

Research Report

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The Role of Isoenzymatic Variation in Delineating Phylogenetic Relationships within *Zea Genus*

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Abstract This research explores the role of isoenzymatic variation in delineating phylogenetic relationships within the genus *Zea*, which includes maize (*Zea mays*) and its wild relatives, the teosintes. The primary aim is to synthesize existing research on isoenzymatic markers to understand their effectiveness in resolving phylogenetic relationships and uncovering genetic diversity within *Zea*. The methodology involved a comprehensive search of peer-reviewed studies focusing on isoenzymatic variation, phylogenetic analysis, and genetic diversity in *Zea*, utilizing databases such as PubMed, Web of Science, and Scopus. Studies were selected based on their relevance, methodological rigor, and contributions to the field. Key findings indicate that isoenzymatic markers are effective in identifying genetic differentiation between maize and teosinte species, supporting the hypothesis of a single domestication event from *Zea mays* ssp. *parviglumis*. Isoenzymatic data also reveal significant genetic diversity within teosinte populations and highlight the role of hybridization and introgression in shaping the genetic landscape of modern maize. When integrated with molecular markers like SSRs, SNPs, and cpDNA, isoenzymatic data provide a more comprehensive understanding of phylogenetic relationships and evolutionary processes within *Zea*. The research underscores the strengths and limitations of isoenzymatic markers, emphasizing their value in functional genetic studies despite their lower resolution compared to DNA-based markers. Recommendations for future research include expanding the geographic and taxonomic scope of isoenzymatic studies, employing advanced analytical techniques, and integrating isoenzymatic data with genomic and proteomic analyses to enhance phylogenetic resolution.

Keywords *Zea genus*; Isoenzymatic variation; Phylogenetic relationships; Genetic diversity; Molecular markers, Genetic differentiation

The genus *Zea*, commonly known as maize or corn, holds significant agricultural and economic importance worldwide. Comprising several species, *Zea* includes both wild and domesticated varieties that have been studied extensively for their genetic diversity and evolutionary relationships. Understanding the phylogenetic relationships within the *Zea genus* is crucial for various applications, including crop improvement, conservation of genetic resources, and insights into evolutionary biology.

Phylogenetic studies play a pivotal role in elucidating the evolutionary pathways and genetic relationships among species. By constructing phylogenetic trees, researchers can trace lineage diversification, identify ancestral traits, and understand speciation events (Schmidt and Bothme, 2009). In the context of *Zea*, phylogenetic analyses have provided essential insights into the domestication process and the genetic basis of important agronomic traits. These studies are foundational for advancing breeding programs and ensuring the sustainability of maize as a staple crop (Doebley, 2004).

Isoenzymes, or isozymes, are multiple forms of an enzyme that catalyze the same biochemical reaction but differ in amino acid sequence and often in their electrophoretic mobility. These enzyme variants arise from different gene loci or alleles and serve as valuable markers for genetic variation. Isoenzymatic analysis has been employed extensively in plant genetics due to its reliability, simplicity, and ability to reveal polymorphisms that are informative for genetic and phylogenetic studies (Nevo, 2001; Smith and Smith, 2004).

This research aims to synthesize the existing literature on the role of isoenzymatic variation in delineating phylogenetic relationships within the *Zea genus*. By examining the findings from various studies, this research

seeks to highlight the contributions of isoenzymes to our understanding of *Zea* phylogeny and to discuss the strengths and limitations of this approach, also aims to identify gaps in the current research and suggest directions for future studies that could integrate isoenzymatic data with other phylogenetic tools to provide a more comprehensive understanding of *Zea* evolution.

1 Literature Review

1.1 Historical context of *Zea* phylogenetic research

Maize (*Zea mays*) is one of the world's most important crops. The study of its phylogenetic relationships is not only theoretically significant but also provides crucial information for breeding programs. As early as the early 20th century, scientists began to focus on the classification and phylogenetic relationships of maize and its wild relatives (García-Martínez and Martínez-Izquierdo, 2003). Through morphological, cytological, and molecular marker techniques, researchers gradually revealed the phylogenetic relationships among different species of maize. However, with the advancement of molecular biology technologies, especially genome and molecular marker techniques, research on the phylogenetic relationships of maize has entered a new phase.

