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Design and In Silico Evaluation of a PCR Marker for Fall Armyworm Resistance

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Abstract

Fall armyworm (*Spodoptera frugiperda*) has become a significant constraint in corn (*Zea mays* L.) production, necessitating the development of molecular tools to support the identification of potential resistance-associated loci. This study presents the preliminary development and in silico validation of a candidate gene-derived molecular marker targeting a putative chromosome 8 locus associated with previously reported resistance-linked regions. Primer design was based on the NC_050103.1 genomic reference sequence, supported by existing genome-wide association study (GWAS) evidence. The designed primer pair was evaluated through in silico PCR, which produced a predicted amplicon corresponding to the targeted genomic region. Sequence analysis showed 88.24% identity with the reference sequence and homology to a *Zea mays* clone sequence (EU969166.1), supported by BLAST results indicating 95% similarity, an E-value of 3e-44, and 75% query coverage. Multiple sequence alignment using ClustalW showed varying levels of nucleotide identity among the reference sequence, homologous clone, in silico product, and experimentally derived amplicon. Identity values ranged from 82.58% to 100%, indicating partial conservation of the targeted locus across sequences. Conserved regions were observed, although moderate divergence suggests possible genetic variation or closely related genomic loci within chromosome 8. Overall, the findings provide preliminary evidence that the designed marker may target a partially conserved chromosome 8 genomic region potentially associated with resistance-linked loci. Further validation across diverse corn germplasm is required to assess its specificity, stability, and suitability for marker-assisted selection in fall armyworm resistance studies.

Keywords: Maize (*Zea mays* L.), Fall Armyworm (*Spodoptera Frugiperda*), Candidate Gene, Molecular Marker, PCR Amplification, Sequence Alignment

1. Introduction

Corn (*Zea mays* L.) remains a globally important cereal crop used for food, feed, and industrial purposes; however, its production is increasingly threatened by fall armyworm (*Spodoptera frugiperda*), an invasive pest that causes serious foliar damage and yield losses in tropical and subtropical regions (Goergen et al., 2016). Current control practices rely heavily on chemical management, but these approaches are often costly, environmentally unsustainable, and vulnerable to the development of insecticide resistance (Day et al., 2017). In response, host plant resistance and molecular marker development offer more sustainable and precise strategies for identifying corn genotypes with potential resistance traits. This study focuses on the in silico development and preliminary validation of a candidate gene-derived molecular marker targeting a putative chromosome 8 locus in corn, using bioinformatic tools, PCR-based validation, sequence alignment, and homology assessment.

Corn is a major cereal crop with broad agricultural and economic significance, yet its productivity is constrained by insect pests, especially the fall armyworm (*Spodoptera frugiperda*). This pest has become widespread in tropical and subtropical regions and has been reported to cause substantial damage to corn production systems (Goergen et al., 2016). Conventional pest control remains largely dependent on chemical inputs, but this approach raises concerns related to environmental effects, production costs, and the emergence of insecticide resistance (Day et al., 2017). These limitations highlight the need for alternative pest management approaches that are more sustainable, cost-effective, and compatible with long-term crop improvement.

Host plant resistance is recognized as a sustainable strategy for managing fall armyworm in corn. However, resistance traits are often quantitative and governed by multiple genes, making phenotypic selection difficult and less precise. Molecular markers address this limitation by supporting early and accurate identification of resistant genotypes. Candidate gene-based markers are particularly valuable because they target genomic regions with known or predicted biological roles in plant defense pathways. When supported by genome-wide association studies, these markers become more relevant for crop improvement because they are anchored on loci associated with phenotypic variation. Warburton et al. (2022) identified

several loci associated with fall armyworm resistance in corn, including regions on chromosome 8, suggesting the possible involvement of this chromosome in defense-related responses.

