












Genetic diversity analysis of seed phenotypic traits of 302 mung bean germplasm resources

Yupeng Zhang¹  Jiafei Liu¹  Linchao Jing¹  Donghui Ding¹  Wei Chang¹ 
Lingling Cao¹  Wan Zhang¹  Jibao Chen¹  Shuqiong Yang^{1*} 

¹College of Life Science and Agricultural Engineering, Nanyang Normal University, 473061, Nanyang, China. E-mail: sqyang@nynu.edu.cn.
*Corresponding author.

ABSTRACT: Owing to rich in vitamins, proteins, mineral elements, and active substances, the highly medicinal and economic value of mung bean seeds have been proven to be beneficial to human. In order to fully identify and innovative utilization of excellent germplasm resources for mung bean yield and quality breeding, the genetic diversity level of eight seed phenotypic traits of 302 mung bean germplasm resources were comprehensively evaluated by diversity analysis, correlation analysis, principal component analysis (PCA), and cluster analysis. The results showed the genetic diversity of eight seed phenotypic traits of 302 mung bean germplasm resources with a Shannon diversity Index ranging from 0.25 to 2.09. For most traits, their coefficient of variation (CV) were more than 10% with the highest being seed shape (41.51%). Correlation analysis showed that the 100-seed weight had extremely positive correlations with seed size, seed length, seed width, and seed thickness. PCA of seed phenotypic traits showed that the cumulative contribution rate of the first three principal components was 78.551%. These principal components included seed width, seed coat gloss and seed color. The 302 mung bean germplasm resources were divided into eight groups by cluster analysis. A total of 140 germplasm resources with large-seed were selected from group I and group II, of which 9 germplasm resources in group II were likely to be high-yield varieties. Overall, this study not only indicated sufficient variation among the studied germplasm resources, but also provided a theoretical basis for the selection, utilization and hybrid breeding of mung bean germplasm resources.

Key words: Mung bean, seed phenotypic traits, genetic diversity, cluster analysis, principal component.

Análise da diversidade genética de características fenotípicas de sementes de 302 recursos de germoplasma de feijão mungo

RESUMO: Devido ao seu caráter rico em vitaminas, proteínas, elementos minerais e substâncias ativas, o valor altamente medicinal e econômico das sementes de feijão-mungo tem provado ser benéfico para o ser humano. A fim de identificar plenamente e utilizar de forma inovadora excelentes recursos de Germoplasma para a produção de feijão-mungo e reprodução de qualidade, o nível de diversidade genética do oito caracteres fenotípicos de sementes de 302 recursos de germoplasma de feijão-mungo foram avaliados de forma abrangente por meio de análise de diversidade, análise de correlação, análise de componentes principais (PCA) e análise de agrupamento. Os resultados mostraram a diversidade genética de oito características fenotípicas de sementes de 302 recursos de germoplasma de feijão-mungo com um Índice de diversidade Shannon variando de 0,25 a 2,09. A análise de correlação mostrou que o peso de 100 sementes apresentou correlações extremamente positivas com tamanho, comprimento, largura e espessura de sementes. A PCA das características fenotípicas das sementes mostrou que a taxa de contribuição cumulativa dos três primeiros componentes principais foi de 78,551%, sendo estes componentes principais a largura da semente, brilho do revestimento e cor da semente. Os 302 recursos de germoplasma de feijão-mungo foram divididos em oito grupos por análise de agrupamento. Foram selecionados 140 recursos de germoplasma com sementes grandes dos grupos I e II, dos quais nove recursos de germoplasma do grupo II provavelmente seriam variedades de alto rendimento. De forma geral, este estudo não só indicou variação suficiente entre os recursos de germoplasma estudados, como também forneceu uma base teórica para a seleção, utilização e melhoramento híbrido dos recursos de germoplasma de feijão-mungo.

Palavras-chave: Feijão-mungo, características fenotípicas de sementes, diversidade genética, análise de agrupamento, componente principal.

INTRODUCTION

Mung bean (*Vigna radiata* L.) is a cultivated species of the genus *Vigna* in Leguminosae. As one of the origin centers of mung bean, China has a history of cultivation and consumption for more than 2000 years (KANG et al., 2022). Mung bean has been long popular because of its short growth period, long sowing period, strong stress resistance, symbiotic nitrogen fixation, and soil improvement ability (CHEN et al., 2020a; MWANGI et al., 2021). As one of medicinal

and edible crop, mung bean not only has highly economic value, but also plays an irreplaceable role in the optimization and adjustment of agricultural planting structure and the in-depth promotion of high-quality seed project (ZHU et al., 2019).

