

Evaluation Of Mung Bean (*Vigna Radiata*) Varieties For Resistance To Bruchid (*Callosobruchus Spp*) Infestation Using The “No Choice Test”

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Abstract

Mung beans (*Vigna radiata*) commonly known as green grams, are an important legume crop cultivated for food and as a source of income. Mung bean seeds are highly susceptible to infestation by bruchids (*Callosobruchus spp*), which can cause significant post-harvest losses, hence impacting on the global food security. Bruchid infestation control remains a priority, and overreliance on chemical pesticides contributes to environmental concerns due to the potential harm to non-target species such as soil and water quality. Investigating natural resistance mechanisms in mung beans can contribute to sustainable and eco-friendly pest management practices. This study aimed at identifying mung bean varieties resistant to storage bruchids using the 'No Choice' test by determining the seed damage. Twenty-three mung bean varieties, both wild and local, were obtained from KALRO Katumani and evaluated against pulse beetle (*Callosobruchus maculatus*) under laboratory conditions. Fifty seeds of each test sample were placed in separate petri dishes. Five male and female pairs of 0–24-hour-old adults of the beetle were released into each petri dish, covered to prevent insect escape and allow air circulation. The observations were made after 72 hours on oviposition preference by determining the number of eggs laid and percentage seed damage by counting the seeds with one or more holes from the total. The mung bean variety V100-35226 exhibited the highest resistance to bruchid infestations, with minimal seed damage. Other varieties with slight resistance included AMVU-1612, AMVU-1601, AMVU-1603, and V100-1802. The results highlighted significant differences in resistance levels among the mung bean varieties, indicating that some varieties possess natural resistance mechanisms to bruchid infestation.

Keywords: Mung beans (*Vigna radiata*), Bruchids (*Callosobruchus spp*), 'No Choice' test', Integrated Pest management

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I. Introduction

Mung beans are an important legume crop grown throughout Asia and Africa (Prajapati *et al.*, 2022). In Kenya, they are commonly cultivated in counties such as Machakos, Makeni, Kitui, Tharaka Nithi, Embu, and Meru (Muchomba *et al.*, 2023). Mung beans play a crucial role as a food security crop due to their nutritional quality and ability to thrive in arid and semi-arid lands (ASALs) (Nyongesa *et al.*, 2019). They are widely used as food since they are rich in protein and other essential nutrients, thus feature prominently in traditional and modern cuisines. Additionally, mung beans are used as a source of income for farmers (Pataczek *et al.*, 2018).

However, mung bean crops are frequently affected by pests and diseases, most notably the bruchid beetle (*Callosobruchus maculatus*), which causes significant damage and yield losses (Resources *et al.*, 2017). Bruchids, or pulse beetles, are storage pests of worldwide importance to mung beans (Mbeyagala *et al.*, 2017). They attack several pulse crops including mung beans, cowpeas, and pigeon peas. Severe infestations can lead to grain losses of up to 100 percent within six months of storage (Poornasundari & Thilagavathy, 2015). The bruchid beetles lay their eggs on mung bean pods, and the larvae feed on the seeds, reducing their quality and viability.

Several approaches have been employed to control bruchids in mung bean crops, such as synthetic pesticides, biological control agents, and breeding for resistance (Harshitha *et al.*, 2022). In Kenya, farmers often apply chemicals to preserve mung beans against bruchid infestation during storage (Wangu, 2021). However, the use of chemical products on food grains during storage has adverse effects on human and animal health, as well as on other organisms, leading to biodiversity loss and extensive environmental contamination (Divekar *et al.*, 2022b). Additionally, developing countries are increasingly adopting to the use of resistant grain varieties to control stored grain weevils as an alternative to chemical treatments (Yewale *et al.*, 2020). The evolution of resistance in plants involves a variety of secondary metabolites such as alkaloids, terpenes, amines,

glucosinolates, cyanogenic glucosides, quinones, phenolics, peptides, and polyacetylenes that serve as defensive shields against phytophagous herbivores (Divekar *et al.*, 2022a). Understanding these mechanisms is crucial for developing effective breeding strategies.

Integrated pest management (IPM) strategies continue to evolve, incorporating new research findings and technologies. The integration of biological control agents, resistant varieties, and improved storage practices provides a holistic approach to managing bruchid infestations (Kumar *et al.*, 2015). Additionally, educating farmers on best practices for pest management and storage can enhance the effectiveness of these strategies.

