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Variation in Morphological and Agronomic Characteristics of Quartin Clover (*Trifolium quartinianum*) Accessions

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Abstract: *Trifolium quartinianum* is the most productive and widely adapted clover to different soil conditions. However, in-trust collections of this legume maintained at the International Livestock Research Institute in Addis Ababa, Ethiopia, have never been characterized. Agronomic and phenotypic traits were studied in 34 accessions of *T. quartinianum* at Debre Zeit in the Ethiopian Rift Valley, to identify promising accessions for use as feed for livestock. Eight groups of similar accessions were identified based on morphological characters and five groups on agronomic characters. Growth habit, stipule length, seed length, flower colour, seed colour, days to 50% flowering, yield, leaf: stem ratio and plant height were the major traits that contributed to the separation.

Key words: *Trifolium quartinianum* · Clover · Morphological and Agronomic characters

INTRODUCTION

Shortage of feeds and poor quality of available feeds limits livestock production in the Sub-Saharan Africa. Intensification involves incorporating high quality forages such as *Trifolium quartinianum* which can be integrated into rainfed tropical highland crop-livestock systems [1], to mitigate this problem. *T. quartinianum* is the most productive of the native Ethiopian clovers with vigorous growth that can produce 7800 kg ha^{-1} dry matter within three months when growing conditions are favorable [2]. It has higher seed production capacity compared to other *Trifolium* species and may adapt to a wide range of soils from heavy to clay vertisols and nitosols to loams and sandy loams [3]. It tolerates seasonal water logging. *Trifolium* species have potential to improve natural or sown pastures in the tropical highlands of Africa [4]. Grasses, when grown in mixture with *Trifolium* are known to accumulate more dry matter (DM), crude protein (CP) and *in vitro* digestible dry matter (IVDDM) than grass monoculture [5]

Friedericks *et al*. [6] evaluated five annual clover species from Ethiopia and Kenya for biomass production under different moisture condition and reported higher biomass production in *T. tembense*, *T. quartinianum* and *T. decorum* than *T. rueppellianum* and *T. steudneri*, in a green house. They recommended that the high biomass producing species should be evaluated in field trials to determine their use as pasture legumes to support livestock production.

The in-trust germplasm collections maintained at the International Livestock Research Institute in Addis Ababa contains more than 35 accessions of *T. quartinianun* that have never been characterized. Grouping accessions of *T. quartinianum* according to their morphological and agronomical similarities and dissimilarities can be used to assist users to select germplasm best suited to their needs. This will also aid in the selection of accessions with superior traits for multiplication and for further nutritional assessment. The objective of the study was to describe the diversity in 34 *T. quartinianum* accessions for agronomic and phenotypic traits and to identify promising accessions for further use as feed for ruminant animals.

MATERIALS AND METHODS

The study was conducted in the sub-humid highlands at Debre Zeit, Ethiopia, (8°44'N, 38°58'E and 1850 m above sea level) on a light clay-loam soil (30% clay, 35% silt, 34% sand and 5% loam) and pH of 6.7. The mean annual rainfall is 800 mm and is in bimodal distribution pattern with short rainy season occurring between March and April while the main rainy season is from mid-June to mid-September.

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Table 1: Morphological and agronomic and characters evaluated in *Trifolium quartinianum* accessions