Germplasm resources are the basis of crop research, and the analysis of genetic diversity of germplasm resources is the fundamental basis and premise of crop breeding and improvement. In recent years, with the development of science and technology, many methods have been applied to the study of crop genetic diversity, including

morphology, physiology, ecology, molecular biology, etc. Among them, the study of crop genetic diversity through morphological or phenotypic traits is the simplest and most intuitive method (LIU et al., 2007). HAO et al. (2020) identified and evaluated 13 agronomic traits of 160 chickpea germplasm resources, eventually screening out 63 early-maturing, short-stalked, non-branched, multi-legged, large-seed, and high-yield specific germplasm resources. LIU et al. (2014) analyzed the genetic diversity of phenotypic traits of 103 *Gossypium arboreum* germplasms, which found that some *G. arboreum* germplasms from Vietnam, Guizhou, Guangxi, and Yunnan were interlaced in genetic clustering, while the germplasms from India were almost independent of other groups. By investigating 11 phenotypic traits of 177 sweet potato landrace resources, YAO et al. (2022) clarified the genetic diversity degree of sweet potato landrace resources in China and their phenotypic differences in different periods. The determination of plant genetic variation and diversity via phenotypic traits will be conducive to understanding the level of genetic variation in a short amount of time.

The abundant germplasm resources are the basis for further research of mung bean, understanding and mastering the genetic diversity of mung bean seed traits also lays the foundation for further research on mung bean. CHEN et al. (2020b) used the grey correlation analysis method to comprehensively evaluate the field traits of 24 new mung bean varieties from southern Guangxi, and analyzed the correlation between yield and other traits. LIU et al. (2008) constructed a primary core collection of 719 mung bean germplasms based on 14 agronomic traits using 5,072 mung bean germplasms as materials. QIAO et al. (2015) evaluated 5 qualitative and 10 quantitative traits of 352 mung bean germplasms from 15 countries and regions, of which 338 germplasms displayed rich genetic variation in agronomic traits. With the development of statistics, multiple statistical analysis methods have been comprehensively adopted to analyze the relationship between mung bean resource traits (CHEN et al., 2020a; GAO et al., 2020), while few reports have focused on their seed phenotypic traits. In this study, the makeup of genetic relationships among 302 mung bean germplasm resources was determined by analyzing their descriptive and quantitative genetic characteristics. The full understanding the basic seed phenotypes of 302 mung bean germplasm resources will provide a theoretical basis for collection, preservation, innovative and usage of mung beans.

MATERIALS AND METHODS

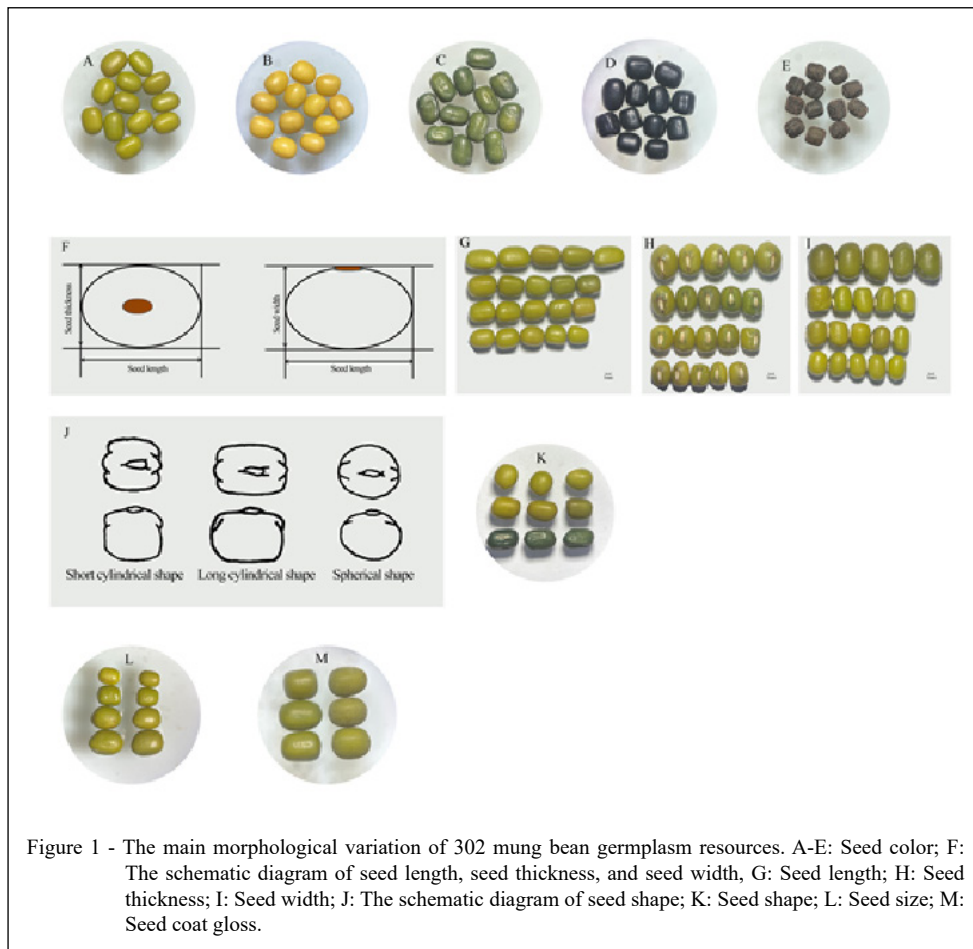
Plant material collection and preparation

