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GENETIC DIVERSITY OF STINGING NETTLE (URTICA DIOICA) BY AGRO MORPHOLOGICAL MARKERS

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ABSTRACT: *14 quantitative traits were studies from 30 genotypes of Urtica dioica grown under shade-nert at National University of Lesotho in a Completely Randomized Block Design (RCBD) with three replicates. The total variation amongst and within the treatments was detected using Analysis of Variance (ANOVA), F-test 5% significance level. The pattern analysis was deployed to detect the relationship amongst the accessions. The ANOVA showed a highly significant difference (P< 0.01) among the accessions on all phenotypic traits measured as obtained from analysis of variance. The LSD comparison of the means for various traits ranked them in descending order. The Hierarchical Cluster Analysis (HCA) identified two main groups (group A and B) with significant genetic distance between both the accessions and morphological traits. The groups were further sub-grouped into i, ii, iii and iv to show similarities among the accessions. The Principal Component Analysis (PCA) showed that plant height, length of internodes, number of leaves and number of nodes are the most important traits in determining the variation amongst accessions.*

KEY WORDS: *U. dioica*, stinging nettle, genetic diversity, hierarchical cluster analysis, principal component analysis

INTRODUCTION

Urtica dioica (Stinging nettle), is a herbaceous flowering perennial plant that grows up to 2 meters in height [1]. It is found in cool areas with high soil moisture of America, Australia, Asia, Europe, and Africa such as South Africa, Morocco, Libya and Lesotho [2]. In Lesotho, the plant is widely distributed throughout the country. *U. dioica* is considered a weed in modern agriculture and grows easily as a wild plant [3]. It is characterized by heart shaped, toothed, serrated leaves growing opposite each other, covered with hollow stinging hairs referred to as trichomes which contain formic acids and histamine that causes redness, itching, irritation on the skin when in contact with [4]. The stems of *U. dioica* are green to purple, erect and covered with trichomes just like the leaves [1].

U.dioica grows in fertile soils with high organic matter content such as around the kraals, under the trees, and o the home garden plots. Kregiel et al (2018) reported that *U.dioica* prefers open or partly shady habitats with adequate moisture and are often found in forests, by rivers or streams and on roadsides. They propagate well in phosphorus-rich and nitrogen-rich soils that

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have recently been disturbed and generally originate in soils rich in inorganic nitrates and heavy metals hence the presence of *U.dioica* acts as an indicator of high soil fertility [5].

Production of *U.dioica* in agri-food setting has been studied in Austria [6], Finland, [7], German [8] and varying yields of fibre have been observed. *U.dioica* has food and fiber provision and medicinal benefits. It is recommended for treatment of different human diseases among others, muscle and joint pain, Eczema, Arthritis, Gout, Anemia, Hay fever, Urinary tract infections, Enlarged prostate and Tendonitis [9] and [5]. In Lesotho, *U.dioica* gained its popularity as a wild vegetable and also as medicinal herb. *U.dioica* leaves and roots have since been used as a medicinal remedy and edible fresh or dried parts of nettle plants have been extensively used in food preparation [10]. Despite its economic importance, the plant has not been considered for domestication in Lesotho. There is limited information on scientific attributes of this plant and its diversity within the country. Documentation of the germplasm has not been done.

Di Virgilio et al (2015) reported that *U.dioica* varies in terms of morphological characteristics. There are more than 30 species of *U.dioica* discovered in the entire world [12]. The variations that exist are a result of environmental heterogeneity factors like rainfall, temperature, altitude and the dosage of the Ultra Violet rays and various selection pressures [13]. Phenotypic traits of *U.dioica* such as, plant height, leaf length, leaf width and hairiness have been used and scored in studying the diversity in morphology of *U.dioica*s [14].

Phenotypic characterization involves the use of morphological markers, which are visually accessible traits and be recorded straight from the field such as colors, shapes, growth habits and pigmentation [2]. Phenotypic traits are utilized as conventional tools when analyzing diversity since they use simple, easy to score and affordable methods [15]. Morphological characterization is done for effective utilization of those accessions with desired phenotypic traits, thus, this kind of characterization of diversity among *U.dioica* accessions is an initial step towards its genetic improvement [16].

The main objective of the study was to determine the morphological diversity among *U.dioica* accessions in Lesotho and understand the genetic diversity that exist within the *U. dioica* species in Lesotho.

METHODOLOGY

The studied species

Urtica dioica is often known as the common nettle or stinging nettle, is a herbaceous perennial plant of a family *urticaceae*. It is cross pollinated plant and grows up to 2 meters high. This plant can spread vegetatively through rhizomes and stem cuttings forming dense colonies [17]. It also propagates well through seeds. It has the toothed leaves that are borne oppositely along the stems. These leaves are covered with numerous stinging and sometimes non-stinging trichomes. *U.dioica* can be either monoecious or dioecious depending on the subspecies. It bears tiny green or white flowers as clusters in the leaf axis and stem tips. This plant produce

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copious amount of seeds. *U.dioica* has a complex pattern across major part of species distribution range consisting of widespread tetraploids cytotype, scattered diploids and sporadically occurring triploids and pentaploids. There is no differences in genome size found so far, the genome size corresponds to 2n=26 at diploid level [18].

Study area

The study was conducted in selected areas of Lesotho (Figure 1), in which the study subjects were identified located using GPS. The 30 locations were selected where *U.dioica* could be observed and harvested for further analysis. The case study lies between 1580m and 2370 in altitude (Table 1). The 30 locations include various districts of Lesotho namely Leribe, Berea, Maseru, Mafeteng and Mohale's Hoek.