Characters	Abbreviations	Units	Definition of characters
Plant height	Platht	cm	Measured at 50% flowering using a ruler
Stem thickness	Stmthk	mm	At the base of the fifth node, identified by counting from the apex of the main stem was measured at 50% of flowering
Leaflet length	Leflgt	mm	Length of terminal leaflet at 50% of plants flowering
Leaflet width	Lefwdt	mm	Width of terminal leaflet at 50% of plants flowering
Leaf shape	Lefshp		Leaf length divide by leaf width
Internode length	Indlgt	mm	At 50% flowering, by measuring the length of the stolon divided by number of nodes on it
Petiole length	Petlgt	mm	Taking the same leaf, length of the leaf stalk is measured using a ruler
Stipule length	Stplgt	mm	Widest part of the stipule is measured using a ruler
Stipule width	Stpwdt	mm	Measured at 50% flowering
Flower number	Flwnum		Number of florets per head
Flower diameter	Flwdia	mm	Diameter across a fully mature flower
Flower length	Flwlgt	mm	Measurement made on a fully expanded flower
Flower width	Flwwdt	mm	Taking the same pedal the widest part is measured
Peduncle length	Pedlgt	mm	Measuring length of flower stalk using a ruler at 50% flowering
Pod length	Podlgt	mm	Measuring length of pod using a 180aliper
Pod width	Podwdt	mm	Measuring pod of pod using a 180aliper
Seed length	Sedlgt	mm	Length of fully developed seed
Seed width	Sedwdt	mm	Width of fully developed seed
Number of seed\pod	Sedpod		Counting number of seeds from mature pod
Seed weight	sedwt	g	Weight of 100 seeds
Length of calyx lobe	Clxlob	mm	Length of the calyx lobe at 50% flowering
Days to 50% maturity	Flow50	days	Days from sowing date to 50% of plants with flowers per plot
Days to 75% Maturity	Flow75	days	Days from sowing date to 75% of plants with flowers per plot
Plant growth pattern	Grwpat		Observed at 50% flowering; open=1, closed = 2
Plant growth habit	Grwhab		Plant growth habit at 50% flowering; erect=1, semi-erect=2, decumbent=3, Prostrate =4.
Stem hardness	Stmhad	days	Observed at 50% flowering; Soft=1, medium soft=2, Hard=3
Stem colour	Stmclr	Score	Rating; 1=green, 2=green/brown and 3=brown at 50% flowering
Stem hairs	Stmhir	Score	Observed at 50% flowering; smooth=1, sparse=2, medium=3, dense=4, very dense=5
Flower color	Flwclr		Observed at 50% flowering on a fully expanded flower; purple=1, violet=2, red purple=3, purple violet=4
Seed colour	Sedclr	Score	Observed at after harvesting; Brown=1, yellow green-=2, yellow orange=3, grey brown=4, grey orange=5, grey green=6
Yield	Yld	kg\ha	Area of 50cm x 50cm harvested using a quadrat at 50% flowering and dried at 60°C for 2 days
Plant height per day	Phd	m/d	Total height divided by the number of days to 50% flowering
Leaf to stem ratio	Lefstrt		Weight of dry leaf divide by weight of dry stem
Leaf dry matter per day	Ldmd	kg/day	Total dry weight of the leaf divide by number of days to 50% flowering
Stem dry matter per day	Sdmd	kg/day	Total dry weight of the stem divide by number of days to 50% flowering

Thirty four accessions belonging to *T. quartinianum* collected from different areas of Ethiopia were sown at the start of the rains in plots measuring 2 x 1m arranged in a randomized block design with three replicates and a spacing of 25 cm between rows. Seeds were sown at the rate of 8 kg ha^{-1}. All plots were clean weeded as soon as weeds appeared. To boost phosphorus, all plots received diamonium phosphate (DAP) at a rate of 100 kg ha^{-1} six weeks after emergence. Agronomic and morphological attributes were measured when the plants reached 50% flowering (Table 1). A quadrate of 50 cm x 50 cm randomly selected from the plot cut at ground level was used for biomass determination. A sub sample of 200 g was taken and divided into two fractions; leaves and stems and oven dried at 60°C for two days. This sub sample was used to calculate dry matter yield and leaf: stem ratio. Twenty seven morphological and eight agronomic characters of accessions of *T. quartinianum* were measured (Table 1), on ten plants selected randomly from each of the three replicates.

Principal component analysis (PCA) and cluster analysis (CA) were applied for both morphological and agronomic traits data sets to group similar accessions. Correlations were calculated for each dataset and when the correlation coefficient (r) between two variables was greater than 0.7, then one of the two variables was included in the subsequent analyses of PCA and CA using Genstat Discovery Version Software. Analysis of variance was also carried out for both agronomic and morphological traits to determine if the accessions were significantly different ($P < 0.05$).