In total, 302 mung bean germplasm resources were provided by the Institute of Crop Science of Chinese Academy of Agricultural Sciences, and planted in the experimental farm of Nanyang Normal University, Nanyang City, Henan Province. After threshing, the collected seeds were dried to a moisture content of 6 ~ 8%, and stored at 4°C with the 30 ~ 50% relative humidity. After randomly screening, the 100 normal mature seeds with uniform size from each germplasm resource were prepared for the genetic diversity of seed phenotypic traits.

Measurement items and methods

In this study, eight seed phenotypic traits of 302 mung bean germplasm resources, which including the seed size, seed color, seed shape, seed coat gloss, 100-seed weight, seed length, seed width, and seed thickness were measured and analyzed (Figure 1). Three seed phenotypic traits of seed color, seed shape, and seed coat luster were determined by the visual observation according to the principle of maximum similarity, and each germplasm resource was observed three times. The 100-seed weight of each germplasm resource were measured using the electronic balance. Three phenotypic traits of seed length, seed width, and seed thickness were measured by electronic digital caliper, 10 normal mature seeds were randomly selected from each germplasm resource, and the trait values of each seed were measured and the average number was calculated. According to the 100-seed weight, the seed size trait was determined as small with the 100-seed weight < 4g, medium with $4g \leq 100\text{-seed weight} < 6g$, large with $6g \leq 100\text{-seed weight} < 8g$, extra-large with $8g \geq 100\text{-seed weight}$. The above traits were measured and counted based on the Descriptors and Data Standard for Mung bean [*Vigna radiata* (L.) Wilczek] (CHENG et al., 2006).

In order to facilitate the statistical analysis of seed phenotypic data, the quality traits were evaluated by numbers (Table 1). Each quantitative character was graded separately, the first grade $X_i = (X - 2s)$ increased by 0.5 s for each middle grade until level 10 was $X_i = (X - 2s)$. The relative frequency (P_i) of each level was calculated, and then, P_i was used to calculate the diversity index of each characteristic. The diversity index was calculated by the Shannon-Wiener diversity index (H') (DIKSHIT et al., 2013; ORTIZ-BURGOS., 2016), and the formula was as follows:



$$H' = - \sum_{i=1}^n P_i \ln P_i \quad (1)$$

\ln was the natural logarithm and P_i was the relative frequency of each level (the percentage of the total number of materials in the i th level of a trait).

Data analysis

All the seed phenotypic data were processed using SPSS 25.0, the mean value (\bar{X}),

standard deviation (S), and coefficient of variation (CV) of each trait were calculated, respectively. The correlation among phenotypic traits was calculated using Pearson's correlation coefficient, the results were subjected to two-way ANOVA analysis of variance and compared at $P < 0.05$ and 0.01 . The principal components of phenotypic traits were extracted using dimensionality reduction analysis and factor analysis (SPSS 25.0). Cluster analysis was performed on the values of 8 grain phenotypic traits according to the UPGMA method. Finally, the

Table 1 - Coden designed for seed qualitative traits in mung bean.

Traits	Codon of qualitative traits				
Seed size	1. Small	2. Medium	3. Large	4. Extra-large	
Seed coat gloss	1. Dull	2. Glossy			
Seed shape	1. Long cylindrical shape		2. Short cylindrical shape		3. Spherical shape
Seed color	1. Yellow	2. Green	3. Brown	4. Blue cyan	5. Black

frequency distribution map, correlation heat map, principal component map, and clustering map were drawn by Origin 2021 software using the parameters.

RESULTS

Diversity analysis of seed phenotypic characters of Mung bean

The genetic diversity of seed phenotypic traits of 302 mung bean germplasm resources was analyzed. Among all the mung bean germplasm resources analyzed, the seed color of 95.4% germplasm resources was green, only a few germplasm resources were yellow (9), brown (2), blue-green (1), and black (3) (Figure 2A). The germplasm resources with glossy seed coat and dull seed coat accounted for 80.79% and 19.21%, respectively (Figure 2B). As for the seed shape, the long round, short round, and spherical accounted for 60.27%, 33.77% and 5.96%, respectively (Figure 2C). The number of germplasm resources with medium and large seed size was roughly the same (49.67% and 46.03%), followed by extra-large (2.98%) and small (1.32%) (Figure 2D). The variation range of 100-seed weight was 2.72 ~ 9.38 g, the 100-seed weight of most germplasm resources ranged between 4.55 g and 7.53 g (87.09%), 5.96% of germplasm resources less than 4.55 g, and 6.95% of germplasm resources greater than 7.53 g (Figure 2E). The variation range of seed length was 3.60 ~ 6.39 mm, 96.36% of germplasm resources were concentrated in the range of 4.40 ~ 5.95 mm, 2.65% of germplasm resources less than 4.40 mm and 0.99% of germplasm resources more than 5.95 mm (Figure 2F). The seed width of 267 germplasm resources in the range of 3.67 ~ 4.30 mm, which accounting for 88.41 % of all germplasm resources (Figure 2G). The seed thickness of most germplasm resources (93.38%) ranged from 3.67 ~ 4.50 mm (Figure 2H).