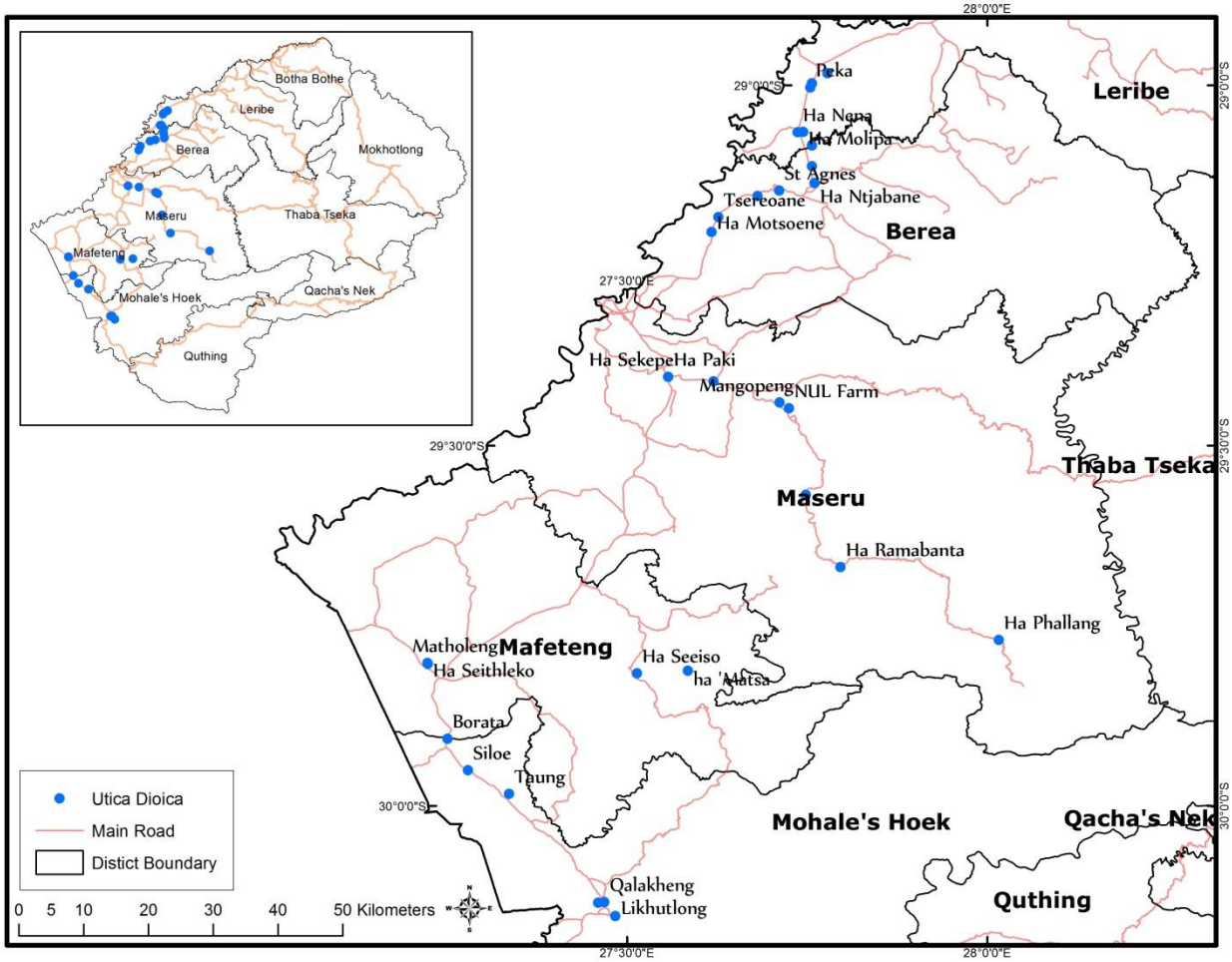

Figure 1: the map for collection areas for *U.dioica*

Plant material collection and regeneration

A total of 30 *U.dioica* accessions with seeds were collected from different regions in the country. The coordinates and elevations of collection of all each accession were recorded from the GPS (Table 1). The green *U.dioica* plants bearing ripened fruits were collected. The plants were collected at fruit maturity stage as identified by easily falling off of seed/fruits from the plant upon shacking. These *U.dioica* plants were taken to the Plant Science laboratory, Faculty

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of Agriculture, National University of Lesotho. The plants were dried at room temperature for 5 days. The seeds from different accessions were then harvested separately and packaged for planting and evaluation of phenotypic characteristics.

Seeds from different accessions were collected and were well labeled in the paper bags as separate accessions. The seeds were then kept in cool place for planting. The seeds for all 30 accessions were planted in a Complete Randomized Block Design under the shade net where each accession was replicated 3 times. Each accession was planted on the plot size of 2 rows of 2m length spaced 30 cm apart, the spacing of 15 cm between plants was maintained. Planting was under the shade net (80% of light penetration) using sterile vermiculite as growth media. After 3 weeks of germination, the plants were thinned to the normal spacing used for leafy vegetables [19]. A total of 90 plots were thus planted.

Table 1 The collection sites for U. dioica in Lesotho and their coordinates including altitudes

Accession name/site	Accession	Latitude	Longitude	Altitude
Ha Phallang	1	29'46'12S	28'0'58 E	2370
Ha Ramabanta	2	29'40'8 S	27'47'47 E	2352
Ha Moitsupeli	3	29'34'7 S	27'44'54 E	1950
Ha Motsoene	4	29'12'13 S	27'37'3 E	1540
Tsereoane	5	29'10'59 S	27'37'36 E	1580
Lekokoaneng	6	29'9'14 S	27'40'53 E	1700
St Agnes	7	29'8'45 S	27'42'40 E	1580
Ha Ramonaheng	8	29'5'27 S	27'45;25 E	1650
Ha Ntjabane	9	29'8'9 S	27'45'38 E	1620
Ha Mphele	10	29'6'44 S	27'45'24 E	1580
Ha Nena	11	29'3'55 S	27'44'13 E	1610
Ha Molipa	12	29'3'53 S	27'44'41 E	1580
Peka	13	29'0'10 S	27'45'17 E	1590
Ha Makhaketsa	14	28'59'49 S	27,45,24 E	1590
Tabola	15	28'58'0 S	27'46'43 E	1590
Matholeng	16	29'48'7 S	27'13'23 E	1680
Ha Seithleko	17	29'48'11 S	27'13'24 E	1670
Siloe	18	29'57'3 S	27'16'45 E	1660
Taung	19	29'59'1 S	27'20'10 E	1600
Likhutlong	20	30'9'11 S	27'29'2 E	1600
Lalane M hoek	21	30'8'5'S	27'27'35 E	1530
Qalakheng	22	30'8'2 S	27'28'6 E	1540
Borata	23	29'54'26 S	27'15'2 E	1710
ha 'Matsa	24	29'48'45 S	27'32'4 E	1920
Ha Seeiso	25	29'48'59 S	27'30'50 E	1860
Ha Sekepe	26	29'24'17 S	27'33'26 E	1610
Ha Makhalanyane	27	29'24'40 S	27'37'12 E	1620
Mangopeng	28	29'26'25 S	27'42'42 E	1620
NUL Farm	29	29'26'54 S	27'43'30 E	1650
Mazenod Ha Paki	30	29'24'17 S	27'33'26 E	1610

