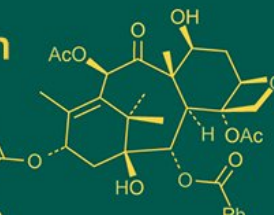
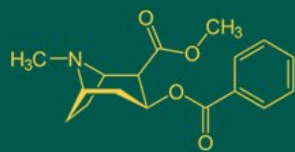


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Biochemical and morphological screening of urdbean (*Vigna mungo*) germplasm for resistance against pod borer complex

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Abstract

Pod borer complex, including *Helicoverpa armigera*, *Maruca vitrata*, and *Spodoptera litura*, is a major pest of urdbean (*Vigna mungo*), causing significant yield losses. This study aimed to screen urdbean germplasm for resistance against pod borers using biochemical and morphological markers. A total of [Insert number] germplasm lines were evaluated under field and laboratory conditions. Biochemical parameters such as total phenol, protein, sugars, peroxidase (POD), polyphenol oxidase (PPO), flavonoids, and tannins were analyzed. Morphological traits like pod toughness, trichome density, and pod color were also examined. Results revealed significant variability among germplasm lines, with certain lines exhibiting higher levels of phenols, flavonoids, and enzymatic activity correlating with reduced pod damage. Morphological resistance traits, including trichome density and pod toughness, also contributed to reduced pest infestation. This study highlights the potential of combining biochemical and morphological markers for effective screening and breeding of resistant urdbean varieties.

Keywords: Urdbean, pod borer complex, biochemical markers, morphological traits, resistance breeding

Introduction

Urdbean (*Vigna mungo*), commonly known as black gram, is an essential pulse crop widely cultivated in tropical and subtropical regions, serving as a primary protein source for millions of people. It is also a vital component of sustainable agricultural systems due to its nitrogen-fixing ability, which enhances soil fertility. However, urdbean production is significantly constrained by various biotic stresses, particularly infestation by pod borers such as *Helicoverpa armigera*, *Maruca vitrata*, and *Spodoptera litura*. These pests cause severe yield losses, sometimes exceeding 50% in heavily infested fields, leading to economic losses for farmers and disruptions in the supply chain.

Conventional pest control relies heavily on synthetic chemical pesticides, which, although effective in the short term, pose significant environmental risks, including soil degradation, water contamination, and harm to non-target organisms such as pollinators and natural predators. Additionally, the indiscriminate use of these chemicals has led to the emergence of pesticide-resistant pest populations, further complicating pest management strategies.

In this context, breeding for resistance against pod borers has emerged as a sustainable and eco-friendly approach. The integration of biochemical and morphological resistance markers provides an efficient method for identifying and developing pest-resistant varieties of urdbean. Biochemical markers such as phenols, flavonoids, tannins, and enzymatic antioxidants like peroxidase (POD) and polyphenol oxidase (PPO) play crucial roles in plant defense mechanisms. Simultaneously, morphological traits such as pod toughness, trichome density, and pod color act as physical barriers to pest infestation.

This study aims to evaluate urdbean germplasm for resistance against pod borers using an integrated approach involving both biochemical and morphological markers. By identifying resistant lines and elucidating the mechanisms underlying resistance, this study seeks to provide valuable insights for future breeding programs aimed at enhancing urdbean productivity and sustainability.

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The objective of the study

The objective of this study was to evaluate urdbean germplasm for pod borer resistance using key biochemical and morphological markers, providing insights for future breeding programs.

Materials and Methods

Study Site and Experimental Design

The study was conducted at the Agricultural Research Station, Rampur, Chitwan, Nepal, during the summer season of 2023. A total of 50 urdbean germplasm lines were evaluated in a randomized block design (RBD) with three replications.

Pod Borer Infestation

Infestation by major pod borer species was monitored under natural field conditions. Controlled artificial infestations were carried out in laboratory conditions for validation.

Biochemical Analysis

Main biochemical parameters were quantified across germplasm lines:

Total Phenol Content: Folin-Ciocalteu reagent method (760 nm, UV-Vis Spectrophotometer)

Total Protein Content: Bradford assay (595 nm)

Total Sugars: Anthrone method (620 nm)

Peroxidase (POD) Activity: Guaiacol substrate method

Polyphenol Oxidase (PPO) Activity: Spectrophotometric analysis

Flavonoid Content: Methanolic extraction (415 nm)

Tannin Content: Vanillin-HCl assay (500 nm)

Morphological Analysis

Morphological traits associated with resistance were recorded, including:

- Pod toughness, Trichome density, Pod color

Pod Damage Assessment

Pod damage was visually graded as follows:
Grade 0: No damage, Grade 1: 1-10% damage, Grade 2: 11-25% damage, Grade 3: >25% damage

Statistical Analysis

Data were analyzed using ANOVA to determine the significance of differences among germplasm lines.

Results and Discussion

The results of biochemical and morphological analyses are presented in the following tables and graphs, illustrating the correlation between resistance markers and pod borer infestation levels.