Our results showed that the coefficients of variation (CVs) and variation ranges of seed phenotypic traits were not exactly the same. On the contrary, they had high variability and rich diversity. (Table 2). Among the tested germplasm resources, the CVs of eight seed phenotypic traits ranged from 5.82% to 41.51%, and the mean CV was 19.07%. The CV of seed shape was the largest (41.51%), which indicating that the seed shape of the tested germplasm resources changed greatly. The CV of seed width was the smallest, about 5.82 %, which indicating that the seed width of 302 mung bean germplasm resources was similar. The diversity index of eight traits ranged from 0.25 to 2.09, with an average of 1.29. Among them, the diversity index of 100-seed weight was the highest (2.09), which indicating that the diversity of 100-seed

weight was the mostly abundant. The diversity index of seed color was the lowest (0.25), which indicating that the richness of seed color diversity was the lowest. The CV and diversity index of seed phenotypic traits of tested germplasm resources were significantly different, which indicating that the rich genetic diversity of seed phenotypic traits of 302 mung bean germplasm resources.

Correlation analysis of seed phenotypic characters of Mung bean

The correlation analysis of eight seed phenotypic traits of 302 mung bean germplasm resources showed that 16 correlation pairs were found, of which 10 pairs were extremely significantly positively correlated, 5 pairs were extremely significantly negatively correlated, and 1 pair was significantly negatively correlated (Figure 3). Among these eight seed phenotypic traits of mung bean, the 100-seed weight was extremely significantly positively correlated with seed length, seed width, seed thickness, and seed size, while it displayed a significantly negative correlation with seed shape and seed color. The seed size was extremely significantly positively correlated with seed length, seed width, and seed thickness, however, it showed an extremely significantly negative correlation with seed shape. Seed length displayed an extremely significantly positive correlation with seed width and seed thickness, meanwhile, seed width was extremely significantly positively correlated with seed thickness. Seed shape was extremely significantly negatively correlated with seed length, seed width, and seed thickness. Seed colors only showed an extremely significantly negative correlation with 100-seed weight, while seed coat gloss was not correlated with any other seed phenotypic traits.

PCA of seed phenotypic characters of Mung bean

Principal component analysis was performed on the seed phenotypic traits of 302 mung bean germplasm resources (Table 3 and Figure 4). According to the principle of eigenvalue greater than 1, three principal components were selected, and the cumulative contribution rate was 78.551%. These three principal components covered most quantitative traits of mung bean germplasm resources. Among them, the initial eigenvalue of principal component 1 (PC1) is 4.235, and the contribution rate was 52.942%, which was the main contributor. Among the principal components, seed width, seed thickness, seed length, 100-seed weight and seed size had the largest influence, and the