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Experimental design

A phenotypic evaluation was conducted on 30 accessions all at flowing stage. The influence of these genotypes on 14 phenotypic traits was determined using ANOVA and LSD. The method of data collection from [14] was implemented. Fourteen morphological characters were considered for characterization of the *U.dioica*. Twenty plants with the representative appearance per plot of the accession were used to quantify the phenotypic trait. Leaf length and width were measured from base to tip and at the widest point in centimeters, respectively. The ratio of leaf length to width was calculated and used to assess the elongate nature of leaves. Stinging hairs (trichomes) were counted under a magnifying glass directly as on $1cm^2$ sample area of leaf surface centered on the midrib in a more or less central location along the length of the lamina. Leaf area (LA) was determined using a simple mathematical method where $1cm^2$ grid where the number of square covering the leaf were counted. Plant height (PH) was measured using a ruler in centimeters. Number of leaves (NL) per plant were counted from all nodes. The three longest internodes were sampled per plant were measured in centimeters. The shape of the leaves was determined [14]. The plant fresh weight (PFW) was measured using a digital scale. Dry weights (DW) were determined for individual oven-dried plants using digital scale [20]. Entire plant fresh weight, the 20 selected plants from the plot were cleaned off to remove the excess dirt.The whole plants were weight on the digital scale (Biobase-BA2204B). For Dry weights, the whole plot plants were oven dried at 50^oC overnight and weight on the digital weigh scale (Biobase-BA2204B). The photosynthetic pigments i.e. chlorophyll 'a', chlorophyll 'b' and carotenoids in leaves were estimated as per the method of [21].

Data processing and analysis

The mean values for the observed parameters were subjected to Analysis of Variance. The significance difference between attributes from different accessions was determined with two way ANOVA using GENSTAT statistical package version 18.2. The means were separated by Least significance difference at $p< 0.01$ to establish the significance level [22]. All data was logarithmically transformed prior to analysis.In order to get the pictorial view of the existing variation amongst the genotypes, the hierarchical clustering analysis (HCA) was performed using Statistical Package for Social Sciences version 20 (SPSS 20) for grouping the accessions and attributes according to their similarities. The principal component analysis (PCA) was obtained from Clustvis online web for visualizing clustering of multivariate data. The PCA was used to construct the bi-plot used to visualize the distribution in patterns and identify the clustering of *U dioica* in relation to morphometric traits. Dendrogram was constructed using the the unweighted-pair group method using arithmetic averages (UPGMA) clustering of pair-wise similarity distances among the genotypes in SPSS 20. This established genetic relationships and distance amongst the accessions and the attributes.

RESULTS AND DISCUSSIONS

Variation in morphometric traits among the accessions.

Minimum, maximum, mean, standard deviation (SD), coefficient of variance (CV), F test and degree of significance of different quantitative traits are presented in Table 2. According to the Fisher (LSD) test, ANOVA showed a highly significant difference between (Table 3)

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accessions studied for all quantitative traits (P value = < 0.01) indicating that the traits studied can be useful for accessions identification. The coefficients of variation show values ranging from 8.18 to 22.8 for all studied traits. Although the genetic diversity in *U. dioica* in the ecosystems is not well studied, it is expected to observe variation in morphology amongst the accessions as influenced by differences in geomorphological parameters of temperature, altitude and as well as foliage harvesting intensity. In studies such as those related to wild medicinal plants like Jute Mallow [23] and *Sideritis scardia criseb* [24], variation in quantitative traits was recorded amongst the accessions. The imperative change in altitude often affects genotype adaptation thus influencing the overall morphology of the plant species [25]. In addition, grazing patterns have been recorded to affect the distribution of the biotypes of *U.dioica* [26].

Table 2 Analysis of Variance (ANOVA) (The anova table was obtained from GENSTAT statistical package version 18.2 (PC/Windows 7).

Variable	Df	MSS	F value	P value
Plant height	29	36.04	11.81	< 0.01
Fresh weight	29	08.81	01.76	0.03
Dry weight	29	00.01	12.73	< 0.01
No. leaves	29	27.25	08.22	< 0.01
Leaf length	29	01.25	04.91	< 0.01
Leaf breadth	29	00.48	01.88	< 0.01
Leaf area	29	03.25	08.52	< 0.01
Carotenoids	29	00.03	04.81	< 0.01
Chlorophyll a	29	21.76	05.30	< 0.01
Chlorophyll b	29	11.81	06.46	< 0.01
Stem diameter	29	00.04	00.85	0.69
No. trichomes	29	40.69	13.20	< 0.01
No. nodes	29	06.81	08.22	< 0.01
Length of 3 nodes	29	08.23	14.26	< 0.01

MSS = mean sum of square, DF= degrees of freedom

LSD ranking reveled differences in the phenotypic traits amongst the accessions (Table 3). Ha Nena and Ha Makhaketsa accessions had highest plant height, number of nodes, length of internodes, number of leaves and dry weight values. While Ha Paki and Mangopeng had lowest values of the traits. It is worth noting that the highest plant height recorded for this study is still shorter than the minimum recorded plant height of cultivated *Urtica dioica* that grows up to 30-150 cm tall [27].

Ha Ramonaheng and Ha Nena had highest values of fresh weight of 8.2g and 3.5g respectively. Ha Nena and Ha makhaketsa highest dry weights of 0.3g and 0.2g respectively. According to literature the leaf area of cultivated *Urtica dioica* is directly related to the fresh weight and or dry weight [28]. This does not include the reference to wild nettles since differences in indices between the wild nettle and cultivated nettle has not been studied.

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Ha Seeiso and Ha Nena had highest values of leave length of 4.2cm and 4.7cm separately while Ha Seithleko, Ha Molipa and Ha Paki had the lowest values of leave length of 2cm, 2cm and 1cm respectively. Ha Nena and Siloe had highest values of leave breadth and leaf area of 3.2cm and 2.9 and 5.7cm^2 and 5.3cm^2 separately, while Ha Sekepe and Ha Seithleko had the smallest value of leave breadth of 1.6cm and 1.5cm respectively and 1.7cm^2 and 1.3cm^2. These leaves retained the similar shape of pointed, heart-shaped to the reported leaves of cultivated nettle which had bigger leaf size of 2.54 - 15.24 cm long and 5.08 wide [29].

