Ethiop. J. Agric. Sci. 30(2) 81-88 (2020)

Agronomic Traits of *Trifolium* Species in Different Agro-ecologies of Benishangul-Gumuz Region

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አህፅሮት

ይህ ጥናት የትሪፎልየም ዝርያዎችን የመላመድ አቅመችዉን ለመገምገም በሰሜን ምዕራብ ኢትዮጵያ ቤንሻንጉል-ጉሙዝ ክልል በሁለት የግብርና ሥነ-ምህዳሮች ዉስጥ የተደረገ ጥናት ነዉ። ሙከራው የተካሄደው በአሶሳ የግብርና ምርምር ማዕከል በተመረጡ ከፍተኛ፣ የመካከለኛና ዝቅተኛ መሬት ግብርና ሥነ-ምህዳሮችን ለመወከል የተመረጡት በተከታተይ ቶንጎ፣ አሶሳ እና የካምሽ ንዑስ ምርምር ጣቢያዎች ነው። አምስት ትሪፎልየም ዝርያዎች ተገምግመዋል። የተቀናጀ ትንተና ውጤት እንደሚጠቁመው በዝርያዎች መካከል የደረቅ ምርት ልዩነት የጎላ እንደነበርና ከተገምገሙ ዝርያዎች መካከል T. quantinum 6301 ዝርያ ከፍተኛ የደረቅ ምርት ይሰጠል። ከአሶሳ እና ቶንጎ ይልቅ ከፍተኛው የደረቅ ምርት በካማሽ ተመዝግቧል። በአጠቃላይ ደረቅ ምርት ላይ የተመሠረተ መረጃ መሠረት በአሶሳ እና ቶንጎ T. quantinum 6301 ዝርያ እና በካማሽ T. steudenary 9720 ዝርያ በአንጻራዊ ሁኔታ የተሻሉ በመሆናቸው ለአካባቢያቸው እንደ አማራጭ አበዘር መኖ ሰብሎች ይመከራሉ።

Abstract

A study was conducted to evaluate Trifolium species for their agronomic traits in two agro-ecologies of Benishangul-Gumuz region, North Western Ethiopia. The experiment was carried out at Tongo, Assosa, and Kamash forage research stations of Assosa Agricultural Research Center, which were purposively selected to represent highland, mid and lowland agro-ecologies respectively. Five Trifolium genotypes (T. steudeneri 9720, T. decorum 9447, T. quantinum 6301, T. tembense 7102, and T. steudeneri 9712) were evaluated in a randomized complete block design with three replications. Plant height at forage harvesting, forage dry matter yield and leaf to stem ratio were significantly ($P < 0.001$) affected by environment (E) and G x E (G and E interaction). The highest mean forage dry matter yield was recorded at Kamash, than Assosa and Tongo. The result of combined analysis indicated that, genotype (G) had significant ($P < 0.001$) effect on forage dry matter yield and T. quantinum 6301 genotype was the best yielder among the genotypes evaluated. Total dry matter yield was significantly different ($P < 0.001$) among genotypes at Tongo and Assosa. Generally, based on forage dry matter yield data, T. quantinum 6301 was well performed at Assosa and Tongo than other genotypes, T. steudenary 9720 was relatively better adapted at Kamash, and they are recommended as an alternative legume forage crops for the respective locations.

Introduction

Improved forage crops play an important role in sustaining the livelihoods of small and medium scale farmers, mainly because of their positive effects on livestock production and contribution to economic and environmental sustainability. On the other hand, some natural pasture components grow fast and accumulate high proportion of fiber that reduces their intake and digestibility, which reduces their usefulness for livestock nutrition (Alemayehu, 2002). Indeed in Benishangul-Gumuz regional state (BGRS) finding adequate feed in dry season is a challenge that smallholder farmers are facing in the region due to the tall nature of the *Hypernia* species which dominate these grasslands. The quality of grasses generally declines as plants mature and become more fibrous with low crude protein, resulting in accumulation of poor quality biomass, which is slowly digestible and low in nutrients. This biomass is usually consumed by wild fire in dry season. This results in low productivity, long calving intervals, susceptibility to diseases and high livestock mortality. Alemayehu *et al*. (2017) indicated the possibility for conserving the available green feeds in rainy season as silage, but the protein content of the ensiled material was below the physiological requirement of animals. Thus, leguminous forages like *Trifolium* could be an important source of protein supplement to fill the gap in such livestock production systems.

