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Estimation of genetic variability, heritability, and genetic advance for the agronomic traits of stinging nettle (*Urtica dioica*)

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Abstract
A total of 30 wild Urtica Dioica accessions was collected from the localities f Lesotho. In an experiment arranged in Complete Randomized Design with three replicates, observations were recorded for 15 traits; Plant-height (cm), Fresh-weight (g), Dry-weight (g), Number of leaves, Leaf length (cm), Leaf breadth (cm), Leaf area (cm^2), total Carotenoids ($mg.g^{-1FW}$), total Chlorophyll-a ($mg.g^{-1FW}$), total Chlorophyll-b ($mg.g^{-1FW}$), Stem-diameter (mm), Number of trichomes, Number of nodes and Length of 3 nodes (cm). The Analysis of variance revealed highly significant difference among the tested genotypes of stinging nettle at (P<0.01) level of significance. The highest phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GPV) were obtained for stem diameter and the least were obtained from carotenoids and chlorophylls. Plant height, had the highest heritability estimate and genetic advance. There is genetic variability and high heritability of attributes in stinging nettle. All observed traits can be targeted for selection.

Keywords: stinging nettle, phenotypic coefficient of variation, genotypic coefficient of variation, heritability, genetic advance, phenotypic variance, genotypic variance

Introduction
There are about 30-45 species of genus Urtica in the family Urticaceae which are predominantly distributed on the temperate zones (Bharmauria *et al*., 2009) [3]. In Lesotho, it is prevalent in all parts of the country though it is evenly distributed. Stinging nettle (Urtica dioica) is a wild dioecious perennial plant distributed in the entire world, which has many medical properties (Haghpanah *et al*., 2016) [8].
U. dioica is characterized by fine toothed oblong or ovulate with dark green color above and pale underneath and the stems are quadrangular erect and green which can either be pithed or hollow all covered by stinging hair called trichomes (Bourgeois *et al*., 2016) [4]. *U. Dioica* is very important plant in various industries such as agriculture, cosmetics, textiles, medicine and food (Adhikari *et al*., 2016) [1]. As food, *U. Dioica* is considered one of the most highly nutritious and high functional value as its leaves contain proteins, fats, carbohydrates, vitamins, minerals and trace elements (Sait *et al*., 2015). Rutto *et al.*, 2013 [22, 20] reported that *U. dioica* does not only possess antibacterial properties but also the antioxidant, antimicrobial, antifungal and antiviral properties.
Genetic variability in isolated plants tends to be accumulated when plants adapt to various environmental conditions. The clear understanding of the genetic diversity of medical plants such as *U. dioica* may lead to optimal exploitation of them (Sarwat *et al*., 2008) [23]. Specifically the knowledge of genetic variability is very important in the improvement of such plant species and it helps to establish a proper breeding programme (Hub, 2011) [10]. Differences in adaptation patterns in U dioica have been attributed the genetic variability amongst the local germplasm. The study by Bharmauria *et al.*, 2009 [3] established changes in altitude affected revealed that genetic variability in *U. dioica*; the leaves from high altitudes possessed lesser number of trichomes compared with low altitude. Moreover the leave samples from lower altitude had less concentrations of phenolic acids compared with high altitude. The altitude characteristics such as rainfall, temperature, dosage of UV rays and various selection pressures are playing important role in determining the kind of genetic material adaptable to the region. Thus it is concluded that phenotypic plasticity and gene isolation are dependent on both geographical distance and environmental conditions (Sexton *et al*., 2014) [24].
Heritability is referred to as the measure of correspondence between phenotypic values and genetic values, which play a vital role in breeding of plants by expressing the reliability of the

phenotype to the breeding value (Dyulgerova and Valcheva, 2014) [6]. High heritability helps in effective selection for a particular trait. There are two ways of estimating heritability; broad-sense heritability and narrow-sense heritability, on single plant, individual plot or mean of entry (Ogunniyan and Olakojo, 2015) [18]. The importance of heritability is that it helps in the proper allocation of time and resources for desired traits and to achieve maximum genetic gain (Ogunniyan and Olakojo, 2015) [18]. Heritability was classified as low (below 30%), medium (30-60%) and high (above 60%) (Johnson *et al*., 1955) [13] and by Islam *et al*., 2015) [12].