Lekokoaneng accession had stem diameters of average of 0.8cm, while Ha Seithleko and Ha Paki accessions had the thinnest stems of 0.1cm in diameter for both accessions. Qalakheng, Ha Molipa and NUL accessions farm had more counts of trichomes on the leaves, 26.3, 26 and 26 respectively, while Ha Paki and Ha Motsoene accessions had lowest number of trichomes of 11.3 and 10.7 respectively on their leaves. The presence of trichomes have been reported in Urtica dioica as biting villi, the acid containing leaf modifications [6].

The photosynthetic pigments namely; Carotenoids, Chlorophyll a and Chlorophyll b were highest with Tabola and Matholeng accessions and they were lowest with Peka and Ha Mphele accessions. The findings on Chlorophyll content contradict the previous research findings on photosynthetic pigments variation in *U. dioica* with altitude. Kim and Donohue, (2013) records an increase with chlorophyll content with an increase in altitude. The highest accession in chlorophyll content and the lowest accession in this study have the similar altitude of 1590m.

Table 3: Table of means for U. dioicas' phenotypic traits

Accession	PH	FW	DW	NL	LL	LB	LA	TC	TCa	TCb	SD	NT	NN	LI
Ha Phallang	11.9b	1.5b	0.1b	15.3b	3.6b	2.6a	3.7b	0.9b	22.0b	13.1b	0.1b	21.7b	7.7b	6.3c
Ramabanta	7.5d	1.1b	0.1b	12.7b	3.3b	2.5a	3.3b	1.0a	25.0a	14.8a	0.1b	21.0b	6.3c	4.8d
Ha Moitsupeli	11.7b	1.0b	0.1c	14.0b	2.8b	2.2a	2.3c	1.1a	25.6a	16.3a	0.1b	21.7b	7.0b	6.4c
Ha Motsoene	8.5c	1.2b	0.1b	14.7b	2.8b	2.3a	2.3c	0.9b	20.7b	13.0b	0.2b	10.7d	7.3b	3.3e
Tsereoane	10.5c	1.0b	0.1c	16.0b	3.2b	2.8a	3.3b	0.9b	22.6b	12.9b	0.2b	23.7a	8.0b	4.8d
Lekokoaneng	15.3b	0.7b	0.1b	18.7b	2.4c	1.7b	2.0c	1.0a	26.8a	15.8a	0.8a	18.3c	9.3a	5.7d
St Agnes	12.6b	0.7b	0.1b	16.0b	2.6b	2.0b	2.7c	1.0a	23.5b	13.9b	0.2b	18.3c	8.0b	6.6c
Ramonaheng	9.0c	8.2a	0.3a	13.3a	2.2c	1.8b	1.3d	1.0a	25.7a	15.8a	0.1b	19.0c	6.7c	4.5e
Ha Ntjabane	12.3b	0.9b	0.1c	15.3c	3.4b	2.4a	3.7b	1.0a	23.8b	14.8a	0.2b	20.7b	7.7b	7.2b
Ha Mphele	13.4b	0.6b	0.1c	18.0b	2.7b	2.0b	1.3d	0.7b	17.8c	10.5b	0.1b	24.0a	9.0a	5.6d
Ha Nena	21.0a	3.5a	0.3a	21.3a	4.7a	3.2a	5.7a	1.0a	24.8a	15.3a	0.3b	15.3c	10.7a	8.8a
Ha Molipa	5.6c	0.3c	0.1c	11.3c	2.0c	1.7b	2.3c	0.9a	23.4b	14.4b	0.1b	26.0a	5.7c	3.2f

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Peka	10.1c	0.7b	0.1c	13.3c	3.0b	2.4a	3.0b	0.6c	15.5c	8.9c	0.1b
17.3c	6.7c	5.0d									
Makhaketsa	19.9a	2.9a	0.2b	20.7a	3.5b	2.7a	5.3a	0.8b	19.8b	12.0b	0.3b
18.3c	10.3a	10.0a									
Tabola	12.1b	1.1b	0.1b	15.3c	3.1b	2.0b	3.7b	1.1a	28.1a	17.0b	0.1b
22.3b	7.7b	5.7d									
Matholeng	12.1b	1.1b	0.1b	16.7b	3.1b	2.1b	2.3c	1.1a	29.0a	18.4b	0.2b
22.7b	8.3c	5.2d									
Ha Seithleko	7.4c	0.4c	0.1b	11.3b	2.0c	1.5b	1.7c	0.9a	23.0b	14.2b	0.1b
18.3c	5.7c	3.6e									
Siloe	12.9b	0.9b	0.1c	14.7b	3.3b	2.9a	5.3a	0.9a	23.2b	13.1b	0.2b
22.7b	7.3b	6.9c									
Taung	14.0b	1.0b	0.1b	14.0b	3.4b	2.6a	4.7a	0.9b	21.6b	12.5b	0.2b
23.0a	7.0b	7.4b									
Likhutlong	11.4c	0.3c	0.1c	12.7c	2.1c	1.6b	2.0c	1.0a	22.7b	13.8b	0.1b
25.7a	6.3c	5.4d									
Lalane	11.7b	0.9b	0.1b	13.3b	3.5b	2.3a	3.0b	1.0a	22.7b	13.6b	0.2b
23.7a	6.7c	6.5c									
Qalakheng	10.8c	1.0b	0.1b	12.0b	3.3b	2.2a	3.3b	0.8b	19.8b	11.4b	0.2b
26.3a	6.0c	6.2c									
Borata	12.5b	1.1b	0.1b	12.7b	3.2b	2.4a	3.0b	0.8b	20.7b	11.8b	0.1b
24.3a	6.3c	7.2b									
Ha Seeiso	13.5b	1.0b	0.1b	12.0a	4.2a	2.8a	4.0a	1.0a	24.1b	14.6b	0.2b
24.7a	6.0c	8.5a									
Ha Sekepe	11.4c	0.4c	0.1b	12.7b	2.5c	1.6b	2.0c	0.9b	21.8b	13.1b	0.1b
22.7b	6.3c	5.0d									
Makhalanyane	9.0c	0.5c	0.1c	10.0b	2.8b	1.9b	2.0c	0.9b	21.4b	13.1b	0.1b
24.0a	5.0c	4.9d									
Mangopeng	4.9c	0.2c	0.1c	10.7b	2.4c	1.8b	2.0c	0.9a	22.6b	14.1b	0.1b
24.3a	5.3c	2.7f									
NUL Farm	9.1c	0.5c	0.1c	11.3b	3.3b	2.3a	3.7b	0.9b	21.0b	13.1b	0.1b
26.0a	5.7c	5.5d									
Ha Paki	3.7d	0.0d	0.1c	6.0d	1.0d	1.8b	2.0c	1.0a	24.4a	15.1a	
0.01c	11.3d	3.0d	1.7f								
Ha 'Matsa	11.7b	1.0b	0.1b	12.7a	3.3b	2.3a	3.1b	0.9b	21.1b	12.3b	0.2b
24.8a	6.3c	6.6c									
LSD	3.7	5.5	0.04	4.2	1.0	1.0	1.2	0.2	4.6	3.7	0.5
3.4	2.1	1.5									
CV%	15.3	18.6	27.2	12.8	16.9	22.8	20.6	8.82	8.90	9.85	13.1
8.18	12.8	13.2									

