Adaptability, forage yield and nutritional quality of alfalfa (*Medicago sativa*) genotypes

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**ABSTRACT**

Livestocks fed with high-quality forage deliver better milk and meat, which could benefit the nutritional health of their keepers and consumers. Six alfalfa genotypes were assessed across four locations, Arba Minch, Areeka Mante Dubo, Bonga and Hawassa Dilla, for adaptability, forage dry matter (DM) yield, crude protein (CP) concentration, nutrient composition and digestibility characteristics from August 2016 to January 2019. The genotypes were evaluated in a randomized complete block design with four replications. Plant height at forage harvest was significantly (P < 0.001) varied across the environment among genotypes. Nutrient components, such as neutral detergent fiber, acid detergent fiber and lignin, hemicelluloses, in vitro DM digestibility and relative feed value, were significantly (P < 0.01) different for genotype variations. The highest mean DM yield was recorded for FGI-9001 followed by FGI-3054. The highest plant height was recorded for FGI-8091 (60.13 cm), while the shortest genotype was FGI-1011. CP yield was recorded above the threshold level for all genotypes in the test. Highest in vitro DM digestibility and relative feed value with the lowest neutral detergent fiber, acid detergent fiber, and lignin and cellulose contents were recorded for FGI-9001. Tested alfalfa genotypes varied in terms of forage yield and nutritional quality at different locations. Thus, the result indicates the potential of genotypes, indicating the promotion to advanced varietal evaluation stages including animal test and release as better alternatives for use in the farming system in rain fed condition and irrigation.

1. Introduction

Livestock production is one of the major components of agriculture in Ethiopia, and it is highly dependent on the quantitative and qualitative adequacy of feed resources (Mengistu et al., 2017). According to the central statistical agency (CSA, 2018), the total livestock population in Ethiopia is estimated to be 193.23 million units, excluding beehives, with cattle making up 60.39 million, sheep 31.30 million, goats 32.74 million, horses 2.01 million, donkeys 8.85 million, mules 0.46 million, camels 1.42 million and poultry 56.06 million. The livestock sector contributes 15–17% to Ethiopia’s GDP, 35–40% to agricultural GDP and 37–87% to household incomes (GebreMaiam et al., 2013). To reduce the nutritional constraints on livestock productivity, the use of adaptive, high yielding, and drought-tolerant improved forages of high quality is recommended (Mengistu et al., 2017).

Alfalfa (*Medicago sativa* L.) is characterized by its ability to produce forage every 35–40 days by storing energy in the crown to support re-growth after cutting (Undersander et al., 2011). It adds nitrogen to the soil by symbiotic nitrogen fixation in bacterial nodules on the roots, and can also withstand long periods of water deficit by halting its vegetative growth and accessing water from greater depths through its deep root system (Annicchiarico & Pecetti, 2010). These properties make alfalfa among some of the most widely grown forage crops in the world. The crop can produce 24 t/h dry matters annually and it is estimated to be 25% more than pasture (Richard, 2011). Evidences indicated that feeding alfalfa improves productivity of animals; particularly, milking cows supplemented with alfalfa could produce over 50–100% additional yield. A practical example is that a Horro cow is reported to produce 1.7 liters of milk on average at peak lactation, while it gives 4 liters of milk when fed and managed properly (Tolera et al., 2012). There are various genotypes of alfalfa...
selected for winter hardiness, drought resistance, tolerance to heavy grazing, pests and diseases (Frame, 2005). While selecting alfalfa genotypes in Ethiopia, it is important to focus on the environmental adaptation, herbage dry matter yield potential and seed bearing ability of the genotypes. Thus, this project was primarily designed to evaluate the yield and nutritional quality of six alfalfa genotypes introduced by the Forage Genetics International plc (FGI), USA and planted in Southern Ethiopia.

