ASSESSMENT OF GENETIC DIVERSITY IN *LEMNA MINOR* (L.) USING RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) MARKERS AND IT 'S NUTRITIONAL ANALYSIS

Dissertation submitted in partial fulfillment of the requirements for the award of the degree of Master of Science in

BOTANY

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BONAFIDE CERTIFICATE

This is to certify that the dissertation entitled **"Assessment of Genetic Diversity in *Lemna minor* (L.) using Random Amplified Polymorphic DNA (RAPD) Markers and its Nutritional Analysis"** submitted by **Ms. A. T Milin Sera,** M.Sc. Botany, St. Teresa's College (Autonomous), Ernakulam in partial fulfillment for the M. Sc. Degree, is the bonafide record of the research work under taken by her in this institution under my guidance and supervision.

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DECLARATION

I hereby declare that the work which is being presented in the dissertation, entitled **"Assessment of Genetic Diversity in *Lemna minor* L. Using Random Amplified Polymorphic DNA (RAPD) markers & it's Nutritional Analysis"** in fulfillment of the requirements for the award of the degree of **Master of Science in Botany and submitted to St. Teresa's College (Autonomous), Ernakulam is an** authentic record of my own work carried out during M.Sc. period under the supervision of Smt. I. K. Nishitha.

The matter embodied in this dissertation has not been submitted by me for the award of any other degree of this or any other University/ Institute.

Place: Ernakulam

Date: Signature of the Candidate

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ABSTRACT

Lemna is a genus of aquatic plant commonly known as duckweed, are small floating plants found in ponds, lakes, and slow-moving streams. *Lemna minor* L. is a well-known species among this genus. *Lemna* species are known for their rapid growth and ability to multiply quickly, making them important in certain ecosystems for nutrient cycling and as food for aquatic organisms. Using Random Amplified Polymorphic DNA (RAPD) markers, the genetic diversity of *L. minor* L. was assessed. Five genotypes of *L. minor* L. were gathered from various parts of Kerala. The plant populations were designated by the names LE 1 (Lemna 1) to LE 5 (Lemna 5). The UPGMA techniques for creating dendrograms were utilized to estimate the phylogenetic tree using Numerical Taxonomy and Multivariate Analysis System (NTSYS). It was found that the RAPD marker system was helpful for identifying variance and conducting genetic diversity investigations. *L. minor* L. is increasingly recognized for its potential as a fodder in livestock nutrition. Due to its high protein content, it serves as an excellent source of dietary protein. Additionally, it contains essential vitamins and minerals necessary for optimal animal health. *Lemna'*s rapid growth rate and ability to thrive in diverse aquatic environments make it a sustainable and cost-effective feed option. Its palatability and digestibility further enhance its suitability as a supplementary or primary feed source for various livestock species.

Key words: RAPD - Random Amplified polymorphic DNA; UPGMA - Unweighted Pair Group Method with Arithmetic mean; NTSYS - Numerical Taxonomy and Multivariate Analysis System.

INTRODUCTION

Angiosperms, a broad category of plants in the phylum Angiospermae (or Magnoliophyta), are commonly referred to as flowering plants. Their creation of flowers, which usually include reproductive organs like ovaries, stamens, and petals, is what distinguishes them. With more than 300,000 species, the angiosperm family of land plants is the most diverse and plentiful. With its dominance over most vegetation types and provision of vital supplies for human life, angiosperms are the most diversified group of plants. They can be distinguished from other plant groups by their enclosed ovules and flowering structures (Bahadur *et al*., 2015).

Aquatic angiosperms are flowering plants that have adapted to live and thrive in aquatic environments. These plants have specialized adaptations to survive in waterlogged or fully submerged conditions, often with roots, stems, leaves, and reproductive structures modified for life underwater. While having a high degree of morphological and functional diversity, aquatic angiosperm diversity is comparatively lower than that of their terrestrial relatives. In aquatic angiosperms, reduced speciation and higher extinction rates are the causes of the low diversity. Although the majority of the transition from land to water occurred in the last 25 million years, it began early in the history of angiosperms. Throughout the Cenozoic period, lineages within aquatic angiosperms gradually accumulated and their rates of diversification remained relatively low and consistent. The low diversity of aquatic angiosperms today is thought to be explained by the harsh environmental conditions and restricted global surface of the aquatic habitat (Andrea *et al*., 2022).

Lemna is an important genus in the family *Lemnaceae*, commonly called duckweeds, comprising aquatic monocotyledonous plants. They are small fresh water plants and are found all throughout the world, from temperate to tropical areas (Wang et al., 2010). These are vascular floating macrophytes that grow quickly and proliferate quickly. They are found in wetlands all over the world. True stems and leaves are absent in these plants. The body of the plant is typically made up of fronds, which are

flattened, expanded leaves that can float on the water's surface or be slightly immersed (El-Kholy et al., 2015). Fronds are responsible for photosynthesis and reproduction. The increasing number of roots is species-specific and they adhere to the lower surface of the fronds (Stomp, 2005).

***Lemna minor* L.** or duckweed, is a quickly spreading plant that provides vital nutrients and human-beneficial secondary metabolites (Eda *et al*., 2022). It is a versatile aquatic plant with various applications. It is rich in essential nutrients like proteins, carbohydrates, and fats, making it a valuable source of alternative food (Gürer & Yapar, 2022). Additionally, Lemna minor L. shows potential in phytoremediation by absorbing nutrients and pollutants from aquatic ecosystems, making it useful in environmental cleanup efforts (Mar *et al*., 2004). Studies have also highlighted its role as a bio-indicator for heavy metal pollution analysis, showcasing its ability to accumulate metals like Mn, Zn, and others from water and sediment, with higher concentrations found in the root tissues (Del Pilar Arroyave, 2004). Furthermore, Lemna minor L. has been identified as a promising source for the development of drugs and pharmaceutical products due to its biosynthetic accumulation of compounds with biological activity, emphasizing its potential in pharmaceutical applications (Kastratović *et al*., 2015).

The *L. minor* L. plant has a very simple morphology. It is a very small, free-floating aquatic plant with fronds (leaves) that are typically oval to round in shape. The fronds are usually 1 to 5 millimeters in length and have a single root (rootlet) that hangs in the water. The upper surface of the frond is generally bright green, and the plant reproduces primarily through vegetative propagation, where daughter fronds form from the edge of the mother frond. The fronds of Lemna minor contain a simple vascular system and are thickened slightly to allow for buoyancy. In favorable conditions, populations of the plant can double in just a few days, leading to dense carpets on the surface of ponds or still water bodies, where they can inhibit the penetration of light and therefore affect the growth of submerged plants. Even though Lemna minor is a flowering plant, flowers are rarely observed, and sexual reproduction is uncommon.

Furthermore, *L. minor* L. was found to have a shiny, crystalline surface when exposed to cadmium, indicating its ability to absorb heavy metals and serve as a bioindicator in contaminated environments (Zhang *et al*., 2023).

Another species in the genus of *Lemna* with unique morphological and physiological characteristics is *Lemna aequinoctialis* Welw. *Lemna aequinoctialis* belongs to two lineages, which can be considered distinct species according to molecular study. *Lemna aequinoctialis* Welw., a new alien species of duckweed, was found and documented through recent studies carried out in Ukraine (Tetiana et al., 2022). *Lemna minor* and *Lemna aequinoctialis* have comparable ecological requirements, and the distinctions between the two species have been verified by morphological and molecular investigations.

Lemna plays a crucial role in aquatic ecosystems and has significant importance in various aspects of environmental and scientific research. It can be used to create value-added goods and alternative foods since it has important nutrients and secondary metabolites that are good for people. The plant has attracted attention as forage due to their high productivity and high protein content. It also helps absorb excess nutrients like nitrogen and phosphorus, which can otherwise lead to water pollution and algal blooms. The plant is capable of thriving in a variety of water bodies and hence can be utilized as an effective biological indicator of water quality, aiding in the assessment of environmental conditions. These aquatic plants are effective bioremediation resources and have been used to treat waste water from both domestic and industrial sources. The plant is also used for studies on flowering, photosynthetic apparatus, hormone and amino acid production (Manuela et al., 2020). *Lemna* species also provide possibilities as lignified biomass sources for fuel generation and as a complete protein source that uses little resources (Manuel et al., 2022).

In order to distinguish between species, genotyping by sequencing has been used in taxonomic studies of the genus *Lemna*. *Lemna minuta* is a species of the genus whose identity, range, biology, effects, and prevention/control are covered in a datasheet.

Genetic Diversity refers to a wide range of variability that arises between genotypes with regard to the overall genetic composition of a single species or between species. Evolutionary changes are based on genetic diversity. A plant species' genetic diversity and its distribution are influenced by various factors, such as its breeding system, evolution, and environment. For genetic variety to be used and conserved effectively, a deeper comprehension of genetic diversity and its distribution is necessary.

The molecular underpinnings of the fundamental biological processes in plants must be understood in order to effectively conserve, manage, and use plant genetic resources (PGR). The evaluation of genetic diversity is often carried out at the molecular level by a variety of laboratory-based methods, including direct measurement of levels of variation through allozyme or DNA analysis. There are several different types of DNA molecular markers used in molecular investigations of genetic variation. A genomic locus is called a molecular marker if its existence clearly separates the chromosomal feature it represents from the surrounding areas at the 3' and 5' extremities. A genomic locus can be discovered using a probe or specialized initiator (primer).

Since increasing population pressure, agricultural land urbanization, and rapid modernization are the main causes of food insecurity in the developing world, the significance of plant genetic diversity (PGD) is now understood. Workers are most interested in genetic variety. There are significant concerns about the declining genetic variability and diversity among crop plant species, and it will be difficult to enhance crop varieties going forward.

Molecular markers are identifiable DNA sequences located at specific positions within an organism's genome. These markers can be used to track genetic variation, map genes, identify individuals, and study evolutionary relationships.

Each and every organism has its own DNA sequence. Variations are mutations that happen in an organism's DNA sequences, and they cause the emergence of new alleles in a population. Polymorphism is the existence of two or more alternative variants of a certain DNA sequence that can exist in various people or communities.

Balanced polymorphism is the state in which non-identical alleles for a trait are preserved at frequencies higher than 1% in a population. The degree of polymorphism in a species' gene pool determines both its chances of surviving and its capacity for evolution. The majority of plants have significant genetic variation in their natural populations, and these variations that set each plant apart.

A gene or DNA sequence that has a defined position on a chromosome and is always linked to a certain gene or trait is called a genetic marker. A genetic marker can be a long DNA sequence, such as mini- and microsatellites, or a short one, such as a single nucleotide polymorphism (SNP). DNA polymorphism is shown by molecular markers.

Allozymes and other physical and biochemical features have long been used to quantify genetic diversity within a species' gene pool. But these days, allozymes are Insufficient to evaluate genetic diversity among populations. These population-level investigations are now feasible because to a variety of molecular-based methods, such as RFLP (Restricted Fragment Length Polymorphism), ISSR (Inter Simple Sequence Repeat), and RAPD (Random Amplified Polymorphic DNA).

Because of their simplicity and speed, RAPDs are among the molecular markers that have been utilized most frequently in genetic studies. Every technique has benefits and drawbacks of its own. The degree to which these techniques can address genetic Distinctions, the kinds of data they provide, and the extent of their applicability. Molecular markers find application in a wide range of fields, including population history, genetic mapping, paternity testing, cultivar identification, marker-assisted crop breeding, and the detection of mutant genes linked to hereditary disorders.