PH=Plant height, FW=Fresh weight, DW= Dry weight, NL=Number of leaves, LL=Leaf length, LB=Leaf Breadth, LA=Leaf Area, TCN=Total carotenoids, TCa=Total Chlorophyll a, TCb=Total chlorophyll b, SD=Stem Diameter, NT=Number of Trichomes, NN=Number of nodes, LI=Length of 3 Internodes, LSD = least significant difference

The big leave area and related attributes, directly affect the yields of important nutrients. The leaves are reported to be rich in minerals and micronutrients and they are comparable to those found in spinach [30, 7]. It is rich in Vitamin (C, K and A), Magnesium, Iron, Calcium, Zinc, Manganese and Potassium. There are also reports of cultivated stinging nettle in the world [6, 7, 8, 31, 32, 33 and 34]. Leaves of nettles have gained popularity is food dishes and also used as supplement of nutritional value.

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Principal component and cluster analysis for phenotypic traits

With the purpose of shrinking a complex data of phenotypic traits to a smaller, interpretable and meaningful data, the principal component analysis was implemented. This was undertaken to understand which traits are backing in genetic variation. There were 14 principal components that contributed to the variation among the variables but only four principal components (PC1, PC2, PC3 and PC4) will be dealt with in this study because their eigenvalues are greater than 1 as shown in Table 4 below. The overall variance for the first four principal components constituted 90.16% of the total variation. The first level of variation was about 42.52%, the second was 22.80% the third was 10.75%, and the last one was 8.20% which summed up to the total variation of 90.16% (Table 4).

Table 4 Cumulative variance of eigenvalues of the 14 principal components of U. dioica accessions.

Component	Initial Eigenvalues			Extraction Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	5.953	42.520	42.520	5.953	42.520	42.520
2	3.192	22.799	65.319	3.192	22.799	65.319
3	1.505	10.748	76.067	1.505	10.748	76.067
4	1.148	8.197	84.264	1.148	8.197	84.264
5	.825	5.891	90.155			
6	.484	3.457	93.612			
7	.357	2.548	96.160			
8	.227	1.623	97.783			
9	.168	1.203	98.986			
10	.071	.505	99.491			
11	.035	.252	99.744			
12	.025	.179	99.923			
13	.011	.077	100.000			
14	5.077E-008	3.626E-007	100.000			

The parameters that contributed most on the variation in principal component 1 were plant height 0.94, length of 3 internodes 0.89, number of nodes 0.86 and number of leaves 0.86. The total chlorophyll a 0.96, chlorophyll b 0.95 and total carotenoids 0.93 correlated with principal component 2. The parameters that described principal component 3 were No of trichomes on the leave 0.47, leaf area 0.47, leaf length 0.41 and leaf breadth 0.40. The fourth principal component constituted by No of trichomes of 0.68 and stem diameter 0.43 (Table 5).

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Table 5 Four Principal components of the phenotypic traits from different accessions of *U.dioica* and their loadings (The principal component analysis was obtained from Statistical Package for Social Sciences (SPSS) IBM version 20).

Phenotypic trait	Component			
1 2 3 4				
Plant height	0.94	0.38	-0.12	0.17
Fresh weight	0.32	0.30	-0.20	-0.50
Dry weight	0.82	0.11	-0.11	-0.24
Number of leaves	0.85	0.18	-0.36	0.16
Leave length	0.82	-0.19	0.42	-0.01
Leaf breadth	0.75	-0.25	0.37	-0.29
Leaf area	0.78	-0.16	0.42	-0.20
Total Carotenoids	-0.08	0.94	0.29	-0.02
Total Chlorophyll a	-0.03	0.96	0.25	0.34
Total chlorophyll b	-0.06	0.96	0.22	-0.21
Stem Diameter	0.48	0.33	-0.37	0.48
No of trichomes	0.06	-0.24	0.56	0.62
No of nodes per plant	0.85	0.18	-0.36	0.16
Length of 3 internodes	0.88	-0.12	0.21	0.14
Percentage variance	42.52%	22.80%	10.75%	8.20%

The UPGMA cluster analysis obtained is shown in (Figure 3). PC1 contributed 97.2% while PC 2 contributed 1.4%. The biplot shows, number of trichomes, total chlorophyll ‘b’ and total chlorophyll ‘a’ to have clustered together implying there is a genetic linkage amongst these traits. As shown in table 3, accessions with high number of trichomes are also rich in photosynthetic pigments, however are low in carotenoids. Number of leaves, plant height, no of nodes and length of internodes have been grouped together which simply means that the tallest *U.dioica* had more leaves. Leaf area, leaf breadth and leaf width also grouped together meaning all the attributes pertaining to leaf size were grouped together. Dry weight and stem diameter were grouped together. Total carotenoids and fresh weight were grouped together. The groupings were also evidenced by the Dendrogram (Figure 3).

