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RESEARCH PAPER

Phenotypic characterization of common bean (*Phaseolus vulgaris* L.) accessions conserved at the Genetic Resources and Biotechnology Institute

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Abstract

The purpose of this study was to access variability in qualitative and quantitative traits among ten accessions that were obtained from Genetic Resources and Biotechnology Institute (GRBI) in Zimbabwe. The accessions were planted in a Randomized Complete Block Design, with three replicates. The qualitative and quantitative characteristics were recorded using the International Board for Plant Genetic Resources (IBPGR) common bean descriptor list. The quantitative data for the number of days to fifty percent flowering, plant height, days to maturity, seed size, grain yield and number of seeds per pod were analysed using hierarchical cluster analysis Minitab Release 12.22 version and GenStat Discovery versions 14 software. Cluster analysis divided genotypes in two main groups when the dendrogram was partitioned at the 26:22 % similarity levels. Cluster A comprised of 2 bean genotypes and cluster B comprised of 8 genotypes distributed in 2 sub-groups. Genetic similarity among the accessions varied significantly depending on the genotype pairs, groups and sub-groups. The genetic similarity was lower between accessions of different clusters (A and B) and higher between accessions within each cluster. Significant differences ($P<0.01$) were observed among the accessions for the number of pods per/plant, number of seeds per pod, length and seed size. Based on different morphological characters exhibited by different accessions, it could be concluded that morphological diversity exists among the ten accessions of common beans. It can also be concluded that there are no duplicate accession among the ten common bean accessions.

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Introduction

The common bean (*Phaseolus vulgaris* L.) is among the five leguminous food crops grown worldwide (Kaplan and Lynch, 1999). It is the second most significant origin of human dietary protein and the third most important source of calories of all agricultural crops in eastern and Southern Africa (Spence, 2006). The *Phaseolus vulgaris* L. has two eco-geographical gene pools, Mesoamerica and Andes with multiple domestications (Kwak and Gepts, 2009). A lot of the genetic variability of this species is kept as ex-situ, outside the canters of origin, in genebanks (Borda 2011). In Zimbabwe, the collected germplasm that is maintained at the GRBI plays an important role in generating new crop varieties with high yield potential and resistance to biotic and abiotic stresses. The knowledge about the extent of genetic diversity, identification, differentiation and characterisation of genotype, provides an information tool for the detection of duplicates in the collection, hence an establishment of Gene bank core collections. This is also done in order to identify accessions with particular interest for plant breeders and researchers.

Characterization of germplasm is an important first step in breeding efforts and it benefits plant breeders in selecting proper parental materials (Cilliers *et al*., 2000; Sarutayophat *et al*., 2007). In Zimbabwe the utilization of common bean accessions by plant breeders in their breeding programs is restricted due to lack of information about these genetic resources, (Valls, 2007). There are a considerable number of common beans conserved at the GRBI in Zimbabwe. This study focuses on characterising the common bean accessions using phenotypic characters to determine desirable agricultural traits. This allows the breeder to identify valuable traits or potentially valuable genotypes quickly. Data generated from characterization is as well employed in collection management and this encompasses verifying that an accession has not been mislabelled during harvesting, collection, monitoring of contamination through source and determining future handling procedures. The aim of the study is to measure variability in qualitative and quantitative characteristics among ten local landraces of common bean conserved at the GRBI.

Materials and methods

Study site

Morphological characterization of the common bean accessions was conducted at the Crop Breeding Institute's (CBI) Harare Research Farm. CBI is situated at the Department of Research and Specialist Services (Latitude 18° 11" South, longitude 31° 28" East), Mean annual rainfall ranges from 700 mm to 959 mm and average summer temperatures are between 24°C to 26°C. The soils are well-drained red clay loam.

Experimental Design trial establishment and measurements

The ten common bean accessions were planted in a Randomized Complete Block Design with three replications. Each accession was sown in a four rows, two meter long. Inter-plant spacing of 0.15m and inter-row, row spacing of 0.45m was used. The central two rows were used for data collection and analysis. The crop was rain fed.