RAPD - Random Amplified Polymorphic DNA markers are preferred among popular molecular markers because they can identify polymorphisms in nucleic acid sequences. For random amplification of polymorphic DNA, it is an independent, straightforward, fast, affordable method that takes a tiny amount of DNA. PD pronounces this method as rapid. It's a kind of PCR reaction in which the amplified DNA segments are chosen at random. Since the primer will bind to the targeted gene

at an unknown location in the sequence, knowledge of the targeted gene's DNA sequence is not necessary for RAPD analysis, which is primarily used in molecular marker studies related to genetic diversity. This makes the method popular for comparing the DNA of biological systems that have not received much attention from the scientific community or in systems where relatively few DNA sequences are compared. The key to the achievement is the use of many genetic markers, which just needed a small quantity of DNA and didn't require cloning. Any type of molecular characterisation of the species' genome, including sequencing, is done using the RAPD method. Markers are longer oligonucleotide primers, typically 10 BP, that bind to complementary sequences throughout the genome to create band profiles. When the area between the opposing primer sides is within the amplifiable distances, PCR amplification takes place.

The following benefits have led to the widespread usage of RAPD markers:

- DNA probes and sequence data are not required for the synthesis of specific primers.
- It is fast, easy to use, and efficient because it doesn't require any blotting or hybridization processes.
- Only little amounts of DNA (about 10 mg) are required for each reaction, and it can be automated.
- A large quantity of fragments.
- Easily obtainable are arbitrary primers
- When compared to other marker technologies, the unit costs per test are minimal.

OBJECTIVES

1. To survey and collect sample materials of *Lemna minor* L. from various regions of Kerala.
2. To isolate genomic DNA from the collected sample populations.
3. To conduct PCR amplification of isolated genomic DNA using RAPD molecular markers and genetic diversity analysis of collected samples.
4. To analyse the nutritional quality of *L. minor* L.

REVIEW OF LITERATURE

GENUS *LEMNA*

The genus *Lemna* belongs to the family Lemnaceae, consisting of aquatic monocots known for their small size, rapid growth, and diverse morphologies. *Lemna* species, such as *Lemna minor* and *Lemna aequinoctialis*, are widely distributed and studied for their physiological, molecular, and ecological characteristics. *Lemna minor*, also called duckweed, is recognized for its resilience, fast growth, and nutritional value, making it a potential source of alternative food and beneficial compounds for health and cosmetics. On the other hand, *Lemna aequinoctialis* has been identified in various regions, including Europe and Ukraine, showcasing distinct physiological traits and genetic lineages. Research on *Lemna* species has focused on flowering protocols, hormone biosynthesis, and genetic improvement for applications in phytoremediation, fuel production, and protein sources (Shiga *et al*., 2023) (Tetiana, *et. al*., 2022) (Eda, *et. al*., 2022). Duckweeds are characterized by their rapid growth and ability to reproduce quickly, making them important in ecological studies and applications in wastewater treatment. They belong to the family *Araceae* and are considered to be one of the smallest flowering plants. Duckweeds are characterized by their round to oval-shaped leaves, which are often small and non-photosynthetic. Duckweeds play significant roles in various ecosystems, including freshwater bodies such as ponds, lakes, and wetlands.Using accurate weather forecasts is crucial in various practical applications.

Different species of the genus *Lemna* include *Lemna aequinoctialis*, *Lemna minor, Lemna minuta, Lemna valdiviana, and Lemna perpusilla. Lemna aequinoctialis* was identified in Ukraine as a new alien species, while *Lemna minor* is native to Ukraine (Shiga *et al*., 2023). *Lemna minuta*, *Lemna valdiviana*, and *Lemna yungensis* are part of the *Lemna* section Uninerves, with *Lemna yungensis* being suggested to be synonymized with *Lemna valdiviana* based on genetic studies (Tetiana, *et. al*., 2022). *Lemna perpusilla* and *Lemna minor* exhibit species-specific resistance to herbivores,

with differences in defense strategies against duckweed *weevils* observed (Manuela, *et. al.,* 2020). Additionally, new invasive *Lemna* species like *Lemna minuta*, *Lemna obscura, Lemna perpusilla, Lemna turionifera,* and *Lemna valdiviana* have been discovered in Bulgaria, indicating long-term species transfer in the region (Gi-Yeul, *et. al*., 2022).

***LEMNA MINOR* L.**

Lemna minor L., commonly known as duckweed, is a versatile aquatic plant with various applications. Research has highlighted its rich chemical composition, including essential nutrients like proteins, carbohydrates, and fats, along with beneficial secondary metabolites, making it a valuable source of alternative food (Min, *et. al.,* 2023). Furthermore, studies have explored its medicinal properties, such as analgesic, antipyretic, and antioxidant effects, indicating its potential in herbal medicine and cosmetics development (Mahoney, *et. al*., 2022). Additionally, *Lemna minor* has been found to contain allelopathic chemicals like phenolic acids, which can influence the growth and competition of other organisms in its environment, showcasing its ecological significance (Sibel, *et. al*., 2022). Moreover, *Lemna minor* has shown promise in wastewater treatment, utilizing dairy processing wastewater to generate biomass while remediating the water, offering a sustainable approach to wastewater management (Gostynska, *et. al*.,2022).

A floating freshwater aquatic plant, *Lemna minor* has one, two, three, or four leaves, each of which has a single root that hangs in the water. Plants split apart and become distinct individuals when additional leaves develop on them. There are 1-2 cm long root. Oval in shape, light green, 1-6 mm long, 0.5 mm broad, with three (rarely five) veins and tiny air gaps to help float, are the leaves. It mostly divides itself vegetatively to reproduce. Rarely, a single ovule and two stamens are seen within a cup-shaped, membranous scale that measures about 1 mm in diameter. The seed has 8–15 ribs and measures 1 mm in length. *L. minor* is largely dispersed to new locations by birds.

USES

Lemna, specifically *Lemna minor*, has various uses based on the research data available. It is utilized for medicinal purposes, such as treating measles opacity, rubella itching, edema, and oliguria, due to its flavonoid content (Min, *et. al*.,2023). Additionally, *Lemna minor* shows promise in wastewater treatment, particularly in dairy processing wastewater, where it can help in nutrient uptake and biomass generation (Mahoney, *et. al*.,2022). Furthermore, *Lemna* species, including *Lemna minuta*, have been studied for their environmental remediation capabilities, showing high efficiency in removing pollutants like Ag, Au, and As from acid mine waters, indicating their potential in environmental cleanup efforts (Manuel, *et. al*., 2022). Overall, *Lemna's* diverse applications span from medicinal uses to wastewater treatment and environmental remediation, showcasing its versatility and potential benefits across various fields.

Lemna minor L., has diverse applications based on research findings. It has been traditionally utilized for medicinal purposes such as analgesic, antipyretic, and antioxidant effects (Min, *et. al*., 2023). Furthermore, *Lemna minor* L. shows promise in phytotechnology for removing heavy metals like Cd, Pb, and Cr from polluted water, with high removal efficiencies observed (Mahoney, *et. al*., 2022). Additionally, *Lemna minor* L. has been explored for its potential in treating dairy processing wastewater, offering a novel approach to wastewater treatment while generating valuable biomass (Sibel, *et. al*., 2022). Moreover, *Lemna minor* L. is rich in essential nutrients, proteins, carbohydrates, fats, and secondary metabolites, making it a valuable source of alternative food and potential for developing herbal medicines and cosmetics (Enas, *et. al*., 2022). The plant's chemical composition has been extensively analyzed, identifying numerous constituents that can contribute to its medicinal and nutritional value (Eda *et al*., 2022).

NUTRITIONAL ANALYSIS IN *LEMNA*

Nutritional analysis of *Lemna*, a freshwater macrophyte, reveals its potential as a sustainable feed source. *Lemna minor* L. shows optimal growth rates at pH 7, with high protein content at pH 7 and 8, and lipid fraction peaking at pH 9 and 10 (Ullah, Gul, Khan, Rehman, *et. al*., 2022). *Lemnaceae*, including *Lemna*, are rich in carotenoids like zeaxanthin, lutein, and β-carotene, as well as vitamin E and protein, making them promising for human nutrition (Stephanie, *et. al*., 2022). Additionally, *Lemna gibba* and *Ulva lactuca* from Mexico exhibit significant nutrient variations, with *Lemna* as a protein source and *Ulva* as an energy source, both containing essential amino acids, inulin, and antioxidants (Raj & Shankar, 2022). In polluted water reservoirs of Pakistan, *Lemna minor*'s biomass production, carbohydrate, lipid, protein, and mineral contents are influenced by varying N and P concentrations, with higher nutrient levels enhancing its nutritional composition (Martha, *et. al*.,2018).

NUTRITIONAL ANALYSIS OF *LEMNA MINOR* L.

Nutritional quality analysis of *Lemna minor* reveals its potential as a high-quality feed source. *Lemna minor*'s growth rate and protein content are influenced by factors like pH, nutrient concentrations, and plant-microbe interactions. Studies show that *Lemna minor* exhibits optimal growth at pH levels between 6-10, with higher protein content at pH 7 and 8 (Zenir *et al*., 2022). Additionally, nutrient concentrations of N and P significantly impact biomass production and nutritional composition, with the combined application of NP enhancing protein, carbohydrate, and lipid content (Ullah *et al*., 2022). Furthermore, the plant's interaction with microorganisms can mitigate adverse effects of elevated CO2 levels, supporting a higher nutritional quality of plant biomass under such conditions (Ullah, Gul, Khan, Akhtar, *et al*., 2022) (Herawati *et al*., 2020). Incorporating fermented *Lemna minor* in tilapia feed enhances growth performance and nutritional quality, with the best results observed at a 2.5% inclusion rate (Adams *et al*., 2022).

Research has shown that *Lemna minor* has a high protein content, making it a valuable protein-rich feed component for animal nutrition (Inguanez *et al*., 2023). Additionally, *Lemna minor* has been evaluated for its nutritional value, revealing it as a potential protein-rich source extracted using green technologies like high-power ultrasound (Devlamynck *et al*., 2021). Furthermore, *Lemna minor* has been considered as a novel food, with its whole plant material containing water, protein, and fiber, suitable for consumption as a vegetable (Gürer & Yapar, 2022). These findings highlight *Lemna minor*'s substantial protein content, making it a promising alternative protein source with various applications in animal feed and human nutrition.

Quantitative analysis of protein in *Lemna minor* has been studied in various contexts. *Lemna minor* was found to contain 11 amino acids among 112 identified constituents, indicating the presence of proteins in this plant (Zhang *et al*., 2023). Comparative analyses with *Arabidopsis thaliana* showed that *Lemna minor* exhibited better quenching efficiencies, suggesting improved light utilization, which could be linked to potential post-translational modifications in *Lemna* proteins (Liebers *et al*., 2023; Liebers, Hommel, Gruebler, *et al*., 2023). Additionally, a study on the protective effect of *Lemna minor* extract on lung protein oxidation in mice indicated a modulation of protein carbonyl content and other oxidative stress markers, highlighting the potential of *Lemna minor* in regulating protein oxidation (Karamalakova *et al*., 2022). Therefore, *Lemna minor* shows promise for further exploration in quantitative protein analysis due to its diverse constituents and potential protein-related modifications.