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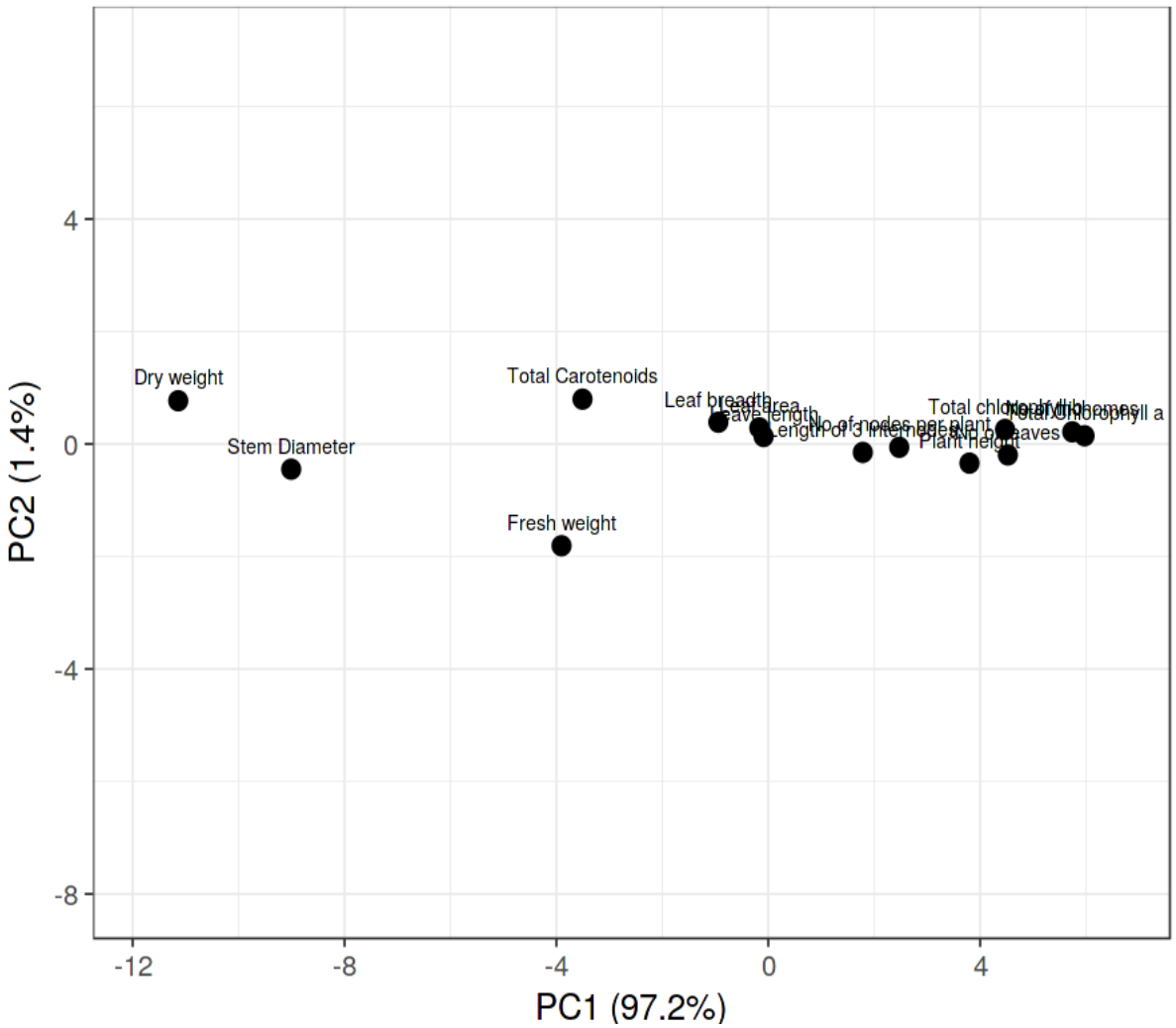

Figure . 2: Two-dimensional plot of PC1 (principle component) versus PC2 for 14 morphological traits of U.dioica (The principal component biplot were obtained from clustvis online web for visualizing clustering of multivariate data).

The phenotypic traits were first clustered into 2 groups A and B. Group B was sub grouped in to two groups; iii and iv (Figure 4). The attributes that appeared under iii were Fresh weight and Total Carotenoids while under subgroup iv there were dry weight and stem diameter. Thicker and longer stems are usually targeted for increased biomass yield in cultivated *U.dioica* (32). The stems of *U.dioica* are not hollow, they are characterized by lignified xylem tissue that makes the bulk of the weight of the tissues. These are usually the harvested parts for fiber [32]. Carotenoids are photosynthesizing pigments useful for carbon metabolism when the green pigments is depleting. These molecules are heavier in weight than chlorophyll forms, thus are major determinants of plants fresh weight. Both the carotenoids and stem thickness determine the plant weight [35]. Moreover in one study it has been observed that the chlorophyll content increase with an increase in altitude. However the morphological characters such as leaf are and plant height remains minimum with an increase in altitude [25].

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Group A was sub grouped into i and ii. Total chlorophyll a, number of trichomes, number of leaves, plant height and chlorophyll b fell under subgroup i while leave length, leaf area, leaf breadth were sub grouped together. Number of nodes per plants and length internodes were sub grouped together. The genetic distance between these leaf/foliage attributes and the plant weight related traits is bigger represented by >10 rescaled distance cluster combine, however within the sub groups there is no much genetic distance. Still that variation within the sub groups is still important.

