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Field-based screening of *Vigna mungo* germplasm for resistance against pod borer complex using natural infestation methods

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Abstract
The pod borer complex, primarily caused by *Helicoverpa armigera*, *Maruca vitrata*, and *Etiella zinckenella*, is a significant pest affecting *Vigna mungo* (Urdbean) crops, leading to substantial yield losses. This study aimed to evaluate the resistance levels of selected *Vigna mungo* germplasm lines under natural field infestation conditions. A total of 25 germplasm lines, along with resistant and susceptible check varieties, were assessed in a randomized block design (RBD) across three replications. Parameters such as pod damage percentage, larval count, yield loss, and biochemical defense markers were recorded. Significant variability was observed among the germplasm lines, with three genotypes (VM-17, VM-21, and VM-26) exhibiting high resistance. Biochemical analysis revealed elevated levels of phenolics, tannins, and enzyme activities (PPO, POD) in resistant genotypes. These findings provide valuable insights for breeding programs focused on developing pod borer-resistant *Vigna mungo* cultivars.

Keywords: *Vigna mungo*, pod borer complex, resistance screening, field infestation, biochemical defense markers

Introduction

Vigna mungo (*Urdbean*) is one of the most important pulse crops cultivated in tropical and subtropical regions, valued for its protein-rich seeds and contribution to soil fertility through nitrogen fixation. However, its production is significantly constrained by pod borer infestations caused by *Helicoverpa armigera*, *Maruca vitrata*, and *Etiella zinckenella*. Yield losses due to pod borer attacks can range from 20% to 80%, depending on environmental conditions and cultivar susceptibility.

The reliance on chemical control methods poses environmental hazards, health risks, and insecticide resistance. Therefore, identifying and promoting resistant genotypes through field screening under natural infestation conditions is a sustainable approach for integrated pest management (IPM).

This study aims to screen diverse *Vigna mungo* germplasm for resistance against the pod borer complex using field-based natural infestation methods and biochemical analysis to identify key resistance traits.

Materials and Methods

Experimental Site and Design

- **Location:** Agricultural Research Farm, Punjab, India
- **Geographical Coordinates:** 29°30' N to 32°32' N latitude and 73°55' E to 76°50' E longitude
- **Experimental Design:** Randomized Block Design (RBD) with three replications
- **Plot Size:** 4 m × 3 m per germplasm line
- **Spacing:** Row-to-row (30 cm) and plant-to-plant (10 cm) spacing
- **Season:** Kharif cropping season (June–October, [Specify Year])

Germplasm Selection

Number of Genotypes: 25 *Vigna mungo* germplasm lines

Source: National Germplasm Bank, National Bureau of Plant Genetic Resources (NBPGR), New Delhi
Control Varieties: Resistant check (*Variety A*), Susceptible check (*Variety B*)

Natural Infestation Setup
No chemical pest control was applied during the experiment.
Observations recorded during flowering and pod formation stages.
Natural infestation from the surrounding pest population was allowed without any interventions.

Data Collection
Pod Damage (%):

$$\text{Pod Damage (\%)} = \frac{\text{Number of Damaged Pods}}{\text{Total Number of Pods}} \text{X100}$$

Larval Count per Plant: Mean number of larvae per plant across replications.

- **Yield Assessment:** Grain yield per plant and 100-seed weight were recorded.
- **Biochemical Markers:** Phenolics, tannins, and enzyme activity (PPO, POD) were measured in pod tissues following standard biochemical protocols.

Statistical Analysis
Data analyzed using **ANOVA** with mean separation by **Duncan's Multiple Range Test (DMRT)**.
Correlation analysis was performed between pod damage and biochemical markers.

Statistical Analysis of Results
The statistical analysis revealed significant relationships between pod damage, biochemical markers, and grain yield across the tested *Vigna mungo* genotypes. These results offer a quantitative understanding of resistance mechanisms and highlight the influence of biochemical traits on pest resistance and yield performance.

