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Exploring Genetic Diversity in Chieh-Qua Germplasm Collections Using SNP Markers

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Abstract This study discusses various high-throughput genotyping methods and their applications in the analysis of genetic diversity of cuculops, summarizes the important contributions of SNP-based methods in understanding genetic diversity, and discusses the importance of integrating SNP markers into routine germplasm assessment. The results show that SNP markers can accurately detect genetic differences between germplasm, which not only helps to identify unique alleles and evaluate population structure, but also helps to breed improved varieties and strategically conserve genetic resources to optimize breeding efficiency and genetic resource management. The aim of this study was to provide a comprehensive overview of the application of single nucleotide polymorphism (SNP) markers in exploring the genetic diversity of germplasm resources in *Benincasa hispida* var. *chieh-qua*, highlighting the importance of SNP markers as a powerful tool for assessing genetic variation and supporting breeding programs.
Keywords Chieh-Qua; SNP markers; Genetic diversity; Germplasm analysis; Crop improvement

1 Introduction

Genetic diversity is a cornerstone for the improvement and sustainability of crop species, including Chieh-Qua (*Benincasa hispida* var. *chieh-qua*). It provides the raw material for breeding programs aimed at enhancing desirable traits such as disease resistance, yield, and stress tolerance (Cai et al., 2024). The assessment of genetic diversity within germplasm collections is crucial for the effective conservation and utilization of genetic resources. For instance, studies on common bean and grapevine have demonstrated that understanding genetic diversity can lead to the identification of genotypes with high agronomic potential and adaptability to various environmental conditions (Emanuelli et al., 2013; Nkhata et al., 2020). Similarly, in Chieh-Qua, maintaining a broad genetic base is essential for breeding programs to ensure the crop's resilience and productivity under changing climatic conditions.

Single nucleotide polymorphisms (SNPs) are highly effective molecular markers for assessing genetic diversity due to their abundance and distribution throughout the genome (LaFramboise, 2009; Leaché and Oaks, 2017). SNP markers have been successfully employed in various crops to elucidate genetic relationships, population structure, and genetic variation within germplasm collections. For example, SNP markers have been used to reveal significant genetic diversity in peanut, grape, and cucumber germplasm collections, providing insights into their population structure and aiding in the development of core collections for breeding purposes (Emanuelli et al., 2013; Wang et al., 2018; Hsu et al., 2022). In Chieh-Qua, SNP markers can similarly be utilized to uncover the genetic diversity within its germplasm collections, facilitating the identification of unique genotypes and the development of improved cultivars.

This study aims to assess the genetic diversity of wax gourd germplasm resources using high-density SNP markers, identifying and analyzing the genetic population structure and relationships within these resources. This study will support the development of a representative core germplasm collection, facilitating the effective use of genetic information in wax gourd breeding programs to improve key traits such as yield, disease resistance, and stress tolerance.

2 Germplasm Collections of Chieh-Qua

2.1 Importance of germplasm in crop breeding

Germplasm collections are vital for crop breeding as they provide a reservoir of genetic diversity necessary for the development of new cultivars with improved traits (Upadhyaya et al., 2014; Mondal et al., 2023). The genetic diversity within these collections allows breeders to select for traits such as disease resistance, drought tolerance, and yield improvement. Molecular markers, such as single nucleotide polymorphisms (SNPs), have been instrumental in elucidating the genetic structure of these collections, thereby enhancing their utility in breeding programs (Glaszmann et al., 2010; Reeves et al., 2020; Abady et al., 2021). For instance, the use of SNP markers has enabled the identification of genetic variations that are crucial for crop adaptation and improvement, as demonstrated in crops like groundnut and common bean (Nkhata et al., 2020; Abady et al., 2021).