Plant characters and data recording

The phenotypic characterization was done using the International Plant Genetic Resources Institute (IPGRI) descriptor list for common bean as the resource guide. Data for qualitative and quantitative characteristics was recorded on an individual plant basis using ten randomly selected plants per plot. The ten plants were selected from the two inner rows using the systematic random sampling method per plot, tagged and scored consistently over all characters.

The phenotypic characters that were recorded in this study were cotyledon colour, hypocotyls colour, germination period, growth habit, terminal leaflet shape, days to 50% flowering, colour of flowers standard, days to 95% pod maturity, immature pod pigmentation, pod length, number of pods per plants, number of seeds per pod, seed size, seed shape, 100

seed weight, seed texture, seed colour and seed pattern as described in the IPGRI descriptor
(1995.)

Statistical analysis

Data was analyzed using the GenStat Discovery Version 3.0 for quantitative characteristic. Duncan's multiple-range test (P>0.05) was used for mean separation. Variation in both qualitative and quantitative characteristics was analyzed using hierarchical cluster analysis Minitab Release 12.22 versions (Minitab, 1998).

Results

Variations in morphological characteristics

The dendrogram revealed the results of the hierarchical clustering of common bean accessions using their phenotypic characteristics (Fig. 1). A dendrogram is a binary tree over the common bean accessions being clustered, with each branch indicating the distance between the two accessions.
The nodes of the hierarchy tree (dendrogram) indicated the levels of similarities that had occurred.
The results displayed by the dendrogram showed that the ten common bean accessions were grouped into two (major) groups.

Table 1. Source of germplasm.

Collector's number	Accession number	Local name	Place of collection	Altitude
MS11	3810	Sugar bean	Ziwa- Nyanga	18 ° 09. 624 South
MS 13	3812	Chidhlau	Ziwa- Nyanga	18 ° 09. 624 South
MS15	3814	Katonga small	Ziwa- Nyanga	18 ° 09. 624 South
MS16	3815	Unknown	Ziwa- Nyanga	18 ° 09. 624 South
MS20	3817	Pfumai	Mukoyi-Honde Valley	18 ° 30.141 South
MS22	3819	Red bean	Mukoyi- Honde Valley	18 ° 30.141 South
MS28	3824	Sugar bean 4	Mukoyi- Honde Valley	18 ° 30.141 South
MS30	3826	Pfumisai	Hauna-Honde Valley	18 ° 23.945 South
MS32	3828	Sugar bean 2	Hauna-Honde Valley	18 ° 23.945 South
MS36	3831	Purple mottled	CBI	18° 11 South

These two main groups A and B were partitioned at the 26.22% similarity level, grouping influenced by the number of days by 50% flowering, growth habit, number of days to 50% germination, number of pods per plants and numbers of seeds per pod.

The dissimilarity distance level between the two major groups was 68.324 (Table 2.). Accessions MS16 and MS36 were in group A. These accessions flowered and matured late (36 to 41 days to flowering) and with a range of 83 to 84 days to maturity. MS16 and MS36 had a range of seven to eight days to 50% germination and were also susceptible to lodging (11 to 12%) respectively. Both accessions had the same growth habit with moderate vigour, formed pods throughout the plant and had terminal leaf shapes, (sub-globose).

Accessions MS28, MS15, MS11, MS22, MS32, MS30, MS20 andMS13 were in group B. Similarities in this group were in the number of days to 50% germination, cotyledon colour, terminal leaf shape, colour of standard flower, number of days to flowering, seed size, seed shape and seed texture.

When the dendrogram was divided at the 39.03% similarity and 56.469 distance levels, two sub-groups were obtained as shown in Fig.1. These were designated I and II. It was noted that there was an apparent decrease in similarity or apparent increase in dissimilar from the first sub-group I to II sub-group.