Breeding for host plant resistance against bruchids represents a promising approach to integrated pest management in mung beans. By selecting and breeding mung bean varieties with inherent resistance to bruchid infestation, breeders can develop cultivars that are less susceptible to pest damage. This approach not only reduces the reliance on chemical insecticides but also promotes sustainable agricultural practices that enhance food security and environmental conservation (Somta *et al.*, 2008).

Several studies have demonstrated the efficacy of breeding programs in developing bruchid-resistant mung bean varieties. Traits such as seed hardness, seed coat thickness, and biochemical compounds have been identified as key factors contributing to bruchid resistance in mung beans. Through conventional breeding techniques and modern biotechnological approaches, researchers have successfully incorporated these resistance traits into elite mung bean cultivars (Rao *et al.*, 2018).

In addition to genetic resistance, good agronomic practices can also play a crucial role in bruchid management. Practices such as timely harvesting, proper drying, and effective storage can help minimize bruchid infestations and reduce post-harvest losses. Ensuring proper ventilation and sanitation in storage facilities is essential for preventing the build-up of bruchid populations and maintaining seed quality (Singh *et al.*, 2017).

There is a pressing need to comprehend the mechanisms underlying mung bean resistance to bruchids in order to develop more sustainable pest control strategies. This study aimed at identifying mung bean varieties resistant to storage bruchids by determining the seed damage using a 'No Choice' test. This test provides insights into natural resistance varieties that can inform sustainable resistant strain breeding as a pest management practice.

II. Materials & Methods

This study employed precise methodology to assess the resistance of twenty-three (23) mung beans varieties against bruchid beetles (*Callosobruchus maculatus*). Resistance to bruchids was evaluated using a “no-choice” bioassay. This method involved confining the bruchid beetles with a single type of mung beans variety, thereby removing any alternative choices and allowing for a direct assessment of each variety's resistance. This approach is essential for determining the intrinsic ability of each bean variety to withstand pest infestation.

Sample Collection

Twenty-three distinct varieties of mung beans, encompassing both local and wild types, were obtained from KALRO Katumani in Machakos County. These varieties were provided by the World Vegetable Centre. The mung beans seeds were carefully selected to represent healthy and vigorous samples, providing a reliable basis for the study's experiments. The focus on both local and wild varieties adds depth to the research, offering insights into the potential differences that affects their response to the experimental exposure. The initial culture of the bruchid beetles, *Callosobruchus maculatus* (Fabricius), were obtained from KALRO Katumani. This beetle is a well-known pest that infests stored legumes, making it a critical subject of the study, specifically on mung beans. The identification and verification of the *Callosobruchus* species were meticulously performed by an entomologist specialist at KALRO Katumani. Accurate species identification was paramount to ensure that the experimental outcomes are valid and applicable, particularly in studies involving pest management and resistance.

Table 1. Mung beans Varieties for Bruchid Resistance Trial

AMVU 1601	AMVU 1608	AMVU 1627	KS 20
AMVU 1602	AMVU 1612	AMVU 1630	KAT 00301
AMVU 1603	AMVU 1614	V100 1709	KAT 00308
AMVU 1604	AMVU 1616	V100 1802	KAT 00309
AMVU 1605	AMVU 1618	V100 35226	Local Meru
AMVU 1606	AMVU 1619	N 26	

Source: KALRO Katumani

Study Site

The research was conducted across two primary locations, Tharaka University located in Tharaka Nithi County Kenya and the Insect Pest Management Laboratory at KALRO Katumani located in Machakos County Kenya. Each site was selected based on its unique facilities and contributions to the study.

Host Preference Bioassay of Bruchids (*Callosobruchus maculatus*)

The evaluation of mung beans varieties for resistance to storage bruchids (*Callosobruchus maculatus*) infestation was conducted to identify lines with superior resistance, moderate resistance, and susceptibility. The bioassay involved a “no-choice” host preference study on twenty-three (23) selected mung beans varieties (Table 1). Each mung beans variety was represented by 50 seeds, which were counted and placed in separate Petri dishes labelled with their variety code (e.g., AMVU 1601, V100 1709, Local Meru). Five pairs of *Callosobruchus maculatus* (bruchids) were introduced into each Petri dish containing 50 seeds of a specific mung beans variety. The Petri dishes were maintained in a room with controlled temperature and pressure, specifically at $28\pm 2^{\circ}\text{C}$ and $92\pm 3\%$ relative humidity, which are conducive conditions for bruchid infestation (Figure 1). The adult *Callosobruchus maculatus* were then allowed to lay eggs, and observations were made on their oviposition preference.

