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Review Article

Metabolite Profiling of Mung Bean (*Vigna radiata*) Varieties Using UV-Vis Spectroscopy: A Tool for Breeding Resilience

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Abstract

Mung beans (*Vigna radiata* L.), commonly known as green grams, are a vital legume crop with significant nutritional and economic importance. As a resilient crop suited to arid and semi-arid conditions, mung beans contribute to food security and income generation, particularly in resource-constrained regions. However, maximizing their potential requires a focus on breeding resilient varieties capable of withstanding various biotic and abiotic stresses.This study utilized UV-Vis spectroscopy to perform metabolite profiling of 23 mung bean varieties, including wild and local accessions sourced from KALRO Katumani. Metabolites quantified included proteins, carbohydrates, phenols, tannins, and flavonoids using standards such as gallic acid, catechol, and albumin serum. Significant differences ($p \leq 0.05$) were observed in the metabolite profiles across the varieties, as indicated by one-way analysis of variance (ANOVA). These results highlight the diversity in metabolite composition among the varieties, which can serve as potential biomarkers in breeding programs. By identifying metabolite variations, such as high levels of phenols and tannins associated with stress responses, this study emphasizes the role of metabolomics in developing resilient mung bean varieties. The findings support the integration of metabolite profiling in breeding strategies to enhance the crop's overall adaptability and sustainability. Leveraging these insights can contribute to reducing reliance on chemical inputs, promoting eco-friendly agricultural practices, and securing global food systems through the cultivation of robust mung bean varieties..

Keywords: Mung beans; Metabolites; Bruchids; UV-VIS Sectrophotometry

Introduction

Mung beans (Vigna radiata L.), an essential legume crop in Asia and Africa, are not only a vital source of nutrition but also exhibit resilience in harsh environmental conditions such as arid and semi-arid lands [5]; [3]. The crop's ability to withstand such conditions makes it an important candidate for breeding strategies aimed at enhancing agricultural sustainability and resilience.

The metabolite profile of mung beans encompasses a variety of primary and secondary compounds, which are essential for seed quality, nutritional value, and medicinal properties. Mung beans are recognized for their high content of vital nutrients, making them a significant dietary staple worldwide. They are particularly abundant in carbohydrates, including starch and dietary fiber, which contribute to sustained energy release and digestive health [7]. Oligosaccharides, such as raffinose and stachyose, present in mung beans, further enhance their nutritional profile by providing prebiotic benefits, which support gut health [8].

Proteins are another key component of mung beans, accounting for approximately 23% of their dry weight. These proteins are particularly valuable due to their well-balanced amino acid profile, with high levels of essential amino acids such as lysine and leucine, which are crucial for human health [6]. The biological value of mung bean proteins highlights their importance in vegetarian diets and food security initiatives. Additionally, mung beans are rich in essential vitamins and minerals, including vitamin B1 (thiamine), vitamin B2 (riboflavin), niacin, vitamin C, and folic acid, all of which are vital for energy metabolism, immune function, and cellular health [6]. Mung beans also provide key minerals such as iron, phosphorus, magnesium, and potassium, essential for bone health, muscle function, and overall physiological balance.

Beyond basic nutritional components, mung beans are distinguished by a variety of secondary metabolites that possess significant bioactive properties. Polyphenolic compounds, including flavonoids, phenolic acids, and tannins, are abundant in mung beans and contribute to their antioxidant and anti-inflammatory activities [11]. These compounds help neutralize free radicals, reducing oxidative stress and protecting cells from damage related to chronic diseases. Saponins, another class of bioactive glycosides, exhibit cholesterol-lowering effects and show potential anticancer properties in experimental settings [11]. Phytosterols, plant-derived compounds structurally similar to cholesterol, also play a role in reducing cholesterol levels, promoting cardiovascular health [12].