1.2 Isoenzymatic variation in plants

Isoenzymes, as a type of polymorphic marker, have been widely used in plant genetic diversity and phylogenetic studies. Research on isoenzymatic variation began in the 1960s when researchers separated and identified isoenzymes in different plant populations using electrophoresis (Matsuoka et al., 2002). Since then, numerous studies have found that isoenzymatic variation not only reflects genetic differences between plant populations but also reveals their evolutionary history and phylogenetic relationships (René Hortelano et al., 2011).

In maize and its wild relatives, significant progress has also been made in the study of isoenzymatic variation. Studies have found that different species of maize and its relatives exhibit clear polymorphisms in isoenzymes such as esterases and peroxidases. These polymorphisms can serve as effective markers for identifying populations and studying phylogenetic relationships (Galal et al., 2013).

1.3 Comparative methods used in phylogenetic studies

Comparative methods are crucial for revealing phylogenetic relationships in phylogenetic studies. Common comparative methods include morphological comparison, molecular marker analysis, and genome comparison. In morphological comparison, researchers infer phylogenetic relationships by comparing the morphological characteristics of different plants, such as leaf shape and inflorescence (Orton et al., 2017). However, because morphological characteristics are easily influenced by environmental factors, this method has limitations in revealing deep phylogenetic relationships.

Molecular marker analysis is one of the most commonly used methods in phylogenetic studies. By analyzing isoenzymes, polymorphic DNA (such as RAPD, SSR), and gene sequence variations, researchers can more accurately reveal genetic relationships and evolutionary history among plant populations (Tsanev et al., 2000). Additionally, genome comparison methods provide more comprehensive phylogenetic information by comparing the genome structures and sequences of different species (Silva et al., 2020).

1.4 Significance of isoenzymatic markers in genetic studies

Isoenzymatic markers have significant importance in plant genetic studies. Isoenzymatic markers have high polymorphism, which can reflect genetic differences between populations (Moeller and Tiffin, 2005). Isoenzymatic markers are easy to operate, as they can be obtained through electrophoresis, making them widely applicable in large-scale population studies (Gómez-Anduro et al., 2011). Finally, isoenzymatic markers can reveal the evolutionary history and phylogenetic relationships of populations, providing important references for breeding programs (Hartings et al., 2002).

In maize and its wild relatives, studies on isoenzymatic markers have not only revealed genetic differences between different populations but also their phylogenetic relationships. For example, studies have found that maize and teosinte (*Zea mexicana*) exhibit high similarity in isoenzymes, providing strong evidence for their phylogenetic relationship (Angelov, 2003).

2 Isoenzymatic Variation in *Zea*

2.1 Explanation of isoenzymes and their genetic basis

Isoenzymes, is a different molecular forms of an enzyme that catalyze the same chemical reaction but vary in amino acid sequence and kinetic properties. These variations arise from gene duplications followed by divergence, which can be attributed to mutations, gene conversion events, or evolutionary pressures that favor different enzyme forms under varying environmental conditions. In plants, isoenzymes are encoded by multiple gene loci, each potentially comprising multiple alleles, thus contributing to genetic diversity within and between species. For example, the enzyme alcohol dehydrogenase (ADH) in *Zea mays* is known to exist in multiple isoenzymatic forms, which can be identified through their distinct electrophoretic mobilities.

The genetic basis of isoenzymes lies in the duplication and subsequent divergence of ancestral genes. These genes can evolve to perform similar functions but may differ in their expression patterns, subcellular localization, or responses to environmental stimuli. Such genetic variability allows plants to adapt to diverse environmental conditions, thereby conferring a selective advantage. In *Zea*, isoenzymatic variation has been particularly well-studied in the context of domestication and adaptation, providing insights into the evolutionary processes that have shaped the genetic makeup of modern maize and its wild relatives, the teosintes.

2.2 Methods of detecting and analyzing isoenzymatic variation

Detecting and analyzing isoenzymatic variation involves several biochemical and electrophoretic techniques. The most commonly used method is starch gel electrophoresis, which separates isoenzymes based on their charge and size. This technique involves grinding plant tissue to extract proteins, which are then subjected to electrophoresis in a starch gel. The gel is subsequently stained with specific substrates that react with the isoenzymes, producing distinct bands that correspond to different isoenzymatic forms. Each band represents a different allele at a given locus, allowing researchers to infer the genetic diversity within and among populations.

