produced from secondary rhizome node and c-enlarged view of primary rhizome. Ls-leaf sheath, Pt-petiole, Rn-rhizome node, Rt-root, Srh-secondary rhizome and Trh-tertiary rhizome.

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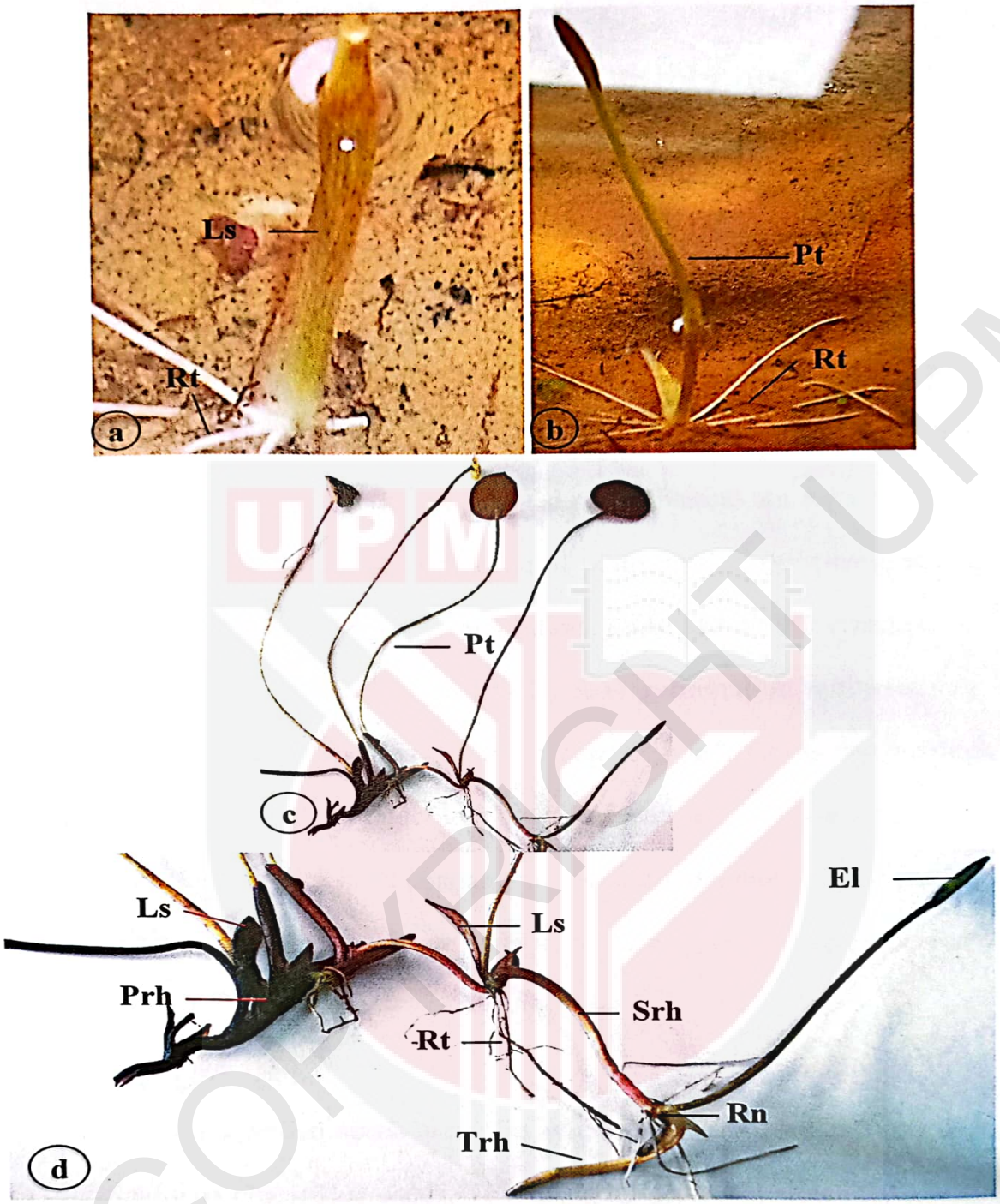


Plate 25: Development of leaves and secondary rhizome from second node of primary rhizome. a-leaf sheath with roots produced, b-elongation of plumule into enrolled young leaf, c-three leaves produced from primary rhizome and one leaf emerged from secondary rhizome and d-enlarged view of primary rhizome having erected leaf sheath, secondary rhizome have two nodes and tertiary rhizome. El-enrolled leaf, Ls-leaf sheath, Prh-primary rhizome, Pt-petiole, Rn-rhizome node, Rt-roots, Srh-secondary rhizome and Trh-tertiary rhizome.

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4.4 Reproductive propagation of *Nelumbo nucifera*

4.4.1 Seed germination

Germination was 100% for treated or scarified seeds, while only 96% for non-scarified seeds. The treatments are significantly different for period of seeds germination as shown in Figure 4. All the 24 scarified seeds need only seven to ten days to imbibed and split the seed coat in order to allow emergence of plumule. Twenty two scarified seeds (92%) split the seed coat within seven to eight days. Only two or 8% scarified seeds are split within ten days. The period of scarified seeds germination are relatively short compared to non scarified seeds. The resistant to germination is caused by the thick shell or seed coat which is almost impermeable to water penetration. The scarification process removed the protective or thick seed coat and allows water penetration and imbibition of the seeds. Therefore, scarified seeds are able to overcome its dormancy period within seven to ten days.

All 24 non scarified seeds are splits within seven to 77 days. The dormancy period is longer for non scarified seeds. The seeds immersed in water for almost three months in order to allow the germination. There are only 11% or 3 seeds are split within seven to eight days. Majority non scarified seeds (14 seeds or 58%) are overcome its dormancy period within 76 days and six seeds broken its dormancy period within 75 to 77 days. The treatment is effective for speeding up the germination on *Nelumbo nucifera* seeds that have very thick seed shell or coat.

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Table 3: Summary of seed development stages (from the time seeds are immersed in water until seed detached from plant).

Seeds immersed in water	Treatment/ Percentage (%) of seed germination	Germination (days)/ development stages (day from seed immersed in water until leaf emerge)						
		Swollen seed and seed coat broken	Emergence of plumule	Elongation of plumule, emergence of roots & rhizome	First leaf open	Produce more roots and rhizome	Seed detachment	
							In substrate	In water
24	**Scarified** 45.8 (11seeds) 45.8 (11 seeds) 8.3 (2 seeds) (100 % germination)	7 8 10	After one day seed coat split	18-19 (10.92-14.6 cm)	8-9 (3.5-4.6 cm)	After 7 days first and second leaves open	3-5 days after transplant	18-23 days
24	**Non scarified** 8.3 (2 seeds) 4.2 (1 seeds) 12.5 (3 seeds) 58.3 (14 seeds) 12.5 (3 seeds)) (96 % germination)	7 8 75 76 77	After one day seed coat split	18-19 (8.4-9.8 cm)	8-9 (3.12-5.5cm)	After 7 days first and second leaves open	3-5 days after transplant	18-23 days

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The seeds that scarified and non scarified only possesses difference in the time that taken for breaking dormancy, while the germination stages after the seeds coat splits are same to all seeds.

