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Molecular characterisation and phylogeographic patterns of *Maruca vitrata,* on black gram in rainfed and SAT regions of Andhra Pradesh

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The legume spotted pod borer, *Maruca vitrata* Fabricius, is a significant pest of leguminous crops in tropical and subtropical regions, particularly in Andhra Pradesh, India. This study was conducted during *Rabi* 2023-2024 at the Department of Entomology, Sri Krishnadevaraya College of Horticultural Sciences, Ananthapuram, to investigate the genetic variability and phylogeographic patterns of *M. vitrata* populations across Rainfed and Semi-Arid Tropical (SAT) regions of Andhra Pradesh, providing insights into how environmental conditions influence pest adaptation. A total of 10 larvae were collected from key black gram growing areas in Rainfed (Nellore, Vijayawada, Guntur, Bapatla & Tirupathi) and SAT (Kadapa, Anantapur & Prakasam) regions. DNA was isolated and the mitochondrial cytochrome-c oxidase subunit I (mtCOI) gene was amplified and sequenced, yielding an average sequence length of 700 bp. Phylogenetic analysis revealed distinct genetic clustering, with SAT populations exhibiting greater genetic divergence, forming separate clades from those in Rainfed regions. This divergence is indicative of adaptation to the harsher climatic conditions of SAT regions. Sequence alignment revealed unique haplotypes in SAT samples, contrasting with a more conserved genetic sequence in Rainfed populations. The unique genetic makeup of SAT populations suggests they may respond differently to control measures, such as insecticides or biocontrol agents, compared to their counterparts in Rainfed regions. Moreover, the high genetic diversity observed in SAT regions raises concerns about the potential for developing insecticide resistance. This study provides critical insights into the evolutionary dynamics of *M. vitrata*, on black gram crop in Andhra Pradesh.

Keywords: Black gram, Rainfed, Haplotypes, Larvae, Molecular identification

The legume spotted pod borer, *Maruca vitrata*, is a significant agricultural pest with a broad distribution across tropical and subtropical regions, particularly in Africa and Asia. This pest is notorious for causing severe damage to economically important leguminous crops such as, pigeon pea (*Cajanus cajan*), cowpea (*Vigna unguiculata*), mung bean (*Vigna radiata*), and black gram (*Vigna mungo*), leading to significant yield losses[1,2]. The larvae of *M. vitrata* feed on floral buds, flowers, and developing pods, directly impacting crop yield and quality. The pest's adaptability to various climatic conditions and its wide host range further complicate management efforts, making it a formidable challenge for agricultural production in the affected regions[3]. Molecular tools, particularly DNA barcoding using the mitochondrial cytochrome-c oxidase subunit I (mtCOI) gene, have emerged as powerful and reliable methods for species identification and population genetic studies[4]. These techniques enable precise species delimitation and facilitate the study of intraspecific genetic diversity, which is crucial for understanding the evolutionary dynamics of pest populations and developing localised pest management strategies[5,6].

Traditional morphological methods often fall short in accurately identifying *Maruca* species due to their close resemblance, which hampers the effectiveness of pest management strategies[7]. This identification challenge has significant implications for the implementation of targeted control measures. However, molecular tools, particularly DNA barcoding using the mitochondrial cytochrome-c oxidase subunit I (mtCOI) gene, have emerged as powerful and reliable methods for species identification and population genetic studies. Recent advancements in next-generation sequencing (NGS)

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and phylogenetic analyses have further enhanced the ability to investigate the evolutionary history and geographic structuring of *M. vitrata* populations[8].

In *M. vitrata*, the differences between rainfed and SAT regions can drive genetic divergence, influencing factors such as pest virulence, host plant preferences, and resistance to control measures[9,10]. In rainfed regions, where crop production is highly dependent on unpredictable rainfall patterns, *M. vitrata* populations may exhibit genetic traits that confer adaptability to fluctuating moisture levels and associated changes in host plant availability[11]. In SAT regions; the evolutionary pressures are different but equally significant. The extreme climatic conditions can lead to the selection of traits that enhance survival under water-limited conditions, such as altered metabolic rates or behavioural changes that reduce water loss[12]. These adaptive responses can result in genetically distinct populations that are better suited to the SAT environment, further complicating pest management efforts[13]. By comparing the genetic profiles of populations from these two regions, one can identify potential barriers to gene flow, areas of high genetic diversity, and regions where pest populations are particularly robust or vulnerable. The study of *M. vitrata* populations across rainfed and SAT regions of Andhra Pradesh is critical for understanding the pest′s adaptation to diverse agro-ecological conditions.