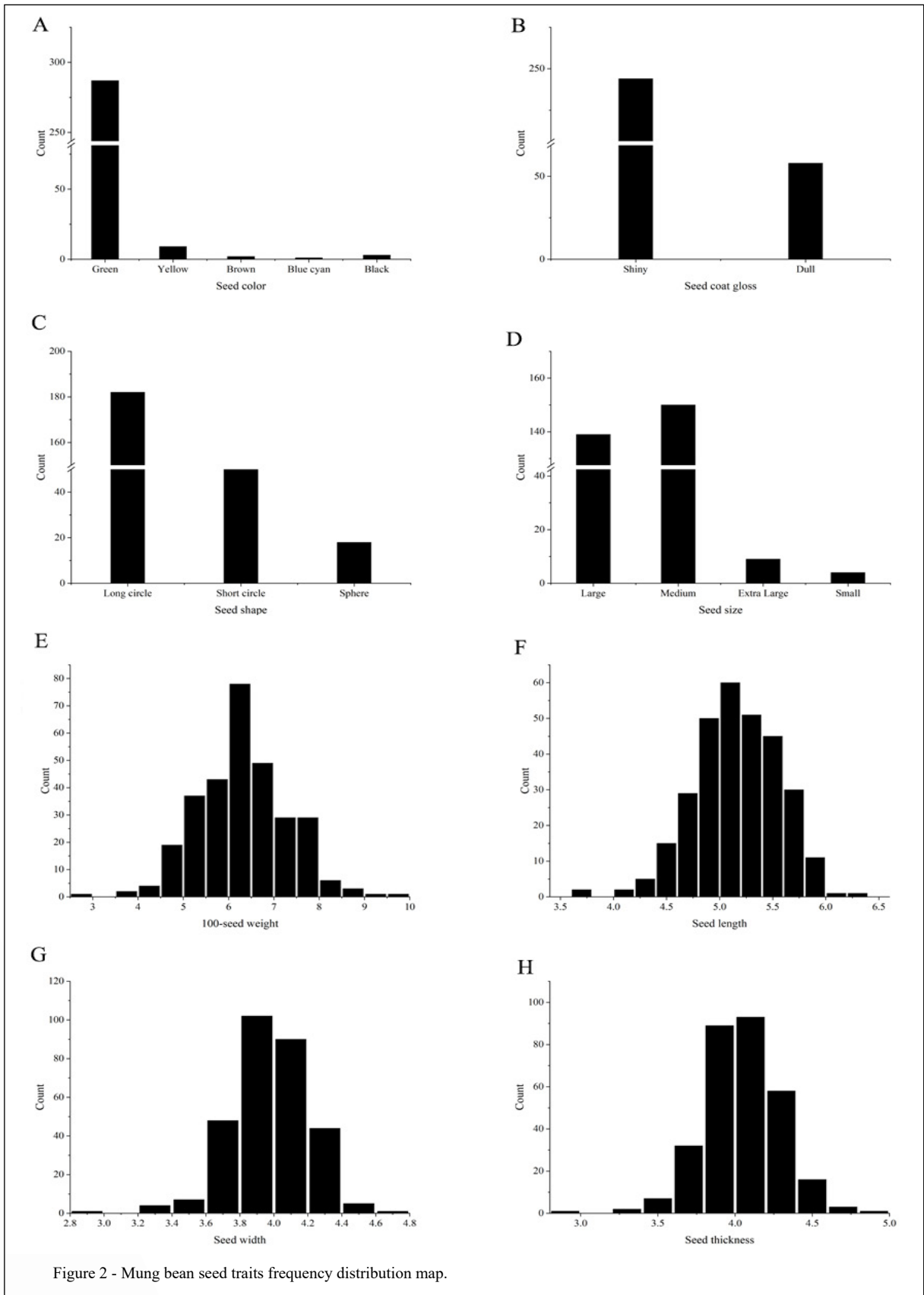


Figure 2 - Mung bean seed traits frequency distribution map.

Table 2 - Variation of seed phenotypic traits of 302 mung bean germplasm resources.

Traits'	Max	Min	Average	SD	CV/%	Diversity index
100-seed weight	9.38	2.90	6.04	1.00	16.63	2.09
Seed length	6.39	3.60	5.16	0.40	7.78	2.05
Seed width	4.60	2.83	3.98	0.23	5.82	1.93
Seed thickness	4.84	2.84	4.04	0.25	6.14	1.84
Seed size	4	1	2.51	0.58	23.10	0.86
Seed color	5	1	2.01	0.37	18.54	0.25
Seed shape	3	1	1.46	0.61	41.51	0.84
Seed coat gloss	2	1	1.19	0.39	33.10	0.49

eigenvectors were 0.928, 0.919, 0.903, 0.885 and 0.843, respectively. Among them, the load of 100-seed weight and seed size was larger. Therefore, the first factor was used as the principal component of yield. These indicators are closely related to yield: the greater of seed width, seed thickness, and seed length value, the greater of the seed size and 100-seed weight, the higher of the potential yield.

The initial characteristic value of PC2 was 1.047, which having a contribution rate of 13.086 %. The seed coat gloss, seed shape, and seed color contributed to this component, with their vectors were 0.913, 0.361, and 0.218, respectively. Therefore, the second factor was used as the principal component of seed coat glossiness. Compared with

dull mung bean, the mung bean with the seed of dark green and shiny, large and neat, round shape was better. Therefore, this factor should be properly controlled in the further breeding.

The initial eigenvalue of PC3 was 1.002, and the contribution rate was 12.523 %, which was driven by seed color. The vector was 0.798, and the seed color load was the largest. Therefore, the third factor was regarded as the principal component of seed color. The plant pigment biosynthetic pathway was relatively conservative, which was closely related to the enzymes in the flavonoid biosynthetic pathway. So, breeders should think over the relationship of seed color and nutritional quality in the process of breeding.