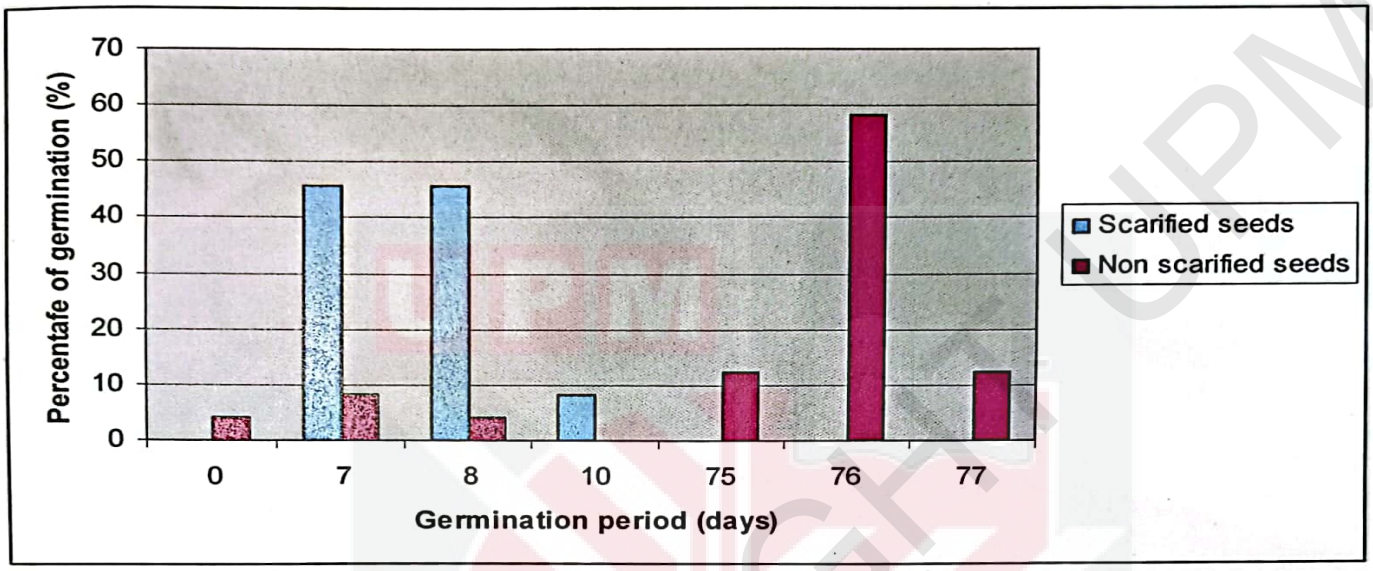


Figure 4: Period and percentage germination for scarified and non scarified seeds.

4.4.2 Germination stages

In the first four days of imbibition the seed coat become wrinkled, the embryo begins to swell within seven to ten days for scarified seeds and seven to 77 days for non-scarified seeds (Plate 26a-26b). After the swelling is completed, the seed coat shows sign of splitting and it was splits longitudinally starting from dimple end (Plate 26c). The seed germination pattern of this plant is hypogeous (lack expansion of hypocotyl, only the epicotyl emerged above the ground and cotyledon remain below ground). After the seed coat split, two cotyledons then separate and the folded-over peduncle of green embryo can be seen between it.

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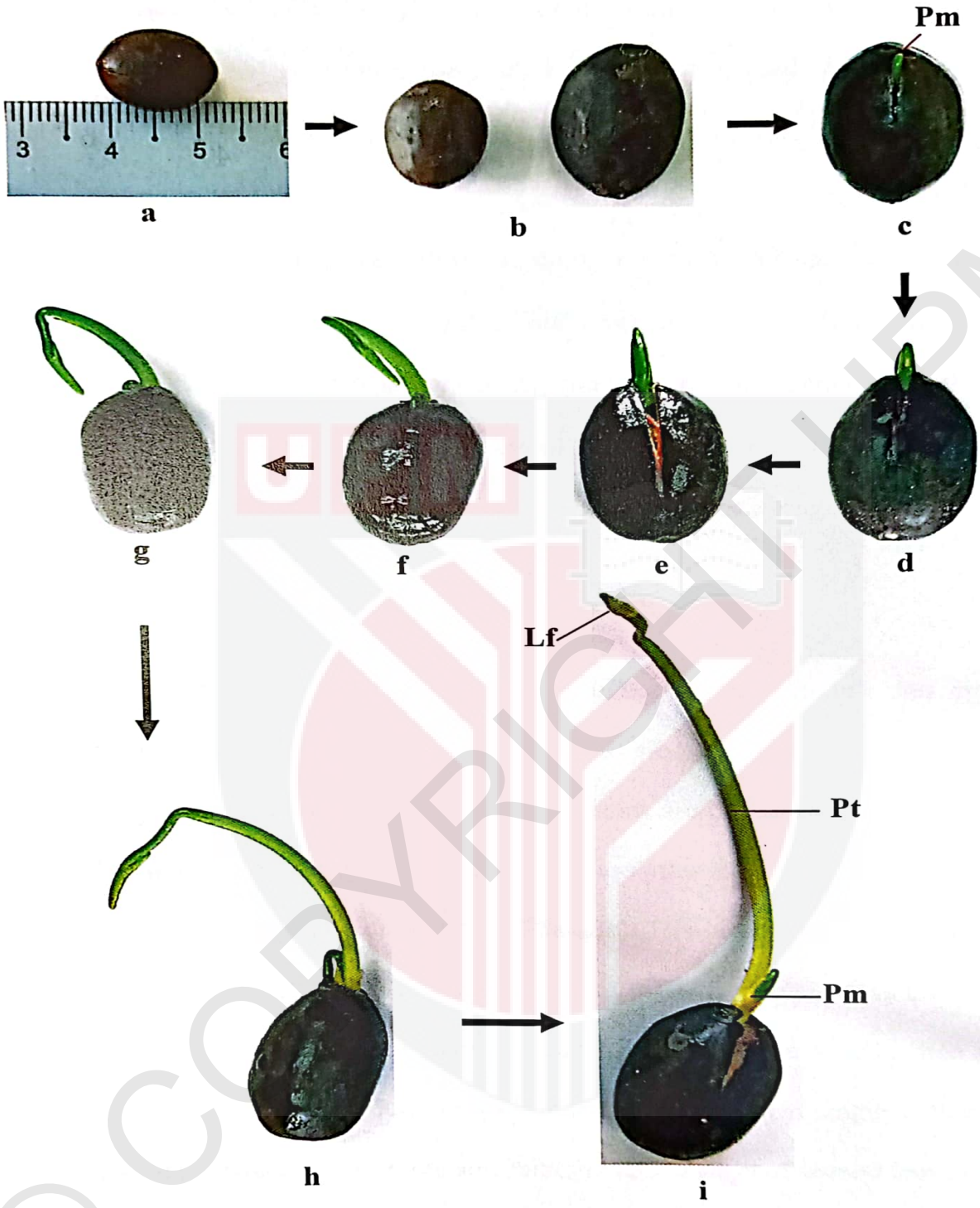


Plate 26: Seed germination stages. a, b-seed swollen, c-seed coat split longitudinal and two cotyledons then separate and the folded over peduncle of green embryo can be seen, d, e-emergence of plumule and folded over peduncle grows out of the seed coat, f, g-hook shaped seedling and h, i-petiole straightens vertically and elongates. Lf-leaf, Pm-plumule and Pt-petiole.