RESULTS

Environmental Conditions During the Study Period: The total rainfall recorded for the growing period, June-October, 2008 was 898 mm, which exceeded the 15-year, mean for that period by 176 mm. However, the June rainfall came two weeks later than expected and was below normal.

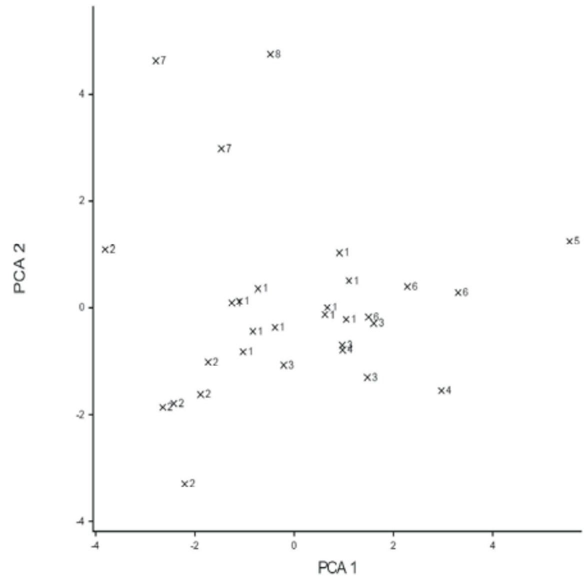

Fig. 1: Accessions grouped into eight morphological groups in a plot of the first principal component against the second principal component

Morphological Characters: Eight well-defined groups of *T. quartinianum* were identified using the first two components of the principal component analysis (PCA) and cluster analysis (CA) was truncated at 87% clustering threshold (Figures, 1 and 2). Groups one to eight comprised 35.3%, 17.6%, 11.8%, 14.7%, 2.9%, 11.8%, 2.9% and 2.9% of the total accessions, respectively.

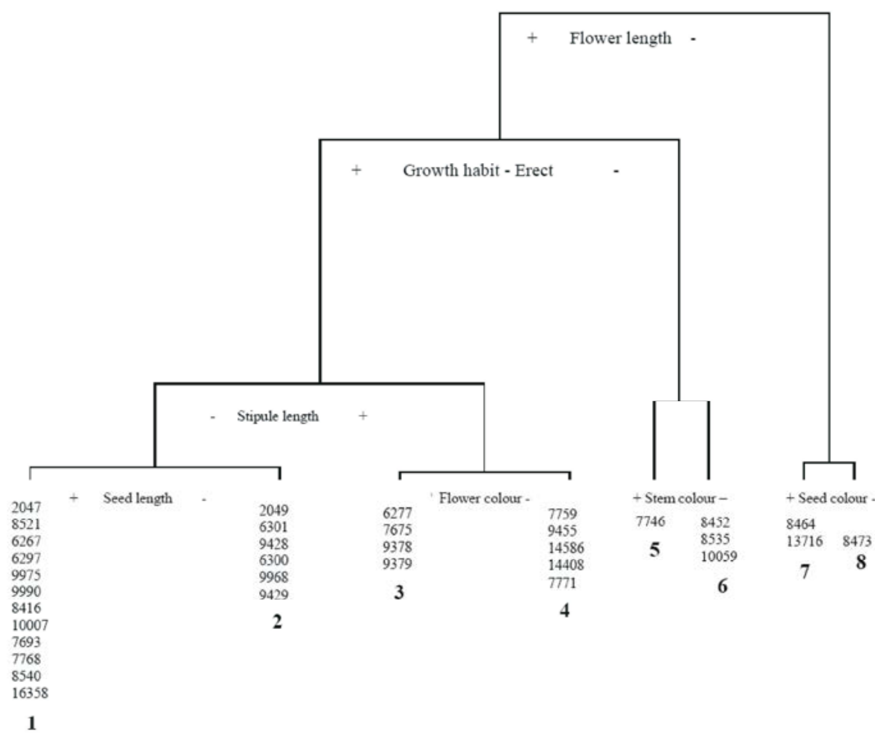

Fig. 2: Cluster analysis dendrogram with 19 morphological characters of 34 accessions of *T. quartinianum* based Euclidean distance

