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Research paper

Biochemical characterization and multivariate analysis of different genotypes of mungbean (*Vigna radiata* (L.) R. Wilczek)

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ABSTRACT

Mungbean is an important short-duration annual crop primarily grown for food and fodder purposes in Asian countries. Present investigation aimed to explore the biochemical diversity by conducting quantitative phytochemical tests including total phenol, flavonoid, tannin, cardiac glycosides and alkaloid content and to check the antioxidant potential of leaf, stem, pod and seed samples of 26 mungbean genotypes were sown in randomized block design during June to August, 2021 with three replications under similar agronomical conditions. Significant variations were observed in genotypes with respect to quantification assays and plant parts. A strong positive correlation was observed between phenol content and antioxidant potential, and between flavonoid content and glycoside content. Moreover, multivariate approaches including PCA and HCA were performed to characterize, evaluate and categorize mungbean genotypes based on similarities and dissimilarities among them. A first four PCs accounted 90.47% of cumulative variance and loading plot suggested significant contribution of all variables to total variance of PC1 and PC2. Cluster analysis distinguished highly variable genotypes as S-3, S-5, S-6, GM-6, SKNM-1608, SKNM-1801, SKNM-1808 and SKNM-1809, and all of them were found to be rich in bioactive constitution. These genotypes can be recommended for use as parents in future plant breeding programs, and can also be used in selecting prominent functional mungbean landraces for daily diet purposes.

Key words: Antioxidant activity, Biochemical characterization, Hierarchical luster analysis, Principal component analysis

INTRODUCTION

Mungbean (*Vigna radiata* (L.) R. Wilczek) is an important diploid (2n = 22) annual crop of *Fabaceae* family and is best known for its shorter life cycle, higher productivity, nutrition rich comestibles and economically and ecologically sound farming systems). High-quality proteins, carbohydrates and fats are the key components of mungbean, along with vitamins, mineral elements, and folates. Moreover, various secondary metabolites add functional value to mungbean for its antioxidant, antitumor, anti-melanocyte and antidiabetic properties (Khant *et al*., 2021). It is primarily grown for food and fodder purposes in tropical and subtropical regions of Asia including India, Bangladesh, Pakistan, China, Myanmar, Thailand, Philippines, and Indonesia. Of these, India is the leading producer, accounting for 54% of global production and 65% of the acreage on its own (Gupta *et al*., 2021; Pratap *et al*., 2021). Native mungbean distribution was most reported in Rajasthan, Orissa, Andhra Pradesh, Maharashtra, Uttar Pradesh, Karnataka and Tamil Nadu (Jena *et al*., 2017). Despite accounting for 18.07% of total pulses area coverage and 11.48% total pulses production in India, the average mungbean yield is poor and requirements are met through import from other countries (Samyuktha *et al*., 2020). These challenges may have arisen due to lack of genetic diversity, vanishing of agronomically important genes, poor harvest indices, susceptibility to diseases and pests, abiotic and biotic stress, and limited investments in developing new varieties (Kumar *et al*., 2015). For the sustainable production of mungbean, genetic characterization was crucial which helps in the development of new varieties tolerant to different stresses.

A steady supply of genetic variations is essential for the effective crop improvement since intense breeding practices lead to genetic homogeneity in the population which makes them vulnerable to stresses. Hence, criteria for selection of parental stock must be based on genetic dissimilarity in addition to agronomic value. The protection of variety under the Protection of Plant Variety and Farmer Act 2001 also mandates that the variety had to be distinguishable from others, uniformly express distinctive characters among plants of population and exhibit consistency in the expression of distinctive characters across time and space. This eventually requires an assessment of genetic variations (Jadhav *et al*., 2021). One of the common methods used for genetic diversity analysis was the biochemical characterization (Jain *et al*., 2014), which can be rendered by valorizing bioactive components such as phenols, flavonoids, tannins, glycosides and alkaloids from plant samples. These metabolites have proven health benefits against severe medical issues likes diabetes, acute inflammation, cancer, malaria, gastrointestinal, cardiovascular and sexually transmitted diseases (Mwamatope *et al*., 2023). Phenolics, in particular, are effectual antioxidants capable of scavenging free radicals in the human body by radical chain breaking and metal chelation, as well as exhibiting antiseptic, anti-tumor and immunomodulatory properties. Likewise, flavonoids have been reported to the best remedy for bacterial and viral infections (Nyero *et al*., 2023). Alkaloids and glycosides are also eminent anti-inflammatory, cardiotonic, anaesthetics, and analgesic agents (Heinrich *et al*., 2021).

