European Journal of Agriculture and Forestry Research
Vol.9, No.3, pp. 1-12, 2021
Print ISSN: ISSN 2054-6319 (Print),
Online ISSN: ISSN 2054-6327(online)

# MORPHOLOGICAL CHARACTERIZATION AND ESTIMATION OF GENETIC PARAMETERS IN SOYA-BEAN (*GLYCINE MAX* (L.) MERR.) CULTIVARS GROWN IN LESOTHO

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**ABSTRACT***: Soya-bean cultivars grown in Lesotho have not been characterized morphologically to distinguish them. A study was conducted in Lesotho, with objectives of (i)distinguishing the cultivars of soya-beans, (ii) estimating genetic distances among cultivars, (iii) determining the morphological markers with high discriminatory power and (iv) estimating genetic and phenotypic variance among cultivars. Experiment was laid-out using randomized completely block design with 28 treatments and three replications. Data collected using IPGRU descriptor were stem determination, pubescence presence, pubescence density, pubescence colour, pubescence type, leaflets size and leaflet shape. Data were subjected to analysis of variance, cluster analysis and principal component analysis. Analysis of variance revealed a highly significant difference among soya-bean cultivars for pubescent type and pubescent density, and only significant for leaf size and leaf colour. No significant difference was obtained for leaf shape and stem determination. Cluster analysis was able to group cultivars into two groups which further divided into sub-groups. Sub-groups again were divided into smaller groups. Outlier was also obtained. Highest genotypic variance was obtained in pubescence density and pubescence type, while lowest genotypic variance was observed in leaflets shape, leaf size and pubescence colour. Pubescence density and stem determination revealed high phenotypic variance. Leaf size and pubescence colour expressed lowest phenotypic variances. High heritability was expressed in pubescence type and pubescence density. Low heritability was experienced in leaflets shape and stem determination. Highest genetic advance was shown by leaf size, pubescence type, leaflets shape and pubescence density. The lowest genetic advance was experienced with pubescence colour.*

**KEYWORDS:** *Glycine max*, morphological markers, cluster analysis, principal component analysis, phenotypic variance, genotypic variance.

## INTRODUCTION

Soya-bean (*Glycine Max* (L.) Merril) is one of the most important crop in the world ranking fourth after rice, maize and wheat as evidenced by area under which it is harvested and produced (Fried *et al.*, 2018). In the past 40 years, soya-bean production increased by 400 folds, with United States of America producing 51%, Brazil 20%, Argentina and China 10% each (Malek and Raffi, 2014). It originated in Manchuria, North of China and was disseminated to Asia, Europe, United Stated of America, Latin America and Africa during 7$^{th}$ century (Matsunami *et al*., 2004). This crop is one of the oldest cultivated crops utilized by Chinese as a source of food for human and feed for

animal consumption in 2500 BC. World-wide, a large proportion of soya-bean is used predominantly as animal feed in a grain or vegetative form (Malek and Raffi, 2014). It was only discovered by Western World as a rich source of proteins and oil in the 19th Century. It is a highly nutritious legume crop containing 36 – 54% crude protein, 15 – 25% oil, carbohydrates 31 – 37%, crude fibre 9 – 11%, 4 – 7% ash and dietary (Ali *et al*. 2016; Lindt and Lightfoot, 2016). Besides, soya-bean have therapeutic components, namely lactose, free fatty acids, anti-oxidants, folic acids, vitamin B and isoflavones (Chave`z-Servia *et al*., 2016).