Starting from the smaller sub-groups upwards accessions MS30 and MS32, in sub-group I were the most similar, hence were the first to be joined (Fig. 1). They were 78.70 % similar and were separated by a distance level of 19.720.

Table 2. Clustering of common bean accessions into major and sub-groups based on different levels of dissimilarity coefficient.

Group	Level of split	Characteristics	Accession numbers
Major group A	26.66%	This group consist of large seeds which belong to Andean South American genotype based on the seed size and 100 seed weight. Cotyledon colour (all green), number of days to 50 % to flowering (41 to 36 days) and number of days to 95 % maturity (range of 83 to 84 days) , and seed shape (both kidney shaped), lodging susceptibility(12 to 11 %). Terminal leaf shape (sub-globose).	MS16, MS36
Major group B	26.66%	This group consisted of both the Mesoamerican and intermediate seed size. Grouping of accessions with the same tendency for growth habit, four accessions had (Type I), two (type III) and two (Type IV). Number of days to 50 % flowering range from 31 to 39 days, number of pods per plants (ranged from 9 to 11) and numbers of seeds per pod five accessions had (four) two (five seeds) and one accession had six seeds per pod.	MS13, MS20, MS30, MS32, MS22, MS11, MS15, MS28
Sub-group I	39.02%	This group consist of intermediate seeds which fall in between the Andean South American and Mesoamerican. Cotyledon colour (all green), standard flower colour (white, pink), number of days to 95 % maturity accessions MS13 and MS20 had 82days. Accessions MS30 (74 days) MS32 (76 days). Number of pods per plants, accessions (range seven to eleven) and seed pattern (Striped).	MS13, MS20, MS30, MS32, MS22
Sub-group II	39.02%	This group consist of small seeds which belong to the Mesoamerican genotype. , Cotyledon colour accession MS15 and MS28 (green) and MS11 (purple). Terminal leaf shape, two (sub-hastate) and MS11 (sub-globose), number of days to 50 % of flowering (range of 37 and 38 days), MS15 and MS11 (ranged 76 and 77 days) number of days to 95 % maturity and MS28 had 83 % and All the exhibited good levels of resistance to the major diseases occurring in the field including rust.	MS15, MS28, MS11,

The characteristics that separated them were percentage to lodging and the disease severity. MS32 was tolerant to Bean Common Mosaic Virus (BCMV) and Aschochyta Leaf Blight (ASC) diseases; and it was susceptible to rust. The accession was susceptible to lodging. MS30 had lodging percentage of twelve and it was highly susceptible to BCMV, Common Bacterial Blight (CBB) as compared to MS 32. The accession was also susceptible to rust.

Accession MS22 was grouped together with MS13 at the 39.0 2% similarity level and the distance dissimilarity was 56.469. MS22 constituted sub-group I in major group B. Accession MS22 was distinguished from the other four accessions MS13, MS20, MS30, and MS32 by flower colour (light pink) an indeterminate bush habit with weak main stem which had prostate branches with a moderate vigor and formed pods throughout the plant, number of days to 95% maturity (77 days), plain deep red seed pattern, red immature pod pigmentation, MS22 was tolerant to diseases like ALS, ASC, CBB, and rust. It was highly susceptible to BCMV and was resistant to Anthracnose (ANT) compared to the other four accessions (MS13, MS20, MS30 and MS32).

The next accessions to be grouped together were MS15 and MS28 as shown in Fig. 1. They were joined at the 53.21 % similarity level and the distance between them was 43.326. These two accessions were separated by hypocotyls color, standard flower colour, growth habit, number of days to 95 % maturity, disease severity, immature pod pigmentation, seed size, seed pattern, seed shape and number of seeds per pod.

Table 3. Separation mean for quantitative characteristics.