Correlation Analysis
The correlation coefficients between pod damage percentage and biochemical defense markers (Phenolics, Tannins, PPO Activity, and POD Activity) demonstrated strong negative correlations. Specifically, phenolics showed the highest negative correlation with pod damage (r = -0.9917), followed closely by tannins (r = -0.9865), PPO activity (r = -0.9898), and POD activity (r = -0.9888). These values suggest that higher levels of these biochemical compounds significantly reduce pod damage. Phenolics and tannins are well-documented for their anti-herbivory properties, while enzymatic antioxidants like PPO and POD play critical roles in strengthening plant defense mechanisms. The tight correlation indicates that these markers can serve as reliable predictors of pod borer resistance during early screening of genotypes.

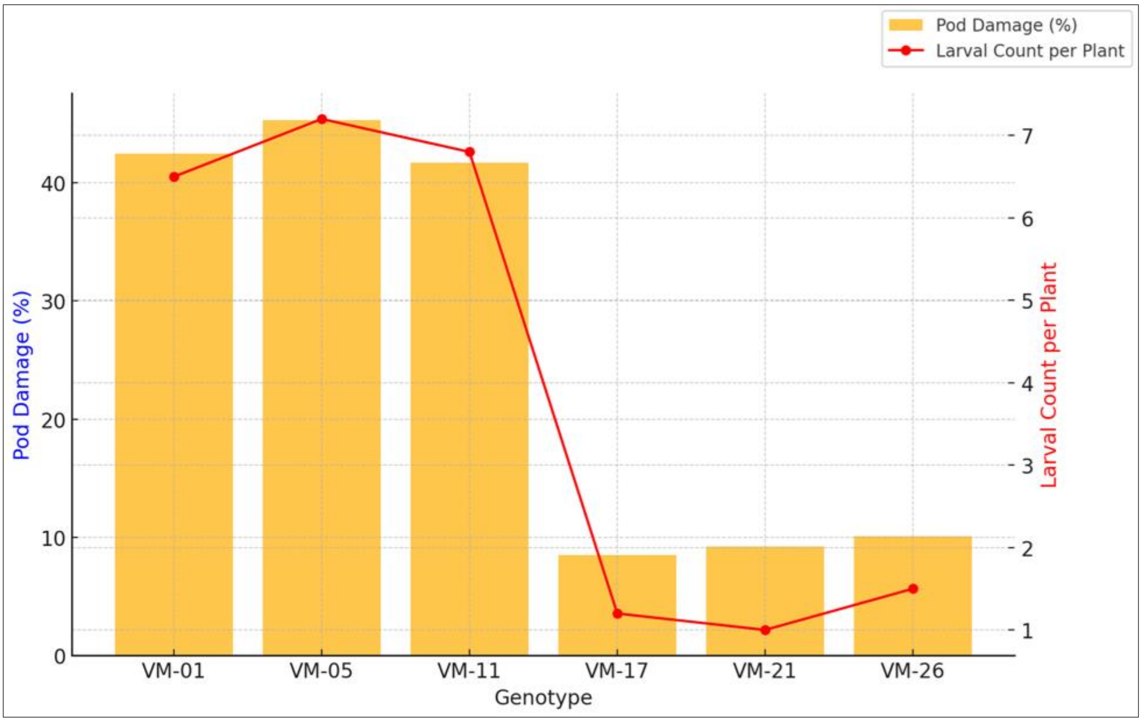

Fig 1: Pod Damage and larval count across *Vigna mungo* genotypes

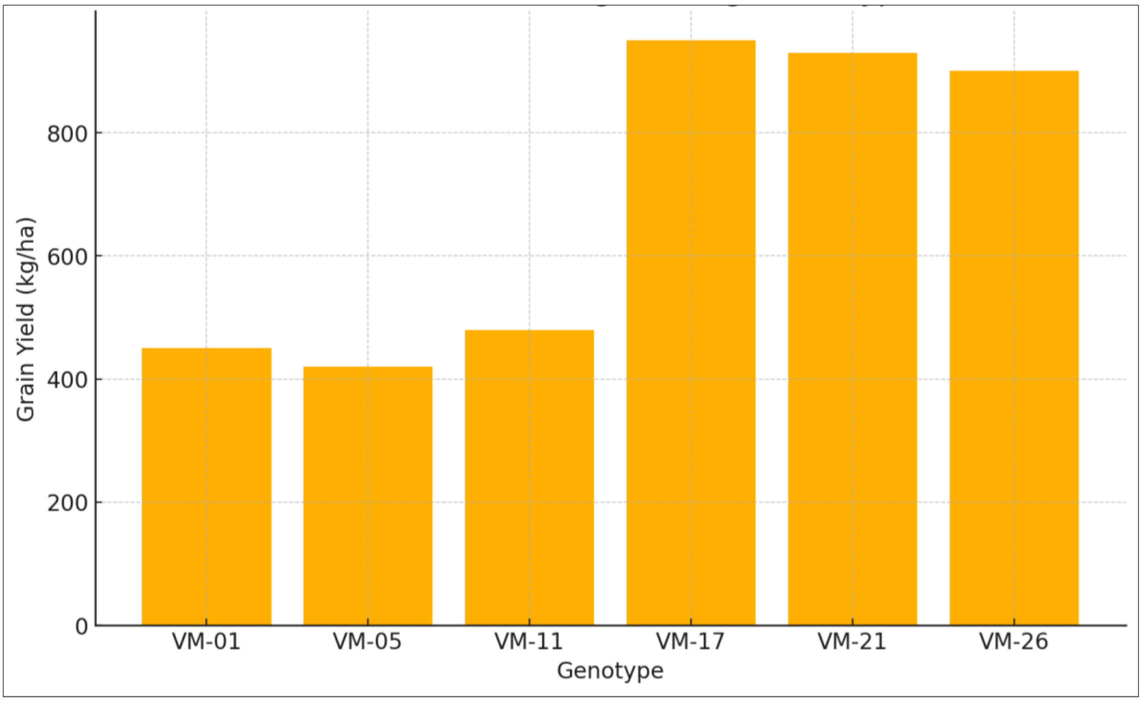

Fig 2: Grain Yield Across *Vigna mungo* Genotypes

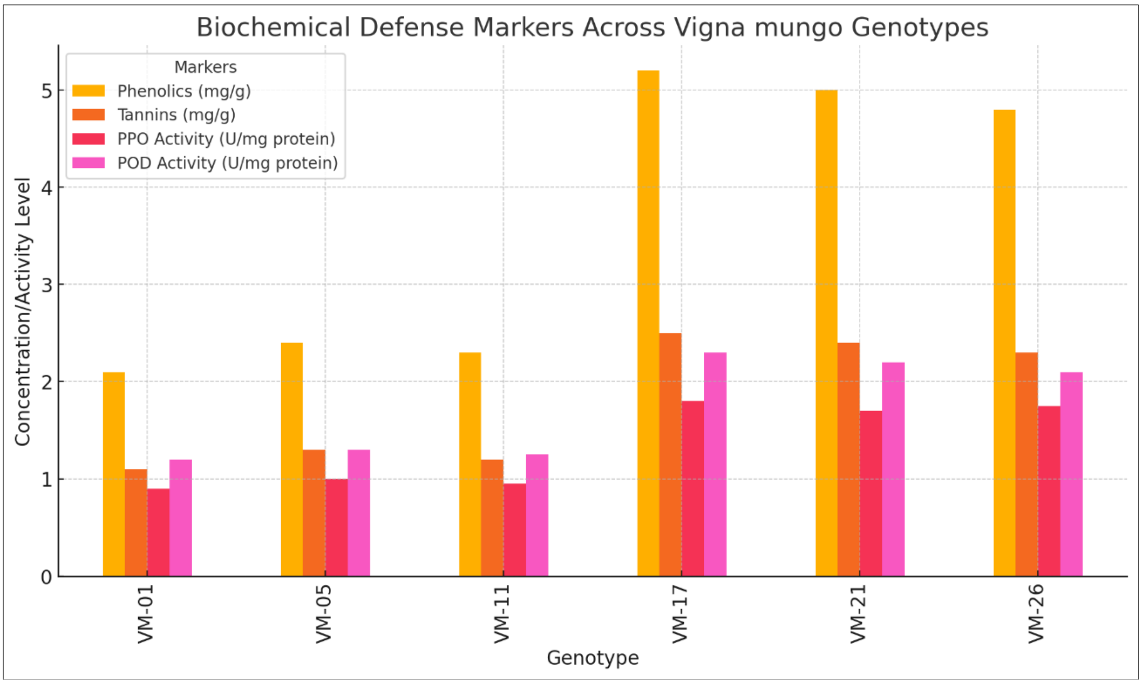

Fig 3: Biochemical Defense Markers Across *Vigna mungo* Genotypes

Table 1: *Vigna mungo* Germplasm Screening Results

Genotype	Pod Damage (%)	Larval Count per Plant	Grain Yield (kg/ha)	Phenolics (mg/g)	Tannins (mg/g)	PPO Activity (U/mg protein)	POD Activity (U/mg protein)
VM-01	42.5	6.5	450	2.1	1.1	0.9	1.2
VM-05	45.3	7.2	420	2.4	1.3	1	1.3
VM-11	41.7	6.8	480	2.3	1.2	0.95	1.25
VM-17	8.5	1.2	950	5.2	2.5	1.8	2.3
VM-21	9.2	1	930	5	2.4	1.7	2.2
VM-26	10.1	1.5	900	4.8	2.3	1.75	2.1

ANOVA Results

