Genetic Diversity of Potato (*Solanum tuberosum* L.) Cultivars Grown in Lesotho as Determined by Morphological Markers

Motlatsi Eric Morojele[1*]; Moteka Molefali[2]; Motanyane Stanislaus Motake[3]

Department of Crop Science, National University of Lesotho, P. O. Roma, 180 Lesotho
*Corresponding Author

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Abstract— *Irish potato is the only tuber crop grown by farmers in Lesotho, particularly in the foothills where environment is conducive for its growth and high yield. Potato seeds are imported from South Africa without verifying for authenticity, fraudulence and intellectual property rights. The objectives of the study were to: (i) estimate genetic distance among potato cultivars using morphological markers, (ii) determine discriminatory power of morphological markers in distinguishing potato cultivars. The study was conducted at National University of Lesotho experimental farm, Roma. Experiment was laid out using Randomized Complete Block Design with eight treatments and three replications. Treatments were cultivars; Mondial, Panamera, Taurus, Tyger, Tyson, Valor, Avalanche and Innovator. Data on 21 markers were collected using potato descriptor, thereafter analyzed using Genstat (Version 17) to perform cluster analysis and principal component analysis. Results of cluster analysis revealed variability among potato cultivars. Cultivars constituted 1 major group, which in turn divided into two sub-group. Two sub-groups further sub-divided three times forming sub-subgroups and outliers. First six principal components contributed 97% of variation among cultivars. Characters with high discriminatory power were marketable tubers, root fresh weight, tuber fresh weight, number of main stems, leaf dry weight, leaf fresh weight and total yield. In conclusion, cluster analysis has group cultivars according to their similarities and principal component analysis revealed characters with high discriminatory power.*

Keywords— Cluster Analysis; Genetic Diversity; Principal Component Analysis; Solanum Tuberosum L.

I. INTRODUCTION

Irish Potato (*Solanum tuberosum* L.) is originated from Peru, Bolivia and Andes in the Western South America [1]. Peru is considered as the center of origin, diversity and its wild relatives [2,3]. They were domesticated in the Andes and Southern Peru approximately 10 000 years ago [2,4]. Potatoes were introduced by Spaniards from Peru to Spain during trading, after which it was introduced in Europe where it became an important food crop in Ireland. Southern and Eastern Asia also showed the most rapid expansion over the past century [5]. Since then, its world-wide distribution increased tremendously making it the fourth largest starchy food crop following wheat, rice and maize. China is now the largest potato producing country in the world with the total annual yield of 60 million tons on 4 million ha and productivity of 15 tons ha^{-1} [6]. Approximately 85% of this potato is produced in the Northern part of China because of low temperature and suitability for growing potato crop [7].

Irish Potato crop is an annual, herbaceous dicotyledonous commonly propagated vegetatively, although it can also be propagated through seeds known as true potato seeds. Nonetheless, it can be grown as a perennial in selected environments [8]. Morphologically, potato can be characterized by erect stem which grows up to a height of one meter with alternate compound leaves of three to five pairs of leaflets arranged in a downward position [9]. Potato can be distinguished by its ability to develop tubers under short days and cool nights, and knowledge of genetic diversity, identification and characterization of cultivars provides an informative tool for the detection of duplicates in the collection, effective extension, better characterization and use in breeding programs [10]. In addition, characterization of potato genetic diversity is also important for tracing fraud, duplication, violation of cultivar protection, intellectual property right and ascertaining proper use of trademark [11,12].

In the past, morphological characterization was the most powerful tool in description, classification and evaluation of genetic resources. With time, morphological was superseded by biochemical and DNA-based molecular markers [10]. Nonetheless, morphological markers are still a powerful method where genetic distance of cultivars are far apart, and has a limitation where cultivars are closely related and share parentage

In the beginning of the past century, characterization was accomplished in potatoes using morphological markers, even though these markers were complex and greatly influenced by the environment [13,14]. In morphological characterization, descriptor for potato is available and compiled by International Plant Genetic Resource Unit of Food and Agriculture Organization (1982) to guide throughout the process. Descriptor is based on morphological traits such as leaf, stem, flower and tuber characteristics. Data regarding morphological characterization are generated throughout the growing season as potato cultivars are growing under field conditions [15], after which appropriate statistical tool is applied for analysis and virtualization leading to better comprehension.