Multivariate strategies such as principal component analysis and cluster analysis might be the effective tool for quantitative estimation of biochemical diversity, as multivariate statistics allow personnel to sum up their data and reduce the number of variables required to explain those data (Sahoo *et al*., 2022). Likewise, multivariate approaches are used to characterize, evaluate and categorize plant genetic resources when dealing with large number of accessions deployed to study numerous traits (Sood *et al*., 2015). Principal component analysis reduces the multivariate data into biplot that may further used for grouping material, while cluster analysis is the pivotal factor for comprehending the genetic and evolutionary relations among different accessions (Gupta *et al*., 2021). Present investigation aimed to conduct biochemical characterization of the different mungbean genotypes to assess the biochemical diversity. Further, two multivariate techniques, Principal Component Analysis and Cluster Analysis were performed to identify and classify the groups of genotypes and to determine the variability among them. Our research may open up new prospects for mungbean breeders and growers to encourage the development of mungbean genetic stock with higher amount of bioactive components.

MATERIALS AND METHODS

Plant material: The experimental material comprised of twenty genotypes of mungbean procured from the Pulses Research Station, Sardarkrushinagar Dantiwada Agricultural University, Gujarat, and six genotypes collected from the market of different regions of Gujarat and cultivated for evaluation in Randomized Block Design (RBD) with three replications during June to August 2021. Each entry was accommodated in single row plot of 5 m length with inter and intra row spacing 45 cm and 15 cm, respectively. At maturity the leaves, stems, pods, and seeds were collected for testing purposes. The collected samples were assiduously washed with distilled water and oven-dried at 50˚C for 24 h. The dried samples were milled

Table 1. List of genotypes with their sample code

S. No.	Sample Code	Genotype	S. No.	Sample Code	Genotype
1	S-1	Western Mahavir (from market)	14	D-8	SKNM-1608
2	S-2	Vaishakhi Research Mungbean (from market)	15	D-9	SKNM-1701
3	S-3	Neel^{++} Research Mungbean (from market)	16	D-10	SKNM-1703
4	S-4	Avani-65 Research Moong (from market)	17	D-11	SKNM-1704
5	S-5	Nidhi Seeds (from market)	18	D-12	SKNM-1705
6	S-6	Guj-4 (from market)	19	D-13	SKNM-1801
7	D-1	GM-3	20	D-14	SKNM-1802
8	D-2	GM-4	21	D-15	SKNM-1803
9	D-3	K-851	22	D-16	SKNM-1806
10	D-4	GMM-5	23	D-17	SKNM-1808
11	D-5	GM-6	24	D-18	SKNM-1809
12	D-6	SKNM-1516	25	D-19	SKNM-1810
13	D-7	SKNM-1605	26	D-20	SKNM-1812

to fine powder by grinder and stored in evacuated containers at low temperature until further use. The names of genotypes are listed in Table 1 along with sample code.

Extract preparation: Plant extracts were prepared by cold maceration technique. The 10 g dry powder of each sample was dissolved in 100 ml methanol solvent (1:10). Then, the mixtures were kept on orbital shaker at room temperature for 72 h with constant shaking for maceration. Subsequently, the macerated mixtures were filtered with Whatman filter paper No. 1. and the excess methanol from filtrate was evaporated with rotary evaporator to obtain a completely dry crude extract. Percentage Yield was calculated by following formula:

$$\text{Yield (\%)} = \frac{\text{Weight of extract after solvent evaporation}}{\text{Weight of powdered samples}} \times 100$$