2. Materials and methods

2.1. Description of the study site

Adaptability, forage yield and nutritional quality of six alfalfa genotypes were evaluated from August 2016 to January 2019 at Arba Minch, Areka Mante Dubo, Hawassa Dilla and Bonga in Southern Ethiopia, under rain fed condition. The materials were planted in August 2016 in all locations and evaluated through 2017 and 2018 for yield and nutritive values and extended to 2019 for some rows disease occurrence. Arba Minch center is located at 6°06’47″N and 37°35’10.5″E at an altitude of 1206 metres above sea level. The texture of a composite soil (0–30 cm) sample collected from the experimental site was a sandy loam with pH 6.2, available phosphorus 14.47, total nitrogen 0.29, organic carbon 1.19 and organic matter 1.63. The pH of experimental soil is within the range for productive soils (FAO, 2000). The soils at ArbaMinch location were medium in available phosphorus (Mamo et al., 1991), medium (Landon, 1991) to high (Mamo et al., 1991) in N fertility class and in the medium range for organic carbon (Herrera, 2005) which is satisfactory for good growth and yield for alfalfa. Mante Dubo research substation, where the experiment was conducted, in Areka agricultural research center is located at 6°4′N, 37°41′E and an altitude of 1772 meter above sea level. The soil at the center is formed from pyroclastic rocks and is clay in texture (Esayas, 2003). Dilla is located at 6°43’N and 38°44’E at an altitude of 1470 meter above sea level. The site is characterized by wet/moist Kolla and highly recognized by its agro-forestry cropping system (Worku et al., 2014). Bonga research center is located at 7°19’N, 36°13’E and 1723 meter above sea level. The climatic data including rainfall, maximum and minimum temperature of four locations during four (2016, 2017, 2018 & 2019) years are presented in Figure 1.

2.2. Experimental design and treatments

Six alfalfa genotypes (FGI-8091, FGI-3054, FGI-1011, FGI-5282, FGI-0916 and FGI-9001) were laid out in a randomized complete block design with four replications in 4×4m (16 m²) plots with 20 cm row spacing. The seeds were drilled in rows during the main cropping season at four experimental locations. All recommended field management practices were applied to all plots in a similar manner. Fertilizer is applied at planting in the form of NPS compounds with a rate of 19%N, 37%P2O5, 7% S.

Data collection

Days to 50% flowering, plant height, tiller number per plant data collection were undertaken using two central rows. A sample of 300 gram was collected from each plot and taken to the laboratory to estimate the dry matter yield and nutritional quality of the genotypes. Relative feed value (RFV) was calculated using the formula RFV = DDM (% DM) x DMI (%BW)/1.29 (Uttam et al., 2010), where the digestible dry matter (DDM) derived from acid detergent fiber (ADF) and the dry matter intake (DMI) as a percent of the body weight) derived from neutral detergent fiber.

Figure 1. Rainfall, maximum and minimum temperature of the experimental locations during 2016, 2017, 2018 and 2019.
(NDF). DDM = 88.9-(0.779*ADF) and DMI = 120/NDF. Hemicellulose and cellulose contents of the genotypes were calculated using the formula NDF minus ADF and ADF minus acid detergent lignin (ADL), respectively. Crude protein yield and DDM yield were calculated using the formula CPY = CP% x DMY (t/ha) and DGY = IvDMD % x DMY (t/ha), where CPY is crude protein yield, CP% is crude protein content in percent, DMY is dry matter yield in ton per hectare, DGY is digestible yield, IvDMD% is in vitro dry matter digestibility content in percent.

2.3. Statistical analysis

R software (R Core Team, 2017) package was used to compute analysis of variance (ANOVA) and means of significant variation was separated using least significance difference (LSD) at 5% probability level.

For forage CP and digestible DM yield measurements, genotypes, year, location and their interactions were considered as independent variables in the model indicated as:

\[ Yijkl = \mu + Gi + Ej + Yk + (Gi \times Ej \times Yk) + Bl(j) + eijkl, \]

where: Yiijkej = response variable; 
\( \mu \) = overall mean; 
Gi = genotypic effect; 
Ej = environmental effect; 
Yk = year effect; 
Gi \times Ej \times Yk = interaction effect of genotype, environment and year; 
Bl(j) = block effect; and 
eijkl is the random error.