Carbohydrates play a significant role in *Lemna minor*. Studies have shown that *Lemna minor* contains various carbohydrates like acid arabinogalactan and pectin, with residues of galactose, arabinose, glycuronic acids, and other sugars present in different fractions (Ullah, Gul, Khan, Akhtar, *et al*., 2022) (Dianati, *et. al*., 2010) (Günter *et al*., 2004). Additionally, the presence of conventional carbohydrates like glucose and lactose affects the uptake of phenol by *Lemna minor*, impacting its metabolic processes (Day & Saunders, 2004). Furthermore, *Lemna minor* has been found to incorporate chlorophenols into its tissues, forming chlorophenyl glycosides like 2,4-dichlorophenyl-beta-D-glucopyranoside and related compounds, indicating its ability to uptake and transform xenobiotics (Khasina, *et. al*., 2003). Overall,

carbohydrates in *Lemna minor* not only contribute to its structural components but also influence its interactions with organic compounds and xenobiotics.

Studies have shown that *Lemna minor* is rich in various essential oils, phenols, and vitamins like vitamin A and vitamin D (Doğan *et al*., 2022). Furthermore, research indicates that the presence of vitamins in *Lemna minor* contributes to its antioxidant properties, suggesting its potential use in food and medicine industries (Adolfo, *et. al*.,2011). Vitamin A and vitamin D are present in *Lemna minor* feed for fancy carp, as outlined in Zhang *et al*., 2023. Additionally, *Lemna minor* is a good source of vitamin C, as it is traditionally used as a vitamin C supplement (Zhang, *et. al*., 2017). Furthermore, the study by Zhang *et al*. identified various amino acids, including essential ones that can act as precursors for vitamin synthesis in *Lemna minor*. Therefore, *Lemna minor* is rich in a variety of vitamins, making it a valuable nutritional source with potential medicinal benefits.

Research indicates that *Lemna minor* has a high accumulation potential for various macro elements. *Lemna minor* has been found to accumulate significant amounts of macro elements such as phosphorus (P), sulfur (S), sodium (Na), potassium (K), calcium (Ca), and magnesium (Mg) (Tatar *et al*., 2019). Additionally, *Lemna minor* has been observed to accumulate elements like potassium, chlorine, sulfur, calcium, and magnesium from agricultural wastewaters, which can be a concern in long-operating recirculation systems (Devlamynck *et al*., 2021). Furthermore, studies on heavy metal pollution in lake environments have shown that *Lemna minor* has a high capacity for the accumulation of manganese (Mn) and tends to accumulate higher concentrations of zinc (Zn) in its root tissue compared to sediment.

Studies has shown that *Lemna minor* has a high accumulation potential for a range of micro elements, including phosphorus (P), antimony (Sb), barium (Ba), cobalt (Co), iron (Fe), lead (Pb), manganese (Mn), mercury (Hg), silver (Ag), and zinc (Zn) (Jewell *et al*., 2023). Additionally, studies have demonstrated that *Lemna minor* can uptake metals like chromium (Cr), manganese (Mn), copper (Cu), and zinc (Zn) from water, showing the plant's ability to internalize these elements in its tissues (Tatar *et al*., 2019).

Studies have shown that duckweed biomass can be a potential source of lipids (Goswami *et al*., 2022). Lipid content in *Lemna minor* can be extracted using various solvent systems, with the highest yield achieved using hexane-ethanol extraction (Chakrabarti *et al*., 2018). The lipid fraction in *Lemna minor* mainly consists of sterols, fatty acids, and triglycerides (Ullah *et al*., 2022). Furthermore, the lipid content in duckweed can vary based on cultivation conditions, with lipid output ranging from 4% to 6% of dry biomass (Tatiana, *et. al*., 2019). These findings highlight the presence of lipids in *Lemna minor*, making it a valuable resource for lipid extraction and potential applications in various industries. *Lemna minor* contains various types of lipids, including sterols, fatty acids, triglycerides, and phospholipids. Sterols are a significant component of the lipid fraction in *Lemna minor*, as indicated by the study on lipid extraction methods using different solvent systems (Rina, *et. al*.,2018). Additionally, the lipid content extracted from *Lemna minor* includes fatty acids, which are essential components of the plant's lipid profile (Hafiz, *et. al*., 2022). Furthermore, *Lemna minor*'s lipid composition comprises triglycerides, contributing to its overall lipid content and nutritional value (Yulia, *et. al*.,2018). Phospholipids are also present in *Lemna minor*, with studies showing changes in phospholipid composition and fatty acid percentages under the influence of pesticide adjuvants. Overall, *Lemna minor*'s lipid profile is diverse, encompassing various lipid types essential for its growth and function.

The plant contains dietary fibres as one of its essential components. One specific type of pectic polysaccharide found in *Lemna minor* is lemnan, which is a pectin with a sugar chain mainly composed of D-galacturonic acid, galactose, arabinose, xylose, and D-apiose. Lemnan belongs to the apiogalacturonan type pectin family and exhibits an immunomodulatory effect by activating the system of phagocytosis (Eda, *et. al*.,2022). The presence of these dietary fibres in *L. minor* contributes to its nutritional value and potential health benefits, making it a valuable source of *Lemna* alternative food with promising applications in health, nutrition, and potentially in the development of value-added products (Hafiz, *et. al*.,2022).

The moisture content in *Lemna minor* can vary based on different factors. Some researchers found that doses of benzyladenine influenced the water content in *Lemna*

minor plants, with concentrations ranging from 0.002 to 2.0 ppm increasing water content, while a concentration of 5.0 ppm decreased it. Additionally, a preparation method for *Lemna minor* protein feed involved drying the plant material until the water content reached 80%.

Additionally, *Lemna minor* has been found to contain phenolic compounds, such as phenolic acids, flavonoids, isoflavonoids, and coumarins, with varying concentrations among different species of duckweed (Adolfo, *et. al*.,2011).

Lemna minor, commonly known as duckweed, can accumulate various toxic elements. Studies have highlighted its ability to cope with contaminants like uranium (U) and perfluorooctanoic acid (PFOA) (Annelise, *et. al*., 2023), copper (Cu) (Begoña, *et. al*., 2022), cadmium (Cd), lead (Pb), and chromium (Cr) (Enas, *et. al*., 2022), as well as palladium (Pd) (Souleimen, *et. al*.,2021). These toxic elements can have detrimental effects on *Lemna minor*, impacting growth, chlorophyll content, antioxidant capacity, and overall health. The plants response to these contaminants varies, with different elements showing varying levels of bioaccumulation and toxicity. *Lemna minor* has demonstrated potential in phytoremediation, effectively removing heavy metals like Cd, Pb, and Ni from contaminated water (Mohd, *et. al*.,2020). Overall, *Lemna minor* serves as a valuable model organism for studying the ecotoxicity of various toxic elements and their impact on plant health.

USE AS FODDER

Duckweed (*Lemna* sp.) has been studied as a possible cattle and aquaculture feed source because of its high protein and nutritional composition. It has been discovered to be an important source of protein for fish, swine, chickens, cows, sheep, and goats (Reindert *et al*., 2021). Duckweed can produce a feed source with a 35% DM protein content and efficiently extract nutrients from agricultural wastewaters (Marcin *et al*., 2019). Duckweed's usage as a feed additive may be restricted, nevertheless, as it can gather harmful metals and chemicals from the aquatic environment (Karolinny *et al*., 2013). To optimise duckweed's use as a feed additive, more study is required to improve growth medium technologies and evaluate

production chain hazards (Yuli *et al*., 2019). *Lemna* sp. exhibits promise as a possible source of fodder overall, but in order to guarantee its safety and efficacy as a feed ingredient, great care should be taken during its cultivation and processing (Sanjeev *et al*., 2014).

Kunda *et al*., 2008, conducted an analysis of three periphyton substrates, feeding, and fertilisation strategies for raising fish yield in Bangladeshi fertilised fish ponds. This study addresses the usage of *Lemna* as a potential feed substrate in addition to comparing various feeding strategies for fish development in fertilised fish ponds. Fish production in Bangladeshi ponds was assessed in relation to *Lemna* species and other periphyton substrates. This study offers information on the use of *Lemna* as a feed source for aquaculture, indicating that it may also be used as a viable choice for fish and other livestock.

GENETIC DIVERSITY

The term "genetic diversity" describes the differences in genetic traits that exist both within and among populations of organisms. It contributes significantly to species variety, ecosystem diversity, and landscape diversity. It is a significant part of biodiversity. Numerous methods, including DNA polymorphism analysis, morphological marking, cytological marking, and allozyme analysis, can be used to quantify genetic diversity (Aremu, 2017). These methods give scientists effective instruments to investigate genetic variety at several scales, ranging from molecular to morphological and biochemical (Shen *et al*., 2001) (Coit, 1960). Molecular markers, including SSRs, AFLPs, RAPDs, and RFLPs, have emerged as effective instruments for determining the genetic diversity of both domesticated and wild plant species (Albrecht, 2015). By overcoming the drawbacks of earlier marker systems, they make it possible to evaluate genetic diversity intra-specifically through pedigree analysis and germplasm grouping. Understanding genetic variety is essential for breeding programmes, crop improvement, and animal protection because it affects these fields chances of success in the future.

Numerous species in the genus *Lemna*, such as *Lemna aequinoctialis*, *L. minor*, and *L. gibba*, have had their genetic diversity examined. Physiological and molecular investigations of *L. aequinoctialis* showed two unique tendencies in the development of flower organs, corresponding to two different physiological groups (Shiga *et al*., 2023). Based on physiological, morphological, and molecular traits, the studies conducted by researchers supports the existence of two lineages within the *Lemna aequinoctialis* complex. Two unique physiological groups were identified by analysing the morphology and physiological characteristics of *Lemna aequinoctialis* sensu lato strains from various locations. These patterns in floral organ development, protogyny and adichogamy, were found to be present. Two lineages that correspond to these physiological categories have been identified through molecular analysis using loci of chloroplast DNA, indicating that they can be considered distinct species.

The study conducted by Senevirathna *et al*., 2023, examined the population genetic structure of two cryptic duckweed species in Alberta, Canada: *Lemna minor* and *L. turionifera*, using genotyping-by-sequencing. The two species were clearly distinguished from one another; *L. minor* had at least three genetically separate populations within a limited geographic area, but *L. turionifera* showed no indication of genetically distinct populations. Variables related to surface water quality were shown to have an impact on the distribution of the two *Lemna* species. At the locations where it was discovered, a greater variety of water chemistry factors suggested that *L. turionifera* was more adaptable to varying environmental conditions. Conversely, the water chemistry profiles of the three genetically diverse *L. minor* groups varied. The results emphasise the significance of recording and keeping an eye on *Lemna* species, especially in areas where they co-occur, because of the significant variations in their geographic ranges and levels of genetic differentiation.

In this study conducted by Bog *et al*., 2020, *Lemna minuta*, *Lemna valdiviana*, and *Lemna yungensis* are the three species that make up *Lemna* section *Uninerves*. Genotyping-by-sequencing (GBS) molecular taxonomy investigations have demonstrated that *L. minuta* is easily distinguished from *L. valdiviana*/ *L. yungensis*. Nonetheless, no genetic differentiation was discovered between *L.*

valdiviana and *L. yungensis*, suggesting their genetic homology. The authors propose that *Lemna valdiviana* and *Lemna yungensis* be used as synonyms for each other in light of these findings. For all the clones that were originally identified as *L. valdiviana* and *L. yungensis*, the older description of *L. valdiviana* by Philippi is regarded as the legitimate common name. With this ruling, there are now only 36 species in the *Lemnaceae* family. In the genus *Lemna*, genotyping-by-sequencing (GBS) shown to be more effective for species delimitation than barcoding based on PCR amplifications.