Accessions	Days to 50% Flowering	No. of seeds/pod	Mean 100SW	Number of Days to Maturity
MS28	39.33^{ef}	4.83^{d}	32.34^{b}	82.67^{b}
MS30	32.67^{ab}	4.10^{bc}	40.42^{cde}	74.33^{a}
MS32	33.67^{bc}	4.00^{abc}	43.09^{cdef}	75.67^{a}
MS36	38.67^{ef}	4.26^{c}	31.71^{b}	84.00^{a}
MS11	35.33^{cd}	4.86^{d}	37.37^{bc}	76.67^{a}
MS13	34.00^{bc}	3.60^{ab}	44.39^{def}	81.67^{b}
MS15	37.67^{de}	5.53^{e}	22.22^{a}	77.33^{a}
MS16	41.00^{f}	3.56^{a}	48.30^{f}	84.33^{b}
MS20	31.00^{a}	3.93^{abc}	45.30^{ef}	81.67^{b}
MS22	37.33^{de}	4.00^{abc}	39.07^{cd}	76.67^{a}
Min.	31	3.56	22.22	74.33
Max.	41	5.53	48.30	84.33
Mean	36.07	4.27	38.42	79.50
LSD 5%	1.527	4.416	4.638	1.733
S.e	0.89	0.265	2.704	1.010
P-value	**	**	**	**
CV%	2.4	6.2	7.0	1.6

Note: $^{a, b, c, d, e.f.}$ Within column, figures with the same letter of the alphabet within a column are not significantly different by Duncan's multiple-range test ($P > 0.05$). ns = not significant, * $P<0.05$, ** $P<0.01$, ***$P<0.001$.

MS15 had a white pink standard flower colour; an indeterminate climbing growth habit with a moderate vigor and formed pods throughout the plant. MS15 had 77 days to 95 % maturity, immature pod pigmentation which was black, and ovoid seed size. The accession had six seeds per pod, a striped seed pattern, and an ovoid seed shape. MS28 had a standard flower colour that was light purple white, an indeterminate bushy growth habit with weak main stem, moderate vigor and formed pods throughout the plant. MS28 had 83 days to 95 % maturity, immature pod pigmentation which was purple in colour and intermediate seed size. The accession also had five seeds per pod, a mottled seed pattern and a little rhomboid spotting seed shape. There was variation in seed colour both within and among the entries. It was interesting to note that where there was variation within a genotype. These two accessions (MS15 and MS28) were similar in the following characteristics; cotyledon colour (green), terminal leaf shape (sub-hastate), number of days to 50 % to flowering (range of 37 and 38 days), lodging susceptibility (about eleven and twelve percent lodged).

Accession MS11 was joined with MS15 and MS28 at the 42.20 % similarity level. The distance dissimilarity was 53.523. Accessions MS11, MS15 and MS28 constituted sub-group II in major group B. Accession MS11 was distinguished from the other two by cotyledon colour (purple), hypocotyls colour (purple), standard flower colour (white pink), number of days to 50 % flowering (35 days), immature pod pigmentation (red), seed shape (kidney), seed texture (rough to wrinkled) and number of seeds per pod (five). However, all the three accession were similar in the following characteristics; terminal leaf shapes (sub-globose) and all accessions were resistant to rust.

Analysis of variance

Variability was noted in most of the different quantitative characteristics recorded as shown by the *P* values of less than 0.01 for the number of days to

50% flowering, number of seeds per pod, 100 seeds weight and number of days to 95% maturity. The accessions showed a moderate variability for a number of days to 50% germination and plant height ($P<0.05$).

The mean days to 50% flowering were significantly different among the accessions ($P<0.01$) (Table 3). The average days taken 50% flowering for the ten accessions were 33 days, with accession MS20 recording the earliest number of days (31 days). Accession MS16 was the latest flowering accession (41 days). The accession MS20 was significantly different from MS22, MS13, MS16 and MS32 with respect to mean days to 50% flowering as shown in Table 3. MS11 had a mean day to 50% flowering that was different from accessions MS28, MS36 and MS30.

