RESEARCH ARTICLE

SABRAO Journal of Breeding and Genetics
57 (3) 1050-1059, 2025
http://doi.org/10.54910/sabrao2025.57.3.17
http://sabraojournal.org/
pISSN 1029-7073; eISSN 2224-8978

NUTRITIONAL COMPONENT ANALYSIS OF MUNG BEAN (*VIGNA RADIATA* L.)

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SUMMARY

Mung bean (*Vigna radiata* L.), being rich in protein, starch, and bioactive compounds, has become more recognized as a nutritious and healthy food crop. Mining germplasm resources with higher contents of nutritional factors will further improve their efficient use. In the present study, assessing the crude protein, total starch, total polyphenol and flavonoid, and vitexin/isovitexin contents occurred in 400 accessions of mung bean core collection. The results showed that overall, the crude protein content ranged from 21.26% to 31.14% with an average of 25.91%, whereas the total starch content ranged from 34.24% to 59.82% with an average of 40.19%. The total polyphenol content (2.07–5.89 mg/g) was higher than the total flavonoid content (1.13–2.76 mg/g), observing a positive correlation between these two factors. A significant positive correlation also emerged between the contents of vitexin (0.18–2.28 mg/g) and isovitexin (0.15–1.97 mg/g). Similarly, the results also specified that the wild germplasm had higher levels of nutritional components, except for starch. This study will lay a foundation for enhancing the utilization of mung bean resources in developing new varieties with high contents of bioactive compounds.

Keywords: Mung bean (*V. radiata* L.), crude protein, total starch, total polyphenol and flavonoid, vitexin and isovitexin

Key findings: The study showed higher variations for vitexin/isovitexin and flavonoid contents in mung bean (*V. radiata* L.) genetic resources. The contents of crude protein, total flavonoids, total polyphenols, vitexin, and isovitexin were notable at higher rates in the wild germplasm than breeding cultivars, with no obvious relationships among the contents of nutritional components, except for vitexin and isovitexin.

Communicating Editor: Dr. Prakit Somta

Manuscript received: December 05, 2024; Accepted: March 31, 2025.

Citation: Wang S, Wu M, Hu Q, Jiang C, Wang S, Yao Y, Wang L (2025). Nutritional component analysis of mung bean (*Vigna radiata* L.). *SABRAO J. Breed. Genet.* 57(3): 1050-1059. http://doi.org/10.54910/sabrao2025.57.3.17.

INTRODUCTION

Mung bean (*Vigna radiata* [L.] Wilczek), belonging to the family Leguminosae and genus *Vigna*, is one of the most important edible legumes. It is often a useful environmentally resilient crop because it can grow in diverse types of soils and environments. Additionally, mung bean is a short-duration crop with the ability to grow in drought and alkali soils (Mohammed *et al*., 2017). Originally cultivated in Asia, mung bean, a versatile crop, now has spread to dry regions of Africa, Europe, and Australia, as well as in the warmer regions of Canada and the USA (Dahiya *et al*., 2015). In China, mung bean is a widely used plant in intercropping and rotation systems all over the country because of its short growth period and ability to fix nitrogen. However, the total planting area decreased in past years, owing to the low economic efficiency and the impact and competition of imported mung beans (Tian *et al*., 2021).

As a nutritious and healthy food, mung bean is rich in high-quality proteins and excellent carbohydrates, vitamins, minerals, and fibers. It also provides almost all amino acids necessary for daily dietary needs (Deng and Wang, 2010). Moreover, hydrolyzed mung bean proteins have easy absorption and can enhance the absorption of different minerals (Ali *et al*., 2013; Hashiguchi *et al*., 2017). Mung bean starch, primarily comprising amylose with exceptional thermal stability, serves as an ideal raw material for producing vermicelli, noodle paste, ice cream, and jelly products (Li *et al*., 2011).