Isoenzymatic analysis can be further refined using techniques such as isoelectric focusing, which separates proteins based on their isoelectric points, and two-dimensional gel electrophoresis, which combines isoelectric focusing with SDS-PAGE to achieve high-resolution separation of complex protein mixtures. Advances in mass spectrometry have also facilitated the identification and characterization of isoenzymes by providing precise information on their molecular weights and peptide sequences. Additionally, molecular cloning and sequencing of isoenzyme genes have allowed researchers to investigate the underlying genetic variation and evolutionary history of these enzymes in *Zea*.

In phylogenetic studies, isoenzymatic data are often analyzed using statistical methods to assess genetic distances and construct phylogenetic trees. Techniques such as Nei's genetic distance and Rogers' distance are commonly used to quantify genetic divergence based on isoenzymatic variation. These measures are then subjected to clustering algorithms, such as UPGMA (Unweighted Pair Group Method with Arithmetic Mean) or neighbor-joining, to generate phylogenetic trees that depict the evolutionary relationships among taxa. The robustness of these trees can be evaluated through bootstrapping, which involves resampling the data to estimate the confidence intervals for each branch of the tree.

2.3 Significance of isoenzymatic variation in phylogenetic studies

Isoenzymatic variation has significant implications for phylogenetic studies, particularly in the context of the genus *Zea*. One of the primary advantages of using isoenzymes as phylogenetic markers is their ability to reveal genetic differences at the protein level, which can provide complementary information to DNA-based markers. This is especially valuable in cases where genetic variation at the DNA level is limited or difficult to detect. Isoenzymes have been successfully used to elucidate phylogenetic relationships within *Zea*, helping to clarify the evolutionary history and domestication pathways of maize and its wild relatives.

Studies have shown that isoenzymatic variation can effectively distinguish between different species and subspecies within *Zea*. Early research by Doebley et al. (1984) used isoenzymes to differentiate between maize and its closest wild relative, teosinte, revealing distinct genetic profiles that supported their classification as

separate taxa. Further studies have expanded on this work, using isoenzymes to investigate the genetic structure of maize landraces and improved cultivars. These studies have provided valuable insights into the genetic diversity of maize, identifying specific isoenzymatic markers associated with desirable agronomic traits, such as disease resistance and drought tolerance.

Isoenzymatic variation has also been instrumental in tracing the domestication history of maize. By comparing isoenzymatic profiles of modern maize varieties with those of ancient maize and teosinte populations, researchers have been able to reconstruct the domestication process and identify key genetic changes that accompanied the transition from wild to cultivated forms. For example, studies by Smith and Smith (1989) demonstrated that certain isoenzymatic alleles present in modern maize are absent in wild teosinte populations, suggesting selective pressures during domestication that favored specific enzyme forms.

In addition to its utility in phylogenetic analysis, isoenzymatic variation provides insights into the adaptive evolution of *Zea*. Isoenzymes can exhibit differential expression in response to environmental stressors, such as temperature extremes, water availability, and pathogen attacks. This adaptive flexibility is crucial for the survival and reproduction of plants in diverse ecological niches. By studying isoenzymatic variation, researchers can identify genetic loci that confer adaptive advantages, contributing to our understanding of plant resilience and informing breeding programs aimed at improving crop performance under changing environmental conditions.

Despite its advantages, the use of isoenzymatic variation in phylogenetic studies is not without limitations. Isoenzymes represent only a fraction of the total genetic variation within a species, and their resolution may be lower than that of DNA-based markers, such as single nucleotide polymorphisms (SNPs) or microsatellites. Moreover, isoenzymatic analysis requires fresh or properly stored tissue samples, which can be challenging to obtain for rare or historical specimens. Nevertheless, when used in conjunction with other phylogenetic markers, isoenzymes provide a powerful tool for elucidating the genetic relationships and evolutionary history of *Zea*.

3 Phylogenetic Relationships within *Zea*

3.1 Overview of the *Zea* genus and its species

The genus *Zea*, belonging to the Poaceae family, encompasses some of the most vital plant species in agriculture and human history. *Zea* is primarily known for *Zea mays*, commonly known as maize or corn, which is a staple crop worldwide. The genus also includes four species of teosinte: *Zea diploperennis*, *Zea perennis*, *Zea luxurians*, and *Zea nicaraguensis*, along with subspecies of *Zea mays*, such as *Zea mays* ssp. *parviglumis* and *Zea mays* ssp. *mexicana*. These teosintes are wild grasses native to Mexico and Central America and are the closest wild relatives of maize. The evolutionary relationship between maize and teosinte is a subject of extensive research due to its significance in understanding domestication processes and the genetic foundation of crop development.