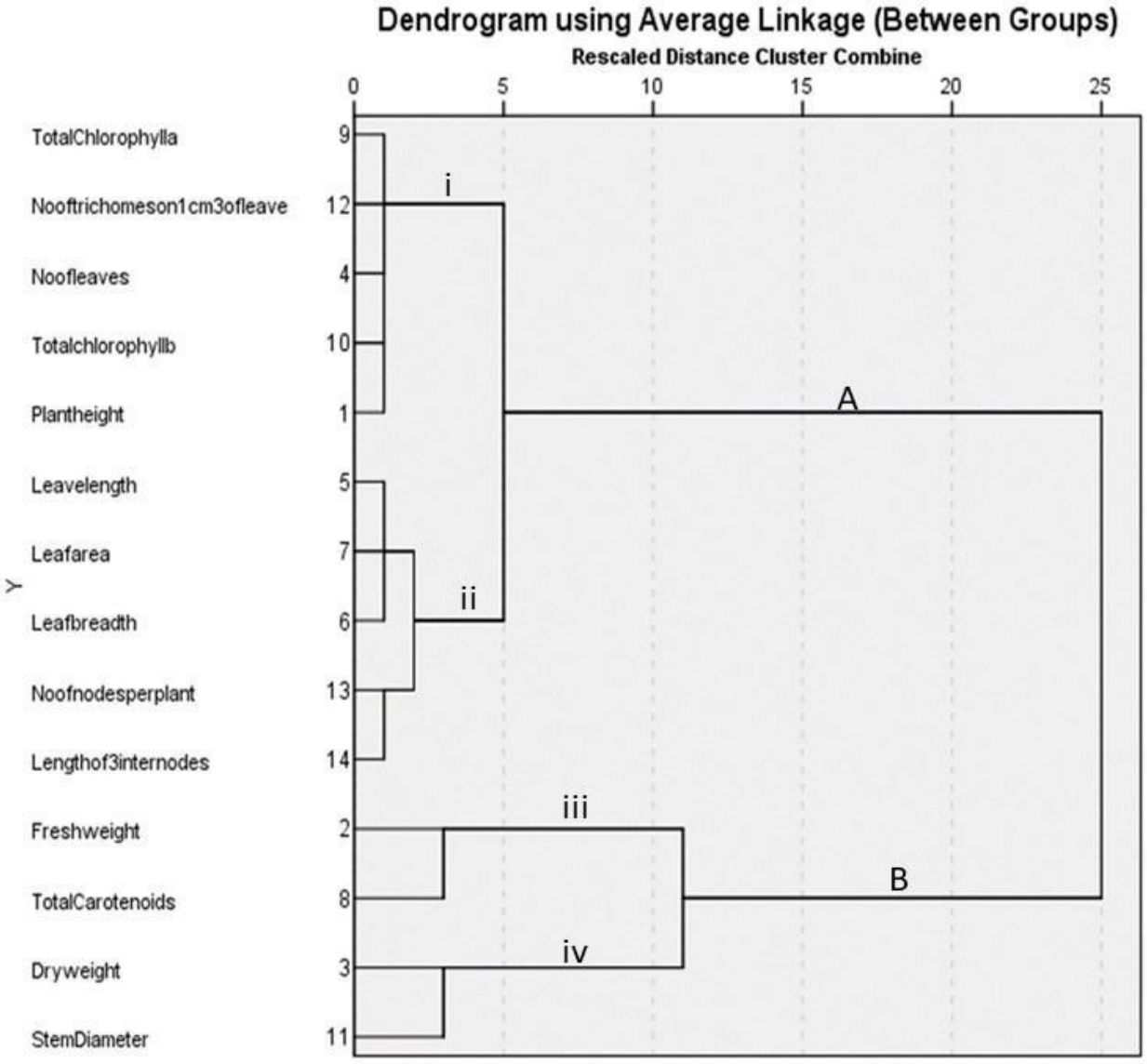

Figure 3 Dendrogram for clustering of the phenotypic traits of U.dioica (The Dendrogram on figure 4 was obtained from Statistical Package for Social Sciences (SPSS) IBM version 20).

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Principal component and Cluster analysis for accessions

Based on the morphological data transformed into a binary matrix, the cluster analysis was performed and graphically displayed through biplot (Figure 5) and dendrogram (Figure 6). The most contributing principal components for areas of collection; PC1 and PC2 were plotted. PC1 contributed 48.5% to the variance while PC2 contributed 21.4%.

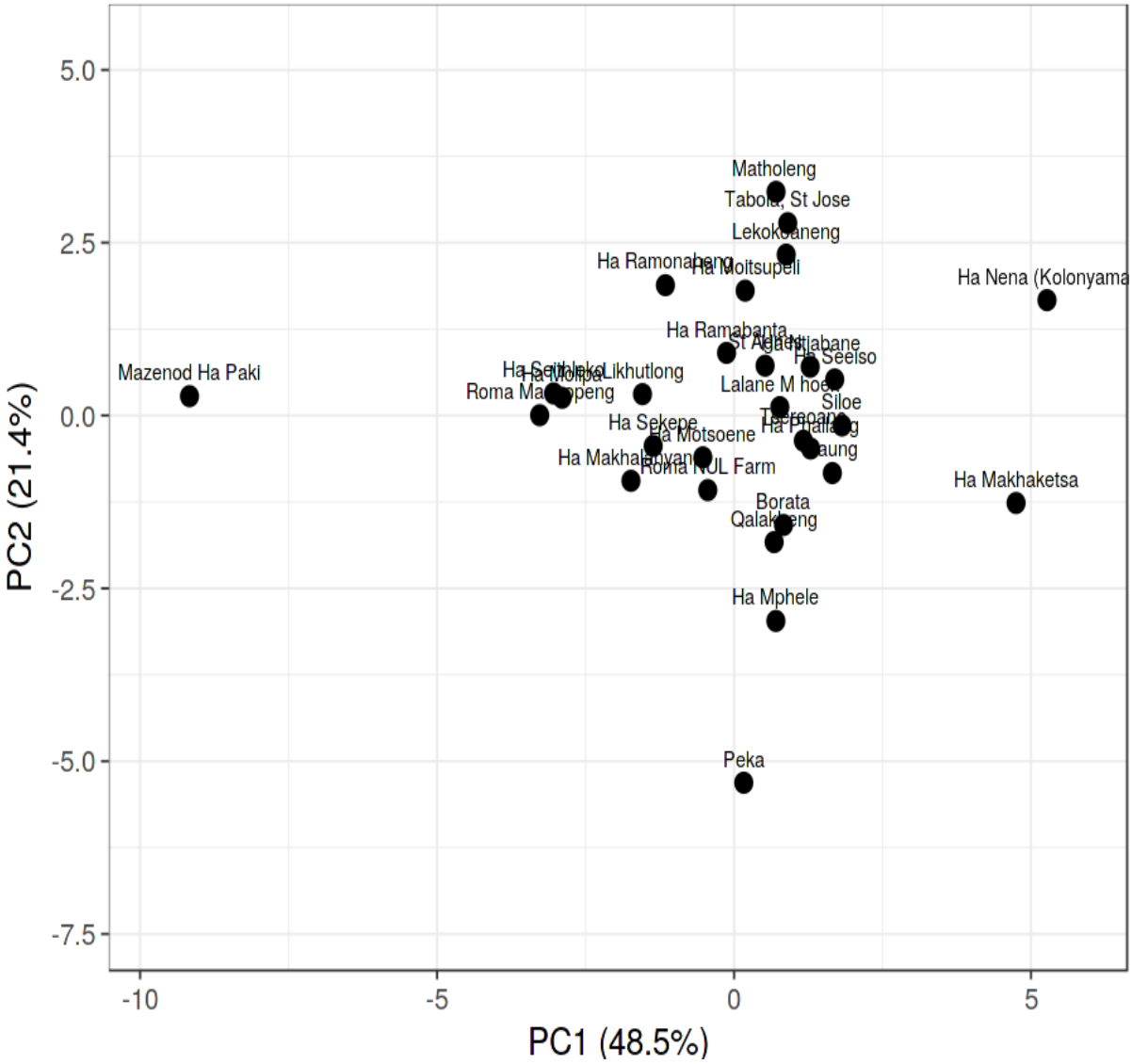

Figure. 4: Two dimensional plot of PC1 (principle component) versus PC2 for the 30 accessions of U.dioica

The cluster analysis of the accessions shows two main clusters A and B. Group B only had one accession; Mazenod Ha Paki characterized by lowest fresh and dry weights that do not correlate with their total carotenoid content. Group A was made of Ha Ramonaheng and the rest of the Group A accessions were characterized by highest values of plant height, number of nodes, internodes, number of leaves, and dry weight values. This Group B was further sub-

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grouped into three groups; i, ii and iii where Ha Ramonaheng accession was an outlier in that group.