The Chinese pharmacopoeia '*Bencao Gangmu*' considered mung bean as a kind of medicine for heat-clearing, detoxification, and other medical functions (Hou *et al*., 2019). Recent studies also proved that mung bean had numerous potential health benefits, including hypoglycemic (Liyanage *et al*., 2018), hypolipidemic and antihypertensive (Lopes *et al*., 2018), anticancer and anti-melanogenesis, and hepatoprotective effects (Gupta *et al*., 2018).

Considerable research has progressed on the chemical composition of mung bean seeds, especially on polyphenolics and flavonoids (Giusti *et al*., 2008; Katiyar and Dixit, 2011). China has the largest gene pool for mung bean germplasm, and the core collection of this crop had grown and proven to be a representative sample of the entire collections 10 years ago (Wang *et al.,* 2009). However, only a few of their accessions underwent evaluation for nutritional compounds, which is a disadvantage of efficient use of those germplasms in breeding programs. Therefore, in the following study, 400 mung bean core collections bore assessment for the contents of nutritional compounds. The main targets are to select elite germplasm resources for improving the production and processing of this crop and provide a sound genetic base for future breeding programs and the development of processed products.

MATERIALS AND METHODS

Experimental materials

A total of 400 accessions of the mung bean core collection from the Chinese Genebank, used in this study, consisted of 381 Chinese domestic genotypes from 25 provinces, with 19 as exotic, namely, 17 from Japan and one each from Madagascar and the Philippines (Table 1). The 400 accessions included 282 landraces, 114 breeding cultivars, and four wild genotypes. According to the seed coat color, they break down into five groups, i.e., green (359), dark green (6), black (10), yellow (10), and brown (15) (Figure 1). All these mung bean accessions' sowing ensued in the winter of 2022 in Hainan Province (18°14′ N, 109°59′ E), with their harvest continuing in early 2023. For each accession, selected 30 g seeds sustained grinding into powder using an electric seed crusher (Beijing Liren Technology Co., Ltd.) and then passed through an 80-mesh sieve before further analysis.

Table 1. Origin information of 400 mung bean germplasm.

Source	Quantity	Source	Quantity
Hebei,CN	59	Heilongjiang,CN	6
Henan,CN	41	Shaanxi,CN	6
Inner Mongolia,CN	35	Gansu,CN	5
Shandong,CN	32	Guangdong,CN	3
Taiwan,CN	29	Hainan,CN	3
Hubei,CN	27	Sichuan,CN	3
Jiangsu,CN	27	Guizhou,CN	2
Shanxi,CN	25	Jiangxi,CN	2
Jilin,CN	24	Ningxia,CN	2
Beijing,CN	17	Tianjin,CN	2
Japan	17	Chongqing,CN	2
Anhui,CN	11	Philippines	1
Liaoning,CN	9	Madagascar	1
Hunan,CN	8	Zhejiang,CN	1

Figure 1. Different colors of mung bean.

Reagents

Purchasing sulfuric acid, potassium sulfate, copper sulfate, boric acid, sodium hydroxide, methyl red indicator, bromocresol green indicator, ethanol, and glacial acetic acid buffer *(P*H 5) came from Sigma-Aldrich (St. Louis, Missouri, USA). The GOPOD reagent buffer, heat-resistant α-amylase, glucoamylase, and glucose standard solution (100 μg/0.1 mL) came from NEOGEN Corporation (Lansing, Michigan, USA).

Experimental procedure

Determination of the crude protein content

The study determined crude protein content using the Kjeldahl method (KDY-9830 Kjeldahl nitrogen analyzer) and the Chinese food safety standard (GB5009.5-2016) (Du *et al*., 2018). Briefly, 1.00 g (accurate to 0.001 g) powder of each accession reached mixing with 1.5 g copper sulfate, 4.5 g potassium sulfate, and 10 mL concentrated sulfuric acid in an 80-ml tube. Then, the solution received incubation at 420 °C for 1 h. The transparent digestion solution, cooled down to room temperature, gained 10 mL of distilled water before completing the distillation, absorption, and titration steps. After approximately eight minutes, recording data followed on crude protein content.