Within soya-bean crop species, there are many cultivars that differ from each other in one or more characteristics of economic importance (Babu *et al*., 2018). This variation among cultivars created in a great wealth of germplasm evolving over time from wild relative, landrace, obsolete cultivars, modern cultivars and hybrids (Charanj *et al*., 2018; Faria *et al*., 2016). The genetic variability in soya-bean is created by both natural causes and man-made efforts. Natural causes such as natural mutations, genetic recombination and polyploidy have made insignificant impact in the genetic variability (Hamawaki *et al*., 2012; Ali *et al*., 2016). Conversely, man-made variability has resulted in enormous variation which is directed to the benefit of mankind. Man-made efforts include conventional breeding, induced mutation and genetic engineering (Loko *et al.*, 2018). The companies involved in the improvement of soya-bean through afore-mentioned methods protect their intellectual property rights which are enacted. International Union of the Protection of New Varieties of Plants (1991) introduced the protection law and came up with concept of distinctness, uniformity and stability. In South Africa, Plant Breeders Rights and Plant Improvement Act are enacted. This is to make the cultivars produced by each company exclusive and protected from abuse and fraudulence. In order to identify and distinguish the soya-bean cultivars, many methods are employed including morphological, biochemical, molecular and DNA-based methods.

Many soya-bean cultivars are introduced into Lesotho by farmers, retailers, seed companies and research organizations to be grown without characterization, authentication and without compliance of plant breeder' rights. One cultivar may be mistaken for another or the same cultivar bear different names depending on the agent that brought it in the country. The soya-bean generations include obsolete, primitive, isogenic, landraces and hybrid and genetically modified. All of these have to be distinguished by the farmers using morphological markers for adaptation in different environments and yield potential. It is therefore with this reason that the study is undertaken to rectify these mistakes. The objectives of the study are manifold; (1) distinguish the cultivars of soya-beans, (2) estimate genetic distances among cultivars, (3) determine the morphological markers with high discriminatory power and, (4) estimate genetic and phenotypic variance among cultivars.

## MATERIALS AND METHODS

### Study area

The study was conducted at Roma Campus of the National University of Lesotho which is situated 34km south west of Maseru, the capital city of Lesotho. The coordinates for Roma campus are $29^0$ 26' 48 south latitude and $27^0$ 42' 29 east longitudes, with an altitude of 1610 m above sea level. Temperature increases gradually in August from $20^o$ C during the day and $14^o$ C during the night

to January when it reaches the highest temperature of 35$^{o}$C during the day and 23$^{o}$C at night, thereafter it declines to -7$^{o}$C from May to July. The average annual rainfall is 750mm commencing in October and reach the peak in February. Normally, it is dry from May to August. Snowfall is experienced from May to July. Hailstorm may occur at any time during the growing season, particularly in summer, autumn and spring,

**Site description**

Roma valley is broad and fertile area surrounded by sand stone cliffs towards the east. The soil type consists of Berea series (Plinthaquic dystruchrepts). Top soil is a sandy loam with hue of 10 YRS, 4/3 while sub-soil is dark yellowish sandy clay loam with hue of 10yrs 4/4. The soil analysis results revealed pH 3.63 with phosphorus of 0.033 and 0.000 at 0ppm, 0.197 at 1ppm, 0.223 at 2ppm, 0.329 at 3ppm and 0.525 at 4ppm.

**Experimental design**

The research was conducted over a period of two growing seasons, namely; November 2019 to April 2020 and October 2020 to March 2021. The experiment was carried out in a Randomized Complete Block Design with 28 treatments (soya-bean genotypes) and three replications. The size of the field was 36m x 17.2m equivalent to 619.2m$^2$ which was divided into 3 blocks where each block had 28 plots. Each plot had 4 rows with the length of 4m each. The inter-row and intra-row spacing were 0.9m and 0.15m, respectively. Soya-bean seeds used in experiment were obtained from Department of Agricultural Research- Ministry of Agriculture and Food Security, Lesotho.

**Agronomic practices**

The land was first prepared using a tractor mounted mould-board plough, after which a disc harrow was used to level the seedbed and break the clods. A compound fertilizer of 2:3:2 (22) + Zn was applied by hand over the field at the rate of 250kg ha$^{-1}$ as basal dressing. Top-dressing was not applied. Sowing of seeds was done by hand. The field was irrigated twice a week due to prolonged drought that prevailed. Weeding was done by hand-hoeing thrice during the growing period of the soya- beans to control nutsedge (*Cyprus esculentum* L.) which was very problematic. Cape Mount rifles (*Mylabris spp.*) feeding on flowers of the plants was controlled chemically by applying ripcord (Cypermethin).