Materials and Methods

Sample collection

This study was conducted during Rabi 2023-2024 at Department of Entomology, Sri Krishnadevaraya College of Horticultural Sciences, Ananthapuram to investigate the genetic variability and phylogeographic patterns of *M. vitrata* populations across rainfed and Semi-Arid Tropical (SAT) regions of Andhra Pradesh, providing insights into how environmental conditions influence pest adaptation. Field surveys were conducted across multiple legume-growing rainfed (Bapatla, Guntur, Vijayawada, Nellore & Tirupati) and SAT (Anantapur, Kadapa & Prakasam) regions of Andhra Pradesh to collect *M. vitrata* larvae **(**Fig. 1**)**. The selection of sampling sites was based on the prevalence of leguminous crops and the historical presence of *M. vitrata* infestations.

Larvae were taxonomically identified prior to collection based on their morphological characteristics. The body is semitransparent, light yellow in colour, with distinct black spots on each segment, although the intensity of spotting may vary[14] (Fig. 2). These spots typically fade before pupation. The larvae undergo five instars, lasting between 8 to 10 days[15], and in some cases up to 16 days[16], depending on climatic conditions and host plant species. Specimens were collected from black gram using the handpicking method. The collected larvae were immediately preserved in 95% ethanol and transported to the laboratory for further analysis[17].

DNA isolation and amplification

Genomic DNA was isolated from individual larvae using the DNAase Qiagen kit, following a modified protocol to ensure high yield and purity. DNA concentration and quality were assessed using a NanoDrop spectrophotometer, with a concentration

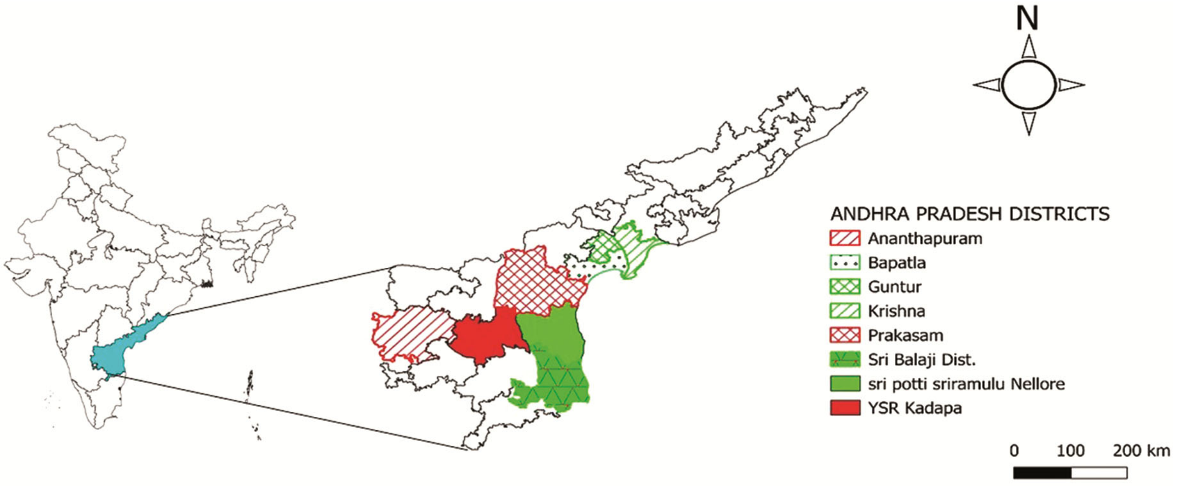

Fig. 1 — *Maruca vitrata* samples collected from rainfed (Bapatla, Guntur, Vijayawada, Nellore & Tirupati) and SAT (Anantapur, Kadapa & Prakasam) regions of Andhra Pradesh. [Only for education purpose, not applicable for legal purpose]