NO CHOICE TEST BRUCHID BIOASSAY

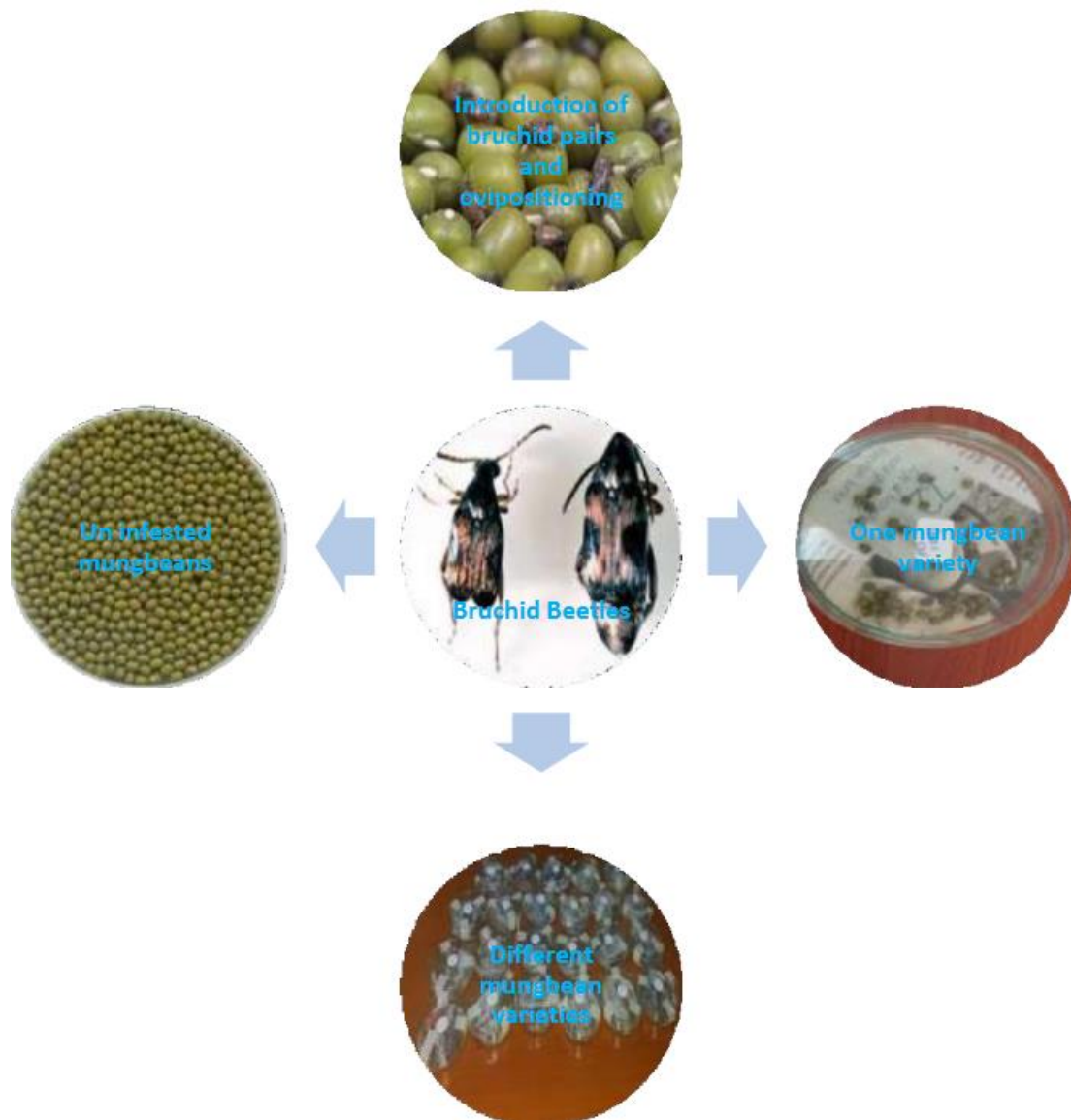


Figure 1. No Choice Test Bruchid Bioassay

To determine the percentage of seed damage, test sample seeds from each variety's replication petri dishes were evaluated after 30 days. Seeds with one or more holes were considered damaged and separated from the total sample for counting. The percentage of damaged seeds was then calculated using the formula proposed by Adams and Schuten (1978) (Equation 1).

$$\text{Percentage seed damage} = \frac{\text{Number of seeds damaged}}{\text{Total Number of seeds counted}} \times 100. \dots\dots\dots(1)$$

The data collected on the number of damaged seeds and the percentage of seed damage were analysed to determine the resistance levels of the mung beans varieties.

Statistical analysis

Statistical analysis was conducted to compare the levels of seed damage across different varieties. The evaluation of the significant differences in seed damage among mung bean varieties was conducted using Tukey's Honest Significant Difference (HSD). The varieties were assigned letters (a, b, c,n) to denote significant differences in resistance levels.