**Data collection**

Five soya-bean plants along the two middle rows in each plot were randomly selected and tagged with small card by thread, from where all measurements were taken throughout the growing period. There were eight morphological parameters recorded, namely; sterm determination, pubescence presence, pubescence density, pubescence colour, pubescence type, leaflets size and leaflet shape. International Plant Genetic Resources Unit (1981) soya-bean descriptor was used to collect the data.

**Data analysis**

Data collected were subjected to analysis of variance using GENSTAT Version 20 to establish the differences among soya-bean cultivars, after which least significant difference was employed.

Cluster analysis was also performed to estimate genetic distance and principal component analysis (PCA) was generated to determine characters with high discriminatory powers among the cultivars.

## RESULTS

### Analysis of variance

Analysis of variance depicted in table 1 revealed highly significant ($P<0.01$) among the soya-bean cultivars for pubescence density and pubescence type, and only significant ($P<0.05$) for pubescence colour and leaf size. No significant difference was found among cultivars for leaflet size and presence of pubescence.

The presence of pubescence and number of leaflets do not vary in these cultivars, meaning that their scores were uniform for all of these cultivars and they showed no variation hence they were insignificant. These cultivars were all pubescent with three leaflets per petiole. Stem determination varies among cultivars, in such a way that there are indeterminate plants, semi-determinate plants and few determinate plants, thus stem determination is significant ($P<0.05$) in characterizing variation of the cultivars. Pubescence varies also in terms of type, density and colour among these cultivars and the plants show differences in the pubescence characteristics. Pubescence parameters are highly significant in variation among the cultivars, hence pubescence colour is significant ($P<0.05$) and pubescence density as well as pubescent type being highly significant ($P<0.001$) parameters in exhibiting the variance.

The leaflets also vary in terms of shape and size but most significantly in terms of leaflet size whereby the cultivars had varying sizes of the leaflets that are mostly medium sized with areas from $71cm^2$ to $149cm^2$. Besides, some are small in size with an area below $71cm^2$. There are no large sized leaflets in all the 28 cultivars but the small and medium sizes varied significantly, the leaf size was significant ($P<0.05$). The leaflets of these cultivars were almost uniform in shape with most leaflets being broad and ovate in shape, a few of these cultivars showing an intermediate shape of leaflets in more than one replication and some cultivars showing a narrow lanceolate shape in utmost one replication for these reasons the leaflet shape is not significant.

Table 1. Analysis of variance for morphological markers

| Source of variation | df | Mean squares for soya-bean parameters | | | | | |
|---|---|---|---|---|---|---|---|
| | | Leaf shape | Leaf size | Pubescence colour | Pubescence Density | Pubescence type | Stem determination |
| Replication | 2 | 0.333 | 0.9048 | 0.0833 | 4.321 | 0.333 | 0.334 |
| Cultivars | 27 | 1.665 | 0.8325* | 0.7809* | 9.815** | 3.430** | 2.702 |
| Error | 54 | 1.617 | 0.5097 | 0.4290 | 2.210 | 0.4568 | 2.358 |
| Total | 83 | | | | | | |
| Cv (%) | | 1.7 | 3.9 | 3.4 | 6.0 | 4.1 | 1.9 |
| Means | | 6.52 | 4.619 | 1.583 | 6.50 | 2.690 | 5.81 |
| LSD | | 2.082 | 1.1687 | 1.0722 | 2,434 | 1.1064 | 2.514 |