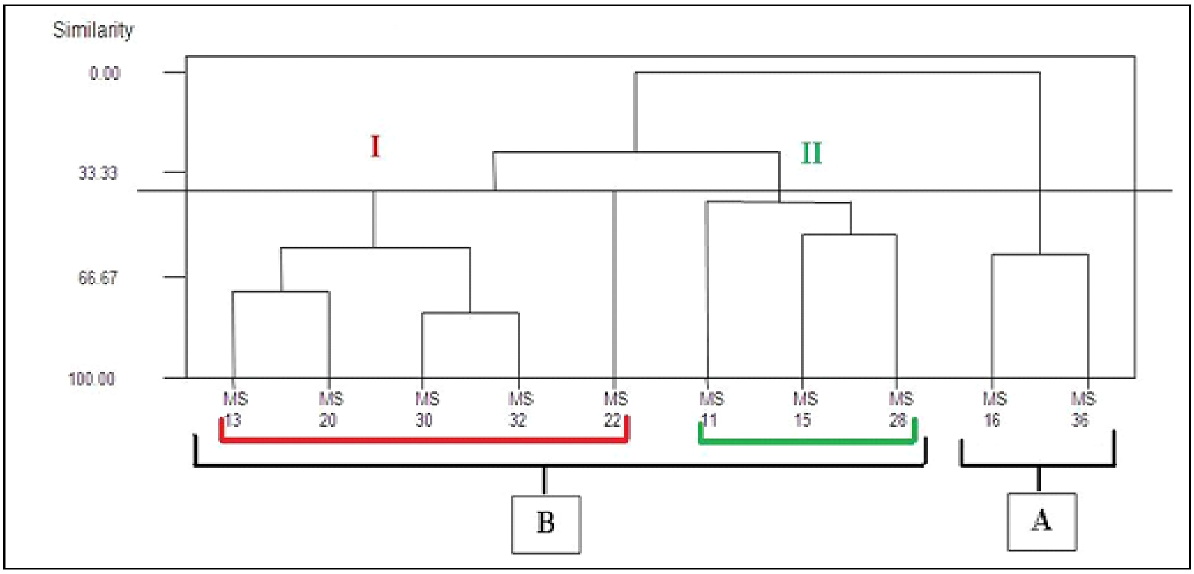

Fig. 1. Hierarchical clustering of ten common bean accessions based on the phenotypic characteristics.

The mean number of seeds per pod was significantly different among the accessions ($P<0.01$). The mean number of seeds per pod for all the accession was 4.27. Accession MS15 had the highest number of seeds per pod (5.53) whilst accession MS16 had the lowest (3.56). Accession MS15 was significantly different from MS11, MS36 and MS32. MS30 was significantly different from MS13, MS20, MS16 and MS15.

The 100 seed weight means were significantly different among the accessions ($P<0.01$). The mean for 100 seed weight for all the ten accessions was 38.42 g. Accessions MS16 had the highest 100 seed weight of 48.30 g. Accession MS15 had the least 100 seed weight of 22.22 g. All accession means were significantly different from each other except for accession MS28 which was not significantly different from MS36.

The mean number of days to 95% pods maturity was significantly different among the accessions ($P<0.01$). The accession MS30 was the earliest maturing variety with 74 days whilst accessions MS16 and MS36 were the later maturing varieties with 84 days. MS11 was significantly different from MS20, MS13, MS16 and MS30 with respect to 95% pods maturity. Accessions MS16 was significantly different from MS22, MS36 and MS32.

Discussion

Genetic diversity is a prerequisite for the genetic improvement of a crop. The rational use of germplasm collections requires a good knowledge about their characteristics (Stoilova and Pereira. 2013). Characterisation of accessions is traditionally based upon morphological and agronomic traits, which is of high interest for plant breeders.

The results displayed by the dendrogram showed that ten common bean accessions could be grouped into two sub-groups when the dendrogram is cut at about 39.02% similarity level. The clusters were designated I and II (Fig.2). The major groups were designated A and B, A does not consist of any sub-group, whilst major group B consisted of two sub-groups I and II (Table 2). However, it was noted that there was an apparent decrease in similarity or apparent increase in dissimilar from the first group A to group B. The apparent decrease in similarity could be explained in terms of increasing genetic distances between the accessions as well it could be a result of out–crossing with other genotypes.