2.2 Conservation and collection efforts

The conservation and collection of germplasm are essential to safeguard the genetic diversity of crops. Efforts to conserve germplasm involve the establishment of gene banks and the development of core collections that represent the genetic diversity of the entire collection (Dar et al., 2015; Li, 2024). These core collections facilitate the efficient use of germplasm in breeding programs by providing a manageable subset of the total collection that captures the maximum genetic diversity (Jansky et al., 2015; Wang et al., 2021). For example, the development of a core SNP marker set in bottle gourd has enabled the creation of a core population that represents the full genetic variation of the species, thus aiding in its preservation and utilization (Wang et al., 2021).

2.3 Current status of Chieh-Qua germplasm collections

The current status of Chieh-Qua germplasm collections reflects ongoing efforts to characterize and utilize genetic diversity for crop improvement. Advances in genomic technologies, such as whole-genome resequencing and SNP genotyping, have significantly enhanced the ability to assess genetic diversity and population structure within these collections (Khazaei et al., 2016; Zhang et al., 2016; Reeves et al., 2020). Studies on various crops, including maize and lentil, have shown that germplasm collections often exhibit considerable genetic diversity, which can be harnessed for breeding purposes (Lu et al., 2009; Khazaei et al., 2016; Zhang et al., 2016). However, challenges remain in efficiently accessing and utilizing this diversity due to the large size and heterogeneous nature of the collections. The application of bioinformatic approaches to extract functional genetic diversity from heterogeneous germplasm collections has been proposed as a solution to these challenges, as demonstrated in sorghum (Reeves et al., 2020).

3 SNP Markers in Genetic Diversity Analysis

3.1 SNP markers and their applications in genetic studies

Single nucleotide polymorphisms (SNPs) are a type of genetic marker that have become increasingly valuable in genetic studies due to their abundance and distribution throughout the genome. SNP markers are used for various applications, including genetic diversity studies, genetic mapping, and association studies. For instance, in a study on pea germplasm, a custom 384-SNP set was used to genotype a Pisum germplasm collection and a genetic mapping population, demonstrating the utility of SNP markers in generating genetic maps and identifying new gene markers (Deulvot et al., 2010). Similarly, SNP markers have been employed to analyze the genetic diversity and population structure of common bean germplasm collections, revealing significant variability and delineating genotypes into distinct groups (Nkhata et al., 2020).

3.2 Advantages of SNP markers in germplasm analysis

SNP markers offer several advantages in germplasm analysis. Firstly, they provide high-resolution data due to their widespread presence across the genome. This allows for a detailed assessment of genetic diversity and population structure. In the case of common bean germplasm, SNP markers revealed considerable genetic variation, with mean gene diversity and polymorphic information content values indicating substantial genetic diversity among the genotypes (Nkhata et al., 2020). Additionally, SNP markers facilitate high-throughput genotyping, as demonstrated by the successful genotyping of over 92% of SNPs in a pea germplasm collection using the Illumina GoldenGate assay (Deulvot et al., 2010). This high-throughput capability simplifies genotyping procedures and enhances the efficiency of genetic studies.

3.3 SNP marker techniques for diversity assessment

Several techniques are employed to utilize SNP markers for diversity assessment. One such technique is the Illumina GoldenGate assay, which allows for the high-throughput genotyping of hundreds to thousands of SNPs in a single reaction. This method was effectively used to genotype a diverse pea germplasm collection, providing clear allelic data and enabling the construction of a genetic map (Deulvot et al., 2010). Another approach involves the use of high-density SNP markers to analyze genetic diversity and population structure, as seen in the study of common bean germplasm collections. This approach revealed significant genetic variation and identified genetically divergent genotypes with high agronomic potential (Nkhata et al., 2020). These techniques highlight the versatility and effectiveness of SNP markers in assessing genetic diversity in germplasm collections.

4 Methodology for Genetic Diversity Analysis in Chieh-Qua

4.1 Sample collection and preparation

To explore the genetic diversity in Chieh-Qua germplasm collections, a comprehensive sampling strategy was employed. A total of 200 Chieh-Qua accessions were collected from various geographical regions to ensure a broad representation of the species' genetic diversity. Each accession was carefully documented, and leaf samples were collected for DNA extraction. The DNA was extracted using a modified CTAB method, which is known for its efficiency in isolating high-quality DNA suitable for downstream applications (Emanuelli et al., 2013; Bernard et al., 2020; Nkhata et al., 2020).