Teosintes exhibit a range of morphological and genetic diversity, offering valuable insights into the evolutionary pathways leading to modern maize. *Zea mays* ssp. *parviglumis* is recognized as the direct ancestor of domesticated maize, while other teosinte species contribute to understanding the genetic variability and adaptability of the genus. The study of these relationships not only illuminates the domestication and evolution of maize but also aids in the conservation and utilization of genetic resources for crop improvement.

3.2 Historical context of phylogenetic studies in *Zea*

Phylogenetic studies in *Zea* have a long history, beginning with morphological analyses and progressing to molecular techniques. Early studies relied on morphological traits to distinguish between species and infer relationships. However, these methods had limitations due to the plasticity of morphological traits influenced by environmental factors.

The advent of molecular techniques in the late 20th century revolutionized phylogenetic studies in *Zea*. Isoenzymatic analyses were among the first molecular methods used, providing insights into genetic variation at the protein level. These studies revealed significant diversity within *Zea* and highlighted the close relationship between maize and teosintes.

Subsequent studies employed DNA-based markers, such as RFLPs, SSRs, and SNPs, to further resolve phylogenetic relationships. The integration of these molecular markers with isoenzymatic data has provided a more comprehensive understanding of the evolutionary history and genetic diversity within *Zea*.

3.3 Key phylogenetic markers and their relevance

Isoenzymatic markers have played a crucial role in delineating phylogenetic relationships within *Zea*. Isoenzymes are advantageous because they are relatively easy to assay and provide a direct measure of genetic variation at the protein level. Early studies using isoenzymes, Doebley et al. (1984) study, identified significant genetic differentiation between maize and teosinte species, supporting the hypothesis of multiple domestication events and subsequent gene flow between wild and cultivated populations.

Beyond isoenzymes, several other phylogenetic markers have been pivotal in studying *Zea*'s evolutionary relationships. Chloroplast DNA (cpDNA) markers have provided valuable information on maternal lineage and biogeographical patterns. cpDNA studies have confirmed the monophyletic origin of maize and its closest relationship to *Zea mays* ssp. *parviglumis*, while also highlighting the role of *Zea mays* ssp. *mexicana* in contributing to genetic diversity through hybridization (Sánchez et al., 1999).

Nuclear DNA markers, including SSRs and SNPs, have enabled high-resolution analyses of genetic diversity and population structure. SSR markers, with their high polymorphism and co-dominant inheritance, have been extensively used to study the genetic relationships and diversity within *Zea*. These markers have helped identify distinct genetic clusters corresponding to different teosinte species and maize landraces, illustrating the complex patterns of domestication and gene flow (Matsuoka et al., 2002).

SNP markers, identified through NGS technologies, have revolutionized phylogenetic studies by providing dense genetic maps and facilitating genome-wide association studies (GWAS). SNP analyses have corroborated earlier findings of maize's closest relationship to *Zea mays* ssp. *parviglumis* and have identified numerous genomic regions associated with domestication traits. These studies have highlighted the role of both selective sweeps and introgression in shaping the genetic architecture of maize (Van Heerwaarden et al., 2011).

In addition to molecular markers, morphological and ecological data continue to contribute to understanding phylogenetic relationships within *Zea*. Traits such as plant architecture, kernel morphology, and flowering time provide complementary information that, when integrated with molecular data, enhances the resolution of phylogenetic analyses.

4 Findings from Isoenzymatic Studies

4.1 Summary of key studies on isoenzymatic variation in *Zea*

Isoenzymatic studies have provided significant insights into the phylogenetic relationships within the genus *Zea*. These studies utilize isoenzymes as markers to assess genetic diversity and evolutionary relationships among species and subspecies. One of the seminal works in this field was conducted by Doebley et al. (1984), who analyzed isoenzymatic variation across multiple *Zea* species. They utilized starch gel electrophoresis to separate and identify different isoenzymes, revealing considerable genetic differentiation between maize (*Zea mays* ssp. *mays*) and its wild relatives, the teosintes (Doebley et al., 1984).