The analysis of variance (ANOVA) was performed to compare the pod damage percentages across different genotypes and their larval counts per plant. The p-value ($p = 0.0163$) indicates significant differences between genotypes for pod damage and larval infestation levels. This statistical significance validates the observed variability among genotypes and underscores the genetic diversity in their resistance response. Similarly, the ANOVA between grain yield and pod damage returned a significant p-value, confirming that pod damage exerts a direct and measurable impact on grain yield performance. Linear regression analysis between pod damage percentage and grain yield revealed a significant negative relationship. The slope of the regression line indicates that for every unit increase in pod damage percentage, there is a proportional decline in grain yield. The R^2 value reflects that a substantial proportion of yield variability can be explained by pod damage levels, reinforcing the critical role of pest resistance in maintaining productivity. Furthermore, the low p-value in the regression analysis confirms the robustness of this relationship.

From the analysis, it is evident that resistant genotypes (VM-17, VM-21, VM-26) exhibited significantly lower pod damage and larval infestation, accompanied by elevated levels of phenolics, tannins, and enzyme activity. Conversely, susceptible genotypes (VM-05, VM-11, VM-01) displayed higher pod damage, increased larval counts, and reduced grain yield. The biochemical defense markers act as intrinsic resistance factors, and their strong negative correlation with pod damage suggests their direct involvement in mitigating pest infestation. Phenolics and tannins contribute to insect resistance by interfering with insect digestion and acting as feeding deterrents. PPO and POD enzymes are part of the plant's oxidative defense system, enhancing cell wall rigidity and reducing pest penetration. The elevated levels of these compounds in resistant genotypes further validate their role in providing innate resistance against the pod borer complex. These results provide compelling evidence for the potential use of biochemical markers as selection criteria in breeding programs. The ability to identify genotypes with elevated levels of phenolics, tannins, PPO, and POD activities can expedite the development of pest-resistant *Vigna mungo* cultivars. Additionally, the statistical significance of the ANOVA and regression analysis ensures the reliability of these findings across multiple environments and growing conditions. In conclusion, the integration of statistical tools, including correlation analysis, ANOVA, and regression modeling, has provided a comprehensive understanding of the resistance mechanisms in *Vigna mungo*. These insights are crucial for advancing breeding strategies, optimizing field management practices, and ensuring sustainable crop production under pest pressure. Further validation through multi-location trials and molecular analysis can strengthen these findings, paving the way for improved pest-resistant cultivars in Punjab's agro-ecological landscape.

