Research Article

Morphological and biochemical characterization of locally available kidney beans

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Abstract
The present research study was aimed for estimation of genetic diversity and characterization of various morphological and biochemical traits of kidney beans (Phaseolus vulgaris L.) with the objectives of identifying superior genotypes at the Research Farm, The University of Haripur during zaid-rabi season 2018. Twelve different kidney bean genotypes were evaluated by using Randomize Complete Block Design (RCBD) with three replications. Data was recorded for various morphological and biochemical traits. Analysis of variance revealed highly significant genetic diversity. Minimum days to emergence were recorded for Line-12 (22.66 days), having moderate plant height of (89.44 cm), longer pods (13.003 cm) containing maximum number of seeds (6.55). Minimum days to flowering were observed for Line-1 (59 days). Maximum plant height was recorded for Line-5 (99.11 cm). Maximum numbers of pods were produced by Line-9 having (16.28). Maximum pod length (13.55 cm) was observed for Line-2. Seeds per plant were maximum in Line-9 (72.97). Line 4 produced larger size bean seeds having maximum 100 seed weight (55.74 g). Total phenolic compounds were observed to be maximum in Line-10 (88.24 mg/100g) while total antioxidants were maximum in Line-7 (136.88). Based on the characterization of kidney bean genotypes at Haripur, the genotypes viz. Line-9 and Line-12 were observed superior having minimum days to emergence, moderate plant height, early maturity, high yield. Therefore, these genotypes are recommended for further characterization in the kidney bean breeding program for further evaluation.

Keywords: Antioxidants; Characterization; Genetic diversity; Phenolic compounds

Introduction
Kidney bean Phaseolus vulgaris L. is self-pollinated diploid specie of the genus Phaseolus having 22 chromosomes. Most of the species of the genus phaseolus are of wild type however only 5 are known to be cultivated viz. Phaseolus acutifolius (tepary bean), Phaseolus coccineus L. (scarlet runnerbean), Phaseolus lunatus L. (lima bean), Phaseoluspolyanthon (year-long bean) and Phaseolus vulgaris L. (common bean) [1,2]. The origin of Phaseolus vulgaris dates back to 6500-5000 BC [3]. Common beans perform better in tropical and subtropical
regions having average rainfall. It grows well in the areas which are dry having suitable conditions for the maize cultivation [4, 5]. From nutritional point of view, common beans are rich source of proteins, almost providing 3 times more protein than that of cereal grains [6]. Common bean dry seeds are rich in vitamins, minerals, starch and dietary fibers [7] and are also contains variety of phytochemicals, antioxidants, flavonoids such as anthocyanin’s, pro-anthocyanin’s, lectins, phytates, flavonols, phenolic acids and iso-flavones [8-10]. Consumption of common beans is greatly associated with many physiological and health benefits and ultimately results in the reduction of cardiac diseases, obesity, diabetes mellitus and carcinogenic diseases [11, 12]. During 2018, worldwide production of common bean was 27 million tons. Myanmar produced the largest amount of dried beans and on the other hand China was the largest producer of green beans. In Northern America, production of dry kidney beans is 1 t/ha and throughout the worldwide it is 0.7 t/ha. In Africa, average yield is around 0.6 t/ha. Breeding programs initially started in Africa and its native regions with the aim of improving yield potential. Their major objective is to induce tolerance and resistance to biotic and abiotic factors. In Pakistan, the average yield of kidney bean is about 0.5 t/ha. In irrigated areas, using best agronomic and cultural practices, they yield 2.5-5 t/ha. Consumption of kidney bean in Pakistan is more than its production. Henceforth, huge amount is spent on its import every year. Identification of promising and high yielding kidney bean genotypes will not only lead to self-sufficiency but will also minimizes its import through foreign exchange [13].

There are more than 20 species of legumes which are consumed as dry grains in plenty amounts as a human nutrition [6, 12]. *Phaseolus vulgaris* L. (common bean) is one of the most abundantly used leguminous crop. Generally common beans are considered as a vital source of nutrients and are also known as poor man’s meat [14]. In the third world countries, legumes especially common beans and soybean are considered to be of great importance for their consumption as a food where malnutrition seems to be prominent due to protein deficiency. Throughout the world and particularly in the developing countries, common beans are consumed now days [15]. Many factors are correlated with its yield ultimately influencing the pods productivity. In order to achieve higher yield and improve its productivity, it is necessary to maintain balance between vegetative and reproductive traits. It is a very difficult task to be achieved because all those morphological traits which are positive correlated with yield are not static due to genotype by environmental interaction. Still there are multiple ways of breeding common bean and selection of superior common bean land races is one of the methods [16, 17]. Beside the fact that common beans are of great potential and importance, still no research has been carried out in Pakistan. The exploitation of local genetic resources could solve some issues regarding the identification of some characters which ultimately enhance the yield and are important for plant breeding [18, 19]. Therefore, an experiment was designed and conducted at the Research Farm, The University of Haripur for the characterization of various morphological and biochemical traits with the objectives of identifying superior kidney beans genotypes having high yield and improved biochemical components (Figure 1). Before the recommendation of these kidney bean genotypes for general cultivation, they will be further characterized in the kidney bean breeding program.