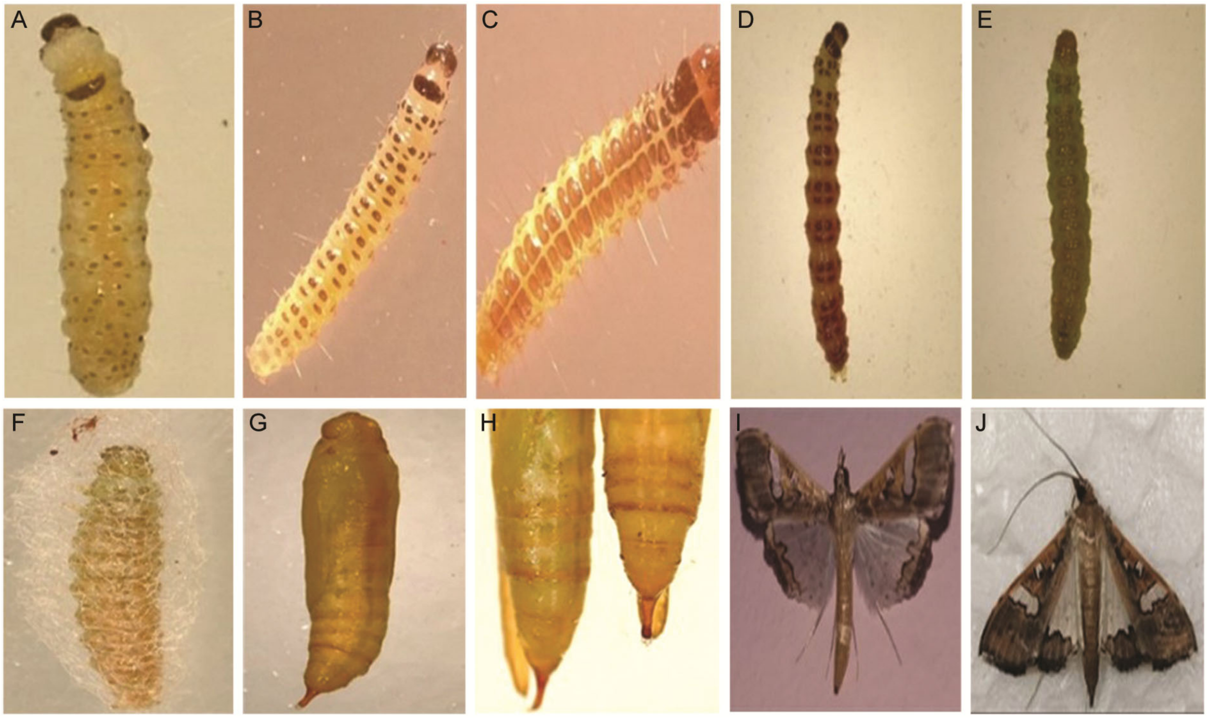

Fig. 2 — Different life stages of Spotted pod borer *M. vitrata* on black gram.

range between 50 to 200 ng/µL deemed optimal for PCR amplification (ProFlex™ PCR System, 96-well; A30756). The mitochondrial cytochrome-c oxidase subunit I (*mtCOI*) gene was selected for amplification due to its high degree of conservation and widespread use in phylogenetic studies. The primers LCO 1490 and HCO 2198 were utilized to amplify a 700 bp fragment of the *mtCOI gene*, which has been demonstrated to effectively differentiate closely related species[4].

Gel electrophoresis and PCR product purification

The PCR products were resolved on a 1.5% agarose gel stained with ethidium bromide, and the resulting bands were visualised underUV light using a gel documentation system (SYNGENE Gene flash, U.K.). A 100 bp DNA ladder was used to estimate the size of the amplified products. The PCR products were confirmed by gel electrophoresis and then purified using the Qiagen Gel Extraction Kit for downstream sequencing[18.]The purified DNA was quantified and checked for purity using a NanoDrop spectrophotometer before being sent for sequencing[19].

Sequencing and phylogenetic analysis

Sequencing was performed at Barcode Bio Sciences, Bangalore, using the Sanger sequencing method. The raw sequence data were edited and aligned using BioEdit 7.2.5, and sequence identity was confirmed through BLAST searches against the NCBI database. Finally, sequences were submitted to the National Center for Biotechnology Information (NCBI). Later Phylogenetic relationships were inferred using the Maximum Likelihood method implemented in Bio-R software[20,21]. The phylogenetic trees were constructed with 1000 bootstrap replicates to assess the robustness of the nodes.

Results and Discussion

The phylogenetic analysis of *M. vitrata* populations from SAT (Kadapa, Anantapur, Prakasam) and rainfed regions (Nellore, Vijayawada, Guntur, Bapatla) of Andhra Pradesh reveals distinct genetic clustering that reflects the environmental and geographical factors influencing these regions. The phylogenetic tree shows clear differentiation between populations from SAT and rainfed regions. The SAT regions exhibit significant genetic divergence, forming distinct clades separate from those of the rainfed regions. This divergence is indicative of the unique evolutionary pressures faced by *M. vitrata* in the semi-arid tropics (SAT), which include harsher climatic conditions, water scarcity, and potentially different host plant interactions. In contrast, the rainfed regions, characterised by more stable and