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After one day seed coat split, the folded peduncle the seed shell have length range from 0.6 to 0.9 cm and pulls the enrolled first leaf after it (Plate 26d-26f).

Within four days the emerged plumule, elongates until 3.11 cm. The seedlings look like a sharp hook with 3 cm length (Plate 26g) and posses slightly red color enrolled leaf (27c-27d). Petiole has spiny surface and it slowly straightens vertically and elongates up to 4 to 5 cm. During the elongation of first young leaves, the seed produced second plumule with 0.76 to 0.9 cm length (Plate 26h-26i). The elongation of plumule into young enrolled leaf, took almost 18 to 19 days.

Throughout the elongation of first plumule, the second plumule has petiole length that range from 7 to 8 cm. As the elongation of first leaves continued it possess length 8.97 to 10.03 cm and primary roots and rhizomes are produced (Plate 27a-27b). The enrolled young leaves slowly open within nine days after elongation of plumule and leaves starts expand into green colour (Plate 28a-28b). Diameter of the first leaves is 3.34 to 4.52 cm, while length of petiole is 12 to 13 cm. After five to six days the second leaves was open with 3.21 to 4 cm diameter and 10.72 to 11 cm length, then third plumule was emerged. The third plumule was elongates up to 6.7 cm length within 4 to 5 days. The seeds produced more roots and rhizome after 7 days of second leaves open and third plumule elongates (Plate 28c). Primary roots are white colour and have smooth surface (Plate 28d) and its produce roots hairs within 4 days after emerged (Plate 28e).

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Plate 27: Seed germination stages. a-seedling with two enrolled young leaves, primary roots and rhizome, b-enlarged view of primary roots and rhizome, c-front view of enrolled leaf (magnification 1.0X) and d-side view of enrolled leaf with sharp apex. Lf-leaf, Pt-petiole, Rh-rhizome and Rt-roots. (Scale bar = 1mm)

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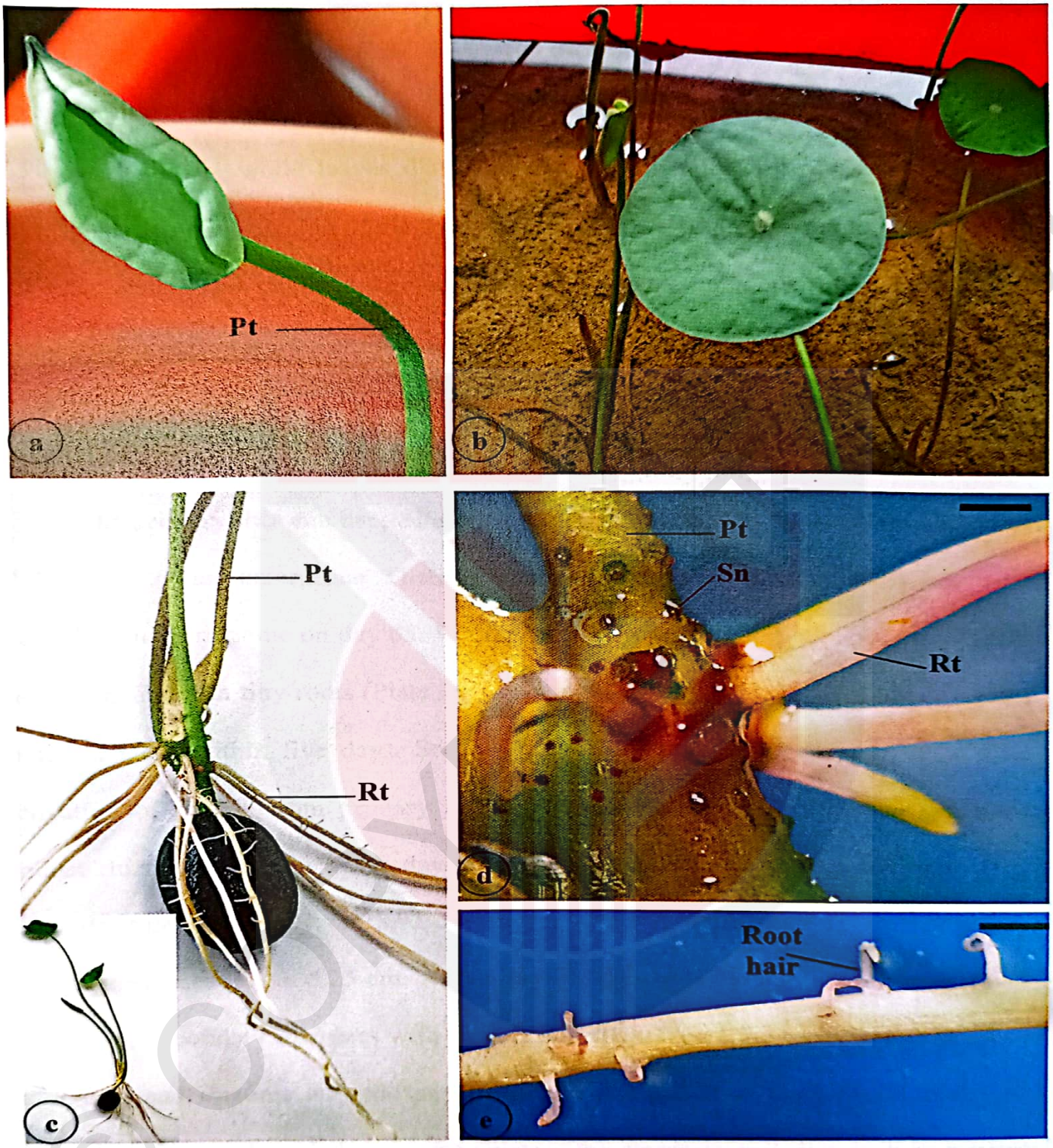


Plate 28: Development of first leaf, roots and rhizome. a, b-leaf slowly open, c-emergence of more roots and rhizomes, d-enlarged view of primary roots (magnification 1.0X) and e-emergence of root hairs (magnification 1.0X). Pt-petiole, Rt-roots and Sn-spine. (Scale bar = 1mm).

Third leaves were open around 22 days after first leaves open. The first and second leaves continued elongate and expand. At day 49, the first leaves have petiole length 17.90 to 19.14 cm, while leaves diameters are 5.23 to 5.99 cm. Meanwhile, second leaves possess petiole length 17.61 to 19 cm with leaves diameter range from 4.89 to 5.02 cm. The fourth plumule was emerged at day 52 after more rhizome and roots produced. Rhizomes are occurs between petiole and it have smooth surface (Plate 29a-29b). The fourth leaves were open after nine days of it emergence.