** Highly significant (P<0.01); * Significant (P<0.05)

**Cluster analysis**

The dendrogram generated from data collected revealed two major groups of cultivars with one outlier, P64T39R (Fig. 1). One major group was designated A and the other B. Group A consisted of LDC 5.3, NA 5509R and P71T74R. This group was further divided into sub-groups A(i) and A(ii); with sub-group A(i) comprising of LDC 5.3 and NA 5509R, while sub-group A(ii) had P71T74R only. Group B consisted of 24 cultivars, with two sub-groups named; B(i) and B(ii). Sub-group B (i) consisted of LS 6851 R, DM5901R, LS 6164, NS 6448, P61T38R, SSS6560, SSS5052, DM 6968, LS 6860, LS 6161, DM 5351, SSS5449, LDC5.9, PAN 1663 and P48T48R. This sub-group furthermore divided into three sub-sub-groups, named B(i)a, B(i)b and B(i)c. Sub-sub-group B(i)a entailed LS 6851 R, DM5901R, LS 6164, NS 6448, P61T38R, SSS6560, SSS5052, DM 6968, LS 6860 and LS 6161. Sub-sub-group B(i)b comprised DM 5351 and SSS5449, while sub-sub-group B(i)c consisted of LDC5.9, PAN 1663 and P48T48R. On one hand, Sub-group B(ii) was comprised of DM 5953R, PAN 1555R, LS 6868, PAN1575, NS 5909, PAN 1521, PAN 1644R, DM6.81R and DM 5302. This group furthermore divided into two sub-sub-groups and an outlier. The sub-sub-groups were named B(ii)a and B(i)b. Sub-sub-group B(ii)a consisted DM 5953R, PAN 1555R and LS 6868 while sub-sub-group B(ii)b comprised PAN1575, NS 5909, PAN 1521, PAN 1644R and DM6.81R. DM 5302R was an outlier. These sub-sub-groups were further divided into other smallest groups.

