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In-field screening of elite Urdbean genotypes for pod borer complex tolerance using pest damage scoring, biochemical markers, and PCR-based molecular analysis

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Abstract
Urdbean (*Vigna mungo*), an important pulse crop, suffers significant yield losses due to infestation by the pod borer complex, including *Helicoverpa armigera* and *Maruca vitrata*. This study aimed to screen elite urdbean genotypes for tolerance to pod borer infestation using an integrated approach combining pest damage scoring, biochemical marker analysis, and PCR-based molecular techniques. Field experiments were conducted under natural infestation conditions, and pest damage was evaluated using a standard damage scoring scale. Biochemical assays were performed to quantify defense-related markers, including chitinase, polyphenol oxidase (PPO), and peroxidase (POD) activities. PCR analysis targeted resistance-associated genes using specific molecular markers. The results revealed significant variation among genotypes in pest damage levels, biochemical defense responses, and the presence of resistance-associated genes. This study provides a robust platform for identifying pest-tolerant genotypes and emphasizes the importance of integrating morphological, biochemical, and molecular tools for sustainable pest management in urdbean breeding programs.

Keywords: Urdbean, pod borer complex, pest damage scoring, biochemical markers, PCR analysis, resistance screening

1. Introduction

Urdbean (*Vigna mungo*), commonly known as black gram, is a vital pulse crop cultivated across tropical and subtropical regions, contributing significantly to human nutrition due to its high protein content and agricultural sustainability [1]. However, the productivity of urdbean is severely hampered by biotic stresses, particularly damage caused by the pod borer complex, including *Helicoverpa armigera* and *Maruca vitrata* [2]. These pests directly affect reproductive structures, causing significant yield losses ranging from 30% to 80% depending on environmental factors and infestation severity [3].

Chemical insecticides remain the primary control measure against pod borers, but their over-reliance has led to pesticide resistance, environmental pollution, and adverse effects on non-target organisms [4]. Therefore, the development of host plant resistance (HPR) offers an ecologically sustainable and cost-effective solution for pest management [5].

Screening urdbean germplasm for resistance to pod borers requires a multidisciplinary approach, combining morphological screening, biochemical marker analysis, and molecular tools to comprehensively evaluate tolerance mechanisms [6]. Field-level screening using pest damage scoring systems provides a reliable method to identify tolerant genotypes under natural infestation conditions [7]. Additionally, biochemical markers such as chitinase, polyphenol oxidase (PPO), and peroxidase (POD) play critical roles in plant defense mechanisms [8].

At the molecular level, Polymerase Chain Reaction (PCR) analysis using specific molecular markers enables the identification of resistance-associated genes, facilitating marker-assisted breeding (MAS) programs [9]. Combining these tools provides a robust platform for identifying and characterizing pest-tolerant urdbean genotypes, which can be further utilized in breeding programs for developing resistant varieties. This study aims to screen elite urdbean genotypes for pod borer complex tolerance using pest damage scoring, biochemical marker analysis, and PCR-based molecular analysis under natural field conditions.

2. Materials and Methods

2.1 Plant Material and Field Experimentation

Fifteen elite urdbean (*Vigna mungo*) genotypes, including ten resistant and five susceptible lines, were procured from certified germplasm banks such as the National Bureau of Plant Genetic Resources (NBPGR, New Delhi), International Crops Research Institute for the Semi-Arid Tropics (ICRISAT, Hyderabad), and the United States Department of Agriculture (USDA) Germplasm Collection Bank (Griffin, Georgia, USA). These genotypes were selected based on their historical performance against pod borer infestations and genetic diversity. The field experiment was conducted in a randomized complete block design (RCBD) with three replications to ensure statistical robustness. Plants were grown under natural infestation conditions during the peak reproductive stage, and standard agronomic practices, including fertilization, irrigation, and pest control (excluding chemical insecticides), were followed to maintain optimal growth conditions. Pest damage assessment was performed using a standardized visual damage scoring system (1–9 scale), where a lower score indicated higher tolerance. Larval density per plant and pod damage percentage were recorded manually at regular intervals during the flowering and pod development stages. Plant growth parameters such as height, number of pods per plant, and grain yield per genotype were documented for comparative analysis.

2.2 Biochemical and Molecular Analysis

Leaf and pod samples were collected from both resistant and susceptible genotypes at peak infestation stages for biochemical and molecular analysis. Total protein extraction was performed using the Tris-HCl buffer method (pH 7.5) supplemented with phenylmethylsulfonyl fluoride (PMSF) as a protease inhibitor. Biochemical assays were conducted to quantify the activity of defense-related enzymes, including chitinase, polyphenol oxidase (PPO), and peroxidase (POD), using spectrophotometric analysis. The protein concentration in each sample was determined using the Bradford assay. For molecular analysis, genomic DNA was extracted using the CTAB method, followed by Polymerase Chain Reaction (PCR) using resistance-specific primers targeting genes associated with pod borer resistance. Amplified PCR products were separated on a 1.5% agarose gel, stained with ethidium bromide, and visualized using a gel documentation system. Statistical analysis of field and laboratory data was performed using Analysis of Variance (ANOVA) at a significance level of $p < 0.05$. Pearson's correlation coefficient was calculated to determine the relationships between pest damage scores, biochemical markers, and molecular marker expression profiles.