III. Results And Discussions

The evaluation of seed damage across various mung beans lines demonstrated differential resistance to bruchid (*Callosobruchus maculatus*) infestation. The V100-35226 line exhibited minimal seed damage at 2.16%, indicating superior resistance to bruchid attack. Other lines with high resistance included AMVU-1612 and AMVU-1601, with seed damage recorded at 7.16% and 7.83%, respectively. Additionally, AMVU-1603 and V100-1802 displayed relatively low damage levels at 10.5% and 12.33%, respectively (Table 2). Moderate resistance was observed in lines AMVU-1602 (18.16%) and AMVU-1619 (19.50%). In contrast, varieties AMVU-1630 (24.83%) and V100-1709 (30.33%) exhibited moderate damage levels, suggesting intermediate resistance (Table 2). Lines exhibiting higher susceptibility included AMUV-1604 (43.16%), AMUV-1605 (35.66%), AMUV-1606 (37.66%), and AMVU-1608 (30.33%), indicating significantly reduced resistance. The line AMVU-1618 showed even higher susceptibility with a damage level of 46.0%. The most susceptible lines were KAT-00309 (64.66%), KAT-00308 (63.33%), KAT-00301 (59.16%), AMVU-1627 (59.16%), KS-20 (55.0%), Meru (55.83%), and N_26 (55.83%) (Table 2). These lines experienced the highest levels of seed damage, indicating extreme susceptibility to bruchid infestations. The mung bean varieties demonstrated significant variations in seed damage due to bruchid infestation, exhibiting the diversity in their resistance levels. To evaluate the significant differences in seed damage among mung bean varieties, post hoc analysis using Tukey's Honest Significant Difference (HSD) was conducted. Varieties such AMUV-1604, AMUV-1605, and AMUV-1608 were assigned the same letter, indicating that their levels of seed damage were not significantly different from each other. Although their specific seed damage percentages vary, they are statistically similar in their resistance to bruchid infestation. However, these varieties differ significantly from more resilient lines such as V100-35226 and AMVU-1612, which have much lower seed damage levels and are assigned different letters in the analysis (Table 2).

Table 2. Screening of mung beans varieties resistant to storage bruchids (*Callosobruchus maculatus*) infestation

Variety Code	No. of seeds	No. of Bruchid Pairs	No. of Damaged seeds	Percentage seed damage	Turky's ASD
AMVU-1601	50	5	3.33	7%	7.83 ^{hi}
AMVU-1602	50	5	15.67	31%	18.16 ^{hfgi}
AMVU-1603	50	5	4.67	9%	10.5 ^{hgi}
AMVU-1604	50	5	34	68%	43.16 ^{bc}
AMVU-1605	50	5	29.33	64%	35.66 ^{dcc}
AMVU-1606	50	5	30.33	61%	37.66 ^{dc}
AMVU-1608	50	5	26	52%	30.33 ^{dfee}
AMVU-1612	50	5	3	6%	7.16 ^{hi}
AMVU-1614	50	5	27.67	55%	33.3 ^{dfee}
AMVU-1616	50	5	27	54%	33.0 ^{dfee}
AMVU-1618	50	5	36	72%	46.0 ^{bc}
AMVU-1619	50	5	18.67	37%	19.50 ^{hfige}
AMVU-1627	50	5	46.33	99%	59.16 ^{ba}
AMVU-1630	50	5	24	48%	24.83 ^{dfige}
KS-20	50	5	45	90%	59.16 ^{ba}
KAT-00301	50	5	47.67	95%	63.33 ^a
KAT-00308	50	5	49.33	99%	64.66 ^a
KAT-00309	50	5	49.67	99%	55.00 ^{ba}
Local Meru	50	5	45.33	91%	55.83 ^{ba}
N-26	50	5	46.33	93%	55.83 ^{ba}
V100-1709	50	5	26.67	53%	30.33 ^{dfee}
V100-1802	50	5	6	12%	12.33 ^{hgi}
V100-35226	50	5	0.33	1%	2.16 ⁱ

****ASD =Average Seed Damage**

These findings effectively highlighted the range of resistance among the mung bean varieties. The comparison also aligns with previous findings, such as those reported by Mukuru *et al.* (2015) and Kimani *et al.* (2016), where various legume cultivars displayed similar patterns of differential resistance when exposed to bruchids. This consistency reinforces the observed heterogeneity in bruchid resistance among the mung bean lines. Table 2 provides a detailed breakdown of the seed damage across various mung bean lines, further supporting the graphical representation and statistical analysis of the results.