All potato cultivars grown by farmers in Lesotho are brought from South Africa, which in turn obtain them from overseas or breed them within the country. They are imported into the country of Lesotho without following proper protocols of evaluating for distinctness, registration and adaptability. No method of characterization of potato has been established to date. As a result, some cultivars are mistaken for others, fraudulence is committed by people who multiply the seeds and sell them without a license from the owner, retailers use a different name for the same cultivar to disguise as if they are the ones breeding them and lastly, some are not easy to distinguish by visual appraisal. At the end of the day, such closely related cultivars are mixed when planting, thereby losing their distinctness and other economically important traits. This study is therefore undertaken with the following objectives; (i) to estimate genetic distance among eight potato cultivars using morphological markers, (ii) to determine the discriminatory power of the different morphological markers in distinguishing potato cultivars.

II. MATERIALS AND METHODS

2.1 Study area:

The study was conducted at National University of Lesotho experimental farm which is domiciled in Roma valley, about 34 km Southeast of Maseru, capital city of Lesotho. Lesotho is situated in Southern African region. The coordinates are 29^{o}26’’48’S latitude and 27^{o}42’’12’91E longitude. The altitude is 1,610 m above sea level.

Soils within the Roma valley are predominantly Berea and Tsiki series [16]. Berea series consists of fine-loamy, mixed, mesic family of Plinthaquic Dystrochrepts, with gradient of ≤ 2% slope. Tsiki comprises soil texture ranges from sandy to loamy, with sand content ranging from 50.8% to 67.7%, silt from 13.3% to 35.9% and clay from 13.3% to 20 %. Soil pH ranges from 5.02 to 5.22 (slightly acidic), organic matter ranges from 2.5 to 4.5 % [17].

Climate is temperate with average annual precipitation of 850mm, of which approximately 85% of it occurs from October reaching a peak in February, after which it declines rapidly to April [18]. Winter season is dry and cold with extreme low temperature of -100^{o}C. Summer season is hot and humid, with highest temperature of 35.5^{o}C in the lowlands and 24^{o}C in the highlands [19]. High winds of up to 20 meters per second sometimes occur during summer season. Thunderstorms, frost, snow and hailstorms are experienced in Roma area [20].

2.2 Experimental Design:

Eight potato cultivars used in this study were obtained from Wesgrow Potatoes (Pty) Ltd located at Christiana in South Africa. It is a reputable company known for production of high-quality potato seeds that are disease free. The potato cultivars used were Mondial, Panamera, Taurus, Tiger, Tyson, Valor, Avalanche and Innovator.

The experiment was laid out using Randomized Complete Block Design (RCBD) with eight treatments (cultivars) and three replications. Dimensions of the main plot were 51.5m length x 10.8m width giving a total area of 556.2m^2. The main plot was divided into 24 sub-plots spaced 0.5m apart and each measuring 6m × 3.6 m consisting of 5 rows that were 0.90m apart. Potato seeds were planted 0.70m between planting stations giving a population of 30 plants per plot and 240 plants for the whole experiment.

2.3 Agronomic practices:

The field was cultivated using tractor mounted plough digging in the soil to the depth of 25-30cm, after which the plots were leveled using disc harrow. Treatments were applied on the sub-plots according to trial plan. Medium sized and well sprouted tubers were planted on the sides of ridges which were dug using the spade. The planting depth was maintained at 0.25m. Wonder 2:3:2 [14] granular basal fertilizer was applied at the rate of 20kg per 556.2m^2. Fertilizer was placed inside the furrow, while the potato seed was placed on the side of the furrow to avoid direct contact between fertilizer and seeds. Later, both fertilizer and seed were covered with soil. Planting was performed by hand. Weeding was done four times due to high level of weed infestation. Cyperthrin 200 insecticide was applied against blister beetle (*Milabris oculata*) insect using the knap sack sprayer. Irrigation was performed using hose-pipe once in a week.