The composition and concentration of these metabolites in mung beans are influenced by various factors, including genetic variation, environmental conditions, and post-harvest processing techniques [10]. Different cultivars exhibit distinct secondary metabolite profiles, which reflect their responses to environmental stresses and selective breeding efforts aimed at improving desirable traits. Understanding these complex metabolite profiles can inform breeding strategies to enhance crop yield, nutritional quality, and the development of biofortified varieties with additional health benefits [12]. Breeding programs that focus on metabolite-rich cultivars may contribute to crops that are better adapted to climate change and more resilient to pests like bruchids [8].

Metabolite profiling plays a critical role in advancing these breeding efforts. Secondary metabolites such as phenols, tannins, and other bioactive compounds act as a natural defense mechanism in plants against biotic stresses, including pests like bruchid beetles *(Callosobruchus spp*) [1]. These metabolites not only protect plants but also provide insights into their adaptability and survival under stress conditions. By analyzing these profiles, researchers can identify key metabolites associated with resilience traits, which serve as biomarkers for selecting and breeding robust mung bean varieties [4,6].

UV-V is spectroscopy is an efficient tool for metabolite profiling as it enables the quantification of secondary metabolites and helps establish their correlation with desirable agronomic traits [5]. In the case of mung beans, such analyses have the potential to revolutionize breeding programs by providing a scientific basis for selecting varieties that are not only nutritionally superior but also resilient to environmental and pest pressures. This contributes to the development of high-quality, resistant mung bean varieties that reduce post-harvest losses and reliance on chemical pesticides [2,4].

By leveraging metabolomics approaches, breeding programs can integrate metabolite-based selection into traditional and modern methodologies. This alignment fosters sustainable agricultural practices, ultimately enhancing food security and supporting the livelihoods of smallholder farmers who depend on mung beans as a staple and cash crop.

Materials and Methods

The metabolite profiles of 23 mung bean (Vigna radiata) varieties obtained from the Kenya Agricultural and Livestock Research Organisation (KALRO) Katumani were analyzed using UV-V is spectrophotometry to quantify key compounds such as proteins, carbohydrates, phenols, tannins, and flavonoids. This analysis provided a detailed comparison of the nutritional and phytochemical properties across the varieties, offering valuable insights into their potential resistance to bruchids (Callosobruchus spp.).

For the analysis, mung bean seeds from the 23 varieties were ground into a fine powder and subjected to solvent extraction, with ethanol used for phenols and flavonoids, and methanol used for proteins and carbohydrates. The powdered seeds were mixed with the solvent, vortexed for 5 minutes, and incubated for 24 hours at room temperature. After centrifugation, clear supernatants were collected for further analysis.

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	

Table 1: Mung beans Varieties for Bruchid Resistance Trial.

Source: KALRO Katumani.

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.

Results and Discussions

UV-Vis spectrophotometry, combined with standard curves for each metabolite, enabled the precise quantification of the metabolites. The standards used included; Bovine serum albumin (BSA) was used to create the standard curve for protein quantification based on the Bradford assay method. Glucose was used as the standard to quantify total carbohydrates using the anthrone method.

Concentration(µg/g)	2	4	10	20	30	40
Phenol A (765nm)	0.323	0.778	1.846	1.9784	2.7664	3.506
Tannins A (725nm)	0.113	0.119	0.137	0.248	0.348	0.407
Flavonoids A (510nm)	0.124	0.165	0.344	0.743	0.945	1.344
Proteins A (595nm)	0.0966	0.175	0.6282	0.8506	1.2722	1.5943
Carbohydrates A(490nm)	0.0119	0.0151	0.0407	0.0668	0.0834	0.1187

Table 2: Standard Curve Estimations of Selected Metabolites.

Gallic acid was used to create a standard curve for total phenolic content based on the Folin-Ciocalteu method. Tannic acid was used as the standard for quantifying total tannins while Quercetin was used as the standard for flavonoid quantification.

For phenols, absorbance was measured at 765 nm, with increasing concentrations showing a consistent rise in absorbance values. Similarly, tannins were quantified by measuring absorbance at 725 nm, flavonoids at 510 nm, proteins at 595 nm, and carbohydrates at 490 nm. The absorbance data were plotted against the known concentrations, and the trend lines derived from these plots facilitated the quantification of unknown samples.