Plate 1. Seed coat colour of the ten common bean accessions.

The most similar accessions in this trial were MS30 and MS32 in the sub- group I and were the first to be joined together. They were observed to be 78.70% similar and were separated by a distance level of 19.720. This led to an inference that there were no duplications among the accessions. However, a distance of 19.720 that separated these two accessions might be a result of predominantly self-pollinated mating system on the common bean (Graham *et al*,. 1997), which causes low rates of gene flow among populations, which in turn results in a spatial differentiation. Moving from one sub-group to another, there was an apparent decrease in similarity which resulted in all accessions grouping to form one large cluster at 0% similarity level (Fig.1), dissimilarity distance index of 19.720.

The overall dissimilarity between the two major groups which were partitioned at the 39.02% level of similarity in the, number of days to 50% flowering, number of days to 95% maturity, growth habit, and seed size could be explained in-terms of phenotypic traits that were subjected to natural, artificial selection since environmental conditions and farmer's selection criteria lead to divergences between landraces.

This clearly discernible difference in phenotypic traits was observed among the ten accessions that were collected from the two different locations in Zimbabwe; Nyanga and Honde Valley. These differences can be attributed to wide genetic differences between the two groups due to differences in parental races amongst the two groups; Andean origin and Mesoamerican origin based on seed weight.

This study confirms the existence of morphological variability in the collection of common bean at GRBI in Zimbabwe and this could be a result of several years of natural and artificial selections by farmers for better adaptation to local growing conditions, with different bean types being preferred by farmers in different regions (Harlan, 1976). Among different traits, seed traits have been found most important in common bean and major determinants of commercial acceptability of varieties (Park *et al.,* 2000; Bisht *et al*., 2014; Rana *et al*., 2014). Seed traits have also been considered highly heritable traits, therefore important in breeding programmes (Singh *et al*., 2007; Blair *et al.,* 2010).

The pigments responsible for the wide variations in colour of beans seed coats are flavonoids (Beninger *et al*., 1999). Modifying genes interact with the colour genes to form various pigments in bean seed coats (Beninger *et al*., 1999). Variation in seed coat colours within accessions can be explained in terms of mutation and segregation. According to Musvosvi (2009), variation in seed colour within an accession may be due to a mutation which is followed by segregation as this is very common with common bean seed colour variation among accessions. This may also mean that their progenitors are different. This shows that (seed coat colour within accessions) genes were still largely heterozygous for several loci and seed coat colour genes were not yet fixed. Variations in seed coat colour among accessions can be explained in terms of epistasis (modifying gene effect). Modifying genes have an intensifying effect or darkening influence upon pale colours formed by the action of the colour genes but do not impact colour in them (Beninger *et al*., 1999).The genetic diversity among and within landraces makes them a valuable resource as potential donors of genes for the development and maintenance of modern crop varieties (Karp *et al*., 1997). The colour, shape, and size of seeds are special attention for consumers. The most preferred colour is mottled seed in Zimbabwe with medium to large size and farmers are willing to pay higher prices for such grains.

Plate 2. Variation in seed colours.

The phenological stages of number of days to 50% flowering and number of days to 95% pods maturity are important for early and uniform maturity. The number of days to 50% flowering was observed to be between 31 days to 42 days. Accession MS20 was the earliest flowering within 31 days and indicated a determinate bush type of beans. The latest accession was MS16 within 42 days as a result of indeterminate growth habit. Early flowering characteristic is a very important component to grain yield in common bean production and results in early harvesting. Therefore early flowering had been recognised as a good agronomic attribute of crops for early maturity, uniformity of yield and crop production in general (Kumanga *et al.,* 2003).