For quality traits, since only a composite sample per treatment was taken from each location, location was considered as a replicate, and hence the data were subjected to the following model:

\[ Yij = \mu + Gi + Ej + eij, \]

where: Yi refers to the response of forage quality traits; 
\( \mu \) = overall mean; 
Gi = effect of genotypes i; 
Ej = environmental effect (replicate); and 
eij is the random error.

3. Results and Discussion

3.1. Rainfall and temperature during production period

Years of study were different both in the amount and distribution of precipitation and in the minimum and maximum temperatures. The study materials were planted in August 2016 and the data collection was begun in 2017 through 2018, and January 2019 was added for finalizing the disease occurrence. Planting year 2016 and production year 2017 were characterized with the highest precipitation (Arba Minch 750–1000 mm, Bonga >2000 mm, Hawassa Dilla >1500 mm and Areka Mante Dubo>1250 mm) and 2018 and 2019 with lowest <250 mm (Figure 1). During the same period, the minimum-to-maximum average temperature was ranging from 20°C to 30°C at Arba Minch, 15°C to 20°C Bonga, 14°C to 25°C Hawassa Dilla and 16°C to 27.5°C at Areka ManteDubo experimental locations. Alfalfa production mainly depends on the moisture condition and optimum temperature requirements. This was observed in dry matter yield (t/ha) dropping from 12.88 in 2017 to 5.19 in 2018 (85.2%) at ArbaMinch, from 5.13 in 2017 to 3.25 in 2018 (47.7%) at Bonga and from 13.48 in 2017 to 2.49 in 2018 (136%) at Areka Mante Dubo.

3.2. Plant height and forage yield at harvest

The mean value of plant height for all locations in all rounds of forage harvesting stage is presented in Table 2. The overall mean plant height was ranging from 56.14 to 60.13 cm. The highest \( (p < 0.05) \) plant was FGI-8091 with the height of 75.14 cm at Arba Minch, whereas the shortest genotype was FGI-0916. At Areka, the tallest plant was FGI-0916 (65.97 cm), while the shortest was FGI-9001 (49.98 cm). The tallest and shortest genotypes at Bonga and Hawassa were FGI-8091 (71.4 cm), FGI-5282 (63.9 cm) and FGI-1011 (36.95 cm) and FGI-0916 (-30.88 cm), respectively. Variation among tested genotypes in plant height could be due to genetic and environmental factors, such as soil characteristics, moisture conditions, temperature, humidity, pest and disease occurrence, and management of the trial field. Environmental factors influencing the growth of crops at various stages of development (Fahad et al., 2017) were mainly due to the wider response of genotypes to the environment. The consistency of genotypes across environments enables breeders to make effective evaluation of genotypes with minimum cost in a few environments (Adebola et al., 2018). In this regard, the present study showed consistency in plant height for genotype FGI-8091 followed by FGI-3054 and FGI-0916. Genetic variation among genotypes in the trial, response of genotypes to environmental factors and their interactions could be major reasons for the variation of plant height in the current study, and it was previously reported for different genotypes of alfalfa in which the current average result lay in the range (Kebede et al., 2017; Wayu & Atsbha, 2019).

Selection of better yielding genotypes in one environment may not enable identification of genotypes that
can repeat nearly similar performance in other environments (Kebede et al., 2017). Mean dry matter yields of alfalfa genotypes across locations over the years are presented in Table 3. The mean value was ranging from 6.49 to 7.87 t/ha in which a higher dry matter value was recorded for FGI-9001 followed by FGI-3054. The result also showed that the highest alfalfa dry matter yield was recorded at Arba Minch in 2017 compared to other testing sites with the lowest yield at Dilla. The minimum yield in 2018 over locations may be due to lower moisture condition (Figure 1) and that indicated 2017 was better in climatic requirements for Alfalfa production. The dry matter yield variability at different locations in the present study may be due to the variation in climatic condition and genotype effect. The noted low yield at Hawassa Dilla was due to disease incidence (Figure 2). The average dry matter yield of 7.01 t/ha recorded in the present study was by far better than some national and international reports (Awad & Bakri, 2009; Afsharmanesh, 2009; Befekadu & Yunus, 2015; Gashaw et al., 2015). Growth stage, cut number, moisture conditions at harvest and processing method are the most important causes of variation for yield of alfalfa (Veronesi et al., 2010). Maximum yield on alfalfa is achieved at reproductive maturity when the nutritive value of the forage is at a minimum (Collins & Fritz, 2003). The yield of the present study was better than alfalfa national average dry matter yield (3–5 t/ha) over the main cropping season, according to crop variety registration issue number 19 (MOANR, 2016).

