International Journal of Science, Environment and Technology, Vol. 14, No 4, 2025, 424 – 432
ISSN 2278-3687 (O)
2277-663X (P)

PRELIMINARY ASSESSMENT OF GENETIC HOMOGENEITY IN INVASIVE WATER HYACINTH USING THE *rbcL* MARKER

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Abstract: Water hyacinth (*Pontederia crassipes*) is one of the most invasive aquatic plant species, having been introduced to India in 1896 as an ornamental plant. Despite its significant ecological impact, research on its genetic variation has been limited. This preliminary study was conducted in Powai Lake, Mumbai, from September 2024 to January 2025 to assess the genetic variation of water hyacinth using the *rbcL* marker. Four samples were collected from various shoreline sites and categorised into two morphotypes based on morphological characteristics. DNA extraction, PCR amplification, and sequencing were conducted, and pairwise genetic distances were calculated using the Maximum Composite Likelihood model. A phylogenetic tree was constructed employing the Maximum Likelihood method with 1,000 bootstrap replicates, and Analysis of Molecular Variance (AMOVA) was performed using Arlequin software. The findings reveal minimal genetic variation among the water hyacinth populations in Powai Lake, likely attributed to clonal reproduction. This observed genetic uniformity suggests that a single chemical or biological control strategy may suffice for effective management of the infestation in Powai Lake.
Keywords: AMOVA, Clonal reproduction, Genetic variation, Powai Lake, *RbcL*, Water hyacinth

Introduction

Water hyacinth (*Pontederia crassipes),* formerly known as *Eichhornia crassipes*, is a major invasive aquatic weed in tropical and subtropical water bodies. Native to the Amazon, it has spread across Latin America, the Caribbean, Africa, Southeast Asia, and the Pacific (Jafari, 2010). According to Wilson *et al*. (2005), *P. crassipes* was introduced worldwide during the late 19th and early 20th centuries. In India, water hyacinth has become a significant ecological concern, rapidly colonising freshwater bodies, although its exact introduction date remains undocumented to date.

Powai Lake (19°08′N, 72°54′E), located in suburban Mumbai, spans approximately 2,100 hectares, with depths ranging from 3 meters at the periphery to 12 meters at its deepest point. Constructed in 1891 by the Mumbai Municipality, the lake was initially designed as a drinking water reservoir (Surya S. *et al*., 2018). The condition of Powai Lake has been deteriorating for

*Received July 24, 2025 * Published Aug 2, 2025 * www.ijset.net*

a long time, making its water unsuitable for drinking. Mitter C. (2024) and Surya S. *et al*. (2018) studied the water quality parameters of Powai Lake, and their findings indicate that most of the parameters exceed permissible levels. Mitter C (2024) reported that the Biochemical Oxygen Demand (BOD) of the water in Powai Lake is 46.45 ± 3.4875 mg/litre. Such a high BOD suggests a large amount of organic matter in the lake, which promotes the rapid growth of water hyacinth (*P. crassipes*).

Water hyacinth is found abundantly throughout the year in Powai Lake. It exhibits distinct morphological variations depending on environmental conditions. In isolated conditions, it forms swollen petiole bases (floats) and kidney-shaped lamellae. In contrast, when forming dense mats, the plants develop long, straight petioles without floats and rounded lamellae. When rooted in the substrate, they display a similar morphology (Olive, 1894). In this study, the former is referred to as Morph A, and the latter is Morph B. Water hyacinths form dense mats on the water surface, which has a higher impact on the ecosystem. Fewer plants in a lake, pond, or river may enhance fish production, but when they form a closed mat, they severely impact aquatic ecosystems by blocking sunlight, depleting dissolved oxygen, and causing hypoxia, leading to fish kills and biodiversity loss (Gowanloch, 1944).

Even though it is widely distributed, genetic studies on water hyacinth remain limited. Research by Da Cunha *et al*. (2022) in Brazil using the Genotype-By-Sequencing (GBS) technique found that *P. crassipes* exhibited a lower average pairwise genotypic distance compared to *Pontederia azurea*, indicating lower genetic variation. Additionally, the inbreeding coefficient was more negative in *P. crassipes*, suggesting populations exhibit greater heterozygosity, possibly due to frequent clonal reproduction than in *P. azurea*.