The study is situated within the broader challenge of sustaining corn production amid increasing pest pressure. Globally, fall armyworm has become a major invasive pest affecting corn-growing regions, particularly in tropical and subtropical areas (Goergen et al., 2016). At the production level, reliance on chemical control creates economic and environmental concerns, especially when repeated pesticide use contributes to resistance development and increased production costs (Day et al., 2017). For corn-producing communities and agricultural research institutions, molecular tools that support resistance screening may contribute to more sustainable pest management and improved breeding decisions.

The study is conceptually grounded in molecular marker-assisted crop improvement, candidate gene-based marker development, and genome-wide association study-guided locus selection. Candidate gene-derived markers are based on the principle that genomic regions with known or predicted functional relevance may be used to identify traits associated with plant defense. In this study, the target chromosome 8 locus was selected because it was linked to fall armyworm resistance in prior genome-wide association evidence (Warburton et al., 2022). The use of bioinformatics tools further supports this conceptual basis, as Primer-BLAST enables primer design while sequence analysis and homology assessment help validate target specificity. BLAST was used for homology assessment and sequence comparison (Altschul et al., 1990).

Although fall armyworm resistance in corn has been linked to multiple genomic regions, practical marker development remains necessary to support future resistance screening and marker-assisted selection. Phenotypic selection alone is limited because resistance is complex, quantitative, and influenced by multiple genes. The study responds to this gap by developing and preliminarily validating a candidate gene-derived molecular marker targeting a putative chromosome 8 region associated with previously reported resistance-linked loci (Warburton et al., 2022). The rationale is to generate preliminary molecular evidence that may help guide future validation across diverse corn germplasm and strengthen marker-assisted approaches for fall armyworm resistance studies.

This study aligns most directly with SDG 2 – Zero Hunger because it contributes to crop improvement efforts that may support more resilient corn production. It also relates to SDG 12 – Responsible Consumption and Production by exploring a molecular approach that may reduce dependence on chemical pest control, which is often associated with environmental impacts and increasing production costs (Day et al., 2017). The study also supports SDG 9 – Industry, Innovation and Infrastructure through the application of bioinformatics, primer design, PCR-based validation, and molecular marker development in agricultural biotechnology. Its connection to SDG 15 – Life on Land is evident in its potential contribution to more sustainable pest management practices that may lessen ecological pressure from repeated chemical control.

The study contributes to agricultural, environmental, technological, and socio-economic sustainability. Agriculturally, it supports the development of molecular tools that may improve the identification of corn genotypes associated with resistance to fall armyworm. Environmentally, the study supports the search for pest management alternatives that may reduce dependence on chemical control, which has been associated with environmental impacts and insecticide resistance (Day et al., 2017). Technologically, it demonstrates the use of in silico primer design, PCR validation, BLAST analysis, and sequence alignment as integrated tools for crop biotechnology. Socio-economically, improved resistance screening may eventually support farmers by contributing to more stable crop production and reduced pest management costs, although further validation is still needed before the marker can be considered robust for practical breeding applications.

2. Material and methods

2.1 Research Design

The study employed a molecular marker development and validation approach using both bioinformatic and laboratory-based procedures. A candidate genomic region located on chromosome 8 of corn was selected as the target locus because of its reported association with fall armyworm resistance based on genome-wide association studies (Warburton et al., 2022). The research design integrated in silico primer design, computational validation, PCR amplification, sequencing, and comparative sequence analysis to assess whether the designed marker could target the intended genomic region.

2.2 Locale of the Study

The study was conducted in two phases: phenotyping and genotyping. The phenotyping component was carried out under greenhouse conditions using maize accessions obtained from the collection of Cagayan. The genotyping component involved laboratory-based DNA extraction, PCR amplification, and sequence processing, together with in silico analyses using online genomic and protein analysis platforms.

2.3 Respondents of the Study

The biological materials used were sampled corn accessions, which served as sources of genomic DNA for PCR amplification and sequence analysis. The focus of the study was the development and validation of a molecular marker rather than the collection of data from human participants.