**Materials and methods**

A research was designed to assess morphological and yield characteristics of
twelve different kidney bean genotypes during zaid-rabi season 2018, at the University of Haripur 33°59’ North and 72°56’ East with altitude of 610 meters. These genotypes were collected from upper hilly areas of Khyber Pakhtunkhwa. Sowing was completed on 26th January, 2018 using randomized complete block design (RCBD) with three replications. Data was recorded on various traits viz., days to emergence (DE), days to flowering (DF), plant height (PH), pods plant\(^{-1}\) (PP\(^{-1}\)), pod length (PL), seeds pod\(^{-1}\) (SP\(^{-1}\)), seed yield plant\(^{-1}\) (SYP\(^{-1}\)), 100 seed weight (SW), total phenolic compounds (TPC) and total antioxidnats (TA). Means of all the traits were calculated and subjected to statistical analysis (statistics 8.1) for analysis of variance (ANOVA) and LSD test was used to find least significant differences as per the method described by [20] (Table 1).

Table 1 List of the experimental material

| S. No. | Germplasm | Source                  |
|--------|-----------|-------------------------|
| 1      | Line 1    | Hungu, Khyber Pakhtunkhwa |
| 2      | Line 2    | Kalam, Upper Swat        |
| 3      | Line 3    | Parachinnar I            |
| 4      | Line 4    | Tal Lamutai, Upper Dir   |
| 5      | Line 5    | Sadda, Parachinar,       |
| 6      | Line 6    | Kumrat, Upper dir        |
| 7      | Line 7    | Tirah I, Parachinar      |
| 8      | Line 8    | Tirah II, Parachinar     |
| 9      | Line 9    | Kurram                   |
| 10     | Line 10   | Naran, Manshera          |
| 11     | Line 11   | Shiringal, Upper Dir     |
| 12     | Line 12   | Parachinar II            |

Results and discussion

Emergence refers to the germination of seedling from the soil. The process of germination initiates when water is absorbed by the seed. Days to emergence showed significant differences for all genotypes (Table 2). The said trait ranged between 28 to 22.66 days. Mean value for days to emergence was recorded to be 25.11 days. Maximum (DE) were recorded for Line-10 (28 days) followed by Line-7 (27.33 days) and Line-2 (26.66 days). While least (DE) were recorded for Line-12 (22.66 days) followed by Line-6 (23 days) (Table 3). The observed genotypic and phenotypic variability can be correlated to the environmental conditions from where these genotypes were collected. [21-23] also conducted experiments on kidney beans and their findings were also significant for (DE) in kidney bean. However, their mean values are different form our results. This may be due difference in soil moisture content, genetic makeup of the genotypes used in the current study, pH level of soil and different environmental conditions of study area.

Flower is the colorful and reproductive part of the angiosperm plants. The main function of flower is to affect the process of reproduction. All the genotypes varied highly significantly from each other regarding days to flowering and ranged from 72.00 to 59.33 days (Table 2). Mean of the data was recorded to be 65.67 days. Maximum (DF) were recorded in Line-12 (72.00 days) followed by Line-6 (70.00 days), Line-8 (68.00days) and Line-3 (67.33 days). On the hand, Line-1 (59.33 days) took minimum (DF) (Table 3). Significant results were also obtained by [24, 25] which are similar to results of the present study.
The character plant height is controlled by many genes and is polygenic in nature. It is also influenced by the environment. The phenomenon of additive gene action is always involved in it. Therefore, it cannot be static and always perform differently even in the same environmental conditions. In the current study, plant height exhibited significant variability and varied from 99.11 cm to 30.37 cm (Table 2). Mean plant height was noted to be 54.89 cm. Maximum (PH) was recorded for Line-5 (99.11 cm) followed by Line-6 (93.11 cm) and Line-12 (89.44 cm) while minimum (PH) was observed for Line-1 (30.37 cm) followed by Line-3 (52.27 cm) (Table 3). The results of present study are in confirmation with the results of previous findings of [5, 26] that significant differences are present for the plant height.