A study by Ren *et al*. (2005) investigating the genetic variation of *P. crassipes* populations in China using RAPD markers revealed a low mean Nei's gene diversity of 0.046 ± 0.0145. The Analysis of Molecular Variance (AMOVA) indicated that 83.9% of the genetic variation exists within populations, while 88.1% is distributed within localities, highlighting substantial genetic diversity at the local level despite overall low variation.

Zhang *et al*. (2010) performed an extensive population genetic survey of water hyacinth (*P. crassipes*) using AFLP markers. They gathered samples from both the native range in South America and various introduced regions, including Asia, Africa, Europe, North America, Central America, and the Caribbean. Their findings revealed a total of 49 distinct clones. Notably, they observed that the introduced populations displayed significantly lower genetic

diversity when compared to those in the native range, with approximately 80% of the introduced populations being dominated by a single clone.

While there have been some global studies on genetic variation in water hyacinths, there is a significant gap in research specifically focusing on this topic in India. The genetic variation within invasive species like *P. crassipes* plays a crucial role in their adaptability and ecological success. Despite their widespread distribution, previous studies indicate that water hyacinth populations typically exhibit low genetic variation due to their clonal reproduction. However, occasional genetic variation can arise from mutations or rare instances of sexual reproduction. Investigating the genetic structure of water hyacinth populations in Powai Lake could provide valuable insights into their modes of spread, adaptability, and potential management strategies. This study aims to analyse the genetic variation of water hyacinth in Powai Lake using the *rbcL* gene as a molecular marker. The *rbcL* is a chloroplast gene that encodes the large subunit of Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase (RuBisCO). It is widely utilised in phylogenetic and genetic variation studies due to its presence in all plants and its moderate evolutionary rate (Patwardhan *et al*., 2014). Additionally, it examines the genetic relationship between two observed morphs: Morph A (rounded lamella, long petiole without float) and Morph B (kidney-shaped lamella, short petiole with float), offering insights into their taxonomic distinction and evolutionary linkage.

Materials and Methods

Sample Collection

The current study was undertaken between September 2024 and January 2025, focusing on the diverse ecosystem of Powai Lake. Samples were collected from the shore of the water body. Selected plants were photographed to capture taxonomically important features of the plant, like flowers and leaves. Pieces of the apical meristematic tissue or young leaves were taken by clean forceps and were preserved in 95% ethanol in a 2 mL microcentrifuge tube (Axygen Biosciences, Central Avenue, Union City, USA). Photographs and tissue samples were labelled following a strategic method to track them during laboratory processing. Alcohol used in the preservation of tissues was changed after 24 hours with a fresh one. All samples were brought to the laboratory, and tissue samples were stored in a refrigerator at -20^0C.

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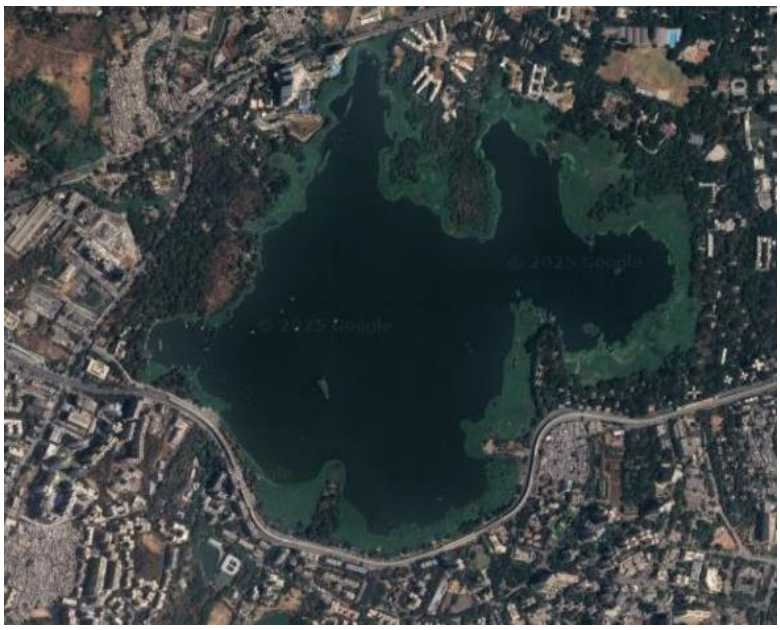
Fig 1. Satellite image of Powai Lake (image source – Google Maps)