2.4 Sample and Sampling Procedure

The maize accessions were obtained from the collection of Cagayan and planted for screening under greenhouse conditions. Two phenotyping trials were conducted to determine the potential resistance of the accessions to fall armyworm. For genotyping, leaf samples were collected from young plants for DNA extraction. Primer selection considered standard parameters such as GC content, melting temperature, and the absence of secondary structures. Primer pairs with compatible annealing temperatures were prioritized to allow simultaneous amplification in a block PCR run.

2.5 Research Instrument

The study used bioinformatic tools and molecular laboratory procedures as the main research instruments. Primer-BLAST was used to design primers targeting the candidate genomic region NC_050103.1 on chromosome 8 of the corn genome. BLAST was used to assess sequence identity and similarity against publicly available databases (Altschul et al., 1990). The ExPASy Translate Tool was used to translate nucleotide sequences into predicted protein sequences (ExPASy, n.d.), while PHYRE2 was used for protein homology modeling (Kelly et al., 2015). Multiple sequence alignment was conducted using the ClustalW algorithm implemented in MEGA X to compare the reference sequence, homologous sequence, in silico PCR product, and experimentally obtained sequence (Tamura et al., 2021).

2.6 Data Gathering Procedure

The data gathering procedure began with the selection of a chromosome 8 candidate genomic region associated with fall armyworm resistance (Warburton et al., 2022). Primers were designed using Primer-BLAST, followed by in silico PCR analysis to determine the expected amplification profile. The predicted product corresponded to a chromosome 8 region at chr8:172209729–172209913. Laboratory PCR was then performed using genomic DNA from sampled corn accessions as template. The reaction mixture included template DNA, forward and reverse primers, PCR buffer mix, dNTPs, Taq polymerase, and sterile nano-pure water. Amplification was carried out through initial denaturation, repeated cycles of denaturation, annealing, and extension, followed by final extension. The resulting PCR products were separated through agarose gel electrophoresis, stained with a nucleic acid dye, and visualized under UV illumination to determine the presence and size of the expected amplicons. Purified PCR products were then subjected to Sanger sequencing, and the resulting sequences were analyzed through BLAST, translation, protein homology modeling, and multiple sequence alignment.

2.7 Data Analysis Techniques

The study used computational and comparative sequence analysis techniques. In silico PCR was used to confirm the predicted amplification site and product length of the designed primer. BLAST analysis was used to determine sequence similarity, identity, query coverage, and homology between the amplified product and publicly available maize sequences (Altschul et al., 1990). Protein homology was assessed by translating the nucleotide sequence using the ExPASy Translate Tool (ExPASy, n.d.) and generating predicted models through PHYRE2 (Kelly et al., 2015). Multiple sequence alignment using ClustalW in MEGA X was applied to compare the target reference sequence, homologous sequence, in silico PCR result, and experimentally obtained sequence, allowing the study to evaluate sequence conservation, nucleotide identity, and correspondence among the analyzed sequences (Tamura et al., 2021).

3. Results and Discussion

3.1 Molecular Marker Development and Validation

3.1.1 Primer Targeting and Amplification Pattern

The designed primer successfully targeted a genomic region on chromosome 8 of the corn genome. In silico prediction identified the expected amplification site within the intended locus, and the observed amplification supported the primer's ability to bind to the targeted genomic region. The designed primer pair produced an expected product length of 185 bp, with the forward and reverse primers showing suitable length, melting temperature, GC content, and complementarity values for amplification. This result indicates that the primer design strategy was effective in directing amplification toward the intended chromosome 8 region. Since this locus was previously associated with fall armyworm resistance through GWAS, the successful targeting of this region supports the potential relevance of the marker for future molecular studies and resistance screening in corn (Warburton et al., 2022).