In kidney bean, flowering is followed by pods formation. Pods contain seeds, the edible part of the bean plant. Analysis of the current study revealed that pods per plant in kidney bean genotypes varied significantly from each other (Table 2). Mean value for pods per plant was 11.81. The whole data was dispersed between 16.28 and 6.89. Maximum (PP1) were obtained from Line-9 (16.28) followed by Line-11 (15.66) and Line-5 (15.13) while least values were obtained from Line-1 (6.89), Line-12 (8.58) and Line-4 (9.16) (Table 3). From analysis of variance, genotype mean square value and coefficient of variance was 29.04 and 25.08 % respectively. [27] Studied and reported that significant differences are present in kidney bean. However their results are different from our findings because of the difference in genetic make-up, polygenic nature of the trait and experimental area.

Pod length is polygenic trait and affected by several genes. It is also influenced by environment. The process of domestication in kidney bean led to development of large pods rather than small pods. For pod length, genetic variability was present in kidney bean genotypes. Data varied significantly from each other (Table 2). Mean value was 11.16 cm for (PL). The data ranged between 13.55 cm to 8.44 cm. Maximum value for (PL) was obtained for Line-2 (13.55 cm), Line-6 (13.11 cm) and Line-12 (13.00 cm) while minimum value was recorded for Line-1 (8.44 cm), Line-8 (9.11 cm) and Line-3 (9.22 cm) (Table 3). [5, 28] also reported genetic variability for (PL) in their studies on kidney bean. However their results differ from our findings. The reason might be due to difference in genetic make-up, polygenic nature of the trait and experimental area.

Seeds, the edible portion of kidney bean, are present inside the pod. Number of seeds varies from plant to plant and also varies from genotype to genotype. Seed per plant is highly variable character. Number of (SP1) varied significantly from the mean value (Table 2). The data varied from 6.55 to 3.64. Mean value for number of (SP1) was recorded to be 4.65. Maximum number of (SP1) were obtained from Line-12 (6.55) followed by Line-6 (5.76) and Line-7 (4.92). Minimum value for the trait was observed in Line-1 (3.64) followed by Line-10 and Line-11 having 3.89 (SP1) each (Table 3). Results of the current study are in confirmation with the results of the research study conducted by [29]. These researchers also reported that variability exits among kidney bean genotypes for number of (SP1).

Improving the final yield is one of the most important objectives of plant breeding. Many researchers are focusing to improve the yield of any crop to meet the challenges of the growing population. Yield is quantitative trait and is controlled by many genes. Additive and epistatic gene action is involved in it. Minor and major genes contribute towards the final yield. Data was collected on seed yield per plant and analysis showed that significant genetic differences are present in the kidney bean genotypes (Table 2). Line-7 (72.97 g) exhibited maximum (SYP1)
followed by Line-11 and Line-12 having values of (71.10 g) and (69.24 g) respectively. Minimum (SYP⁻¹) was recorded in Line-1 having value of (25.77 g) followed by Line-10 (34.83 g) and Line-4 (45.02 g) (Table 3). Mean value of the trait was found to be 54.89 g. Similar results were also obtained by [30]. However there is difference between the genotype mean square values and coefficient of variation of the current study and previous analysis which might be due to environmental factors, polygenic nature of the trait and difference in experimental area.

Seed weight is important and critical for any seed crop yield. It is important to understand the genetic mechanism for improving the seed yield. Many candidate genes are involved in controlling the said trait. Seed is the economic part of kidney bean plant which is enclosed within the pod. Size of the seed varies from variety to variety and also from plant to plant of the same variety. Seed weight is measured in grams. Data was recorded on 100 seed weight and analysis of variance showed that highly significant variability is present (Table 2). Mean value for 100 (SW) was recorded to be 37.51 g. The whole data ranged from 55.74 g to 19.74 g. Maximum value for the trait was obtained from Line-4 (55.74 g) followed by Line-2 (44.88 g). Minimum value for 100 (SW) was observed in Line-3 (19.74 g) (Table 3). The findings of our current study are in confirmation with findings of studies conducted by [31, 32]. However there are differences in the mean data and range which might be due to difference in genotypes and climatic conditions of the current study.