Determination of the total starch content

The total starch content's determination used the GOPOD method and the AACC 76-13.01 standard (Megazyme Total Starch Content Determination Kit) (Li *et al*., 2021). Specifically, 0.100 g of powder from each accession was mixed with 0.2 mL 80% ethanol in a 10-ml tube, using intense shaking to mix thoroughly. Then, adding 2 mL of DMSO (dimethyl sulfoxide), shaking continued before heating in boiling water for 5 min, with 0.1 mL of heat-resistant α-amylase immediately added and shaken again. Heating in boiling water

took 6 min, followed by putting in 50 °C water. Then, the pouring of 4 mL of glacial acetic acid buffer *(P*H 5) occurred before shaking and adding 0.1 mL of glucoamylase. The solution reached rigorous mixing and placing in 50 °C water for 30 min before transferring to a 50-mL volumetric flask, with 7-ml diluted sample solution moved into a centrifuge tube for 4-min centrifugation (224.8 g rcf). Finally, taking out 0.1 mL of the supernatant attained mixing with 3 mL of GOPOD (dimethyl sulfoxide) buffer and heated in 50 °C water for 20 min. Afterward, the 0.2-mL supernatant, as placed into an enzyme plate, acquired analysis (Corning Inc.).

Determination of flavonoid and polyphenol contents

The detection of total flavonoid and total polyphenol contents employed a Spectra Max Plus 384 microplate reader and extraction method (Wang *et al*., 2022). Briefly, 0.10 g powder of each accession was mixed with 8 mL of 70% methanol for a 15-min ultrasonic extraction (KQ3200 ultrasonic cleaner). Then, centrifuging at 10,000 rpm for 10 min had a room temperature (5430R high-speed refrigerated centrifuge) before collecting the supernatant for further analysis.

The steps to obtain total flavonoid include: taking 1 mL of supernatant to mix with 0.75 mL 7% $NaNO_2$; the mixed solution left standing for 3 min and then added with 0.75 mL 3% $AlCl_3$; again, leaving the mixed solution for 3 min before mixing with 0.5 mL 4% NaOH solution and diluted with 0.6 mL distilled water; the final solution left undisturbed for 5 min before analysis using a microplate reader to determine the absorbance at 510 nm. Rutin served as the standard and gained quantification according to the external standard method, expressing the results as mg rutin/g dry weight.

For total polyphenol, steps were as follows: 0.4 mL supernatant mixed with 0.25 mL of 1 M F-C reagent and 3 mL distilled water; the solution left undisturbed for 5 min and then mixed with 0.75 mL 7% $NaCO_3$ solution, diluted with distilled water (for a final volume of 6 mL), and incubated for 1 h at room temperature in the dark, using a labeled enzyme; the absorbance at 765 nm was measured; gallic acid, as the standard, reached quantification according to the external standard method, with results expressed as mg GAE/g dry weight.

Determination of the vitexin and isovitexin contents

Determining the vitexin and isovitexin contents utilized a high-performance liquid chromatography system (Shimadzu HPLC) (Chen *et al*., 2006). Quickly, 0.20 g mung bean powder underwent thorough mixing with 8 mL of 70% methanol in a 10-mL centrifuge tube for a 10-min sonication, then centrifuged at 10,000 rpm for 15 min. Finally, a 2 mL aliquot of the supernatant received filtering through a 0.22 μm membrane before analysis using a high-performance liquid chromatography system (Shimadzu HPLC).

Statistical analysis

Excel 2010, Origin 2021, and R Studio applications were used for all data analyses, respectively.