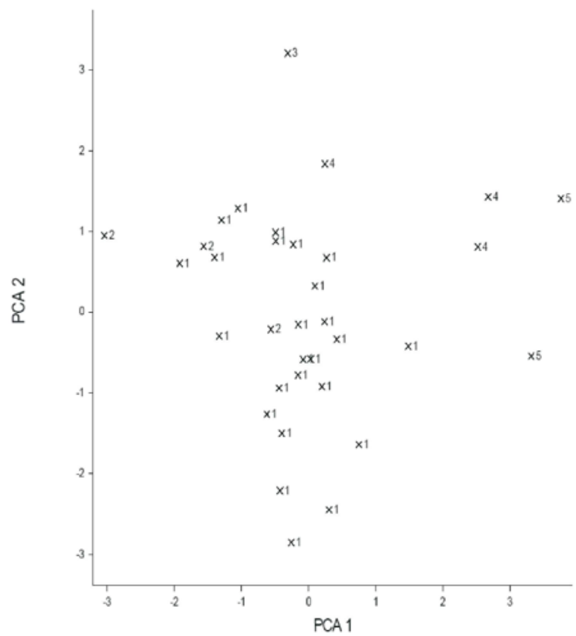

Fig. 3: Accessions grouped into four agronomical groups in a plot of the first principal component against the second principal component

The total variation explained by the first five respective PCA was 21.17%, 14.67%, 12.80%, 8.44% and 8.08%. According to McGarigal *et al.* [7] when correlation matrix is used in the principal component analysis, the latent vector coefficients are directly proportional to the correlations between the original variables and the principal components. In this study, correlation matrix was used. The trait flower length, growth habit, stipule length, seed length, flower colour, stem colour and stem colour contributed most to the separation of groups (Fig. 2).

Accessions in the eight groups showed diversity in morphological characters, however, all accessions were open in their growth pattern with soft and hairless stems. Group 1 comprised of 12 accessions (35.3 % of total collection) with average length of flower, stipule and seeds longer and thicker stem than accessions of group 2. Members of this group originated from varying altitudes, 1460- 2500 m above sea level, with varying rainfall, temperature and soil type. Group 2 comprised six accessions (17.6% of total collection) with the longer flower and petiole than accessions of groups 7 and 8. accessions of this group had grey brown seeds similar to accessions of group 3. Six accessions of this group originated from high altitudes areas (2270 -2450 m_ a_s_l) and only one from altitude similar to Debre Zeit, with varying rainfall from 1100 -1400 mm per year and varying temperature and soil type. Group 3 had four accessions comprising 11.8% of all accessions assessed with stem thickness similar to accessions of group four and seven and less than an accession of group 5. The stipule length was similar to members of groups 1, 2, 6 and 7 but less than groups 4, 5 and 8. Flower length was similar to group 1 but less than for accessions of groups 4 and 5 and more than the rest of the other groups. Accessions of this group originated from altitude of 1870 – 2400 m a s l and rainfall of 800 - 1300 mm per year and mean temperature of 16 – 19.2 °C. Group 4 had five accessions with stipule and flower longer than accessions of group 3. The flower colour was red purple. Accessions of this group originated from varying altitudes, temperature rainfall and soils. Group 5 had only one accession, ILRI 7746. This accession had thicker stem compared to members of group 6 and greater than accessions of group 1 except for longer inter nodes and long and narrow leaflets. Group 4 had only one accession with decumbent growth habit, slender stems, short petiole, fewer flowers, short flower and fewer seeds per pod compared to the accessions of other clusters.

Flower width, calyx lobe and pod length measurements were removed from the analysis because of high correlation with other traits. Flower width was highly correlated to flower diameter and flower length, calyx lobe with flower diameter and petiole length and pod length with pod width and number of seeds per pod.