Due to a variety of reasons including population structure, hybridization, polyploidy, genome size variation, and ecological adaptability, *Lemna* species show a high degree of genetic diversity. Comprehending the genetic diversity present in *Lemna* is imperative in order to explicate their evolutionary background, ecological functioning, and their uses in domains like biotechnology and environmental restoration.

MOLECULAR MARKERS

Molecular markers are frequently employed in research on genetic diversity. They offer important information regarding the genetic diversity and unique traits of different plant species (Bahar *et al*., 2023). These studies have frequently used a variety of molecular markers, including Simple Sequence Repeats (SSRs) and Single Nucleotide Polymorphisms (SNPs), because of their co-dominancy, high polymorphism, repeatability, and specificity. Because SSRs are co-dominant, extensively dispersed throughout the genome, and incredibly informative, they are especially well-liked. The genetic diversity of many plant species, including common food plants like potatoes, maize and tomatoes, has been evaluated using these markers (Aditi *et al*., 2023). SSR markers have also been used to evaluate the genetic diversity of other plant species, including Indian mustard and Persian walnut (Hadama, 2023). The conservation and use of genetic resources have been aided by the use of molecular markers, particularly SSRs, which have shown to be an invaluable tool in identifying genetic diversity and interactions within and between plant species.

Many plant species can be identified by their genetic diversity using molecular markers. Numerous marker types have been created, such as Sequence Characterised Amplified Regions (SCARs), Single Nucleotide Polymorphisms (SNPs), and Simple Sequence Repeats (SSRs). These markers are useful resources for researching genetic diversity because of their benefits, which include co-dominance, high polymorphism, and reproducibility. Additional markers like as Amplified Fragment Length Polymorphism (AFLP), Inter Simple Sequence Repeats (ISSR), and Restriction Fragment Length Polymorphism (RFLP) are also frequently employed. These markers may identify different kinds of DNA mutations and are unaffected by external conditions. Furthermore, markers like Genotyping-By-Sequencing (GBS) and Expressed Sequence Tags (ESTs) have been created to support crop development and genetic resource conservation ((Ruíz-Sánchez *et al*., 2023). Chloroplast markers, nuclear markers, ISSRs, and SSRs are frequently employed to assess genetic diversity in bamboo species. In general, molecular markers are essential for recognising and comprehending the genetic diversity seen in plants.

Plant conservation and environmental adaption are greatly aided by genetic diversity. Plant genetic diversity can be effectively analysed with the use of molecular genetic markers such RFLPs, RAPDs, and SSRs. Plant species have long employed cytoplasmic genome-derived DNA markers, such mtDNA and cpDNA, to identify geographic origins and population difference. For assessing genetic diversity and population organisation in plants, AFLP markers are useful tools. RNA-seq and other transcriptome-based enrichment techniques are commonly utilised to find SNPs and reduce genomic complexity for the generation of molecular markers. Analysing SNP data from sequencing can reveal important information about the genetic diversity and connections between various accessions and species (Vu *et al*., 2021).

Using both molecular and morphological data, the study conducted by Corniquel & Mercier, 1997, explores the evolutionary links among *Lemnaceae* (duckweeds). It offers important insights into the use of DNA markers for phylogenetic study within the *Lemnaceae* family, even if it predates some of the more current developments in molecular marker techniques (Corniquel & Mercier, 1997).

Numerous studies have produced molecular markers for *Lemna* species. The transcriptome sequencing was used to create a comprehensive set of SSR markers for *Lemna gibba* (Lili *et al*., 2020). Chloroplast microsatellite markers for *Lemna minor* were characterised by Wani *et al*. (Gowher *et al*., 2014), offering helpful resources for population genetic research. Fernanda *et al*. examined the cross-amplification of nuclear microsatellite markers in this species and discovered high rates of polymorphism and cross-amplification (Fernanda *et al*., 2019). According to Wang *et al*.'s suggested sequence of polymorphism-based DNA barcoding system for *Lemnaceae*, a potential universal marker for species-level identification was developed, it was the atpF-atpH noncoding spacer (Wenqin *et al*., 2010). Furthermore, *Lemna minor* and *Spirodela polyrhiza* microsatellite markers have been created, and the majority of these markers exhibit polymorphism. These studies offer important resources for population genetics, species identification, and genetic diversity assessment in *Lemna* species.

RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) MARKERS

Random Amplified Polymorphic DNA markers, or RAPD markers, are molecular tools that are used to analyse genetic variation in a variety of plant species. These markers are created by utilising short primers to amplify random genomic DNA sequences in PCR. After that, the amplified fragments are divided and examined to see if any polymorphisms between the various genotypes exist or not. *Menoreh durian* accessions, onion cultivars, soybean genotypes, and green gram genotypes have all had their genetic diversity studied using RAPD markers (Ghodake *et al*., 2023) (Nandariyah *et al*., 2022) (Anandhinatchiar *et al*., 2023). Important details about the genetic diversity, linkages, and clustering patterns among these plant species have been gleaned from the RAPD study results. Because RAPD markers are polymorphic, diverse genotypes can be classified into different genetic groups and unique alleles can be identified. Because RAPD markers are polymorphic, it is possible to identify individual alleles and categorise genotypes into different genetic groups. In a variety of plant taxa, RAPD markers have shown to be useful instruments for molecular

classification, marker-assisted breeding, and crop improvement (Abubakar *et al*., 2022).

The study by Mane-Deshmukh *et al*., 2023, aimed to characterize and validate the genetic diversity of thirty onion genotypes using agro-morphological traits and RAPD markers. Agro-morphological foliage attitude, bulk colour, and bulk shape were studied. Four RAPD markers (OPB-09, OPA-16, OPB-16, and OPA-09) were used to assess genetic diversity, with 98.21% average polymorphism observed. UPGMA analysis revealed significant genotypic differences and two major clusters of onion genotypes. The study provides valuable insights for breeding strategies and varietal development in onion cultivars.

RAPD markers have been extensively utilized in *Lemna* genetic studies. Studies have employed RAPD analysis to assess intergeneric, interspecific, and intraspecific polymorphism in *Lemnaceae* species in Russia, revealing varying levels of genetic distances (Xu *et al*., 2018). Additionally, AFLP marker techniques have been applied to identify *Lemna* species, with successful recognition of 10 out of 13 species based on genetic clustering (Martirosyan *et al*., 2008). Furthermore, RAPD markers have been employed to evaluate genetic diversity in *Lemna sativum* genotypes, showing a total polymorphism of 67.7% with specific similarity coefficients between different genotypes (Bog *et al*., 2010). These findings highlight the significance of RAPD markers in elucidating genetic diversity and relationships within *Lemna* species, showcasing their utility in molecular studies of *Lemnaceae*.

RAPD (Random Amplified Polymorphic DNA) markers are frequently employed in phylogenetic analysis, population genetics, and genetic diversity research. These are PCR-based markers that use arbitrary, brief primers to amplify randomly selected genomic regions.

MATERIALS AND METHODS

PLANT MATERIALS

Five genotypes of *Lemna minor* L. were gathered from 5 locations in Kerala (Table 2). The aquatic plant conservatory at KSCSTE—the Malabar Botanical Garden and Institute for Plant Science in Calicut, Kerala, India, was subsequently used to successfully preserve these species. LE 1 (Lemna 1) to LE 5 (Lemna 5) are the names of plant populations (Table 2). The germplasm was kept in good condition with appropriate irrigation and growth-promoting factors. As a result, all five samples comprised a healthy plant population that was always available for research.

Plate 1: *Lemna minor* L.

KINGDOM	**PLANTAE**
CLADE	**ANGIOSPERMAE**
DIVISION	**MONOCOTYLEDONAE**
ORDER	**ALISMATALES**
FAMILY	**ARACEAE**
GENUS	***LEMNA***
SPECIES	***MINOR***
BINOMIAL NAME	***LEMNA MINOR* L.**

Table 1: Scientific Classification of *Lemna minor* L.

Small, free-floating aquatic plants in the genus *Lemna* are referred to as duckweeds. The fronds on the plants are small, flat, oval to spherical structures that might be one or more in number. Usually green in colour, these fronds float on the water's surface. While some species may have short stolons or roots, they do not have stems. Their roots are hair-like and fine, and they reach below the water's surface. The main purposes of these roots are anchoring and nutrient absorption. *Lemna* fronds, which usually measure a few millimetres to a few centimetres, are among the smallest of all flowering plants. They float on the water with ease in part because of their small size. They procreate vegetatively, mostly by asexual processes like fragmentation and budding. Their capacity to establish dense colonies in aquatic settings is facilitated by their quick reproduction. Despite being flowering plants, *Lemna* species are often overlooked because of how little their blossoms are seen. The tiny blooms are typically concealed by a spathe. They can be found in ponds, lakes, marshes, and meandering streams, among other freshwater environments. They can withstand a variety of environmental conditions, but they do best in environments that are high in nutrients.

SL. No.	Location	Latitude	Longitude
LE1	Puzhavathu, Changanassery, Kerala 686101, India	9.4405	76.532
LE2	Kodamthuruth Gramapanchayath, Alappuzha	9.794	76.307
LE3	Chenthrapini, Thrissur Kerala 680687	10.358	76.131
LE4	Panangad, Ernakulam 682505	9.894	76.328
LE5	Malabar Botanical Garden, Kozhikode	11.243	75.827

Table 2: Details of *L. minor* L. samples collected from different regions of Kerala for genetic diversity analysis