The crude protein yield, digestible yield and RFV of the tested alfalfa genotypes are presented in Table 4. Crude protein yield variation (p < 0.05) among genotypes was ranging from 2.4 to 2.91 t/ha. FGI-9001 obtained higher crude protein yields than its counterparts in the test. The digestible yields variation (p > 0.05) ranged from 2.11 to 2.7 with a mean of 2.64 t/ha. The highest FGI was for FGI-9001 (211.2) followed by FGI-8091 (192.1) and FGI-0916 (193.3), whereas the lowest RFV was for genotype FGI-1011 (159.1). Recorded variation (p < 0.05) among alfalfa genotypes for RFV was ranging from 159.1 to 211.2 with the mean value of 184.85. RFV reveals the potential intake and fiber digestibility of legumes (Undersander & Moore, 2002) and reflects how well an animal will eat and digest a particular forage species when it is fed as the only source of energy (Kazemi et al., 2012). The RFV index observed for the cultivars evaluated was higher than a threshold level (RFV = 151) (Redfearn & Zhang, 2011) and by far better than the result reported before (Gashaw et al., 2015; Geleti et al., 2014; Kebede et al., 2017). Animal feed with a higher RFV index of more than 100 is considered to be a higher quality feed.

Days to flowering and tiller number per plant are presented in Table 1. Days to 50% flowering which was not significantly (P > 0.05) varied among genotypes over year across locations ranged from 94.38 to 96.25. Tested genotypes also did not show significant variations in tiller number per plant with a mean value of 9.55.

Figure 2. Disease effect on dry matter yield of alfalfa planting in August 2016.
Table 1. Mean values of days to 50% flowering (DTF (50%)) and tiller number per plant (TNPP) of different locations planting in August 2016

| Genotype   | 2017  | 2018  | Mean  | 2017  | 2018  | Mean  |
|------------|-------|-------|-------|-------|-------|-------|
| FG1-0916   | 94.98 | 96.27 | 95.62 | 5.87  | 12.75 | 9.31  |
| FG1-1011   | 94.98 | 95.02 | 95   | 6.87  | 13.88 | 10.38 |
| FG1-3054   | 94.98 | 93.77 | 94.38 | 5.87  | 11.75 | 8.81  |
| FG1-5282   | 94.98 | 95.02 | 95   | 6.62  | 14.25 | 10.44 |
| FG1-8091   | 93.73 | 98.77 | 96.25 | 5.37  | 13.25 | 9.31  |
| FG1-9001   | 93.73 | 95.02 | 94.38 | 6.37  | 11.75 | 9.06  |
| Mean       | 94.56 | 95.65 | 95.1  | 6.16b | 12.94a| 9.55  |

CV% = 2.8, LSD_{0.05} variety (V) = NS, Year (Y) = NS, VxY = NS

Table 2. Mean plant height variation of alfalfa genotypes across testing locations planting in August 2016

| Genotype     | Arba Minch | Areka | Bonga | Hawassa | Mean |
|--------------|------------|-------|-------|---------|------|
| FG1-9001     | 73.83 b    | 69.77 abd | 69.77 abcd | 63.24  | 61.58 |
| FG1-3054     | 71.76 abc  | 68.19  bcd | 68.19  bcd | 59.41  | 59.14 |
| FG1-5282     | 71.43 abc  | 63.74 a | 63.24 b | 58.49  | 58.49 |
| FG1-8091     | 75.14 a    | 71.44a b | 71.44a b | 60.13  | 57.54 |
| FG1-1011     | 68.41 bcd a| 57.02 d | 57.02 d | 57.19  | 57.19 |
| FG1-9016     | 71.47 abc  | 65.97 c | 67.07  cd | 58.84  | 58.84 |
| Mean         | 72.01      | 59.45 | 68.49 | 33.51  | 58.36 |