Total phenol conten: The total phenol concentration of the test samples was assessed using the Folin-Ciocalteu method, reported by Singleton and Rossi (1965) with minor modifications. Briefly stated, 500 µl of the sample (1 mg/ml) was mixed with 500 µl of Folin-Ciocalteu reagent and 10 ml of distilled water. Thereafter, 3 ml solution of sodium carbonate (20%, Na_2CO_3) was added to the mixture and total volume made up to 25 ml with distilled water. The mixture was then allowed to rest at room temperature in dark for 30 min. Later, the absorbance was recorded at 765 nm in spectrophotometer (Lasany UV-VIS Spectrophotometer Model: LI-294). Following the steps outlined above, the standard gallic acid (100 µg/ml to 1000 µg/ml) was also processed to obtain the calibration curve. All the experimental tasks were conducted in triplicates to avoid errors. The phenolic concentration of each sample was expressed as milligrams of gallic acid equivalent/gram of sample (mg of GAE/g of sample).

Total flavonoid content: The total flavonoid content in each sample was assayed by Aluminium chloride ($AlCl_3$) method as given by Hossain and Shah (2015) with few modifications. 50 µl of 10% $AlCl_3$ was added to the 500 µl of sample (1 mg/ml) and mixed well. Then, 50 µl of 1M potassium acetate (CH_3COOK) was added and total volume of 5 ml made with distilled water. Later, incubation was given to the mixture for 30 min in dark at room temperature. The absorbance of the sample solutions recorded at 415 nm. Quercetin (100 µg/ml to 1000 µg/ml) was used as a reference flavonoid and processed similarly to plot the calibration curve from which the total flavonoid content could be chronicled. The tests were carried out in triplicates to avoid errors. The results were presented as milligram of quercetin equivalent/gram of sample (mg QE/g of sample).

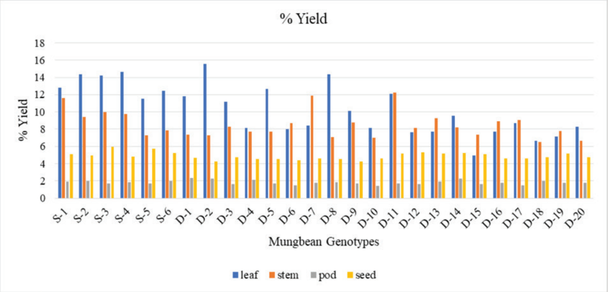

Fig. 1. Extraction yield in percentage

Total tannin content: The folin-Ciocalteu method was employed to determine the total tannins as described by Haile and Kang (2019). As stated in the procedure, 100 µl of plant extract (1 mg/ml) was mixed with 5 ml of distilled water, 500 µl of Folin-Ciocalteu reagent, and 1 ml of 35% sodium carbonate (Na_2CO_3). The total volume was made up to 10 ml by adding distilled water. The solution was well shaken and kept in dark at room temperature for 30 min. Later, the absorbance was measured at 700 nm. Tannic acid solution (10 µg/

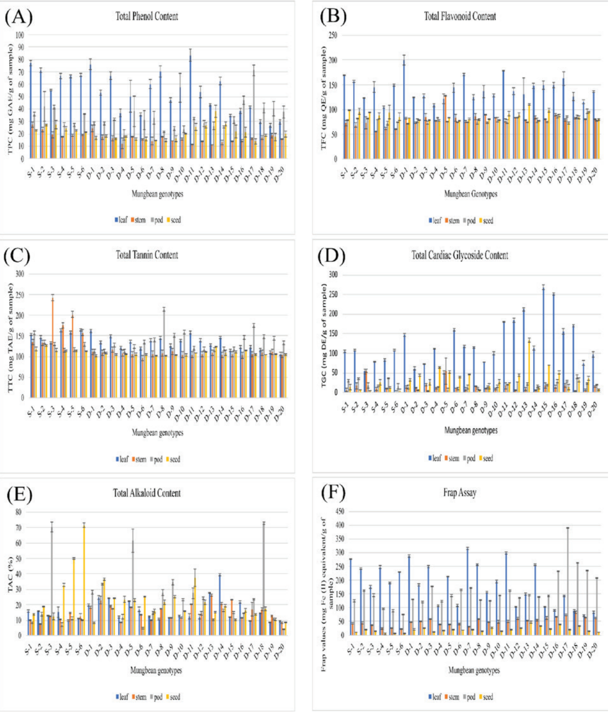

Fig. 2. Biochemical parameters: (A) Total phenol content, (B) Total flavonoid content, (C) Total tannin content, (D) Total cardiac glycoside content, (E) Total alkaloid content, and (F) Antioxidant activity (FRAP assay)