3.1.2 In Silico Prediction and Experimental Amplicon

The in silico PCR analysis predicted an amplification product located at chr8:172209729–172209913, corresponding to a 185 bp chromosome 8 region. However, the experimentally obtained amplicon measured 153 bp, which was shorter than the predicted product. Despite this difference, the sequenced fragment still corresponded to the intended genomic region,

suggesting that the primer amplified a related sequence within the target locus. The difference between the predicted and experimental product lengths may be associated with sequence variation in primer binding sites, polymorphisms among corn genotypes, or partial amplification of a homologous region. Although the amplified fragment was shorter than expected, the result still supports the primer's specificity for a chromosome 8 genomic region and provides preliminary evidence that the marker can detect a related locus within the corn genome.

3.1.3 In Silico and Sequence-Based Validation

The in silico PCR result confirmed that the designed primer was expected to amplify a chromosome 8 locus within the selected genomic region. This predicted amplification corresponded to the genomic area associated with fall armyworm resistance reported by Warburton et al. (2022). The sequence-based validation further supported the connection between the amplified product and the intended target region. The agreement between the computational prediction and the selected target region supports the accuracy of the primer design process. This strengthens the preliminary validity of the marker because its target site was not randomly selected but was anchored on a previously reported resistance-linked genomic region. The result suggests that the marker may be useful for further validation in fall armyworm resistance-related molecular studies.

3.1.4 BLAST and Protein Homology Assessment

BLAST analysis showed that the amplified sequence had significant homology with a *Zea mays* clone sequence identified as EU969166.1. The result showed 95% similarity, an E-value of 3e-44, and 75% query coverage. These values indicate that the PCR product was derived from the maize genome and had substantial similarity with a known maize sequence. Protein homology modeling produced several candidate models with different confidence and coverage values, and the selected model showed 22.5% confidence and 48% coverage. These findings support the specificity of the amplification and reduce the likelihood of contamination or non-target amplification. The BLAST result confirms that the designed primer amplified a corn genomic region consistent with the intended locus. Although some PHYRE2 models had higher confidence but lower coverage, the selected model provided the best balance between confidence and sequence representation, making it the most appropriate option for subsequent interpretation.

3.1.5 Multiple Sequence Alignment and Percent Identity

Multiple sequence alignment showed conserved nucleotide regions among the reference sequence NC_050103.1, the homologous sequence EU969166.1, the experimentally obtained amplicon, and the in silico PCR product. Pairwise comparison revealed varying levels of nucleotide identity. The reference sequence showed 88.24% identity with the experimentally obtained amplicon and 88.0% identity with the in silico-derived sequence. The reference sequence also showed higher similarity with the homologous clone sequence at 97.77%. The experimentally sequenced product showed 83.02% identity with the homologous clone sequence and 88.24% identity with both the reference and in silico sequences. The in silico sequence showed 82.58% identity with the homologous clone sequence. These results indicate that the amplified region corresponds to a partially conserved chromosome 8 locus. The presence of conserved segments supports the conclusion that the primer amplified a homologous maize genomic region, while the observed differences suggest possible allelic variation, polymorphism among corn germplasm, or the presence of closely related paralogous sequences. Overall, the alignment results support the preliminary specificity of the marker while also showing that further validation is necessary before it can be applied confidently in marker-assisted selection.

3.2 Overall Interpretation of Marker Applicability

The combined results from primer targeting, in silico PCR, experimental amplification, BLAST analysis, protein homology modeling, and sequence alignment indicate that the designed marker can amplify a maize genomic region related to the intended chromosome 8 target. The marker demonstrated partial conservation across analyzed sequences, with identity values showing both similarity and moderate sequence divergence. These findings suggest that the designed marker has potential value as a preliminary tool for studying chromosome 8 regions associated with fall armyworm resistance. However, because the marker showed sequence variation and produced an experimentally shorter amplicon than predicted, it should still be considered putative. Further validation across broader and more diverse corn germplasm is necessary to confirm its specificity, stability, and usefulness for resistance screening and molecular breeding applications.