LSD_{0.05} G = NS, E = NS, GxE = 6.41, CV% = 7.8, P < 0.0001

Table 3. Mean dry matter yield (DMY t/ha) variation of alfalfa genotypes over year across locations planting in August 2016

| Genotypes | Arba Minch | Bonga | Areka | Hawassa | Genotype | Mean |
|-----------|------------|-------|-------|---------|----------|------|
| FG1-0916  | 10.18      | 4.43  | 7.31  | 5.08    | 3.53     | 3.99 |
| FG1-1011  | 12.30      | 4.97  | 8.64  | 5.03    | 2.99     | 3.71 |
| FG1-3054  | 13.69      | 5.17  | 9.43  | 5.05    | 3.31     | 4.17 |
| FG1-5282  | 12.80      | 5.41  | 9.10  | 5.25    | 3.22     | 3.85 |
| FG1-8091  | 12.12      | 5.45  | 8.79  | 5.02    | 3.12     | 3.81 |
| FG1-9001  | 16.19      | 5.68  | 10.94 | 5.38    | 3.33     | 4.11 |
| Environment Mean | 12.88 | 5.19 | 9.03 | 5.13 | 3.25 | 3.94 |
| LSD_{0.05} | 1.41      | NS    | 1.04  | NS      | NS       | NS   |
| CV%       | 13.4       | 24.1  | 20.22 | 7.9     | 19.6     | 14.85 |

4. Conclusion

Six alfalfa genotypes evaluated for adaptability, dry matter yield and nutritional composition in four locations of Southern Ethiopia during August 2016 to January 2019. Alfalfa genotypes in the test were best adapting the locations through the undulating condition of rainfall and temperatures (minimum and maximum) to produce optimum dry matter content of the presented alpha genotypes ranged from 300.8 to 400.5 g/kg dry matter with a mean value of 369.6 g/kg dry matter. It was reported that high-quality alfalfa contains >190 g CP/kg dry matter, <310 g ADF/ kg dry matter, 400 g NDF/kg dry matter and >151 RFV (Redfearn & Zhang, 2011). Variation in the nutritive chemical composition of alfalfa could be due to harvesting management, genotype and harvesting frequency (Wwayu & Atsghha, 2019). The genotypes in the trial showed supreme crude protein content compared with the threshold level (Geleti et al., 2014; Kebede et al., 2017) and over qualifies the standard (CP>190 g/kg DM) for making high-quality alfalfa hay for livestock and poultry according to the USDA standard.

CP = crude protein, NDF = neutral detergent fiber, ADF = acid detergent fiber, IVMD = in vitro dry matter digestibility, Hecell = hemi-cellulose, cell = cellulose, LSD = least significant difference, CV% = coefficient of variation, NS = not significant, means with common letter superscripts in row are not significantly different (P > 0.05)
yield. Six alfalfa genotypes could be competent for quality supply in Ethiopian crop livestock farming system feed requirement and could qualify the nutritional demand of animals in the country. Better in vitro dry matter digestibility result is observed and it improves the low-quality feed of the area. Among six materials, FGI-9001 genotype was found to be better in all parameters in this study and recommended to be produced in testing environments and similar agro-ecologies. The materials were found to be best fit to adapt to the area and promising to be promoted to advanced varietal selection and animal test for the production in rain fed condition and better to check in irrigation supplements.

**Acknowledgements**

The authors are thankful to the Forage Genetics International (FGI) USA for their financial and seed support in implementing the study. Southern Agricultural Research Institute for financial and the technical assistants of all testing locations are also acknowledged for their support during the field research work. The technical assistants at the nutrition laboratory of the National Veterinary Institute and Hawassa College of Agriculture are also acknowledged for their support in the chemical analysis of the feed samples.