There are 240 to 300 clover species of the genus *Trifolium* species are ubiquitous in natural grasslands in cooler climates (Allen and Allen, 1981). Although clovers rank second in forage productivity and feeding value to alfalfa (*Medicago sativa)* in western countries, few *Trifolium* species are cultivated due to their recent domestication (Davies and Young, 1967). Forty *Trifolium* species are reported to be found in Sub Saharan Africa (SSA) of which 25% are endemic to Ethiopia (Akundabweni, 1984b). Two-thirds of the clover species in Ethiopia are annuals and the remainder perennials; biennials are not found in Ethiopia (Akundabweni, 1984a; Getnet*et al*., 2006). The Ethiopian highlands are reported to be one of the world's most important centers of genetic diversity for clovers (Akundabweni, 1984b).

Clovers grow rapidly when the growing conditions are favorable and produce a large amount of dry matter. This can be conserved as hay, which can be used to increase the quality of straw-based diets and to overcome seasonal feed shortages (Akundabweni, 1984a). Some species grow naturally in valley bottoms which are not cultivated due to seasonal water logging. Thus, they can be managed to increase forage production without competing with food crops for land. Some of the African clovers are adapted to acid and low phosphorous soils. Similarly, clovers can be grown on land that would otherwise lie fallow as part of crop rotation system. Their ability to fix atmospheric nitrogen will help to improve the fertility of the soil and thus increase the yield of succeeding crop (Akundabweni, 1984a).

The cultivation of high quality forages with a high yielding ability, adaptable to biotic and abiotic environmental stresses is one of the possible options to increase livestock production under smallholder farmers conditions (Tessema, 1999). The performance of forage species vary across locations due to differences in soil types, temperature and amount and distribution of rainfall. Thus, testing the adaptability and yield potential of different forage crops across various agro-ecological zones is very important to identify the best-bet accessions for utilization. Therefore, the objective of this study was to evaluate the agronomic performance of *Trifolium species and* accessions under different environmental conditions of BGRS, Western Ethiopia, and to select those with better environmental adaptation and high dry matter yield.

Materials and Methods

Study area

The trial was conducted under field conditions at Assosa, Tongo, and Kamash forage research stations of Assosa Agricultural Research Center during the main cropping season. The test locations represent the highland, mid and lowland areas ranging in altitude from 1000 to 2200 meter above sea level. The farming system of the study areas is Agro-pastoral. Descriptions of the test environments are indicated in Table 1.

Table 1: Descriptions of the test environments for geographical position

Parameter	Study site		
	Tongo	Assosa	Kamash
Latitude	09.9°45'N	10°30'N	09. °30'N
Longitude	34°44'E	034°20'E	35°45'E
Altitude(m)	1600-2200	1500-1550	1000-1350
Annual rainfall(mm)	1316	1316	1150
Daily minimum Temperature (^{0}C)	17.5	16.75	25
Daily maximum Temperature (^{0}C)	28	27.9	30

Experimental treatments and design

The five genotypes of *Trifolium* species (*T. Steudenary* 9720, *T. Decorum* 9447, *T. quantinum* 6301, *T. tembense* 7102, and *T. steudenary* 9712) were collected from International livestock research institute (ILRI). The accessions were planted in 3m x 4m plot using a randomized complete block design with three replications at the beginning of the main rainy season. Seed was sown by drilling in rows spaced 30 cm between rows, at a depth of 3 cm. The total experimental area was 13 m × 26 m (338m^2) with individual plot size of 12m^2 and spacing between plots and replications of 1.5 and 2 m, respectively at each testing environments. The treatments were sown according to their recommended seeding rates of 3-5 kg/ha. The diammonium phosphate (DAP) and urea fertilizer were not applied.