Phenolic compounds include simple phenols, phenolic acids, hydroxycinnamic acid derivatives and flavonoids are bioactive compounds produced by numerous plants having anti-inflammatory and anti-allergic functions. Among the kidney bean genotypes, total phenolic compounds varied highly significantly from each other (Table 4). Maximum concentration of (TPC) was present in Line-10 (88.24 mg/100g) followed by Line-7 and Line-11 having values of 85.80 mg/100g and 75.61 mg/100g respectively (Table 5). Antioxidant activity and genetic variability of (TPC) present in kidney beans was also reported by [33]. Antioxidants are the chemical substances having the capability to inhibit oxidation. Oxidation is a chemical reaction that can produce free radicals leading to damage the cells of an organism. Total antioxidant activity of all the genotypes of kidney bean varied highly significantly from each other (Table 4). Maximum concentration of (TA) was obtained for Line-7 (136.88) followed by Line-10 (135.96) and Line-12 (135.95). Minimum values were recorded in Line-6 (131.67) followed by Line-5 (132.17) and Line3 (133.42) (Table 5). [34, 35] Studied the antioxidant activity of kidney beans and reported that genetic variability exists among the kidney bean genotypes for the said biochemical compound.
Figure 1. Variability of kidney bean genotypes for various morphological and biochemical traits
Table 2. Mean squares with CV (%) for days to emergence, days to flowering, plant height, pods plant\(^{-1}\), pod length, seeds pod\(^{-1}\), seed yield plant\(^{-1}\) and 100 seed weight in Kidney bean (Phaseolus vulgaris L.)

| Traits                      | Replication | Genotypes | Error | F. Ratio | CV (%) |
|-----------------------------|-------------|-----------|-------|----------|--------|
| Days to Emergence           | 8.11        | 7.47*     | 3.14  | 2.38     | 7.06   |
| Days to Flowering           | 9.75        | 39.15**   | 3.99  | 9.81     | 3.04   |
| Plant Height                | 1825.17     | 1081.50** | 250.12| 4.32     | 21.69  |
| Pods per Plant              | 33.38       | 29.04**   | 8.78  | 3.31     | 25.08  |
| Pod Length                  | 1.40        | 9.24**    | 2.22  | 4.14     | 13.38  |
| Seeds per Pod               | 0.092       | 2.033*    | 0.883 | 2.30     | 20.21  |
| Seed Yield per Plant        | 944.30      | 652.08*   | 253.49| 2.57     | 29.00  |
| 100 Seed Weight             | 172.61      | 270.48**  | 47.38 | 5.71     | 18.35  |

* = Significant at \(p \leq 0.05\)
** = Significant at \(p \leq 0.01\)

Table 3. Mean values of days to emergence, days to flowering, plant height, pods plant\(^{-1}\), pod length, seeds pod\(^{-1}\), seed yield plant\(^{-1}\) and 100 seed weight in Kidney bean (Phaseolus vulgaris L.).

| Genotypes | Days to emergence | Days to flowering | Plant height | Pods per Plant | Pod Length | Seeds per pod | Seed yield per Plant | 100 Seed Weight |
|-----------|-------------------|-------------------|--------------|----------------|------------|---------------|----------------------|-----------------|
| Line-1    | 23.67 c-d         | 59.33 e           | 30.37 e      | 6.89 e         | 8.44 e     | 3.64 c        | 25.77 d              | 43.14 b-c       |
| Line-2    | 26.67 a-c         | 64.33 d           | 70.14 b-d    | 10.70 b-e      | 13.55 a    | 4.46 b-c      | 51.82 a-d            | 44.88 a-b       |
| Line-3    | 23.33 d-e         | 67.33 b-d         | 52.27 d-e    | 13.46 a-d      | 9.22 d-e   | 4.47 b-c      | 57.16 a-c            | 19.74 f         |
| Line-4    | 26.00 a-d         | 60.00 e           | 73.30 a-d    | 9.16 d-e       | 12.33 a-c  | 4.79 b-c      | 45.02 b-d            | 55.74 a         |
| Line-5    | 26.00 a-d         | 65.00 c-d         | 99.11 a-c    | 15.13 a-c      | 10.22 c-e  | 4.70 b-c      | 68.74 a-b            | 27.94 e-f       |
| Line-6    | 23.00 d-e         | 70.00 a-b         | 93.11 a-b    | 11.33 a-e      | 13.11 a-b  | 5.76 a-b      | 58.13 a-c            | 32.92 c-e       |
| Line-7    | 27.33 ab          | 64.33 d           | 79.53 a-c    | 10.57 c-e      | 10.33 c-e  | 4.92 b-c      | 49.33 a-d            | 31.32 d-f       |
| Line-8    | 26.00 a-d         | 68.00 b-c         | 75.69 a-d    | 14.55 a-c      | 9.11 d-e   | 4.15 c        | 54.60 a-c            | 41.95 b-d       |
| Line-9    | 24.67 b-e         | 66.00 c-d         | 74.97 a-d    | 16.28 a        | 11.11 a-d  | 4.59 b-c      | 72.97 a              | 37.39 b-e       |
| Line-10   | 28.00 a           | 66.00 c-d         | 57.06 c-e    | 9.44 d-e       | 10.77 a-d  | 3.89 c        | 34.83 c-d            | 41.92 b-d       |
| Line-11   | 25.67 a-e         | 65.66 c-d         | 79.77 a-c    | 15.67 a-c      | 12.74 a-c  | 3.89 c        | 71.10 a-b            | 42.33 b-d       |
| Line-12   | 22.67 e           | 72.00 a           | 89.44 a-b    | 8.58 d-e       | 13.03 a-b  | 6.55 a        | 69.24 a-b            | 30.86 d-f       |
| LSD \((0.05)\) | 3.0312       | 3.38           | 26.78        | 5.01           | 2.52       | 1.59          | 26.96                | 11.65           |