**Disclosure statement**

The authors have no conflict of interest.

**Funding**

This study was supported by Forage Genetics International PLC (FGI), USA

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**Public interest statement**

Adaptability, forage yield and quality evaluation of six alfalfa genotypes in southern Ethiopia is crucial to identify optimum yielding quality forages for the contribution of feed supply in the local and regional level. It was identified that the feed utilization practices in Ethiopia mainly depending on open grazing (55.23%) with improved forages only 0.67%. Public report on feed inventory and feed balance in Ethiopia showed negative balance on feed dry matter, feed metabolizable energy and feed crude protein supply. Thus, this paper highlights the information on some special materials with high quality and optimum dry matter yield among six alfalfa genotypes. And also the paper points the importance of including these materials in crop livestock farming system of Ethiopia to achieve the set goals in terms of animal feed supply in quality and quantity.

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**Table 4.** Mean crude protein yield (CPY t/ha), digestible yield (DGY t/ha) and relative feed value (RFV) of alfalfa genotypes planting in August 2016

| Genotype   | CPY | DGY | RFV     |
|------------|-----|-----|---------|
| FGI-0916   | 2.40 | 2.48 | 193.3<sup>b</sup> |
| FGI-1011   | 2.45 | 2.11 | 159.1<sup>d</sup> |
| FGI-3054   | 2.70 | 2.6  | 184.4<sup>bc</sup> |
| FGI-5282   | 2.60 | 2.7  | 168.9<sup>b</sup> |
| FGI-8091   | 2.48 | 2.65 | 192.1<sup>b</sup> |
| FGI-9001   | 2.91 | 2.37 | 211.2<sup>a</sup> |
| Mean       | 2.59 | 2.64 | 184.85   |
| LSD<sub>0.05</sub> | 0.35 | NS   | 22.68    |
| CV%        | 16.46 | 16.06 | 8.15     |

**Table 5.** Ash, crude protein (CP), fibers (ADF and NDF), lignin (ADL) and in vitro dry matter digestibility (IVDMD) of alfalfa genotypes in gram per kilogram dry matter planting in August 2016

| Genotype | Ash  | CP  | NDF  | ADF  | ADL  | IVDMD | Hecell | Cell |
|----------|------|-----|------|------|------|-------|--------|------|
| FGI_9001 | 128.9| 300.8| 326.9| 192.6| 51.3| 873.6<sup>a</sup>| 134.3<sup>b</sup>| 141.3|
| FGI_5282 | 126.5| 400.5| 389.4<sup>ab</sup>| 236.1<sup>ab</sup>| 65.3<sup>ab</sup>| 839.8<sup>ab</sup>| 153.3<sup>b</sup>| 170.8|
| FGI_8091 | 131.7| 398.1| 349.8<sup>bc</sup>| 216.4<sup>c</sup>| 52.9<sup>cd</sup>| 849.5<sup>bc</sup>| 133.5<sup>b</sup>| 163.4|
| FGI_1011 | 122.2| 403.1| 408.5<sup>bc</sup>| 250.2<sup>d</sup>| 70.5<sup>d</sup>| 828.6<sup>bc</sup>| 158.3<sup>b</sup>| 179.7|
| FGI_3054 | 130.3| 355.1| 359.8<sup>bc</sup>| 229.5<sup>ab</sup>| 54.3<sup>cd</sup>| 819.2<sup>c</sup>| 130.3<sup>b</sup>| 175.2|
| FGI_0916 | 132.4| 360.1| 345.1<sup>bc</sup>| 222.4<sup>b</sup>| 61.2<sup>bc</sup>| 751.7<sup>bc</sup>| 122.7<sup>b</sup>| 161.2|
| Mean     | 128.7| 369.6| 363.3| 224.5| 59.3| 827.1<sup>b</sup>| 138.7| 165.3|
| LSD<sub>0.05</sub> | NS   | NS   | 39.26| 23.53| 8.63| 46.74| 2.9 | NS  |
| CV%      | 4.72 | 13.58| 7.71 | 6.95 | 9.67| 3.75 | 14.09 | 7.75 |
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