Data collection

Data were collected on plant height at harvesting forage stage, leaf to stem ratio and forage dry matter yield. Plant height at forage harvesting was taken on six plants randomly selected from each plot and measured using a steel tape from the ground level to the highest leaf. For determination of biomass yield, genotypes were harvested at 10% blooming stage using a quadrant which has 1m^2 areas. Weight of the total fresh biomass yield was recorded from each plot in the field and 500 g sample was taken from each plot to the laboratory and a sub-sample of known fresh weight was oven-dried for 72 hours at a temperature of 65 ^{0}C to determine dry matter yield. Leaf to stem ratio was calculated from the dried weight of leaf and stem.

Statistical analysis

Analysis of variance (ANOVA) procedures of SAS general linear model (GLM) was used to analyses the quantitative data (SAS, 2002). LSD test at 5% significance was used for comparison of means. The data were analyzed using the following model:

Yijk = μ + Gi + Ej + GEij + Bk + e ijk

Where, Yijk = dependent variables,

μ = grand mean,

Gi = effect of genotype i,

Ej = effect of environment j, j = Assosa, Tongo and Kamash

GEij = is the interaction effect of genotype i and environment j

Bk = effect of block k, and

e ijk = random error effect of genotype i, environment j, interaction effect of genotype i and environment j, and block k.

Results and Discussion

Environment and interaction effect on performance of Trifolium species

Combined analysis of variance for measured agronomic traits of *Trifolium* species tested under three agro-ecologies of Benishangul-Gumuz regional state (BGRS) is presented in Table 2. The result showed that genotype (G), environment (E) and G x E interaction had significant ($P < 0.001$) effect on forage dry matter yield. The significant effect of G x E interaction on forage dry matter yield indicated inconsistency in the performance of the genotypes across the environments and the need for assessing performance in order to identify *Trifolium* genotypes with stable and superior yield across the environments. This implies genotypes selected for superior performance under one set of environmental conditions may perform poorly under different environmental conditions. In concurrence to the result of this study, Dixon and Nukenine (1997) also pointed out that interaction is a result of changes in a cultivar's relative performance across environments due to differential responses of the genotypes to various edaphic, climatic and biotic factors. Although, this variation might be due to, the difference among the agro-ecologies in altitude, soil types, temperature and differences in both amount and distribution of annual rainfall and other agro-climatic factors. The result of this study in line with the finding of Gezahagn *et al*. (2016) who reported that where environmental differences are greater, it may be expected that the G x E interaction will also be greater. Environments and G x E interaction displayed significant ($P < 0.001$) variations for plant height and leaf to stem ratio. Generally, the result of this study indicated that, environmental factors significantly influenced the yield performance and adaptation patterns of *Trifolium* genotypes. Therefore, evaluations of yield performance and adaptation patterns of *Trifolium* genotypes in multiple environments are very important step in agronomic evaluation and selection of better adapted and high yielding species and varieties.

Table 2. Combined analysis of variance for measured agronomic traits of *Trifolium* species tested across three locations/environments

Trait	Mean square		G X E	Mean	CV
	Genotype	Environment			
Plant height (cm)	ns	***	***	70.78	27.76
Forage DM yield (t/ha)	***	***	***	4.67	29.10
Leaf to stem ratio	ns	***	***	0.44	33.75