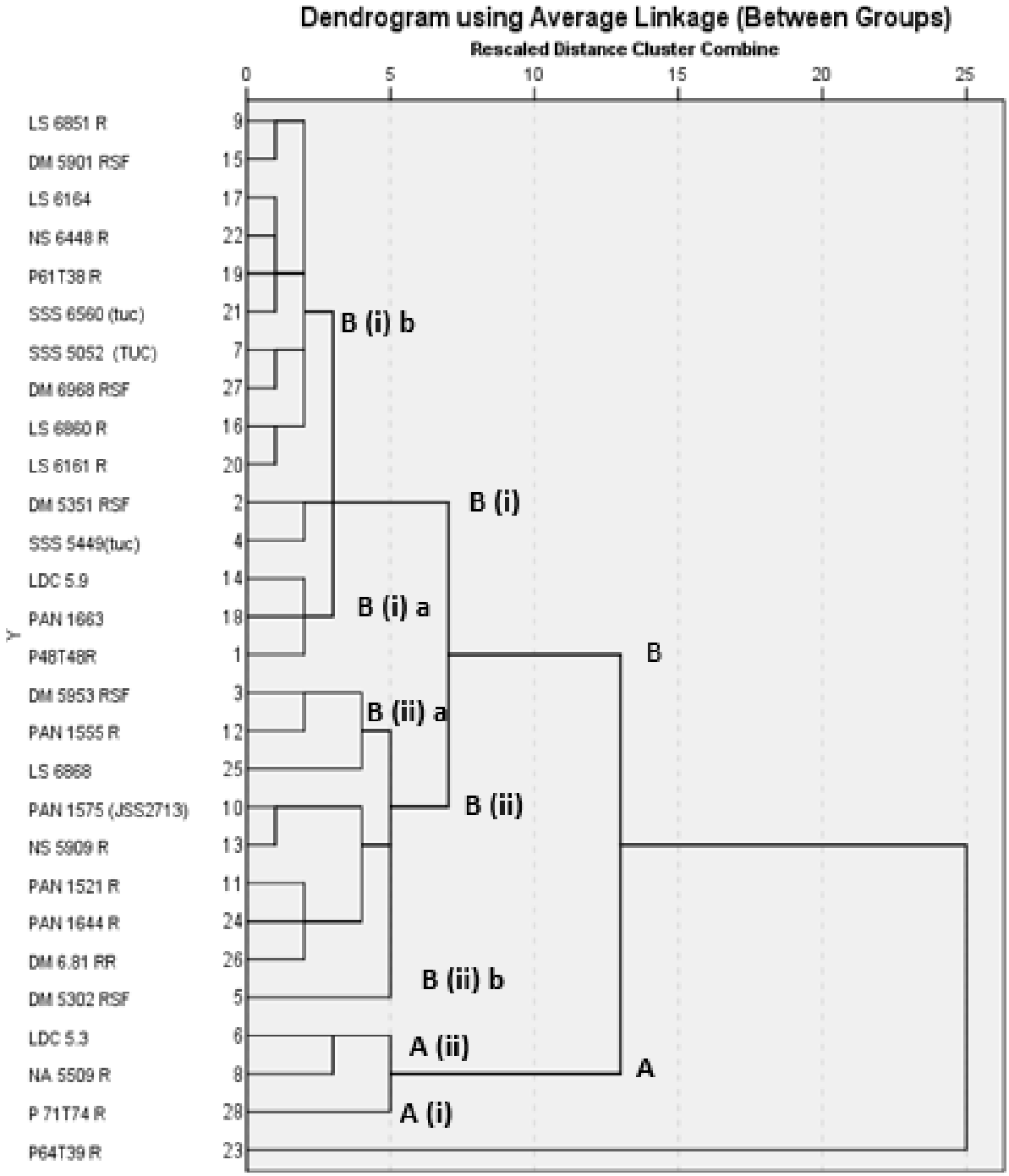


FIGURE1. Cluster analysis showing genetic distances

**Principal component analysis**

Principal component analysis (PCA) was applied to identify the characters which caused major variation among cultivars (Table 2). Out of six (6) principal components generated, only the first

two components constituting 62.885 % of total variation were considered in the analysis. The first and second components accounted for 34.476% and 26.409%, respectively. The characters with their respective eigen-value (in brackets) responsible for separation along the first principal component analysis were pubescence density (0.846), leaflets shape (0.839), stem determination (0.553). leaflets size (0.429), pubescence colour (-0.313) and pubescence type (-0.246). The second principal component analysis were influenced by the following characters with their respective eigen-value; pubescence type (-0.783), pubescence colour (0.725), leaflets size (0.521), stem determination (-0.505), pubescence density (0.182) and leaflets shape (0.076) shown in Table 3.

Table 2. Principal components showing variation contributed by characters.

| Principal Component | Variation | | |
|---|---|---|---|
| | Total | % of Variance | Cumulative % |
| 1 | 2.069 | 34.476 | 34.476 |
| 2 | 1.705 | 28.409 | 62.885 |
| 3 | 0.894 | 14.902 | 77.787 |
| 4 | 0.614 | 10.228 | 88.016 |
| 5 | 0.454 | 7.569 | 95.585 |
| 6 | 0.265 | 4.415 | 100.000 |

Table 3: Principal component matrix

| Characters | Component | |
|---|---|---|
| | 1 | 2 |
| Stem Determination | 0.553 | -0.505 |
| Pubescence Density | 0.846 | 0.182 |
| Pubescence Colour | -0.313 | 0.725 |
| Pubescence Type | -0.246 | -0.783 |
| Leaflets Size | 0.429 | 0.521 |
| Leaflets Shape | 0.839 | -0.076 |

**Genotypic and genotypic variance**

The genotypic and phenotypic variance, their coefficient of variation, heritability in the broad sense and genetic advance are presented in Table 4 below. The highest genotypic variance was obtained in pubescence density (2.535), followed by pubescence type (0.991), while the lowest genotypic variance was observed in leaflets shape (0.016), followed by leaf size 0,1076 and pubescence colour (0.1173). Pubescence density and stem determination revealed high phenotypic variance of 4.745 and 2.478, respectively. Leaf size and pubescence colour expressed lowest phenotypic variances of 0.617 and 0.5461, respectively.