Number of days to 95% pods maturity among accessions (Table 2.); accession MS 30 took the shortest period (74 days) of days to 95% pod maturity whilst accession MS 16 and MS 36 took the longest period with 84 days. Breeders prefer accessions with early maturity for crossing with traits such as rust resistance, high pod and seed number. Late maturity promotes continuous picking and is an advantageous to smallholder farmers as a food source and not for commercial purposes. According to Muasya *et al.,* (2002a), seeds from earlier pods achieve physiological maturity earlier than seeds from later maturity pods. In common bean, seeds from earlier pods tend also to achieve maximum seed quality earlier than those from later pods within a crop (Muasya *et al*., 2002b). Seeds from earlier pods take a longer time for moisture content to decline between physiological maturity and harvest (Muasya *et al.,* 2002a) and may thus be more susceptible to deteriorating conditions during maturation drying. Farmers prefer short season varieties with 74 to 84 days to maturity with wide adaptation capacity. The results published by Muasys *et al*., (2002b) confirmed the strong emphasis on developing earlier maturing common bean cultivars because of shorter growing season and less risk of frost at higher attitudes. Number of seeds per pod is the most important characters for yield production. The high value of seed per pod can be utilized for improvement of beans potential productivity.

The seed weight is important quality trait, as well as main yield component and market characteristic (Vasic *et al.,* 2010; Kelly *et al.,* 1998). The mean of 100 seed weight for all the ten accessions was 38.42g. Accession MS16 had the highest 100 seed weight of 48.30g, indicated that among the accessions they were Andean South America genotype that have large seeded beans with more than 40g per 100 seeds. Whilst accession MS15 had the least 100 seed weight of 22.22g which also indicated the Mesoamerican genotypes among the collection, which are small seeded 25g to 39g. Medium seeded beans which ranges between 25 g to 40 g per 100 seeds define introgression between the gene pools through breeding programs (Gepts *et al.,* 1988). Within accessions conserved at the GRBI, all three groups are present; small (1), medium (4) and large seeded bean (5) (Table 3). This result suggests that Zimbabwe common bean germplasm collection is a rich source of material for use in the improvement of the crop. Farmers choose seeds of specific size and shape for sowing and in that way indirectly affect the selection and phenotype of local landraces (Papa and Gepts, 2003). According to Vasic *et al.,* (2010), it is important to collect local landraces and accession, which are source of significant genetic variability.

The results of this study indicated that some of the common bean accessions exhibited good levels of resistance to the major diseases occurring in the field. Their adoption would, therefore, increase bean production, example accession MS 22, MS 11, MS 15 and MS 28. According to CIAT (1987) accessions with the scoring rate from one to three are in the category of being resistance to diseases. There were no visible symptoms on these accessions therefore the germplasm can be used as parents or commercial varieties. Accession MS 11, MS 15, MS 22 and MS 28 were resistant to most of those diseases except the Bean Common Mosaic Virus. The accessions or germplasm can be used as a source of resistance to certain diseases.

Conclusions

Based on the differences in phenotypically characters expressed by the different accessions, it can be concluded that significant variability in qualitative and quantitative characteristics exists among the ten common bean genotypes obtained from the GRBI. The ten accessions showed variation in number of days to fifty percent flowering, number of seeds per pod, and number of days to 95% maturity and 100 seed weight. These phenotypic differences could mean that the accessions are of different genetic makeup, therefore could be exploited, enriched and or directly utilized in breeding programs. The present and future utilization of the genetic resources conserved largely depends on what is known about the accessions. The generated information therefore will definitely contribute to a more effective utilization of the common bean germplasm collection. From the results, variation in seed colour ranged from white, black, cream, golden yellow and brown as shown in Plate 2. There was variation in seed colour both within and among the accessions. The variation in seed colour of beans shows the complexity of the genetic synthesis of seed coat colour. It can be concluded that there are no duplicate accessions as evidenced by the most similar accessions, which are only 78.70% similar. This scientific information obtained from this study is useful for breeding programs, researchers and farmers.

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