Genetic advance is the relationship of the response to selection and heritability of desired traits (Hussain *et al*., 2016) [11]. Genetic advance together with heritability are very important in the prediction of the gain under selection than the heritability alone (Islam *et al*., 2015) [12] and thus high genetic advance and high heritability lead to proper selection for desired traits. It is therefore correct to consider genetic advance as one of the important selection parameters that can be used by the breeders in the selection program (Dyulgerova and Valcheva, 2014) [6].

In this study, the aim is to determine the genetic variability, heritability, and genetic advance in *U. dioica* collected in Lesotho and to evaluate suitable selection criteria for further breeding.

Materials and Methods

The study was conducted at the National University of Lesotho, Crop science Department which is situated at Roma, Lesotho in 2020-2021. The University of Lesotho is located at about 34 km South East of Maseru, the capital town of Lesotho. The university is situated at 1610 m above the sea level.

Plant material collection and regeneration

U. dioica plants with seeds were collected in different agro ecological zones of Lesotho. The coordinates and elevations of collection of all each accession were recorded from the GPS (Table 1). The *U. dioica* plants were dried at room temperature in the Crop Science laboratory. 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.

A total of 30 accessions was collected from different places in Lesotho, and they were planted from their seeds 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.

Table 1: Collection sites for U. dioica and their coordinates

Accession name	Accession	Latitude	Longitude	Elevation
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

Data Collection:

The morphological data was collected for a sample of 20 plants per plot at flowering stage. plants were selected at random from each plot, and morphological data was measured and recorded from them (Sahin *et al*., 2019) [21].

For leaf size related attributes, five fully developed, highly synthetic leaves were selected per plant for observation. Leaf length and width were measured from base to tip and at the widest point using a ruler, respectively. The averages of leaf length, width per plot were recorded as actual values per plot

(accession). The ratio of leaf length to width was calculated and used to assess the elongate nature of leaves.
Leaf area was determined using 1cm grid where the number of squares covering the leaf were counted. The partial squares that covered at least half of the leaf, were added to the fully covered squares. Plant height was measured using a ruler. Number of leaves per plant were counted from all nodes. The three longest internodes were measured using a ruler. The shape of the leaves was determined as described by (Sahin *et al*., 2019) [21].
Stinging hairs (trichomes) on the leaves were counted directly using the magnifying glass.
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 plats were oven dried at 50°C overnight and weight on the digital weigh scale (Biobase-BA2204B)
The photosynthetic pigments i.e., total chlorophyll; chlorophyll 'a', chlorophyll 'b' and carotenoids in leaves were estimated as per the method of Hiscox and Israelstam (1979) [9].
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. Means separation and ranking in an ascending order was carried using Least Significant difference. Mean Square phenotype (MSp) and Mean Square error (MSe) were obtained from the Anova table. Means were used to calculate the following genetic parameters; genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), genotypic variance(σ^2g), phenotypic variance(σ^2p), environmental variances (σ^2e), heritability (h^2) (Kapanigowda *et al*., 2013) [14].
The estimation of phenotypic and genotypic correlation coefficients from corresponding variance and covariance components were calculated as per (Miller *et al*., 1958) [16].

$$r_p = \frac{pcov\ x.y}{\sqrt{\delta^2 px.\delta^2 py}}$$

$$r_g = \frac{pcov\ x.y}{\sqrt{\delta^2 gx.\delta^2 gy}}$$

where,
r_p is phenotypic correlation coefficient,
r_g is genotypic correlation coefficient between characters x and y,
Pcov xy is phenotypic covariance
Gcov xy is genotypic covariance between characters x and y.