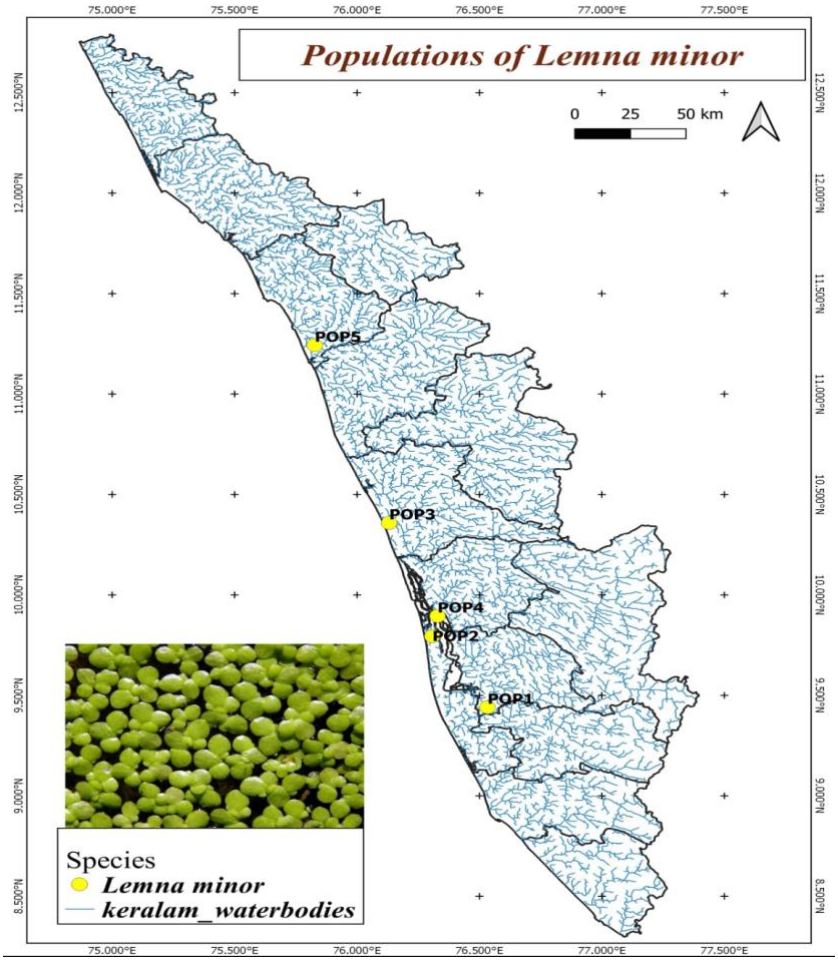

Plate 2: *L. minor* L. in Kerala waterbodies

PREPARATION OF GENOMIC DNA FROM PLANT TISSUE

Samples of tender, healthy leaves were taken from every accession. They were rinsed well and pat dried with sterile filter paper. After that, one gram of leaf material was weighed, then chopped it into pieces in a mortar that has already cooled. Prior to extraction, polyvinylpyrrolidone (PVP) was added to the CTAB Extraction Buffer and it was preheated. Using a mortar and pestle, the tissues were pulverized. Then added 500–1000 µl of extraction buffer (Table 3), it was stirred to form a slurry, and then transferred to a 2 ml centrifuge tube. The tubes were incubated in water bath for 45 minutes at 65°C, with periodic stirring.

After the liquid was cooled down to the room temperature, 70µl of the chloroform: isoamyl alcohol (24:1) combination was added, it was stirred thoroughly to create an emulsion, and centrifuged for 15 minutes at 25°C at 12,000 rpm. Transferred the top aqueous phase to a fresh tube and filled it with 1/10 of the CTAB/NaCl solution. After that the mixture was gently stirred and the same amount of chloroform was added to it, then centrifuged for ten minutes at 4°C at 12,000 rpm. Transferred the top aqueous phase to a fresh eppendorf tube, added the same volume of chloroform, and centrifuged for ten minutes at 4°C at 12,000 rpm. After that, it was carefully mixed by inverting the tubes, then transferred the supernatant to a new eppendorf tube and added cold 100% isopropanol through the walls. For one hour, the mixture was kept at 20°C. Then centrifuged for 15 minutes at 4°C at 7850 rpm. Dried the collected pellets in the air after they were centrifuged with twice cold 70% ethanol at 1000 rpm for five minutes. Pellets were suspended in 200 µl of TE buffer. In a water bath, tubes were incubated at 37°C for one to two hours after the addition of 4µl of RNase.

Incorporated 500 µl of phenol: Chloroform: Isoamyl mixture (24:25:1), gently mixed, and centrifuged for 10 minutes at 4°C at 10,000 rpm. Collected the supernatant into a new eppendorf tube, then carefully mixed with 500 µl of chloroform. Centrifuged for ten minutes at 1000 rpm. Collected the supernatant and added double the volume of cooled ethanol into a new 1.5 ml eppendorf tube and 0.1 liter of 7.5 M sodium acetate.

Overnight, stored the sample at -20 °C. Centrifuged the pellets for 15 minutes at 4 °C at 10,000 rpm to collect the pellets. After that the pellet were washed in cold 75% ethanol and air dried. Resuspended the pellets again in around 50 µl of TE buffer (Table 3). The pellets that had been resuspended were collected and kept at -20 °C.

SOLUTIONS	COMPOSITION	QUANTITY
Tris buffer pH8	Tris 1M H_2O	12.11 gm 100ml
EDTA	Na_2EDTA H_2O	18.61 gm 100ml
CTAB Extraction buffer pH 8.0 (stored at room temperature)	CTAB 2% W/V Tris buffer 100 mM Na_2EDTA 20 mM PVP 1% NaCl 1.4 M H_2O	2gm 10 ml 4 ml 1 gm 8.2 gm 100 ml
TE buffer	Tris buffer 10mm Na_2EDTA 1 M H_2O	1 ml 0.2 ml 100 ml
Sodium acetate	Sodium acetate 3 M H_2O	24.61 gm 100 ml

Table 3: Stock solution required for genomic DNA extraction

PCR AMPLIFICATIONS

Five accessions of *Lemna minor* L. genomic DNA were amplified by PCR utilizing Random Amplified Polymorphic DNA (RAPD) primers, which was created by Bserve Biotechnologies in accordance with the Operon Technologies, Inc. USA sequence.

A 25µl volume was used for PCR amplification procedures, which included 1µl of template DNA, 12.5µl of Takara Master Mix, 1µl of 10pmol primers, and enough Milli Q water to get the volume to 25µl. All of the reagents were combined with a quick spin. A Bio-Rad thermal cycler was used to carry out the amplification process.

10-mer short oligonucleotide primers chosen at random from the operon series were used for RAPD amplification. The steps in the RAPD amplification sequence were as follows: 39 cycles of 1 minute at 36°C and 2 minutes at 72 °C were followed by 1 cycle of 2 minutes at 95 °C, 35 °C, and 75 °C. A 7-minute extension at 72°C occurred after the last cycle. Samples were stored at 12°C.

Primer	Sequence	No of base pairs	GC content %
OPA02	5'-TGCCGAGCTG-3'	10	70
OPC17	5'-TTCCCCCCAG-3'	10	70
OPG17	5'-ACGACCGACA-3'	10	60
OPG02	5'-GGCACTGAGG-3'	10	70
OPC08	5'-TGGACCGGTG-3'	10	70

Table 4: List of RAPD primers used in the study

PCR steps	Temperature (in ℃)	Time (in minutes)
Initial denaturation	94	2
Denaturation	95	2
Annealing	35	2
Extension	72	2
No. of cycles of denaturation, annealing, extension	39 cycles at 93, 36, 72	1,1 and 2
Final extension	72	7

Table 5: Conditions for PCR reaction

QUANTIFICATION AND VISUALIZATION OF DNA

Using a nanodrop spectrophotometer (Multiskan sky), optical density (O.D.) at A260 and A280 was measured in order to quantify DNA. The software called nanodrop (Thermo Scientific, USA) was used to record the samples concentration and purity. The samples of DNA were diluted to a 50 ng/µl concentration and kept for usage at -20°C. To prepare DNA samples, 6X loading dye was mixed with 1X final concentration. To assess the quality of the isolated DNA, 1µl loading dye (Table 6) and 5µl of isolated genomic DNA were loaded onto a 0.8% agarose gel stained with ethidium bromide. The gel was then electrophoresed in 1X TAE buffer (Table 6) for 30 minutes at 100V. The gels were captured on camera using a Bio-Rad Gel Documentation system.

Solution	Composition	Quantity
TAE Buffer (10X) pH 8	Tris base Acetic acid Na_2EDTA 0.5 Mm H_2O	21.6 gm 11 gm 8 ml 100 ml
Gel loading buffer	Bromo phenol blue 0.25% Xylene cyanole 0.25 % Sucrose 40%(w/v) H_2O	250 gm 250 gm 40 gm 100 ml
Ethidium bromide	Ethidium bromide H_2O	1gm 100 ml

Table 6: Stock solution required for agarose gel electrophoresis

DATA ANALYSIS

The bands were qualitatively scored using gel photos derived from RAPD analysis. Data were recorded in a binary matrix sheet with "0" denoting absence and "1" denoting presence, based on clearly repeatable bands from each accession that were scored as unit characters. The data set contained both polymorphic and monomorphic bands to demonstrate an objective assessment of genetic variation. The software package Numerical Taxonomy and Multivariate Analysis System (NTSYSpc) (version 2.02) was utilized to evaluate the binary data that was acquired from the marker systems.

The “J” coefficient was chosen in NTSYS, and a dendrogram was created using UPGMA (Unweighted Pair Group Method with Arithmetic Mean) to analyze diversity patterns and interpret data.

PIC (Polymorphism Information Content)

Due to the polymorphism that the dominant marker revealed, the data were elaborated on a binary matrix of the presence or absence of bands. The following general equation was used to estimate the Polymorphism Information Content (PIC), where frequency of bands presents as P and the frequency of absents as q;

PIC = $1-(P^2-Q^2)$ (Nunes dos Santos, K., *et.*al,2019).

By dividing the number of isolate alleles where the band was discovered by the total number of isolates, the frequency of an allele can be determined. To evaluate genetic diversity, PIC is a useful metric. When measuring a genetic marker's information for linkage studies, PIC value is frequently employed. It is possible to assess the degree of gene variation whereby using the PIC index:

A locus with a PIC >0.5 is considered to have great variety

PIC <0.25 denotes a low level of diversity at the locus

PIC of 0.25 to 0.5 suggests that the locus has an intermediate level of diversity

PIC analyses can be used to assess markers, allowing the best marker to be chosen for phylogenetic and genomic mapping.

EMR (EFFECTIVE MULTIPLEX RATIO)

The EMR of a primer is "the product of the fraction of polymorphic loci and t he number of polymorphic loci of the individual assay."
It is the result of multiplying the fraction of polymorphic fragments (β) by the total n umber of fragments per primer (n).

E=nβ

where β = total number of polymorphic DNA and n = total number of bands (Chesnokov, *et al*., 2015).

MARKER INDEX

The marker index MI was computed for each of the five markers in order to assess the overall utility of a particular marker system. Rather than the degree of polymorphism found, the high MI is a reflection of the markers ability to assess a greater number of bands simultaneously. PIC and EMR produce MI (Varshney *et al*., 2005).

MI = PIC * EMR

Where PIC = Polymorphism information content & EMR = Effective Multiplex Ratio

ESTIMATION OF CARBOHYDRATE BY THE ANTHRONE METHOD

Principle:

Carbohydrates were dehydrated by conc.H_2SO_4 to form furfural. Active form of the reagent was anthranol, the enol tautomer of anthrone, which reacts by condensing with the carbohydrate furfural derivative to give a green color in dilute and a blue color in concentrated solutions, which was determined colorimetrically. The blue - green solution showed absorption maximum at 620 nm (Richterich, *et. al.*,1969).

Methodology:

(a) Materials required:

(i) Equipment's: UV Spectrophotometer, Vortex mixer, Water Bath.

(ii) Chemicals/Reagents: Anthrone Reagent, Glucose.

(iii) Glass wares: Test-tube, Test-tube stand, Pipettes, Beaker, Ice Test tube caps, Tissue paper, Wash bottle.

(b) Preparation of Reagents:

(i) **Anthrone reagent:** Dissolved 2g of Anthrone in 1 liter of concentrated H_2SO_4. Used freshly prepared reagent for the assay.

(ii) **Glucose stock solution:** 50µg glucose per mL distilled water.

[Note: Can include other carbohydrates of the same concentration if desired]

(c) Procedure:

1.) Different volumes of glucose solutions from 200 to 100µl (10 -50µg) were pipetted out from the supplied stock solution into a series of test tubes and were made up to the volume to 10mL with distilled water.