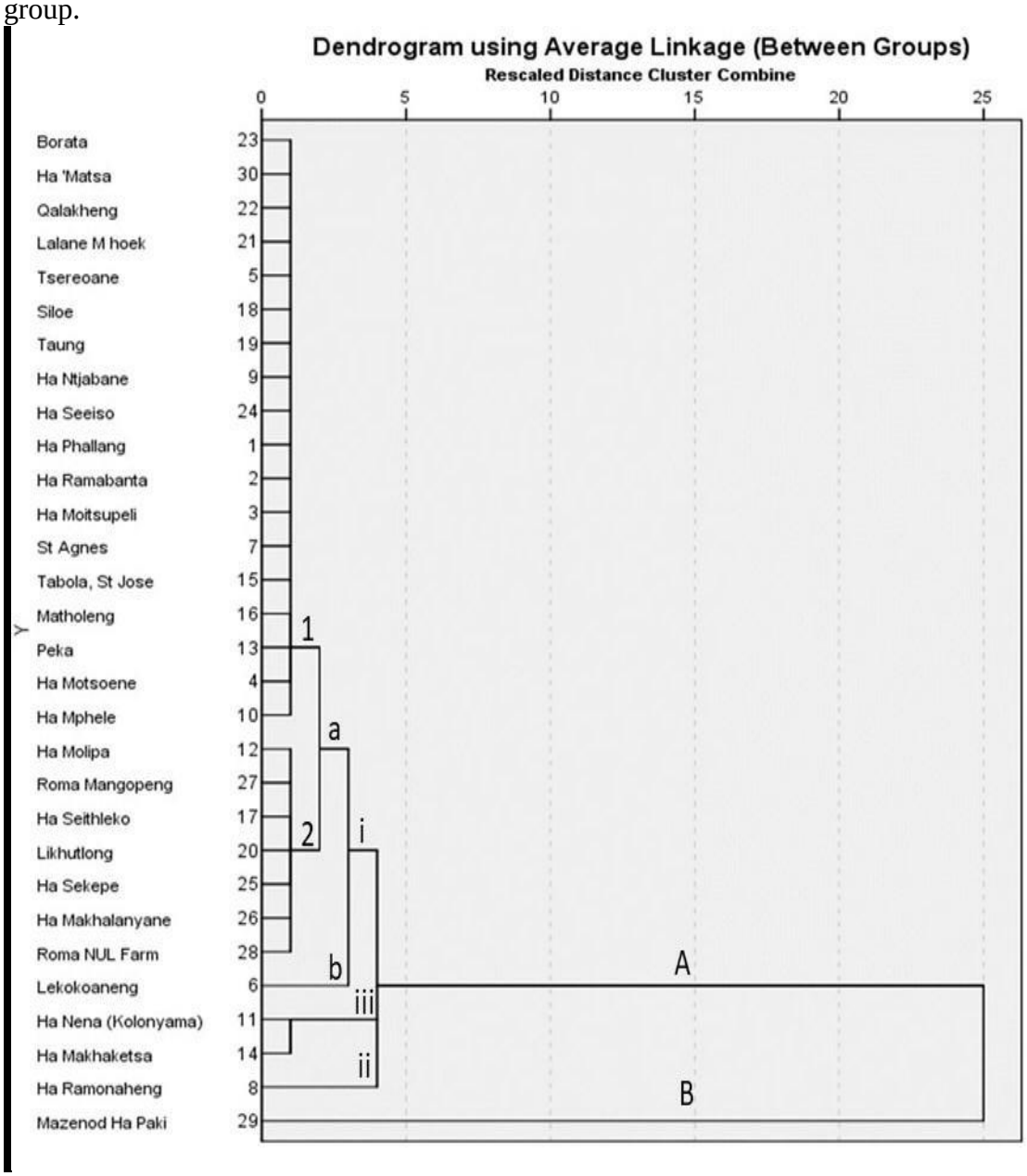

Figure. 5: the Dendrogram for the 30 accessions of U.dioica (The Dendrogram was obtained from Statistical Package for Social Sciences (SPSS) IBM version 20).

There is observed genetic variation with different locations as influenced by varying environments, this findings are in line with [36]. However the genetic distances between the subgroups is not big indicating very close environmental relationships between the places. The variation that exists between the groups is still genetically significant. During the course of environmental adaptation process, the isolated plant populations tend to accumulate genetic variations [37]. The medicinal plant and cultivated plant; *U. dioica,* is a perennial herb with

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reproduction through seeds and rhizomes [38]. It is also adapted over diverse environments exhibiting variation in morphological traits as determine by various environmental factors.

In this study, the means for the phenotypic traits as quantified for all accessions is variable from accession to accession (Table 2). The differences that exist between the accessions are not merely environmental but genetic as shown by ANOVA indicating significant difference between accessions in all the traits. Genetic diversity within and among the species of plants can be influenced by environmental factors such as climate gradients related to temperature and precipitation [39] and a range of ecological factors [40]. Bharmauria et al (2009) also found out that *U.dioica* accessions varied due to environmental factors such as ecology and precipitation. For instance, grazing habits and illegal collection of *U.dioica* play an important role in the variation in the presence of trichomes as morphological features.

U.dioica can be domesticated for medical purposes (such as relieve of arthritis, rheumatism, muscular pain) and as food (nettle leaves as leaf vegetable, nettle tea, nettle spices). *U.dioica* is a wild plant that has found difficulty in domestication due to the fact that it possesses the trichomes, which sting as their defensive mechanism but owing to its functions to human beings [41]. Pollard et al (2009) indicated that the number of trichomes found in both leaves and stem in *U.dioica* can vary based on the presence and absence of the herbivores. The number of trichomes was found to be high on the grazing land than on the non-grazing land. It is thus evident that *U.dioica* morphology varies depending on the diverse range of environmental factors ranging from climatic and socio-political factors.

CONCLUSION

Two main groups exist based on the similarity of morphology within the *U.dioica* germplasm in Lesotho with the second biggest group exhibiting three distinct sub groups. The major determining factor on grouping this germplasm is the presence of trichomes that is characterized by high photosynthetic capacity, secondly by the plant height that is associated with increased foliage characters such as number of leaves and internodes, and lastly by the fresh weight a characteristic associated with the accessions with high carotenoids content.

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