The primary rhizome keep elongates until it produced first node (Plate 29c) after 57 days of seedling emergence. There is emergence of leaf sheath and roots from first node of primary rhizome on day 65. Within seven days, leaf with petiole emerged from the leaf sheath with tiny roots (Plate 29d). The petiole from primary rhizome elongates up to 8.23 cm within five days. Secondary rhizome was produced eight days after elongation of petiole from primary rhizome (Plate 29e) and the secondary rhizome produce rhizome node after seven days of emergence. The entire leaves from seeds were keeps elongate until 21.04 to 23.73 cm on the 70th day. At the day 73, the secondary rhizome possesses length 5.25 cm. At the day 75, the seeds having three to four leaves primary and secondary rhizomes with roots (Plate 30a). When, the seeds start to produce more roots and rhizome an endosperm shrinked and attach to plant, while seed coat removed, and caused seed detachment (Plate 30b). Seeds in soil detached from plant within 3 to 4 days. While seeds detached in water within 18 to 23 days.

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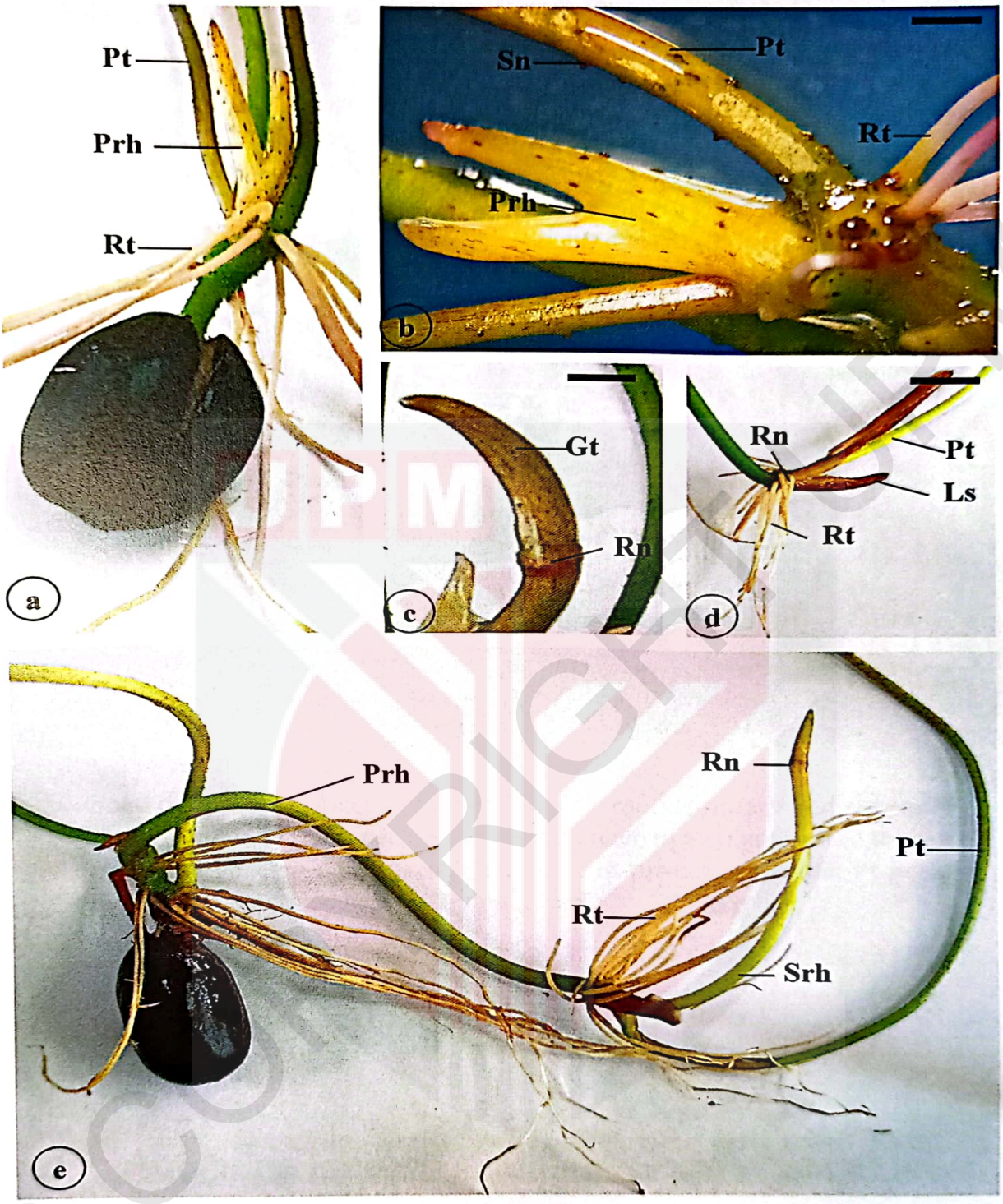


Plate 29: Primary and secondary rhizome development stages. a-primary rhizomes emerged at the middle of petiole, b-enlarged view of primary rhizome (magnification 1.0X), c-first rhizome node of primary rhizome (magnification 1.0X), d-emergence of roots and petiole from leaf sheath at first node of primary rhizome and e-elongation of primary rhizome and produced secondary rhizome and new leaf. Gt-growing tip, Ls-leaf sheath, Prh-primary rhizome, Rt-roots, Srh-secondary rhizome and Sn-spine.