Agronomic Characters: The scatter plot of the first principal component (PC) versus second and cluster analyses showed five distinct groupings for agronomic characters assessed (Fig. 3). Of the 34 accessions, the first group had 26 accessions, the second 3, the third 1, fourth 3 and the fifth 2. The variance expressed by the first four respective PCA of the total was 47.18, 27.80, 14.67 and 6.48. The characters plant height, time from planting to 50% flowering, yield and leaflet length contributed positively to the separation of the accessions into clusters (Fig. 4). The time from planting to 50% flowering and yield were the first two PC, accumulated 74.98 % of the total variance, relatively high value compared to those of morphological data. The accessions in group 1 flowered 16.1 weeks after planting, group two 17.4 and group three 15.9, group four 13.5 and group five 13.9 weeks after planting. The order of leafiness among the accessions in the groups was 4> 3>2 >1. The groups with more stems also flowered late compared with the leafy ones that flowered early.

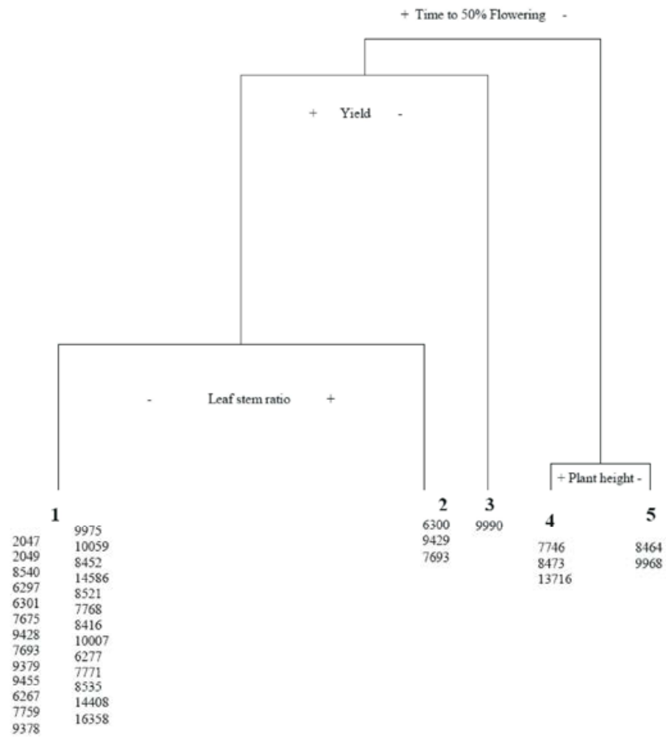

Fig. 4: Cluster analysis dendrogram with five agronomic characters of 34 accessions of *T. quartinianum* based on Euclidean distance

Leaf dry matter per day (Ldmd) and stem dry matter per day (Sdmd) were highly correlated to yield and plant height per day was correlated to plant height, hence these traits were not used in the analysis. Other characters that were dropped included: days to 75% flowering and leaf width because they were highly correlated to 50% flowering.

The environmental condition of the place of origin played a major role in the grouping of accessions. Accessions in group 1 which comprised 28.57 % of the total accessions originated from altitudes of 2300 -2500 masl with a seasonal temperature range of 15 – 16 °C, while those in group 2 that comprised of 54.28% of total accessions thrived well in medium altitude areas (1880 – 2010 m_a_s_l) and temperature of 19 °C.

DISCUSSION

The rainfall and temperature experienced during the growing period were suitable for growth and development, despite the fact that rainfall is always expected in mid June delayed to the last week of that month. Rainfall stopped early on October affecting yield of those accessions that flowered late.

Morphological and Agronomic Characters: Diversity was observed for morphology and agronomic traits among the 34 accessions of *T. quartinianum* assessed. All the accessions in this study were collected from different places in Ethiopia. According to Morris and Greene [8], sub-Saharan Africa serves as a native habitat for about 15% of the 225-250 species of *Trifolium* found worldwide. The majority of these are native to the Eritreo-Arabian Province primarily in the subalpine and alpine highlands of Ethiopia, Sudan, Eritrea, Kenya, Tanzania and Uganda. All are reported to show high levels of genetic diversity due to their wide geographic distribution [9]. However, *T. quartinianum* were collected from Ethiopia and may have a low level of genetic diversity.