Another key study by Goodman and Stuber (1983) focused on the isoenzymatic variation within maize and teosinte populations. Their work highlighted the extensive genetic diversity present within teosinte populations and underscored the close genetic relationship between maize and *Zea mays* ssp. *parviglumis*. This study was instrumental in establishing the hypothesis that *Zea mays* ssp. *parviglumis* is the most likely progenitor of domesticated maize.

Subsequent studies have built on these foundational works, employing more sophisticated techniques to analyze isoenzymatic variation. For instance, studies by Sánchez et al. (1999) and Matsuoka et al. (2002) have used isoenzymatic data in conjunction with other molecular markers to provide a more comprehensive understanding of

the phylogenetic relationships within *Zea*. These studies have consistently shown that isoenzymatic markers are effective in distinguishing between species and subspecies, thus providing valuable tools for phylogenetic analysis.

4.2 Major findings regarding phylogenetic relationships

The primary findings from isoenzymatic studies have significantly contributed to our understanding of phylogenetic relationships within *Zea*. One of the most consistent findings is the close genetic relationship between maize and *Zea mays* ssp. *parviglumis*. Isoenzymatic analyses have shown that these two taxa share a high degree of genetic similarity, supporting the hypothesis that *Z. mays* ssp. *parviglumis* is the direct ancestor of domesticated maize (Doebley et al., 1984; Matsuoka et al., 2002).

Another significant finding is the genetic distinctiveness of *Zea mays* ssp. *mexicana* and other teosintes. Isoenzymatic data have revealed that *Z. mays* ssp. *mexicana*, while closely related to maize, exhibits distinct genetic markers that differentiate it from *Z. mays* ssp. *parviglumis*. This suggests that *Z. mays* ssp. *mexicana* may have contributed to the genetic diversity of maize through hybridization events, but it is not the primary progenitor (Goodman and Stuber, 1983).

4.3 Comparative analysis of isoenzymatic data with other phylogenetic markers

while isoenzymatic data have provided valuable insights into the phylogenetic relationships within *Zea*, it is essential to compare these findings with data obtained from other phylogenetic markers to obtain a comprehensive understanding. Molecular markers such as SSRs, SNPs, and cpDNA have been extensively used in phylogenetic studies and provide complementary information to isoenzymatic data.

SNPs (single nucleotide polymorphisms) are another powerful tool for phylogenetic analysis. SNP markers provide a high-density genetic map, enabling precise identification of genetic variation and evolutionary relationships. Studies using SNP markers have corroborated the findings from isoenzymatic and SSR analyses, confirming the close relationship between maize and *Z. mays* ssp. *parviglumis*. SNP data have also identified regions of the genome that have undergone selection during domestication, providing insights into the genetic basis of key domestication traits (Van Heerwaarden et al., 2011).

Chloroplast DNA (cpDNA) markers provide information on maternal lineage and are useful for studying biogeographical patterns. cpDNA studies have shown that maize and *Z. mays* ssp. *parviglumis* share a common maternal ancestor, supporting the hypothesis of a single domestication event. Sánchez et al. (1999) studies have also highlighted the role of hybridization in the evolutionary history of *Zea*, with evidence of gene flow between maize and various teosinte species (Sánchez et al., 1999).

When compared with these molecular markers, isoenzymatic data have certain advantages and limitations. Isoenzymatic markers are relatively easy to assay and provide direct measures of genetic variation at the protein level. However, they may not capture the full extent of genetic diversity present at the DNA level. Molecular markers such as SSRs, SNPs, and cpDNA provide higher resolution and greater detail, allowing for more precise phylogenetic analyses. Nevertheless, isoenzymatic markers offer valuable complementary information and can be particularly useful in studies where DNA-based techniques are limited.

5 Implications for Future Research

5.1 Potential for further isoenzymatic studies in *Zea*

The role of isoenzymatic variation in delineating phylogenetic relationships within the genus *Zea* has proven to be both insightful and valuable. However, there remains significant potential for further isoenzymatic studies to deepen our understanding of the evolutionary history and genetic diversity within this genus. Future research could expand on the existing body of knowledge by exploring isoenzymatic variation across a broader range of *Zea* species and subspecies. This would involve conducting comprehensive surveys that include lesser-studied teosinte species and diverse maize landraces from different geographical regions. Such studies could uncover new isoenzymatic markers that provide additional resolution in phylogenetic analyses and reveal patterns of genetic diversity and adaptation in different environmental contexts (Doebley et al., 1984).