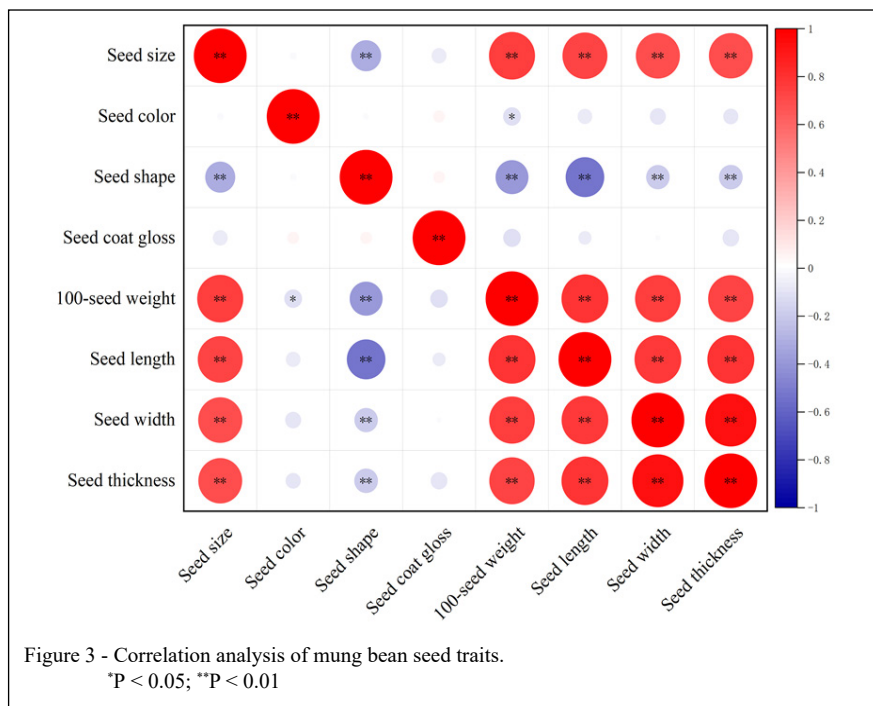


Table 3 - Eigenvectors and cumulative contribution rates of each principal component.

Traits	-----Eigen vectors-----		
	PC1	PC2	PC3
Seed width	0.928	0.089	-0.101
Seed thickness	0.919	0.003	-0.097
Seed length	0.903	-0.129	0.176
100-seed weight	0.885	-0.145	0.051
Seed size	0.843	-0.069	0.113
Seed coat gloss	-0.003	0.913	0.084
Seed color	-0.137	0.218	0.798
Seed shape	-0.374	0.361	-0.583
Eigenvalue	4.235	1.047	1.002
Contribution rat (%)	52.942	13.086	12.523
Cumulative (%)	52.942	66.028	78.551

Cluster analysis of seed phenotypic characters in Mung Bean

Cluster analysis of eight seed phenotypic traits of 302 mung bean germplasm resources showed that they were clustered into eight groups (group I ~ VIII) with the genetic distance of 2.18 (Figure 5). The group I contained 131 germplasm resources, accounting for 43.3% of the total germplasm resources. The mean 100-seed weight of this group

was 7.004g. The group II contains 9 germplasm resources, accounting for 3.0% of the total germplasm resources. The mean 100-seed weight of this group was 8.558g. The 100-seed weight was one of the important factors affecting the yield, so the 140 germplasm resources of group I and group II were likely to be high-yield mung bean varieties.

There were 132 germplasm resources were clustered in the group III, which accounting for 43.7%