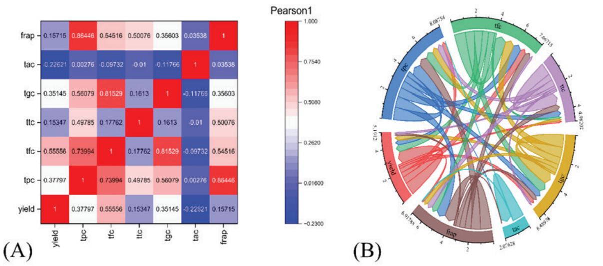

Fig. 3. Pearson's correlation coefficient between phytocomponents and antioxidant activity represented by (A) Heatmap and (B) Chord diagram

ml to 100 µg/ml) was used as the reference standard to construct the standard calibration curve. The procedure was carried out in triplicates to prevent subjective errors. The total tannin content was expressed as milligrams of tannic acid equivalent/gram of sample (mg TAE/g of sample).

Determination of cardiac glycosides: The cardiac glycoside concentration in the test samples was quantified spectrophotometrically as described by Solich *et al*. (1992) with minor revisions. The Baljet's reagent was freshly prepared by mixing of 1% picric acid and 10% NaOH in 19:1 proportion. Further, 500 µl of plant extract was added to the 500 µl of Baljet's reagent, and the mixture was kept undisturbed for 1 hour at room temperature. After incubation, the mixture was diluted with 9 ml of distilled water and well shaken. The absorbance of the reaction mixture was recorded at 495 nm. All the tasks were performed in triple replicates to avoid errors. The cardiac glycoside digoxin (100 µg/ml to 1000 µg/ml) used as reference standard to obtain regression curve and equation, eventually from which the total cardiac glycoside concentration was computed, and the results were expressed as milligrams of Digoxin equivalent/gram of sample (mg DE/g of sample).

Total alkaloid content: Total alkaloid content in test samples was determined using the procedure explained by the Senguttuvan *et al*. (2014) with some modifications. The 40 ml of 20% acetic acid was carefully added to the 1 g powder of each sample placed in a separate beaker and covered to kept undisturbed for 8 hours. The mixture was then filtered, and the collected filtrate was heated in hot water bath to reduce it to one-fourth. Moreover, concentrated ammonia solution was gradually added to this mixture until precipitate formed. The precipitates were then allowed to settle for filtration, and the weight of filtered principates was noted. Finally, the alkaloid content was expressed as a percentage computed from the below equation:

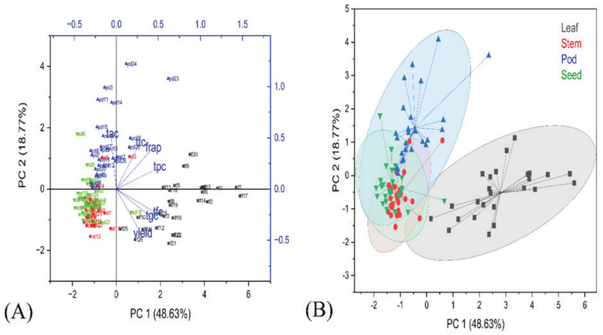

Fig. 4. Principal component analysis: (A) PCA Biploat and (B) PCA Cluster plot

$$\text{Percentage of total alkaloid content (\%)} = \frac{\text{Weight of Residue}}{\text{Weight of Sample Taken}} \times 100$$