Furthermore, advancing the methodological approaches used in isoenzymatic studies could yield more precise and comprehensive data. Techniques such as two-dimensional gel electrophoresis and mass spectrometry could be employed to detect a wider array of isoenzymes with greater sensitivity and accuracy. These advanced methods could help identify novel isoenzymatic variants and their functional implications, offering deeper insights into the genetic and evolutionary dynamics within *Zea*. Additionally, combining isoenzymatic data with proteomic analyses could provide a more holistic view of the genetic and phenotypic diversity in *Zea* species (Goodman and Stuber, 1983).

5.2 Recommendations for integrating isoenzymatic data with other phylogenetic tools

To maximize the utility of isoenzymatic data in phylogenetic studies, it is essential to integrate these data with other phylogenetic tools. Combining isoenzymatic markers with molecular markers such as SSRs, SNPs, and cpDNA can enhance the resolution and robustness of phylogenetic analyses. This integrated approach allows for cross-validation of findings and provides a comprehensive view of genetic relationships and evolutionary processes. Matsuoka et al. (2002) research isoenzymes offer insights into functional genetic variation, SSRs and SNPs provide high-resolution data on genetic structure and diversity at the DNA level.

One practical recommendation is to use a multi-marker approach in phylogenetic studies of *Zea*. Researchers should design studies that simultaneously analyze isoenzymatic, molecular, and morphological markers. This approach can identify congruent patterns across different data types, thereby strengthening phylogenetic inferences. Additionally, integrating genomic data from next-generation sequencing technologies can uncover the genomic regions underlying isoenzymatic variation, linking genetic and phenotypic diversity. Van Heerwaarden et al. (2011) would not only enhance our understanding of phylogenetic relationships but also provide insights into the genetic basis of key traits and their evolutionary significance.

Furthermore, developing bioinformatics tools and databases that facilitate the integration and analysis of multi-marker datasets would be highly beneficial. These resources could include standardized protocols for data collection, storage, and sharing, as well as analytical frameworks for combining isoenzymatic, molecular, and morphological data. Such tools would promote collaboration and data sharing among researchers, accelerating the progress of phylogenetic studies in *Zea* and other plant genera (Sánchez et al., 1999).

5.3 Identification of gaps in current research and suggestions for future studies

Despite significant advancements, several gaps remain in the current research on isoenzymatic variation and phylogenetic relationships within *Zea*. One major gap is the limited geographic and taxonomic scope of many studies. Most research has focused on a few well-studied species and populations, primarily from Mexico and Central America. Expanding research to include more diverse populations from different regions, including South America and the Caribbean, could reveal new patterns of genetic diversity and evolutionary history. This expanded scope is crucial for a comprehensive understanding of the genus *Zea* and its adaptive potential in various environmental contexts (Goodman and Stuber, 1983).

Another gap is the relatively limited functional characterization of isoenzymatic variation. While isoenzymes are valuable markers for genetic diversity, understanding their functional roles and ecological significance remains a challenge. Future studies should aim to link isoenzymatic variation with specific physiological and ecological traits, such as stress tolerance, disease resistance, and reproductive strategies. This functional perspective would provide deeper insights into the adaptive significance of genetic variation and its role in the evolutionary success of *Zea* species (Hufford et al., 2012).

There is a need for longitudinal studies that track changes in isoenzymatic variation over time. Such studies could investigate the effects of environmental changes, such as climate change and habitat fragmentation, on genetic diversity and phylogenetic relationships within *Zea*. Longitudinal data would provide valuable insights into the dynamics of genetic diversity and adaptation, informing conservation strategies and breeding programs (Doebley, 2004).

Integrating traditional knowledge and practices into scientific research could enhance the understanding and conservation of genetic diversity in *Zea*. Indigenous communities have long histories of cultivating and managing

maize and its wild relatives, possessing valuable knowledge about their diversity and uses. Collaborative research that incorporates indigenous knowledge and participatory approaches could uncover new insights into the genetic and ecological dynamics of *Zea* and support the conservation of both genetic and cultural heritage (Olsen and Wendel, 2013).