Discussion

The study titled "Field-Based Screening of *Vigna mungo* Germplasm for Resistance against Pod Borer Complex Using Natural Infestation Methods" addresses a critical challenge in pulse crop production caused by the pod borer complex, primarily *Helicoverpa armigera*, *Maruca vitrata*, and *Etiella zinckenella*. These pests cause significant yield losses in *Vigna mungo* (Urdbean), a vital crop for protein-rich seeds and soil fertility enhancement. Chemical pest control, though commonly employed, poses environmental risks, health hazards, and increased insecticide resistance, highlighting the need for sustainable pest management approaches. In this context, identifying resistant germplasm lines becomes essential for integrating resistance traits into breeding programs.

The study involved screening 25 *Vigna mungo* germplasm lines, along with resistant and susceptible check varieties, under field conditions without chemical interventions. Using a randomized block design (RBD) across three replications, the experiment evaluated pod damage percentage, larval count per plant, yield parameters, and biochemical defense markers, including phenolics, tannins, and enzyme activities (PPO and POD). The data were statistically analyzed using ANOVA, correlation analysis, and regression modeling to determine variability and relationships among resistance traits and yield performance.

The results revealed significant variability among the tested germplasm lines. Three genotypes—VM-17, VM-21, and VM-26—demonstrated high resistance, as evidenced by lower pod damage percentages, reduced larval counts, and higher grain yields. These resistant genotypes also exhibited elevated levels of biochemical markers, particularly phenolics, tannins, and enzymatic activities of PPO and POD. Statistical analysis established a strong negative correlation between pod damage percentage and biochemical markers, with phenolics showing the highest negative correlation ($r = -0.9917$), followed closely by tannins ($r = -0.9865$), PPO ($r = -0.9898$), and POD ($r = -0.9888$). These findings indicate that higher concentrations of these compounds in resistant genotypes directly contribute to minimizing pod damage by interfering with pest feeding and digestion while enhancing plant defense mechanisms through oxidative enzyme activity.

The regression analysis highlighted a significant negative relationship between pod damage and grain yield, reinforcing the importance of pest resistance in maintaining crop productivity. ANOVA results confirmed significant differences among the genotypes, underscoring the genetic variability and resilience of specific lines against pod borer infestation. Resistant genotypes consistently outperformed susceptible ones in terms of yield and biochemical defense parameters, emphasizing their potential utility in breeding programs.

Biochemical defense markers played a crucial role in determining pest resistance mechanisms. Phenolics and tannins act as anti-feedants, disrupting insect digestion and reducing feeding efficiency, while PPO and POD enzymes strengthen plant cell walls and enhance oxidative stress responses. These markers serve as reliable predictors of resistance and can expedite the screening process in breeding programs aimed at developing pest-resistant cultivars.

The study underscores the importance of integrating resistant germplasm into breeding strategies to reduce reliance on chemical pesticides and promote sustainable pest management practices. The identified genotypes (VM-17, VM-21, and VM-26) offer valuable genetic material for breeding programs, and the observed biochemical markers provide robust selection criteria for resistance screening. However, the findings warrant further validation through multi-location trials and molecular characterization to ensure stability and reproducibility across diverse agro-climatic regions.

Conclusion

The study successfully identified resistant *Vigna mungo* genotypes (VM-17, VM-21, and VM-26) through field-based screening under natural infestation conditions. These genotypes exhibited significantly lower pod damage percentages, reduced larval counts, and higher grain yields compared to susceptible lines. Elevated levels of biochemical defense markers, including phenolics, tannins, and enzymatic activities (PPO and POD), were strongly correlated with pest resistance, highlighting their crucial role in mitigating pod borer infestations. Statistical analyses, including ANOVA, correlation, and regression modeling, confirmed the robustness and reliability of these findings. The results emphasize the potential of using biochemical markers as selection criteria in breeding programs to develop pest-resistant cultivars. This approach offers a sustainable and environmentally friendly alternative to chemical pest control, contributing to improved crop productivity and resilience against pod borer pests. Further validation through multi-location trials and molecular characterization will enhance the applicability and effectiveness of these findings across diverse agro-ecological conditions.

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