Variance components
The estimation of phenotypic and genotypic coefficients of variation (Burton and de Vane (1953) [5]

Environmental variance (σ^2e) = MSe/r

Phenotypic variance (σ^2p) = ($\sigma^2g+\sigma^2e$)

Genotypic variance (σ^2g) = MSp – MSe/r

Where:
MSe = Mean square error
MSp = Mean square phenotype
r = Replication

$$\text{Phenotypic coefficients of variation (PCV)} = \frac{\sqrt{\sigma^2 px}}{x} \times 100$$

$$\text{Genotypic coefficients of variation (GCV)} = \frac{\sqrt{\sigma^2 gx}}{x} \times 100$$

Where:
σ^2p = Phenotypic variance
σ^2g = Genotypic variance
x = Grand mean of a character

Heritability (H^2)
In order to estimate broad sense heritability, the method which was described by Allard (1960) [2], was adopted on genotype mean basis as shown below. Heritability of broad sense (h^2) is described on the formula as the percentage of the ratio of the variance based on genotype (g) to the variance based on phenotype (p).

$$h^2B = \frac{\sigma^2 g}{\sigma^2 p} \times 100$$

Where:
h^2 = broad sense heritability
σ^2p = variance due to phenotype
σ^2g = variance based on genotypic

Genetic advance as percent of mean (GAM)
The estimation of genetic advance (GA) and mean percentage (GAM) with assumption selection of superior 5% of the genotypes Allard (1960) [2], Singh and Chaudhury (1985) [25]

$GA = K \times h^2$

where:
GA = Expected genetic advance
K- Constant = 2.063 at 5% selection intensity
h^2 - heritability in broad sense.
The genetic advance as percentage of mean (GAM) was computed as:

$$GAM(\%) = \frac{GA}{x} \times 100$$

Where:
GAM = Genetic advance as percentage of mean
GA = Expected genetic advance
X = Grand mean of a character

Results and Discussion
Genetic Variability among stinging nettle genotypes (Analysis of variance)
The Analysis of variance (Table 2) for plant height (cm), fresh weight (g), dry weight (g), number of leaves, leaf length (cm), leaf breadth (cm), leaf area (cm^2), carotenoids ($mg.g^{-1FW}$), chlorophyll 'a' ($mg.g^{-1FW}$), chlorophyll 'b' ($mg.g^{-1FW}$), stem diameter (cm), number of trichomes, number of nodes, length of 3 nodes (cm), showed highly significant difference among the tested genotypes of stinging nettle at (P<0.01) level of significance. The results showed that the tested

genotypes of *U. dioica* varied from each other in various characteristics and thus implying high inherent genetic variability. The study by Bharmauria *et al*., 2009 [3] also showed that there was significant difference between *U. dioica* genotypes with varying altitudes. Genetic variation in any given population is critical to effectively select and manage yield improvement programs (Ndukauba *et al*., 2015) [17].

In this present study there is wide range of the means from all the measured attributes. This is a clear indication of wider genetic variation between the genotypes of *U. dioica*. In addition the ANOVA (table 2) clearly shows the significant difference between the accessions. The plant height of *U. dioica* genotypes ranged from 3.7cm to 21cm. fresh weight ranged from 0.9g to 3.5g. Dry weight ranged from 0.01g to 0.3g. Number of leaves ranged from 6 to 21. Leaf length ranged from 1.0cm to 3.7cm. Leaf breath ranged from 1.8cm to 2.7cm. Leaf area ranged from 2.0cm^2 to 5.7cm^2. Total Carotenoids ranged from 0.9nm to 1.1nm. Total chlorophyll 'a' ranged from 15.5 mg.g^{-1FW} to 29.0 mg.g^{-1FW}. Total Chlorophyll 'b' ranged from 8.9 mg.g^{-1FW} to 18.4 mg.g^{-1FW}. Stem diameter ranged from 0.01cm to 0.3cm. No. trichomes ranged from 10.7 to 26. No. nodes ranged from 3.0 to 10.7. Length of 3 nodes ranged from 1.7 to 10.0. The results simply show that the wide genetic variation in the genotypes of *U. dioica* can be used as source of genetic material for *U. dioica* improvement for various objectives such as medical purposes and as food.