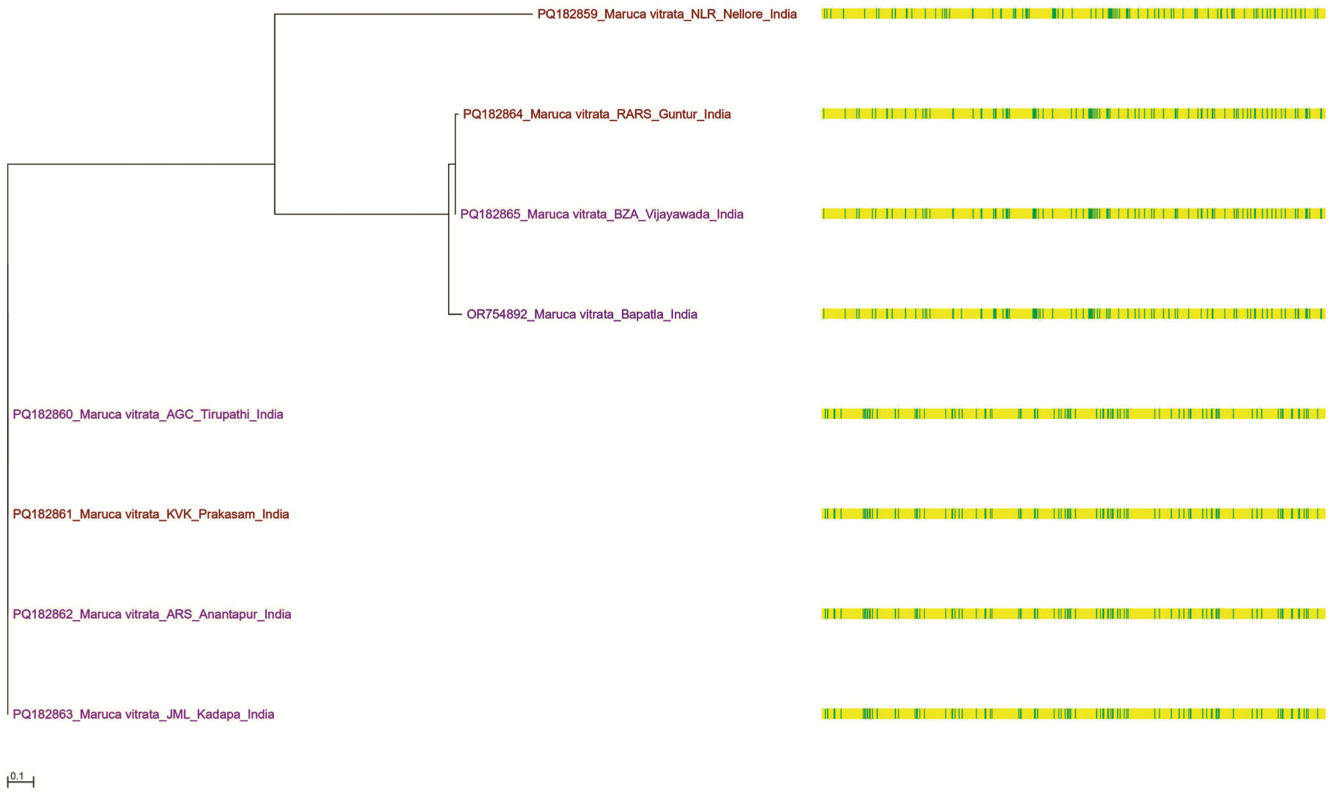

Fig. 3 — Phylogenetic relationships among *Maruca vitrata* populations from rainfed and SAT regions on black gram crop in Andhra Pradesh.

moderate environmental conditions, show less genetic divergence among their *M. vitrata* populations. The samples from Nellore, Vijayawada, Guntur, and Bapatla form a closer cluster, suggesting a higher degree of genetic similarity and possibly more gene flow between these populations.

The sequence alignment further supports the phylogenetic findings, highlighting the genetic variability between the populations. In SAT regions, particularly Kadapa (PQ182863), Anantapur (PQ182862), and Prakasam (PQ182861), the alignment shows a higher frequency of sequence variations (highlighted in yellow), suggesting these populations have unique haplotypes. These variations may be adaptive responses to the challenging environmental conditions of the SAT regions, such as extreme temperatures and limited water availability. On the other hand, the rainfed regions, including Nellore (PQ182859), Vijayawada (PQ182865), Guntur (PQ182864), and Bapatla (OR754892), display more conserved genetic sequences with fewer variations (Fig. 3). This conservation likely reflects the more homogeneous environmental conditions of these regions, which do not exert as strong selective pressures on the populations. The genetic divergence observed in the SAT regions suggests that *M. vitrata* populations in these areas may have developed unique ecological adaptations. For instance, these populations might exhibit different behavioural or physiological traits that enhance survival under the specific environmental stresses of SAT regions. The presence of unique haplotypes in these regions also indicates limited gene flow between SAT and rainfed regions, possibly due to differing ecological niches. In contrast, the closer genetic relationship among populations from rainfed regions implies that these areas may be more interconnected, through agricultural practices that facilitate gene flow. The reduced genetic divergence in these populations suggests that they may respond more uniformly to environmental changes or pest management strategies.