2.4 Data collection and analysis:

Data were collected using descriptor of potato (*Solamun tuberosum* L.) compiled by International Board of Genetic Resources Unit (1982) and revised by Kawochar and Mohammed (2015) [21]. Three plants in a plot were tagged where all the measurements were taken every time recording was performed. The characters of potato plants measured to distinguish cultivars based on plant parts were:

- Stem: number of main stems, stem dry weight, stem fresh weight, stem diameter, root fresh weight;
- Leaf: leaf dry weight, leaf fresh weight, number of leaves, leaf width, leaf length, leaflet length, leaflet width;
- Tuber: marketable tubers, tuber fresh weight, total yield, tuber fresh weight, large size tubers, chlorophyll content, unmarketable tubers, medium size tubers;
- Root: root dry weight.

Data collected were captured and entered into Microsoft Excel, after which data were analyzed using GENSTAT (version 17) software to generate cluster analysis and principal component analysis.

III. RESULTS AND DISCUSSION

3.1 Cluster analysis:

Data generated from twenty-one traits were used to draw a cluster analysis (Dendrogram) (Fig. 1) below, which established genetic distance among eight potato cultivars. The cultivars used in this study were Panamera, Innovator, Valo, Tyson, Mondial, Taurus, Tyger and Avalanche.

Cluster analysis (Fig.2) consisted of one main group which was divided into two sub-groups, namely; 1 and 2. Sub-group 1 comprised sub-sub-group A which further sub-divided into A (i) which still further sub-divided into sub-sub-group A (iii) containing Innovator, A (iv) Avalanche and (v) Tyson, A(ii) contained Tyger. Sub-group B was an outlier. Sub-group 2 sub-divided into sub-sub group C and D. D became an outlier, whereas C further still divided into i and ii being Panamera and Taurus.

At a higher level of hierarchy, cluster analysis (Fig.1) showed a closer relationship among the cultivars, but as more and more traits were applied the cultivars separated into distinct groups, individuals and positioned as outliers. This implied that their genetic make-up differed greatly from each other. The outliers exhibited that they had many traits that separated them from others and make them stand alone. Conversely, those cultivars which clustered together at a very low level of hierarchy expressed high degree of similarity, thus most of the genes that they had were similar. The results of this study resonated with findings of some researchers who generated dendrogram using morphological traits of 42 potato germplasm collected from Ethiopia [23]. It grouped 42 potato cultivar into four main clusters, and reported high degree of similarity between two cultivars among Ethiopian nationally released cultivars. Similarly, a study was conducted on 30 potato cultivars, which were formed into six clusters [24]. The inter cluster distances were higher than the average intra cluster distances, which indicated wide genetic diversity among cultivars of different groups than those of the same cluster. Again, another study conducted it was revealed that 20 potato cultivars studied constructed clusters and had higher mean values for desirable traits [25]. It was further observed that the inter-cluster genetic distance was greater that the intra cluster for all clusters indicating that considerable amount of genetic diversity existed between cultivars of different groups.