6 Concluding Remarks

This research has highlighted the significant role of isoenzymatic variation in delineating phylogenetic relationships within the genus *Zea*. Through the analysis of key studies, it is evident that isoenzymatic markers provide valuable insights into the genetic diversity and evolutionary history of *Zea* species, particularly maize (*Zea mays* ssp. *mays*) and its wild relatives, the teosintes, and also underscores the close genetic relationship between maize and *Zea mays* ssp. *parviglumis*, supporting the hypothesis of a single domestication event. Isoenzymatic studies have consistently shown high levels of genetic similarity between these taxa, reinforcing the idea that *Z. mays* ssp. *parviglumis* is the direct ancestor of domesticated maize.

Moreover, isoenzymatic data have revealed the genetic distinctiveness of *Zea mays* ssp. *mexicana* and other teosinte species. These findings suggest that while *Z. mays* ssp. *mexicana* may have contributed to the genetic diversity of maize through hybridization events, it is not the primary progenitor. The genetic differentiation between *Z. mays* ssp. *mexicana* and *Z. mays* ssp. *parviglumis* highlights the complex evolutionary dynamics within the genus *Zea*.

The research emphasizes the extensive genetic diversity present within wild teosinte populations. This diversity is crucial for understanding the evolutionary processes that have shaped *Zea* species and for conserving genetic resources vital for future crop improvement efforts. Isoenzymatic markers have proven effective in distinguishing between different species and subspecies, providing a robust tool for phylogenetic analysis.

However, the research also identifies limitations in using isoenzymatic markers alone for phylogenetic studies. While isoenzymes provide direct measures of genetic variation at the protein level, they may not capture the full extent of genetic diversity present at the DNA level. This limitation necessitates the integration of isoenzymatic data with other molecular and morphological markers to achieve comprehensive and accurate phylogenetic analyses.

Isoenzymatic variation has played a pivotal role in advancing our understanding of the phylogenetic relationships within *Zea*. The use of isoenzymatic markers has provided valuable insights into the genetic structure and evolutionary history of maize and its wild relatives. These markers have been instrumental in confirming the close relationship between maize and *Z. mays* ssp. *parviglumis*, highlighting the genetic distinctiveness of *Z. mays* ssp. *mexicana*, and revealing the extensive genetic diversity within teosinte populations.

One of the key strengths of isoenzymatic markers is their ability to detect functional genetic variation. Unlike DNA-based markers that may only reflect neutral genetic variation, isoenzymes can provide insights into the adaptive significance of genetic diversity. This functional perspective is particularly important for understanding the ecological and evolutionary dynamics within *Zea*, as it sheds light on the traits that have contributed to the success and adaptability of different species and subspecies.

However, the research also acknowledges the limitations of isoenzymatic markers. The relatively low resolution and limited scope of these markers necessitate their use alongside other molecular tools to achieve a comprehensive understanding of phylogenetic relationships. Integrating isoenzymatic data with molecular markers such as SSRs, SNPs, and cpDNA can enhance the resolution and robustness of phylogenetic analyses. This integrative approach allows for cross-validation of findings and provides a more holistic view of genetic relationships and evolutionary processes.

Future research should focus on expanding the geographic and taxonomic scope of isoenzymatic studies. Including a broader range of *Zea* species and populations from diverse regions can uncover new patterns of genetic diversity and evolutionary history. Additionally, advancing the methodological approaches used in isoenzymatic studies, such as employing two-dimensional gel electrophoresis and mass spectrometry, can yield more precise and comprehensive data.

Longitudinal studies that track changes in isoenzymatic variation over time are also needed to understand the effects of environmental changes on genetic diversity and phylogenetic relationships within *Zea*. Such studies can provide valuable insights into the dynamics of genetic diversity and adaptation, informing conservation strategies and breeding programs.

Furthermore, integrating traditional knowledge and practices into scientific research can enhance the understanding and conservation of genetic diversity in *Zea*. Indigenous communities possess valuable knowledge about the diversity and uses of maize and its wild relatives. Collaborative research that incorporates indigenous knowledge and participatory approaches can uncover new insights into the genetic and ecological dynamics of *Zea* and support the conservation of both genetic and cultural heritage.

Conflict of Interest Disclosure

The author affirms 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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