Antioxidant activity (FRAP assay): Antioxidant activity of all the test samples was analysed by the FRAP (Ferric reducing antioxidant power) assay as stated by Benzie and Strain (1996) with minor revisions. Sodium acetate buffer (300 mM; pH 3.6), 10 mM TPTZ in 40 mM HCL, and 20 mM $FeCl_3.6H_2O$ mixed in a proportion of 10:1:1, respectively, to prepare the FRAP reagent. The reagent mixture was then kept in hot water bath for incubation at 37˚C for 30 min. Thereafter, 500 µl of extract solution (1 mg/ml) was added to the 4 ml of FRAP reagent. Additionally, 10 ml of distilled water added to the mixture and then incubated for 30 min at room temperature in dark. The absorbance of reaction mixtures was recorded at 593 nm in spectrophotometer. The similar procedure was followed for standard iron (II) sulphate ($FeSO_4.7H_2O$) (100 µg/ml to 1000 µg/ml) to construct calibration curve. The above procedure was repeated 3 times for each sample and standard to avoid errors. The results were presented as mg Fe (II) equivalent/gram of plant material.

Statistical analysis: All the experimental tests were executed in triplicates and the results were displayed as Mean ± SD. Statistically significant differences between values for various extracts were determined by the one-way analysis of variance (ANOVA) with Tukey's test. Additionally, Pearson's correlation between quantified phytocomponents and antioxidant activity was computed and multivariate analysis was performed to create principal components analysis (PCA) biplot and cluster dendrograms using OriginPro®2023b.

RESULTS AND DISCUSSION

Extraction yield

The extractive yield of leaf samples ranged from 4.93% to 15.53% with lowest yield observed in genotype D-15 while highest in D-2 (Fig. 1). However, in case of stem, the yield varied from 6.53% (D-18) to 12.2% (D-11), whereas in pods and seeds it ranged from 1.43% (D-10) to 2.31% (D-1) and 4.24% (D-2) to 5.93% (S-3), respectively. Significant differences were observed between the extractive yield of leaf, stem, pod, and seed samples ($p < 0.05$), but not between the yield values of different genotypes ($p > 0.05$), indicated consistency in values of different genotypes for all the plant parts. The results revealed higher yield in leaf samples, followed by stem and seed, and lower in pod sample.

Total phenol content

The concentration of phenols in different plant parts of all the genotypes varied significantly ($p < 0.05$) (Fig. 2(A)), as it was found between 26.75 ± 1.52 (D-19) to 83.42 ± 5.08 mg GAE/g of sample (D-11) in leaves, 11.32 ± 0.00 (D-13) to 27.28 ± 2.19 mg GAE/g of sample (S-1) in stem, 19.03 ± 4.54 (D-4) to 71.32 ± 4.50 mg GAE/g of sample (D-17) in pods, and 13.95 ± 2.11 (D-17) to 38.16 ± 5.02 mg GAE/g of sample (D-13) in seeds. The results also indicated significant variations in the phenol content of different genotypes for all the plant parts. Comparable results were obtained in mungbean seeds evaluated by Plainsirichai *et al.* (2021) with TPC content ranging from 44.72 to 57.20 mg GAE/100 g of Dry Weight. The results obtained were considerably higher than the phenol content reported by Shi *et al.* (2016) in seeds of 20 mungbean genotypes, ranging from 2.05 ± 0.44 to 2.38 ± 0.34 mg/g. Another study also reported considerable phenol content as soluble fraction ranging from 1.78 to 4.10 mg GAE/g and insoluble-bound phenolic content ranging from 0.78 to 1.5 mg GAE/g in different mungbean accessions (Wang *et al.*, 2021). Nine Shrilankan mungbean varieties assessed for the phenolic profile, and the results found between 170 ± 10 to 250 ± 10 mg GAE/100 g (Yang *et al.*, 2020).

Comparatively, leaf samples showed high phenol concentration, while stem samples had the lowest. Strangely, pod samples showed higher phenol content than seeds. Such a high proportion of phenols in the leaves and pods suggests that they have positive health benefits on livestock populations as they promote growth, nutritional intake, quality of milk as well as meat, and also lowers methane emissions in the forage portions (Johnson *et al.*, 2022).