Leaf to stem ratio

The leaf to stem ratio at forage harvesting of *Trifolium* species tested under three agro-ecologies is indicated in Table 3. Leaf to stem ratio was significantly (P < 0.001) influenced by environment and highest value of leaf to stem ratio recorded at Tongo than Kamash and Assosa agro-ecologies. This result implies, highest leaf to stem ratio was obtained from the cooler condition and this might be due the function of the longer periods of physiological growth of plants in cooler environment with increase defoliation frequency stimulating leaf growth at the expense of stem production. The leaf to stem ratio has significant implications on the nutritive quality of the forage as leaves contain higher levels of nutrients and less fiber than stems. The result indicated that the leaf to stem ratio is an important factor affecting diet selection, quality and intake of forage (Smart *et al.*, 2004). The leaf to stem ratio is associated with high nutritive value of the forage because leaf is generally of higher nutritive value (Tudsri *et al.*, 2002) and the performance of animals is closely related to the amount of leaf in the diet. Therefore, according to the report of these authors the *Trifolium* genotypes grown at Tongo is more nutritious than *Trifolium* genotypes grown at Assosa and Kamash due to the leaf to stem ratio was higher at Tongo than Assosa and Kamash. Genotype had significant (P < 0.001) effect on leaf to stem ratio at Tongo and highest value recorded for *T. steudnery* 9712 than other genotype except *T. steudenary* 9720 (*T. steudenary* 9712 = *T. steudenary* 9720 = *T. tembense* 7102 > *T. decorum* 9447 = *T. quantinum* 6301). The result of combined analysis showed that leaf to stem ratio was not significant (P < 0.05) among the tested genotypes. Similarly, genotypes had no significant (P < 0.05) effect on leaf to stem ratio at Assosa and Kamash locations.

Table 3. Mean leaf to stem ratio of *Trifolium* species tested in three agro-ecologies of BGRS

Accession	Location/Environments			Combined analysis
	Assosa	Tongo	Kamash	
T. steudenary 9720	0.30	0.76^{ab}	0.26	0.38
T. decorum 9447	0.41	0.36^{c}	0.35	0.39
T. quantinum 6301	0.36	0.35^{c}	0.57	0.40
T. tembense 7102	0.36	0.67^{b}	0.43	0.44
T. steudenary 9712	0.34	0.79^{a}	0.32	0.43
Mean	0.35^{b}	0.59^{a}	0.39^{b}	0.41
CV	0.06	9.67	28.56	48.65
P-value	0.7069	0.0001	0.2036	0.9310

Plant height at harvesting stage

The plant height at forage harvesting stage of tested *Trifolium* species under three agro-ecologies of BGRS is indicated in Table 4. Plant height at forage harvesting was significantly (P < 0.001) different among locations and *Trifolium* genotypes planted at Kamash and Tongo had significant plant height than Assosa. This variation could be due to the differences in edaphic, climatic and biotic conditions of the location and genetic behavior of the tested genotypes. Mean plant height at forage harvesting of *Trifolium* genotypes were significantly (for Assosa P < 0.001; for Tongo P < 0.01) different at Assosa and Tongo. The highest mean plant height was obtained from *T. quantinum* 6301 and *T. steudenary* 9720 genotype at Assosa, while *T. quantinum* 6301 genotype produced the highest plant height at forage harvesting than other genotypes at Tongo. Taller plant heights in Kamash resulted in better biomass yields. This is due to the fact that longer plants possess relatively more leaves and branches that may result in increase in biomass yield. According to annual Basque Research (Cited in Agyeman et al., 2014), plants growing under water limiting conditions tend to grow taller in an effort to scramble for below nutrients around the growing environments. The result of combined analysis for plant height at forage harvesting indicated that there was nosignificant (P > 0.05) difference among the genotypes. The mean plant height at forage harvesting of *Trifolium* genotypes obtained at highland agro-ecology (Tongo) was longer than the mean value (32cm) reported by Tadesse *et al.* (2013) for *Trifolium* genotypes under the highland agro-ecologies of Holetta.