Phenotypic coefficient of variance of all traits studied were higher than those of genotypic coefficient of variance with major differences revealed in pubescence colour (46.678% and

21.636%), stem determination (27,0941% and 5.980%) and leaflets shape (19.599% and 1.945%). The least difference between genotypic and phenotypic variance was exhibited by pubescence type (44.733% and 37.007%).

High heritability in the broad sense was expressed in pubescence type (68.439%), followed by pubescence density (53.425%). Low heritability in the broad sense was experienced in leaflets shape (0.979) and stem determination (4.871%). Highest genetic advance was shown by leaf size (61.09%), followed by pubescence type (52.1741%), then leaflets shape (39.53%) and pubescence density (36.9818%), while, the lowest genetic advance was experienced with pubescence colour (20.6545%).

Table 4. Estimation of genetic parameters of seven morphological markers; Genotypic coefficient of variation (GCV), Phenotypic coefficient of variation (PCV), Genetic advance (GA).

| Characters | Genotypic variation | Phenotypic variation | Grand mean | Heritability | GCV% | PCV% | GA |
|---|---|---|---|---|---|---|---|
| Shape leaflets | 0.016 | 1.633 | 6.52 | 0.979 | 1.940 | 19.599 | 39.53 |
| Leaf size | 0.1076 | 0.617 | 4.619 | 17.439 | 7.102 | 17.006 | 61.093 |
| Pubescence colour | 0.1173 | 0.5461 | 1.583 | 21.480 | 21.636 | 46.678 | 20.655 |
| Pubescence density | 2.535 | 4.745 | 6.50 | 53.425 | 24.501 | 33.512 | 36.882 |
| Pubescence type | 0.991 | 1.448 | 2.690 | 68.439 | 37.007 | 44.733 | 52.174 |
| Determinate stem | 0.1207 | 2.478 | 5.81 | 4.871 | 5.980 | 27.0941 | 27.187 |

## DISCUSSION

### Cluster analysis

Cluster analysis generated from six morphological markers eventually formed 6 sub-sub-groups at a very low level as evidenced in Figure 1 above, indicating that 28 soya-bean cultivars exhibited large divergence. On the other hand, the sub-groups showed differences in soya-bean cultivars within each sub-group, thus there were high degrees of similarities among cultivars and low degree of dissimilarities in morphological markers. Conversely, among the groups, the degree of dissimilarities was high while degree of similarities was low. The cluster analysis implied that those falling in one sub-group share most genes. It may be that their progenitors are the same. The different groupings of cultivars revealed different combinations of genes implying that they originated from different progenitors. The results were consistent with other researchers whose findings also revealed major groups, sub-groups and sub-sub-groups of soya-bean cultivars studied in Bangladesh (Malek *et al*., 2014) and Brazil (Ojo *et al*., 2012); with the degrees of similarities being high within the sub-groups and low among the sub-sub group.

**Principal component analysis**

Six morphological characters employed to distinguish soya-bean cultivars were sufficient to differentiate twenty-eight soya-bean cultivars. No one character was able to distinguish cultivars but a combination of three and more differentiated many cultivars, for example pubescence density, leaflets shape and stem determination as in figure 4. Some characters had infinitesimal influence in discriminating soya-bean cultivars but in combination with others, they made an impact that created differentiation. The afore-mentioned characters with high discriminatory power could be applied where cultivars are closely related whereas those with low discriminatory power could be used with distantly related cultivars. The results are resonating with other researchers. Shaahu *et al*. (2013) conducted a study to determine discriminatory power of pod height, number of pods per plant, number of branches, colour of flowers, number of kernels per pod and number of pods per plant. Their findings revealed pod height, colour of flowers and number of pods per plant to have high separation power. Similarly, Dubey *et al*. (2018) studied 16 morphological markers for distinguishing power and found variation in their influence with some expressing high discriminatory power while others revealed mediocracy and infinitesimal separation of soya-bean cultivars. Similarly, the afore-mentioned studies demonstrated the inability of one to three characters to adequately differentiate cultivars. Moreover, they emphasized that as the number of characters increased, the more the differences among the soya-bean cultivars were observed.