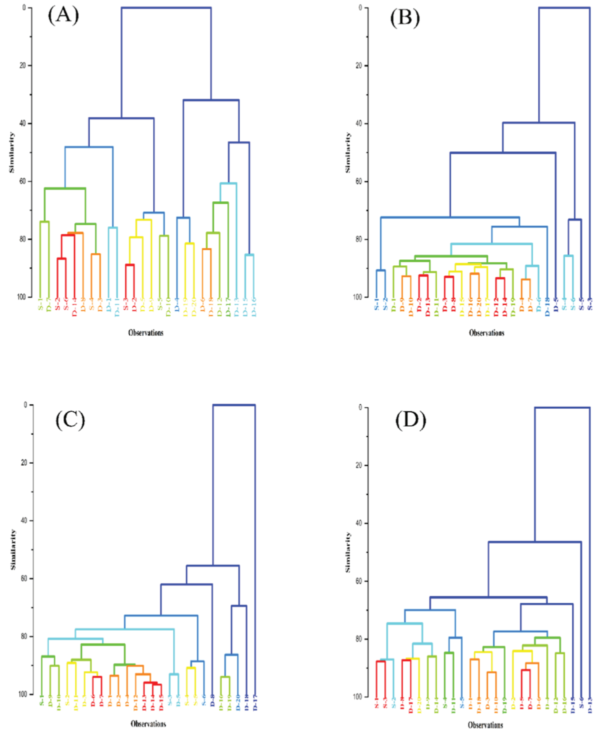

Fig. 5. Cluster Dendrogram of Mungbean genotypes with respect to (A) Leaf, (B) Stem, (C) Pod and (D) Seed Sample

Total flavonoid content

The mungbean genotypes were found to be rich in flavonoids. Scientific reports claim that consuming foods high in flavonoids may offer protection against human diseases caused by oxidative stress (Shukla *et al.*, 2020; Sharma and Modi, 2023). All samples of leaf, stem, pod and seeds showed significant differences ($p < 0.05$), with variations spread from 104.83 ± 1.82 to 199.97 ± 9.38 mg QE/g of sample in leaf, 56.22 ± 0.24 to 127.61 ± 1.05 mg QE/g of sample in stem, 70.94 ± 0.24 to 96.08 ± 4.91 mg QE/g of sample in pod, and 73.02 ± 2.37 to 109.97 ± 1.27 mg QE/g of sample in seed (Fig. 2(B)). The values also differed significantly amongst the various genotypes. Numerous earlier investigations have reported significant variations among different mungbean cultivars (Wang *et al.*, 2021; Shi *et al.*, 2016; Plainsirichai *et al.*, 2021; Priti *et al.*, 2021). Comparable variations have also been observed among different cultivars of other legumes including cowpea, fababean and chickpea (Yusnawan *et al.*, 2021; Khan *et al.*, 2015). Highest flavonoid content was found in leaf, stem, pod and seed samples of genotype D-1, D-5, D-19, and D-13, respectively. The leaf samples showed higher flavonoids in comparison to others.

Total tannin content

Abundant tannin concentration was observed in all parts of the mungbean genotypes. Values of tannin content differed significantly among genotypes and between plant parts ($p < 0.05$), ranging from 106.3 ± 1.70 to 164.44 ± 2.94 mg TAE/g of sample in leaf, from 96.67 ± 6.76 to 242.59 ± 4.21 mg TAE/g of sample in stem, from 112.59 ± 3.39 to 214.44 ± 0.00 mg TAE/g of sample in pod, and from 101.48 ± 0.64 to 127.04 ± 1.70 mg TAE/g of sample in seeds (Fig. 2(C)). The highest tannin content was observed in genotypes S-6, S-3, and S-2 for leaves, stems, and seeds, respectively, and in genotype D-8 for pods, indicating higher tannin content among commercially available genotypes. Likewise, significant variations were also observed in tannin content across leaf and seed samples of different *Vicia faba* L. genotypes, ranging from 13.76 to 55.23 mg CAE/100 g and 13.51 to 18.29 mg CAE/100 g, respectively (Mohamed and Abd-El Hameed, 2014). However, in seed samples of Fenugreek accessions, the tannins were found between 30.21 to 74.54 CAE/100 g (Al-Maamari *et al*., 2016).