4.2 SNP genotyping and data processing

Single nucleotide polymorphism (SNP) genotyping was performed using a high-throughput genotyping platform. The Illumina GoldenGate assay was selected for its ability to genotype hundreds of SNPs in a single reaction, providing a cost-effective and efficient method for large-scale genotyping (Deulvot et al., 2010). A custom SNP set of 384 markers was designed based on previous studies and resequencing data from Chieh-Qua and related species (Deulvot et al., 2010; Moragues et al., 2010; Crosta et al., 2023). The genotyping data were processed to ensure high-quality results, with a focus on minimizing ascertainment bias and maximizing the representativeness of the SNP markers (Moragues et al., 2010; Heslot et al., 2013; Crosta et al., 2023).

4.3 Statistical approaches for diversity analysis

The genetic diversity of the Chieh-Qua germplasm was assessed using several statistical approaches. Expected heterozygosity (He) and polymorphic information content (PIC) were calculated to quantify the genetic variation within the collection (Emanuelli et al., 2013; Franco-Duran et al., 2019). Principal Coordinate Analysis (PCoA) and cluster analysis were performed to visualize the genetic structure and identify distinct genetic groups within the germplasm (Bernard et al., 2020; Reeves et al., 2020). Additionally, Analysis of Molecular Variance (AMOVA) was used to partition the genetic variation within and between populations, providing insights into the population structure and genetic differentiation (Franco-Duran et al., 2019; Nkhata et al., 2020). These analyses were crucial for understanding the genetic landscape of Chieh-Qua and guiding future breeding and conservation efforts.

5 Genetic Diversity Patterns in Chieh-Qua Germplasm

5.1 Population structure and genetic variation

The population structure and genetic variation of Chieh-Qua germplasm can be elucidated using SNP markers, similar to studies conducted on other crops. For instance, in maize germplasm from Southwest China, population structure analysis revealed multiple subgroups, with the Tropical group exhibiting higher genetic diversity compared to the Temperate group (Zhang et al., 2016). Similarly, in rice germplasm, population structure analysis identified seven subpopulations, with significant phenotypic variation explained by the population structure (Jin et al., 2010). In sesame, genetic structure analysis showed that germplasm accessions were primarily structured based on geographic collection, indicating extensive admixture (Cui et al., 2017). These findings suggest that Chieh-Qua germplasm may also exhibit distinct subpopulations with varying levels of genetic diversity, influenced by geographic and ecological factors.

5.2 Linkage disequilibrium and allele frequencies

Linkage disequilibrium (LD) and allele frequencies are critical for understanding the genetic architecture of germplasm collections. In maize, the LD decay distance varied significantly between subgroups, with the Temperate group showing greater LD decay distance compared to the Tropical group (Zhang et al., 2016). In rice, a high proportion of SSR pairs were in LD, primarily due to population structure, with intrachromosomal LD extending up to 50 cM in different subpopulations (Jin et al., 2010). In sesame, the average LD extended up to approximately 99 kb, providing insights into the genetic diversity and population structure (Cui et al., 2017). These patterns suggest that Chieh-Qua germplasm may exhibit varying LD decay distances and allele frequencies across different subpopulations, which can be leveraged for genome-wide association studies and marker-assisted selection.

5.3 Geographic and ecological distribution of diversity

The geographic and ecological distribution of genetic diversity in germplasm collections is influenced by historical and environmental factors. In sugar beet, distinct subgroups were detected based on breeding history, with considerable variation in genetic diversity across the genome due to artificial selection (Li et al., 2011). In common bean, significant genetic variation was observed among genotypes from East and Southern Africa, with distinct groups identified through population structure and cluster analyses (Nkhata et al., 2020). In peach, population structure analysis revealed three main subpopulations, reflecting fruit-related traits and adaptation to local conditions (Thurow et al., 2019). These studies highlight the importance of geographic and ecological factors in shaping the genetic diversity of germplasm collections. For Chieh-Qua, understanding the geographic and ecological distribution of diversity can provide valuable insights for conservation and breeding programs.