RESULTS

Crude protein content

Overall, the crude protein (CP) content ranged from 21.26% to 31.14%, with an average of 25.91% and a coefficient of variation of 7.64% among all mung bean accessions. About 96.79% of those accessions had CP contents from 22% to 30%. The frequency distribution of the CP was consistent *(P* > 0.05) with a normal distribution (Figure 2). Three mung bean accessions with CP content exceeding 30% originated from Jiangsu (2 acc.) and Liaoning (1 acc.). Only 2.5% of the accessions had a CP content lower than 22.0%. Accessions from Tianjin had the highest CP content (26.72%), followed by Chongqing (26.49%). Accessions with the lowest average of CP (25.11%) were from Sichuan.

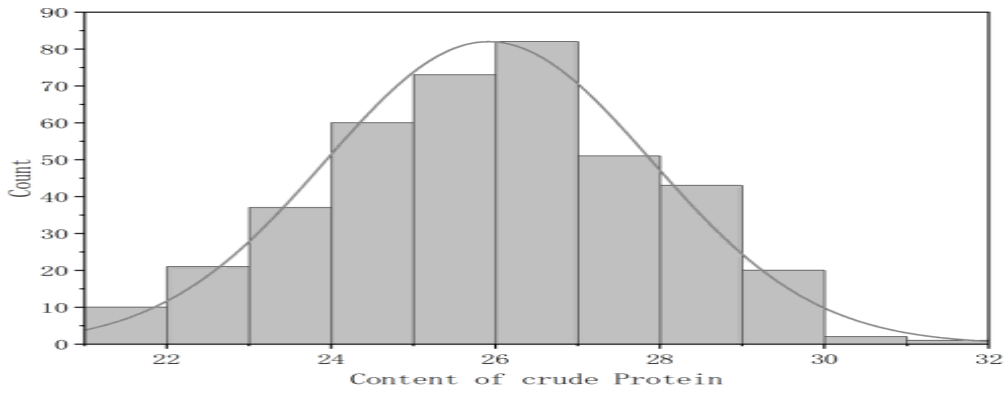

Figure 2. Frequency distribution of the crude protein content in 400 mung bean accessions.

No significant *(P* > 0.05) difference for CP existed between the domestic (25.93%) and exotic mung bean accessions (25.69%). However, the average CP was the topmost in wild accessions (27.16%), followed by breeding cultivars (26.14%) and landraces (25.80%). Non-significant *(P* > 0.05) differences for CP were evident among the yellow (26.4%), green (25.93%), brown (25.79%), black (25.50%), and dark green (24.84%) seeds of mung bean.

Total starch content

Mostly, the total starch (TS) content ranged from 34.24% to 59.82%, with an average of 40.19% and a coefficient of variation of 13.98% among all accessions. The frequency distribution of the TS was inconsistent *(P* < 0.05) with a normal distribution, and 60.75% of those accessions distributed from 35% to 40%. Seven accessions showing higher TS than 56.00% were from Jilin, Jiangsu, Hebei, Guizhou, Heilongjiang, Hubei, and Shaanxi, respectively. Accessions from Hunan had the lowest average TS (35.86%).

Non-significant *(P* > 0.05) differences appeared between local (40.48%) and foreign (40.57%) mung bean accessions for average TS. The average TS was the highest in the landraces (40.83%), followed by breeding cultivars (36.39%) (Figure 3). For different seed colors, the values for average TS were yellow (42.35%) > green (40.58%) > brown (38.84%) > dark green (38.62%) > black (38.62%).

Total flavonoid content

The total flavonoid (TF) content varied from 1.13 to 2.76 mg/g, with an average of 1.95 mg/g and a coefficient of variation of 16.98%. The frequency distribution of TF was inconsistent with a normal distribution *(P* < 0.05), and 67.75% of the accessions ranged from 1.4 to 1.8 mg/g for TF. Three accessions with TF exceeding 2.6 mg/g originated from Japan (1 acc.) and Hebei (2 acc.). In addition, seven accessions (5 from Hebei and 2 from Jilin) had TF values less than 1.2 mg/g.