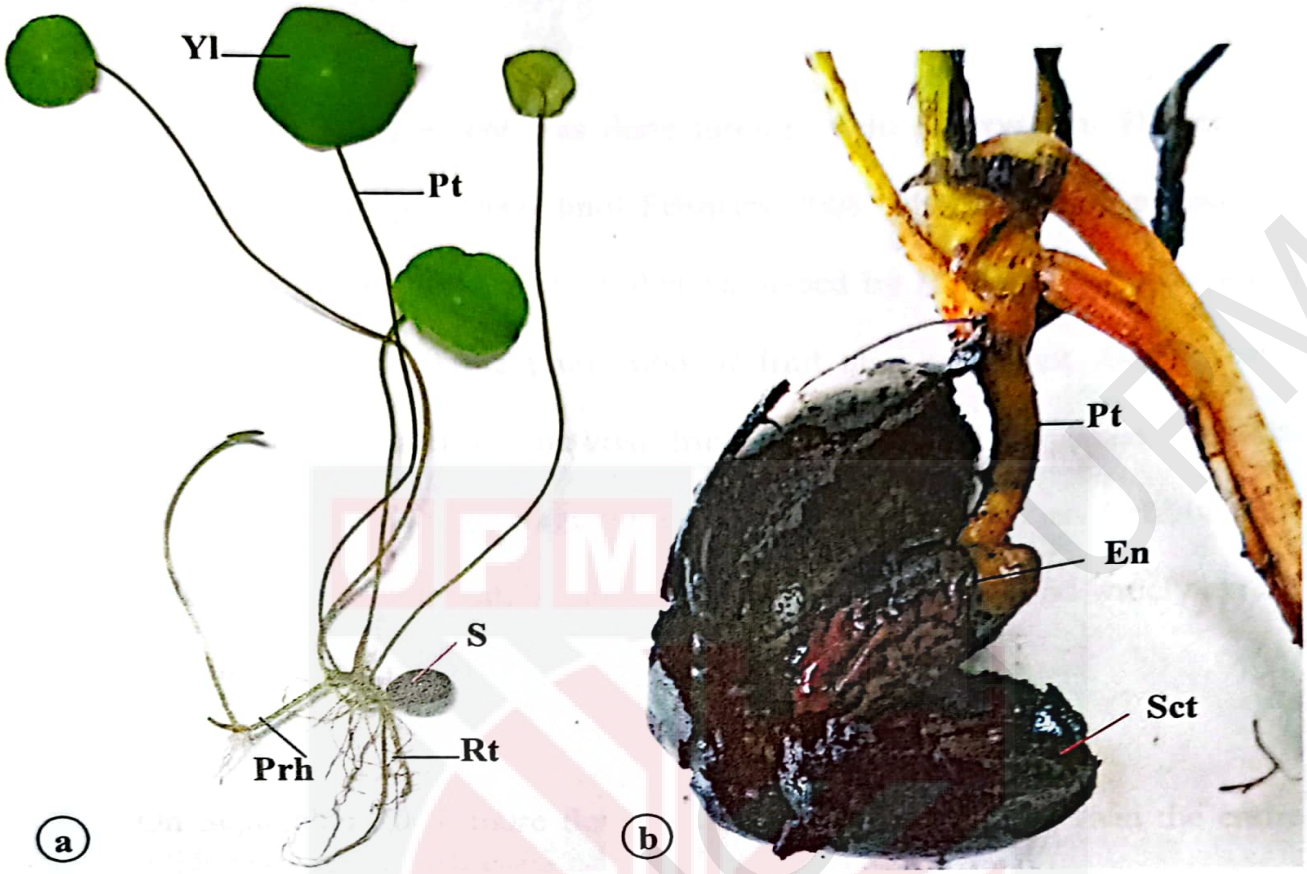


Plate 30: Seed detachment. a-seed produced leaves, roots, primary and secondary rhizomes and b-endosperm shrinked ad attached to plant, while the seed coat removed. En-endosperm, Rt-roots, Prh-primary rhizome, Pt-petiole, S-seed, Sct-seed coat and Yl-young leaf.

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4.4.3 Flower and fruit development

Flowers and fruits development was done through field observation. Flower was produced throughout August 2007 until February 2008. This flowering period was continued, this is due to the dormancy that possessed by *N. nucifera* seeds. When flowering period continued the production of fruit also consistent. During early August 2007, high number of matured fruits produced and dispersed it seeds. Statistic shows, only 35% or average three seeds per fruit are possess fertility. This might be due to lack of nutrient, because these plant are naturally and widely spread in slow moving water channel.

On September 2007, more flower buds ad flower develops. Then the entire flowers fall and develop into young fruit at the early of October 2007. The young fruits then become matured and dispersed it seeds on November 2007. On November 2007, high numbers of matured leaves were observed. More young fruits then develop into matured fruits, and flower was highly concentrated during December 2007. The rainy season during December 2007, provide a hostile environment and support the seeds to break down it dormancy period. End of December 2007, a lot of flowers, fruit and leaves were produced.

The production of flower and fruits are keep occurring. The observation was continued to January 2008 until February 2008, and still flower and fruits are produced.

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CHAPTER 5

DISCUSSION

5.1 Habitat

Through this study *Nelumbo nucifera* plants are distributed in slow moving or stagnant water channel that has temperature 25°C to 39.9°C. According to Greg and Speichert (2000) these plants adapt in humid weather and start to grow when the temperature is 15.5°C and need temperature of around 26.67°C to bloom the flower. An extreme temperature could overcome by this species because the flower can regulates it temperature to form own microclimate within 30°C to 35°C (Seymour *et al.*, 1998; Sharma and Goel, 2000 and Storey, 2001). This species are widely associated with many other aquatic and terrestrial plants, moreover be a shelter for birds, snails and snake. Sharma and Goel (2000) were reported this species only associated with aquatic livings. Summary of present and previous study of *Nelumbo nucifera* habitat are presented in Table 4.

5.2 Vegetative and reproductive morphology of *Nelumbo nucifera*

Nelumbo nucifera leaves are solitary and emergent. Although many studies have been reported the leaves are floating (Mulhberg, 1982; Cronk and Fennessey, 2001; Kamarudin and Latiff, 2002; Narayanan, 2003; Daniel, 2006 and Simpsom, 2006) only limited studies mention that this species have emergent leaves (Jones and German, 1993; Green, 2002). Mulhberg (1982) and Kamarudin and Latiff (2002) reported the diameter of leaves are 30 – 60 cm, but the leaves observed in this present study are only 25 – 35 cm and comparatively smaller.

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Table 4: Summary of present and previous study of *Nelumbo nucifera* habitat.

Habitat characteristic	Present study	Schneider *et al.* (1990)	Seymour *et al.* (1998)	Greg and Speichert (2000)	Sharma and Goel (2000)	Storey (2001)	Watling *et al.* (2006)	Yi and Lin (2006)
Natural and constructed water bodies	Temporary wetland, slow moving or stagnant water channel	-	-	-	-	-	-	-
Weather	Adapted to extreme weather (hot and rain)	-	-	Humid	-	-	-	-
Water °C	25.2-29.7°C	27.3-29.3°C	30-35°C	15.55 - 26.67°C	30-35°C	30-35°C	41-45°C	30-35°C
Dissolve oxygen (DO)	1.66-1.83 ppm	-	-	-	-	-	-	-
Water pH	5.99-6.23, slightly acidic	-	-	Sensitive to low pH	-	-	-	-
Association/ shelter	Aquatic and terrestrial plants including *Lindernia* sp., *Tribolium* sp., *Nymphaeae pubescens*, *Limnocharis flava*, *Monochoria vaginalis*, *Eleocharis acutangula*, *Eriocaulon trancatum*, *Mimosa pudica*, *Urticularia aurea*, snail, snake and birds	-	-	-	Leaves become shelter for aquatic lives such as fish, frogs, snails and snakes.	-	-	Fish

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Although more studies are concentrated on leaves part, but very limited studies was done on the leaves surfaces (adaxial and abaxial), yet limited information about the presents of stomata. In this study, the leaves adaxial and abaxial surface is waxy with smooth margin and stomata are highly present on adaxial surface. The adaxial surface of young leaves is pale green in colour with pink net veined, while the abaxial surface are pink colour. The young leaves have sharp leaf tip, because when the leaves emerged from mother plant or seeds, it enrollred and as it develop the leaves open and cause the oval shape pattern resemble at the abaxial surface of young leaves. The old and dry leaves are an umbrella-shaped. The petiole observed (Bintulu) was also shorter compared to length that have been reported to be 183 cm by Jones and German, 1993; Greg and Speichert, 2000. The petiole have shiny surface with spines and 10 round shaped air lacunae surrounded small air lacunae. Wax compounds are produced in petiole and this species have fibrous roots.

The rhizome is a thickened, prostrate, underground modification of stem provided with distict nodes and internodes. Most of the time rhizome remains underground in a dormant condition, when exposed to hostile environment the terminal bud and some of the axillary buds grow up into long or short leafy aeriel shoot which again die down after a few month (Dutta, 1980). *Nelumbo nucifera* rhizomes are having thickened internodes that are connected by nodes. The rhizome of *Nelumbo nucifera* is shaped like a long sausage with hollow portion and is connected like sausage on string (Mukherjee, 1996). There is nine major lacunae or air canal sorrounded by small lacunes, that arranged at the edge of rhizome. Summary of present and previous study on vegetative structures of *Nelumbo nucifera* are illustrated in Table 5.