Cardiac glycosides

The mean values for cardiac glycosides were presented in Fig. 2(D) showed statistically significant difference among all the test samples ($p < 0.05$). The values ranged from 50.95 ± 4.12 (D-5) to 268.1 ± 5.77 mg DE/g of sample (D-15) in leaf samples, from 2.38 ± 2.18 (S-6) to 55.24 ± 4.36 mg DE/g of sample (S-3) in stem samples, from 3.33 ± 0.82 (D-3) to 40.00 ± 5.71 mg DE/g of sample (D-18) in pod samples, and from 2.86 ± 1.43 (S-3) to 133.81 ± 4.36 mg DE/g of sample (D-13) in seed samples. Leaf samples showed higher glycoside content, while stem, pod and seed sample were found be low in glycoside concentration. Significant differences observed in total cardiac glycoside values among all the genotypes indicated the presence of noteworthy variation in the genetic stock of mungbean.

Total alkaloid content

Alkaloid content values represented in the Fig. 2(E) differed significantly between various plant parts and among different genotypes ($p < 0.05$). Moreover, significant interactions were also found between samples from different plant parts and genotypes ($p < 0.05$). The alkaloid concentration ranged from 8.5 ± 0.30% (D-19) to 39.57 ± 0.67% (D-14) in leaf samples, from 7.63 ± 0.21% (S-2) to 26.07 ± 0.74% (D-13) in stem samples, from 4.33 ± 0.25% (D-20) to 72.83 ± 0.57% (D-18) in pod samples, and from 8.5 ± 0.26% (D-20) to 71.57 ± 1.45% (S-6) in seed samples of tested mungbean genotypes. The findings suggested rich alkaloid concentration in pod and seed samples. Nevertheless, all plant parts of genotype D-20 were found quite low in alkaloid concentration.

Antioxidant activity – FRAP assay

Antioxidant potential of mungbean genotypes was expressed as FRAP value, which was presented in Fig. 2(F). The significant difference was observed between the frap values of different plant parts ($p < 0.05$), but the values were found be statistically consistent among the different genotypes ($p > 0.05$). The interaction between different genotypes and different plant parts also showed significant difference ($p < 0.05$). The frap values in leaf sample ranged from 71.18 ± 2.77 (D-19) to 313.96 ± 4.51 mg Fe (II) equivalent/g of sample (D-7), whereas they ranged from 23.12 ± 1.50 (S-6) to 83.68 ± 4.17 mg Fe (II) equivalent/g of sample (D-18) and 74.65 ± 0.24 (S-6) to 389.51 ± 1.27 mg Fe (II) equivalent/g of sample (D-17) in stem and pod samples, respectively, and from 4.51 ± 3.37 (S-4) to 46.74 ± 3.96 (D-13) mg Fe (II) equivalent/g of sample in seed samples. Additionally, the outcomes suggested most prominent antioxidant activity in leaves and pods of the mungbean genotypes, but relatively lesser in seed samples. Strong positive correlation was observed between phenol content and antioxidant activity ($r^2 = 0.815$; $p < 0.05$), and total flavonoid content and total glycoside content ($r^2 = 0.864$; $p < 0.05$). However moderate correlation was observed for flavonoid and tannin content with frap assay. Chord diagram represents correlation between phytocomponents and antioxidant activity (Fig. 3).

Multivariate analysis (principal component analysis)

An attempt was made to differentiate between the mungbean genotypes by using multivariate approach of data analysis to elucidate relations between different phytochemical contents and antioxidant activity as well as to learn more about the variables that primarily affect similarities and dissimilarities among the samples. The collected data from all the biochemical tests was subjected to Principal Component Analysis (PCA) to identify the differences among mungbean accessions and the resulting principal component scores were plotted to generate outcomes. The first four principal components PC1, PC2, PC3 and PC4 accounted for 48.63%, 18.77%, 13.51%, and 9.55% of the total variance, respectively, representing about 90.47% of the cumulative variance. The absolute value determines the significance and contribution of particular component to the overall variance, while, the extent to which every variable is associated with each principal component is denoted by coefficient of absolute vectors (Sanni *et al*., 2012). PCA biplot of scores and loading of 26 mungbean genotypes with four plant parts leaves, stem, pod, and seed are displayed in Fig. 4, PC1 was positively correlated with TPC, TFC, frap, TGC, yield and TTC respectively, however, it was negatively correlated with TAC. In case of PC2, it was positively correlated with TAC, TTC, frap and TPC, and negatively correlated with yield, TFC and TGC. Loading plot suggested that all the variables contributed significantly to the total variance of PC1 with Eigenvector value ranging from 0.275 to 0.500 except for TAC (-0.06). Likewise, all the variables equivalently contributed to the total variance of PC2 (TAC; 0.528, TTC; 0.444, yield; -0.444, FRAP; 0.392, TGC; -0.272, TFC; -0.238 and TPC; 0.206). In case of PC3 and PC4, the main contribution was given by TAC (0.720) and Yield (0.772), respectively.