**Genotypic and phenotypic variance**

There was a large difference between phenotypic coefficient of variation and genotypic coefficient of variation for all the traits in all the cultivars, indicating high influence of environmental factors on the expression of the traits and low probability of genetic gain. Traits influenced by environment cannot be transferred from generation to generation, unlike those that are influenced by additive gene action. The influence of environment on traits is of no significant in breeding programs and are normally neglected. However, genotypic coefficient of variation is of paramount importance in the breeding programs because it gives a wider divergence from which a breeder can make a selection to improve the traits of interest. The wider genetic coefficient of variation observed in this study implied more genes available for soya-bean improvement. A wide genotypic and phenotypic variance was also evident in a study conducted by Hamawaki *et al*. (2012) on genotypic and phenotypic variance of soya-bean genotypes, which led to a wider choice for use in breeding programs.

**Heritability**

Heritability in the broad sense was high for all traits except leaflets shape and stem determination. This implied that early generation testing can be performed resulting in faster improvement of traits achieved within a short time with few generations. High heritability was also revealed in pubescence type and pubescence density. Leaf size and pubescence colour had moderate heritability meaning that improvement would not be as fast as the previous two traits and more time would be required to achieve targeted value. Again, the number of generations required to improve these traits would be more. Besides, heritability dictates the method of breeding that the plant breeder has to adopt (Painkra *et al*., 2018). Similarly, Charanj *et al*. (2018) and Faria *et al*.

(2016) in their studies found a moderate to high heritability coefficient implying that improvement in the traits of interest will be faster with less number of generations.

**Genetic advance**

Genetic advance was found to be high in most of the traits studied; ranging from 36.8818% to 61.093% except for pubescence colour and stem determination which obtained moderately low genetic advance value of 20.6545 and 27.094%, respectively. Genetic advance shows the rate at which the trait can be improved. The ones with high genetic advance improve faster while the ones with low genetic advance takes a long period of time to reach a desired level of the trait. The genetic advance of 36.881% to 61.093% illustrated progress which would be obtained within one generation of selection for these traits. The results further indicated the effectiveness of the selection made on the traits. Sulistyo and Mejaya, (2018) and Malek and Farri, (2014) conducted similar studies and obtained results consistent with the current study. There were traits with very high genetic advance and heritability of 52% to 73% and 35% to 59%, respectively in their studies.

**Implication of the study**

The study exhibited a great variability among the soya-bean cultivars that can be exploited for further improvements of desired traits in plant breeding programme. Besides, the farmers have a wide choice from which they can choose from depending on their interests. Similarly, consumers' preferences can also be met by certain cultivars for quality, nutritional value and organoleptic tests. A pool of agronomic traits can be drawn from cultivars which can be fully utilized for the benefits of the farmers. Furthermore, the study has added a lot of information to the body of knowledge by showing genetic distances among the soya-bean cultivars grown in Lesotho which were unknown before it was conducted. The most distinguishing characters which caused variations were identified and ranked accordingly. Both phenotypic and genotypic variability were determined among the cultivars.

## CONCLUSION

Based on the results of cluster analysis performed, several groups were formed showing similarities and dissimilarities among the soya-bean cultivars. This was a normal pattern followed by most crops with genetic variability. There was a large difference between phenotypic coefficient of variation and genotypic coefficient of variation for all the traits indicating high influence of environmental factors on the expression of the traits and low probability of genetic gain. Heritability in the broad sense was high for all traits except leaflets shape and stem determination. It ranged from 36.8818% to 61.093% except for pubescence colour and stem determination which obtained moderately low genetic advance value of 20.6545 and 27.094%, respectively. Genetic advance shows the rate at which the trait can be improved.

**Acknowledgements**

The authors would like to acknowledge Research and conference committee of the National University of Lesotho for research funding.

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