Table 2: ANOVA Table (The Anova table was obtained from GENSTAT statistical package version 18.2 (PC/Windows 7)

Source of variation	df	MSS	F value	P value	Means	
					Minimum	Maximum
Plant height	30	36.03	11.81	< 0.01	3.7	21
Fresh weight	30	8.81	1.76	0.03	0.9	3.5
Dry weight	30	0.01	12.73	< 0.01	0.01	0.3
No. leaves	30	27.25	8.22	< 0.01	6	21
Leaf length	30	1.25	4.91	< 0.01	1.0	3.7
Leaf breadth	30	0.48	1.88	< 0.01	1.8	2.7
Leaf area	30	3.25	8.52	< 0.01	2.0	5.7
Carotenoids	30	0.03	4.81	< 0.01	0.9	1.1
Chlorophyll 'a'	30	21.76	5.3	< 0.01	15.5	29.0
Chlorophyll 'b'	30	11.81	6.46	< 0.01	8.9	18.4
Stem diameter	30	0.04	0.85	0.69	0.01	0.3
No. trichomes	30	40.69	13.2	< 0.01	10.7	26
No. nodes	30	6.81	8.22	< 0.01	3.0	10.7
Length of 3 nodes	30	8.23	14.26	< 0.01	1.7	10.0

df = degree of freedom, MSS = Mean Sum of Squares

Variance components

Table 3 shows genotypic variance (δ^2g), phenotypic variance (δ^2p), environmental variance (δ^2e), broad sense heritability (H^2) genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV) and GAM for traits of the stinging nettle lines. Estimated variance components for all the measured traits revealed that the PCV was higher in magnitude than the GCV, which implies that environmental effect influenced expression of all the traits. Although there was a difference between PCV and GCV, it was not that significant in all the traits indicating the gene additive effect controlling the observed attributes.

The highest phenotypic coefficient of variation was obtained for stem diameter (96.64), followed by dry weight (76.89) and leaf area (60.92) and the least was obtained from carotenoids (20.34), chlorophyll 'a' (20.89), chlorophyll 'b' (25.32) and fresh weight (26.25). Khan *et al*., 2009 [15] emphasized that high phenotypic coefficient of variation indicates the occurrence of a larger possibility of making proper selection for the trait under consideration, which depends mostly on the available variability. It is, therefore, expected that stem diameter, leaf area and dry weight have greater ability to be selected. Alternatively, there is narrow scope for selection of photosynthetic pigments and fresh weight.

According to Ene *et al*., 2016 [7], the genotypic coefficient of variation (GCV) is just a measure of genetic variability that exists in diverse quantitative traits. The highest genotypic coefficient of variation was found from stem diameter (96.57), followed by dry weight (75.89) and leaf area (59.74) and the least was obtained from carotenoids (19.66), chlorophyll 'a' (20.25), chlorophyll 'b' (24.68) and fresh weight (23.94). Yadav *et al*. (2009) [28] showed that high GCV designates there is variation in the genes for the traits, which can be utilized for proper selection.

Heritability (H^2)

The heritability estimate is a measure of phenotypic variation within a population, which is brought by the variation caused by the genetic make-up of the individual within population, high heritability helps in effective selection for a particular trait (Ullah *et al*., 2012) [27]. The *U. dioica* traits studied in this study showed high heritability estimates, which ranged from 83.2% to 97.7% table 3. Among the traits, plant height, dry weight, number of trichomes and length of internodes had the highest heritability percent of 97% as approved by Patel *et al*., (2012) [19] while Stem diameter had the lowest heritability of 68.9%. %. According to this data, there is inverse relationship between heritability and PVC for stem diameter. This implies that the high PVC in stem diameter observed means stem diameter is influenced by environmental factors which makes it unreliable to be used for selection, which is supported by its low observed heritability compared to other parameters.