Mung bean samples were analyzed by obtaining their absorbance values at the respective wavelengths. These values were then compared to the corresponding standard curves, allowing for the interpolation of the specific concentration of each metabolite in the sample. This approach provided a reliable estimation of the metabolite profiles, which is crucial for identifying variations among mung bean varieties.

Variety code	Treatment code	Phenols µg/g	Tannins µg/g	Flavonoids µg/g	Proteins µg/g	Carbohydrates µg/g
AMVU-1601	AMVU_A	11.222^{bac}	7.5020^{ba}	18.8705^{ba}	30.615^{bac}	23.659^{ba}
AMVU-1602	AMVU_B	9.860^{ebdfc}	6.3775^{edc}	17.1439^{bdce}	30.207^{bdac}	22.602^{bac}
AMVU-1603	AMVU_C	11.014^{bdac}	7.1807^{bc}	18.0072^{bac}	31.905^{ba}	24.481^{a}
AMVU-1604	AMVU_D	6.217^{hjlki}	4.4418^{jik}	13.8345^{i}	29.274^{ebdac}	16.790^{gih}
AMVU-1605	AMVU_E	7.224^{hjki}	5.0924^{hjgi}	14.6978^{ehi}	25.601^{ebdhgf}	20.373^{dec}
AMVU-1606	AMVU_F	6.365^{hjlki}	4.9317^{hji}	14.4101^{ei}	25.096^{ebdhgf}	16.983^{gfih}
AMVU-1608	AMVU_G	8.675^{ehgf}	5.6386^{hegdf}	16.0647^{deg}	27.874^{ebdgcf}	19.326^{gfdec}
AMVU -1612	AMVU_H	12.093^{a}	7.8876^{a}	19.3741^{a}	31.517^{ba}	25.072^{a}
AMVU-1614	AMVU_I	7.372^{hjgki}	5.1566^{hjgi}	15.2734^{deh}	$25.926^{ebdhgcf}$	17.432^{gfieh}
AMVU-1616	AMVU_J	8.024^{hjgkif}	5.3173^{hjgif}	15.4892^{deh}	$26.503^{ebdhgcf}$	18.170^{gfeh}
AMVU-1618	AMVU_K	5.921^{mjlki}	4.6104^{hjik}	13.5468^{ik}	24.159^{eihgf}	15.956^{ijh}
AMVU-1619	AMVU_L	9.208^{edgfc}	6.1526^{edf}	16.6403^{def}	28.956^{ebdac}	20.128^{fdec}
AMVU-1627	AMVU_M	3.641^{mn}	3.6787^{lk}	12.1079^{l}	21.742^{ij}	13.933^{kj}
AMVU-1630	AMVU_N	9.060^{edgf}	5.9598^{egdf}	16.2806^{defg}	28.343^{ebdacf}	19.936^{fdec}
KAT-00301	KAT_O	4.322^{mkn}	3.7751^{lk}	12.2518^{l}	22.536^{ihg}	14.062^{kij}
KAT-00308	KAT_P	3.493^{mn}	3.6145^{lk}	11.1727^{ml}	19.336^{ij}	12.874^{k}
KAT-00309	KAT_Q	3.197^{n}	3.3574^{l}	12.0216^{m}	17.270^{j}	12.425^{k}
KS-20	KS_20	5.744^{mlk}	4.4498^{jik}	13.2590^{ik}	23.942^{eihgf}	15.635^{kijh}
Local Meru	Meru	5.507^{mlkn}	4.3454^{jlk}	12.9712^{ikl}	23.293^{ihgf}	14.993^{kijh}
N-26	N_26	8.375^{ehgif}	5.3574^{hjgif}	12.5396^{il}	28.555^{ebdacf}	17.405^{gfieh}
V100-1709	V100_R	8.201^{ehjgif}	5.4458^{hgif}	15.8489^{de}	$26.792^{ebdhgcf}$	18.459^{gfeh}
V100-1802	V100_S	10.363^{ebdac}	6.6345^{bdc}	17.5755^{bdc}	32.520^{a}	21.669^{bdc}
V100-35226	V100_U	11.624^{ba}	8.0562^{a}	18.6547^{ba}	31.817^{ba}	24.210^{a}
STD		4.635	4.011	2.833	1.411	2.670
SEM		0.558	0.483	0.341	0.170	0.321
F-Value		6.4	12.7	12.24	18.56	17.69
P-Value		0.0001	0.0001	0.0001	0.0001	0.0001

Table 3: Metabolite Profile Analysis.