The average TF values of brown, dark green, black, yellow, and green mung bean seeds were 1.88, 1.80, 1.76, 1.71, and 1.65 mg/g, respectively. The average TF of wild accessions (2.05 mg/g) was clearly higher *(P* < 0.01) than that of breeding cultivars (1.68 mg/g) and landraces (1.66 mg/g) (Figure 4). Non-significant *(P* > 0.05) differences occurred between the exotic (1.74 mg/g) and local (1.67 mg/g) mung bean accessions.

Total polyphenol content

The total polyphenol (TP) content ranged from 2.07 to 5.89 mg/g, with an average of 3.30 mg/g and a coefficient of variation of 12.80% among all accessions. The frequency distribution of the TP was inconsistent with a normal distribution *(P* < 0.05), and 76.5% of the accessions distributed between 3.0 and 3.9

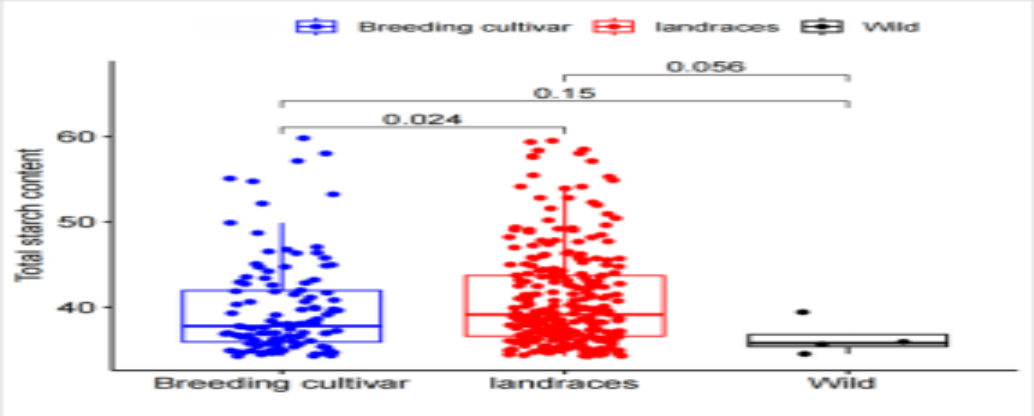

Figure 3. Comparison of total starch contents in different types of mung bean accessions.

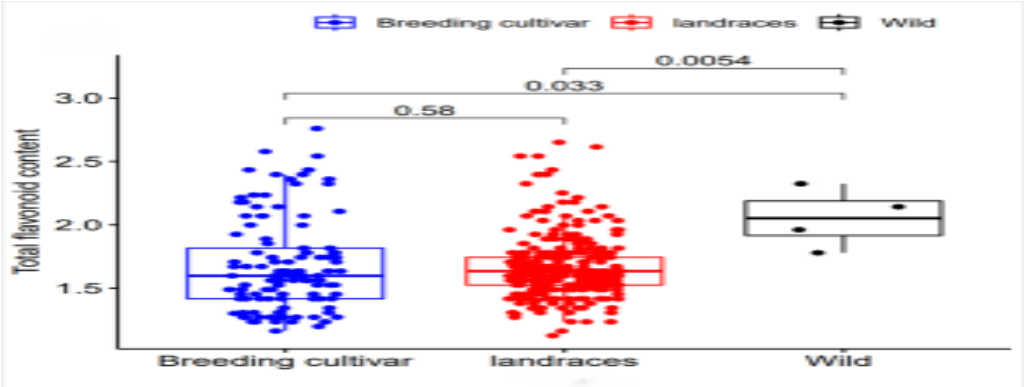

Figure 4. Comparison of total flavonoid contents in different types of mung bean accessions.

mg/g. Two accessions with TP greater than 5 mg/g were both from Inner Mongolia. However, for the average value of TP, both accessions from Shanxi and Inner Mongolia had the supreme average TP content (3.46 mg/g).