The cluster plot (Fig. 5) delineates the homogeneity shared by different accessions based on phytochemical composition. A total of four clusters were suggested, as samples were coalesced depending on plant part. Cluster 1 and 2 consist of samples obtained from leaf and stem part of different mungbean accessions, respectively, whereas Cluster 3, and 4 were made up of samples derived from pod and seed of mungbean accessions. The current investigation found considerable variation in metabolic composition and antioxidant potential between different plant parts, especially leaf and pod, as they found to be distinct from others, while overlapping was observed between seed and stem samples. Furthermore, all the genotypes varied significantly based on phytoconstituent composition of each plant part, though close grouping was found among different genotypes based on composition of seed samples and stem samples. Among leaf samples, genotypes D-11 (lf17) and D-14 (lf20) found to be distinguished from others, might be due to higher phenol flavonoid, alkaloid and frap values. However, in case of stem samples genotype S-3 (st3) and D-5 (st11) discriminated from others, these results are congruent with highest flavonoid, tannin, and glycoside content found in them. The cluster of pod samples distinguished genotype D-17 (pd23) and D-18 (pd24) from all others. All the metabolites were found highest in both the genotypes, this might account for their distinctiveness. Lastly, in seed samples, genotypes S-6 (sd6) and D-13 (sd19) were found to be distinguished. They both presented highest values in all assays.

Hierarchical Cluster Analysis (HCA) dendrogram was created to determine the similitude between clustered genotypes and to identify potential grouping within the genotypes. HCA dendrogram (Fig. 5) supported previous conclusions. According to data from all the four dendrograms constructed based on the various plant parts, i.e., leaf, stem, pod and seed samples, the 26 tested genotypes showed significant variability. No particular pattern was observed in the grouping, although majority of the genotypes obtained from the commercial market fell into close clusters, while genotypes procured from agriculture university tend to be fall in similar clusters. Additionally, dendrograms distinguish S-3, S-5, S-6, D-5, D-8, D-13, D-17 and D-18 from other genotypes that may indicate high variability in them as they all have been reported to be highly rich in metabolites. The results were also congruent with the forementioned PCA analysis.

CONCLUSION

A total of 26 mungbean (*Vigna radiata* (L.) R. Wilczek) genotypes were procured and sown under similar agronomic environment to quantify phytoconstituents and ascertain the effects of biochemical composition on the genetic diversity of mungbean germplasm. All tested biochemical assays showed remarkable variations among different genotypes, with each plant part contributing significantly to the observed diversification. A strong positive correlation was observed between phenol content and antioxidant potential, and between flavonoid content and glycoside content. The quantitative outcomes were supported by the multivariate PCA and HCA analysis. PCA cluster plot and cluster dendrograms discriminated most representative genotypes as S-3, S-5, S-6, GM-6, SKNM-1608, SKNM-1801, SKNM-1808 and SKNM-1809, and all of them were found be rich in phenol, flavonoid, tannins, cardiac glycosides, alkaloids, sugar and antioxidant properties. These genotypes can be recommended for use as parents in future breeding programs of crop improvement, and can also be used in selection of prominent mungbean landraces for daily diet purposes and formulation of potential functional foods against various chronic diseases. Future research using biochemical markers in combination with molecular markers is advised with additional mungbean cultivars and samples from several harvest with variable concentration ranges.

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