Detailed comparative analysis of *M. vitrata* populations from SAT and rainfed regions of Andhra Pradesh highlights the significant genetic diversity driven by environmental factors. The presence of genetically distinct populations suggests that a one-size-fits-all approach to pest management may not be effective. Instead, region-specific strategies that consider the unique genetic makeup of *M. vitrata* populations are essential for achieving sustainable control[22]. For example, the high genetic similarity among southern populations suggests that control measures effective in one region may be applicable across other regions in southern India. However, the genetic divergence observed in populations from northern regions, such as Delhi, suggests that different management strategies may be required to control *M. vitrata* in these areas[19]. The detection of genetic diversity within *M. vitrata* populations also raises concerns about the potential for insecticide resistance development. Populations with high genetic diversity are more likely to harbour resistance alleles, which can spread through gene flow and compromise the effectiveness of control measures. Monitoring genetic diversity and resistance alleles in *M. vitrata* populations should be an integral part of integrated pest management (IPM) strategies to mitigate the risk of resistance and ensure the long-term efficacy of control methods[23].

By examining mitochondrial genome data, researchers have gained insights into the genetic variation across different regions, informing strategies to mitigate the pest's impact through targeted interventions[1]. In Andhra Pradesh, India, the agricultural zones are broadly classified into rainfed and Semi-Arid Tropical (SAT) regions, each presenting unique environmental challenges that influence the population dynamics and genetic structure of *M. vitrata*. Rainfed regions are characterised by their reliance on seasonal rainfall, leading to high variability in water availability, which can cause periods of drought stress interspersed with intense rainfall. These environmental conditions not only affect crop health but also exert selective pressures on pest populations, potentially leading to distinct genetic adaptations. In contrast, the SAT regions are characterised by their hot and dry climate, with erratic and scant rainfall, which further complicates agricultural practices[24]. The harsh conditions of SAT regions are likely to impose even stronger selective pressures on *M. vitrata*, possibly leading to greater genetic differentiation and the emergence of specialised ecotypes

Moreover, the phylogeographic structuring observed in this study provides insights into the historical biogeography of *M. vitrata* in India. The divergence between rainfed and SAT region populations could be indicative of historical climatic barriers or differences in host plant distribution that have shaped the evolutionary trajectory of this pest. Understanding these historical factors is crucial for predicting how *M. vitrata* populations might respond to future environmental changes, such as shifts in climate or agricultural practices[25,26]. Furthermore, exploring the role of horizontal gene transfer or hybridization events in generating genetic diversity within *M. vitrata* populations could offer new perspectives on the evolution of this pest[27,28]. By tailoring management strategies to the genetic and ecological characteristics of *M. vitrata* populations, it is possible to enhance the effectiveness of control measures and reduce the economic impact of this pest on leguminous crops in India[6]. The molecular characterisation of *M. vitrata* populations in these regions, using advanced techniques such as mitochondrial DNA analysis, provides insights into how these pests have evolved in response to the distinct environmental conditions of rainfed and SAT zones[29,30]. For instance, climatic factors such as temperature and humidity have been shown to influence the distribution and genetic diversity of agricultural pests[30]. The genetic variability observed in pest populations across different agro-ecological zones is often a reflection of these environmental stresses.

Conclusion

In conclusion, understanding the genetic differences between *M. vitrata* populations in SAT and rainfed regions is crucial for developing effective pest management strategies. The unique genetic makeup of the SAT populations suggests that they may respond differently to control measures, such as insecticides or biocontrol agents, compared to their counterparts in rainfed regions. Therefore, region-specific pest management strategies tailored to the genetic characteristics of *M. vitrata* in each region are recommended. For example, the unique haplotypes found in SAT regions may be targeted for the development of specialised biocontrol agents or resistant crop varieties that are better suited to the conditions of these areas. In rainfed regions, where populations are more genetically similar, a more uniform approach to pest management may be effective. Future research should focus on exploring the functional significance of the observed genetic variations and their implications for pest management in different agro-ecological zones.

Conflict of interest

The authors declare no competing interests.

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