The average TP of yellow mung bean seeds (3.24 mg/g) was slightly lower than that of green seeds (3.29 mg/g) *(P* < 0.05), dark green (3.44 mg/g) *(P* > 0.05), brown (3.53 mg/g) *(P* < 0.01), and black (3.62 mg/g) *(P* < 0.01) seeds. The average TP was the ultimate in wild accessions (3.93 mg/g), followed by landraces (3.31 mg/g) and breeding cultivars (3.26 mg/g). No significant *(P* > 0.05) differences emerged between the domestic (3.30 mg/g) and exotic (3.32 mg/g) accessions (Figure 5).

Vitexin and isovitexin contents

The vitexin (VX) content ranged from 0.18 to 2.28 mg/g and the isovitexin (IVX) content ranged from 0.15 to 1.97 mg/g, with coefficients of variation at 38.69% and 39.29%, respectively. Both the frequency distributions of VX and IVX were consistent with a normal distribution *(P* > 0.05). Three accessions with VX and IVX exceeding 2.1 and 1.9 mg/g, respectively, originated from Shandong, Taiwan, and Madagascar. One accession with a VX value less than 0.2 mg/g came from Henan, whereas three accessions with an IVX less than 0.2 mg/g had origins from Henan, Hebei, and Jiangsu, respectively. Accessions from Tianjin had the highest average of VX and IVX (1.43 and 1.25 mg/g, respectively) (Figure 6).

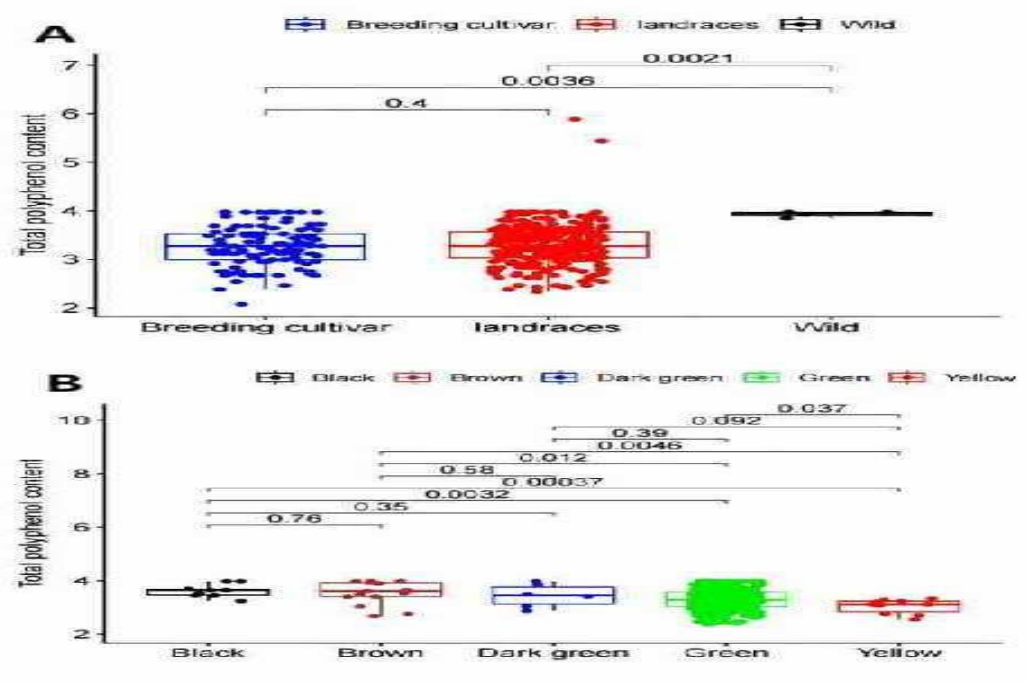

Figure 5. Comparison of total polyphenol contents of mung bean accessions that differed in terms of type (A), and seed color (B).