3. Results

3.1 Pest Damage Scoring

Table 1: Pest Damage Scoring and Larval Count Analysis

Genotype	Damage Score (1–9)	Larval Count (per plant)
Resistant-1	2.3	1.5
Resistant-2	2.8	1.7
Susceptible-1	8.5	6.2

The results presented in Table 1 highlight significant variability in pest damage scores and larval counts among the urdbean genotypes screened under field conditions. Resistant genotypes (Resistant-1 and Resistant-2) exhibited consistently lower damage scores (2.3 and 2.8, respectively) and fewer larvae per plant (1.5 and 1.7, respectively) compared to susceptible genotypes (Susceptible-1), which recorded a damage score of 8.5 and an average larval count of 6.2. These findings indicate a clear correlation between genotype resistance levels and reduced pest infestation. The statistical analysis ($p < 0.05$) confirmed the significance of the observed differences, suggesting that resistant genotypes possess inherent tolerance mechanisms against pod borer infestation. The low pest damage scores in resistant genotypes can be attributed to structural and biochemical defenses, which limit pest feeding and reproduction. Conversely, susceptible genotypes showed higher larval density, signifying an absence or reduced efficiency of these defense mechanisms. These findings align with previous studies on pigeon pea and chickpea, where resistant varieties demonstrated reduced pest damage and lower larval survival rates due to enhanced biochemical defenses.

3.2 Biochemical Marker Analysis

Table 2: Biochemical Marker Analysis

Genotype	Chitinase (U/mg)	PPO (U/mg)	POD (U/mg)
Resistant-1	85.4	62.1	55.3
Resistant-2	78.2	58.4	50.7
Susceptible-1	25.6	18.7	15.4

Table 2 presents the enzymatic activity levels of key biochemical defense markers, including chitinase, polyphenol oxidase (PPO), and peroxidase (POD), across the selected urdbean genotypes. Resistant genotypes (Resistant-1 and Resistant-2) displayed significantly higher enzymatic activities compared to susceptible genotypes (Susceptible-1). Chitinase activity was recorded at 85.4 U/mg and 78.2 U/mg in Resistant-1 and Resistant-2, respectively, while it was substantially lower in Susceptible-1 (25.6 U/mg). Similarly, PPO and POD activities followed the same trend, with resistant genotypes exhibiting significantly higher enzyme levels.

These elevated enzyme activities suggest an active biochemical defense response in resistant genotypes, contributing to their ability to counteract pest attacks. Chitinase is known to degrade the chitinous exoskeleton of insect pests, while PPO and POD are involved in oxidative stress responses and lignification, creating physical barriers against pest penetration. The statistical analysis confirmed the significance of these differences ($p < 0.05$), supporting the hypothesis that these enzymes play a crucial role in urdbean's defense mechanisms.

A strong positive correlation was observed between enzymatic activity levels and reduced pest damage scores, indicating a direct relationship between enhanced biochemical defenses and pod borer tolerance. These results are consistent with findings in chickpea and soybean, where increased chitinase and POD activities were associated with pest resistance.

3.3 PCR Analysis

Resistance-associated markers were amplified in resistant genotypes but absent in susceptible ones.

Combining observations from Table 1 and Table 2, a clear trend emerges: genotypes with lower pest damage scores and larval counts exhibited higher enzymatic activities. Resistant genotypes demonstrated an integrated defense strategy involving both structural barriers and enhanced biochemical responses.

The significant correlation ($p < 0.05$) between pest damage scores and enzyme activities suggests that chitinase, PPO, and POD can serve as biochemical markers for pest resistance screening in urdbean breeding programs. These markers offer a reliable and reproducible method for identifying and validating pest-tolerant genotypes.

Overall, the results emphasize the importance of integrating pest damage scoring and biochemical marker analysis for robust screening of urdbean genotypes against pod borer infestation. These findings contribute to a deeper understanding of the molecular and biochemical mechanisms underpinning pest resistance in urdbean and provide valuable insights for breeding programs aimed at developing pest-tolerant varieties.

4. Discussion

The present study aimed to evaluate elite urdbean (*Vigna mungo*) genotypes for tolerance against the pod borer complex using a multi-dimensional approach, including pest damage scoring, biochemical marker analysis, and PCR-based molecular analysis. The findings revealed significant variations among the genotypes in terms of pest damage, biochemical responses, and molecular marker expression, providing valuable insights into the underlying mechanisms of resistance.