Johnson *et al*., (1955) [13], Islam *et al*., (2015) [12] classified heritability as low (below 30%), medium (30-60%) and high (above 60%) and therefore heritability of stem diameter, which is the lowest in the study, is still high according to this classification. High heritability values (table 3) indicate that the *U. dioica* traits under study are less influenced by environment in their expression and that means when breeding for stinging nettle, all the parameters under study can be utilized for selection.

Genetic advance as percent of mean (GAM)

The genetic advance is an estimate that checks the progress expected due to selection on the relevant population. In order

to access a more effective trait selection, it is crucial to put together heritability accompanied by genetic advance than heritability alone (Ullah *et al*., 2012) [27]. The genetic advance of all traits was slightly different as the difference is insignificant. In this study, plant height (17.9), dry weight (17.95), number of trichomes (17.96) and length of internodes (17.69) were observed to have highest genetic advance while the least was observed in stem diameter (12.69 and leaf breadth (15.50). High heritability and high genetic advance for a considered trait shows that it is administered by additive gene action and thus offers the most effective range for selection (Tazeen *et al*., 2009; Ndukauba *et al*., 2015) [26, 17]. From table 3, it is observed that heritability and genetic advance are both at almost highest level in all the traits which simply means that most *U. dioica* traits can provide a broad condition for selection.

Table 3: Variance components for the 14 agronomic traits in the U. dioica lines

Traits	MSp	MSe	σ^2g	σ^2e	σ^2p	H^2	PCV	GCV	GA	GAM
Plant height	36.04	3.05	35.70	1.02	36.72	97.20	53.87	53.35	2.01	17.91
Fresh weight	8.81	5.00	8.26	1.67	9.92	83.20	26.25	23.94	1.72	14.30
Dry weight	0.01	0.01	0.01	0.01	0.01	97.40	76.89	75.89	2.01	17.95
Number of Leaves	27.25	3.32	26.89	1.11	27.99	96.10	37.79	37.03	1.98	17.69
Leaf length	1.25	0.26	1.22	0.09	1.31	93.50	38.14	36.88	1.93	17.22
Leaf breadth	0.48	0.26	0.46	0.09	0.54	84.10	33.44	30.67	1.74	15.50
Leaf area	3.26	0.38	3.21	0.13	3.34	96.20	60.92	59.74	1.99	17.72
Carotenoids	0.03	0.01	0.03	0.01	0.03	93.40	20.34	19.66	1.93	17.20
Chlorophyll ‘a’	21.76	4.10	21.31	1.37	22.68	94.00	20.89	20.25	1.94	17.30
Chlorophyll ‘b’	11.81	1.83	11.60	0.61	12.21	95.00	25.32	24.68	1.96	17.50
Stem diameter	0.04	0.05	0.04	0.02	0.05	68.90	96.64	96.57	1.42	12.69
Number of trichomes	40.69	3.08	40.34	1.03	41.37	97.50	30.06	29.68	2.01	17.96
Number of Nodes	6.81	0.83	6.72	0.28	7.00	96.10	37.79	37.03	1.98	17.69
Length of Internodes	8.23	0.58	8.17	0.19	8.36	97.70	50.71	50.13	2.02	17.99

MSp = Mean Square phenotype, MSe = Mean Square error, σ^2p =Phenotypic variance, σ^2g=Genotypic variance, h^2=Heritability in broad sense, σ^2e = Environmental variance, PCV is phenotypic covariance, GCV is genotypic covariance, GA = genetic advance and GAM = genetic advance as percent of mean

Conclusion

It was therefore concluded that the tested genotypes of *U. dioica* showed statistically high significant difference at (P<0.01) level of significance revealing presence of substantial amount of genetic variability in stinging nettle found in Lesotho. The percentage heritability was high in all the characters of *U. dioica* meaning all the characters were heritable. There was relatively low difference between the PCV and GCV of all characters of *U. dioica*, which means that both genotypes and the environment had influence on the variation between the characters of *U. dioica*.

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