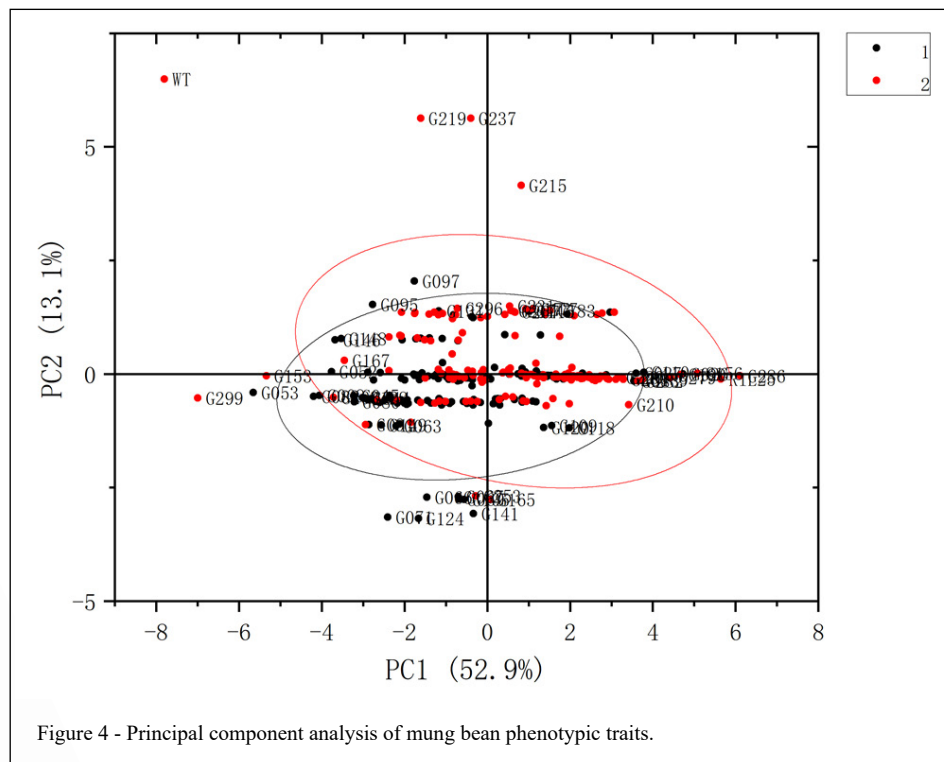
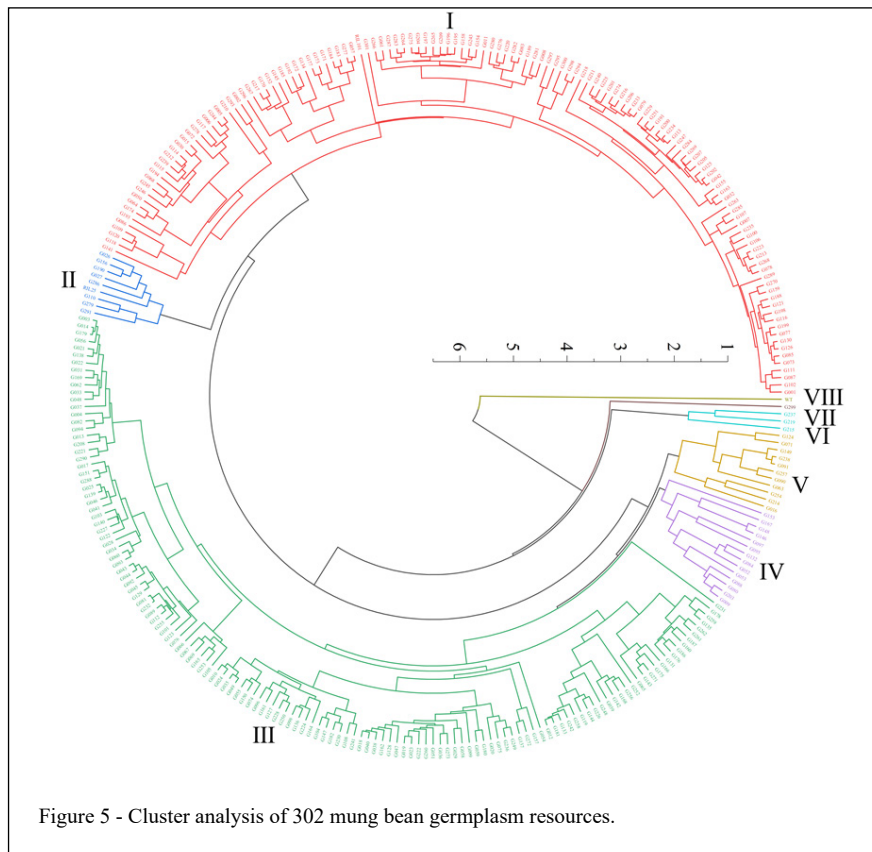


Figure 4 - Principal component analysis of mung bean phenotypic traits.



of the total resources. Group IV and group V contained 14 and 11 germplasm resources respectively, which accounting for about 8.3% of the total resources. The mean 100-seed weight of these three groups was 5.215g, and the mung bean germplasm resources with brown and yellow seed coats also appeared. Compared with other group members, the seed phenotypic traits of 157 mung bean germplasm resources in these three groups were general, so they could be stored as common germplasm resources in mung bean germplasm resource bank.

The number of germplasm resources contained in the group VI, VII, and VIII were 3, 1, and 1 respectively, which only accounting for about 1.7% of the total germplasm resources. Compared with the other groups, these three groups with members were clustered at a distance, the rare black and blue-green mung beans also appeared in the group VI and VIII. The 100-seed weight of the group VIII members was only 2.726g. In summary, the three groups of members were far different from other groups in seed phenotypic traits. These rare color mung bean materials could be used for in-depth study of mung bean medicinal research.

DISCUSSION

Genetic diversity of germplasm resources mainly refers to the differences in genic composition among individuals within a group, which also differs to varying degrees among different varieties of the same species. Even among different individuals of the same variety, diversity also has a great influence on the breeding and improvement in cultivars (LIU et al., 2022). As the foundation for the evolution and development of species, the genetic diversity analysis of germplasm resources also provides a premise for mung bean germplasm resource innovation and variety breeding. Plant phenotypic traits are the external manifestations of biodiversity. Because of their advantages of being more intuitively and conveniently evaluated and identified among germplasm resources, which make them have been widely used in the genetic diversity study of various crop germplasm resources (MAHMOODI et al., 2021; PENG et al., 2020).