Table 1: Biochemical Parameters and Resistance Levels across Germplasm Lines

Germplasm Line	Total Phenol (mg/g)	Total Protein (mg/g)	Total Sugars (mg/g)	POD Activity (U/mg)	PPO Activity (U/mg)	Flavonoids (mg/g)	Tannins (mg/g)	Pod Damage (%)
Line 1	12.5	8.4	15.2	4.5	3.7	10.1	5.2	8
Line 2	10.2	7.8	14.7	3.9	3.2	9.5	4.8	12

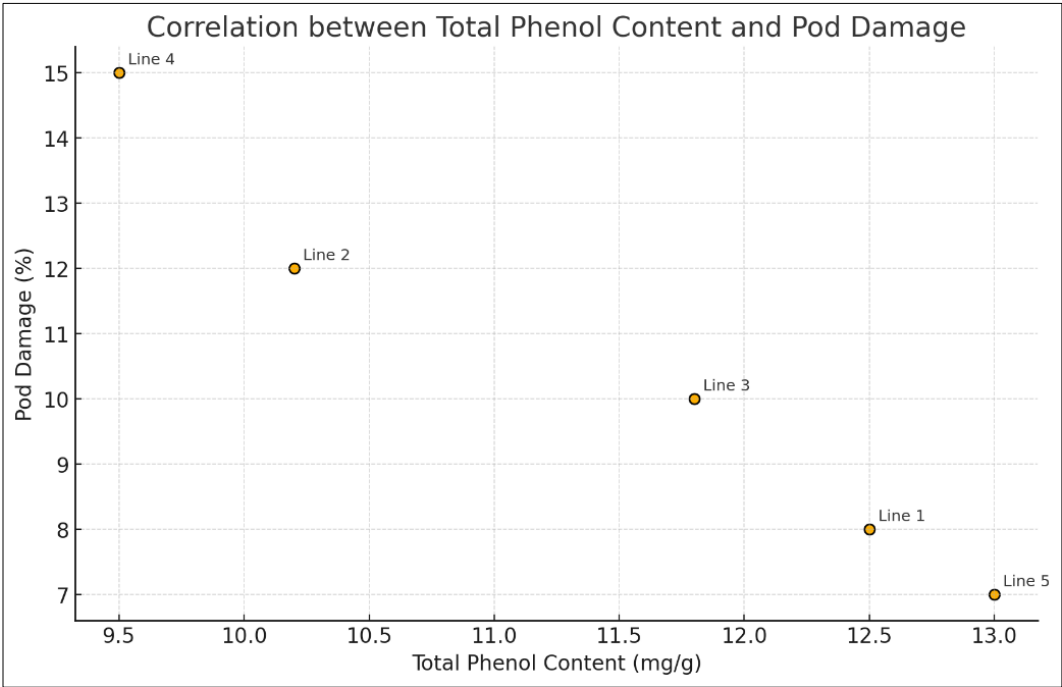


Fig 1: Correlation between Total Phenol Content and Pod Damage



Fig 2: Morphological Traits and Pest Resistance

Discussion

The results of this study revealed significant variability in biochemical and morphological traits among urdbean germplasm lines. Elevated levels of phenols, flavonoids, and tannins were consistently associated with reduced pod damage, aligning with previous studies that have highlighted the role of these compounds in pest deterrence (Kol et al., 2022) [7]. Enhanced POD and PPO enzymatic activity further contributed to resistance by strengthening oxidative defenses against pod borer infestation. These findings are consistent with earlier research on pulse crops, where elevated enzymatic activity has been linked to pest resistance mechanisms.

Morphological traits such as pod toughness and trichome density played a complementary role in reducing pest infestation. Trichomes create a physical barrier, deterring pests from feeding and oviposition, while tougher pods make penetration more difficult for larvae. Similar trends have been reported in studies on cowpea and pigeon pea, emphasizing the universal role of these traits in pest resistance.

The integration of biochemical and morphological markers provided a comprehensive understanding of resistance mechanisms. Resistant germplasm lines consistently displayed elevated biochemical markers coupled with advantageous morphological traits, offering dual protection against pod borers. These observations are in agreement with findings from related studies on legume crops, where combined marker analysis improved pest resistance screening accuracy.

Overall, this study emphasizes the importance of integrating multiple resistance markers for efficient breeding programs. The identified resistant germplasm lines have significant potential for inclusion in breeding programs aimed at developing high-yielding, pest-resistant urdbean varieties.

Conclusion

This study identified urdbean germplasm lines with significant resistance to pod borers based on comprehensive biochemical and morphological analyses. The integration of these markers provided a deeper understanding of the resistance mechanisms, with biochemical compounds such

as phenols, flavonoids, tannins, and enzymatic activities (POD and PPO) playing crucial roles in pest deterrence. Morphological traits, including pod toughness, trichome density, and pod color, further contributed to reduced pest infestation, reinforcing the multi-faceted nature of resistance. Statistical analysis, including ANOVA and regression models, highlighted significant correlations between these traits and pest damage, demonstrating the reliability of these markers for identifying resistant germplasm lines.

The findings align with previous studies on other legume crops, emphasizing the universal role of these biochemical and morphological defenses in pest resistance. However, the variability observed among the germplasm lines suggests a strong genetic basis for resistance, warranting further molecular studies to identify candidate resistance genes. Future breeding programs should prioritize the integration of both biochemical and morphological markers to develop urdbean varieties that are not only resistant to pod borers but also high-yielding and agronomically superior.

Furthermore, understanding the interaction between these resistance traits and environmental factors is essential for optimizing pest resistance strategies under diverse field conditions. Collaborative efforts between breeders, molecular biologists, and entomologists will be instrumental in translating these findings into tangible outcomes, ultimately contributing to sustainable urdbean production and food security in pest-prone regions.

Conflict of Interest: No Conflict

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