Figure 2 shows the susceptibility and resistance of different mung bean varieties. The highly resistant lines are V100-35226 while the highly susceptible ones are KAT-00309 and KAT-00308. This pattern aligns with previous studies by Mukuru *et al.* (2015) and Kimani *et al.* (2016), which reported similar differential resistance among legume cultivars when exposed to bruchid infestation. These findings highlight the diverse resistance profiles within the mung bean lines and emphasize the importance of selecting and breeding for bruchid-resistant varieties.

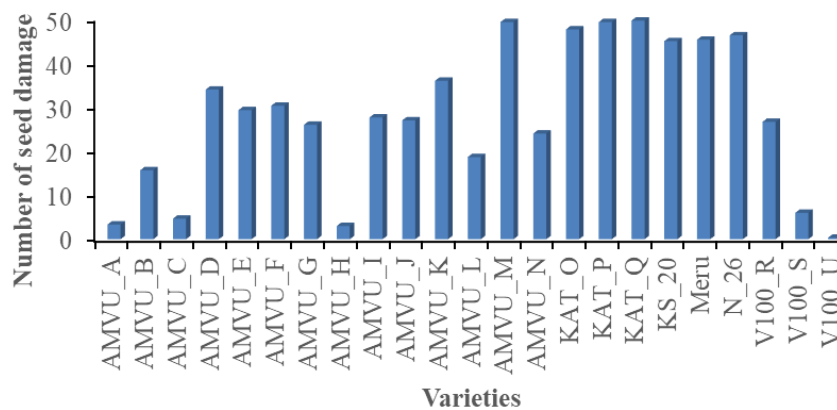


Figure 2. Seed damage among different mung bean varieties

The evaluation of mung bean lines against *Callosobruchus maculatus* infestation revealed significant variations in resistance levels, as indicated by the extent of seed damage across different varieties. Among the tested lines, V100-35226 demonstrated the highest resistance with minimal seed damage at 2.16%, while other lines such as AMVU-1612 and AMVU-1601 also exhibited relatively low damage levels at 7.16% and 7.83%, respectively. Conversely, lines like KAT-00309, KAT-00308, and AMVU-1627 displayed the highest susceptibility with seed damage percentages ranging from 55.0% to 64.66%.

The observed differences in seed damage highlight the diverse resistance capabilities among mung bean varieties against bruchid infestation. This diversity is crucial for selecting and breeding varieties that can withstand pest pressures, thereby reducing economic losses and enhancing food security. The results are consistent with previous studies that have documented similar differential resistance patterns in legume cultivars exposed to bruchid infestation (Mukuru *et al.*, 2015; Kimani *et al.*, 2016).

The statistical analysis confirmed significant differences in resistance levels among the tested mung bean lines, with distinct groups identified based on seed damage percentages. Varieties within the same group exhibited comparable resistance, while those in different groups showed statistically significant variations. This categorization provides valuable insights into the genetic and biochemical factors influencing mung bean resistance to bruchids, paving the way for targeted breeding efforts aimed at developing resilient cultivars.

These findings underscore the importance of integrating resistance screening into mung bean breeding programs and agricultural practices. By identifying and promoting resistant varieties like V100-35226 and AMVU-1612, farmers can effectively manage bruchid infestations without relying heavily on chemical pesticides. Moreover, understanding the metabolite profiles associated with resistance, can provide deeper insights into the biochemical mechanisms underlying mung bean resistance.

IV. Conclusion

The study identified significant variations in bruchid resistance among different mung beans varieties. The variety V100-35226, along with AMVU-1612 and AMVU-1601, exhibited high resistance to bruchid infestations. The most susceptible lines were KAT-00309 KAT-00308, KAT-00301, AMVU-1627, KS-20, Meru, and N_26. These lines experienced the highest levels of seed damage, indicating extreme susceptibility to bruchid infestations.

There were significant differences in the resistance levels among the tested mung bean lines, which implies genetic and biochemical factors influencing mung bean resistance to bruchids. Therefore, the findings of this study form a baseline for targeted breeding efforts aimed at developing resilient breeds, integrating resistance screening into mung bean breeding programs and agricultural practices, and identifying and promoting resistant varieties like V100-35226 and AMVU-1612, to effectively manage bruchid infestations without overreliance on chemical pesticides.

V. Recommendation

The study recommends the integration of highly resistant varieties, such as V100_U, into breeding programs to improve overall resistance to bruchid infestations. This approach will enhance crop yield and seed quality by minimizing damage.

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Conflicts of Interest: The authors declare no conflict of interest.

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