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Table 5: Summary of present and previous study on vegetative structures of *Nelumbo nucifera.*

Vegetative structures	Present study	Denffer *et al.* (1965)	Muhlberg *et al.* (1982)	Jones and German (1993)	Greg and Speichert (2000)	Cronk and Fennessey (2001)	Green (2002)	Kamarudin and Latiff (2002)	Narayanan (2003)
Leaves	Solitary, emergent, round, plate shaped net veined, dark green, 25-35 cm diameter	-	Floating, 30-60 cm diameter	Solitary, emergent, round, plate shaped and net veined	Bowl like	Floating	Blue green	Floating, 30-60 cm diameter	Floating
Adaxial and abaxial surface	Waxy with smooth margin	Adaxial waxy	-	-	-	-	-	-	Adaxial waxy
Petiole	Green colour, 91.63 -114.4 cm in length, 0.8-1 cm diameter, shiny surface with spines	-	-	183 cm	183 cm	-	-	-	-
Rhizome	Oval smooth, blank spots, light yellow with 16-20 cm length and 3.6-7.0 cm diameter	-	-	-	-	-	-	-	-
Roots	Fibrous	-	-	-	-	-	-	-	-

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From the observation, the flower is hermaphrodite having both androecium (male reproductive part) and gynoecium (female reproductive part). *Nelumbo nucifera* flowers are very fragile and pinkish white. Other studies reported flowers pale red (Muhlberg, 1982), white or dark pink blossoms (Jones and German, 1993; Mukherjee, 1996; Greg and Speichert, 2000; Kamarudin and Latiff, 2002; Narayanan *et al.*, 2003) and yellow (Green, 2000). The flowers are cup shaped (Klein, 1987) or tulip shape (Green, 2000) or bowl like (Greg and Speichert, 2002). The petals are similarly shaped and having waxy surface because it impermeable to water.

Petals vary per flower 13 to 17 similar as stated by Jones and German (1993). According to Muhlberg (1982), and Klein (1987) the petals range from 16 to 160. The numbers of petal that been reported relatively very high compared to present study, this might be due to this plant are natural occurring in the study area and deficient of supplement nutrients or lack of proper management. When the petals are small, the blossom wide also becomes smaller. In this study, stomata are observed in the apex, edge, surface and base of petals. According to Dutta (1980) petals are considered as floral leaves or modified vegetative leaves, usually brightly coloured but in the simpler form. Astroscelereid is found in the mid ribs of petals base. Sepals are green when young and resemble as small leaves at flower base. Many studies have been done on *N. nucifera* but limited information stated about the sepal. Through this study, occurrence of stomata on sepal surface and apex was observed this is due to sepals are considered as floral leaves or modified vegetative leaves that presences at the lower most whorl of the flower (Dutta, 1980).

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Flower hold by stalk that usually known as peduncle. Peduncle are long, similar as stated by Lawrence (1951), Jones and German (1993) and Simpsom (2006). The peduncle is almost similar to petiole in term of surface and length. The peduncle also have shiny surface with spines, but the spines in peduncle are sharper and hook like. The peduncle and petiole only could differentiate with the lacunae design. In peduncle the major lacunae only six and it shaped slightly triangular and arranged in order, however wax are also occur in peduncle. Summary of present and previous study on reproductive structures (flower and peduncle) of *Nelumbo nucifera* are presented in Table 6.

This species are undergone self pollination or autogamy. According to Lawrence (1951), Porter (1967), Narayanan (2003) Bidlack *et al.* (2006), Daniel (2006) and Simpsom (2006) yellow receptacle occurs in the middle of flower, it is spongy with numerous sunken cavities that contains individual pistil and unovulate carpel sunken. *Nelumbo nucifera* has superior ovary because the ovary occupies the highest portion on the receptacle or thalamus, while stamens, petals and sepals are separately and successively inserted below the ovary. Such a flower is known as hypogynous. According to Dutta (1980) stamens are stated as modified vegetative leaves, but stamens are unlike leaves in all respects. The homology with leaves can de made out from certain flowers, thus there is transition from sepals to petals and from petals to stamens (Dutta, 1980). Stamens consist of filament, anther and vegetative tips similar to Schneider *et al.* (1990), Jones and German (1993), Daniel (2006) and Simpsom (2006).

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Table 6: Summary of present and previous study on reproductive structures of *Nelumbo nucifera.*

Reproductive structures	Present study	Muhlberg (1982)	Klein (1987)	Jones & German (1993)	Greg & Speichert (2000)	Sharma and Goel (2000)	Green (2002)
Flowers	Solitary, emergent, bowl like, pinkish white, 13.4-15.9 cm in wide, 13-17 petals per flower	Pale red,16 - 160 petals per flower	Cup shaped, 16 -160 petals per flower	Dark pink or white, 13-17 petals per flower	Dark pink or white	Bowl like	Tulip White, yellcw, pink or red,10.16-30.5cm in wide
Petal	Oval, pink surface with white base, 3.22-11.4 in length cm, 1.02-5.7 cm width	-	-	-	-	-	-
Upper and beneath surface of petal	Waxy with parallel veins	-	-	-	-	-	-
Peduncle	Light green, shine surface with spines, 80.5-110.7 cm in length 0.5-0.6 cm diameter	-		3 -6 feets	6 feets	6 feets	6 feets
Sepal	Oval , like small leaf, green, smaller than petals	-	-	-	-	-	-

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Stamens elongated, 98-150 per flower, 2.10 - 2.80 cm in length, filament 0.97 - 1.40 cm in length, anther 0.84 - 1.20 cm in length and vegetative tips 0.20 - 0.50 cm in length. Many studies stated the stamens are spiral, but through this study the stamens observed are elongated. Studies have been reported the stamens are numerous (Lawrence, 1951; Porter, 1967; Schneider *et al.*, 1990; Jones and German, 1993; Green, 2000; Greg and Speichert, 2002 and Daniel, 2006). Schneider *et al.* (1990) was stated the vegetative tips are able to regulate the flower temperature, this is might be due to it surface with cusp that has thermogenic properties. Summary of present and previous study on reproductive structures (receptacle and stamens) of *Nelumbo nucifera* are presented in Table 7.

The seed pods are funnel-shaped similar to Jones and German (1993), Green (2000), Sharma and Goel (2000) and Greg and Speichert (2002), however, Klein (1987) was stated the seed pods are refer as seed capsules. Seed pods also refer as thalamus which is swollen end of the axis with the floral leaves (sepals and petals) inserted on it (Dutta, 1980). The seed pods are unique because the carpel patterns within and between seed pods are vary and none of the carpel are possess to be same. The carpels possess an important value as decorative stuff (Goel *et al.*, 2001). Seeds are oval shaped, smooth surface, blackish brown and tardily dehiscent berry. The seeds have two cotyledons that possess this species as dicotyledonous. Not all the ovule that forms in the sunken pistil are develops into seeds, some ovule are failed to form seeds and only empty seed (seed coats only) are formed. Sometimes the seeds are well developing but infertile. Summary of previous and present study of seed pod and seeds structures of *Nelumbo nucifera* are presented in Table 8.