The analysis of the metabolite profiles in the 23 mung bean varieties revealed significant variability in the concentrations. The data show a range of metabolite levels, with some varieties exhibiting higher concentrations in certain compounds compared to others. Phenolic content varied significantly across the mung beans varieties, with concentrations ranging from 3.197 µg/g in 'KAT-00309' (KAT-Q) to 12.093 µg/g in 'AMVU-1612' (AMVU-H). The highest phenol concentration was observed in 'AMVU- 1612' (12.093 µg/g), followed closely by 'V100 35226' (V100-U) and 'AMVU-1601'. Varieties such as 'AMVU-1604', 'AMVU-1606', 'AMVU-1618', and 'KAT-00308' displayed the lowest phenolic content, with values significantly lower than the high phenol groups. Tannin levels also exhibited considerable variability, with the highest concentration found in 'V100-35226' (8.0562 µg/g) and the lowest in 'KAT-00309' (3.3574 µg/g). Flavonoid concentrations spanned a broad range, from 11.1727 µg/g in 'KAT-00308' to 19.3741 µg/g in 'AMVU-1612'. The highest flavonoid content was found in 'AMVU-1612' and 'AMVU-1601', which might indicate higher antioxidant properties in these varieties. AMVU-1627', 'KAT-00308', and 'KAT-00309' showed the lowest flavonoid levels. Protein concentrations varied from 17.270 µg/g in 'KAT-00309' to 32.520 µg/g in 'V100-1802'. The highest protein content was observed in 'V100-1802', 'AMVU-1603', and 'AMVU-1612', suggesting these varieties could be more nutritious in terms of protein content. Varieties like 'KAT-00309', 'AMVU-1627', and 'KAT-00301' had the lowest protein levels, indicating potential differences in their protein profiles. Carbohydrate content ranged from 12.425 µg/g in 'KAT-00309' to 25.072 µg/g in 'AMVU-1612'. The highest carbohydrate concentrations were seen in 'AMVU-1612' and 'V100-35226', which could suggest these varieties are more energy-dense. Lower carbohydrate levels were noted in varieties such as 'KAT-00309', 'AMVU-1627', and 'KAT-00308'.

The statistical analysis, indicated by the F-values (6.4 for phenols, 12.7 for tannins, 12.24 for flavonoids, 18.56 for proteins, and 17.69 for carbohydrates) and the corresponding p-values (all ≤ 0.0001), strongly supports the significant differences in metabolite concentrations among the varieties. This suggests that the observed variations are not due to random chance and that the metabolite profiles of the varieties are distinctly different. These findings underline the importance of metabolite profiling as a tool for identifying biochemical traits that could contribute to the resilience of mung beans, especially in the context of pest resistance. The standard error of mean (SEM) values further confirm the reliability and consistency of the measurements across replicates.

Conclusion

ANOVA results revealed significant differences in the metabolite profiles (proteins, carbohydrates, phenols, tannins, and flavonoids) among the mung beans varieties, with extremely low P values (< 0.0001). These findings indicate that each mung beans variety has a distinct biochemical composition, which is essential for targeted breeding and nutritional evaluations. Post hoc analyses further clarified which varieties exhibited significantly different metabolite levels, guiding the selection of mung beans for specific dietary and health benefits.

Recommendation

Harness the significant differences in metabolite profiles to breed mung beans varieties that meet specific nutritional and health criteria. Selecting varieties based on their unique biochemical composition can optimize their dietary value and health benefits

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