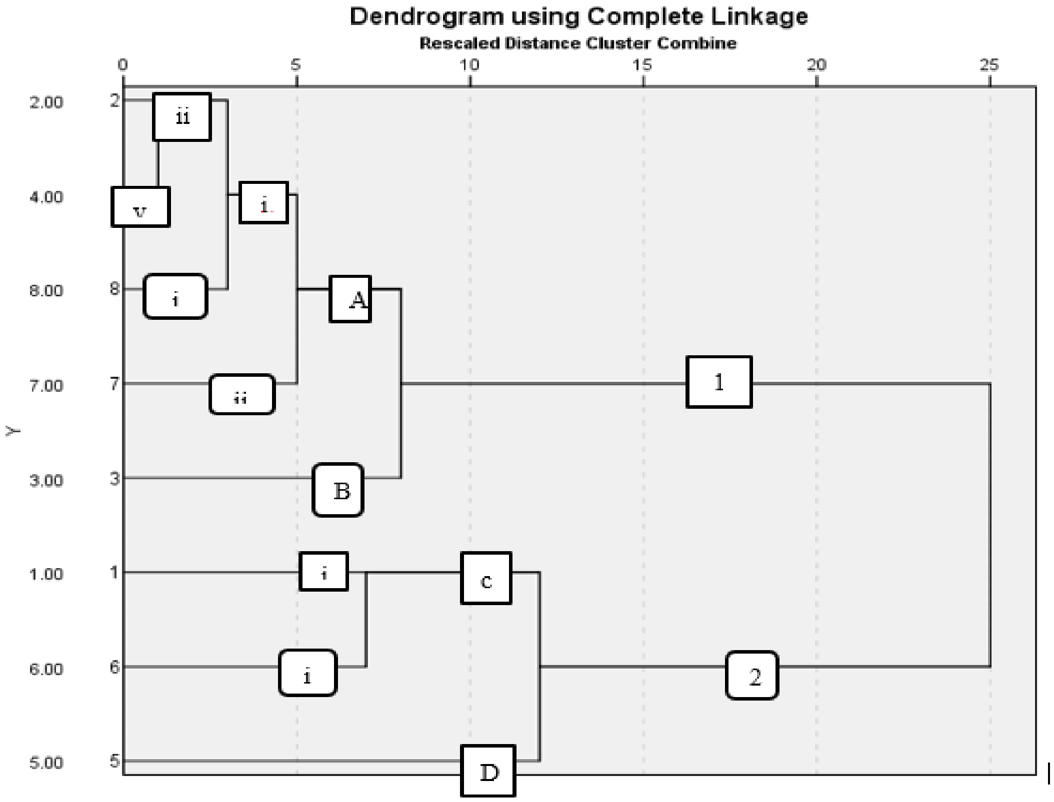

FIGURE 1: Dendrogram displaying genetic distance among potato cultivars; Panamera (1.00), Innovator (2.00), Valor (3.00), Tyson (4.00), Mondial (5.00), Taurus (6.00), Tyger (7.00), and Avalanche (8.00).

3.2 Principal Component Analysis:

Table 1 below depicts the contribution of each principal component towards the genetic variability in eight potato cultivars. Six principal components accounted for 97.490% of the total variation in distinguishing the potato cultivars. Principal Component 1, 2, 3, 4, 5, and 6 contributed 42%, 17%, 14%, 10%, 6% and 5%, respectively, implying that they were adequate to distinguish the eight potato cultivars in this study. Principal component analysis subjected 21 characters to distinguish potato cultivars and apply their discriminatory power among eight potato cultivars, after which they were ranked accordingly. Principal component 1 consisted of marketable tubers, root fresh weight, tuber fresh weight, number of main stems, leaf dry weight, leaf fresh weight, total yield, stem dry weight, stem fresh weight, large size tubers and leaf length. Principal component 2 comprised leaf width and stem diameter, while PC 3 consisted of unmarketable tubers, medium size tubers and leaflet length. Principal component 4 and PC 5 consisted of leaflet width and root dry weight, respectively as depicted on Table 2 below.

These traits had a high discriminating power that enabled the potato cultivars to be distinguished. Nonetheless, no single or two traits were able to distinguish any potato cultivars necessitating more than three characters to be applied in order to differentiate some but not all, hence the number of traits had to be increased in order to separate more cultivars. This is consistent with a study that assessed the total variation among 24 potato cultivar for 23 quantitative and six qualitative traits [25]. The first eight principal components accounted for 90.26% of the total genetic variation. The first eight components were retained in analysis because Eigen values were greater than 1. The other factors having Eigen values greater than 1 were ignored due to Gutten's lower bound principle. Furthermore, in the evaluation of diversity among potato cultivars using agro-morphological and yield components, it was observed that there was 80.1% of the total variation among traits [26]. The first PC comprised tuber yield, tuber weight, dry matter content and harvest index. They suggested that the principal component was very important for differentiating highly related clones and parents for breeding. Similarly, a study of principal component analysis of twelve potato cultivars was conducted, and only the two component axes had eigenvalues up to 1.0% representing cumulative variance of 84.1% and therefore suggested that the important traits considered effective in the investigation with respect to agronomic traits were yield per plant, number of tubers per plant, tuber weight per plant, plant height, plant emergence and leaves per plant [27].