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Table 7: Summary of present and previous study on reproductive structures (receptacle and stamens) of *Nelumbo nucifera*.

Reproductive structures	Present study	Lawrence (1951)	Porter (1967)	Schneider *et al.* (1990)	Jones and German (1993)	Green (2000)	Greg and Speichert (2002)	Daniel (2006)	Simpsom (2006)
Receptacle	Yellow, spongy, cone shaped	Spongy , numerous sunken pistil contain individual pistil	Spongy , numerous sunken pistil contain individual pistil	Spongy , numerous sunken pistil contain individual pistil	-	-	-	Uniovulate carpel sunken	-
Stamens	Elongated	Spiral	Spiral	Spiral	Spiral	Spiral	Spiral	Spiral	Spiral
Filament	White, 0.97-1.4 cm	-	-	Thin or narrow	Thin or narrow	Thin or narrow	-	Thin or narrow	Thin or narrow
Anther	0.84-1.2 cm	-	-	Latrorse or introrse	Latrorse or introrse	Latrorse or introrse	-	Latrorse or introrse	Latrorse or introrse
Vegetative tips	0.2-0.5 cm	-	-	Appendage and tetra-sporangiate	Appendage and tetra-sporangiate	-	-	Appendage and tetra-sporangiate	Appendage and tetra-sporangiate
Number of stamen per flower	98 - 143	Numerous	Numerous	Numerous	Numerous	Numerous	Numerous	Numerous	Numerous

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Table 8: Summary of present and previous study on reproductive structures (seed pods and seeds) of *Nelumbo nucifera*.

Reproductive structures	Present study	Porter (1967)	Klein (1987)	Narayanan (2003)	Bidlack *et al.* (2006)	Daniel (2006)	Simpsom (2006)	Wan (2006)
Seed pod	Funnel shaped, young –green; matured-dark brown	-	Seed capsule, brown	-	-	-	-	-
Seed pod length	3.4 – 5.3 cm	-	-	-	-	-	-	-
Seed pod diameter	3.4 – 7.2 cm	-	-	-	-	-	-	-
Carpel shape	Vary	-	-	-	-	-	-	-
Carpel diameter	0.6 -2.3 cm	-	-	-	-	-	-	-
Seed	Round or oval, dark brown or black, smooth	Oval, brown, smooth	Oval, brown, smooth	Exalbuminous, leathery, tardily dehiscent berry and nut like	Exalbuminous, leathery, tardily dehiscent berry and nut like	Exalbuminous, leathery, tardily dehiscent berry and nut like	Exalbuminous, leathery, tardily dehiscent berry and nut like, Blackish brown	Exalbuminous, leathery, tardily dehiscent berry and nut like
Seed length	1.2 -1.6 cm	-	-	-	-	-	-	-
Seed diameter	0.8 -2.0 cm	-	-	-	-	-	-	-
Seeds per pod	7-20	-	-	-	20-31	20-31	-	20-31

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5.3 Vegetative and reproductive propagation of *Nelumbo nucifera*

Usually vegetative propagation is produced leaf and expands the plants quickly compare to reproductive propagation. In this study, vegetative propagation by using rhizome took almost four weeks to produce first leaves. While reproductive propagation by using seeds that undergone scarification process took only 11 days.

In this study, reproductive propagation seems to be effective for this species. In natural environments at Bintulu Old Airport rhizomes may produced in large amount and the emergence of plants through rhizome are high because some plant are emerged on land, this is definitely because of rhizome elongation until beneath of land. Through the observation, leaf that produced from rhizome is emerged from leaf sheath. The rhizome are germinated, as long as there is appropriate humid and temperature occur. The reproductive propagation naturally, the seeds may scarify through soil surface. Therefore, there is continuous production of plants and fruits at study area after dispersal of seeds.

According to Goel *et al.* (2001) the scarified seeds could germinate within three to four days whereas normal seeds take 10 to 15 days. Meanwhile, Green (2000) stated the normal seeds could break its dormancy period within 30 days. However, through this study seeds that have undergone scarification process were germinated within 7-10 days but normal or non scarified seed took 77 days to absorbed water and germinate because it having very hard seed coat. Period of seed germination only vary until the seed coat

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broken. Once the seeds starts germinate the period for emergence of leaves, roots and rhizome are the same.

The characteristic of wild plants is the seeds are not germinated at the same time, naturally the wild plants need to keep some seeds dormant or reserve in case the first sprouts face unfavorable growing condition (Pagels, 2001). However, in this study all scarified seeds were germinated at the same day. It could be because the seeds are able to overcome its dormancy period when seed coat becomes thin. The day after scarified seeds immersed in water, the seeds have increase in size, this is due to the intake of water which hydrates the dried cotyledons and dormant embryo within the seed coat (Pagels, 2001).

The first four leaves are produced after 105 days, according to Pagels (2000) the seed produced first four floating leaves about 30 days after the seed has sprouted. The seeds contain enough nutrients to sustain without extra nutrition until first four leaves emerged (Green, 2000). There is slightly a change in the colour of water in seed container, either light tan or cloudy white. The tan comes from the tannin in the seed, the cloudiness from bacteria feeding on the exudation from the seed interior (Greg and Speichert, 1993).

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CHAPTER 6

CONCLUSION

In this study, most of the vegetative and reproductive morphology of *Nelumbo nucifera* from temporary wetland, Bintulu Old Airport are same as described by Denffer *et al.* (1965), Jones and German (1993), Green (2002), Sharma and Goel (2000), Greg and Speichert (2002) and Simpsom (2006). The plants are simply adapted to hot weather because the flower can regulates the temperature according to environmental condition. *Nelumbo nucifera* associated with many other flora and fauna.

The propagation was done through rhizome and seeds. Reproductive propagation through seeds are more effective in this study because the emergence of first leaf are earlier than leaf from rhizome. The rhizome development stages and seed development stages are shown in Figure 5 and 6. The rhizome development take around six months to produce 4 leaves, roots and secondary rhizome.

Seeds are scarified in order to speed up the germination. The germinated seeds produced plumule within 7 to 11 days, while normal seeds immersed in water took almost 3 months, then only its manage to break down the dormancy and enable the plumule to emerge.