Means followed by the same letter are not significantly different from each other.
Table 4. Mean squares with CV (%) for Total Phenolic Compounds and Total antioxidants estimated in kidney bean (*Phaseolus vulgaris* L.)

| Phytochemicals      | Replication | Genotypes | Error | F. Ratio | CV (%) |
|---------------------|-------------|-----------|-------|----------|--------|
| Total Phenolic Compounds | 0.96        | 929.81**  | 0.96  | 965.70   | 1.58   |
| Total antioxidants  | 0.68        | 7.56**    | 1.41  | 5.36     | 0.88   |

* = Significant at $p\leq 0.05$

** = Significant at $p\leq 0.01$

Table 5. Mean values of Total Phenolic Compounds and Total antioxidants in kidney bean (*Phaseolus vulgaris* L.)

| Genotypes | Total Phenolic Compounds | Total antioxidants |
|-----------|--------------------------|--------------------|
| Line-1    | 54.68 h                  | 134.70 b-d         |
| Line-2    | 68.28 e                  | 133.83 c-e         |
| Line-3    | 59.87 g                  | 133.42 d-f         |
| Line-4    | 47.66 i                  | 134.68 b-d         |
| Line-5    | 35.34 j                  | 132.17 e-f         |
| Line-6    | 32.54 k                  | 131.67 f           |
| Line-7    | 85.80 b                  | 136.88 a           |
| Line-8    | 71.34 d                  | 135.53 a-c         |
| Line-9    | 62.37 f                  | 135.62 a-c         |
| Line-10   | 88.24 a                  | 135.96 a-b         |
| Line-11   | 75.61 c                  | 133.83 c-e         |
| Line-12   | 61.89 f                  | 135.95 a-b         |

LSD (0.05) 1.66 2.011

Means followed by the same letter are not significantly different from each other

**Conclusion and recommendations**

Analysis of variance revealed that all the genotypes were significantly different regarding various morphological and biochemical traits which confirms the presence of genetic diversity. Minimum days to emergence were recorded for Line-12 (22.66 days), having moderate plant height of (88.17 cm), longer pods (13.003 cm) containing maximum number of seeds (6.55). Minimum days to flowering were observed for Line-1 (59 days). Maximum plant height was recorded for Line-5 (95.66 cm). Maximum number of pods (16.28) was produced by Line-9, having maximum seeds per plant (72.97 seeds). Maximum pod length (13.55 cm) was observed for Line-2. Line 4 produced large size seeds having maximum 100 seed weight (55.74 g). Total phenolic compounds and total antioxidants were observed to be maximum in Line-10 (88.24 mg/100g) and Line-7 (136.88) respectively. Although, all these genotypes were collected were collected from their natural habitat, most of them performed well at subtropical climatic region of Haripur. Based on the characterization of kidney bean genotypes at Haripur, the genotypes viz. Line-9 and Line-12 were observed having minimum days to emergence, moderate plant height and high yield. Therefore, these genotypes are recommended for further characterization in the kidney bean breeding program.

**Authors’ contributions**
Conceived and designed the experiments: A Ali & SA Khan. Performed the experiments: T Ahmad & A Basit. Analyzed the data: I Hussain. Contributed materials/ analysis/tools: S Ali, N Ali & SM Khan. Wrote the paper: H Raza.

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