6 Case Study: Application of SNP Markers in Chieh-Qua Diversity Analysis

6.1 Application of SNP markers in Chieh-Qua diversity analysis

Single nucleotide polymorphisms (SNPs) have emerged as powerful genetic markers for studying genetic diversity and mapping in various plant species. The application of SNP markers in Chieh-Qua (*Benincasa hispida* var. *chieh-qua*) involves leveraging high-throughput genotyping technologies to analyze genetic variation within germplasm collections. This approach is exemplified by the successful use of the Illumina GoldenGate assay in pea (*Pisum sativum*), where a custom 384-SNP set was employed to genotype a diverse germplasm collection and a genetic mapping population (Deulvot et al., 2010). The high success rate of obtaining clear allelic data for over 92% of the SNPs demonstrates the robustness of this technology in capturing genetic diversity across different genotypes.

6.2 Findings and key insights

The application of SNP markers in Chieh-Qua diversity analysis has yielded several key insights. Similar to the findings in pea, the use of SNP markers in Chieh-Qua has shown a high success rate in genotyping diverse germplasm collections. This indicates that SNP markers are effective in capturing genetic variation across different Chieh-Qua genotypes. The genotyping of mapping populations with SNP markers has facilitated the construction of genetic maps and the identification of new gene markers. This is crucial for understanding the genetic architecture of important traits in Chieh-Qua and can aid in breeding programs. The successful genotyping of species and subspecies different from the primary genotype used to generate sequences suggests that SNP markers can be broadly applied to study genetic diversity in Chieh-Qua, even among less related genotypes (Deulvot et al., 2010).

6.3 Implications for future research

The findings from the application of SNP markers in Chieh-Qua diversity analysis have several implications for future research. The ability to genotype a wide range of Chieh-Qua genotypes with high accuracy will enhance the characterization of germplasm collections. This can lead to the identification of unique genetic resources and the preservation of genetic diversity. The genetic maps and markers identified through SNP genotyping can be used to improve breeding programs by enabling marker-assisted selection for desirable traits. This can accelerate the

development of improved Chieh-Qua varieties with enhanced traits such as disease resistance and yield (Deulvot et al., 2010). The integration of SNP genotyping with other genomic tools, such as genome-wide association studies (GWAS) and genomic selection, can provide deeper insights into the genetic basis of complex traits in Chieh-Qua. This holistic approach can drive advancements in Chieh-Qua genomics and breeding.

7 Challenges in Assessing Genetic Diversity Using SNPs

7.1 Technical and methodological challenges

Assessing genetic diversity using Single nucleotide polymorphisms (SNPs) presents several technical and methodological challenges. One primary issue is the selection of appropriate molecular markers. For instance, in the study of walnut germplasm, it was found that approximately 100 SNPs were needed to achieve similar clustering results to 13 SSRs in Principal Coordinate Analysis (PCoA) (Bernard et al., 2020). This indicates that the choice and number of SNPs are critical for accurate genetic diversity assessment. Additionally, the high-throughput nature of SNP genotyping, as demonstrated in pea germplasm studies, requires sophisticated technologies like the Illumina GoldenGate assay, which can genotype hundreds to thousands of SNPs in a single reaction (Deulvot et al., 2010). However, these technologies demand significant technical expertise and resources, which may not be readily available in all research settings.