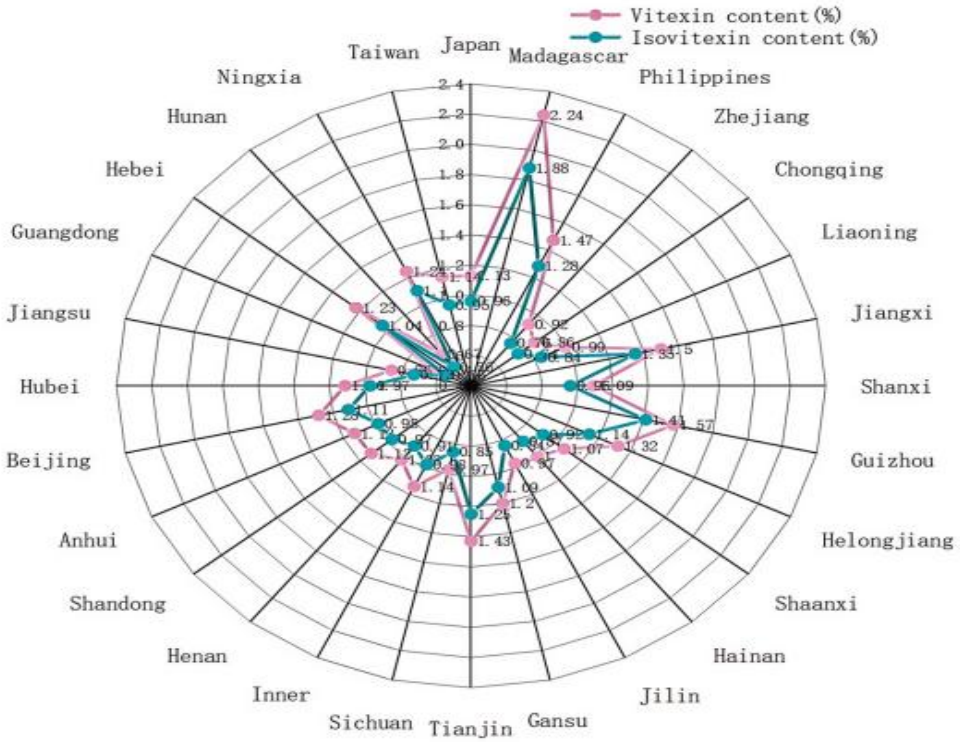

Figure 6. Average vitexin and isovitexin contents in 400 mung bean accessions with different origins.

The average VX and IVX values were the maximum in yellow seeds (1.53 and 1.39 mg/g, respectively), followed by brown seeds (1.35 and 1.15 mg/g, respectively), whereas they were the lowest in green seeds (1.08 and 0.93 mg/g, respectively). The higher *(P* < 0.05) average values were notable in wild accessions (1.81 and 1.5 mg/g, respectively) compared with breeding cultivars (1.09 and 0.94 mg/g, respectively). Non-significant *(P* > 0.05) differences manifested between the foreign (1.32 and 1.11 mg/g, respectively) and the domestic (1.09 and 0.94 mg/g, respectively) accessions (Figure 7).

Correlation analysis of the nutritional compounds

No observable association (R = −0.00) between CP and TS values in mung bean accessions existed (Table 2). Both slight correlations were noteworthy among TS, TP, and VX (R = 1.18, 0.15), IVX (R = 0.20, 0.16), as well as the correlations between TF and TP (R = 0.32), VX (R = 0.17), and IVX (R = 0.18). However, a significant positive correlation (R = 0.99) was evident between the VX and IVX in mung bean accessions.

DISCUSSION

Factually, mung beans have higher nutritional values and various physiologically active compounds. However, only a few studies on the assessment of nutritional factors in Chinese mung bean collection were available. In the presented study, researchers assessed the crude protein, total starch, polyphenol, flavonoid, and vitexin and isovitexin contents in a set of 381 accessions of Chinese mung bean core collection and 19 exotic accessions. The results showed a higher crude protein content with an average value of 25.91%, which was similar with a previous study (25.69%) in mung bean genotypes (Zhu *et al*., 2005). It suggested the content of protein in mung bean germplasm was relatively stable, and this study result is relatively reliable.