2.) Tube 1 was considered as the blank and from tubes 2 to 9 were used for construction of the standard curve. Tubes from 10-15 were the plant samples taken.

3.) To each of the tube added 5mL of the anthrone reagent and was mixed well by using a vortex mixture.

4.) The tubes were then cooled and covered with marbles/caps on top.

5.) Then incubated at 90℃ for 17 minutes or can be use boiling water bath for 10 minutes.

6.) Cooled to room temperature and measured the optical density at 620 nm against a blank.

7.) Prepared the standard curve of absorbance vs. μg glucose for calculating the concentration of carbohydrates in the plant sample.

(iv) **Calculation:** Determined the amount of glucose in the plant sample by plotting the value obtained at A620 in the standard curve with absorbance on Y-axis and μg of Glucose on X-axis. (Layne, 1975; Plummer, 1990).

ESTIMATION OF PROTEIN BY LOWRYS METHOD

Principle:

The principle behind the Lowry method of determining protein concentrations lies in the reactivity of the peptide nitrogen[s] with the copper [II] ions under alkaline conditions and the subsequent reduction of the Folin Ciocalteau phosphomolybdic phosphotungstic acid to hetero polymolybdenum blue by the copper-catalyzed oxidation of aromatic acids. The Lowry method is sensitive to pH changes and therefore the pH of assay solution should be maintained at 10 - 10.5. The Lowry method is sensitive to low concentrations of protein. Some researchers suggested the concentrations ranging from 0.10 - 2 mg of protein per ml, while others suggested the concentrations ranging from 0.005 - 0.10 mg of protein per ml. The major disadvantage of the Lowry method is the narrow pH range within which it is accurate. However, for very small volumes of samples, there will be little or no effect on pH of the reaction mixture. A variety of compounds will interfere with the Lowry procedure. These include some amino acid derivatives, certain buffers, drugs, lipids, sugars, salts, nucleic acids and sulphydryl reagents (Matulis, *et. al.*,1995).

Methodology:

(a) Reagents: A; 2% Na_2CO_3 in 0.1 N NaOH, B; 1% NaK Tartrate in H_2O, C; 0.5% $CuSO_4.5\ H_2O$ in H_2O.

Reagent I: 48 ml of A, 1 ml of B & 1 ml of C, Reagent II: 1-part Folin-Phenol [2 N]: 1 part water

(b) Materials required:

(i) Equipment's: UV Spectrophotometer, Water Bath.

(ii) Glass wares: Test-tube, Test-tube stand, Pipettes, Beaker, Tissue paper, Wash bottle.

BSA Standard - 5 mg/ 10 ml

(c) Procedure:

1.) 0.2 ml of BSA working standard in 5 test tubes and made up to 1 ml using distilled water.

2.) The test tube with 1 ml distilled water was used as the blank.

3.) Added 4.5 ml of Reagent I to each test tube and incubated for 10 minutes.

4.) After incubation added 0.5 ml of Reagent II and incubated for 30 minutes.

5.) Then measured the absorbance at 660 nm.

(d) Calculation: Determined the amount of protein in the plant sample by plotting the value obtained at A660 at the standard curve with absorbance on Y-axis and concentration of BSA on X-axis (Price, *et. al*.,1999).

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RESULT

Genomic DNA Isolation

Lemna minor samples from five natural populations were used to extract genomic DNA. Table 7 provides information on the DNA samples concentration and purity. Plate 3 shows how these isolates were qualitatively estimated.

Sl. No.	Plant ID	Concentration of Genomic DNA (ng/µl)	Purity (A260/A280)
1	LE-01	276	1.70
2	LE-02	325	1.78
3	LE-03	397	1.73
4	LE-04	202	1.67
5	LE-05	542	1.81

Table 7: Quantitative Estimation of Genomic DNA of *L. minor*

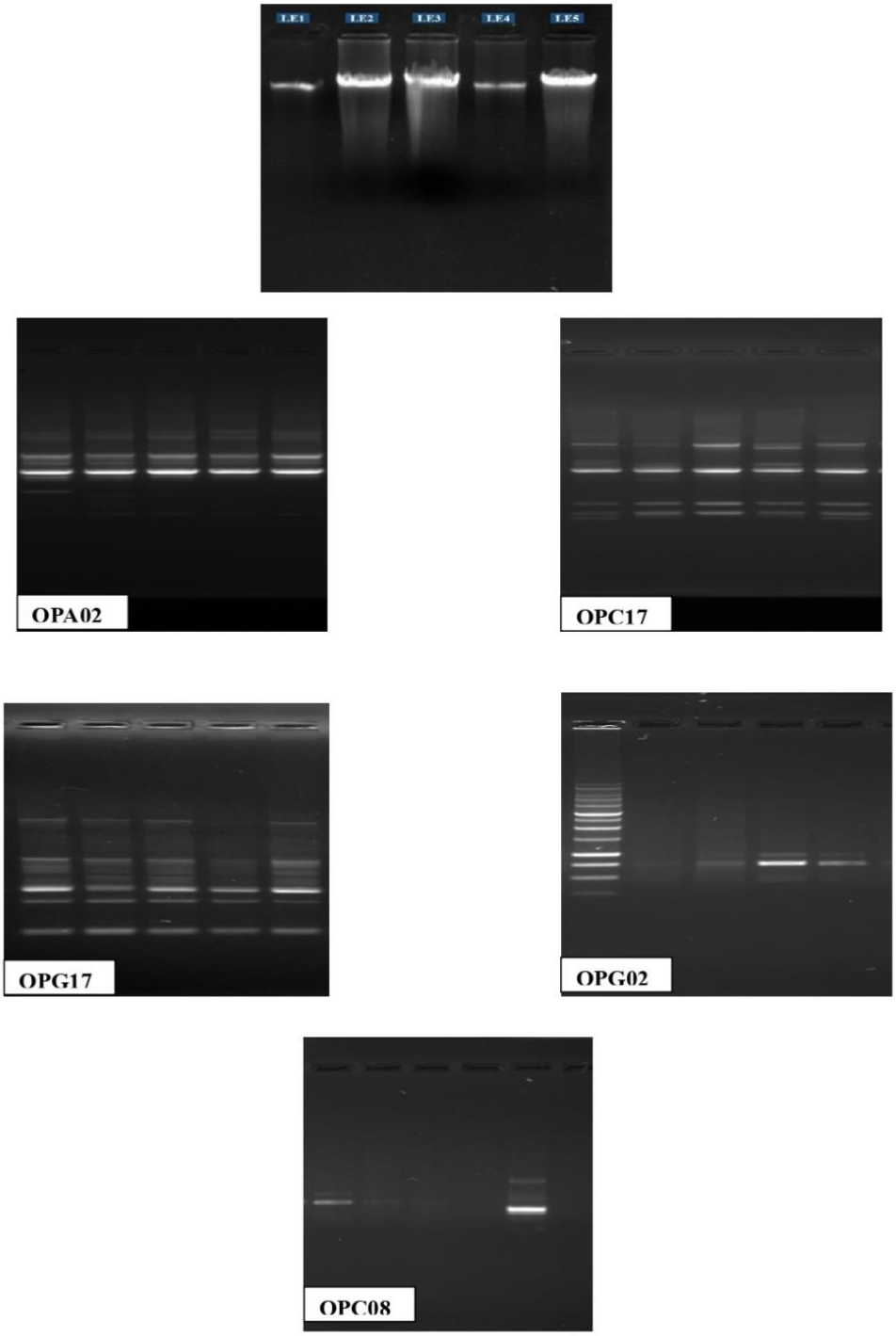

Plate 3: Gel image of genomic DNA of *L. minor*

RAPD BAND ANALYSIS

The current study examined the genetic diversity of *Lemna minor* populations in different locations of Kerala. Using RAPD primers, samples from five populations were amplified. Populations were clearly divided into several clusters according to the banding pattern produced by RAPD primers, demonstrating genetic variation among them. With RAPD, diversity estimates offer helpful information for comprehending the genetic structure of the plant. Five RAPD primers were used in this investigation to test pooled DNA accessions of *Lemna minor* for polymorphisms.

Using five RAPD primers, the PCR analysis of five research samples produced 47 scoreable bands. Each primer produced 9.4 bands on average. OPC17 and OPG02 yielded the most bands (14) out of the five RAPD primers, while OPC08 (4) yielded the fewest bands. OPA02 and OPG17 primers yielded 5 and 10 bands, respectively.

RAPD Primer	**Number of bands**	**No. of Polymorphic bands**	**Percentage of Polymorphism**	**PIC**	**EMR**	**MI**
OPA02	05	05	100%	0.318	25	7.965
OPC17	14	11	78.5 %	0.239	154	36.806
OPG17	10	10	100 %	0.361	100	36.1
OPG02	14	14	100 %	0.24	196	47.04
OPC08	04	02	50 %	0.21	8	1.686
Total	47	42	428.5 %	1.368	483	129.597
Average	9.4	8.4	85.7%	0.273	96.6	25.919

Table 8: Data for RAPD primers used for analyzing 05 accessions of *L. minor*

42 of the 47 scoring bands have been found to be polymorphic, and 8.4 was the average number of polymorphic bands. It was found that OPG02, OPC17, and OPG17 had more polymorphism bands than OPA02 and OPC08, which had 05 and 02 polymorphic bands, respectively. OPA02, OPG17, and OPG02 had high levels of polymorphism (100%) while OPC08 had the lowest amount of polymorphism (50%). It turned out that the average degree of polymorphism was 85.7%.

The primers yielded PIC (Polymorphism Information Content) values ranging from 0.21 (OPC08) to 0.361 (OPG17), with an average of 0.273 across all primers. Given that the PIC value of OPG17 was closest to 0.361, it was determined that this primer would be more useful for research in this species. The number of polymorphic bands and the total number of bands were multiplied to get the Effective Multiplex Ratio (EMR), which was highest for primer OPG02 (196) and lowest for primer OPC08 (8). EMR and PIC were multiplied to determine the marker index. With a value of 47.04, RAPD primer OPG02 exhibits the greatest marker index, while primer OPC08 displays the lowest (1.686). (Table 8).

CLUSTER ANALYSIS

In Figure 1, a dendrogram produced by the RAPD primer cluster analysed using UPGMA (Unweighted Paired Group Method using Arithmetic Averages) software displayed. Based on Jaccard's coefficient of genetic similarity, the dendrogram was created. Entire genotype was divided into two main clusters in this dendrogram: CLUSTER 1 and CLUSTER 2. There were four populations in the first cluster: LE1, LE2, LE4, and LE5. With populations LE2, LE4, and LE5, the first cluster split into two clades, while the second clade further split into two. There was only one population of LE3 in the second cluster.