The groupings of accessions according to morphological characters were skewed toward one large cluster comprising 85.7% of the accessions. The long flower and long petiole exhibited by this group has grazing and pollination implications. Long petioles display the leaf blades well above the terminal bud to form the upper canopy. At early stages, animals graze mostly leaf blades and petioles and may not remove the terminal bud allowing it to continue producing new re growth. Since nectar is secreted by the glands located at

Table 2: Agronomic characters of top10 best performing accessions based on yield Accession number

Trait	8535	16358	7771	6301	6300	10059	6277	14408	9378	8521	LSD
Days to 50% flowering	111	115	111	122	122	111	111	104	112	112	
Plant height (cm)	38	36.7	39.3	40.5	38.9	43	40.4	42	41	38	6.255
Yield, kg/ha	8667	8312	7463	6853	6816	6713	6698	6520	6508	6461	ns
Length of leaflet (mm)	52.4	56.3	72.6	44.1	53.4	67.7	60.9	60.6	63.1	61.5	7.891
Width of leaflet (mm)	12.8	13.4	13.7	15.6	17.1	11.5	19	14.8	15.1	15.8	2.304
Leaf to stem ratio	0.53	0.61	0.61	0.31	0.37	0.55	0.66	0.79	0.53	0.50	0.1696
Plant height per day cm/day	0.38	0.45	0.38	0.36	0.40	0.36	0.31	0.37	0.35	0.40	

the corolla tube's base, the length of the flower (corolla tube) is a determinant factor in the ability of bees and other insects to reach the nectar and thus a factor in pollination. Bees are likely to encounter problems when accessing nectar from members of this group compared to members of other groups. Accessions in this group were tall and erect growing, which means that they can be used for hay or silage because most of them were also high yielding. However, if these accessions can be grazed then proper grazing schemes should be employed to allow sufficient rest period and residual leaf area for carbohydrate production after grazing.

The results showed that accessions 8463, 8473 and 13716 have similar morphological and agronomic traits and, therefore, are closely related. These accessions were collected along pathways from the same district and animals may have been involved in their dispersal. Similar accessions may have been collected in different environments after dispersal from a common point. The pods and mature calyx of *T. quartinianum* tend to have a coarse surface and points which may attach to a passing animal and be transported to other places. Some seeds may be harvested as weeds together with those of crops and be distributed with the crop seeds.

The high yielding accessions are in agronomic group 1 and 2. Plants of these accessions were later flowering and also appeared to have good water use efficiency. In this study, the accessions were subjected to the same rainfall conditions and in limiting water conditions, those accessions that produced high yields used water efficiently.

Time to 50% flowering was an important and variable trait. This trait appears to be important in legumes and is highly variable in *Centrosema brasilianum* [10]. When 50% of the plants are flowering, both cell content and structural components are balanced. Accessions in group three (Fig. 4) flowered early and can be harvested at least four weeks before the late flowering ones in those areas where rainfall is not limiting. The longer growing period of late maturing accessions allowed them to accumulate more biomass. This phenomenon is explained by Freidrecks *et al.* [6], Kahurananga and Tsehay [11], who stated that yield differential of *Trifolium* is a function of length of growing period.

In this trial acession 6301, which is known to consistently produce high biomass [12], did not reach its potential (Table 2) because it was affected by moisture stress. However, the biomass accumulations of the top 10 accessions were not significantly different from each other (Table 2). Most of the late flowering accessions produced lower than expected yield compared to mid flowering accession because of early onset of the end of rains before flowering and this affected the yield drastically.

Plants of accessions with the highest yield flowered 111 days after planting while low yielding accessions flowered early, about 91days after planting. The normal height for *T. quartinianum* is 20-40 cm [9]. The groupings of accessions did not relate to the environmental conditions of collection site. According to Burlie *et al.* [13] information about germplasm collecting site is important because those conditions are associated with patterns of genetic variability.

CONCLUSION

There were eight morphologic and agronomic traits contributed most to the separation of accessions. Ten high yielding accessions are identified for altitudes in the range of 1700 to 2500 m above sea level. Most of the accessions showed similar in morphological characteristics.

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