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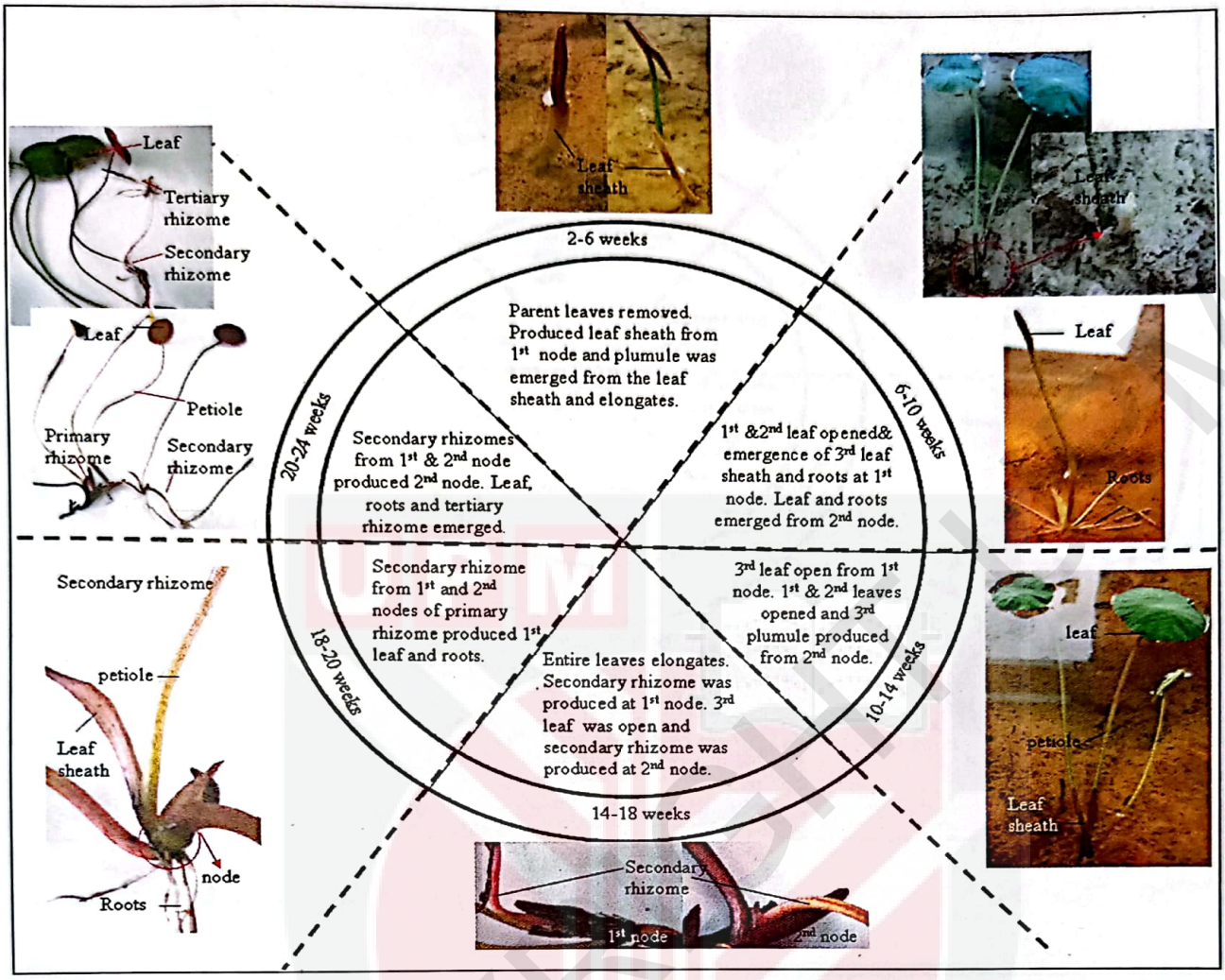


Figure 5: Rhizome development stages

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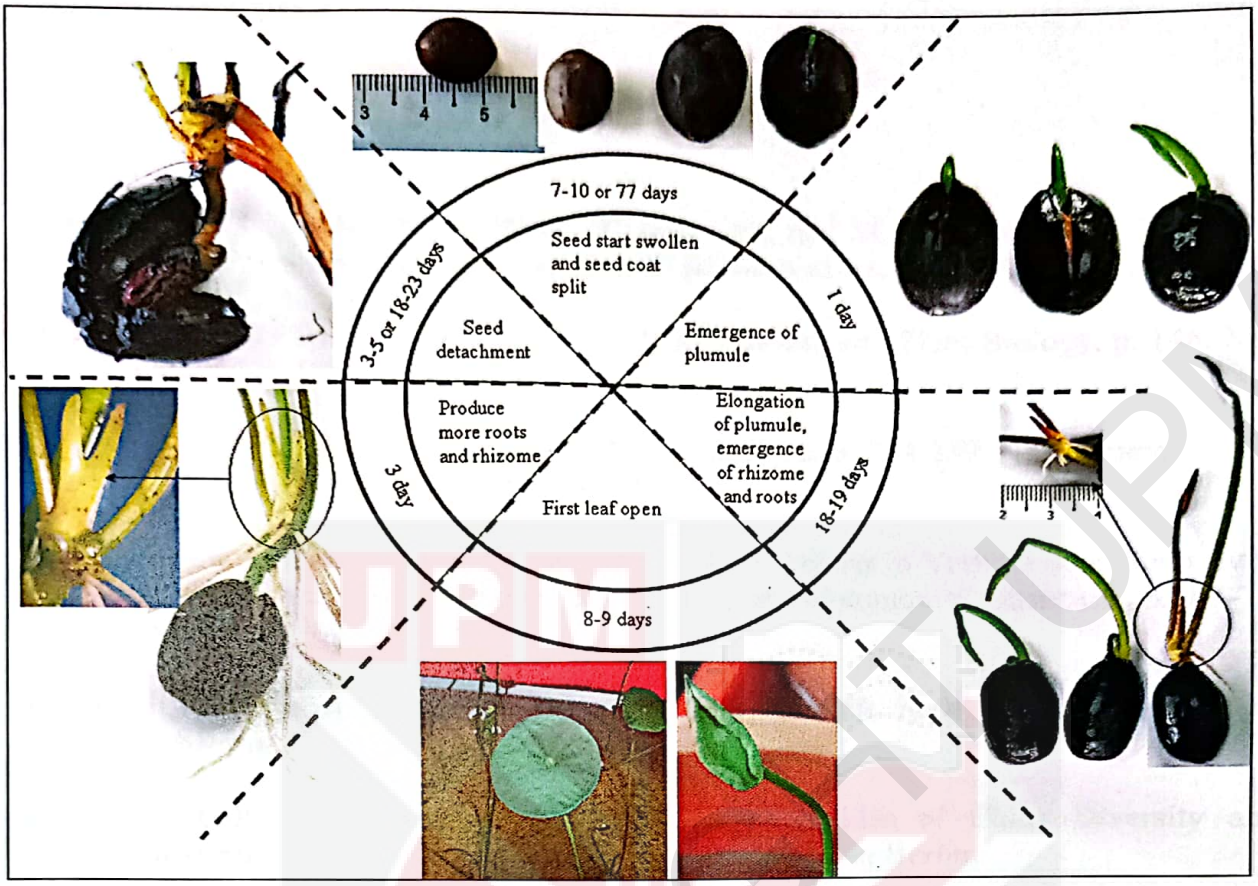


Figure 6: Seed development stages

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PUBLICATION OF THE PROJECT UNDERTAKING

This is to certify that I have no objection to publish the project entitled "Vegetative and Reproductive Propagation of *Nelumbo nucifera*" by the supervisor in a joint authorship. However, it has to be evaluated by the Faculty of Agriculture and Food Sciences, Universiti Putra Malaysia Bintulu Campus and published in the form approved by the Faculty.

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C L YOGES A/P CHANDRAM

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