Table 4. Mean plant height at harvesting stage (cm) of *Trifolium* species tested in three agro-ecologies of BGRS

Accession	Location			Combined analysis
	Assosa	Tongo	Kamash	
T. steudenary 9720	59.40[a]	48.48[bc]	133.33	72.00
T. decorum 9447	42.30[b]	61.33[b]	105.11	58.67
T. quantinum 6301	66.01[a]	88.83[a]	81.00	73.57
T. tembense 7102	27.20[c]	38.33[c]	108.56	45.70
T. steudenary 9712	47.17[b]	38.50[c]	116.11	59.22
Mean	48.42[b]	55.10[b]	108.82[a]	61.83
CV	2.92	19.16	27.07	53.51
P-value	0.0001	0.0019	0.6341	0.1439

Forage dry matter yield

The forage dry matter yield of *Trifolium* species tested across three agro-ecologies of BGRS is indicated in Table 5. Forage dry matter yield of *Trifolium* genotypes significantly (P < 0.001) influenced by environment and *Trifolium* genotypes planted at Kamash was best yielder than Assosa and Tongo. This implies that Kamash location has better soil and climatic conditions for *Trifolium* growing than Assosa and Tongo locations. This might be attributed to the differences among the environments in altitude, soil types, temperature and variation in both amount and distribution of annual rainfall and other agro-climatic factors.Although, the result might be due to the forage dry matter yield is directly related to plant height at forage harvesting stage, because when the plant height at forage harvesting increases forage dry matter yield also increase. The result of combined analysis showed that forage dry matter yield was significantly different (P < 0.001) among the genotypes and this might be genetic difference of the *Trifolium* genotypes. Forage dry matter yield was significantly (P < 0.001) influenced by genotype at Assosa and Tongo and *T. quantinum* 6301 genotype gave the highest forage dry matter yield at both locations. Contrast to the result obtained at highland (Tongo), Tadesse *et al.* (2013) reported that *T. tembense* produced better yield (4.0 t ha^{-1}) than *T. quartinianum* (2.8 t ha-1) in the highland of Holetta. The difference in forage dry matter yield among reports could be attributed to the stage of maturity at the time of harvesting, management and effect of agro-ecologies. The overall mean (4.25 t ha^{-1}) for forge dry matter yield of the five *Trifolium* genotypes was greater than the overall mean value (2.5 t ha-1) reported by Tadesse *et al.* (2013) for tested *Trifolium* genotypes (*T. quartinianum, T. decorum, T. tembense, T. rueppellianum* and *T. steudenary*).

Table 5. Mean forage DM yield (t/ha) of *Trifolium* species tested in three agro-ecologies of BGRS

Accession	Location			Combined analysis
	Assosa	Tongo	Kamash	
T. steudenary 9720	3.86[b]	1.74[b]	6.98	4.06[a]
T. decorum 9447	3.64[b]	2.27[b]	5.52	3.75[a]
T. quantinum 6301	5.11[a]	4.88[a]	4.91	5.03[a]
T. tembense 7102	1.49[c]	0.93[c]	6.16	2.31[b]
T. steudenary 9712	3.99[ab]	0.95[c]	6.17	3.82[a]
Mean	3.62[b]	2.16[c]	5.95[a]	4.25
CV	0.27	18.24	17.79	47.94
P-value	0.0001	0.0000	0.2611	0.0004

Conclusion

From this study it can be concluded that, the overall performance of all *Trifolium* genotypes was better at lowland agro-ecology than mid and highland agro-ecologies and this indicating that *Trifolium* expressed its genetic potential under lowland altitude than mid and highland altitude in Benishangul-Gumuz region state. Genotype *Trifolium quantinum* 6301 was well performed at Assosa and Tongo than other genotypes and genotype *Trifolium steudenary* 9720 was relatively better adapted at Kamash. Thus these genotypes were recommended to the specific locations. In general further works should be done on performance of animals fed these genotypes to reach firm recommendations.

Acknowledgements

The authors are grateful to Ethiopian Institute of Agricultural Research for financial support and Assosa Agricultural Research Center staffs specially Livestock Research Process technical and field assistance for their honesty and hearty assistance.

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