4. Conclusion & Recommendations

This study presented the preliminary in silico development and evaluation of a candidate gene-derived molecular marker targeting a putative chromosome 8 locus in corn (*Zea mays* L.). Primer design based on NC_050103.1 and supported by previously reported GWAS evidence (Warburton et al., 2022) produced a predicted chromosome 8 amplicon through in silico PCR. Sequence characterization showed that the amplified fragment shared 88.24% identity with the target reference sequence and demonstrated homology with a Zea mays clone sequence (EU969166.1), supported by BLAST results showing 95% similarity, an E-value of 3e-44, and 75% query coverage. ClustalW multiple sequence alignment showed variable nucleotide identity ranging from 82.58% to 100%, indicating conserved regions but also moderate divergence that

may reflect genetic variation, polymorphism, or related genomic regions. Overall, the findings provide preliminary evidence that the designed marker may target a chromosome 8 genomic region

Further validation should be conducted across a broader and more diverse set of corn germplasm to confirm the specificity, stability, robustness, and practical applicability of the developed marker. Additional testing is necessary before the marker can be confidently used for marker-assisted selection in fall armyworm resistance studies.

The marker should be considered putative at this stage because the observed sequence divergence and moderate identity levels indicate possible genetic variation within the target locus or amplification of related genomic regions. The study provides only preliminary evidence, and the marker still requires further validation before it can be established as a reliable tool for fall armyworm resistance screening.

Compliance with ethical standards

The author declares that there are no conflicts of interest in relation to this study. The research was conducted without any commercial of financial relationships that could be interpreted as a potential conflict of interest. The author gracefully acknowledges Cagayan State University for its continuous support and encouragement during the course of this study. Deep appreciation is also extended to the Department of Agriculture – Cagayan Valley Research Center for granting access to their laboratory facilities and for the invaluable technical assistance provided. The completion of this research would not have been possible without their generosity and support.

REFERENCES

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology, 215*(3), 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2.

Day, R., Abrahams, P., Bateman, M., et al. (2017). Fall armyworm: Impacts and implications for Africa. *Outlooks on Pest Management, 28*(5), 196–201. https://doi.org/10.1564/v28_oct_02.

Doyle, J. J., & Doyle, J. L. (1990). Isolation of plant DNA from fresh tissue. *Focus, 12*, 13–15.

ExPASy. (n.d.). *Translate tool (Expasy Translate)*. Swiss Institute of Bioinformatics. https://web.expasy.org/translate/

Goergen, G., Kumar, P. L., Sankung, S. B., Togola, A., & Tamò, M. (2016). First report of outbreaks of fall armyworm in Africa. *PLOS ONE, 11*(10), e0165632. https://doi.org/10.1371/journal.pone.0165632.

Kelly, L. A., Mezulis, S., Yates, C. M., Wass, M. N., & Sternberg, M. J. E. (2015). *The Phyre2 web portal for protein modeling, prediction and analysis*. Nature Protocols**, 10**(6), 845–858. https://doi.org/10.1038/nprot.2015.053.

Sievers, F., & Higgins, D. G. (2018). Clustal Omega for accurate multiple sequence alignment. *Protein Science, 27*(1), 135–145. https://doi.org/10.1002/pro.3290.

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution, 38*(7), 3022–3027. https://doi.org/10.1093/molbev/msab120.

Warburton, M. L., Woolfolk, S. W., Smith, J. S., Hawkins, L. K., Castaño-Duque, L., LeBar, M. D., & Williams, W. P. (2022). Genes and genetic mechanisms contributing to fall armyworm resistance in corn. *The Plant Genome*. https://doi.org/10.1002/tpg2.20311.

Ye, J., Coulouris, G., Zaretskaya, I., Cutcutache, I., Rozen, S., & Madden, T. L. (2012). Primer-BLAST: A tool to design target-specific primers for PCR. *BMC Bioinformatics, 13*, 134. https://doi.org/10.1186/1471-2105-13-134.