For average protein content, non-significant differences were prominent between accessions from different origins. However, it was a little higher in breeding cultivars (26.14%) than in landraces (25.80%), indicating the protein content's enhancement during the breeding process. Black mung bean is very popular among consumers, where reports stated it with a fairly high protein content (25.13% to 28.48%) (Ai *et al*., 2023). However, this promising study detected a low protein content in black seeds, mainly owing to the limited number of accessions for black seeds.

Moreover, observed in the study was a much lower content of starch (34.24% to 59.82%), as compared with a previous study (53.1% to 61.17%) (Jin *et al*., 2013). This might be due to the different origins of the mung bean accessions and the different extraction methods used because they employed the 3, 5-dinitrosalicylic acid (DNS) colorimetric method. This study did not find any apparent trends for the total starch among the accessions from different sources. A similar result (1.13 to 2.76 mg/g) emerged for total flavonoid, as reported in previous studies (1.51 ± 0.08 mg/g) (Liu *et al*., 2020), but a much higher value for total polyphenol (2.07 to 5.89

Table 2. Pearson's correlation coefficients among quality-related traits in 400 mung bean accessions.

Traits	cp	TS	TF	TP	VX	IVX
TS	-0.0042					
TF	0.04	0.0042				
TP	-0.029	0.027	0.32***			
VX	-0.034	0.18***	0.17***	0.15**		
IVX	-0.039	0.2***	0.18***	0.16	0.99***	
HSW	-0.024	-0.03	-0.12*	-0.12*	-0.2	-0.23***

Note: HSW: 100-seed weight; CP: crude protein; TS: total starch; TF: total flavonoid; TP: total polyphenol; VX: vitexin; IVX: isovitexin. *: *P* > 0.05, **: *P* < 0.01, ***: *P* > 0.001.

mg/g) in this research appeared than a previous report in germinated mung bean seeds (Zhang *et al*., 2022). This could refer to the gradual degradation of polyphenol during the germination of mung beans.

Notably, a high positive correlation between VX (0.18–2.28 mg/g) and IVX (0.15–1.97 mg/g) was consistent with previous studies (1.50 mg/g of VX and 1.92 mg/g of IVX) (Cao *et al*., 2011). If soaking mung bean seeds for 24 h and using 80% (v/v) ethanol/water as solvent, the VX and IVX values could increase (Kang *et al*., 2015). Except for protein and starch, the other components were relatively low in mung bean seeds. Therefore, the detection methods might affect the exact data, as reported in different studies; however, it will be useful for the same batch of samples.

Earlier reports stated the nutrition traits in soybean and mung bean have control mainly by micro-effect polygenes, which acquire influences from environmental conditions, resulting in a particular degree of randomness and variability in results (Ma and Li, 2008; Ning *et al*., 2005; Masari *et al*., 2017). However, we only detected the seeds from one ecoregion here, and to precisely evaluate the accumulation of nutritional factors on mung bean, more systematic trials should progress in the future. Therefore, further planting and assessment on those accessions with higher content of each nutritional factor in the presented study will continue in diverse environments to investigate the appropriate environmental conditions for increasing the synthesis of these nutritional compounds.

CONCLUSIONS

In this relevant study, 400 mung bean accessions underwent analysis for their crude protein and total starch contents and functional compounds. The results showed significant variations among the mung bean accessions for different nutritional factors. Except for total starch, wild mung beans had higher contents of nutritional factors than cultivated ones. Yellow seeds had a fairly low content of total polyphenol and flavonoid, while having the highest content of vitexin and isovitexin than any other seeds. The mung bean accessions with the higher content of nutritional factors will be helpful for potential use in breeding programs.

ACKNOWLEDGMENT

Authors thank the National Key R&D Program of Ministry of Science and Technology (2023YFD1200705/ 2023YFD1200700), the National Natural Science Foundation of China (32241042), and the China Agriculture Research System of MOF and MARA (CARS-08) for financial support.

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