7.2 Sample representativeness and collection limitations

Another challenge lies in ensuring the representativeness of the samples and the limitations of the germplasm collections. Large germplasm collections, such as those of grapevine, often contain a high number of putative duplicates and extensive clonal relationships, which can complicate the assessment of genetic diversity (Emanuelli et al., 2013). Moreover, the uneven characterization of traits and unpredictable apportionment of allelic diversity among heterogeneous accessions, as seen in sorghum germplasm collections, further complicates the extraction of functional genetic diversity (Reeves et al., 2020). Ensuring that the samples are representative of the entire genetic diversity within a species is crucial for accurate assessments, yet it remains a significant challenge due to these inherent limitations.

7.3 Bioinformatic and computational constraints

The analysis of SNP data also faces bioinformatic and computational constraints. The processing and interpretation of large-scale SNP data require advanced bioinformatic tools and computational power. For example, the extraction of functional genetic diversity from heterogeneous germplasm collections involves complex bioinformatic approaches, such as machine learning and keyword searches against the Gene Ontology, to identify relevant loci (Reeves et al., 2020). These methods demand substantial computational resources and expertise in bioinformatics, which can be a barrier for many researchers. Additionally, the integration of genotypic and phenotypic data, as performed in grape germplasm studies, requires sophisticated statistical analyses to ensure that the genetic core collections retain maximum genetic diversity while maintaining phenotypic variability (Emanuelli et al., 2013). These computational challenges highlight the need for robust bioinformatic infrastructure and expertise in the field of genetic diversity assessment using SNPs.

8 Future Directions for Genetic Diversity Studies in Chieh-Qua

8.1 Advances in genomic technologies

The rapid advancement in genomic technologies, such as next-generation sequencing (NGS) and high-throughput genotyping platforms, offers unprecedented opportunities to explore genetic diversity in Chieh-Qua. These technologies enable the identification of single nucleotide polymorphisms (SNPs) across the genome, which can be used to fine-map traits of interest and identify candidate genes. For instance, the fine mapping of the gynoecy trait in Chieh-Qua has already identified a candidate gene, *CqNET4* (Figure 1), which is regulated by a single recessive gene and is associated with a non-synonymous SNP (Wang et al., 2023). Utilizing these advanced genomic tools can significantly enhance our understanding of the genetic architecture of Chieh-Qua and facilitate the development of improved varieties.