The pest damage assessment showed clear differences between resistant and susceptible genotypes. Resistant genotypes (Resistant-1 and Resistant-2) consistently exhibited lower damage scores and reduced larval counts per plant when compared to susceptible genotypes (Susceptible-1). These observations suggest that resistant genotypes possess inherent defense mechanisms, either structural or biochemical, that deter pod borer infestation. Lower pest damage in resistant genotypes could be attributed to factors such as thicker pod walls, increased trichome density, or biochemical compounds that act as deterrents to pest feeding. In contrast, susceptible genotypes showed higher larval densities, indicating a failure in activating defense responses upon pest attack. These findings are consistent with earlier studies conducted on chickpea and pigeon pea, where similar trends in reduced pest damage and larval density were observed in resistant varieties. The statistical significance ($p< 0.05$) of the observed differences in pest damage scores reinforces the reliability of these results and highlights the genetic basis of pest tolerance in urdbean.

The biochemical analysis further supported the observed phenotypic variations between resistant and susceptible genotypes. Enzymatic activities of key biochemical markers, including chitinase, polyphenol oxidase (PPO), and peroxidase (POD), were significantly higher in resistant genotypes compared to susceptible ones. Chitinase, an enzyme responsible for breaking down chitin in insect exoskeletons, was found in higher concentrations in resistant lines, indicating its active role in restricting pest growth and reproduction. PPO and POD, both involved in oxidative stress management and lignin biosynthesis, were also significantly upregulated in resistant genotypes. These enzymes contribute to reinforcing cell walls and creating toxic environments for pests, thereby preventing further damage. The strong correlation between high enzyme activity and reduced pest damage scores suggests that these biochemical markers are closely linked to pest resistance mechanisms. These results align with earlier findings in chickpea and soybean, where increased chitinase and peroxidase activities were associated with reduced pest damage and higher resistance levels.

At the molecular level, PCR analysis revealed the presence of resistance-associated markers in resistant genotypes, which were either absent or faintly expressed in susceptible lines. The successful amplification of resistance-associated genes, particularly those linked to PR proteins and protease inhibitors, indicates the genetic basis of resistance in these genotypes. PR proteins are known to disrupt pest feeding and digestion processes, while protease inhibitors interfere with the activity of insect gut enzymes, reducing pest fitness and survival. The absence of these molecular markers in susceptible genotypes highlights the genetic vulnerability of these lines, reinforcing the importance of molecular screening in identifying and validating resistant genotypes.

The integration of field-based pest damage scoring, biochemical markers, and molecular tools in this study allowed for a comprehensive evaluation of pod borer resistance mechanisms in urdbean. The correlation between low pest damage scores, high biochemical marker activity, and the presence of resistance-associated genes underscores the complementary nature of these tools in pest resistance screening. Genotypes exhibiting low damage scores, higher enzymatic activities, and positive amplification of resistance genes emerged as strong candidates for pest-resistant breeding programs.

Furthermore, the statistical analysis revealed significant positive correlations between biochemical marker activities and pest damage scores, highlighting their reliability as indicators of pest resistance. Chitinase, PPO, and POD activities were found to be directly proportional to the degree of resistance, emphasizing their utility as biochemical markers for breeding purposes. These findings contribute to a growing body of evidence supporting the role of biochemical defense mechanisms in pest resistance across legume crops.

This study also highlights the limitations of relying on a single screening approach for pest resistance. Field-based assessments provide practical insights but may be influenced by environmental factors, while biochemical and molecular tools offer deeper insights into the underlying mechanisms but require validation across diverse environments. Combining these approaches ensures a more robust and reliable identification of pest-tolerant genotypes.

In comparison with previous studies on chickpea, pigeon pea, and soybean, the findings of this study align with established knowledge regarding the role of PR proteins, protease inhibitors, and antioxidant enzymes in plant defense. However, this study uniquely integrates field, biochemical, and molecular screening methods, providing a holistic approach to resistance evaluation. Such integrated methodologies are essential for addressing complex pest resistance mechanisms and enhancing breeding efficiency in pulse crops.

5. Conclusion

This study successfully evaluated elite *Vigna mungo* (urdbean) genotypes for tolerance against the pod borer complex using an integrated approach combining pest damage scoring, biochemical marker analysis, and PCR-based molecular techniques. The results revealed significant differences between resistant and susceptible genotypes, with resistant varieties (*Resistant-1* and *Resistant-2*) consistently exhibiting lower pest damage scores, reduced larval counts, and higher biochemical defense enzyme activities, including chitinase, polyphenol oxidase (PPO), and peroxidase (POD). Molecular analysis confirmed the presence of resistance-associated markers, further validating the observed phenotypic and biochemical responses.

The correlation analysis demonstrated a significant positive relationship between enzymatic activity and reduced pest damage, indicating that these biochemical markers are reliable indicators of resistance. Genotypes with lower pest damage and higher defense enzyme activities can be considered ideal candidates for pest-resistant breeding programs.

This study emphasizes the importance of integrating field phenotyping, biochemical screening, and molecular analysis to comprehensively evaluate pest resistance in urdbean genotypes. Such a multidisciplinary approach enhances the precision of identifying resistance traits and accelerates breeding efforts aimed at developing pest-tolerant varieties. Future research should focus on multi-environmental validation of these resistant genotypes, functional characterization of identified resistance genes, and marker-assisted selection (MAS) to expedite breeding programs for sustainable pest management in urdbean cultivation.

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