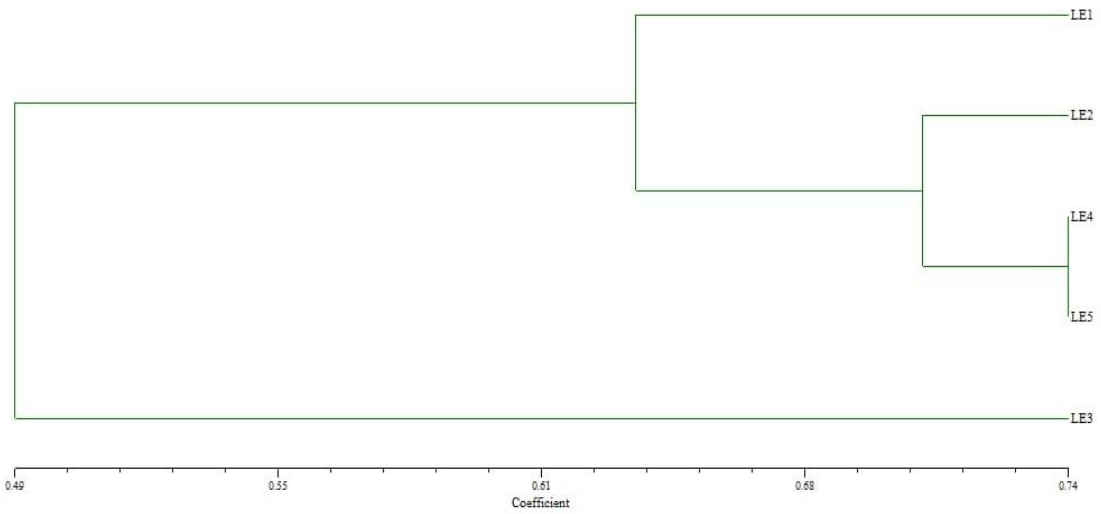

Figure 1: Dendrogram based on Jaccards coefficient of genetic similarity (UPGMA) showing genetic distance

Rows/ Columns	LE 1	LE 2	LE 3	LE 4	LE 5
LE 1	1.0000000				
LE 2	0.6571429	1.0000000			
LE 3	0.4285714	0.4444444	1.0000000		
LE 4	0.6315789	0.6842105	0.5555556	1.0000000	
LE 5	0.6216216	0.7222222	0.5428571	0.7368421	1.0000000

Table 9: Similarity index table showing Jaccards coefficient of genetic similarity

The genetic similarity coefficient (Jaccard's coefficient, J) of five populations of *L. minor*, were determined by cluster analysis, they varies from 0.428 to 0.736. With

a Jaccard value of genetic similarity of 0.736, populations 4 and 5 stand out, since they had the highest degree of similarity, and indicated a tight genetic affinity. Likewise, population 2 has high degree of genetic resemblance to population 5, can be seen by their strong genetic link (Jaccard coefficient of 0.722). Populations 1 and 3, on the other hand, have different genetic profiles from the others and, in the study, form a unique cluster. The similarity between populations 1 and 3 was still quite low despite their divergence; a Jaccard's coefficient of 0.428 suggested that they are less genetically connected to populations 2, 5, and 4. Here, only a moderate similarity was seen among these five *L. minor* populations.

The populations LE4 (collected from Panangad, Ernakulam) and LE5 (collected from Malabar Botanical Garden, Kozhikode) showed the most similarity, according to the similarity index analysis. The value of the Jaccards similarity index of these populations can be used to understand the genetic diversity. The populations LE4 and LE5 had the highest similarity score of 0.736. A genetic diversity value of 0.264 (1-0.736) was calculated between these two populations, which was found in the manner by subtracting the value of similarity index between the populations from one (where 1 is the maximum value of the Jaccard similarity index). Comparably, the populations LE1 and LE3, which displayed the least similarity, had a genetic diversity score of 0.572. Consequently, among these groups, the populations LE1 (collected from Changanassery, Kottayam) and LE3 (collected from Chenthrapini, Thrissur) demonstrated the most diversity.

NUTRITIONAL ANALYSIS

Carbohydrate Estimation:

The amount of glucose in the *L. minor* sample was determined by plotting the value obtained by colorimetric or spectrophotometric method at A620 on a standard curve with absorbance (OD value) on Y-axis and concentration of glucose (µg of Glucose) on X-axis.

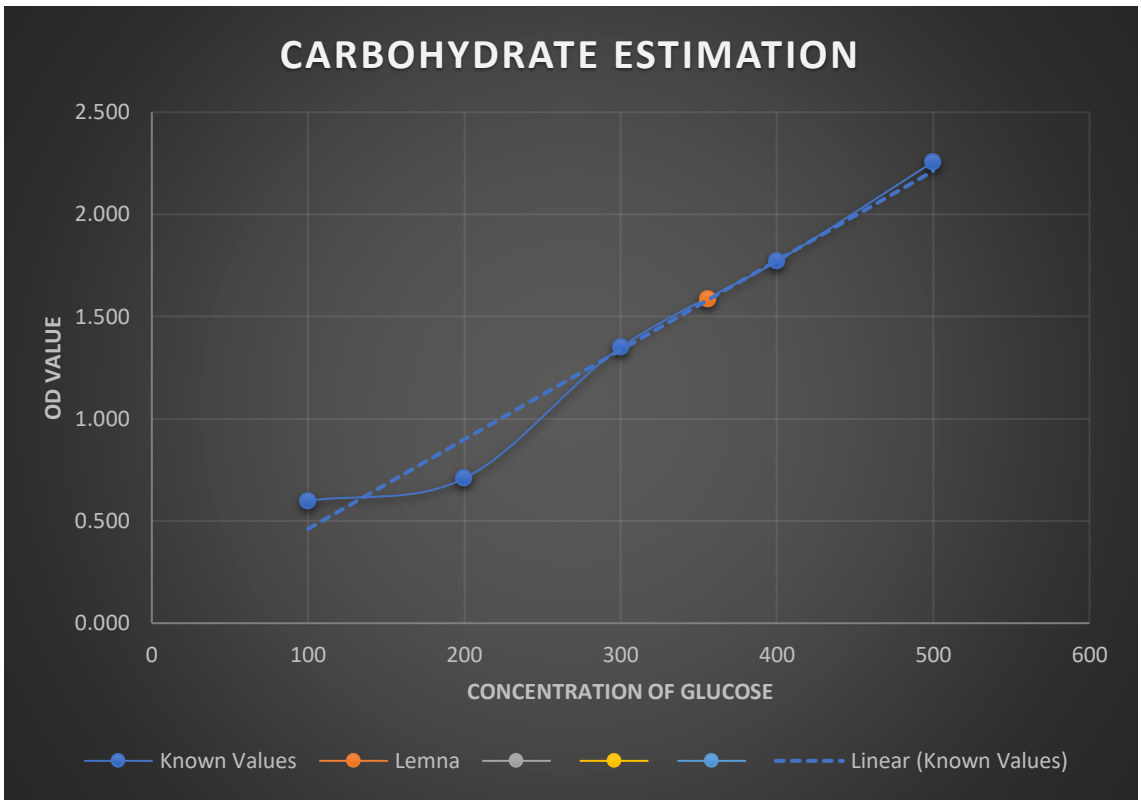

Figure 2: Graph of carbohydrate estimation

RESULT: The concentration of carbohydrate was estimated as 1 gm in 3.5 mg/gm of plant sample.

Protein Estimation:

The amount of protein present in the *L. minor* sample was estimated from the standard graph of protein with concentration of BSA (µg) on X axis & absorbance (OD value) at Y axis, by plotting the value observed at A660 by colorimetric or spectrophotometric analysis.

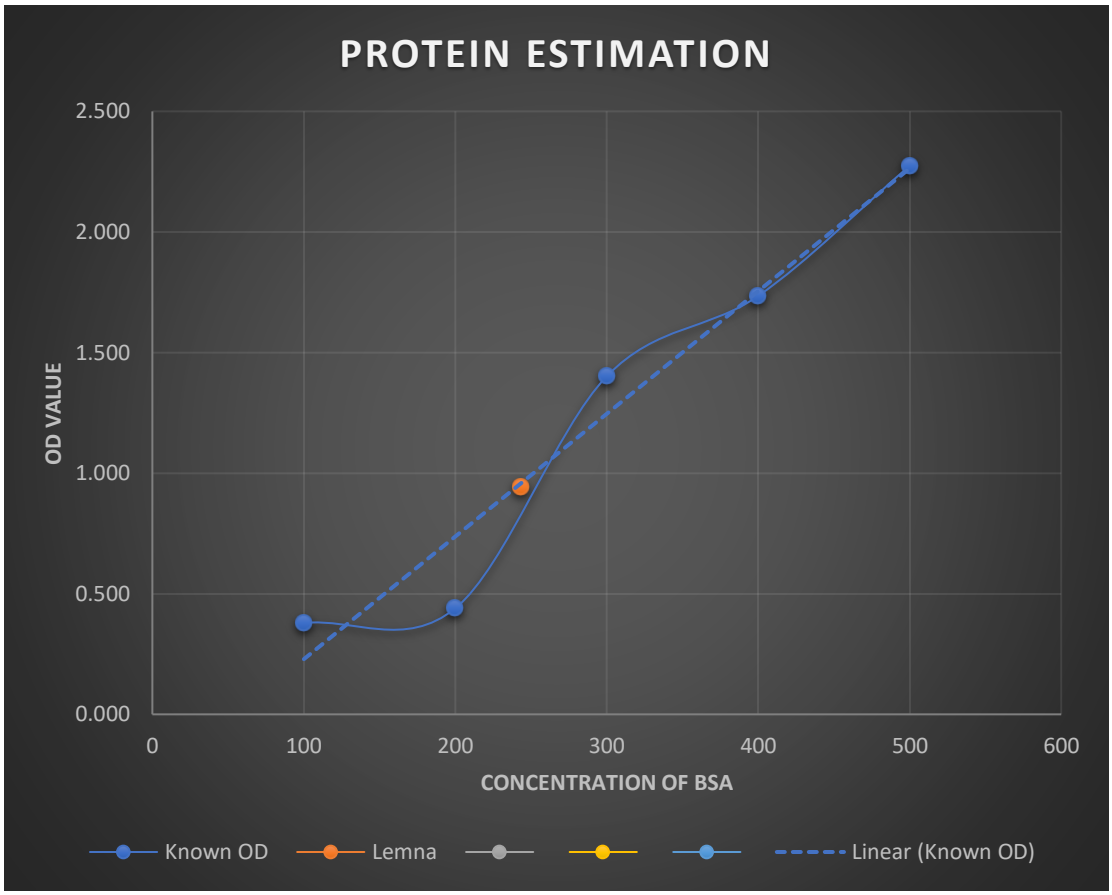

Figure 3: Graph of protein estimation

RESULT: The concentration of protein was estimated from the graph as 1 gm in 2.4 mg/gm of the plant sample.

DISCUSSION

Lemna minor holds significant importance due to its versatile properties. It serves as a potent phytoremediator, effectively removing pollutants like methylene blue from wastewater (Muhammad, *et al*., 2021). Additionally, *Lemna minor* is rich in essential nutrients and secondary metabolites, making it a valuable source of alternative food and potential ingredient for herbal medicines and cosmetics. Studies have shown that the plant interacts closely with microbial organisms, impacting its fitness and phenotype in varying environmental conditions. Furthermore, *Lemna minor* demonstrates high potential for wastewater treatment, as seen in its cultivation on dairy processing wastewater, offering a sustainable approach for both wastewater remediation and biomass generation (Mahoney, *et al*., 2022). Overall, Lemna minor's phytoremediation capabilities, nutritional value, microbial interactions, and wastewater treatment potential underscore its significance in environmental, health, and economic contexts.

