Genetic Diversity of Tropical Plant Species Revealed by DNA Metabarcoding Using Oxford Nanopore Sequencing

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Abstract

Genetic diversity is a fundamental component for understanding plant adaptation, conservation, and ecosystem stability. DNA metabarcoding has emerged as an effective molecular approach for assessing plant genetic diversity at the community level. This study aimed to evaluate the genetic diversity of tropical plant species using DNA metabarcoding combined with Oxford Nanopore sequencing technology. Total genomic DNA was extracted from pooled plant samples and amplified using universal plant barcode primers. Sequencing was performed using the Oxford Nanopore platform, and the resulting reads were analyzed to identify taxonomic composition and genetic variation. The results demonstrated that Nanopore-based metabarcoding successfully detected diverse plant taxa and revealed substantial genetic variation among samples. This approach provides a rapid and cost-effective method for assessing plant genetic diversity, with significant implications for biodiversity monitoring and conservation strategies.

Keywords: plant genetics, genetic diversity, DNA metabarcoding, Oxford Nanopore, tropical plants

Introduction

Genetic diversity plays a crucial role in plant survival, adaptation, and evolutionary processes. High genetic variation enables plant populations to respond to environmental changes, resist diseases, and maintain ecosystem functions. In tropical ecosystems, where biodiversity is exceptionally high, comprehensive assessments of plant genetic diversity are essential for effective conservation and management.

Conventional methods for assessing plant diversity rely heavily on morphological identification, which can be time-consuming and prone to misidentification, particularly in species-rich environments. Molecular approaches, such as DNA barcoding and metabarcoding, have become powerful alternatives for species identification and genetic diversity analysis. Plant metabarcoding commonly

targets conserved genomic regions such as *rbcL*, *matK*, and ITS, allowing simultaneous identification of multiple taxa from mixed samples.

Recent advances in third-generation sequencing technologies, particularly Oxford Nanopore sequencing, offer advantages including long read lengths, real-time data generation, and portability. These features make Nanopore sequencing a promising tool for large-scale plant genetic studies. This study aimed to assess plant genetic diversity using DNA metabarcoding coupled with Oxford Nanopore sequencing as a rapid and efficient molecular approach.

Materials and Methods

Sample Collection and DNA Extraction

Plant samples representing mixed tropical vegetation were collected from selected sampling sites. Fresh leaf tissues were pooled and stored at −20 °C prior to DNA extraction. Total genomic DNA was extracted using a commercial plant DNA extraction kit following the manufacturer's protocol. DNA quality and concentration were assessed using spectrophotometry.

PCR Amplification

DNA metabarcoding amplification was performed using universal plant barcode primers. Prior to amplification, DNA templates were diluted tenfold. PCR reactions were carried out in a total volume of 25 μL containing KAPA HiFi HotStart ReadyMix 2×, forward and reverse primers (10 μM), DNA template, and nuclease-free water. PCR conditions consisted of an initial denaturation at 95 °C for 3 min, followed by 35 cycles of denaturation at 98 °C for 30 s, annealing at 57 °C for 30 s, and extension at 72 °C for 30 s, with a final extension at 72 °C for 1 min.

Library Preparation and Sequencing

PCR products from replicate reactions were pooled and purified using AMPure beads. Purified amplicons were used for library preparation according to the Oxford Nanopore sequencing protocol and subsequently sequenced using a Nanopore platform.

Bioinformatic Analysis

Raw sequencing reads were quality-filtered and trimmed prior to downstream analysis. Taxonomic assignment was performed by comparing sequences against a reference database using standard bioinformatic pipelines. Genetic diversity was evaluated based on sequence variation and taxonomic richness.

Results

Oxford Nanopore sequencing generated a substantial number of high-quality reads suitable for metabarcoding analysis. Multiple plant taxa were successfully identified from the mixed samples, demonstrating the effectiveness of the selected barcode primers. Genetic variation was observed among detected taxa, indicating high genetic diversity within the sampled plant communities. The long-read capability of Nanopore sequencing enabled improved resolution in taxonomic assignment compared to short-read approaches.

Discussion

The results of this study highlight the potential of Oxford Nanopore-based DNA metabarcoding as a reliable tool for assessing plant genetic diversity. The method efficiently captured a wide range of plant taxa and provided insights into genetic variation within tropical plant communities. Compared to conventional sequencing platforms, Nanopore sequencing offers flexibility and scalability, making it suitable for biodiversity studies in resource-limited settings.

The observed genetic diversity underscores the importance of molecular approaches in complementing traditional biodiversity assessments. However, further optimization of primer selection and bioinformatic pipelines is necessary to improve taxonomic resolution and reduce sequencing errors inherent to long-read technologies.

Conclusion

DNA metabarcoding using Oxford Nanopore sequencing represents a powerful approach for analyzing plant genetic diversity. This method enables rapid, accurate, and comprehensive assessment of plant communities and has strong potential applications in conservation genetics, ecological monitoring, and plant biodiversity research.

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