In this study, eight seed phenotypic traits of 302 mung bean germplasm resources were comprehensively identified by diversity analysis,

correlation analysis, PCA, and cluster analysis. Mung bean germplasm resources have a rich genetic background and a high genetic diversity. Genetic diversity analysis showed that the variation value of quality traits was greater than that of quantitative traits, similar result on 170 mung bean germplasm resources from Liaoning also was obtained by evaluating 34 qualitative traits and 12 quantitative traits (HE et al., 2022). The CV is widely used to describe the degree of phenotypic variation, if the CV is greater than 10% for a particular trait, meaning a great difference in that trait among different accessions (GÖTZE et al., 2017; BEDEIAN & MOSSHOLDER, 2000). Among the eight seed phenotypic traits, the CV of five descriptive traits were greater than 10%, which indicating that the characteristics of mung bean germplasm resources had a high level of diversity and showed potential for variety improvement. In the future breeding work, attention should be paid to the simultaneous improvement of wild and cultivated varieties and the discovery of high-quality genes.

Most plant agronomic traits are controlled by micro-effect multiple genes, and there are different degrees of correlation between different agronomic traits (XI et al., 2008). Changes in one trait can also lead to changes in other traits. Therefore, in the process of breeding, the minor traits can be improved simultaneously while improving the main agronomic traits (GUO et al., 2018). Correlation analysis of seed phenotypic traits showed that the 100-seed weight of 302 mung bean germplasm resources was extremely significantly positive correlation with seed size, seed length, seed width, and seed thickness, meanwhile the correlation between seed width and seed thickness was the strongest. These results indicated that there is a close relationship between the seed phenotypic traits of mung bean, the 100-seed weight can be changed by improving seed length, seed width, and seed thickness. High yields were the vital agronomic trait for mung bean, the mung bean yield should be increased to promote the coordinated development of various seed phenotypic traits.

In the process of multi-objective breeding, PCA is a multivariate statistical analysis technique, which is helpful for improving breeding levels and parent selection effects (MAJI & SHAIBU, 2012; PLACIDE et al., 2015). The results of PCA in this study showed that the first three principal components reflected most of the information on eight seed phenotypic traits of 302 germplasm resources, and the cumulative contribution rate was 78.551%. These three principal components had an impact on seed yield and nutritional quality, so breeders should

pay attention to these associated characteristics. In previous studies, PCA has been used to show that the sugar yield, sugar content, leaf number, and ring number per vascular bundle contributed the most to 10 quantitative traits in sugar beet (LIU et al., 2022). The first four principal components of durum wheat explained 71% of CV, which displayed a great influence on the germplasm phenotype (AMBATI et al., 2020). With respect to the breeding of mung bean germplasm resources, the purpose should be clear, and rational selection should be conducted in terms of the yield, nutritional quality, and disease resistance.

Based on the eight seed phenotypic traits, cluster analysis of 302 mung bean germplasm resources was performed to categorize the types of mung bean germplasm resources from different sources. At the same time, parents with complementary traits should be selected in accordance with breeding objectives to make parent selection more scientific and reasonable for mung bean breeding (ODONG et al., 2011). In this study, 302 mung bean germplasm resources were categorized into eight groups. The 100-seed weight of group I and group II were much larger than that of other groups, meanwhile the 100-seed weight was closely related to the yield. Therefore, the members of these two groups were likely to be high-yield mung bean germplasm resources and could be used for breeding high-yield mung bean varieties. At the same time, there are also studies reported that there is an inseparable relationship between seed weight and nutritional quality (ZHAO et al., 2022). The mean 100-seed weight of group I and group II was 7.781g. Owing to the protein and flavonoids in mung bean had certain detoxification effect (TIAN et al., 2017), these germplasm resources with higher 100-seed weight could be used as raw materials for functional health food production. Compared with other group members, the germplasm resources of group VI, group VII, and group VIII were clustered at a distance, indicating that these three group members were significantly different from other group members in seed phenotypic traits. In terms of seed color, black mung bean materials appeared in group VI and group VIII. Previous study has shown that the color of mung bean seed coat is closely related to flavonoids (LIAN et al., 2020), so these three group members could be used as raw materials for food production and selective breeding in healthcare applications.

CONCLUSION

The 302 mung bean germplasm resources evaluated in this study displayed the rich genetic

diversity, which could provide excellent germplasm resources for the yield and quality breeding of mung bean. Genetic diversity index of eight seed phenotypic traits of 302 germplasm resources was higher in 100-seed weight, so 100-seed weight trait is one of the most important seed traits in mung bean. According to the cluster analysis in this experiment, the germplasm resources in group I and group II can be used for the high-yield breeding and the functional health food production. The rare black mung bean germplasm resources in group VI and group VIII can be used for food production and selective breeding in healthcare applications.

ACKNOWLEDGEMENTS

This research was supported by the Natural Science Foundation projects of Nanyang Normal University (2019ZX027 and 2022PY014), Natural Science Foundation of Henan province (232300420213), and the Key Scientific Research Project of Higher Education of Henan Province (24A210021).

DECLARATION OF CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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