Lemna minor holds significance due to its genetic diversity, impacting various aspects. Studies revealed that *L. minor* populations exhibit intraspecific genetic differences, with natural populations consisted of multiple distinct clones (Senevirathna *et al*., 2023). This genetic diversity was crucial for the species adaptability to different environmental conditions, aided in its successful invasion of new ecosystems (Paolacci *et al*., 2021). Furthermore, the genetic structure of *L. minor* has been shown to vary significantly from another cryptic duckweed species, *L. turionifera*, emphasized the importance of monitoring *Lemna* species where they co-occur (Bog *et al*., 2022). Additionally, the genome sequencing of *L. minor* has provided insights into its biological processes and potential applications in biomass production, highlighting its importance in various fields such as agriculture, phytoremediation, and energy production (Gürer & Yapar, 2022).

Genetic diversity assessment was crucial for understanding the genetic makeup and variability within a species. By using RAPD molecular markers, researchers

analysed the DNA of different individuals within a population of *Lemna minor* and identified unique genetic variations (Muhammad *et al*., 2016). This information provided insights into the evolutionary history, population structure, and potential adaptations of *Lemna minor*. Additionally, genetic diversity assessment aided in conservation efforts by identifying populations with higher genetic variability, which can be more resistant to environmental stressors or better able to adapt to changing conditions. RAPD molecular markers have been widely used for genetic diversity assessment in various species, including *Lemna minor*. These markers were simple and cost-effective to be used, making them accessible for researchers studying genetic diversity in smaller or less-studied species (Varshney *et al*., 2006). Furthermore, the use of RAPD molecular markers allowed for the detection of genetic polymorphisms and the assessment of allelic diversity within populations of *Lemna minor* (Soriano, 2020). This information was valuable for understanding the overall genetic health of a population and the conservation strategies to ensure the long-term survival of *Lemna minor* can be identified. In conclusion, the utilization of RAPD molecular markers for genetic diversity assessment in *Lemna minor* was an important tool, helped in understanding the species genetic variability and its implications for its evolutionary trajectory, population dynamics, and conservation strategies (Maniruzzaman *et al*., 1970). This method provided valuable insights into the genetic structure and diversity of the *Lemna minor* population, which aided in conservation efforts and developing breeding programs (Varshney *et al*., 2006). In conclusion, the usage of RAPD molecular markers in genetic diversity assessment of *Lemna minor* allows researchers to analyse the DNA of individuals within a population and identify unique genetic variations, ultimately providing valuable insights into the species evolutionary history, population structure, and potential adaptations. Additionally, this information can be used in conservation efforts to identify populations with higher genetic variability that may be more resilient to environmental stressors (Muhammad *et al*., 2016).

An examination of the genetic diversity of five populations of *L. minor* plant species was conducted in this study. These five plant populations were collected from various parts of Kerala, including the districts of Kottayam, Kozhikode, Alappuzha,

Thrissur, and Ernakulam. Five RAPD primers were used to evaluate their diversity: OPA02, OPC17, OPG17, OPG02, and OPC08. These accessions showed a limited degree of diversity, according to a dendrogram made using the band analysis of the samples and cluster analysis. The dendrogram showed two major clusters: cluster 1 contained the clade-divided populations LE1, LE2, LE4, and LE5, whereas cluster 2 contained the population LE3. The populations LE4 & LE5 have the highest degree of similarity, according to the Jaccards coefficient similarity study. However, there was a lot of variances in the populations LE1 and LE3. The similarity analysis of *L. minor* from multiple locations in Kerala revealed a small diversity and only moderate resemblance between them.

The scope of understanding genetic diversity between *Lemna minor* species encompasses the study of genetic variation and its implications for the species biology and ecology, with applications to conservation, research, and bioremediation. **Ecological Interactions**; understanding how different *Lemna minor* populations interact with their environment and other species is important. For instance, adaptability to various contaminants, as indicated by their ability to accumulate substances like uranium, could vary between populations (Favas *et al*., 2014). **Conservation Biology**; assessing genetic diversity can guide conservation strategies to maintain or enhance the genetic health of populations. **Phytoremediation**; since *Lemna minor* shows potential in uranium accumulation for phytoremediation, understanding genetic diversity could optimize this process by identifying and breeding the most efficient accumulators (Favas *et al*., 2014). **Phylogenetics** and **Evolutionary Studies**; genetic analysis can shed light on the evolutionary relationships within and between species, helping to understand their historical development and adaptive strategies. **Agricultural** and **Horticultural Research**; for species related to or cross-compatible with *Lemna minor*, genetic diversity studies might inform breeding programs for desirable traits, including disease resistance. As an analogy, in eggplants, genetic mapping is used to identify resistance to bacterial wilt caused by *Ralstonia solanacearum* (Huet, 2014). **Toxicity** and **Allelopathy**; different genotypes within *Lemna minor* may respond differently to toxins, as observed

in studies where plant extracts affected the growth of *Lemna* plants in varying ways (Ali *et al*., 2005).

In summary, understanding the genetic diversity between *Lemna minor* species is crucial to fully appreciate the species ecological functions, conservation needs, agricultural potential, and evolutionary history.

Quantitative nutritional analysis of *Lemna minor* reveals its potential as a valuable feed source. *Lemna minor* growth rate peaks at pH 7, with the highest protein content at pH 7 and 8, while lipids are abundant at pH 9 and 10 (Zenir *et al*., 2022). The plants nutritional quality was influenced by varying nutrient concentrations, with higher protein, lipid, and carbohydrate contents observed at specific N and P levels (Ullah *et al*., 2022). Additionally, *Lemna minor* contained great amount of essential minerals like Ca, Mg, Fe, Mn, Zn, and trace elements, making it a promising source for animal feed (Zhang *et al*., 2023 & Ullah, *et al*., 2022). Furthermore, the plant contained flavonoids like apigenin and luteolin-7-O-glucoside, contributed to its medicinal potential (Devlamynck *et al*., 2021). Overall, *Lemna minor*'s nutritional profile underscores its significance as a sustainable and nutrient-rich alternative feed source for livestock and fishery industries.

This study quantified the amount of carbohydrates and protein in *Lemna minor* species. The Lowrys method was used to estimate the protein, while the Anthrone method was used to determine the carbohydrates. Plotting the absorption value of the sample at A620 on the standard carbohydrate graph curve, allowed for the determination of the amount of carbohydrate. Similar to this, the amount of protein was calculated by charting the samples absorbance at A660 on the protein standard graph. The plant sample was found to contain a concentration of 1gm of carbohydrate in 3.5 mg/gm of plant sample. On the other hand, a protein concentration of 1 gram in 2.4 mg/gm of plant sample was discovered. It was turned out that the *Lemna minor* plant sample had a high protein and carbohydrate content, making it appropriate for use as fodder or for other suitable applications.

The scope of studying the nutritional analysis of *Lemna minor* encompasses various aspects, including its potential as a sustainable food source, its nutritional value

for both human and animal consumption, and its role in addressing food security and environmental sustainability challenges. **Nutritional composition**; researches focused on determining the precise nutritional composition of *Lemna minor*, including its protein, carbohydrate, lipid, vitamin, mineral, and fiber contents. This analysis provided insights into its potential as a nutritious food source (Ji & Stomp, 2009). **Human nutrition**; studies investigated the suitability of *Lemna minor* as a dietary supplement or functional food for humans due to its high protein content and nutritional profile (Sree *et al*., 2019). **Animal feed**; researchers examined the potential of *Lemna minor* as a feed ingredient for livestock, poultry, and aquaculture due to its high protein content and nutritional quality. **Nutritional recovery**; studies explored the use of *Lemna minor* in wastewater treatment systems for nutrient removal and recovery, thereby improving water quality and mitigating environmental pollution (Äzim & Little, 2008). **Environmental sustainability;** researchers investigated the environmental benefits of cultivating *Lemna minor*, such as its ability to sequester carbon, reduce nutrient runoff, and provide habitat for aquatic organisms.

By studying the nutritional analysis of *Lemna minor* within these contexts aim to contribute to sustainable food production, environmental protection, and public health improvement.

SUMMARY & CONCLUSION

This study concentrated on *Lemna*, a small aquatic angiosperm that is also referred to as duckweed. *Lemna minor* L., one of its common species, was the subject of the inquiry. An examination of the plants nutritional and genetic diversity was conducted. Five populations of *L. minor* plant species were gathered from various localities of Kerala for the genetic diversity research. The districts of Kottayam, Kozhikode, Alappuzha, Thrissur, and Ernakulam were among the places. After their DNA was isolated, five RAPD primers were employed to assess their diversity. OPA02, OPC17, OPG17, OPG02, and OPC08 were the primers used. Followed by the primer hybridization, gel electrophoresis was conducted to separate the DNA, and the gel documentation method was used to visualize the DNA bands. Number of polymorphic and non-polymorphic bands were identified via the band image analysis. Then computed their PIC, EMR, and MI values.

According to the results, 42 out of the 47 scoring bands exhibited polymorphism. On the basis of the band analysis, a dendrogram was then made. Two main clusters were visible in the dendrogram: cluster 1 held the clade-divided populations LE1, LE2, LE4, and LE5, whereas cluster 2 held the population LE3. Based on the band analysis of the samples and cluster analysis, it was indicated that these accessions showed a small degree of diversity.

The similarity analysis used the Jaccard coefficient approach. This revealed the populations with the highest degree of resemblance as LE4 and LE5. On the other hand, there was significant differences between the populations LE1 and LE3. There was only moderate similarity and little degree of variation among the *L. minor* samples collected from different parts of Kerala in this study.

The similarity index study provided that the populations with the greatest degree of resemblance as LE4 (collected from Panangad, Ernakulam) and LE5 (collected from Malabar Botanical Garden, Kozhikode). The populations LE4 & LE5 showed the lowest genetic diversity with value of 0.264. The populations LE1

(collected from Changanassery, Kottayam) and LE3 (collected from Chenthrapini, Thrissur) showed greatest dissimilarity. These two populations showed the greatest diversity with a value of 0.572.

The dendrogram created utilizing the band analysis of the samples and cluster analysis revealed a limited degree of diversity among these five accessions of *Lemna minor* L. from various sites in Kerala.

Lemna is a nutrient rich plant. This aquatic plant has the nutrients such as, protein, fiber, lipids, carbohydrates, vitamins (including A, C, and K), minerals including both micro & macro (like calcium, iron, and magnesium), and antioxidants. The nutritional value of this plant and its possible uses in the food industry can be better understood by the quantitative examination of these components. The principal nutrient components that underwent quantitative investigation in this study were protein and carbohydrates. The amount of protein and carbohydrates in *Lemna minor* species were measured in this study. The protein was estimated using the Lowrys method, while the carbohydrates were estimated using the Anthrone method.

These techniques often entailed extracting the plant sample and using a spectrophotometer to measure the absorbance at the appropriate wavelength. The amount of carbohydrates was calculated by plotting the samples absorption value at point A620 on the standard carbohydrate graph curve. Likewise, the protein content was determined by graphing the absorbance of the sample at A660 on the protein standard graph. The amount of carbohydrates in the plant sample was found out to be 1 gram in 3.5 mg/gm of plant sample. However, it was found that the protein concentration was 1 gram in 2.4 mg/gm of plant sample. It was turned out that the *Lemna minor* plant sample was suitable for use as fodder or other similar applications due to its high protein and carbohydrate content.

A quantitative analysis of the protein and carbohydrate content of the plant as well as an understanding of all other nutrient types present in the same plant by investigating with the available datas allowed for new insights into the plant's nutritional quality and potential use as a source of food for aquatic animals.

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