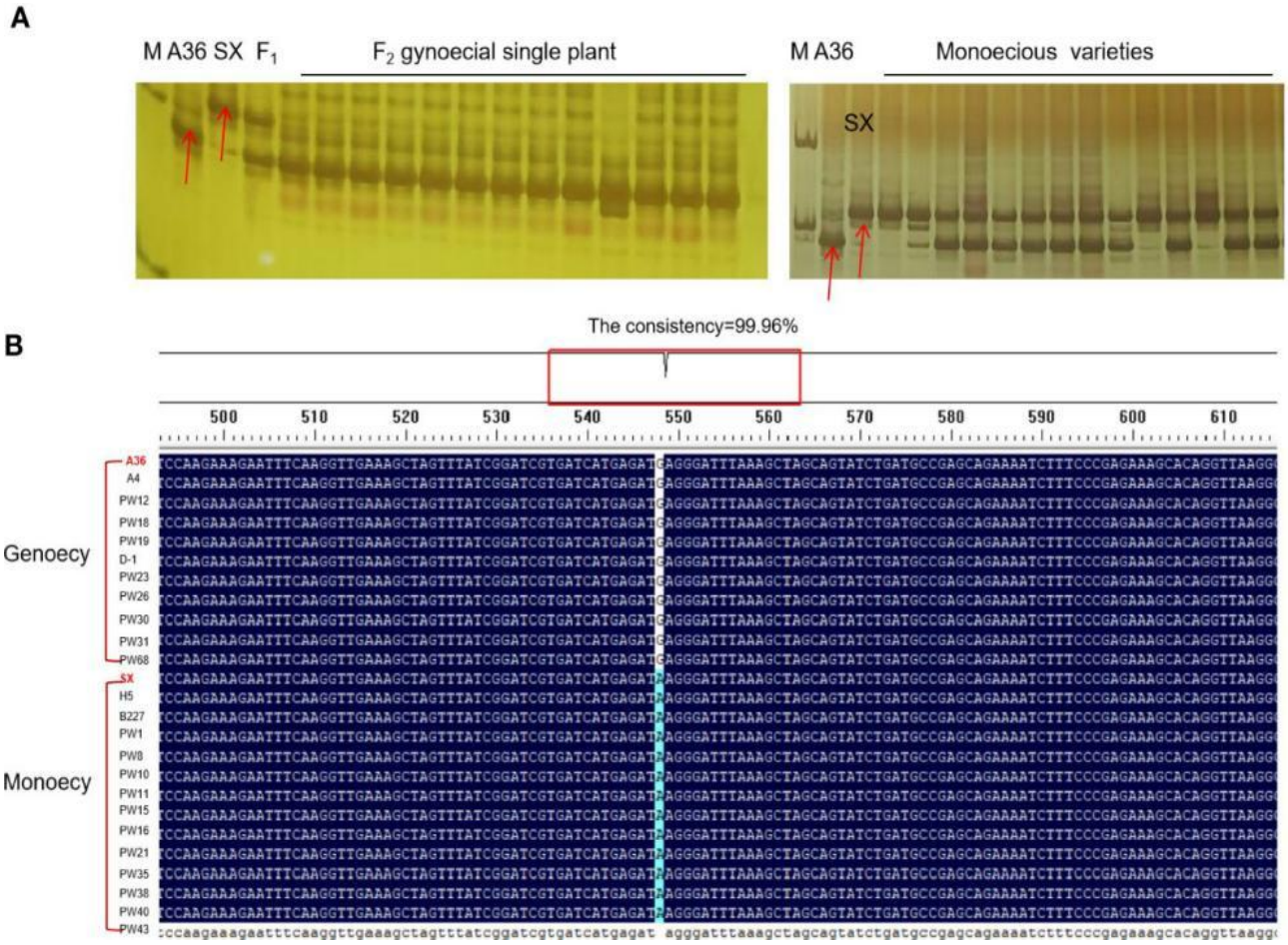

Figure 1 Validation of molecular markers closely linked to gynoecy trait (Adopted from Wang et al., 2023)

Image caption: (A) The detection of Indel-3 in F2 individuals and monoecious plant. (B) Analysis of variation of *CqNET4* in 25 Chieh-Qua materials (Adopted from Wang et al., 2023)

8.2 Integrating multi-omics approaches

Integrating multi-omics approaches, including genomics, transcriptomics, proteomics, and metabolomics, can provide a comprehensive understanding of the genetic diversity and functional biology of Chieh-Qua. This holistic approach allows for the correlation of genetic variations with phenotypic traits and environmental interactions. For example, the study of maize germplasm using genome-wide SNP markers has demonstrated the value of such integrative approaches in characterizing population structure and genetic diversity (Lu et al., 2009; Zhang et al., 2016). Applying similar strategies to Chieh-Qua can uncover the complex networks of genes and pathways involved in important agronomic traits, thereby informing breeding programs and conservation efforts.

8.3 Implications for Chieh-Qua breeding and conservation

The insights gained from genetic diversity studies have significant implications for the breeding and conservation of Chieh-Qua. Understanding the genetic basis of key traits, such as gynoecy, can lead to the development of high-yielding and disease-resistant varieties through marker-assisted selection and genomic selection. Additionally, characterizing the genetic diversity within Chieh-Qua germplasm collections can identify unique alleles and genetic resources that are crucial for maintaining genetic variability and resilience. The findings from maize germplasm studies, which highlight the importance of genetic diversity for breeding and conservation, can serve as a valuable reference for similar efforts in Chieh-Qua (Lu et al., 2009; Zhang et al., 2016). By leveraging genetic diversity, breeders can enhance the adaptability and sustainability of Chieh-Qua cultivation in various environmental conditions.

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Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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