DNA isolation, PCR and Sequencing

Genomic DNA was extracted from the stored samples using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). DNA quantification was performed using a NanoDrop ND1000 spectrophotometer. The DNA was diluted to a final concentration of 200 ng/µl for further use. A portion of the *rbcL* gene was amplified in a 25 µL reaction volume containing 2 µL of 250 µM dNTP, 0.5 µL 25 mM $MgCl_2$, 0.5 µL 10 pM primers *rbcL*a-F - ATGTCACCACAAACAGAGACTAAAGC and *rbcL*a-R-GTAAAATCAAGTCCACCRCG each, 1U *Taq* Polymerase (Kapa Biosystems, USA) using a T100 Thermal Cycler (Bio-Rad Laboratories). All PCR products were visualized on 1.2% agarose gel. Selected samples were used for bidirectional sequencing using a Sanger sequencing platform (ABI 3730xl genetic analyzer, Applied Biosystems, Foster City, USA).

Genetic Analysis

Sequence alignment and assembly were carried out using CodonCode Aligner v12.0.1 (CodonCode Corporation). Nucleotide sequences were aligned and submitted to BOLD under project code WHPL (Process ID WHPL001-25 to WHPL004-25) and to GenBank with accession numbers (Accession No. PV158398 to PV158401). ECPL1 and ECPL2 were classified as Morph-A (characterised by the absence of floats, long petioles, and rounded

lamella), while ECPL3 and ECPL4 were classified as Morph-B (characterised by the presence of floats, short petioles, and kidney-shaped lamella).

The evolutionary history was inferred using the Maximum Likelihood method with the Tamura-Nei model (Tamura & Nei, 1993). The percentage of trees in which the associated taxa clustered together is indicated next to the branches. Initial tree(s) for the heuristic search were automatically generated using the Neighbor-Joining and BioNJ algorithms, based on a pairwise distance matrix estimated under the Tamura-Nei model. The topology with the highest log-likelihood value (-837.01) was selected.

These analyses included four nucleotide sequences, considering codon positions 1st, 2nd, 3rd, and non-coding regions. The final dataset comprised 580 positions. Evolutionary analyses were conducted using MEGA11 (Tamura *et al*., 2021). A locus-by-locus AMOVA was performed for 63 polymorphic loci using Arlequin v3.5.2.2 (Excoffier & Lischer, 2010).

Result and Discussion

Upon alignment, the analysis revealed 574 conserved sites and 3 variable sites from a total of 580 loci (aligned sequences are given in supplementary data). The nucleotide composition consisted of 30.3% thymine (T), 19.4% cytosine (C), 27.7% adenine (A), and 22.6% guanine (G).

Pairwise distances are shown in Table 1. The pairwise genetic distance results reveal a clear genetic differentiation pattern among the samples. ECPL1 is genetically distinct from the other samples, with a pairwise genetic distance of 0.0058 ± 0.0034 when compared to ECPL2, ECPL3, and ECPL4. This suggests that ECPL1 may represent a separate genetic lineage. ECPL2, ECPL3, and ECPL4 have a genetic distance of zero, indicating that they are genetically identical (or nearly identical). This suggests that these three samples belong to the same genetic group or clonal lineage with no observed genetic variation among them. The zero genetic distance between ECPL2, ECPL3, and ECPL4 suggests that they may have originated from the same population.

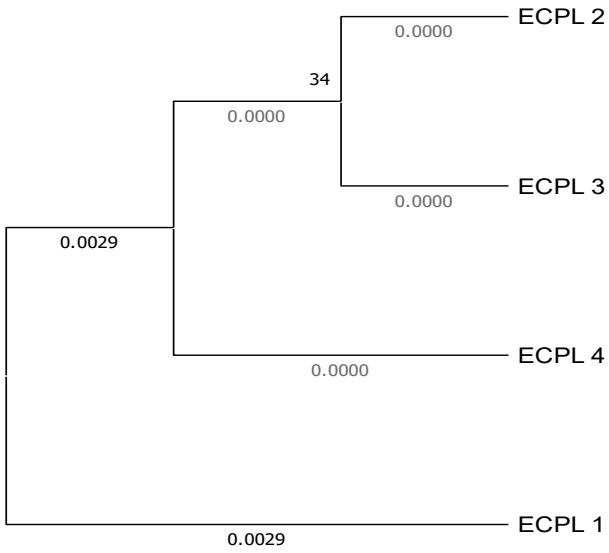
Table 1. Pairwise distances

Sample 1	Sample 2	Distance	Std. Error
ECPL1	ECPL2	0.0058	0.0034
ECPL1	ECPL3	0.0058	0.0034
ECPL2	ECPL3	0.0000	0.0000