TABLE 1
CONTRIBUTION OF EACH PRINCIPAL COMPONENT TO THE VARIATION IN THE POTATO CULTIVARS

Principal component	Initial Eigenvalues	Total % of variance	Cumulative %
1	9.448	42.943	42.943
2	3.752	17.056	59.999
3	3.13	14.228	74.227
4	2.315	10.525	84.752
5	6.828	6.828	91.579
6	1.3	5.91	97.49
7	0.552	2.51	100

TABLE 2
COMPONENT MATRIX

Parameters	Component				
	1	2	3	4	5
1. Marketable tubers	0.948				
2. Root fresh weight	0.927				
3. Tuber fresh weight	0.918				
4. Number of main stems		0.894			
5. Leaf dry weight	0.884				
6. Leaf fresh weight	0.88				
7. Total yield	0.87				
8. Stem dry weight	0.832				
9.Tuber fresh weight	0.795				
10. Stem fresh weight	0.767				
11. Large size tubers	0.712				
12. Leaf length	0.658				
13. Number of leaves	0.611				
14. Chlorophyll content			-0.872		
15. Leaf width		0.658			
16. Stem diameter		0.635			
17. Unmarketable tubers				-0.801	
18. Medium size tubers			0.716		
19 Leaflet length			0.68		
20. Leaflet width				0.736	
21. Root dry weight					0.675

3.3 Loadings (Rotated factors):

Table 3 below revealed six rotated factors. Regarding rotated factors, leaf fresh weight (0.942), total yield (0.935), tubers dry weight (0.924), total yield (0.935), tubers dry weight (0.924), tubers fresh weight (0.915), marketable tubers (0.901), large size (0.761), number of main stems (0.735), root fresh weight (0.711), leaf dry weight (0.697), number of leaves (0.655) all had high positive loadings on the first factor, and low loadings on the second, third, fourth and sixth. Stem fresh weight (0.905), leaf length (0.891), stem dry weight (0.834) and tuber medium size (0.756) had high positive loadings on the second factor and low positive loading on the first and fifth. The third and fourth had both negative and positive loadings except for the sixth which only had negative loading. Leaf width (0.903) and stem diameter (0.696) had high positive loading the third factor had low positive loading. The unmarketable tuber (0.885) and tuber small size (0.762) had high positive loading but leaflet length had negative (-0.835) loading on the fourth factor and positive loading on the first, second and fifth. Root dry weight (0.968) had high positive loading on the first, second and fifth factor but negative on third, fourth and sixth. Leaflet width (– 0.793) had high negative loading on the sixth factor and positive loading on the first, second and fifth except third and fourth where it had both negative and positive loadings.

TABLE 3
ROTATED MATRIX

Parameters	Components					
	1	2	3	4	5	6
Leaf fresh weight	0.942					
Total yield	0.935					
Tuber dry weight		0.924				
Tuber fresh weight	0.915					
Marketable tubers	0.901					
Large size tubers		0.761				
Number of main stems	0.735					
Root fresh weight		0.711				
Leaf dry weight		0.697				
Number of leaves		0.655				
Stem fresh weight		0.905				
Leaf length		0.891				
Stem dry weight		0.834				
Medium size tubers		0.756				
Leaf width			0.903			
Chlorophyll content			-0.864			
Stem diameter			0.696			
Unmarketable tubers			0.885			
Leaflet length				-0.835		
Small size tubers				0.762		
Root dry weight						0.968
Leaflet width						-0.793

IV. CONCLUSIONS

The results revealed that there was a wide variation among eight potato cultivars which can be exploited in the breeding. The variation existed in vegetative, reproductive, seed and physiological maturity features. Cultivars can be tested under varying environmental conditions to screen for the most suitable for specific localities. Mondial showed to be the only cultivar with large tuber size, followed by Panamera and Taurus, respectively. Characters with high discriminatory power were marketable tubers, root fresh weight, tuber fresh weight, number of main stems, leaf dry weight, leaf fresh weight, total yield and stem dry weight.

DATA AVAILABILITY STATEMENT

Data will be shared upon request.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

NUL: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review and editing.

DECLARATION OF COMPETING INTEREST

The authors of this manuscript declare that there is no conflict of interest or competing interest.

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