ECPL1	ECPL4	0.0058	0.0034
ECPL2	ECPL4	0.0000	0.0000
ECPL3	ECPL4	0.0000	0.0000

The phylogenetic tree confirms the genetic distance results. ECPL3 and ECPL4 cluster together with a bootstrap value of 34 and a branch distance of zero, meaning they are genetically indistinguishable. ECPL2 is also grouped with this cluster, again with a branch distance of zero, further supporting their genetic similarity. ECPL1 is positioned separately from the other samples, with a branch distance of 0.029, reinforcing its distinct genetic divergence. This phylogenetic relationship suggests that ECPL1 is an outlier or has undergone separate evolutionary changes compared to the ECPL2-ECPL3-ECPL4 group.

Fig. 2 Phylogenetic tree



Note: Bootstrap values and branch length are given above and below the branch, respectively.

The AMOVA results provide a statistical measure of genetic variation within and among populations. The AMOVA results are presented in Table 2. The variance among populations is zero, meaning there is no significant genetic differentiation between Morph A and Morph B. This is likely because ECPL2, ECPL3, and ECPL4 are genetically identical, and the only source of genetic variation comes from within the population. The variance within populations is 100%, indicating that all observed genetic variation comes from differences within a single genetic pool rather than between separate groups. The Fixation Index (F_{SI}) is 0.0000, with a p-

value of 1.0000, further confirming that there is no significant genetic structuring among the samples.

Table 2. Results of Analysis of Molecular Variance (AMOVA)

Source of Variation	Sum of Squares	Variance Components	Percentage Variation
Among Populations	15.7500	0.0000	0.0000
Within Populations	31.5000	15.7500	100.0000
Total	47.2500	15.75000	

This study confirms that the population of *P. crassipes* (water hyacinth) in Powai Lake exhibits low genetic variation. The results are generally consistent with the findings of Da Cunha *et al*. (2022), Ren *et al*. (2005), and Zhang *et al.* (2010). The observed low genetic variation suggests that a uniform response to control methods may be expected, potentially increasing the effectiveness of a single chemical or biological control strategy. However, further studies on environmental factors and resistance mechanisms are necessary before implementing such methods. This research lays the groundwork for developing molecular approaches to effectively manage water hyacinths. Further studies can explore molecular methods for controlling water hyacinth infestations in aquatic environments.

Our findings reveal a striking genetic homogeneity among the water hyacinth populations sampled from Powai Lake, as indicated by the rbcL marker. However, we must remain mindful of potential genetic heterogeneity across different regions of the lake. Water hyacinth is renowned for its clonal propagation, yet localised environmental pressures or multiple introduction events could give rise to subtle genetic variations. To uncover such fine-scale genetic structures, future research should utilise more variable molecular markers, such as microsatellites or AFLP, and expand spatial sampling throughout the lake.

While this research offers significant insights into the genetic variation of *P. crassipes*, it also presents certain limitations. The sample size in our study was relatively small due to challenges

in sampling, which may have impacted the reliability of our results. To strengthen the validity of our findings, future studies should aim for a larger sample size. Furthermore, employing multiple genetic markers will enhance our ability to assess and compare genetic variation more comprehensively.

Conclusion

Based on a comprehensive analysis of the available data, it can be concluded that the genetic variation observed in water hyacinth (*P. crassipes*) is minimal, indicating a high degree of genetic uniformity within this species. The results from the Analysis of Molecular Variance (AMOVA) further reinforce this conclusion, revealing that both identified morphotypes of *P. crassipes* not only share similar phenotypic characteristics but are also genetically identical at a molecular level. This suggests that environmental factors may have a more significant impact on morphological differentiation than genetic divergence within this invasive aquatic plant species.

Acknowledgement

The authors sincerely thank the Paul Hebert Centre for DNA barcoding and Biodiversity Studies, Chhatrapati Sambhajinagar and Progenome Lifescience Pvt. Ltd., Chhatrapati Sambhajinagar, for providing laboratory facilities and support throughout the study. We are also grateful to the field staff and research assistants who aided in sample collection at Powai Lake, Mumbai. This research was conducted without specific funding from public, commercial, or not-for-profit sectors.

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